Chapter 14
An Overview of the Epigenetic Landscape of the Male Germline

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Abstract The challenges faced in agriculture for improving crop yield and overcoming natural barriers are becoming more complex due to environmental changes and population growth. Improving agriculture will, in many ways, require a better understanding of the genetics and epigenetics behind plant adaptation and inheritance of desirable traits. In order to do so, it is essential to understand the mechanisms of germline regulation. The epigenetic mechanisms that orchestrate chromatin remodeling include DNA methylation, histone modifications, and small RNAs that act in synergy to modulate gene expression and regulatory elements. In pollen, these mechanisms are still poorly understood, but nevertheless, are coming to light.

14.1 Introduction

For centuries, crop improvement has been one of the crucial goals for humanity survival. Due to climate change and the growing rate of human population, better and faster strategies for increasing crop yield are necessary to feed human populations for the years to come. Crossing plants for acquiring desirable traits has been the main strategy to accomplish this task. As breeders select desirable phenotypes and not the type of underlying molecular variation, these traits can be either genetic or epigenetic (Springer 2013).

Another approach used by breeders is to introduce new alleles through mutagenesis or transgenic modification. Additionally, epigenetic mechanisms can shape transgenic performance, either by silencing inserted transgenes or by modulating the epigenetic status of a particular gene; consequently, all these strategies could be used to acquire desirable traits (Springer 2013).

In flowering plants, gametes develop within the floral primordia that arise from postembryonic stem cells of the shoot and floral meristems, keeping some undifferentiated cells from early embryogenesis until floral determination

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(Feng et al. 2013). Additionally, the floral primordia have the ability to generate somatic tissues, such as leaves and somatic branches. Consequently, plant germ cells might be exposed to somatic modification and transmit these somatic marks to the next generation (Feng et al. 2013; Schmidt et al. 2015).

The paternal germline derives from a pollen mother cell (PMC) that undergoes two divisions, meiosis I and meiosis II, resulting in four haploid microspores (Fig. 14.1). An additional asymmetric mitotic division subsequently results in the

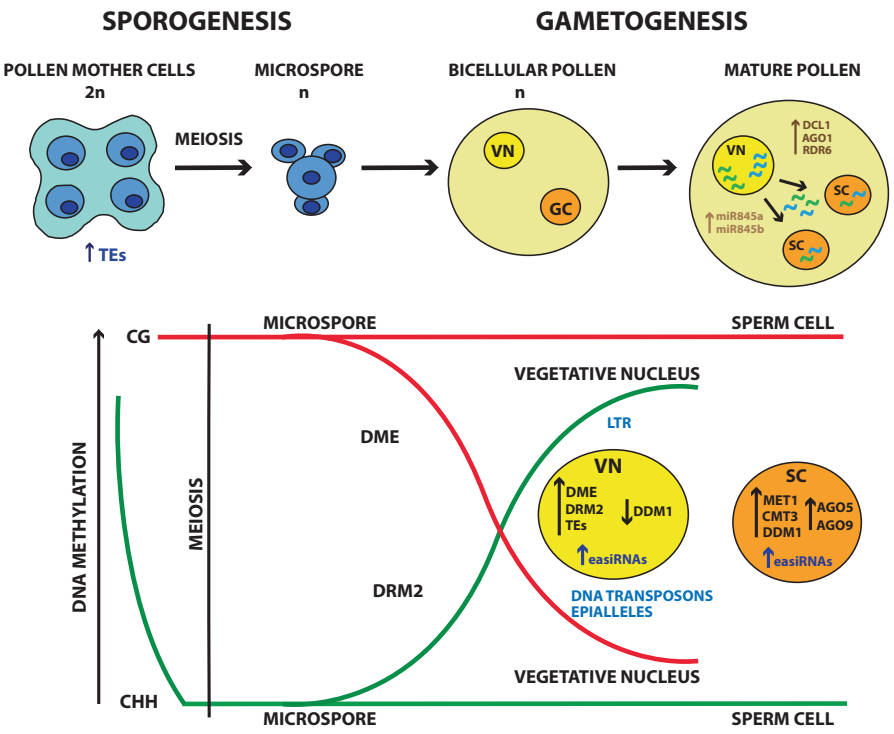

Fig. 14.1 *Arabidopsis thaliana* male gametogenesis epigenetic reprogramming. In the flower, the PMC is produced in a position-dependent manner from somatic cells in the male reproductive tissues. Meiosis takes place and generates microspores; the microspore undergoes asymmetrical division to give rise to the vegetative nucleus (VN) and the generative cell (GC). The GC divides again to create two sperm cells (SC), which leads to the mature pollen. The mCHH levels decline from the microspore to the SC; nevertheless, the level of mCG remains stable after meiosis, which is consistent with the expression of genes such as *MET1*, *CMT3,* and *DDM1* involved in DNA methylation. Transcription of TEs started to accumulate in the VN but not in SC. The VN loses mCG and restores mCHH especially at LTR retrotransposons. In the mature pollen, miR845a and miR845b play role in the biogenesis of 21 and 22nt easiRNAs by targeting retrotransposons. *DCL1*, *AGO1*, and *RDR6* are up-regulated, as well as *AGO5* and *AGO9* are also up-regulated in mature pollen. easiRNAs accumulate in SC at the same time that VN losts heterochromatin and TEs start to reactivate, due to the activity of DDM1 and DME. easiRNAs are generated in the VN and travel to the SC, where they target TEs, also 24-siRNAs from transposable elements flanking imprinted genes accumulate in SC

formation of the generative cell (germ cell—GC) and vegetative cell (VC) that exit the cell cycle in G_0. The generative cell divides again, producing isomorphic sister sperm cells (SC) enveloped in the cytosol of the larger vegetative cell (Fig. 14.1). The vegetative cell, a terminally differentiated cell type, eventually undergoes directional growth to form the pollen tube. The pollen tube is a morphological feature that guides the delivery of both sperm cells to the ovule, where double fertilization of the egg and central cell gives origin to the developing embryo, and endosperm, respectively. The central cell is diploid, hence the endosperm is a triploid extra-embryonic tissue where gene imprinting and dosage occurs, processes required for proper seed development (McCormick 1993; Berger and Twell 2011).

Chromatin remodeling is the dynamic process by which chromatin structure is modified restricting or allowing access to genomic DNA and regulatory elements, and thereby controlling gene expression. Epigenetic modifications affecting chromatin properties include DNA methylation, histone modifications, and chromatin modifiers, as well as microRNAs (miRNAs) and small interfering RNAs (siRNA). Epigenetic variation in plants can be inherited by the next generation through germline transmission, leading to phenotypic effects (Jablonka and Raz 2009). During male gametogenesis there is a decrease in global gene expression, at the same time pollen-specific transcripts raise, somatic transcripts are selectively silenced, possibly due to miRNA activity (Honys and Twell 2004). Moreover, functionally different transcripts arise from the vegetative nucleus (VN) and sperm cells, while VN is enriched with pollen tube growth and pollen germination transcripts (Pina et al. 2005), the SC undergo a long DNA replication phase that last until fertilization, with the predominance of transcripts dedicated to DNA repair, cell cycle transition, and ubiquitin-mediated protein degradation (Borges et al. 2008).

Another layer of regulation modulating locus accessibility is the covalent attachment of a methyl group to a cytosine. DNA methylation (mC) is associated with genetic regulation, cell memory, silencing of transposable elements, genomic imprinting, and repression of pseudo-elements coming from duplicate sequences (Bird 1995; Yoder et al. 1997; Colot and Rossignol 1999). Methylation patterns are established and maintained via an appropriate functional DNA methylation machinery. DNA methylation can be inherited across cell division without changes in DNA sequence, therefore it is defined as an epigenetic modification. An important aspect of DNA methylation in plants is that it can arise in three sequence contexts: CG, CHG, and CHH, in which H can be A, T, or C (Kawashima and Berger 2014).

To guarantee the integrity of the genome for the next generation, the germline should be free of errors. In addition, germline reprogramming is a key to allow totipotency in the zygote. Reprogramming erases epigenetic signatures acquired in response to the environment and during organismal development. Without reprogramming, epigenetic marks will be inherited across generations and allow epialleles (alternative chromatin states) to be inherited and accumulate across generations. This can have adverse effects, such as the release of silenced TEs (transposable elements) that may be harmful to the integrity and homeostasis of the genome (Martienssen and Colot 2001; Lippman et al. 2003; Slotkin et al. 2009;

Borges et al. 2012; Calarco et al. 2012). On the other hand, epialleles can also be beneficial, and their epigenetic inheritance can lead to evolutionary adaptations (Johannes et al. 2008, 2009; Weigel and Colot 2012).

The idea that the environment influences heredity exists in the evolutionary view for centuries. In the early nineteenth century, the evolutionist Jean-Baptiste Lamarck proposed the "Theory of inheritance of acquired characteristics," wherein the use or disuse of an organ led to its amplification or atrophy and the next generation inherits the phenotype (Springer 2013; Blake and Watson 2016). The theory proposed by Lamarck makes special sense in the Plant Kingdom, since plants generate germ cells from somatic tissue, potentially accumulating long-term environmental influences while in animals, the organism saves a dedicated germ cell line for this purpose (Springer 2013; She and Baroux 2015; Blake and Watson 2016).

Epigenetic inheritance is widespread, this phenomenon could be partially explained because sperm cells reprogramming occurs in asymmetric cytosine -CHH-methylation, while after fertilization CHH methylation is reestablished by small RNAs that come from the maternal side and disseminated through the embryo (Calarco et al. 2012; Ibarra et al. 2012). Moreover, throughout this process, small RNAs play an important role in modulating transcriptional and translational dynamics from individual developmental stages (Borges et al. 2011).

Inheritance of epigenetic changes through the germline (i.e., transgenerational) also occurs in unicellular and other multicellular organisms. DNA methylation is often associated with the inheritable changes in genomic expression leading to diversity and adaptation. In plants, DNA methylation is established and maintained by DNA methyltransferases. METHYLTRANSFERASE1 (MET1) is responsible for symmetric CG methylation after DNA replication by recognizing hemimethylated CG sites (Law and Jacobsen 2010). CHROMOMETHYLTRANSFERASE3 (CMT3) and CROMOMETHYLTRANSFERASE2 (CMT2) maintain CHG and CHH methylation via the chromo and BAH domains that recognize methylated histone H3 tails. CHG methylation is mostly correlated with H3K9 (histone H3 lysine 9) methylation (Du et al. 2012). Conversely, the H3K9 methyltransferases KRYPTONITE (KYP), SU(VAR)3-9 HOMOLOG 5 (SUVH5), and SUVH6 bind to CHG and CHH methylation to catalyze H3K9me2 (Du et al. 2015). De novo DNA methylation in all contexts is catalyzed by DOMAINS REARRANGED METHYLTRANSFERASE2 (DRM2) (Cao and Jacobsen 2002).

Plants also display a complex and still not completely understood pathway in which de novo DNA methylation is triggered by small RNAs (sRNAs), the RNA dependent DNA methylation (RdDM) pathway. Briefly, RNA polymerase IV-dependent transcripts, mostly from TEs (Castel and Martienssen 2013), are converted to double-stranded RNA by RDR2 (RNA dependent RNA polymerase II) and cleaved into 24nt siRNAs by DCL3 (DICER-LIKE 3). Following processing, the 24nt-siRNAs are loaded into AGO (ARGONAUTE) effector complexes, including AGO4, AGO6, and AGO9. Next, RNA polymerase V produces longer noncoding transcripts used as scaffolds for recruiting additional RdDM factors, including 21, 22, and 24nt siRNA-loaded ARGONAUTE proteins and several accessory proteins that are still not well understood, involving canonical and non-canonical pathways. Finally, these interactions direct the recruitment of DRM1

(DOMAINS REARRANGED METHYLTRANSFERASE1) and DRM2 which methylate DNA in the three contexts (Hamilton and Baulcombe 1999; Cao et al. 2003; Henderson and Jacobsen 2007; Zhong et al. 2014; Borges and Martienssen 2015).

For standard DNA methylation, the SNF2 nucleosome remodeler DDM1 (DECREASE DNA METHYLATION1) is required (Jeddeloh et al. 1999; Lippman et al. 2004). DDM1 works by moving along the DNA and altering nucleosome composition and placement, allowing other proteins to gain access to heterochromatic DNA (Ryan and Owen-Hughes 2011). DDM1 mediates DNA methylation in all contexts independently of the RdDM pathway by refuting the linker histone H1 (Zemach et al. 2013; Lyons and Zilberman 2017).

Among the chromatin regulating factors, the epigenetic state is also mediated by histones and histone post-translational modifications (PTMs) that dynamically change alongside DNA methylation to mark and reprogram the genome. Histones are the architectural proteins that pack the DNA into nucleosomal units (Henikoff et al. 2004). There are five histone families—H1, H2A, H2B, H3, and H4—which are subject to PTMs. Histones and their modifications became the focus of research interest as a result of the discovery of the histone code and its significance for chromatin modulation. The histone code is the result of covalent PTMs: methylation, acetylation, phosphorylation, ubiquitination, and poly-ADP-ribosylation that takes place at the N-terminal tails (and also the C-terminal tail of H2A) of histones. The outcome of PTMs can influence gene expression by altering chromatin structure or recruiting other histone modifiers (Jenuwein and Allis 2001).

Histone variants are also subjected to PTMs. They are substitutes for the core canonical histones that can confer specific structure and function to the nucleosome (Mariño-Ramírez et al. 2005). Canonical histones are expressed during the S-phase of the cell cycle and incorporated to chromatin in a DNA replication-dependent manner, while histone variants are expressed through the cell cycle in a replication-independent mode (Bernatavichute et al. 2008; Law and Jacobsen 2010). Histone variants are expressed at different developmental stages and are connected to specific processes. For example, in Arabidopsis pollen, MGH3 is a male gamete-specific H3 variant (Okada et al. 2005) that integrates the regulatory pathway of germ cell cycle progression (Brownfield et al. 2009). Furthermore, histone H3 variants replace canonical H3 in both vegetative and sperm cells (Ingouff et al. 2007; Schoft et al. 2009).

The reprogramming of the vegetative nucleus leads to the accumulation of small RNAs and activation of transposons in the gametes, reinforcing the germline imprinting events and transposon silencing (Slotkin et al. 2009; Hsieh et al. 2009). Conventionally, heterochromatin is considered transcriptionally inactive, while euchromatin is transcriptionally active. In the last decades, this concept has changed due to the abundance of heterochromatic transcripts found in the germline cells, involved in TEs control and germ-cell fate (Creasey and Martienssen 2010). Euchromatin correlates with low levels of mCG (Lister et al. 2008), while heterochromatin is highly methylated in all contexts (Henderson and Jacobsen 2007). Heterochromatin in plants consists mostly of transposable elements and related tandem repeats (Lippman et al. 2004). In the germline, the PMC has reduced heterochromatin and shows TE (transposable elements) transcriptional reactivation

(Yang et al. 2011; She and Baroux 2015). On the other hand, the vegetative nucleus heterochromatin is decondensed while the sperm cells have tight condensed heterochromatin (Calarco et al. 2012).

Evolution can be driven by TEs, ubiquitous elements within the eukaryotic genome that have the ability to control gene expression and generate mutagenesis through transposition (Chuong et al. 2017). In 1961, Barbara McClintock showed in maize that the transposable elements Activator and Suppressor Mutator could cycle between active and silent states and be inherited through generations. These elements are frequently controlling color genes, allowing the genetic identification of both *cis* (transposons) and *trans*-acting (transposase) regulatory factors (McClintock 1961).

It was in plants that TE-related silencing across generations was described for the first time (McClintock 1957). TEs are subjected to epigenetic silencing, presumably due to the harmful outcome correlated with its activity (Lippman et al. 2004). TEs have the ability to interrupt gene function, damage the chromosome, increase in copy number, and overpass host gene number (Creasey and Martienssen 2010). In plant chromosomes, meiotic recombination frequency alters dramatically in gene-dense euchromatin and suppressed within centromeres, enriched by TEs. In Arabidopsis, centromeric and pericentromeric regions are enriched for CG DNA methylation (Stroud et al. 2014), which contributes to TEs silencing.

Transgenerational epigenetic inheritance is more common in plants than in animals, which undergoes robust germline reprogramming (Soppe et al. 2000; Manning et al. 2006; Martin et al. 2009; Durand et al. 2012). Yet despite the fact that genes, transgenes, and TEs remain methylated over generations in plants, some epigenetic reprogramming does occur during sexual reproduction (Heard and Martienssen 2014; Kawashima and Berger 2014).

14.2 Epigenetic Mechanisms in Pollen

Germ cells developed mechanisms to guarantee the proper resetting of epigenetic marks and chromatin remodeling prior to the transmission to the next generation. Silencing of transposable elements and heterochromatin formation are important pathways in this process. Epigenetics marks are also involved in mechanisms beyond reprogramming: defending the genome against TEs on one hand, and having functional centromeres on the other.

14.2.1 Small RNAs in Pollen

In pollen, small RNAs are important components of the plant epigenetic reprogramming machinery, altering transcriptional and translational dynamics (Borges et al. 2011). The miRNA pathway is important to regulate multiple biological functions

such as development, the response to biotic and abiotic stress, as well as hormone response (Bartel 2004; Chen 2005; Martin et al. 2010; Khraiwesh et al. 2012; Sunkar et al. 2012). miRNAs act by cleaving their specific complementary mRNA targets and are also able to repress translation (Chen 2005; Brodersen et al. 2008). MicroRNAs are present and active in mature pollen; additionally, there is a consistent expression of genes connected to the miRNA pathway such as *DCL1* (*DICER-LIKE1*), *AGO1* (*ARGONAUTE1*), and *RDR6* (*RNA DEPENDENT RNA POLYMERASE6*) (Kidner and Martienssen 2005; Grant-Downton et al. 2009). In sperm cells, genes related to the small RNA pathway and DNA methylation—*MET1*, *DDM1*, *AGO9*, and *AGO5*—are enriched in mature pollen compared to sporophytic tissues (Borges et al. 2008; Slotkin et al. 2009).

According to the parental conflict theory that could be described as the struggle between maternal and paternal genome dosage (Moore and Haig 1991), paternally inherited microRNAs might provide a direct mechanism to regulate maternally expressed inhibitors of embryo growth (Spielman et al. 2001). Indeed, it is possible that in Arabidopsis SC small RNAs are delivered during fertilization. For example, transcripts from *SSP* (*SHORT SUSPENSOR*) accumulate in *Arabidopsis* SC and are translationally suppressed before fertilization, yet translated only in early zygotic development (Bayer et al. 2009). Paternal miRNA may be delivered at fertilization, playing important roles such as signaling molecules or triggering early zygotic patterning and endosperm development, providing an efficient reprogramming mechanism in the early development (Borges et al. 2011).

The reactivation of TEs in the vegetative nucleus leads to the accumulation of small interfering RNAs (siRNAs), while the accumulation of TE-derived siRNA can lead to TE silencing in sperm cell, targeting gene silencing in gametes (Slotkin et al. 2009). In the VN, *DDM1* is down-regulated, allowing expression of transposons, whose transcripts are subsequently processed by the RNA interference (RNAi) pathway into 21nt siRNAs, which are then also found in the SC. There is probably an unknown mechanism of communication between the VN and the SC, considering that the 21nt-siRNAs produced in the VN target TEs in the SC, where they are highly methylated and transcriptionally silenced, leading to the possibility that these 21-siRNA are mobile and transmitted from VN to SC (Slotkin et al. 2009; Martienssen 2010).

During epigenetic reprogramming in *Arabidopsis* pollen, the biogenesis of 21 and 22nt easiRNA (epigenetically activated siRNA) takes place. easiRNA is another class of secondary siRNA derived from transcriptionally reactivated transposable elements, and still poorly understood. In wild-type VN and *ddm1* mutant inflorescence, easiRNAs accumulate from the retrotransposon *ATHILA6* 3'UTR (untranslated region) (Slotkin et al. 2009). These small RNAs accumulate in sperm cells at the same time that the heterochromatin from VN is lost and TEs start to reactivate (Slotkin et al. 2009). In *ddm1* mutants, *DDM1* levels are down-regulated and methylation of H3K9 is replaced by methylation of H3K4, DNA methylation is lost and TEs start to become active, triggering the biogenesis of easiRNA (Nuthikattu et al. 2013; Creasey et al. 2014). Among other TEs, *Gypsy* and *Copia* retrotransposons are targeted by miRNAs, particularly by miR845a (21nt) and miR845b (22nt) and

generate easiRNAs in *Arabidopsis* pollen (Borges et al. 2018). Potentially, these molecules are generated in the vegetative nucleus, where TEs are reactivated and easiRNAs travel to the sperm cells, targeting TEs to promote genome stability of the next generation (Martinez et al. 2016). Intriguingly, the well-known miR156, miR159, miR172, and miR859 were also recognized to generate secondary siRNA from TEs mRNA targets, likely able to target TEs (Ronemus et al. 2006; Creasey et al. 2014).

Another class of small RNAs that plays a role in the male gamete is phased siRNA (phasiRNA). They are produced in the germinal cells and persist throughout pollen differentiation and maturation (Zhai et al. 2015). Secondary phased siRNAs are triggered by 22nt miRNA generating 21nt and 24nt phasiRNA. In monocotyledons, these sRNAs are generated from *PHAS* precursors, transcribed by RNA polymerase II, subsequently cleaved by miR2118 to generate 21nt-phasiRNAs and by miR2275 to generate 24nt-phasiRNA. The *PHAS* 3'mRNAs are then converted into a double-stranded RNA by RDR6 and processed by DCL4 and 5 (Song et al. 2012a, b). In grasses, this RNA class is prevalent in anthers, during early development and meiosis (Zhai et al. 2011; Arikit et al. 2013; Komiya et al. 2014). In rice and maize, 21nt-phasiRNAs accumulate in anthers before meiosis, during cell fate specification, while 24nt-phasiRNAs accumulate during meiosis (Nonomura et al. 2007; Zhai et al. 2015). Additionally, phasiRNAs are essential for male fertility (Zhai et al. 2015; Kakrana et al. 2018); however, no targets have been found so far for this class of sRNA, leaving the biological role of these intriguing molecules an open question.

Another interesting possibility for sRNA function in the germline is the parental epigenetic contribution to the next generation, where sRNAs from one parent could be required to silencing incoming TEs from the other (Klattenhoff and Theurkauf 2008). Heterochromatin reprogramming, like genome imprinting, could produce a parent-specific defensive barrier against interspecific and interploidy hybridization. Also, it is possible that sRNAs from the male germline are delivered into the next generation, and once more, bringing the Lamarckian inheritance to the spot, since the activation of many TEs may respond to environmental cues (Creasey and Martienssen 2010).

Small RNAs play important roles in pollen development and maintenance. However, the complex network of interrelation among the different pathways remains unknown. With the aid of the new sequencing techniques, novel classes of regulatory molecules and layers of regulation are beginning to unravel.

14.2.2 DNA Methylation

Methylation of cytosine residues plays important roles in the maintenance of genomic stability, control of gene expression, and imprinting (Law and Jacobsen 2010). Epigenetic consequences of DNA methylation include modification of alternative splicing and transcription. These effects can respond to environmental

cues in a reversible way (Richards 2011) without changes in DNA sequence (Jablonka and Raz 2009; Law and Jacobsen 2010).

Throughout male gametogenesis, DNA methylation patterns undergo reprogramming. There is a decrease in the mCHH levels from the microspore stage to the sperm cell stage while mCG levels remain stable (Fig. 14.1). Moreover, in contrast to the sperm cells, the vegetative nucleus loses mCG and restores mCHH at specific TE loci. These changes correlated with the expression of DNA methylation enzymes: the chromatin remodeler DDM1, which is involved in DNA methylation in heterochromatic regions, is found in SC, but not in VN; MET1 and CMT3 are expressed only in SC; DRM2 and DME (DEMETER), a DNA glycosylase enzyme involved in DNA demethylation, are expressed in the VN (Kawashima and Berger 2014). These observations reinforce the idea of the presence of specialized reprogramming machinery in the male germline.

RdDM is one of the pathways that guide DNA methylation on the male germline. RdDM is highly complex and the major small RNA-mediated epigenetic pathway in plants (Hamilton and Baulcombe 1999; Qi et al. 2006). RdDM has many biological functions, including transcriptionally repressing genes and transposons, related to intercellular communication as well as in stress response and reproduction (Borges et al. 2012; Calarco et al. 2012). The complex maintenance machinery ensures the perseverance of established mC through cell division and across generations (Law and Jacobsen 2010; Matzke and Mosher 2014; Lewsey et al. 2016). In Arabidopsis, for example, DNA methylation patterns after 30 generations of single seed descent were found to exhibit a rate of CG methylation per site change per generation considerable higher than nucleotide mutation (Schmitz et al. 2011).

The reprogramming of CG methylation in the vegetative nucleus is not clear; however, the mechanism overlays with chromatin remodelers. CG methylation is reduced in the vegetative nucleus, likely because of the reduced expression of *MET1* (Jullien et al. 2012). Furthermore, *DDM1* the main regulator of constitutive heterochromatin and TEs is not expressed in the VN (Slotkin et al. 2009). Moreover, H3K9me2 plays an important role aiding in the silencing of TEs in sperm cells, yet is not found in VN. Methylation in both somatic and pollen cells is maintained through similar mechanisms; however, the maintenance of mCG is more efficient in pollen, even though CG methylation level is similar among vegetative, sperm, and leaf cells (Hsieh et al. 2016). The lack of the H3K9me2 mark, required by CMTs enzymes to play its role, implies that mCHH and mCHG in the VC may mostly rely on the RdDM pathway (Hsieh et al. 2016).

Variation of methylation between pollen and soma could be an inevitable outcome of unique selective pressures. On one hand, gametes have the potential to undergo unlimited cell divisions, which will keep a strong selection to retain efficient methylation maintenance. On the other hand, somatic cells will divide limited times which demands just enough methylation activity to maintain TEs in control and other methylation functions from collapsing. These differences may occur because of the maintenance fluctuations rather than the developmental reprogramming (Hsieh et al. 2016).

In the vegetative nucleus, DME, ROS1 (REPRESSOR OF SILENCING1), DML2 (DEMETER-LIKE2), and DML3 (DEMETER-LIKE3) are expressed (Schoft et al. 2011). DME is required for demethylation of TEs and tandem repeats that surround the imprinted maternally expressed genes *MEA* (*MEDEA*) and *FWA (FLOWERING WAGENINGEN)* that are usually expressed from the maternal allele in the endosperm but are also expressed in the VN (Schoft et al. 2011). Moreover, several hypomethylated regions are targeted by ROS1/DML2/DML3 and distinctive hypomethylated regions by DME in the VN and microspore, suggesting that these DNA glycosylases are responsible for the loss of mCG in the VN (Calarco et al. 2012).

In rice sperm cells, nearly all classes of chromatin-modifying genes are up-regulated (Russell et al. 2012). Furthermore, there is evidence that somatic alterations in rice DNA mC patterns are inherited and maintained in the germline possibly through the DOMAINS REARRANGED METHYLTRANSFERASE (DRM) pathway, increasing the evidence that transcriptional expression is fine-tuned by mC in a plastic manner, and suggesting that the Lamarckian inheritance concept could be right in this instance (Akimoto et al. 2007).

The differential methylation patterns leading to the upsurge of epialleles occur naturally or as a response to environmental cues. In either way, non-Mendelian segregation of epialleles can be observed when these alleles undergo paramutation, an allelic interaction in which one allele leads to a heritable change in the expression of the homologous allele (Della Vedova and Cone 2004). These phenomena illustrate the importance of epigenetic variation and paramutation in phenotypic variation (Greaves et al. 2012; Hövel et al. 2015).

In the SC, when some epialleles are in a pre-methylated state at the CG context, these same alleles are hypomethylated in the leaf of the parental line. One possible explanation is that CG hypermethylation at some loci (Becker et al. 2011; Calarco et al. 2012) is the default state at undifferentiated cells that will give rise to gametophytes, depending exclusively on MET1 for its maintenance, which will pass on to the germline, but its stability requires RdDM and 24nt siRNA accumulation (Borges and Martienssen 2013).

In an interesting experiment, EpiRILs (epigenetic recombinant inbred lines) were constructed by crossing Arabidopsis with distinct DNA methylation profiles, *ddm1* mutant and wild-type plants, then backcrossing the progeny by single seed descendants. The reactivated hypomethylated chromosomal segments generated by these mutants were tracked across at least eight generations, resulting in a high heritability for complex traits such as flowering time and plant height, without selection (Johannes et al. 2009).

The possibility to track epialleles led the way for identification of epiQTLs (epigenetic quantitative trait loci) where a QTL influences the chromatin state in either cis or trans, while classical genetics analysis of QTLs takes into account phenotypic variations due to changes in DNA sequences. Therefore, integrating these two approaches—genetics and chromatin-level information—now provides a more comprehensive view to generate, and track the maintenance of, phenotypes over time (Johannes et al. 2008).

14.2.3 Histone Variants and Modifications

The Arabidopsis pollen mother cell is characterized by a global dynamic change in the nucleosomal organization and chromatin modifications, the differential fate in mature pollen cells rely on the chromatin organization—VC has a large and diffuse nucleus, compared to the SC smaller and condensed nucleus (She and Baroux 2015). The correct assembly and accessibility of chromatin also depends on histone variants and on the covalent PTMs of histones.

Both in animals and plants, the histone variant H3.3 replaces H3.1 at transcribed *loci* where it replaces H3.1 during transcriptional elongation (Tagami et al. 2004; Okada et al. 2005; Ausió 2006; Wollmann and Berger 2012; Stroud et al. 2012; Biterge and Schneider 2014; Jiang and Berger 2017). Furthermore, H3.3 organizes chromatin both in transcribed *loci* and in promoter regions (Shu et al. 2014). These dynamic alterations make it easier for global changes in chromatin structure and histone modification to occur (Wollmann et al. 2012). During Arabidopsis pollen development, the H3.1 five copies and the H3.3 three copies show differential expression (Ingouff et al. 2007; Borg and Berger 2015), both H3.1 and H3.3 are present in the microspore chromatin, after division H3.1 is not found in mature pollen. The chromatin from SC is almost entirely consisted of the H3.3 and H3.10 variants (Borg and Berger 2015). However, it is not expected that in SCs H3.1 is absent, since a new phase of DNA replication takes place before fertilization (Durbarry et al. 2005), suggesting that H3.1 synthesis is separated from proliferation during SC development. Therefore, through male gametogenesis, other regulatory pathways appear to control the dynamic expression of H3 isoforms, shaping the unique chromatin landscape from the male germ cells (Borg and Berger 2015).

Pioneering studies in the monocot lily described a broad range of specific male gamete histone variants that replace H2A, H2B, H3, and H4 somatic histones, such as gH2A, gH2B, gH3, gH4 (Ueda and Tanaka 1995; Ueda et al. 2012; Yang et al. 2016), gcH2A, gcH3 (Xu et al. 1999), leH3, soH3-1, and soH3-2 (Okada et al. 2006). Nevertheless, the biological role of these variants remains to be fully understood. However, the acquisition of histone variants specific to the germline reinforces the idea of distinctive chromatin functions between the SC and the VC (Yang et al. 2016). In the lily chromatin, H3K4 (histone 3 lysine 4) is hypermethylated in the GC and hypomethylated in the VN, while H3K9me2 is weekly distributed in the GC, probably H3 variants play role in distinctive chromatin assembly among the cell types during pollen development, as well as in male-specific transcriptional activation (Okada et al. 2006).

The SC-specific histones appear to be unique among species, for example, Arabidopsis genome contains 15 histone H3 genes, among them CENH3 and H3.10, also known as MALE GAMETE-SPECIFIC HISTONE3 (MGH3), are found in centromere and sperm cell chromatin, respectively, whereas the rice genome displays 16, including the MGH3 homolog H3.709 (Borg and Berger 2015). Moreover, SCs from rice express a distinctive and diverse set of histones H2B (Russell et al. 2012). Despite the apparent conservation of histone H3 male gamete-

specificity, there are minor, but important differences found in the basic amino acids of the N-terminal domain, the target region for most H3 PTMs (Russell et al. 2012; Borg and Berger 2015). For example, the R26-K27-S28 motif, location of important modifications, is not conserved in the rice histone variant H3.709. In this histone variant, this motif is present but contains a nine amino acid long insertion that is nonexistent in other histones such as H3.1, H3.3, and MGH3 (Borg and Berger 2015). Gamete-specific proteins diverge fast and their adaptive evolution could drive speciation via generation of fertilization barriers (Swanson and Vacquier 2002).

In Arabidopsis SC chromatin, MGH3 is under the control of a male germline-specific MYB transcription factor DUO1 (Rotman et al. 2005) that is expressed at the beginning of the pollen development. DUO1 is required for the regulatory network that controls SC differentiation within the mitotic entry of the germ cell, MGH3 activity follows DUO1 expression after microspore division (Brownfield et al. 2009). The expression of both DUO1 and MGH3 before meiosis II implies that the regulatory network that controls the germ cells specification begin soon after asymmetric division (Rotman et al. 2005; Okada et al. 2005; Borg et al. 2009), besides MGH3 specific and abundant expression in the SC suggests that this histone variant may play important role in chromatin structure in the germline (Borg et al. 2009). MGH3 promoter contains four DUO1 binding motifs (wAACCGy), and two of them are required for MGH3 activation by DUO1 in the germline (Borg et al. 2011). At the same time, DUO1 also controls the expression of a duet of zinc-finger proteins DAZ1/DAZ2 (DUO1-ACTIVATED ZINC FINGER1/DUO1-ACTIVATED ZINC FINGER2), key to intermediate germ cell mitotic entry and gamete differentiation (Borg et al. 2014).

The histone variant CENH3 is a main component of centromeres in eukaryotes and it is important for kinetochore assembly and chromosome segregation (Henikoff and Furuyama 2012). The Arabidopsis centromeric heterochromatin of the vegetative nucleus undergoes decondensation and loses the histone variant CENH3, the H3K9me2 mark, and centromeric identity (Schoft et al. 2009). In addition, the VN exits the cell cycle after microspore division (Borg et al. 2009). CENH3 does not undergo post-translational modification, which may contribute to the loss of centromeric heterochromatin in the vegetative nucleus (Schoft et al. 2009). Furthermore, H3K27me1 is still present in centromeric regions in the VN, but still retains non-CG methylation leading to transcriptional silencing probably through control of the RdDM/DRM2 pathway and 24nt siRNAs generated from centromeric regions (Schoft et al. 2009). DRM2 is expressed specifically in the vegetative nucleus, but not in the sperm cell (Calarco et al. 2012).

In Arabidopsis vegetative nucleus, SDG4 (SET DOMAIN GROUP4) is one of the enzymes responsible for the maintenance of methylation in H3K4 and H3K36—marks related with active euchromatin—and regulates the expression of genes that play role in pollen germination and pollen tube elongation (Cartagena et al. 2008). Likewise, SET DOMAIN GROUP2 (SDG2) mediates H3K4 trimethylation in the VN to control pollen germination and pollen tube elongation as well. Moreover, SDG2 is required for the expression of the transposable element *ATLANTYS1* in the VN (Pinon et al. 2017).

The linker histone H1 globally reduces heterochromatic DNA methylation in all contexts (Zemach et al. 2013). H1 is present in SC and absent in the VC, yet does not increase heterochromatic methylation in pollen (Hsieh et al. 2016). In heterochromatic TEs, the increased efficiency of mCG might be because of the reduced levels of H1, probably with a specific mechanism that differs from genes and euchromatic regions, where loss of H1 does not facilitate mCG (Hsieh et al. 2016).

During chromatin reorganization in PMC from Arabidopsis, there is an eviction of the linker histone H1 (She and Baroux 2015), consistent with chromatin decondensation, followed by an increase in nuclear size and reduction of the heterochromatin content. This is a *ddm1*-like phenotype, where TEs are activated after the loss of heterochromatin (Slotkin et al. 2009) and may assist the rapid CENH3 turnover in the PMC (Schubert et al. 2014). Furthermore, in the PMC, there is a reduction in the heterochromatin domains, likewise, a decrease of the H3K27me1 mark. Reduction of H3K27me3 (a repressive mark) and increase of H3K4me2 (a permissive mark) suggest a distinctive epigenetic landscape. SDG2 may also play role in the PMC epigenetic landscape (She and Baroux 2015).

Acetylation of lysine residues on the N-terminal tail of histones neutralizes their positive charge, decreasing the affinity for the negatively charged DNA strand, changing the conformation of chromatin and therefore altering gene accessibility. Hyperacetylated histones are usually correlated with gene activation, while hypoacetylation with gene silencing. HDAs (histone deacetylases) act together with corepressors in multiprotein chromatin modifiers complexes (Mehdi et al. 2016; Perrella et al. 2016). In *Arabidopsis*, some members of the HDA family are associated with the silencing of transposable elements, transgenes, and ribosomal RNA (Lippman et al. 2003; Probst et al. 2004). This family also plays a role in both euchromatin and heterochromatin, and may inhibit de novo DNA methylation in CG context (Hristova et al. 2015; Zhang et al. 2015). Moreover, these enzymes are involved in male fertility in maize (Forestan et al. 2018). Histone acetylation may participate in the germline epigenetic reprogramming, although its role still remains to be investigated.

14.3 Transposable Elements

TEs comprises Class I—retrotransposons which replicate through RNA and cDNA—that can be divided into LTR (long terminal repeats) and non-LTR, and Class II—DNA transposons which replicate via a DNA intermediate—that does not necessarily require transcription of the DNA elements (Underwood et al. 2017). In Arabidopsis, the LTR retrotransposon family Athila occupies 2.7% of the genome and is one of the building blocks of the centromere and the center of Arabidopsis epigenetic regulation, potentially playing an important role in speciation (Slotkin 2010). Athila elements, along with other TEs, are epigenetically reactivated in the VN, in part due to the lack of DDM1 (Slotkin et al. 2009). Additionally, Athila is not

controlled by sRNAs in the plant body, nevertheless in the female gametophyte is (Olmedo-Monfil et al. 2010). Taken together, the reactivation of Athila in the pollen and its regulation in other tissues clearly suggest a distinct regulation mechanism and a specific biological function in the pollen, possibly to make the necessary substrate—mRNA—to generate easiRNA to the effective silencing of TEs in the next generation (Slotkin et al. 2009).

In maize and *Arabidopsis*, TEs become active in the PMC, accompanied by a reduction in heterochromatin and changes in histone modifications (Wang and Köhler 2017). Additionally, TEs accumulate only in the VN and not in the SC (Borges et al. 2008), accompanied by novel transposition events in pollen DNA, but not in the subsequent progeny, thus reinforcing the notion that they are not active in the SC (Creasey and Martienssen 2010). Dynamic changes in mC during male gametogenesis include increases in non-CG methylation in the VN, and siRNAs homologous to the retrotransposons LTRs (long terminal repeats) are found in the vegetative nucleus, while 21 and 24nt siRNAs are found in sperm cells. In the SC, non-canonical RdDM pathways modify these elements (Borges et al. 2012). In rice, the same mechanisms may be present, as genes from distinct RNA silencing pathways are upregulated (Russell et al. 2012). The sources of TEs control during plant reproduction comprise changes in DNA methylation along with small RNA in specific tissues or cell types (Slotkin et al. 2009; Calarco et al. 2012).

A cooperation between H3K9me2, non-mCG dependent on CMT2, CMT3, and RdDM is established to maintain TE expression under control (Stroud et al. 2014); therefore, an upregulation of TEs in the male meiocyte indicates that DNA and H3K9 methylation are reduced before meiosis. Moreover, TE activation in pollen does not lead to genome instability and TE transposase activity, suggesting the presence of another layer of regulation to keep these elements from harming the genome (Slotkin et al. 2009; Calarco et al. 2012; Creasey et al. 2014).

Another potential mechanism to control TEs in pollen could be through the still poorly understood tRNA derived fragment (tRF) pathway. tRFs have been identified in different species and cell types, ranging from 13 to 30 nucleotides long; these molecules are processed from mature tRNAs in 5' tRFs, 3'CCA tRFs, and tRNA halves (Lee et al. 2009; Alves et al. 2017; Martinez et al. 2017; Schorn et al. 2017), although the biogenesis pathway for most tRFs is still unknown. These sRNAs are able to target TEs both in mouse stem cells and *Arabidopsis* pollen. In mouse, 3'CCA tRFs are able to target and inhibit retrotransposons by binding retrotransposons primer site, which is where a tRNA can bind and prime their reverse-transcription. Therefore, tRFs competing for the primer site can inhibit the transcription of these elements (Schorn et al. 2017). Pollen-specific 19 nucleotides 5'tRFs target TE mRNAs in *Arabidopsis*. Furthermore, the accumulation of 19nt-5'tRF in reproductive tissue/pollen is conserved among plants and there is evidence that suggests that 5'tRFs in pollen are processed by DCL1 (Martinez et al. 2017).

Arabidopsis sperm cells retain CG and CHG methylation while CHH methylation is lost, accompanied by extensive epigenetic remodeling of the VN cell (Slotkin et al. 2009). TE reactivation occurs in Arabidopsis, maize, and rice pollen, and could indicate a conserved mechanism among land plants (Nobuta et al. 2007; Slotkin

et al. 2009). The VN undergoes extensive histone variant substitution, losing canonical histones and CENH3, likely contributing to TE activation (Ingouff et al. 2007; Schoft et al. 2009). In rice sperm cells, nearly all classes of chromatin-modifying genes are up-regulated (Russell et al. 2012), and somatic changes in mC are inherited and maintained in the germline (Akimoto et al. 2007).

14.4 Imprinting

Imprinting is a phenomenon where one of the parental alleles is preferentially expressed over the other and has the potential to generate advantageous traits but still is poorly understood. This epigenetic singularity leads to parent-of-origin differentiated expressed alleles inheritance in several plant species, including maize, rice, and *Arabidopsis* (Luo et al. 2011; Waters et al. 2011; Pignatta et al. 2014). In plants, it occurs mostly in the endosperm, and hundreds of imprinted genes have been identified so far (Gehring et al. 2011; Luo et al. 2011; Wolff et al. 2011; Zhang et al. 2016; Yuan et al. 2017). After fertilization, the endosperm is originated from a triploid cell, containing the diploid maternal cell and one haploid sperm cell. The expected ratio of maternal and paternal expression is 2:1, therefore imprinted genes could differ from the probability where maternally expressed genes (MEGs) or paternally expressed genes (PEGs) diverged the expected ratio. Imprinting can be determined by suppression or activation of MEGs or PEGs. Studies have shown that MEGs are preferentially expressed in the endosperm while PEGs could be detected in the endosperm as well as in other tissues during development, suggesting that PEGs and MEGs could be regulated by different mechanisms (Waters et al. 2013; Pignatta et al. 2014; Zhang et al. 2016).

PEGs may be involved on the postzygotic hybridization barrier in the endosperm, indicating a major role in plant speciation (Wolff et al. 2015). In rice, a set of PEGs regulates endosperm development and nutrient metabolism, improving seed development and offspring fitness (Yuan et al. 2017; Pignatta et al. 2018).

Imprinted genes are usually bordered by TEs—which are frequently highly methylated—and could be affected by TEs methylation machinery that possibly overlaps the genes edges (Martienssen et al. 2004; Radford et al. 2011). It is not clear how regulation of imprinted parental genes occurs, but studies suggested that TEs could be the trigger for this phenomenon (Martienssen et al. 2004; Gehring et al. 2009; Wolff et al. 2011).

In Arabidopsis VN, TEs are target by DME (DEMETER), ROS1 (REPRESSOR OF SILENCING1), DML2 (DEMETER-LIKE2), and DML3 (DEMETER-LIKE3)—DNA demethylation enzymes—causing them to lose CG methylation (Lister et al. 2008; Calarco et al. 2012). In the SCs, 24nt easiRNAs corresponding to some of these elements accumulate, especially in TEs regions that flank MEGs (Calarco et al. 2012), probably playing role in the RdDM pathway from those cells. To illustrate this complex mechanism, there are examples such as *SDC* (*SUPPRESSOR OF DRM2/CMT3*) that is active only when the flanking sequences

are not methylated (Henderson and Jacobsen 2007), and the PEG *PHE1* (*PHERES1*) that is expressed only when a tandem repeat downstream of the coding region is methylated (Makarevich et al. 2008). In the VN, tandem repeats flanking both genes lose methylation. In the SC, these regions also lose mCG, although retain mCHH and accumulate 24nt easiRNAs, while imprinted genes are protected from the global loss of methylation.

The multidomain protein complex FACT (facilitates chromatin transaction) interacting with nucleosome components to initiate and elongate transcripts also is involved with DME at imprinted genes in Arabidopsis (Ikeda et al. 2011). Mediated by the linker histone H1, DME requires FACT for DNA demethylation especially in TEs regions with high CG content and nucleosome activity, enriched for heterochromatin marks, such as H3K27me1 and H3K9me2. So far, this mechanism is known to occur in the female central cell, but not for the male VN. This observation is particularly interesting because both cell types are separated from its somatic precursor by one cell division and have decondensed chromatin (Frost et al. 2018), demonstrating the specific epigenetic regulation mechanisms developed by maternal and paternal germlines.

14.5 Environmental Response and Inheritance

Plants are able to modulate gene expression to fine-tune biotic and abiotic stress responses. The rise of temperature triggered by climate change is deeply affecting plant farming worldwide: for example, the estimation is that for each 1 °C of increase in temperature, there will be a 10% decrease in rice yield (Peng et al. 2004).

Pollen grains are exceptionally delicate, particularly sensitive to elevated temperatures, and the mechanisms that underlie this stress response are still poorly understood. Heat stress response in tomatoes triggers the accumulation of small non-coding RNA (sncRNAs), transfer RNAs (tRNAs), and small nucleolar RNAs (snoRNAs) during post-meiotic and mature stages of pollen development (Bokszczanin et al. 2015). In *Arabidopsis*, the increase in temperature reduces the expression of the gene *SGS3* (*SUPPRESSOR OF GENE SILENCING3*), involved in the RNA interference (RNAi) pathway, therefore decreasing the accumulation of siRNAs. Moreover, heat stress induces a transgenerational epigenetic inheritance (Zhong et al. 2013). During pollen development, heat stress response can also trigger shifts in global DNA methylation together with methyltransferase expression (Solís et al. 2012). In *Brassica napus* microspores, DNA methylation levels and TEs activity change during heat stress (Li et al. 2016). Arabidopsis epigenetic silencing of transposable elements can also endure the consequences of heat stress through the RdDM pathway (McCue et al. 2015; Matsunaga et al. 2015). However, there is no evidence that the mechanism that regulates these alterations is of adaptive value (Lamke and Baurle 2017).

Twenty-four nucleotide hc-siRNAs (heterochromatic siRNA) derived from TE could be involved in pollen development and epigenetic regulation of the stress

response (Bokszczanin et al. 2015). The 24nt hc-siRNAs also participate in the RdDM machinery (Calarco et al. 2012; Zhou et al. 2018), associated with transcriptional gene silencing, they act by modulating DNA and histones modifications, while the 21nt siRNAs and microRNAs play role in transcriptional and post-transcriptional regulation (Brodersen et al. 2008). During heat stress in tomato, there is a loss of abundance of 22nt-sncRNAs in post-meiotic and mature pollen, which may be due to reduction in the production or degradation of these sRNAs. These 22nt-sncRNAs likely play a similar role as the 21nt-siRNA generated from TEs in Arabidopsis, also the difference in the length of sncRNAs in the different stages of pollen development is due to their different functions (Bokszczanin et al. 2015).

Environmental cues can lead to changes in gene expression by alterations in chromatin structure at specific responsive genes and/or the biogenesis of small RNAs (Hirsch et al. 2013). The majority of epigenetic stress-related alterations are only detected in somatic cells and rapidly disappear, although methyl cytosine (mC) and H3K27me3 (trimethyl histone H3 lysine 27) induced by stress can last one stress-free generation (Lamke and Baurle 2017).

During pollen development, microgametogenesis is the stage where mitosis occurs. Mitotic inheritance of epigenetic traits can be explained through the interplay among small RNA, maintenance DNA methyltransferases, and other chromatin modifiers, working together to retain the epigenetic information into the next cell division, preserving tissue integrity and correct function.

Variation in epigenetic marks, such as gain or loss of DNA methylation on a specific gene, can lead to silencing or activation of the affected gene altering its phenotype (Bond and Baulcombe 2015). There are a few examples that illustrate heritable epimutation in plants: the famous *Linaria vulgaris* example, in which the floral symmetry changes due to hypermethylation and transcriptional silencing of *Lcyc* (*Linaria cycloidea-like*) (Cubas et al. 1999), as does fruit color in the tomato locus *Colorless non-ripening* (*Cnr*) (Manning et al. 2006). An additional alteration that may affect *L. vulgaris* phenotype is a depletion of a TE approximately 10 kb from the *Lcyc* gene; however, it is not clear how this depletion could affect the phenotype. Besides, in many cases TEs mediate this epigenetic silencing, for example, at the *hcf106* (*high chlorophyll fluorescence106*) locus in maize (Martienssen et al. 1990), at the melon transcription factor gene *CmWIP* (Martin et al. 2009) and *Arabidopsis FWA* (Soppe et al. 2000), resulting in gene silencing in *cis*. *Cis*-regulatory elements are frequently within or near the target loci, while *trans*-regulatory elements play a regulatory role in a distant position from where they are transcribed, such as small RNAs. Small RNAs can cause epimutation by silencing the Arabidopsis gene *FOLT1* (FOLATE TRANSPORTER 1) (Durand et al. 2012) and homologous genes are methylated by RdDM pathway. However, most epialleles cause no phenotype and can only be detected by molecular means. From an evolutionary biology perspective, an extra layer of generation of heritable variation within complex traits may explain the rapid adaptation to environmental changes seen in natural populations (Pál and Miklós 1999). As yet, there is no

evidence that these epigenetic variations are subject to natural selection or have adaptive value (Manning et al. 2006; Hirsch et al. 2013).

Epialleles can also be induced by environmental challenges, such as biotic or abiotic stress. The heritability of these epigenetic alterations might be an interesting adaptive mechanism. External changes can lead to modifications in gene expression by alterations in chromatin structure at specific responsive genes and/or the biogenesis of small RNAs (Hirsch et al. 2013). The majority of epigenetic stress-related changes are only detected in somatic cells and, after a few days, these effects disappear. Although there are a few observations demonstrating the heritability of the epigenetic marks mC and H3K27me3 after stresses such as hyperosmotic, iron deficiency, bacterial infection, chemical stressors, and caterpillar herbivory, these transgenerational epigenetic alterations are reset after one stress-free generation (Lamke and Baurle 2017).

Some hypomethylated epialleles can be stably inherited, but after a few generations the methylation levels can be restored by an RNAi dependent pathway, because sperm cells can retain mCG and mCHG during differentiation, while a lower level of mCHH is retained during mitosis (Teixeira et al. 2009; Calarco et al. 2012). Methylation levels are restored by DRM2 guided by pollen 24nt siRNA in the VN prior to fertilization (Calarco et al. 2012; Ingouff et al. 2017).

On one hand, epialleles often arise throughout stress conditions, on the other, they arise naturally on a given population. There are numerous features in germline reprogramming to make sure that the next generation is going to be viable and fertile; however, it is not known how and why this natural variation occurs, also when they are fixed in the population and what their advantages in terms of adaptability are.

14.6 Perspectives

Rapid introgression of desired traits is the ultimate goal for increasing the quality of crops. Enhancing productivity by improving yield with larger seeds, more branches, and more fruits is imperative to feed the population worldwide. So far, breeders rely mostly on genetic techniques and test-crossing on the field to achieve this goal. With expanding molecular biology and big data techniques, a new world of epigenetic features is now beginning to unravel. The possibility to understand how epialleles, methylation levels, and other epigenetic mechanisms underlying desirable crop traits are inherited across generations is imperative to teach us how to manipulate them and to achieve the best crop production. Part of this modulation happens in the male germline that acquired complex and intricate chromatin regulation mechanisms. The differences between the vegetative nucleus and sperm cells are remarkable and we just have started to shed light on the germline regulation and male inheritance. More studies on these mechanisms are needed to understand the complex world of the male germline.

References

Akimoto K, Katakami H, Kim H-J et al (2007) Epigenetic inheritance in rice plants. Ann Bot 100:205–217

Alves CS, Vicentini R, Duarte GT et al (2017) Genome-wide identification and characterization of tRNA-derived RNA fragments in land plants. Plant Mol Biol 93:35–48. https://doi.org/10.1007/s11103-016-0545-9

Arikit S, Zhai J, Meyers BC (2013) Biogenesis and function of rice small RNAs from non-coding RNA precursors. Curr Opin Plant Biol 16:170–179

Ausió J (2006) Histone variants - the structure behind the function. Brief Funct Genomic Proteomic 5:228–243. https://doi.org/10.1093/bfgp/ell020

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Bayer M, Nawy T, Giglione C et al (2009) Paternal control of embryonic patterning in *Arabidopsis thaliana*. Science 323:1485–1488. https://doi.org/10.1126/science.1140693

Becker C, Hagmann J, Müller J et al (2011) Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. Nature 480:245–249. https://doi.org/10.1038/nature10555

Berger F, Twell D (2011) Germline specification and function in plants. Annu Rev Plant Biol 62:461–484

Bernatavichute YV, Zhang X, Cokus S et al (2008) Genome-wide association of histone H3 lysine nine methylation with CHG DNA methylation in *Arabidopsis thaliana*. PLoS One 3:e3156. https://doi.org/10.1371/journal.pone.0003156

Bird AP (1995) Gene number, noise reduction and biological complexity. Trends Genet 11:94–100. https://doi.org/10.1016/S0168-9525(00)89009-5

Biterge B, Schneider R (2014) Histone variants: key players of chromatin. Cell Tissue Res 356:457–466. https://doi.org/10.1007/s00441-014-1862-4

Blake GET, Watson ED (2016) Unravelling the complex mechanisms of transgenerational epigenetic inheritance. Curr Opin Chem Biol 33:101–107

Bokszczanin KL, Krezdorn N, Fragkostefanakis S et al (2015) Identification of novel small ncRNAs in pollen of tomato. BMC Genomics 16:714. https://doi.org/10.1186/s12864-015-1901-x

Bond DM, Baulcombe DC (2015) Epigenetic transitions leading to heritable, RNA-mediated de novo silencing in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 112:917–922. https://doi.org/10.1073/pnas.1413053112

Borg M, Berger F (2015) Chromatin remodelling during male gametophyte development. Plant J 83:177–188. https://doi.org/10.1111/tpj.12856

Borg M, Brownfield L, Twell D (2009) Male gametophyte development: a molecular perspective. J Exp Bot 60:1465–1478. https://doi.org/10.1093/jxb/ern355

Borg M, Brownfield L, Khatab H et al (2011) The R2R3 MYB transcription factor DUO1 activates a male germline-specific regulon essential for sperm cell differentiation in Arabidopsis. Plant Cell 23:534–549. https://doi.org/10.1105/tpc.3.3.317

Borg M, Rutley N, Kagale S et al (2014) An EAR-dependent regulatory module promotes male germ cell division and sperm fertility in Arabidopsis. Plant Cell 26:2098–2113. https://doi.org/10.1105/tpc.114.124743

Borges F, Martienssen RA (2013) Establishing epigenetic variation during genome reprogramming. RNA Biol 10:490–494

Borges F, Martienssen RA (2015) The expanding world of small RNAs in plants. Nat Rev Mol Cell Biol 16:727–741

Borges F, Gomes G, Gardner R et al (2008) Comparative transcriptomics of Arabidopsis sperm cells. Plant Physiol 148:1168–1181. https://doi.org/10.1104/pp.108.125229

Borges F, Pereira PA, Slotkin RK et al (2011) MicroRNA activity in the Arabidopsis male germline. J Exp Bot 62:1611–1620

Borges F, Calarco JP, Martienssen RA (2012) Reprogramming the epigenome in Arabidopsis pollen. Cold Spring Harb Symp Quant Biol 77:1–5. https://doi.org/10.1101/sqb.2013.77.014969

Borges F, Parent J-S, Van Ex F et al (2018) Transposon-derived small RNAs triggered by miR845 mediate genome dosage response in Arabidopsis. Nat Genet 50:186–192

Brodersen P, Sakvarelidze-Achard L, Bruun-Rasmussen M et al (2008) Widespread translational inhibition by plant miRNAs and siRNAs. Science 320:1185–1190. https://doi.org/10.1126/science.1159151

Brownfield L, Hafidh S, Borg M et al (2009) A plant germline-specific integrator of sperm specification and cell cycle progression. PLoS Genet 5:e1000430. https://doi.org/10.1371/journal.pgen.1000430

Calarco JP, Borges F, Donoghue MTA et al (2012) Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. Cell 151:194–205. https://doi.org/10.1016/j.cell.2012.09.001

Cao X, Jacobsen SE (2002) Role of the Arabidopsis DRM methyltransferases in de novo DNA methylation and gene silencing. Curr Biol 12:1138–1144. https://doi.org/10.1016/S0960-9822(02)00925-9

Cao X, Aufsatz W, Zilberman D et al (2003) Role of the DRM and CMT3 methyltransferases in RNA-directed DNA methylation. Curr Biol 13:2212–2217. https://doi.org/10.1016/j.cub.2003.11.052

Cartagena JA, Matsunaga S, Seki M et al (2008) 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 315:355–368. https://doi.org/10.1016/j.ydbio.2007.12.016

Castel SE, Martienssen RA (2013) RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. Nat Rev Genet 14:100–112. https://doi.org/10.1038/nrg3355

Chen X (2005) MicroRNA biogenesis and function in plants. FEBS Lett 579:5923–5931. https://doi.org/10.1016/j.febslet.2005.07.071

Chuong EB, Elde NC, Feschotte C (2017) Regulatory activities of transposable elements: from conflicts to benefits. Nat Rev Genet 18:71–86

Colot V, Rossignol JL (1999) Eukaryotic DNA methylation as an evolutionary device. BioEssays 21:402–411

Creasey KM, Martienssen RA (2010) Germline reprogramming of heterochromatin in plants. Cold Spring Harb Symp Quant Biol 75:269–274. https://doi.org/10.1101/sqb.2010.75.064

Creasey KM, Zhai J, Borges F et al (2014) miRNAs trigger widespread epigenetically activated siRNAs from transposons in Arabidopsis. Nature 508:411–415. https://doi.org/10.1038/nature13069

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401:1–5

Della Vedova CB, Cone KC (2004) Paramutation: the chromatin connection. Plant Cell 16:1358–1364. https://doi.org/10.2307/3871316

Du J, Zhong X, Bernatavichute YV et al (2012) Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants. Cell 151:167–180

Du J, Johnson LM, Jacobsen SE, Patel DJ (2015) DNA methylation pathways and their crosstalk with histone methylation. Nat Rev Mol Cell Biol 16:519. https://doi.org/10.1038/nrm4043

Durand S, Bouché N, Perez Strand E et al (2012) Rapid establishment of genetic incompatibility through natural epigenetic variation. Curr Biol 22:326–331

Durbarry A, Vizir I, Twell D (2005) Male germ line development in Arabidopsis. duo pollen mutants reveal gametophytic regulators of generative cell cycle progression. Plant Physiol 137:297–307. https://doi.org/10.1104/pp.003301

Feng X, Zilberman D, Dickinson H (2013) A conversation across generations: soma-germ cell crosstalk in plants. Dev Cell 24:215–225. https://doi.org/10.1016/J.DEVCEL.2013.01.014

Forestan C, Farinati S, Rouster J et al (2018) Control of maize vegetative and reproductive development, fertility, and rRNAs silencing by histone deacetylase 108. Genetics 208:1443–1466. https://doi.org/10.1534/genetics.117.300625

Frost JM, Kim MY, Park GT et al (2018) FACT complex is required for DNA demethylation at heterochromatin during reproduction in Arabidopsis. Proc Natl Acad Sci 115:E4720–E4729. https://doi.org/10.1073/pnas.1713333115

Gehring M, Bubb KL, Henikoff S (2009) Extensive demethylation of repetitive elements during seed development underlies gene imprinting. Science 324:1447–1451. https://doi.org/10.1126/science.1171609

Gehring M, Missirian V, Henikoff S (2011) Genomic analysis of parent-of-origin allelic expression in *Arabidopsis thaliana* seeds. PLoS One 6:23687. https://doi.org/10.1371/journal.pone.0023687

Grant-Downton R, Le Trionnaire G, Schmid R et al (2009) MicroRNA and tasiRNA diversity in mature pollen of *Arabidopsis thaliana*. BMC Genomics 10:643. https://doi.org/10.1186/1471-2164-10-643

Greaves IK, Groszmann M, Ying H et al (2012) Trans chromosomal methylation in Arabidopsis hybrids. Proc Natl Acad Sci 109:3570–3575. https://doi.org/10.1073/pnas.1201043109

Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286:950–952

Heard E, Martienssen RA (2014) Transgenerational epigenetic inheritance: myths and mechanisms. Cell 157:95–109

Henderson IR, Jacobsen SE (2007) Epigenetic inheritance in plants. Nature 447:418–424

Henikoff S, Furuyama T (2012) The unconventional structure of centromeric nucleosomes. Chromosoma 121:341–352. https://doi.org/10.1007/s00412-012-0372-y

Henikoff S, Furuyama T, Ahmad K (2004) Histone variants, nucleosome assembly and epigenetic inheritance. Trends Genet 20:320–326

Hirsch S, Baumberger R, Grossniklaus U (2013) Epigenetic variation, inheritance, and selection in plant populations. Cold Spring Harb Symp Quant Biol 77:97–104

Honys D, Twell D (2004) Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol 5:R85. https://doi.org/10.1186/gb-2004-5-11-r85

Hövel I, Pearson NA, Stam M (2015) Cis-acting determinants of paramutation. Semin Cell Dev Biol 44:22–32

Hristova E, Fal K, Klemme L et al (2015) HISTONE DEACETYLASE6 controls gene expression patterning and DNA methylation-independent euchromatic silencing. Plant Physiol 168:1298–1308. https://doi.org/10.1104/pp.15.00177

Hsieh TF, Ibarra CA, Silva P et al (2009) Genome-wide demethylation of Arabidopsis endosperm. Science 324:1451–1454. https://doi.org/10.1126/science.1172417

Hsieh P-H, He S, Buttress T et al (2016) Arabidopsis male sexual lineage exhibits more robust maintenance of CG methylation than somatic tissues. Proc Natl Acad Sci U S A 113:15132–15137. https://doi.org/10.1073/pnas.1619074114

Ibarra CA, Feng X, Schoft VK et al (2012) Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. Science 337:1360–1364

Ikeda Y, Kinoshita Y, Susaki D et al (2011) HMG domain containing SSRP1 is required for DNA demethylation and genomic imprinting in Arabidopsis. Dev Cell 21:589–596. https://doi.org/10.1016/J.DEVCEL.2011.08.013

Ingouff M, Hamamura Y, Gourgues M et al (2007) Distinct dynamics of HISTONE3 variants between the two fertilization products in plants. Curr Biol 17:1032–1037

Ingouff M, Selles B, Michaud C et al (2017) Live-cell analysis of DNA methylation during sexual reproduction in Arabidopsis reveals context and sex-specific dynamics controlled by noncanonical RdDM. Genes Dev 31:72–83. https://doi.org/10.1101/gad.289397.116

Jablonka E, Raz G (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q Rev Biol 84:131–176

Jeddeloh JA, Stokes TL, Richards EJ (1999) Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat Genet 22:94–97. https://doi.org/10.1038/8803

Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074–1080. https://doi.org/10.1126/science.1063127

Jiang D, Berger F (2017) Histone variants in plant transcriptional regulation. Biochim Biophys Acta Gene Regul Mech 1860:123. https://doi.org/10.1016/j.bbagrm.2016.07.002

Johannes F, Colot V, Jansen RC (2008) Epigenome dynamics: a quantitative genetics perspective. Nat Rev Genet 9:883–890

Johannes F, Porcher E, Teixeira FK et al (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genet 5:e1000530. https://doi.org/10.1371/journal.pgen.1000530

Jullien PE, Susaki D, Yelagandula R et al (2012) DNA methylation dynamics during sexual reproduction in *Arabidopsis thaliana*. Curr Biol 22:1825–1830. https://doi.org/10.1016/j.cub.2012.07.061

Kakrana A, Mathioni SM, Huang K et al (2018) Plant 24-nt reproductive phasiRNAs from intramolecular duplex mRNAs in diverse monocots. Genome Res 28:1333–1344. https://doi.org/10.1101/gr.228163.117

Kawashima T, Berger F (2014) Epigenetic reprogramming in plant sexual reproduction. Nat Rev Genet 15:613–624

Khraiwesh B, Zhu J-K, Zhu J (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819:137–148. https://doi.org/10.1016/j.bbagrm.2011.05.001

Kidner CA, Martienssen RA (2005) The role of ARGONAUTE1 (AGO1) in meristem formation and identity. Dev Biol 280:504–517. https://doi.org/10.1016/j.ydbio.2005.01.031

Klattenhoff C, Theurkauf W (2008) Biogenesis and germline functions of piRNAs. Development 135:3–9. https://doi.org/10.1242/dev.006486

Komiya R, Ohyanagi H, Niihama M et al (2014) Rice germline-specific Argonaute MEL1 protein binds to phasiRNAs generated from more than 700 lincRNAs. Plant J 78:385–397

Lamke J, Baurle I (2017) Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol 18:1–11

Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 11:204–220. https://doi.org/10.1038/nrg2719

Lee YS, Shibata Y, Malhotra A, Dutta A (2009) A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev 23:2639–2649. https://doi.org/10.1101/gad.1837609

Lewsey MG, Hardcastle TJ, Melnyk CW et al (2016) Mobile small RNAs regulate genome-wide DNA methylation. Proc Natl Acad Sci 113:E801–E810. https://doi.org/10.1073/pnas.1515072113

Li J, Huang Q, Sun M et al (2016) Global DNA methylation variations after short-term heat shock treatment in cultured microspores of *Brassica napus* cv. Topas. Sci Rep 6:38401. https://doi.org/10.1038/srep38401

Lippman Z, May B, Yordan C et al (2003) Distinct mechanisms determine transposon inheritance and methylation via small interfering RNA and histone modification. PLoS Biol 1:E67. https://doi.org/10.1371/journal.pbio.0000067

Lippman Z, Gendrel A-V, Black M et al (2004) Role of transposable elements in heterochromatin and epigenetic control. Nature 430:471–476. https://doi.org/10.1038/nature02651

Lister R, O'Malley RC, Tonti-Filippini J et al (2008) Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell 133:523–536. https://doi.org/10.1016/J.CELL.2008.03.029

Luo M, Taylor JM, Spriggs A et al (2011) A genome-wide survey of imprinted genes in rice seeds reveals imprinting primarily occurs in the endosperm. PLoS Genet 7:1002125. https://doi.org/10.1371/journal.pgen.1002125

Lyons DB, Zilberman D (2017) DDM1 and Lsh remodelers allow methylation of DNA wrapped in nucleosomes. elife 6:1–20. https://doi.org/10.7554/eLife.30674

Makarevich G, Villar CBR, Erilova A, Köhler C (2008) Mechanism of PHERES1 imprinting in Arabidopsis. J Cell Sci 121:906–912. https://doi.org/10.1242/jcs.023077

Manning K, Tör M, Poole M et al (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 38:948–952. https://doi.org/10.1038/ng1841

Mariño-Ramírez L, Kann MG, Shoemaker BA, Landsman D (2005) Histone structure and nucleosome stability. Expert Rev Proteomics 2:719–729. https://doi.org/10.1586/14789450.2.5.719
Martienssen R (2010) Small RNA makes its move. Science 328:834–835. https://doi.org/10.1126/science.1187959
Martienssen RA, Colot V (2001) DNA methylation and epigenetic inheritance in plants and filamentous fungi. Science 293:1070–1074
Martienssen R, Barkan A, Taylor WC, Freeling M (1990) Somatically heritable switches in the DNA modification of Mu transposable elements monitored with a suppressible mutant in maize. Genes Dev 4:331–343
Martienssen R, Lippman Z, May B et al (2004) Transposons, tandem repeats, and the silencing of imprinted genes. Cold Spring Harb Symp Quant Biol 69:371–379
Martin A, Troadec C, Boualem A et al (2009) A transposon-induced epigenetic change leads to sex determination in melon. Nature 461:1135–1138. https://doi.org/10.1038/nature08498
Martin RC, Liu P-P, Goloviznina NA, Nonogaki H (2010) microRNA, seeds, and Darwin?: diverse function of miRNA in seed biology and plant responses to stress. J Exp Bot 61:2229–2234. https://doi.org/10.1093/jxb/erq063
Martinez G, Panda K, Köhler C, Slotkin RK (2016) Silencing in sperm cells is directed by RNA movement from the surrounding nurse cell. Nat Plants 2:16030
Martinez G, Choudury SG, Slotkin RK (2017) tRNA-derived small RNAs target transposable element transcripts. Nucleic Acids Res 45:5142–5152
Matsunaga W, Ohama N, Tanabe N et al (2015) A small RNA mediated regulation of a stress-activated retrotransposon and the tissue specific transposition during the reproductive period in Arabidopsis. Front Plant Sci 6:48. https://doi.org/10.3389/fpls.2015.00048
Matzke MA, Mosher RA (2014) RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. Nat Rev Genet 15:394–408
McClintock B (1957) Controlling elements and the gene. Cold Spring Harb Symp Quant Biol 21:197–216. https://doi.org/10.1101/SQB.1956.021.01.017
McClintock B (1961) Some parallels between gene control systems in maize and in bacteria. Am Nat 95:265–277. https://doi.org/10.1086/282188
McCormick S (1993) Male gametophyte development. Plant Cell 5:1265–1275. https://doi.org/10.1105/tpc.016659
McCue AD, Panda K, Nuthikattu S et al (2015) ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation. EMBO J 34:20–35. https://doi.org/10.15252/embj.201489499
Mehdi S, Derkacheva M, Ramström M et al (2016) The WD40 domain protein MSI1 functions in a histone deacetylase complex to fine-tune abscisic acid signaling. Plant Cell 28:42–54. https://doi.org/10.1105/tpc.15.00763
Moore T, Haig D (1991) Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 7:45–49. https://doi.org/10.1016/0168-9525(91)90230-N
Nobuta K, Venu RC, Lu C et al (2007) An expression atlas of rice mRNAs and small RNAs. Nat Biotechnol 25:473–477
Nonomura K-I, Morohoshi A, Nakano M et al (2007) 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 19:2583–2594. https://doi.org/10.1105/tpc.107.053199
Nuthikattu S, McCue AD, Panda K et al (2013) The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21-22 nucleotide small interfering RNAs. Plant Physiol 162:116–131. https://doi.org/10.1104/pp.113.216481
Okada T, Endo M, Singh MB, Bhalla PL (2005) Analysis of the histone H3 gene family in Arabidopsis and identification of the male-gamete-specific variant AtMGH3. Plant J 44:557–568. https://doi.org/10.1111/j.1365-313X.2005.02554.x
Okada T, Singh MB, Bhalla PL (2006) Histone H3 variants in male gametic cells of lily and H3 methylation in mature pollen. Plant Mol Biol 62:503–512. https://doi.org/10.1007/s11103-006-9036-8

Olmedo-Monfil V, Durán-Figueroa N, Arteaga-Vázquez M et al (2010) Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature 464:628–632. https://doi.org/10.1038/nature08828

Pál C, Miklós I (1999) Epigenetic inheritance, genetic assimilation and speciation. J Theor Biol 200:19–37. https://doi.org/10.1006/jtbi.1999.0974

Peng S, Huang J, Sheehy JE et al (2004) Rice yields decline with higher night temperature from global warming. Proc Natl Acad Sci 101:9971–9975. https://doi.org/10.1073/pnas.96.11.5952

Perrella G, Carr C, Asensi-Fabado MA et al (2016) The histone deacetylase complex 1 protein of Arabidopsis has the capacity to interact with multiple proteins including histone 3-binding proteins and histone 1 variants. Plant Physiol 171:62–70. https://doi.org/10.1104/pp.15.01760

Pignatta D, Erdmann RM, Scheer E et al (2014) Natural epigenetic polymorphisms lead to intraspecific variation in Arabidopsis gene imprinting. elife 3:3198. https://doi.org/10.7554/eLife.03198

Pignatta D, Novitzky K, Satyaki PRV, Gehring M (2018) A variably imprinted epiallele impacts seed development. PLoS Genet 14:1–18. https://doi.org/10.1101/339036

Pina C, Pinto F, Feijó JA, Becker JD (2005) Gene family analysis of the Arabidopsis pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. Plant Physiol 108:744–756. https://doi.org/10.1023/A:1024866716987

Pinon V, Yao X, Dong A, Shen W-H (2017) SDG2-mediated H3K4me3 is crucial for chromatin condensation and mitotic division during male gametogenesis in Arabidopsis. Plant Physiol 174:1205–1215. https://doi.org/10.1104/pp.17.00306

Probst AV, Fagard M, Proux F et al (2004) Arabidopsis histone deacetylase HDA6 is required for maintenance of transcriptional gene silencing and determines nuclear organization of rDNA repeats. Plant Cell 16:1021–1034. https://doi.org/10.1105/tpc.018754

Qi Y, He X, Wang X-J et al (2006) Distinct catalytic and non-catalytic roles of ARGONAUTE4 in RNA-directed DNA methylation. Nature 443:1008–1012. https://doi.org/10.1038/nature05198

Radford EJ, Ferrón SR, Ferguson-Smith AC (2011) Genomic imprinting as an adaptative model of developmental plasticity. FEBS Lett 585:2059–2066

Richards EJ (2011) Natural epigenetic variation in plant species: a view from the field. Curr Opin Plant Biol 14:204–209

Ronemus M, Vaughn MW, Martienssen RA (2006) MicroRNA-targeted and small interfering RNA–mediated mRNA degradation is regulated by argonaute, dicer, and RNA-dependent RNA polymerase in Arabidopsis. Plant Cell 18:1559–1574. https://doi.org/10.1105/tpc.106.042127.1

Rotman N, Durbarry A, Wardle A et al (2005) A novel class of MYB factors controls sperm-cell formation in plants. Curr Biol 15:244–248. https://doi.org/10.1016/J.CUB.2005.01.013

Russell SD, Gou X, Wong CE et al (2012) Genomic profiling of rice sperm cell transcripts reveals conserved and distinct elements in the flowering plant male germ lineage. New Phytol 195:560–573

Ryan DP, Owen-Hughes T (2011) Snf2-family proteins: chromatin remodellers for any occasion. Curr Opin Chem Biol 15:649–656. https://doi.org/10.1016/j.cbpa.2011.07.022

Schmidt A, Schmid MW, Grossniklaus U (2015) Plant germline formation: common concepts and developmental flexibility in sexual and asexual reproduction. Development 142:229–241

Schmitz RJ, Schultz MD, Lewsey MG et al (2011) Transgenerational epigenetic instability is a source of novel methylation variants. Science 334:369–373

Schoft VK, Chumak N, Mosiolek M et al (2009) Induction of RNA-directed DNA methylation upon decondensation of constitutive heterochromatin. EMBO Rep 10:1015–1021

Schoft VK, Chumak N, Choi Y et al (2011) Function of the DEMETER DNA glycosylase in the *Arabidopsis thaliana* male gametophyte. Proc Natl Acad Sci 108:8042–8047. https://doi.org/10.1073/pnas.1105117108

Schorn AJ, Gutbrod MJ, LeBlanc C, Martienssen R (2017) LTR-retrotransposon control by tRNA-derived small RNAs. Cell 170:61–71.e11

Schubert V, Lermontova I, Schubert I (2014) Loading of the centromeric histone H3 variant during meiosis–how does it differ from mitosis? Chromosoma 123:491–497. https://doi.org/10.1007/s00412-014-0466-9

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. Front Plant Sci 6:294. https://doi.org/10.3389/fpls.2015.00294

Shu H, Nakamura M, Siretskiy A et al (2014) Arabidopsis replacement histone variant H3.3 occupies promoters of regulated genes. Genome Biol 15:R62. https://doi.org/10.1186/gb-2014-15-4-r62

Slotkin RK (2010) The epigenetic control of the Athila family of retrotransposons in Arabidopsis. Epigenetics 5:483–490. https://doi.org/10.4161/epi.5.6.12119

Slotkin RK, Vaughn M, Borges F et al (2009) Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. Cell 136:461–472

Solís MT, Rodríguez-Serrano M, Meijón M et al (2012) DNA methylation dynamics and MET1a-like gene expression changes during stress-induced pollen reprogramming to embryogenesis. J Exp Bot 63:6431–6444. https://doi.org/10.1093/jxb/ers298

Song X, Li P, Zhai J et al (2012a) Roles of DCL4 and DCL3b in rice phased small RNA biogenesis. Plant J 69:462–474. https://doi.org/10.1111/j.1365-313X.2011.04805.x

Song X, Wang D, Ma L et al (2012b) Rice RNA-dependent RNA polymerase 6 acts in small RNA biogenesis and spikelet development. Plant J 71:378–389. https://doi.org/10.1111/j.1365-313X.2012.05001.x

Soppe WJJ, Jacobsen SE, Alonso-Blanco C et al (2000) The late flowering phenotype of fwa mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. Mol Cell 6:791–802

Spielman M, Vinkenoog R, Dickinson HG, Scott RJ (2001) The epigenetic basis of gender in flowering plants and mammals. Trends Genet 17:705–711

Springer NM (2013) Epigenetics and crop improvement. Trends Genet 29:241–247

Stroud H, Otero S, Desvoyes B et al (2012) Genome-wide analysis of histone H3.1 and H3.3 variants in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 109:5370–5375. https://doi.org/10.1073/pnas.1203145109

Stroud H, Do T, Du J et al (2014) Non-CG methylation patterns shape the epigenetic landscape in Arabidopsis. Nat Struct Mol Biol 21:64–72. https://doi.org/10.1038/nsmb.2735

Sunkar R, Li Y-F, Jagadeeswaran G (2012) Functions of microRNAs in plant stress responses. Trends Plant Sci 17:196–203. https://doi.org/10.1016/J.TPLANTS.2012.01.010

Swanson WJ, Vacquier VD (2002) The rapid evolution of reproductive proteins. Nat Rev Genet 3:137–144. https://doi.org/10.1038/nrg733

Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y (2004) Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 116:51–61. https://doi.org/10.1016/S0092-8674(03)01064-X

Teixeira FK, Heredia F, Sarazin A et al (2009) A role for RNAi in the selective correction of DNA methylation defects. Science 323:1600–1604

Ueda K, Tanaka I (1995) The appearance of male gamete-specific histones gH2B and gH3 during pollen development in *Lilium longiflorum*. Dev Biol 169:210–217. https://doi.org/10.1006/dbio.1995.1138

Ueda K, Ono M, Iwashita J et al (2012) Generative cell-specific activation of the histone gH2A gene promoter of *Lilium longiflorum* in tobacco. Sex Plant Reprod 25:247–255. https://doi.org/10.1007/s00497-012-0194-3

Underwood CJ, Henderson IR, Martienssen RA (2017) Genetic and epigenetic variation of transposable elements in Arabidopsis. Curr Opin Plant Biol 36:135–141. https://doi.org/10.1016/j.pbi.2017.03.002

Wang G, Köhler C (2017) Epigenetic processes in flowering plant reproduction. J Exp Bot 68:797–807

Waters AJ, Makarevitch I, Eichten SR et al (2011) Parent-of-origin effects on gene expression and DNA methylation in the maize endosperm. Plant Cell 23:4221–4233. https://doi.org/10.1105/tpc.111.092668

Waters AJ, Bilinski P, Eichten SR et al (2013) Comprehensive analysis of imprinted genes in maize reveals allelic variation for imprinting and limited conservation with other species. Proc Natl Acad Sci U S A 110:19639–19644. https://doi.org/10.1073/pnas.1309182110

Weigel D, Colot V (2012) Epialleles in plant evolution. Genome Biol 13:249. https://doi.org/10.1186/gb-2012-13-10-249

Wolff P, Weinhofer I, Seguin J et al (2011) High-resolution analysis of parent-of-origin allelic expression in the Arabidopsis endosperm. PLoS Genet 7:1002126. https://doi.org/10.1371/journal.pgen.1002126

Wolff P, Jiang H, Wang G et al (2015) Paternally expressed imprinted genes establish postzygotic hybridization barriers in *Arabidopsis thaliana*. elife 4:1–14. https://doi.org/10.7554/eLife.10074

Wollmann H, Berger F (2012) Epigenetic reprogramming during plant reproduction and seed development. Curr Opin Plant Biol 15:63–69. https://doi.org/10.1016/j.pbi.2011.10.001

Wollmann H, Holec S, Alden K et al (2012) Dynamic deposition of histone variant H3.3 accompanies developmental remodeling of the Arabidopsis transcriptome. PLoS Genet 8:28–31. https://doi.org/10.1371/journal.pgen.1002658

Xu H, Swoboda I, Bhalla PL, Singh MB (1999) Male gametic cell-specific expression of H2A and H3 histone genes. Plant Mol Biol 39:607–614. https://doi.org/10.1023/A:1006162120037

Yang H, Lu P, Wang Y, Ma H (2011) The transcriptome landscape of Arabidopsis male meiocytes from high-throughput sequencing: the complexity and evolution of the meiotic process. Plant J 65:503–516. https://doi.org/10.1111/j.1365-313X.2010.04439.x

Yang H, Yang N, Wang T (2016) Proteomic analysis reveals the differential histone programs between male germline cells and vegetative cells in Lilium davidii. Plant J 85:660–674. https://doi.org/10.1111/tpj.13133

Yoder JA, Walsh CP, Bestor TH (1997) Cytosine methylation and the ecology of intragenomic parasites. Trends Genet 13:335–340

Yuan J, Chen S, Jiao W et al (2017) Both maternally and paternally imprinted genes regulate seed development in rice. New Phytol 216:373–387. https://doi.org/10.1111/nph.14510

Zemach A, Kim MY, Hsieh P-H et al (2013) The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. Cell 153:193–205. https://doi.org/10.1016/J.CELL.2013.02.033

Zhai J, Jeong DH, de Paoli E et al (2011) MicroRNAs as master regulators of the plant NB-LRR defense gene family via the production of phased, trans-acting siRNAs. Genes Dev 25:2540–2553. https://doi.org/10.1101/gad.177527.111

Zhai J, Zhang H, Arikit S et al (2015) Spatiotemporally dynamic, cell-type-dependent premeiotic and meiotic phasiRNAs in maize anthers. Proc Natl Acad Sci U S A 112:3146–3151. https://doi.org/10.1073/pnas.1418918112

Zhang S, Zhan X, Xu X et al (2015) Two domain-disrupted hda6 alleles have opposite epigenetic effects on transgenes and some endogenous targets. Sci Rep 5:17832. https://doi.org/10.1038/srep17832

Zhang M, Li N, He W et al (2016) Genome-wide screen of genes imprinted in sorghum endosperm, and the roles of allelic differential cytosine methylation. Plant J 85:424–436. https://doi.org/10.1111/tpj.13116

Zhong S-H, Liu J-Z, Jin H et al (2013) Warm temperatures induce transgenerational epigenetic release of RNA silencing by inhibiting siRNA biogenesis in Arabidopsis. Proc Natl Acad Sci U S A 110:9171–9176. https://doi.org/10.1073/pnas.1219655110

Zhong X, Du J, Hale CJ et al (2014) Molecular mechanism of action of plant DRM de novo DNA methyltransferases. Cell 157:1050–1060. https://doi.org/10.1016/j.cell.2014.03.056

Zhou M, Palanca AMS, Law JA (2018) Locus-specific control of the de novo DNA methylation pathway in Arabidopsis by the CLASSY family. Nat Genet 50:865–873. https://doi.org/10.1038/s41588-018-0115-y