

Focus: Molecular Memory

Epigenetic memory in plants

Mayumi Iwasaki* & Jerzy Paszkowski**

Abstract

Epigenetics refers to heritable changes in patterns of gene expression that occur without alterations in DNA sequence. The epigenetic mechanisms involve covalent modifications of DNA and histones, which affect transcriptional activity of chromatin. Since chromatin states can be propagated through mitotic and meiotic divisions, epigenetic mechanisms are thought to provide heritable ‘cellular memory’. Here, we review selected examples of epigenetic memory in plants and briefly discuss underlying mechanisms.

Keywords epialleles; epigenetics; memory; plants; transposons

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Introducing epigenetics

The term ‘epigenetics’ combines two words ‘epigenesis’ and ‘genetics’ and was coined by Conrad H. Waddington in 1942. He defined epigenetics as “the branch of biology that studies the causal interaction between genes and their products, which brings the phenotype into being” (Waddington, 1942) and proposed the concept of the epigenetic landscape as a metaphor for cell differentiation (Waddington, 1957). At various points during the progression toward their final differentiated states, changes occur in cells according to genetic and/or environmental factors. For this process to occur, altered features of the cells must be memorized after each cell division. Epigenetics has since been redefined several times. Nowadays, it is commonly taken to mean the study of mitotically and/or meiotically heritable changes in patterns of gene expression that occur without alterations in DNA sequence. Current epigenetic studies are often focused on chemical modifications of chromatin and their roles in active transcription and transcriptional silencing. Chemical modifications of chromatin alter both DNA and histone proteins.

DNA methylation is a covalent modification of DNA, and although it is found across many genera, its crucial role in epigenetic regulation of transcription is best documented in plants and mammals. DNA hydroxymethylation is another DNA modification recently discovered in mammals. It is possible that this modification represents an intermediate of DNA demethylation, but it may also contribute to epigenetic regulation. Histone proteins are subjected to

various covalent modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. In addition, incorporation of histone variants and relocation of nucleosomes can also affect chromatin structure and its function in transcriptional regulation.

Non-coding RNAs, including small RNAs, frequently influence the distribution patterns of epigenetic marks and can thus act in a sequence-specific manner to regulate gene expression at both transcriptional and post-transcriptional levels. In plants, certain small RNAs direct DNA methylation at their homologous regions in a process known as RNA-directed DNA methylation (RdDM).

It is well documented that the interplay of epigenetic marks determines particular chromatin states essential to the regulation of various biological processes. In plants, years of work aiming to understand the molecular mechanisms underlying paramutation, gene imprinting, suppression of transposons, and silencing of transgenic loci led to the discovery of epigenetic regulation that contribute to heritability: memorization as well as mitotic and meiotic transmission of particular transcriptional states.

In the first part of this review, we will briefly discuss examples of ‘epigenetic memory’ in the regulation of plant development—modifications that are reset at each generation allowing progeny to recapitulate developmental steps of their parents. In the second part, we provide selected examples of epigenetic contributions to transgenerational inheritance in plants, as well as illustrative examples of stable epialleles found in nature or induced experimentally. Finally, we address the somewhat controversial topic of environmentally induced transgenerational changes in epigenetic memory.

Mitotically heritable epigenetic memory—resetting marks between generations

Imprinting—memory of parental origin

Genomic imprinting is a phenomenon that leads to differential allelic expression depending on whether a gene was inherited through a female or male gamete. Genomic imprinting is well documented for seed plants and for mammals but is thought to have evolved independently (Feil & Berger, 2007). In both groups of organisms, imprinting occurs in embryo-nourishing tissues: the endosperm in plants and the placenta in mammals (Kohler & Weinhofer-Molisch, 2010).

Double fertilization in flowering plants is a specific process involving multicellular male and female gametophytes, pollen grain

The Sainsbury Laboratory, University of Cambridge, Cambridge, UK

*Corresponding author. Tel: +44 1223 761159; E-mail: mayumi.iwasaki@slcu.cam.ac.uk

**Corresponding author. Tel: +44 1223 761159; E-mail: jerzy.paszkowski@slcu.cam.ac.uk

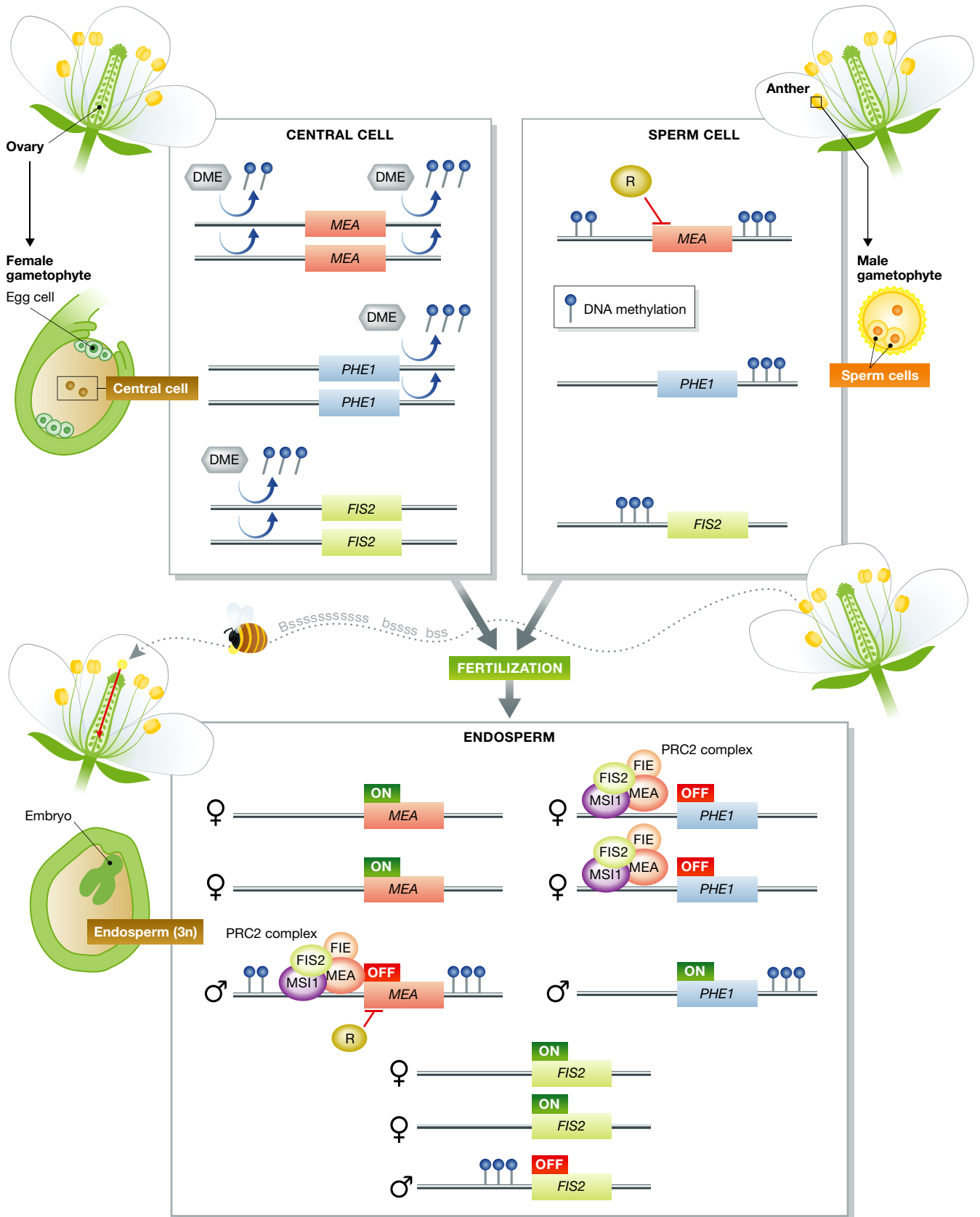


Figure 1. Schematic illustration of parental imprinting.

In females, the central cell DME removes DNA methylation from maternally expressed genes, *MEA* and *FIS2*, and from the paternally expressed gene *PHE1*. DNA methylation at these loci is maintained in the male gametophyte. During fertilization, the central cell fuses with one sperm cell to form the endosperm. In endosperm, maternal alleles of *MEA* and *FIS2* are expressed. The PRC2 complex including *MEA* and *FIS2* binds to the promoter of the paternal allele of *MEA* and mediates silencing by catalyzing H3K27 tri-methylation. Another unknown repressor (R) may be required for repression of the paternal allele of *MEA*. The PRC2 complex mediates silencing of the maternal allele of *PHE*. In addition to the PRC2 complex, maternal removal of DNA methylation downstream of *PHE* gene is required for silencing of its maternal allele.

and embryo sac, respectively. The pollen grain contains two sperm cells. One sperm cell fuses with an egg cell and a second fuses with the bi-nucleated central cell of the embryo sac, leading to development of the embryo and the triploid endosperm, respectively (Fig 1). The endosperm, thought to be functionally analogous to the placenta in mammals, supports and nourishes the embryo during seed development and/or seed germination (Ingram, 2010).

During gametogenesis, imprinted gene alleles are epigenetically silenced either maternally or paternally. The epigenetic memory of parental origin persists beyond fertilization and results in differential transcriptional activity of maternal and paternal alleles in the developing endosperm. Since the endosperm is a terminal tissue, imprinting features of specific genes cannot be transmitted to the next generation and are thus not reset.

In plants, two epigenetic marks of DNA methylation and histone methylation are involved in the regulation of imprinting. DNA demethylase DEMETER (DME), which has DNA glycosylase activity directed toward methylated cytosines, is present in the central cell and removes methylated cytosines from maternally expressed genes (MEGs) such as *MEA*, *FIS2*, and *FWA*, leading to transcriptional activation of their maternal alleles (Choi *et al*, 2002, 2004; Gehring *et al*, 2006; Morales-Ruiz *et al*, 2006). DNA methyltransferase MET1 also regulates maternally imprinted genes. In somatic tissues, DNA methylation is maintained by MET1; however, expression of MET1 is suppressed in the central cell during female gametogenesis, and this seems to contribute to DNA hypomethylation of MEGs (Jullien *et al*, 2006b, 2008).

Further factors regulating imprinting include the evolutionarily conserved polycomb group proteins. *Arabidopsis* polycomb complex PRC2, consisting of *MEA*, *FIE*, *FIS2*, and *MSI1*, catalyzes H3K27 trimethylation, and this repressive histone mark leads to the suppression of paternal alleles of MEGs or the maternal alleles of paternally expressed genes (PEGs) (Kohler *et al*, 2005; Baroux *et al*, 2006; Jullien *et al*, 2006a; Makarevich *et al*, 2006).

A certain subset of imprinted genes undergoes dual regulation by PRC2 and DME. For example, silencing of a maternal allele of *PHE1* (PEG) involves hypomethylation of repeats located in the 3' region of *PHE1* as well as binding of PRC2 to the gene promoter (Makarevich *et al*, 2008). Recent genome-wide analysis has revealed antagonistic distributions of DNA methylation and H3K27 tri-methylation, and it was suggested that DNA methylation prevents PRC binding while its removal allows PRC2 to bind histones and catalyze H3K27 tri-methylation (Weinhofer *et al*, 2010).

In maize, certain imprinted genes such as *MEE1* and *FIE2* are differentially methylated in endosperm but not in gametes, illustrating that differential methylation patterns are established after fertilization (Gutierrez-Marcos *et al*, 2006; Jahnke & Scholten, 2009). In *Arabidopsis*, it was shown that the regulation of

imprinted *MEA* expression by DME and MET1 may also occur indirectly (Wohrmann *et al*, 2012). These results suggest the existence of additional epigenetic signals besides methylation that contribute to establishing imprinting marks. The mechanisms involved in imprinting are summarized in Fig 1.

Although imprinting is widely conserved among plant species, its biological significance is not clear. One hypothesis explaining its origin is that imprinting is a by-product of transposon (TEs) silencing. Indeed, in *Arabidopsis*, the majority of imprinted genes harbor TEs or repeated sequences in their flanking regions (Wolff *et al*, 2011). In the endosperm, the activity of DME combined with the absence of MET1 results in hypomethylation of TEs and biogenesis of TE-derived small RNAs (Mosher *et al*, 2009). These small RNAs may relocate to the embryo reinforcing TE silencing there (Hsieh *et al*, 2009; Bauer & Fischer, 2011). Transcriptionally active TEs in the endosperm may also affect expression of neighboring genes. Therefore, imprinting observed in the endosperm could be linked to activation of TEs (Gehring *et al*, 2009; Hsieh *et al*, 2009; Zemach *et al*, 2010).

A hypothesis explaining the evolutionary maintenance of imprinting is that of parent conflict, which proposes that genomic imprinting evolved via competition between parents in the allocation of resources to their progeny. Several male individuals can contribute to the offspring of one female, and maximizing flow of resources to their own offspring is of paternal interest. In contrast, maternal resources are distributed equally to offspring. Therefore, PEGs would stimulate growth and thus increase seed size, whereas MEGs would limit growth (Haig & Westoby, 1989; Wilkins & Haig, 2003; Kohler & Weinhofer-Molisch, 2010). Indeed, several imprinted genes are found to be involved in endosperm development and in the control of seed size in *Arabidopsis* (Grossniklaus *et al*, 1998; Kiyosue *et al*, 1999; Kohler *et al*, 2003), and nutrient uptake and allocation in maize (Costa *et al*, 2012; Xin *et al*, 2013).

Further evidence supporting the parent conflict theory is the observation that a 2:1 maternal to paternal genome ratio in the endosperm is required for proper seed development and that imbalanced parental genome dosage alters seed size. In *Arabidopsis*, increasing paternal genome dosage in the endosperm by pollination of a diploid plant with pollen derived from a tetraploid (2m: 2p) results in larger seeds. In contrast, increasing the maternal genome dosage by pollination of a tetraploid plant with haploid pollen (4m:1p) results in smaller seeds (Scott *et al*, 1998; Tiwari *et al*, 2010). A recent study showed that most small RNAs found in the developing endosperm are expressed from the maternal genome (Mosher *et al*, 2009), and levels of these siRNAs are responsive to parental genome dosage. It has also been suggested that maternal siRNAs mediate parental genome balance and gene expression during endosperm development (Lu *et al*, 2012).

Vernalization—memory of winter

Unlike the development of animals, in which most organs are formed during embryogenesis, the organogenesis in plants continues throughout the entire life span. There are mechanisms in plants that adjust form and flexibility in developmental timing according to the ambient environment. In particular, environmental control of the timing of developmental changes often requires a certain delay between the environmental trigger and the initiation of a differentiation process. Consequently, a prolonged memory of the trigger is needed. One such well-studied developmental process is the vernalization response, in which cold exposure of winter annual plants synchronizes flowering to the optimal season. Vernalized plants thus appear to propagate a ‘memory of winter’ during most of their vegetative development (Chouard, 1960).

Molecular mechanisms of vernalization have mainly been studied in *Arabidopsis* where the flowering suppressor, *FLC*, plays a central role. *FLC* encodes a MADS box transcription factor that inhibits flowering in a dose-dependent manner (Michaels & Amasino, 1999; Sheldon *et al.*, 1999). *FLC* is expressed throughout the early vegetative development of vernalization-sensitive *Arabidopsis* strains, prior to the exposure to prolonged cold. After a certain cold period, *FLC* is silenced and flowering can be initiated according to environmental cues characteristic of a particular season (temperature, day length, etc.). Remarkably, the chromatin properties of the *FLC* gene are modified dynamically depending on the environmental phases of plant growth to reflect states before cold exposure, during cold exposure, and after cold exposure (Michaels & Amasino, 2000; Kim *et al.*, 2009).

Before cold exposure The expression of *FLC* is reset at every generation. This means that the memory of parental vernalization is erased prior to vernalization of the progeny thus allowing *de novo* adjustment of the flowering time. *FLC* resetting is associated with its transcriptional reactivation during embryogenesis (Sheldon *et al.*, 2008; Choi *et al.*, 2009), and several factors are involved in *FLC* activation. First, the FRI complex acts as an activator of *FLC* by binding to the *FLC* promoter and contributing to induction of *FLC* transcription (Johanson *et al.*, 2000). In addition, the PAF1 complex associates with RNA polymerase II and influences transcription elongation (Oh *et al.*, 2004). EFS, a component of PAF1 complex, recruits FRI at the *FLC* locus, and both ERF and FRI are required for H3K4 tri-methylation and H3K36 dimethylation (Zhao *et al.*, 2005; Xu & Shen, 2008; Ko *et al.*, 2010). The COMPASS-like complex, including the Trithorax family proteins ATX1 and ATXR7, mediates H3K4 tri-methylation (Saleh *et al.*, 2008; Tamada *et al.*, 2009). PAF1 may coordinate these activities by recruiting COMPASS (Krogan *et al.*, 2003) as such tight cooperation of similar complexes has been shown in yeast. The SWR1 complex, which is involved in H2A.Z deposition, is also required for full activation of *FLC* expression (Choi *et al.*, 2007).

During cold exposure Transcription of *FLC* is gradually silenced during prolonged cold treatment, and this is associated with PRC2-mediated H3K27 tri-methylation (Bastow *et al.*, 2004). The PRC2 complex regulating *FLC* expression consists of VRN2, SWN, FIE, and MSI1 and thus differs from the imprinting complex described in the previous section (De Lucia *et al.*, 2008). Although the core PRC2 associates with the *FLC* locus before cold exposure, PRC2 associates with plant homeodomain (PHD) proteins only during prolonged low

ambient temperatures. This gives rise to the PRC2-PHD complex, which targets a specific nucleation region of the *FLC* locus, resulting in increased H3K27 tri-methylation (Sung & Amasino, 2004; Sung *et al.*, 2006b; Greb *et al.*, 2007; De Lucia *et al.*, 2008).

Two long non-coding RNAs, COLDAIR and COOLAIR, also seem to be involved in vernalization. COLDAIR, transcribed from the first intron of *FLC*, accumulates during cold treatment and interacts physically with PRC2 (Heo & Sung, 2011). This suggests that COLDAIR acts as a scaffold to target PRC2 to the *FLC* locus, similar to the involvement of HOTAIR in PRC2-mediated silencing in humans (Zhao *et al.*, 2008). COOLAIR, also induced in the cold period, is an antisense non-coding RNA relative to the *FLC* transcript that seems to enhance silencing of *FLC* (Swiezewski *et al.*, 2009). Noticeably, regulation of the *FLC* locus is an important example of regulation of chromatin by long non-coding RNA.

After cold exposure When warm temperatures return, *FLC* remains silent and this state is mitotically inherited due to the presence of PRC2-PHD over the entire region of *FLC* (De Lucia *et al.*, 2008). As a result, H3K27 tri-methylation spreads to the whole region of *FLC* and this epigenetic silencing mark is stable during the rest of the plant’s life cycle (Finnegan & Dennis, 2007; Angel *et al.*, 2011). The stability of vernalization also depends on other factors, including VRN1 and LHP1; the latter is a homolog of HP1 in animals (Levy *et al.*, 2002; Mylne *et al.*, 2006; Sung *et al.*, 2006a).

Importantly, the duration of the cold period is critical to the final stability of *FLC* silencing. Just how the duration of the cold period is registered in plants remains an open, fascinating question. VIN3, one of the PHD proteins associated with PRC2, may play a role. The expression of VIN3 is stimulated by cold, and this increase in transcript levels may be correlated with the duration of the cold treatment, apparently antiparallel to the decrease in *FLC* transcripts (Sung & Amasino, 2004; Greb *et al.*, 2007). Thus, the increasing abundance of VIN3-PRC2 may act as a molecular measure of the cold period. However, the accumulation of VIN3 transcripts is only transient, diminishing rapidly after the cold period. This suggests that the initial memory of cold duration, possibly triggered only by VIN3, is converted to a more stable state by other mechanisms.

Notably, studies of vernalization at the level of single cells combining ChIP, *FLC* reporter gene, and mathematical modeling revealed that each cell can be switched autonomously between ‘active’ and ‘silenced’ states (Angel *et al.*, 2011). At the end of the cold period, the accumulation of H3K27 tri-methylation at the nucleation region of *FLC* in a subset of random cells switches them into a stable silenced state. Importantly, the probability for a given cold-exposed cell to switch to a silenced state increases with the duration of the cold period. Therefore, the quantitative nature of vernalization is determined by a subpopulation of cells in which *FLC* is stably silenced (Angel *et al.*, 2011; Song *et al.*, 2012). An overview of *FLC* regulation is presented in Fig 2.

Acclimation—abiotic stress memory

Mechanisms of transcriptional epigenetic regulation are known to be involved in plant stress responses. For example, when rice seedlings are submerged, the levels of H3K4 methylation and H3 acetylation increase on the submergence-inducible genes *ADH1* and *PDC1* (Tsuji *et al.*, 2006). In *Arabidopsis*, drought stress changes histone modifications at the drought stress-inducible loci *RD29A*, *RD29B*,

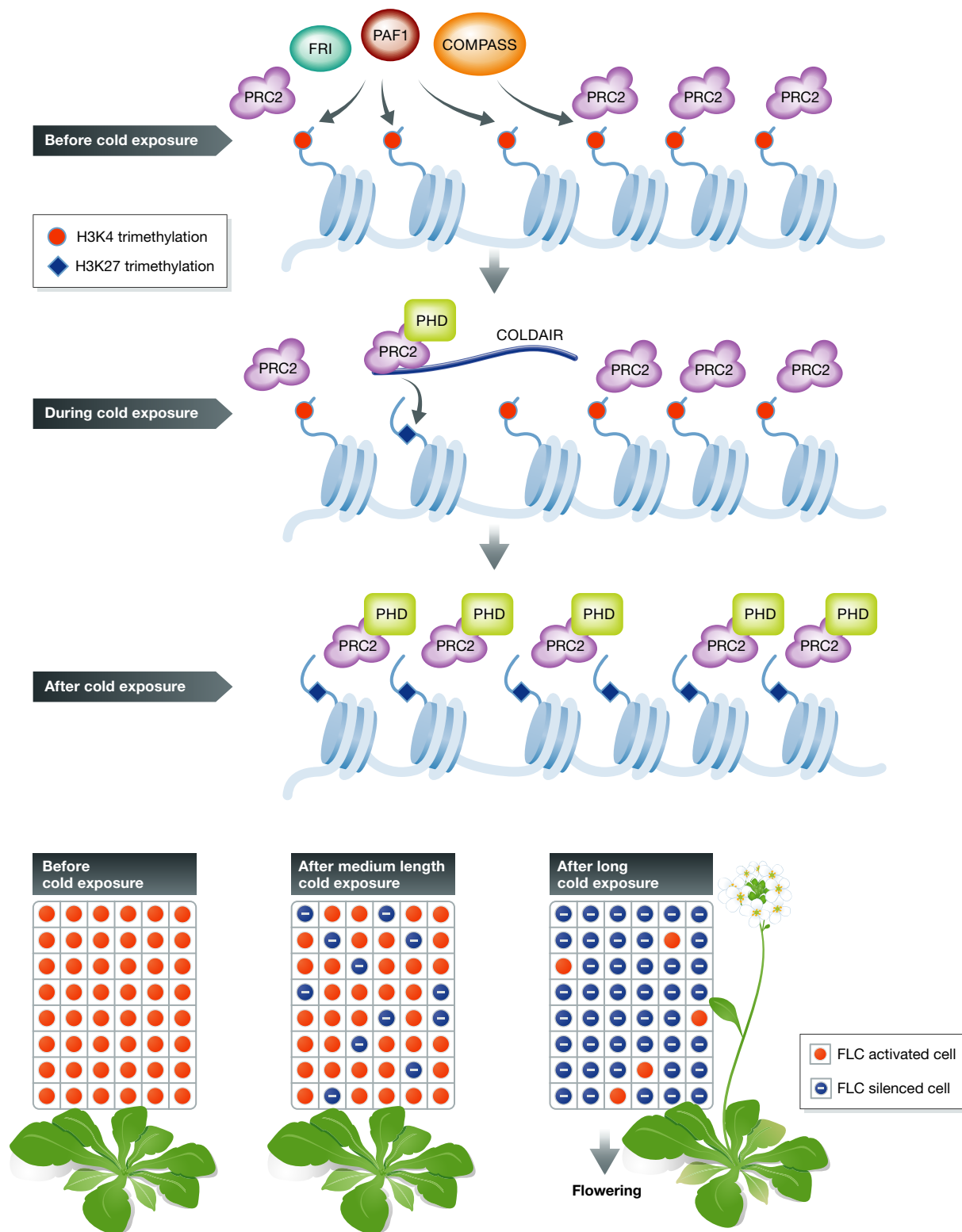


Figure 2. FLC regulation.

FRI, PAF1, and COMPASS-like complexes are involved in activation/reset of *FLC* at every generation. During cold exposure, the PRC2-PHD complex and non-coding RNA COLDAIR are recruited at the nucleation region of *FLC* and catalyze H3K27 tri-methylation. After return to higher temperatures, PRC2-PHD associates across the entire region of *FLC* leading to cell-autonomous stable transcriptional silencing. After prolonged cold exposure, the number of cells in which *FLC* is stably silenced increases.

RD20, and *At2g20880* (Kim *et al.*, 2008). The expression levels of HDA6 and HDA19, members of the histone deacetylase family (HDACs), increase during environmental stresses such as low temperature, wounding, or hormonal signals, suggesting that these HDACs regulate stress-associated target genes (Zhou *et al.*, 2005).

Small RNAs also seem to play an important role in stress responses. For example, salt stress in *Arabidopsis* induces the production of siRNAs from overlapping gene pairs of *P5CDH* and *SRO5* that in turn influence salt stress tolerance (Borsani *et al.*, 2005).

There are several examples of stress affecting DNA methylation. In maize, cold stress induces hypomethylation of *ZmM11* in roots (Steward *et al.*, 2002). White clover and industrial hemp treated with heavy metals display hypomethylation of specific loci in their roots (Aina *et al.*, 2004). The biological significance of these changes in methylation is not clear, though, and since reduced levels of DNA methylation are only found in roots, they cannot be passed to the next generation.

In addition to the implication of epigenetic regulation in immediate stress responses, such mechanisms have also been suggested to be involved in long-term stress adaptation. This can be illustrated by the exposure of plants to long-term cold (2°C for 3 days), a treatment that increases future freezing tolerance. Such plant hardening has been defined as cold acclimation. Cold-treated *Arabidopsis hda6* mutants are not only less tolerant to freezing than cold-treated wild-type plants but also resist cold acclimation, which suggests the involvement of HDA6-mediated chromatin modifications in the acclimation process (To *et al.*, 2011).

Memory of pathogen attack—systemic acquired resistance

The first exposure of a plant to a pathogen can induce long-lasting, systemic immunity against subsequent pathogen attacks; this is now known as systemic acquired resistance (SAR) (Vlot *et al.*, 2008). SAR involves the plant hormone salicylic acid (SA) (Loake & Grant, 2007) and the downstream signaling protein NPR1 (Durrant & Dong, 2004), which are both essential for SAR. During SAR, the transcription of SA-responsive genes is activated, including genes encoding antimicrobial pathogenesis-related proteins (PR) (Ryals *et al.*, 1996). Elevated levels of SA induce changes in chromatin modification at these target genes. For example, the levels of H3 acetylation, H4 acetylation, and H3K4 methylation are increased at the *PR-1* promoter (Butterbrodt *et al.*, 2006). It is still not clear to which extent these modifications contribute to the stability of SAR in terms of enhanced memory of the initial pathogen attack. However, it has been suggested that histone modification and/or histone replacement by histone variants may prime pathogen responsive genes for rapid activation during subsequent pathogen attacks.

WRKY genes encode transcription factors that are also induced by pathogen infection or SA treatment (Asai *et al.*, 2002; Dong *et al.*, 2003). It has been shown that local pathogen infections induce changes in histone modifications at promoters of several *WRKY* genes and that this also occurs in leaves distant from the infection sites. Interestingly, although the levels of active histone marks such as H3 acetylation and H3K4 methylation increase, the genes remain silent. It has been postulated that these modifications are primed for amplified transcriptional responses during subsequent pathogen attacks, thus implicating histone modifications in possible mechanisms of memory in SAR (Jaskiewicz *et al.*, 2011).

A further epigenetic mechanism that may contribute to memory in SAR involves histone variant H2A.Z. As one of the most conserved eukaryotic histone variants, H2A.Z is enriched at the transcription start sites of genes, and it has been suggested that its incorporation contributes to gene activation, transcriptional memory, heterochromatic silencing, and thermal sensing (Dhillon *et al.*, 2006; Brickner *et al.*, 2007; Zlatanova & Thakar, 2008; Kumar & Wigge, 2010; Light *et al.*, 2010). In *Arabidopsis* mutants deficient in the SWR1 complex, which is required for H2A.Z deposition, a large number of genes induced in SAR are constitutively expressed (March-Diaz *et al.*, 2008). Since deposition of H2A.Z is associated with transcriptional memory and rapid reactivation of genes, H2A.Z may be important for priming genes induced in SAR.

Meiotically heritable epigenetic memory—the formation of epialleles

In this section, we will consider examples where certain loci are converted to alternative and relatively stable epigenetic states that are transmitted between generations in the form of heritable epialleles. We also discuss epigenetic mechanisms possibly involved in epiallelic switching—using examples of experimentally induced epialleles—and address the question of environmentally triggered deposition of transgenerational epigenetic memory.

Experimentally induced epialleles

In plants, DNA methylation is an epigenetic mark for which meiotic inheritance has been clearly demonstrated. DNA methylation is restricted to cytosines and is found in plants in multiple sequence contexts: CG, CHG, and CHH (H stands for A, C, or T), in contrast to mammals where DNA is found almost exclusively on CG sequences. Mechanisms maintaining CG methylation through the DNA replication cycle are well characterized in plants and mammals and involve similar DNA methyltransferases, MET1 and DNMT1, respectively. During replication, these enzymes recognize hemimethylated DNA and add methylation to cytosines of the newly synthesized strand using the old, methylated strand as a guide. Consequently, CG methylation patterns are faithfully maintained throughout mitotic or meiotic cell divisions. However, if CG methylation patterns are altered, the aberrant methylation will also be propagated (Saze *et al.*, 2003; Mathieu *et al.*, 2007; Law & Jacobsen, 2010).

Non-CG methylation, a characteristic of plants, is maintained by the redundant activities of DNA methyltransferases CMT3 and DRM2, and other associated activities. CMT3, a plant-specific chromomethylase, catalyzes non-CG methylation in cooperation with histone modifications, especially H3K9 methylation. DRM2 is guided by siRNAs in a process of RdDM. In addition, the chromatin remodeling protein DDM1 is required as evidenced by *ddm1* mutants where the levels of DNA methylation in all sequence contexts are decreased (Law & Jacobsen, 2010).

The maintenance of proper CG methylation patterns is important for plant development and is thus most faithfully inherited. *met1* and *ddm1* mutants have decreased levels of CG methylation and show severe developmental phenotypes, while mutants defective in non-CG methylation have only minor developmental alterations. Certain phenotypes in *met1* or *ddm1* mutants can be explained by

the loss of DNA methylation at particular genes, a process that results in the generation of hypomethylated epiallelic variants. For example, the *FWA* gene that acts as a flowering repressor is normally transcriptionally silenced in the sporophyte by CG methylation of its promoter. In *met1* or *ddm1* mutants, CG methylation is lost and transcriptional activation of *FWA* results in a late flowering phenotype (Soppe *et al.*, 2000). Interestingly, the hypomethylated state of *FWA* is stably maintained, and its normal methylation status cannot be regained even after *MET1* or *DDM1* are provided in backcrosses (Kankel *et al.*, 2003). This can be explained by the loss of the methylation template in the promoter of the *FWA* gene.

Using these properties of *MET1* and *DDM1*, two populations of epigenetic recombinant inbred lines (epiRILs) were constructed (Johannes *et al.*, 2009; Reinders *et al.*, 2009; Teixeira *et al.*, 2009). Both epiRIL populations were initiated from F1 hybrids between isogenic wild type and *met1* or *ddm1* mutants. Genetically identical parents were highly divergent epigenetically due to the methylation deficiencies of the mutants. Individuals homozygous for wild-type allele (*MET1* or *DDM1*) were selected in the F2 generation, and these plants were inbred for 7–8 generations by single-seed descent (where the *ddm1*-derived F1 hybrid was backcrossed to wild type before inbreeding). DNA methylation analyses performed after inbreeding demonstrated that hypomethylation of distinct chromosomal segments derived from the mutant backgrounds was stably inherited over many generations in the presence of *MET1* or *DDM1*. However, re-methylated regions derived from the mutant backgrounds were also found in both epiRIL populations. These regions were associated with siRNAs, suggesting that re-methylation occurs through an RdDM pathway (Teixeira *et al.*, 2009). Interestingly, various novel phenotypic traits were observed during the inbreeding process. Certain traits such as delayed flowering were stably inherited, but most traits were unstable, probably due to dynamic methylation changes during inbreeding. It remains unknown what properties determine the stability of DNA methylation at some loci but not others. This is an important question that needs clarification to allow the prediction of genes that can be epigenetically altered in a stable, heritable fashion and those that would rapidly return to their original epigenetic state.

Natural epialleles

Besides experimentally induced epialleles, there are several examples of naturally occurring stable epialleles. In toadflax (*Linaria vulgaris*), different flower shapes are found ranging from bilaterally symmetrical to radial forms. This phenotypic variability is caused by variable levels of methylation of the promoter of the *CYCLOIDEA* gene (Cubas *et al.*, 1999).

The tomato Colorless non-ripening (*Cnr*) variant displays bright, immature patches on its fruits due to spontaneous hypermethylation at the *CNR* locus (Manning *et al.*, 2006). In melon, DNA methylation spreading from a transposon induces transcriptional silencing of the *CmWIP1* gene that controls sex determination and thus varying proportions of male and female flowers (Martin *et al.*, 2009). A recent example of a natural epiallele was revealed by studies of genetic incompatibility between *Arabidopsis* accessions. The incompatibility was due to epigenetic characteristics of duplicated *AtFOLT* genes where a particular rearrangement of one *AtFOLT* locus promoted DNA methylation of the second copy through an RdDM pathway (Durand *et al.*, 2012).

It is not clear whether environmental cues contributed to the establishment of these natural epialleles. However, the frequent observation of TE or TE-related sequences in the vicinity of genes forming natural epialleles suggests that transposon-derived *cis* elements could be involved in the acquisition of epiallelic properties for individual genes.

Transposons, environmental stress, and epigenetic variation

TEs are found in chromosomes of most organisms and often constitute a major component of the genome in multicellular eukaryotes. Most TEs are epigenetically silenced, but some TEs are transcriptionally activated in mutants defective in epigenetic regulation. In addition, transcription of TEs can be activated by stress, a process that occurs over a wide evolutionary range from bacteria to mammals (Capy *et al.*, 2000).

Barbara McClintock was the first to observe that environmental stresses can activate movement of TEs, a finding that has been extensively supported in later work (McClintock, 1984; Wessler, 1996; Grandbastien, 1998). This ability of TEs to display such environmental sensing is illustrated by the following examples: *Tnt1* and *Tto1* are LTR-type retroelements in tobacco, and their transposition is induced by wounding or pathogen attack (Takeda *et al.*, 2001; Perez-Hormaeche *et al.*, 2008). The *Bs1* LTR-type retroelement in maize was shown to transpose after virus infection (Mottinger *et al.*, 1984; Johns *et al.*, 1985). For *ONSEN*, an LTR-type retroelement in *Arabidopsis*, transcription is induced by heat stress, and *ONSEN* transposes in siRNA-defective mutants (Ito *et al.*, 2011). All the above examples involve the most abundant TEs belonging to the class I retroelements that transpose by a ‘copy and paste’ mechanism. However, there are also a few examples of class II DNA transposons that transpose by a ‘cut and paste’ mechanism following stress exposure. For example, the frequency of excision of the Ac/Ds type transposon *Tam3* is enhanced at low temperature in *Antirrhinum majus* (Harrison & Fincham, 1964; Carpenter *et al.*, 1987).

Barbara McClintock postulated that activation of TEs reflects a response of the genome to a challenge (McClintock, 1984). Several examples of TEs playing a crucial role in gene regulation and genome evolution support this hypothesis (Slotkin & Martienssen, 2007; Fedoroff, 2012). It has been suggested that environmentally activated TEs create new genetic and epigenetic variability that, when under selection, could contribute to enhanced adaptive potential of plants subjected to stresses (Mirouze & Paszkowski, 2011; Bucher *et al.*, 2012) (Fig 3).

Recent studies have directly demonstrated that newly inserted TEs can indeed provide stress-responsive regulation to adjacent genes. In rice, it was shown that the active DNA transposon *mPing* preferentially inserts into 5' flanking regions of genes and not into exons. Transcription of a subset of genes harboring an *mPing* insertion in the promoter region was found to be induced by cold or salt stress (Naito *et al.*, 2009).

In *Arabidopsis*, new copies of *ONSEN* preferentially insert into genic regions rather than to the heterochromatic regions where the majority of TEs are located. It has been shown that the LTR of *ONSEN* has a heat-responsive element that is activated by transcriptional heat stress responses (Cavrak *et al.*, 2014). Consequently, genes in the vicinity of or harboring newly inserted *ONSEN* copies become heat responsive (Ito *et al.*, 2011). A further study showed

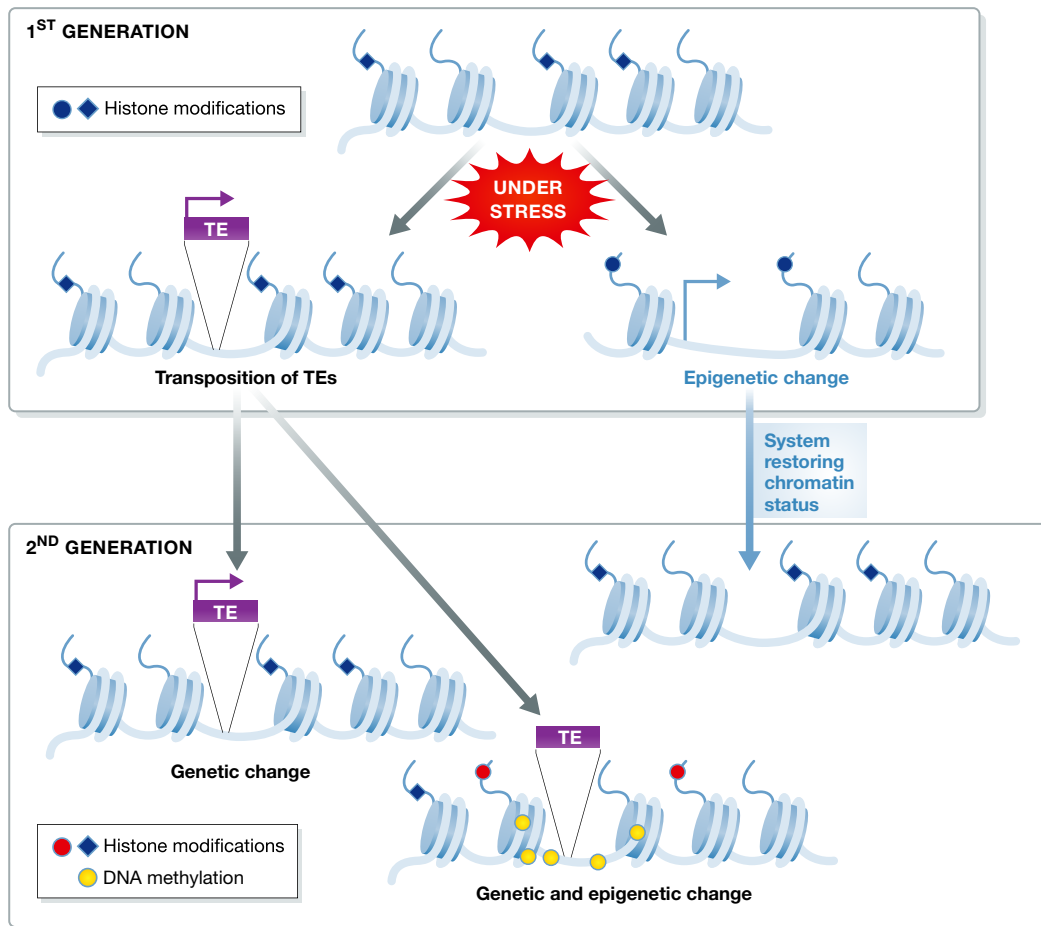


Figure 3. Environmentally induced genetic and epigenetic variations.

Stress induces activation of transposons and epigenetic changes at various silent genomic loci, including heterochromatic regions. Activated transposons may transpose and generate genetic variation. New insertions of transposons also generate epigenetic variation in the vicinity of the new insertions. In contrast, epigenetic changes are mostly transient due to restoration of the pre-stress chromatin status. Therefore, transgenerational transmission of stress-induced epigenetic changes is very restricted.

that phenotypic variation in a particular Italian strain of blood oranges around Mount Etna is caused by the insertion of an LTR retrotransposon in the promoter of *Ruby*, a gene that encodes a transcriptional activator of anthocyanin biosynthesis. The LTR retrotransposon in the promoter confers cold responsiveness on the *Ruby* gene in fruits, thus determining the temperature-dependent coloration of blood oranges (Butelli *et al*, 2012).

Environmentally induced transgenerational epigenetic memory

The concept that adaptive traits can be acquired by an individual and inherited by its progeny was proposed by Jean-Baptiste Lamarck, but later gave way to the Darwinian theory of evolution. After the discovery of epigenetic mechanisms of inheritance and especially recent studies suggesting transgenerational inheritance of acquired traits in plants and animals, the previously abandoned Lamarckian theory has regained limited attention.

In *Arabidopsis*, it was demonstrated that UV-C radiation or introduction of the bacterial elicitor flagellin induces a higher frequency of somatic homologous recombination, and this 'induced' state is transmitted in a dominant manner as a newly acquired trait to the progeny (Molinier *et al*, 2006). A similar study performed in tobacco

demonstrated that a tobacco mosaic virus (TMV)-induced systemic signal increases somatic recombination rates. The progeny of TMV-infected plants also showed a higher frequency of recombination (Boyko *et al*, 2007). Further studies showed that SAR can be transmitted to the next generation in tomato and *Arabidopsis* (Luna *et al*, 2012; Rasmann *et al*, 2012; Slaughter *et al*, 2012).

Although there are many more examples in plants suggesting inheritance of environmentally induced traits, the issue remains controversial (Boyko & Kovalchuk, 2011; Mirouze & Paszkowski, 2011; Paszkowski & Grossniklaus, 2011; Pecinka & Mittelsten Scheid, 2012). This is mainly due to the absence of defined molecular mechanisms that could account for such phenomena, although the involvement of epigenetic regulation has been repeatedly suggested.

The prospect that environmental stresses can lead to the emergence of transgenerationally heritable epigenetic traits in plants may be associated with negative consequences. Despite the very tempting possibility that such mechanisms could potentially contribute to adaptive advantage, it may also be the case that accumulation of epigenetic information reflecting the 'stress memories' of previous generations could impair responses to current environmental

challenges. Moreover, bona fide examples of transgenerational transmission of environmentally induced traits are still quite scarce, which is surprising given the centuries of plant domestication and human driven selection for use in agriculture and horticulture. During much of this time, Lysenko (Gordin, 2012) was the only proponent of the inheritance of acquired traits. Therefore, it is conceivable that an as yet unknown mechanism hinders the inheritance of environmentally induced epigenetic traits (Fig 3).

Recently, a forward genetic screen in *Arabidopsis* apparently revealed such a system. Two chromatin regulators DDM1 and MOM1 were found to act redundantly in preventing the transmission of stress-induced transcriptional changes to progeny of the stressed plants. In *ddm1 mom1* double mutants, transcriptional signatures induced by stress were found in the subsequent generation (Iwasaki & Paszkowski, 2014). Thus, such DDM1- and MOM1-mediated or other mechanisms of chromatin resetting could prevent or act very restrictively on transgenerational transmission of environmentally induced epigenetic traits.

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Conflict of interest

The authors declare that they have no conflict of interest.

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