Epigenetic Memory in Plants

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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.

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 towards 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 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 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 (Figure 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 towards 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; Choi et al. 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; Jullien et al. 2008).

Further factors regulating imprinting include the evolutionally conserved polycomb group proteins. Arabidopsis polycomb complex PRC2, consisting of MEA, FIE, FIS2 and MSI1, catalyzes H3K27 tri-methylation and this repressive histone mark leads to the suppression of paternal alleles of MEGs or the maternal alleles of paternally expressed genes (PEGs) (Baroux et al. 2006; Jullien et al. 2006a; Kohler et al. 2005; 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 has been shown that the regulation of imprinted *MEA* expression by DME and MET1 may occur also 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 Figure 1.

Although imprinting is widely conserved among plant species, its biological significance is not clear. One hypothesis explaining the its origin is that imprinting is a byproduct 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 result in hypomethylation of TEs and biogenesis of TEs derived small RNAs (Mosher et al, 2009). These small RNAs may relocate to the embryo reinforcing TEs silencing there (Bauer & Fischer, 2011; Hsieh et al, 2009). 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; Kohler & Weinhofer-Molisch 2010; Wilkins & Haig 2003). 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 lifespan. 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 (Kim et al. 2009; Michaels & Amasino 2000).

1) 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 (Choi et al. 2009; Sheldon et al. 2008) 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 trimethylation and H3K36 dimethylation (Ko et al, 2010; Xu & Shen, 2008; Zhao et al, 2005). The COMPASS-like complex, including the Trithorax family proteins ATX1 and ATXR7, mediates H3K4 trimethylation (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).

2) 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 (De Lucia et al. 2008; Greb et al. 2007; Sung & Amasino 2004; Sung et al. 2006b).

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.

3) 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 (Angel et al. 2011; Finnegan & Dennis 2007). 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 (Greb et al. 2007; Sung & Amasino 2004). 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 modelling 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 Figure 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*, *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 *ZmMI1* 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 are 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 (Brickner et al. 2007; Dhillon et al. 2006; Kumar & Wigge 2010; Light et al. 2010; Zlatanova & Thakar 2008). 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 contents: 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 hemi-methylated 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 (Law & Jacobsen 2010; Mathieu et al. 2007; Saze et al. 2003).

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 generating 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 points suggest 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 (Grandbastien 1998; McClintock 1984; Wessler 1996). This TEs abilities of "environmental sensing" are illustrated by the following examples: Tnt1 and Tto1 are LTR-type retroelements in tobacco and their transposition is induced by wounding or pathogen attack (Perez-Hormaeche et al. 2008; Takeda et al. 2001). The Bs1 LTR-type retroelement in maize was shown to transpose after virus infection (Johns et al. 1985; Mottinger et al. 1984). 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* (Carpenter et al. 1987; Harrison & Fincham 1964).
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 (Fedoroff 2012; Slotkin & Martienssen 2007). 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 (Bucher et al, 2012; Mirouze & Paszkowski, 2011) (Figure 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 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 (Figure 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.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

Aina R, Sgorbati S, Santagostino A, Labra M, Ghiani A, Citterio S (2004) Specific hypomethylation of DNA is induced by heavy metals in white clover and industrial hemp. *Physiologia Plantarum* **121:** 472-480

Angel A, Song J, Dean C, Howard M (2011) A Polycomb-based switch underlying quantitative epigenetic memory. *Nature* **476:** 105-108

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature* **415:** 977-983

Baroux C, Gagliardini V, Page DR, Grossniklaus U (2006) Dynamic regulatory interactions of Polycomb group genes: MEDEA autoregulation is required for imprinted gene expression in Arabidopsis. *Genes Dev* **20:** 1081-1086

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* **427:** 164-167

Bauer MJ, Fischer RL (2011) Genome demethylation and imprinting in the endosperm. *Curr Opin Plant Biol* **14:** 162-167

Borsani O, Zhu J, Verslues PE, Sunkar R, Zhu JK (2005) Endogenous siRNAs derived from a pair of natural cis-antisense transcripts regulate salt tolerance in Arabidopsis. *Cell* **123:** 1279-1291

Boyko A, Kathiria P, Zemp FJ, Yao Y, Pogribny I, Kovalchuk I (2007) Transgenerational changes in the genome stability and methylation in pathogen-infected plants: (virus-induced plant genome instability). *Nucleic Acids Res* **35:** 1714-1725

Boyko A, Kovalchuk I (2011) Genome instability and epigenetic modification--heritable responses to environmental stress? *Curr Opin Plant Biol* **14:** 260-266

Brickner DG, Cajigas I, Fondufe-Mittendorf Y, Ahmed S, Lee PC, Widom J, Brickner JH (2007) H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. *PLoS Biol* **5:** e81

Bucher E, Reinders J, Mirouze M (2012) Epigenetic control of transposon transcription and mobility in Arabidopsis. *Curr Opin Plant Biol* **15:** 503-510

Butelli E, Licciardello C, Zhang Y, Liu J, Mackay S, Bailey P, Reforgiato-Recupero G, Martin C (2012) Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. *Plant Cell* **24:** 1242-1255

Butterbrodt T, Thurow C, Gatz C (2006) Chromatin immunoprecipitation analysis of the tobacco PR-1a- and the truncated CaMV 35S promoter reveals differences in

salicylic acid-dependent TGA factor binding and histone acetylation. *Plant Mol Biol* **61:** 665-674

Capy P, Gasperi G, Biemont C, Bazin C (2000) Stress and transposable elements: co-evolution or useful parasites? *Heredity (Edinb)* **85 (Pt 2):** 101-106

Carpenter R, Martin C, Coen ES (1987) Comparison of Genetic Behavior of the Transposable Element Tam3 at 2 Unlinked Pigment Loci in Antirrhinum-Majus. *Molecular & General Genetics* **207:** 82-89

Cavrak VV, Lettner N, Jamge S, Kosarewicz A, Bayer LM, Mittelsten Scheid O (2014) How a retrotransposon exploits the plant's heat stress response for its activation. *PLoS Genet* **10:** e1004115

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

Choi K, Park C, Lee J, Oh M, Noh B, Lee I (2007) Arabidopsis homologs of components of the SWR1 complex regulate flowering and plant development. *Development* **134:** 1931-1941

Choi Y, Gehring M, Johnson L, Hannon M, Harada JJ, Goldberg RB, Jacobsen SE, Fischer RL (2002) DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in arabidopsis. *Cell* **110:** 33-42

Choi Y, Harada JJ, Goldberg RB, Fischer RL (2004) An invariant aspartic acid in the DNA glycosylase domain of DEMETER is necessary for transcriptional activation of the imprinted MEDEA gene. *Proc Natl Acad Sci U S A* **101:** 7481-7486

Chouard P (1960) Vernalization and Its Relations to Dormancy. *Annual Review of Plant Physiology and Plant Molecular Biology* **11:** 191-238

Costa LM, Yuan J, Rouster J, Paul W, Dickinson H, Gutierrez-Marcos JF (2012) Maternal control of nutrient allocation in plant seeds by genomic imprinting. *Curr Biol* **22:** 160-165

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* **401:** 157-161

De Lucia F, Crevillen P, Jones AM, Greb T, Dean C (2008) A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. *Proc Natl Acad Sci U S A* **105:** 16831-16836

Dhillon N, Oki M, Szyjka SJ, Aparicio OM, Kamakaka RT (2006) H2A.Z functions to regulate progression through the cell cycle. *Mol Cell Biol* **26:** 489-501

Dong J, Chen C, Chen Z (2003) Expression profiles of the Arabidopsis WRKY gene superfamily during plant defense response. *Plant Mol Biol* **51:** 21-37

Durand S, Bouche N, Perez Strand E, Loudet O, Camilleri C (2012) Rapid establishment of genetic incompatibility through natural epigenetic variation. *Curr Biol* **22:** 326-331

Durrant WE, Dong X (2004) Systemic acquired resistance. *Annu Rev Phytopathol* **42:** 185-209

Fedoroff NV (2012) Presidential address. Transposable elements, epigenetics, and genome evolution. *Science* **338:** 758-767

Feil R, Berger F (2007) Convergent evolution of genomic imprinting in plants and mammals. *Trends Genet* **23:** 192-199

Finnegan EJ, Dennis ES (2007) Vernalization-induced trimethylation of histone H3 lysine 27 at FLC is not maintained in mitotically quiescent cells. *Curr Biol* **17:** 1978-1983

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

Gehring M, Huh JH, Hsieh TF, Penterman J, Choi Y, Harada JJ, Goldberg RB, Fischer RL (2006) DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. *Cell* **124:** 495-506

Gordin MD (2012) How Lysenkoism Became Pseudoscience: Dobzhansky to Velikovsky. *Journal of the History of Biology* **45:** 443-468

Grandbastien MA (1998) Activation of plant retrotransposons under stress conditions. *Trends in Plant Science* **3:** 181-187

Greb T, Mylne JS, Crevillen P, Geraldo N, An H, Gendall AR, Dean C (2007) The PHD finger protein VRN5 functions in the epigenetic silencing of Arabidopsis FLC. *Curr Biol* **17:** 73-78

Grossniklaus U, Vielle-Calzada JP, Hoeppner MA, Gagliano WB (1998) Maternal control of embryogenesis by MEDEA, a polycomb group gene in Arabidopsis. *Science* **280:** 446-450

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

Haig D, Westoby M (1989) Parent-Specific Gene-Expression and the Triploid Endosperm. *American Naturalist* **134:** 147-155

Harrison BJ, Fincham JRS (1964) Instability at Pal Locus in Antirrhinum Majus .I. Effects of Environment on Frequencies of Somatic + Germinal Mutation. *Heredity* **19:** 237-&

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. *Science* **331:** 76-79

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

Ingram GC (2010) Family life at close quarters: communication and constraint in angiosperm seed development. *Protoplasma* **247:** 195-214

Ito H, Gaubert H, Bucher E, Mirouze M, Vaillant I, Paszkowski J (2011) An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature* **472:** 115-119

Iwasaki M, Paszkowski J (2014) Identification of genes preventing transgenerational transmission of stress-induced epigenetic states. *Proc Natl Acad Sci U S A* **111:** 8547-8552

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

Jaskiewicz M, Conrath U, Peterhansel C (2011) Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep* **12:** 50-55

Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, Bulski A, Albuisson J, Heredia F, Audigier P, Bouchez D, Dillmann C, Guerche P, Hospital F, Colot V (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet* **5:** e1000530

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C (2000) Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science* **290:** 344-347

Johns MA, Mottinger J, Freeling M (1985) A low copy number, copia-like transposon in maize. *EMBO J* **4:** 1093-1101

Jullien PE, Katz A, Oliva M, Ohad N, Berger F (2006a) Polycomb group complexes self-regulate imprinting of the Polycomb group gene MEDEA in Arabidopsis. *Curr Biol* **16:** 486-492

Jullien PE, Kinoshita T, Ohad N, Berger F (2006b) Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting. *Plant Cell* **18:** 1360-1372

Jullien PE, Mosquna A, Ingouff M, Sakata T, Ohad N, Berger F (2008) Retinoblastoma and its binding partner MSI1 control imprinting in Arabidopsis. *PLoS Biol* **6:** e194

Kankel MW, Ramsey DE, Stokes TL, Flowers SK, Haag JR, Jeddeloh JA, Riddle NC, Verbsky ML, Richards EJ (2003) Arabidopsis MET1 cytosine methyltransferase mutants. *Genetics* **163:** 1109-1122

Kim DH, Doyle MR, Sung S, Amasino RM (2009) Vernalization: winter and the timing of flowering in plants. *Annu Rev Cell Dev Biol* **25:** 277-299

Kim JM, To TK, Ishida J, Morosawa T, Kawashima M, Matsui A, Toyoda T, Kimura H, Shinozaki K, Seki M (2008) Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in Arabidopsis thaliana. *Plant Cell Physiol* **49:** 1580-1588

Kiyosue T, Ohad N, Yadegari R, Hannon M, Dinneny J, Wells D, Katz A, Margossian L, Harada JJ, Goldberg RB, Fischer RL (1999) Control of fertilization-independent endosperm development by the MEDEA polycomb gene in Arabidopsis. *Proc Natl Acad Sci U S A* **96:** 4186-4191

Ko JH, Mitina I, Tamada Y, Hyun Y, Choi Y, Amasino RM, Noh B, Noh YS (2010) Growth habit determination by the balance of histone methylation activities in Arabidopsis. *EMBO J* **29:** 3208-3215

Kohler C, Page DR, Gagliardini V, Grossniklaus U (2005) The Arabidopsis thaliana MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. *Nat Genet* **37:** 28-30

Kohler C, Weinhofer-Molisch I (2010) Mechanisms and evolution of genomic imprinting in plants. *Heredity (Edinb)* **105:** 57-63

Krogan NJ, Dover J, Wood A, Schneider J, Heidt J, Boateng MA, Dean K, Ryan OW, Golshani A, Johnston M, Greenblatt JF, Shilatifard A (2003) The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol Cell* **11:** 721-729

Kumar SV, Wigge PA (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. *Cell* **140:** 136-147

Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet* **11:** 204-220

Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C (2002) Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. *Science* **297:** 243-246

Light WH, Brickner DG, Brand VR, Brickner JH (2010) Interaction of a DNA zip code with the nuclear pore complex promotes H2A.Z incorporation and INO1 transcriptional memory. *Mol Cell* **40:** 112-125

Loake G, Grant M (2007) Salicylic acid in plant defence--the players and protagonists. *Curr Opin Plant Biol* **10:** 466-472

Lu J, Zhang C, Baulcombe DC, Chen ZJ (2012) Maternal siRNAs as regulators of parental genome imbalance and gene expression in endosperm of Arabidopsis seeds. *Proc Natl Acad Sci U S A* **109:** 5529-5534

Luna E, Bruce TJ, Roberts MR, Flors V, Ton J (2012) Next-generation systemic acquired resistance. *Plant Physiol* **158:** 844-853

Luo M, Bilodeau P, Koltunow A, Dennis ES, Peacock WJ, Chaudhury AM (1999) Genes controlling fertilization-independent seed development in Arabidopsis thaliana. *Proc Natl Acad Sci U S A* **96:** 296-301

Makarevich G, Leroy O, Akinci U, Schubert D, Clarenz O, Goodrich J, Grossniklaus U, Kohler C (2006) Different Polycomb group complexes regulate common target genes in Arabidopsis. *EMBO Rep* **7:** 947-952

Makarevich G, Villar CB, Erilova A, Kohler C (2008) Mechanism of PHERES1 imprinting in Arabidopsis. *J Cell Sci* **121:** 906-912

Manning K, Tor M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat Genet* **38:** 948-952

March-Diaz R, Garcia-Dominguez M, Lozano-Juste J, Leon J, Florencio FJ, Reyes JC (2008) Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in Arabidopsis. *Plant J* **53:** 475-487

Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Dogimont C, Bendahmane A (2009) A transposon-induced epigenetic change leads to sex determination in melon. *Nature* **461:** 1135-1138

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

Mcclintock B (1984) The Significance of Responses of the Genome to Challenge. *Science* **226:** 792-801

Michaels SD, Amasino RM (1999) FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11:** 949-956

Michaels SD, Amasino RM (2000) Memories of winter: vernalization and the competence to flower. *Plant Cell and Environment* **23:** 1145-1153

Mirouze M, Paszkowski J (2011) Epigenetic contribution to stress adaptation in plants. *Curr Opin Plant Biol* **14:** 267-274

Molinier J, Ries G, Zipfel C, Hohn B (2006) Transgeneration memory of stress in plants. *Nature* **442:** 1046-1049

Morales-Ruiz T, Ortega-Galisteo AP, Ponferrada-Marin MI, Martinez-Macias MI, Ariza RR, Roldan-Arjona T (2006) DEMETER and REPRESSOR OF SILENCING 1 encode 5-methylcytosine DNA glycosylases. *Proc Natl Acad Sci U S A* **103:** 6853-6858

Mosher RA, Melnyk CW, Kelly KA, Dunn RM, Studholme DJ, Baulcombe DC (2009) Uniparental expression of PolIV-dependent siRNAs in developing endosperm of Arabidopsis. *Nature* **460:** 283-286

Mottinger JP, Johns MA, Freeling M (1984) Mutations of the Adh1 gene in maize following infection with barley stripe mosaic virus. *Mol Gen Genet* **195:** 367-369

Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, Bernatavichute YV, Jacobsen SE, Fransz P, Dean C (2006) LHP1, the Arabidopsis homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of FLC. *Proc Natl Acad Sci U S A* **103:** 5012-5017

Naito K, Zhang F, Tsukiyama T, Saito H, Hancock CN, Richardson AO, Okumoto Y, Tanisaka T, Wessler SR (2009) Unexpected consequences of a sudden and massive transposon amplification on rice gene expression. *Nature* **461:** 1130-1134

Oh S, Zhang H, Ludwig P, van Nocker S (2004) A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the Arabidopsis FLC/MAF MADS box gene family. *Plant Cell* **16:** 2940-2953

Paszkowski J, Grossniklaus U (2011) Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Curr Opin Plant Biol* **14:** 195-203

Pecinka A, Mittelsten Scheid O (2012) Stress-induced chromatin changes: a critical view on their heritability. *Plant Cell Physiol* **53:** 801-808

Perez-Hormaeche J, Potet F, Beauclair L, Le Masson I, Courtial B, Bouche N, Lucas H (2008) Invasion of the Arabidopsis genome by the tobacco retrotransposon Tnt1 is controlled by reversible transcriptional gene silencing. *Plant Physiol* **147:** 1264-1278

Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol* **158:** 854-863

Reinders J, Wulff BB, Mirouze M, Mari-Ordonez A, Dapp M, Rozhon W, Bucher E, Theiler G, Paszkowski J (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic Arabidopsis epigenomes. *Genes Dev* **23:** 939-950

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996) Systemic Acquired Resistance. *Plant Cell* **8:** 1809-1819

Saleh A, Alvarez-Venegas R, Yilmaz M, Le O, Hou G, Sadder M, Al-Abdallat A, Xia Y, Lu G, Ladunga I, Avramova Z (2008) The highly similar Arabidopsis homologs of

trithorax ATX1 and ATX2 encode proteins with divergent biochemical functions. *Plant Cell* **20:** 568-579

Saze H, Mittelsten Scheid O, Paszkowski J (2003) Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. *Nat Genet* **34:** 65-69

Scott RJ, Spielman M, Bailey J, Dickinson HG (1998) Parent-of-origin effects on seed development in Arabidopsis thaliana. *Development* **125:** 3329-3341

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The FLF MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. *Plant Cell* **11:** 445-458

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

Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B (2012) Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. *Plant Physiol* **158:** 835-843

Slotkin RK, Martienssen R (2007) Transposable elements and the epigenetic regulation of the genome. *Nat Rev Genet* **8:** 272-285

Song J, Angel A, Howard M, Dean C (2012) Vernalization - a cold-induced epigenetic switch. *J Cell Sci* **125:** 3723-3731

Soppe WJ, Jacobsen SE, Alonso-Blanco C, Jackson JP, Kakutani T, Koornneef M, Peeters AJ (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

Steward N, Ito M, Yamaguchi Y, Koizumi N, Sano H (2002) Periodic DNA methylation in maize nucleosomes and demethylation by environmental stress. *J Biol Chem* **277:** 37741-37746

Sung S, Amasino RM (2004) Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. *Nature* **427:** 159-164

Sung S, He Y, Eshoo TW, Tamada Y, Johnson L, Nakahigashi K, Goto K, Jacobsen SE, Amasino RM (2006a) Epigenetic maintenance of the vernalized state in Arabidopsis thaliana requires LIKE HETEROCHROMATIN PROTEIN 1. *Nat Genet* **38:** 706-710

Sung S, Schmitz RJ, Amasino RM (2006b) A PHD finger protein involved in both the vernalization and photoperiod pathways in Arabidopsis. *Genes Dev* **20:** 3244-3248

Swiezewski S, Liu F, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. *Nature* **462:** 799-802

Takeda S, Sugimoto K, Kakutani T, Hirochika H (2001) Linear DNA intermediates of the Tto1 retrotransposon in Gag particles accumulated in stressed tobacco and Arabidopsis thaliana. *Plant J* **28:** 307-317

Tamada Y, Yun JY, Woo SC, Amasino RM (2009) ARABIDOPSIS TRITHORAX-RELATED7 is required for methylation of lysine 4 of histone H3 and for transcriptional activation of FLOWERING LOCUS C. *Plant Cell* **21:** 3257-3269

Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, Ciaudo C, Cruaud C, Poulain J, Berdasco M, Fraga MF, Voinnet O, Wincker P, Esteller M, Colot V (2009) A role for RNAi in the selective correction of DNA methylation defects. *Science* **323:** 1600-1604

Tiwari S, Spielman M, Schulz R, Oakey RJ, Kelsey G, Salazar A, Zhang K, Pennell R, Scott RJ (2010) Transcriptional profiles underlying parent-of-origin effects in seeds of Arabidopsis thaliana. *BMC Plant Biol* **10:** 72

To TK, Nakaminami K, Kim JM, Morosawa T, Ishida J, Tanaka M, Yokoyama S, Shinozaki K, Seki M (2011) Arabidopsis HDA6 is required for freezing tolerance. *Biochem Biophys Res Commun* **406:** 414-419

Tsuji H, Saika H, Tsutsumi N, Hirai A, Nakazono M (2006) Dynamic and reversible changes in histone H3-Lys4 methylation and H3 acetylation occurring at submergence-inducible genes in rice. *Plant Cell Physiol* **47:** 995-1003

Vlot AC, Klessig DF, Park SW (2008) Systemic acquired resistance: the elusive signal(s). *Curr Opin Plant Biol* **11:** 436-442

Waddington CH (1942) The epigenotype. *Endeavour***:** 18-20

Waddington CH (1957) *he strategy of the genes. A discussion of some aspects of theoretical biology.*: London: George Allen & Unwin.

Weinhofer I, Hehenberger E, Roszak P, Hennig L, Kohler C (2010) H3K27me3 profiling of the endosperm implies exclusion of polycomb group protein targeting by DNA methylation. *PLoS Genet* **6**

Wessler SR (1996) Turned on by stress. Plant retrotransposons. *Curr Biol* **6:** 959-961

Wilkins JF, Haig D (2003) What good is genomic imprinting: the function of parent-specific gene expression. *Nat Rev Genet* **4:** 359-368

Wohrmann HJ, Gagliardini V, Raissig MT, Wehrle W, Arand J, Schmidt A, Tierling S, Page DR, Schob H, Walter J, Grossniklaus U (2012) Identification of a DNA methylation-independent imprinting control region at the Arabidopsis MEDEA locus. *Genes Dev* **26:** 1837-1850

Wolff P, Weinhofer I, Seguin J, Roszak P, Beisel C, Donoghue MT, Spillane C, Nordborg M, Rehmsmeier M, Kohler C (2011) High-resolution analysis of

parent-of-origin allelic expression in the Arabidopsis Endosperm. *PLoS Genet* **7:** e1002126

Xin M, Yang R, Li G, Chen H, Laurie J, Ma C, Wang D, Yao Y, Larkins BA, Sun Q, Yadegari R, Wang X, Ni Z (2013) Dynamic expression of imprinted genes associates with maternally controlled nutrient allocation during maize endosperm development. *Plant Cell* **25:** 3212-3227

Xu L, Shen WH (2008) Polycomb silencing of KNOX genes confines shoot stem cell niches in Arabidopsis. *Curr Biol* **18:** 1966-1971

Zemach A, Kim MY, Silva P, Rodrigues JA, Dotson B, Brooks MD, Zilberman D (2010) Local DNA hypomethylation activates genes in rice endosperm. *Proc Natl Acad Sci U S A* **107:** 18729-18734

Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT (2008) Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* **322:** 750-756

Zhao Z, Yu Y, Meyer D, Wu C, Shen WH (2005) Prevention of early flowering by expression of FLOWERING LOCUS C requires methylation of histone H3 K36. *Nat Cell Biol* **7:** 1256-1260

Zhou C, Zhang L, Duan J, Miki B, Wu K (2005) HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. *Plant Cell* **17:** 1196-1204

Zlatanova J, Thakar A (2008) H2A.Z: view from the top. *Structure* **16:** 166-179

Figure Legends

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.

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.

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.

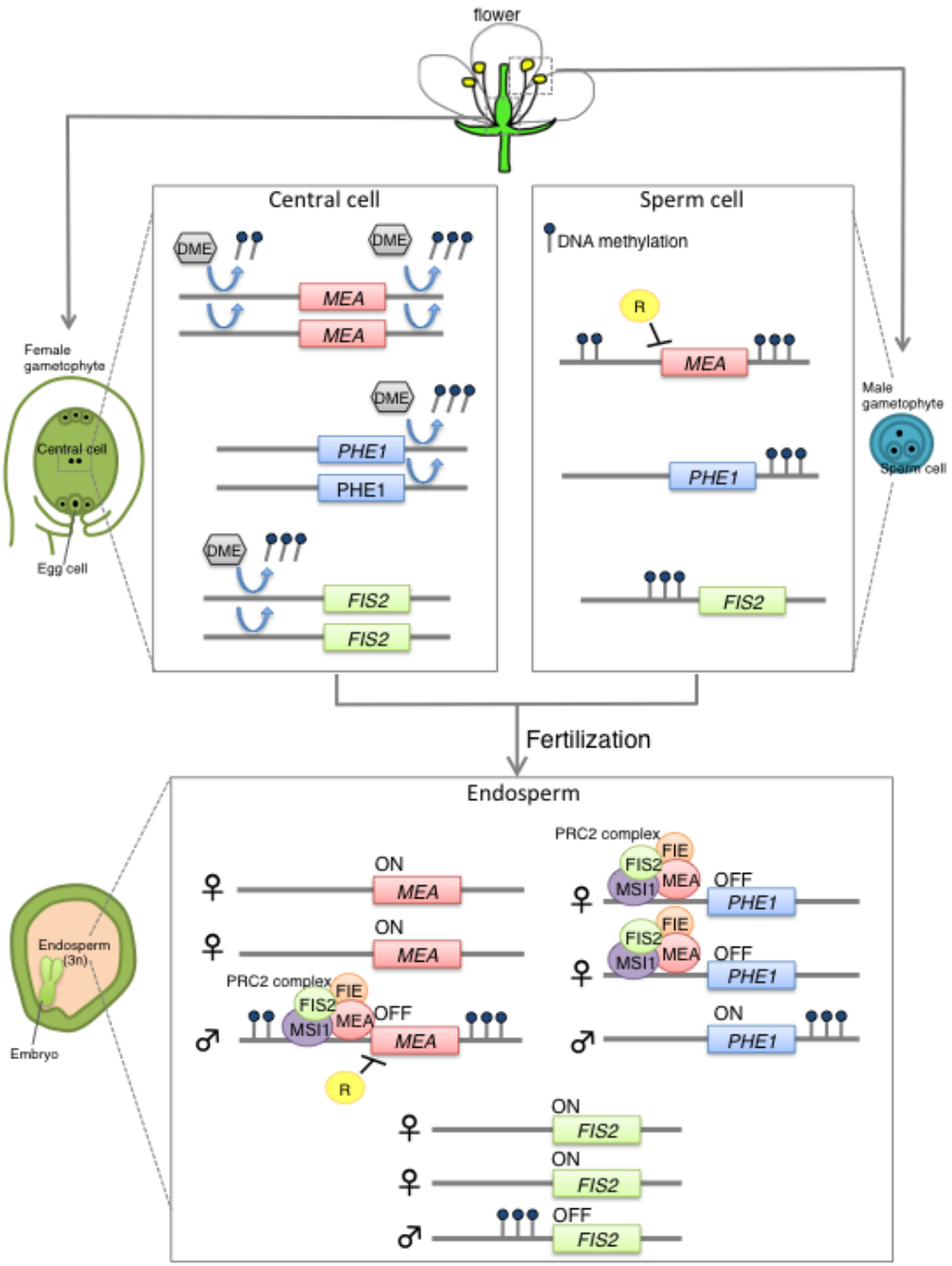
flower
Central cell
Sperm cell
DNA methylation
DME
MEA
PHE1
FIS2
R
Female gametophyte
Central cell
Egg cell
Male gametophyte
Sperm cell
Fertilization
Endosperm
Endosperm (3n)
Embryo
PRC2 complex
FIE
FIS2
MSI1
MEA
ON
OFF

Figure 1

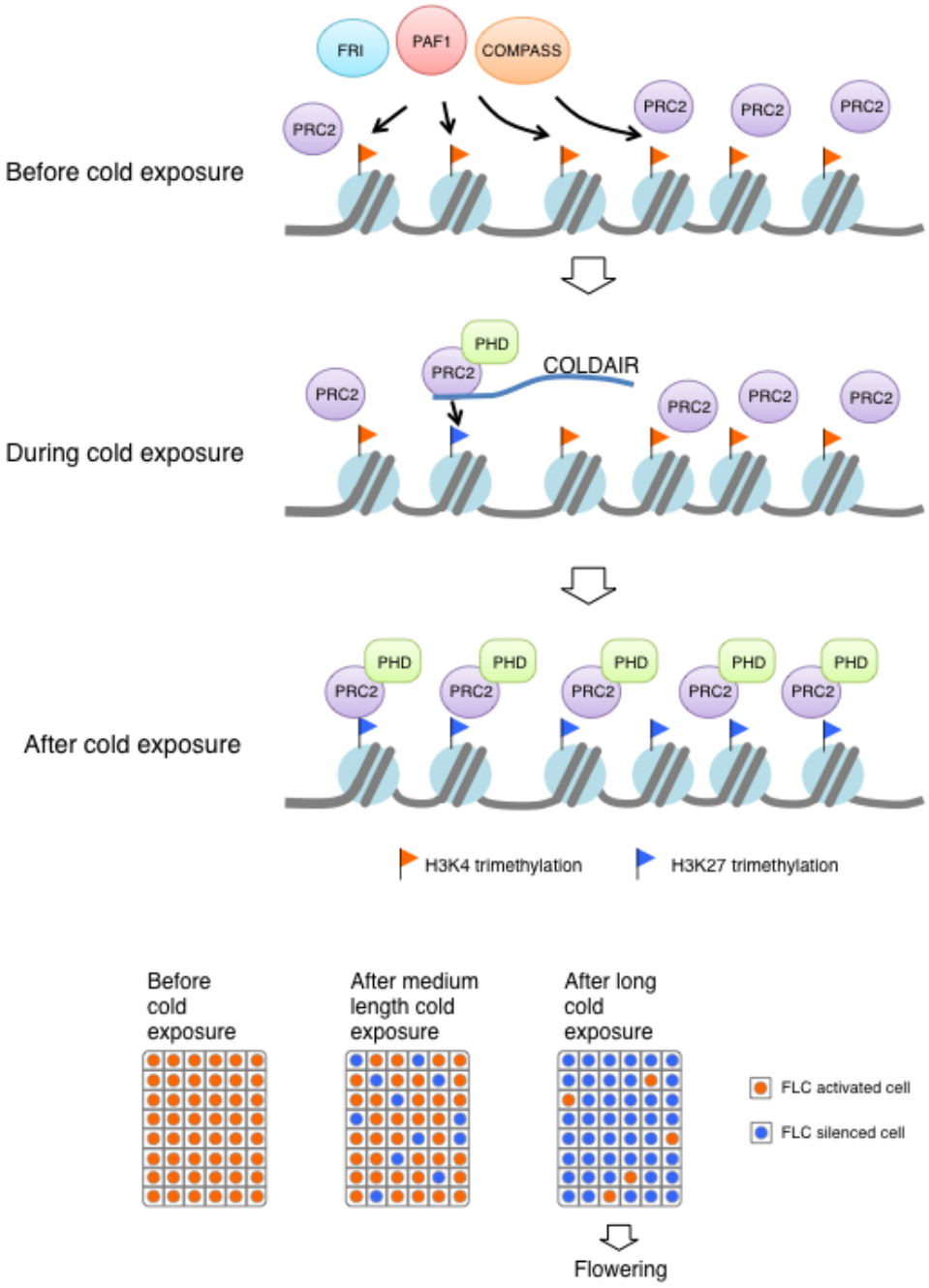
FRI
PAF1
COMPASS
PRC2
PRC2
PRC2
PRC2
Before cold exposure
PHD
PRC2
COLDAIR
PRC2
PRC2
PRC2
PRC2
During cold exposure
PHD
PRC2
PHD
PRC2
PHD
PRC2
PHD
PRC2
PHD
PRC2
After cold exposure
H3K4 trimethylation
H3K27 trimethylation
Before cold exposure
After medium length cold exposure
After long cold exposure
FLC activated cell
FLC silenced cell
Flowering

Figure 2

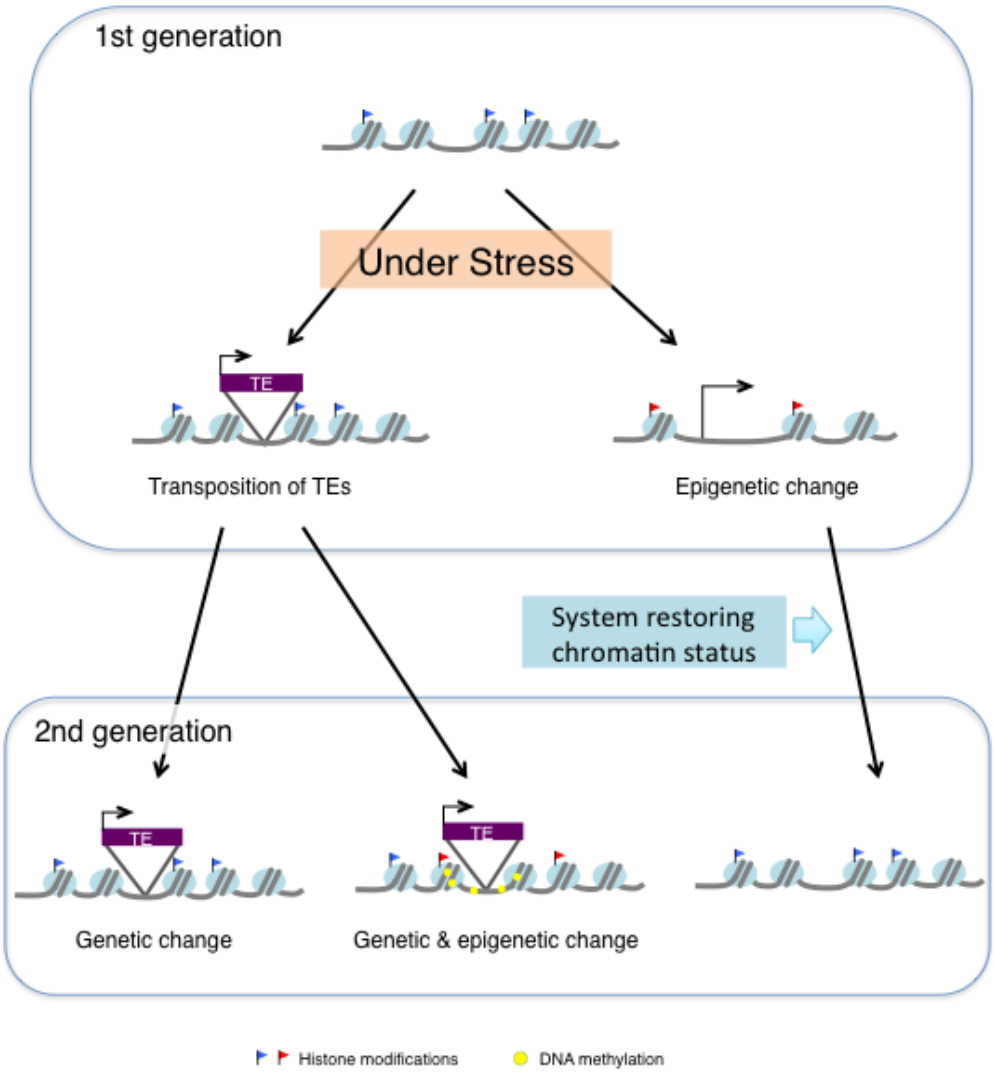
1st generation
Under Stress
TE
Transposition of TEs
Epigenetic change
System restoring
chromatin status
2nd generation
TE
TE
Genetic change
Genetic & epigenetic change
Histone modifications
DNA methylation

Figure 3