Transgenerational inheritance of induced changes in the epigenetic state of chromatin in plants

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There is growing experimental evidence from both animals and plants that changes in the environment can have profound effects on the epigenetic state of chromatin in nuclei. The epigenetic state of chromatin and the cell-specific transcription profile of genes are mitotically stable and, sometimes, can be transmitted across generations. Plants often show stable transgenerational inheritance of induced alterations of epigenetic states that are associated with morphologically or physiologically distinctive phenotypes. This pattern of inheritance may be due to the fact that germ cells produced by terminal differentiation and to the absence of appreciable epigenetic reprogramming during the life cycle. Recent advances in mass sequencing technology have accelerated the decoding of the epigenomes of various tissues and cell types and provided new insights into the dynamics of epigenetic changes during the plant life cycle and in response to environmental challenges. As plants have a sessile nature, the epigenetic regulation of genes and transposable elements in response to environmental stresses might be crucial for the generation and inheritance of phenotypic variations in plants in natural populations.

Key words: *Arabidopsis thaliana*, DNA methylation, environmental stress, transgenerational epigenetic inheritance, transposable elements

INTRODUCTION

The epigenetic regulation of gene expression is mediated by a variety of covalent modifications of the nucleotides and chromatin, such as methylation of cytosine residues in the DNA and post-translational alterations of core histone proteins, and also by production of small RNA molecules (Law and Jacobsen, 2010; Saze et al., 2012). Epigenetic modifications can be inherited in both a short-term (mitotic) and long-term (meiotic) manner to achieve an active or silent state in particular genes without changes to the primary DNA sequences. Specific enzymes are involved in the stable propagation of epigenetic marks during DNA replication. Importantly, the epigenetic marks are heritable but potentially reversible, which allows dynamic regulation of gene activities in response to environmental stimuli.

Cytosine methylation and covalent modifications of histone tails are key epigenetic modifications that are important for coordination of genomic integrity and proper gene

regulation during plant development. In plant genomes, cytosine methylation can occur in all sequence contexts (i.e. CG, CHG, CHH; H = A, T or C) and is controlled by specific DNA methyltransferases (Matzke et al., 2009; Law and Jacobsen, 2010; Saze et al., 2012). *De novo* methylation of unmethylated DNA is directed by an RNA interference-based mechanism named RNA-directed DNA methylation (RdDM) (Chan et al., 2005). In addition, the SWI2/SNF2-like chromatin remodeling factor DECREASE IN DNA METHYLATION 1 (DDM1) maintains CG/non-CG methylation and H3 lysine 9 methylation (Lippman et al., 2004). These marks are essential for the epigenetic silencing of transposable elements (TEs) (Kakutani et al., 2004; Law and Jacobsen, 2010). Shotgun bisulfite sequencing of the *Arabidopsis thaliana* genome and high-throughput sequencing of small RNAs showed that TEs and repeats accumulated in the pericentromeric regions are the primary target of DNA methylation and small interfering RNAs (siRNAs); these genomic regions are induced to form heterochromatin, a condensed and inactive chromatin structure (Cokus et al., 2008; Lister et al., 2008; Feng et al., 2010; Zemach et al., 2010). In addition to DNA methylation of TEs and repeats, plant

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Fig. 1. A model for the differential epigenetic coordination of genes and Transposable elements (TEs). CG methylation (represented by black Ms) of both genes and TEs is maintained by METHYLTRANSFERASE1 (MET1). Non-CG methylation (red Ms) and Histone H3K9 methylation are directed by CHROMOMETHYLASE3 (CMT3) and KRYPTONITE (KYP), respectively. DECREASE IN DNA METHYLATION1 (DDM1) is required for maintenance of CG, non-CG and H3K9 methylation at TEs (Lippman et al., 2004). DDM1 is also required to prevent hypermethylation of some genic loci such as *SUPERMAN* or *BONSAI* (Saze, 2008). The pathway that directs non-CG methylation and H3K9 methylation to genic regions becomes apparent in the absence of INCREASE IN BONSAI METHYLATION1 (IBM1) (Inagaki et al., 2010; Miura et al., 2009; Saze et al., 2008) or MET1 (Cokus et al., 2008; Lister et al., 2008; Mathieu et al., 2007). DEMETER (DME) and other DNA demethylases are recruited by small-interfering RNAs (siRNAs; gray bars) and enhance production of siRNAs and RNA-directed DNA Methylation (RdDM) at TEs (Zhu, 2009). For more details on RdDM, see reviews by Law and Jacobsen (2010), Matzke et al. (2009), and Saze et al. (2012).

genomes have methylation of CG sites within actively transcribed genes (Zhang et al., 2006; Zilberman et al., 2007); gene body methylation shows evolutionary conservation in plants and animals (Feng et al., 2010; Zemach et al., 2010). Although the function of the gene body methylation is still enigmatic, DNA methylation has been shown to be preferentially targeted to the nucleosomes of exons in animals and plants, suggesting that DNA methylation has a role in exon definition (Chodavarapu et al., 2010). A recent analysis of the *Arabidopsis* genome showed that methylated genes are longer than unmethylated genes; methylated genes are also more functionally important and evolve more slowly (Takuno and Gaut, 2012).

In addition to epigenetic silencing mechanisms that primarily target TEs and repeat sequences, plants have evolved pathways that prevent accumulation of heterochromatic epigenetic modifications (Fig. 1). H3K9 methylation and non-CG methylation generally accumulate at repeat sequences in the pericentromeric regions (Bernatavichute et al., 2008; Cokus et al., 2008; Lister et al., 2008). The INCREASE IN BONSAI METHYLATION 1 (IBM1) gene encodes a putative H3K9 demethylase that acts specifically against H3K9 methylation and non-CG methylation of gene bodies in euchromatic regions (Saze et al., 2008; Miura et al., 2009; Inagaki et al., 2010). By contrast, the DNA demethylation pathway mediated by DNA glycosylase/lyase-type enzymes preferentially targets repetitive loci producing siRNAs (Zheng et al., 2008; Zhu, 2009); this

targeted demethylation enhances production of siRNAs and reinforces the silencing of TEs (Gehring et al., 2009; Hsieh et al., 2009) (see below).

Recent advances in mass sequencing technologies combined with bisulfite analysis for DNA methylation or with Chromatin Immuno-Precipitation Sequencing (ChIP-Seq) have made it feasible to obtain the “epigenomic landscape” of particular tissues or cell types in plants (Li et al., 2008; Lister et al., 2008; Kaufmann et al., 2010; Ha et al., 2011). In this review, I mainly focus on recent progress in our understanding of the differential epigenetic regulation of genes and TEs throughout the plant life cycle, particularly in the model species *Arabidopsis*. Additionally, I review the evidence on the heritability of epigenetic changes, which has a potential impact on long-term phenotypic variation in plant populations.

EPIGENETIC CONTROL OF TES

The function of DNA methylation in plant genomes has been directly examined by analysis of loss-of function mutants of epigenetic modifiers such as *DDM1* and *METHYLTRANSFERASE 1* (*MET1*), a maintenance CG methylase (Vongs et al., 1993; Kankel et al., 2003; Saze et al., 2003). These studies demonstrated that the mutants induce genome-wide changes in both genetic and epigenetic contexts, which often result in the induction of developmental abnormalities (Kakutani et al., 2004; Saze, 2008). Some of the developmental abnormalities associ-

ated with the *ddm1* mutation were due to new insertions of both DNA transposons and retrotransposons within development regulator genes (Miura et al., 2001; Tsukahara et al., 2009). The *met1* mutation was similarly associated with reactivation of a copia-type retrotransposon (*ATCOPIA93*) and a *CACTA* transposon (both also reactivated by *ddm1*) although the effects are limited to the TEs (Mirouze et al., 2009). The distinct responses of the TE families to the mutations might be due to multiple layers of repressive epigenetic marks that prevent TEs from transposition. Indeed, some TEs show enhanced transposition ability in *ddm1* or *met1* plants that also carry mutations in RNAi factors or histone modifiers (Kato et al., 2003; Mirouze et al., 2009; Tsukahara et al., 2009).

Interestingly, a relatively recent amplification of the *COPIA93* family seems to have occurred in *A. lyrata*, a close relative of *A. thaliana*. Most of these retrotransposons are integrated into centromeric satellite repeat sequences, which are gene-poor regions; this target preference would be expected to be less harmful to genes and might also be beneficial to the long-term survival of the TEs in the population (Tsukahara et al., 2009). In contrast, some types of transposons were shown to integrate into gene unit, which could alter expression patterns (Ito et al., 2011). The presence of conserved epigenetic silencing mechanisms for TEs suggests that bursts of transposon amplification would generally be harmful to the integrity of the host genome, but could allow the plants to respond to rapid changes in the environment by provide a genetic resource for restructuring genes (see below).

CONTROL OF TES DURING PLANT REPRODUCTION

It has become apparent that control of TEs by small RNAs plays an important role during plant development and reproduction (Mosher and Melnyk, 2010). The silencing of TEs by ARGONAUTE 9 (AGO9) controls female gamete formation in *A. thaliana* (Olmedo-Monfil et al., 2010). Mutations in AGO9 and other RNAi factors cause differentiation of multiple female gametic cells and reactivation of TEs in the egg and synergid cells before fertilization. In plants, double fertilization gives rise to the embryo and to the endosperm that nourishes the developing embryo. The endosperm displays a parent-of-origin specific pattern of expression of some genes (genomic imprinting) that is essential for proper embryogenesis; this imprinting is regulated by asymmetric DNA methylation patterns in maternal and paternal alleles (Ikeda and Kinoshita, 2009; Jullien and Berger, 2009). The DNA demethylase DEMETER (DME) demethylates maternal alleles in the endosperm, ensuring maternal allele-specific expression of imprinted genes (Kinoshita et al., 2004; Gehring et al., 2006; Hsieh et al., 2011). DNA

methylation profiling of the *Arabidopsis* endosperm genome showed that the reduction in DNA methylation occurs throughout the genome, but particularly at TE sequences (Gehring et al., 2009; Hsieh et al., 2009). CG methylation in the *dme* endosperm is higher in TEs and genes than in the wild type endosperm, while non-CG methylation is reduced in the *dme* endosperm compared to the wild type. The latter might be a consequence of a lower level of TE-mediated production of siRNAs that normally reinforce non-CG methylation and silencing of TEs in the endosperm and possibly also the embryo (Hsieh et al., 2009). Furthermore, in parallel with the global reduction in DNA methylation in the endosperm, a large increase in the production of siRNAs from maternal chromosomes was observed in developing seeds (Mosher et al., 2009, 2011).

Intriguingly, an analogous mechanism may operate in anthers during pollen maturation. A pollen grain has one vegetative cell nucleus (VN) that is not transmitted to the next generation, and two sperm cell nuclei (SN): one SN fertilizes the egg cell, the other fuses to the central cell of the embryo sac to give rise to the endosperm. The VN shows extensive decondensation of centromeric chromatin along with a reduction in H3K9 methylation (Schoft et al., 2009). TEs are activated and transposed in the VN; this behavior coincides with downregulation of DDM1 and active expression of DME in the VN (Slotkin et al., 2009; Schoft et al., 2011). The small RNAs produced in the VN may be transported to the gametes through cytoplasmic connection and direct silencing of their TEs (Borges et al., 2011; McCue et al., 2011, 2012).

These studies have provided new insights into the function of germline companion cells with regard to the control of TEs in the genome through their production of siRNAs to inactivate TE activity in gametes. Interestingly, transgene-derived and endogenous siRNAs, derived principally from TEs and methylated regions, can both travel systemically through the phloem and direct RdDM to homologous target genes in remote cells (Dunoyer et al., 2010a, 2010b; Molnar et al., 2010; Melnyk et al., 2011). The details of the transportation mechanism that moves mobile siRNAs through the nuclear envelope to chromatin is still unclear; however, such mobile siRNAs offer a possible mechanism for “synchronization” of the epigenetic state of TEs not only in somatic cells but also in gametes to prevent germline transmission and propagation of active TEs in the population.

STRESS RESPONSES AND EPIGENETIC CHANGES

There has been a number of observations in plants and animals showing that environmental stresses can induce genetic and epigenetic changes that may be a source of phenotypic variation in the population (Boyko and

Kovalchuk, 2011; Feil and Fraga, 2012; Mirouze and Paszkowski, 2011). In plants, epigenetic changes can drastically affect plant morphology and physiology (Cubas et al., 1999; Manning et al., 2006; Martin et al., 2009; Lira-Medeiros et al., 2010; Paun et al., 2010). However, the underlying molecular mechanisms/pathways by which environmental cues influence the epigenetic status of chromatin remain elusive. Furthermore, the transgenerational effects of stress-induced epigenetic changes have been questioned so far in plants (Pecinka and Mittelsten Scheid, 2012). For example, in *A. thaliana*, a prolonged period of cold treatment of plants, called vernalization, induces repression of a MADS box gene *FLC* (*Flowering Locus C*) and promotes flowering (Amasino, 2005; Baurle and Dean, 2006; Dennis and Peacock, 2007). The repression of *FLC* is mediated by PcG complex that deposit H3K27 methylation on *FLC* locus. The epigenetic state of *FLC* is stably transmitted through mitosis, but not meiosis, and is reset in every generation. Environmental stress such as prolonged heat treatment can cause a transient de-repression of the silencing of transgenes and of endogenous TEs in constitutive heterochromatin (Lang-Mladek et al., 2010; Pecinka et al., 2010; Tittel-Elmer et al., 2010). Interestingly, although the de-repression was associated with a loss of heterochromatic structure, it was not accompanied by changes in epigenetic modifications such as DNA methylation or histone modification, and was restored relatively quickly to the initial silent state. The efficiency of restoration is impaired in plants with mutations affecting aspects of chromatin assembly, indicating that the *de novo* nucleosome assembly pathway at heterochromatic loci is essential for the re-establishment of the initial silencing state (Pecinka et al., 2010). Similarly, transient reactivation and movement of a copia-type endogenous retrotransposon (named *ONSEN*) can also be induced in plants with an impaired RNA interference machinery that are placed under a heat stress (Ito et al., 2011; Matsunaga et al., 2012).

STABILITY OF EPIGENETIC ALLELES AND TRANSGENERATIONAL EPIGENETIC INHERITANCE OF PHENOTYPIC VARIATIONS INDUCED BY MUTATIONS OF EPIGENETIC MODIFIERS

Epigenetic alleles (epi-alleles) induced by mutations of epigenetic modifiers are sometimes associated with changes in gene expression that lead to abnormal phenotypes during development (Henderson and Jacobsen, 2008; Saze, 2008). The epi-allele formation is often related to a destruction of epigenetic control of TEs (Lippman et al., 2004; Kinoshita et al., 2007; Saze and Kakutani, 2007; Fujimoto et al., 2008). Once established, the altered epigenetic state can be maintained through mitoses and often across generations. In plants,

DNA methylation patterns can be maintained through several rounds of DNA replication by maintenance DNA methylase and DNA binding proteins, as has also been found in mammals (Bostick et al., 2007; Sharif et al., 2007). In addition, histone-based inheritance of the epigenetic state might occur during DNA replication (Moazed, 2011). The transgenerational stabilities of epi-alleles were examined in epigenetic recombinant inbred lines (epiRILs) of *A. thaliana* generated by crossing *met1* or *ddm1* mutants to the isogenic wild type strain (Johannes et al., 2009; Reinders et al., 2009; Teixeira et al., 2009). In general, DNA methylation patterns showed stable inheritance and the epigenetic marks on chromosomes were transmitted over several generations. However, in *ddm1*-derived epiRILs, a progressive DNA remethylation occurred at repeat sequences associated with siRNAs. Unexpectedly, in *met1*-derived epiRILs, nonparental DNA methylation patterns not related to the methylation profiles of the parental chromosomes were identified. This intriguing phenomenon in *met1*-derived epiRILs might be the result of an RNAi-based remethylation mechanism or a “back-up” mechanism of DNA methylation upon the genome-wide loss of CG methylation (Mathieu et al., 2007). The epiRIL populations exhibited continuous phenotypic variation for important complex quantitative traits including flowering time, plant height, biomass, and responses to both abiotic and biotic stresses (Johannes et al., 2009; Reinders et al., 2009). Furthermore, some of these phenotypic variations were stably inherited by the next generation, confirming the potential of epigenetic variation to contribute to heritable phenotypic variation.

Recent studies examined the spontaneous loss or gain of DNA methylation and its stability across generations in *A. thaliana* experimental lines propagated by single-seed descent for more than 30 generations (Becker et al., 2011; Schmitz et al., 2011). CG methylation varied in gene-rich regions of the genome, whereas CG and non-CG methylation of TEs were relatively stable. The stable inheritance of DNA methylation at TEs may occur because of a close association of 24-nucleotide siRNAs to TEs that reinforces *de novo* DNA methylation. Schmitz et al. (2011) estimated a minimum level for epi-mutation of 4.5×10^{-4} methylation polymorphisms per site per generation; this estimate is orders of magnitude higher than the estimated genetic mutation rate of 7×10^{-9} base substitution rate per site per generation in the same *Arabidopsis* experimental population (Ossowski et al., 2010; Becker et al., 2011). Alteration of DNA methylation patterns at some loci were associated with changes in gene expression levels, whereas other loci did not show a significant alteration in expression in response to the methylation changes, indicating the complexity of the effects of DNA methylation and other epigenetic marks on gene transcription behavior.

CONCLUSION AND PERSPECTIVES

There is growing evidence that environmentally induced heritable changes to the epigenome are common in both plants and animals (Carone et al., 2010; Feil and Fraga, 2012; Seong et al., 2011; Daxinger and Whitelaw, 2012). However, because of the plastic nature of the epigenome, it is still not clear whether such environmentally induced changes are a reflection of a temporary acclimation to the environment or have evolutionary consequences, i.e., alteration of the primary DNA sequence. It is known that 5-methyl cytosine (5mC) has a potentially high rate of mutagenic changes (i.e., spontaneous deamination of 5mC results in C to T substitution) (Turner, 2009). In addition, stress-induced activation and insertion of TEs in or near genes may cause modulation of cellular transcriptomes as well as new gene formation by exonization (Cordaux and Batzer, 2009; Matsunaga et al., 2012). The “domestication” of TEs in the genome could thus be essential for future genomic stability particularly after structural reorganization events in the genome such as polyploidization and hybrid formation (Chen et al., 2008; Chang et al., 2010; Pignatta et al., 2010; Fujimoto et al., 2011; Greaves et al., 2012; Shen et al., 2012). In addition to genomic DNA, it is possible that small RNA molecules could act as a transmitter of extragenic information on the surrounding environment to the progeny. For example, virus-derived small-interfering RNAs can mediate a transgenerational antiviral response in *Caenorhabditis elegans*, showing the inheritance of an “acquired trait” across generations (Rechavi et al., 2011). Although it is yet unclear in plants, translocation of mobile small RNAs from somatic cells to shoot meristems and eventually to germlines could affect epigenetic inheritance of phenotypic variations (Martienssen, 2010). Thus, unveiling the molecular mechanisms of transgenerational epigenetic inheritance is an important future research area. In addition, molecular evidence for pathways and factors via which environmental cues influence chromatin structure will provide further insights into stress-induced epigenetic changes and their transgenerational consequences, which should occur frequently under natural environment.

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