REVIEW PAPER

Epigenetic variation and environmental change

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Abstract

Environmental conditions can change the activity of plant genes via epigenetic effects that alter the competence of genetic information to be expressed. This may provide a powerful strategy for plants to adapt to environmental change. However, as epigenetic changes do not modify DNA sequences and are therefore reversible, only those epimutations that are transmitted through the germline can be expected to contribute to a long-term adaptive response. The major challenge for the investigation of epigenetic adaptation theories is therefore to identify genomic loci that undergo epigenetic changes in response to environmental conditions, which alter their expression in a heritable way and which improve the plant's ability to adapt to the inducing conditions. This review focuses on the role of DNA methylation as a prominent epigenetic mark that controls chromatin conformation, and on its potential in mediating expression changes in response to environmental signals.

Key words: Adaptation, DNA methylation, epigenetics, stress response.

Introduction

Epigenetic mechanisms alter the probability or competence of genetic information to be expressed in a heritable but still reversible way. This is mediated by changes in chromatin structure that alter the accessibility of a genetic region for the transcription machinery, or by changes in turnover rates of selected transcripts. In many, but not all, cases these changes are implemented by small RNAs or longer non-coding RNAs that serve as sequence- or locus-specific guides for DNA methylation, chromatin modification, or transcript degradation/amplification mechanisms.

While epigenetic changes can influence mutation and recombination rates, epigenetic target loci do not change their DNA sequence. A local epigenetic modification, as long as it is maintained, therefore alters the conversion of genetic information into a phenotype, while reversal to the original epigenetic state restores the previous status quo. This provides plants with an efficient tool to alter gene function in specific cell types, developmental stages, or under specific environmental conditions, and to pass on the altered epigenetic state during somatic cell division or even via the germline to subsequent generations. Depending on the epigenetic modification, this can lead to the silencing of a previously active gene or to the activation of a functional but so far silent genetic region. Reversible epigenetic modifications include histone marks, in particular methylation, acetylation, or phosphorylation marks at histone tails, and methylation of cytosines. Changes in DNA methylation are the easiest to detect and most precisely positioned indicators and modifiers of epigenetic change, which influence gene expression directly or in combination with histone marks.

DNA methylation pathways in plants

In the model system Arabidopsis thaliana, cytosine methylation occurs in three sequence contexts, mediated by DNA methyltransferases that are guided to their targets by methylation patterns, histone marks, small RNAs, or non-coding scaffold transcripts. The most prominent methylation marks are found at CG sites, where they are faithfully propagated by maintenance DNA METHYLTRANSFERASE1 (MET1), a
plant homologue of the mammalian DNA methyltransferase 1 (Dnmt1), which has a strong affinity for hemi-methylated cytosines. Non-symmetrical cytosine methylation in a CHH context (H representing C, T, or A) is largely controlled by the RNA-directed DNA methylation (RdDM) pathway with 24 nucleotide small interfering RNAs (siRNAs) acting as guides for de novo DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2). The RdDM pathway predominantly controls repeats in heterochromatic regions and in dispersed transposons, and related sequences in euchromatic regions (Matzke et al., 2009).

Non-coding RNAs and histone marks provide a guiding function for DNA methyltransferases, assisting them in identification of their targets. For DRM2-mediated de novo methylation, this involves two plant-specific RNA polymerases, Pol IV and Pol V, which are only found in plants and which have both evolved from Pol II. Pol IV, which initiates biogenesis of small RNAs, is guided to its target regions by a dual lysine methyl reader protein, DNA-BINDING TRANSCRIPTION FACTOR 1/SAWADEE HOMEODOMAIN HOMOLOG 1 (DTF1/SHH1), which identifies targets by probing for both unmethylated lysine 4 residues at histone H3 (H3K4) and for methylated H3K9 modifications (Law et al., 2013; Zhang et al., 2013). Pol V, which assists in targeting of the siRNA complex, is guided to its target loci by the DDR chromatin-remodelling complex consisting of DEFECTIVE IN MERISTEM SILENCING 3 (DMS3), DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1 (DRD1), and RNA-DIRECTED DNA METHYLATION 1 (RDM1) (Zhong et al., 2012) and by two homologues of the histone lysine methyltransferase, suppressor of variegation 3–9 [SU(VAR)3–9], SUVH2 and SUVH9, with SRA (SET-and RING-ASSOCIATED) domains that bind methylated DNA (Johnson et al., 2014). Pol V assists in the recruitment of DRM2 as part of ARGONAUTE4 (AGO4) effector complexes by producing a non-coding scaffold transcript that base-pairs with siRNAs, which results in specific methylation of the template strand by DRM2 (Zhong et al., 2014) (Fig. 1A).

Not all RdDM target loci are controlled by Pol V transcription, as we can distinguish between Pol IV- and Pol V-dependent (type I) loci, and Pol IV-dependent but Pol V-independent (type II) loci. AGO4 co-localizes with Pol V in the nucleolar processing centre but not in the nucleoplasm, where it associates with Pol II (Gao et al., 2010). Pol II and Pol V therefore have locus-specific AGO4 recruitment functions. Pol II also plays a locus-specific role in siRNA amplification. At intergenic low-copy-number repeat sequences, Pol II produces scaffold transcripts adjacent to silenced loci that help to recruit Pol V, and Pol II recruits Pol IV to these loci assisting in amplification of siRNA pools (Zheng et al., 2009). The selection of a genomic region as a RdDM target will therefore be influenced by the presence of a pool of homologous siRNAs, by local transcription of scaffold transcripts at or in the vicinity of the locus, and by DNA methylation and histone marks at the locus.

A third DNA methyltransferase, CHROMOMETHYLASE3 (CMT3), which is exclusively found in plants, predominantly controls CHG methylation (Jackson et al., 2002) in combination with histone methylation marks (Cao et al., 2003). CMT3...
contains a chromodomain that binds methylated H3K9 marks, which are generated by the partially redundant activity of histone methyltransferases SUVH4, SUVH5, and SUVH6, which contain a methylC-binding domain. CHG methylation is therefore maintained by a self-enforcing loop of cytosine and H3K9 methylation enzymes (Johnson et al., 2002). Loss of histone methylation by transcription-associated histone replacement or demethylation (Inagaki et al., 2010) breaks this circle, also leading to loss of CHG methylation. At some loci, RdDM pathway functions counter-balance transcription-associated loss of histone methylation and stabilize CMT3-controlled CHG-specific methylation (Enke et al., 2011). Chromomethylases (CMTs) that bind to histone methylation marks have only been identified in embryophytes (Noy-Malka et al., 2014). Most CMTs analysed so far, including CMT3, preferentially methylate CHG targets. CMT2, however, methylates both CHG and CHH targets (Stroud et al., 2014), acting co-operatively with (Stroud et al., 2014) or independent of (Zemach et al., 2013) the RdDM pathway.

The analysis of distinct genomic loci has helped to establish mechanistic models that allocate specific functions to the different DNA methyltransferases. MET1 has mainly been discussed in the context of its maintenance function for CG methylation marks, providing more stable epigenetic patterns than the target loci of the RdDM pathway, which show a higher level of epigenetic variation in Arabidopsis accessions (Schmitz et al., 2013). The role of MET1, however, is not strictly limited to maintenance of CG methylation. At least at some target regions, MET1 has been shown to affect non-CG methylation as well, for example as a co-ordinator of methylation of stem–loop structures (Gentry and Meyer, 2013) (Fig. 1B). An indirect MET1-specific effect on non-CG methylation has also been observed at certain loci with CMT2-controlled CHH and CMT3-controlled CHG methylation, which derive from Gypsy elements (Fig. 1C, D). These loci lose their H3K9 methylation in a met1 mutant, which results in a loss of CHG and CHH methylation marks (Stroud et al., 2013). Loss of MET1 can generate hypomethylated, active epi-alleles, which are stably transmitted to the next generation (Watson et al., 2014).

DNA demethylation pathways in plants

De novo and maintenance methylation in plants is balanced by cytosine demethylation under the control of base excision repair pathways involving the 5-methylcytosine DNA glycosylase REPRESSOR OF SILENCING 1 (ROS1) and its homologues DEMETER (DME), DEMETER-LIKE 2 (DML2), and DML3. After 5mC removal and incision of the DNA backbone, the unmethylated cytosine is restored following 3’phosphate removal, DNA polymerization, and DNA ligation (Penterman et al., 2007). Like DNA methylation, DNA demethylation is linked to histone modification systems, and enzymatic activity and regulation of demethylating complexes is better understood than their target selection criteria. Changes in histone marks are used to recruit demethylation functions or to inhibit de novo methylation functions. The histone acetylase Increase DNA Methylation 1 (IDM1), for example, binds to methylated loci with low lysine (H3K4) and arginine (H3R2) methylation levels, and acetylates H3K18 and H3K23 sites to recruit DNA demethylases (Qian et al., 2012). The histone demethylase increase in BONSAI Methylation 1 is recruited to transcribed regions where it demethylates H3K9me2 marks. This removes the binding targets for the chromodomain of CMT3, leading to selective loss of CHG methylation marks that are no longer restored after replication (Inagaki et al., 2010).

In the literature, DNA methylation is often exclusively discussed in the context of gene repression, which does not take into account the complex interaction between the different methylation and demethylation systems. In a met1 mutant, for example, RdDM functions are activated, while expression of the ROS1 demethylase is eliminated and DML2 and DML3 transcript levels are reduced (Mathieu et al., 2007). Mutation of several RdDM pathway functions also reduces ROS1 activity (Li et al., 2012), illustrating that the RdDM pathway can also have an activating role via maintaining ROS1 expression.

Biological effects of DNA methylation

Changes in DNA and histone methylation influence gene expression, in particular transcription (Huettel et al., 2006), splicing (Regulski et al., 2013), and polyadenylation (Tsuchiya and Eulgem, 2013), but they also affect DNA repair (Yao et al., 2012), recombination (Mirouze et al., 2012), and meiotic crossover in euchromatic regions (Melamed-Bessudo and Levy, 2012). The multiple mechanistic effects make it difficult to differentiate between direct changes mediated by DNA methylation and their secondary effects. While the literature is full of reports that correlate DNA methylation and specific phenotypes, there are many fewer reports that demonstrate a direct role for DNA methylation in the transcriptional regulation of one or several distinct target loci, which are responsible for a defined effect or phenotype. Examples of mechanisms and phenotypes under direct control of DNA methylation include parental imprinting (Huh et al., 2008), floral symmetry (Cubas et al., 1999), flowering time (Soppe et al., 2000), pigmentation (Stam et al., 2002), fruit ripening (Manning et al., 2006), sex determination (Martin et al., 2009), and stomatal development (Tricker et al., 2012; Yamamuro et al., 2014). Seed yield, determined by energy use efficiency, was the first quantitative trait associated with distinct, heritable DNA methylation patterns (Hauben et al., 2009). Flowering time and primary root length are two other complex quantitative traits linked to DNA methylation patterns at differentially methylated regions (DRMs). Methylation patterns of some DRMs are heritably altered in epigenetic mutants, which suggest that they are specific targets of an epigenetic system that enhances expression variability. Accordingly, many DRMs display a considerable level of variability in natural Arabidopsis populations (Cortijo et al., 2014).

Stress-induced epigenetic changes

While epigenetic Arabidopsis mutants have proven useful to test the significance of epigenetic functions in stress responses (Yao et al., 2012; Popova et al., 2013), we have to be careful when drawing conclusions about a direct role for epigenetic
functions, especially when using epigenetic mutants that display a range of phenotypes due to secondary effects. Mutation of the **MET1** gene, for example, inhibits expression of DNA demethylases and leads to the establishment of histone H3K9 methylation and RNA-directed methylation marks in new genomic regions (Mathieu et al., 2007). This generates a variety of stochastic epi-mutations and phenotypes, many of which probably do not represent direct **MET1** targets but reflect randomly established novel epigenetic marks. Another factor that complicates the comparison of epigenetic mutants and wild-type lines is background differences in gene expression profiles frequently observed among different plant lines due to epigenetic diversity (Havecker et al., 2012). The use of epigenetic mutants to link phenotypic effects to distinct epigenetic changes is further complicated by the mutagenic consequence of certain epigenetic alterations, which induce genetic changes that could be mistaken for stable epi-mutations. This is exemplified by the **bal** variant that was isolated from an inbred **ddml** mutant background and that contains a 55 kb duplication within the **RPP5** (recognition of *Peronospora parasitica* 5) locus, which includes a cluster of disease Resistance (R) genes. Duplication is accompanied by hypermethylation and up-regulation of **SNC1** (**SUPPRESSOR OF NPR1-1, CONSTITUTIVE 1**), which co-ordinately activates **RPP5** locus R genes and induces a distinct dwarfism and curled-leaf phenotype (Yi and Richards, 2009). It is unclear if these changes represent a random, independent event, or if recombination and mutation rates at the **RPP5** locus are increased by **DDM1** deletion. If hypomethylation induced by mutation of **DDM1** or other methylation functions stimulates recombination and mutation events at distinct loci, this could lead to genetic changes of identical regions in different DNA methylation mutants that could be mistaken for epi-mutations.

To identify direct epigenetic targets for stress effects among a background of epi-alleles and genetic mutations, it will therefore be important to link expression changes at potential epigenetic target loci in epigenetic mutants with corresponding epigenetic changes in response to the stress effect. An example for this strategy is the discovery of epigenetic target loci that are activated in response to bacterial pathogens (Dowen et al., 2012). Indications for a role for DNA methylation in biotic stress responses came from infection studies of methylation mutants **met1-3** and **ddc** (**ddm1-2 dmr2-2 cmt3-11**), which showed enhanced resistance to pathogenic and avirulent strains of *Pseudomonas syringae*. A screen for DMRs in wild-type plants, in response to bacterial infection, identified methylation changes at DMRs that correlated with activation of pathogen response genes. While methylation differences were relatively modest due to the high background of unaffected tissue that was not involved in the local response to bacterial infection, they were significant to identify distinct target regions for pathogen-induced DMRs. These mainly comprised changes in CG and CHH marks in intergenic regions and at 5′ and 3′ boundaries of protein-coding genes. Infections with virulent and avirulent strains induced similar changes at CG and CHG sites but different changes at CHH sites, which suggests that certain non-symmetrical methylation marks are modified in a stress-specific way. Hypomethylation at non-genic regions correlated with a moderate increase in transcript abundance of proximal genes, while transcript levels were more strongly increased for genes with hypomethylated coding regions. Genes affected by hypomethylation in the wild type after infection were also misregulated in **met1-3** and **ddc** mutants, which implies that all three methyltransferases were involved in their transcriptional control (Dowen et al., 2012).

Various biotic (Boyko et al., 2007) and abiotic stress conditions (Kovarik et al., 1997) have now been shown to correlate with changes in DNA methylation profiles. We still, however, lack clear evidence for a model case demonstrating that a stress-specific epigenetic modification is transmitted to subsequent generations, improving the progeny’s capability to cope with the relevant stress (Pecinka and Mittelsten Scheid, 2012). Some reports demonstrate heritable changes in DNA methylation at distinct loci in response to stress but do not show the relevance of these loci to stress tolerance (Kou et al., 2011; Zheng et al., 2013). Others detect a correlation between stress conditions and overall or tissue-specific methylation changes in putative stress response genes but do not report on the heritability of these changes (Steward et al., 2002; González et al., 2013). Factors that make it difficult to assess the relevance of defined epigenetic changes in stress adaptation are the lack of control over the combined effects of multiple stress conditions a population has been exposed to and the high level of epigenetic variability in populations (Woo and Richards, 2008; Becker et al., 2011; Groszmann et al., 2011).

It is also unclear if epigenetic changes at distinct loci are the direct consequence of changing environmental conditions or if they are the secondary consequences of other stress-induced changes. In this context, it is worth noting that certain environmental stress conditions alter the expression levels of epigenetic regulators. The Geminivirus Rep protein, for example, reduces transcript levels of the **NbMET1** and **NbCMT3** methyltransferase genes in *Nicotiana benthamiana* (Rodriguez-Negrete et al., 2013), and in *Arabidopsis, MET1* and **DDM1** transcript levels are down-regulated in response to biotic stress or salicylic acid (Dowen et al., 2012), and various stress conditions increase transcript levels of histone deacetylases **HDA6** (To et al., 2011) and **HDA19** (Zhou et al., 2005). At least for certain loci that are sensitive to heritable epigenetic variation in response to environmental conditions, the local concentration of regulatory factors may therefore mediate environmental influences on epigenetic patterns. Environmental effects that alter the concentration of DNA methyltransferases, their interacting histone modifiers, or potentially their regulatory siRNA or transcripts (Lakhotia, 2012; Di Ruscio et al., 2013), may induce epigenetic changes at loci that are sensitive to quantitative changes of key regulators of methylation. Even transient exposure to stress conditions may add to epigenetic diversity if it influences efficiency and fidelity of epigenetic maintenance.

### Transposable elements: mediators of epigenetic response

Transposable elements (TEs) and their derivatives, which make up more than half of the DNA in many species, play a prominent role in the epigenetic regulation of adjacent genes,
and in the transmission of epigenetic memory effects due to the conversion of epigenetic states in response to environmental change (McClintock, 1984; Mirouze and Paszkowski, 2011; Fedoroff, 2012). TEs are controlled by different, frequently interacting epigenetic pathways that determine the stability and fidelity of their transcriptional repression, activation, and re-setting (Lippman et al., 2003; Zemach et al., 2013).

TEs can be activated by stress conditions leading to transient (Tittel-Elmer et al., 2010), cell-specific (Matsunaga et al., 2012), or widespread (Dowen et al., 2012) expression. Activation of TEs can alter expression of adjacent genes and of genes adjacent to new integration sites, into which new TE copies have transposed. Environmental conditions influence the activity of TEs if these contain specific stress response elements, and they influence the activation of TEs if they change their epigenetic state (Johannes et al., 2009; McCue et al., 2012). Examples of stress-responsive TEs that insert into genic regions are \textit{mPing}, a miniature inverted-repeat rice TE, and the \textit{Arabidopsis OSEN} retroelement. Amplified copies of \textit{mPing}, which are produced after cold and salt stress, preferentially insert into 5’ regions of genes, avoiding potential mutagenic damage via insertion into exons (Naito et al., 2009). \textit{ONSEN} has acquired a heat-responsive element that regulates its activation (Cavrak et al., 2014) and that induces heat responsiveness in genes adjacent to its new insertion sites (Ito et al., 2011).

How useful is an epigenetic stress memory?

The responsiveness of DNA methylation patterns to environmental stress (Finnegan, 2002) has been suggested to act as a molecular switch for evolutionary adaptation of plants to environmental change (Kou et al., 2011). In many cases, however, the continuous activity of stress-responsive genes will be undesirable due to secondary effects or the associated energy burden. This may make it advantageous for stress response pathways with secondary effects to remain active only for the duration of the inducing stress. Under this concept, epigenetic changes should be more useful if they did not cause permanent expression of target genes but rather they enabled the gene to respond more quickly and efficiently to frequently re-occurring stress conditions. To detect these kinds of epigenetic changes, we would face the much harder task of searching for changes in transcriptional competence and/or response time to secondary challenges, not for changes in expression levels.

Under continuous stress conditions, it may be advantageous if epigenetic changes lead to continuous activity of stress response genes that were previously only temporarily active. A potential example where durable changes in environmental conditions could have caused continuous activation of stress response genes may be mangrove populations that grow in close vicinity in riverside or salt marsh locations, respectively. The two populations differ more significantly in their methylation patterns than in DNA sequence. Plants in the salt marsh population, which display shrub-like phenotypes, have a lower level of methylation diversity than the tree-like plants in the riverside population (Lira-Medeiros et al., 2010). This may reflect a loss of epigenetic flexibility in response to permanent adaptation to salt stress. If this assumption was correct, one would expect to identify active genes in salt marsh populations that are associated with variable methylation patterns in riverside populations, and that are responsible both for improved salt tolerance and changes in plant architecture.

While heritable epigenetic changes may be advantageous to adapt to continuous changes in environmental conditions, a transmission of any stress-induced epigenetic state would probably compromise plant growth and development. Plants have therefore developed several layers of control mechanisms that revert activated epig-alleles to their silent states. Heritability and transmission efficiency of epigenetic patterns are target specific and dependent on different epigenetic functions. The siRNA pathway plays an important role in restricting retrotransposition triggered by environmental stress. The heat-stress-activated \textit{copia}-type \textit{ONSEN} retrotransponson is silenced in the next generation (Ito et al., 2011) but remains active in plants with compromised siRNA biogenesis. Hypomethylation patterns of RdDM-dependent TEs and their derivatives are faithfully restored within a few generations (Teixeira et al., 2009), while other hypomethylation patterns are stably retained over at least eight generations (Johannes et al., 2009). DDM1 and Morpheus’ Molecule1 (MOM1) have recently been shown to act redundantly to restore silencing of some loci that are activated by heat stress (Iwasaki and Paszkowski, 2014). This does, however, only affect \textasciitilde{}10\% of all stress-activated genes, which suggests the presence of one or several other resetting mechanisms that prevent trans-generational transmission of epigenetic changes.

Current models and discussions for plants are dominated by the RdDM pathway, and many publications exclusively refer to DNA methylation being established by the guiding function of small RNAs that are generated and transported by RdDM pathway components. While, at least for \textit{A. thaliana}, it is certainly correct that DNA methylation of most genomic regions is controlled by the RdDM pathway, we should not ignore the presence of RdDM-independent DNA methylation targets (Singh et al., 2008; Havecker et al., 2012; Sasaki et al., 2012; Gentry and Meyer, 2013; Zemach et al., 2013; Watson et al., 2014). Methylation at some RdDM-independent target loci requires specific epigenetic functions, including HDA6, DDM1, or MET1. These may act as mediators of environmental change if certain stress conditions influence their steady-state levels and if this affects maintenance and stability of their methylation targets.

Outlook

Work on the model system \textit{A. thaliana} has helped to define epigenetic pathways, targets, and their interactions with various stress conditions. With the rapid completion of genome sequencing projects for various species and the increased...
resolution of epigenetic maps, we can now investigate species-specific differences in the representation and distribution of epigenetic targets and their control mechanisms. The following questions still remain to be answered. How does a genetic locus become a DNA methylation target and what determines if its DNA methylation pattern is controlled by an RNA-dependent DNA methylation pathway, by an RNA-independent pathway, or by a combination of both? Which of these DNA methylation targets produce distinct epi-alleles that are heritable and that contribute to epigenetic diversity? Which of these heritable epigenetic patterns change expression levels and which alter expression competence? Do plant species differ in the composition and representation of target loci for the different DNA methylation pathways, and does this affect their potential to generate epigenetic diversity? How does this influence a plant’s potential to cope with stress or to adapt to changing environmental conditions? Considering its relatively low proportion of TEs and TE-derived genes, it is uncertain if A. thaliana is the best model system to investigate the interplay between epigenetic control of gene activity and a changing environment. We may obtain more relevant examples for epigenetic adaptation from species which faced gradual changes in their local environment, to which they could respond over several generations, as illustrated by the morphological changes in the mangrove populations mentioned above. Another fascinating example of epigenetic adaptation has been reported for a Diplacus species complex in Southern California that changes its flower morphology and colour when adapting to different pollinator populations. Within a geographical transition region containing coastal Diplacus puniceus plants with red flowers pollinated by hummingbirds and inland Diplacus australis plants with yellow flowers pollinated by insects, intermediate populations with orange flowers are found. Over a period of 12–15 years, individual plants in this transition zone change in colour and morphology from a yellow, insect-pollinated phenotype to a red bird-pollinated phenotype. The new phenotype is heritable but reverts at a rate of 1–2%, which confirms the epigenetic nature of the morphological change, induced by unknown environmental factors (Hirsch et al., 2012).

A search for appropriate epigenetic model systems will help us to assess the significance of epigenetic changes in adaptation to rapidly changing environments, which will ultimately also become highly relevant for the development of novel crops. Considering the historical focus in crop breeding on high yield and uniform development, it is likely that wild plant species have retained a more powerful epigenetic potential than crop lines—another good reason to rethink the current stringent focus of many research programmes on ‘commercially useful’ species.

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