DNA Methylation and Demethylation in Plant Immunity

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Abstract

Detection of plant and animal pathogens triggers a massive transcriptional reprogramming, which is directed by chromatin-based processes, and ultimately results in antimicrobial immunity. Although the implication of histone modifications in orchestrating biotic stress–induced transcriptional reprogramming has been well characterized, very little was known, until recently, about the role of DNA methylation and demethylation in this process. In this review, we summarize recent findings on the dynamics and biological relevance of DNA methylation and demethylation in plant immunity against nonviral pathogens. In particular, we report the implications of these epigenetic regulatory processes in the transcriptional and co-transcriptional control of immune-responsive genes and discuss their relevance in fine-tuning antimicrobial immune responses. Finally, we discuss the possible yet elusive role of DNA methylation and demethylation in systemic immune responses, transgenerational immune priming, and de novo epiallelism, which could be adaptive.

INTRODUCTION

PAMP: pathogen-associated molecular pattern

PTI: PAMP-triggered immunity

TGS: transcriptional gene silencing

TEs: transposable elements

Priming: state of increased alertness or plant defense potentiation

Transgenerational immune priming: immune responses induced in the progeny of infected/elicited plants

PTGS post-transcriptional gene silencing

The first layer of active defense, known as PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI), relies on the perception of PAMPs or MAMPs (microbe-associated molecular patterns) by surface receptors defined as pattern-recognition receptors (PRRs) (52). PAMP perception is followed by the activation of immune responses, which results in basal immunity (9). As a counter-defense, adapted pathogens produce effectors that are secreted inside host cells to dampen PTI, thereby allowing microbial multiplication (25, 29). As a counter-counter defense, pathogen effectors—or their effects on host targets—can be perceived by disease resistance proteins, the activation of which often results in a potent immune response referred to as effector-triggered immunity (ETI) (52, 74). Importantly, activation of both PTI and ETI relies on a massive transcriptional reprogramming that is tightly controlled by chromatin-based regulatory mechanisms (88, 94, 104), and several recent reviews have covered the role of histone modifications in this process (14, 27, 113). Here, we focus on DNA methylation, which refers to the addition of a methyl group to cytosines and is a conserved form of epigenetic regulation involved in transcriptional gene silencing (TGS) and maintenance of genome integrity through transposon taming. Using recent studies in the model plant *Arabidopsis thaliana*, we examine the biological relevance of DNA methylation, as well as demethylation, in plant immunity against nonviral pathogens. We report on the role of these two epigenetic pathways in plant disease resistance and in the transcriptional and co-transcriptional control of immune-responsive genes. Furthermore, we present the dynamics of DNA methylation changes during plant immune responses and discuss their role in fine-tuning the expression of defense genes that are associated with transposable elements (TEs) and repeats. Finally, we discuss the possibility of a yet elusive role of small RNAs, DNA methylation and demethylation in systemic immune responses, transgenerational immune priming, and de novo epiallelism that may be adaptive.

DNA Methylation in *Arabidopsis*

DNA methylation targets tandem and interspersed repeats, generally derivatives of TEs (80, 90). At these loci, DNA methylation occurs in three different sequence contexts: symmetrical CG dinucleotides (usually highly methylated at 80–100%); symmetrical CHG, where H corresponds to A, T, or C (methylated at 20–100%); and asymmetrical CHH (usually lowly methylated at 10% or less) (21, 70). DNA methylation can also be found at gene bodies, exclusively at CG residues. In this particular case, DNA methylation function is currently unclear, hence the focus on repeat-associated DNA methylation—in promoters and introns—in this review.

Establishment of DNA Methylation at Transposable Elements and Repeats

Substantial knowledge on the establishment of DNA methylation at transposons and repeated sequences was gained during the past decade through the study of various systems, in particular *FLOWERING WAGENINGEN* (*FWA*) transgene silencing upon *Agrobacterium*-mediated transformation (18, 78), virus-induced gene silencing (VIGS) targeted at the *FWA* endogene (10), and *Arabidopsis* mutants in which TEs are mobilized and must be resilenced (75, 82, 89).

The first step of TE and repeat silencing is the conversion of a PolII transcript recognized as aberrant (the nature of this aberration is unclear but could be related to transcript over-accumulation) into a double-stranded RNA (dsRNA) by the RNA-dependent RNA polymerase RDR6. dsRNAs are subsequently processed by Dicer-like 2 and 4 (DCL2 and DCL4) into 21–22-nt small interfering RNAs (siRNAs) (75, 82, 89, 108) that can mediate post-transcriptional gene silencing (PTGS) and/or transcriptional gene silencing (TGS). In PTGS, 21–22-nt siRNAs

are loaded into an RNA-induced silencing complex (RISC) containing ARGONAUTE 1 (AGO1) or AGO2 and mediate the slicing of sequence complementary mRNA targets (11). In TGS, 21–22-nt siRNAs are presumably loaded into AGO6 to direct this effector at TEs through base pairing with transcripts generated by the plant-specific PolV; methylation of the DNA template strand is catalyzed by the de novo methyltransferase DOMAINS REARRANGED METHYLASE 2 (DRM2; possibly DRM1 as well) in all sequence contexts (**Figure 1**) (32, 37, 82, 128). This pathway is thus referred to as the PolV-RNA-directed DNA methylation (PolV-RdDM) pathway (78). This pre-establishment phase transitions to a PolIV-RdDM–mediated stabilization phase where single-stranded RNAs (ssRNAs) are produced by PolIV (also referred to as NRPD1), another plant-specific polymerase that transcribes methylated DNA (78). The RNA-dependent RNA polymerase 2 (RDR2) presumably uses these ssRNAs as templates to produce dsRNAs that are processed into 24-nt siRNAs by DICER-LIKE 3 (DCL3). siRNAs are then loaded into RISCs containing AGO4, AGO6, or AGO9 (43, 84). PolV transcripts interact with AGO4/6/9-loaded homologous siRNAs, and DNA methylation of the PolV DNA template strand is catalyzed by DRM2 (and possibly DRM1) through its binding to AGO4/6/9 (129).

RdDM: RNA-directed DNA methylation

Heterochromatin: densely packed chromatin

Maintenance of DNA Methylation

Once established, DNA methylation marks are maintained during DNA replication and cell division by different pathways, depending on the sequence context of the methylated cytosine. Briefly, CG methylation is maintained by METHYLTRANSFERASE1 (MET1) and CHG methylation by CHROMOMETHYLASE2 and 3 (CMT2 and CMT3); the maintenance of asymmetric CHH methylation requires either CMT2 or RdDM, depending on the loci that are targeted (**Figure 1**) (31, 78). Furthermore, the putative chromatin remodelers DECREASE IN DNA METHYLATION (DDM1) (54, 115) and DEFECTIVE IN RNA-DIRECTED DNA METHYLATION (DRD1) (56) presumably allow access of the DNA methylation machineries to long heterochromatic TEs and short euchromatic TEs, respectively (125).

The methyltransferase MET1 acts on CG sites that are hemimethylated after DNA replication. MET1 is recruited to catalyze methylation of the other DNA strand by VARIANT IN METHYLATION (VIM) proteins, which recognize hemimethylated sites through their SET- AND RING-ASSOCIATED (SRA) domain (61).

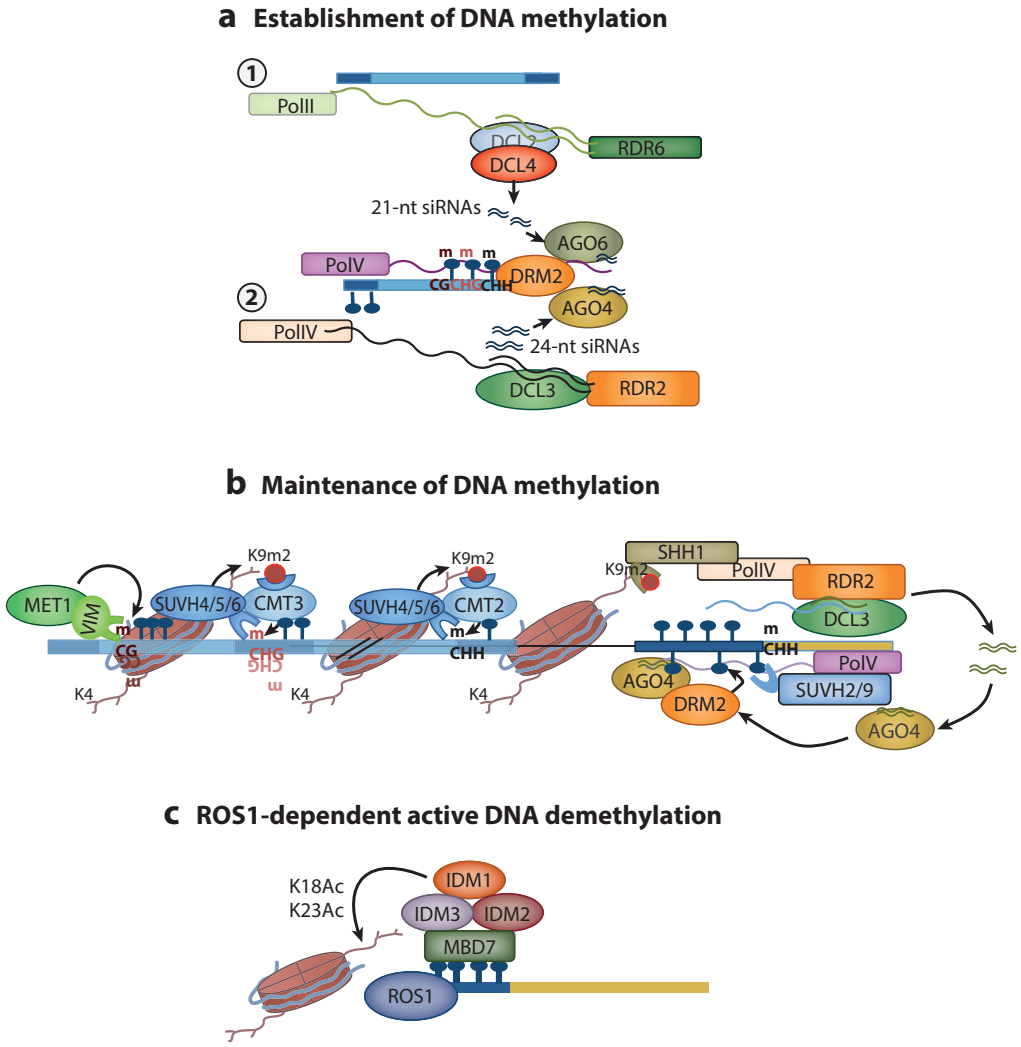
At long TEs, which are rich in H3K9me2 (dimethylation of the lysine 9 of histone 3) and are usually located in pericentromeric heterochromatin, CMT3 and CMT2 redundantly catalyze non-CG methylation (109). SU(VAR)3–9 HOMOLOG 4 (SUVH4)/KRYPTONITE KYP, SUVH5, and SUVH6 histone methyltransferases recognize methylated CHG through their SRA domains and mediate the deposition of H3K9 methylation (31). H3K9me2 marks are in turn bound by the chromodomain of CMT3/CMT2, which can catalyze DNA methylation, thereby creating a self-reinforcing loop of DNA and histone methylation (31). At these targeted sites, CHH methylation is mediated by CMT2 and maintained through a reinforcement loop between CHH methylation and H3K9me2 marks (31, 110, 125).

At the edges of TEs and at smaller TEs, which usually display less H3K9me2 and present gene chromatin features (125), CHG methylation is deposited mostly by CMT3, and CHH methylation by the PolIV-RdDM pathway as described during the establishment phase. Just like CMT2/3-mediated DNA methylation, RdDM-directed CHH methylation is stabilized by a self-reinforcing loop at two different levels: SAWADEE HOMEODOMAIN HOMOLOG 1 (SSH1) binds to H3K9me2 histone modifications, which are dependent on DNA methylation at these sites, and recruits PolIV (60), whereas the two catalytically inactive homologs SUVH2 and SUVH9 proteins bind directly to CHH methylation to recruit PolV (51).

DNA Demethylation in *Arabidopsis*

Arabidopsis encodes four paralogous DNA demethylases, namely REPRESSOR OF SILENCING 1 (ROS1), DEMETER (DME), DME-LIKE2 (DML2), and DML3, which can actively erase DNA methylation in all methylated cytosine (mC) contexts through a base excision repair mechanism (1, 85). The recruitment of ROS1 to its targets is mediated, at least in part, by the methyl CpG-binding domain (MBD) protein MBD7, which binds to highly methylated regions and recruits the histone acetyltransferase INCREASED DNA METHYLATION 1 (IDM1) to the methylated DNA. The resulting IDM1-dependent acetylation of histone H3 at lysines 18 and 23 (H3K18ac and H3K23ac) further facilitates ROS1 recruitment to these regions. Importantly, this process is essential to limit the spread of DNA methylation at TE boundaries (**Figure 1**) (58, 66, 67, 98, 99).

Active DNA demethylation contributes to a drastic reduction in DNA methylation in the vegetative nucleus (the male gamete companion cell) of mature pollen and in the central cell (the female gamete companion cell), giving rise to the demethylated endosperm (20, 39, 46). As for vegetative tissues, steady state expression levels of hundreds of loci are the result of the maintenance of methyl-marks and their persistent pruning by ROS1, DML2, and DML3 (41, 91, 97). Importantly, 80% of the regions targeted by ROS1/DML2/DML3 are located near annotated



genes or even overlap with their 5′ and 3′ ends (96, 97). Thus, a role in maintaining boundaries of DNA methylation targets was proposed for ROS1/DML2/DML3 (96, 97, 130). Given that regions close to the transcriptional start are more prone to demethylation by ROS1, it has also been suggested that this demethylase maintains the methylation at low levels within promoters, enabling potentially fast modulation of expression (97, 127, 130, 131), and this could be particularly relevant for the regulation of stress-responsive genes.

Interestingly, RdDM and demethylation are highly active at a TE embedded in the promoter of *ROS1*; this TE acts as a methylstat by sensing DNA methylation levels and tightly controls *ROS1* basal expression (64, 122). As a result, an increase in RdDM activity enhances *ROS1* expression, whereas a reduced DNA methylation level represses *ROS1* expression. The existence of such a sensitive regulatory and compensatory mechanism further points to a potential role of *ROS1* as a fine-tuner of stress-responsive gene expression.

Importantly, active DNA demethylation can be coordinated with passive demethylation, in particular in tissues in which *MET1* and *CMT3* are not expressed, such as the central cell (53). In leaves and seedlings, steady state levels of DNA methylation were shown to be, at some loci, the result of active DNA demethylation mediated by ROS1, DML2, and DML3 (97, 130). Similar to the central cell, one could imagine that this activity is also contributed by passive demethylation in tissues where *MET1*—and/or other factors involved in maintenance of DNA methylation—are expressed at low levels, such as in cotyledons (5) or during endoreduplication, which can notably occur in the context of host-parasite interactions (19, 23, 121).

Endoreduplication: phenomenon by which DNA replicates in the absence of cell division; frequent in tissues such as trichomes/epidermis

Transposable Elements as Epigenetic Modules of Regulation for Defense Gene Expression

TEs cannot be seen only as mobile deleterious mutagens, as they can also have a positive regulatory role, even as degenerated forms, on nearby gene expression (7, 68, 69). Several examples showing

Figure 1

(*a*) Establishment of DNA methylation. ① In PolV-RdDM (RNA-directed DNA methylation), RDR6 recognizes PolII-derived transposable element (TE) transcripts and the resulting double-stranded RNA (dsRNA) is processed by DCL2 and DCL4 into 22- and 21-nt small interfering RNAs (siRNAs), respectively. These siRNAs are loaded in AGO6, which directs DRM2 (and/or DRM1) and subsequent de novo methylation at TEs presumably in a PolV transcript–dependent manner. ② The resulting partially methylated locus is transcribed by PolIV and RDR2 uses this transcript to generate a dsRNA. This dsRNA is processed by DCL3 into 24-nt small RNAs that are subsequently loaded in AGO4 (and/or AGO6/9) to mediate and stabilize de novo methylation in all sequence contexts. (*b*) Maintenance of DNA methylation. At both long and short TEs, the VIM proteins recognize hemimethylated CGs and recruit MET1, which subsequently catalyzes methylation on the unmethylated strand during replication. CHG methylation is deposited by CMT3 upon binding to the H3K9me2 mark. As a reinforcement loop, SUVH proteins (KYP) bind to the methylated CHG to deposit H3K9 methylation. At long TEs, CMT2 catalyzes CHH methylation, whereas at short TEs or genes, CHH methylation is perpetuated by the RdDM pathway. H3K9me2 marks recruit PolIV via SHH1. A dsRNA is produced by RDR2 from a PolIV-dependent transcript and subsequently processed by DCL3 in 24-nt siRNAs. At short TEs, these siRNAs are loaded in AGO4 (and/or AGO6/9) and interact in a sequence-specific manner with a PolV transcript to recruit DRM2, which catalyzes CHH methylation. (*c*) ROS1-dependent active DNA demethylation. ROS1-dependent demethylation occurs at least in part at the boundaries of TEs to limit DNA methylation spreading. MBD7 binds to highly methylated, CG-dense regions physically associates IDM2 and IDM3. Both IDM2 and IDM3 interact directly with IDM1, a histone acetyltransferase that acetylates H3K18 and H3K23. These histone modifications provide a chromatin environment that is presumably favorable for the recruitment of ROS1, which in turn restricts DNA methylation spreading at TE boundaries through an active DNA demethylation process.

SA: salicylic acid

coexpression of TEs and genes have been reported, in particular for LTR elements (LTRs serve as promoters for the element they are associated with and are present at both extremities of the element) and derived solo LTRs. For example, the rice LTR retrotransposon "renovator" is present in the promoter region of the *Pit* resistance gene in specific rice cultivars. This insertion enables the expression of the rice blast resistance gene and, importantly, confers resistance to *Magnaporthe grisea* (44). The finding that TEs are targets of DNA methylation added another layer to their regulatory potential. In fact, as such silencing targets, they can have a negative impact on the expression of the nearby genes but can also act as epigenetic modules regulating gene expression. This is particularly relevant for immune-responsive genes, whose basal and biotic stress–induced expression need to be tightly regulated, as discussed below.

Impact of DNA Methylation on Plant Disease Resistance

Although a biological role of DNA methylation has been initially characterized in plant developmental processes and in resistance against DNA viruses by mediating TGS of viral genomes (13, 16, 38, 103, 118, 123), recent studies have provided evidence that this pathway also modulates immune responses against nonviral pathogens (2, 30, 71, 72, 124). Inactivation of the PolV-dependent pathway was, for instance, shown to enhance disease resistance against the hemibiotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto* DC3000) (124), which is controlled by the salicylic acid (SA) defense pathway. These phenotypes were associated with an earlier and enhanced, but not constitutive, induction of the SA-marker gene *pathogenesis-related 1* (*PR1*) in PolV mutants challenged with *Pto* DC3000 or treated with SA (124). Enhanced presence of active histone marks was also observed at the *PR1* promoter in PolV mutants, suggesting that the chromatin is prepared for a rapid and pervasive transcription in response to pathogens in these mutant backgrounds. This points to a role for PolV in repressing priming, a phenomenon sensitizing plant cells for the activation of immune responses (114, 116). Other studies have also reported a strong enhanced resistance toward *Pto* DC3000 in mutants impaired in the maintenance of CG (i.e., *met1* mutant), CG and CHH (i.e., *met1 nrdp2* mutant, where *nrpd2* is mutated for a shared subunit of PolV and PolIV), or CHG and CHH methylation (e.g., *drm1 drm2 cmt3* mutant, referred to as *ddc* mutant) (30, 124). Importantly, these phenotypes were not restricted to the control of *Pto* DC3000 multiplication, as an enhanced resistance to the obligate biotrophic pathogen *Hyaloperonospora arabidopsidis* isolate WACO9 was also observed in *ddc* and *drm1drm2kyp* (*ddk*) mutants (72, 73). Furthermore, a strong constitutive expression of *PR1* was detected in the *met1 nrpd2* mutant (30, 124), which was associated with the presence of cell death within and around leaf secondary veins. This result suggested that DNA methylation prevents vascular propagation of phytopathogens, which was experimentally validated by showing that the *ddc* and *met1 nrpd2* mutants exhibit less bacterial propagation in *Arabidopsis* leaf vasculature (124). Collectively, these results indicate that loss of DNA methylation pathways either primes—upon removal of CHH methylation—or constitutively derepresses—upon removal of CG, CG/CHH, or CG/CHG methylation—the SA-dependent defense response, thereby restricting the growth of biotrophic pathogens. This conclusion is congruent with early observations showing that the demethylating agent 5-azadeoxycytidine also enhances resistance against the biotrophic bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (4).

DNA methylation was also shown to suppress the development of crown gall tumors induced by the soilborne biotrophic pathogen *Agrobacterium tumefaciens* in *Arabidopsis* (40). Indeed, strongly enhanced *Agrobacterium*-mediated tumor growth was found in both *ddc* and *ago4* mutants, indicating that non-CG methylation likely prevents tumor growth formation. Interestingly, tumor growth restriction is unlikely due to the transcriptional gene silencing of integrated oncogenes,

as a low methylation level was detected at these sequences in wild-type tumors (40). Instead, growth restriction is more likely due to the induced methylation of plant genes that act as positive regulators of tumor growth formation.

The resistance of RdDM-defective mutants has also been assessed in response to necrotrophic fungi, which are controlled by the jasmonic acid (JA) defense pathway (71). Indeed, with the exception of *nrpd1* mutants, *nrpe1*, *nrpd2*, *rdr2*, *drd1*, *ago4-2*, and *drm1drm2* exhibited an increased susceptibility to *Botrytis cinerea*. All these mutants, including *nrpd1* mutants, were also more susceptible to *Plectosphaerella cucumerina* (71). Furthermore, these phenotypes were associated with a compromised induction of the JA-responsive marker gene *PDF1.2a* in *nrpe1* and *nrpd2* mutants (both defective for PolV activity), and to a smaller extent in the *nrpd1* mutant (defective for PolIV activity), infected with *P. cucumerina* (71). Altogether, these results indicate that the RdDM pathway positively regulates resistance against necrotrophic fungi presumably by promoting JA signaling during infection. This phenomenon could be due to the potentiation of SA signaling observed in RdDM-defective mutants (124), which likely inhibits the JA defense pathway through the classical antagonistic interaction between SA and JA signaling (14, 116).

Active DNA Demethylation Positively Regulates Resistance Against Biotrophic Pathogens

The role of the demethylase *ROS1* in disease resistance has been initially characterized during *Pto* DC3000 infection. Indeed, a loss-of-function mutation in *ROS1* enhances vascular spreading of a GFP (green fluorescent protein)-tagged *Pto* DC3000 strain in *Arabidopsis* leaf secondary veins (124). This indicates that *ROS1* restricts vascular propagation of *Pto* DC3000 in secondary veins, which is consistent with the intense expression of *ROS1* in these tissues (124). In addition, *PR1* induction was attenuated in *ros1* mutant plants treated with flg22, indicating that *ROS1* acts as a positive regulator of SA-dependent defense response during PTI (124).

The function of *Arabidopsis* active DNA demethylation in disease resistance was further characterized in response to *Fusarium oxysporum*, a devastating vascular hemibiotrophic fungal pathogen that infects a wide range of economically important crops. Interestingly, *Arabidopsis ros1dml2dml3* (*rdd*) triple mutants—but not the single *ros1*, *dml2*, and *dml3* mutants—exhibited enhanced disease susceptibility to *F. oxysporum* (62). Surprisingly, *nrpe1* and *ago4* mutants were also more susceptible to *F. oxysporum* (64, 122). These results also suggest that a subset of functionally relevant immune genes might be regulated in a similar way in *nrpe1*, *ago4*, and *rdd* mutants to control *F. oxysporum* infection.

Impact of DNA Methylation and Demethylation on the Expression of Defense Genes

DNA methylation and demethylation impact plant disease resistance, and this is likely due to the regulation of numerous immune-responsive genes. Depending on where DNA methylation is located (either in the promoter or in the introns of these genes), two different types of control were found to be exerted, which we describe in this section.

Regulation of immune-responsive genes containing transposable elements/repeats in their promoter regions. Consistent with a negative effect of DNA methylation on gene expression, six *Arabidopsis* disease resistance genes carrying repeats in their vicinity were found derepressed in unchallenged *met1 nrpd2* mutants (124). Among them, *RESISTANCE METHYLATED GENE 1* (*RMG1*) was strongly induced in wild-type plants treated with flg22 and exhibited high expression

levels in untreated *met1 nrpd2* mutant (124). Interestingly, *RMG1* contains two helitron-derived repeats in its promoter, a distal repeat that is strongly methylated in all mC contexts and a proximal repeat that is unmethylated in the wild type but hypermethylated in all mC contexts in *ros1* mutants. The hypermethylation detected in the *ros1* mutant is directed by RdDM and occurs at the border of the proximal repeat, as previously reported at canonical *ROS1* targets (130). The patch of hypermethylation detected in the *ros1* mutant likely prevents the transcriptional activation of *RMG1*, as its induction was severely impaired in *ros1* mutants elicited with flg22. Collectively, these results indicate that the transcriptional status of *RMG1* is controlled by a dual and antagonistic control: siRNA-directed DNA methylation represses its basal expression, likely to prevent the fitness cost, whereas active DNA demethylation facilitates its pathogen-triggered induction by constitutively pruning DNA methylation at the boundaries of its proximal repeat, which likely contains functional *cis*-regulatory elements (**Figure 2**). The latter regulatory mechanism might be further amplified by the downregulation of some TGS factors occurring during plant defense,

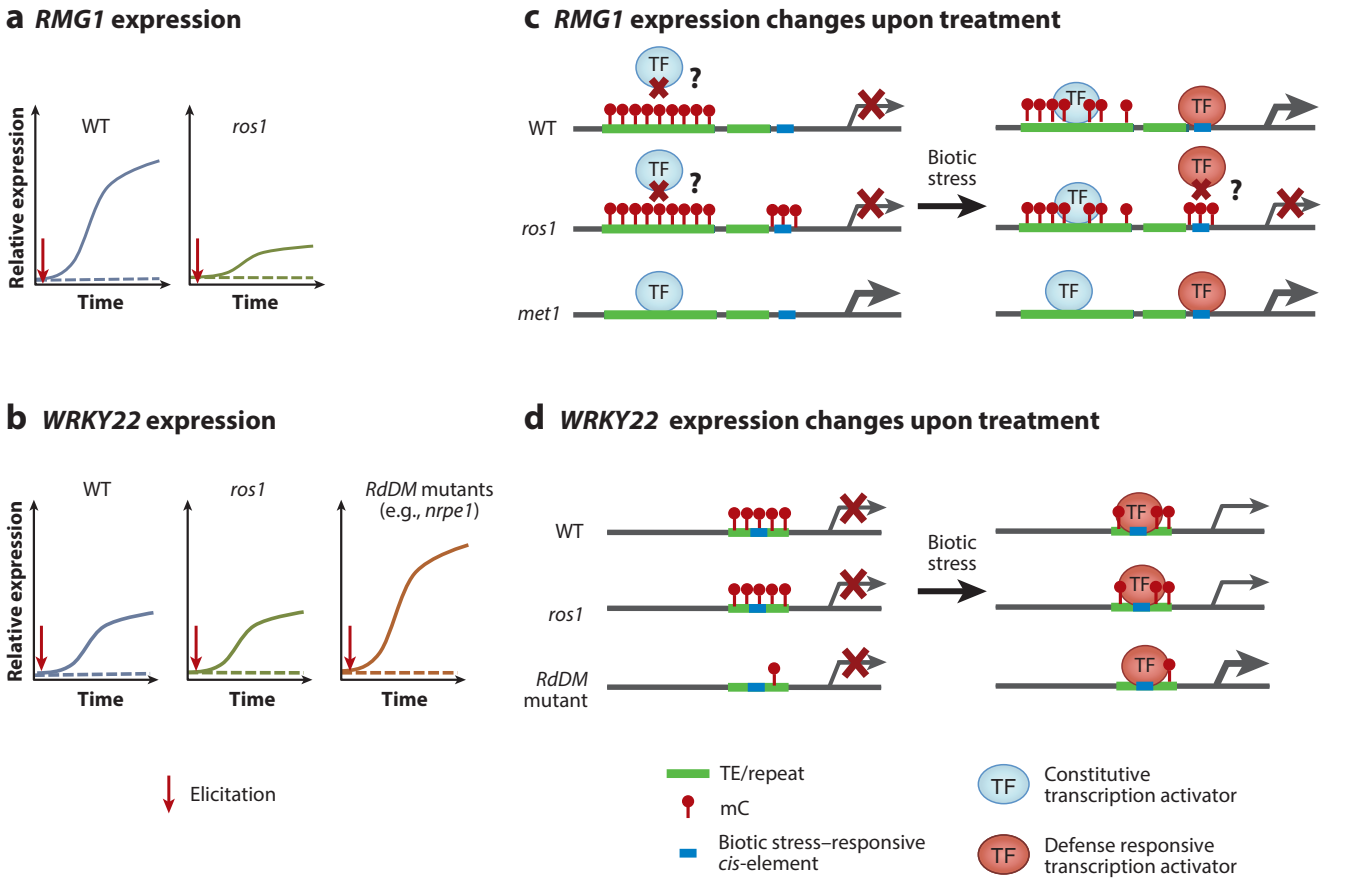


Figure 2

Role of DNA methylation and demethylation in the transcriptional control of *RMG1* and *WRKY22* in response to biotic stress. Graphical representation of (*a*) *RMG1* and (*b*) *WRKY22* relative expression levels over time in the indicated lines, at basal state (*dashed lines*) or upon elicitation with flg22 (*solid lines*). A red arrow represents the elicitation time. Schematic overview of (*c*) *RMG1* and (*d*) *WRKY22* methylation and expression changes upon flg22 treatment. It is noteworthy that the changes in *RMG1* and *WRKY22* expression observed in the mutants may not be due to DNA methylation changes in *cis* at the promoter, but instead (or in addition) may be the result of DNA methylation changes and expression changes at other loci. Abbreviations: mC, methylated cytosine; TE, transposable element; TF, transcription factor; WT, wild type.

as discussed below (124). Similar regulatory mechanisms were found at other defense genes that are induced in response to *F. oxysporum*. For instance, the disease-resistant gene *RPP7* contains repeats in its promoter region that are hypermethylated in *ros1dml2dml3* (*rdd*) mutants (62).

APA: alternative polyadenylation

For other immune-responsive genes that are targeted by siRNAs/DNA methylation, the lack of methylation observed in DNA methylation-defective mutants at these loci was not sufficient to alter their basal expression. This is, for instance, the case for the transcription factor *WRKY22*, which exhibits RdDM-dependent DNA methylation in its promoter region and whose transcript levels were unchanged in untreated DNA methylation–defective mutants (47, 124). However, a primed and sustained induction of *WRKY22* was detected in *nrpd2*, *nrpe1*, and *ddc* seedlings treated with flg22, supporting a role for DNA methylation in repressing the expression of this gene during elicitation (124). Therefore, by contrast with *RMG1*, which is constitutively derepressed in DNA methylation–defective mutants, transcription activators of *WRKY22* are likely not present or not active in unchallenged conditions (**Figure 2**). Induction of *WRKY22* would thus require the concomitant activation of its transcription activators as well as dynamic changes in chromatin marks (e.g., DNA demethylation) and/or the presence of active histone marks (71). In addition, induction of *WRKY22* was not affected in the *ros1* mutant treated with flg22, indicating that *ROS1*-dependent active demethylation is not required for its transcriptional activation.

Overall, these studies indicate that DNA methylation and/or demethylation control the basal and/or biotic stress–induced expression of some defense genes containing TEs/repeats in their promoter regions. However, despite the presence of known *cis*-elements in the promoter regions that are methylated or actively demethylated, there is still no experimental evidence indicating that DNA methylation/demethylation in plants could interfere with the accessibility of binding sites for transcription factors, as recently demonstrated in mouse embryonic stem cells (28).

Regulation of immune-responsive genes containing intronic transposable elements. Recent studies unraveled a role for DNA methylation in the transcriptional regulation of genes through the control of alternative polyadenylation (APA), a process by which transcripts containing more than one polyadenylation site lead to the production of transcript variants. This process has emerged as an important mechanism of gene regulation, and in some cases APA occurs through DNA methylation and/or histone modifications (26, 77).

The INCREASE IN BONSAI METHYLATION1 (IBM1) gene encoding a histone demethylase that protects CG body–methylated genes from heterochromatinization possesses an intronic DNA/H3K9 methylated non-TE sequence. Reduction of DNA methylation at this patch results in the global decrease of the IBM1 full-length transcript and the production of an alternative transcript that is inactive (24, 102). This mechanism is regulated by the chromatin-binding proteins IBM2/ANTISILENCING1 (ASI1)/SHOOTGROWTH1 (SG1) and ENHANCED DOWNY MILDEW 2 (EDM2) (22, 63, 105, 120).

IBM2 and EDM2 regulate IBM1 and three other targets through an APA mechanism. Importantly, these targets include the resistance gene *RPP7* (63, 105, 111, 120). *EDM2* encodes a nuclear-localized protein that positively regulates *RPP7* expression and disease resistance to *H. arabidopsidis* isolate Hiks1 (*Hp*Hiks1) (36, 111). *RPP7* contains a patch of methylation in a long intronic *COPIA-R7* TE element that is highly methylated and condensed (111) (**Figure 3**). In *edm2* and *ibm2*, the *RPP7* full-length transcript level was strongly decreased, whereas a short isoform called ECL (exon1-containing LTR-terminated transcript), which originated from the same promoter but stopped in the first intron at a cryptic polyadenylation site in the 5′ LTR of the *COPIA-R7*, accumulated (111) (**Figure 3**). Furthermore, a reduction in H3K9me2 in the *kyp suvh5 suvh6* triple mutants also reduces *RPP7* full-length transcript accumulation and resistance against *Hp*Hiks. This points to the importance of H3K9me2 and likely of DNA methylation in this

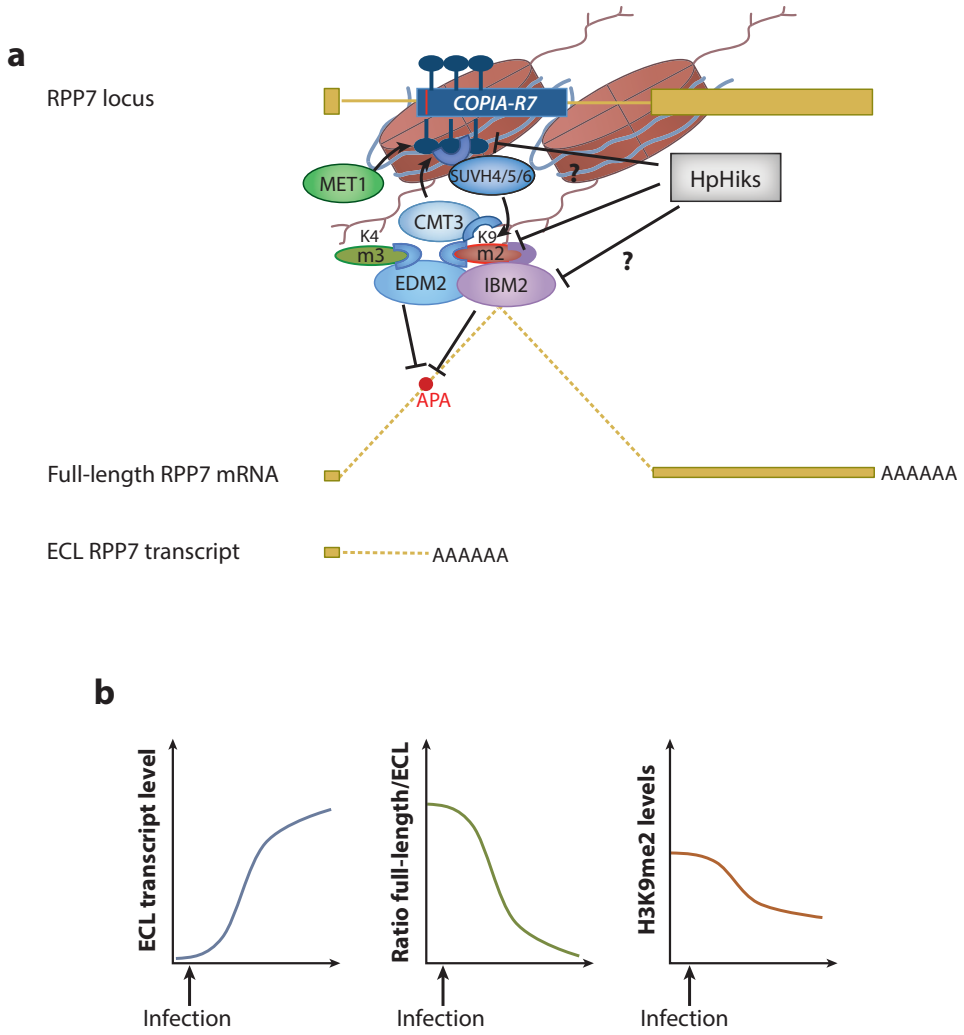


Figure 3

Regulation of alternative polyadenylation (APA in *RPP7* transcripts. (*a*) IBM2 and EDM2 are recruited to an intronic transposable element (TE) (Copia-R7; *blue box*), which is methylated (*lollipops*) and is located in the first intron of the *RPP7* gene. At COPIA-R7, MET1 and CMT3 catalyze the methylation of DNA and SUVH4/5/6 induces H3K9me2 marks. Both DNA methylation and H3K9me2 are required for the proper accumulation of RPP7 full-length transcripts. EDM2 binds to a combination of both active (H3K4me2) and repressive (H3K9m2) marks, which may confer its binding specificity to intragenic TEs and could also confer the binding specificity to IBM2. IBM2 presumably binds to H3K9me2 marks through its BAH (bromo-adjacent homology) domain and to the RPP7 transcript through its RRM (RNA recognition motif). The dashed lines represent the intronic sequence. The recruitment of IBM2 and EDM2 through the methylation patch results in the production of a full-length mRNA with distal polyadenylation. When DNA methylation and/or H3K9me2 levels are reduced, the recruitment of EDM2 and IBM2 is likely compromised, resulting in the co-transcription and accumulation of the ECL [exon 1-containing LTR (long terminal repeat)-terminated] transcript. The ECL transcript originates from the same promoter but stops in the first intron at a cryptic polyadenylation site in the 5′ LTR of Copia-R7. HpHiks infection negatively impacts H3K9me2 levels; however, the underlying mechanism remains elusive: HpHiks could either interfere with the maintenance of H3K9/DNA methylation directly or with EDM2 activity. (*b*) Infection by HpHiks concomitantly induces a reduction of H3K9me2 levels and an upregulation of ECL transcript levels. This changes the balances between the two RPP7 transcripts isoforms, one active and the other inactive, impacting the defense response.

regulation (111). Interestingly, EDM2 binds in vitro to a combination of both active and repressive marks, which might confer EDM2 binding specificity to intragenic TEs (112). Mutation in *IBM2* neither affects the methylation level of the large intron nor the expression of *RPP7*, suggesting that *IBM2* functions through a post-transcriptional mechanism. Thus, EDM2 and IBM2 allow proper transcription of the full-length *RPP7* transcript, possibly through inhibition of cryptic early polyadenylation signals or by affecting splicing machinery (63, 111, 120).

Dynamics of DNA Methylation Changes in Response to Biotic Stresses

A global DNA hypomethylation was initially reported in *Arabidopsis* one day postinoculation with a virulent *P. syringae* strain, and these effects were associated with demethylation of centromeric repeats and decondensation of heterochromatin (95). Significant insights into DNA methylation changes in response to bacteria were more recently gained by studying high-resolution genome-wide methylation changes in *Arabidopsis* in response to virulent and avirulent *Pto* DC3000 strains (30). This study first revealed a link between DNA methylation changes and transcriptional reprogramming at immune-responsive genes. In the case of *Pto* DC3000 infection, the differentially mCs (DmCs) were enriched at gene-rich regions and composed primarily of CG and CHH (30). Importantly, a strong enrichment of DmCHHs was observed at regions flanking protein-coding genes, which is reminiscent of targeting by active DNA demethylases. Furthermore, methylation at *Pto* DC3000–responsive DmCHHs did not occur in response to SA or to *Pto* DC3000 (*AvrPphB*), suggesting that dynamic regulation of CHH might be associated with bacterial-induced disease development (30).

Specific trends in DNA methylation changes were also observed in response to *Pto* DC3000 (*AvrPphB*) and to SA, which trigger a preponderance of hypermethylated and hypomethylated DmCs, respectively. For instance, megabase-scale hypermethylation and hypomethylation were observed at pericentromeric regions in response to *Pto* DC3000 (*AvrPphB*) and SA treatments, respectively (30). Furthermore, genes proximal to hypoDMRs (differentially methylated regions) exhibited the strongest differential transcript abundance compared with all genes, suggesting a potential role for demethylation in transcriptional activation. SA treatment was also found to trigger demethylation and transcriptional reactivation of TEs, and, in some instances, similarly altered expression was observed at nearby genes.

The dynamics of DNA methylation at specific immune-responsive genes and TEs was recently reported after flg22 treatment (124). The transcriptional activation of some TEs and defense-related genes was associated with a moderate CHH demethylation, and, for some of them, this process was dependent on the active demethylase ROS1 (124). Interestingly, a recent genome-wide DNA methylation profiling analysis conducted in human dendritic cells infected with *Mycobacterium tuberculosis* also provided evidence that bacterial challenge triggers a rapid and potentially active DNA demethylation process that facilitates the transcriptional activation of thousands of immune-responsive genes (93). Overall, these studies support a role for dynamic methylation changes in modulating the transcriptional status of TEs as well as defense genes that are linked to TEs/repeats.

Although gene body methylation occurs in expressed genes, very little is known about the dynamics and impact of these methylation patterns on gene expression. Gene body methylation changes were detected in response to biotic stresses and could be important for gene regulation (30). Indeed, a strong correlation was observed between gene body demethylation and increased transcript abundance. This phenomenon may contribute to the differential accumulation of alternative spliced transcripts as CHG acceptor site methylation was shown to repress splicing efficiency in maize (100). The induced accumulation of specific alternative spliced transcripts can be

important to trigger proper immune responses, as demonstrated at some functionally relevant disease resistance loci (42). It is also possible that a subset of these biotic stress–sensitive genes exhibit altered methylation at intronic TEs, thereby producing alternative polyadenylated transcripts. The dynamics and relevance of APA have been characterized at the *RPP7* locus, where the level of H3K9me2—and thus potentially of DNA methylation—progressively decreased in the course of *Hpa*Hiks infection. This decrease correlated with a progressive increase in ECL transcripts (**Figure 3**) (111). This mechanism likely allows the fine-tuning of *RPP7* regulation by ensuring solely a transient induction of *RPP7* functional transcripts during pathogen response, thereby preventing trade-off effects caused by a sustained accumulation of RPP7-coding transcripts. Future DNA methylation/H3K9me2 profiling in response to unrelated pathogens and elicitors will further unveil the extent to which this regulatory mechanism can control the expression of immune-responsive genes at the whole-genome level.

Dynamic Regulation of Transcriptional Gene Silencing Factors During Plant Immunity

Dynamic changes in the expression of TGS factors have been reported in *Arabidopsis* in response to biotic stresses. For instance, an increase in *MET1*, *DRM2*, *CMT3*, and *AGO4* transcript levels was detected in *Arabidopsis* crown gall tumors and was associated with a global hypermethylation (40). These results suggest that the enhanced expression of these epigenetic factors might be part of a plant-induced defense response to prevent *Agrobacterium*-induced tumor development. Alternatively, it could be caused by modulation of hormonal pathways (e.g., cytokinin) and enhanced cell division occurring during tumor formation.

By contrast, messenger RNAs for some factors involved in de novo methylation and maintenance of CHH (e.g., AGO4, PolIV, and PolV subunits) and of CG (e.g., MET1) methylation were downregulated in *Arabidopsis* adult leaves treated with flg22 (124). Interestingly, most of these factors were coregulated and showed a transient downregulation at 3 h and 6 h after flg22 treatment, which correlated with the upregulation of defense marker genes controlled by RdDM. Downregulation of RDR6 mRNAs was additionally detected in response to flg22, suggesting that the pre-establishment phase of RdDM might be transiently inactivated during PTI (8). Furthermore, the downregulation of *AGO4* and *Nuclear RNA Polymerase E5* (*NRPE5*) mRNAs was also accompanied by a decrease in AGO4 and NRPE5 protein levels, along with a moderate CHH demethylation at some RdDM targets such as the retroelement *AtSN1* (124). In addition, flg22 induces a decrease in *ROS1* mRNA levels that might occur as a consequence of the downregulation of RdDM activity, which is known to impact the feedback regulation on *ROS1* expression, as previously discussed. Therefore, these results suggest that flg22 might inhibit DNA methylation, at least in part, in tissues where TGS factors are expressed prior to elicitation (64, 67, 122). This phenomenon might occur in response to not only flg22 but also to an SA challenge, as downregulation of *MET1*, *DDM1*, and other RdDM factor genes is also observed in response to this phytohormone and may contribute to the SA-triggered megabase-scale hypomethylation mentioned above (30, 124).

WRKY: transcription factors that can bind to W-box *cis*-elements and activate or repress transcription of immune-responsive genes

Yu et al. (124) also took advantage of a transcriptional reporter line of *AGO4* to provide evidence that the flg22-triggered repression of *AGO4* occurs, at least in part, at the transcriptional level (43). Furthermore, a promoter analysis of coregulated and flg22-sensitive TGS factor genes revealed an over-representation of three *cis*-regulatory elements in their promoter regions. Among these regulatory elements, the W-box motif, which is the binding site for WRKY transcription factors,

was identified (104). Therefore, some WRKY proteins may be responsible for the flg22-induced transcriptional shutdown of TGS factors, but further investigation is needed to test this hypothesis.

Cambium: tissue located between xylem and phloem and formed by unspecialized, actively dividing cells

Can DNA Methylation and Demethylation Orchestrate Short and Long Distance Immune Responses, Transgenerational Immune Priming, de novo Epiallelism, and Genetic Changes?

It is clear at present that DNA methylation plays a role in the plant biotic stress response and that transiently induced DNA methylation changes contribute to its short-term plasticity. However, its contribution to middle- and long-term adaptation to the environment, by mediating, for instance, stable genome modifications, remains to be elucidated. This question deserves attention because plant germ cells are produced, contrary to the way germline cells are produced in mammals, towards the end of the plant development in the floral organs, which theoretically could allow the epigenetic changes occurring during the vegetative growth to be inherited in the progeny. In addition, mitotic inheritance could allow some more middle-term adaptation throughout the lifetime of the plant and in newly developing tissues. In this section, we discuss the possibility that DNA methylation and small RNAs, through their respective properties (heritability and mobility), could contribute to these changes in addition to modulating active immune responses in specific tissues, the plant leaf vasculature and the reproductive tissues, to control the spreading of pathogens.

Can DNA methylation and demethylation control immunity around leaf vasculature?

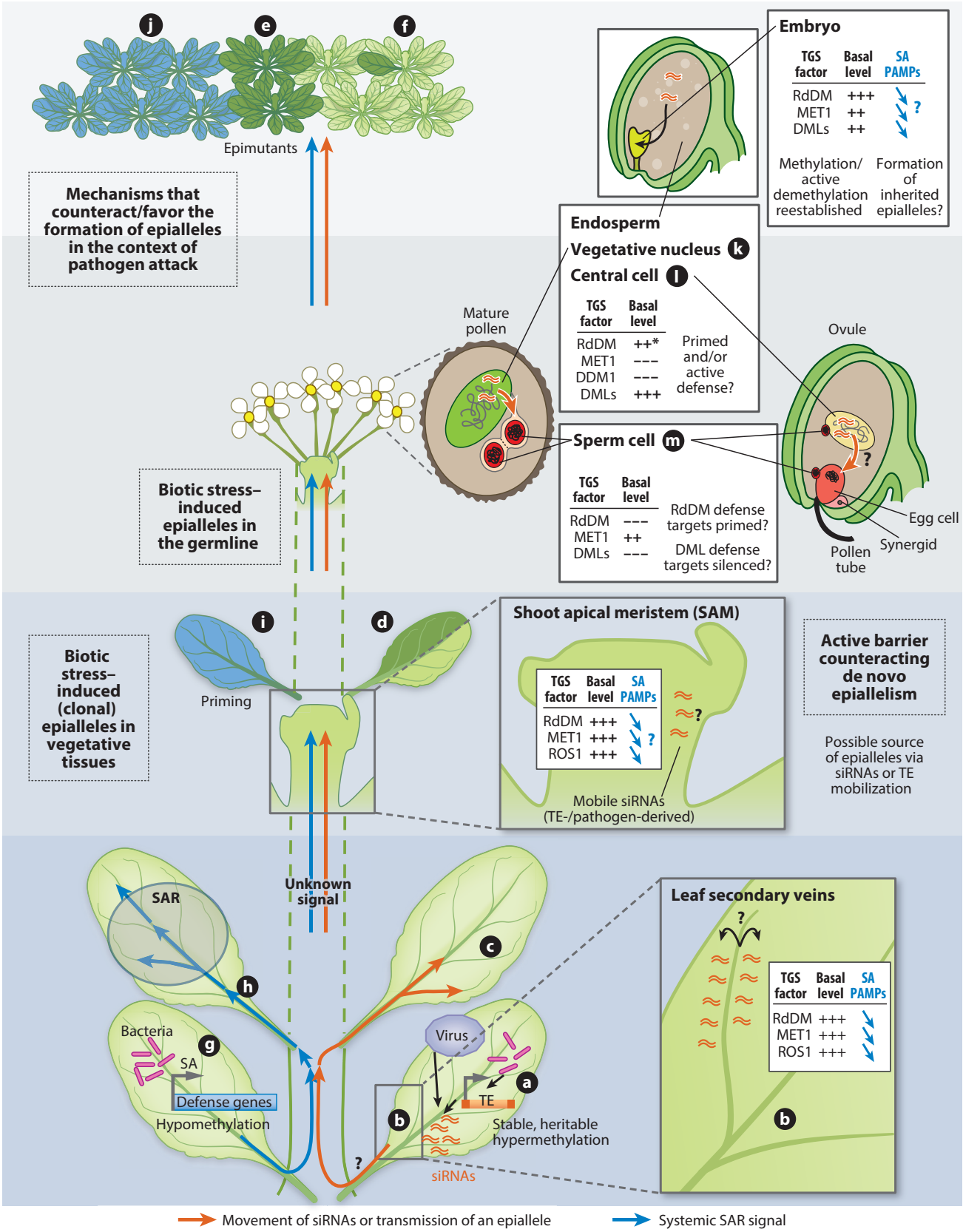
DNA methylation profiling studies conducted in response to biotic stresses revealed only mild methylation changes when whole *Arabidopsis* leaf samples were used; this may suggest that DNA methylation changes occur in specific tissues and/or cell types (30, 124). Consistently, a transgenic line reporting the transcriptional activity of the gypsy retrotransposon AtGP1, which is targeted by RdDM, revealed a flg22-induced reactivation within and around *Arabidopsis* leaf vasculature (124). This phenomenon was pronounced at the level of secondary veins and at the base of midveins, which represent the only tissues in which cells are still dividing at late stages of leaf development and where proper maintenance of DNA methylation is thus critical. Accordingly, factors involved in the establishment and maintenance of DNA methylation are well expressed in leaf vasculature, including the vascular cambium (3, 55). Therefore, the flg22-induced downregulation of some of these factors (30, 124) may explain the transcriptional reactivation of AtGP1 observed in vascular tissues, which could further be facilitated by a concomitant ROS1-dependent active demethylation (Note that it cannot be excluded that the flg22-induced transcriptional reactivation of AtGP1 could also be facilitated by stress-responsive *cis*-elements within its LTR regions) (3, 124). More generally, the decrease in RdDM activity occurring during plant defense could contribute to the derepression and/or poised induction of defense-related RdDM targets (e.g., *RMG1*) within and around the vasculature, thereby ensuring a strong antimicrobial immunity in these tissues. Furthermore, it is possible that the reactivation of some TEs in the vasculature leads to the production of *trans*-active mobile siRNAs that would have the potential to silence modulators of plant defense in cells that surround sites of TE reactivation. The idea of such short-distance non-cell-autonomous silencing effect is supported by previous findings showing that (*a*) 21-nt siRNAs can move from cell-to-cell to direct non-cell-autonomous PTGS (34), (*b*) the transcriptional reactivation of TEs produces 21-nt mobile siRNAs that trigger PTGS in neighboring cells (76, 108), (*c*) the transcriptional reactivation of an Athila TE produces a 21-nt siRNA that can regulate a stress-responsive gene in *trans* (81), and (*d*) an enhanced accumulation of TE-derived 21-nt siRNAs occurs in response to SA (30). This putative non-cell-autonomous regulatory mechanism

could thus ensure the formation of an immune cell layer around the vasculature. This would limit pathogen spreading from xylem vessels to mesophyll cells and vice versa (**Figure 4**).

Can DNA methylation and demethylation protect gametes and embryos from pathogen infection? Several studies have provided evidence for TE demethylation and derepression in the companion cell of the male gamete, the pollen vegetative cell (VN) (15, 46, 108). These phenomena occur through concomitant passive and active demethylation processes. Importantly, TE derepression results in the production of mobile siRNAs that can resilence TEs in pollen sperm cells, and/or during/after fertilization, as discussed below (15, 46, 53, 76, 108). A similar mechanism was also proposed in the central cell, the companion cell of the female gamete, which produces TE-derived siRNAs that might mediate TE resilencing in the egg cell (**Figure 4**) (46). The

Figure 4

Hypothetical mechanisms by which biotic stress could contribute to transgenerational immune priming and epigenetic reprogramming during gametogenesis and embryogenesis, and how plants could preserve the integrity of gametes and of the embryo during infection. (*a*) Small interfering RNAs (siRNAs) can be produced in response to biotic stresses, such as bacteria (*pink ellipses*), or salicylic acid (SA), which can induce the differential expression of transposable elements (TEs) in *Arabidopsis* leaves, in part through the downregulation of transcriptional gene silencing (TGS) factors. Such transcriptional reactivation of TEs can trigger the production of RDR6-dependent TE-derived siRNAs. Alternatively, siRNAs can be produced from exogenous pathogens/parasites such as viruses through virus-induced gene silencing (VIGS). (*b*) Once produced, these biotic stress–induced siRNAs have the potential to trigger silencing in a non-cell-autonomous manner either in cells that surround the site of primary siRNA production (e.g., cells nearby leaf secondary veins) or (*c*) in distal tissues by long-distance transport and movement of siRNAs through the phloem up to the shoot apical meristem (SAM) or to germline tissues. (*d*) This phenomenon may lead to the formation of clonal somatic epialleles produced from meristematic tissues (SAM, the vascular cambium, or floral meristems). An example of such a putative epiallele initially produced in the SAM and revealed in a newly emerging leaf after cell division is depicted in dark green. (*e*,*f*) siRNA-directed methylation of such epialleles in the SAM may be amplified during reproduction and transgenerationally inherited, leading to fully penetrant and stable epialleles. Other siRNA-directed epialleles could be generated later (e.g., during reproduction) and transgenerationally inherited with a lower penetrance, as observed in the progeny of plants subjected to *FWA* promoter VIGS or, alternatively, with a fully penetrant phenotype possibly depending on the context of the methylated cytosines. In addition, stress-induced mobilization of TEs could lead to de novo epiallelism. (*g*) In parallel, plant defense genes controlled by TEs can be expressed following TGS release in response to bacteria/SA at the infection site. (*h*,*i*) Persistent SAR (systemic acquired resistance) signals could mediate TGS release in distal tissues that perceive the signal, such as in systemic leaves, and similarly contribute to inherited epigenetic changes (*blue leaves*) in the SAM, emerging leaves, and germline tissues, leading to priming of the defense response. (*j*) This signal of an unknown nature could also inhibit the reestablishment of DNA methylation and active demethylation processes occurring during embryogenesis (*see blue arrows in the SAM and embryo*). On the one hand, such a release of epigenetic reprogramming during embryogenesis could favor the formation of the transgenerationally inherited epialleles described in panels *e* and *f*. On the other hand, the persistence of the signal could result in maintained repression of TGS factors, leading to a more homogeneous effect than the variegated effects caused by specific siRNA-induced epialleles. (*k*,*l*,*m*) Besides these biotic stress–induced transgenerational effects, constitutive epigenetic reprogramming in reproductive tissues might also contribute to disease resistance, thereby preventing potentially vertical transmission of pathogens. For instance, the low expression of *DDM1* and *MET1* in the pollen vegetative nucleus and in the central cell, together with the high expression of *DML*s (Demeter-like DNA demethylases) might trigger a primed or constitutive defense in these gamete companion cells by elevating the expression of some defense-related methylated targets or by facilitating their rapid induction in the presence of pathogens. Similarly, the strong expression of *DML*s and the concomitant transient downregulation of RdDM factors in the endosperm during early embryo development (*RdDM factors are well expressed in the central cell and vegetative nucleus, as illustrated in the figure, but transiently repressed in the endosperm during early embryonic development) might contribute to a primed defense response in these tissues to protect the embryo against pathogen infections. At the same time, the lack of *DML* expression in sperm cells triggers a robust silencing of plant defense genes that are controlled by DMLs and thus potentially preserves the integrity of the male gamete against constitutive defense responses that could have important costs on fertility. Furthermore, the genome-wide decrease in CHH methylation and the low expression of some RdDM factors in sperm cells might additionally ensure a primed activation of defense genes that are specifically controlled by RdDM (e.g., *WRKY22*) during plant defense responses. Abbreviation: PAMPs, pathogen-associated molecular patterns.



endosperm, which is the fertilization product of the central cell, also exhibits global hypomethylation in comparison to the embryo (45, 46, 108). Furthermore, low expression levels of *MET1* and RdDM factors were detected, as well as the concomitant derepression of TEs and imprinted genes, in the endosperm, during an early phase of embryo development (6, 53, 117). Therefore, derepression of TEs in the endosperm may also result in the production of TE-derived siRNAs that could similarly resilence TEs in the embryo; this scenario is supported by the detection of CHH hypermethylation at endosperm-demethylated TEs in the rice embryo (126). Similarly, a subset of immune-responsive genes that carry TEs/repeats in their promoter regions could well be demethylated and therefore exhibit constitutive and/or poised expression in the gamete companion cell as well as in the endosperm. This would crucially protect the gametes and the embryo from pathogen infection (**Figure 4**). In addition, the production of mobile TE-derived siRNAs, which result from TE reactivation in the gamete companion cells and in the endosperm, could also mediate de novo methylation and/or PTGS of defense genes in germ cells: This would thus lower their basal expression, thereby optimizing the fitness of the gametes. Silencing of these immune-responsive genes might be even further reinforced in sperm cells, where *DME* and its paralogs are not expressed, thereby leading to siRNA-directed hypermethylation of *DML* targets (15).

SAR: systemic acquired resistance

During embryogenesis, the reestablishment of DNA methylation at TEs/imprinted genes was reported, and likely occurs during and/or after fertilization, when *DRM2*, *MET1*, and *CMT3* are highly expressed (53). This phenomenon may also involve maternal 24-nt siRNAs produced from the endosperm and seed coat or paternally inherited 24-nt siRNAs produced from the VN cell (15, 87). In addition, a restoration of active DNA demethylation is achieved in the embryo: It presumably directs demethylation of incoming TEs and imprinted genes that displayed CG hypermethylation in the sperm cells as a result of the lack of *DML* expression in these cell types (15). Therefore, as for imprinted genes, a subset of immune-responsive genes might progressively regain a dual and antagonistic regulatory mechanism during embryogenesis, which is mediated by a concomitant DNA methylation and active demethylation processes, as proposed for the leaf vasculature (124). These genes might thus be poised for expression in the embryo and rapidly transcribed in the presence of seedborne pathogens or upon perception of a systemic defense signal (30, 124). These speculative active defense mechanisms would be well adapted not only to protect gametes and embryo against pathogen infections but also to limit vertical transmission of pathogens, which is a critical step in the epidemiology of plant disease and in microorganism ecology, as it ensures pathogen survival and dispersal (79).

Can DNA methylation and demethylation control transgenerational immune priming and de novo epiallelism? Systemic acquired resistance (SAR) is an inducible broad-spectrum immune response in plants that prevents infection in distal tissues through a priming mechanism (35). Interestingly, this response also occurs in the progeny of *Arabidopsis* plants challenged with bacterial and viral pathogens (57). For example, the progeny of plants treated with either a *Pto* DC3000 (*AvrRpt2*) or the chemical SAR inducer β-amino-butyric acid (BABA) displayed enhanced resistance to *Pto* DC3000 compared to the progeny of mock-treated plants, and this phenotype was associated with primed induction of SA-dependent marker genes (107). In another study, the progeny of plants repetitively challenged with *Pto* DC3000 exhibited a primed induction of SA marker genes and these effects were associated with increased resistance to *Pto* DC3000 and *H. arabidopsidis* compared to the progeny of mock-treated plants (72). Although a maternal effect and the accumulation of secondary metabolites and proteins in the seeds of stressed parental plants cannot be excluded, the maintenance of priming over one stress-free generation suggests that these effects might be recorded in the form of DNA methylation and/or other epigenetic modifications that would be transmitted via the gametes. The fact that

transgenerational SAR was abolished in mutants defective in CHG methylation (i.e., *kyp* mutants) and in de novo methylation/maintenance of CHH methylation argues for this hypothesis (72, 73). However, these results should be cautiously interpreted, as mutations in these epigenetic factors modulate immune responses in infected/elicited tissues (mentioned above). Therefore, specific inactivation of these factors in the gametes would be a better approach to assess the relevance of DNA methylation/demethylation in pathogen-induced transgenerational effects.

Epialleles: versions of a gene generated by an epimutation

epiRILs: epigenetic recombinant inbred lines

At this stage, we envisage two possible mechanisms that could contribute to transgenerational immune priming. The first possible mechanism involves biotic stress–induced production of small RNAs, which could notably occur as a result of TE reactivation. This would trigger the accumulation, in challenged tissues, of mobile siRNAs that could be transmitted through the phloem to germ cells and thus passed to the next generation to modulate the expression of defense genes sharing sequence homologies with the transgenerationally inherited siRNAs (**Figure 4**). The idea of such siRNA-directed long-distance non-cell-autonomous silencing effect is supported by previous findings showing that *Arabidopsis* 24-nt siRNAs can move from shoot to root to mediate DNA methylation in the recipient cells (65, 83, 86).

The second possible mechanism, which is not mutually exclusive with the first, is through persistent systemic SAR signals (e.g., SA) that would further release TGS in distal germline tissues (receptor tissues of the SAR signal). This would lead to not only the production of TE-derived mobile siRNAs but also the derepression/primed induction of defense-related RdDM targets. Such a scenario would be particularly relevant in the female germ cells and in the embryo, where an active DNA demethylation process is reestablished and a gradual remethylation occurs during or after fertilization (15, 53). Therefore, persistent systemic SAR signals releasing epigenetic reprogramming during gametogenesis and embryogenesis might cause, or have significant consequences on, the production of epialleles in subsequent generations.

It is important to note that the same two mechanisms could similarly contribute to SAR throughout plant development, such as in systemic leaves, by targeting the shoot apical meristem (SAM), which gives rise to newly emerging leaves, or the systemic leaves directly.

The detailed mechanisms involved in biotic stress–induced transgenerational changes remain to be identified and characterized. A first step toward the understanding of such mechanisms is to identify biotic stress–induced DNA methylation changes at the level of the SAM and determine whether those changes could be inherited through division and further maintained throughout differentiation in somatic and reproductive tissues, and, subsequently, in the progenies of stressed plants. It should be emphasized that the putative DNA methylation changes would have to impact defense gene transcription, or induce a primed state at these loci, to be relevant in defense. Identification of such relevant epialleles could also be achieved through the use of natural accessions of *Arabidopsis* by identifying epigenetic quantitative trait loci (epiQTLs) for disease resistance or SAR, or through the use of epigenetic recombinant inbred lines (epiRILs), in which variation is attributed to DNA methylation changes but not to genetic changes (50, 101). The latter approach has already been used to show that heritable variation in DNA methylation can cause significant variation in plant response to SA, JA, and pathogen treatments (59, 101), and the causal epialleles could be further mapped in the future. A second step would be to dissect the mechanisms by which siRNAs direct transgenerational epigenetic changes at meristematic tissues in the context of SAR. In that respect, understanding the buffering regulatory mechanisms by which plants counteract transgenerational inheritance of epigenetic states is particularly relevant. Such mechanisms exist and likely represent a major barrier for stress-induced heritable changes (49) and for the mobilization of TEs over generations, as observed in the context of the heat-stress response (17, 47). The use of genome-wide associations in *Arabidopsis* natural accessions could also be instrumental

in identifying epigenetic factors that modulate these epigenetic reprogramming processes, which are likely released or reinforced in accessions subjected to different pathogen constraints. This approach notably allowed the identification of *CMT2* as an important adaptive factor of temperature seasonality that inhibits tolerance to heat stress and positively regulates tolerance to *Pseudomonas viridiflava* (33, 106).

Can DNA methylation and demethylation impact expression of defense genes by directing genetic changes? A first mechanism by which DNA demethylation could direct genetic changes with a consequential impact on gene expression would be by facilitating TE mobilization. The newly inserted copies could generate novel gene regulatory networks by inserting in the vicinity of protein-coding genes and modulating their expression. For instance, the stress-sensitive LTR retrotransposon *Onsen* provides functional heat stress–responsive regulatory elements to two genes upon its transposition nearby (47). Furthermore, it was recently shown that an insertion of *Onsen* into an abscisic acid (ABA)-responsive gene created a mutant allele of this gene that could be inherited and conferred salt tolerance, showing that even the mutagenic effect of a TE could potentially be adaptive (48). Finally, epigenetically induced mobilization of the retrotransposon *EVD* results in intense transcription of the TE (and its neocopies), which triggers siRNA production. These siRNAs target TGS at both *EVD* and an *EVD*-derived solo LTR located in the promoter region of the disease resistance gene *RECOGNITION OF PERONOSPORA PARASITICA 4* (*RPP4*) (73). Although transcriptional reactivation of *Onsen*, *EVD*, and other TEs has been reported in response to biotic stresses (30, 124), there is so far no evidence indicating that these elements could transpose during plant immunity.

Another mechanism by which DNA methylation could induce genetic changes, with a possible consequential impact on gene expression, is through deamination of mC, which often gives rise to thymine in different organisms, including *Arabidopsis* (92). Indeed, this phenomenon might contribute to the appearance of de novo *cis*-regulatory elements in the methylated regions of plant defense gene promoters and thus potentially enhance their responsiveness to pathogens. In addition, given that some components of the *Arabidopsis* homologous recombination machinery are recruited to defense gene promoters during SAR and that *Tobacco mosaic virus* not only enhances homologous recombination frequency in tobacco-infected plants but also induces genetic changes at hypomethylated defense-related genes (12, 57, 119), it is possible that biotic stress–induced DNA methylation changes, occurring within or near promoter-derived repeats, contribute to genetic variation at defense gene promoters during evolution, thereby modulating their capacity to respond to pathogens. Future investigations will be required to assess the role of such epigenetically directed genetic changes on the evolution of plant genomes and on the generation of expression variants under pathogen constraints.

SUMMARY POINTS

1. TEs and TE remnants provide epigenetic modules that can fine-tune defense gene expression.
2. DNA methylation impacts plant disease resistance: DNA methylation–defective mutants are more resistant to some biotrophs and more susceptible to some necrotrophs. Active DNA demethylation positively regulates antimicrobial disease resistance, particularly at the level of leaf vasculature.

3. DNA methylation and active DNA demethylation processes act antagonistically on the transcriptional control of some immune-responsive genes. This regulatory mechanism likely ensures a low basal expression of immune-responsive genes in the absence of pathogens (limiting trade-off effects) and a rapid and pervasive induction of these genes upon pathogen detection.
4. DNA methylation changes are dynamically controlled during biotic stresses and may contribute to expression changes at TEs or defense-related genes associated with TEs/repeats.
5. Some functionally relevant TGS factors are downregulated during PTI and SA-dependent defense response, and this phenomenon is associated with the derepression of some RdDM targets.
6. *RPP7* is regulated by APA at an intronic, methylated TE.
7. The moderate DNA methylation changes observed in response to biotic stresses might be due to tissue-specific DNA methylation changes, for example, at the level of leaf vasculature. The intense expression of RdDM factor genes and of *ROS1* in the leaf vasculature prior to elicitation/infection might contribute to this phenomenon.
8. A memory of pathogen stress has been observed across generations, but it is not known whether this phenomenon is recorded in the form of DNA methylation or other heritable modifications of the chromatin.

FUTURE ISSUES

1. What are the dynamics of biotic stress–induced DNA methylation changes in tissues/cell types in which TGS factors are expressed prior to elicitation/infection?
2. Do DNA methylation and demethylation interfere with the chromatin accessibility of binding sites for transcription activators during plant immunity?
3. What is the extent to which intronic TEs control defense gene expression?
4. Do pathogens or their mimics trigger mobilization of TEs in specific tissues/cell types?
5. Do biotic stresses induce epialleles in the shoot apical meristem and in subsequent generations? If so, which of those could be adaptive?
6. What are the mechanisms by which plants counteract/favor siRNA-directed transgenerational inheritance of epigenetic states during plant immunity?
7. Do epigenetic regulatory processes occurring during gametogenesis and embryogenesis protect gametes and embryos from pathogen infections? Do they also control vertical transmission of pathogens?
8. Do DNA methylation/demethylation factors represent adaptive factors of plant pathogen response in nature by modulating epigenetic and epigenetic-directed genetic changes at immune-responsive genes?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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NOTE ADDED IN PROOF

Reference 89a was published during the proof stage of the present review and provides some experimental evidence that some transcription factors in *Arabidopsis* can be methylation sensitive.

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124. DNA methylation and active demethylation antagonistically control immune-responsive gene expression and orchestrate antibacterial defense in *Arabidopsis*.

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Errata

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