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Epigenetic Mechanisms in Nematode–Plant Interactions

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Abstract

Epigenetic mechanisms play fundamental roles in regulating numerous biological processes in various developmental and environmental contexts. Three highly interconnected epigenetic control mechanisms, including small noncoding RNAs, DNA methylation, and histone modifications, contribute to the establishment of plant epigenetic profiles. During the past decade, a growing body of experimental work has revealed the intricate, diverse, and dynamic roles that epigenetic modifications play in plant–nematode interactions. In this review, I summarize recent progress regarding the functions of small RNAs in mediating plant responses to infection by cyst and root-knot nematodes, with a focus on the functions of microRNAs. I also recapitulate recent advances in genome-wide DNA methylation analysis and discuss how cyst nematodes induce extensive and dynamic changes in the plant methylome that impact the transcriptional activity of genes and transposable elements. Finally, the potential role of nematode effector proteins in triggering such epigenome changes is discussed.

INTRODUCTION

Plant-parasitic nematodes are widely distributed obligate parasites that impose serious problems in agricultural production worldwide. Cyst and root-knot nematodes are the most economically important species (36). These root parasites initiate and maintain complex relationships with host plants that result in transforming terminally differentiated root cells into permanent feeding sites. Feeding sites formed by cyst nematodes (syncytia) and root-knot nematodes (giant-cells) are relatively different and reflect the evolutionary differences between nematode genera (2, 93). Functional syncytia and giant-cells are vital for nematode feeding and development and completion of the life cycle (**Figure 1**). Feeding-site formation is mediated through a suite of nematode effector proteins, which are expressed specifically in the esophageal gland cells and injected into plant root cells via a needle-like apparatus called the stylet. Once secreted into root cells, nematode effectors alter numerous host cellular processes, synchronously reflected by alterations in the expression of a large number of genes (24, 26, 66). Although the mechanisms underlying gene expression alterations associated with feeding-site differentiation and formation are partially understood, epigenetic mechanisms have been shown to play important roles (27). This is consistent with the demonstrated role of various epigenetic modifications in establishing cell-fate specification and organ identity (35, 67).

Epigenetic control of gene expression associates with various regulators, including noncoding small RNAs, DNA methylation, and histone modifications, which coordinately contribute to gene regulation. Plant small RNAs are typically 21–24-nucleotide (nt) RNA molecules generated from double-stranded RNAs (dsRNAs) through the action of DICER-LIKE proteins (DCLs) (39, 97). Small RNAs can be classified into two main classes that include the 21-nt microRNAs (miRNAs) and the 21/24-nt epigenetically active small RNAs (88). miRNAs are involved in post-transcriptional gene silencing (TGS) through base pairing with their target genes that contain perfect or near-perfect complementary sequences. miRNA target-gene binding triggers mRNA degradation or translational repression (3). The 21/24-nt-long epigenetically active small RNAs are involved in TGS of genes and transposable elements (TEs) by establishing DNA methylation through the RNA-directed DNA methylation (RdDM) pathway (61, 88).

DNA methylation is a common epigenetic mark that is highly stable and heritable. In plants, DNA methylation exists in the CG, CHG, and CHH sequence contexts, which are established by different enzymes. DNA METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLASE 3 (CMT3) are responsible for maintaining methylation status at CG and CHG sites, respectively (9, 45). Contrary to CG and CHG methylation, which are maintained during DNA replication, CHH methylation is carried out de novo during each cell cycle (9, 45). DOMAINS REARRANGED METHYLTRANSFERASE2 (DRM2) in conjunction with the RdDM pathway is required for establishing de novo DNA methylation in all three sequence contexts (7–9). In this process, DRM2 is directed to specific DNA loci, mainly through the canonical RdDM pathway, where the 24-nt siRNA molecules are loaded into a protein complex containing ARGONAUTE4 (AGO4) and AGO6 that guides DRM2 to target sequences to generate DNA methylation de novo in the CG, CHG, or CHH sequence contexts. A noncanonical RdDM pathway that involves the 21–22-nt siRNAs and AGO2 can also guide DRM2 to establish DNA methylation but at a relatively low level (61). Although cytosine methylation is a metastable epigenetic mark, it can be actively erased through the activity of a specific subfamily of DNA glycosylases or passively lost after DNA replication (102).

Another epigenetic mechanism of gene expression control includes post-translational histone modifications. Histone proteins are the subject of a multitude of biochemical changes, particularly methylation and acetylation, which repeatedly occur at lysine (K) residues. These modifications

Syncytium: a multinucleated feeding structure formed by cyst nematodes through successive cell-to-cell fusion events that involve up to two hundred cells

Giant-cells: feeding cells formed by root-knot nematodes that are reprogrammed to enlarge several hundred times through repeated mitosis without cell division

Nematode effector: nematode protein secreted into host cells to enable nematode parasitism

Epigenetic modifications: the biochemical modifications of DNA and attached proteins but without changes in the underlying DNA nucleotide sequences

DNA methylation: the addition of a methyl group (CH_3) to cytosine bases at the C5 position to form 5-methylcytosines

miRNAs: miRNAs are typically 21-nt noncoding RNA molecules that post-transcriptionally downregulate their target genes, which contain perfect or near-perfect complementary sequences

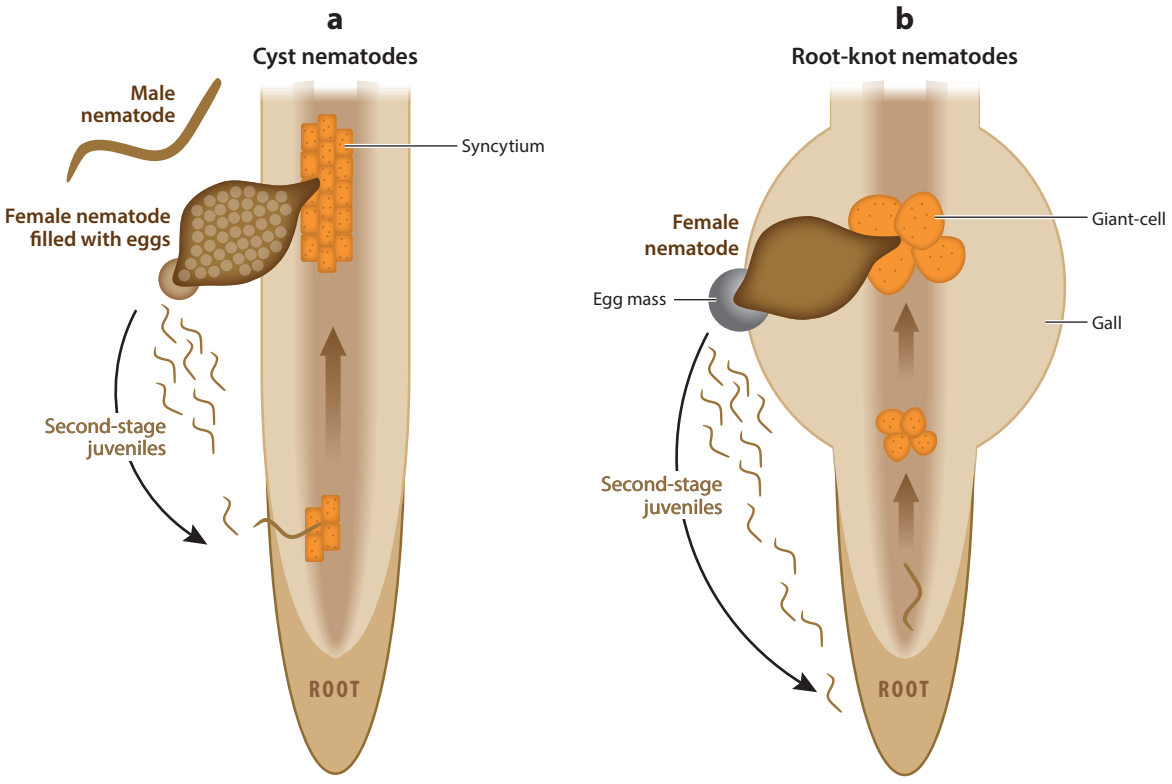


Figure 1

Life cycles of cyst and root-knot nematodes. (*a*) Life cycle of cyst nematodes. After hatching from eggs, the second-stage juveniles (J2s) of cyst nematodes penetrate plant roots and migrate intracellularly and then became sedentary. Sedentary infective J2s select compatible vascular cells and induce cell-to-cell fusion events in more than a hundred cells to form one large multinucleated syncytium, which is used as a permanent feeding site. Infective J2 nematodes feed from the syncytium and undergo three molts to mature into adult females or males. The males became motile and move toward the adult females and fertilize them. Adult females transform into cyst-like structures filled with hundreds of eggs, which hatch in suitable conditions. (*b*) Life cycle of root-knot nematodes. After hatching, infective juveniles of root-knot nematodes penetrate plant roots at the elongation zone, migrate intercellularly, and then select between typically three and seven compatible cells in the vascular cylinder to induce the formation of giant-cells once they became sedentary. Sedentary infective J2s stimulate the selected cells to enlarge several hundred times through repeated mitosis without cytokinesis to form giant-cells. Giant-cell formation also stimulates surrounding cells to divide and increase in size, leading to the formation of knot-like structures called galls at the site of infection. Similar to cyst nematodes, the infective root-knot nematode juveniles feed from the giant-cells and undergo three molts to develop into adult females or males. The female lays hundreds of eggs in a gelatinous matrix. Unlike cyst nematodes, which reproduce through outcrossing, most economically important root-knot nematodes reproduce through mitotic parthenogenesis.

result in substantial changes in chromatin structure and gene transcription (44). For example, acetylation of histones H3 and H4 is recognized as euchromatic marks and frequently correlated with active gene transcription (46). In contrast, methylation of H3K9 and H3K27 is recognized as heterochromatic marks and frequently associated with gene silencing (46). Similar to cytosine methylation, these marks can be actively removed through the activity of histone demethylases and histone deacetylases. The interplay between histone-modifying enzymes determines histone methylation/acetylation status, which can impact chromatin accessibility to transcription factors and, ultimately, gene activation or repression (20).

Galls: knot-like structures induced by root-knot nematodes at the infection sites that result from division and hypertrophy of cells surrounding the giant-cells

There is growing evidence indicating that various components of epigenetic mechanisms can sense and respond to pathogen infection by regulating gene expression at transcriptional and post-transcriptional levels. Indeed, pathogen infection can alter small RNA production, histone structure, and DNA methylation patterns (11, 17, 29, 81, 87, 103). During the past decade, several studies exploring various components of epigenetic regulators have yielded intriguing insights into the complexity and significance of epigenetic mechanisms in establishing plant–nematode interactions. Because of the dynamic nature of epigenetic modifications, these modifications can provide host plants with efficient, fast-acting, and reversible cellular responses to infection. However, parasitic nematodes can co-opt epigenetics as an alternative regulatory mechanism to overcome the fundamental genetic regulatory mechanism of host plants. Here, I review the current knowledge about epigenetic mechanisms during plant–nematode interactions and discuss how epigenetic modifications impact the outcome of the interaction.

NEMATODE-RESPONSIVE SMALL RNAs

Analysis of *Arabidopsis* mutants impaired in the production of small RNAs provided the first clue that small RNA may have regulatory functions during cyst nematode parasitism of host plants. RNA-dependent RNA polymerase (*rdr*) and DICER-like (*dcl*) mutants exhibited a general trend of reduced susceptibility to the beet cyst nematode *Heterodera schachtii* (28). These mutants showed similar responses to infection by the root-knot nematode *Meloidogyne javanica* (84). Likewise, analysis of ARGONAUTE mutants (*ago1-25*, *ago1-27*, *ago2-1*, *ago1-27/ago2-1*) revealed reduced susceptibility to the root-knot nematode *Meloidogyne incognita* (64), suggesting that global disruption of small RNA biogenesis interferes with successful nematode parasitism. Recently, high-throughput sequencing of small RNAs isolated from *Arabidopsis* galls formed by *M. incognita* resulted in the identification of significant numbers of siRNA clusters that were differentially expressed between the nematode-induced galls and noninfected root tissues at 7 or 14 days post infection (dpi) (63). These clusters were grouped into two main classes (20–22 nt and 23–24 nt) and mapped to protein-coding genes. The heterochromatic 23–24-nt siRNA class was the most abundant and accumulated preferentially in gene promoters and to a lesser degree in the gene body regions. Interestingly, the majority of these clusters showed higher abundance in the galls at 7 or 14 dpi as compared with noninfected roots, suggesting that *M. incognita* infection activates the machinery of siRNA biogenesis and silencing (63). Linking the abundance of the heterochromatic siRNA clusters with the expression levels of the associated genes pointed to a possible role of these clusters in regulating gene expression in the galls, most probably through the RdDM pathway. In contrast to the 23–24-nt siRNAs, the 20–22-nt clusters were mainly found in gene body regions and showed lower abundance in the galls compared with uninfected roots. A similar observation of the distribution of these two classes of siRNAs was reported in the three-day-old galls formed by *M. javanica* on *Arabidopsis* roots (5), highlighting a striking parallel between small RNA biogenesis and accumulation in the galls formed by two different root-knot nematode species.

Plant transcriptional reprogramming upon nematode infection seems to rely on a considerable number of miRNA genes. Following the early study of Hewezi et al. (28), who documented the expression changes of 30 miRNA genes in *Arabidopsis* upon infection by *H. schachtii*, several reports on differential expression of miRNAs upon nematode infection were published. For example, Li et al. (50) identified 40 differentially expressed miRNA genes in soybean in response to infection by the soybean cyst nematode (SCN; *Heterodera glycines*). A more recent study identified 60 SCN-responsive miRNA genes (91), suggesting a key role for miRNA regulation in plant–cyst nematode interactions.

Differential accumulation of miRNAs in response to root-knot nematode infection has also been reported. Analyzing differentially expressed miRNAs in early-developing galls of *M. javanica* yielded a set of 62 differentially expressed miRNAs with 11 genes upregulated and 51 genes downregulated (5). Although the functional significance of this downregulation remains to be investigated, this finding is in agreement with an earlier report showing a general trend of miRNA downregulation during the early stages of cyst nematode infection (28). Differentially expressed miRNAs were also identified in fully developed galls of *M. incognita* in *Arabidopsis*. A set of 24 differentially expressed miRNAs were detected and the temporospatial expression patterns of some of these miRNAs in the galls were confirmed using promoter–reporter fusions (64). In another recent study, a large number of miRNAs were identified from *M. incognita*–infected tomato roots at various disease development stages (40). Of note is that the majority of the miRNAs identified in these studies are highly conserved in plant species. Taking into consideration the wide host range of root-knot nematodes, this finding may underscore the importance of these miRNAs in regulating common gene networks essential for gall formation. Another interesting note is that many of the identified miRNAs are responsive to both cyst and root-knot nematodes and exhibit common or opposite patterns of regulation upon infection. This finding may reflect the similarity in infection processes and also highlights the remarkable differences in gene regulation between syncytium and giant-cells.

FUNCTIONS OF miRNAs IN PLANT–CYST NEMATODE INTERACTION

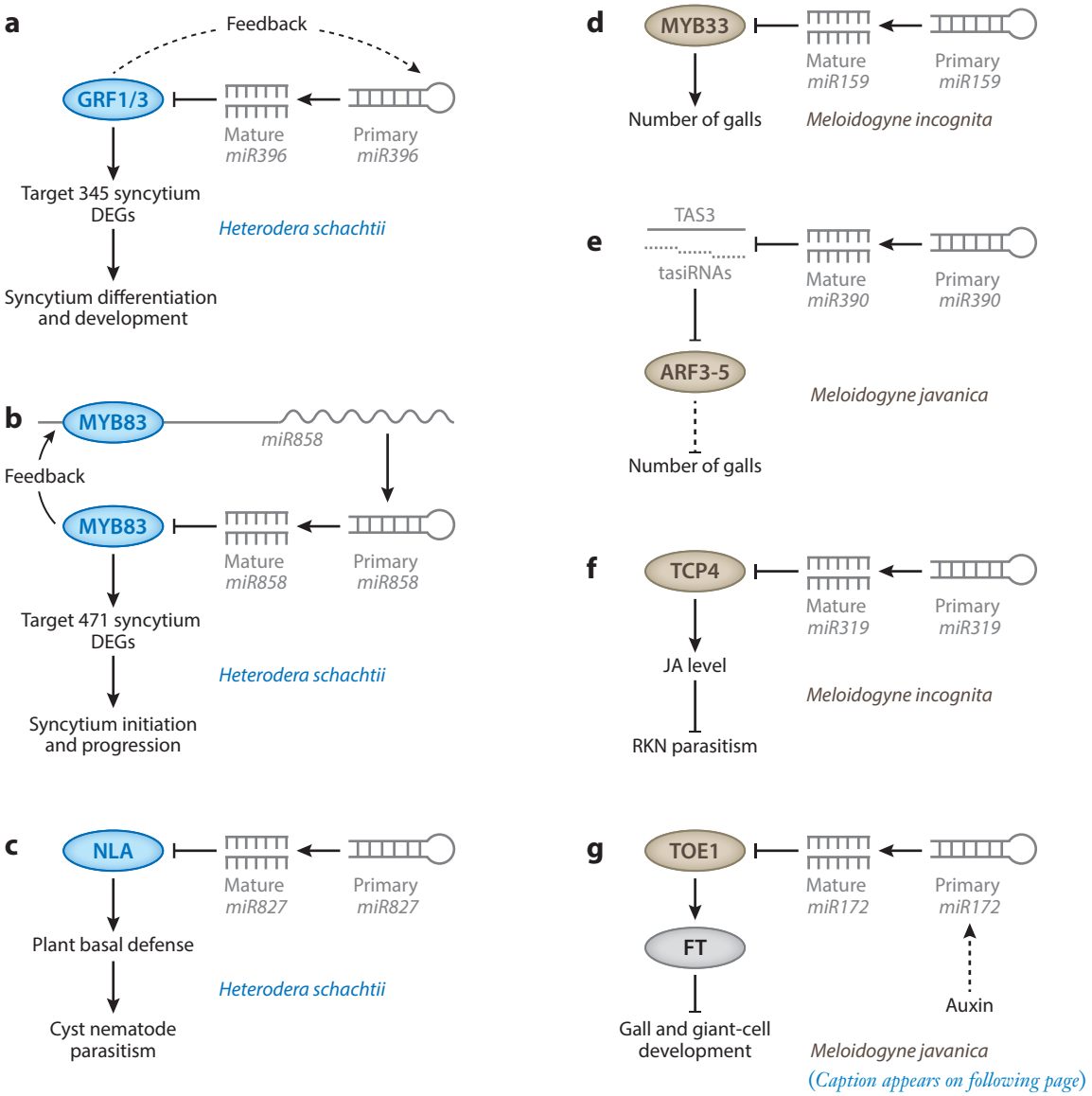
miR396

Although a significant number of miRNAs were identified as nematode-responsive genes, a limited number of miRNAs have been functionally characterized. *Arabidopsis* miR396 was the first miRNA to be functionally characterized in great detail (30). miR396 expression in the *Arabidopsis* syncytium pointed to a role in the transition from the syncytium formation phase to the maintenance phase. In this transition, miR396 downregulation defines the start of the syncytium formation phase and a following strong induction corresponds to the beginning of the maintenance phase (30). Of the seven growth-regulating transcription factors (*GRFs*) known to be post-transcriptionally regulated by miR396 in *Arabidopsis*, only *GRF1* and *GRF3* were demonstrated to be the targets of miR396 in the syncytium. When interfering with the expression of miR396 or its *GRF1/3* targets, the syncytium did not properly develop, leading to arrested nematode development at the J2 and J3 stages and reduced plant susceptibility to *H. schachtii*. These findings indicate that coordinated expression of miR396 and its *GRF* targets is vital for syncytium differentiation and development, and interference with the expression equilibrium of this regulatory module impedes successful nematode parasitism. The coordinated expression of miR396 and its *GRF* targets is mediated via a reciprocal feedback loop in which miR396 and *GRF1/3* regulate the expression of each other (25). Importantly, gene expression analysis indicated a key regulatory function of these transcription factors in establishing the syncytium transcriptome (30). Careful examination of GRF-regulated genes emphasized a conceivable role of these transcription regulators in synchronizing the interplay between various developmental events and defense responses at the nematode feeding site (54). This possibility was recently confirmed after determining the direct targets of GRF1 and GRF3 using chromatin immunoprecipitation (ChIP)-Seq (78). Interestingly, 345 of the 1,510 identified direct targets of GRF1 or GRF3 were among the syncytium differentially expressed genes and have functions related to cell differentiation and development and defense responses (S. Piya & T. Hewezi, unpublished data). Thus, *H. schachtii* recruits miR396 to rewire defense and development pathways during two distinct stages of syncytium initiation and maintenance by switching GRF1 and GRF3 on and off (**Figure 2*a***). Interestingly, a recent study by Noon et al. (72) provided

evidence that the miR36/GRF regulatory mechanism is conserved in the soybean–*H. glycines* interaction. These results indicate that findings in the *Arabidopsis*–*H. schachtii* model pathosystem can be translated into economically important systems and that components of the miR396/GRF regulon could be targeted for producing novel resistant materials against cyst nematodes in crop plants.

miR858

As detailed above, miRNA-targeted transcription factors have a remarkable ability to adjust cellular metabolism and physiology for new functions because of their capability to directly regulate



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Schematic representation summarizing the functions of a set of miRNA genes during plant interactions with cyst and root-knot nematodes. (*a*) miR396 negatively regulates the expression of GRF1 and GRF3 transcription factors, which directly regulate the expression of 345 genes in the syncytium of *Heterodera schachtii*. The expression of miR396 and GRF1/3 is adjusted through a feedback regulatory mechanism. (*b*) miR858 negatively regulates the expression of the transcription factor MYB83, which directly regulates the expression of 471 genes in the *H. schachtii* syncytium. The expression of miR858 and *MYB83* is adjusted through a feedback regulatory system. (*c*) miR827 downregulates NITROGEN LIMITATION ADAPTATION (*NLA*) in the syncytium of *H. schachtii* to suppress the basal defense response and facilitate nematode parasitism. (*d*) miR159 negatively regulates the expression of *MYB33* in the giant-cells of *Meloidogyne incognita*. The mechanism through which miR159-mediated *MYB33* downregulation enhances plant susceptibility remains to be determined. (*e*) miR390-mediated biogenesis of *TAS3*-derived tasiRNAs (*trans*-acting, small-interfering RNAs) negatively regulate the expression of Auxin Response Factors (*ARF3–5*) in the giant-cells of *Meloidogyne javanica*. Downregulation of these factors in the giant-cells is believed to contribute to plant susceptibility. (*f*) miR319 negatively regulates the expression of *TCP4*, which functions as a positive regulator of jasmonic acid (JA) biosynthesis. Increased miR319 expression resulted in the reduction of the JA level and, hence, increased plant susceptibility to *M. incognita*. (*g*) Auxin-induced miR172 negatively regulates the expression of the transcription factor TOE1. Downregulation of *TOE1* in the giant-cells of *M. javanica* alleviates its inhibitory effects on the FLOWERING LOCUS T (*FT*) gene, which may regulate aspects of giant-cell differentiation via an unknown mechanism. Abbreviations: DEGs, differentially expressed genes; RKN, root-knot nematode.

the expression of a large number of target genes. miR858 is one of few miRNAs in *Arabidopsis* that target several transcription factors. MYB83 was recently identified as the main MYB transcription factor targeted by miR858 in the syncytium of *H. schachtii* (77). Post-transcriptional repression of *MYB83* by miR858 in the syncytium was restricted to the early syncytium development stage, highlighting the importance of this regulatory system during the beginning and progression of nematode infection. Manipulating the expression of miR858 and MYB83 using gain- and loss-of-function approaches proved the function of MYB83 as a positive regulator of nematode parasitism of *Arabidopsis*. RNA-seq analysis revealed that MYB83 regulates 1,286 genes in the syncytium; 471 of them contain a MYB83 *cis*-binding motif in their promoters and, hence, are regarded as direct target genes (77). Gene ontology analysis of MYB83-regulated genes revealed their involvement in cellular processes crucial for syncytium development and function that include glucosinolate biosynthesis and defense response, hormone signaling pathways, cell wall modifications, and sugar transport. Similar to the miR396-GRF regulatory system, MYB83 was found to positively regulate the expression of miR858 through a feedback regulatory circuit to finely balance its own transcript level and therefore the activity of downstream target genes in the syncytium (**Figure 2*b***). The functional characterization of miR396 and miR858 revealed how miRNAs and their targeted transcription factors simultaneously fine-tune the expression of each other, providing interesting insights into the tight control over gene expression at the nematode feeding sites.

miR827

Sedentary plant-parasitic nematodes spend a prolonged period of their life cycle in direct contact with host plants that require active suppression of host defense responses. Recruiting plant miR827 provides the nematode with the ability to specifically and permanently negate defense signaling in the nematode feeding sites to facilitate infection and nematode development (**Figure 2*c***) (32). miR827 is encoded by a single gene and targets a ubiquitin E3 ligase gene, also known as NITROGEN LIMITATION ADAPTATION (*NLA*). miR827 was highly induced in the early feeding cells as well as in fully developed syncytia. This activation was associated with post-transcriptional silencing of *NLA* in the syncytium during all sedentary parasitic stages. The functional analyses uncovered a surprising role of NLA in activating basal defense response, as several *PR* genes were induced several-fold in the overexpression lines compared with wild-type plants under noninfected conditions. The importance of silencing *NLA* for successful nematode

parasitism was demonstrated by showing that miR827 overexpression–mediated *NLA* downregulation caused *Arabidopsis* plants to become more susceptible to *H. schachtii*. Conversely, increasing the expression of *NLA* through inactivation of its negative regulator or via overexpression of a nondegradable transcript significantly lowered nematode susceptibility. To further understand the mechanism through which miR827-mediated downregulation of *NLA* impacts nematode susceptibility, the authors identified the interacting protein substrates of NLA using yeast two-hybrid (Y2H) screens. Interestingly, among the identified substrates, many of them are known to be implicated in defense responses, including PR4, thaumatin superfamily protein, β-1,3-endoglucanase, papain family cysteine protease, mitogen-activated protein (MAP) kinase 17, and MAP kinase 2 (32). Finally, it may be important to mention that the miR827-NLA regulatory system is involved in controlling plant response to nitrate and phosphate deficiency (38, 52, 74, 75). Thus, the implication that this regulatory system increases acquisition and utilization of nitrogen and phosphorus in nematode feeding sites is a possibility that needs to be explored.

FUNCTIONS OF miRNAs IN PLANT–ROOT-KNOT NEMATODE INTERACTIONS

The functional sequences of differential expression of miRNAs in the galls induced by root-knot nematodes were also investigated in a number of studies. Medina et al. (64) investigated the role of four differentially expressed miRNAs (miR159, miR319, miR398, and miR408) in establishing the compatibility of the interaction between *Arabidopsis* and *M. incognita*. Using overexpression and/or T-DNA insertional mutagenesis approaches, only miR159 was found to alter plant susceptibility to *M. incognita*. This phenotype was attributed to the intense in situ hybridization signals corresponding to mature miR159 molecules in the giant-cells and adjacent cells. The high abundance of miR159 in giant-cells was correlated with a substantial decrease in the expression of its target gene *MYB33* transcription factor (**Figure 2*d***). However, it remains unknown how miR159-mediated downregulation of *MYB33* in giant-cells modulates nematode parasitism.

miR390 was one of the most abundant miRNAs in galls induced by *M. javanica* in *Arabidopsis* (5). miR390 is known to control the biogenesis of *TAS3*-derived tasiRNAs (*trans*-acting, small-interfering RNAs), which bind the transcripts of three auxin response factors (*ARF3–5*) and mediate their degradation (60). Promoter reporter lines showed that both miR390 and its *TAS3* target are highly induced in the giant-cells and galls during the early infection stages. Mutant lines of miR390 and *TAS3* displayed significant reduction in the numbers of galls. The effect of miR390 and *TAS3* on nematode susceptibility was attributed to *TAS3*-derived tasiRNA-mediated regulation of *ARF3* expression level. This was demonstrated by showing that a GUS (β-glucuronidase) reporter line containing a wild-type variant of *ARF3* was not activated in the giant-cells, whereas a line containing a tasiRNA-resistant variant was activated. These results provided evidence for the involvement of a miR390/TAS3 regulatory module in repressing auxin signaling mediated by ARF3 and probably ARF4 and ARF5 in the giant-cells, despite the fact that downstream components of these factors in the giant-cells remain to be identified (**Figure 2*e***).

Jasmonic acid (JA) is a well-known systemic signaling molecule that plays key roles in the interactions between root-knot nematodes and host plants (16, 68). miR319 was identified as JA-responsive miRNA and found to modulate plant susceptibility to *M. incognita* via its target *TCP4* (TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR 4) (101) (**Figure 2*f***). miR319 overexpression enhanced plant susceptibility, whereas *TCP4* overexpression produced the opposite effects of reduced susceptibility. Quantifying the levels of endogenous JA in overexpressed plants revealed that reduced plant susceptibility of *TCP4* overexpression lines was associated with notable increases in JA level (101), a finding consistent with the role of TCP4 in JA biosynthesis (85).

Recently, it has been demonstrated that miR172, one of the most highly conserved miRNA genes in plants, was highly activated in the galls and giant-cells induced by *M. javanica* in *Arabidopsis* and other host plants, including tomato and pea (12). Functional assays revealed that miR172 in the *Arabidopsis–M. javanica* pathosystem functions via the negative regulation of its AP2-like transcription factor target *TOE1* (12). Inactivation of miR172 and *TOE1* overexpression showed a notable reduction in the size of giant-cells as well as in the total number of galls per plant. These data established that activation of miR172-mediated downregulation of *TOE1* is required for proper cell-fate differentiation of giant-cells and galls. Expression analysis of FLOWERING LOCUS T (*FT*) gene, a defined direct target of TOE1 (100), suggested that downregulation of *TOE1* may be required to alleviate its inhibitory effects on *FT*, leading to *FT* accumulation in the giant-cells (**Figure 2*g***). Additional analysis of a loss-of-function mutant allele of *FT* further suggested its implication in gall and giant-cell development. Although the exact function of TOE1 and FT in giant-cell development is unidentified, they may regulate developmental and morphological pathways similar to those occurring during flowering. Díaz-Manzano et al. (12) found that different members of the *miR172* gene family contain canonical auxin response *cis*-elements in their promoters and, accordingly, were regulated by auxin during nematode infection, which is consistent with the regulation of miRNA172 by ARFs (83). This finding indicates that the miRNA172-TOE1-FT regulatory cascade is instructed by auxin concentration and signaling.

The demonstrated functions of miR390-*TAS3-ARF3* and miR319-*TCP4* regulatory modules during root-knot nematode infection, along with the relevance of miR172 responsiveness to auxin treatment, shed light on a possible role of these miRNAs in mediating interplay between auxin and JA signaling pathways in the giant-cells, a hypothesis that deserves further investigation.

LONG NONCODING RNAs

Another class of regulatory noncoding RNAs is the long noncoding RNAs (lncRNAs; >200 nt). lncRNAs have been recently identified in many plant species and found to contribute to important regulatory functions in different developmental programs and stress responses (41, 56, 69, 79). These lncRNAs can function as *cis*-regulatory elements, precursors of small RNAs, scaffolds for protein complexes, mediators of the RdDM pathway, and target mimics of miRNAs (79). However, limited information about the accumulation of lncRNAs in response to nematode infection is currently available. Recently, Li et al. (51) identified 565 lncRNAs in tobacco (*Nicotiana tabacum*) that are responsive to *M. incognita* infection. However, the regulatory functions of these differentially accumulated lncRNAs remain to be investigated. Identifying lncRNA molecules in the syncytium and giant-cells and further exploring their functional roles will improve our understanding of the regulatory function of noncoding RNAs during plant–nematode interaction.

DNA METHYLATION

Using deep sequencing of bisulfite-treated DNA isolated from soybean roots inoculated with SCN, Rambani et al. (80) generated high-coverage DNA methylation profiles at single-nucleotide resolution. The analysis uncovered key features of the root methylome undergoing susceptible interaction with SCN. SCN infection significantly altered the root methylome, with hypomethylation being more predominant than hypermethylation. This is in agreement with several reports showing that active DNA demethylation is induced during the compatible interactions in various pathosystems (11, 13, 31, 57, 99, 103). The analysis also revealed that 6% of the identified differentially methylated regions (DMRs) was concurrently methylated in various sequence contexts. This finding suggests that DNA methylation in each of the CG, CHG, and CHH sequence contexts generally occurs independent of the others, but a coordinated cytosine methylation

lncRNAs: endogenous long (>200 nt) noncoding RNAs that originate from transcriptional loci and have diverse regulatory functions

Methylome: high-resolution DNA methylation patterns in the genome of an organism

of targeted regions may occur and would be of biological importance during SCN infection. The methylome analysis also indicated that differential DNA methylation occurs preferentially in recently duplicated genes as compared with ancient genes. Thus, SCN-induced differential methylation may contribute to gene dosage equilibrium of recently duplicated genes, which are assumed to be more involved in defense-related function compared with their ancient copies. Importantly, SCN-induced hyper- and hypomethylation were found to impact a significant number of genes previously reported as syncytium differentially expressed genes. These genes are implicated in biological processes known to impact syncytium structure and nematode pathogenesis, including defense response, hormone signaling, cytoskeleton dynamics, cell wall biogenesis, signal transduction, gene regulation, epigenetic modifications, and ubiquitination. This study provided the first proof of the involvement of DNA methylation in plant–cyst nematode interaction through conceivable transcriptional regulation.

The association between DNA methylation patterns and gene expression changes during two distinct stages of nematode infection corresponding to syncytium development and maintenance phases was investigated in *Arabidopsis* after *H. schachtii* infection (29). Similar to SCN, *H. schachtii* dramatically altered root methylomes at both infective stages with widespread hypomethylation, which accounted for more than 90% of all DMRs. The analysis also provided novel insights into the dynamics and specificity of DNA methylation patterns during cyst nematode infection. Each infective stage was accompanied by distinct patterns of DNA methylation that are preferentially linked to specific regions of protein-coding genes and TEs. Hypermethylation of TEs and gene promoters was generally associated with increased abundance of the 24-nt siRNA class with effects detected for methylation sequence context and infection stage (time point). Widespread demethylation in TEs occurs in a context-specific manner and is associated with certain TE families, as previously reported for microspores and sperm cells in *Arabidopsis* (6). It may be important to mention that a significant number of hypermethylated regions induced by *H. schachtii* were not associated with an increase in siRNA abundance. Lister et al. (53) found that DNA methylation and siRNA accumulation were not constantly linked, suggesting that DNA methylation can be induced independent of siRNA.

The importance of DNA methylation changes induced by *H. schachtii* in protein-coding genes was revealed by the significant overlaps between the differentially methylated genes and both the differentially expressed genes and those genes found to change expression in the syncytium. Determining nematode susceptibility of T-DNA insertional mutants of several promoter- and gene body–methylated genes provided insights into a possible role of syncytium differentially methylated genes in mediating plant–nematode interaction. Although the influence of DNA methylation in gene promoters is known to impact gene transcription, the exact role of gene body methylation remains a matter of debate. In this regard, gene body methylation of nematode-responsive genes may be associated with the suppression of intragenic transcription initiation and/or transposon insertion in actively transcribed genes (62, 70). Gene body methylation may also contribute to enhancing splicing efficiency (104). However, these hypothetical functions remain to be proved experimentally.

The proximity of protein-coding genes to TEs is another way in which the expression of these genes can be controlled (95). The methylome analysis of *H. schachtii*–infected roots (29) also pointed to a possible role of TE methylation, particularly the CHH context, in priming the expression of nearby genes. For instance, CHH hypo-DMRs occurred favorably in TEs that are located within 3 kb of the closest genes. Unexpectedly, *H. schachtii*–induced hypomethylation of TEs was correlated with low expression of nearby genes. A similar association was reported in *Arabidopsis* undergoing biotic and abiotic stresses (48, 86). Loss of DNA methylation of these TEs may activate TE-originated cryptic transcripts that could interfere with the transcriptional

activity of the adjacent genes, leading to limited expression. Another possibility is that demethylation of these TEs may facilitate the induction of nematode-regulated genes at a later stage of infection, as DNA methylation in some cases can precede transcriptional changes (29, 86).

The transcriptional activity of TEs during plant–nematode interaction and their potential impact on the transcription of neighboring genes were also investigated in the *Arabidopsis–H. schachtii* pathosystem (76) using RNA-seq data. Interestingly, 192 differentially expressed TEs were identified in the nematode-infected roots with the majority belonging to the DNA transposon RC/Helitron and MuDR families. Notably, more than 70% of these differentially expressed TEs were positioned within 5 kb of genes, many of which change expression in the syncytium. These differentially expressed genes coded for functions involved in, e.g., gene transcription control, primary metabolism pathways, plant cell wall modifications, hormone signaling, and defense responses. Thus, similar to other biotic and abiotic stress stimuli (18, 34, 59, 65), cyst nematodes can regulate the transcriptional activity of TEs and nearby nematode-regulated genes, thereby contributing to the transcriptome reprogramming of syncytial cells. It may be worth mentioning that the study of Piya et al. (76) uncovered only the altered expression of highly expressed TEs. RNA-seq data with significantly higher depth and coverage are needed to portray the complete image of the transcriptional activity of TEs in response to nematode infection and their involvement in transcriptional reprogramming of nematode feeding sites.

In another recent study, Ruiz-Ferrer et al. (84) performed a differential accumulation analysis of siRNAs associated with TEs in the galls of *M. javanica* in *Arabidopsis* roots. They found that the 22- and 24-nt siRNA classes are the most dominant and associate primarily with the retrotransposon Gypsy and Copia families. The accumulation of these siRNAs was correlated with dramatic decreases in the transcript levels of a number of retrotransposons (83). Although gene expression quantification and mutant analysis pointed to a role of the canonical and noncanonical RdDM pathways in the repression of retrotransposon elements in the galls, DNA methylation profiles of these elements remain to be elucidated. Together, these studies revealed a role of epigenetic modifications mediated through differential accumulation of siRNAs and repatterning of DNA methylation to control the mobility and proliferation of TEs. This command is required to maintain genome integrity and stability during genome-wide reprogramming occurring in response to nematode infection.

A recent report provided insights into a possible role of Copia-type elements in regulating SCN resistance mediated by the *rhg1-a* resistance gene (4). The authors discovered the presence of a Copia retrotransposon element within the *rhg1-a* gene in certain soybean accessions. Although no such correlation between the presence of this element and *rhg1-a* expression could be established, SCN-induced epigenetic modifications in this element may regulate *rhg1-a* maturation and splicing variants, taking into consideration that the retrotransposon-type tends to be more vulnerable to DNA methylation changes than other types of TEs during nematode infection (29). In this regard, DNA methylation and other epigenetic marks may modulate the recruitment of splicing factors to the pre-mRNA of *rhg1-a*, thereby impacting the elongation rate of Pol II and hence exon inclusion/skipping in mature mRNA.

ROLE OF SOYBEAN CYST NEMATODE RESISTANCE GENES IN EPIGENETIC MODIFICATIONS

Recently, two major genetic loci that contribute to SCN resistance have been described (10, 55): *Rhg1* (for resistance to *H. glycines*) and *Rhg4*. Although the *Rhg1* locus contains three genes within a 31-kb repeat region at chromosome 18, the *Rhg4* locus contains a single gene coding for serine hydroxymethyltransferase (*SHMT*; *GmSHMT08*) at chromosome 8 (55). DNA methylation analysis

of the *Rbg1* locus across different soybean accessions revealed increased DNA methylation in the resistant lines containing three copies of *Rbg1* as compared with the susceptible lines containing only one copy. The hypermethylation patterns were found in all three sequence contexts, particularly upstream and downstream of the three *Rbg1*-encoding genes. However, these data cannot explain the increased expression of these hypermethylated genes upon SCN infection. One possible explanation is that SCN infection induces hypomethylation of these genes specifically in the syncytium cells, leading to cell-type-specific gene activation.

The involvement of SHMTs in one-carbon metabolism, which supports DNA methylation, provided a suggestion for a potential role of RHg4 (GmSHMT08) in reprograming the methylome of soybean roots during SCN infection. Genome-wide DNA methylation analysis of a pair of highly homozygous near-isogenic lines (NILs) differing at the *GmSHMT08* locus substantiated this suggestion (A. Rambani & T. Hewezi, unpublished data). In response to SCN infection, the susceptible isogenic line experienced a coordinated decrease of global DNA methylation in protein-coding genes as well as TEs, a finding consistent with the absence of a functional *GmSHMT08* allele. In contrast, the resistant line showed increased global methylation levels in all sequence contexts. The possible function of RHg4 in DNA methylation changes, whether directly or indirectly, provides insight into a novel disease resistance strategy in crops based on epigenetic mechanisms, which need to be investigated further.

NEMATODE EFFECTORS ALTER PLANT EPIGENOMES

Plant-parasitic nematodes produce an arsenal of secreted proteins functioning as effectors that modulate plant immunity to facilitate parasitism (24, 27). The development of high-throughput sequencing technology along with precise isolation of nematode esophageal gland cells (58), where effector genes are specifically expressed, allowed the identification of a significant number of nematode effector gene candidates from sedentary as well as migratory nematode species. The large majority of these putative effectors have no sequence similarity to proteins in the public domains, and therefore their functions in nematode parasitism remain largely unknown. Nevertheless, a limited number of these putative effectors showed significant sequence homology to key factors involved in epigenetic modifications (15, 21, 37, 71, 73). Examples of these effector genes are included in **Table 1**. One of these effectors is the SCN GLAND1 effector, which showed strong sequence homology to GCN5-related *N*-acetyltransferases (GNATs) from streptomycetes (71). Further phylogenetic analysis suggested that GLAND1 may be acquired through horizontal gene transfer (HGT) from bacteria (71). The analysis also revealed the presence of homologs in the cyst nematode species *Heterodera avenae*, *Globodera rostochiensis*, and *G. pallida*, and the reniform nematode *Rotylenchulus reniformis* (71). Despite the fact that GNATs are the most commonly spread acetyltransferases in different biological systems, only one GNAT effector from *Mycobacterium tuberculosis* has been reported (42). The function of the *M. tuberculosis* GNAT effector in defense suppression (42) is not necessarily applicable to Hg-GLAND1, taking into consideration the wide biological functions of GNATs and the absence of *Hg-GLAND1* expression during the early stage of nematode parasitism (73), when defense suppression is fundamental for infection success (22, 33). Alternatively, nematode GNATs may mimic the histone acetyltransferase activity of plant GCN5, thereby modulating acetylation levels at specific loci and leading to gene expression changes and plant susceptibility. Consistent with a putative role of GNATs in nematode parasitism, the *Phytophthora* effector PsAvh23 was found to function in decreasing the GCN5-mediated H3K9 acetylation to enhance soybean susceptibility (43). Identifying host interacting proteins of nematode GNATs and their targeting loci will reveal how parasitic nematodes manipulate histone acetylation to reprogram gene expression and cause disease.

Table 1 List of putative nematode effector proteins with potential roles in epigenetic modifications

Effector ID/accession	Nematode species	Annotation	References
GLAND1	*Heterodera glycines*	GCN5-related *N*-acetyltransferases, *Streptomyces violaceusniger*	73
c26112_g3_i5|m.33335	*H. glycines*	Set1/Ash2 histone methyltransferase complex subunit ASH2, *Ascaris suum*	21
c28318_g3_i1|m.62844	*H. glycines*	Histone H4 transcription factor, *A. suum*	21
c30889_g2_i2|m.105894	*H. glycines*	Histone deacetylase 1, *A. suum*	21
c29532_g3_i8|m.82591	*H. glycines*	Peregrin, *A. suum*	21
c27537_g1_i1|m.51051	*H. glycines*	Histone acetyltransferase, *Loa loa*	21
c25350_g1_i5|m.26315	*H. glycines*	Histone deacetylase 4, *A. suum*	21
c28191_g3_i4|m.60557	*H. glycines*	Histone-arginine methyltransferase CARM1, *Ophiophagus hannah*	21
c29841_g1_i3|m.88024	*H. glycines*	Histone-lysine *N*-methyltransferase SETD2, *A. suum*	21
c28450_g1_i11|m.64987	*H. glycines*	Histone-lysine *N*-methyltransferase SUV39H2, *A. suum*	21
c28936_g1_i7|m.72148	*H. glycines*	Histone-lysine *N*-methyltransferase, H3 lysine-79 specific, *A. suum*	21
c10299_g1_i1|m.1887	*H. glycines*	Nucleosome-binding factor SPN POB3 subunit, *Aedes aegypti*	21
H-ave_c1132_g1_i1	*Heterodera avenae*	GCN5-related *N*-acetyltransferases	71
GO251019	*Globodera pallida*	SIN3 histone deacetylase, *Physcomitrella patens*	37
G_pal_comp26399_c0_seq1	*G. pallida*	GCN5-related *N*-acetyltransferases	71
GPLIN_000932700; GPLIN_000767700	*Globodera rostochiensis*	GCN5-related *N*-acetyltransferases	15, 71
R_ren_comp42055_c0_seq1	*Rotylenchulus reniformis*	GCN5-related *N*-acetyltransferases	71

Other putative nematode effectors with potential roles in modifying the host epigenome include, for instance, those with strong similarities to the *Ascaris suum* histone H4 transcription factor, which activates histone H4 gene transcription; histone deacetylase 1, which deacetylates lysine residues of core histones (14); Set1/Ash2 histone methyltransferase complex subunit ASH2, which methylates Lys4 of histone H3 (23, 96); peregrin, which is involved in histone H3 acetylation, preferentially at lys23 sites (98); histone-lysine *N*-methyltransferase SUV39H2, which specifically trimethylates Lys9 of histone H3 (1, 82); and the *Aedes aegypti* POB3 subunit of the nucleosome-binding factor SPN, which is involved in chromatin structure and dynamics (19) (**Table 1**).

Although the majority of nematode effectors are without sequence similarity to proteins with known functions in databases, functional characterization of these effectors may reveal roles in host epigenetic modifications, as in the case of the cyst nematode effector 32E03 (94). 32E03 is a typical nematode effector expressed solely in the dorsal gland cell during the parasitic stages. Apart from the presence of a functional bipartite nuclear localization signal, the 32E03 effector showed no significant sequence similarity to proteins in public domains. Inactivation of 32E03 expression using a host-induced gene silencing approach resulted in decreased plant susceptibility, a finding that underscores the importance of this effector for cyst nematode parasitism (94). Colocalization assays and Y2H screens revealed that 32E03 colocalizes and physically interacts with *Arabidopsis* tuin-type histone deacetylase HDT1 and the histone chaperone FKBP53 (94). The previously reported functions of HDT1 and FKBP53 as suppressors of rRNA gene expression (47, 49) suggested a role of the 32E03 effector in regulating the transcriptional activity of rRNA genes during nematode parasitism of *Arabidopsis* plants.

The impact of 32E03 on histone acetylation level was examined both in vivo using transgenic *Arabidopsis* lines expressing 32E03 coding sequences and in vitro using recombinant 32E03 protein (94). Both assays confirmed the function of 32E03 as a histone deacetylase inhibitor that leads to heightened level of histone H3 acetylation at the rDNA chromatin regions. Because increased histone acetylation is generally linked with increased gene expression, the level of 45S pre-rRNA (pre-ribosomal RNA) transcripts was quantified in transgenic *Arabidopsis* lines expressing low or high levels of the 32E03 transcript. Although the rRNA expression level was significantly increased in the 32E03 low-expressing line, it was significantly reduced in the 32E03 high-expressing line. Further analysis linked the contrasted expression levels of rRNA with the opposite response of high- and low-expressing lines to nematode infection (94). The 32E03 low-expressing line showed increased susceptibility to *H. schachtii*, whereas the high-expressing line showed reduced susceptibility. Interestingly, elevated 32E03 expression in *Arabidopsis* triggered RdDM of rDNA, leading to gene downregulation. Together, these intriguing results demonstrate how cyst nematodes use a nuclear-targeted effector to impose epigenetic changes along the rDNA chromatin in a dose-dependent fashion that impact nematode parasitism and plant response. However, several questions remain to be answered. For example, how does an increase in rRNA abundance contribute to nematode parasitism? Is the 32E03-mediated elevated level of histone acetylation associated with other epigenetic modifications such as DNA methylation given that both marks are highly interconnected (89, 90, 92)? Does 32E03 target loci other than FKBP53 for deacetylation through physical or functional association with plant proteins? Do other phytonematodes employ similar mechanisms of epigenetic-based pathogenesis?

SUMMARY POINTS

1. Plant small RNAs, including miRNAs, differentially accumulate in the nematode feeding sites, suggesting a role in transcriptional and post-TGS.
2. Functional characterization of a small set of miRNAs revealed their involvement in cellular processes essential for feeding-site formation and nematode parasitism.
3. Cyst nematode infection induces extensive changes in plant methylomes, which impact the syncytium transcriptome.
4. Cyst and root-knot nematodes produce effector proteins that could alter the plant epigenome to facilitate infection.

FUTURE ISSUES

1. Despite the remarkable progress in our understanding of the regulatory roles of various epigenetic components (small RNAs, miRNAs, DNA methylation, and histone modifications) in plant–nematode interactions, the coordinated functions of these components remain to be addressed. It is becoming clearer that various epigenetic modifications are tightly interconnected. Accordingly, an integrative genomics approach of multi-omics analyses is fundamental to understanding the regulatory and functional connections of different layers of epigenetic modifications.
2. The molecular mechanism that allows plant-parasitic nematodes to induce epigenetic changes in host plants is far from being completely understood. It is very likely that

nematode effectors play pivotal roles in triggering such epigenetic responses to infection. Functional characterization of nematode effectors and identification of host-targeted proteins are expected to reveal the molecular and biochemical bases underlying epigenome reprogramming induced by nematodes in host plants.

3. Additional studies focused on epigenetic changes induced by parasitic nematodes in various host plants are needed to possibly develop epigenetic signatures characteristic to each nematode species. Detailed temporospatial analysis of epigenome changes specifically in the nematode feeding sites is the essential first step toward developing these signatures. Considering the recent development of single-cell sequencing technology, such signatures can be developed with high resolution and specificity.
4. The fact that many epigenetic modifications can be stably inherited for many generations provides new opportunities to alter plant epigenomes using genome-editing approaches and identify newly formed meiotically heritable epialleles that have major impacts on plant response to nematode infection. This opens a completely new avenue to integrate a system of epigenetic selection in breeding programs in which individuals with the anticipated epialleles can be selected.

DISCLOSURE STATEMENT

The author is 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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Errata

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