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MicroRNAs and Their Regulatory Roles in Plant–Environment Interactions

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Abstract

MicroRNAs (miRNAs) are 20–24 nucleotide noncoding RNAs abundant in plants and animals. The biogenesis of plant miRNAs involves transcription of miRNA genes, processing of primary miRNA transcripts by DICER-LIKE proteins into mature miRNAs, and loading of mature miRNAs into ARGONAUTE proteins to form miRNA-induced silencing complex (miRISC). By targeting complementary sequences, miRISC negatively regulates gene expression, thereby coordinating plant development and plant–environment interactions. In this review, we present and discuss recent updates on the mechanisms and regulation of miRNA biogenesis, miRISC assembly and actions as well as the regulatory roles of miRNAs in plant developmental plasticity, abiotic/biotic responses, and symbiotic/parasitic interactions. Finally, we suggest future directions for plant miRNA research.

Contents

INTRODUCTION

MicroRNAs (miRNAs) are a class of endogenous small noncoding RNAs that negatively regulate gene expression. They are produced through a multistep process including transcription, precursor processing, methylation, and assembly of miRNA-induced silencing complex (miRISC). On the basis of sequence complementarity, miRNAs direct target mRNA cleavage, translational repression, and DNA methylation. Although the main steps of miRNA biogenesis and modes of miRNA action were previously established, new insights into how multiple steps of miRNA biogenesis are coupled, how miRNA biogenesis and other cellular processes like precursor messenger RNA (pre-mRNA) splicing intersect, and where miRISC assembly and actions take place have been provided in recent years. miRNAs play crucial roles in plant–environment interactions. Exciting progress has also been made on the functions of miRNAs in response to developmental and environmental cues, including that many miRNAs have previously unrecognized roles in plant–environment interactions and cross-kingdom gene regulation. Here, we review and discuss our growing understanding of the mechanisms and regulation of plant miRNA biogenesis and actions and the roles of miRNAs in plant developmental plasticity, abiotic/biotic responses, and symbiotic/parasitic interactions.

MicroRNA (miRNA): 20- to 24-nucleotide noncoding RNA that regulates gene expression in plants, animals, and some viruses

miRNA-induced silencing complex (miRISC): the ribonucleoprotein complex formed by an AGO protein and a miRNA

MECHANISMS AND REGULATION OF PLANT MICRORNA BIOGENESIS, MICRORNA-INDUCED SILENCING COMPLEX ASSEMBLY, AND MICRORNA TURNOVER

Transcription of MicroRNA Genes into Primary MicroRNAs

miRNA biogenesis in plants, like that in animals, begins with the transcription of miRNA genes (MIRs) into primary miRNAs (pri-miRNAs) by RNA polymerase II (Pol II) (111, 243). Most

MIRs, usually located in intergenic regions, are independent transcription units, although a few MIRs, located in the intronic sequences of protein-coding genes, can be cotranscribed with their host genes (183, 253, 257).

Cis-regulatory elements and *trans*-acting regulators play crucial roles in transcriptional regulation of MIR expression. Besides the TATA box core promoter element (243), a panel of *cis*-regulatory elements is enriched in MIR promoter sequences in *Arabidopsis* and rice (155, 292). Regulation of MIR expression by transcription factors was first illustrated by the regulation of *MIR156* and *MIR172* expression by SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors. In *Arabidopsis* and maize, miR156 expression declines as miR172 expression increases during the juvenile to adult transition of shoot development (38, 236). Derepression of SPL9 and SPL10, two of the ten SPL transcription factors that are targeted by miR156, allows enrichment of these two SPLs on *MIR172b* promoter and transcriptional activation of *MIR172* (236). Interestingly, derepression of SPL9 and SPL10 also promotes transcription of *MIR156a* to antagonize the decline of miR156 expression and stabilizes the output expression of miR156 (236). In *Arabidopsis*, researchers subsequently determined that the transcription factor APETALA2 (AP2) promotes *MIR156e* transcription but represses *MIR172b* transcription (263); FUSCA3 promotes the transcription of *MIR156a* and *MIR156c* (223); and POWERDRESS promotes the transcription of *MIR172a*, *MIR172b*, and *MIR172c* (274). Class II and class III homeodomain leucine zipper (HD-ZIP) transcription factors cooperate to repress transcription of *MIR165/166* in the establishment of leaf adaxial-abaxial polarity (157). A growing number of transcription factors are reported to dynamically regulate MIR transcription in response to stimuli or stresses. They are not summarized in this review owing to space limitations. Transcription factors that regulate specific MIR expression are often targeted by their cognate miRNAs, forming feedback regulatory loops (74, 236, 263). Whereas some transcription factors specifically regulate the transcription of certain MIRs, other transcription factors affect MIR transcription generally. NEGATIVE ON TATA LESS 2 (227) and CELL DIVISION CYCLE 5, a subunit of MOS4-ASSOCIATED COMPLEX (MAC) (281), are associated with Pol II to facilitate transcription of MIRs. Elongator also colocalizes with Pol II and facilitates Pol II occupancy on MIRs (57). Mediator, a protein complex that relays signals from transcriptional factors to Pol II and thereby functions as a general transcriptional coactivator, is required for MIR transcription generally (111).

In addition to *cis*-regulatory elements and *trans*-acting regulators, epigenetic mechanisms that regulate MIR transcription are emerging. By depositing acetylation marks on histone H3 lysine 14, the histone acetyltransferase GENERAL CONTROL NON-REPRESSED PROTEIN 5 promotes transcription of MIRs (110). By regulating the nucleosome occupancy on MIR promoters and the accessibility of active or repressive transcription regulators to MIR promoters, the ATP-dependent SWR1 chromatin remodeling complex exerts positive or negative effects on the transcription of MIRs (36). It remains an open question how MIRs are distinguished from other Pol II–transcribed genes so that some transcription factors or epigenetic mechanisms specifically influence MIR transcription.

DICER-LIKE (DCL): double-stranded RNA-cleaving enzyme that processes miRNA precursors into miRNAs in plants

Processing of Primary MicroRNAs

The 5′ capped and 3′ polyadenylated long pri-miRNA forms an imperfect fold-back structure and is sequentially processed into a stem-loop structured precursor miRNA (pre-miRNA) and then a duplex consisting of the guide strand (mature miRNA) and the passenger strand (miRNA*). Different from pri-miRNA processing by two families of RNase III endonucleases, Drosha and Dicer, in the nucleus and in the cytoplasm, respectively, in animals (78), the two steps of pri-miRNA processing in plants are both catalyzed by DICER-LIKE 1 (DCL1), a homolog of Dicer, in the nucleus (116, 173). Association of DCL1 with MIR loci suggests that pri-miRNAs are

processed cotranscriptionally (57). Although most miRNAs are 21 nucleotides (nt) long, the 22-nt isoforms of miRNAs are often produced owing to alternative or imprecise processing of pri-miRNAs by DCL1 (124). Other than DCL1, DCL3 is able to process pri-miRNAs and produce a class of 24-nt-long miRNAs in rice (240).

The RNA-binding proteins HYPONASTIC LEAVES1 (HYL1) (76, 115, 220) and SERRATE (SE) (146, 148, 259) associate with DCL1 to form the dicing complex, which is visualized as D-bodies in the nucleus (59, 196). HYL1 promotes the accuracy of pri-miRNA processing (48, 115, 262). SE may promote the accuracy of pri-miRNA processing and stimulate DCL1 activity (48, 94), but recent studies argue for a scaffolding function of SE (148, 233, 261, 298).

In addition to HYL1 and SE, studies in the past two decades uncovered a plethora of factors that regulate different aspects of pri-miRNA processing. Transcription factors CELL DIVISION CYCLE 5 (281), NEGATIVE ON TATA LESS 2 (227), and Elongator (57) interact with the dicing complex to promote pri-miRNA processing. Because they also bind Pol II and positively regulate the transcription of MIRs, they are considered to couple MIR transcription and pri-miRNA processing. Furthermore, MAC7, a subunit of MAC implicated in transcription elongation, positively regulates pri-miRNA processing by promoting the recruitment of HYL1 to D-bodies (98). SMALL1 (SMA1), a splicing factor, promotes MIR transcription and positively regulates pri-miRNA processing (128). CYCLING DOF TRANSCRIPTION FACTOR 2 negatively regulates pri-miRNA processing by sequestering DCL and HYL1 (203), and the transcription factor PHYTOCHROME-INTERACTING FACTOR 4 negatively regulates pri-miRNA processing by destabilizing DCL1 during dark to red light transition (204). Unexpectedly, CHROMATIN REMODELING FACTOR 2 promotes MIR transcription but also unwinds pri-miRNAs and inhibits pri-miRNA processing through interacting with SE (233). TOUGH (188), PLEIOTROPIC REGULATORY LOCUS1 (a conserved WD-40 protein in MAC) (280), DAWDLE (a forkhead-associated domain-containing protein) (149, 264), as well as MAC3A and MAC3B (two U-box type E3 ubiquitin ligases in MAC) (126) also interact with DCL1 and promote pri-miRNA processing. However, they do not affect MIR transcription. They bind pri-miRNAs and might stabilize pri-miRNAs and assist DCL1 and HYL1 to get access to pri-miRNAs.

Many plant MIRs contain introns that need to be removed by splicing. Splicing may also regulate the generation of pri-miRNAs originated from introns (23). Thus, tight coupling between splicing and pri-miRNA processing would be ideal for efficient pri-miRNA processing. Indeed, many factors play dual roles in both splicing and pri-miRNA processing, and many splicing factors interact with or colocalize with the dicing complex and regulate pri-miRNA processing. DCL1, HYL1, and SE not only play key roles in pri-miRNA processing, but also participate in splicing (118, 182), although DCL1 and HYL1 mediate distinct splicing events compared with SE. Two cap-binding proteins, CBP20 and CBP80 (109, 118); splicing-related factors STABILIZED1 (14), GLYCINE-RICH RBP 7 (114), HIGH OSMOTIC STRESS GENE EXPRESSION 5 (HOS5), ARGININE/SERINE-RICH SPLICING FACTOR (RS)40, and RS41 (31); THO2 in the THO/TREX complex (63); PRE-MRNA-PROCESSING PROTEIN (PRP)39b, PRP40a, PRP40b, and LETHAL UNLESS CBC 7 RL (LUC7rl) (113); and SMA1 (128) are all involved in both splicing and pri-miRNA processing. HOS5, RS40, and RS41 interact with DCL1 and HYL1 and regulate pri-miRNA processing in part by facilitating intron removal from nascent transcripts of intron-containing MIRs (31). CBP20, CBP80, PRP39b, PRP40a, PRP40b, and LUC7rl interact with SE. They may regulate pri-miRNA processing by ensuring correct selection of 5′ splice site of the first intron (181, 182), which stimulates exonic pri-miRNA processing but inhibits intronic pri-miRNA processing (17, 113). SMA1 interacts with DCL1 and SE and promotes pri-miRNA processing by positively influencing splicing of intron-containing

pri-miRNAs (128). Recent studies found that pri-miRNA processing is affected in mutants for SICKLE (276) and RNA DEBRANCHING ENZYME 1 (135), proteins involved in the degradation of intronic lariat RNAs. Intronic lariat RNAs, formed as by-products of pre-mRNA splicing, compete with pri-miRNAs for binding to the dicing complex and negatively regulate pri-miRNA processing (135). Thus, the effects of splicing on pri-miRNA processing are not limited to intron-containing MIRs and MIRs residing within the introns of protein-coding genes.

Other factors that regulate pri-miRNA processing include MODIFIER OF SNC1, 2 (MOS2) (241) and SHORT VALVE 1 (STV1) (125), which bind to pri-miRNAs and facilitate the recruitment of pri-miRNAs by the dicing complex. Different from most miRNA biogenesis factors, MOS2 and STV1 do not interact with the dicing complex. However, the formation of D-bodies requires MOS2. THO2, a subunit of the THO/TREX complex, plays a similar role as MOS2 and STV1, although it may also regulate pri-miRNA processing through influencing splicing (63).

XAP5 CIRCADIAN TIMEKEEPER regulates miRNA biogenesis by promoting the transcription of DCL1 (58). In addition to directly regulating pri-miRNA processing, CYCLING DOF TRANSCRIPTION FACTOR 2 also indirectly regulates miRNA biogenesis by upregulating the expression of multiple pri-miRNA processing factors (203). SMA1 also enhances miRNA biogenesis by facilitating the splicing of *DCL1* pre-mRNA (128). *DCL1* mRNA levels are under negative feedback regulation by miR162, which directly targets *DCL1* (244). KARYOPHERIN ENABLING THE TRANSPORT OF THE CYTOPLASMIC HYL1 promotes miRNA biogenesis through promoting the nuclear import of HYL1 (288). In addition, a few factors modulate the levels of posttranslational modifications on pri-miRNA processing factors and regulate their activities. C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1 (CPL1), functioning redundantly with CPL2, dephosphorylates HYL1, thereby facilitating HYL1 localization to D-bodies, enhancing HYL1 activity, and promoting accurate pri-miRNA processing (150). CPL1 also dephosphorylates HOS5 and promotes HOS5 localization to nuclear speckles (31). In young vegetative and reproductive tissues, HOS5 is required for dephosphorylation of HYL1 by CPL1 and CPL2 (105). In addition to CPL1 and CPL2, SUPPRESSOR OF MEK 1, cooperating with PROTEIN PHOSPHATASE 4, dephosphorylates HYL1, stabilizing HYL1 and enhancing pri-miRNA processing (201). In responses to abscisic acid (ABA) treatment, SUPPRESSOR OF MEK 1 is induced to antagonize phosphorylation of HYL1 by MPK3 and prevent HYL1 destabilization (201). HYL1 is also dephosphorylated and miRNA biogenesis is reactivated during recovery from prolonged darkness (2). Besides MPK3, SNF1-RELATED PROTEIN KINASE 2 also phosphorylates HYL1 and SE. However, in contrast to MPK3, HYL1 phosphorylation mediated by SNF1-RELATED PROTEIN KINASE 2 favors HYL1 accumulation in ABA responses (251). Taken together, pri-miRNA processing is a highly coordinated and precisely regulated process that involves many protein–protein and protein–RNA interactions. So far, it is unclear where exactly pri-miRNA processing takes place. Because many pri-miRNA processing factors localize in D-bodies, it was suggested that D-bodies are where pri-miRNA processing occurs (59, 196). However, the large number of pri-miRNA processing events in each nucleus and the limited number of detectable D-bodies argues against this.

Following pri-miRNA processing, SE is expelled from the dicing complex, and HUA ENHANCER 1 (HEN1) replaces SE to bind the DCL1/HYL1 complex and catalyzes 2′-O-methylation at the 3′-ends of miRNA duplex, which promotes the stability of miRNAs (9, 265).

Assembly of MicroRNA-Induced Silencing Complex

The methylated miRNA duplex needs to be loaded into an ARGONAUTE (AGO) protein to form the effector complex miRISC. The strand with lower 5′-end thermodynamic stability is usually selected as the guide strand (53, 212). HYL1, CPL1 (53, 150), HOS5, RS40, and RS41

ARGONAUTE (AGO) protein: effector protein of small RNA-mediated gene expression regulation, which incorporates small RNAs and is guided to target sites by small RNAs

promote correct selection of the guide strand (31). Upon loading, the miRNA duplex unwinds, and the miRNA strand is incorporated into an AGO protein. The 5′ nucleotide largely determines the choice of AGO proteins (158). In *Arabidopsis*, the vast majority of miRNA strands start with 5′ uridine and are preferentially loaded into AGO1, the founding member of the AGO family (158). However, miRNA duplex structures are also important for selection of AGO proteins, and miRNAs can be loaded into other members of AGO proteins (284). The miRNA* strand is usually degraded. However, miRNA*s could also be enriched and loaded into AGO proteins to repress gene expression in specific plant tissues and/or under stressed conditions (246).

Assembly of AGO1-miRISC requires the molecular chaperone HEAT SHOCK PROTEIN 90 (HSP90) (90). CYCLOPHILIN 40 transiently associates with the AGO1-HSP90 complex and promotes AGO1 loading (89). ENHANCED MIRNA ACTIVITY 1 and TRANSPORTIN 1, two importin-beta family members involved in nuclear import of protein cargoes, do not change the cytoplasm-nucleus distribution of AGO1, but negatively and positively regulate AGO1 loading, respectively (41, 228). AGO1 level also affects miRISC assembly. AGO1 homeostasis entails negative feedback regulation by AGO1-bound miR168 (218, 219). Interestingly, a 22-nt isoform of miR168, which is generated owing to structural flexibility of pre-miR168, is preferentially loaded into AGO10 to silence *AGO1* expression and antagonize AGO1 accumulation via triggering production of secondary small interfering RNAs (siRNAs) (88).

In the earlier model, miRNA duplexes are exported partly by HASTY to the cytoplasm and then loaded into AGO1 (173). However, a recent study suggests a revised model where AGO1 loading occurs in the nucleus (18). A nuclear localization signal and a nuclear export signal (NES) direct nucleocytoplasmic shuttling of AGO1, and miRNAs can be loaded into AGO1 even when AGO1 is nuclear retained owing to mutation of the NES (18). In addition, HSP90, a molecular chaperone that assists AGO1 loading (90), coimmunoprecipitates with nuclear-retained AGO1 (18). The facts that the NES is dominant over the nuclear localization signal and wild-type AGO1 is present mostly in the cytoplasm raise the question of how unloaded AGO1 can be retained in the nucleus for loading. According to computational predictions, the NES of AGO1 is hidden before loading of miRNAs into AGO1, allowing AGO1 to localize in the nucleus. miRNA loading triggers the exposure of NES, allowing loaded AGO1 to be exported to the cytoplasm by CRM1/EXPORTIN1 (18).

MicroRNA Turnover

miRNA duplexes that fail to be methylated are degraded by a DEDDy-type 3′ to 5′ exoribonuclease ATRIMMER 2 in *Arabidopsis* (229). Methylated free miRNAs and AGO-bound miRNAs can also undergo degradation. Three steps lead to degradation of methylated miRNA: (*a*) 3′ truncation of methylated, free, or AGO1-bound miRNA; (*b*) 3′ uridylation of truncated miRNA; and (*c*) degradation of truncated and uridylated miRNA (267, 268).

The 3′ to 5′ SMALL RNA DEGRADING NUCLEASE 1 (SDN1) is able to truncate 2′-O-methylated RNA oligonucleotides and 2′-O-methylated AGO1-bound miRNAs in vitro (185, 267). Simultaneous mutations in *SDN1* and *SDN2* not only result in reduced accumulation of 3′ truncated miRNAs in the *hen1* mutant, in which miRNAs lose 2′-O-methylation-dependent protection, but also decrease the levels of 3′ truncated miR165 and miR166 in the wild-type background (267). These findings suggest that SDN1 and possibly SDN2 are responsible for 3′ truncation of miRNAs in vivo.

Terminal uridylyl transferases HEN1 SUPPRESSOR1 (HESO1) and UTP:RNA URIDYLYLTRANSFERASE 1 (URT1) catalyze the 3′ uridylation of unmethylated RNA oligonucleotides and AGO1-bound, unmethylated miRNAs in vitro (187, 214, 230, 293). HESO1 and URT1 preferentially uridylate miRNAs with 3′ terminal U and A, respectively. Possibly because

they have different substrate specificities, 3′ tailing is more efficient when both are present. In vivo, it is likely that URT1 first monouridylates unmethylated miRNAs and HESO1 then polyuridylates those miRNAs (214). However, compared with *utr1* mutant, mutation in *HESO1* compromises 3′ tailing of a greater number of miRNAs, suggesting that HESO1 can carry out 3′ tailing in collaboration with other terminal uridylyl transferases or on its own (214).

The identity of exonucleases that degrade polyuridylated miRNAs in plants is currently unknown. On the basis of the findings in green algae, yeasts, and mammals, SUPPRESSOR OF VARICOSE and RIBOSOMAL RNA-PROCESSING PROTEIN 6-LIKE (RRP6L), the *Arabidopsis* homolog of DIS3-LIKE 2 and RRP6, respectively, are potential candidates for degradation of polyuridylated miRNAs (268).

MECHANISMS AND REGULATION OF PLANT MICRORNA ACTIONS

Target Messenger RNA Cleavage

Pairing between most plant miRNAs and their target mRNAs results in cleavage of their target miRNAs within the region of pairing, yielding 5′ and 3′ cleavage fragments (3, 67). The PIWI domain of AGO proteins, which forms a fold analogous to RNase H, constitutes the catalytic center (195, 271). *Arabidopsis* AGO1, AGO2, AGO4, AGO7, and AGO10 have the cleavage activity (11, 28, 97, 160, 179).

Detection of miRISC 3′ cleavage products in membrane-bound polysome fractions provides important evidence that target mRNA cleavage occurs on the rough endoplasmic reticulum (ER) (124). Furthermore, reduction of the association of AGO1 with membranes in a mutant for *3-HYDROXY-3-METHYLGLUTARYL CoA REDUCTASE 1*, an enzyme required for the biosynthesis of essential membrane components, coincides with compromised target mRNA cleavage (22), suggesting that ER localization is required for miRNA-mediated target mRNA cleavage.

Most 5′ and 3′ cleavage products are degraded by exonucleases. The uncapped 3′ cleavage products become suitable substrates for 5′ to 3′ exoribonucleases. A fraction of the 3′ cleavage products, including those showing unique sequence features and those generated from genes of specific functional groups (190), are degraded by cytoplasmic EXORIBONUCLEASE4 (198), whereas the rest are degraded by yet-to-be identified exonucleases. The capped 5′ cleavage products are first uridylated by HESO1 (189) and then degraded by RISC-INTERACTING CLEARING 3′-5′ EXORIBONUCLEASE 1 (RICE1) and RICE2 (289). The RNA exosome cofactors SKI2, SKI3, and SKI8 are also required for the degradation of uridylated 5′ cleavage products (20). However, CSL4, a noncatalytic component of the RNA exosome, and RRP6L, a nuclear subunit that associates with the RNA exosome, are dispensable for the degradation of miRISC 5′ cleavage products (189).

The cleavage products of transcripts targeted by 22-nt miRNAs, instead of being degraded, are stabilized by SUPPRESSOR OF GENE SILENCING 3 and converted by RNA-DEPENDENT RNA POLYMERASE 6 into double-stranded RNAs. The double-stranded RNAs are subsequently processed by DCL4 into 21-nt phased secondary siRNAs (phasiRNAs), which can, like miRNAs, guide AGO1 to cleave their target mRNAs (60).

Translational Repression

miRNAs can also downregulate gene expression through translational repression. Whereas target mRNA cleavage results in reduction in the levels of target mRNAs, translational inhibition leads to reduced accumulation of proteins translated from target mRNAs. The occurrence of both target mRNA cleavage and translational repression causes a disproportional decrease in protein levels

versus mRNA levels. In animals, a high degree of sequence complementarity between miRNA and its target favors target mRNA cleavage, whereas mismatched miRNA/target sequence is more often associated with translational repression (55). In plants, most miRNAs and their targets have near-perfect sequence complementarity. However, translational repression is widespread and can predominate (21). HYL1, also called DRB1, is required for miRNA biogenesis and miRNA-mediated target mRNA cleavage, whereas DRB2, another DRB family member that interacts with DCL1, determines miRNA-mediated translational repression. DRB2 represses HYL1 expression to facilitate the selection of the translational repression mode (186). Other mechanisms that promote selection of the mode of translational repression remain unknown.

AGO1-, AGO7-, and AGO10-miRISCs can carry out translational repression (21, 83). The mechanisms of translational repression executed by AGO1-miRISCs differ depending on the position of miRNA target sites (92). AGO1-miRISCs targeting 5′ untranslated regions (UTRs) block ribosome recruitment and translation initiation, whereas AGO1-miRISCs targeting open reading frames can block ribosome movement and translation elongation (92). Although these RISCs affect different steps of protein translation, their repressive effects on protein translation both likely result from steric hindrance. The association of AGO1 and miRNAs with polysomes supports the steric hindrance theory (117, 127). AGO1-miRISCs that bind 3′ UTRs also repress translation initiation. However, unlike 5′ UTR-binding AGO1-miRISCs, which repress both cap-dependent and cap-independent translation, 3′ UTR-binding AGO1-miRISCs repress only cap-dependent translation (92). Thus, 3′ UTR-binding AGO1-miRISCs must affect a step unique to cap-dependent translation.

Multiple factors that selectively regulate translation repression have been identified, including KATANIN (21), VARICOSE (21), SUO (260), and ALTERED MERISTEM PROGRAM1 (AMP1) (127). AMP1 is a protein integrated into the membrane of rough ER and prevents association between miRNA target transcripts and polysomes bound to ER membrane, thereby blocking translation of miRNA target transcripts (127). VARICOSE is homologous to the mammalian decapping activator Ge-1. SUO is a large protein that colocalizes with the decapping activator DCP1 (260). Identification of VARICOSE and SUO as factors that regulate miRNA-mediated translational repression suggests that mRNA decapping is involved in the process. KATANIN is a subunit of the microtubule-severing enzyme, suggesting that microtubule dynamics somehow contribute to miRNA-mediated translational repression in plants.

The requirement of ER membrane protein AMP1 to sequester miRNA target transcripts from membrane-bound polysomes provides important evidence that miRNA-mediated translational repression takes place on the ER (127). The requirement of the P-body-localized proteins VCS and SUO for mRNA decapping and the association of AGO1 with rough ER and P-bodies suggest that some components of P-bodies may influence miRNA-mediated translational repression on the ER or P-bodies are also sites of miRNA-mediated translational repression (127, 245).

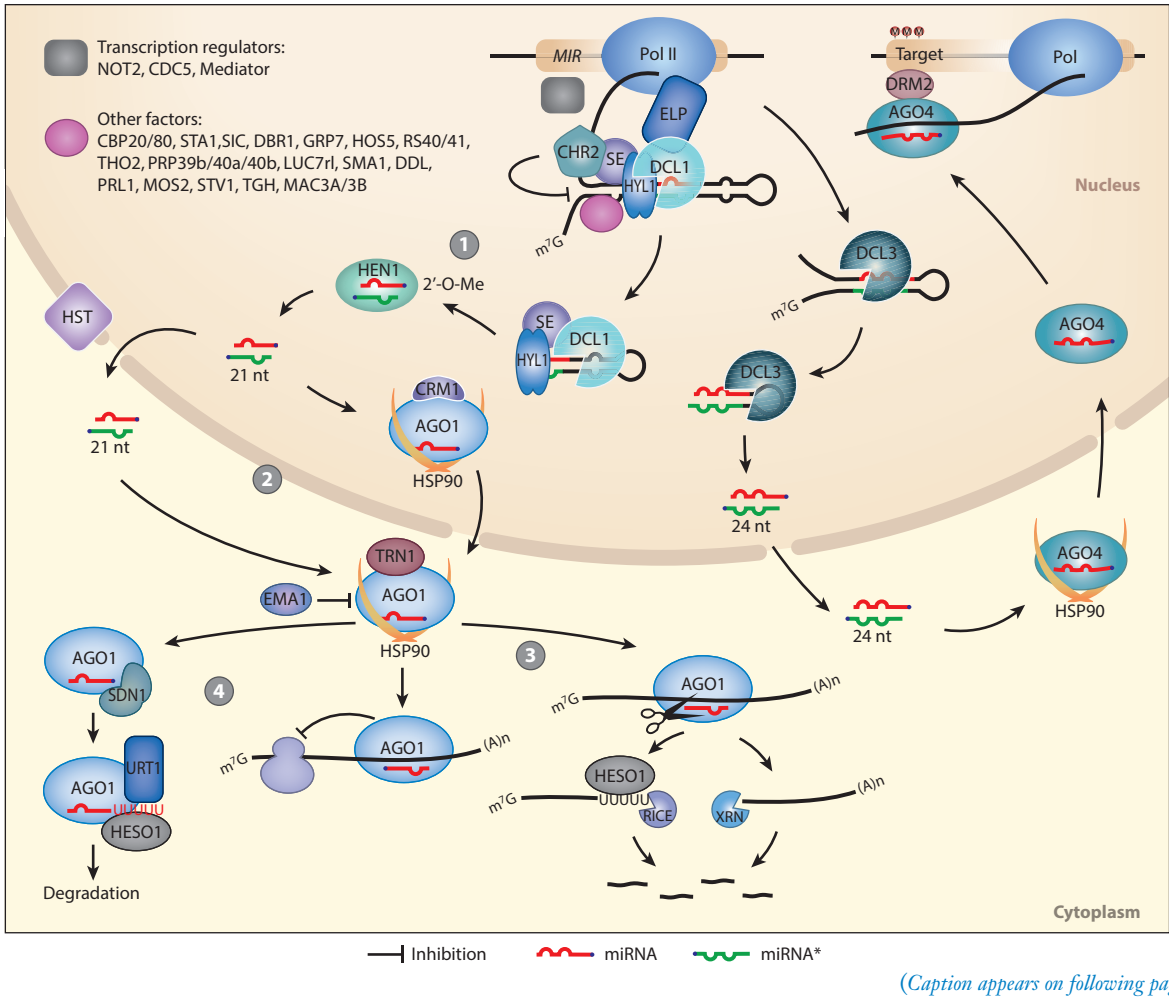
DNA Methylation

The number of studies on miRNA-dependent DNA methylation is limited. However, two different studies provide compelling evidence that miRNAs are capable of directing DNA methylation. Bao et al. (8) found that complementarity between *PHB* and *PHV* mRNA and miR165/166 is required for methylation of *PHB* and *PHV* genes. In rice, a proportion of pri-miRNAs can be processed into canonical miRNAs (21 nt) and long miRNAs (24 nt) by DCL1 and DCL3, respectively. These long miRNAs, like heterochromatic siRNAs in the RNA-directed DNA methylation pathway, are sorted into the effector AGO4 and, possibly by base pairing with target loci-derived transcripts, direct DNA methylation (240). These findings suggest that directing DNA methylation is another mode of miRNA action, which is not at the posttranscriptional level,

but at the transcriptional level, adding further complexity to miRNA-mediated regulation of gene expression. Future studies should investigate the universality of this mode of miRNA action. The major steps of miRNA biogenesis and actions and factors involved in these steps are illustrated in **Figure 1**.

Regulation of MicroRNA Activities

Not only miRNA biogenesis, but also miRNA activities are under tight regulatory control. AGO proteins other than AGO1 sequester certain miRNAs and block miRNA activities. AGO10 is expressed in shoot and floral meristem cells and later in the adaxial side of the cotyledons and leaf primordia. During shoot apical meristem development, it specifically sequesters miR165/166, prevents repression of HD-ZIP III gene expression by miR165/166 in AGO10-expressing regions, and promotes shoot apical meristem development (296, 297). Association of miR165/166 with AGO10 even triggers the degradation of miR165/166, leading to reduced accumulation of miR165/166 (267). Upon virus infection, AGO18 sequesters miR168 to derepress AGO1 to enhance antiviral RNA interference in rice (239). Similarly, miR528 is sequestered by AGO18,



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Models depicting miRNA biogenesis (①), miRISC assembly (②), and miRNA actions and turnover (③, ④). Models depicting miRNA biogenesis, miRISC assembly, and miRNA actions and turnover. MIRs are first transcribed by RNA Pol II into pri-miRNAs. Pri-miRNAs are then, via two steps, processed by DCL1 or DCL3 into 21-nt and 24-nt miRNA duplexes, respectively. HYL1 and SE facilitate pri-miRNA processing by forming a dicing complex with DCL1. Pri-miRNA processing occurs in a transcription- and splicing-coupled manner, with many transcription regulators and splicing factors regulating this process. Proteins that modulate the levels, posttranslational modifications, or activities of key pri-miRNA processing factors also regulate pri-miRNA processing. HEN1 catalyzes 2′-O-Me of the 3′ ribose of miRNA duplexes. Whereas 24-nt miRNAs are loaded into AGO4 likely in the cytoplasm and then reenter the nucleus to mediate DNA methylation, 21-nt miRNA duplexes are exported by HST for miRISC assembly in the cytoplasm to direct target mRNA cleavage and translational repression on the endoplasmic reticulum. Alternatively, 21-nt miRNAs are loaded into AGO1 in the nucleus and then exported by CRM1 (EXPO1). The cleavage products of miRISC are degraded by XRN4 and RICE1/2. miRNAs turnover entails SDN1-mediated 3′ truncation and UTP:RNA URT1- and HESO1-mediated 3′ uridylation. The balance between miRNA biogenesis and miRNA turnover determines the steady-state levels of miRNAs. Abbreviations: 2′-O-Me, 2′-O-methylation; AGO1/4, ARGONAUTE1/4; CBP20/80, CAP-BINDING PROTEIN 20/80; CDC5, CELL DIVISION CYCLE 5; CHR2, CHROMATIN REMODELING FACTOR 2; DBR1, RNA DEBRANCHING ENZYME 1; DCL, DICER-LIKE; DDL, DAWDLE; DRM2, DOMAINS REARRANGED METHYLTRANSFERASE 2; ELP, ELONGATOR PROTEIN; EMA1, ENHANCED MIRNA ACTIVITY 1; GRP7, GLYCINE-RICH RBP 7; HEN1, HUA ENHANCER 1; HESO1, HEN1 SUPPRESSOR1; HOS5, HIGH OSMOTIC STRESS GENE EXPRESSION 5; HSP90, HEAT SHOCK PROTEIN 90; HST, HASTY; HYL1, HYPONASTIC LEAVES 1; LUC7rl, LETHAL UNLESS CBC 7 RL; MAC3A/3B, MOS4-ASSOCIATED COMPLEX 3A/3B; miRNA, microRNA; miRNA*, the strand that pairs with miRNA; *MIR*, miRNA gene; miRISC, miRNA-induced silencing complex; MOS2, MODIFIER OF SNC1, 2; NOT2, NEGATIVE ON TATA LESS 2; nt, nucleotide; Pol II, polymerase II; pri-mRNA, primary microRNA; PRL1, PLEIOTROPIC REGULATORY LOCUS1; PRP, PRE-MRNA-PROCESSING PROTEIN; RICE 1/2, RISC-INTERACTING CLEANING 3′-5′ EXORIBONUCLEASE 1/2; RS, ARGININE/SERINE-RICH SPLICING FACTOR; SE, SERRATE; SDN1, SMALL RNA DEGRADING NUCLEASE 1; SIC, SICKLE; SMA1, SMALL1; STA1, STABILIZED1; STV1, SHORT VALVE 1; TGH, TOUGH; TRN1, TRANSPORTIN 1; URT1, URIDYLYLTRANSFERASE 1; XRN4, EXORIBONUCLEASE4.

leading to derepressed expression of L-ASCORBATE OXIDASE, increased production of reactive oxygen species (ROS), and enhanced plant resistance to viruses (238).

Target mimicry also serves as one mechanism that negatively regulates miRNA activities. The noncoding RNA *INDUCED BY PHOSPHATE STARVATION1* (*IPS1*) is complementary to miR399 but cannot be cleaved by it, owing to a mismatched loop at the miRNA cleavage site. Under phosphate-starved conditions, *IPS1* can be induced to sequester miR399 and prevent persistent cleavage of *PHOSPHATE 2* (*PHO2*) mRNA by accumulating miR399, achieving homeostatic control of shoot phosphate contents (64). Genome-wide computational analysis further identified a number of long noncoding RNAs and protein-coding transcripts as endogenous miRNA target mimics in *Arabidopsis* and rice (91, 237).

BIOLOGICAL FUNCTIONS OF MICRORNAS IN PLANT–ENVIRONMENT INTERACTIONS

Target mimicry: a regulatory mechanism for inhibition of miRNA activity by decoying RNAs binding to and sequestering miRNAs via complementary sequences

miRNAs are key regulators of plant development. There are excellent reviews on the roles of miRNAs in plant development (32, 42, 101, 207). Here, we summarize the functions of miRNAs in the regulation of phenotypic plasticity, abiotic/biotic responses, and symbiotic/parasitic interactions.

Biological Functions of MicroRNAs in Developmental Plasticity

miRNAs not only act as the master regulators of plant development but also are involved in the regulation of phenotypic plasticity triggered by various environmental stimuli, such as light, temperature, and nutrients.

Light. miR156, the most evolutionarily conserved miRNA, targets a subset of *SPL* genes (6). Apart from functioning in plant developmental phase transitions (225), the miR156-*SPL*s module also acts as a negative regulator of shade-avoidance syndrome, an adaptive strategy of plants to avoid shade from canopy or compete for light with their neighbors (30). In *Arabidopsis*, shade can activate *PHYTOCHROME INTERACTING FACTOR*s that directly bind to and transcriptionally repress the expression of *MIR156*s, leading to upregulation of *SPL*s. SPLs mediate diverse morphological changes that are associated with enhanced shade-avoidance syndrome responses (242).

Upon UV-B radiation in *Arabidopsis*, miR396 can be induced to repress its target *GROWTH REGULATING FACTOR*s (*GRF*s). By targeting *GRF*s, miR396 mediates the inhibition of leaf growth, which is an adaptive strategy of plants to arrest the cell cycle, allowing time to repair UV-B-induced DNA damage (29, 99).

In rice, miR2118 targets a long noncoding RNA, *PHOTOPERIOD-SENSITIVE GENIC MALE STERILITY 1 TRANSCRIPT* (*PMS1T*), and triggers the production of phasiRNAs under long-day conditions, leading to photoperiod-sensitive male sterility. A single-nucleotide polymorphism from the locus encoding *PMS1T* might mediate the recognition of *PMS1T* by miR2118 and photoperiod-sensitive male sterility in Nongken 58S, a valuable germplasm that is initially used in the two-line hybrid rice breeding (56).

Shade-avoidance syndrome: typically includes increased height and leaf angle, decreased branching and leaf blade area, and early flowering

Photoperiod-sensitive male sterility: plant mutant whose male fertility is regulated by photoperiod, generally as male-sterile under long-day conditions but fertile under short-day conditions

Temperature. In *Arabidopsis*, miR156 and miR172 are temperature responsive and coordinately fine-tune flowering time after exposure to low ambient temperature (120). This is one of the most striking thermoadaptive strategies of plants in response to temperature fluctuations. miR156 and miR172 have been used to define a well-known age-dependent flowering pathway under normal growth conditions (208, 225). The decline in miR156 levels with age results in upregulation of *SPL*s, which accelerate flowering by directly activating the expression of key flowering genes such as *LEAFY*, *FRUITFULL*, and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (226, 236, 248). SPLs also directly activate *MIR172* to promote flowering by inducing the floral inducer *FLOWERING LOCUS* (*FT*) via miR172-mediated repression of *AP2* (103, 153). In response to low ambient temperature, miR156 is upregulated and mainly represses *SPL3*. Repression of *SPL3* leads to downregulation of *FT* and *FRUITFULL* and the delay in flowering (108, 120). Coincident with upregulation of miR156, miR172 is downregulated. Downregulation of miR172 in turn activates *AP2* to repress *FT*, bringing about adaptive inhibition of flowering (120). Downregulation of miR172 results mainly from reduced accumulation of FCA, an RNA binding protein that can bind to pri-miR172 and promote the processing of pri-miR172 (102).

Nitrate and aluminum. Several miRNA regulatory modules play important roles in the modification of root system architecture (RSA) in response to various environmental stimuli. Much developmental plasticity of RSA is achieved through miRNA-mediated modulation of auxin signaling (15). Conserved miR167 negatively regulates auxin signaling by targeting *AUXIN RESPONSE FACTOR 8* (*ARF8*). Under high nitrate conditions in *Arabidopsis*, miR167 level goes down to allow *ARF8* to accumulate in the pericycle and lateral root cap, thereby enhancing auxin signaling to promote lateral root initiation but to inhibit root elongation. This represents a strategy used by plants to fine-tune their developmental response to nutrients (69) (**Figure 2**). Consistent with increased auxin signaling, the auxin receptor *AUXIN SIGNALING F-BOX 3* (*AFB3*) gene is induced during early nitrate response in *Arabidopsis*. However, some metabolites formed during nitrate reduction and assimilation can induce miR393 expression to repress *AFB3* expression (221). Thus, AFB3-mediated increase of auxin signaling and RSA modification in response to nitrate changes are transient. In contrast to miR393 induction during nitrate metabolism in *Arabidopsis*, miR393 is downregulated upon toxic aluminum stress in barley. Alleviated repression

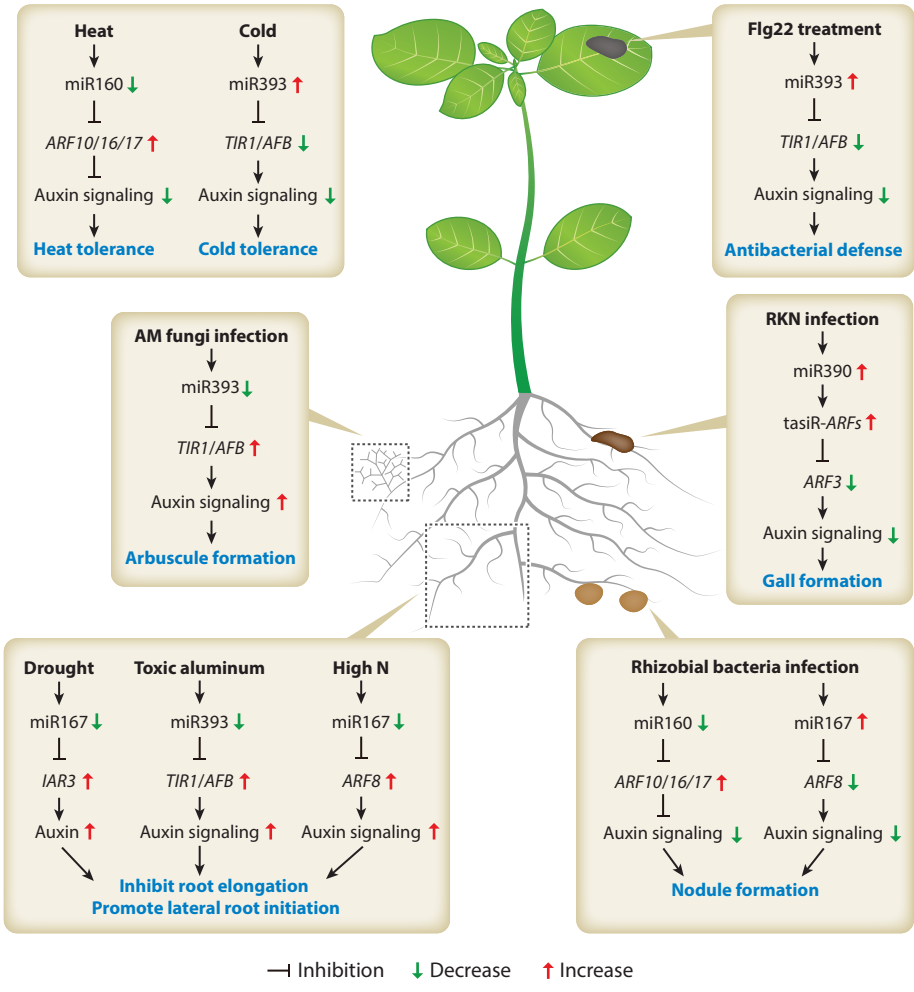


Figure 2

miRNA modules integrating environmental stimuli and auxin pathways. The miRNAs miR160, miR167, miR390, and miR393 respond to environmental stimuli and fine-tune auxin signaling via targeting auxin pathway genes to confer heat/cold tolerance, antibacterial defense, symbiotic nodule/arbuscule formation, parasitic gall formation, and plasticity of root system architecture. *TIR1/AFB* encode auxin receptors; *IAR3* encodes an indole-3-acetic acid–Ala hydrolase that releases bioactive auxin from an inactive storage form; flg22, a 22–amino acid peptide from eubacterial flagellin, can be perceived by host plants to trigger basal immunity; AM fungi generate symbiotic interactions with host plants by forming arbuscules; and RKN generate parasitic interactions with host plants by forming a feeding structure termed a gall. Abbreviations: AM, arbuscular mycorrhizal; *ARF*, *AUXIN RESPONSE FACTOR*; *IAR3*, *IAA-ALA RESISTANT 3*; miRNA, microRNA; N, nitrogen; RKN, root-knot nematode; tasiR, *trans*-acting small interfering RNA; *TIR1/AFB*, *TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX*.

of the miR393 target auxin receptor genes *TRANSPORT INHIBITOR RESPONSE1* (*HvTIR1*) and *HvAFB* enhances auxin signaling and contributes to inhibition of root elongation by aluminum stress (7) (**Figure 2**). By modulating developmental plasticity of RSA, miRNA-mediated integration of environmental stimuli and plant hormone signaling, such as auxin signaling, may promote plant adaptation to dynamically changing environments (**Figure 2**).

Biological Functions of MicroRNAs in Abiotic Stress Responses

Being sessile, plants are constantly exposed to adverse conditions, such as extreme temperatures, high salinity, drought, and nutrient deficiency. These abiotic stresses are the major factors limiting the geographical distribution of plants and crop yield. To reduce the adverse effects of these abiotic stresses, plants have evolved specific mechanisms allowing them to tolerate and survive stressed conditions. miRNA-mediated regulation of gene expression is an important mechanism of plant response to abiotic stresses.

Heat and cold. Plant response to heat stress involves induction of HSPs to protect cellular proteins from denaturation (87). Plants can also undergo heat stress priming, wherein plants acquire higher stress tolerance after exposure to a moderate stress (159). Multiple miRNAs participate in these heat responses. *Arabidopsis* miR398 is involved in the basic heat tolerance response. Two heat shock transcription factors induce *MIR398* expression, and miR398 in turn represses its target genes *COPPER/ZINC SUPEROXIDE DISMUTASE 1* (*CSD1*), *CSD2*, and *COPPER CHAPERONE OF CSD*, whose repression improves heat tolerance by increasing expression of heat shock transcription factors and HSPs. Compared with wild type, transgenic plants expressing miR398-resistant *CSD1*, *CSD2*, and *COPPER CHAPERONE OF CSD* are more sensitive to heat and show a sharp decrease in heat shock transcription factors (71). In cotton (*Gossypium hirsutum*), the downregulation of miR160 mediates the inhibition of auxin signaling via activation of targeting *ARF10/16/17*, leading to cotton anther fertility under heat stress (**Figure 2**). Whereas miR160 is downregulated in heat-tolerant cotton, its overexpression increases heat sensitivity (47).

The miR156-SPLs module may contribute to heat-stress priming. In *Arabidopsis*, recurring heat stress induces miR156, which functions to maintain heat stress memory. In this process, SPLs might act as transcriptional repressors to suppress some genes involved in heat stress memory (199). Another adaptive strategy mediated by miRNAs is found in the heat-tolerant crop sunflower, which has evolved a specific miR396-*HaWRKY6* regulatory module that may function in high-temperature protection during development. Indeed, transgenic plants expressing miR396-resistant *HaWRKY6* have impaired heat tolerance (68).

In plants, cold stress induces a different set of responses. In sugarcane and rice, cold stress induces the evolutionarily conserved miR319. Overexpression of miR319 downregulates *TEOSINTE BRANCHED1*, *CYCLOIDEA*, *PROLIFERATING CELL NUCLEAR ANTIGEN BINDING FACTOR* genes and enhances cold tolerance in both species (210, 256), suggesting that miR319 acts as a positive regulator of cold tolerance. Cold-inducible miR393 also positively regulates cold tolerance in switchgrass by targeting the auxin receptor gene *TIR1/AFB* (**Figure 2**). Overexpression of miR393 or mutation of *TIR1/AFB* enhances cold tolerance, which is accompanied by increased expression of cold-responsive genes (145).

Drought. To adapt to drought conditions, plants modify their RSA by inhibiting primary root growth and increasing development of lateral roots for maximum water assimilation (70). As a well-known morphogenic trigger for lateral root formation (15), accumulation of the bioactive auxin indole-3-acetic acid (IAA) contributes to the plastic changes in RSA. In *Arabidopsis*, miR167a is downregulated, and its target *IAA-ALA RESISTANT 3*, which encodes an IAA-Ala hydrolase that releases IAA from an inactive storage form of auxin, is derepressed to promote IAA accumulation and development of lateral roots (112) (**Figure 2**).

miR165/166 act as important regulators of plant growth and development via targeting transcripts encoding HD-ZIP III. These miRNAs also negatively control drought tolerance. Downregulation of miR165/166 confers enhanced drought resistance in *Arabidopsis* and rice, which

could be a result of HD-ZIP III–mediated elevation of ABA levels (252, 279). In *Arabidopsis*, drought also downregulates miR169 to allow the induction of *NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT ALPHA 5* (*NFYA5*). Overexpression of *NFYA5* enhances drought tolerance, whereas the *nfya5* mutant and miR169-overexpressing plants are hypersensitive to drought, suggesting that NFYA5 confers drought tolerance. NFYA5 likely does so by mediating a stress-responsive transcriptional cascade (130). The soybean miR169-*GmNFYA3* module also functions in drought tolerance, suggesting conserved regulatory roles for miR169-*NFYA* modules in plant drought resistance (164). Apart from downregulating miR169, plants have evolved other mechanisms to ensure the induction of NFYA5 upon drought. In *Arabidopsis*, the *NFYA5* antisense gene *ENHANCING RING FINGER* (*NERF*) can produce siRNAs that have sequences similar to miR169 but cannot direct cleavage of *NFYA5* mRNA. By competing with miR169, *NERF* siRNAs prevent miR169-mediated repression of *NFYA5* expression. This mechanism contributes to *NFYA5*-mediated drought tolerance, as evidenced by high accumulation of NFYA5 and enhanced drought tolerance in *NERF* overexpression lines and the opposite phenotype in *NERF* knockdown lines (66). Recently, Du et al. (51) demonstrated that miR169i and miR169l are induced and positively regulate *NFYA5* expression via translational activation in response to dehydration shock in *Arabidopsis*.

Nutrient deficiency. To grow successfully under a variety of conditions, plants must be able to survive nutrient deficiencies; moreover, to produce high yields with minimal inputs, crop plants must efficiently use nutrients. Under low-nutrient conditions, a handful of miRNAs have been identified as master regulators mediating posttranscriptional regulation of nutrient homeostasis. Identification of these miRNAs broadens our understanding of the molecular mechanism of nutrient-starvation responses and can inform approaches to improve crop nutrient use efficiency.

Some miRNA regulatory modules affect general nutrient uptake. It was recently discovered that miR166 targets *RICE DOF DAILY FLUCTUATIONS 1* (*RDD1*), which encodes a Dof transcription factor. miR166-mediated regulation results in a diurnally oscillating expression pattern of *RDD1*. Overexpression of miR166-resistant *RDD1* significantly increases nutrient ion (NH_4^+, PO_4^{3-}, and K^+) uptake and accumulation under low-nutrient conditions (93), suggesting that manipulation of miR166-*RDD1* may be applied in rice breeding for high nutrient use efficiency.

In contrast, other modules affect specific nutrients. miRNA-mediated regulation affects uptake of macronutrients such as phosphate, nitrogen, and sulfur as well as micronutrients such as copper.

Phosphate. Phosphorus (Pi) is an essential macronutrient for macromolecule biosynthesis, energy transfer, enzymatic activity, and signal transduction in all living organisms (209). Moreover, Pi levels often limit agricultural yields. The first miRNA found to be involved in Pi-starvation response in *Arabidopsis* is miR399 (65). Under Pi-sufficient conditions, the putative ubiquitin-conjugating enzyme PHO2 adds polyubiquitin to the Pi transporter PHOSPHATE TRANSPORTER 1 (PHT1) and targets PHT1 for degradation (86), thereby maintaining optimum Pi uptake. Low-Pi stress strongly induces miR399 in *Arabidopsis* and rice to downregulate *PHO2*. Downregulation of *PHO2* by miR399 increases the level of PHT1 and thus increases Pi acquisition and translocation (5, 10, 65, 85). Overexpression of miR399 under normal conditions causes overaccumulation of Pi in shoots and symptoms of Pi toxicity, similar to mutations in *PHO2* in *Arabidopsis* and rice (5, 65, 85).

In plants subjected to Pi starvation, miR399 accumulates in vascular tissues where *PHO2* is expressed (5), and reciprocal grafting experiments demonstrated that miR399 can move from shoot to root (137, 170). This indicates that miR399 functions as a systemic signal to regulate Pi uptake and translocation under low-Pi conditions. Movement of miR399 may be crucial in the early Pi

starvation response because expression of *MIR399* during this early response in *Arabidopsis* roots is low compared with expression in shoots (137). As Pi starvation progresses, increasing accumulation of miR399 in both roots and shoots leads to excessive uptake of Pi; negative regulators may function to antagonize miR399-mediated Pi acquisition and translocation. One such negative regulator is *IPS1*, a noncoding RNA harboring a sequence motif complementary to miR399. In *Arabidopsis*, Pi starvation can induce expression of *IPS1*. Owing to the central bulge at miR399-*IPS1* base-pairing region, *IPS1* sequesters, instead of being cleaved by, miR399 (64, 193). *IPS1* knockout plants overaccumulate Pi in shoots (193), suggesting crucial regulatory roles of *IPS1* in maintaining Pi homeostasis during Pi starvation.

miRNA-target module: the regulatory unit of miRNA-mediated posttranscriptional/transcriptional regulation on targeting gene

Evolutionarily conserved miR827 is also strongly induced by Pi deprivation in several plants (75, 84, 138). In *Arabidopsis*, miR827 targets *NITROGEN LIMITATION ADAPTATION* (*NLA*), which encodes a ubiquitin E3 ligase. Similar to the miR399-*PHO2* module, the miR827-*NLA* module also regulates ubiquitination of the Pi transporter PHT1 to balance Pi uptake and translocation during Pi starvation (104, 139, 172). Of note, in contrast to the miR827-*NLA* module in *Arabidopsis*, rice miR827 does not target the *NLA* homolog but targets two members of the *PHT5* family (138, 273), which function as vacuolar Pi transporters to mediate Pi storage or remobilization in rice and *Arabidopsis* (135, 142, 222). More recently, on the basis of target prediction analysis, Lin et al. (140) found that miR827 conservatively targets *PHT5* homologs in most angiosperms, but it preferentially targets *NLA* homologs in Brassicaceae and Cleomaceae, suggesting that the target of miR827 has shifted from *PHT5* to *NLA* during evolution. This new miRNA-target module has adopted a different regulatory mechanism but still participates in the same biological process as its original module, which indicates the importance of miR827 for regulation of cellular Pi homeostasis.

Nitrogen and sulfur. Nitrogen is another limiting factor for plant growth and crop productivity. Nitrate deficiency induces changes in miRNA expression in multiple plants (62, 96, 171). *Arabidopsis* exhibits downregulation of miR169a and upregulation of its target *NFYA* family members upon nitrate deficiency. The miR169-*NFYA* module likely regulates the adaptive response of nitrate uptake systems, as evidenced from the observation that overexpression of miR169 reduces expression of multiple nitrate transporter genes and causes an early senescence phenotype (290). The same regulatory role of miR169-*NFYA* in response to nitrate starvation was found in wheat, where the module also shows response to low phosphate availability. Overexpression of *TaNFYA-B1* significantly increased both nitrate and phosphate uptake and grain yield in a field experiment with different levels of nutrient supply (180), suggesting the great potential of the miR169-*NFYA* module in breeding crops targeting high yield with less fertilizer input. Actually, more miRNA modules may act as engineering targets for enhancing nutrient usage efficiency in plants, given their consistent responses to both nitrate and phosphate limitations, including the miR827-*NLA* and miR444-*ANR1* modules (104, 175, 254).

Sulfur availability also regulates plant growth, vigor, and crop yield. In response to sulfur deficiency, miR395 is strongly induced in both *Arabidopsis* and rice to target members of ATP sulfurylases and SULFATE TRANSPORTER2;1, both of which are key factors of the sulfate metabolism pathway and mediate sulfate assimilation and uptake or transport (100, 106, 107, 136, 269). Plants overexpressing miR395 display S-starvation symptoms in both *Arabidopsis* and rice (106, 136, 269), further suggesting that miR395 acts as a negative regulator of plant sulfur response and metabolism. This raises the question of why a negative regulator is induced in response to sulfur deficiency. Because SULFUR LIMITATION 1, the transcriptional activator of sulfate transporters and ATP sulfurylases (106, 152), mediates transcriptional activation of miR395, miR395 may be induced to maintain optimal levels of sulfate transporters and ATP sulfurylases to achieve

sulfur homeostasis under low sulfur condition. miR395 can also be induced by redox signaling under sulfur-deficient conditions in *Arabidopsis* (95), suggesting complex regulation of miR395 expression in response to sulfur deprivation.

Copper. Copper is an essential micronutrient for plants. It plays important roles in photosynthetic and respiratory electron transport, ROS scavenging, cell wall metabolism, and ethylene perception (177). Plants have evolved a set of miRNAs to downregulate dispensable copper-containing proteins when copper is limited, thus saving copper for essential biological processes such as photosynthesis. Copper-responsive miRNAs include miR397, miR398, miR408, and miR857 (1, 249). In response to copper deficiency, all these miRNAs are transcriptionally induced by the transcription factor SPL7, which plays a conserved role in copper sensing, like its green alga homolog COPPER RESPONSE REGULATOR 1 (71, 250, 278). Induction of miR398 leads to downregulation of CSD1 and CSD2 (13, 205, 249), two copper-containing proteins important for ROS scavenging and oxidative stress tolerance in *Arabidopsis* (205). miR397, miR408, and miR857 downregulate dispensable copper-containing proteins plantacyanin and laccase (1). In contrast to unicellular green alga, higher plants lack the COPPER RESPONSE REGULATOR 1–mediated and iron-dependent cytochrome c_6 pathway for photosynthesis during copper deficiency and have a greater demand for copper supply (156). Evolved SPL7-miRNA copper-containing protein modules in higher plants may represent an adaptive regulatory cascade to satisfy the photosynthetic requirement for copper under copper-deprivation conditions. Accordingly, overexpression of miR408 enhances photosynthesis, growth, and seed yield in diverse higher plants (169).

Biological Functions of MicroRNAs in Biotic Stress Responses

In nature, plants are constantly attacked by pathogens including fungi, bacteria, viruses, and insects. miRNAs are important players that mediate plant immune responses to biotic stresses. So far, at least 21 miRNA-target modules are involved in plant defense against pathogens. Many miRNA pathway mutants, such as *dcl1*, *hen1*, and *ago1*, exhibit compromised resistance to bacteria or viruses (133, 161, 163).

Antibacterial and antifungal immunity. Upon attack by bacteria and fungi, host plants can recognize conserved pathogen-associated molecular patterns (PAMPs) and activate basal defense PAMP-triggered immunity (PTI). In *Arabidopsis*, miR393 is transcriptionally induced by PAMP flagellin (flg22) to downregulate the levels of the F-box auxin receptors TIR1, AFB2, and AFB3; dampen auxin signaling; and benefit plant defense against the virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (162) (**Figure 2**). miR160a is induced and contributes to PTI by targeting ARFs and increasing callose deposition (200). In rice, miR398b is induced in defense against the blast fungus *Magnaporthe oryzae* and contributes to PTI (132). Rice subspecies-specific miR7695 targets the alternatively spliced transcript of *NATURAL RESISTANCE-ASSOCIATED MACROPHAGEPROTEIN 6* and can confer plant resistance against *M. oryzae* (27). In soybean roots, miR393 and miR166 are induced when the fungus-like pathogen *Phytophthora sojae* invades and are involved in PTI (235). Although many miRNAs are induced to repress negative regulators of plant defense, some are downregulated to help strengthen plants against pathogens. In *Arabidopsis*, miR398b and miR773 are downregulated by flg22 to enhance callose deposition and plant resistance against bacteria (200). Upon bacterial and fungal infection, miR400 is downregulated (174, 283), leading to enhanced expression of *PENTATRICOPEPTIDE REPEAT1/2* genes, both of which encode mitochondria-localized pentatricopeptide repeats and likely contribute to

PTI by controlling ROS metabolism (213). In rice, miR164a is downregulated, and its target transcription factor OsNAC60 is derepressed to enhance defense response against *M. oryzae* (234).

Pathogens fight against PTI by delivering effector proteins into plant cells. To resist the invasion of pathogens, plant cells employ R proteins to recognize effector proteins and initiate stronger effector-triggered immunity (ETI) (35, 176). In contrast to miR393, which binds AGO1 and participates in PTI (162), miR393* is induced by avirulent *P. syringae* pv. *tomato* DC3000 carrying the effector *avrRpt2*. This miRNA is preferentially loaded into AGO2 and contributes to ETI by promoting secretion of antimicrobial PATHOGENESIS-RELATED PROTEIN via repression of *MEMB12* expression. MEMB12 is a Golgi-localized, SOLUBLEN-ETHYLMALEIMIDE SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTOR protein (285). Similarly, miR863-3p is induced by DC3000 expressing *avrRpt2* and helps increase secretion of PATHOGENESIS-RELATED PROTEIN, although it binds AGO1 and represses *ATYPICAL RECEPTOR-LIKE PSEUDOKINASE 1* and *2* to achieve this effect (165). In barley, the miR398-*CSD1* module can contribute to an *R*-gene *MILDEW RESISTANCE LOCUS A* (*MLA*)-mediated ETI. Upon infection by barley powdery mildew fungus, activation of *MLA* can downregulate miR398 to derepress its target gene *CSD1*, thereby enhancing CSD1-mediated ROS accumulation and cell death (247).

However, R-protein-triggered ETI has a fitness cost for plants and thus is prevented in the absence of pathogen attack and attenuated after defense (211). A panel of miRNAs targets the transcripts of *R* genes and triggers production of phasiRNAs, preventing R-protein-triggered autoimmunity in the absence of pathogen infection (**Figure 3**). It was initially found that the 22-nt miRNAs miR2118, miR1507, and miR2109 can target and cleave the mRNAs of *nucleotide binding and leucine-rich repeat* (*NB-LRR*) genes (the largest group of plant *R* genes) and trigger production of phasiRNAs in *Medicago* (275). Subsequently, it was proven that miRNA-mediated regulation of *R*-gene expression is a conserved mechanism (44, 287). miR6019/6020 targets *TIR-NB-LRR immune receptor N* gene in tobacco (123), miR482/miR2118/miR5300 target *NB-LRR*s with coiled-coil domains in tomato (168, 194), and miR9863 targets *MLA* alleles in barley (141). Upon pathogen infection, pathogen-derived effector proteins repress these miRNA-mediated silencing cascades, and ETI is activated (45). After defense, these miRNAs are derepressed, and *R* genes are repressed again to prevent excessive immunity (**Figure 3**). Interestingly, these miRNA-*R*-gene modules are implicated in age-dependent ETI. Genome-wide elevation of *R*-gene expression during tomato and tobacco growth correlates with reduction of *R*-gene-associated small RNAs (sRNAs) (including miRNAs and phasiRNAs). As a result of the gradual decrease of miR6019/6020 and increased expression of *NB-LRR*s, mature plants are more resistant to tobacco mosaic virus (46).

Antiviral immunity. Similar to bacterial and fungal infections, viral infection also gives rise to alterations in miRNA levels or activities, which are often implicated in the induction of RNA silencing pathway components. Multiple miRNAs including miR535, miR390, and miR171 and a subset of *OsDCL*s and *OsAGO*s are induced when *Rice stripe virus* (RSV) infects rice (50). miR393 and selected rice RNA-dependent RNA polymerases are induced by *Rice dwarf virus* (50). In another study, when RSV infects rice, the monocot-specific miR444 is induced to alleviate transcriptional repression of *RNA-DEPENDENT RNA POLYMERASE1* through targeting three $MIKC^C$-type MADS box proteins, promoting production of antiviral siRNAs and virus resistance (224). Rice AGO18, a cleavage-deficient AGO protein, can be induced by RSV and then bind and sequester miR168, thus releasing its target *AGO1* to confer broad-spectrum virus resistance (239). Apart from affecting expression of RNA silencing components, alterations in miRNA levels or activities may modulate other cellular processes and contribute to plant defense against viruses. For

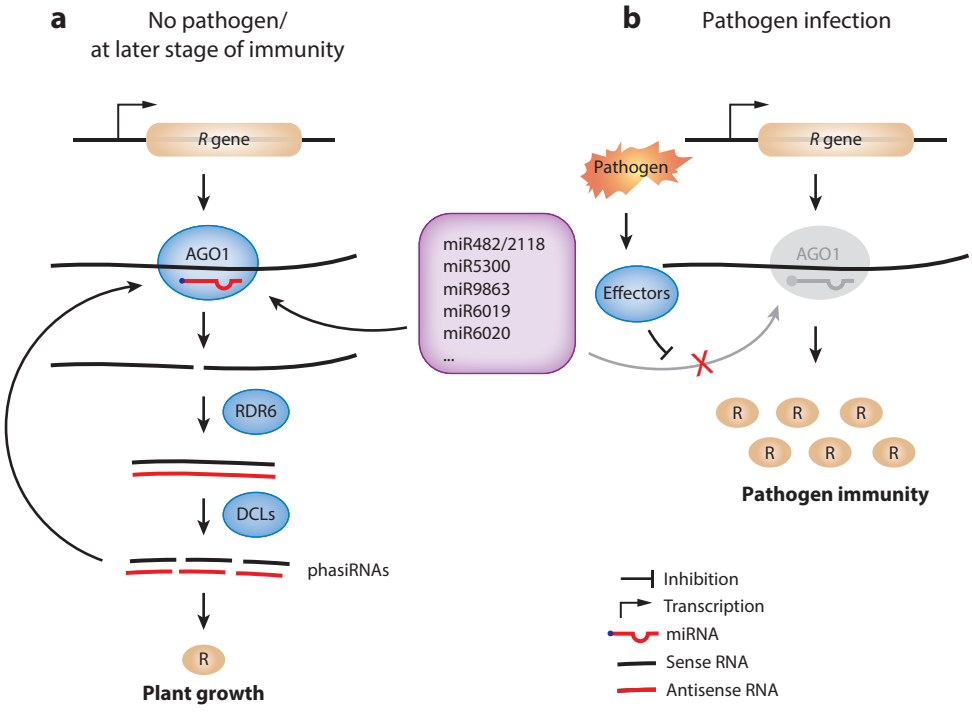


Figure 3

miRNA-mediated regulation of *R* genes. A set of 22-nucleotide miRNAs can target *R* genes and trigger production of phasiRNAs to strengthen *R*-gene silencing in many plants. (*a*) In the absence of pathogen or at a later stage of immunity, these miRNA-*R*-gene modules can be activated to prevent autoimmunity or overinduced immunity, both of which have fitness costs for plants. (*b*) Upon pathogen infection, the silencing mediated by these 22-nucleotide miRNAs is repressed owing to the action of pathogen-effector proteins, resulting in the activation of *R* genes to trigger effector-triggered immunity. Abbreviations: AGO1, ARGONAUTE1; DCL, DICER-LIKE; miRNA, microRNA; phasiRNA, phased secondary small interfering RNA; RDR6, RNA-DEPENDENT RNA POLYMERASE 6.

instance, upon RSV infection, the monocot-specific miR528 is sequestered by AGO18, leading to alleviated repression of its target *L-ASCORBATE OXIDASE*. Elevation of ascorbate oxidase levels increases ROS accumulation and enhances antiviral defense (238).

Anti-insect immunity. In *Arabidopsis*, the miR156-*SPL9* module positively regulates plant resistance to cotton bollworm. High levels of miR156 at the juvenile phase repress *SPL9*, a target of miR156. As SPL9 protects JASMONATE-ZIM DOMAIN protein 3, a repressor of jasmonic acid signaling pathway, from degradation, repression of *SPL9* activates the jasmonic acid signaling pathway and enhances plant defense against insects (151). In *Nicotiana attenuate*, AGO8 is important for plant resistance to larvae of *Manduca sexta* and may mediate protection against *M. sexta* larvae in association with miRNAs (178). In *Cucumis melo*, multiple miRNA-target modules mediate resistance to aphids by repressing the auxin signaling pathway (191).

Cross-kingdom regulation of gene expression. Interestingly, plants employ the miRNA pathway to boost their own immunity and export their miRNAs into some pathogens to silence factors important for virulence and attenuate pathogens. When the hemibiotrophic fungus *Verticillium dahliae* infects *Arabidopsis* and cotton, miR166 and miR159 are induced by and exported

into the fungal hyphae to repress expression of Ca^{2+}-dependent *CYSTEINE PROTEASE-1* and *ISOTRICHODERMIN C-15 HYDROXYLASE*, respectively (282), whose knockout resulted in *V. dahliae* mutants with weak virulence and unable to cause wilt disease (282). Export of sRNAs, including miRNAs, was recently found to be accomplished by plant secretion of extracellular vesicles that can be taken up by fungal cells (25).

miRNA-mediated cross-kingdom regulation: a trans-species regulatory phenomenon of miRNAs in which miRNAs from one species can move and target mRNAs of another interacting species

Biological Functions of MicroRNAs in Symbiotic Processes

To cope with nutrient starvation, legume plants establish symbiotic interactions with soil rhizobial bacteria and arbuscular mycorrhizal fungi, leading to formation of nitrogen-fixing nodules and mycorrhizal arbuscules, respectively. Beneficial to plants, nitrogen-fixing nodules convert free nitrogen to biologically available nitrogen, whereas mycorrhizal arbuscules help plants absorb mineral nutrients efficiently. Their organogenesis involves complicated cellular reprogramming events. miRNA-mediated regulation on either symbiosis-related genes or auxin signaling provides an additional layer of control in fine-tuning the reprogramming events.

Symbiosis-related genes. A complex signaling network controls nodulation. The NOD FACTOR–mediated signaling pathway promotes nodule development, whereas the AUTOREGULATION OF NODULATION (AON) signal–mediated negative feedback system prevents energy-consuming overnodulation (49). Involvement in nodulation was identified first for miR169. miR169 targets the *Medicago truncatula* homolog of *HAP2 MtHAP2-1*, which encodes a symbiosis-specific transcription factor, and spatially refines the mRNAs of *MtHAP2-1* to the nodule meristematic zone. This is essential for differentiation of nodule cells, as transgenic plants expressing miR169-resistant *MtHAP2-1* have impaired nodule growth (39). Infection with rhizobial bacteria induces miR172, which positively regulates nodulation in soybean, *Lotus japonicus*, and common beans (82, 167, 232). During nodulation in soybean, induction of miR172 represses its target *NODULE NUMBER CONTROL 1*, a transcriptional repressor of the early nodulin gene *ENOD40*, and derepresses expression of *ENOD40*, promoting nodule organogenesis. AON signaling to prevent excessive nodulation suppresses miR172 expression (232). Thus, the miR172–*NODULE NUMBER CONTROL 1* regulatory module represents a link between the NOD factor and AON signaling pathways and helps determine nodule numbers in legume plants. Prevention of excessive nodulation is also achieved through induction of miR171. During nodulation in *L. japonicus* and arbuscular mycorrhizal symbiosis in *Medicago*, miR171 is induced to repress *NODULATION SIGNALING PATHWAY 2* (*NSP2*), which encodes a plant-specific GRAS gene family member required for nodule formation. By downregulating *NSP2*, the induction of miR171 prevents overnodulation or overcolonization of roots by mycorrhizal fungi (43, 119). Furthermore, miR393j-3p, which is induced via nodulation, directly targets and represses another nodulin gene, *ENOD93* in soybean, to prevent excessive nodulation (255). In contrast to miRNAs described above, miR396a has reduced expression in response to mycorrhizal fungi infection in *Medicago*. This is accompanied by increased expression of its target *GRF4* (12). Inactivation of miR396 promotes whereas its overexpression inhibits root colonization by mycorrhizal fungi, suggesting that miR396 functions as a negative regulator of formation of mycorrhizal arbuscules.

Auxin signaling. miRNAs posttranscriptionally repress expression of the TIR1/AFB family of auxin receptors and multiple ARFs. miRNA levels are fine-tuned to dictate specific auxin sensitivity at different stages of nodule development and in different nodule tissues. In soybean, miR160 and miR167, which target repressors *ARF10/16/17* and the activator *ARF8*, respectively, act as positive and negative regulators of auxin sensitivity. At the early stage of nodule development, low

auxin sensitivity favors nodule primordia formation (206). To achieve low auxin sensitivity at this stage, miR160 levels are kept low while miR167 is induced (166, 231) (**Figure 2**). Overexpression of miR160 or suppression of miR167 by target mimicry increases auxin sensitivity, resulting in significantly reduced nodule formation (215, 231). However, during nodule maturation, high auxin sensitivity is preferred, and nodules ensure it by accumulating high levels of miR160 (166). The miR393d-*TIR1/AFB3* module negatively regulates soybean nodule development. Nodule tissue-specific expression of miR393d dictates *TIR1/AFB3* expression levels and auxin sensitivity of different nodule tissues (26).

Legume nodules can be categorized into two types based on the persistence of apical meristem: determinate (such as in soybean, *L. japonicus*) and indeterminate (such as in *Medicago*, pea). Two studies revealed that miRNA may dictate differential auxin sensitivity and exert different effects on the development of different types of nodules. miR390 regulates auxin sensitivity by targeting *TAS3* mRNA and triggering production of secondary siRNAs, namely *trans*-acting siRNA (tasiR)-ARFs, that cleave mRNAs of *ARF2/3/4*. Genetic disruption of the miR390-*TAS3*-ARFs module increases auxin sensitivity, promoting rhizobial infection and nodulation in *Medicago* (81). However, owing to depletion of REL3, a key component involved in tasiR-ARF biogenesis, disruption of tasiR-ARF production in *L. japonicus* results in increased sensitivity to auxin transport but reduced auxin sensitivity and repressed nodulation (131). Formation of mycorrhizal arbuscules might require high auxin sensitivity. During mycorrhization in tomato, *Medicago*, and rice, miR393 is downregulated while its target genes encoding TIR1/AFB auxin receptors are upregulated (**Figure 2**), and overexpression of miR393 results in undeveloped mycorrhizal arbuscules (54).

Biological Functions of MicroRNAs in Parasitism Processes

To establish parasitic interactions, parasitic plants or worms form specialized feeding organs to draw nutrients from host plants. Multiple miRNAs are involved in this process. In *Arabidopsis*, miR827 and miR396 contribute to the formation of syncytium, a feeding structure induced by parasitic cyst nematodes. During initial induction and development of syncytium, miR827 is strongly upregulated to suppress basal defense of the host plant via repression of its target *NLA* gene, which encodes a ubiquitin E3 ligase that plays a role in activating the immune system (80). Meanwhile, miR396 is downregulated to derepress expression of its target *GRF1/3*, which encodes a positive regulator of syncytial gene expression and syncytium formation (79). In *Arabidopsis*, miR390 and miR159 contribute to formation of gall, another feeding organ resulting from parasitic interaction between root-knot nematodes and plants. During gall formation, high levels of miR390 accumulate, triggering production of *TAS3*-derived tasiRNAs that silence *ARF3*, leading to repression of auxin signaling (**Figure 2**). Downregulation of *TAS3*-derived tasiRNAs in a *TAS3* mutant significantly decreased the number of galls formed, suggesting that the miR390-*TAS3-ARF3* module is critical for gall formation (24). miR159 gradually accumulates during gall development and restricts expression of its target *MYB33*. The mutant knockout miR159abc is less susceptible to root-knot nematodes, suggesting that miR159 promotes gall development (154).

Not only are host miRNAs used to establish parasitism, but host plants also receive miRNAs from the parasitic plant to facilitate parasitism. The parasitic plant *Cuscuta campestris* delivers a series of 22-nt miRNAs to host *Arabidopsis*, triggering production of secondary siRNAs in haustoria, a feeding structure resulting from plant-plant parasitic interaction. Many targets are associated with plant pathogenesis, suggesting that these *C. campestris* miRNAs may function as virulence factors to remodel host gene expression and facilitate establishment of this parasitic interaction (192). **Table 1** summarizes all identified functional miRNA-target modules in plant–environment interactions.

Table 1 miRNA-target modules in plant–environment interactions

miRNA	Target	Biological functions	Species
miR156	*SPL*	Flowering under abiotic stress (40, 108)	Rice, *Arabidopsis*
		Shade-avoidance syndrome (242)	*Arabidopsis*
		Heat stress memory (199)	*Arabidopsis*
		Phosphate-deficiency response (122)	*Arabidopsis*
		Insect resistance (151)	*Arabidopsis*
		Drought tolerance (4)	*Medicago*
		Age-dependent vernalization (16, 294)	*Arabis alpina*, *Cardamine flexuosa*
miR159	*MYB/HIC-15*	Pathogen immune response (52, 282)	Cotton, *Arabidopsis*
		Plant–nematode interaction (154)	*Arabidopsis*
miR160/miR167	*ARF/IAR3*	Drought tolerance (112, 144)	*Arabidopsis*
		Root responses to nitrate (69)	*Arabidopsis*
		Nodule development (166, 215)	Soybean
		Heat stress tolerance (47)	Cotton
miR163	*EAMT*	Pathogen immune response (37)	*Arabidopsis*
miR164	*NAC4/NAC60*	Pathogen immune response (121, 234)	*Arabidopsis*, rice
miR165/miR166	*HD-ZIPIII/CLP-1/RDD1*	Pathogen immune response (258, 282)	Cotton, *Arabidopsis*
		Drought/cold tolerance (184, 252, 279)	*Arabidopsis*, rice
		Nodule development (19)	*Medicago*
		Ion uptake and accumulation under low-nutrient conditions (93)	Rice
miR168	*AGO1*	Pathogen immune response (73, 216, 217, 239, 266)	Rice, tobacco, *Malus hupehensis*, *Arabidopsis*
		Abiotic stress response (129)	*Arabidopsis*
miR169	*NFYA/HAP2*	Drought tolerance (51, 130, 164, 286)	*Arabidopsis*
		Nodule development (39)	*Medicago*
		Nutrient-deficiency response (180)	Wheat
		Pathogen immunity response (77, 134)	*Arabidopsis*, rice
miR171	*SCL*	Nodule development (43)	*Lotus japonicus*
miR172	*AP2*	Ambient temperature-responsive flowering (102, 120)	*Arabidopsis*
		Nodule development (82, 167, 232)	Soybean, *L. japonicus*, common bean
miR319	*TCP*	Cold tolerance (210, 256)	Sugarcane, rice
		Drought/salt tolerance (295)	Creeping bentgrass
		Pathogen immunity response (277)	Rice
		Plant–nematode interaction (291)	Tomato
miR390	*TAS3*	Nodule development (81)	*Medicago*
miR393	*TIR1/AFB*	Pathogen immunity response (162)	*Arabidopsis*
		Root responses to nitrate/aluminum stress (7, 221)	*Arabidopsis*, barley
		Arbuscule/nodule development (26, 54)	*Arabidopsis*, soybean
		Salt/cold tolerance (33, 145)	*Arabidopsis*, switchgrass
		Seed germination/seedling initiation under submergence (72)	Rice
	ENOD93	Nodule development (255)	Soybean

(*Continued*)

Table 1 (*Continued*)

miRNA	Target	Biological functions	Species
miR393*	*MEBM12*	Pathogen immunity response (285)	*Arabidopsis*
miR395	*APS/SULTR2:1*	Sulfate-deficiency response (95, 106, 107, 136, 269)	*Arabidopsis*, rice
miR396	*GRF*	Syncytium formation response to parasitic nematodes (79)	*Arabidopsis*
		Leaf growth upon UV-B irradiation (29)	*Arabidopsis*
		Pathogen immunity response (197)	*Medicago*
		Mycorrhization development (12)	*Medicago*
	WRKY6	Heat stress tolerance (68)	Sunflower
miR398	*CSD1/CSD2/CCS1*	Oxidative stress tolerance (205)	*Arabidopsis*
		Copper-deficient response (13, 249, 250)	*Arabidopsis*
		Heat stress tolerance (71)	*Arabidopsis*
		Pathogen immunity response (247)	Barley
miR399	*PHO2/UBC24*	Phosphate-deficiency response (34, 64, 65, 75, 85, 137, 143, 170, 172)	*Arabidopsis*, rice, common bean, barley
miR400	*PPR*	Pathogen immunity response (174)	*Arabidopsis*
miR408	*LAC/PLA-TACYANIN*	Copper-deficiency response (1)	*Arabidopsis*
		Salt/drought/cold/oxidative/osmotic-stress responses (61, 147)	*Arabidopsis*, wheat
miR444	*MADS*	Pathogen immunity response (224)	Rice
		Nitrate/phosphate-deficiency responses (254)	Rice
miR482	*R* genes	Pathogen immunity response (168)	Tomato
miR528	*AO/LAC*	Salt and nitrate-deficient response (270)	Creeping bentgrass
		Pathogen immunity response (238)	Rice
		Lodging resistance under nitrogen-luxury conditions (202)	Maize
miR529	*SPL*	Oxidative-stress tolerance (272)	Rice
miR827	*NLA/SPX-MFS*	Phosphate-deficient response (75, 104, 138, 139, 273)	Barley, rice, *Arabidopsis*
		Immune response to nematode (80)	*Arabidopsis*
miR863-3p	*ARLPK1/2*	Pathogen immunity response (165)	*Arabidopsis*
miR2118	*PMS1T*	Photoperiod-sensitive male sterility (56)	Rice
miR5300	*R* gene	Pathogen immunity response (168)	Tomato
miR6019/ miR6020	*N* (*R* gene)	Pathogen immunity response (123)	Tobacco
miR7695	*NRAMP6*	Pathogen immunity response (27)	Rice
miR9863	*MLA1*(*R* gene)	Pathogen immunity response (141)	Barley

Abbreviations: *AFB*, *AUXIN SIGNALING F-BOX*; *AGO1*, *ARGONAUTE 1*; *ARLPK1/2*, *ATYPICAL RECEPTOR-LIKE PSEUDOKINASE1/2*; *AO*, *L-ASCORBATE OXIDASE*; *AP2*, *APETALA2*; *APS*, *ATP SULFURYLASE*; *ARF*, *AUXIN RESPONSE FACTOR*; *CCS1*, *COPPER CHAPERONE OF CSD1*; *CLP-1*, *CYSTEINE PROTEASE-1*; *CSD*, *COPPER/ZINC SUPEROXIDE DISMUTASE*; *ENOD93*, *EARLY NODULIN GENE93*; *FAMT*, *FARNESOIC ACID METHYLTRANSFERASE*; *GRFs*, *GROWTH REGULATING FACTORs*; *HAP2*, *APETALA2 PROTEIN HOMOLOG*; *HD-ZIP III*, *HOMEODOMAIN LEUCINE ZIPPER III*; *HIC-15*, *ISOTRICHODERMIN C-15 HYDROXYLASE*; *IAR3*, *INDOLE-3-ACETIC ACID–ALA RESISTANT 3*; *LAC*, *LACCASE*; miRNA, microRNA; *MLA1*, *MILDEW RESISTANCE LOCUS A1*; *MYB*, *MYELOBLASTOSIS*; *NFYA*, *NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT ALPHA*; *NLA*, *NITROGEN LIMITATION ADAPTATION*; *NRAMP6*, *NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 6*; *PHO2*, *PHOSPHATE 2*; *PMS1T*, *PHOTOPERIOD-SENSITIVE GENIC STERILITY 1 TRANSCRIPT*; *PPR*, *PENTATRICOPEPTIDE REPEAT*; *RDD1*, *RICE DOF DAILY FLUCTUATIONS 1*; *SCL*, *SCARECROW-LIKE*; *SULTR2:1*, *SULFATE TRANSPORTER 2;1*; *SPL*, *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE*; *SPX-MFS*, *S YG1/P bo81/X PR1-MAJOR FACILITATOR SUPERFAMILY*; *TAS3*, *TRANS-ACTING SIRNA3*; *TCP*, *TEOSINTE BRANCHED1*, *CYCLOIDEA*, *PROLIFERATING CELL NUCLEAR ANTIGEN BINDING FACTOR*; *TIR1*, *TRANSPORT INHIBITOR RESPONSE1*; *UBC24*, *UBIQUITIN-CONJUGATING ENZYME 24*.

SUMMARY POINTS

1. MicroRNA (miRNA) biogenesis entails transcription of miRNA genes (MIRs) into primary miRNAs (pri-miRNAs) that are cotranscriptionally processed into miRNA duplexes by DICER-LIKE (DCL) proteins. Splicing factors and lariat RNAs are involved in the regulation of pri-miRNA processing, pointing to crosstalk between or coupling of splicing and pri-miRNA processing.
2. miRNA duplexes are methylated, and the guide strand is incorporated into ARGONAUTE (AGO) proteins to form the miRNA-induced silencing complex (miRISC). Conventional wisdom holds that miRNA loading into AGO proteins occurs in the cytoplasm. However, a revised model depicts nuclear miRNA loading and nuclear export of loaded AGO1.
3. Plant miRNAs display near-perfect complementarity with their targets and direct target messenger RNA (mRNA) cleavage. However, miRNA-mediated translational repression is also prevalent. The endoplasmic reticulum is an important site for target mRNA cleavage and translational repression. Certain miRNAs direct DNA methylation in the nucleus.
4. miRNA turnover involves 3′ truncation of miRNAs by SMALL RNA DEGRADING NUCLEASE 1 (SDN1), 3′ uridylation of truncated miRNAs by HEN1 SUPPRESSOR1 (HESO1) and URIDYLYLTRANSFERASE 1 (URT1), and degradation of polyuridylated miRNAs by unknown exonucleases.
5. miRNA-mediated developmental plasticity is a common strategy for plants to cope with various environmental stimuli from light, temperature, and nutrients. miRNAs act as important environment-responsive regulators, which confer adaptive changes in phenotype and promote plant evolution and adaptation.
6. Plants have evolved miRNA modules to sophisticatedly regulate abiotic stress-tolerance responses, some of which are evolutionarily associated with plant environmental adaptation.
7. miRNAs can either directly regulate defense responses or act as a molecular switch to coordinate plant growth and immunity by targeting *R* genes.
8. miRNAs can respond to both symbiotic and parasitic signaling and reprogram a series of molecular networks to either promote or inhibit the development of symbiotic and parasitic organs.

FUTURE ISSUES

1. What are the sequence/structural features within pri-miRNAs that determine the efficiency and accuracy of processing by dicing complex? Do and, if so, how do RNA modifications regulate pri-miRNA processing?
2. Are D-bodies the organization centers of MIRs and pri-miRNA processing? Does the formation of D-bodies reflect condensation of miRNA processing factors into phase-separated droplets?

3. To what degree and how does splicing regulate pri-miRNA processing? Does and, if so, how does miRNA biogenesis influence pre-mRNA splicing?
4. Many miRNA processing factors regulate the processing of a selected subset of pri-miRNAs. What determines the specificity of different miRNA processing factors?
5. What are the mechanisms that determine the choice of miRNA action mode?
6. Why do only 22-nt miRNAs trigger the production of secondary small interfering RNAs (siRNAs)?
7. How do miRNAs sense environmental stimuli and adjust their accumulation?
8. What is the natural variation pattern of miRNA-target regulons that associate with plant–environment interactions among plant species? Do and, if so, how do they contribute to environmental adaptation?

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