REVIEW

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Functions of microRNAs and related small RNAs in plants

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MicroRNAs (miRNAs) and short interfering RNAs (siRNAs), 20- to 27-nt in length, are essential regulatory molecules that act as sequence-specific guides in several processes in most eukaryotes (with the notable exception of the yeast *Saccharomyces cerevisiae*). These processes include DNA elimination, heterochromatin assembly, mRNA cleavage and translational repression. This review focuses on the regulatory roles of plant miRNAs during development, in the adaptive response to stresses and in the miRNA pathway itself. This review also covers the regulatory roles of two classes of endogenous plant siRNAs, ta-siRNAs and nat-siRNAs, which participate in post-transcriptional control of gene expression.

Small RNAs fall into two categories, miRNAs and siRNAs, which are distinguished by their biogenesis, not by their action[1]. miRNAs derive from long, single-stranded RNAs (ssRNAs) that have the ability to fold and form imperfectly double-stranded RNAs (dsRNAs), which are processed sequentially by RNase III proteins of the Drosha/Dicer family[1–4]. Conversely, siRNA populations are processed by members of the Dicer family from long, perfectly dsRNAs that derive from transcription of inverted repeat sequences, convergent transcription of sense-antisense gene pairs or synthesis by RNA-dependent RNA polymerases (RDRs)[1–4]. miRNAs always act in *trans* by regulating mRNAs that exhibit strong complementarity to the 5′ end of the miRNA sequence, a pairing referred to as seed complementarity in animals[5]. miRNAs cannot regulate the genes from which they originate because they are identical, not complementary, to their precursor RNAs. On the other hand, siRNAs can potentially act in both *cis* and *trans* by targeting the elements from which they derive as well as unlinked elements that exhibit substantial complementarity to their sequence, similar to the *trans* action of miRNAs[1]. miRNAs have primarily been shown to repress gene expression at the post-transcriptional level[1,2,4,6], although a link between miRNA complementarity and DNA methylation has been observed for one plant miRNA[7]. In contrast, roles for siRNAs during both transcriptional and post-transcriptional gene repression have been firmly established[6,8–10].

siRNA biogenesis and action

During viral infection in plants, dsRNAs are produced by viral- or cellular-encoded RDRs and are processed to siRNAs by cellular DICER-LIKE enzymes (DCLs)[11,12]. siRNAs deriving from the negative strand of viral RNAs can guide cleavage of complementary positive-strand viral RNAs, resulting in a strong decrease in virus accumulation, a cellular immune response referred to as viral recovery[6]. siRNAs deriving from one virus also can target complementary RNAs from related viruses, resulting in cross-protection to the second virus[6]. However, many viruses encode proteins that can suppress the siRNA pathway at various steps, allowing a successful virus infection[13]. Coinfection of one virus that can suppress the siRNA antiviral response and a second virus that cannot impede the siRNA antiviral response often results in a successful infection for both viruses, a phenomenon referred to as viral synergism, where one virus benefits from the siRNA-suppressing activity of another virus[14]. Transgenes can also activate the plant siRNA-based immune response, likely by producing RNAs that mimic viral RNA structure or titer, resulting in the production of dsRNAs and subsequently siRNAs that direct transgene mRNA degradation[6,15]. Transgene-derived siRNAs can also direct the repression of complementary endogenous mRNAs, a phenomenon referred to as cosuppression[16].

siRNAs do not derive exclusively from exogenous dsRNA sources. At least three types of endogenous siRNAs exist in plants. *Trans*-acting siRNAs (ta-siRNAs) derive from long noncoding ssRNAs that are cleaved by miRNAs to produce truncated RNAs, which are transformed to dsRNAs by the cellular RNA-dependent RNA polymerase RDR6 and processed, in 21-nt increments, by the DCL enzyme DCL4[17–22]. ta-siRNAs deriving from the positive and negative strands act in *trans* by guiding the cleavage of endogenous mRNAs, similar to miRNAs. ta-siRNAs deriving from the negative strand can also act in *cis* by guiding the cleavage of their precursor RNAs; in this way, they participate in feedback regulation of the pathway. The second type of siRNAs, nat-siRNAs, derives from natural antisense RNAs. A region of the dsRNA, resulting from the transcription of overlapping genes[23], is recognized and processed in a DCL2-dependent manner to a single, 24-nt nat-siRNA species, which directs the DCL1-dependent cleavage of additional 21-nt nat-siRNAs. Although dsRNAs are assumed to result from annealing *cis*-antisense RNA pairs, RDR6 is required for nat-siRNA accumulation. So far, nat-siRNAs have been shown to act only in *cis* by guiding the cleavage of one

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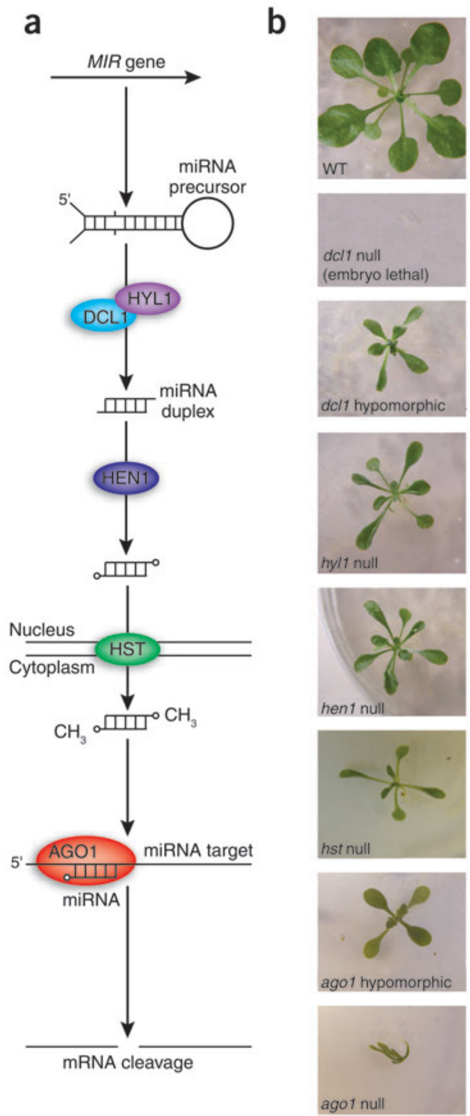

Figure 1 The plant miRNA pathway and miRNA pathway *Arabidopsis thaliana* mutants. (**a**) Simplified schematic of the plant miRNA pathway. (**b**) Phenotypes of wild-type and null and hypomorphic mutant plants impaired in the biogenesis and/or the action of miRNAs. Note that *dcl1* null alleles are embryonic lethal, explaining the absence of homozygous plants.

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of the two mRNAs in the *cis*-antisense pair. The third type of endogenous siRNAs corresponds to heterochromatin and DNA repeats, such as transposons. The production of these primarily 24-nt siRNAs requires RDR2 and DCL3, and the resulting siRNAs guide chromatin remodeling and, in some cases, DNA methylation at the loci from which they derive[11,24,25]. These modifications are thought to maintain the loci in a transcriptionally silenced state.

miRNA biogenesis and action

miRNAs repress expression of endogenous genes in plants and animals, but miRNA biogenesis and action have similarities and differences in these organisms[1,4]. In both plants and animals, miRNAs derive from long ssRNAs that fold and form imperfect hairpin dsRNAs. In animals, miRNA primary precursors (pri-miRNAs) in the nucleus are cut first at the bottom portion of the stem by Drosha, which is assisted by the dsRNA-binding protein Pasha/DGCR8, to produce precursor miRNAs (pre-miRNAs), which are exported to the cytoplasm by Exportin-5 (Exp-5)[1,3,4]. At this point, Dicer, assisted by another dsRNA-binding protein, Log/PACT, cuts the loop of pre-miRNAs and liberates mature miRNA duplexes, which are loaded onto a member of the Argonaute family, owing to the physical association of Argonaute and Dicer. The miRNA duplexes are separated and the miRNA* strand (the RNA from the opposing arm of the miRNA precursor) is destabilized, allowing the mature miRNA-Argonaute complex to interact with partially complementary target mRNAs[1,4]. In most cases, animal miRNAs guide translational repression of their targets. This repression can be accompanied by mRNA decay through a slicer-independent mechanism[26–28]. In at least one case, the animal miRNA miR196, which exhibits a near-perfect homology with its target *Hoxb8*, guides cleavage in the middle of the region of complementarity, similar to siRNA-guided cleavage[29].

In *Arabidopsis thaliana* plants, the two cuts that liberate miRNA duplexes from the fold-back stem loop of miRNA precursors are performed in the nucleus by DCL1, which is assisted by the dsRNA binding protein HYL1[30–35]. The 3′ ends of miRNA duplexes are methylated by HEN1[33,36] and loaded onto AGO1, one of ten AGO proteins in *A. thaliana*, where the mature single-stranded miRNA guides the RNA slicing activity of AGO1 to partially complementary mRNAs[37–39]. The accumulation of several miRNAs is reduced in *hasty*, an *A. thaliana* mutant impaired in the ortholog of Exp-5, but it is unknown whether miRNA duplexes or mature miRNAs are exported by HASTY to the cytoplasm[40]. Indeed, miRNA-guided cleavage of mature mRNAs is thought to occur in the cytoplasm, but miR162-guided cleavage of unspliced *DCL1* has been reported[41], suggesting that AGO1 functions in both the nucleus and the cytoplasm. It is possible that miRNA loading onto AGO1 occurs independently in the two compartments, although another scenario could be that miRNAs loaded onto AGO1 in the nucleus are exported to the cytoplasm by HASTY or another pathway.

mRNA cleavage seems to be the predominant mechanism of miRNA-guided regulation in plants, based on the detection of cleavage products and on the reduction of steady-state levels of full-length mRNAs in plants overexpressing the corresponding miRNA[42,43]. However, because plants that overaccumulate miR172 have unaltered *AP2* mRNA levels but decreased AP2 protein levels, it has been proposed that miR172 guides translation repression of *AP2* mRNA in addition to *AP2* mRNA cleavage[44,45]. More recent work showing that AP2 protein itself participates in negative feedback regulation of *AP2* transcription has made the contribution of miR172-guided *AP2* mRNA cleavage versus translational repression difficult to discern[46]. In addition to post-transcriptional miRNA-guided regulation, two examples of miRNA-guided transcriptional regulation have been suggested. miR165/166 not only guides *PHB* and *PHV* mRNA cleavage, but it also seems to target *PHB* and *PHV* genes for methylation downstream of the miRNA complementarity site[7]. Indeed, in heterozygous plants carrying a wild-type *PHB* allele and a mutant *phb-d* allele encoding an mRNA that is cleaved less efficiently, only the wild-type allele is methylated. Although this mechanism seems to occur in *cis*, the biological role of miRNA-guided DNA methylation is unknown.

miRNAs and plant development

Mutations in key miRNA pathway components lead to pleiotropic developmental defects. The crucial roles of miRNAs in plant development were exemplified in the dramatic and pleiotropic developmental defects of the *A. thaliana* mutants *ago1, dcl1, hen1, hyl1* and *hst*[47–51], which are impaired generally at different points in the miRNA pathway (**Fig. 1**). In particular, null *dcl1* alleles are embryonic lethal, pointing to the essential roles of miRNAs during embryogenesis. Three hypomorphic, but sterile, *dcl1* alleles have been recovered from developmental

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screens, each of them exhibiting a slightly different profile of reduced miRNA accumulation due to distinct point mutations in the RNA helicase domain or a truncation in the second of two dsRNA-binding domains[33,34,52,53]. The phenotypes of hypomorphic *dcl1* mutant plants resemble those of *hen1*, *hyl1* and *hst* null mutants, which also exhibit reduced miRNA accumulation[32,33,35,40]. The developmental defects of hypomorphic *ago1* mutant plants also resemble these mutants[54], but their molecular phenotypes are different. Hypomorphic *ago1* mutant plants do not exhibit reduced miRNA accumulation, but show increased miRNA target accumulation, likely due to reduced miRNA-programmed cleavage efficiency[37,39]. In contrast, *ago1* null alleles have reduced miRNA accumulation and reduced miRNA target cleavage and exhibit dramatic developmental defects that resemble those of transgenic plants in which *DCL1* is strongly inhibited by RNA interference (C. Béclin and H.V., unpublished data). The fact that *ago1* null alleles are not embryonic lethal (unlike *dcl1* null mutants) is likely due to partial functional redundancies among the ten AGO family members of *A. thaliana*. Indeed, although a role for PNH (AGO10) in the miRNA pathway has not been established, *ago1 pnh* double mutants are embryonic lethal[55], supporting this hypothesis. In contrast, DCL1 seems to be the only DCL that processes miRNAs[11,18], whereas redundancies have been observed among DCL2, DCL3 and DCL4 for the production of several siRNA classes[18,21]. The weaker phenotype of *hen1*, *hyl1* and *hst* mutants likely reflects other examples of redundancies (one paralog of *HEN1* and four paralogs of *HYL1* exist in the *A. thaliana* genome[51,56]), or the existence of alternative, unrelated pathways (no paralog of *HST* exists in *A. thaliana*, but miRNAs loaded onto AGO1 may be exported from the nucleus by a different pathway).

Mutations in MIR genes or miRNA target genes lead to specific developmental defects. The determination of the individual developmental roles of several plant miRNAs has been assisted by genetic screens. For example, the precise reason that dominant *phb-d*, *phv-d* and *rev-d* mutant *A. thaliana* plants exhibit polarity defects that result in radialized leaves[57,58] was not revealed until the first miRNAs were cloned from *A. thaliana* and their mRNA targets were computationally predicted based on complementarity and conservation[59]. These experiments revealed that the *HD-ZIPIII* transcription factor mRNAs *PHB*, *PHV* and *REV* are targets of miR165/166, which differ by a single nucleotide, and mapped the dominant *phb-d, phv-d* and *rev-d* mutations to the region complementary to miR165/166[58,60]. Later experiments showed that the *phb-d* and *phv-d* mutations decreased miRNA-directed cleavage efficiency and attributed the developmental defects of *phb-d* and *rev-d* mutants to this change and not to changes in protein activity[58,60,61]. Dominant *jba1-D* and *men* mutants, which both exhibit fasciated stems, have increased expression of *MIR166g* and *MIR166a* genes, respectively, owing to the insertion of a transferred DNA carrying enhancer elements in the vicinity of these miRNA genes[62,63]. Interestingly, miR166 overaccumulation in *jba1-D* and *men* mutants primarily resulted in the downregulation of *PHB*, *PHV* and *CNA/ATHB15* mRNAs, another validated target of miR165/166, but not of *REV* and *ATHB8*, a fifth validated cleavage target of miR165/166, pointing to the importance of the tissue-specific regulation of miRNA expression[62,63]. Indeed, miR165/166 have the potential to be produced by nine different loci (*MIR165a-b* and *MIR166a-g*), and these loci may have different temporal and spatial expression patterns[34].

Genetic screens also have revealed one miRNA that was previously unidentified in cloning efforts. miR319/JAW was discovered in a developmental screen of activation-tagged *A. thaliana* lines[42]. Dominant *jaw-D* mutant plants, which exhibit uneven leaf shape and curvature, have increased expression of the gene *MIR319a* and reduced accumulation of the five TCP mRNAs that are complementary to miR319 (24 *TCP*

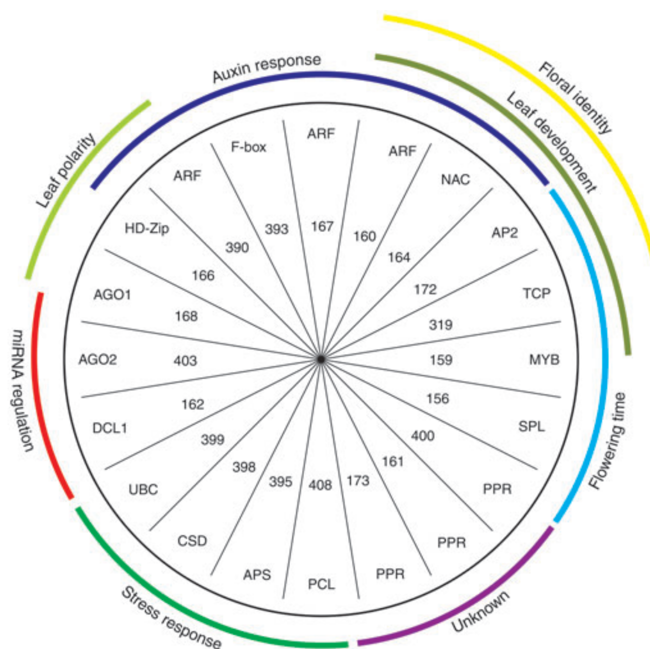

Figure 2 Plant miRNAs regulate overlapping networks. In this diagram, miRNAs (inner circle) are grouped by the targets they regulate (outer circle) and thus their roles (colored lines) in developmental programs, adaptive responses to stress and the miRNA pathway itself. Interactions among miRNA targets likely exist but are not represented on this diagram.

genes exist in *A. thaliana*). Additional miRNAs and their developmental roles have been uncovered in screens. For example, the early flowering *eat-D* mutant and late-flowering *toe1-D* mutants turned out to have increased expression of *MIR172b* and the miR172 target gene *TOE1*, respectively[44]. Finally, the *eep1* mutant, which exhibits early extra petals, is impaired in the production of miR164 from the *MIR164c* locus, one of three loci that can produce miR164 (miR164c differs from miR164a and miR164b in sequence by a single nucleotide), and overaccumulates the miR164 target mRNAs *CUC1* and *CUC2*[64]. So far, *eep1/mir164c* is the only recessive loss-of-function miRNA mutant that has been identified in plants through a forward genetic screen, probably because most plant miRNAs are products of multigene families containing redundant members. Indeed, only *mir164a* mutants, identified by reverse genetics, have altered lateral root development[65], whereas *miR164b* mutants have reduced miR164 levels but no obvious developmental impairments[66].

Insights from miRNA overexpression. Further insights into the specific roles of miRNAs during plant development have been obtained by overexpressing and misexpressing *MIR* genes under the control of the strong CaMV 35S promoter. miRNA overexpression generally leads to pleiotropic developmental defects, probably because most miRNAs regulate multiple targets. For example, plants expressing *35S-MIR156* and *35S-MIR159*, which target members of the *SPL* and *MYB* transcription factor families, respectively, exhibit a late-flowering phenotype[46,67]. They also show decreased apical dominance and male sterility, respectively. Plants expressing *35S-MIR160*, which targets members of the *ARF* transcription factor family, exhibit agravitropic roots and increased lateral rooting[68]. Plants expressing *35S-MIR164*, which targets members of the *NAC* transcription factor family including *CUC1*, *CUC2* and *NAC1*, exhibit a range of embryonic and floral organs fusions (similar to the developmental defects of *cuc1 cuc2* loss-of-function double mutants), vegetative organ fusions and reduced root branching (similar to plants with reduced levels of *NAC1*)[65,66,69]. Plants expressing *35S-MIR166g*, which targets members

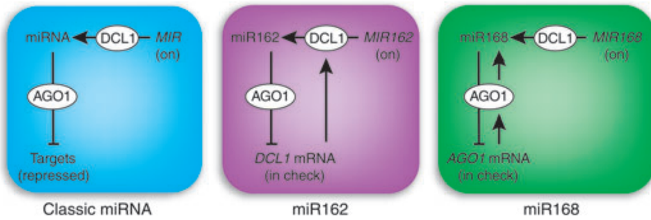

Figure 3 Schematic representation of plant miRNA regulatory mechanisms. Most miRNAs guide target mRNA degradation to allow cells to undergo a new developmental program or adapt to changes in the environment (blue box). To ensure that plants are able to properly develop and respond to stress, key components of the miRNA pathway need to be tightly regulated in every cell. DCL1-mediated production of miR162 and miR162-mediated feedback regulation of DCL1 maintain the DCL1/miR162 homeostasis (orange box), whereas post-transcriptional stabilization of miR168 by AGO1 protein and miR168-guided, AGO1-catalyzed feedback regulation of *AGO1* mRNA allow miR168 and AGO1 levels to be kept in check (green box).

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of the *HD-ZIP* transcription factor family, exhibit radialized leaves, fasciated apical meristems and female sterility[63]; plants expressing *35S-MIR172*, which targets members of the *AP2* transcription factor family, exhibit floral identity defects similar to those of *ap2* loss-of-function mutants, accelerated floral transition and rosette leaves with upward curling[45]. Finally, plants expressing *35S-MIR319*, which targets members of the *TCP* transcription factor family, exhibit uneven leaf shape and curvature and show a modest delay in flowering time[42]. Owing to the simplicity of making *35S-MIR* constructs, it is likely that the limited number of reports describing the effects of miRNA overexpression reflects the absence of obvious developmental consequences, and that these negative results are not reported. If the absence of developmental defects in some *35S-MIR* plants was confirmed, this may reveal that the levels of some miRNAs are not limiting under normal conditions of growth, or that transcriptional or post-transcriptional compensatory mechanisms are able to readjust the levels of mRNA targets. It also is possible that the overexpression of particular miRNAs could be lethal, as suggested by the inability to obtain viable plants that recapitulate the *men* mutant developmental defects by introducing a *35S-MIR166a* transgene[62].

Insights from engineering miRNA-resistant targets. Ectopic expression of mRNA targets carrying silent mutations that disrupt the complementarity between the mRNA and its regulatory miRNA, but do not affect the amino-acid sequence of the protein, allows the role of individual targets to be addressed. However, most miRNA-resistant plants described in the literature do not exhibit specific developmental defects, but instead have dramatic pleiotropic defects, probably because most of the explored targets are transcription factors, which themselves regulate numerous additional genes. Indeed, about two-thirds of the known plant miRNA targets are transcription factors. For example, *miR159-resistant MYB33* (*mir159-MYB33*) plants exhibit reduced size, reduced apical dominance, rounded leaves with reduced petiole lengths, and reduced fertility[70]. *mir160-ARF16* plants exhibit fewer lateral roots and reduced fertility[68], whereas *mir160-ARF17* plants exhibit extra cotyledons, leaf symmetry and shape defects, root growth defects, altered anther development and petal shape, reduced fertility and an overall dwarfed appearance[71]. *mir164-CUC1* and *mir164-CUC2* plants both exhibit leaf shape and polarity defects, extra petals, missing sepals and reduced fertility[66,69], whereas *mir164-NAC1* plants exhibit an increased number of lateral roots[65]. *mir165/166-PHB* and *mir165/166-REV* plants exhibit leaf polarity defects that result in ectopic meristem formation and radialized leaves[60,61]. *mir172-AP2* plants exhibit enlarged floral meristem surrounded by many whorls of stamenoid organs and petals[45]. Finally, *mir319-TCP2* exhibit long hypocotyls, reduced stature and apical dominance, whereas *mir319-TCP4* plants exhibit fused cotyledons, tubular shaped seedlings, bushy rosettes and abnormal inflorescences and, in severe cases, aborted shoot apical meristem development[42].

miRNAs act in networks. Our current knowledge about the regulatory roles of miRNAs and their targets point to fundamental functions in various aspects of plant development, including auxin signaling, meristem boundary formation and organ separation, leaf development and polarity, lateral root formation, transition from juvenile-to-adult vegetative phase and from vegetative-to-flowering phase, floral organ identity and reproduction. Interestingly, most miRNAs do not work independently, but rather are involved in overlapping regulatory networks (**Fig. 2**), pointing to a coordinating role in the fine-tuned adjustment of mRNA levels within these networks. One of the future challenges will be to place additional miRNAs in such networks, in particular those that are likely to have a role in development based on the function of their targets. For example, miR171 targets members of the *SCL* transcription factor family that have a role in hormone signaling and in the radial patterning of shoots and roots[58], and is likely to integrate within the auxin signaling network. It also will be of particular interest to determine the roles of the cluster of *PPR* genes that are targeted by miR161, miR400 and TAS2-derived ta-siRNAs generated through the action of miR173[17,22,58,72], and place these small RNAs in the regulatory network.

miRNAs and plant adaptive responses to stress

miRNAs and abiotic stress. In addition to their roles in development, plant miRNAs seem to have an important function in adaptive responses to abiotic stresses. The first indication for such roles came from bioinformatic miRNA and target gene predictions and miRNA cloning from stressed *A. thaliana* plants, which revealed new miRNAs that had not been cloned previously from plants grown in normal conditions[72,73]. For example, miR395 is not detectable in plants grown under standard conditions, but is induced during low-sulfate stress. miR395 targets ATP sulfurylases (APS) that catalyze the first step of inorganic sulfate assimilation, and the accumulation of APS1 mRNA is decreased under low-sulfate stress[73]. Similarly, miR399 is not detectable in plants grown under standard conditions, but is induced during low-phosphate stress. miR399 targets a ubiquitin-conjugating enzyme (UBC), and *UBC* mRNA accumulation is decreased during low-phosphate stress, which is important to induce the phosphate transporter gene *AtPT1* and attenuate primary-root elongation[74,75]. Overexpression of miR399 leads to downregulation of *UBC*, even under high-phosphate conditions, and induces accumulation of phosphate in these plants. Conversely, *mir399-UBC* plants showed limited induction of *AtPT1* under low-phosphate conditions and limited attenuation of primary-root elongation. Other miRNAs are likely to have roles during stress based on the function of their targets or their pattern of expression. For example, miR398 targets two copper superoxide dismutase enzymes that protect cells against harmful oxidative radicals produced during stress[73]. Furthermore, in poplar trees, miR408 expression is induced by tension and compression stresses in xylem tissues, suggesting that this miRNA has a critical role in the structural and mechanical fitness of woody plants[76].

Interestingly, cloning small RNAs from stressed plants[72] led to the discovery of a new class of endogenous siRNAs, coined nat-siRNAs, which seem to have an important role in stress response. nat-siRNAs derive from the transcription of convergent overlapping genes, and are primarily processed by DCL2 as a single 24-nt species. The founding member of this new class of siRNAs derives from the *P5CDH-SRO5* antisense gene pair[23]. *P5CDH* is constitutively expressed, whereas *SRO5* is induced by NaCl stress. Under high salt stress, the 24-nt nat-siRNA corresponding to the *SRO5* mRNA is produced, targets the *P5CDH* mRNA for degradation and leads

to the production of a population of 21-nt nat-siRNAs. Downregulation of *P5CDH* leads to proline accumulation, which is an important step in the plant's ability to tolerate excess salt. There are about 2,000 antisense gene pairs in *A. thaliana*[77], and many of them have been reported to be regulated by environmental or hormonal stimuli[23]. nat-siRNAs have been detected from several of these antisense pairs of genes, and are inducible under stress conditions, suggesting that, in addition to miRNAs, nat-siRNAs may have an important role in plant adaptive responses to abiotic stresses[23].

miRNAs and biotic stress. In mammals, several examples of miRNA-mediated inhibition or enhancement of viral infection have been reported[78,79]. In plants, neither miRNAs nor endogenous siRNAs has been implicated directly in the response to biotic stress, although a proper functioning of the plant miRNA pathway seems to be beneficial for infection by Red clover necrotic mosaic virus[80] and by *Agrobacterium tumefaciens*[81]. Near-perfect complementarity between endogenous small RNAs and viral genome has been noticed, but there is no experimental validation of their potential influence on viral infection[82]. Interestingly, introduction of miRNA or siRNA complementary sites within human or plant viral genomes has resulted in the selection of mutant viruses[83,84]. These results indicate that viruses not only encode RNA silencing suppressors, but also can escape targeting by accumulating mutations within small RNA target sequences. The fact that some viral suppressors interfere with both the siRNA-mediated immune response and the miRNA pathway is likely due to an effect on steps shared by the miRNA and siRNA pathways[52,85–87]. However, it remains possible that inhibiting the miRNA or endogenous siRNA pathway may be beneficial to some viruses but detrimental to others[80].

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miRNAs regulating the plant miRNA pathway

miRNAs have two major roles in plants: inducing cell differentiation in response to an endogenous stimulus and inducing an adaptive response to a particular exogenous stress. This model implies that the homeostasis of the miRNA pathway needs to be maintained in every cell that expresses miRNAs. DCL1 and AGO1 are two major actors in the miRNA pathway, as inferred by the dramatic phenotypes of *dcl1* and *ago1* mutants. Therefore, it is not surprising that these two key components are themselves regulated by the miRNA pathway. miR162 targets *DCL1*, whereas miR168 targets *AGO1*[35,41,58]. Plants expressing an miR168-resistant *AGO1* mRNA are dwarf and sterile or die at early stages due to degeneration of the shoot apical meristem, pointing to the importance of miR168-mediated regulation of *AGO1* and miRNA-mediated regulation of the miRNA pathway in general[37]. DCL1-mediated production of miR162 and miR162-guided *DCL1* feedback regulation are sufficient to explain the maintenance of the DCL1/miR162 homeostasis[41], but miR168-mediated, AGO1-catalyzed feedback regulation of *AGO1* is not sufficient to explain the maintenance of the AGO1/miR168 homeostasis[37]. Indeed, an additional layer of regulation is required, consisting of the post-transcriptional stabilization of miR168 by AGO1, which together with miR168-guided, AGO1-catalyzed feedback regulation of *AGO1* mRNA allows the levels of miR168 and AGO1 to be kept in check (**Fig. 3**). In addition, *AGO1* and *MIR168* genes seem to be transcriptionally coregulated, allowing the AGO1/miR168 homeostasis to be maintained in every cell where the miRNA pathway is functioning[88]. Interestingly, another member of the AGO family, AGO2, seems also to be regulated by an miRNA, miR403[17]. However, neither the role of AGO2 nor the importance of miR403-mediated regulation of AGO2 has been explored.

Conclusions

In animals, miRNAs are assumed to control directly the expression of more than one third of genes[5,89,90] and to have at some point influenced the expression of nearly the entire genome[91,92]. In plants, the number of known miRNAs and miRNA targets is lower than in animals[93]. Nevertheless, the spectrum of miRNA action seems to be extremely wide, including various aspects of development, several adaptive responses to stresses and the regulation of the miRNA pathway itself. Large-scale cloning efforts have been initiated to identify the complete repertoire of small RNAs in plants, which already have revealed unexpected complexities and overwhelming diversity in small RNA pathways[11,34,94,95]. Deciphering the complete repertoire of small RNA targets as well as their roles will be one of the future challenges in this rapidly growing field.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Erratum: Functions of microRNAs and related small RNAs in plants

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In the version of this article initially published, two labels depicting methylation (CH3) were inadvertently omitted from the miRNA duplex shown below HEN1 in **Figure 1**. In addition, the arcs accompanying the pie chart in **Figure 2** were misaligned. Corrected figures are shown here. These errors have been corrected in the HTML and PDF versions of the article.