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MicroRNA regulation of gene expression in plants

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It has only been a few years since we began to appreciate that microRNAs provide an unanticipated level of gene regulation in both plants and metazoans. The high level of complementarity between plant microRNAs and their target mRNAs has allowed rapid progress towards the elucidation of their varied biological functions. MicroRNAs have been shown to regulate diverse developmental processes, including organ separation, polarity, and identity, and to modulate their own biogenesis and function. Recently, they have also been implicated in some processes outside of plant development.

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Abbreviations

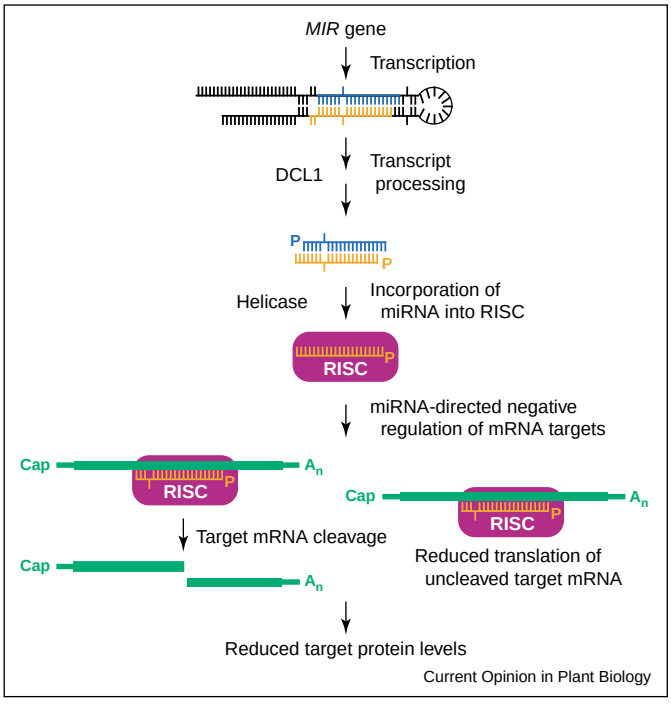
AGO	ARGONAUTE
ap2	*apetela2*
CUC1	CUP-SHAPED COTYLEDON1
DCL	DICER-LIKE
dsRNA	double-stranded RNA
HD-ZIP	homeodomain leucine zipper
HEN1	*HUA ENHANCER1*
HYL1	*HYPONASTIC LEAVES1*
miR	*miRNA-resistant*
miRNA	microRNA
nt	nucleotide(s)
P1/HC-Pro	Protein1/Helper Component-Proteinase
PHB	*PHABULOSA*
PHV	*PHAVOLUTA*
PTGS	post-transcriptional gene silencing
REV	*REVOLUTA*
RISC	RNA-induced silencing complex
rld1	*rolled leaf1*
siRNA	small interfering RNA
TCP	TEOSINTE BRANCHED1, CYCLODEA, and PCF1 and PCF2
toe1	*target of eat1*

Introduction — small RNAs in plants

MicroRNAs (miRNAs) are 21–24 nucleotide (nt) riboregulators that were first discovered as translational attenuators controlling larval development in the nematode *Caenorhabditis elegans* [1]. They are now known to exist in both metazoans and plants [2,3••]. Small interfering RNAs (siRNAs) are similarly sized molecules, first detected in plants [4], that provide specificity to processes known as RNA interference (RNAi) in animals and post-transcriptional gene silencing (PTGS) in plants [5,6]. Both miRNAs and siRNAs act to negatively regulate target mRNAs, but they are distinguished by their genomic origin, the nature of their target genes, and their evolutionary conservation [3••]. siRNAs often originate from the target gene itself, whether this target is derived from a transgene, a virus, a transposon, or an endogenous locus. By contrast, miRNAs are processed from imperfectly paired hairpin precursors, which are generally produced from transcriptional units that are distinct from those of protein-coding genes. Mature miRNAs target protein-coding mRNAs (Figure 1). In animals, miRNAs often display limited complementarity to multiple sites in the 3′ untranslated region (UTR) of the target message and act to repress its productive translation [3••]. In plants, miRNAs generally display near-perfect complementarity to a single site within the target message [7] and can direct the cleavage at this site [8,9••,10••]. However, an animal miRNA that directs target cleavage [11•] and a plant miRNA that acts at the translational level [12••,13••] have been reported, suggesting that the plant and animal systems may be more similar than they first appeared.

Following the biochemical isolation of numerous miRNAs in nematodes, fruit flies, and humans [14–16], four groups of miRNAs were cloned from *Arabidopsis* using similar techniques [17–20]. Altogether, 15 distinct miRNA families were identified in *Arabidopsis* [21]. Most of the miRNAs in this original cohort are conserved in rice, and this facilitates a computational approach for the identification of additional plant miRNAs [22••]. These predicted miRNAs were validated by northern analysis or by detection of the corresponding clones, bringing the count to 22 *Arabidopsis* miRNA families with at least 92 members in total ([22••]; Table 1). As the computational approaches used at present uncover only miRNAs that are conserved in at least two genomes, direct cloning could potentially identify new miRNAs that have more limited distribution or that have atypical features. It is important to note that miRNAs comprise only a small fraction of similarly sized endogenous RNA species found in plants [17,20,21,23••]; a large compilation of cloned small RNAs (both miRNAs and other small RNAs, presumably endogenous siRNAs) can be found at http://cgrb.orst.edu/smallRNA/.

Figure 1



Schematic representation of miRNA biogenesis and function in plants. The transcription of MIR genes gives rise to transcripts that contain an imperfectly paired hairpin region. These transcripts are processed by the DCL1 ribonuclease, perhaps assisted by the HEN1 and HYL1 proteins, into an imperfectly paired dsRNA of about 21 nt that has 2-nt 3′ overhangs. An unidentified helicase probably aids in the selection of the single strand (the miRNA, shown in orange) that is incorporated into the RISC. Within the RISC, the miRNA serves as the specificity determinant to reduce target protein levels, either by cleavage of the target mRNA in the middle of the complementarity site or by repressing its productive translation.

MicroRNA biogenesis and function

Dicer ribonucleases process miRNAs from their precursor hairpins and siRNAs from long double-stranded RNA (dsRNA) precursors, generating dsRNAs of about 21 nt that have 2-nt 3′overhangs [24–27]. One strand of these dsRNAs is selected as the mature miRNA or siRNA, and is incorporated into the RNA-induced silencing complex (RISC) or a RISC-like complex [28–31]. Within this complex, the miRNA or siRNA targets the mRNA for cleavage or represses its productive translation; increased miRNA–mRNA pairing generally favors the cleavage of the target mRNA [32–35]. Dicer enzymes are characterized by an amino-terminal DExH-box RNA helicase domain, a PAZ domain that is also found in ARGONAUTE (AGO) proteins, two RNaseIII domains, and carboxy-terminal dsRNA-binding domains [36]. *Arabidopsis* has four DICER-LIKE (DCL) proteins: DCL3 is needed for endogenous siRNA accumulation, DCL2 is required for the efficient accumulation of certain virus-derived siRNAs [23••], and DCL1 (originally known as CARPEL FACTORY, SHORT INTEGUMENTS1, or SUSPENSOR1) is needed for miRNA accumulation [19,20]. The *dcl1-9* mutant accumulates reduced levels of miRNAs [19,20], but maintains normal PTGS through siRNAs processed from an inverted-repeat transgene [37•]. *dcl1* mutant plants exhibit pleiotropic phenotypes, including embryonic arrest at the globular stage in null alleles and altered leaf shape, delayed floral transition, and female-sterility in partial loss-of-function mutants. These phenotypes attest to the broad range of developmental processes that require DCL1 and, by extension, miRNA activity [36].

Aside from the central involvement of a Dicer ribonuclease, there are differences in miRNA processing between plants and animals. Most animal Dicers lack the nuclear localization signal that is found in *Arabidopsis*, DCL1 [36]. In animals, primary miRNA transcripts are processed in the nucleus by the ribonuclease Drosha into the pre-miRNA hairpin, which is exported to the cytoplasm for Dicer processing [27]. By contrast, *Arabidopsis* DCL1, the closest homolog of animal DICER, is nuclear [23••] and processes the miR159 precursor in the nucleus, suggesting that plant miRNAs are generated in the nucleus [38••].

In addition to *DCL1*, *HYPONASTIC LEAVES1* (*HYL1*) [39•,40•] and *HUA ENHANCER1* (*HEN1*) [19,41] are required for miRNA accumulation and normal develop-

Table 1

MicroRNA targets and method of validation.

MicroRNA (number of *Arabidopsis* loci)	Target protein class (number of predicted target mRNAs in *Arabidopsis*)	Validated target mRNAs	Validation method
miR156/157 (12)	SQUAMOSA-promoter binding (11) [7]	*SPL2* [9••], *SPL3* [79] *SPL10* [40•]	5′ RACE
miR158 (2)	Unknown [7]		
miR159/miR-JAW (6)	MYB (8) [7,19], TCP (5) [63••]	*MYB33*, *MYB65*, *TCP2*, *TCP3*, *TCP4*, *TCP10*, *TCP24* [63••]	5′ RACE, miRNA-resistant target
miR160 (3)	ARF (3) [7]	*ARF10*, *ARF17* [9••]	5′ RACE
miR161 (1)	Pentatricopeptide repeat (9) [7]	***At1g06580*** [40•]	5′ RACE
miR162 (2)	Dicer (1) [78••]	*DCL1* [78••]	5′ RACE
miR163 (1)	Methyl transferases (5) [7]		
miR164 (3)	NAC-domain transcription factor (6) [7,22••]	*CUC1*, *CUC2* [9••], *NAC1*, ***At5g07680***, ***At5g61430*** [61••]	5′ RACE, wheat germ extract, miRNA-resistant target
miR165/166 (9)	HD-ZIP transcription factor (5) [7]	*PHB* [10••], *PHV* [10••], *REV* [62••], *C3HDZIP1* mRNAs [70••]	Wheat germ extract, 5′ RACE, miRNA-resistant target
miR167 (4)	ARF (2) [7,19]	*ARF8* [9••]	5′ RACE
miR168 (2)	AGO (1) [7]	*AGO1* [40•]	5′ RACE, miRNA-resistant target
miR169 (14)	CCAAT-binding factor HAP2-like protein (7) [7]	***At3g05690*** [22••]	5′ RACE
miR170/171 (4)	GRAS-domain transcription factor (3) [7,17,20]	*SCL6-III* [8], *SCL6-IV* [8,9••]	5′ RACE, Agro-inoculation
miR172 (5)	APETALA2-like transcription factor (5) [9••,12••]	*AP2* [9••,12••], *TOE1* [9••,12••], *TOE2* [9••,12••], *TOE3* [9••]	5′ RACE, miRNA-resistant target
miR173 (1)	Unknown [19]		
miR393 (2)	F-box protein (4), bHLH transcription factor (1) [22••]	*TIR1*, ***At1g12820***, ***At3g26810***, ***At4g03190***, ***At3g23690*** [22••]	5′ RACE
miR394 (2)	F-box protein (1) [22••]	***At1g27340*** [22••]	5′ RACE
miR395 (6)	ATP sulfurylase (3) [22••]	*APS4* [22••]	5′ RACE
miR396 (2)	Growth-regulating-factor transcription factor (7), Rhodenase-like protein (1), Kinesin-like protein B (1) [22••]	*GRL1*, *GRL2*, *GRL3*, *GRL7*, *GRL8*, *GRL9* [22••]	5′ RACE
miR397 (2)	Laccase (3), Beta-6 tubulin (1) [22••]	***At2g29130***, ***At2g38080***, ***At5g60020*** [22••]	5′ RACE
miR398 (3)	CSD (2), Cytochrome C oxidase subunit V (1) [22••]	*CSD1*, *CSD2*, ***At3g15640*** [22••]	5′ RACE
miR399 (6)	Phosphate transporter (1) [22••]		

Abbreviations: ARF, AUXIN-RESPONSE FACTOR; CSD, COPPER SUPEROXIDE DISMUTASE; 5′ RACE, 5′ rapid amplification of cDNA ends; *SPL2,* SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE2.

ment in *Arabidopsis*. Like the *dcl1* partial loss-of-function mutants, *hyl* null mutants display reduced miRNA levels but are unaffected in PTGS [39•,40•]. Conversely, miRNA levels increase when *HYL1* is expressed behind the strong Cauliflower mosaic virus 35S promoter [39•]. HYL1 is a nuclear dsRNA-binding protein, and the *hyl1* mutant was originally isolated because it displays multiple phytohormone-response defects [42], which are consistent with the possibility that certain miRNAs modulate hormone signaling.

Like *hyl1* mutants, *hen1* null mutants display reduced miRNA accumulation or altered miRNA size [19,23••, 41], suggesting that HEN1, a novel protein with an amino-terminal dsRNA-binding motif, augments but is not required for miRNA processing. The *hen1* mutation also disrupts the processing of a subset of siRNAs, suggesting that HEN1 functions in both the miRNA and the siRNA pathways [23••,41]. Whereas neither HEN1 nor HYL1 are absolutely required for miRNA biogenesis, the double mutant is infertile [40•], suggesting that these two genes act synergistically.

Members of the AGO family also are implicated in miRNA and siRNA functioning [43]. AGO proteins are defined by the presence of PAZ and PIWI domains [43]; the PAZ domain, which is shared with Dicer, may function to bind RNA [44–46]. As AGO proteins reside in the RISC, the diversity of AGO proteins may contribute functional specificity to various RISC-like complexes. *Arabidopsis* has ten AGO-like genes; mutations in *AGO1*, *AGO4*, *PINHEAD/ZWILLE*, and *ZIPPY* have been characterized. The *ago1* mutant displays pleiotropic shoot architecture defects [47] that overlap with those of *pinhead/zwille* mutants [48,49]. By contrast, *zippy/ago7* mutants develop nearly normally except for an accelerated developmental progression from juvenile to adult [50•]. AGO1, but not PINHEAD/ZWILLE, is required for PTGS [51,52], whereas AGO4 is required for the accumulation and function of certain heterochromatic siRNAs [53•]. *ago1* null mutants show decreased miRNA accumulation, and several mRNAs that are targets for miRNAs over-accumulate in *ago1* hypomorphs [54••], suggesting that at least some functional miRNA RISC-like complexes require AGO1.

Many plant viruses have evolved silencing suppressors that can disable siRNA-based host defenses, and studies of these proteins are providing insights into miRNA functioning. For example, the tombusvirus p19 protein binds siRNA or miRNA duplexes, thus inhibiting the functional incorporation of both siRNAs and miRNAs into the RISC [38••,55–58]; the *Beet yellows virus* p21 protein may act similarly [59]. *Tobacco etch virus* and *Turnip mosaic virus* P1/HC-Pro also affect miRNA accumulation and function [9••,57,60]. P1/HC-Pro does not bind siRNA or miRNA duplexes directly, and thus probably interferes at a point other than p19 [59]. Thus, PTGS suppressors from several unrelated viruses impinge on various points of miRNA functioning in their attempts to block host defenses; these proteins are being used to unravel the various shared and unique components that are required for miRNAs and siRNA function.

Targets of plant microRNAs: identification and validation

The high degree of complementarity between plant miRNAs and their target mRNAs has allowed the identification of targets using algorithms that scan the genome for mRNA–miRNA complementary [7]; refinement of this method has increased the reliability of the prediction [22••]. When a miRNA targets multiple mRNAs, the targeted genes are often members of a gene family, and miRNAs that are conserved between *Arabidopsis* and rice also tend to have conserved targets [7,22••]. Intriguingly, plant miRNAs display a striking propensity to target the mRNAs of transcription factors that have already been established as or are related to key developmental regulators [7]. Other targets include members of the Dicer-like and AGO families, as well as *S*-adenosyl-*L*-methionine-dependent methyl transferases [7], F-box proteins that mediate ubiquitin-dependent protein degradation, and proteins that are involved in sulfate assimilation [22••].

Many computationally identified miRNA–target mRNA pairings have been validated experimentally (Table 1). Four methods have been used to validate plant miRNA targets. *Agrobacterium*-mediated infiltration of test constructs into leaves allows both the miRNA-directed cleavage of candidate mRNAs to be assessed and the rapid evaluation of the effects of mRNA or miRNA mutations [8,9••]. *In-vitro* cleavage assays, in which radiolabeled target RNAs are added to wheat germ extracts that contain endogenous wheat miRNAs, can also be used to study the effects of mutations in candidate targets [10••,61••]. The most common form of miRNA target validation involves ligating an RNA adaptor to uncapped RNAs using T4 RNA ligase. This allows the specific amplification of the 3′-cleavage products of the target mRNAs using 5′-RACE. Cloning and sequencing the resultant PCR amplicons can both verify that a target undergoes miRNA-directed cleavage and determine the nucleotide position at which cleavage commonly occurs [8,9••]. These experiments have revealed that miRNA-directed cleavage occurs in the center of miRNA complementarity, between the residues that pair with the tenth and eleventh nucleotides of the miRNA [8,9••]. Finally, several miRNA–mRNA pairs have been validated by expressing miRNA-resistant versions of target genes in transgenic plants [13••,54••,61••–63••]; these miRNA-resistant genes are discussed in more detail below.

MicroRNAs regulating development: miR165/166 and polarity

The dramatic developmental defects of *dcl1* [36], *hyl1* [42], and *hen1* [64] mutants, which each display altered miRNA accumulation [19,20,39•,40•,41], implicate miRNAs in the control of diverse developmental processes. However, the misregulation of multiple miRNAs and their targets in these mutants complicates the task of ascribing particular roles to individual miRNAs. More specific consequences of disrupting miRNA-based regulation were found upon re-examining existing dominant alleles of miRNA targets, several of which are likely to derive from disrupted miRNA regulation.

Dominant mutations in any of three closely related *Arabidopsis* class-III homeodomain leucine zipper (HD-ZIP) transcription factors, *REVOLUTA* (*REV*), *PHABULOSA* (*PHB*), and *PHAVOLUTA* (*PHV*), result in adaxialization and radialization of leaves and vasculature [62••,65]. These mutations occur within a narrow region of a conserved domain, suggesting that the mutant phenotypes could result from the altered binding of a ligand [65]. The discovery that two related miRNAs, miR165 and miR166, display complementarity to the very sequence that is mutated in the gain-of-function alleles of *PHB* and *PHV* prompted speculation that these alleles are freed from miRNA-directed negative regulation [7]. Indeed, the *PHV* mRNA is cleaved within the miR165/166 complementarity site in wheat germ extract, whereas the *phv-1d* allele is resistant to cleavage [10••]. Moreover, expressing a *REV* mRNA in which the miRNA complementarity site contains 2-nt changes that decrease the complementarity between miR165/166 and *REV* phenocopies a gain-of-function *rev* allele, even though the protein encoded by the gene is not altered [62••]. The same miR165/166 complementarity site mutation in *REV* that was found in *rev-10d* [62••] is found in the semi-dominant *amphivasal vascular bundle1* mutant [66•]. This miR165/166 complementarity site mutation reduces *REV* cleavage and increases the levels of full-length *REV* mRNA [66•]. *In-situ* hybridization suggests that miR165 accumulates on the abaxial side of developing leaves [67••], in a pattern reciprocal to that of *PHB* [65]. The presumably miR165/166-resistant version of *PHB* mRNA that is encoded by the gain-of-function *phb-1d* allele is not restricted to the adaxial

Table 2

miRNA overexpression altering development.

MiRNA	Validated target mRNAs	Consequences of overexpression in *Arabidopsis*
MiR-JAW	*TCP2*, *TCP3*, *TCP4*, *TCP10*, *TCP24*	Uneven leaf shape and curvature, late flowering [63••]
MiR164	*CUC1*, *CUC2*, *NAC1*, ***At5g07680***, ***At5g61430***	Embryonic [73••], floral [61••,73••] and vegetative [61••] fusions
MiR172	*AP2, TOE1*, *TOE2, TOE3*	Early flowering and floral organ identity defects [12••]

domains of developing cotyledons, but is also found abaxially [65]. Together, these results provide strong support for the model in which miR165/166 functions to clear *REV*, *PHB*, and *PHV* mRNAs from cells that give rise to abaxial tissues.

In a striking parallel to the *Arabidopsis phb*, *phv*, and *rev* mutations, dominant mutations in the maize class-III HD-ZIP gene *rolled leaf1* (*rld1*) reduce complementarity to miR166 and are accompanied by leaf adaxialization in older primordia and inappropriate *rld1* accumulation in abaxial leaf tissues [68••]. During leaf development, *rld1* and miR166 are expressed in reciprocal domains that are consistent with a role for miR166 in restricting the presence of *rld1* transcripts within abaxial tissues [68••]. miR166 is expressed in an expanding pattern during leaf development and accumulates in the phloem, suggesting that it is a mobile signal [68••].

One of the characteristic features of miRNAs is that they are evolutionarily conserved [69]. Intriguingly, miR165/166 complementarity sites are found in class-III HD-ZIP transcription factor mRNAs not only in flowering plants, such as *Arabidopsis*, rice [7], and maize [68••], but also in gymnosperms, ferns, lyocopods, and bryophytes [70••]. Moreover, mRNA cleavage products that correspond to the miRNA complementarity sites have been detected in several of these lower plants, suggesting that miR165/166 has been negatively regulating class-III HD-ZIP transcription factors for at least 400 million years [70••].

Other microRNAs that regulate development

Two general types of experiments have been employed to assess individual miRNA functions when forward genetics has not revealed miRNA-resistant target alleles. One type of experiment involves overexpressing the miRNA gene [12••,61••,63••], which is expected to reduce the function of all target mRNAs (Table 2); the second involves expressing target genes that have reduced complementarity [13••,54••,61••–63••], which is expected to confer miRNA-resistance upon the target (Table 3). As redundancy in the genetic code allows complementarity to be reduced without altering the protein-coding potential of the target mRNA, this type of experiment can cleanly dissect the role of miRNA in regulating the target, especially when the endogenous promoter is used. As both of these approaches rely on dominant changes that can be observed following the transformation of wildtype plants, these experiments should be generalizable to all miRNAs in a variety of plant systems.

The biological roles of several *Arabidopsis* miRNAs have been inferred following overexpression studies (Table 2). miR-JAW, a relative of miR159, was originally uncovered through activation-tagging, a technique that allows the isolation of genes that confer phenotypes upon overexpression [71]. This miRNA regulates a subset of *TCP* genes, which encode non-canonical basic Helix-Loop-Helix (bHLH) transcription factors [72]. miR-JAW overexpression results in uneven leaf shape and curvature,

Table 3

MiRNA-resistant target mRNAs altering development.

MiRNA resistant target mRNA	Promoter	miRNA	Phenotype
CUC1	Endogenous	miR164	Shortened rosette leaf petioles, aberrant leaf shape, increase in petal and decrease in sepal numbers [61••]
CUC2	Inducible	MiR164	Abnormal leaves, increased sepal separation [73••]
REV	Endogenous	miR165/166	Radicalized and centralized vasculature, strands of leaf tissue attached to stems [62••]
AGO1	Endogenous	miR168	Spoon-shaped, curly or twisted leaves, increased levels of miRNA-targeted mRNAs [54••]
AP2	35S	miR172	Loss of floral determinacy, late flowering [13••]
TCP4	Endogenous and 35S	miR-JAW	Arrested seedlings with fused cotyledons, lack of SAM, tubular shape [63••]
TCP2	35S	miR-JAW	Long hypocotyls, small leaves, reduced apical dominance [63••]
MYB33	35S	miR159	Up-curled leaves [63••]

delayed flowering, and reduced levels of four *TCP* mRNAs that contain complementarity to miR-JAW [63••]. Expression of a miR-JAW-resistant version of *TCP4* with 6-nt changes in the miRNA complementarity site, but unaltered coding potential, results in embryonic defects that culminate in seedling arrest, whereas expression of a miR-JAW resistant *TCP2* confers longer hypocotyls and reduced apical dominance [63••].

A subset of the *Arabidopsis* NAC-domain transcription factors, including CUP-SHAPED COTYLEDON1 (CUC1), CUC2, NAC1, **At5g07680**, and **At5g61430**, are miR164 targets [7]. miR164 overexpression leads to sepal and stamen fusions [73••] that are reminiscent of the defects of *cuc1 cuc2* double mutants [74,75]. Additional fusions that are not observed in *cuc1 cuc2* mutants are seen in miR164-overexprssing plants, such as rosette leaf fusions, leaf–stem fusions, and stem–pedicle fusions, implicating an miR164 target in addition to *CUC1* and *CUC2* in controlling lateral organ boundaries [61••]. The expression of a miR164-resistant version of *CUC1* from its own promoter causes aberrant leaf development as well as decreased sepal numbers and increased petal numbers [61••]. Expression of a miR164-resistant version of *CUC2* from an inducible promoter also alters leaf development and increases sepal separation [73••]. *CUC1* and *CUC2* mRNAs are normally limited to the regions between developing petal and sepal primordia [75]; it is probable that negative regulation by miR164 is in part responsible for restricting the expression of these mRNAs. The conservation of miR164 complementarity sites [61••] in NAC-domain genes that are required for organ separation in petunia [76] and snapdragon [77] suggest that miR164 function may be general to flowering plants.

Most examples of the regulation of plant mRNAs by miRNAs can be explained by target mRNA cleavage. However, at least one *Arabidopsis* miRNA, miR172, reduces the accumulation of target protein without significantly effecting target mRNA levels, suggesting that this miRNA has a role in inhibiting productive translation [12••,13••]. A screen for genes that alter flowering time when overexpressed yielded *early activation tagged, dominant* (*eat-D*), an early-flowering *apetela2* (*ap2*)-like mutant that overexpresses miR172, and *target of eat1* (*toe1*), a late-flowering mutant that overexpresses a miR172 target *AP2*-like mRNA [12••]. In spite of near-perfect complementarity between miR172 and *AP2* mRNA, miR172 overexpressing plants display reduced AP2 protein levels but normal *AP2* transcript levels, consistent with the idea that miR172 negatively regulates *AP2* translation [12••,13••]. Conversely, expressing a miR172-resistant version of *AP2* increases AP2 protein levels, but *AP2* mRNA levels again remain unchanged [13••]. An unanswered question in miRNA biology is what factors other than complementarity influence whether various miRNAs promote mRNA cleavage or translational attenuation.

MicroRNAs that regulate microRNA biogenesis or function

Interestingly, two miRNAs target the mRNAs of genes that are implicated in miRNA biogenesis (*DCL1*) or function (*AGO1*). The *DCL1* mRNA, which is required for miRNA accumulation [19,20], is targeted by miR162 [78••]. Unlike many validated miRNA–mRNA pairs, a 1-nt bulge in the *DCL1* mRNA is present when miR162 pairs with *DCL1*. Despite this bulge, which occurs across from position 7–8 of the miRNA, the *DCL1* mRNA is cleaved in the middle of the miRNA complementarity site [78••]. (This theme is repeated in *GRL* mRNAs that are targeted by miR396, which also have a 1-nt bulge across from miRNA position 7–8 and are cleaved in the center of the complementarity site; a similarly positioned bulge is found in two of the miR398 targets [22••].) In *dcl1-7* and *hen1-1* plants, miR162 levels are reduced whereas *DCL1* mRNA accumulates to a higher level than in wildtype plants, suggesting that miR162 is part of a negative feedback loop that controls DCL1 levels [78••].

AGO1 and miR168 also seem to participate in a feedback loop. Like *DCL1* [78••], *AGO1* is the only member of its family to possess an extensive miRNA complementarity site [7,54••]. As discussed above, AGO proteins are present in the RISC [43], and loss-of-function *ago1* alleles accumulate miRNA target messages [54••]. Hence, the targeting of *AGO1* mRNA by miR168 could limit AGO1 levels and thus RNA-programmed RISC activity. When *AGO1* complementarity to miR168 is decreased, the *miRNA-resistant AGO1* (*mir-AGO1*) plants display curled leaves that are reminiscent of the leaves of *hen1* and *hyl1* mutants, with the additional phenotype of aberrant cotyledons [54••]. Severely affected plants lack a shoot apical meristem and die before flowering [54••]. When compensatory changes are made in the miR168 sequence, *mir-AGO1* phenotypes are restored to wildtype [54••]. Target mRNA levels increase in both *ago1* hypomorphs and *mir-AGO1* plants, suggesting that excess AGO1 promotes the formation of non-functional RISC sub-complexes [54••].

Conclusions

miRNA-based regulation adds to the cell's complex repertoire of negative regulatory mechanisms. As more miRNAs are discovered and their target genes identified, finding biological roles for these interactions enables greater understanding of gene regulation and of the roles of both the miRNA and its target genes in plant development. Obviously, there is still much to learn. Very little is known about transcriptional and post-transcriptional contributions to the regulation of the *MIR* genes that produce miRNAs. It will be interesting to determine whether the multiple miRNAs within each family have distinct or overlapping roles. The factors other than complementarity that influence target cleavage as opposed to translational attenuation remain to be identified. Despite these, and many other fascinating complexities

that await future investigation, a fundamental paradigm has emerged: plants and other multicellular organisms use the simple process of base-pairing to specifically down-regulate critical messages during development and other processes.

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- • of special interest
- •• of outstanding interest

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