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Translational Inhibition by MicroRNAs in Plants

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Chapter 3

Translational Inhibition by MicroRNAs in Plants

Bin Yu and Hai Wang

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Abstract MicroRNAs (miRNAs) are 21–24 nucleotide riboregulators, which selectively repress gene expression through transcript cleavage and/or translational inhibition. It was thought that most plant miRNAs act through target transcript cleavage due to the high degree of complementarity between miRNAs and their targets. However, recent studies have suggested widespread translational inhibition by miRNAs in plants. The mechanisms underlining translational inhibition by plant miRNAs are largely unknown, but existing evidence has indicated that plants and animals share some mechanistic similarity of translational inhibition. Translational inhibition by miRNAs has been shown to regulate floral patterning, floral timing, and stress responses. This chapter covers recent progress on plant miRNA-mediated translational control.

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3.1 Introduction

MicroRNAs (miRNAs) are 21–24 nucleotide noncoding RNAs that inhibit the expression of genes containing partially complementary sequences at post-transcriptional levels through translational inhibition or target cleavage (Bartel 2004), or sometimes at the transcriptional level through chromatin modification (Bao et al. 2004). Plant miRNAs were first identified in 2002 (Llave et al. 2002a; Mette et al. 2002; Park et al. 2002; Reinhart et al. 2002), approximately a decade after the shorter *lin-4* RNA, the founding member of miRNAs, was identified in *Caenorhabditis elegans* (Lee et al. 1993). Since then, the functions of plant miRNAs in regulating biological processes including development, metabolism, hormone responses, responses to biotic and abiotic stress, and others have been established (Mallory and Vaucheret 2006). Owing to the development of computational prediction algorithms and large-scale sequence techniques, hundreds of miRNAs have been identified in plants (Meyers et al. 2006). The great potential of these miRNAs to regulate thousands of genes has emphasized the importance of a riboregulatory network of gene expression in addition to transcriptional factors.

Although translational inhibition by miRNAs has been reported in plants (Aukerman and Sakai 2003; Chen 2004; Dugas and Bartel 2008; Gandikota et al. 2007), target-cleavage by miRNAs is thought to be the predominant way for miRNAs to act (Bartel 2004). However, recent studies have shown that translational inhibition is widely present in plants (Brodersen et al. 2008). In addition to miRNAs, plants are also enriched with small interfering RNAs (siRNAs). Translational inhibition by siRNAs in plants has been demonstrated recently (Brodersen et al. 2008). This chapter summarizes the recent knowledge about the biogenesis of plant miRNAs and siRNAs, miRNA-mediated translational control in plants, and translational inhibition by siRNAs.

3.2 miRNA and siRNA Biogenesis

3.2.1 Plant miRNA Biogenesis

miRNAs are generated from long transcripts by DNA-dependent RNA polymerase II (pol II) (Bartel 2004). The primary transcripts of miRNA genes, containing a hairpin structure, carry a seven-methyl guanosine (m⁷G) cap at the 5' end and a polyadenosine tail (polyA) at the 3' end (Bartel 2004). Plant miRNA genes are independent transcription units subjected to transcriptional regulation with a few exceptions (Rajagopalan et al. 2006). For instance, miR838 are derived from intron 14 of *DICER-LIKE1* gene (Rajagopalan et al. 2006).

In Arabidopsis and Rice, an RNAase III like domain-containing protein, called Dicer-like 1 (DCL1), releases a single miRNA/miRNA* duplex from the hairpin structure of pri-miRNAs through two-step cleavages in the nucleus excluding

miR822 and miR839, which are generated by DCL4 (Rajagopalan et al. 2006; Ramachandran and Chen 2008b). The miRNA/miRNA* has a 2-nt overhang at the 3' end of each strand and a phosphate group at the 5' end of each strand, which are typical features of RNAase III cleavage products (Bartel 2004). The efficient and accurate processing of pri-miRNAs requires a double-strand RNA binding protein HYPONASTIC LEAVES1 (HYL1) and a zinc finger protein SERRATE (SE) (Dong et al. 2008; Han et al. 2004; Lobbes et al. 2006; Reinhart et al. 2002; Yang et al. 2006a). DCL1, HYL1 and SE form a small nuclear body containing pri-miRNAs, called D-body or SmD3/SmB nuclear bodies (Fang and Spector 2007; Kurihara and Watanabe 2004; Song et al. 2007).

It was reported recently that the loss-of-function of ABH1/CBP80 and CBP20, two subunits of nuclear cap-binding complex, reduces the levels of miRNAs, suggesting the involvement of the multifunctional cap-binding complex in pri-miRNA processing (Chen, 2008; Gregory et al. 2008; Laubinger et al. 2008). In addition, it was proposed that DAWDLE (DDL), a forkhead domain containing protein, might participate in the miRNA biogenesis by stabilizing pri-miRNAs and facilitating their access or recognition by DCL1 (Yu et al. 2008). It has been shown that DDL interacts with DCL1, and lack of DDL reduces the levels of pri-miRNAs, pre-miRNAs, and mature miRNAs (Yu et al. 2008). Interestingly, Smad interacting protein1 (SNIP1), a human ortholog of DDL, interacts with Drosha and participates in the miRNA biogenesis, suggesting that DDL is an evolutionarily conserved factor in the miRNA biogenesis (Yu et al. 2008).

After generation, the miRNA/miRNA* duplexes are methylated on the 2'OH of the 3' terminal ribose on each strand by a protein named HUA EHANCER1 (HEN1) (Yang et al. 2006b; Yu et al. 2005). Currently, it is not clear whether the methylation occurs in the nucleus or cytoplasm. In plants carrying a *hen1* mutation, lack of miRNA methylation reduces miRNA abundance and causes the addition of 1–6 uridines at miRNA 3' terminal, suggesting that miRNA methylation protects miRNAs from degradation and/or uridylation (Li et al. 2005). The genes encoding these enzymes are unknown. A recent study showed that a family of exoribonucleases encoded by the SMALL RNA DEGRADING NUCLEASE (SDN) genes degrades single-stranded mature miRNAs in Arabidopsis (Ramachandran and Chen 2008a). In Arabidopsis, an ortholog of exportin-5 called HASTY exports mature miRNAs to the cytoplasm, although it is not clear whether miRNAs are exported as duplex or single strand (Park et al. 2005). However, mutations in HASTY do not reduce the levels of several miRNAs, suggesting the presence of an alternative exporting mechanisms (Park et al. 2005).

3.2.2 *SiRNA Biogenesis*

Beyond miRNAs, plants are also enriched with siRNAs, which represent 85% of cellular small RNAs (Kasschau et al. 2007; Lu et al. 2006; Rajagopalan et al. 2006; Zhang et al. 2007). The difference between miRNAs and siRNAs is their

origin ([Chen 2005](#)). While miRNA is excised from a partially complementary hairpin structure in the pri-miRNA, siRNA is produced from a hairpin transgene or a perfect complementary long dsRNA converted by RNA dependent polymerases (RDRs) from a single-stranded RNA, or resulted from sense and antisense transcription ([Chen 2005](#)).

A class of 24 nt RNAs produced from transposons and repetitive DNA consists the largest portion (84%) of small RNAs. The function of this class siRNAs includes directing DNA methylation and histon modification ([Mallory and Vaucheret 2006](#)). These siRNAs are excised by DCL3, a homolog of DCL1, from dsRNAs, which are presumably converted by RNA dependent RNA polymerase 2 from transcripts of repeat DNAs ([Xie et al. 2004](#)). The biogenesis of these siRNAs requires the plant specific DNA-dependent polymerase IV, Pol IVa ([Herr et al. 2005](#); [Kanno et al. 2005](#); [Onodera et al. 2005](#)). Pol IVb, another form of Pol IV is required for the function of these siRNAs and is involved in the biogenesis of some of them ([Kanno et al. 2005](#); [Pontier et al. 2005](#)).

Trans-acting siRNAs (ta-siRNAs), consisting ~1% of small RNA, represent another class of siRNAs ([Allen et al. 2005](#); [Vazquez et al. 2004](#); [Yoshikawa et al. 2005](#)). ta-siRNAs are generated from noncoding transcripts. The transcripts are first subjected to miRNA-mediated cleavage. The cleavage fragments are then stabilized by SGS3 and converted to dsRNAs by RDR6, which are processed by DCL4 into 21 nt siRNAs ([Allen et al. 2005](#); [Vazquez et al. 2004](#); [Yoshikawa et al. 2005](#)). The process of ta-siRNA biogenesis also requires SDE5, whose function is unknown, and DOUBLE STRAND RNA BINDING PROTEIN4 (DRB4), which is a homology of HYL1. ta-siRNAs act on genes other than their originating genes ([Adenot et al. 2006](#); [Hernandez-Pinzon et al. 2007](#)).

The third class of siRNAs is natural sense-antisense siRNAs (Nat-siRNAs) identified under biotic or abiotic stresses ([Borsani et al. 2005](#); [Katiyar-Agarwal et al. 2006](#)). They are generated from bidirectional transcripts. RDR6, SGS3, Pol IVa, HYL1, HEN1, and two DCL proteins are involved in the biogenesis of *nat-siRNAs*. Recently, a class of 30–40nt long siRNAs (IsiRNAs) was isolated under biotic stress or specific growth conditions ([Katiyar-Agarwal et al. 2007](#)). The biogenesis of this class siRNAs is dependent on HYL1, HST, RDR6, and Pol IV ([Katiyar-Agarwal et al. 2007](#)).

3.3 ARGONAUTE Proteins

Through base-pairing with the complementary sequence embedded in the targets, small RNAs guide transcriptional and post-transcriptional silencing performed by a ribonucleoprotein complex called RNA induced silencing complex (RISC) ([Bartel 2004](#)). Members of Argonaute (AGO) protein family are the effectors in the RISC complex ([Vaucheret 2008](#)). *AGO1* is the founding member of this gene family identified from a genetic screen for mutants deficient in plant growth ([Bohmert et al. 1998](#); [Vaucheret 2008](#)). It was named because of the resemblance of appearance between *ago1* and a small squid of *Agrounauta* genus ([Bohmert et al. 1998](#)). AGO proteins are conserved among eukaryotes. They contain conserved PAZ, MID, and

PIWI domains in the C-termini. It has been shown that the MID domain associates with the 5' phosphate of small RNAs, the PAZ domain binds to the 3' terminal nucleotide, and the PIWI domain, which contains an RNaseH signature, exhibits endonuclease activity and performs target cleavage.

The numbers of AGO proteins are diversified among different plant species. While unicellular green algae *Chlamydomonas reinhardtii* encodes only two AGOs (Zhao et al. 2007b), Arabidopsis and rice contain 10 and 18 AGOs, respectively (Morel et al. 2002; Nonomura et al. 2007). Among ten AGOs of Arabidopsis, AGO1, AGO7, and AGO4 have the slicing activity. It was found that AGO1 is responsible for most miRNA-mediated mRNA cleavage (Baumberger and Baulcombe 2005), and AGO7 is the slicer of miR390-mediated target cleavage (Montgomery et al. 2008). AGO4 acts redundantly with AGO6 in siRNA-mediated DNA methylation of histone modification (Zheng et al. 2007).

The AGO proteins selectively recruit the miRNA strand with a less stable 5' end from the miRNA/miRNA* duplex into RISC complex. The other strand named miRNA* is then degraded. Intriguingly, deep sequence analysis of small RNAs coimmunoprecipitated with AGO proteins revealed that small RNAs are selectively channeled into RISC complexes according to their 5' terminal nucleotide (Mi et al. 2008; Montgomery et al. 2008; Takeda et al. 2008). AGO1 preferentially binds to miRNAs with a 5' uridine (Mi et al. 2008). AGO2 and AGO4 complexes harbor small RNAs with a 5' adenine, and AGO5 associate with small RNAs with a 5' cytosine (Mi et al. 2008). Substituting the 5' terminal uridine of miR391 or miR171, which are associated with AGO1, with an adenine forces them into an AGO2 complex, indicating an essential role of 5' terminal nucleotides in sorting small RNAs into RISC complex (Mi et al. 2008). However, there are a few exceptions. For instance, miR172 and miR390 with a 5' terminal A are preferentially associated with AGO1 and AGO7, respectively (Mi et al. 2008; Montgomery et al. 2008).

In addition, sequence analysis showed that AGO4 associates with miR172 in vivo, and the AGO4 complex harboring miR172 cleaves miRNA target in vitro (Qi et al. 2006). However, plants lacking of AGO4 do not show miR172-resistant phenotypes, indicating that AGO4 is not required for miR172 function in vivo (Qi et al. 2006). This result raises question about the functional role of AGO4–miR172 association in Arabidopsis (Qi et al. 2006; Vaucheret 2008).

3.4 Translational Inhibition by Small RNAs is Common in Plants

3.4.1 Translational Inhibition by miRNAs

miRNAs regulate gene expression through translational inhibition and/or target cleavage. A key factor determining the functional mechanism of miRNAs is the complementarity between miRNAs and their targets (Bartel 2004). It was observed that a perfect match between miRNAs and their targets promotes target cleavage,

while central mismatch inhibits cleavage and enables translational inhibition (Bartel 2004). Most animal miRNAs have imperfect matches with their targets and repress the translation, while plant miRNAs are highly complementary to their targets and were thought to function predominantly by target-cleavage (Bartel 2004).

However, it was observed recently that translational inhibition by miRNA is a widespread phenomenon (Brodersen et al. 2008). In a genetic screen for Arabidopsis mutants deficient in repressing the expression of a green fluorescent protein (GFP) containing a miR171 target site immediately downstream of the stop codon, two *microRNA action deficient* (*mad*) mutants, *mad5* and *mad6*, in which the levels of GFP protein but not mRNA are increased were isolated (Brodersen et al. 2008). Further analysis of several targets of endogenous miRNAs showed that the protein levels of all these targets are increased in *mad5* and *mad6* compared with those in WT (Brodersen et al. 2008). In contrast, the mRNA levels of most targets remain unchanged (Brodersen et al. 2008). These miRNA targets represent the distribution of target site in the 5' UTR, coding sequence, or 3' UTR of mRNAs and have different degree of complementarity with miRNAs (Brodersen et al. 2008). These results suggested that translational inhibition is a common action mechanism of plant miRNAs regardless of the localization of target site in the target and the degree of complementarity.

3.4.2 Translational Inhibition by siRNAs

Translational inhibition by siRNAs has been established in animals but not in plants. The SUC–SUL (SS) silencing system represses the expression of the endogenous SULFUR mRNA (SUL) in Arabidopsis through the action of DCL4 dependent siRNAs, which are generated from phloem-specific expression of an inverted-repeat (IR). Silencing of SUL results in a vein-centered chlorotic phenotype. Silencing of SUL requires AGO1 protein. Introducing an *ago1-27* mutation into the SUL silencing line represses the silencing without changing the levels of SUL mRNA and siRNAs. In addition, the protein levels of SUL in *ago1-27* are similar to those of WT. These results reveal that like animal siRNAs, plant siRNAs mediate translational inhibition.

In addition, siRNA-mediated translational inhibition is also present in *Chlamydomonas reinhardtii*. When an IR transgene was used to silence MAA7, a tryptophan β -synthase subunit, ~20% transformants, in which MAA7 protein levels are reduced, displayed no significant change of MAA7 mRNA levels (Cerutti, personal communication). Furthermore, disruption of an exportin 5-like protein in these transformants reduces the accumulation of IR siRNAs, resulting in an increased MAA7 protein levels and unchanged MAA7 mRNA levels (Cerutti, personal communication). These data

suggested that IR siRNAs can inhibit translation and raises a question why some siRNAs mediate translation inhibition, while others guide target cleavage (Cerutti, personal communication).

3.4.3 Mechanistic Similarity of Translational Inhibition by miRNAs Between Plants and Animals

Unlike in animals, our understanding on the mechanisms governing translational inhibition by plant miRNAs only begins to emerge. Among ten AGO proteins from *Arabidopsis*, AGO1 and AGO10 were proposed to be involved in translational inhibition (Brodersen et al. 2008). In a hypomorphic *ago1-27* mutant, the protein levels of several target increase dramatically while the mRNA levels increase only moderately, suggesting that AGO1 may contribute to translational inhibition (Brodersen et al. 2008). AGO10 protein is the closed paralog of AGO1 and might function redundantly with AGO1 in some aspects, because the *ago10* mutant has some similar developmental defects with *ago1*, and *ago1 ago10* double mutations cause lethality. In fact, in a frameshift *ago10* mutant, the protein levels of CSD2, a miR398 target, increased disproportionately higher than the mRNA levels, indicating that AGO10 might be a player of translational inhibition (Brodersen et al. 2008).

The deficiency of miRNA-mediated translational inhibition in *mad5* is caused by a mutation in the *KATANINI* (*KTN1*) gene (Brodersen et al. 2008), which encodes the catalytic subunit of 60 kDa (P60) of the microtubule-severing protein (Burk et al. 2001). *KTN1* is an ATPase associated with various cellular activities. *KTN1* severs microtubules in the presence of ATP in vitro (Stoppin-Mellet et al. 2002). Over-expressing *KTN1* in the plant interphase cells releases cortical microtubules and generates motile microtubules that incorporate into bundles, indicating the role of *KTN1* in microtubule dynamics (Stoppin-Mellet et al. 2006). The reduction of protein but not mRNA levels of miRNA targets in the *mad5* and other mutant alleles of *KTN1* suggested a role of microtubule dynamics in the miRNA-mediated translational inhibition but not miRNA-mediated cleavage (Brodersen et al. 2008). The involvement of microtubules in miRNA-mediated gene regulation has been documented in animals. For instance, in *C. elegans*, the reduction tubulins by RNAi disrupted miRNA-guided translational inhibition (Parry et al. 2007); in *Drosophila*, Armitage, a microtubule-associated protein, functions in RISC assembly (Cook et al. 2004). The finding that microtubules are required for miRNA-mediated translational inhibition suggested that plant miRNAs might employ similar mechanisms to direct translational inhibition (Brodersen et al. 2008). In fact, VSC, a component of the decapping complex, is required for plant miRNA-mediated translational inhibition, which resembles the function of the components of animal decapping complex in animals such as DCP1, DCP2, and Ge-1 (Brodersen et al. 2008; Eulalio et al. 2007).

3.5 Roles of miRNA-Mediated Translational Inhibition in Plants

3.5.1 *miR172-Mediated Translational Inhibition*

The first plant miRNA that was observed to act through translational inhibition is miR172, which presents in both eudicotyledons and monocotyledons including *Arabidopsis*, Rice, and others (Aukerman and Sakai 2003; Chen 2004). miR172 represses the expression of a class of *APETALA2* (*AP2*)-like transcription factors (Table 3.1), including AP2, TOE1-3, SMZ, and SNZ, which are involved in controlling multiple biological processes in plants such as development, hormone responses, and disease resistance (Park et al. 2002; Schmid et al. 2005; Schwab et al. 2005). In plants, miR172-mediated translational inhibition has been shown to regulate floral patterning, floral determinacy, and flowering time.

3.5.1.1 Roles of miR172-Mediated Translational Inhibition in Floral Patterning

Most angiosperm flowers possess four types of organs, from outside to inside, sepals, petals, stamens, and carpels. They are arranged in a series of whorls. Among them, stamen and carpel are reproductive organs, and sepals and petals are perianth organs. It was proposed that the floral organ identity is specified through the combinatorial activities of three classes of genes, called *A*, *B*, and *C* genes (Jack 2004). Most of these *A*, *B*, and *C* genes are transcription factors. The *A* genes alone specify sepal, the *A* and *B* genes specify petals, the *B* and genes specify stamen, and the *C* genes alone specify carpel. *A* and *C* genes reciprocally repress each other to restrict their domains of activities (Jack 2004). Recent studies have revealed the important role of translational inhibition by miR172 in specifying organ identity, adding a new layer of regulating network of floral organ identity.

APETALA2 (*AP2*) functions as an *A* gene because loss of function of *ap2* mutation results in the expression of *AGAMOUS* (*AG*), a *C* gene, in the outer two whorls, which causes the replacement of perianth organs by reproductive organs (Jack 2004). AP2 protein contains a miR172-binding site and appears to be regulated by miR172 through translational inhibition. Overexpression of miR172 under the control of a cauliflower mosaic virus (CaMV) 35S promoter reduced the AP2 protein levels without significantly changing the accumulation of AP2 mRNAs, suggesting translational regulation of AP2 by miR172 (Aukerman and Sakai 2003; Chen 2004). Consistent with this, the expression of an *AP2* cDNA (*AP2m3*), in which the binding site of miR172 was abolished, but not wild-type *AP2* cDNA increased AP2 protein levels without affecting *AP2* mRNA levels (Chen 2004). Overexpression of miR172 converted the perianth organs to reproductive organs, which resembles the

phenotypes of *ap2* mutant, indicating loss-of-control of AG by AP2 in the outer two whorls (Aukerman and Sakai 2003; Chen 2004). In AP2m3, the expression of AG is reduced in the inner two whorls, and the reproductive organs are replaced with perianth organs (Chen 2004). These results demonstrated the important role of miR172 in controlling floral patterning through translational inhibition of AP2. Unlike in Arabidopsis, overexpression of miR172 from Arabidopsis in *Nicotiana benthamiana* converts sepal to petal and/or more sepals and petals (Mlotshwa et al. 2006). Although it is not clear how miR172 regulates floral homeotic transformation in *N. benthamiana*, this result indicated that miR172-mediated gene regulation might have different roles in floral patterning in different plant species (Mlotshwa et al. 2006).

In Arabidopsis, the stamen identity determination requires the function of class *B* genes, AP1 and PI, which are present in whorl 2 and 3 (Zhao et al. 2007a). The presence of stamens flanking floral meristem in AP2m3 suggested that the expression domain of AP3 and PI was expanded (Zhao et al. 2007a). In fact, AP3 and PI RNAs are present in all internal stamen primordia in AP2m3 (Zhao et al. 2007a). In addition, PI RNAs also exist in the center of the meristem throughout flower development since its initiation in AP2m3 (Zhao et al. 2007a). These data suggested a role of miR172 in defining the boundary of *B* gene expression domain in whorl 2 and whorl 3 (Zhao et al. 2007a).

The floral meristem terminates after the production of carpel. However, the flowers of AP2m3 plants contain numerous stamens flanking an indeterminate floral meristem, suggesting that miR172 mediated-repression of AP2 is crucial in specifying floral stem cell fate (Zhao et al. 2007a). In Arabidopsis, *WUSCHEL* (*WUS*), a homeodomain transcription factor, functions in regulating floral stem cells (Clark 2001). The *WUS* gene is expressed in a small number of cells underneath the stem cell and identifies the overlying cells as stem cells (Clark 2001). Unlike in the wild-type plants, the expression domain of *WUS* in AP2m3 is expanded into the entire meristem and young organ primordia in the very late stage flowers (Zhao et al. 2007a). Introducing a *wus* mutation into AP2m3 completely abolishes the indeterminate phenotypes (Zhao et al. 2007a). These results confirmed the role of miR172 in regulating floral stem cells through *WUS* pathway (Zhao et al. 2007a).

3.5.1.2 Roles of miR172-Mediated Translational Inhibition in Floral Timing

miR172-mediated translational inhibition also regulates flowering time. *TOE1* and *TOE2* are another two AP2-like transcription factors regulated by miR172 (Aukerman and Sakai 2003). Lack of *TOE1* but not *TOE2* causes slightly early flowering in Arabidopsis (Aukerman and Sakai 2003). However, loss-of-function of both *TOE1* and *TOE2* results in a much earlier flowering phenotype, suggesting that *TOE1* and *TOE2* function redundantly as floral timing repressors (Aukerman and Sakai 2003). Overexpression of miR172 reduces the *TOE1* protein levels without significantly affecting its mRNA level, although the cleavage products of *TOE1* can be detected

(Aukerman and Sakai 2003). Unlike *TOE1*, the *TOE2* mRNA levels are reduced by overexpression of miR172, indicating a role of miR172-mediated target cleavage (Aukerman and Sakai 2003). The miR172-overexpressing plants display an extremely early flowering phenotype (Aukerman and Sakai 2003). In addition, overexpression of miR172 suppresses the later flowering phenotype caused by overexpression of *TOE1* (Aukerman and Sakai 2003). These data suggested a role of mi172 in controlling flowering time through repressing *TOE1* and *TOE2* (Aukerman and Sakai 2003). In addition, the *toe1toe2* does not flower as early as *miR172*-overexpressing plants, indicating that other floral repressors are also targets of *miR172* (Aukerman and Sakai 2003).

3.5.2 *miR156-Mediated Translational Inhibition*

miR156 is another conserved plant miRNA family, which has been shown to function as translational inhibitor. miR156 targets a class of plant specific *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)* transcription factors (Table 3.1), which contain a highly conserved DNA-binding domain (Cardon et al. 1999; Rhoades et al. 2002). Out of 17 SPL genes, 11 are predicted to be targets of miR156 (Rhoades et al. 2002). SPL3, containing a miR156 target site at 3' UTR, is a member of SPL gene family and functions in floral induction as overexpression of SPL3 cDNA lead to an early flowering phenotype (Cardon et al. 1997). Some studies have suggested that miR156/157 regulates the expression of SPL3 through target-cleavage, because overexpression of miR156 reduces the levels of SPL3 mRNA and the transcripts of SPL3 are increased in *dcl1-12* and *hasty*, which are known mutants deficient in miRNA biogenesis (Park et al. 2005; Schwab et al. 2005). In addition, the cleavage products of SPL3 by miR156 have been detected in Arabidopsis (Schwab et al. 2005). However, recent evidence

Table 3.1 Validated microRNAs inhibiting translation of targets

MicroRNA	Targets	Targets regulated through translational inhibition	Function
miR172	AP2-like transcription factors	AP2, TOE1	Floral patterning, floral meristem, and floral timing
miR156/157	SPL transcription factors	SPL3	Floral timing
miR398	COX5b.1,CSD1, CSD2	CSD1, CSD2	Oxidative stress
miR171	SCL transcription factors	SCL6-IV	Unknown
miR834	CIP4	CIP4	Positive regulator of photomorphogenesis

AP2 APETALA2; *SPL* SQUAMOSA PROMOTER BINDING PROTEIN-LIKE; *COX5b.1* Cytochrome Oxidase Subunit 5b.1; *CSD* COPPER SUPEROXIDE DIMUTASE; *SCL* SCARECROW-like; *CIP4* *COP1*-interactive partner 4

suggested that miR156 also represses the expression of SPL3 at translational level (Gandikota et al. 2007). Overexpression of a SPL3 transgene resistant to miR156 or a wild-type SPL3 transgene under the control of 35S promoter in Arabidopsis produced considerable levels of SPL3 transcripts, but only the transcripts resistant to miR156 generated detectable protein levels of SPL3 (Gandikota et al. 2007). In addition, in *mad5*, a miRNA-mediated translational inhibition deficient mutant, the SPL3 protein levels are increased dramatically, while the SPL3 mRNA levels are slightly decreased, suggesting that miR156 negatively regulate SPL3 translation (Brodersen et al. 2008). These results suggested that miR156 is able to regulate the expression of SPL3 through both target-cleavage and translational inhibition.

Overexpression of miR156 caused a later flowering time indicating that miR156 acts as a floral time repressor (Gandikota et al. 2007). Overexpression of a SPL3 transgene resistant to miR156, but not a wild-type SPL3 transgene, induces early flowering time, suggesting that miR156 regulates flowering time through repressing the expression of SPL3 (Gandikota et al. 2007).

3.5.3 miR398-Mediated Translational Inhibition

miR398 represents a class of conserved angiosperm miRNAs and has been shown to function through translational inhibition (Table 3.1). In Arabidopsis, miR398 recognizes two *COPPER SUPEROXIDE DISMUTASE* (*CSD*) genes, *CSD1* and *CSD2*, and a *CYTOCHROME C OXIDASE 5b.1* (*COX5b.1*) gene (Bonnet et al. 2004; Jones-Rhoades and Bartel 2004; Sunkar and Zhu 2004). Due to the lack of an antibody recognizing COX5b.1, it is currently unknown whether miR398 regulate COX5b.1 through translational inhibition. Similar to miR156, miR398 regulates *CSD1* and *CSD2* through both translational inhibition and target cleavage (Dugas and Bartel 2008; Sunkar et al. 2006; Yamasaki et al. 2007). Overexpression of miR398 results in a dramatic reduction of proteins levels of *CSD1* and *CSD2* and a moderate reduction of mRNA levels of *CSD1* and *CSD2*, indicating miR398 regulates *CSD1* and *CSD2* through translational inhibition (Dugas and Bartel 2008). Consistent with this result, the *mad* mutants that are deficient in miRNA-mediated translational inhibition display increased protein levels of CDS1 and CDS2 without significantly changing mRNA levels of *CSD1* and *CSD2* (Brodersen et al. 2008). Overexpression of miR398 reduces the mRNA levels of *CSD1* and *CSD2* indicating that miR398 also functions through target-cleavage (Dugas and Bartel 2008; Sunkar et al. 2006; Yamasaki et al. 2007). Agreeing with this, the mRNA levels of *CSD1* and *CSD2* increased dramatically in *dcl1-12*, in which the levels of miR398 are reduced (Brodersen et al. 2008). Intriguingly, introducing *CSD1* and *CSD2* transgenes with altered miR398-binding site increased the accumulation of mRNAs, but not proteins of *CSD1* and *CSD2*, indicating that altering target site complementarity can change miRNA-mediate cleavage to translational inhibition (Dugas and Bartel 2008).

The function of CSD1 and CSD2 is to protect plants from oxidative stress through neutralizing superoxide radicals by releasing molecular oxygen and hydrogen peroxide. It was suggested that CSD2 might protect plants from oxidative stress generated by photosynthetic activities (Kliebenstein et al. 1998). Their function requires copper as an essential cofactor (Kliebenstein et al. 1998). Several studies have suggested that miR398 regulates CSD1 and CSD2 in response to oxidative stress or copper availability (Sunkar et al. 2006; Dugas and Bartel 2008; Yamasaki et al. 2007). It has been observed that the levels of miR398 are positively regulated by sucrose (Dugas and Bartel 2008). Sugar is able to inhibit photosynthesis, which produces reactive-oxygen species (ROS) (Dugas and Bartel 2008). Therefore, the induction of miR398 by sucrose might reflect that plants reduce the levels of CSD1 and CSD2 through enforcing miR398-mediated regulation in response to reduced oxidative stress (Dugas and Bartel 2008). In addition, the miR398 levels are reduced by copper supplement and are increased by copper limitation suggesting copper regulates the levels of CSD1 and CSD2 through releasing or enforcing miR398-mediated regulation (Dugas and Bartel 2008; Sunkar et al. 2006; Yamasaki et al. 2007).

3.5.4 Other miRNA-Mediated Translational Inhibition

Beyond miR172, miR156, and miR398, some other miRNAs, including miR171 and miR834 are also involved in translational inhibition. miR171 targets several members of the SCARECROW-like (SCL) family of putative transcription factors, which are involved in numerous development processes (Brodersen et al. 2008). It has been shown that miR171 regulates SCL6-IV through target cleavage (Llave et al. 2002b). However, disruption of translational control in *mad* mutants lead to increased protein levels without significant effect on the mRNA levels (Brodersen et al. 2008). miR834 is identified only in Arabidopsis and targets *COP1-interactive partner 4* (CIP4), which is a putative transcription factor (Fahlgren et al. 2007). Both *mad* mutants and *dcl1-12* mutant displayed increased CIP4 protein levels and WT mRNA levels, suggesting miR834 regulates CIP4 through translational inhibition (Brodersen et al. 2008).

3.6 Conclusion

Although it has been established that translational inhibition is a common acting mechanism of plant miRNAs, many aspects of translational inhibition remain unclear. Unlike in animals, the mechanism governing miRNA-mediated translational inhibition is little known. Given the fact that both plant and animal miRNA-mediated translational inhibition requires microtubule network and P-body components, plants and animals might have some mechanistic similarities of miRNA mediated translational inhibition (Brodersen et al. 2008). It was proposed that

the complementarity between miRNAs and targets determines the miRNA activity. However, the same plant miRNA can act through both target cleavage and translational inhibition, raising the question how cells make choices between these two activities (Brodersen et al. 2008). It remains unclear whether these two activities coexist or are temporarily and/or spatially separated (Brodersen et al. 2008). AGO1 performs both cleavage activity and translational inhibition raising a question how another activity is repressed when one activity is ongoing (Brodersen et al. 2008).

Detailed studies on three miRNAs have shown that miRNA mediated translational inhibition are involved in controlling floral patterning, floral timing, and stress responses. Given hundreds of miRNAs have been identified in plants, testing the functional roles of translational inhibition by these miRNAs represents another challenge.

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References

- Adenot X, Elmayan T, Lauressergues D, Boutet S, Bouche N, Gascioli V, Vaucheret H (2006) DRB4-dependent TAS3 trans-acting siRNAs control leaf morphology through AGO7. *Curr Biol* 16:927–932
- Allen E, Xie Z, Gustafson AM, Carrington JC (2005) [microRNA-directed phasing during trans-acting siRNA biogenesis in plants. *Cell* 121:207–221](#)
- Aukerman MJ, Sakai H (2003) Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. *Plant Cell* 15:2730–2741
- Bao N, Lye KW, Barton MK (2004) MicroRNA binding sites in Arabidopsis class III HD-ZIP mRNAs are required for methylation of the template chromosome. *Dev Cell* 7:653–662
- Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116:281–297
- Baumberger N, Baulcombe DC (2005) Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. *Proc Natl Acad Sci U S A* 102:11928–11933
- Bohmert K, Camus I, Bellini C, Bouchez D, Caboche M, Benning C (1998) [AGO1 defines a novel locus of Arabidopsis controlling leaf development. *EMBO J* 17:170–180](#)
- Bonnet E, Wuyts J, Rouze P, Van de Peer Y (2004) Detection of 91 potential conserved plant microRNAs in Arabidopsis thaliana and Oryza sativa identifies important target genes. *Proc Natl Acad Sci U S A* 101:11511–11516
- Borsani O, Zhu J, Verslues PE, Sunkar R, Zhu JK (2005) [Endogenous siRNAs derived from a pair of natural cis-antisense transcripts regulate salt tolerance in Arabidopsis. *Cell* 123:1279–1291](#)
- Brodersen P, Sakvarelidze-Achard L, Bruun-Rasmussen M, Dunoyer P, Yamamoto YY, Sieburth L, Voinnet O (2008) [Widespread translational inhibition by plant miRNAs and siRNAs. *Science* 320:1185–1190](#)
- Burk D H, Liu B, Zhong R, Morrison W H and Ye ZH (2001) [A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* 13:807–827](#)
- Cardon G, Hohmann S, Nettesheim K, Saedler H, Huijser P (1997) Functional analysis of the Arabidopsis thaliana SBP-box gene SPL3: a novel gene involved in the floral transition. *Plant J* 12:367–377

- Cardon G, Hohmann S, Klein J, Nettekheim K, Saedler H, Huijser P (1999) Molecular characterization of the Arabidopsis SBP-box genes. *Gene* 237:91–104
- Chen X (2004) A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science* 303:2022–2025
- Chen X (2005) MicroRNA biogenesis and function in plants. *FEBS Lett* 579:5923–5931
- Chen X (2008) A silencing safeguard: links between RNA silencing and mRNA processing in Arabidopsis. *Dev Cell* 14:811–812
- Clark SE (2001) Cell signalling at the shoot meristem. *Nat Rev Mol Cell Biol* 2:276–284
- Cook HA, Koppetsch BS, Wu J, Theurkauf WE (2004) The Drosophila SDE3 homolog armitage is required for oskar mRNA silencing and embryonic axis specification. *Cell* 116:817–829
- Dong Z, Han MH, Fedoroff N (2008) The RNA-binding proteins HYL1 and SE promote accurate in vitro processing of pri-miRNA by DCL1. *Proc Natl Acad Sci U S A* 105:9970–9975
- Dugas DV, Bartel B (2008) Sucrose induction of Arabidopsis miR398 represses two Cu/Zn superoxide dismutases. *Plant Mol Biol* 67:403–417
- Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, Doerks T, Dorner S, Bork P, Boutros M, Izaurralde E (2007) Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. *Genes Dev* 21:2558–2570
- Fahlgren N, Howell MD, Kasschau KD, Chapman EJ, Sullivan CM, Cumbie JS, Givan SA, Law TF, Grant SR, Dangel JL, Carrington JC (2007) High-throughput sequencing of Arabidopsis microRNAs: evidence for frequent birth and death of MIRNA genes. *PLoS ONE* 2:e219
- Fang Y, Spector DL (2007) Identification of nuclear dicing bodies containing proteins for microRNA biogenesis in living Arabidopsis plants. *Curr Biol* 17:818–823
- Gandikota M, Birkenbihl RP, Hohmann S, Cardon GH, Saedler H, Huijser P (2007) The miRNA156/157 recognition element in the 3' UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. *Plant J* 49:683–693
- Gregory BD, O'Malley RC, Lister R, Urlich MA, Tonti-Filippini J, Chen H, Millar AH, Ecker JR (2008) A link between RNA metabolism and silencing affecting Arabidopsis development. *Dev Cell* 14:854–866
- Han MH, Goud S, Song L, Fedoroff N (2004) The Arabidopsis double-stranded RNA-binding protein HYL1 plays a role in microRNA-mediated gene regulation. *Proc Natl Acad Sci U S A* 101:1093–1098
- Hernandez-Pinzon I, Yelina NE, Schwach F, Studholme DJ, Baulcombe D, Dalmay T (2007) SDE5, the putative homologue of a human mRNA export factor, is required for transgene silencing and accumulation of trans-acting endogenous siRNA. *Plant J* 50:140–148
- Herr AJ, Jensen MB, Dalmay T, Baulcombe DC (2005) RNA polymerase IV directs silencing of endogenous DNA. *Science* 308:118–120
- Jack T (2004) Molecular and genetic mechanisms of floral control. *Plant Cell* 16(Suppl):S1–S17
- Jones-Rhoades MW, Bartel DP (2004) Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Mol Cell* 14:787–799
- Kanno T, Huettel B, Mette MF, Aufsatz W, Jalgot E, Daxinger L, Kreil DP, Matzke M, Matzke AJ (2005) Atypical RNA polymerase subunits required for RNA-directed DNA methylation. *Nat Genet* 37:761–765
- Kasschau KD, Fahlgren N, Chapman EJ, Sullivan CM, Cumbie JS, Givan SA, Carrington JC (2007) Genome-wide profiling and analysis of Arabidopsis siRNAs. *PLoS Biol* 5:e57
- Katiyar-Agarwal S, Morgan R, Dahlbeck D, Borsani O, Villegas A Jr, Zhu JK, Staskawicz BJ, Jin H (2006) A pathogen-inducible endogenous siRNA in plant immunity. *Proc Natl Acad Sci U S A* 103:18002–18007
- Katiyar-Agarwal S, Gao S, Vivian-Smith A, Jin H (2007) A novel class of bacteria-induced small RNAs in Arabidopsis. *Genes Dev* 21:3123–3134
- Kliebenstein DJ, Monde RA, Last RL (1998) Superoxide dismutase in Arabidopsis: an eclectic enzyme family with disparate regulation and protein localization. *Plant Physiol* 118:637–650
- Kurihara Y, Watanabe Y (2004) Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. *Proc Natl Acad Sci U S A* 101:12753–12758

- Laubinger S, Sachsenberg T, Zeller G, Busch W, Lohmann JU, Ratsch G, Weigel D (2008) Dual roles of the nuclear cap-binding complex and SERRATE in pre-mRNA splicing and microRNA processing in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 105:8795–8800
- Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75:843–854
- Li J, Yang Z, Yu B, Liu J, Chen X (2005) Methylation protects miRNAs and siRNAs from a 3'-end uridylation activity in *Arabidopsis*. *Curr Biol* 15:1501–1507
- Llave C, Kasschau KD, Rector MA, Carrington JC (2002a) Endogenous and silencing-associated small RNAs in plants. *Plant Cell* 14:1605–1619
- Llave C, Xie Z, Kasschau KD, Carrington JC (2002b) Cleavage of Scarecrow-like mRNA targets directed by a class of *Arabidopsis* miRNA. *Science* 297:2053–2056
- Lobbes D, Rallapalli G, Schmidt DD, Martin C, Clarke J (2006) SERRATE: a new player on the plant microRNA scene. *EMBO Rep* 7:1052–1058
- Lu C, Kulkarni K, Souret FF, MuthuValliappan R, Tej SS, Poethig RS, Henderson IR, Jacobsen SE, Wang W, Green PJ, Meyers BC (2006) MicroRNAs and other small RNAs enriched in the *Arabidopsis* RNA-dependent RNA polymerase-2 mutant. *Genome Res* 16:1276–1288
- Mallory AC, Vaucheret H (2006) Functions of microRNAs and related small RNAs in plants. *Nat Genet* 38(Suppl):S31–S36
- Mette MF, van der Winden J, Matzke M, Matzke AJ (2002) Short RNAs can identify new candidate transposable element families in *Arabidopsis*. *Plant Physiol* 130:6–9
- Meyers BC, Souret FF, Lu C, Green PJ (2006) Sweating the small stuff: microRNA discovery in plants. *Curr Opin Biotechnol* 17:139–146
- Mi S, Cai T, Hu Y, Chen Y, Hodges E, Ni F, Wu L, Li S, Zhou H, Long C, Chen S, Hannon GJ, Qi Y (2008) Sorting of small RNAs into *Arabidopsis* argonaute complexes is directed by the 5' terminal nucleotide. *Cell* 133:116–127
- Mlotshwa S, Yang Z, Kim Y, Chen X (2006) Floral patterning defects induced by *Arabidopsis* APETALA2 and microRNA172 expression in *Nicotiana benthamiana*. *Plant Mol Biol* 61:781–793
- Montgomery TA, Howell MD, Cuperus JT, Li D, Hansen JE, Alexander AL, Chapman EJ, Fahlgren N, Allen E, Carrington JC (2008) Specificity of ARGONAUTE7-miR390 interaction and dual functionality in TAS3 trans-acting siRNA formation. *Cell* 133:128–141
- Morel JB, Godon C, Mourrain P, Beclin C, Boutet S, Feuerbach F, Proux F, Vaucheret H (2002) Fertile hypomorphic ARGONAUTE (*ago1*) mutants impaired in post-transcriptional gene silencing and virus resistance. *Plant Cell* 14:629–639
- Nonomura K, Morohoshi A, Nakano M, Eiguchi M, Miyao A, Hirochika H, Kurata N (2007) A germ cell specific gene of the ARGONAUTE family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice. *Plant Cell* 19:2583–2594
- Onodera Y, Haag JR, Ream T, Nunes PC, Pontes O, Pikaard CS (2005) Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell* 120:613–622
- Park W, Li J, Song R, Messing J, Chen X (2002) CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. *Curr Biol* 12:1484–1495
- Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS (2005) Nuclear processing and export of microRNAs in *Arabidopsis*. *Proc Natl Acad Sci U S A* 102:3691–3696
- Parry DH, Xu J, Ruvkun G (2007) A whole-genome RNAi Screen for *C. elegans* miRNA pathway genes. *Curr Biol* 17:2013–2022
- Pontier D, Yahubyan G, Vega D, Bulski A, Saez-Vasquez J, Hakimi MA, Lerbs-Mache S, Colot V, Lagrange T (2005) Reinforcement of silencing at transposons and highly repeated sequences requires the concerted action of two distinct RNA polymerases IV in *Arabidopsis*. *Genes Dev* 19:2030–2040
- Qi Y, He X, Wang XJ, Kohany O, Jurka J, Hannon GJ (2006) Distinct catalytic and non-catalytic roles of ARGONAUTE4 in RNA-directed DNA methylation. *Nature* 443:1008–1012

- Rajagopalan R, Vaucheret H, Trejo J, Bartel DP (2006) A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*. *Genes Dev* 20:3407–3425
- Ramachandran V, Chen X (2008a) Degradation of microRNAs by a family of exoribonucleases in *Arabidopsis*. *Science* 321:1490–1492
- Ramachandran V, Chen X (2008b) Small RNA metabolism in *Arabidopsis*. *Trends Plant Sci* 13:368–374
- Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. *Genes Dev* 16:1616–1626
- Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP (2002) Prediction of plant microRNA targets. *Cell* 110:513–520
- Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU (2005) A gene expression map of *Arabidopsis thaliana* development. *Nat Genet* 37:501–506
- Schwab R, Palatnik JF, Riester M, Schommer C, Schmid M, Weigel D (2005) Specific effects of microRNAs on the plant transcriptome. *Dev Cell* 8:517–527
- Song L, Han MH, Lesicka J, Fedoroff N (2007) *Arabidopsis* primary microRNA processing proteins HYL1 and DCL1 define a nuclear body distinct from the Cajal body. *Proc Natl Acad Sci U S A* 104:5437–5442
- Stoppin-Mellet V, Gaillard J, Vantard M (2002) Functional evidence for in vitro microtubule severing by the plant katanin homologue. *Biochem J* 365:337–342
- Stoppin-Mellet V, Gaillard J, Vantard M (2006) Katanin's severing activity favors bundling of cortical microtubules in plants. *Plant J* 46:1009–1017
- Sunkar R, Zhu JK (2004) Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. *Plant Cell* 16:2001–2019
- Sunkar R, Kapoor A, Zhu JK (2006) Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in *Arabidopsis* is mediated by downregulation of miR398 and important for oxidative stress tolerance. *Plant Cell* 18:2051–2065
- Takeda A, Iwasaki S, Watanabe T, Utsumi M, Watanabe Y (2008) The mechanism selecting the guide strand from small RNA duplexes is different among argonaute proteins. *Plant Cell Physiol* 49:493–500
- Vaucheret H (2008) Plant ARGONAUTES. *Trends Plant Sci* 13:350–358
- Vazquez F, Vaucheret H, Rajagopalan R, Lepers C, Gasciolli V, Mallory AC, Hilbert JL, Bartel DP, Crete P (2004) Endogenous trans-acting siRNAs regulate the accumulation of *Arabidopsis* mRNAs. *Mol Cell* 16:69–79
- Xie Z, Johansen LK, Gustafson AM, Kasschau KD, Lellis AD, Zilberman D, Jacobsen SE, Carrington JC (2004) Genetic and functional diversification of small RNA pathways in plants. *PLoS Biol* 2:E104
- Yamasaki H, Abdel-Ghany SE, Cohu CM, Kobayashi Y, Shikanai T, Pilon M (2007) Regulation of copper homeostasis by micro-RNA in *Arabidopsis*. *J Biol Chem* 282:16369–16378
- Yang L, Liu Z, Lu F, Dong A, Huang H (2006a) SERRATE is a novel nuclear regulator in primary microRNA processing in *Arabidopsis*. *Plant J* 47:841–850
- Yang Z, Ebright YW, Yu B, Chen X (2006b) HEN1 recognizes 21–24 nt small RNA duplexes and deposits a methyl group onto the 2' OH of the 3' terminal nucleotide. *Nucl Acids Res* 34:667–675
- Yoshikawa M, Peragine A, Park MY, Poethig RS (2005) A pathway for the biogenesis of trans-acting siRNAs in *Arabidopsis*. *Genes Dev* 19:2164–2175
- Yu B, Yang Z, Li J, Minakhina S, Yang M, Padgett RW, Steward R, Chen X (2005) Methylation as a crucial step in plant microRNA biogenesis. *Science* 307:932–935
- Yu B, Bi L, Zheng B, Ji L, Chevalier D, Agarwal M, Ramachandran V, Li W, Lagrange T, Walker JC, Chen X (2008) The FHA domain proteins DAWDLE in *Arabidopsis* and SNIP1 in humans act in small RNA biogenesis. *Proc Natl Acad Sci U S A* 105:10073–10078
- Zhang X, Henderson IR, Lu C, Green PJ, Jacobsen SE (2007) Role of RNA polymerase IV in plant small RNA metabolism. *Proc Natl Acad Sci U S A* 104:4536–4541

- Zhao L, Kim Y, Dinh TT, Chen X (2007a) miR172 regulates stem cell fate and defines the inner boundary of APETALA3 and PISTILLATA expression domain in Arabidopsis floral meristems. *Plant J* 51:840–849
- Zhao T, Li G, Mi S, Li S, Hannon GJ, Wang XJ, Qi Y (2007b) A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*. *Genes Dev* 21:1190–1203
- Zheng X, Zhu J, Kapoor A, Zhu JK (2007) Role of Arabidopsis AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. *EMBO J* 26:1691–1701