Plant Growth Regulation (2021) 93:1–12
https://doi.org/10.1007/s10725-020-00654-9

REVIEW PAPER

MicroRNA biogenesis in plant

Zihan Gao[1] · Jingtao Nie[1] · Huasen Wang[1]

Received: 3 June 2020 / Accepted: 21 August 2020 / Published online: 19 September 2020

Abstract
MicroRNAs (miRNAs), a type of endogenous non-coding small RNA, with a length of 20 to 24 nt, represses their target gene expression post-transcriptionally. There are a growing number of studies have discovered that miRNAs are found in animals and plants. Plant miRNAs are involved in growth and development, and they play an important role in physiological and biochemical regulation. This review mainly focuses on components of different miRNA processing pathways and how miRNA effectors inhibit the expression of target genes in plants. We discuss not only the various pathways affecting miRNA biogenesis, but also the different forms of miRNA effector pathways. Previous studies found that there have been some primary advances in the formation of miRNAs, we hope to provide a comprehensive introduction to the biogenesis of plant miRNAs.

Keywords Plant miRNAs · Biogenesis · Processing · Protein

Introduction

MicroRNA (miRNA) is a class of eukaryotic endogenous non-coding small RNAs with a length of about 20 to 24 nt, which do not encode proteins and have no open reading frame. In 1993, the first members of miRNA, lin-4 and let-7, were found in *Caenorhabditis elegans*, and they were demonstrated to influence the expression of the target nuclear lin-14 through post-transcription and translation inhibition, thereby affecting the transition from larval L1 development to L2 (Lee et al. 1993; Wightman et al. 1993; Reinhart et al. 2000). Since then, a large number of miRNAs have been found in many plant species, including *Solanum lycopersicum*, *Zea mays, Elettaria cardamomum Maton, Nicotiana tabacum, Arabidopsis thaliana and Oryza sativa* et al. These miRNAs have been shown to play a vital role in the plant development and growth, as well as biological and abiotic stress physiology (Voinnet 2009; Silva et al. 2014; Wang et al. 2015a,2016; Ma et al. 2015; Yu et al. 2015,2017a; Feng et al. 2016; Leng et al. 2017; Lotfi et al. 2017; Zhang et al. 2019; Wu et al. 2019; Feng et al. 2020; Yang et al. 2020; Lakhwani et al. 2020). Like most mRNAs, MIR genes (genes encoding miRNA) are also transcribed by DNA-dependent RNA polymerase II (Pol II) and then undergo a series of transcriptional modifications including splicing of the 5′ cap and 3′ polyadenylation to form primary transcript pri-miRNAs (Kurihara et al. 2004; Kim et al. 2011). These pri-miRNAs containing partially complementary paired hairpin structures are able to be identified and cleaved by multiple protein complexes such as DCL1 (dicer-like1), double-stranded RNA-binding protein HYPONASTIC LEAVES (HYL1), C_2H_2-zinc finger structural protein SERRATE (SE), and G-patch structural protein TGH to form the precursor miRNA (pre-miRNA) (Kurihara et al. 2004; Kurihara et al. 2006; Dong et al. 2008; Ren et al. 2012a).

The 3′end of the processed double-stranded miRNAs (miRNA/miRNA*) can be methylated by HUA Enhancer 1 (HEN1) to prevent double-stranded miRNAs from by uridylation (Li et al. 2005; Yu et al. 2005). These double-stranded RNAs may be transferred from the nucleus to the cytoplasm under the action of HST (HASTY, the plant homolog of exportin-5/Exp5) and other unknown factors (Park et al. 2005). The miRNA* in the double-stranded

Zihan Gao and Jingtao Nie have equally contribute to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10725-020-00654-9) contains supplementary material, which is available to authorized users.

✉ Huasen Wang
whsych66@163.com

[1] State Key Laboratory of Subtropical Silviculture, Laboratory of Plant Molecular and Developmental Biology, College of Forestry and Biotechnology, Zhejiang Agriculture and Forestry University, Zhejiang, China

miRNA is then degraded, leaving only the mature miRNA. The resulting mature miRNA can reduce gene expression via incorporation into ARGONAUTE (AGO1), which combines with other proteins to form the RNA induced silencing complex (RISC). The RISC complex then cleaves mRNAs or represses their translation (Baumberger and Baulcombe 2005). Overall, the maturation process of miRNAs can be summarized into three important aspects: transcription, post-transcriptional processing and the stabilization of miRNA after processing (Fig. 1).

miRNAs play an irreplaceable biological role in plants and can affect various development stages, enabling complex regulation of plant growth as well as responses to biotic or abiotic stresses (Ori et al. 2007; Wu et al. 2009; Guo et al, 2005; Sunkar et al. 2006; Wang et al. 2012; Huang et al. 2018; Anjali et al. 2019). Therefore, a full understanding of miRNA transcription and processing is particularly important. This review mainly focuses on the many proteins involved in the biogenesis of miRNAs and how these miRNA function in downstream target genes (Table S1).

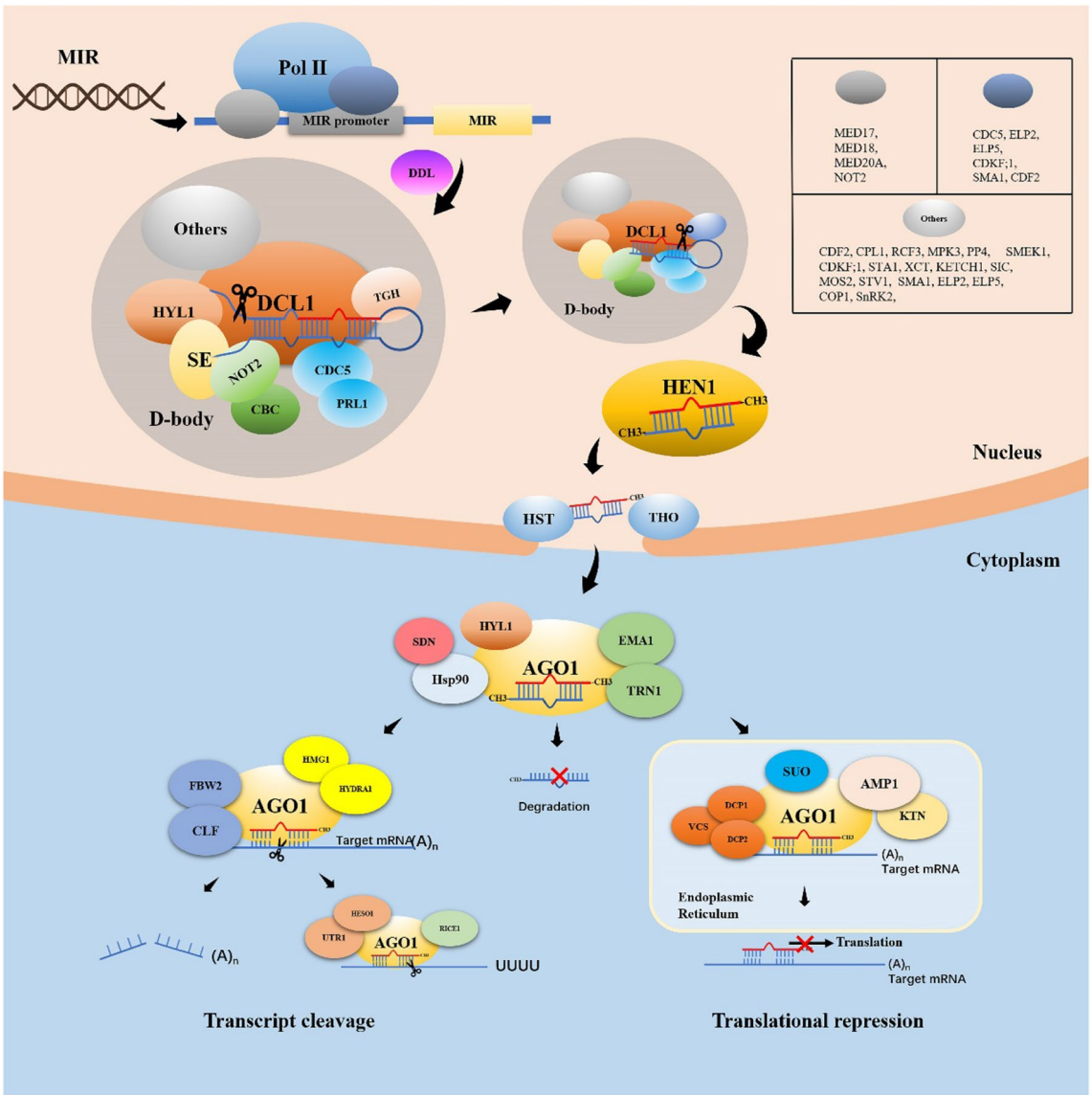

Fig. 1 A diagram of the miRNA biogenesis and effector pathway in plants. miRNAs are transcribed into primary miRNAs (pri-miRNAs) by DNA dependent RNA Polymerase II (Pol II) and then processed into precursor miRNAs (pre-miRNAs) by the action of D-body. The processed double-stranded miRNAs are then methylated by HEN1, and transported from the nucleus to the cytoplasm by HST. The resulting mature miRNA can reduce gene expression via incorporation into AGO1, which combines with other proteins to form the RISC. RISC has two forms of action, one is transcriptional cleavage, the other is translation repression

MIR gene transcription

The initiation of miRNA formation follows the same process as most genes, which involves DNA-dependent RNA polymerase II (Pol II). The initiation process of miRNA transcription, like many other genes, is often regulated by biotic and abiotic factors (Lee et al. 2004; Xie et al. 2005). The pri-miRNA is transcribed by Pol II during a process that is regulated by many transcription factors. For example, MED20A (Mediator20A), MED17 (Mediator17) and MED18 (Mediator18) are able to affect gene transcription by interacting with Pol II. Studies have found that the transcription complexes formed by MED20A, MED17 and MED18 are required for microRNA (miRNA) biogenesis due to the ability of these proteins to recruit Pol II to the promoters of miRNA genes. Interestingly, studies have found that the levels of miRNA and pri-miRNA are significantly decreased in the *Arabidopsis* mutants of *med20a, med17* and *med18* (Chadick et al. 2005; Kim et al. 2011).

In Arabidopsis it was shown that the NOT2_3_5 domain protein NOT2 interacts with Pol II and influences the transcription of miRNAs, while *NO2a-1* and *NO2b-1* mutants have significantly reduced pri-miRNA levels, indicating that NOT2a/b plays a key regulatory role in the transcription of miRNAs (Anand et al. 2007; Wang et al. 2013). In addition, CDC5, a protein containing the MYB domain, can bind to the promoter of MIR genes to affect the transcriptional activity of miRNAs (Zhang et al. 2013a).

Recent research has found that the disease resistance gene SNC1 can interact with the transcriptional co-suppressor TPR1 to reduce miRNA levels, suggesting that the R gene-miRNA-phasiRNA regulatory module plays an important role in plant immune responses (Cai et al. 2018).

Elongation is critical in the process of transcription, and conserved elongation factors play an important role in the transcription in animals and plants. The extension genes ELP2 and ELP5 have been shown to participate in the plant miRNA transcription process, while disruption of elongator reduces RNAPII occupancy at miRNA loci and suppresses pri-miRNA transcription. MIR genes eventually form pri-miRNA through RNA polymerase Pol II and other proteins, and cycling DOF transcription factors (CDF2) can accelerate the transcription of MIRs (Sun et al. 2015). A recent study found that SMA1 can affect the binding of Pol II to the promoter of MIR genes, while SMA1 can bind the promoter region of the gene that encodes the pri-miRNAs, which is necessary for MIR transcription (Li et al. 2018). Transcription of MIR genes play a very important role in the final formation of miRNA and further processing and modification can be carried out only after the transcription is complete.

miRNA post-transcriptional regulation

Processing of primary miRNAs and precursor miRNAs

Pri-miRNAs are processed into mature miRNAs by a series of intricate modifications. The length of the stem loop structure of the pri-miRNA varies, which results in different cleavage forms. Base to loop direction also matters and affects bidirectional processing (Zhu et al. 2013; Cuperus et al. 2010; Bologna et al. 2009; Song et al. 2010). DCL1 (Dicer-like1) was the first protein shown to be directly involved in the processing of pri-miRNA and pre-miRNA (Kurihara et al. 2004). There are four DCL genes (DCL1-4) in Arabidopsis, among which DCL1 plays the most crucial function in the processing of miRNAs, which are significantly decreased in the *dcl1* mutant (Kurihara et al. 2004; Deleris et al. 2006). DCL1 is a homolog of the Drosophila gene Dicer and is similar to the Human Dicer gene (Bernstein et al. 2001; Macrae et al. 2006). DCL1 contains several conserved domains, including an N-terminal helicase domain (DExH-box RNA-helicase-C motifs), unknown domain DUF283, PAZ domain (PIWI/AGRONAUTE/ZWILLE), RNA ribonuclease domains (RNase III, RNase IIIa, RNase IIIb) and 1 or 2 double-stranded RNA-binding domains at the C-terminus. The PAZ domain can bind to the RNA ends, especially double-stranded RNA with a two-nucleotide protrusion at the 3′ end (Tagami et al. 2009; Schauer et al. 2002). Earlier research using miRNA163 as an example demonstrated that DCL1 can participate in the cleavage of pri-miRNA163, with DCL1 acting as the exonuclease (Kurihara et al.2004). The helicase domain provides energy for the cleavage function of DCL1 and is required for the processing of some specific miRNAs (Liu et al. 2012). DDL, is an RNA binding protein that participates in miRNA processing by interacting with pri-miRNA to promote the effective identification of pri-miRNA by DCL1 (Yu et al. 2008). Overall, DCL1 plays a critical role throughout the process of miRNA biogenesis.

After the transcription of a MIR gene, the pri-miRNA transcript is subjected to subsequent cleavage by the DCL1 processing complex and eventually forms a mature miRNA. In addition to the DCL1, there are many other protein factors in the DCL1 processing complex, some of which can interact with DCL1 to affect the accuracy and efficiency of processing.

HYL1 (HYPONASTIC LEAVES1) has been shown to interact with DCL1 to improve the accuracy of pri-miRNA cleavage (Kurihara et al. 2006). Mutants of HYL1 accumulate higher levels of pri-miRNAs and less mature miRNAs, indicating that HYL1 influences the content of mature miRNA by participating in the post-transcriptional processing of pri-miRNA (Dong et al. 2008). The N-terminus of HYL1 contains two double-stranded RNA-binding domains

(dsRBD1 and dsRBD2), and the C-terminus contains protein interaction binding domains (Lu and Fedoroff 2000; Wu et al. 2007; Yang et al. 2010). Previous research has shown that the DsRBD2 domains in HYL1 can form homodimers, which affect the selection of cleavage sites in pri-miRNA and facilitates processing. However, disruption of this homodimer does not interfere with its interactions with other proteins, such as DCL1 and SE (Yang et al. 2010, 2014).

SERRATE is a C2H2 zinc finger protein which can also influence the recognition and cleavage efficiency of pri-miRNA by interacting with DCL1 (Dong et al. 2008). A decrease in miRNA content and an increase in pri-miRNA content were detected in the *se-1* mutant, which has several pleiotropic phenotypes. The *se* mutant exhibits some similar developmental abnormalities as the *hyl1* mutant. SE is localized in the nucleus where it interacts with HYL1 (Yang et al. 2006; Lobbes et al. 2006) and the core region of the SE protein may provide a platform for the interaction between HYL1 and DCL1. However, the interacting region of SE is not in the zinc finger region but in the middle and N-terminal region. Overall, it is thought to act as a scaffold protein, which affects the accuracy of miRNA processing through binding pri-miRNA to HYL1 and DCL1 proteins (Machida et al. 2011).

NOT2 protein can also act as a scaffold during miRNA processing, and mutants deficient in this protein have fewer pri-miRNAs and mature miRNAs. These findings indicate that NOT2 not only influences the formation of miRNAs at transcriptional level but also may influence the processing of miRNAs at post-transcriptional processing level (Anand et al. 2007; Wang et al. 2013).

The cap-binding protein complex (CBC) binds to the caps of all Pol II transcripts (Wen et al. 1998). In addition, CBP80 and CBP20 play a crucial role in the processing of miRNAs. Mutants of *cbp80* and *cbp20* lead to higher levels of pri-miRNA and less mature miRNAs. Similar to SE, CBP80/20 also acts to combine RNA with DCL1 and promote miRNA processing (Kim et al. 2008; Kierzkowski et al. 2009).

TGH is an RNA-binding protein containing domains with high similarity to G-patch and SWAP domains (Compressor-of-White-Apricot) (Calderon-Villalobos et al. 2005). The TGH protein has been found to be a critical part of the DCL1-HYL1-SE processing complex, with data showing that TGH regulates the levels of miRNAs by affecting the efficiency of cleavage of pri-miRNA (Ren et al. 2012a). However, TGH only regulates the abundance of miRNAs by promoting DCL1 cleavage efficiency but does not affect the accuracy of processing (Ren et al. 2012a).

Studies have shown that CDC5 and PRL1 in the MAC complex (MOS4-associated complex) participate in the processing of miRNAs. CDC5 not only affects miRNA genes at the transcriptional level, but also impacts processing steps. However, experiments such as Co-Immunoprecipitation and Bimolecular Fluorescence Complementation have shown that CDC5 can interact with DCL1 and SE, thereby influencing the cleavage efficiency of pri-miRNA and formation of mature miRNAs (Zhang et al. 2013a). PRL1 (an evolutionarily conserved WD-40 protein) can interact with CDC5 as well as with DCL1 and SE to promote the transcription of miRNA and the stability of pri-miRNA, but mutants of PRL1 do not show reduced interaction between CDC5 and DCL1.These results demonstrate that PRL1 may assist CDC5 in its function in the MAC complex and act as a component of the DCL1 complex to enhance primary miRNA processing (Zhang et al. 2014). In addition to the transcriptional level, CDF2 can affect the miRNA at the post-transcriptional level by interacting with DCL1 (Sun et al. 2015).

There are also many proteins that can influence the action of the DCL1 processing complex on pri-miRNA via indirect pathways. Phosphorylation plays a key role in the processing of miRNA, and the accuracy and stability of phosphorylation of HYL1 impact the processing of miRNA. C-terminal domain phosphatase-like 1 (CPL1/FRY2) interacts with HYL1 and SE proteins in the DCL1 processing complex, and CPL1 maintains the phosphorylation of HYL1 to ensure the accuracy of pri-miRNA cleavage, in which SE protein acts as a scaffold to regulate the interaction of HYL1 and CPL1 (Koiwa et al. 2002; Xiong et al. 2002; Manavella et al. 2012). In contrast, mitogen activated protein (MPK3) promotes the phosphorylation of HYL1 to reduce its activity. A significant decrease in miRNA levels was detected in the mutants of *atmpk3* and *atmpk6* (Raghuram et al. 2015). *Arabidopsis thaliana* K homology (KH) domain protein REGULATOR OF CBF GENE EXPRESSION 3 (RCF3) acts as a cofactor for miRNA processing via nuclear interactions with the phosphatases CPL1 and CPL2. RCF3 can interact with CPL1 and CPL2 to promote HYL1 dephosphorylation (Karlsson et al. 2015).

The protein phosphatase PP4 (Protein Phosphatase 4) and SMEK1 (Suppressor of MEK1) complex enables HYL1 dephosphorylation in order to enhance processing of miRNA by antagonizing the MAPK cascade and mediating HYL1 stability. SMEK1 can reduce the phosphorylation of HYL1 by inhibiting the activity of mitogen activated protein kinase MAPK, and also it can recruit PP4 protein to maintain the dephosphorylation of HYL1, thereby ensuring miRNA biogenesis (Su et al. 2017).

Recently, the regulatory subunit 3 (PP4R3A) in the phosphatase PP4 complex, together with two of the redundant catalytic subunit genes, PPX1 and PPX2, was shown to promote miRNA formation (Wang et al. 2019a). *PP4R3A* loss-of-function mutations have been shown to cause lower levels of miRNA, pri-miRNA and HYL1. However, the results of co-IP experiments showed that PP4R3A could not interact directly with HYL1 and SE proteins, suggesting that PP4 facilitates miRNA biogenesis at transcriptional and

post-transcriptional levels by recruiting the microprocessor component HYL1 to MIR genes and nuclear dicing bodies (Wang et al. 2019a). Additionally, SHORT VALVE 1 (STV1), a conserved ribosomal protein, modulates miRNA process by promoting to recruit pri-miRNAs to the DCL1 (Li et al. 2017).

Mature pri-miRNA usually includes a 5′ end cap structure, a 3′ end poly-A tail structure and at least one stable neck loop structure. The cell cyclin-dependent protein kinase CDKF-1 plays a crucial role in the stabilization of pri-miRNA and the *cdkf-1* mutant has lower levels of precursors and mature miRNAs (Hajheidari et al. 2012). In Arabidopsis, STABILIZED1 (STA1), a pre-mRNA processing factor 6 homolog, is also involved in the processing of miRNAs. Similar to other miRNA process-defective mutants, *sta1* mutants accumulate levels of pri-miRNAs and decrease levels of mature miRNAs. STA1 indirectly participates in the processing of miRNAs by directly promoting pri-miRNA splicing and modulating DCL1 at the transcriptional level (Lee et al. 2006; Chaabane et al. 2013). *CMA33* encodes a nuclear localized protein, XAP5 CIRCASIAN TIMEKEEPER (XCT), which can act as a transcriptional factor to regulate miRNA biogenesis by modulating the transcription of DCLs (Fang et al. 2015b).

SIC is a proline-rich protein, which can influence the function of the DCL1 processing complex by interacting with HYL1. The levels of miRNAs in the SIC loss-of-function mutants are significantly reduced (Zhan et al. 2012). Recent research has found that an importin-β-protein (KETCH1) indirectly facilitates processing of pri-miRNA through transport of HYL1 into the nucleus and thus contributes to the formation of the DCL1 processing complex (Zhang et al. 2017a). The main function of the MOS2 protein, which includes G-patch and KOW domains, is RNA binding (Zhang et al. 2005). The *mos2* mutants have lower levels of mature miRNA, though MOS2 can neither interact with proteins in processing complex such as DCL1, HYL1 and SE nor localize to the D-bodies. It is thought that MOS2 may affect miRNA by recruiting pri-miRNA into D-bodies (Wu et al. 2013).

SMALL1 (SMA1), a homolog of the DEAD-box pre-mRNA splicing factor Prp28, participates in miRNA biogenesis by affecting the intron excision process of the MIR gene (Strauss and Guthrie 1994; Li et al. 2018). The transcriptional elongation proteins EPL2 and ELP5, in addition to influencing miRNA content at the transcriptional levels, can also participate in the processing of miRNA by interacting with DCL1 to localize DCL1 to the Dicer-like 1 complex (Fang et al. 2015a). Constitutive photomorphogenic 1 (COP1), a RING-finger E3 ligase, plays a crucial role in photomorphogenesis by destabilizing many light-regulated transcriptional factors and photoreceptors. COP1 can avoid the degradation of HYL1 in the cytoplasm by protease X under high light, thus regulating the processing of miRNA (Seo et al. 2003; Cho et al. 2014). SnRK2 kinase, a response factor for abscisic acid (ABA) and osmotic stress signals, has recently been found to reduce the accumulation of miRNA. SnRK2 phosphorylates HYL1 and SE in the DCL1 processing complex. These results demonstrate a significant role for SnRK2 kinases in the processing of miRNA and reveal a mechanism by which ABA and osmotic stress signaling are linked to miRNA biogenesis (Fujii and Zhu 2009; Yan et al. 2017). The RNA-binding protein FCA can also increase the levels of some miRNAs by affecting the process of pri-miRNA in response to temperature change (Jung et al. 2012).

CHR2/BRM is an ATPase in the SWI/SNF chromosome-remodeling complex, and *chr2* mutants have a pleiotropic phenotype including altered miRNA expression. Recently, CHR2 was found to not only facilitate the production of pri-miRNA at the transcriptional level, but also reduce the accumulation of miRNA at the post-transcriptional level through interactions with the SE protein. The secondary structure of pri-miRNA is altered in the mutants of *chr2*, suggesting that CHR2 may influence the processing of miRNA by remodeling their secondary structures. CHR2 plays a crucial role in the positive and negative regulation of miRNA and is important for maintaining their balance in plants (Farrona et al. 2004; Hurtado et al. 2006; Clapier et al. 2017; Wang et al. 2018).

Further processing of miRNA and transport of pri-miRNA

The pri-miRNA is further modified by the DCL1 processing complex to form pre-miRNA, which is then cleaved to form double-stranded miRNA/miRNA* (Song et al. 2007; Kurihara et al. 2006). In this process, the pre-miRNA processing modification is critical. THO2, which encodes a core subunit of the plant THO/TREX complex, participates in miRNA biogenesis by recruiting miRNA precursors into the miRNA processing complex. The *tho2* mutants have reduced levels of miRNA and increased precursor levels due to a failure to recruit precursors into the DCL1 processing complex (Reed and Cheng 2005; Furumizu et al. 2010; Francisco-Mangilet et al. 2015). Recent research has shown that the nucleotide transferase HESO1 (HEN1 SUPPESSOR1) is able to promote the uridylation of pre-miRNA. In Arabidopsis, HESO1, NTP6 and NTP7 contribute to pre-miRNA cytidylation. The addition of these tails to pre-miRNA can restore the cleaved pre-miRNA to full length, thus facilitating subsequent processing. In addition, uridylation regulated by HESO1 can also degrade pre-miRNAs that cannot be accurately processed (Song et al. 2019).

The final processed double-stranded miRNAs (miRNA/miRNA*) require further stabilization and modification in

order to form mature miRNAs. The HEN1 protein plays a critical role in stabilizing and modifying double-stranded RNA. HEN1 is a methyltransferase with a binding site for double-strand miRNA at its N-terminal and a conserved C-terminal methylation domain. Therefore, HEN1 accelerates the processing of miRNA by improving the stability of double-stranded miRNAs through methylation (Yu et al. 2005).

In animals, the nuclear export receptor protein Exp5 (the nuclear export receptor Exportin5) can transport pre-miRNAs from the nucleus to the cytoplasm (Zeng and Cullen 2004; Lund et al. 2004). In Arabidopsis, the Exp5 homolog HASTY (HST) has been shown to modulate the processing of miRNA. There is a decreased level of miRNA in the Exp5 loss-of-function mutants, though there is insufficient evidence that HST is involved in the transport of miRNA. Because the accumulation of miRNAs in the nucleus and cytoplasm are not detected in mutants of *hst*, HST is thought to act more to stabilize miRNA than to transport it (Park et al. 2005). The THO/TREX complex is involved not only in the transport of mRNAs but also in the transport of miRNAs (Köhler and Hurt 2007). Overall, the exact mechanism of miRNA transport in plants is not fully understood and requires more investigation.

miRNA effector formation and function

RISC complex formation and miRNA loading

miRNAs regulate target gene expression by translational repression and post-transcriptional cleavage via the RISC complex. It was first reported in humans that miRNAs are loaded into the RNA-induced silencing complex (RISC) to modulate post-transcriptional silencing and translational repression (Gregory et al. 2005). Similar to animals, miRNAs in plants also form a RISC complex by interaction with AGO1 proteins. First, pre-miRNA binds to AGO proteins to form pre-RISC, after which the miRNA* in double-stranded RNAs is removed from the complex to form a mature RISC protein complex. The AGO1 protein is the crucial component of the RISC protein complex and is able to modulate its slicing activity (Baumberger and Baulcombe 2005; Eamens et al. 2009). In Arabidopsis, there are ten AGO proteins, among which AGO1, AGO2, AGO4, AGO7 and AGO10 have all been shown to have cleavage activity that indirectly affects the activity of different small RNAs (Mi et al. 2008; Takeda et al. 2008; Montgomery et al.2008; Zhu et al. 2011). In addition to the PAZ domain that binds to the miRNA, the AGO proteins include the PIWI domain, a Mid domain and a cap-binding-like domain (MC domain). The Mid domain can bind to the 5′ phosphate of single-stranded RNA by a divalent cation, and the PIWI domain catalyzes the activity of endoribonuclease (Hutvagner and Simard 2008). AGO1 is involved in the transcriptional suppression and cleavage of miRNAs, and this function is associated with the presence of miRNAs and AGO1 in polyribosomes (Brodersen et al. 2008; Lanet et al. 2009).

The double-stranded RNA binding protein complex DRB1/HYL1 participates in miRNA biogenesis by interacting with AGO1 and modulating strand selection through directional loading of the miRNA duplex into RISC for passenger strand degradation (Eamens et al. 2009). The molecular chaperone Hsp90 indirectly interferes with the function of RISC by interacting with AGO1 and altering its conformation. In addition, Hsp90 contains double-stranded RNA that binds to AGO1 and provides energy for the degradation of the RNA* in double-stranded RNA (Iki et al. 2010). In Arabidopsis, SQN, an orthologue of the immunophilin cyclophilin 40 (Cyp40), is involved in the silencing of miRNA. Many studies have found that SQN can interact with DCL1, HYL1, HEN1 and Hsp90, and works together with AGO1 to regulate the levels of miRNAs (Berardini et al. 2001; Smith et al. 2009). Enhanced MiRNA Active 1 (EMA1) and Transporter 1 (TRN1) encode two importin-β family proteins, which negatively and positively modulate the RISC loading process, respectively. The vast majority of miRNAs can be purified in the effector complex of miRNA in the *ema1* mutants, implying that EMA1 regulates the activity of miRNAs by suppressing the loading of the miRNA into the AGO1 complex (Wang et al. 2011). In contrast to *ema1*, the *trn1* mutant does not reduce the levels of miRNAs, but instead it deceases their activity. Furthermore, the *trn1* mutant does not interfere with the distribution of miRNA and AGO1 in the nucleus and cytoplasm, but it inhibits the interaction between miRNAs and AGO1 (Wang et al. 2011; Cui et al. 2016).

Translational repression

Some miRNA target genes are regulated by miRNAs at the transcript level, while others only have translational alteration (Lanet et al. 2009). This protein translational repression primarily functions through ALTERED MEISTEM PROGRAM1 (AMP1), which can inhibit target mRNA entry into polyribosomes, while AMP1 is localized on the endoplasmic reticulum. Protein translation occurs at the rough endoplasmic reticulum surface, while both AGO1 and miRNAs are present in polyribosomes, which implies that the miRNA translational repression process takes place on the endoplasmic reticulum (Helliwell et al.2001; Li et al. 2013). In addition, there are other protein factors that regulate the miRNA-mediated translational repression. The SUO domain-containing GW protein can promote the translational repression of miRNAs and the microtubule-severing enzyme KATANIN1 (KTN1) also facilitates translational repression. Mutants in these genes

have higher levels of target protein without altered levels of mRNA (Brodersen et al. 2008; Yang et al. 2012). DCP1, DCP2 and VCS co-localize into process bodies (P-bodies) and form a decapping complex in the cytoplasm, which is an important hub for the RISC complex to exercise its translational repression function (Xu et al. 2006; Brodersen et al. 2008).

Transcript cleavage

The RISC complex can inhibit gene expression through cleavage of the target mRNA. FBW2 is an inhibitor of AGO1 and the over-expression of FBW2 reduces the level of AGO1 protein but not AGO1 mRNA in Arabidopsis (Earley et al. 2010). Recently, CLF, which encodes a PRC2 methyltransferase complex, has been found to increase activity and stability of miRNA by preventing ubiquitination of AGO1. CLF directly regulates FBW2, which is a negative regulator of AGO1 (Earley et al. 2010; Ré et al. 2020). In Arabidopsis, HMG1 and HYDRA1 modulate the synthesis of isoprene, which regulates the formation of plant membrane proteins at the post-transcriptional level. Since AGO1 is a membrane protein, deletion of HMG1 can suppress the activity of miRNAs by reducing the formation of AGO1 (Brodersen et al. 2012). The Coenzyme factors RICE1 and RICE2 degrade the uridylation of cleavage targets, which are uridylated by HESO1 and UTR1 to regulate the function of the RISC (Zhang et al. 2017b). miRNAs play a significant role in plants, and function through RISC complex in the above two forms, and there are many regulatory mechanisms involved in its function to be explored.

Degradation of miRNAs

Studies have found that *heso1-2* mutants can partially rescue *hen1-2*-induced miRNA reduction, which indicates that HESO1 is able to uridylate double-stranded miRNAs that are not methylated by HEN1, leading to higher levels of mature miRNAs (Ren et al. 2012b; Zhao et al. 2012). However, the presence of uridylated miRNA in both *hen1* and *heso1* mutants has been found, likely due to the fact that RNA uridine invertases URT1 and HESO1 can 3′ uridylate miRNAs methylated by HEN1. In addition, these proteins can independently perform their functions in multiple terminal nucleotide transferases at the 3′ tailing end of small RNAs and shift the balance between uridylation and trimming (Wang et al. 2015b). AGO1 colocalizes with autophagy-related protein 8a (ATG8a), demonstrating that the autophagy pathway of AGO1 also affects the stability of miRNAs (Derrien et al. 2012).

Other pathways participate in miRNA biogenesis

Epigenetic regulation of miRNA processing

Over the past decade, many studies have revealed the influence of epigenetic modifications on gene expression. Unsurprisingly, miRNA genes have also been shown to be regulated through similar epigenetic mechanisms in both plants and animals. In plants, the histone acetyltransferase General control non-repressed protein 5 (GCN5) can acetylate the H3K14 locus (Grant et al. 1997; Zhang et al. 2008). The acetylation of histones can also promote the transcription of miRNA genes, while the histone acetylation (HDAC) inhibitor TSA can reduce the accumulation of some miRNAs. In addition, the mutants of *gcn5* cause increased expression of *DCL1*, *SE*, *HYL1* and *AGO1*, which accelerates miRNA processing. In general, the histone acetylation/deacetylation epigenetic mechanisms depend on GCN5 interfering with miRNA biogenesis at both the transcriptional and post-transcriptional levels (Tian and Chen 2011; Kim et al. 2009). Additionally, the PRC2 complex can suppress MIR156/157 at the transcriptional level by catalyzing H3K27me3, which is a major epigenetic silencing mechanism in plants (Zhang et al. 2007; Lafos et al. 2011). There are many epigenetic pathways that interfere with the formation of miRNA, such as BRAHMA (BRM), ARABIDOPSIS TRITHORAX7 (ATXR7) and ATP-dependent SWR1 chromatin remodeling complex (SWR1-C) (Xu et al. 2016, 2018).

Alternative splicing regulation of miRNA processing

Alternative splicing (AS) of pri-miRNAs has been reported to influence the processing of miRNA by reducing the cleavage efficiency of the DCL1 complex. Additionally, miRNA transcripts without introns can reduce the accumulation of their respective mature miRNAs (Bielewicz et al. 2013; Schwab et al. 2013). MAC7 in the MOS4-ASSOCIATE COMPLEX influences the ability of HYL1 to localize to dicing bodies and participate in the processing of miRNA. In addition, MAC plays a key role in the splicing of mRNA in animals but only acts to shear some genes in Arabidopsis. However, recent research has found that transcripts with retained introns are significantly accumulated in the *mac* mutant compared to Col-0, which indicates that MAC may interfere with the formation of miRNAs by controlling the alternative splicing of pre-miRNAs (Xu et al. 2012; Zhang et al. 2013b; Jia et al. 2017). Recently, two conserved disassembly factors of the intron-lariat spliceosome (ILs) complex, Increased Level of Polyploidy1-1D (ILP1) and NTC-Related protein 1 (NTR1), have been found to promote miRNA processing by forming a complex to co-regulate the alternative splicing of pri-miRNAs. In addition, ILP1 and

NTR1 improve the transcriptional elongation of MIR genes and interact with DCL1 and SE proteins (Wang et al. 2019b).

Exonuclease regulation of miRNA processing

The SMALL RNA DEGRADING NUCLEASE (SDN) family encodes four 3′-to-5′ exonucleases that promote the degradation of miRNAs. Among them, SDN1 is also able to degrade miRNAs by effectively recognizing their methylation sites at 2′-O-terminal in the 3′-terminal. Subsequent studies have found that SDN1 and SDN2 can cause 3′ truncation of some miRNAs, and AGO10 can participate in the degradation of *SDN1* and *SDN2* transcripts through miRNA165/166 (Yu et al. 2017b).

The DEDDy type 3′-to-5′ nucleic acid exonuclease ATRM2 can degrade unmethylated miRNAs and miRNAs*, while ATRM2 interacts with AGO proteins to participate in the monitoring of unmethylated double-stranded miRNA/miRNA* during the initiation of RISC protein complex formation (Wang et al. 2017). Recent studies have found that the 5′-3′ nucleic acid exonucleases XRN2 and XRN3 can promote the cleavage of pre-miRNA and thus affect miRNA formation (You et al. 2019). RICE1 and RICE2 also act as 3′-5′ exoribonucleases which degrade single-stranded RNAs and thus affect the function of RISC (Zhang et al. 2017b).

Chloroplast-to-nucleus signaling regulation of miRNA processing

CUE1 is a transporter protein localized to the chloroplast, which can transport phosphoenolpyruvate (PEP) into the chloroplast and then metabolize tyrosine to eventually produce vitamin E. Further research has found that vitamin E can regulate the entry of the retrograde chloroplast signaling molecule 3′-phosphoadenosine 5′-phosphate (PAP) to the nucleus and inhibit the activity of the RNA exonuclease XRN2 in the nucleus (Fang et al. 2019). Recent studies have also found that FRY1, a chloroplast-localized protein, can prevent PAP from interfering with the cleavage activity of XRN2/3 (Kim and Arnim 2009; You et al. 2019).

Perspectives

In recent years, the regulatory network of miRNAs in plants has gradually become clearer, and the impact of these networks on plant growth and development are being explored. Future studies will likely find many additional genes which are involved in miRNA biogenesis and function, including potential interactors with the DCL complex. Besides the known proteins, what proteins are involved in the transcription of the MIR gene? What other proteins affect the function of the Dicer-like complex during posttranscriptional processing? In addition to the existing processing pathways, are there any other ways that can participate the miRNA biogenesis? Addressing these issues will lead to a deeper understanding of the miRNA production process and its impact on plant health.

Acknowledgements This work was supported by the Public Projects of Zhejiang Province (No. LGN19C150007), National Natural Science Foundation of China (No. 31701915) and (Grant No. 31872105), and start-up fund from Zhejiang Agriculture & Forestry University (2017FR006). We thank Gang Wu for his advices on our review.

References

Anand A, Krichevsky A, Schornack S, Lahaye T, Tzfira T, Tang Y, Citovsky V, Mysore KS (2007) *Arabidopsis* VIRE2 INTERACTING PROTEIN2 is required for Agrobacterium T-DNA integration in plants. Plant Cell 19(5):1695–1708

Anjali N, Nadiya F, Thomas J, Sabu KK (2019) Identification and characterization of drought responsive microRNAs and their target genes in cardamom (*Elettaria cardamomum* Maton). Plant Growth Regul 87:201–216

Baumberger N, Baulcombe DC (2005) *Arabidopsis* ARGONAUTE1 is an RNA slicer that selectively recruits microRNAs and short interfering RNAs. Proc Natl Acad Sci USA 102(119):28–33

Berardini TZ, Bollman K, Sun H, Poethig RS (2001) Regulation of vegetative phase change in *Arabidopsis* thaliana by cyclophilin 40. Science 291:2405–2407

Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409:363–366

Bielewicz D, Kalak M, Kalyna M (2013) Introns of plant pri-miRNAs enhance miRNA biogenesis. EMBO Rep 14:622–628

Bologna NG, Mateos JL, Bresso EG, Palatnik JF (2009) A loop-to-base processing mechanism underlies the biogenesis of plant microRNAs miR319 and miR159. EMBO J 28:3646–3656

Brodersen P, Sakvarelidze-Achard L, Bruun-Rasmussen M, Dunoyer P, Yamamoto YY et al (2008) Widespread translational inhibition by plant miRNAs and siRNAs. Science 320:1185–1190

Brodersen P, Sakvarelidze-Achard L, Schaller H, Khafif M, Schott G, Bendahmane A, Voinnet O (2012) Isoprenoid biosynthesis is required for miRNA function and af–fects membrane association of ARGONAUTE1 in Arabidopsis. Proc Natl Acad Sci USA 109:1778–1783

Cai Q, Liang C, Wang S, Hou Y, Gao L, Liu L, He W, Ma W, Mo B, Chen X (2018) The disease resistance protein SNC1 represses the biogenesis of microRNAs and phased siRNAs. Nat Commun 9:5080

Calderon-Villalobos LI, Kuhnle C, Dohmann EM, Li H, Bevan M, Schwechheimer C (2005) The evolutionarily conserved TOUGH protein is required for proper development of *Arabidopsis thaliana*. Plant Cell 17:2473–2485

Chaabane SB, Liu R, Chinnusamy V, Kwon Y, Park J, Kim SY, Zhu JK, Yang SW, Lee B (2013) STA1, an *Arabidopsis* pre-miRNA processing factor 6 homolog, is a new player involved in miRNA biogenesis. Nucleic Acid Res 41:1984–1997

Chadick JZ, Asturias FJ, Biochem T (2005) Structure of eukaryotic mediator complexes. Science 30:264–271

Cho SK, Ben Chaabane S, Shah P, Poulsen CP, Yang SW (2014) COP1 E3 ligase protects HYL1 to retain microRNA biogenesis. Nat Commun 5:5867

Clapier CR, Iwasa J, Cairns BR, Peterson CL (2017) Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. Nat Rev Mol Cell Biol 18:407–422

Cui YW, Fang XF, Qi YJ (2016) TRANSPORTIN1 promotes the association of MicroRNA with AGONAUTE1 in *Arabidopsis*. Plant Cell 28:2576–2585

Cuperus JT, Montgomery TA, Fahlgren N, Burke RT, Townsend T, Sullivan CM, Carrington JC (2010) Identification of MIR390a precursor processing-defective mutantsin *Arabidopsis* by direct genome sequencing. Proc Natl Acad Sci USA 107:466–471

Deleris A, Gallego-Bartolome J, Bao J, Kasschau KD, Carrington JC, Voinnet O (2006) Hierarchical action and inhibition of plant dicer-like proteins in antiviral defense. Science 313:68–71

Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, Ziegler-Graff V, Isono E, Schumacher K, Genschik P (2012) Degradation of the antiviral component ATGONAUTE1 by the autophagy pathway. Proc Natl Acad Sci USA 109:15942–15946

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 USA 105:9970–9975

Eamens AL, Smith NA, Curtin SJ, Wang MB, Waterhouse PM (2009) The *Arabidopsis thaliana* double-stranded RNA binding protein DRB1 directs guide strand selection from microRNA duplexes. RNA 15:2219–2235

Earley K, Smith M, Weber R, Gregory BD, Poethig RS (2010) An endogenous F box protein regulates ARGONAUTE1 in *Arabidopsis thaliana*. Silence 1:15

Fang X, Cui Y, Li Y, Qi Y (2015a) Transcription and processing of primary microRNAs are coupled by elongator complex in *Arabidopsis*. Nat Plants 1:15075

Fang X, Shi Y, Lu X, Chen Z, Qi Y (2015b) CMA33/XCT regulates small RNA production through modulating the transcription of dicer-like genes in *Arabidopsis*. Mol Plant 8:1227–1236

Fang X, Zhao G, Zhang S, Li Y, Gu H, Li Y, Zhao Q, Qi Y (2019) Chloroplast-to-nucleus signaling regulates microRNA biogenesis in *Arabidopsis*. Dev Cell 48:371–382

Farrona S, Hurtado L, Bowman JL, Reyes JC (2004) The *Arabidopsis thaliana* SNF2 homolog AtBRM controls shoot development and flowering. Development 131:4965–4975

Feng S, Xu Y, Guo C, Zheng J, Zhou B, Zhang Y, Ding Y, Zhang L, Zhu Z, Wang H, Wu G (2016) Modulation of miR156 to identify traits associated with vegetative phase change in tobacco (*Nicotiana tabacum*). J Exp Bot 67(5):1493

Feng S, Zhang J, Mu Z, Wang Y, Wen C, Wu T, Yu C, Li Z, Wang H (2020) Recent progress on the molecular breeding of *Cucumis sativus* L. in China. Theor Appl Genet 133:1777–1790

Francisco-Mangilet AG, Karlsson P, Kim MH, Eo HJ, Oh SA, Kim JH, Kulcheski FR, Park SK, Manavella PA (2015) THO2, a core member of the THO/TREX complex, is required for microRNA production in *Arabidopsis*. Plant J 82:1018–1029

Fujii H, Zhu JK (2009) *Arabidopsis* mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. Proc Natl Acad Sci USA 106:8380–8385

Furumizu C, Tsukaya H, Komeda Y (2010) Characterization of EMU, the *Arabidopsis* homolog of the yeast THO complex member HPR1. RNA 16:1809–1817

Grant PA, Duggan L, Cote J, Roberts SM, Brownell JE, Candau R, Ohba R, Owen-Hughes T, Allis CD, Winston F, Berger SL, Workman JL (1997) Yeast Gcn5 funtion in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev 11:1640–1650

Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R (2005) Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell 123:631–640

Guo HS, Xie Q, Fei JF, Chua NH (2005) MicroRNA directs mRNA cleavage of the transcription factor NAC1 to downregulate auxin signals for *Arabidopsis* lateral root development. Plant Cell 17:1376–1386

Hajheidari M, Farrona S, Huettel B, Koncz Z, Koncz C (2012) CDKF;1 and CDKD protein kinases regulate phosphorylation of serine residues in the C-terminal domain of *Arabidopsis* RNA polymerase II. Plant Cell 24:1626–1642

Helliwell CA, Chin-Atkins AN, Wilson IW, Chapple R, Dennis ES, Chaudhury A (2001) The *Arabidopsis* AMP1 gene encodes a putative glutamate carboxypeptidase. Plant Cell 13:2115–2125

Huang X, Feng J, Wang R, Zhang H, Huang J (2018) Comparative analysis of microRNAs and their targets in the roots of two cultivars with contrasting salt tolerance in rice (*Oryza sativa* L.). Plant Growth Regul 87:139–148

Hurtado L, Farrona S, Reyes JC (2006) The putative SWI/SNF complex subunit BRAHMA activates flower homeotic genes in *Arabidopsis* thaliana. Plant Mol Biol 62:291–304

Hutvagner G, Simard MJ (2008) Argonaute proteins: key players in RNA silencing. Nat Struct Mol Biol 9:22–32

Iki T, Yoshikawa M, Nishikiori M, Jaudal MC, Matsumoto-Yokoyama E, Mitsuhara I, Meshi T, Ishikawa M (2010) In vitro assembly of plant RNA-induced silencing complexes facilitated by molecular chaperone HSP90. Mol Cell 39:282–291

Jia L, Zhang B, You C, Zhang Y, Zeng L, Li S, Johnson KCM, Yu B, Li X, Chen X (2017) The *Arabidopsis* MOS4-associated complex promotes microRNA biogenesis and precursor messenger RNA splicing. Plant Cell 29:2626–2643

Jung JH, Seo PJ, Ahn JH, Park CM (2012) *Arabidopsis* RNA-binding protein FCA regulates microRNA172 processing in thermosensory flowering. J Biol Chem 287:16007–16016

Karlsson P, Christie MD, Seymour DK, Wang H, Wang X, Hagmann J, Kulcheski F, Manacella PA (2015) KH domain protein RCF3 is a tissue-biased regulator of the plant miRNA biogenesis cofactor HYL1. Proc Natl Acad Sci USA 112:14096–14101

Kierzkowski D, Kmieciak M, Piontek P, Wojtaszek P, Szweykowska-Kulinska Z, Jarmolowski A (2009) The *Arabidopsis* CBP20 targets the cap-binding complex to the nucleus, and is stabilized by CBP80. Plant J 59:814–825

Kim BH, Von Arnim AG (2009) FIERY1 regulates light-mediated repression of cell elongation and flowering time via its 3' (2'), 5'-bisphosphate nucleotidase activity. Plant J 58:208–219

Kim W, Benhamed M, Servet C, Latrasse D, Zhang W, Delarue M, Zhou DX (2009) Histone acetyltransferase GCN5 interferes with the miRNA pathway in *Arabidopsis*. Proc Natl Acad Sci USA 19(7):899–909

Kim S, Yang JY, Xu J, Jang IC, Prigge MJ, Chua NH (2008) Two cap-binding proteins CBP20 and CBP80 are involved in processing primary microRNAs. Plant Cell Physiol 49:1634–1644

Kim YJ, Zheng B, Yu Y, Won SY, Mo B, Chen X (2011) The role of Mediator in small and long noncoding RNA production in *Arabidopsis thaliana*. EMBO J 30:814–822

Köhler A, Hurt E (2007) Exporting RNA from the nucleus to the cytoplasm. Nat Rev Mol Cell Biol 8:761–773

Koiwa H, Barb AW, Xiong L, Li F, McCully MG, Lee BH, Sokolchik I, Zhu J, Gong Z, Reddy M, Sharkhuu A, Manabe Y, Yokoi S, Zhu JK, Bressan RA, Hasegawa PM (2002) C-terminal domain phosphatase-like family members (AtCPLs) differentially regulate *Arabidopsis thaliana* abiotic stress signaling, growth, and development. Proc Natl Acad Sci USA 99:10893–10898

Kurihara Y, Takashi Y, Watanabe Y (2006) The interaction between DCL1 and HY L1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. RNA 12(2):206–212

Kurihara Y, Watanabe Y (2004) *Arabidopsis* micro-RNA biogenesis through dicer-like 1 protein functions. Proc Natl Acad Sci USA 101:12753–12758

Lafos M, Kroll P, Hohenstatt ML, Thorpe FL, Clarenz O, Schubert D (2011) Dynamic regulation of H3K27 Trimethylation during *Arabidopsis* differentiation. PLoS Genet 7:1002040

Lakhwani D, Pandey A, Sharma D, Asif MH, Trivedi PK (2020) Novel microRNAs regulating ripening-associated processes in banana fruit. Plant Growth Regul 90:223–235

Lanet E, Delannoy E, Sormani R, Floris M, Brodersen P, Crete P, Olivier V, Tobaglia C (2009) Biochemical evidence for translational repression by *Arabidopsis* microRNAs. Plant Cell 21:1762–1768

Lee BH, Kapoor A, Zhu J, Zhu JK (2006) STABILIZED1, a stress upregulated nuclear protein, is required for pre-mRNA splicing, mRNA turnover, and stress tolerance in *Arabidopsis*. Plant Cell 18:1736–1749

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

Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN (2004) MicroRNA genes are transcribed by RNA polymerase II. EMBO J 23:4051–4060

Leng X, Wang P, Zhao P, Wang M, Cui L, Shangguan L, Wang C (2017) Conservation of microRNA-mediated regulatory networks in response to copper stress in grapevine. Plant Growth Regul 82:293–304

Li J, Yang Z, Yu B, Liu J, Chen X (2005) Methylation protects miRNAs and siRNAs from a 30-end uridylation activity in *Arabidopsis*. Curr Biol 15:1501–1507

Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, Ji L, Pan Z, Cao X, Mo B, Zhang F, Raikhel N, Jiang L, Chen X (2013) MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in *Arabidopsis*. Cell 153:562–574

Li S, Liu K, Zhang S, Wang X, Rogers K, Ren G, Zhang C, Yu B (2017) STV1, a ribosomal protein, binds primary microRNA transcripts to promote their interacion with the processing complex in *Arabidopsis*. Proc Natl Acad Sci USA 114(6):1424–1429

Li S, Xu R, Li A, Liu K, Gu L, Li M, Zhang H, Zhang Y, Zhang S, Wang Q, Gao G, Li N, Zhang C, Li Y, Yu B (2018) SMA1, a homolog of the splicing factor Prp28, has a multifaceted role in miRNA biogenesis in *Arabidopsis*. Nucleic Acids Res 47:9148–9159

Liu C, Axtell MJ, Fedoroff NV (2012) The helicase and RNaseIIIa domains of *Arabidopsis* DCL1 modulate catalytic parameters during microRNA biogenesis. Plant Physiol 159:748–758

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

Lotfi A, Pervaiz T, Jiu S, Faghihi F, Jahanbakhshian Z, Khorizoghi EG, Fang J, Mahdi Seyedi S (2017) Role of microRNAs and their target genes in salinity response in plants. Plant Growth Regul 82:377–390

Lu C, Fedoroff N (2000) A mutation in the *Arabidopsis* HYL1 gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. Plant Cell 12:2351–2366

Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U (2004) Nuclear export of microRNA precursors. Science 303:95–98

Ma C, Burd S, Lers A (2015) miR408 is involved in abiotic stress responses in *Arabidopsis*. Plant J 84:169–187

Machida S, Chen HY, Yuan YA (2011) Molecular insights into miRNA processing by *Arabidopsis thaliana* SERRATE. Nucleic Acids Res 39:7828–7836

Macrae IJ, Zhou K, Li F, Repic A, Brooks AN, Cande WZ, Adams PD, Doudna JA (2006) Structural Basis for Double-Stranded RNA Processing by Dicer. Science 311:195–198

Manavella P, Hagmann J, Ott F, Laubinger S, Franz M, Macek B, Weigel D (2012) Fast-forward genetics identifies plant CPL phosphatases as regulators of miRNA processing factor HYL1. Cell 151:859–870

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 complex is directed by the 5' terminal nucleotide. Cell 133:116–127

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

Ori N, Cohen AR, Etzioni A, Arnon B, Yanai O, Sharona S, Menda N, Amsellem Z, Efroni I, Pekker I, Alvarez JP, Blum E, Zamir D, Eshed Y (2007) Regulation of LANCEOLATE by miR319 is required for compound-leaf development in tomato. Nat Genet 39:787–791

Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS (2005) Nuclear processing and export of microRNAs in *Arabidopsis*. Proc Natl Acad Sci USA 102:3691–3696

Raghuram B, Sheikh AH, Rustagi Y, Sinha AK (2015) MicroRNA biogenesis factor DRB1 is a phosphorylation target of mitogen activated protein kinase MPK3 in both rice and *Arabidopsis*. FEBS J 282:521–536

Ré D, Cambiagno DA, Arce AL, Tomassi AH, Giustonzzi M, Casati P, Ariel FD, Manavella PA (2020) CURLY LEAF regulates microRNA activity by controlling ARGONAUTE 1 degradation in plants. Mol Plant 13:72–87

Reed R, Cheng H (2005) TREX, SR proteins and export f mRNA. Curr Opin Cell Biol 17:269–273

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403:901–906

Ren G, Chen X, Yu B (2012a) Uridylation of miRNAs by hen1 suppressor in *Arabidopsis*. Curr Biol 22:695–700

Ren G, Xie M, Dou Y, Zhang S, Zhang C, Bin Y (2012b) Regulation of miRNA abundance by RNA binding protein TOUG-H in *Arabidopsis*. Proc Natl Acad Sci USA 109:12817–12821

Schauer SE, Jacobsen SE, Meinke DW, Ray A (2002) DICER-LIKE1: blind men and elephants in *Arabidopsis* development. Trends Plant Sci 7:487–491

Schwab R, Speth C, Laubinger S (2013) Enhanced microRNA accumulation through stemloop-adjacent introns. EMBO Rep 14:615–621

Seo HS, Yang JY, Ishikawa M, Bolle C, Ballesteros ML, Chua NH (2003) LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. Nature 423:995–999

Silva GFF, Silva EM, Azevedo MS, Guivin MAC, Ramiro DA, Figueiredo CR, Carrer H, Peres LEP, Nogueira FTS (2014) microRNA156-targeted SPL/SBP box transcription factors regulate tomato ovary and fruit development. Plant J 78:604–618

Smith MR, Willmann MR, Wu G, Berardini TZ, Möller B, Weijers D, Poethig RS (2009) Cyclophilin 40 is required for microRNA activity in *Arabidopsis*. Proc Natl Acad Sci USA 106:5424–5429

Song J, Wang X, Song B, Gao L, Mo X, Yue L, Yang H, Lu J, Ren G, Mo B, Chen X (2019) Prevalent cytidylation and uridylation of precursor miRNAs in *Arabidopsis*. Nat Plants 5:1260–1272

Song L, Axtell MJ, Fedoroff NV (2010) RNA secondary structural determinants of miRNA precursor processing in *Arabidopsis*. Curr Biol 20:37–41

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 USA 104:5437–5442

Strauss EJ, Guthrie C (1994) PRP28, 'DEAD-box' protein, is required for the first step of mRNA splicing in vitro. Nucleic Acids Res 22:3187–3193

Su C, Li Z, Cheng J, Li L, Zhong S, Liu L, Zheng Y, Zheng B (2017) The protein Phosphatase 4 and SMEK1 complex

dephosphorylates HYL1 to promote miRNA biogenesis by antagonizing the MAPK cascade in *Arabidopsis*. Dev Cell 41:527–539
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
Sun Z, Guo T, Liu Y, Liu Q, Fang Y (2015) The roles of *Arabidopsis* CDF2 in transcriptional and posttranscriptional regulation of primary microRNAs. PLoS Gene 11:1005598
Tagami Y, Motose H, Watanabe Y (2009) A dominant mutation in DCL1 suppresses the *hyl1* mutant phenotype by promoting the processing of miRNA. RNA 15:450–458
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
Tian L, Chen ZJ (2011) Blocking histone deacetylation in *Arabidopsis* induces pleiotropic effects on plant gene regulation and development. Proc Natl Acad Sci USA 98:200–205
Voinnet O (2009) Origin, biogenesis, and activity of plant microRNAs. Cell 136:669–687
Wen Y, Yue Z, Shatkin AJ (1998) Mammalian capping enzyme binds RNA and uses protein tyrosine phosphatase mechanism. Proc Natl Acad Sci USA 95:12226–12231
Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75:855–862
Wang B, Xue Y, Zhang Z, Ding D, Fu Z, Tang J (2016) Transcriptomic analysis of maize kernel row number-associated miRNAs between a single segment substitution line and its receptor parent. Plant Growth Regul 78:145–154
Wang H, Yu C, Fan P, Bao B, Li T, Zhu Z (2014) Identification of two cucumber putative silicon transporter genes in *Cucumis sativus*. J Plant Growth Regul 34(2):332–338
Wang H, Zhu Z, Feng Z, Zhang SG, Yu C (2012) Antisense-mediated depletion of GMPase gene expression in tobacco decreases plant tolerance to temperature stresses and alters plant development. Mol Biol Rep 39(12):10413–10420
Wang L, Song X, Gu L, Li X, Cao S, Chu C, Cui X, Chen X, Cao X (2013) NOT2 proteins promote polymerase II-dependent transcription and interact with multiple microRNA biogenesis factors in *Arabidopsis*. Plant Cell 25:715–727
Wang L, Sun S, Jin J, Fu D, Yang X, Weng X, Xu C, Li X, Xiao J, Zhang Q (2015a) Coordinated regulation of vegetative and reproductive branching in rice. Proc Natl Acad Sci USA 112(50):15504–15509
Wang J, Chen S, Jiang N, Li N, Wang X, Li Z, Li X, Liu H, Li L, Yang Y, Ni T, Yu C, Ma J, Zheng B, Ren G (2019a) Spliceosome disassembly factors ILP1 and NTR1 promote miRNA biogenesis in *Arabidopsis thaliana*. Nucleic Acids Res 47(15):7886–7900
Wang S, Quan L, Li S, You C, Zhang Y, Gao L, Zeng L, Liu L, Qi Y, Mo B, Chen X (2019b) The PROTEIN PHOSPHATASE4 complex promotes transcription and processing of primary microRNAs in *Arabidopsis*. Plant Cell 31:486–501
Wang W, Ye R, Xin Y, Fang X, Li C, Shi H, Zhou X, Qi Y (2011) An importin β protein negatively regulates microRNA activity in *Arabidopsis*. Plant Cell 23:3565–3576
Wang X, Wang Y, Dou Y, Chen L, Wang J, Jiang N, Guo C, Yao Q, Wang C, Liu L, Bin Y, Zheng B, Chekanova JA, Ma J, Ren G (2017) Degradation of unmethylated miRNA/miRNA*s by a DEDDy-type 3' to 5' exoribonuclease Atrimmer 2 in *Arabidopsis*. Proc Natl Acad Sci USA 115:6659–6667
Wang X, Zhang S, Dou Y, Zhang C, Chen X, Yu B, Ren G (2015b) Synergistic and independent actions of multiple terminal nucleotidyl transferases in the 3'tailing of small RNAs in *Arabidopsis*. PLoS Genet 11:1005091
Wang Z, Ma Z, Castillo-González C, Sun D, Li Y, Yu B, Zhao B, Li P, Zhang X (2018) SWI2/SNF2 ATPase CHR2 remodels pri-miRNAs via Serrate to impede miRNA production. Nature 557:516–521
Wu F, Yu L, Cao W, Mao Y, Liu Z, He Y (2007) The N-terminal double-stranded RNA binding domains of *Arabidopsis* HYPONASTIC LEAVES1 are sufficient for pre-microRNA processing. Plant Cell 19:914–925
Wu G, Park MY, Conway SR, Wang JW, Weigel D, Poethig RS (2009) The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. Cell 138:750–759
Wu H, Li B, Iwakawa H, Pan Y, Tang X, Ling-hu Q, Liu Y, Sheng S, Feng L, Zhang H, Zhang X, Tang Z, Xia X, Zhai J, Guo H (2019) Plant 22-nt siRNAs mediate translational repression and stress adaptation. Nature 7806(581):89–93
Wu X, Shi Y, Li J, Xu L, Fang Y, Li X, Qi Y (2013) A role for the RNA-binding protein MOS2 in microRNA maturation in *Arabidopsis*. Cell Res 23:645–657
Xie Z, Allen E, Fahlgren N, Calamar A, Givan SA, Carrington JC (2005) Expression of *Arabidopsis* MIRNA genes. Plant Physiol 138:2145–2154
Xiong L, Lee H, Ishitani M, Tanaka Y, Stevenson B, Koiwa H, Bressan RA, Hasegawa PM, Zhu JK (2002) Repression of stress-responsive genes by FIERY2, a novel transcriptional regulator in *Arabidopsis*. Proc Natl Acad Sci USA 99:10899–20904
Xu F, Xu S, Wiermer M, Zhang Y, Li X (2012) The cyclin L homolog MOS12 and the MOS4-associated complex are required for the proper splicing of plant resistance genes. Plant J 70:916–928
Xu J, Yang JY, Niu QW, Chua NH (2006) *Arabidopsis* DCP1, DCP2 and VARICOSE form a decapping complex required for postembryonic development. Plant Cell 18(12):3386–3398
Xu M, Leichty AR, Hu T, Poethig RS (2018) H2A.Z promotes the transcription of MIR156A and MIR156C in Arabidopsis by facilitating the deposition of H3K4me3. Development 145(2):152868
Xu Y, Guo C, Zhou BY, Li C, Wang H, Zheng B, Ding Y, Zhu Z, Peragine A, Cui Y, Poethig S, Gang Wu (2016) Regulation of vegetative phase change by SWI2/SNF2 chromatin remodeling ATPase BRAHMA. Plant Physiol 172:2416–2428
Yan J, Wang P, Wang B, Hsu CC, Tang K, Zhang H, Hou YJ, Zhao Y, Wang Q, Zhao C, Zhu X, Tao WA, Li J, Zhu JK (2017) The SnRK2 kinases modulate miRNA accumulation in *Arabidopsis*. PLoS Genet 13(4):1006753
Yang L, Liu Z, Lu F, Dong A, Huang H (2006) SERRATE is a novel nuclear regulator in primary microRNA processing in *Arabidopsis*. Plant J 47:841–850
Yang L, Wu G, Poethig RS (2012) Mutations in the GW-repeat protein SUO reveal a developmental function for microRNA-mediated translational repression in *Arabidopsis*. Proc Natl Acad Sci USA 109:315–320
Yang SW, Chen HY, Yang J, Machida S, Chua NH, Yuan YA (2010) Structure of *Arabidopsis* HYPONASTIC LEAVES1 and its molecular implications for miRNA processing. Cell 18:594–605
Yang X, Ren W, Zhao Q, Zhang P, Wu F, He Y (2014) Homodimerization of HYL1 ensures the correct selection of cleavage sites in primary miRNA. Nucleic Acids Res 42:12224–12236
Yang Z, Huang Y, Yang J, Yao S, Zhao K, Wang D, Qin Q, Bian Z, Li Y, Lan Y, Zhou T, Wang H, Liu C, Wang W, Qi Y, Xu Z, Li Y (2020) Jasmonate signaling enhances RNA silencing and antiviral defense in rice. Cell Host Microb 28:89–103
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 USA 105:10073–10078

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

You C, He W, Hang R, Zhang C, Cao X, Guo H, Chen X, Cui J, Mo B (2019) FIERY1 promotes microRNA accumulation by suppressing rRNA-derived small interfering RNAs in *Arabidopsis*. Nat Commun 10(1):1–15

Yu N, Niu QW, Ng KH, Chua NH (2015) The role of miR156/SPLs modules in *Arabidopsis* lateral root development. Plant J 83:673–685

Yu Y, Jia T, Chen X (2017a) The 'how' and 'where' of plant microRNAs. New Phytol 216(4):1002–1017

Yu Y, Ji L, Le BH, Zhai J, Chen J, Luscher E, Gao L, Liu C, Cao X, Mo B, Ma J, Meyers BC, Chen X (2017b) ARGONAUTE10 promotes the degradation of miR165/6 through the SDN1 and SDN2 exonucleases in Arabidopsis. PLoS Biol 15(2):2001272

Zeng Y, Cullen BR (2004) Structural requirements for pre-microRNA binding and nuclear export by exportin 5. Nucleic Acid Res 32:4776–4785

Zhang CJ, Zhou JX, Liu J, Ma ZY, Zhang SW, Dou K, Huang HW, Cai T, Liu R, Zhu JK, He XJ (2013a) The splicing machinery promotes RNA-directed DNA methylation and transcriptional silencing in *Arabidopsis*. EMBO J 32:1128–1140

Zhang L, Ding H, Jiang H, Wang H, Chen K, Duan J, Feng S, Wu G (2019) Regulation of cadmium tolerance and accumulation by miR156 in *Arabidopsis*. Chemosphere 242:125168

Zhang L, Volinia S, Bonome T et al (2008) Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc Natl Acad Sci USA 105:7004–7009

Zhang S, Liu Y, Yu B (2014) PRL1, an RNA-binding protein, positively regulates the accumulation of miRNAs and siRNAs in *Arabidopsis*. PLoS Genet 10:1004841

Zhang S, Xie M, Ren G, Yu B (2013b) CDC5, a DNA binding protein, positively regulates posttranscriptional processing and/or transcription of primary microRNA transcripts. Proc Natl Acad Sci USA 110:17588–17593

Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J, Jacobsen SE (2007) Whole-genome analysis of histone H3 lysine 27 Trimethylation in *Arabidopsis*. PLoS Biol 5:129

Zhang Y, Cheng YT, Bi D, Palma K, Li X (2005) MOS2, a protein containing G-patch and KOW motifs, is essential for innate immunity in *Arabidopsis thaliana*. Curr Biol 15:1936–1942

Zhang Z, Guo X, Ge C, Ma Z, Jiang M, Li T, Koiwa H, Yang SW, Zhang X (2017a) KETCH1 imports HYL1 to nucleus for miRNA biogenesis in *Arabidopsis*. Proc Natl Acad Sci USA 114:4011–4016

Zhang Z, Hu F, Sung MW, Shu C, Castillo-Gonzalez C, Koiwa H, Tang G, Dickman M, Li P, Zhang X (2017b) RISC-interacting clearing 3'-5' exoribonucleases (RICEs) degrade uridylated cleavage fragments to maintain functional RISC in *Arabidopsis thaliana*. eLife 6:24466

Zhan X, Wang B, Li H, Liu R, Kalia RK, Zhu JK, Chinnusamy V (2012) *Arabidopsis* proline rich protein important for development and abiotic stress tolerance is involved in microRNA biogenesis. Proc Natl Acad Sci USA 109(44):18198–18203

Zhao Y, Yu Y, Zhai J, Ramachandran V, Dinh TT, Meyers BC, Mo B, Chen X (2012) The *Arabidopsis* nucleotidyl transferase HESO1 uridylates unmethylated small RNAs to trigger their degradation. Curr Biol 22(8):689–694

Zhu H, Hu F, Wang R, Zhou X, Sze SH, Liou LW, Barefoot A, Dickman M, Zhang X (2011) *Arabidopsis* argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. Cell 145:242–256

Zhu H, Zhou Y, Castillo-González C, Lu A, Ge C, Zhao YT, Duan L, Li Z, Axtell MJ, Wang XJ, Zhang X (2013) Bidirectional processing of pri-miRNAs with branched terminal loops by Arabidopsis Dicer-like1. Nat Struct Mol Biol 20(9):1106–1115