JGG

REVIEW

Plant MicroRNAs and Development

Gang Wu [a,b,*]

[a] The Key Laboratory for Quality Improvement of Agricultural Products of Zhejiang Province, School of Agriculture and Food Sciences, Zhejiang Agriculture & Forestry University, Hangzhou 311300, China
[b] The State Key Laboratory of Plant Physiology and Biochemistry, Zhejiang University, Hangzhou 311058, China

ABSTRACT

MicroRNAs (miRNAs) are a class of about 20–24 nt small non-coding RNAs that can regulate their target gene expression transcriptionally and posttranscriptionally. There are an increasing number of studies describing the identification of new components and regulatory mechanisms involved in the miRNA biogenesis and effector pathway as well as new functions of miRNAs in plant development. This review mainly focuses on the components involved in this pathway, and the developmental defects associated with the corresponding mutations. Some functions of important miRNAs in plant development, together with the modes of miRNA action, are also discussed in this review to describe the recent advance in this area.

KEYWORDS: Plant miRNAs; Biogenesis; Function; Development

INTRODUCTION

MicroRNAs (miRNAs) are a class of about 20–24 nt small non-coding RNAs that can regulate their target gene expression transcriptionally and posttranscriptionally. The founding members of miRNAs, *lin-4* and *let-7*, were initially identified as key regulators of the juvenile-to-adult larval development in *Caenorhabditis elegans* (Lee et al., 1993; Reinhart et al., 2000). A wealth of different miRNAs have been described since then by direct small RNA cloning, computer algorithm prediction and high throughput sequencing in both procaryotes and eucaryotes, a lot of which proved to play pivotal roles in development and growth.

Most plant miRNAs are encoded by sequences previously annotated as intergenic regions. miRNAs are transcribed by DNA dependent RNA polymerase II, recruited by Mediator to promoters of miRNAs (Kim et al., 2011) to form 5′-capped, spliced, and 3′-polyadenylated primary miRNAs (pri-

miRNAs) (Kurihara and Watanabe, 2004; Xie et al., 2005). These pri-miRNAs are then presumably stabilized by an RNA binding protein DAWDLE (DDL) (Yu et al., 2008) and processed into stem loop pre-miRNAs in the Dicing bodies (D-bodies) or small nuclear RNA binding protein D3 bodies (SmD3-bodies) (Kurihara et al., 2006; Fang and Spector, 2007; Fujioka et al., 2007) by a combinatorial action of the RNase III enzyme Dicer-like 1 (DCL1) (Schauer et al., 2002), the double-stranded RNA (dsRNA)-binding protein HYPO-NASTIC LEAVES (HYL1) (Han et al., 2004; Vazquez et al., 2004), the C2H2-zinc finger protein SERRATE (SE) (Grigg et al., 2005; Yang et al., 2006), and the nuclear cap-binding protein complex (CBC) (Kim et al., 2008). The pre-miRNAs are further excised into miRNA/miRNA* RNA duplexes by DCL1. The miRNA/miRNA* RNA duplexes are then methylated on the 3′ ribose of the last nucleotide by a protein called Hua enhancer 1 (HEN1) which shows methyltransferase activity to prevent these RNA duplexes from uridylation and subsequent degradation (Li et al., 2005; Yu et al., 2005). The pre-miRNAs, miRNA/miRNA* RNA duplexes or the mature miRNAs are transported from the nucleus to the cytoplasm by

* Corresponding author. Tel: +86 571 6374 1826.
  E-mail address: wugang@zafu.edu.cn.

the plant exportin-5 orthologue HASTY (HST) and other un-known factors (Park et al., 2005). The guide strand of the miRNA/miRNA* RNA duplexes is then incorporated into an RNA-induced silencing complex (RISC) protein complex containing ARGONAUTE 1 (AGO1) protein as the core component to regulate target gene expression by mRNA cleavage, translational repression or epigenetic modification. The single-stranded miRNA, however, can be degraded by a family of exoribonucleases called SMALL RNA DEGRAD-ING NUCLEASE (SDN), which has the 3′−5′ exonuclease activity to specifically degrade single-stranded miRNAs (Ramachandran and Chen, 2008). In addition to SDN-mediated miRNA degradation, HEN1 SUPPRESSOR1 (HESO1) (Ren et al., 2012a; Zhao et al., 2012), which shows terminal nucleotidyl transferase activity and prefers uridine as the substrate nucleotide, can also target miRNAs for degra-dation. Plant miRNAs usually regulate target expression by a combination of target mRNA cleavage and translational repression (Fig. 1). AGO1 was shown to be the main slicer for target mRNA (Baumberger and Baulcombe, 2005; Qi et al., 2005), and miRNA-targeted mRNAs are upregulated in *ago1* mutants correspondingly (Vaucheret et al., 2004). A recent report showed that SQUINT (SQN), the orthologue of immunophilin cyclophilin 40 (Cyp40) in *Arabidopsis*, is
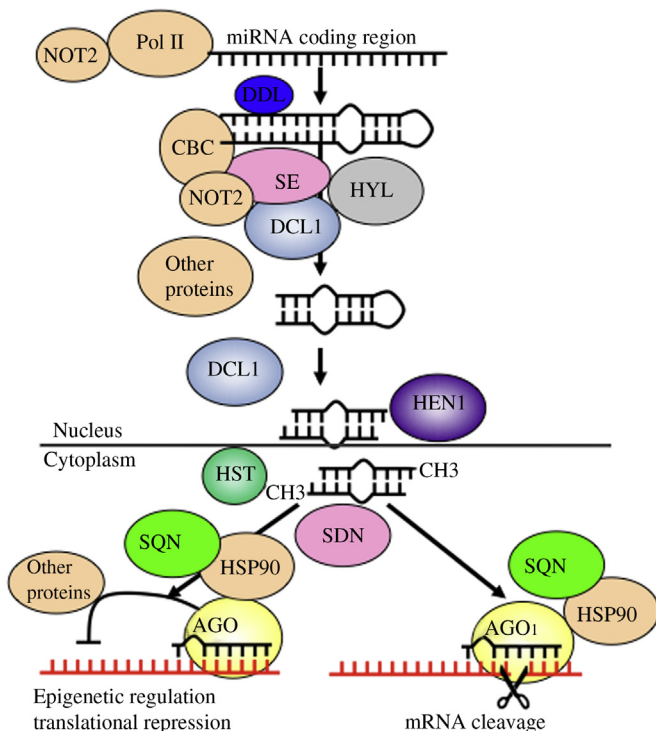

Fig. 1. A diagram of the miRNA biogenesis and effector pathway in plants. miRNAs are transcribed into primary miRNAs (pri-miRNAs) by DNA dependent RNA Polymerase II (Pol II) with the help of NOT2 and Mediator. Pri-miRNAs are stabilized by DDL, and then processed into precursor miR-NAs (pre-miRNAs) by the combinatorial action of DCL1, SE, HYL1, NOT2, and CBC. Pre-miRNAs or mature miRNAs processed by DCL1 are then methylated by HEN1, and transported from the nucleus to the cytoplasm by HST. The guide strand of the RNA duplex is then incorporated into the RISC complex to carry out its function.

required for miRNA-mediated repression by promoting AGO1 activity (Smith et al., 2009). The discrepancy between target mRNA level and corresponding protein level suggests that miRNAs also regulate target expression by translational repression (Aukerman and Sakai, 2003; Chen, 2004; Bari et al., 2006; Gandikota et al., 2007). Translational repression is a widespread mode of plant miRNA action as shown by a forward genetic screen that specifically impairs miRNA-mediated translation repression (Brodersen et al., 2008). Some proteins like AGO1, AGO10, the microtubule-severing enzyme KATANIN, the decapping component VARICOSE (VCS)/Ge-1, 3-hydroxy-3-methylglutaryl CoA reductase (HMG1), sterol C-8 isomerase HYDRA1 (Brodersen et al., 2012), and SUO (Yang et al., 2012) are shown to be required for miRNA-mediated translational repression. How-ever, the mechanism underlying miRNA-mediated trans-lational repression still remains largely unknown in plants.

DEVELOPMENTAL DEFECTS ASSOCIATED WITH MUTATIONS IN THE PROTEINS REQUIRED FOR miRNA BIOGENESIS AND FUNCTION

The pleiotropic phenotypes caused by mutations in the proteins required for miRNA biogenesis and function have long been described before the discovery of miRNA in plants. It was not until the discovery of small RNAs in plants that people started to understand that these pleiotropic defects are attributable to the dysregulation of miRNAs, which usually occur in the miRNA biogenesis and effector pathway.

Proteins involved in miRNA biogenesis

DNA dependent RNA polymerase II (Pol II), Mediator and At-Negative on TATA less 2 (NOT2) proteins

DNA dependent RNA polymerase II produces different classes of RNA, including messenger RNA precursors and numerous stable RNA precursors of small nuclear RNAs, small nucleolar RNAs and non-coding small RNAs including miRNAs (Kurihara and Watanabe, 2004; Xie et al., 2005; Houseley and Tollervey, 2009). Null alleles of Pol II are em-bryo lethal (Onodera et al., 2005), while a weak allele in the gene encoding the second largest subunit of Pol II, NRPB2, is viable. This weak allele displays defects including distorted leaf shape, delayed leaf emergence, reduced floral organ number, abnormal phyllotaxy, and shorter siliques. Further molecular and biochemical analyses show that Pol II is indispensable for endogenous small interfering RNA (siRNA)-mediated transcriptional gene silencing (TGS) at intergenic low-copy-number loci (Zheng et al., 2009). The RNA polymerase II C-terminal domain (Pol II CTD) is subject to phosphorylation by CYCLIN-DEPENDENT KINASE F;1(CDKF;1) and CYCLIN-DEPENDENT KINASE Ds (CDKDs). Mutations in CDKF;1 and CDKD alter the Ser phosphorylation pattern of Pol II CTD, leading to character-istic defects in the transcription and co-transcriptional pro-cessing of a set of miRNAs, siRNAs, and some transcripts encoding key components of small RNA biogenesis pathways