Dicing Bodies[1]

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In eukaryotes, small noncoding RNAs of approximately 21 to 24 nucleotides function as guide molecules in many biological processes, including genome organization and stability, developmental timing and patterning, and antibacterial and antiviral defense (Carrington and Ambros, 2003; Poethig, 2009; Simon and Meyers, 2011). The small RNAs regulate the functions of target DNA or RNA in a sequence-specific manner at either the transcriptional or posttranscriptional level through an RNA-silencing mechanism (Hammond, 2005; Czech and Hannon, 2011). Based on whether RNase III family proteins participate in the biogenesis, the small RNAs are divided into at least two classes: RNase III family protein-dependent small RNAs, including microRNAs (miRNAs) and many small interfering RNAs (siRNAs); and RNase III family protein-independent small RNAs, including PiwiRNAs and secondary siRNAs that are processed from single-stranded precursors in worms (Czech and Hannon, 2011). The mature miRNAs and siRNAs are sorted and loaded specifically with Argonaute (AGO) subfamily proteins, forming the RNA-induced silencing complexes (RISCs) that undergo a specific RNA-silencing mechanism (Ender and Meister, 2010; Fabian et al., 2010). Here, we briefly summarize the molecular basis of miRNA biogenesis pathways and provide an update on nuclear dicing bodies (D-bodies), structures involved in miRNA processing in plant cells. For an overview on siRNAs and other small RNAs, readers are referred to recent excellent articles (Li et al., 2006; Pontes et al., 2006; Ahmad et al., 2010; Chen, 2010a; Law and Jacobsen, 2010; Czech and Hannon, 2011; Simon and Meyers, 2011; Zhang and Zhu, 2011).

THE MIRNA BIOGENESIS PATHWAYS IN ANIMALS AND PLANTS

MiRNAs (approximately 21–22 nucleotides) are a class of small, regulatory RNAs that are found in almost all of the eukaryotes (Reinhart et al., 2000; Lau et al., 2001; Llave et al., 2002; Molnár et al., 2007; Zhao et al., 2007; Chen, 2010b). Like protein-coding genes, miRNA genes in both plants and animals are transcribed by RNA polymerase II into primary transcripts, known as pri-miRNAs. Animal miRNAs are often clustered on the same precursor. These pri-miRNAs are subject to 5′ capping, 3′ polyadenylation, and splicing, as some of the pri-miRNAs may contain introns. A pri-miRNA contains a stem-loop structure: an imperfect double-stranded (ds) RNA hairpin that harbors the mature miRNA (Bartel, 2004; Cai et al., 2004; Lee et al., 2004; Xie et al., 2005; Kim and Nam, 2006; Laubinger et al., 2008; Chen, 2010b). The pri-miRNAs are then processed by two substantial site-specific endonucleolytic events and eventually turned into miRNA duplexes. In animals, the initial step is converting the pri-miRNAs into precursor miRNAs (pre-miRNAs) in the nucleus through the microprocessor, a complex that contains the RNase III family protein Drosha and its partner Pasha/DiGeorge syndrome critical region gene 8, a dsRNA-binding (dsRBD) protein (Denli et al., 2004; Han et al., 2004a; Landthaler et al., 2004; Zeng et al., 2005). The specific sites of the pri-miRNA are recognized by the microprocessor and cleaved by Drosha to generate an approximately 60- to 70-nucleotide, folded pre-miRNA with a two-nucleotide overhang at the 3′ end (Han et al., 2006; Chen, 2010a). Mitrons, some intronic pri-miRNAs, are converted into pre-miRNAs by the RNA-splicing machinery rather than the microprocessor complex (Berezikov et al., 2007; Okamura et al., 2007; Ruby et al., 2007; Flynt et al., 2010). Recently, additional factors, including the nuclear export receptor Exportin1, the cap-binding complex, and ARSENITE-RESISITANCE PROTEIN2, were found to participate in this process (Laubinger et al., 2008; Gruber et al., 2009; Sabin et al., 2009; Buessing et al., 2010). The pre-miRNAs are then recognized by the nuclear export protein Exportin5 and transported to the cytoplasm in a Ran-GTP-dependent manner (Yi et al., 2003; Bohnsack et al., 2004; Lund et al., 2004). The secondary step is to generate the approximately 22- to 23-nucleotide miRNA/miRNA* duplex from the pre-miRNA through a cytoplasmic RNase III-like enzyme and its specific dsRBD partner (Bernstein et al., 2001; Hutvágner et al., 2001). In mammals, the RNase III enzyme Dicer functions in association with the cytoplasmic dsRBD protein TAR RNA-binding protein 2, whereas in *Drosophila*

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melanogaster, Dicer1 with its cofactor Loquacious plays a role in this transition (Chendrimada et al., 2005; Jiang et al., 2005; Saito et al., 2005; Park et al., 2007). The mature RNAs are sorted and loaded with specific AGO proteins to assemble RISCs. Sorting may depend on the interaction between components of biogenesis and effectors, 5′ end nucleotide and thermodynamics of the small RNA duplexes, and/or the structure biases of the AGO family (Khvorova et al., 2003; Schwarz et al., 2003; Gregory et al., 2005; Miyoshi et al., 2005; Rand et al., 2005; Mi et al., 2008; Okamura et al., 2009; Frank et al., 2010).

In contrast to the nuclear cropping and cytoplasmic dicing of pri-miRNAs in animals, both of the steps in pri-miRNA processing occur in the nucleus of plant cells. In addition, a single plant RNase III family protein, DICER-LIKE1 (DCL1), plays similar roles to both nuclear Drosha and cytoplasmic Dicer in animals (Park et al., 2002; Reinhart et al., 2002; Schauer et al., 2002; Kurihara and Watanabe, 2004). Plant miRNA genes are also transcribed by RNA polymerase II, and the primary transcripts are 5′ capped and 3′ polyadenylated (Xie et al., 2005; Kim and Nam, 2006; Chen, 2010a). The pri-miRNAs are cropped into pre-miRNAs with a shorter stem-loop structure, which are further cut into the miRNA/miRNA* duplex. This process requires the interaction of DCL1 with its dsRBD protein partner HYPONSTIC LEAVES1 (HYL1), similar to Drosha and Dicer in animals, which are assisted by a specific dsRBD protein partner (Han et al., 2004a, 2004b; Vazquez et al., 2004; Kurihara et al., 2006; Yang et al., 2010). The C2H2 zinc finger protein SERRATE (SE) also interacts with DCL1 and HYL1 and participates in the transition process (Yang et al., 2006a; Laubinger et al., 2008; Montgomery and Carrington, 2008). HYL1 together with SE promotes the accuracy of miRNA processing (Dong et al., 2008). Many miRNAs are reduced in abundance, while their corresponding pri-miRNAs accumulate in the *dcl1*, *hyl1*, or *se* mutant (Han et al., 2004b; Kurihara and Watanabe, 2004; Vazquez et al., 2004; Yang et al., 2006a). Other proteins such as DAWDLE (an RNA-binding protein) and the nuclear cap-binding complex also act in this process, probably by facilitating DCL1 to access or recognize pri-miRNAs or the loading of miRNA processing factors onto pri-miRNAs (Laubinger et al., 2008; Yu et al., 2008). Recently, plant Mediator was found to participate in miRNA biogenesis by recruiting RNA polymerase II to *MIR* gene promoters and then promoting their transcription (Kim et al., 2011). After the miRNA/miRNA* duplex is released from the precursor, each strand of the duplex is methylated by the small RNA methyltransferase HUA ENHANCER1 (HEN1) in the 2′-OH of the 3′ terminal nucleotide (Li et al., 2005; Yu et al., 2005; Yang et al., 2006b). The methylation is a protection from further polyuridylation and from the subsequent degradation by the exonucleases of the Small RNA-Degrading Nuclease family (Li et al., 2005; Yu et al., 2005; Ramachandran and Chen, 2008).

The 3′ methylated miRNA/miRNA* duplex may be transported by a plant Exportin5 homolog HASTY (HST) or through HST-independent mechanisms to the cytoplasm (Park et al., 2005; Eamens et al., 2009), where RISC can be assembled as in animals. However, the exact form of the exported miRNA and the subcellular localization of plant RISC loading and maturation are still not clear (Voinnet, 2009). Plant RISC may also be assembled in the nucleus. In this scenario, only mature AGO1 with a single-stranded miRNA can be exported to the cytoplasm (Eamens et al., 2009). Plant AGO1 is the major part of RISC and has an endonucleolytic cleavage activity that cleaves complementary mRNAs in the center of the miRNA-mRNA paired region (Vaucheret et al., 2004; Baumberger and Baulcombe, 2005; Qi et al., 2005).

PLANT MIRNA PROCESSING PROTEINS CONCENTRATE IN DISCRETE D-BODIES

Recent progress in live-cell imaging proposed that nuclear chromatin is packaged into a higher order three-dimensional structure that may correlate to the regulation of the genes (Hübner and Spector, 2010; Misteli, 2010). In addition, the interchromatin region in the cell nucleus is highly heterogeneous and contains various nuclear domains or bodies, for example, nuclear speckle, paraspeckle, nucleolus, perinucleolar compartment, Cajal body (CB), cleavage body, gemini of coiled bodies, OPT (for Oct1/PTF/transcription) domain, SAM68 (for Src associated in mitosis of 68 kD) nuclear body, polymorphic interphase karyosomal association, polycomb body, promyelocytic leukemia body (Mao et al., 2011), and plant-specific nuclear bodies, such as cyclophilin, phytochrome, or

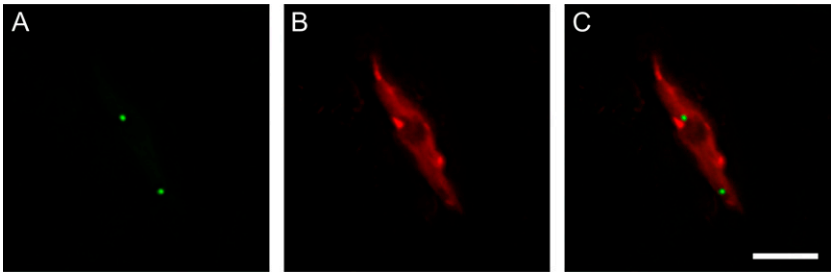

Figure 1. Arabidopsis nuclear D-bodies. A, HYL1-YFP signals in the nucleus of a leaf epidermal cell. B, 4′,6-Diamidino-2-phenylindole staining of the nucleus in A. C, Overlay of A and B. Two D-bodies are observed in the image, one of them close to the nucleolus as shown in C. Bar = 10 μm.

abscisic acid-activated protein kinase-containing nuclear bodies (Shaw and Brown, 2004; Chen et al., 2010). These bodies are present in the nucleus at steady state and dynamically respond to basic cellular processes as well as to diverse metabolic conditions, alterations in cellular signaling, and various forms of stress (Dundr and Misteli, 2010; Mao et al., 2011). Live-cell imaging of plant miRNA processing proteins DCL1 and HYL1, which were fused to fluorescent proteins and expressed in transgenic Arabidopsis (*Arabidopsis thaliana*) plants under the control of their endogenous promoters, revealed that DCL1 was enriched in round nuclear bodies measuring 0.2 to 0.8 μm in diameter as well as being diffusely distributed throughout the nucleoplasm, predominantly excluded from nucleoli (Fig. 1; Fang and Spector, 2007). The number of nuclear bodies present in each nucleus ranged from zero to four, with the majority of the nuclei having one nuclear body. A population of DCL1 bodies (approximately 60%) localize in close proximity to nucleoli in projection images, but three-dimensional deconvolution analysis revealed that they are not within nucleoli. The DCL1 partner protein HYL1 displays a similar localization pattern of nuclear bodies to DCL1 bodies. Colocaliza-

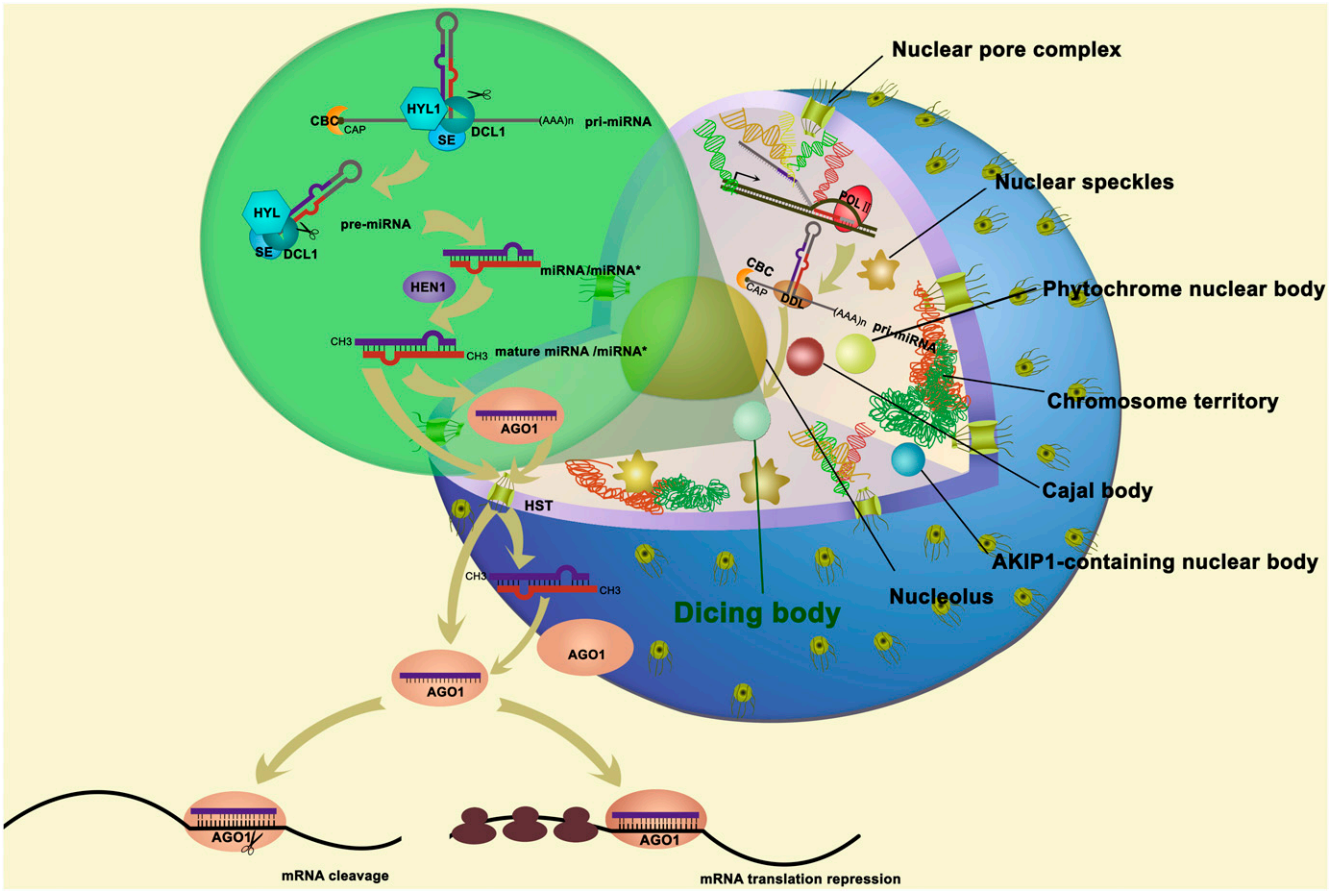

Figure 2. Plant nuclear D-bodies, which contain proteins for miRNA processing and are involved in miRNA biogenesis. In eukaryotic cells, the nucleus is encapsulated in two layers of membranes in which nuclear pore complexes are embedded for transport between the nucleus and the cytoplasm. Chromosomes in the nucleus are organized into chromosome territories. The interchromatin region of the cell nucleus is highly heterogeneous, containing various nuclear domains or bodies. In a plant cell, these nuclear bodies include nucleolus, CB, nuclear speckles, phytochrome nuclear body, AAPK-Interacting Protein1 (AKIP1)-containing nuclear body, and D-body. These bodies have different sizes, shapes, components, dynamics, and functions. D-bodies play a role in the biogenesis of miRNAs. Plant miRNA genes are transcribed by RNA polymerase II to generate pri-miRNAs. The RNA-binding protein DAWDLE presumably stabilizes pri-miRNAs and facilitates DCL1 to access or recognize pri-miRNAs. The nuclear cap-binding complex (CBC) likely facilitates the loading of miRNA processing factors onto pri-miRNAs. The pri-miRNAs are then recruited to D-bodies, which contain DCL1, the dsRBD protein HYL1, and the C2H2 zinc finger protein SE. These pri-miRNAs are then processed into a shorter stem-loop structure called pre-miRNAs and then further into the miRNA/miRNA* duplex. The miRNA/miRNA* duplex is methylated by the small RNA methyltransferase HEN1 in the 2′-OH of the 3′ terminal nucleotide. The mature miRNA/miRNA* may be transported in an HST-dependent or -independent manner through the nuclear pore complex, or the guide strand of mature miRNA/miRNA* is probably selectively loaded into AGO1-RISC in the nucleus and the miRISC is transported into the cytoplasm. The miRISC carries out the silencing reactions through translation repression or mRNA cleavage in the cytoplasm.

tion analysis revealed that DCL1 bodies and HYL1 bodies are the same structures as they colocalize (Fang and Spector, 2007; Song et al., 2007). Unlike DCL1 and HYL1, SE was distributed in nuclear speckles or interchromatin granule clusters containing the Ser/Arg (SR) splicing factor SR33 (Fang et al., 2004; Fang and Spector, 2007). In a small population of cells, the SE signal was also present in HYL1 bodies in addition to its nucleoplasmic distribution. The dual localization patterns of SE both in nuclear speckles and DCL1/HYL1 nuclear bodies may correlate with its dual roles in both splicing and miRNA processing (Fang and Spector, 2007; Laubinger et al., 2008).

The DCL1/HYL1-containing bodies are different from most known nuclear bodies, due to their round shape, size, and average number per nucleus (Shaw and Brown, 2004), but are similar to CBs, as they are round in shape and their distribution is frequently perinucleolar (Nizami et al., 2010). CBs contain components involved in the processing/assembly of small nuclear RNAs, small nucleolar RNAs, and possibly siRNAs (Li et al., 2006; Pontes et al., 2006; Nizami et al., 2010). However, colocalization analysis indicated that DCL1/HYL1-containing bodies are different from CBs (Fang and Spector, 2007; Song et al., 2007), since they show no overlay with the AtCoilin signal, a signature marker of CBs. DCL1/HYL1-containing bodies are called dicing bodies or D-bodies (Fang and Spector, 2007; Fig. 1). In living cells of Arabidopsis plants, D-bodies move in the nuclei in a constrained manner.

HOW ARE D-BODIES FORMED?

Nuclear bodies are membraneless subnuclear organelles. A specific nuclear body is formed in a stochastic or ordered assembly manner (Dundr and Misteli, 2010). In addition, a seeding mechanism has been proposed to assemble, maintain, and regulate particular nuclear bodies (Mao et al., 2011). DCL1 contains two C-terminal dsRBDs. The mutant *DCL1-6*, with truncation of its two dsRBDs, is embryo lethal, while *DCL1-9*, a mutant with truncation of only the second dsRBD, results in infertility and severe defects in the biogenesis of most miRNAs, suggesting an important role of these dsRBDs in miRNA processing (Schauer et al., 2002). Live-cell imaging revealed that DCL1-9 failed to localize to D-bodies but instead distributed diffusely in the nucleoplasm, demonstrating that the dsRBD of DCL1 is critical for its localization to D-bodies. HYL1 contains two N-terminal dsRBDs, and these two dsRBDs are sufficient for pre-miRNA processing and localization to D-bodies (Wu et al., 2007). These results suggested that the dsRBDs in these miRNA processing proteins are essential for their targeting to D-bodies, possibly forming the seed for the assembly of D-bodies.

WHAT ARE THE FUNCTIONS OF D-BODIES?

In vivo tracking of a pri-miRNA using 24 tandem MS2 translational operators (MS2 repeats) and the MS2 coat protein-yellow fluorescent protein (MS2-YFP) system demonstrated that an introduced pri-RNA concentrates in DCL1-containing D-bodies in addition to being present in a diffuse distribution in the nucleoplasm, indicating that the pri-miRNAs can be recruited to D-bodies, where the machinery for their processing is enriched (Fang and Spector, 2007).

The precise and efficient pri-miRNA processing requires protein-protein interactions between the miRNA processing proteins (Kurihara et al., 2006). Using bimolecular fluorescence complementation, Fang and Spector (2007) found that DCL1, HYL1, and SE interact in the nuclear D-bodies in vivo, while the bimolecular fluorescence complementation signal in the surrounding nucleoplasm is very weak. In addition, DCL1 and HYL1 self-interact in the D-bodies. By contrast, DCL1-9 showed no interaction with SE, HYL1, or DCL1. Together, these results suggested a role of D-bodies in the dicing reaction of pri-miRNAs mediated by DCL1 and its interacting partner HYL1 (Fig. 2).

PERSPECTIVES

Apart from DCL1, HYL1, and SE, which localize predominantly or transiently to D-bodies, the miRNA/miRNA* duplex, the methyltransferase HEN1, and the slicer AGO1 also exhibited some localization to D-bodies in addition to their nucleoplasmic and cytoplasmic distribution patterns when examined by colocalization analysis with HYL1 (Fang and Spector, 2007). Therefore, it is of interest to investigate if HEN1 and AGO1 are recruited to D-bodies to methylate the miRNA/miRNA* duplex and load mature miRNAs to AGO1 to assemble the RISC complex in D-bodies. In this case, only mature AGO1 and miRNA containing RISC can be exported to the cytoplasm through nuclear pore complexes (Eamens et al., 2009; Fig. 2). In addition, HYL1 was observed to colocalize with its homolog DRB4 in D-bodies (Y. Fang and D.L. Spector, unpublished data). It was known that DRB4 interacts with DCL4 in vivo and is involved in the biogenesis of siRNAs (Fukudome et al., 2011). Therefore, more extensive studies are needed to learn about the potential roles of D-bodies in orchestrating the processing, sorting, RISC assembly, and functioning of small RNAs.

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