2012

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Ren, Guodong and Yu, Bin, "Critical roles of RNA-binding proteins in miRNA biogenesis in Arabidopsis" (2012). Faculty Publications from the Center for Plant Science Innovation. 164.
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Critical roles of RNA-binding proteins in miRNA biogenesis in Arabidopsis

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Abstract
MicroRNAs (miRNAs) are key regulators of gene expression and play critical roles in modulating metabolism, development and physiology in animals and plants. miRNA levels are transcriptionally and post-transcriptionally controlled for their proper function. Recent studies have shown that RNA-binding proteins play important roles in producing miRNAs by affecting the accurate and/or efficient processing of precursors of miRNAs. Many of these RNA-binding proteins also have roles in general RNA metabolism, indicating potential connections between miRNA biogenesis and other RNA metabolism. Here, we focus on the function of several RNA-binding proteins in miRNA biogenesis in Arabidopsis.

Keywords: RNA-binding protein, miRNA biogenesis, DCL1, HYL1, SE, DDL, TOUGH, CBP80/CBP20, Arabidopsis
SE, HYL1, CBP80 and CBP20

HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE) are the best characterized RNA-binding proteins in miRNA biogenesis in Arabidopsis. HYL1 and SE interact with DCL1 in distinct subnuclear speckles called Dicing-body (D-body) and positively regulate miRNA processing efficiency and accuracy (Fig. 1B).\textsuperscript{16-20} Mutations in HYL1 lead to reduced root growth, hyponastic leaves, reduced fertility and altered hormone responses.\textsuperscript{21} These phenotypes of hyl1 can be restored by DCL1 alleles with increased activity, suggesting that the morphological phenotypes of hyl1 are mainly caused by the reduction of miRNA levels.\textsuperscript{22,23} HYL1 contains two tandem dsRNA-binding domains (dsRBD1 and dsRBD2) at its N-terminal that bind dsRNAs and

Figure 1. miRNA biogenesis in plant. (A) Schematic domain structure of miRNA processing components in Arabidopsis (At) and their respective counterparts/homolog in Homo sapiens (Hs). The N domain and MID domain of SE is based on.\textsuperscript{35} The DUF domains in DDL and SNIP are possibly RNA binding domains.\textsuperscript{41} (B) miRNA biogenesis in plants. MiR genes are transcribed into primary miRNAs (Pri-miRNAs) by Pol II. Recruitment of Pol II to MiR genes is assisted by Mediator complex.\textsuperscript{56} Processing of pri-miRNAs into miRNAs requires two sequential cleavages by DCL1 and is assisted by RNA-binding proteins, which recognize different parts of pri-miRNAs and/or associate with key components of processing complex. After production, miRNA/miRNA* duplex is methylated by HEN1 and miRNA strand is loaded into AGO1 containing RISC complex.
six repeats of 28 amino acids (aa) at its C-terminal that were thought to provide a platform for protein–protein interaction (Fig. 1). Truncated HYL1 containing two dsRBDS alone is sufficient to completely complement hyl1 phenotype, demonstrating that the N-terminal of HYL1 is sufficient for its function whereas the C-terminal of HYL1 may be dispensable for miRNA biogenesis. Structural and biochemical studies reveal that dsRBDS confers dsRNA-binding activity while dsRBDS2 of HYL1 may be required for its dimerization in vivo (Fig. 1 and ref. 24). It is proposed that the HYL1 dimer preferentially recognizes and binds the miRNA* miRNA* duplex region of pri-miRNA to ensure accurate processing by DCL1. Although HYL1 is a plant specific protein, it may function analogously to metazoan DGCR8 and TRBP. Consistent with this idea, HYL1 may also have a role in miRNA strand selection and RISC loading, mimicking a role of TRBP in animals.

SE is another essential component required for accurate and efficient miRNA processing. Consistent with this, the null alleles of se are embryonic lethal while the weak alleles exhibit serrated/hyponastic leaves, reduced fertility and altered phyllotaxy alleles. Unlike DCL1 and HYL1, the localization of SE is not restricted to D-body but rather heterogeneous, suggesting that SE may have roles other than pri-miRNA processing. In fact, SE participates in the splicing of a subset of pre-mRNA. The core region of SE (Residues 194–543; Fig. 1) consisting of N, Mid and zinc finger domains mediates the interaction of SE with DCL1 and HYL1. Intriguingly, the zinc finger domain together the C-terminal unstructured tail of SE binds miRNA precursors and is able to largely restore se-1 phenotypes, arguing against an indispensable role of SE-DCL1 and SE-HYL1 interactions in miRNA biogenesis.

The eukaryotic nuclear cap-binding complex (CBC) is a heterodimer consisting of two subunits, Cap-binding protein 80 (CBP80) and CBP20. In Arabidopsis, CBP80/ABA HYPERSENSITIVE 1 (ABH1) was originally identified as a genetic screen of altered Abscisic acid (ABA, a plant hormone) response mutants. The cbp80/abh1 confers ABA hypersensitivity and increases drought resistance. The cbp80/abh1 and cbp20 null alleles exhibit serrated leaf phenotype, which resembles the weak se alleles, indicating that the CBC complex and SE may have overlapping functions. Indeed, cbp80/abh1 and cbp20 cause defects in pre-mRNA splicing, reduce the levels of miRNAs and increase levels of pri-miRNAs, demonstrating that like SE, the CBC complex is involved in both pre-mRNA splicing and miRNA biogenesis. These results also suggest a role of cap structure in facilitating pri-miRNA processing in addition to its proposed role in pri-miRNA stability control since the CBC complex binds the m7G cap of pri-miRNAs.

**DDL and TGH**

DDL (DDL) encodes a forkhead-associated (FHA) domain-containing protein that interacts with the N-terminal part of DCL1 and positively regulates miRNA biogenesis (Fig. 1). As the FHA domain specifically recognizes and binds phosphorylated peptide, it will be interesting to test whether the FHA domain is required for DDL-DCL1 interaction. If so, then a role of phosphorylation modification of DCL1 can be studied through mapping of its DDL binding motif. Unlike hyl1, se and dcl1 mutants, where pri-miRNA levels are highly accumulated due to inefficient processing, mutations in DDL reduce the levels of pri-miRNAs. However, DDL seems not to contribute to the MIR gene expression since it does not affect the promoter activity of MIR172. It has been proposed that DDL may protect pri-miRNAs from degradation through DDL-pri-miRNA binding. In support of this hypothesis, DDL is an ssRNA-binding protein and binds pri-miRNA162b in vitro. Further work on mapping of the motif of DDL that binds the pri-miRNAs and structural analysis of DDL-pri-miRNA complex will be needed to test the role of DDL in pri-miRNA stability control.

More recently, we revealed that TOUGH (TGH), another ssRNA-binding protein, promotes pri-miRNA processing. TGH contains an uncharacterized DUF1604 domain, a G-patch domain and a SWAP domain, all of which exist in RNA processing related proteins (Fig. 1). Mutations in TGH lead to the pleiotropic phenotypes, which overlap with other miRNA biogenesis defective mutants, e.g., narrow leaf, altered vascularization and phyllotaxy, and reduced fertility. Northern blot as well as small RNA deep sequencing analyses reveal a general reduction of miRNA levels in tgh. In addition, pri-miRNAs levels are moderately increased in tgh. These results suggest that TGH acts on the processing of pri-miRNAs. This is further supported by the fact that tgh diminishes pri-miR162b processing without affecting DCL1 and HYL1 protein levels. TGH physically interacts with DCL1, HYL1 and SE in an RNA-independent manner, suggesting that TGH is a novel component of DCL1 processing complex. TGH binds ssRNAs in vitro and pri-miRNAs in vivo. In addition, we were able to recover pre-miRNA172a and pre-miRNA166a from the TGH complex. Based on these results, we propose that TGH binds the loop or the bulge of pri-miRNAs/pre-miRNAs. The binding of TGH to pri-miRNAs may contribute to the interaction between primiRNA and the DCL1 complex, as primiRNAs are less efficiently bound to the HYL1 complex in tgh-1.

Previous study has shown that TGH interacts with TATA-box binding protein 2 (TB2), and may have a role in activating gene expression. It is possible that TGH gene transcription, co-transcriptional modification and pri-miRNA processing are tightly coupled and TGH may promote MIR gene expression in addition to its role in miRNA processing. Consistent with potential dual roles of TGH in MIR gene expression and pri-miRNA processing, we noticed that pri-miRNA levels in tgh-1 are increased (~1.5–2.5-fold) much less dramatic than those in hyl1 and se mutants (~5–20 and up to 100-fold). In addition, TGH has been shown to colocalize with splicing regulator SRp34, suggesting a role of TGH in splicing. Indeed, TGH homolog protein has been identified as a component of spliceosomal complex through a proteomic approach. However, tgh does not cause any splicing defects of several pre-mRNAs, whose splicing is affected by se and hyl1 (Ren and Yu, unpublished). To this end, we cannot rule out the possibility of TGH acting on the splicing of other mRNAs. Although tgh-1 only causes moderate miRNA reduction, its phenotypes, at least in some aspects, are more severe than those of dcl1-9 and hyl1-2, suggesting that TGH may have additional functions other than miRNA biogenesis.
DDL and TGH also participate in the biogenesis of small interfering RNAs (siRNAs, another class of endogenous regulatory small RNA besides miRNA).41,44 How DDL affects siRNA biogenesis is currently unknown. TGH is required for miRNA822, IR-71 and multiple repeat associated siRNAs (rasiRNAs) which are DCL4-, DCL2- and DCL3-dependent, respectively.44 Consistently, TGH interacts with DCL3 and promotes its processing activity in vitro.44 However, neither AtSN1A nor AtSN1B RNAs, which are transcribed from AtSN1 siRNA locus and its flanking region, respectively, are detectable within the TGH-HA complex,44 consistent with the fact that siRNA s are generated from dsRNAs.

All the RNA-binding proteins discussed above except HYL1 have their cognate homologs in animals (Fig. 1). Smad nuclear interacting protein (SNIP), a human homolog of DDL, interacts with Drosha and regulates the abundance of miRNAs.41 Arsenite-resistance protein 2 (ARS2), a homolog of SE, physically interacts with CBC complex and binds mG capped RNA.47,48 The ARS2-CBC complex interacts with Drosha but not Dicer and acts in the miRNA biogenesis.47,48 However, the ARS2-CBC complex may have roles in the stability of pri-miRNAs since depletion of ARS2 or the CBC complex reduces the levels of pri-miRNAs.47,48 It will be interesting to test the potential involvement of TGH homolog protein in animal miRNA biogenesis. Knockdown of TGH homolog in C. elegans causes either embryonic lethality or developmental defects in genome-wide RNAi screens,49-51 which is consistent with the role of miRNAs in controlling development. RNAi pathway exists in filamentous fungus *Neurospora crassa* (*N. crassa*) but not in budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*).52,53 A BLAST search was able to identify putative homologs of SE, DDL and TGH in *N. crassa* but not in *S. cerevisiae*, consistent with the hypothesis that RNA silencing machinery might originate from the same ancestor and diversify during evolution.54

**Perspective**

Clearly, RNA-binding proteins play critical roles in regulating the cellular levels of miRNAs in plants. A big challenge is to dissect how these RNA-binding proteins co-operate with DCL1 to regulate miRNA biogenesis either globally or individually. Structural modeling of DCL1-RBPs-pri-miRNAs and biochemical determining the effect of these RNA-binding proteins on DCL1 kinetics will be needed to address this challenge. Another challenge is to identify whether the RNA-binding proteins involved in miRNA biogenesis have roles in the metabolism of other RNAs. *dc1*, *se*, *hyl1*, *ddl*, *cbp80*, *cbp20* and *tgh* only have partially overlapping phenotypes, indicating that they may act differentially on various miRNAs and/or have roles other than miRNA biogenesis. Indeed, DCL1, SE and HYL1 differentially control the levels of some miRNAs and contribute differently to the silencing of a subset of unannotated transcriptionally active regions (TARs) as well as transposons.43 In addition, SE, CBP80/20 and HYL1 have been shown to act on pre-mRNA splicing. Identification of RNAs associated with these RNA-binding proteins will be invaluable to a full understanding of their functions and will provide new insights into our integrated view of the cooperation and/or competition of various RNA processing machineries. For instance, high-throughput sequencing and cross-linking immunoprecipitation (HITS-CLIP) analyses of DGCR8 associated RNAs reveal that DGCR8 binds mRNAs, small nucleolar RNAs (snoRNAs) and long noncoding RNAs and regulates their abundance and/or splicing.55

**Disclosure** — No potential conflicts of interest were disclosed.

**Acknowledgments** — We thank David Holding, Meng Xie, Xiangu Liu, and Shuxin Zhang from University of Nebraska-Lincoln for critical reading of the manuscript. The National Science Foundation (MCB-1121193) supported this work.

**References**

16. Fujikawa Y, Utsumi M, Ohba Y, Watanabe Y. Location of a possible


20. Kurihara Y, Takashi Y, Watanabe Y. The interaction between DCL1 and HYL1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. RNA 2006; 12:206- 12; PMID:16428603; doi:10.1216/ma.2146906


23. Tagami Y, Motoose H, Watanabe Y. A dominant mutation in DCL1 suppresses the hyl1 mutant phenotype by promoting the processing of miRNA. RNA 2009; 15:450-8; PMID:19155326; doi:10.1261/rna.1297109


35. Kurihara Y, Takashi Y, Watanabe Y. The interaction between DCL1 and HYL1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. RNA 2006; 12:206- 12; PMID:16428603; doi:10.1216/ma.2146906

