miRNA processing turned upside down

Rebecca Schwab and Olivier Voinnet*

IBMP-CNRS, UPR2357 Université de Strasbourg, 12 rue du Général Zimmer, Strasbourg, France

*Correspondence to: olivier.voinnet@ibmp-ulp.u-strasbg.fr

The EMBO Journal (2009) 28, 3633–3634. doi:10.1038/emboj.2009.334

MicroRNAs are 19–22 nt-long RNAs that regulate eukaryotic gene expression. They are processed from stem-loop containing precursor transcripts by RNase III enzymes of the Dicer family. In this issue of *the EMBO Journal*, a study by Bologna and colleagues investigates the processing of two plant *MIRNA* families with unusually long precursors. Their findings suggest a non-canonical mode of biogenesis in which DCL1, the plant miRNA-producing enzyme, initiates sequential cuts close to the loop at the tip of the stem rather than at its base. It therefore requires the integrity of the upper stem in the precursor, although the structural and/or sequence features that guide DCL1 to its initial binding platform are yet to be identified. Owing to the loop-to-base processing and the unusual length of the stem, several additional small RNA species are produced before the cognate miRNA is excised, a phenomenon that might shed light on the origin of *MIRNA* genes.

Eukaryotic miRNAs originate from primary transcripts with characteristic stem-loop structures consisting of a terminal loop, an upper stem, the miRNA/miRNA* region, and a lower stem (Figure 1). Consecutive cuts at defined positions by class III ribonucleases release the miRNA/miRNA*

duplex, of which the miRNA strand incorporates into an Argonaute (AGO) effector complex. The AGO-bound miRNA then guides sequence-specific silencing of complementary target mRNAs. Plant miRNA stem-loops are longer and more heterogeneous than those of their animal counterparts and they are mostly processed by a single Dicer protein, DICER-LIKE 1 (DCL1) (for review, see Voinnet, 2009). Nonetheless, a study of *Arabidopsis* *MIR163* suggested that an initial cut near the stem base (Kurihara and Watanabe, 2004) is a common feature of plant and metazoan miRNA processing.

Bologna *et al* (2009) investigated the processing of *Arabidopsis* *MIR319a* and found that, unexpectedly, shortening the lower stem below the miRNA sequence had no impact on miRNA production and its biological effects, unlike for other ‘conventional’ precursors, such as that of *MIR172a*. Thus, owing to the unusual length of the stem and the miRNA being located far below the tip, an intuitive processing model entails successive cleavage events by DCL1, starting close to the loop at the tip of the stem and proceeding to the base, in ~21-nt increments (Figure 1A). Deep-sequencing reads of low-abundant small RNAs indeed map to these

postulated byproducts. Moreover, using RACE-PCR, Bologna *et al* (2009) could recover the predicted processing intermediates for several precursors in the miR319 and miR159 families, confirming their unconventional synthesis.

But what determinants direct DCL1 to the position of its initial cut? The sequences and predicted secondary structures of *MIR319* and *MIR159* tips above the first cut appear variable. Moreover, experimentally enlarging or opening the *MIR319a* loop reduced, yet did not abolish, miRNA production. In mammalian miRNA precursors, the double-stranded (ds) RNA-binding (DRB) protein, DGCR8, guides the initial Drosha-mediated cut at the single-stranded RNA-ds RNA junction of lower stems (for review, see Kim *et al*, 2009). HYL1, the DRB partner of DCL1, is similarly required for miR319 production and might thus recognize an analogous structure, but at the stem/loop junction of the tip, rather than at the base, of *MIR319a*. However, experimentally reducing the size of the *MIR319a* loop did not abolish processing.

These uncertainties regarding the characteristics of HYL1/DCL1-binding platforms emphasize the need for much deeper structural investigations of miRNA stems and loops. RNA molecules can also fold through non-Watson-Crick interactions (Leontis and Westhof, 2003), resulting in tertiary arrangements diverging sometimes dramatically from predictions of secondary structure algorithms (e.g. mfold; Zuker, 2003). Genetic experiments might also help in deciphering this question. For instance, swapping the tips of stems both within the *MIR319/159* families and also with other precursors undergoing canonical base-to-loop processing might identify features necessary and/or sufficient to attract the processing machinery.

Upon precursor recognition, DCL1 performs sequential cuts on *MIR319* and *MIR159* upper stems to eventually generate mature miRNAs. Bologna *et al* (2009) investigated the structural importance of the upper stem for those processing steps by closing bulges that improve strand pairing of a normally low-abundant small RNA (sRNA1) produced by DCL1 before miR319 excision. One such modification markedly decreased miR319 accumulation and concomitantly increased sRNA1 abundance (Figure 1B). The authors' interpretation is that bulges in upper stems normally prevent the accumulation of small RNAs other than the miRNA itself,

because processing generates mismatched duplexes that are less stable or less prone to AGO incorporation. Binding to a more stable stem could also, in turn, prevent DCL1 from proceeding towards the stem's base to excise miRNA.

This interpretation, however, assumes that the same enzyme, DCL1, processes both the bulged and closed upper stem variants of *MIR319a*. However, no genetic data were provided to rule out that DCL4, and not DCL1, processes specifically the closed stem variant: DCL4 indeed shows strong affinity to dsRNA stem-loops of high complementarity. Owing to intrinsic differences in the activity of DCL proteins, DCL4- as opposed to DCL1-dependent processing of the modified *MIR319a* precursor could generate a suite of small RNAs offset by one or more nucleotides (Figure 1C). These small RNAs would now bear distinct 5' terminal nucleotides, a feature that significantly impacts small RNA sorting into specific plant AGOs. Incorporation into non-cognate AGO proteins might therefore alter small RNA stabilities. The existence, in plants, of several DCL paralogues that compete for dsRNA substrates thus makes it necessary to systematically assess structural changes to miRNA stem-loops not only in wild-type but also in *dcl* multiple mutants.

In fact, the above issue could be anticipated from a popular model for plant *MIRNA* gene evolution, in which proto-*MIRNA*s arise from perfectly dsRNA fold-back structures generated by inverted gene duplication (Voinnet, 2009). Those structures initially attract multiple DCLs, chiefly DCL4, to produce heterogeneous small RNA species. Acquisition of DCL1 dependence and production of discrete small RNA species would then require accumulation of drift mutations causing fold-back mis-pairing. The preferential recruitment of DCL4, rather than DCL1, through conversion of three bulges into pairing bases in the *MIR319a* upper stem could thus be considered as a 'step back in time', according to the proto-*MIRNA* model. Determining the respective contribution of DCLs in the processing of modified precursors of *MIR319a* and other *MIRNA*s could thus provide a useful handle towards understanding the mechanisms by which *MIRNA* genes evolve in plants.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- 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
- Kim VN, Han J, Siomi MC (2009) Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* **10**: 126–139
- Kurihara Y, Watanabe Y (2004) Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. *Proc Natl Acad Sci USA* **101**: 12753–12758
- Leontis NB, Westhof E (2003) Analysis of RNA motifs. *Curr Opin Struct Biol* **13**: 300–308
- Voinnet O (2009) Origin, biogenesis, and activity of plant microRNAs. *Cell* **136**: 669–687
- Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* **31**: 3406–3415