

RESEARCH HIGHLIGHT

Dicer partners expand the repertoire of miRNA targets

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Abstract

Processing of pre-miRNAs by Dicer is regulated by its dsRNA-binding protein partner, and leads to the generation of alternative miRNA forms with distinct target sets.

Research highlight

Dicer's RNase III endoribonuclease activity is key to the biogenesis of small interfering RNAs (siRNAs) and microRNAs (miRNA) in the siRNA and miRNA pathways, respectively. But Dicer does not act alone in regulating small RNA biogenesis; indeed, its ribonuclease activity is known to be influenced by interactions with other proteins (for a review, see [1]). In *Drosophila*, Dicer's binding partners include two Loquacious (Loqs) protein isoforms, Loqs-PA and Loqs-PB, the depletion of which results in the accumulation of pre-miRNAs [2].

To clarify the role of each of the protein isoforms generated from the *loquacious* locus, Fukunaga *et al.* [3] generated flies that express various combinations of *loqs* transgenes, and investigated the resultant phenotypes and small RNA populations.

Loqs unlock Dicer

Central to Dicer's function are the two RNase III domains, which confer endoribonuclease activity, but its protein structure also includes a double-stranded RNA-binding domain (dsRBD), a Piwi-Argonaute-Zwille (PAZ) domain and a DExD helicase domain. Most Dicer proteins, in various species, have been shown to associate with other dsRBD-containing proteins, such as Loqs, that modulate the activity of Dicer's endoribonuclease domains.

In *Drosophila*, two separate Dicer enzymes, Dcr-1 and Dcr-2, with distinct dsRBD protein partners (see Figure 1a), participate in the miRNA and siRNA pathways. Dcr-1

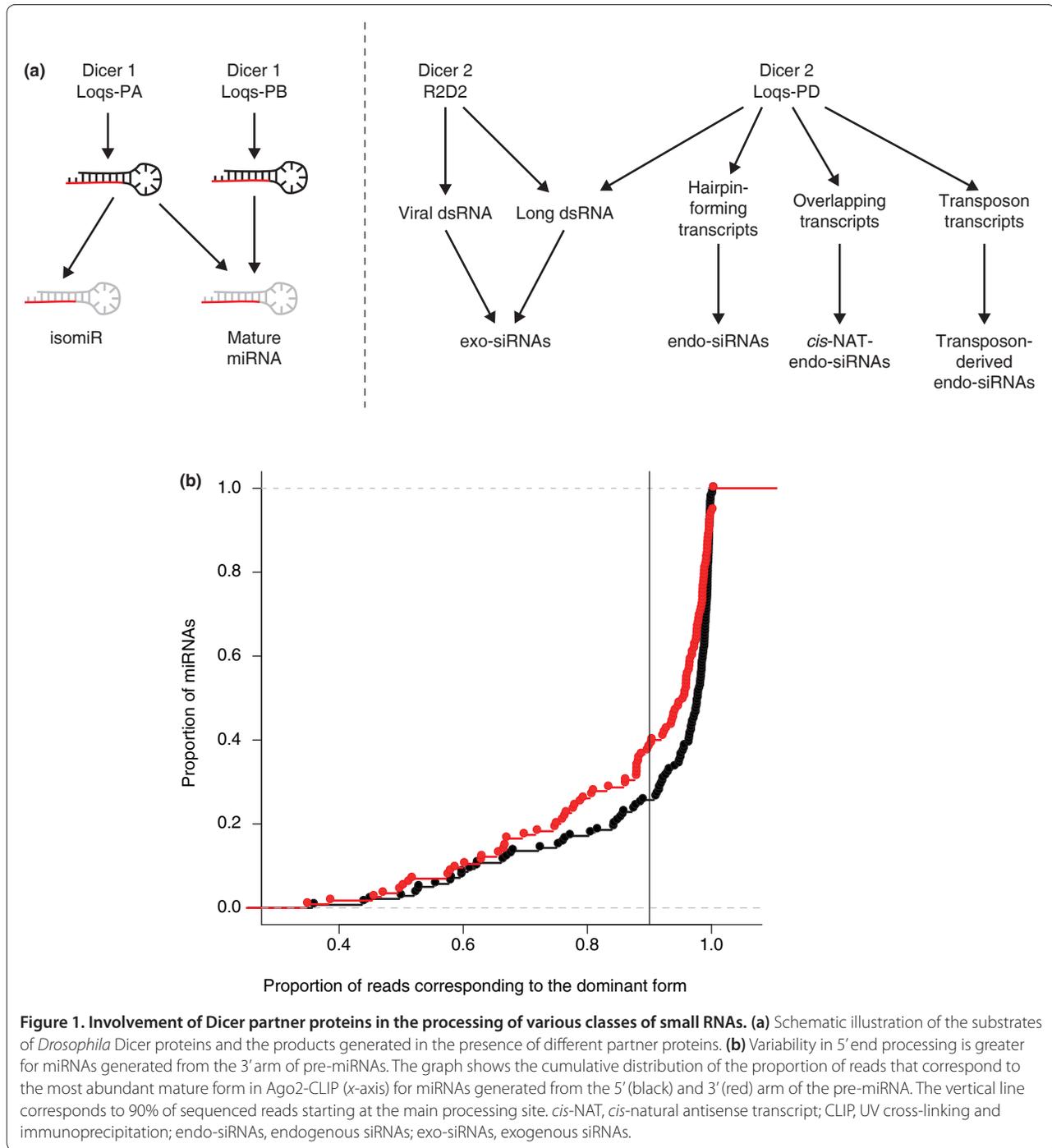
associates with the Loqs-PA and Loqs-PB isoforms of Loqs; association of these isoforms with Dcr-1 promotes cleavage of the enzyme's dsRNA substrate, but they are not required for the loading of mature miRNAs into the Argonaute 1 (Ago-1) protein. Dcr-2 interacts with the R2D2 dsRBD-domain-containing protein [4], which is required for siRNA production and, at the same time, suppresses Dcr-2 pre-miRNA processing activity. Dcr-2 and R2D2 form the loading complex that loads the guide siRNA strand into the Argonaute 2 (Ago-2) protein in a manner that is sensitive to the thermodynamic properties of the ends of the siRNA duplex [5]. Dcr-2 does not exclusively associate with R2D2. During the production of endogenous siRNAs (endo-siRNAs, also known as esiRNAs), Dcr-2 was found to be associated with a third splice variant of Loqs, Loqs-PD. This was initially found to be required for endo-siRNA production in cultured S2 cells [6], but Fukunaga *et al.* now show that it is also essential for processing of exogenous siRNA from long inverted repeats as well as for endo-siRNA accumulation *in vivo* [3].

Isoforms and isomiRs are key to Loqs function

To investigate isoform-specific functions of Loqs, Fukunaga *et al.* [3] used a transgene approach in *loqs* knockout (*loqs^{KO}*) mutant flies to restrict function to either Loqs-PA or Loqs-PB. Both of these isoforms were able to restore the embryonic lethality seen in *loqs^{KO}* mutant flies, a phenotype that itself underscored the role of miRNAs in fly embryonic development and differentiation. However, only Loqs-PB contributed to the maintenance of female germline stem cells, and the wild-type levels of some miRNAs in *loqs^{KO}* ovaries were specifically restored by Loqs-PB but not Loqs-PA. *In vitro*, both Loqs-PA and Loqs-PB decreased the K_m value of Dcr-1. However, Loqs-PB increased the reaction turnover rate, and thus changed the overall enzymatic kinetics, which may explain its ability to restore germline stem cell maintenance in mutant flies.

Interestingly, the role of Loqs-PB in pre-miRNA processing is not restricted to the increase in Dcr-1's substrate affinity and catalysis rate observed in the *in vitro* setting. Analysis of deep-sequencing reads from the

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Loqs-PA and Loqs-PB transgenic *loqs*^{KO} mutant flies revealed differences in mature miRNA lengths, with miR-307a producing a longer form, and miR-9 and miR-316 shorter forms, in the presence of Loqs-PB. miR-307a is derived from the 3' arm of its precursor, which means that its 5' end is defined by Dcr-1. Consequently, the miR-307a seed sequence, which largely defines the target specificity, is determined by the precise position of Dcr-1 cleavage.

In vitro experiments confirmed that Loqs-PB directly influenced the Dcr-1-dependent processing of pre-miR-307a: Dcr-1 alone and Dcr-1 with Loqs-PA produce mainly a 21 nt isomiR, whereas Dcr-1 supplemented with Loqs-PB generates additional 22 and 23 nt mature forms, of which the 23 nt form is canonical. As expected from their differing 5' ends, and consequently distinct seed sequences, synthetic luciferase reporter experiments showed that the 21 nt and 23 nt isomiRs had different

target specificities. Furthermore, in reporter systems and through mRNA sequencing, the authors demonstrated that the 23 nt miR-307a and not the 21 nt isoform suppresses the expression of two predicted targets, *glycerol kinase* and the Trithorax group transcript *taranis*. A more in-depth analysis of the mRNA-seq data generated in the study may reveal additional details about the differences in specificity between isomiRs.

Changing the Loqs has genome-wide implications

To investigate the potential effect of a Loqs-mediated switch between the 23 and 21 nt isoforms of fly miR-307a, we used the EIMMo miRNA target prediction method [7] to predict targets of these two isomiRs. As expected, the canonical 23 nt isoform is predicted to target a quite distinct set of mRNAs compared to the shorter variant that is produced in the absence of Loqs-PB. Gene Ontology analysis [8] of the top 50 predicted targets for the 23 nt isoform revealed mRNAs involved in wing disc development, sex differentiation, axonogenesis and post-embryonic appendage morphogenesis, whereas the 21 nt isoform, produced in the absence of Loqs-PB, was predicted to target genes involved in adult locomotory behavior, cell morphogenesis and chromatin remodeling.

One of the variables estimated by EIMMo is the probability that a match to a miRNA seed region in *Drosophila melanogaster* 3' UTRs is under selection in at least one other fly species. We obtained a higher estimate of this probability for the 23 nt isoform (0.25 compared to 0.1 for the 21 nt-long variant), suggesting that its targets tend to be more conserved.

Perspectives

Modulation of miRNA length and isomiR production by the dsRNA-binding partner of Dicer does not appear to be restricted to the case of Loqs in *Drosophila*. In mammals, the Dicer-interacting protein TRBP also affects the kinetics of the Dicer reaction. In contrast to Loqs, however, TRBP is not a general enhancer of Dicer activity; instead, its effect depends on the structure of the substrate, which determines whether TRBP stimulates or inhibits the efficiency of pre-miRNA processing relative to Dicer alone [9]. In common with Loqs, TRBP influences the position of pre-miRNA cleavage so that a longer isoform is produced, as has been shown in mouse cells *in vitro* [3,9].

However, it remains unclear just how extensive isomiR production is. Similarly, we do not know the extent to which isomiRs extend a miRNA's target repertoire. When considering how to answer these questions, we can make some estimates based on a set of 255 miRNAs obtained in Argonaute 2 cross-linking and immunoprecipitation experiments [10], and by taking into account that modulation of Dicer-dependent processing can only affect the

seed region of the miRNAs that are encoded in the 3' arm of pre-miRNAs. miRNAs encoded in the 3' arm of the pre-miRNA (45%) are generally less frequent than those encoded in the 5' arm. The fraction is even lower (34%) when one considers only the 50 most abundant miRNAs, but the variability in the 5' end processing of 3' arm miRNAs forms is generally higher than in the 5' arm cohort (see Figure 1b).

Some oncogenic miRNAs, such as miR-19a and miR-17, are either predominantly encoded in, or generate a substantial number of reads from, the 3' arms of their respective pre-miRNAs, which results in an isomer rate of between 3 and 7%. Interestingly, miR-17 is similar in its seed sequence to many other miRNAs that are expressed in an embryonic context (for example, the mir-430 family in zebrafish, the miR-290/295 cluster in mouse and the miR-302 family in human), but some of these variants differ in the presence or absence of a few nucleotides at the 5' end. Thus, alternatively processed variants of these miRNAs may have the same sequences, and hence act on the same sites, as canonical forms of other miRNAs.

Conclusions

Given that the relative abundance of isomiRs varies between different cells and tissues, it is clear that they are regulated post-transcriptionally, possibly at the level of Dicer processing. The developmental or tissue-specific regulation of Loqs, TRBP and other small dsRBD-domain-containing partners of Dicer has not yet been studied in detail, but appears to be an interesting avenue to pursue for those seeking to understand the complexity and evolution of the miRNA targetome.

Abbreviations

Dcr, Dicer; dsRBD, double-stranded RNA-binding domain; dsRNA, double-stranded RNA; endo-siRNA, endogenous siRNA; K_{m} , Michaelis-Menten equilibrium constant; Loqs, Loquacious; miRNA, microRNA; nt, nucleotide; PAZ, Piwi-Argonaute-Zwille domain; siRNA, small interfering RNA; TRBP, trans-activation response RNA-binding Protein.

Acknowledgements

We apologize for having to omit many relevant references due to space limitations. Work in the laboratory of MZ is supported by the Swiss National Foundation (grant #31003A_127307).

Published: 29 November 2012

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doi:10.1186/gb-2012-13-11-179

Cite this article as: Jaskiewicz L, Zavolan M: **Dicer partners expand the repertoire of miRNA targets.** *Genome Biology* 2012, **13**:179.