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Before they become associated with Argonaute (AGO) proteins to exert their regulatory effects, microRNAs (miRNAs) exist in a duplex with a complementary miRNA* strand. Until recently, only the miRNA strand was thought to be sorted to AGO, and miRNA*s were thought to be degraded as non-functional by-products of miRNA metabolism. However, three new studies indicate that miRNA*s are abundant, actively sorted into AGO and might have a regulatory function in *Drosophila melanogaster*.

Okamura *et al.*, Czech *et al.* and Ghildiyal *et al.* found that, in contrast to miRNAs, miRNA*s are commonly modified by 2′-*O*-methylation at their 3′ ends. Small interfering RNAs (siRNAs) that bind to AGO2 to mediate silencing are also modified at their 3′ termini. The authors showed that unlike their partner miRNA strands, which are preferentially incorporated into AGO1, miRNA*s strongly associate with AGO2.

Are siRNA factors required for the accumulation and loading of miRNA*s into AGO? Okamura *et al.* and Czech *et al.* used RNAi to knock down canonical miRNA and siRNA factors in S2 cells, and found that miRNA*s were depleted from AGO2 after knockdown of the miRNA factor Dicer 1 (DCR1), as well as after knockdown of the siRNA factor DCR2 and its partner R2D2. Similarly, Ghildiyal *et al.* found that DCR2 and R2D2 were required *in vivo* in adult flies to load miRNA* into AGO2. This suggested that the accumulation and AGO-loading of miRNA*s is dependent on a unique combination of canonical miRNA and siRNA factors.

To investigate potential functions of miRNA*s, Czech *et al.* created miRNA and miRNA* 'sensor constructs' and expressed them in S2 cells in which miRNA and siRNA pathway components had been knocked down. Depletion of the canonical miRNA factor Drosha led to de-repression of the miRNA sensors; however, it also caused de-repression of the miRNA* sensors, which suggests that miRNA*s can repress mRNA targets. Depletion of DCR1 also led to the de-repression of both types of sensors. Consistent with miRNA* loading into AGO2, the miRNA* sensors were de-repressed when AGO2 was depleted, and this was dependent on DCR2 and R2D2. Using clonal analyses in transgenic sensor flies, they also showed that miRNA*s can repress targets *in vivo*.

It is unlikely that both strands of an miRNA duplex simultaneously interact with both AGOs, as Czech *et al.* also found that depletion of either AGO protein leads to an increase in the miRNA strands associated with the other AGO. Moreover, Ghildiyal and colleagues identified miRNA duplexes for which both strands are sorted to only one AGO. These data suggest that miRNA duplexes are independently sorted depending on whether they are viewed from the perspective of the miRNA or miRNA* strand. The three papers highlight a set of key sequence and structural features that determine AGO sorting of the miRNA duplex: sorting to AGO2 is associated with a higher degree of overall base pairing in the duplex, particularly with base pairing at nucleotides 9 and 10 from the 5′ end, whereas the presence of a 5′ U seems to be important for AGO1 sorting.

The finding that both strands of the miRNA duplex are actively sorted and can silence target mRNAs potentially broadens the regulatory influence of these RNA species.

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