SMALL RNAS

Dispensable Dicer

> a conserved miRNA biogenesis pathway that requires AGO2 catalysis

Argonaute (Ago) proteins are effector enzymes in small RNA-mediated gene regulation with conserved catalytic centres. Yet, animal microRNAs (miRNAs) regulate gene expression without the need for Ago-mediated cleavage, so it is puzzling why mammalian AGO2 has retained endonuclease activity. The groups of Hannon and Giraldez now identify a conserved miRNA biogenesis pathway that requires AGO2 catalysis but is independent of the miRNA processing enzyme Dicer.

Cheloufi *et al.* engineered mice with catalytically inactive *Ago2* alleles and found that homozygous mutants suffered anaemia and died shortly after birth. Interestingly, the requirement of AGO2 during embryogenesis seems to depend solely on the expression of AGO2 in the placenta. These results suggest that miRNA-directed target cleavage might be important for postnatal erythrocyte maturation. Indeed, deep sequencing of miRNAs in liver tissue from wild-type animals and AGO2 mutants showed that the level of miR-451 was reduced in mutant fetal liver tissue.

Precursor miR-451 (pre-miR-451) and mature miR-451 have an unusual secondary structure that seems incompatible with processing by the canonical miRNA processing enzyme Dicer. Whereas pre-miR-451 is normally excised by Drosha from its primary transcript (pri-miR-451), the unusually short 17-nucleotide stem region of pre-miR-451 is thought to be too short to be recognized by Dicer. Indeed, expression of pri-miR-451 in embryonic stem cells that are homozygous for Dicer conditional alleles did not cause a change in the levels of miR-451. However, the authors noticed an accumulation of the Drosha cleavage product in AGO2-mutant liver tissue compared with wild-type tissue, suggesting that catalytically active AGO2 is required for cleaving pre-miR-451, as was subsequently confirmed in an *in vitro* assay.

Taking a somewhat different approach, Cifuentes *et al.* set out to identify pathways that might process miRNAs in a Dicer-independent manner by sequencing small RNAs from zebrafish wild-type embryos and Dicer mutants. Several miRNAs, including miR-451, remained unaffected by Dicer loss of function. The authors noticed miR-451's unusual secondary structure, and that small RNAs were derived from a cleavage in the middle of the stem that was incompatible with Dicer function. Instead, this cleavage was reminiscent of the sequence-specific cleavage or 'slicing' by Ago2 in the passenger strand (that is, the strand to be degraded) in small interfering RNAs. To investigate the hypothesis that the catalytic activity of Ago2 might participate in miRNA biogenesis, Cifuentes *et al.* created an Ago2-null zebrafish mutant and showed that the level of miR-451 in mutant embryos was significantly reduced, whereas the level of other miRNAs was unaffected. Erythrocyte maturation was impaired in Ago2 mutants, and this effect could be rescued by providing wild-type Ago2 or mature miR-451.

Both teams engineered a structural mimic of pre-miR-451 that was processed independently of Dicer, suggesting that the secondary structure of the pre-miRNA determines whether it is processed in a Dicer-independent manner. In addition, both teams provide evidence that AGO2-cleaved pre-miRNA intermediates undergo polyuridylation and are subsequently trimmed by a nuclease to generate mature miRNAs — but the factors responsible remain unknown.

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ORIGINAL RESEARCH PAPERS Cheloufi, S. *et al.* A Dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature* **465**, 584–589 (2010) | Cifuentes, D. *et al.* A novel miRNA processing pathway independent of Dicer requires Argonaute2 catalytic activity. *Science* 6 May 2010 (doi:10.1126/science.1190809)
FURTHER READING Hutvagner, G. & Simard, M. J. Argonaute proteins: key players in RNA silencing. *Nature Rev. Mol. Cell Biol.* **9**, 22–32 (2008)