The biology of microRNAs in animals

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Introduction

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- regulate specific mRNAs with sequence complementarity to the microRNA;
- many (10's to 100's) of predicted targets for each microRNA, many (100's) microRNAs.

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2000: *let-7*: a second small RNA (21 nt long), implicated in worm development.

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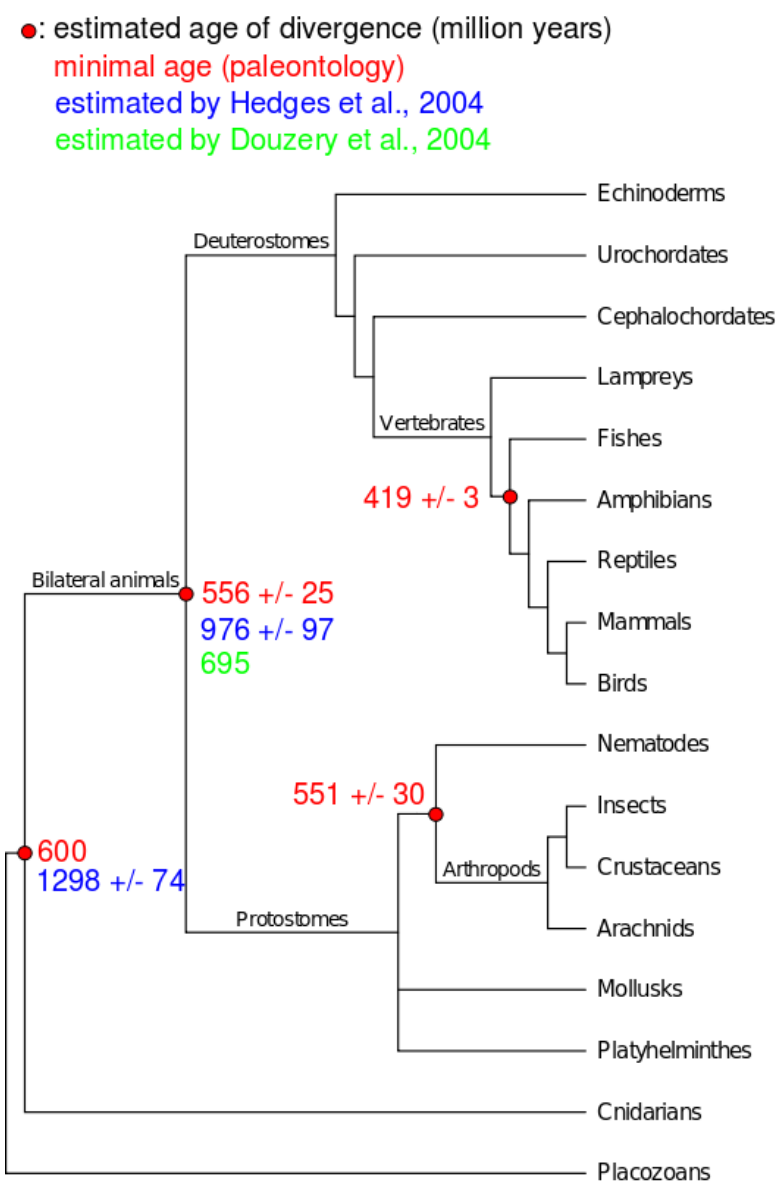

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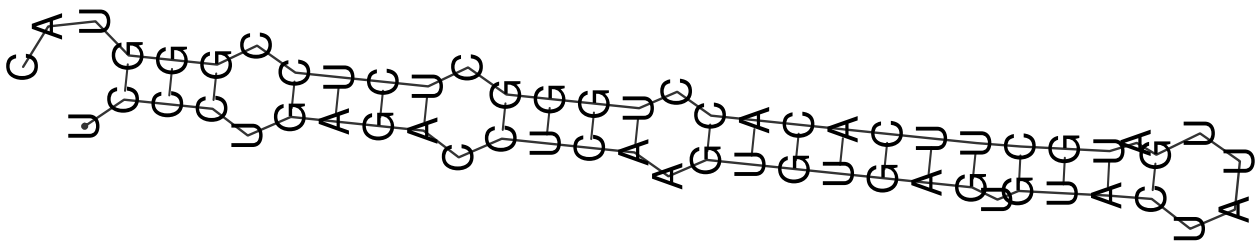

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Since then: in metazoans, metaphytes, *Chlamydomonas*, viruses.

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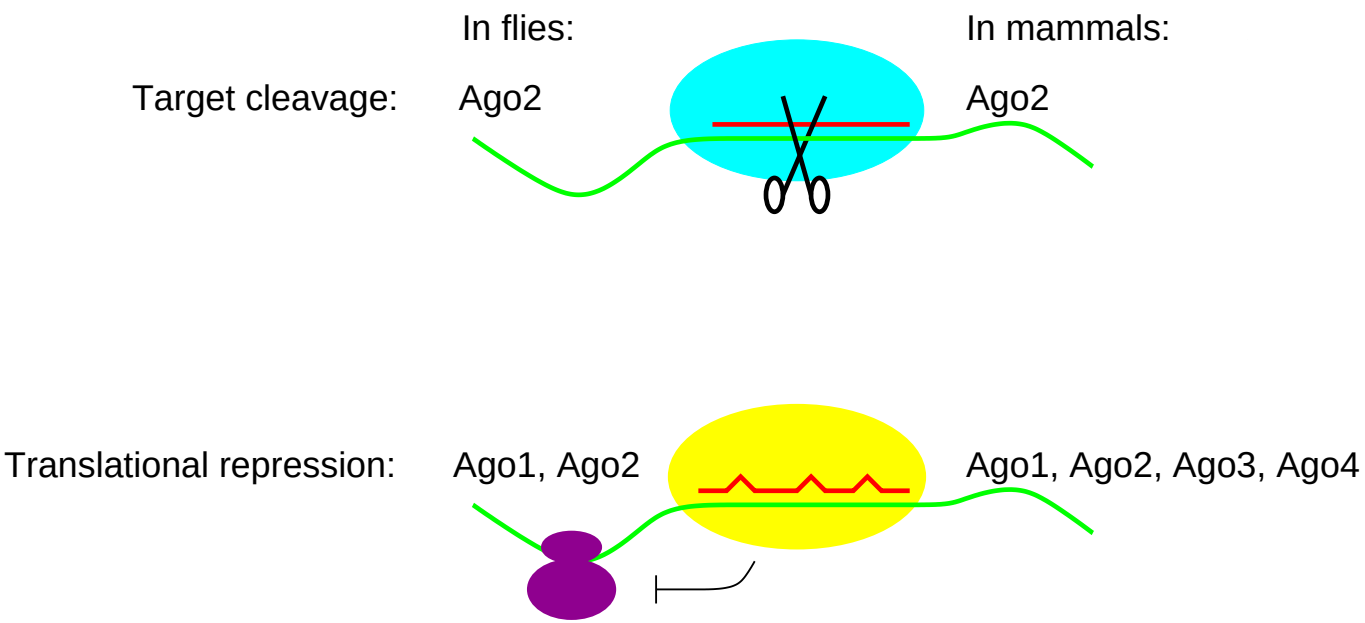

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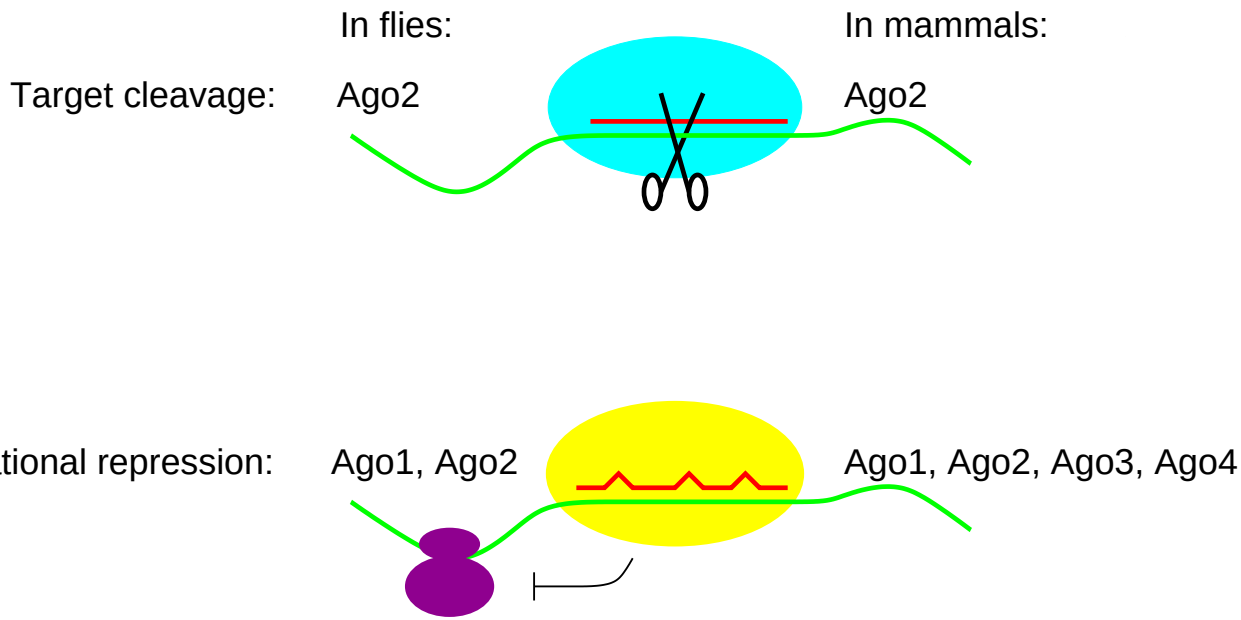

Definition of a functional imperfect complementarity?

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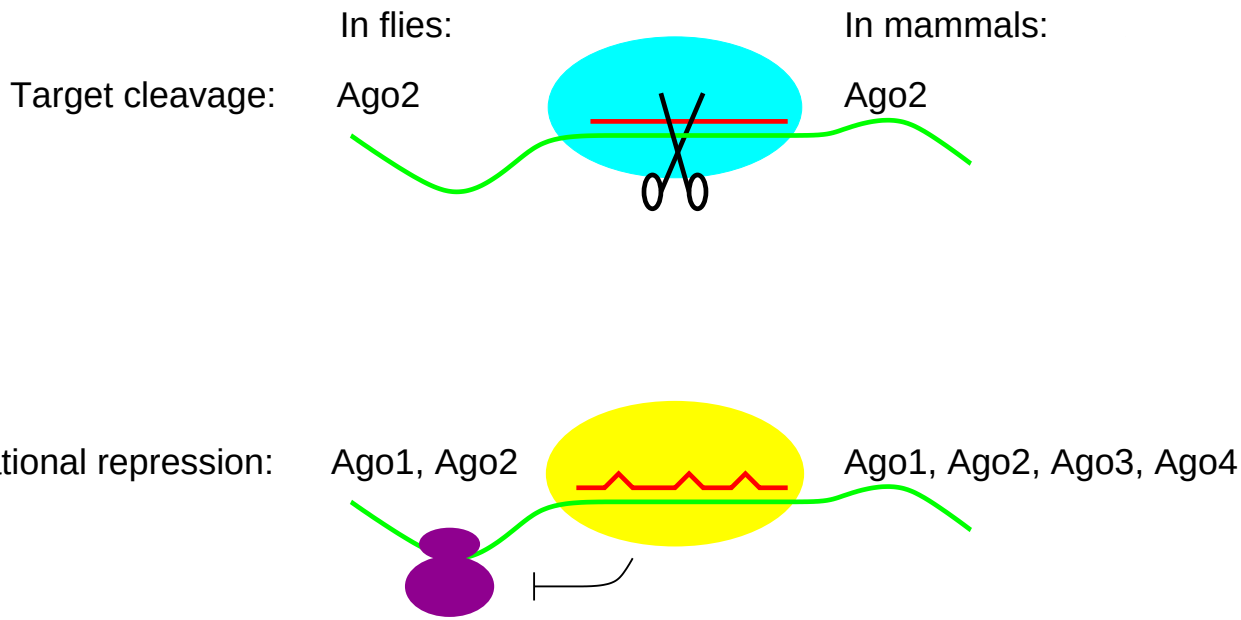

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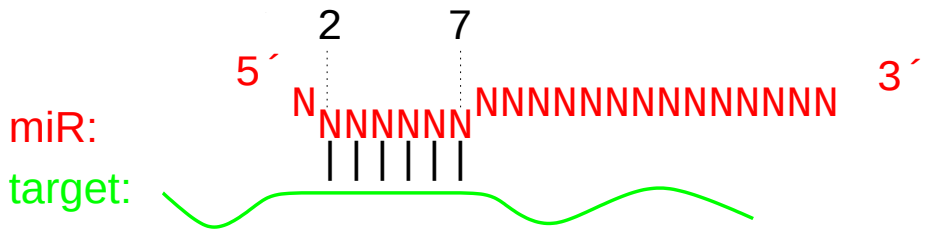

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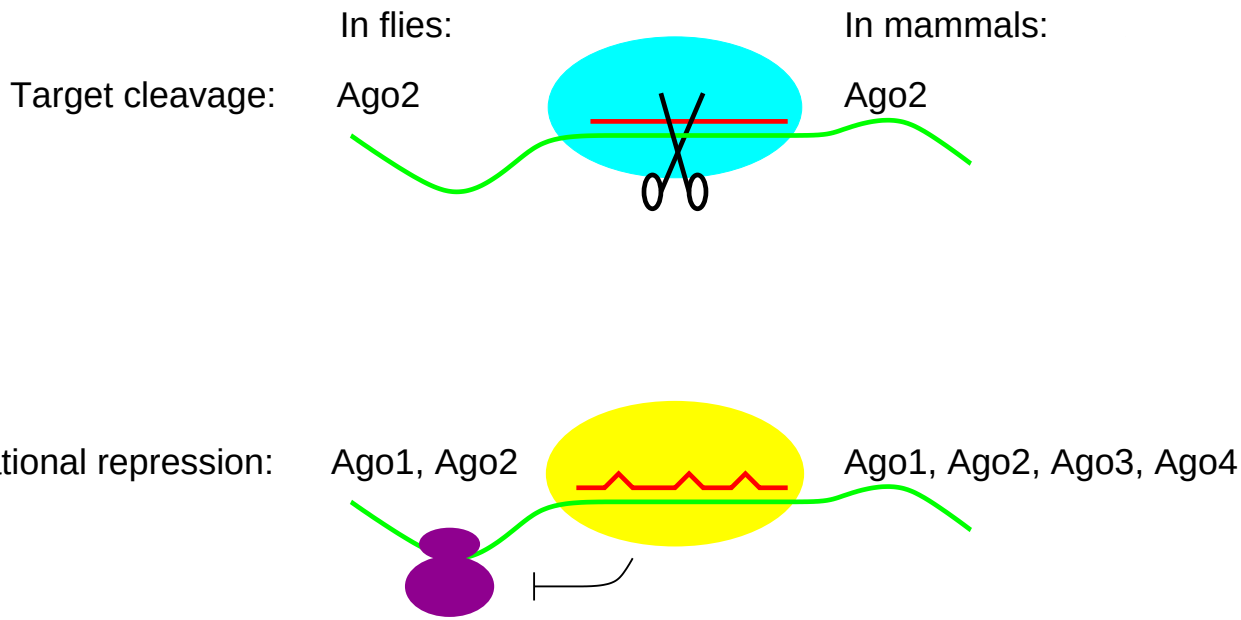

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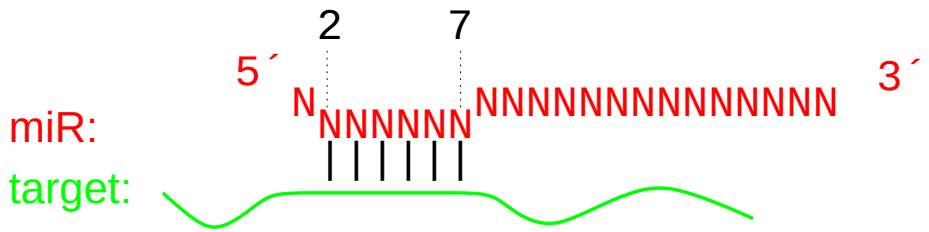

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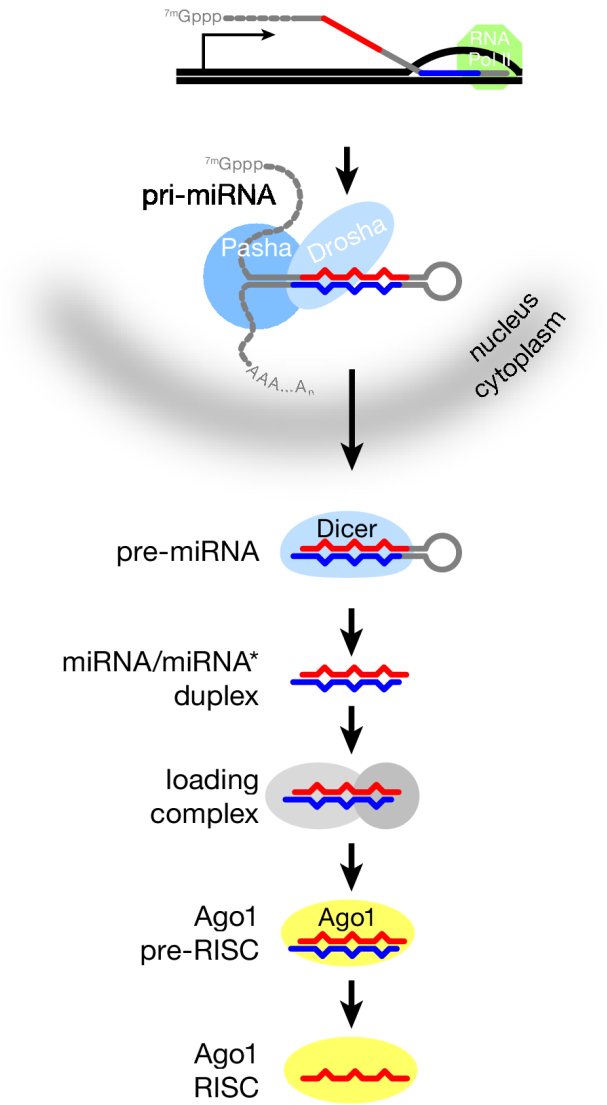
Location of miRNA binding sites: 3´ UTRs? Not that clear!

MicroRNA biogenesis



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  ▸ Asymmetry rule
- Mechanism for target cleavage? For translational repression? ▸ Mechanism

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MiRNAs seem to be implicated in every physiological process in animals.

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Non-conserved miRNAs: prevalence? role in speciation?

Physiological impact of miRNA-mediated regulation?

Supplementary data

- Slicer identification
- The asymmetry rule
- Repression mechanism
- pri-miRNAs
- miRNA identification

The Argonaute family

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- I.P. against several Ago's
- Mouse *Ago2* mutant
- Crystallographic structure (Piwi domain $\approx$ RNase H domain)
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$\Longrightarrow$ Ago2 is the mammalian Slicer

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Other Ago's: other roles ? (translational repression ?);
Drosophila: both Ago1 and Ago2 can cleave, but only Ago2 in physiological conditions.

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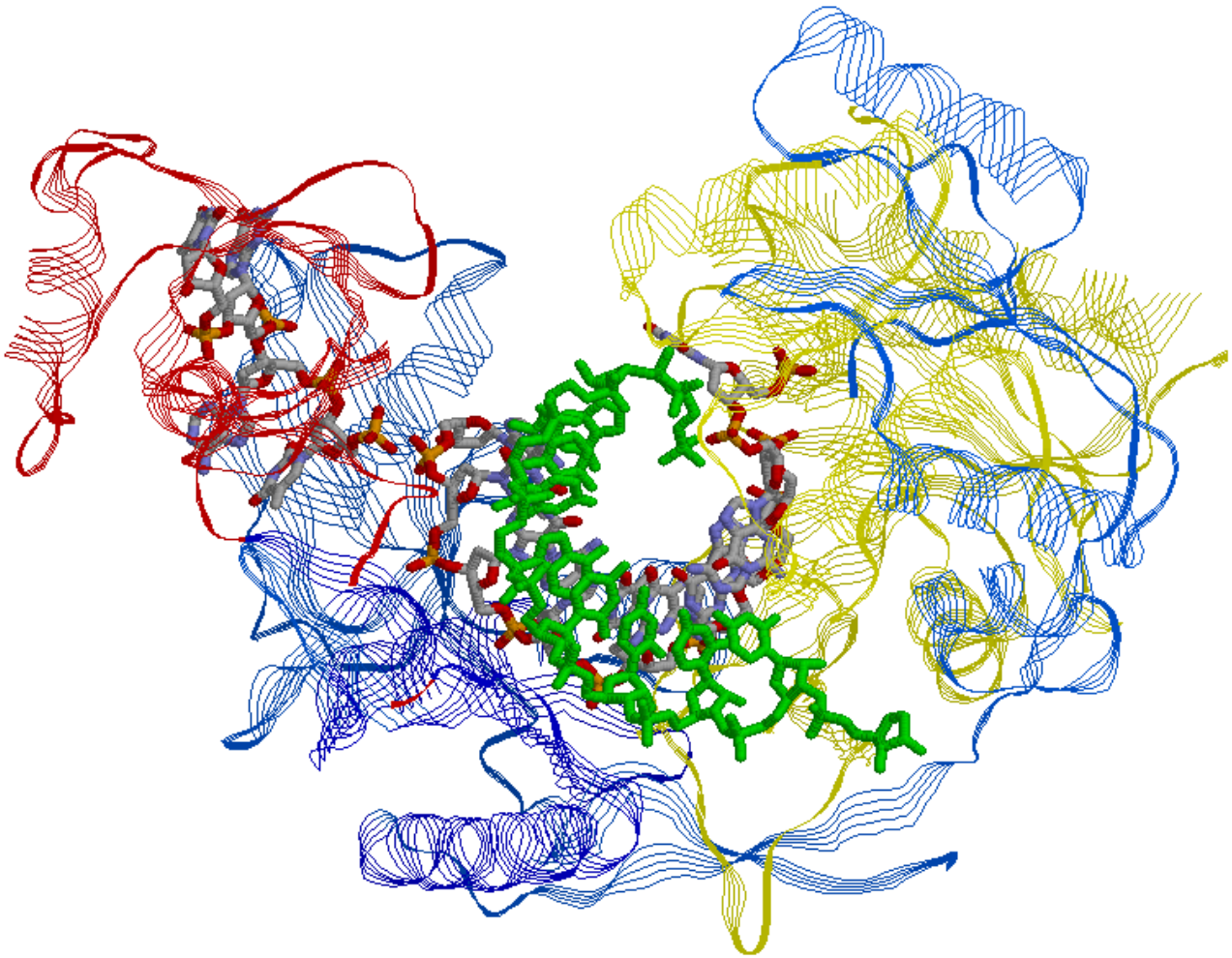

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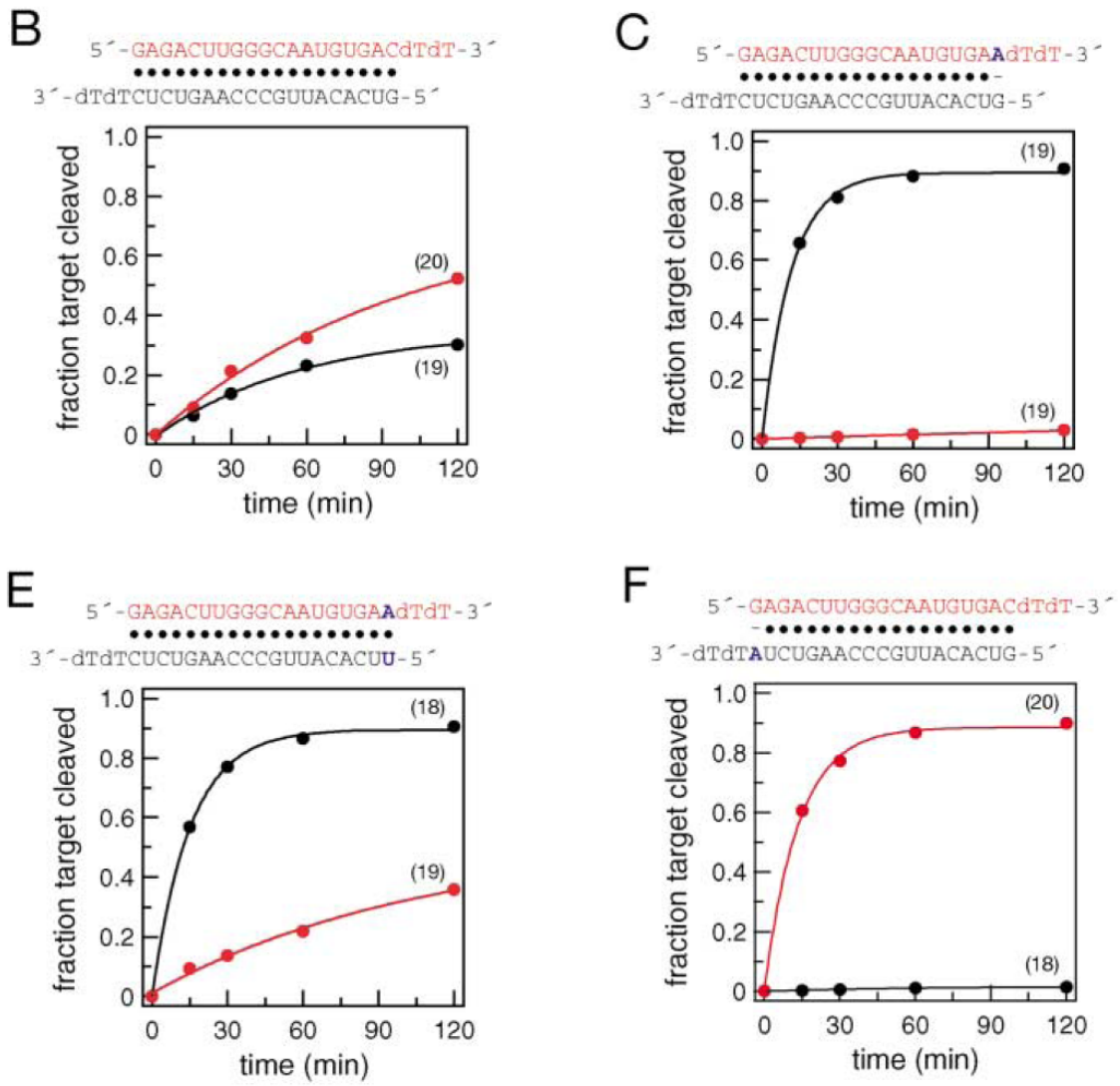

(Schwarz *et al.*, 2003)

The asymmetry rule

Also valid for miRNAs.

▸ Biogenesis

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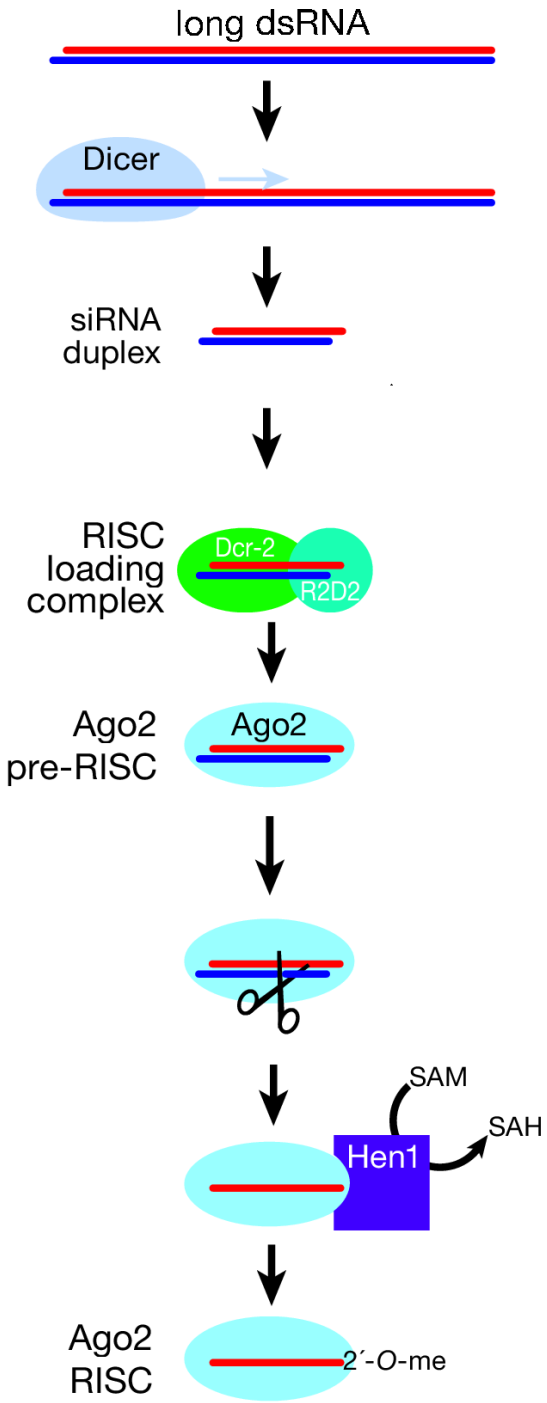
For siRNAs, the asymmetry sensor is the heterodimer Dcr-2/R2D2.



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$\longrightarrow$ Several mechanisms ? One determinant: sequence and structure of the duplex between the small RNA and its target (Alemán *et al.*, 2007).

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Intronic miRNA maturation does not compete with host mRNA splicing.

miRNA identification

Great diversity (17 341 known miRNAs: miRBase 16.0)

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- Computational approach: prediction of secondary structure of candidate pre-miRNAs; screening (stem-loop stability, conservation)

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Each approach has its limitations:

- Genetics: random, very rare.
- Cloning: abundance (low expression, spatially or temporally restricted).
- Computation: choice of selection criteria, sometimes arbitrary (stem-loop stability, phylogenetic conservation), does not identify the extremities of the mature miRNA.

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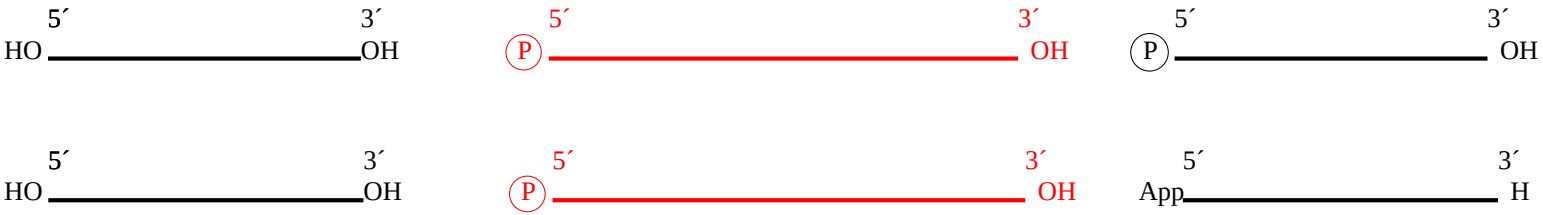

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