

miRNA activity: the role of AGO2

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30/11/2015

Three classes of small RNAs

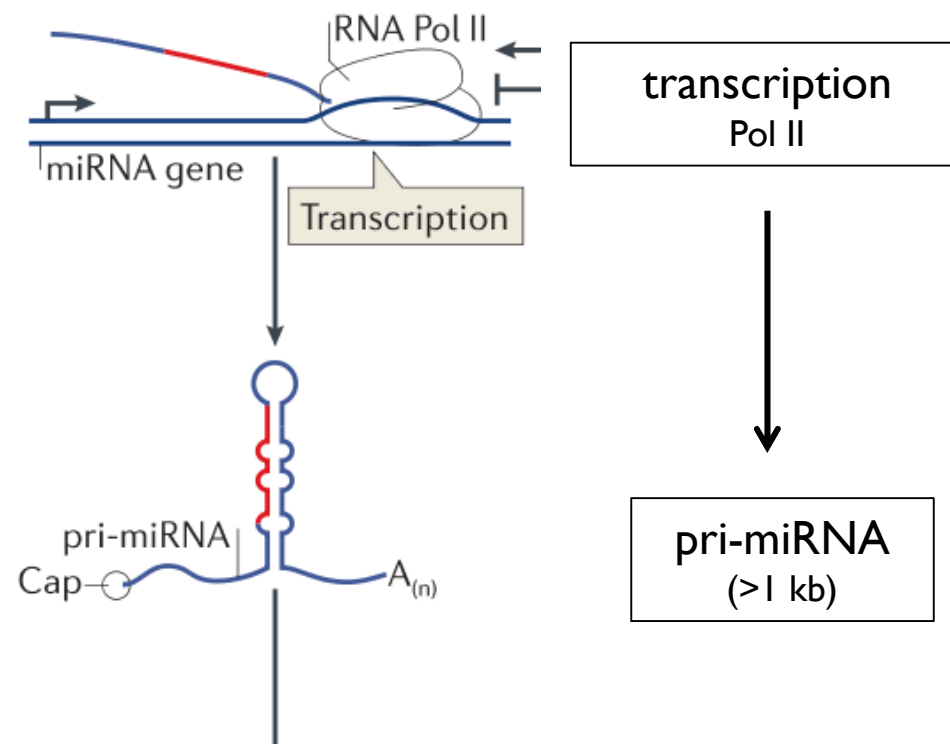
Features	miRNA	siRNA	piRNA
Length	~22 nucleotides	~21 nucleotides	24–30 nucleotides
Processing enzymes	Drosha and Dicer	Dicer	Zucchini and unknown trimming enzymes
AGO subclades	AGO	AGO	PIWI
Mechanism of action	<ul style="list-style-type: none"> • Translational repression • mRNA degradation 	RNA cleavage	<ul style="list-style-type: none"> • Transcriptional or post-transcriptional repression of transposons • Multigenerational epigenetic phenomena in worms
Function	Regulation of protein-coding genes	<ul style="list-style-type: none"> • Regulation of protein-coding genes and transposons • Antiviral defence 	<ul style="list-style-type: none"> • Pre-pachytene piRNA: transposon silencing • Pachytene piRNA: unknown • piRNA-like small RNA in soma: unknown

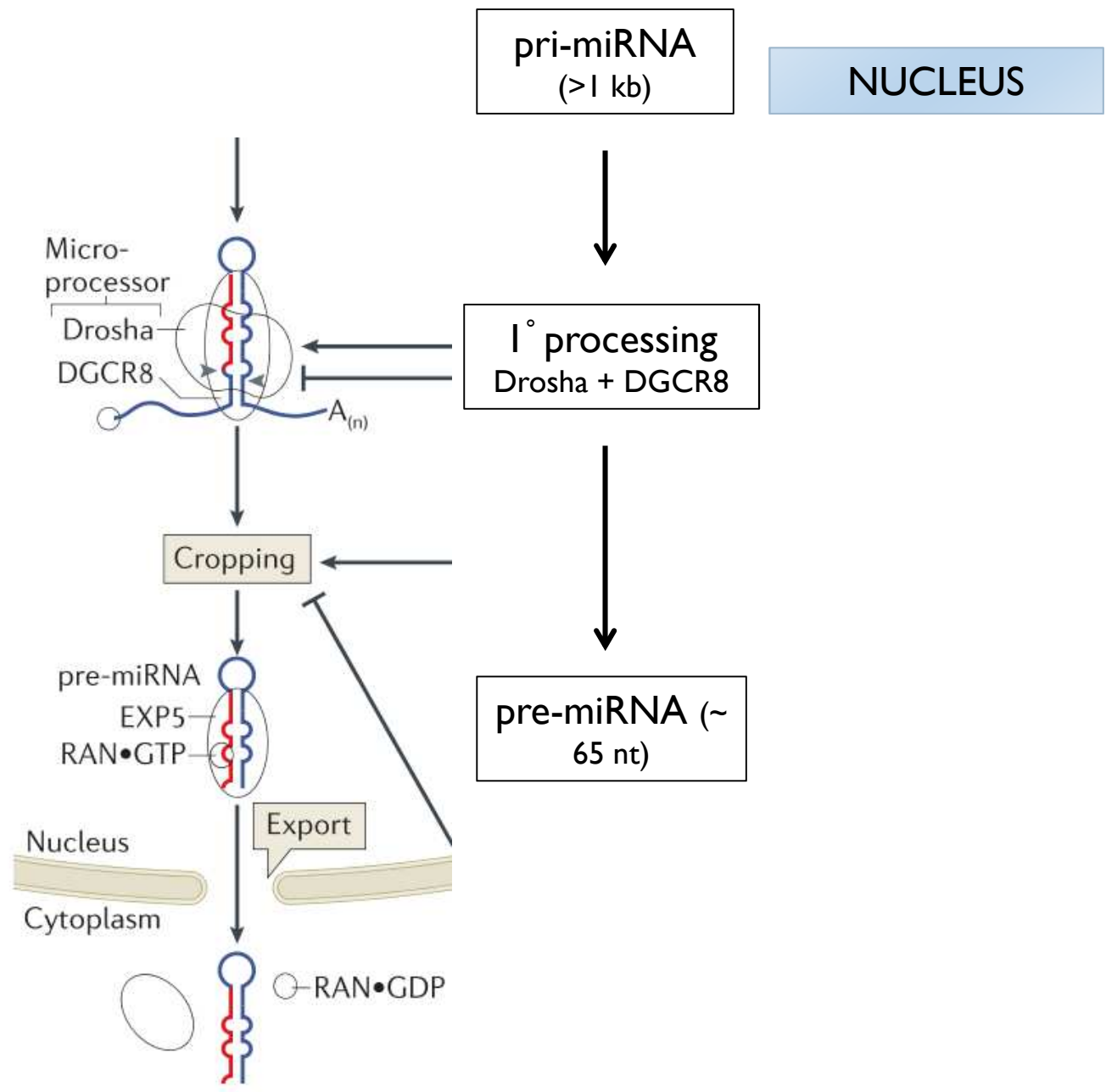
siRNA = small interfering RNA → RNA interference (RNAi)

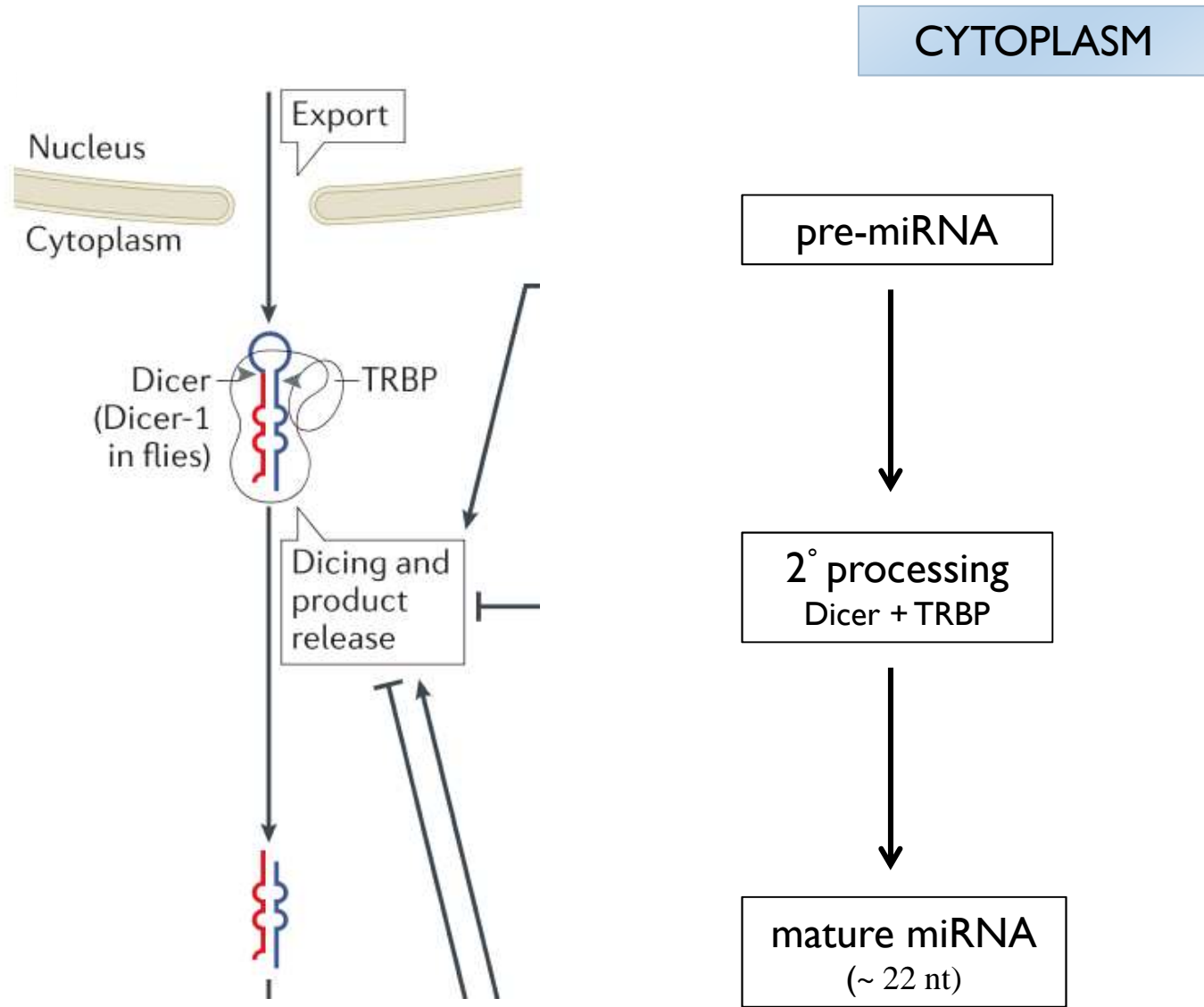


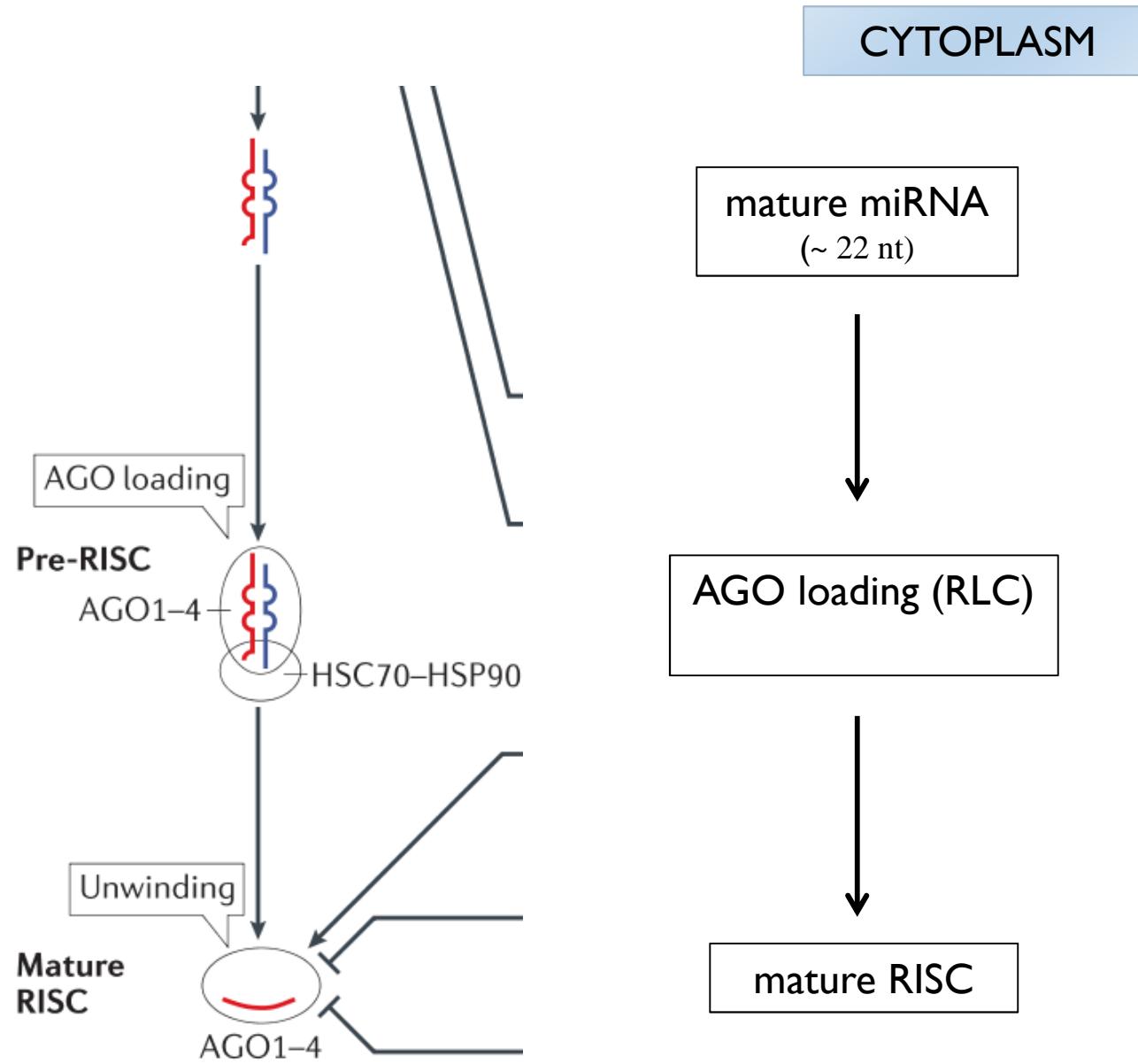
miRNA biogenesis

NUCLEUS









RNA-induced silencing complex (RISC)

RISC is a ribonucleoprotein complex that consists of a small RNA guide strand bound to an Argonaute protein (AGO).

AGO proteins are ubiquitously expressed and associate with miRNAs or siRNAs.

There are four AGO subclade proteins in humans (AGO1-4). Only AGO2 can slice perfectly matched target mRNAs.

Either cleavage of the target or translation repression are depending on the degree of complementarity between guide and target strands.

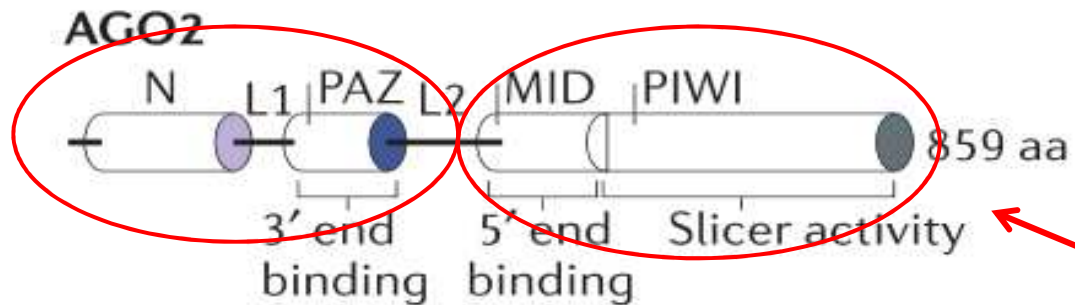
Perfect complementarity -> cleavage (slicing).

imperfect targets -> repression of mRNA translation.



Argonaute 2 (AGO2)

AGOs adopt a bilobal architecture.



N-terminal lobe

(~150 residues)

PAZ domain binds the 3' end of the guide by interactions with the nucleotides 20 and 21.

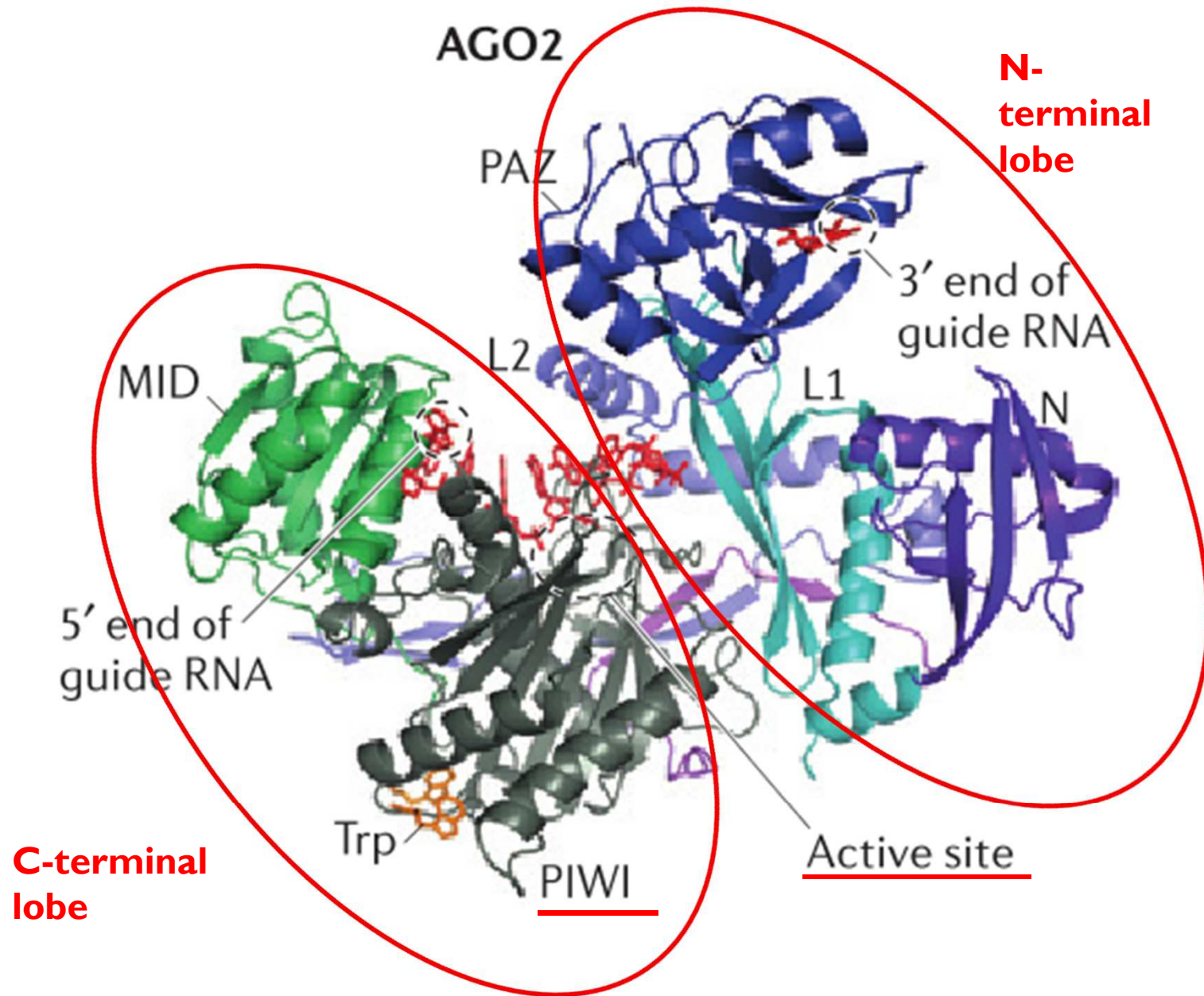
C-terminal lobe

(~400 residues)

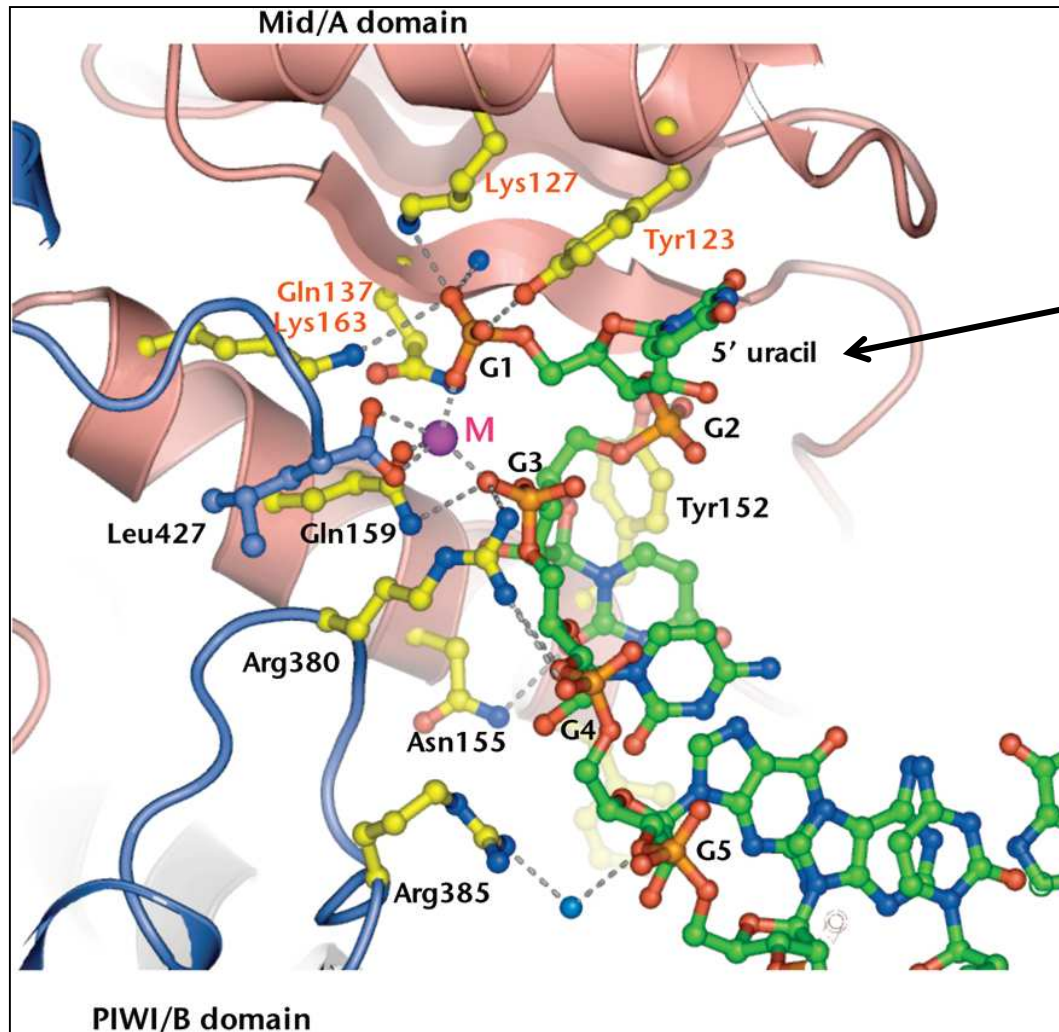
PIWI domain includes the RNase H-like active site

MID domain binds the 5' end of the guide

Both the GI-binding pocket and RNase H-like catalytic site are conserved throughout evolution.



5' phosphate binding pocket



M = Mg²⁺ or Mn²⁺

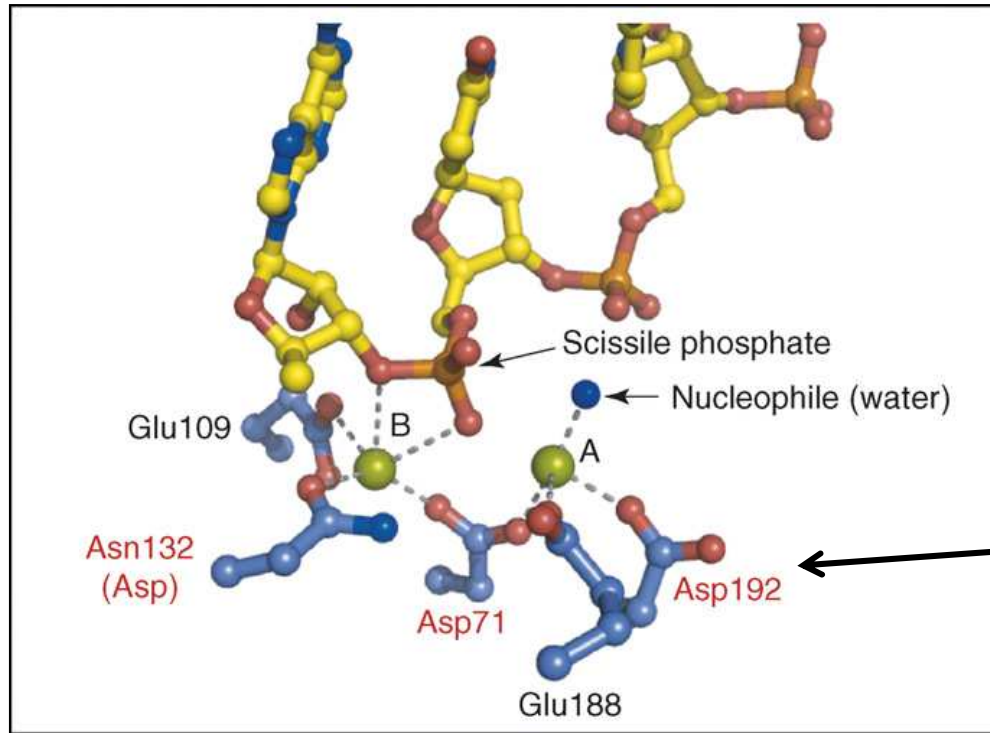
preference for U or A

The 5' phosphate group is important for the stability of the complex.

The 5' phosphate is important for the fidelity of the position of mRNA cleavage.

RNase H catalytic site – a model for Slicer

The trajectory of the RNA directs the target strand into the RNase H-like catalytic site, placing the scissile phosphate, in close proximity to the putative catalytic site (T10 and T11 interacting with G10 and G11).



DDX motif (X it is usually aspartic acid or histidine).

A, B = metal ions

Structure of *Bacillus halodurans*

Mutations of either catalytic aspartic acid in AGO2 abolishes Slicer activity.

Target strand recognition

The “**seed**” **sequence** is sufficient for target recognition (**nucleotides 2-7** of miRNA).

These nucleotides expose the bases for a Watson-Crick interaction with the target strand.

The seed of guide miRNA is pre-arranged in an A-form helix conformation that facilitates efficient scanning of target mRNAs for complementary sequences.

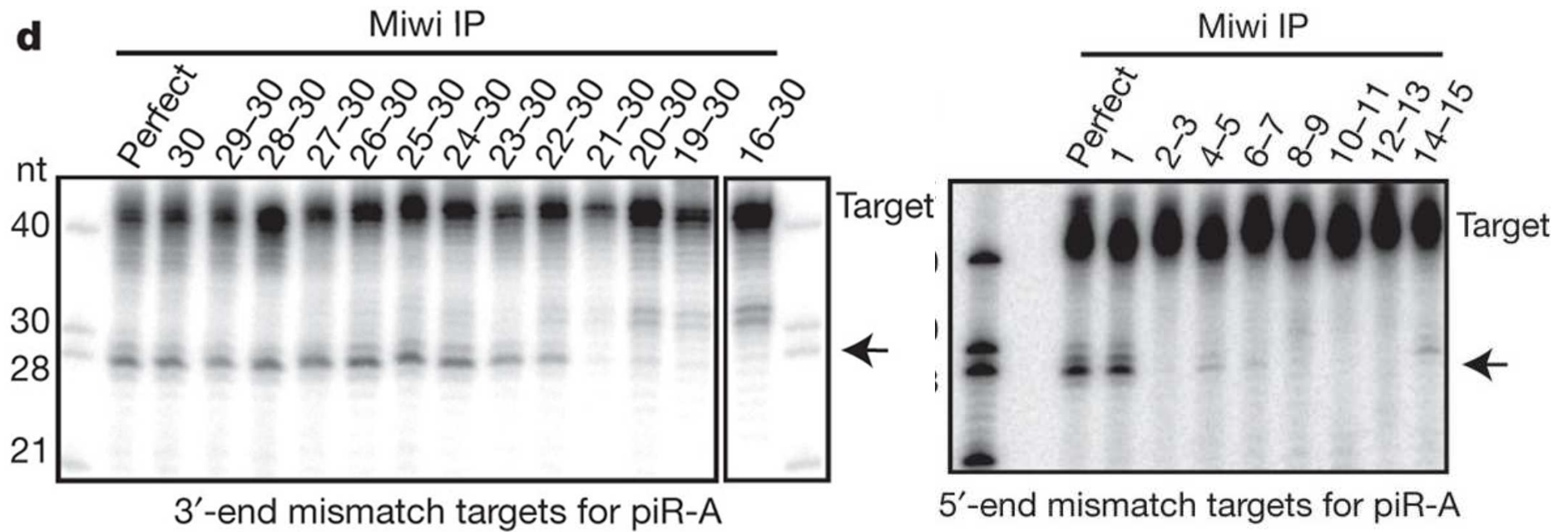
Cleaving of target mRNAs between nucleotide positions 10 and 11 (relative to the 5' end of the guide RNA).





30/11/2015

Miwi is a small RNA-guided RNase (slicer) that requires extensive complementarity for target cleavage in vitro. .



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