

The Hitch Hiker's Guide to

the interfering RNA Galaxy

# DON'T PANIC

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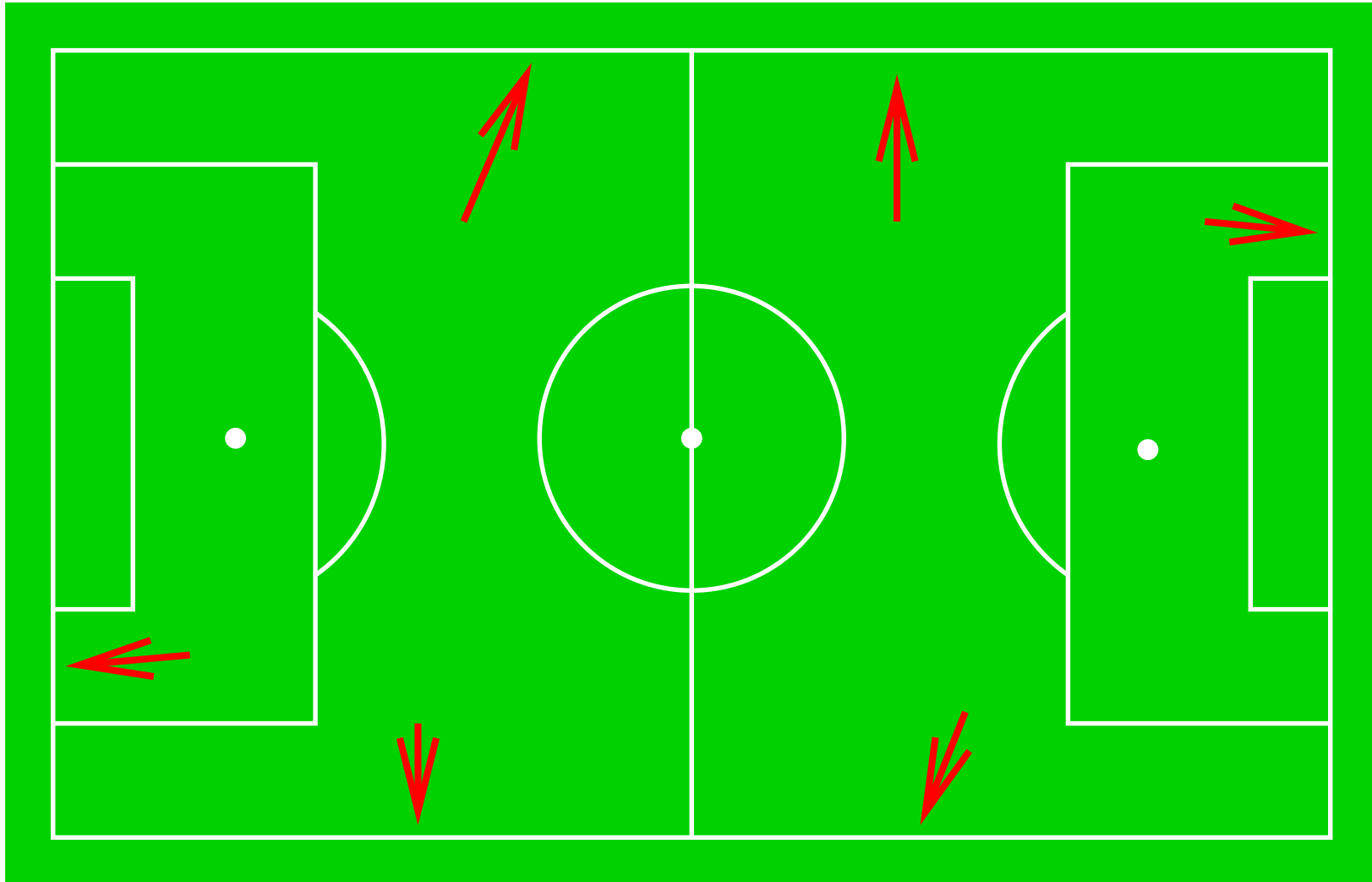
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Bled, February 04

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# Outline



## Outline

interfering RNA - What is dat?

Brief history of RNA interference.

What does it do?

How does it work?

## What is RNA interference?

Recently discovered regulatory level.

Genome immune system.

Caused by short ( $\approx 22$ nt) non-coding RNA molecules.

Two discriminated molecule types, siRNA and miRNA.

Almost identical pathways for miRNA and siRNA.

Post-transcriptional regulation.

Degradation of mRNA or translational inhibition.

Chromatin silencing.

## History of RNA interference.

	miRNA	siRNA
1990		PTGS in plants: Napoli, van der Krol
1992		quelling in fungi: Romano
1993	lin4 in C.elegans: Lee	
1998		RNAi in C.elegans: Fire,Mello
1999		RNA in PTGS: Hamilton and Baulcombe
2000	let7 in C.elegans: Reinhart	
2000	stRNAs: Reinhart	
2000	Conservation of let7:Pasquinelli	
2001		RNAi phrase coined by Elbashir et. al.
2001	about 100 miRNAs found: Tuschl, Ambros, Bartel labs	
2001	Dicer identified:	Bernstein
2002	Breakthrough of the year	in science magazine
now	130 publications	900 publications

## History of RNA interference

RNAi is now widely used by geneticists for gene knock out.

Mechanisms are still poorly understood.

Computational methods for identification of miRNAs, their targets as well as for siRNA design are still in their infancy.

Recently, indications of unspecific attenuation effects connected to RNAi have been described.

## Function of siRNA

Thought to be the genomes immune system.

Plants PTGS protects against viral infection.

Endogenous or exogenous dsRNA.

Nematodes show activation of multiple transposable elements when function of RNAi is lost.

siRNA has a systemic effect in nematodes and plants.

Amplification of response by RNA-directed RNA polymerase, siRNA acts as a primer, e.g. in plants, nematodes.

Amplification mostly is 5' to 3' unidirectional, bidirectional occurs in plants.

(Somewhat longer) siRNAs can also lead to epigenetic effects through chromatin silencing.

Mathematical model

$$D'_i(t) = -aD_i(t) + gC_i(t)$$

$$R'_i(t) = a \sum_{j=i}^n D_j(t) - d_R R_i(t) - bR_i(t)M(t)$$

$$C'_i(t) = bR_i(t)M(t) - (g + d_C)C_i(t)$$

$$M'(t) = h - d_M M(t) - b \sum_{j=1}^n R_j(t)M(t)$$



## Artificial RNA interference

Is used to induce gene knock out in plants, fungi and animals. dsRNA can be introduced by injecting it directly or using vectors.

Long dsRNAs induce unspecific interferon reactions in mammals.

Using short siRNA has been shown to be effective while not triggering interferon response in mammals.

Recently, off-target gene regulation has been reported.

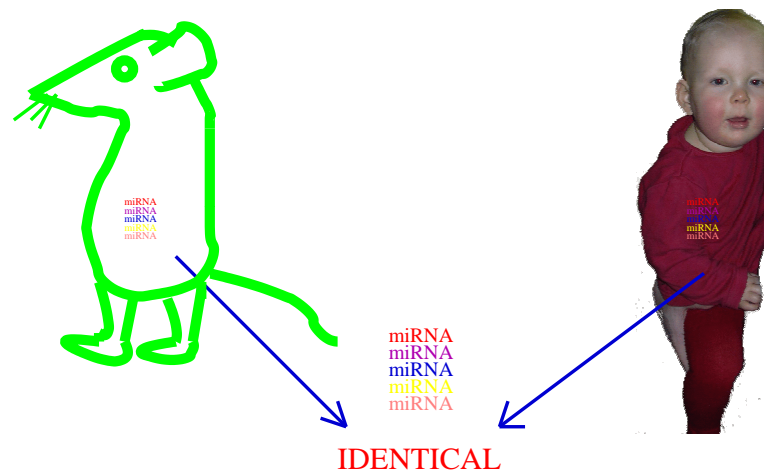
Effect varies between different siRNAs, but is reproducibly equal for same siRNA.

## Function of miRNA

Found in animals, plants and fungi.

Distinct miRNA genes exist.

Highly conserved in closely related species, almost 100% of the  $\approx 250$  human miRNA also found in mouse



## Function of miRNA

miRNA is often used in development and cell differentiation. 66% of *Drosophila* miRNA targets are transcription factors. Binds mostly to 3' UTR of mRNA, but maybe to coding region and 5' UTR as well.

miRNAs play an important role in chromatin reorganization.

At least one miRNA can be found in the Hox cluster.

Surely it must have something to do with Hyaluronan.

Possibly derived from siRNA pathway (although Peter seems to disagree...).

Is proposed to be the answer to the regulatory catastrophe (although Peter says there is no such thing...).

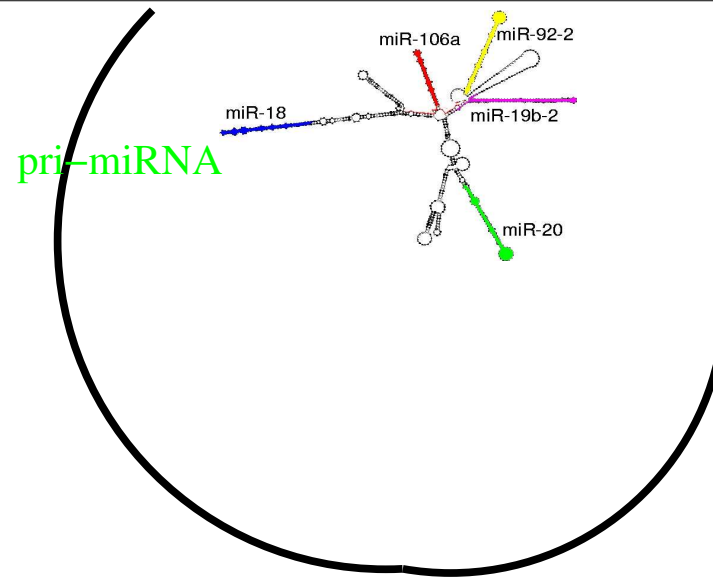
## Differences between miRNA and siRNA

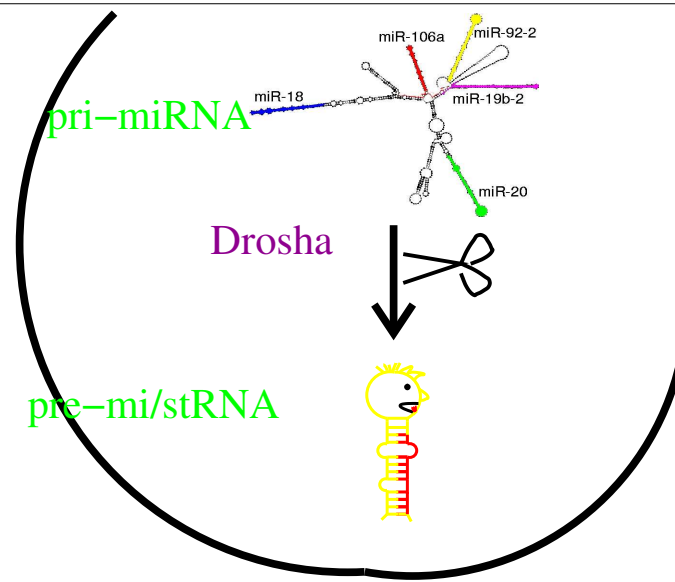
miRNAs are excised from endogenous transcript having the ability to form stable, hairpin structures.

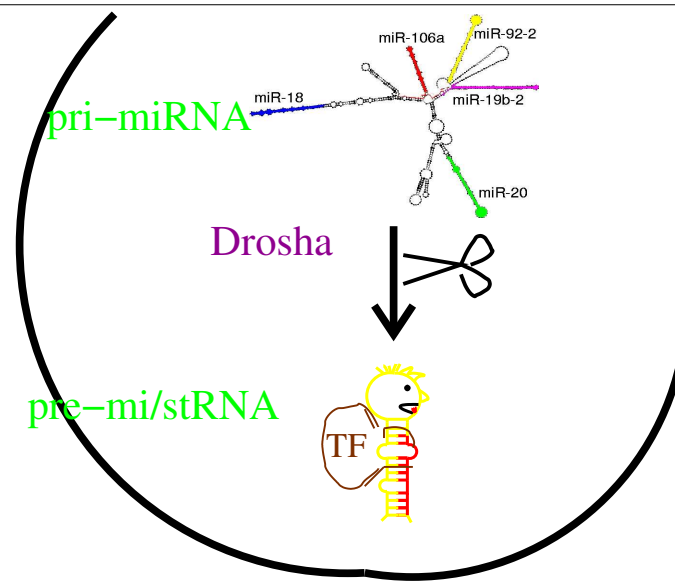
miRNAs are usually highly conserved in closely related and often conserved in distant species.

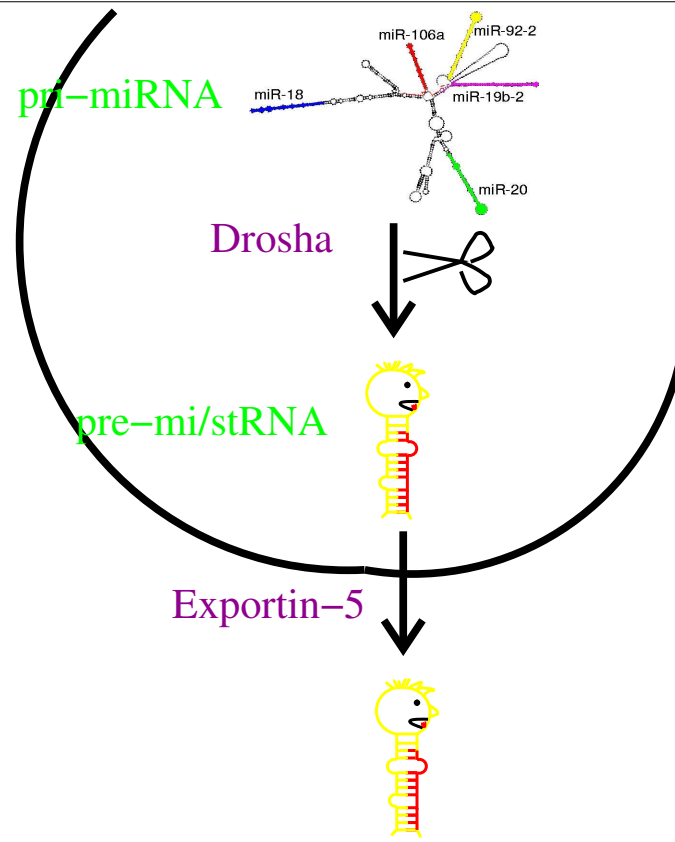
Precursor is processed into one discrete mature miRNA.

miRNAs are encoded in another locus than their targets.

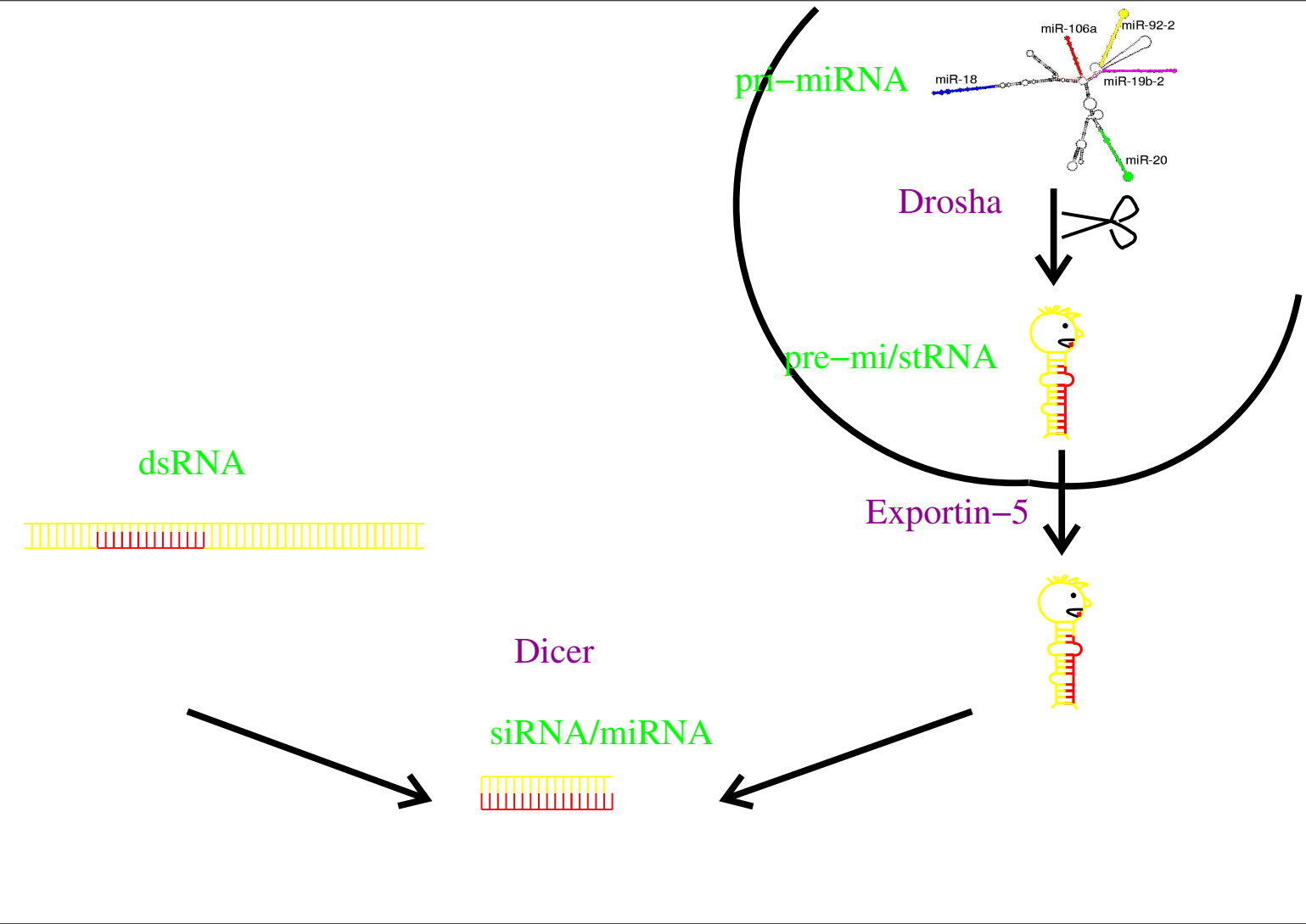






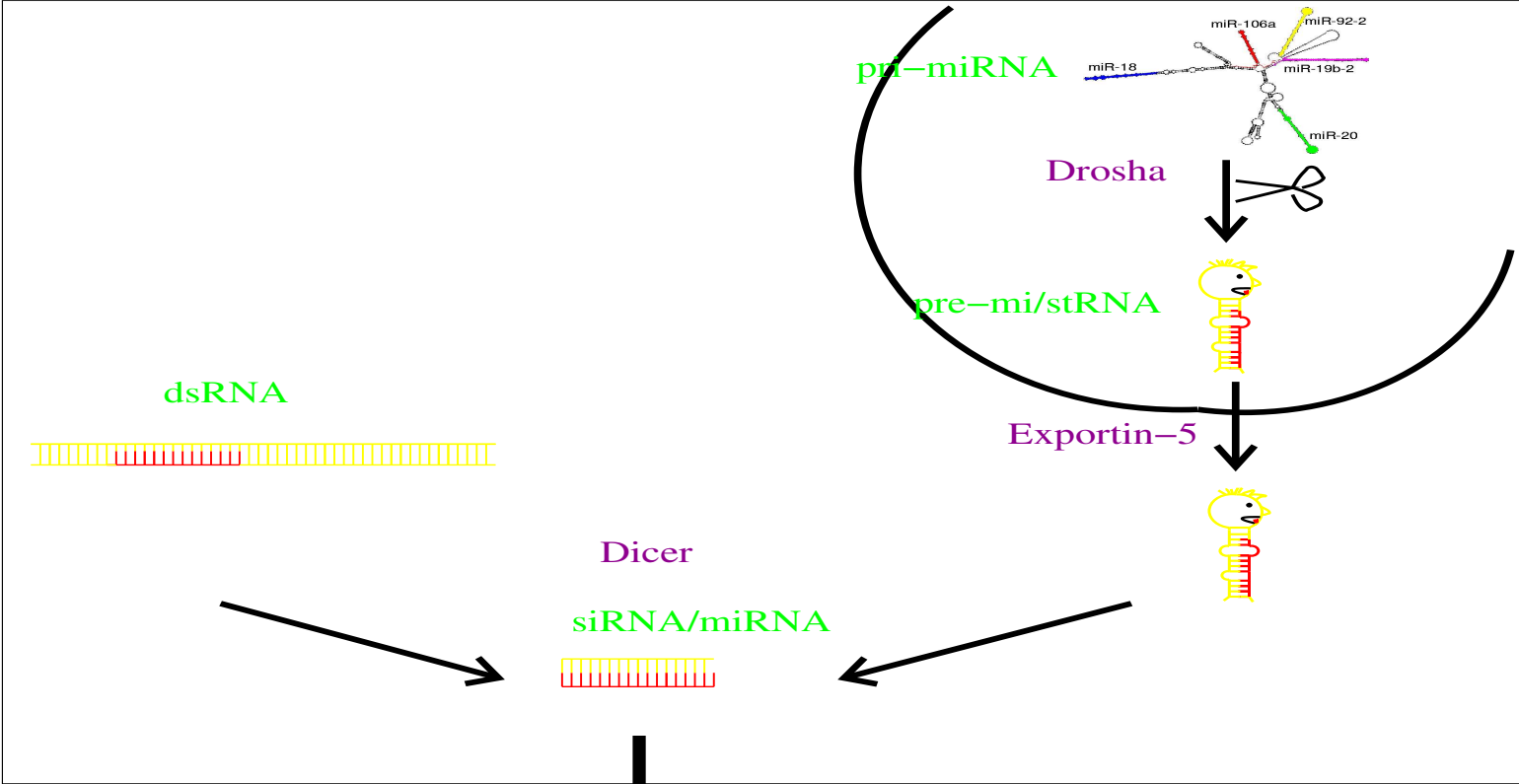






## Dicer

Class 3 RNaseIII type enzyme and ATP-dependent helicase. Binds, cleaves and unwinds dsRNA irrespective of sequence. About 1900 amino acids long and 218 kDa, acts as a dimer. Needs  $Mg^{2+}$  for cleaving. Cleaves double stranded RNAs or hairpins. Cleaves into double stranded RNA's of  $\approx 22$  nucleotide length, can leave overhang of 2 nt 3'. situated in cytosol or in ER, in plants also found in nucleus. Contains PAZ domain as putative link to RISC. 4 members of Dicer family present in Arabidopsis genome, DCL1 knock out inactivates miRNA/siRNA pathway. Possibly longer siRNAs ( $\approx 25$  nt) are cleaved by different dicer enzymes.



Argonautes

Determination which strand of RNA duplex is used in effector step

Thermodynamic guidelines:

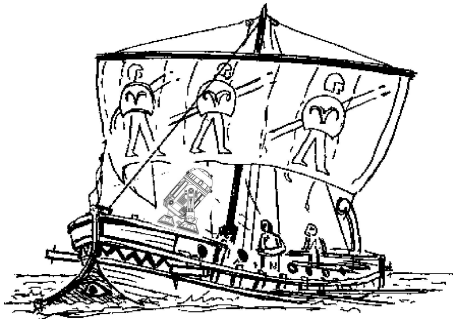
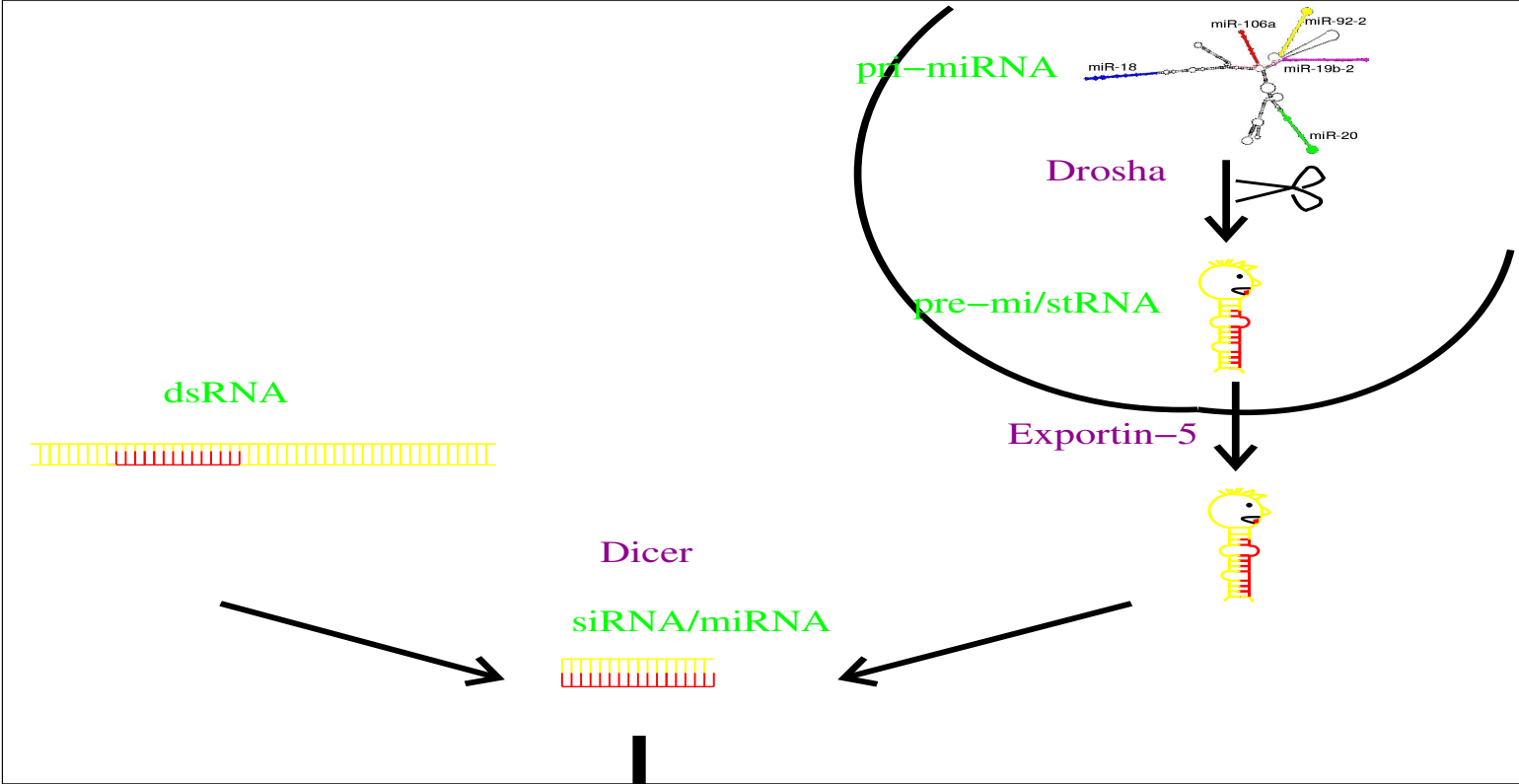
5' end with lower stability is preferably incorporated in RISC/miRNP (Schwarz et. al., Khvorova et. al.)

Assumed to be due to helicase function of Dicer

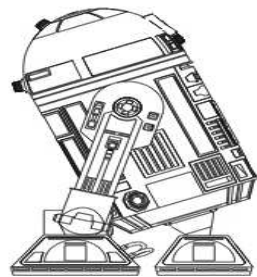
Argonautes like R2D2 of *Drosophila* are thought to link the initiation and effector steps.

Argonaute proteins contain two domains of unknown function, termed PIWI and PAZ.

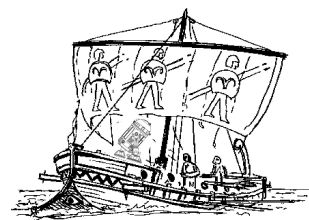
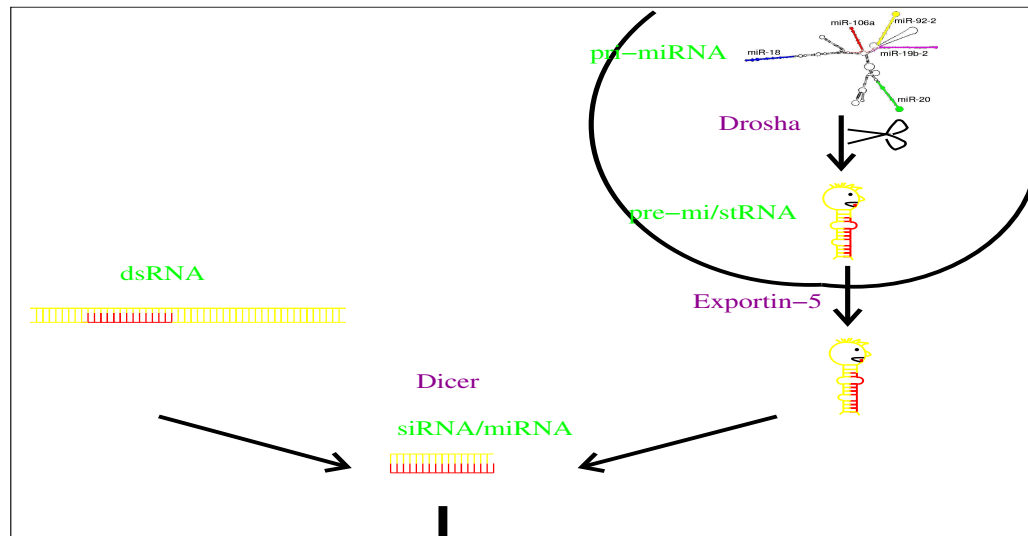
In addition, at least in R2D2 there are two dsRNA binding domains.



Argonautes



R2D2

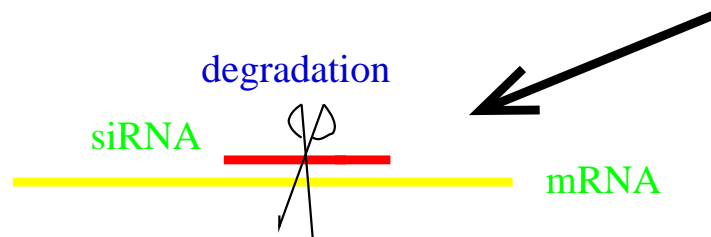


Argonautes



R2D2

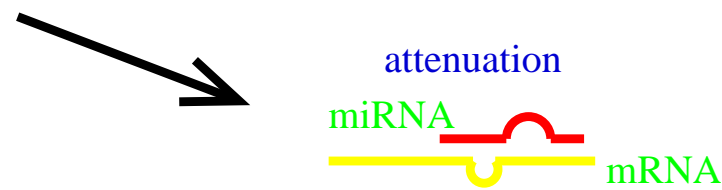
RISC/miRNP



degradation

siRNA

mRNA



attenuation

miRNA

mRNA

## RISC/miRNP

Drosophila: RNA Induced Silencing Complex  $\approx 500\text{kDa}$ .

Contains Argonaute Ago-2, dFXR, Vasa Intronic Gene (VIG) protein and tudor staphylococcal nuclease.

human RISC, 90-160kDa

Contains two Argonaute Proteins, eIF2C1 and eIF2C2

human miRNP, sediments as  $\approx 15\text{S}$  particle

Contains eIF2C2, Gemin4, and Gemin3, a putative RNA helicase

## RISC/miRNP

Both can cleave mRNA or attenuate translation.

Cleavage dependent on complementarity, the more mismatches, the less cleavage probability.

Cleavage enhanced by U at position 10 of siRNA.

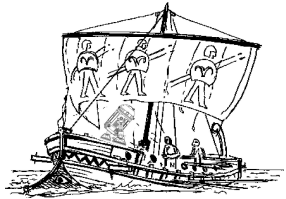
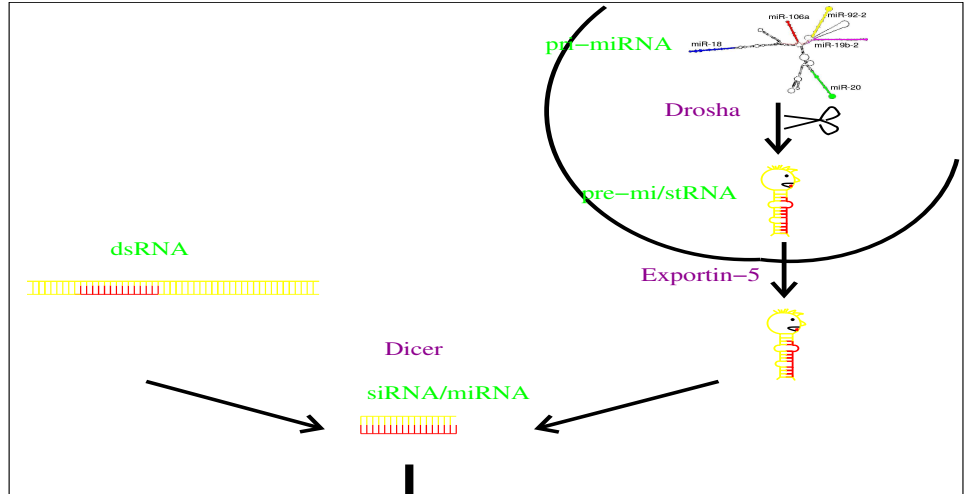
Level of mRNA degradation seemingly dependent on binding energy of the central part of the siRNA-mRNA complex.

Level of mRNA degradation may be independent of mRNA secondary structure on binding site.

Cleavage can be inhibited with 2'-O-Me RNA derivatives.

Maybe associated to Ribosomes even without RNA.



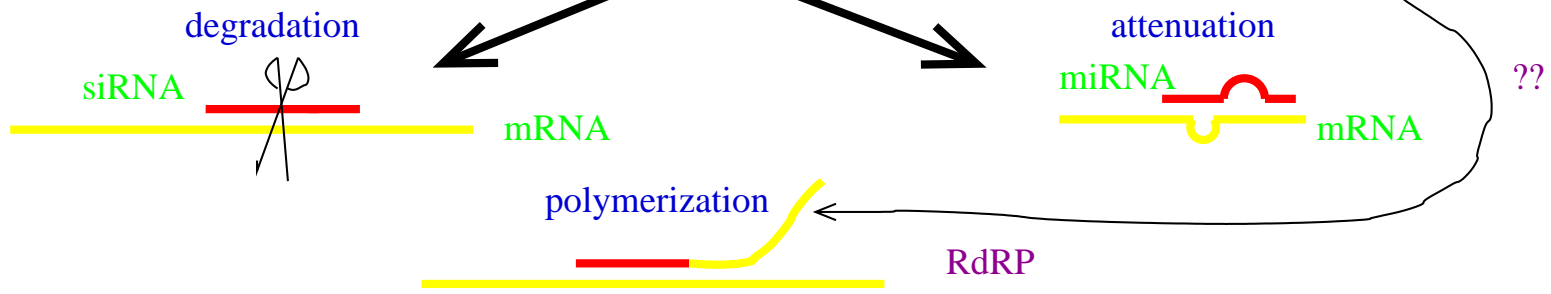


Argonautes



R2D2

RISC/miRNP



degradation

siRNA

mRNA

attenuation

miRNA

mRNA

polymerization

RdRP

??

Proposed question to the ultimate answer:

What is the average number of different proteins regulated by one miRNA?

Thank You