

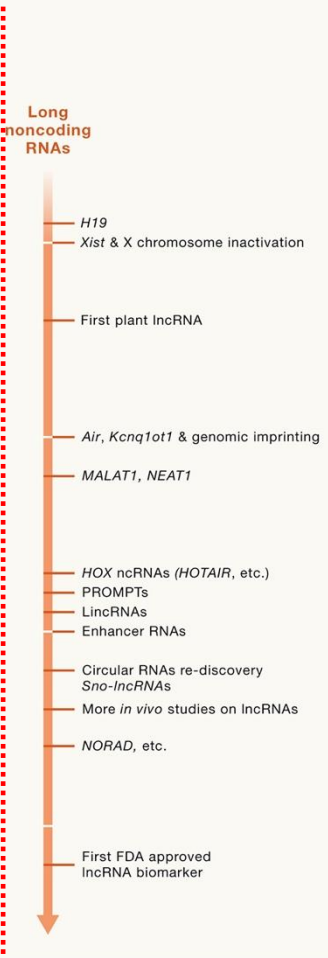
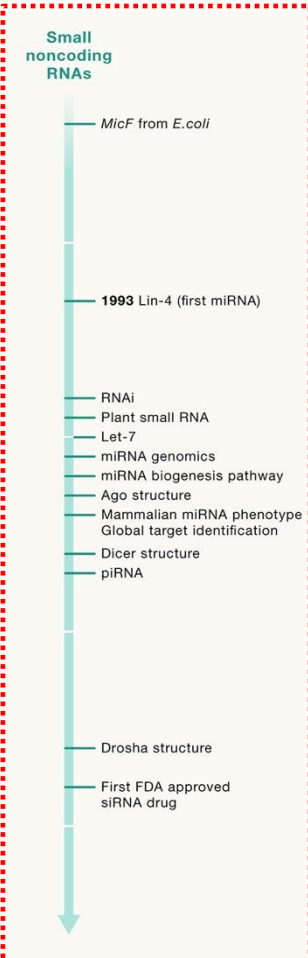
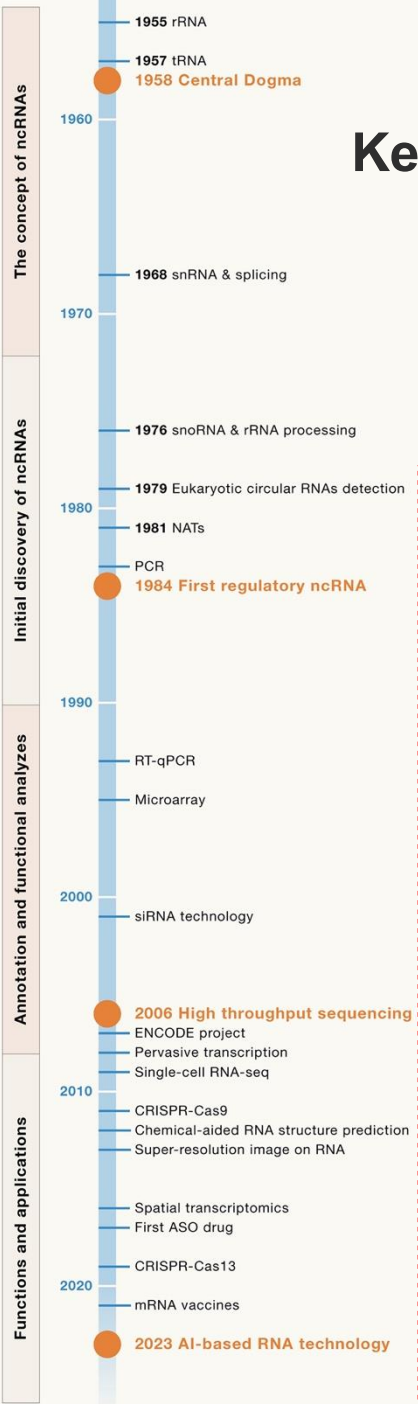
Small RNAs and gene expression regulation

Roles for small RNAs in gene expression regulation

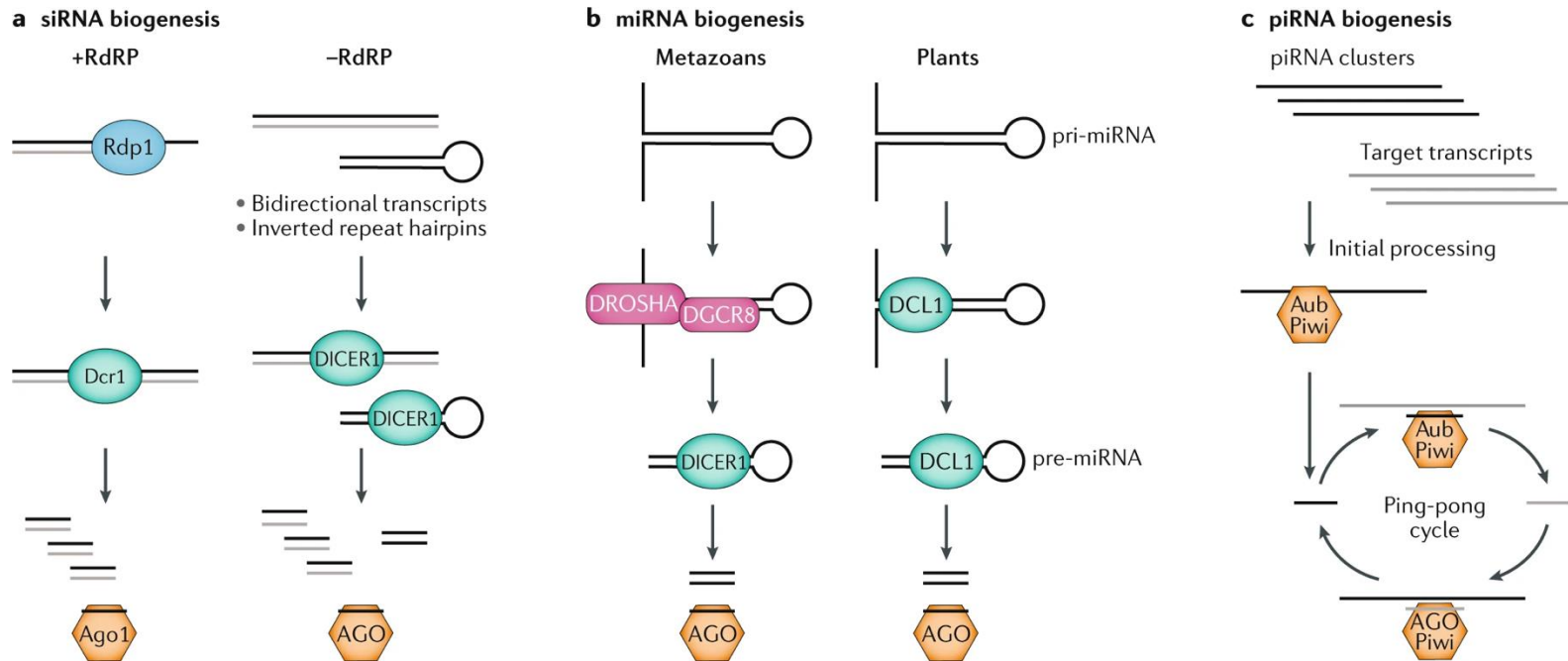
Three classes of small RNA can regulate genes by targeting transcripts. These are:

- **small interfering RNAs (siRNAs)**, which have perfect complementarity to targets and cause transcriptional or post-transcriptional gene silencing;
- **PIWI-interacting RNAs (piRNAs)**, which target transposon transcripts in animal germ lines;
- **microRNAs (miRNAs)**, which are hairpin-derived RNAs with imperfect complementarity to targets and that cause translational repression.

Key discoveries of ncRNAs over decades



Small-RNA biogenesis pathways



- Small interfering RNAs (siRNAs) can be produced either from double-stranded RNA (dsRNA) generated by an RNA-dependent RNA polymerase (RdRP) or from other dsRNA substrates.
- DROSHA and DICER1 possess RNase III domains that are also found in bacteria and have important roles in rRNA processing. Eukaryotic RdRP likely derived from a bacteriophage protein.

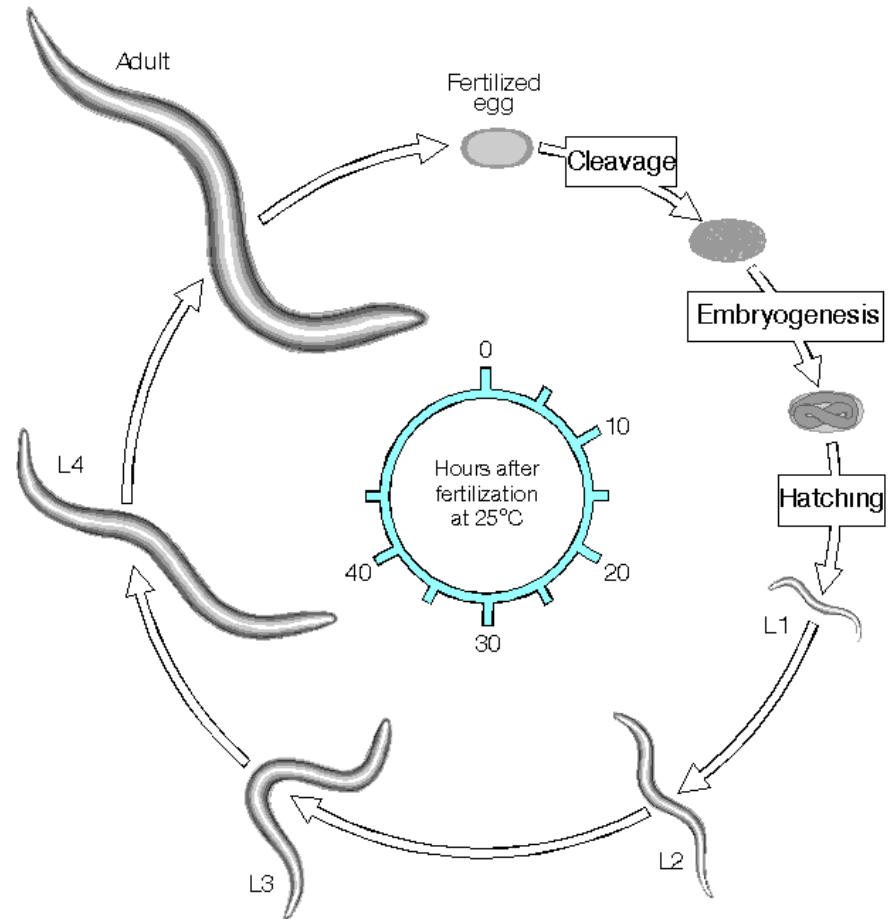
microRNAs: biogenesis and function

1. The Discovery of miRNAs

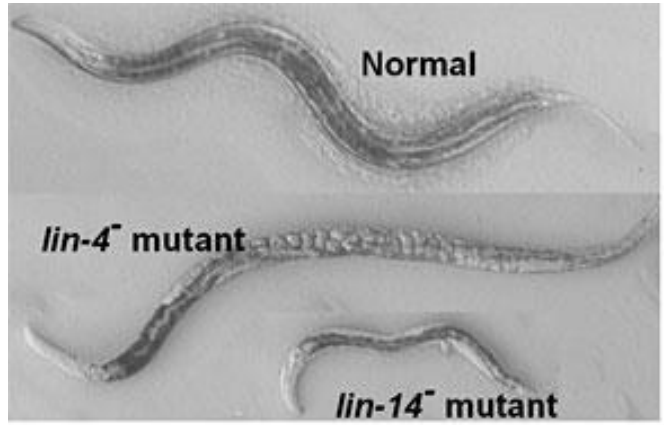
Caenorhabditis elegans larval development

A genetic pathway of **heterochronic genes** in *Caenorhabditis elegans* acts to specify the temporal fates of cells during larval development, thereby controlling the timing and sequence of events in diverse postembryonic cell lineages

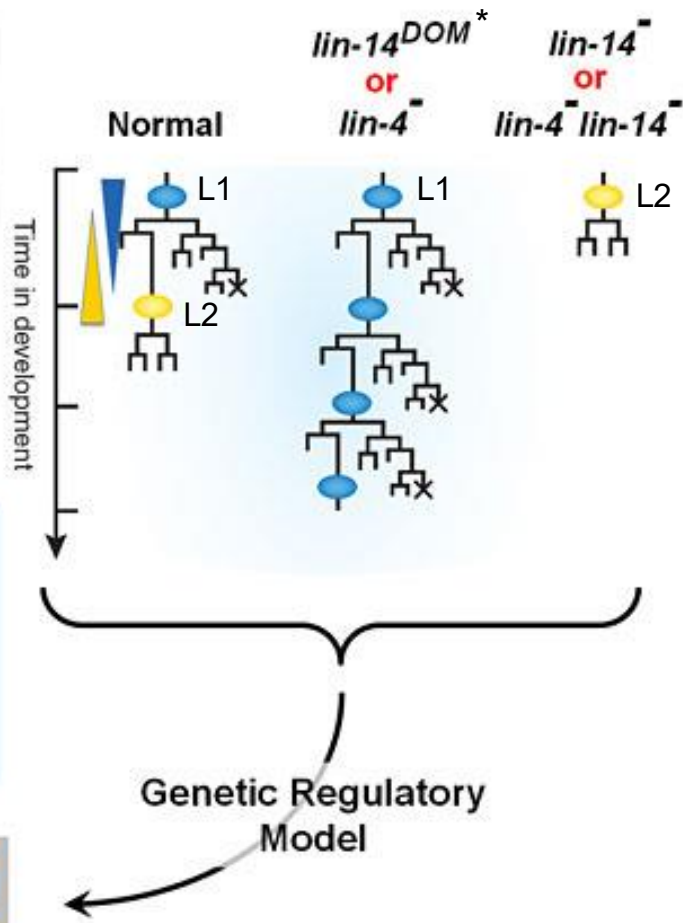
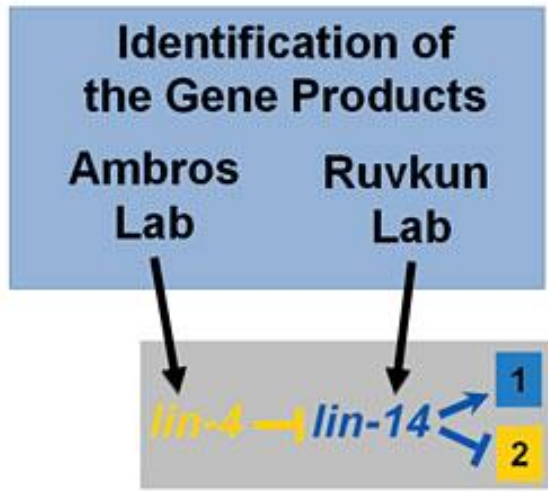
Mutations in the heterochronic genes can cause either precocious development, in which normally late developmental programs are expressed at early larval stages, or retarded development, in which normally early developmental programs are reiterated at later stages .



Mutations in *lin-4* disrupt the temporal regulation of larval development, causing L1 specific cell-division patterns to reiterate at later developmental stages. Opposite developmental phenotypes – omission of the L1 cell fates and premature development into the L2 stage – are observed in worms that are deficient for *lin-14*.



Heterochronic Mutants



**lin14^{DOM}*: mutant of *Lin-14* mRNA lacking its 3'-UTR

Lin4 post-transcriptionally regulates lin-14 expression

LIN-14 protein is normally abundant in the nuclei of late-stage embryos and younger L1 larvae and then is barely detectable by the L2 while lin-14 transcripts are constant throughout development → **Lin-14 is negatively regulated post-transcriptionally**

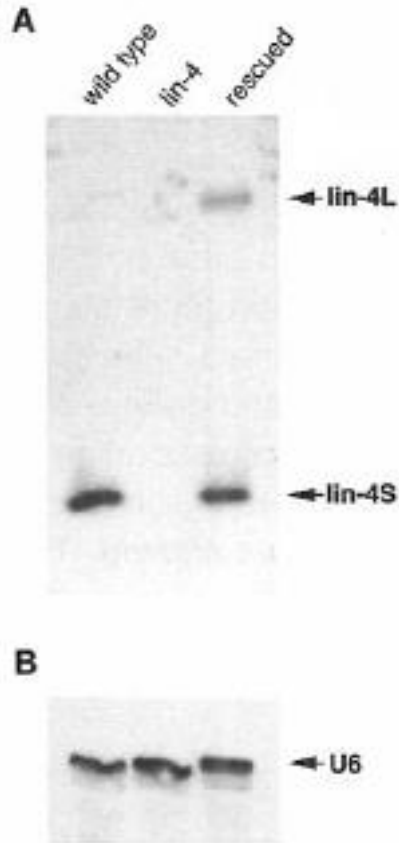
In Lin-4 mutant animals the level of LIN-14 protein remains abnormally high late in development

Mutations to the 3'UTR of Lin-14 mRNA (lin14^{DOM}) phenocopied the lin-4 mutant

The temporal decrease in LIN-14 protein levels requires both lin-4 in trans and lin-14 3'UTR sequences in cis.

→ **lin-4 gene product acts via the lin-14 3'UTR to inhibit, directly or indirectly, the translation of lin-14 mRNA.**

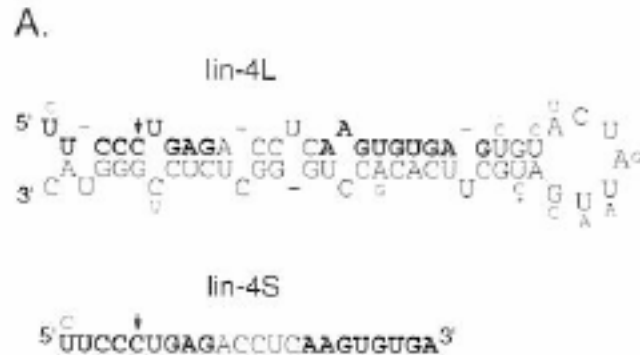
Characterization of lin-4 products



the 693 bp of lin-4 rescuing sequence detected two small transcripts:

Lin-4 L 61 nt

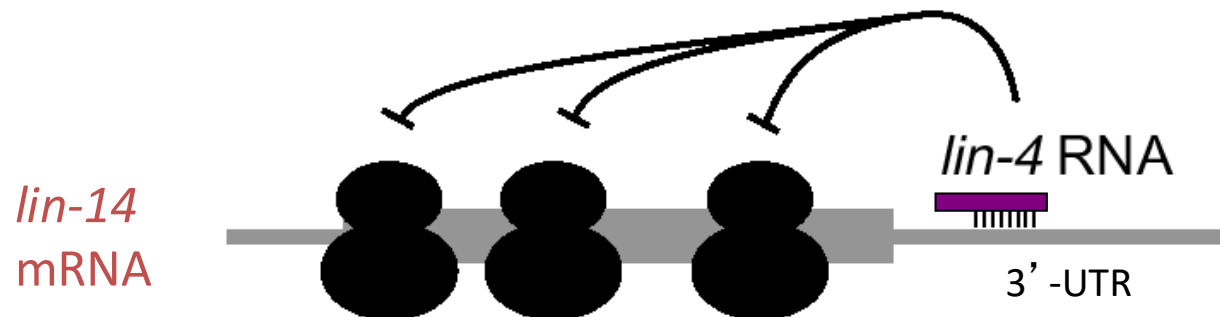
Lin-4 S 22 nt



The “small temporal” RNA *lin-4*

Gene	Mutant phenotype	Gene product
<i>lin-14</i>	Omission L1 fates	Nuclear protein
<i>lin-4</i>	Reiteration of L1 fates	22nt RNA

Negative regulation of *lin-14* protein is required for L1 to L2 transition



Victor Ambros and **Gary Ruvkun** have been awarded the 2024 Nobel Prize in Medicine for “the discovery of microRNA and its role in post-transcriptional gene regulation”.

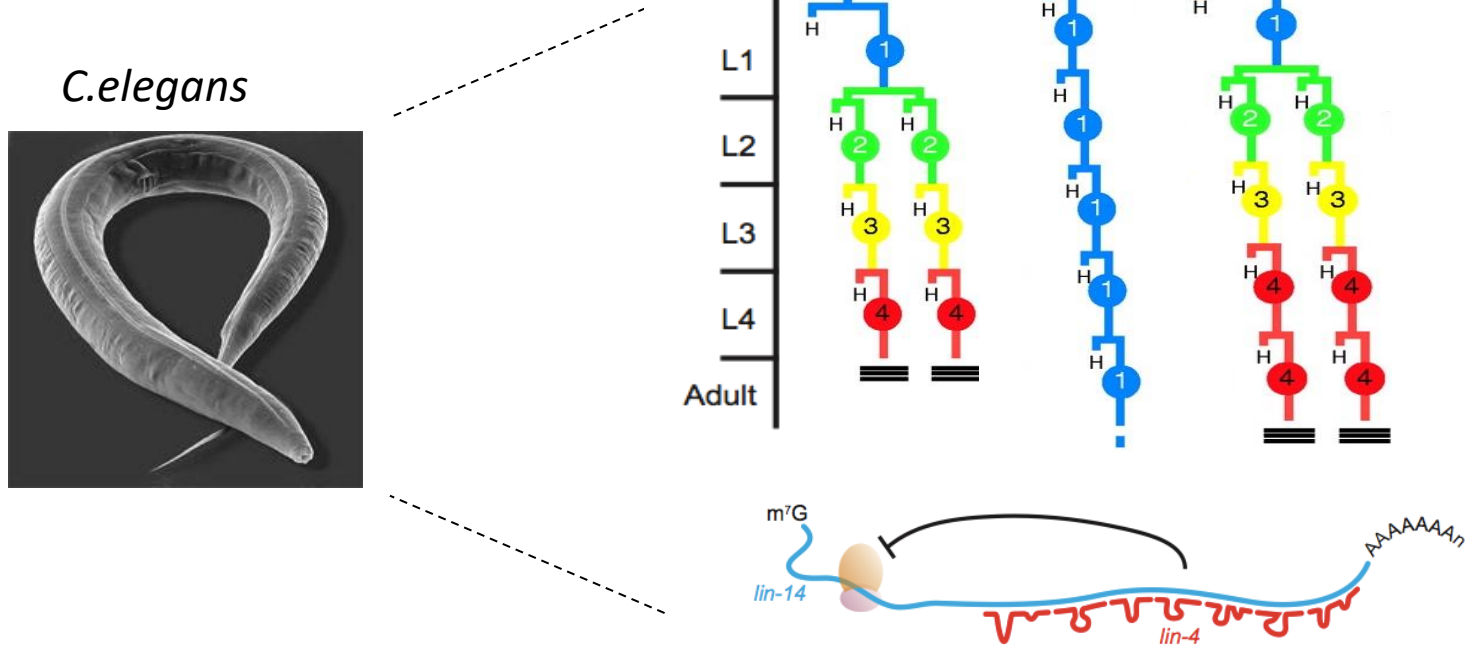


Ill. Niklas Elmehed © Nobel Prize Outreach
Victor Ambros



Ill. Niklas Elmehed © Nobel Prize Outreach
Gary Ruvkun

7 year later.....stRNA let-7



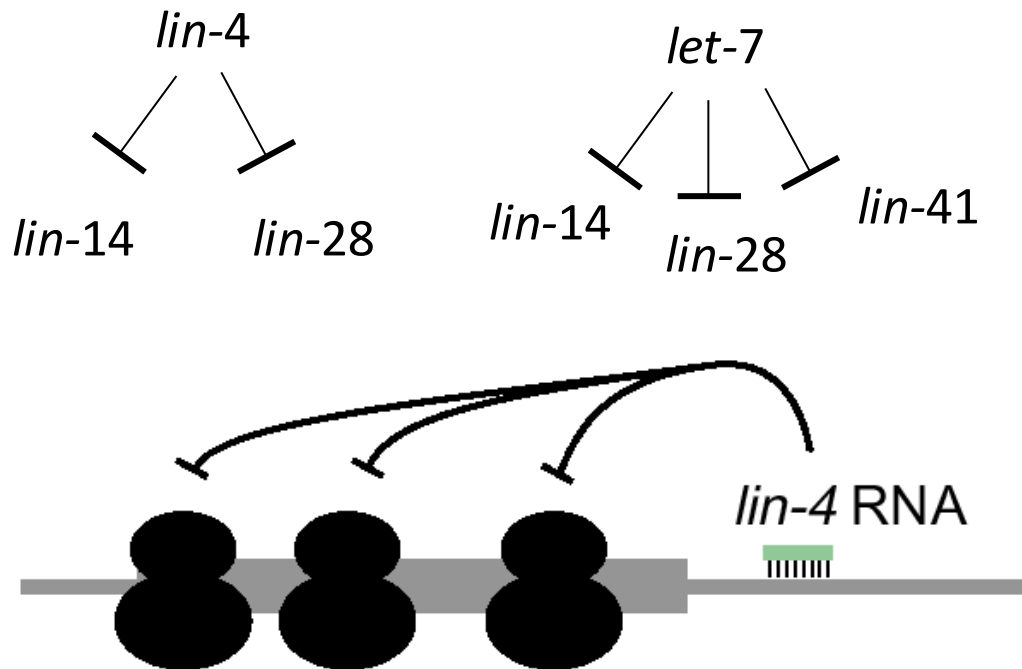
Wightman et al. 1993. *Cell*

Lee et al. 1993. *Cell*

Reinhart et al. 2000. *Nature*

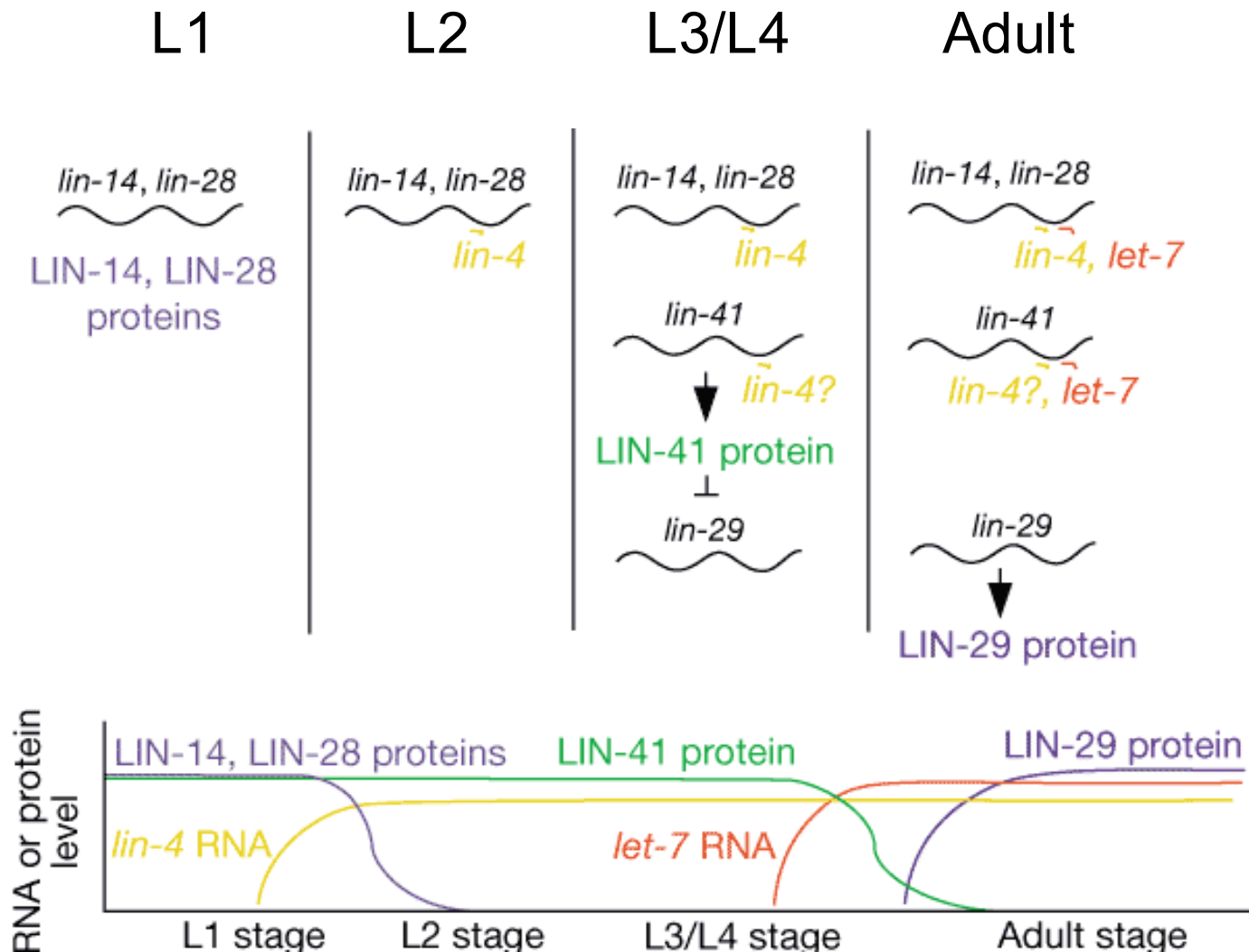
stRNAs regulate gene expression during *C.elegans* development

- *lin-4* and *let-7* are stRNAs that act as translational repressors of gene function by binding to the 3'-UTR



2000

stRNAs regulate gene expression during *C.elegans* development



.....2000.....

let-7 (stRNA) is conserved in all metazoa

Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA

Amy E. Pasquinelli[†], Brenda J. Reinhart^{††}, Frank Slack[‡], Mark O. Martindale[§], Mitzi I. Kurodall, Betsy Maller[‡], David C. Hayward[¶], Eklon E. Ball[¶], Bernard Degnan[‡], Peter Müller^{*}, Jürg Spring^{*}, Ashok Srinivasan^{**}, Mark Fishman^{**}, John Finnerty^{††}, Joseph Corbo^{‡‡}, Michael Levine^{††}, Patrick Leahy^{§§}, Eric Davidson^{§§} & Gary Ruvkun^{*}

Pasquinelli et al., 2000. *Nature*

whereas the nematode and the fly have only one *let-7* miRNA, higher animals (e.g., fishes and mammals) have diverse *let-7* family members

A

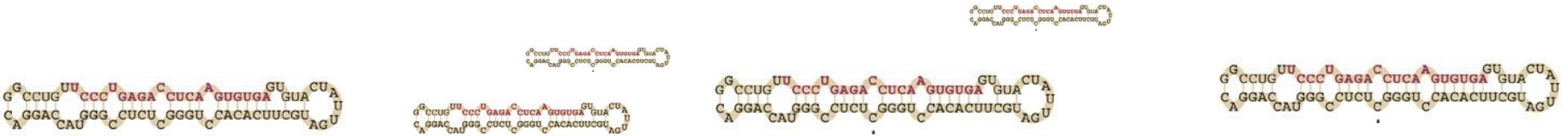
Consensus	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
cel-let-7	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
dme-let-7	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	-
xtr-let-7a	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
dre-let-7a	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
gga-let-7a	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
cfa-let-7a	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
mmu-let-7a	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
hsa-let-7a	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U

B

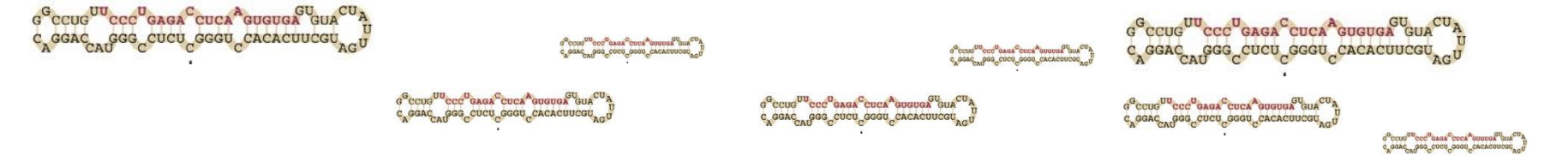
Consensus	u	G	A	G	G	U	A	G	u	A	g	g	U	U	G	u	a	u	a	G	U	U
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
hsa-let-7a-1	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
hsa-let-7a-2	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
hsa-let-7a-3	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
hsa-let-7b	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	G	U	A	G	U	U
hsa-let-7c	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U
hsa-let-7d	A	G	A	G	G	U	A	G	U	A	G	G	U	U	G	C	A	U	A	G	U	U
hsa-let-7e	U	G	A	G	G	U	A	G	G	A	G	G	U	U	G	U	A	U	A	G	U	U
hsa-let-7f-1	U	G	A	G	G	U	A	G	U	A	G	A	U	U	G	U	A	U	A	G	U	U
hsa-let-7f-2	U	G	A	G	G	U	A	G	U	A	G	A	U	U	G	U	A	U	A	G	U	U
hsa-let-7g	U	G	A	G	G	U	A	G	U	A	G	U	U	U	G	U	A	C	A	G	U	U
hsa-let-7i	U	G	A	G	G	U	A	G	U	A	G	U	U	U	G	U	G	C	U	G	U	U
hsa-miR-98	U	G	A	G	G	U	A	G	U	A	A	A	G	U	U	G	U	A	U	G	U	U



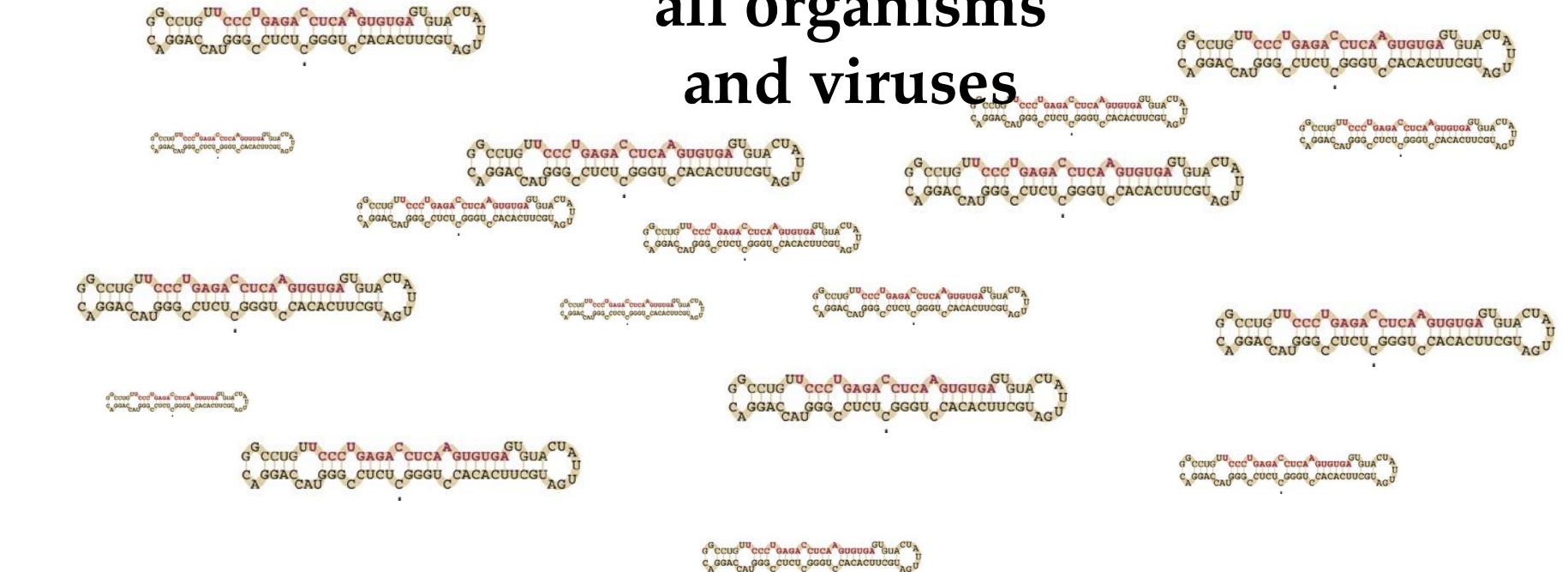
...after 2000



hundreds of microRNAs!



all organisms and viruses



microRNAs

- **complex family of 21-23 nt long small RNAs**
(~1% of higher eukaryotes genes)
- **present in metazoa and plants**
 - 469 D.melanogaster*
 - 437 C.elegans*
 - 2654 H.sapiens*
 - 427 A.thaliana*
- **and mammalian viruses**
 - Herpesviruses (27), EBV (44).....*

they control gene expression by regulating mRNA stability and translation

Human microRNAs

- 2654 distinct microRNAs (20-22 nt) have been identified in humans (data from MiRBase)
- Negatively regulate the translation of mRNAs involved in almost every cellular process.
- MicroRNAs and their targets form complex regulatory networks

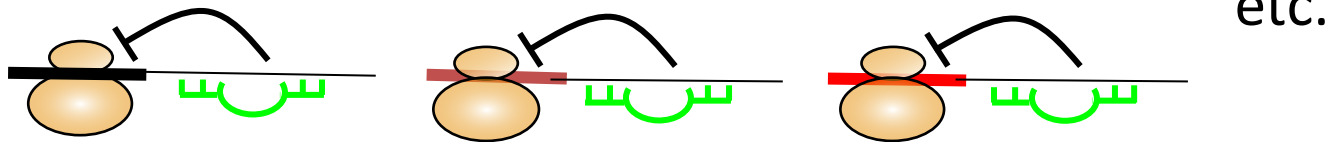
Major advantages of microRNA regulation:

- **Networking** and **fine-tuning** of gene expression
- **Rapid repression**

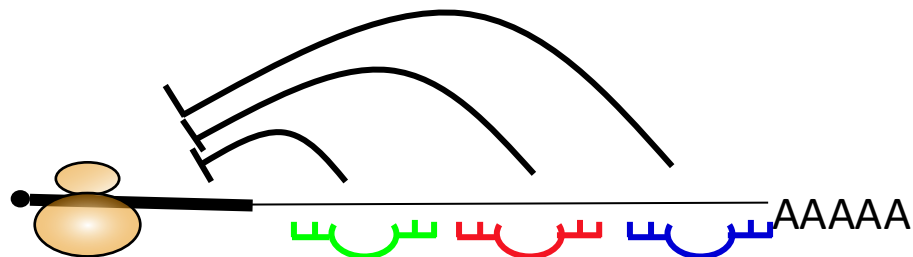
Human microRNAs

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One microRNA can control hundreds different mRNAs

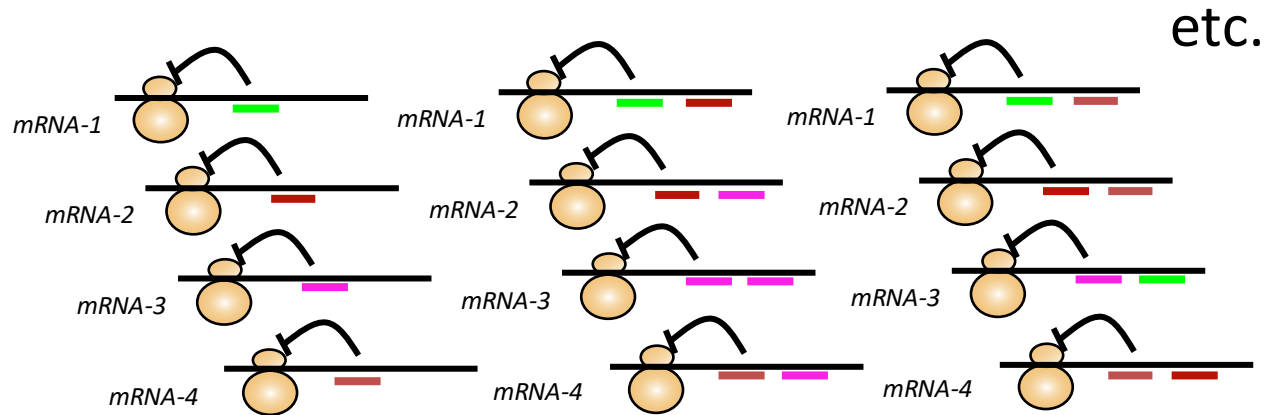


A single mRNA can be controlled by more than one microRNA



Human microRNAs

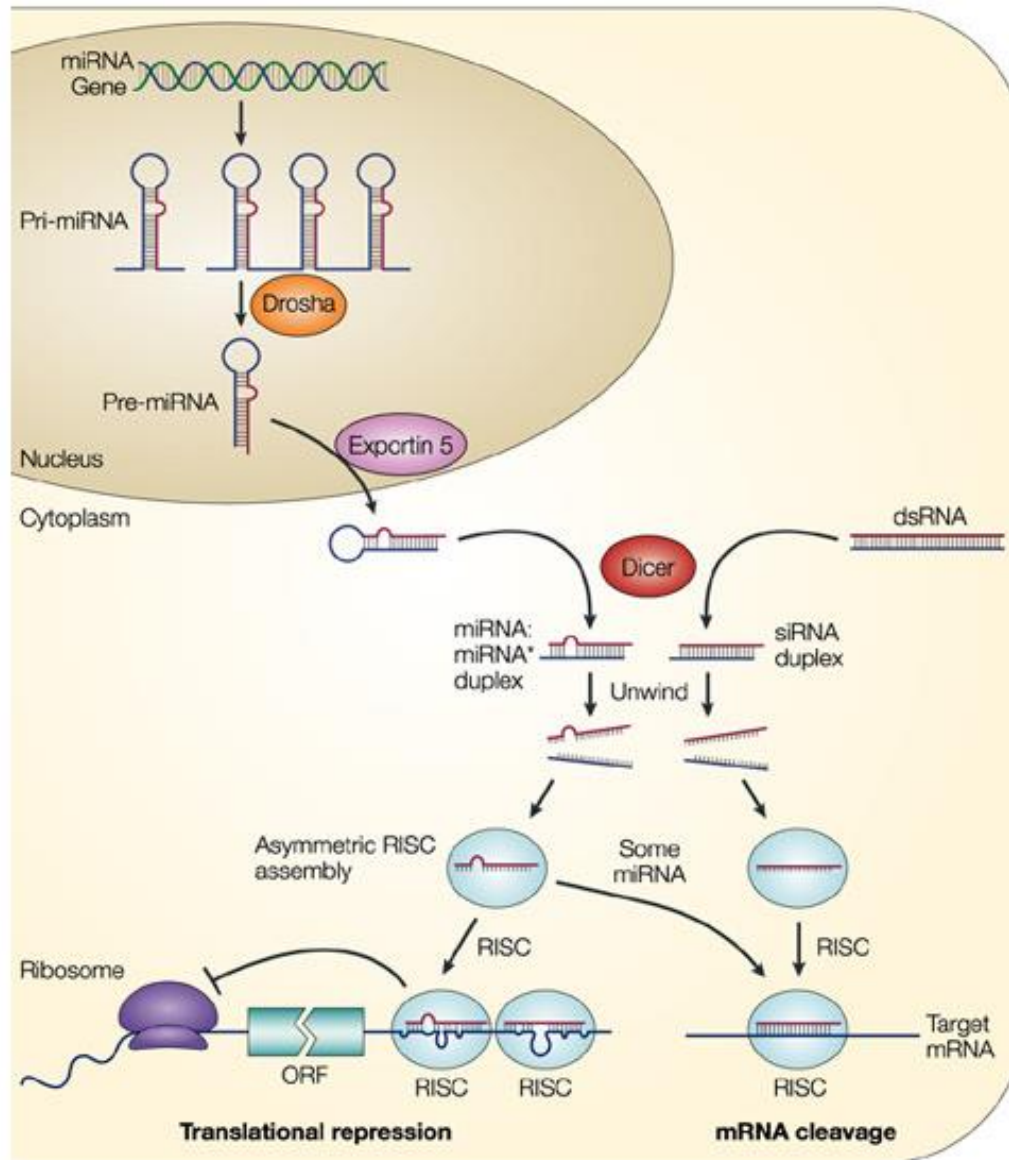
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2. Synthesis

miRNAs and siRNAs

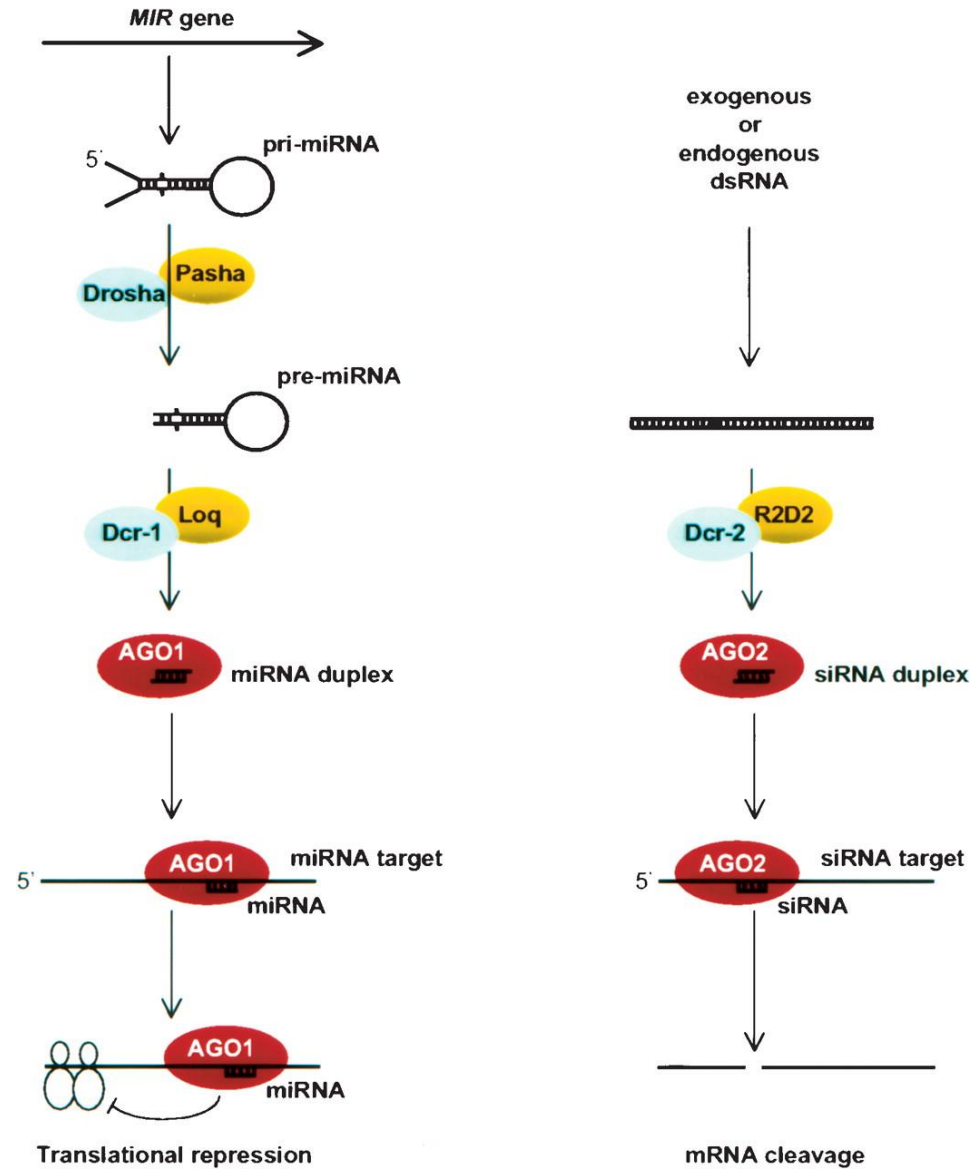
mammals



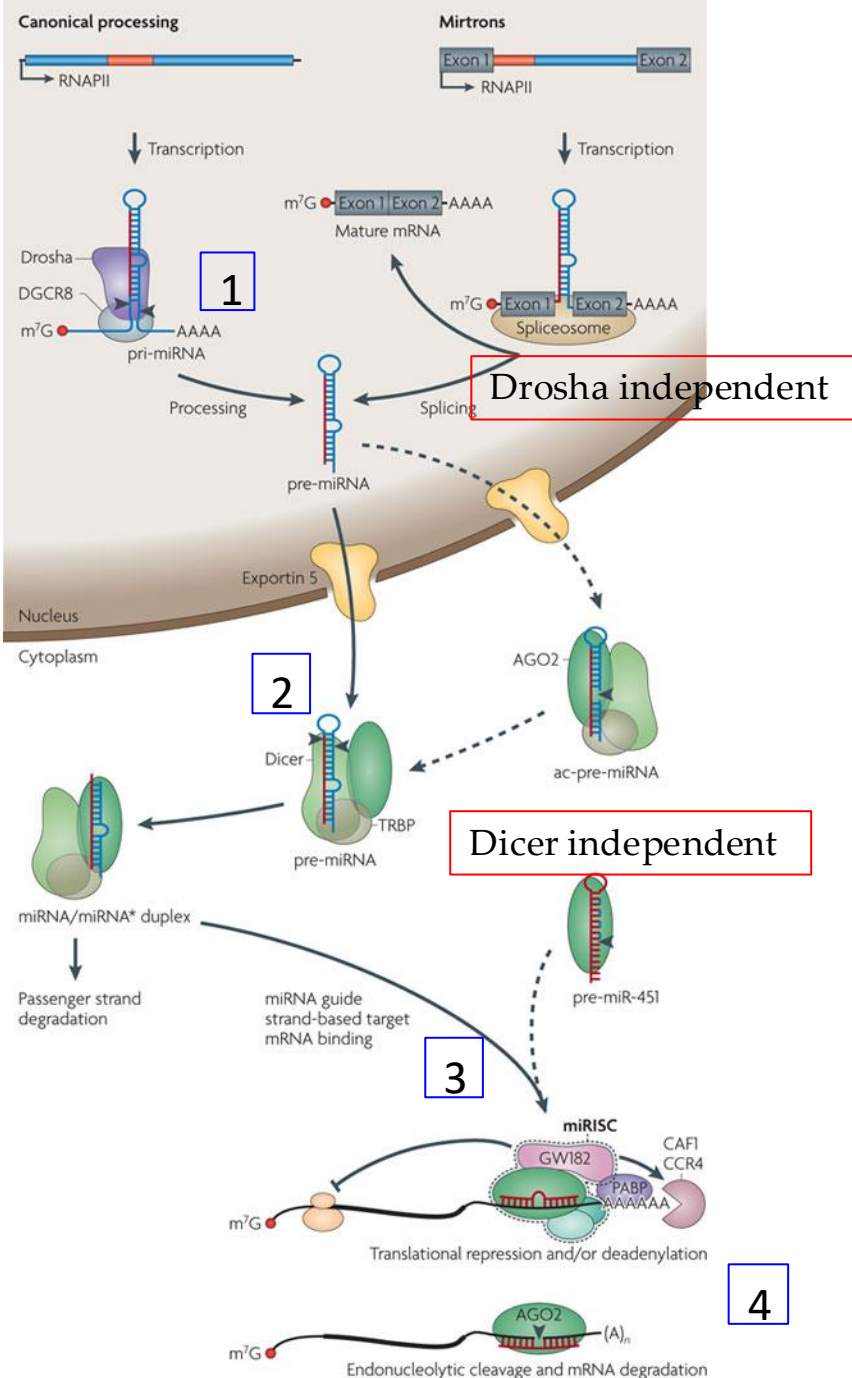
miRNAs and siRNAs



D. melanogaster



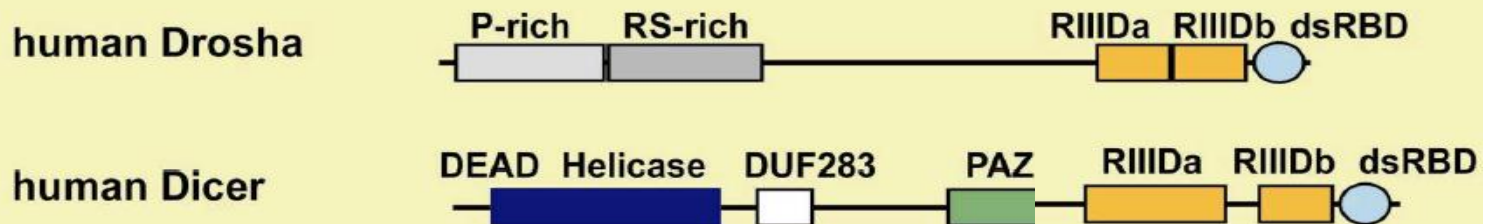
miRNA biogenesis



1. The pri-miRNAs fold into hairpins, which act as substrates for two members of the RNase III family of enzymes, Drosha and Dicer.
2. The product of Drosha cleavage, a ~70-nucleotide pre-miRNA, is exported to the cytoplasm where Dicer processes it to a ~20-bp miRNA/miRNA duplex.
3. One strand of this duplex, representing a mature miRNA, is then incorporated into the miRNA-induced silencing complex (miRISC)
4. As part of miRISC, miRNAs base-pair to target mRNAs and induce their deadenylation and/or translational repression (short complementarity) or cleavage and degradation (perfect complementarity)

Players

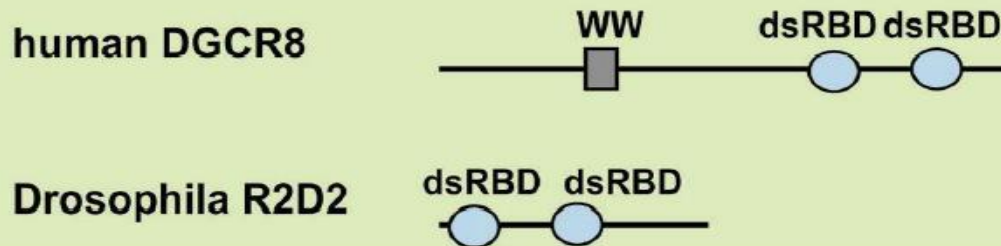
A. RNase III type proteins



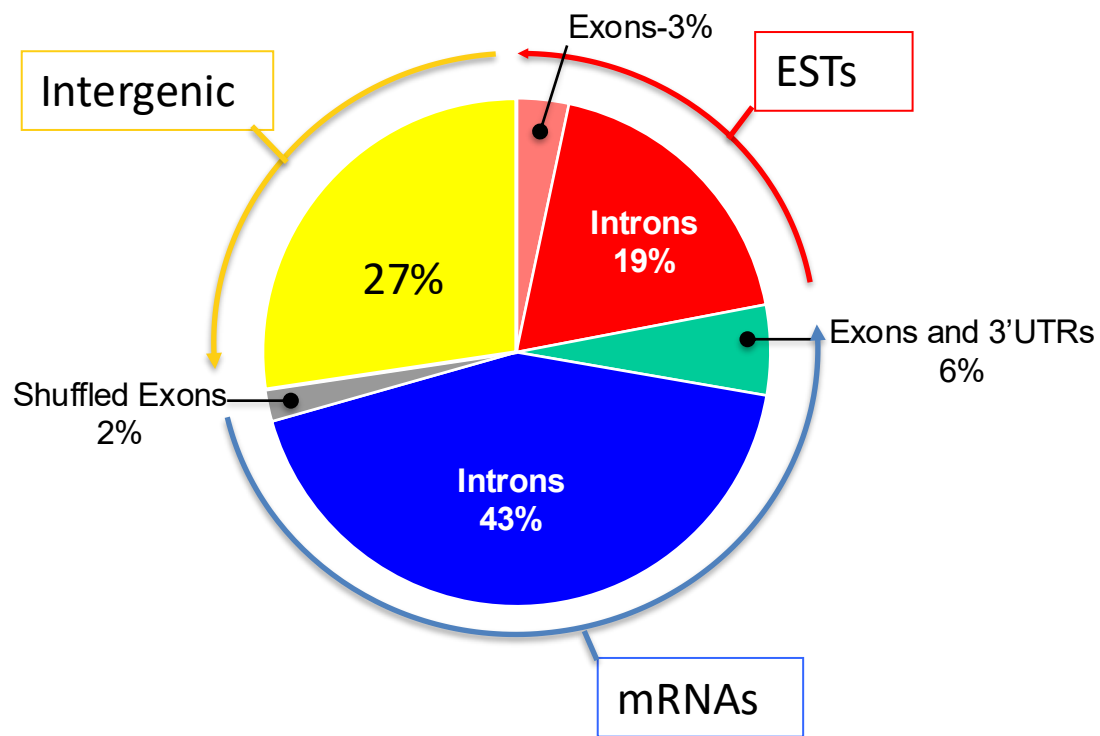
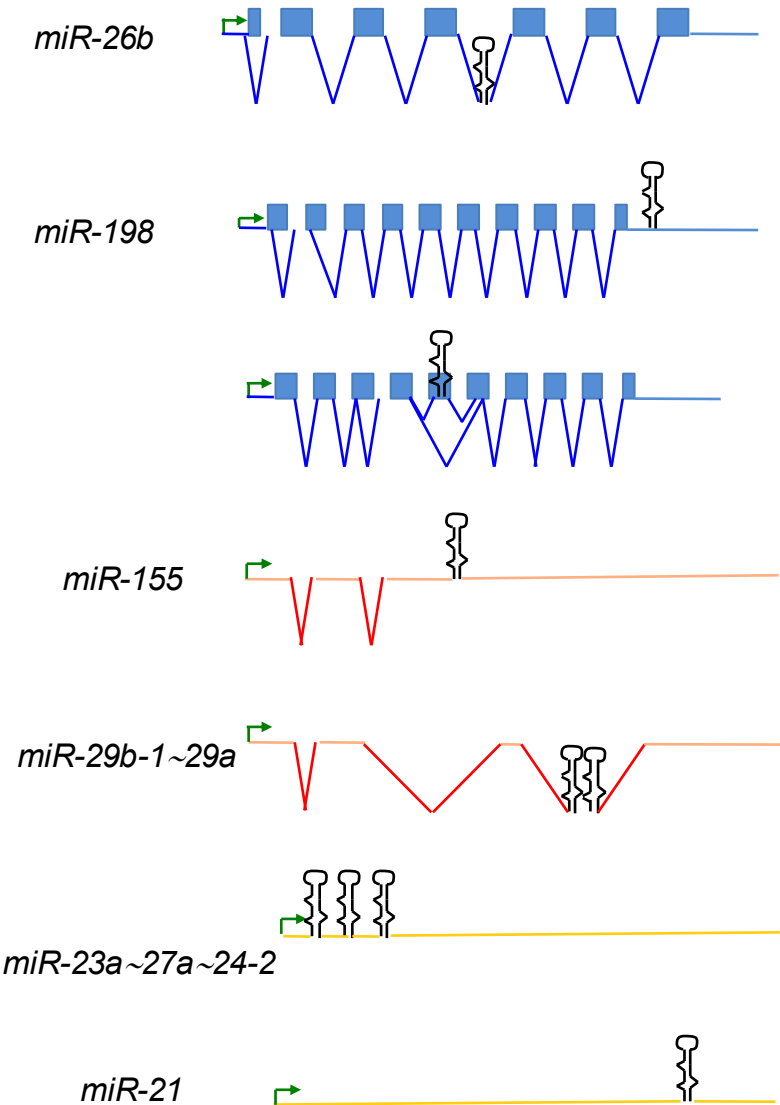
B. Argonaute proteins



C. dsRNA-binding proteins



Genomic organization of human microRNAs



Morlando et al., **NSMB** (2008)

“Drosha” and “Dicer” RNases

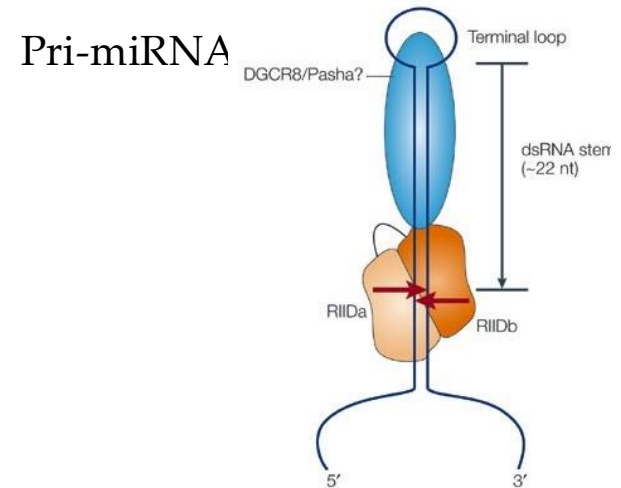
• Two processing events lead to mature miRNA formation in animals. In the first, the nascent miRNA transcripts (pri-miRNA) are processed into ~70-nucleotide precursors (pre-miRNA); in the second event that follows, this precursor is cleaved to generate ~21–25-nucleotide mature miRNAs.



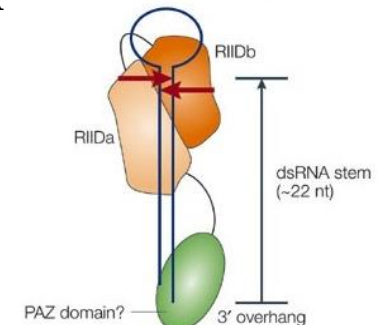
 dsRNA binding domain

 RNase III like domain

 RNA binding domain

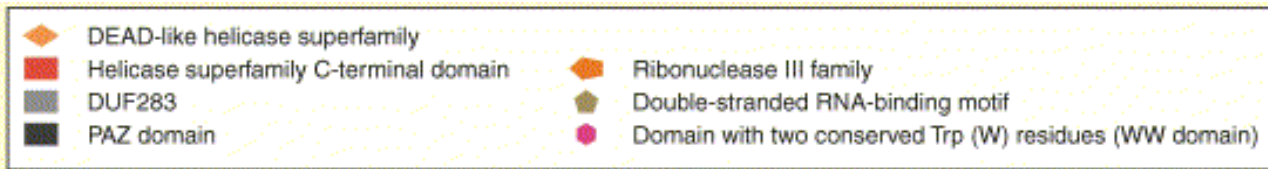
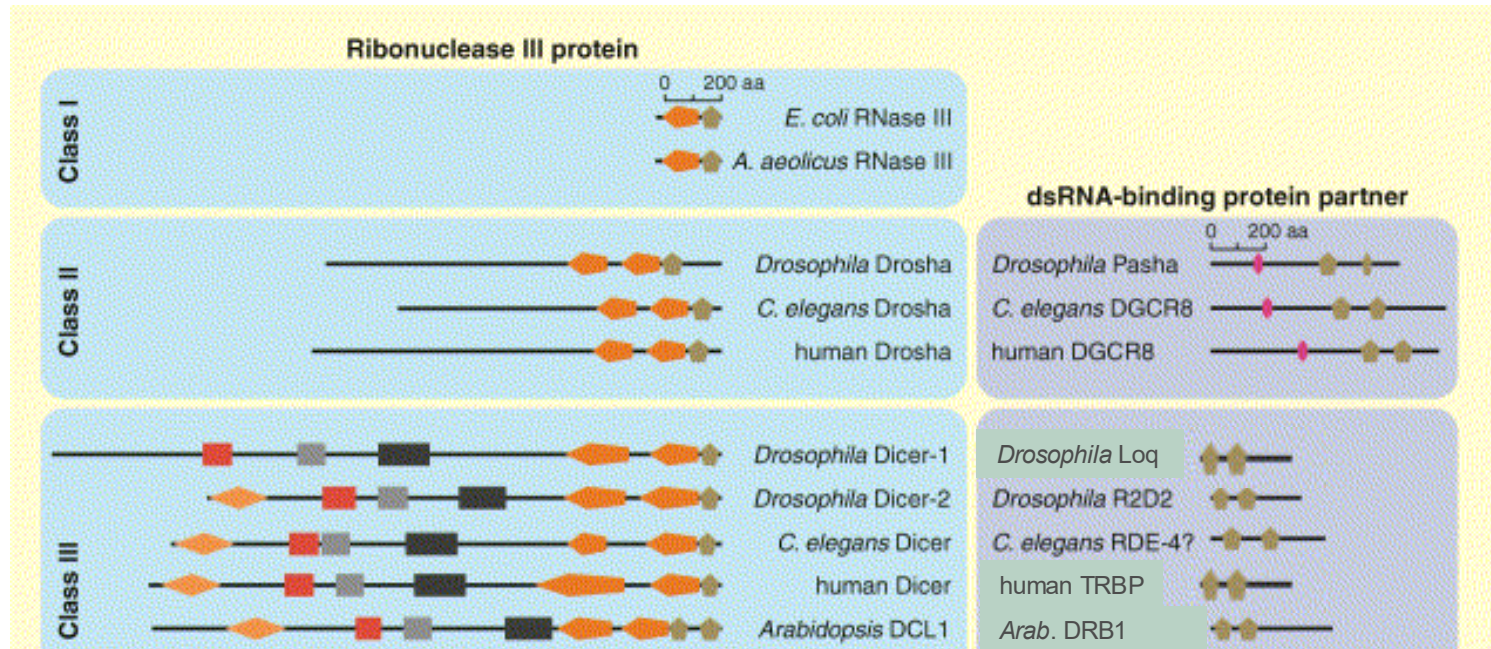


Pre-miRNA



• They generate 2-nt-long 3' overhangs at the cleavage site.

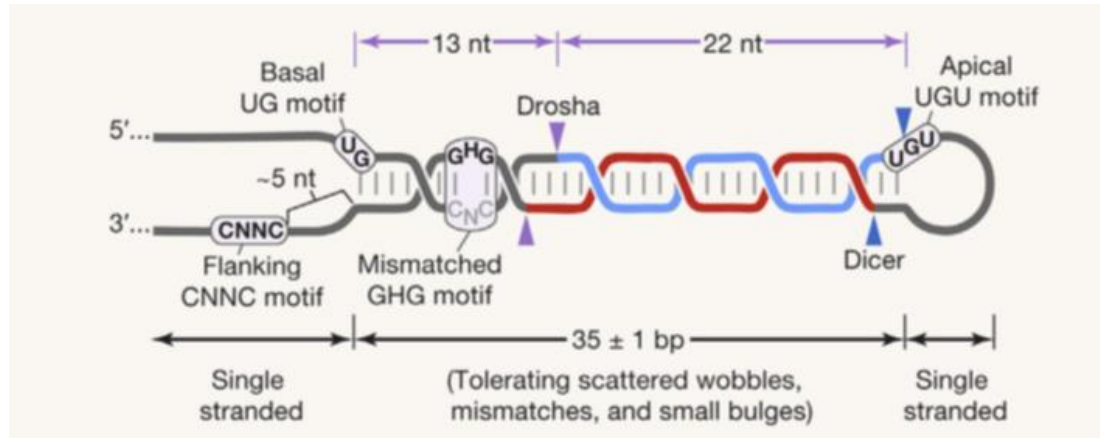
RNase III family



Current Biology

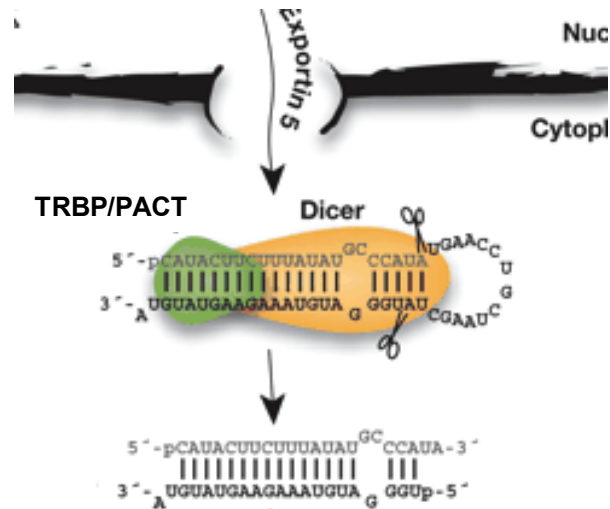
Tomari e Zamore, 2005.

Drosha



- Drosha is predominantly localized in the nucleus and contains two tandem RNase-III domains, a dsRNA binding domain and an N-terminal segment of unknown function.
- It requires **DGCR8 (Pasha)** for pri-mRNA cleavage. The efficiency of Drosha processing depends on the **stem structure** and the **flanking sequence** of the Drosha cleavage site.
- Regardless of the diverse primary sequences and structures of pri-miRNAs, Drosha cleaves these into ~70-bp pre-miRNAs that consist of an imperfect stem-loop structure. Additional features can enhance processing and help specify the sites of cleavage; these include a basal UG motif, an apical UGU motif, a flanking CNNC motif, and a mismatched GHG motif (in which H is A, C, or U).

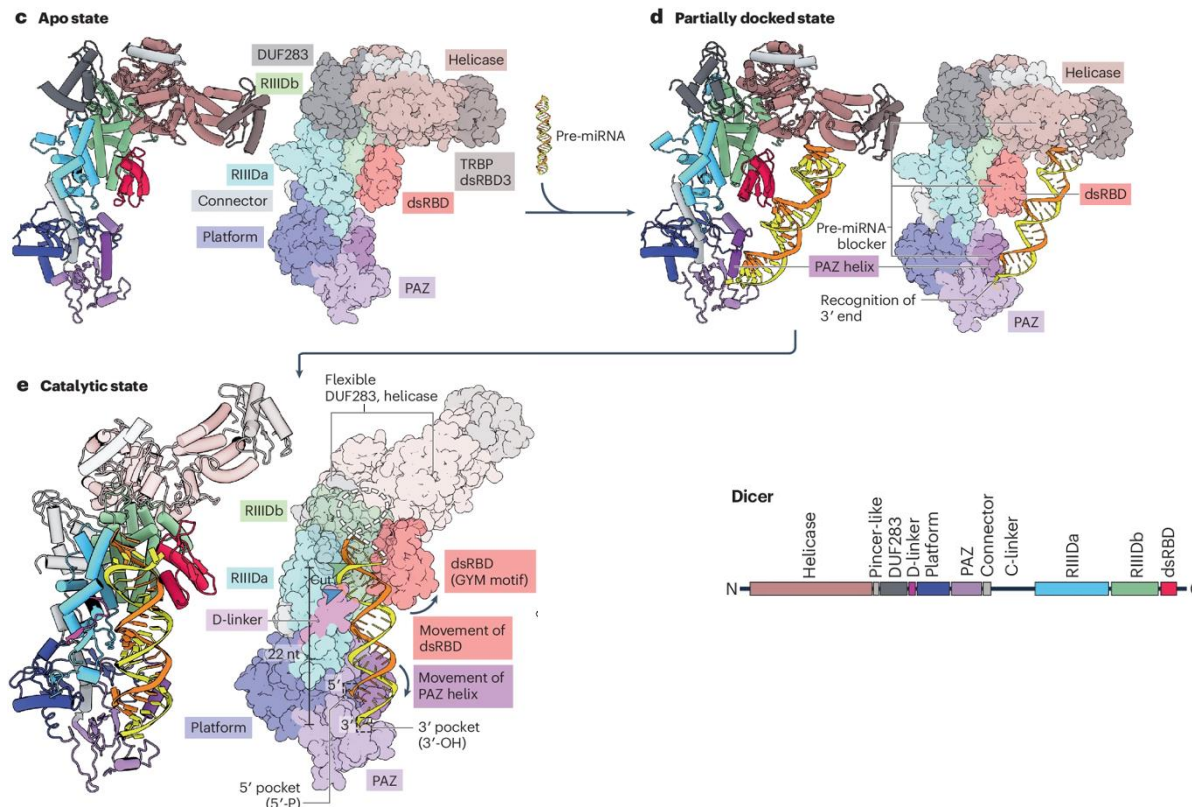
Dicer



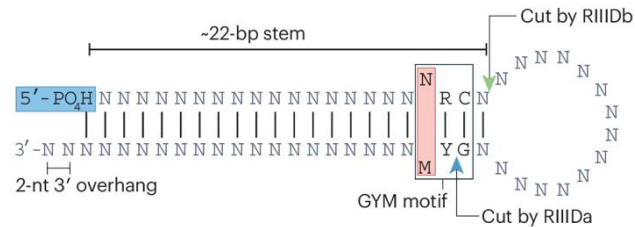
- Following export into the cytoplasm, pre-miRNAs are processed into their mature forms by Dicer. Two dsRNA-binding proteins, TRBP (also known as TARBP2) and PACT (also known as PRKRA), associate with Dicer and aid miRNA production in cells. However, deletion of the two genes does not substantially affect the steady-state level of miRNAs, indicating they have a supportive but not essential role in miRNA biogenesis in mammals.

Pre-miRNA processing by Dicer

Human Dicer has a molecular weight of approximately 200 kDa and a distinctive domain arrangement, which includes an N-terminal DExH/D helicase-ATPase domain, followed by the DUF283 domain, the platform domain, a PAZ domain, two RNase III domain (RIIDs) and a dsRBD at the C terminus. To process a pre-miRNA, Dicer undergoes three major conformational changes. First, the helicase and DUF283 domains become flexible, dislocating from the core of Dicer. Second, the dsRBD swings outward, creating space for recognizing the pre-miRNA stem and the GYM motif. Finally, the PAZ helix is reoriented to anchor the 5' and 3' termini of the pre-miRNA. Once fully docked, the negatively charged backbone of the pre-miRNA lies along the positively charged residues of Dicer. RIIDA and RIIDb then cleave the pre-miRNA.



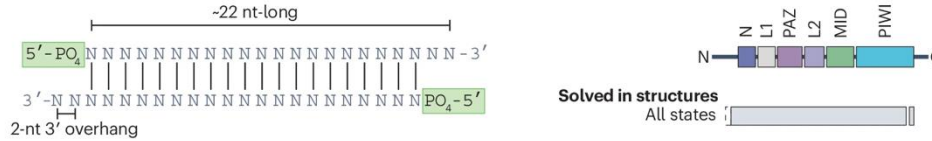
Sequence determinants of pre-miRNA processing



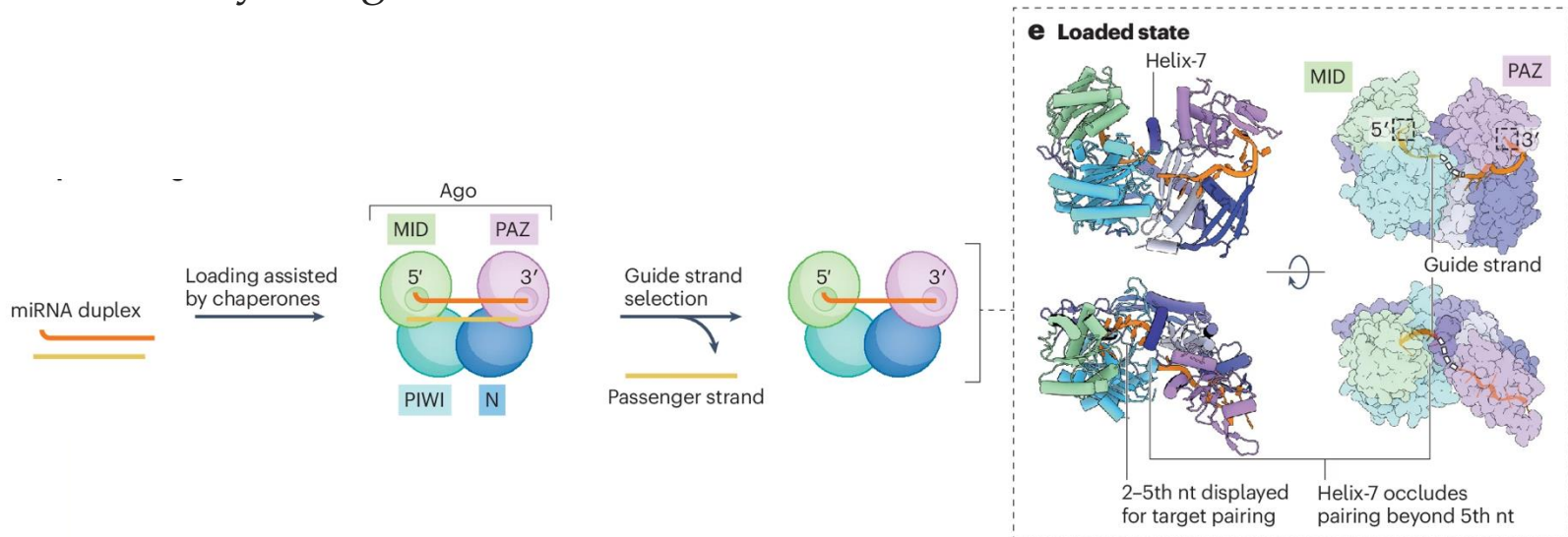
The optimal pre-miRNA structure has a 22-bp stem with a single-stranded apical loop, a 2-nt 3' overhang and a GYM motif. Dicer also recognizes all bases except guanine (H) at the 5' end for cleavage-site decision.

Massively parallel assays on pre-miRNA variants with randomized bases revealed that Dicer recognizes a unique cis-acting sequence element that is crucial for efficient and accurate processing. Importantly, this element, termed the '**GYM motif**' (a paired guanine (G), paired pyrimidine (Y) and mismatched cytosine or adenine (M)) positioned within the cleavage site, is deeply conserved across metazoan lineages. Biochemical and structural analyses have revealed that the dsRBD specifically recognizes the 'M' element of the GYM motif.

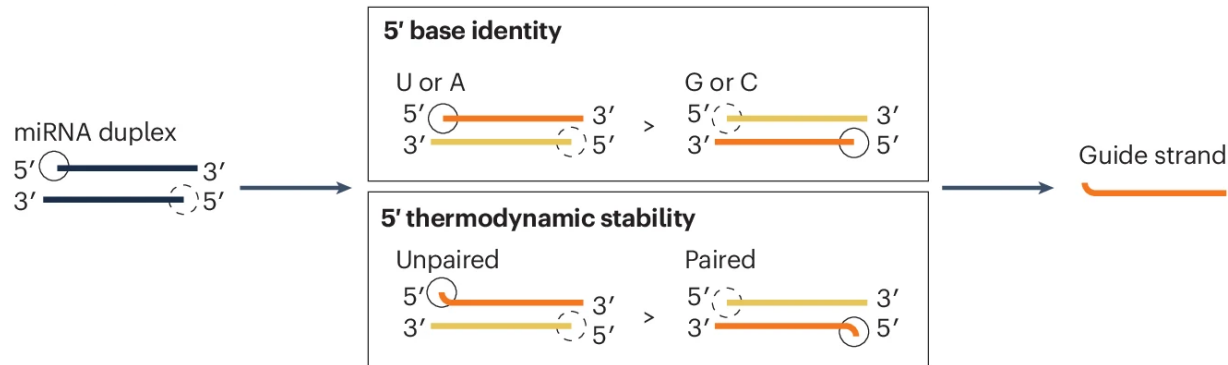
miRNA loading



The Ago proteins are organized into four key domains: the N-terminal (N), PAZ, middle (MID) and PIWI domains. These domains are connected together by two specialized linkers known as L1 and L2. The MID and PAZ domains capture the 5' phosphorylated and 3' ends of miRNAs, respectively. Once the miRNA duplex is loaded onto Ago in a specific orientation, the passenger strand is unwound and expelled by Ago. This unwinding requires mismatches in the central part of the duplex. If a duplex is fully base-paired (as in the case of mir-486 or synthetic siRNAs), the unwinding is dependent on the cleavage of the passenger strand by the slicer activity of Ago2.



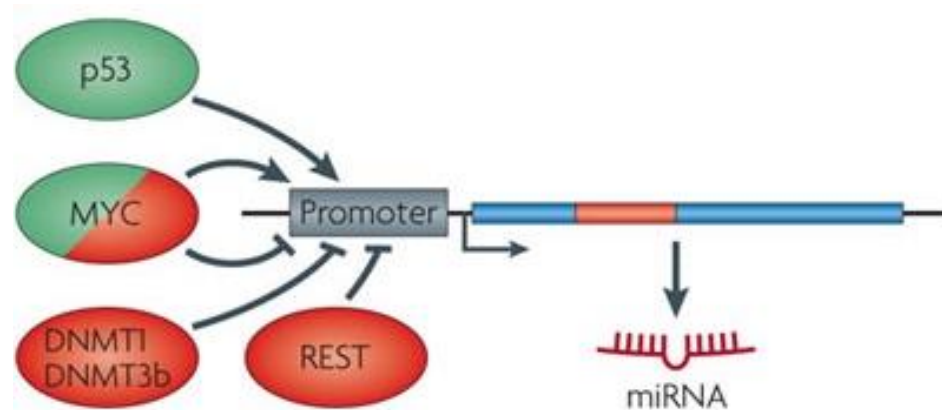
miRNA loading



Strand selection is dictated by two characteristics of the 5' termini of the duplex: base identity and thermodynamic stability. The strand with a U or A at the 5' end is preferred by Ago because the 'nucleotide specificity loop' in the MID domain has charge repulsion to G or C. In addition, the Ago proteins preferentially select the strand with a thermodynamically unstable 5' end as it is more accessible to the MID domain. The mammalian Ago proteins intrinsically recognize the terminal properties of small RNA duplexes. Collectively, these mechanisms ensure the correct orientation of the miRNA for incorporation onto Ago.

Regulation of miRNA gene transcription

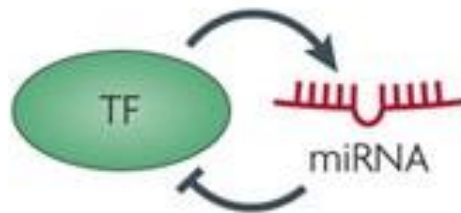
- The promoters of miRNA genes are controlled by transcription factors (TFs), enhancers, silencing elements and chromatin modifications, which is similar to protein-coding genes.
- Many TFs regulate miRNA expression positively or negatively in a tissue-specific or developmental-specific manner



Regulation of miRNA gene transcription

- miRNAs frequently act in regulatory networks with TFs, which can drive or repress the expression of the miRNAs.
- Unilateral or reciprocal-negative feedback loops (single or double loops) result in oscillatory or stable mutually exclusive expression of the TF and miRNA components.

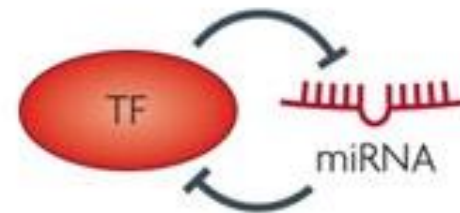
Unilateral negative feedback loops



PITX3
RUNX1
MYB

miR-133b
miR-27a
miR-15a

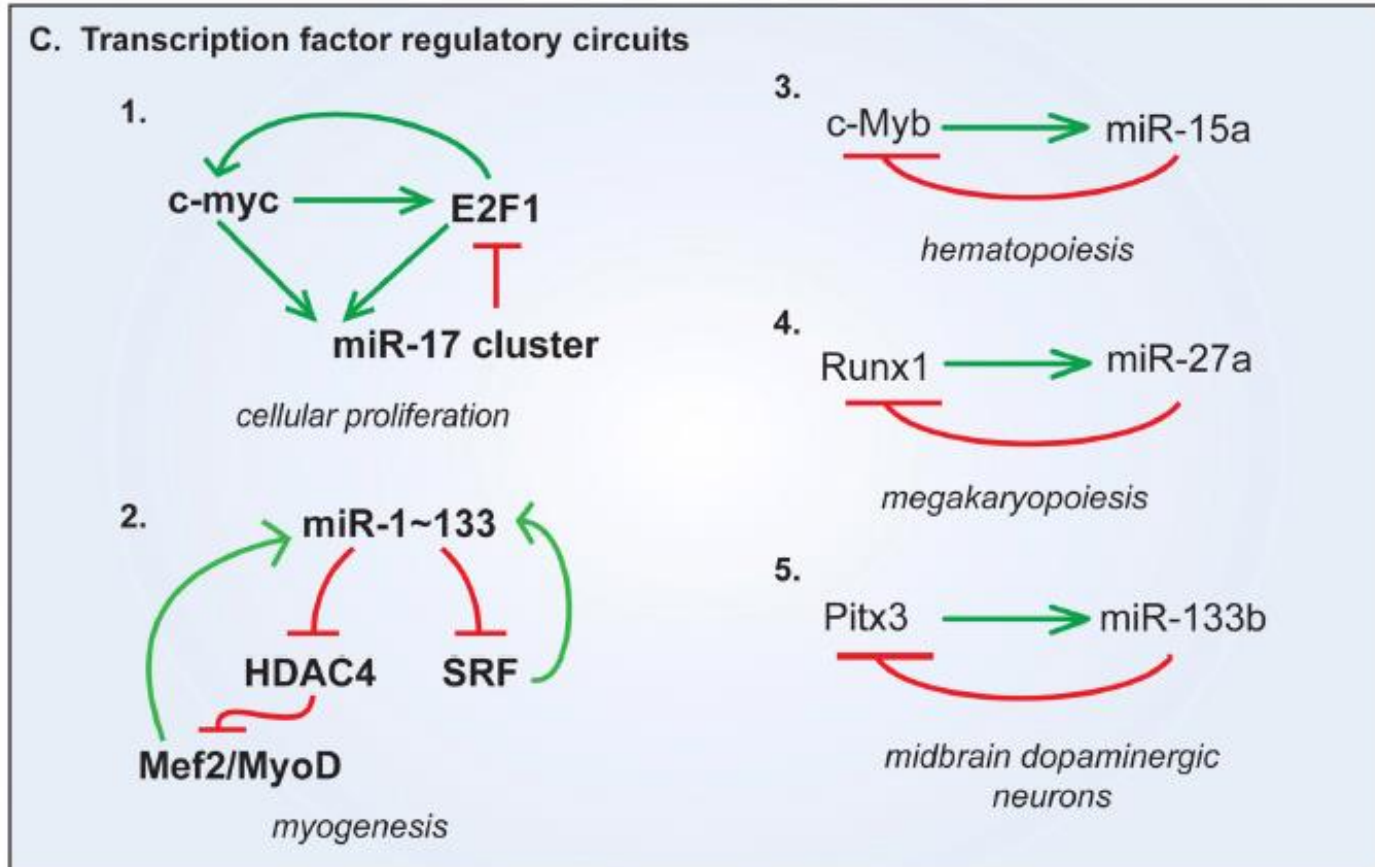
Reciprocal negative feedback loops



HBL-1
YAN
ZEB1

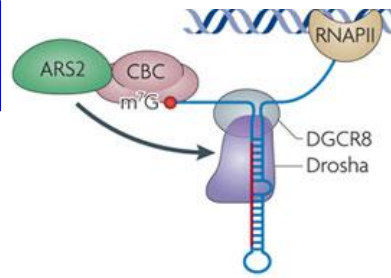
let-7
miR-7
miR-200

Regulatory networks between TFs and microRNAs

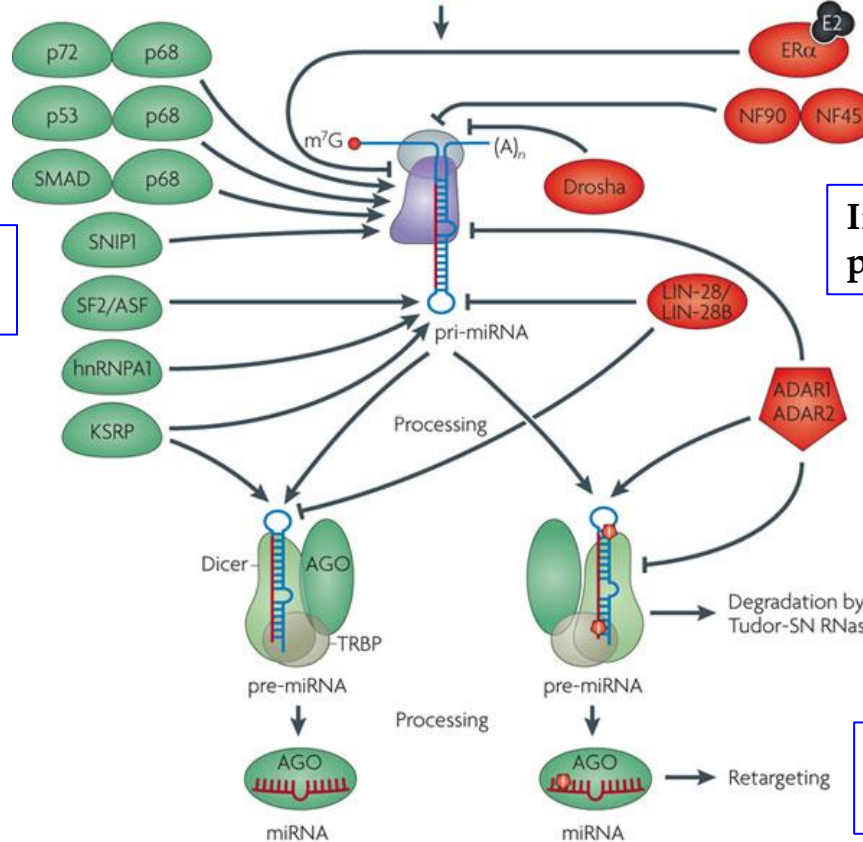


Regulators of miRNA processing

Coupling of pri-miRNA transcription and processing



Stimulation of miRNA processing



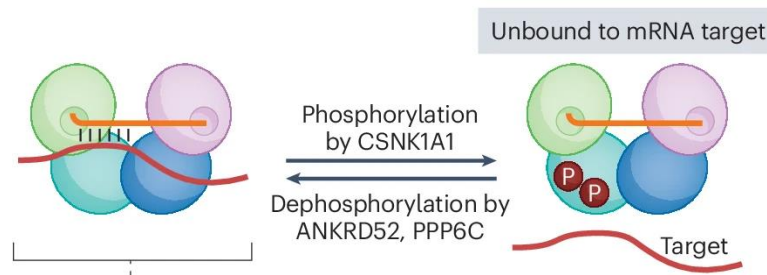
Inhibition of miRNA processing

Degradation

Changing miRNA target specificity

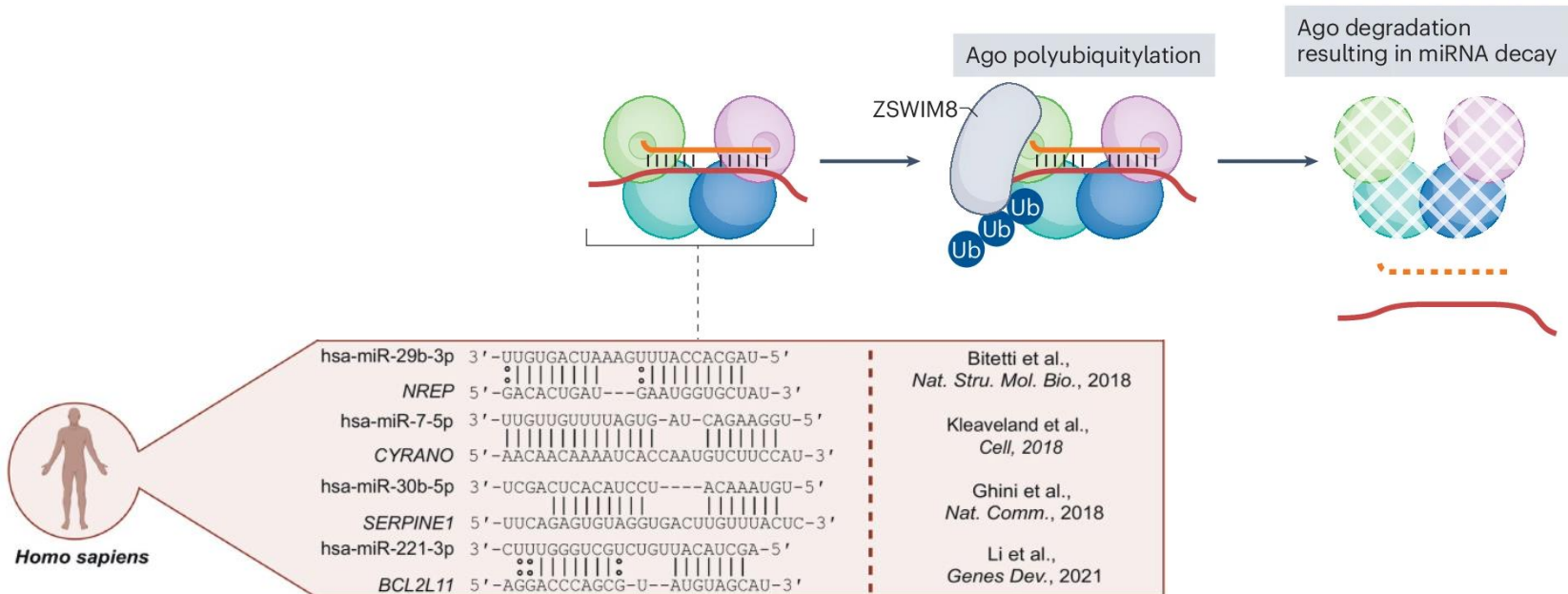
Argonaute post-translational modifications

Ago proteins undergo a range of post-translational modifications that affect their ability to bind to target mRNAs. Most notably, Ago undergoes active phosphorylation and dephosphorylation, which together regulate target binding. When the RISC binds to a target, a set of highly conserved Ser and Thr residues (S824–S834) of Ago are phosphorylated by casein kinase I isoform α (CSNK1A1), leading to inhibition of target binding. By contrast, Ago S824–S834 dephosphorylation by ANKRD52 (also known as PPP6R1) and serine/threonine-protein phosphatase 6 catalytic subunit (PPP6C) can restore target association. Perturbing this process considerably disrupts mRNA targeting, highlighting its pivotal role in maintaining RISC activity.



Argonaute post-translational modifications

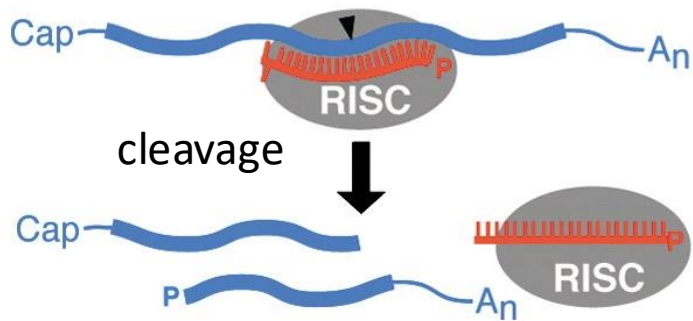
Target mRNAs that form extensive base-pairing with the miRNA trigger **target-directed miRNA decay (TDMD)**. ZSWIM8 recognizes and ubiquitylates (Ub) Ago, leading to Ago degradation and subsequently to the degradation of the miRNA. Certain endogenous and viral transcripts, as well as artificial transcripts, were reported to trigger miRNA decay. A notable example is the decay of miR-7-5p, which is mediated by a lncRNA, *Cyrano*, which contains a site that is extensively paired with miR-7-5p.



3. Mode of action

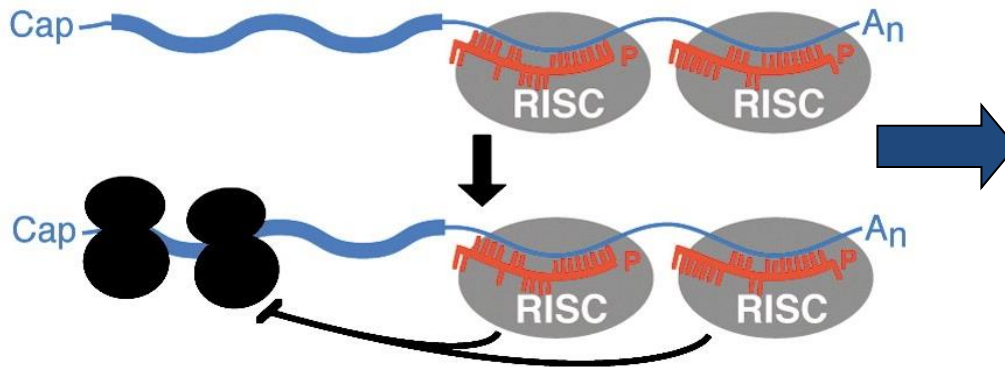
Post-transcriptional repression by microRNAs

A Extensive complementarity in coding region or UTR



mRNA degradation
(plant)

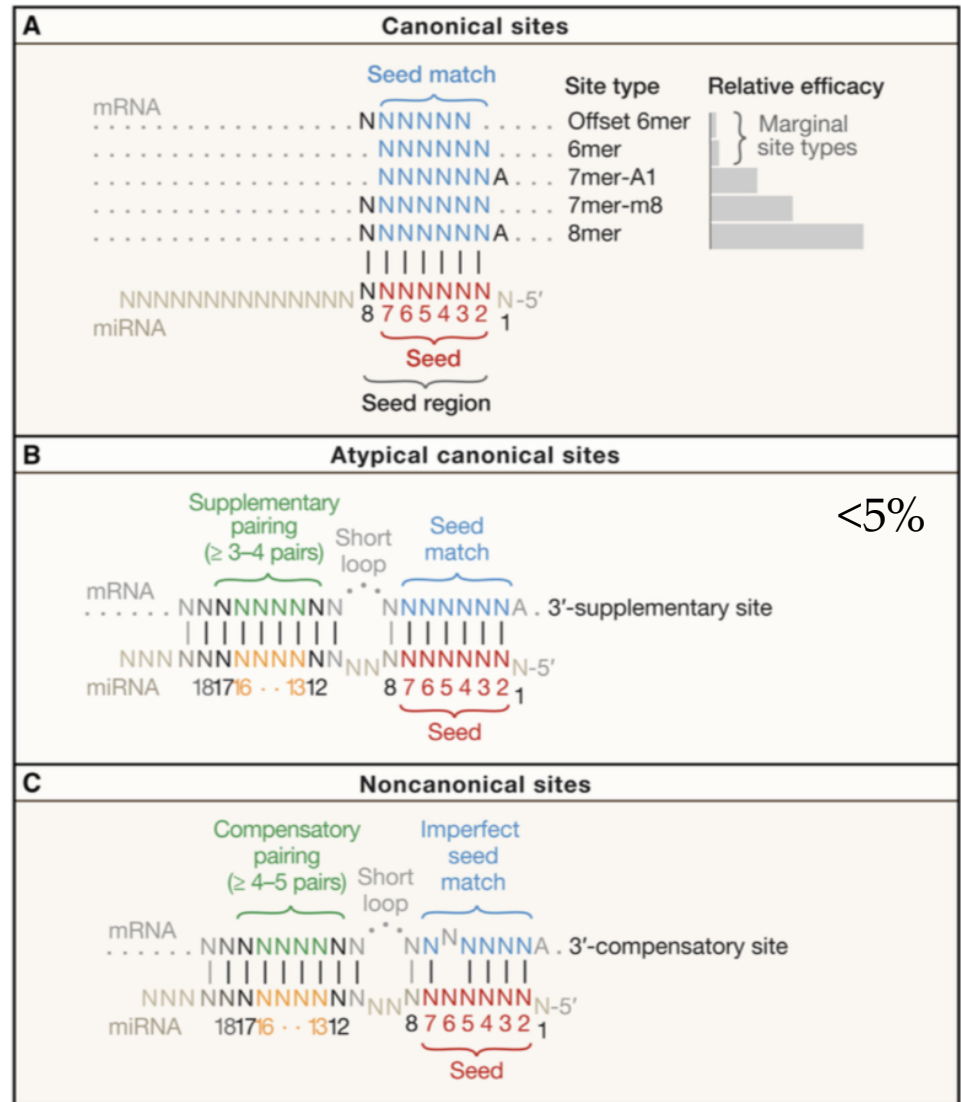
B Short complementary segments in 3'-UTR



Translational Repression/
mRNA degradation
(metazoa)

Post-transcriptional repression by microRNAs

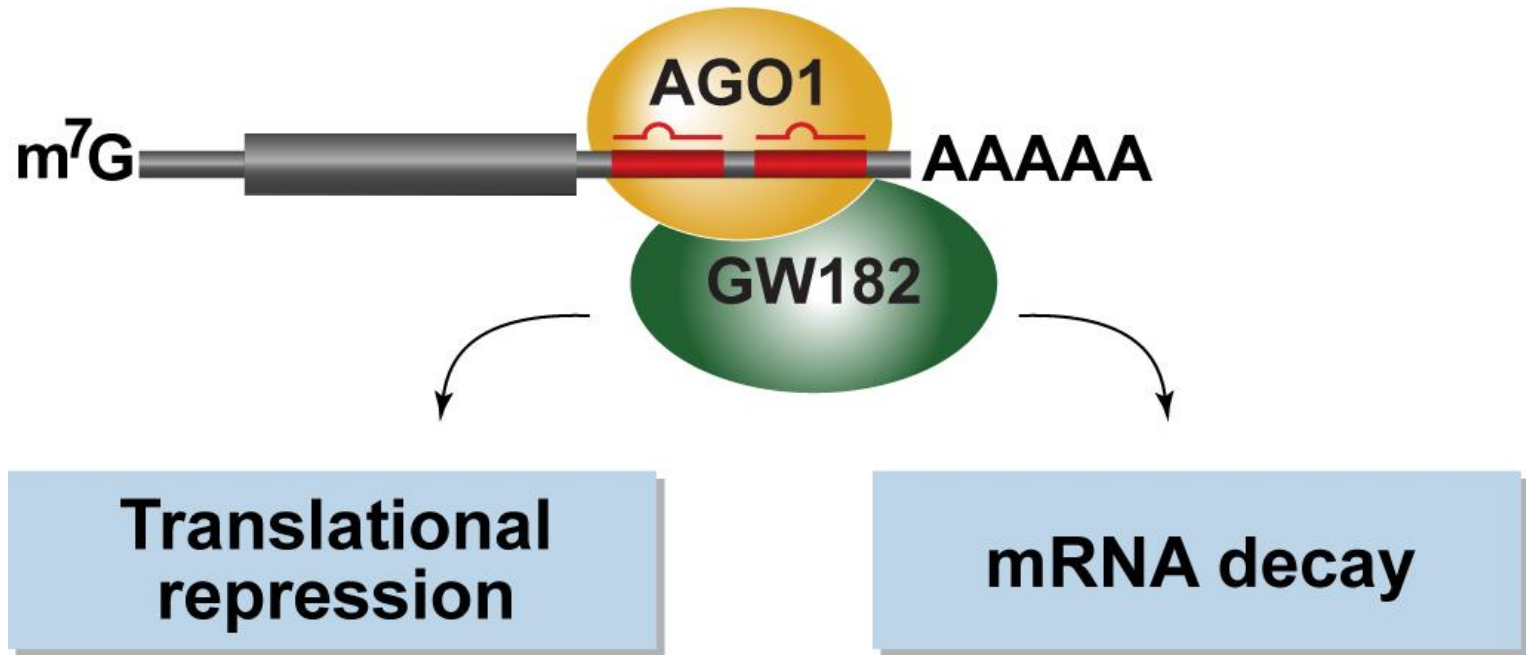
The 5' -sequence is called “seed” region (from the base 2 to base 8 or 9) is particularly important for target site recognition (A). A small fraction of the **canonical sites** (<5%) benefit from pairing to the 3' region of the miRNA (B). The **noncanonical sites** do not have six contiguous Watson-Crick pairs to the seed region (C). Compensating for the imperfect seed match is extensive pairing to the 3' region of the miRNA.



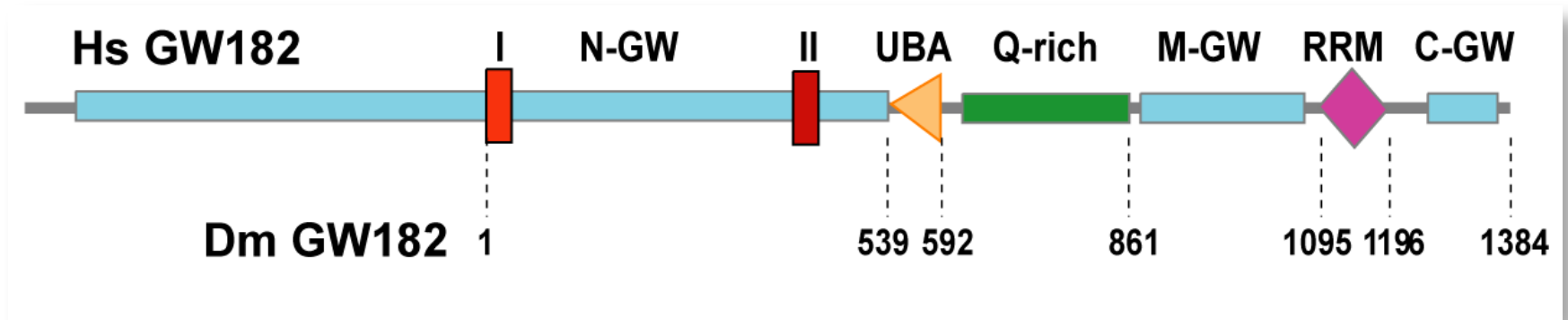
Post-transcriptional repression by microRNAs

- miRNA-mediated target mRNA decay
- miRNA-mediated translational repression

GW182-AGO interaction is essential for silencing



GW182 (TNRC6): a P-body marker in metazoans



UBA: ubiquitin associated domain

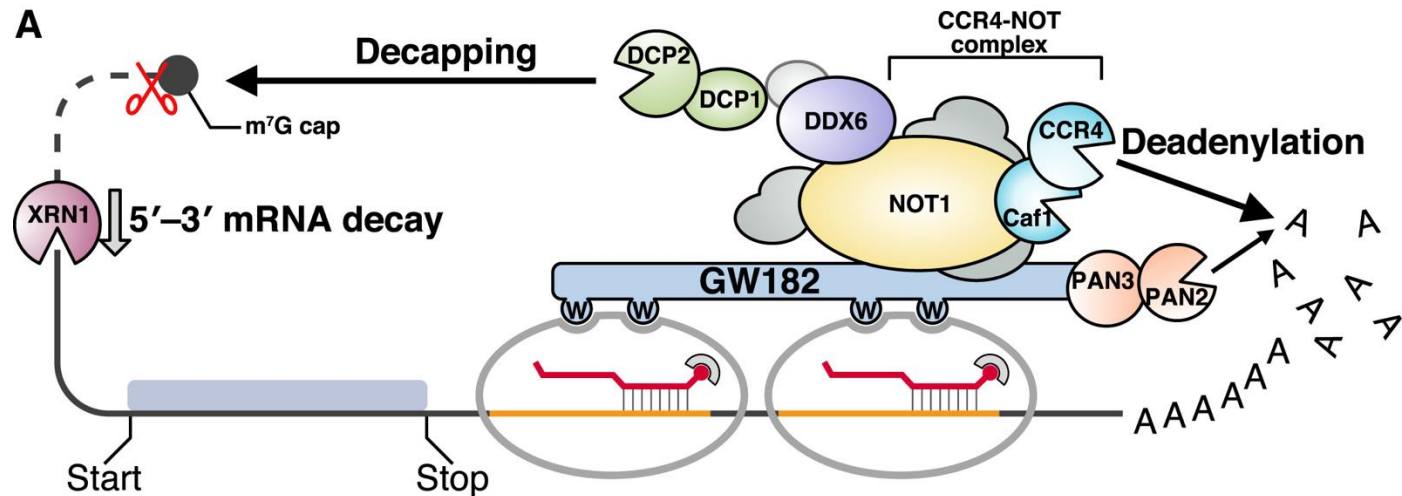
RRM: RNA recognition motif

GW: glycine and tryptophan repeats

I and II: conserved motifs

Identified as an interactor of Ago

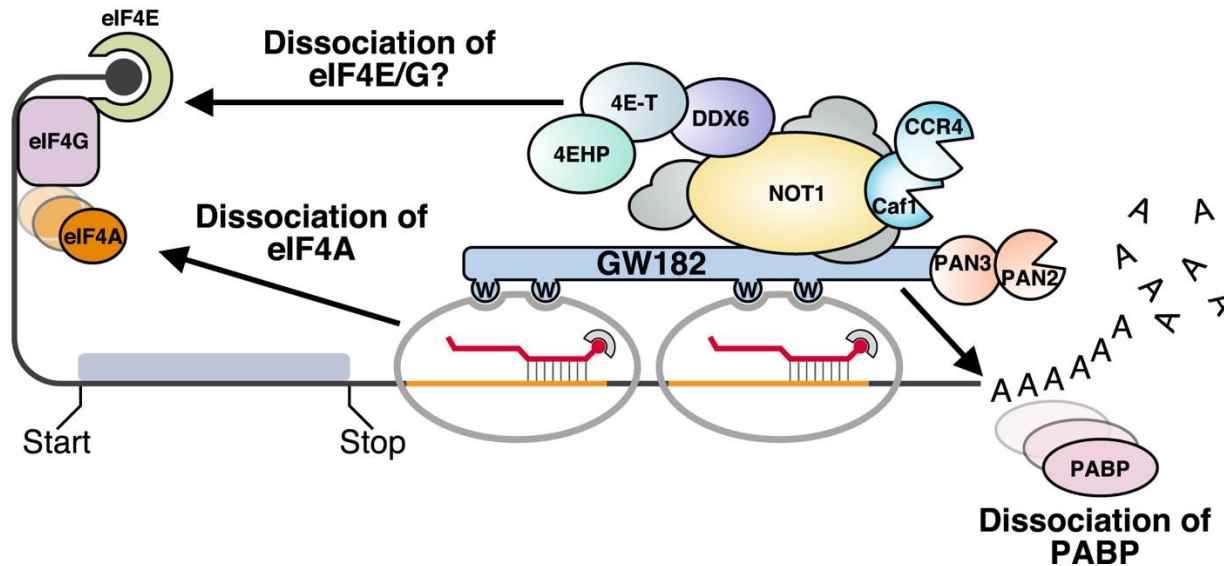
miRNA-mediated target mRNA decay



miRNA-mediated target mRNA decay involves deadenylation, decapping, and exonucleolytic degradation of the target mRNA, which is orchestrated by the GW182 protein

GW182 has an N-terminal domain with GW repeats and a C-terminal silencing domain. The GW repeats of GW182 interact with the tandem tryptophan-binding pockets on the PIWI domain of AGOs. GW182 functions as a scaffold that promotes the dissociation of PABP from the poly(A) tail of the mRNA and instead promotes the recruitment of two deadenylation complexes known as CCR4-NOT and PAN2-PAN3. The recruitment of the CCR4-NOT complex not only deadenylates the target transcript but also serves as a scaffold for the recruitment of decapping factors.

miRNA-mediated translational repression

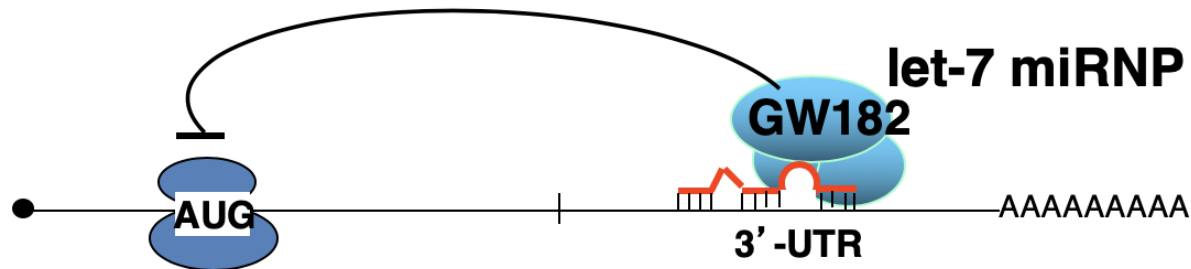


Several mechanisms for the repression of translation initiation have been proposed, including PABP displacement, recruitment of translation inhibitors targeting eIF4E or eIF4G, and eIF4A dissociation.

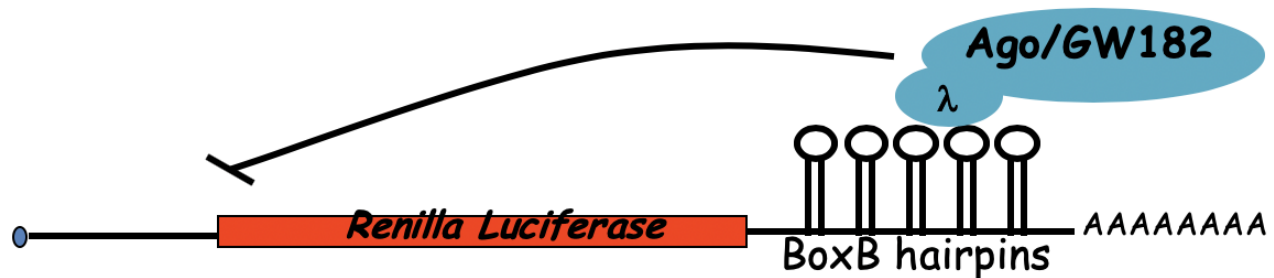
NOT1, a component of the CCR4-NOT complex that associates with RISC via GW182, interacts with the translational inhibitor and decapping enhancer DDX6. This interaction promotes miRNA-mediated repression even when deadenylation is blocked, suggesting that DDX6 is involved in miRNA-mediated translational repression as well as mRNA decay. 4E-T, an eIF4E-binding protein that interacts with DDX6, and the cap-binding protein 4EHP (eIF4E2) are involved in miRNA-mediated translational repression.

mRNA reporters to study miRNA-mediated repression

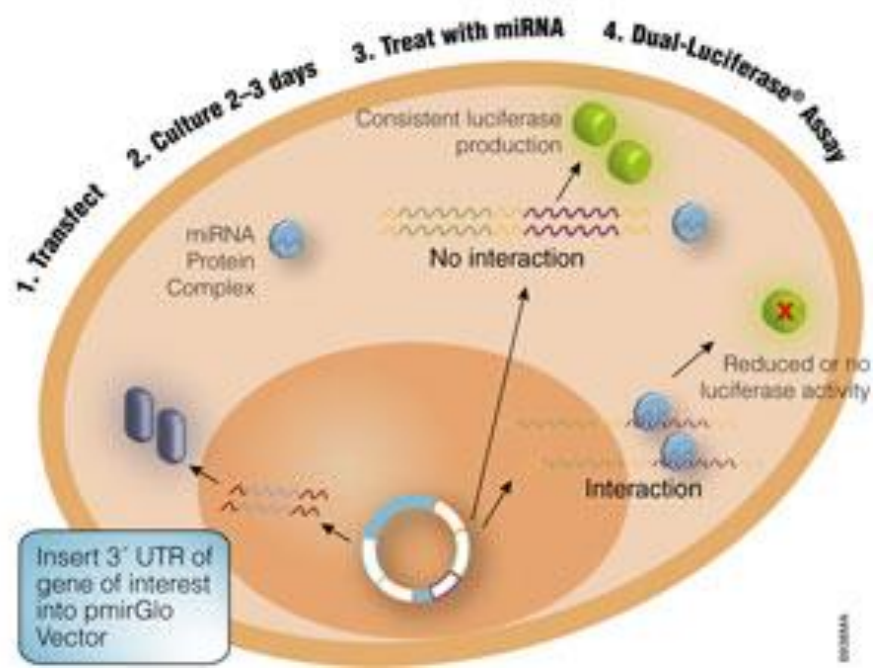
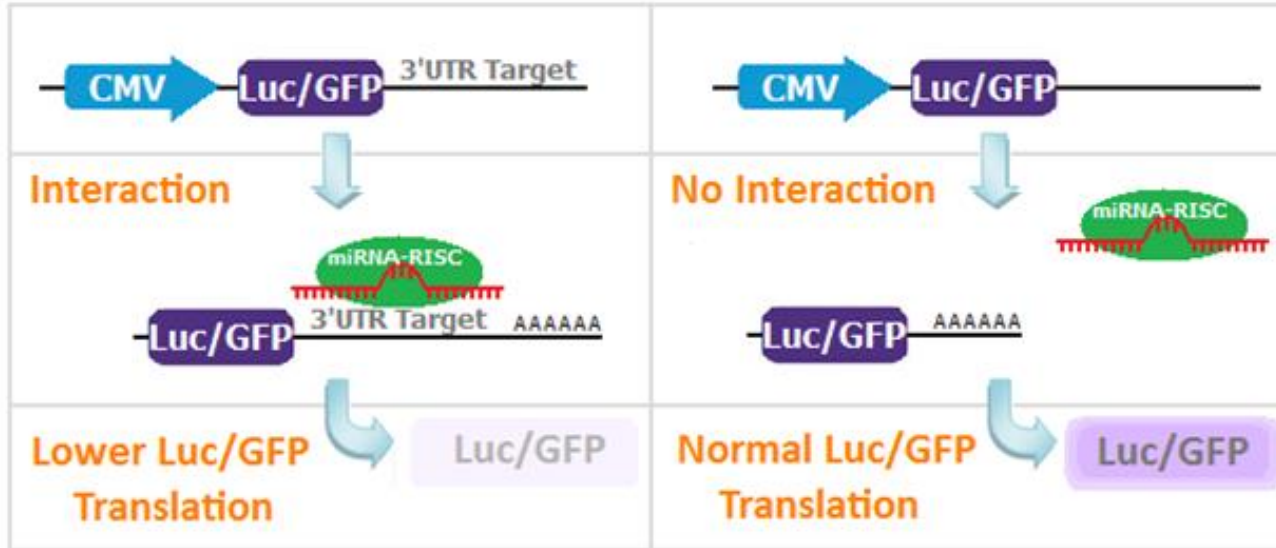
- Reporters responding to the endogenous let-7 miRNP



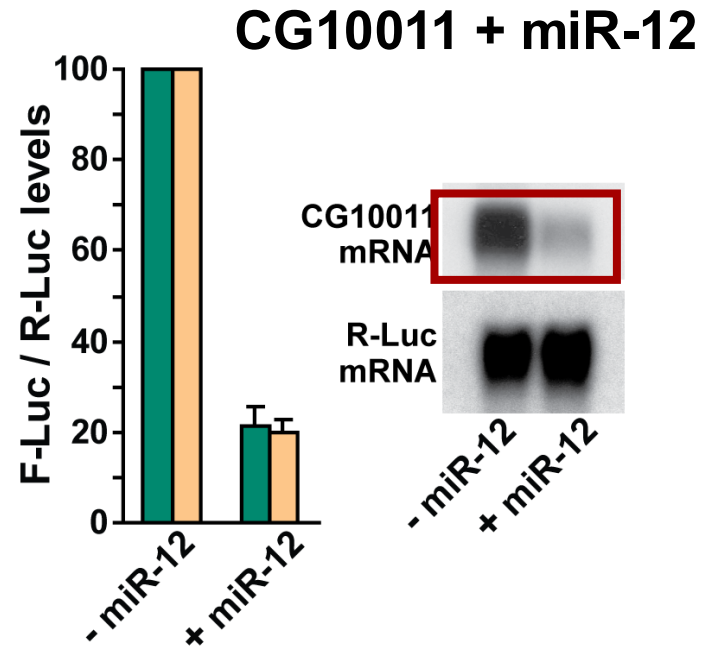
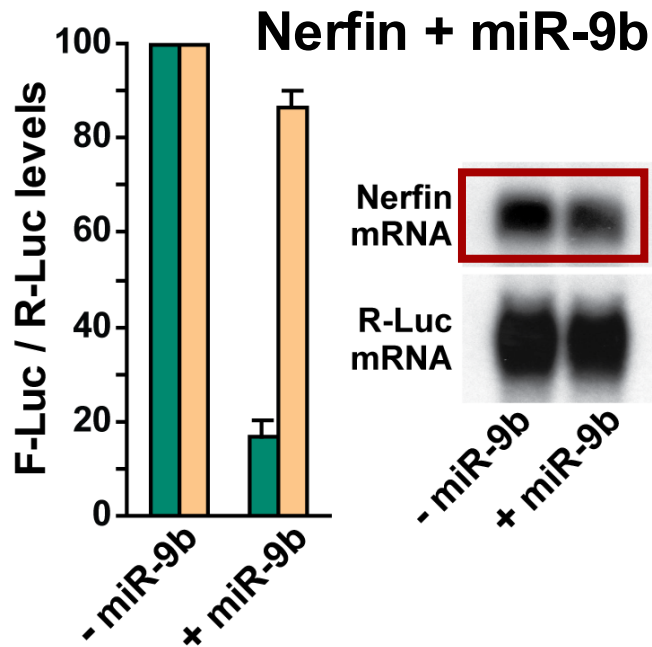
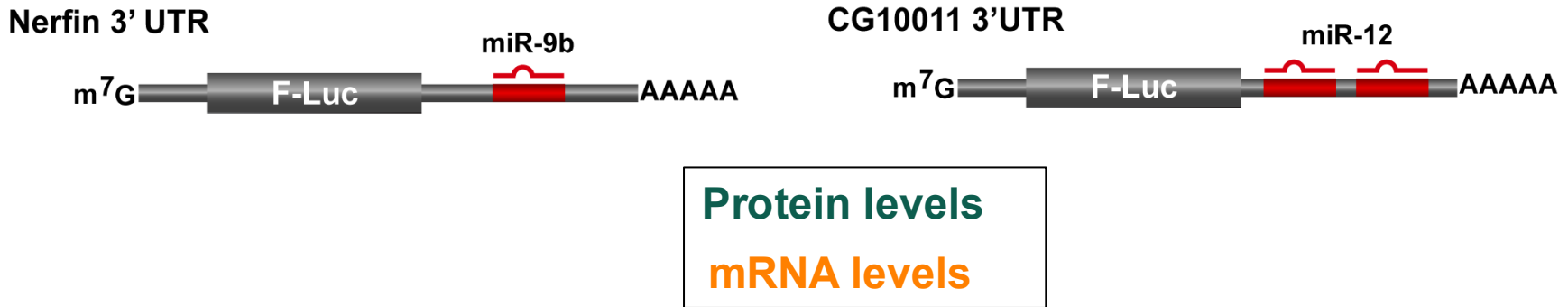
- Reporters responding to the miRNP protein tethering



RNA reporters to verify microRNA targets



Silencing by miRNAs involves translational repression and / or mRNA decay



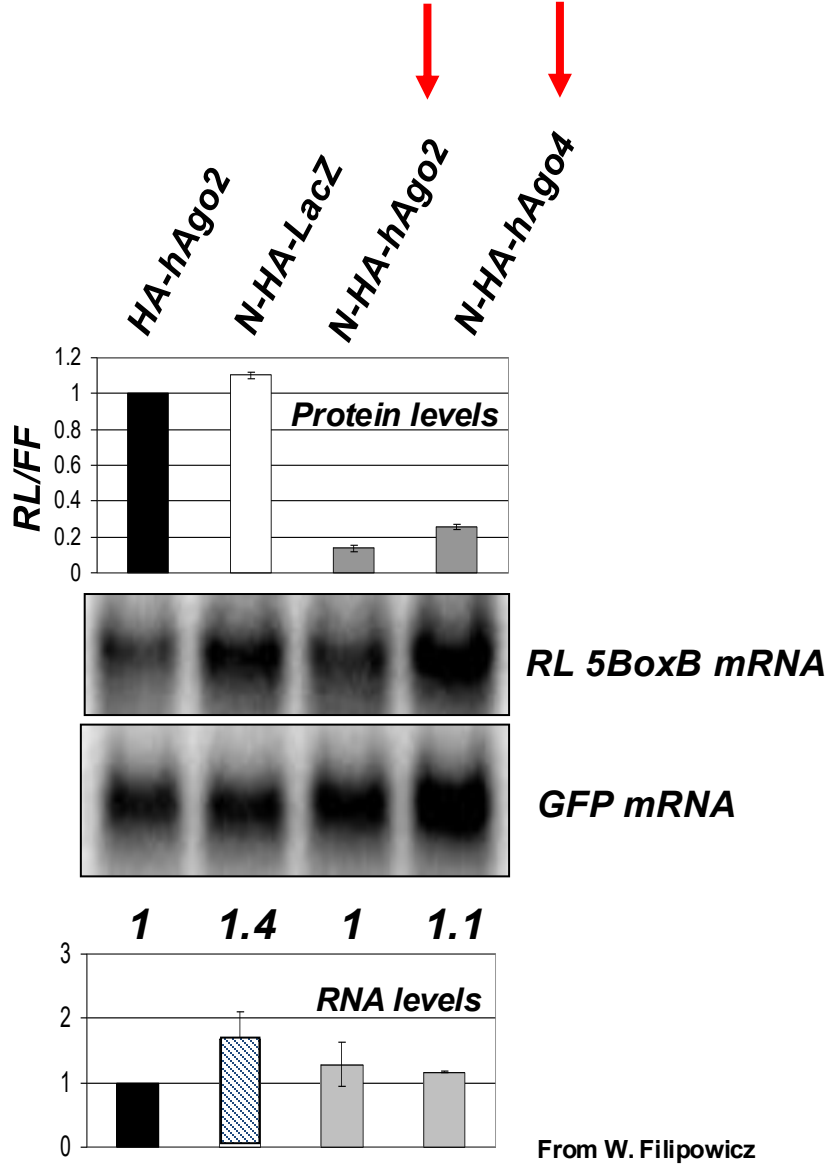
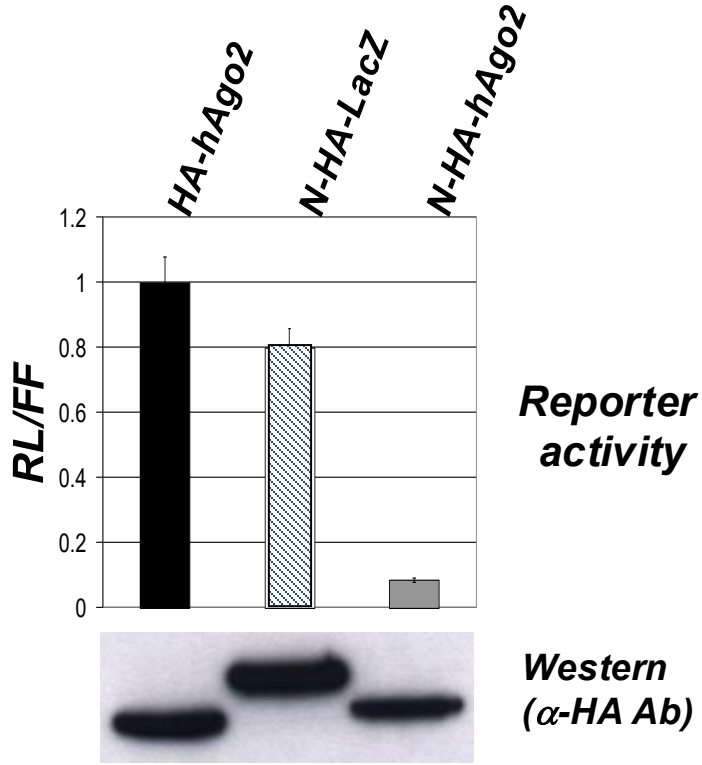
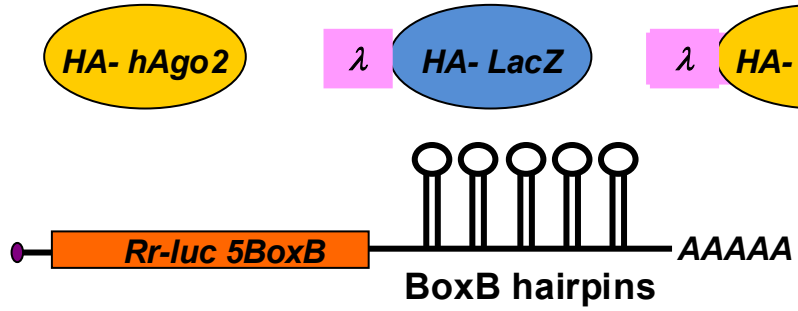
Slicer independent miRNA-mediated decay

- Introducing a specific miRNA into a cell decreases the levels of transcripts with potential binding sites for the miRNA (Lim et al., 2005; Giraldez et al., 2006).

- ✓mRNA with **rapid decay rates** may appear to be solely **translationally repressed** since the mRNA turnover is already fast. In contrast, **long-lived mRNAs** may be more susceptible to an **increase in decay rates** by miRNA repression.

- ✓Translation and general mRNA decay can be differentially regulated in response to stresses or developmental stages. RNA modifications and RNA binding proteins might affect whether miRNAs trigger translation repression or degradation.

Tethering of human Argonautes to the reporter mRNA mimics the effect of miRNAs



GW182 is required for silencing by miRNAs

Nerfin 3' UTR



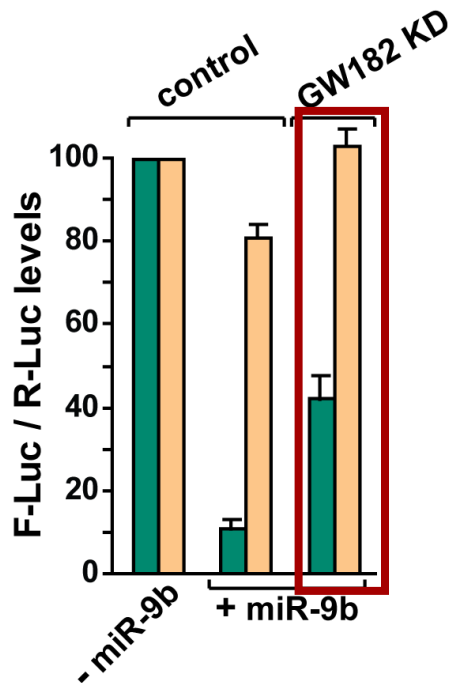
CG10011 3'UTR



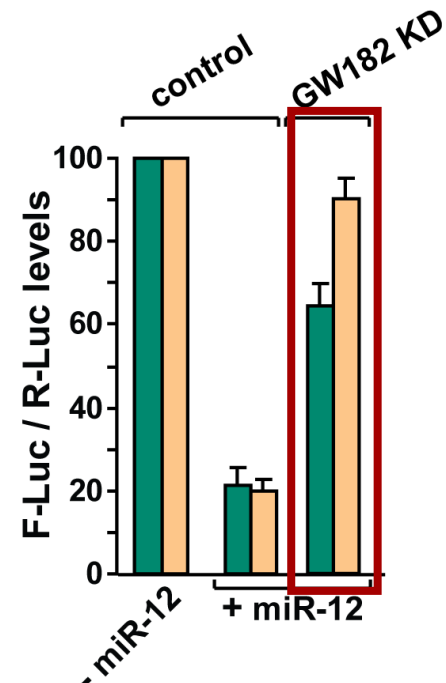
Protein levels

mRNA levels

Nerfin + miR-9b

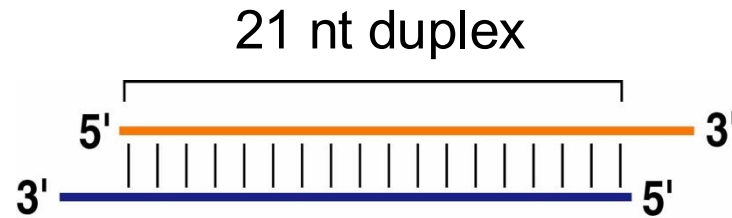


CG10011 + miR-12

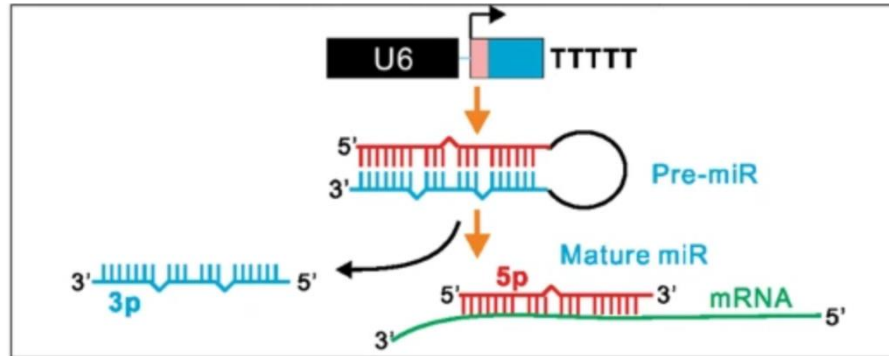


miRNA expression system

- RNA duplex

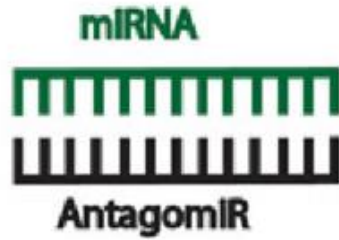


- Pol III vector



miRNA inhibitors

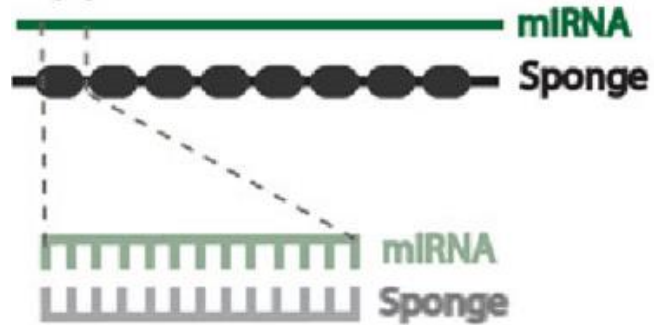
(A)



(B)



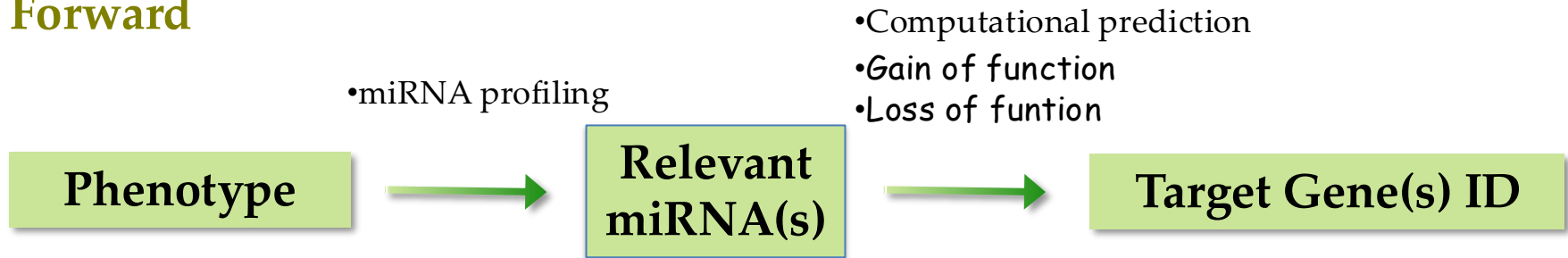
(C)



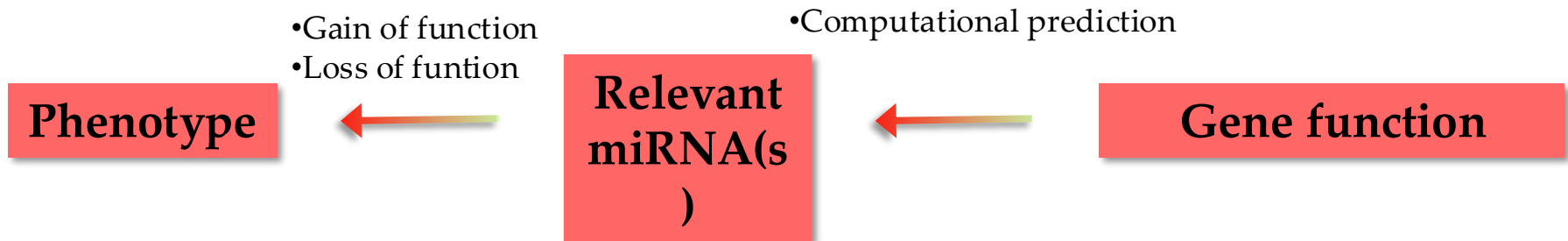
4-Target identification

Target identification

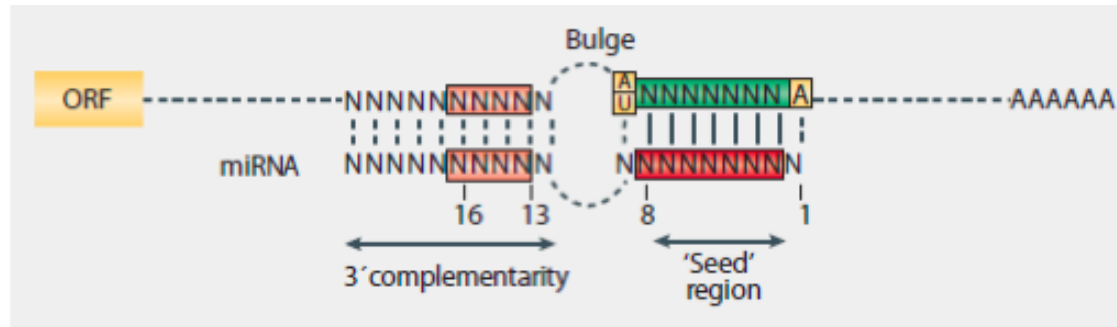
1) Forward



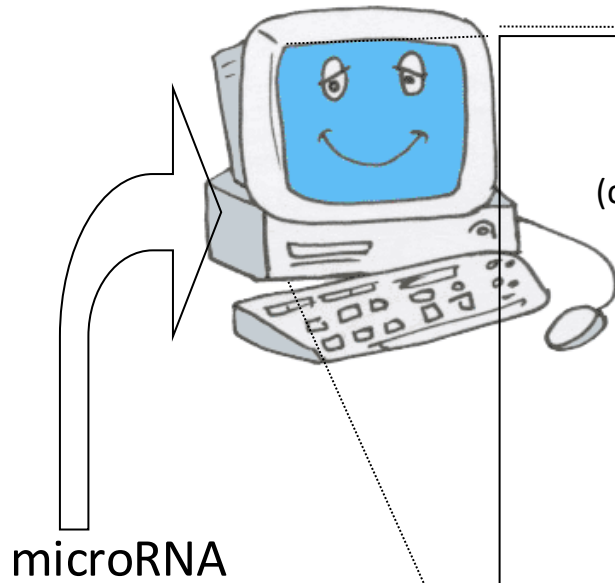
2) Reverse



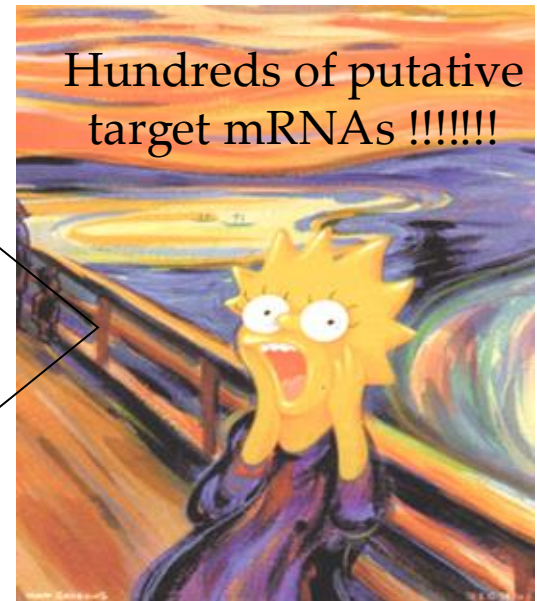
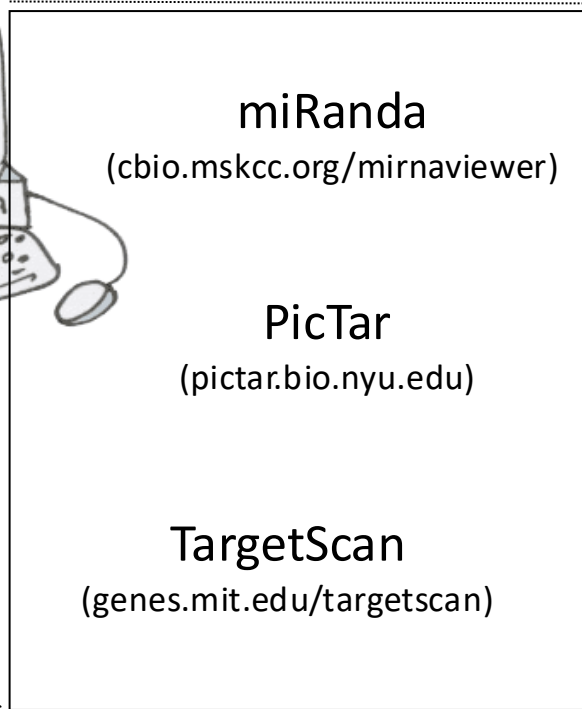
Identification of microRNA targets



1) Forward



microRNA



Identification of microRNA targets

miR-223 targets

Human | miR-223

311 conserved targets, with a total of 321 conserved sites and 80 poorly conserved sites.

Table sorted by total context score [Sort table by aggregate P_{CT}]

GetTable sorted by total context score rved sites are not shown [View top predicted targets, irrespective of site conservation]

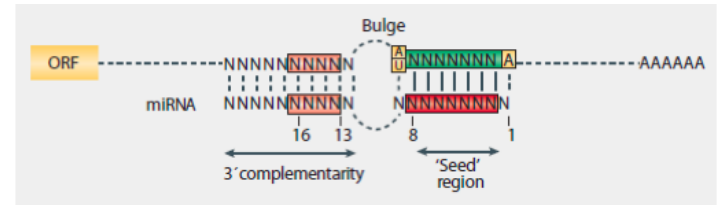
The table shows at most one transcript per gene, selected for having the highest aggregate P_{CT} (or the one with the longest 3' UTR, in case of a tie). [Show all transcripts]

Target gene	Representative transcript	Gene name	Conserved sites				Poorly conserved sites				Representative miRNA	Total context+ score	Aggregate P _{CT}	Previous TargetScan publication(s)	Links to sites in UTRs
			total	8mer	7mer-m8	7mer-1A	total	8mer	7mer-m8	7mer-1A					
FBXW7	NM_001013415	F-box and WD repeat domain containing 7	3	3	0	0	1	0	0	1	hsa-miR-223	-1.05	0.87	2003, 2005, 2007, 2009	Sites in UTR
SP3	NM_001017371	Sp3 transcription factor	1	1	0	0	1	1	0	0	hsa-miR-223	-0.78	0.47	2005, 2007, 2009	Sites in UTR
PAX6	NM_000280	paired box 6	1	1	0	0	1	0	1	0	hsa-miR-223	-0.66	0.47		Sites in UTR
C13orf31	NM_001128303	chromosome 13 open reading frame 31	1	1	0	0	1	0	1	0	hsa-miR-223	-0.62	0.32		Sites in UTR
PURB	NM_033224	purine-rich element binding protein B	1	1	0	0	1	1	0	0	hsa-miR-223	-0.57	0.59	2005, 2007, 2009	Sites in UTR
SYNRG	NM_001163544	synergin, gamma	1	1	0	0	1	1	0	0	hsa-miR-223	-0.56	0.55	2009	Sites in UTR
RHOB	NM_004040	ras homolog gene family, member B	1	1	0	0	1	0	1	0	hsa-miR-223	-0.56	0.52	2007, 2009	Sites in UTR
APC	NM_000038	adenomatous polyposis coli	1	0	1	0	1	1	0	0	hsa-miR-223	-0.55	0.28	2009	Sites in UTR
ECT2	NM_018098	epithelial cell transforming sequence 2 oncogene	1	0	1	0	1	0	1	0	hsa-miR-223	-0.54	0.36	2009	Sites in UTR
PRDM1	NM_001198	PR domain containing 1, with ZNF domain	1	0	1	0	2	0	1	1	hsa-miR-223	-0.53	0.41	2005, 2007	Sites in UTR
GALNTL4	NM_198516	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 4	1	1	0	0	0	0	0	0	hsa-miR-223	-0.53	0.46		Sites in UTR
RIBC1	NM_144968	RIB43A domain with coiled-coils 1	1	1	0	0	1	1	0	0	hsa-miR-223	-0.53	0.34	2009	Sites in UTR
SEPT6	NM_015129	septin 6	1	1	0	0	1	0	1	0	hsa-miR-223	-0.52	0.51	2005, 2007, 2009	Sites in UTR
SLC8A1	NM_001112800	solute carrier family 8 (sodium/calcium exchanger), member 1	1	1	0	0	1	0	0	1	hsa-miR-223	-0.51	0.57	2005, 2007, 2009	Sites in UTR
SLC4A4	NM_001098484	solute carrier family 4, sodium bicarbonate cotransporter, member 4	1	1	0	0	1	0	1	0	hsa-miR-223	-0.50	0.48	2009	Sites in UTR
ADCY7	NM_001114	adenylate cyclase 7	1	0	1	0	2	0	1	1	hsa-miR-223	-0.48	0.61	2009	Sites in UTR
SLC37A3	NM_032295	solute carrier family 37 (glycerol-3-phosphate transporter), member 3	2	0	1	1	0	0	0	0	hsa-miR-223	-0.47	0.68	2005, 2007, 2009	Sites in UTR
OLFM1	NM_006334	olfactomedin 1	1	1	0	0	0	0	0	0	hsa-miR-223	-0.45	0.40		Sites in UTR
SEPT10	NM_144710	septin 10	1	1	0	0	0	0	0	0	hsa-miR-223	-0.44	0.46	2009	Sites in UTR
ATP7A	NM_000052	ATPase, Cu++ transporting, alpha polypeptide	1	1	0	0	1	0	0	1	hsa-miR-223	-0.44	0.57	2005, 2007, 2009	Sites in UTR

.....more

Identification of microRNA targets

Let7/4458/4500 family (share same seed region)



Human l let-7/98/4458/4500

1072 conserved targets, with a total of 1203 conserved sites and 162 poorly conserved sites.

Table sorted by total context score [Sort table by aggregate P_{CT}]

Genes with only poorly conserved sites are not shown [View top predicted targets, irrespective of site conservation]

The table shows at most one transcript per gene, selected for having the highest aggregate P_{CT} (or the one with the longest 3' UTR, in case of a tie).

[Show all transcripts]

Target gene	Representative transcript	Gene name	Conserved sites				Poorly conserved sites				Representative miRNA	Total context+ score	Aggregate P _{CT}	Previous TargetScan publication(s)	Links to sites in UTRs
			total	8mer	7mer-m8	7mer-1A	total	8mer	7mer-m8	7mer-1A					
C14orf28	NM_001017923	chromosome 14 open reading frame 28	3	2	1	0	1	0	0	1	hsa-let-7g	-1.25	> 0.99	2007, 2009	Sites in UTR
FIGNL2	NM_001013690	fidgetin-like 2	3	3	0	0	0	0	0	0	hsa-miR-4458	-1.07	> 0.99	2009	Sites in UTR
HMGA2	NM_003483	high mobility group AT-hook 2	6	1	3	2	1	0	0	1	hsa-miR-4458	-1.04	> 0.99	2005, 2007, 2009	Sites in UTR
LIN28B	NM_001004317	lin-28 homolog B (C. elegans)	4	2	2	0	1	0	0	1	hsa-let-7d	-0.98	> 0.99	2007, 2009	Sites in UTR
TRIM71	NM_001039111	tripartite motif containing 71	2	2	0	0	0	0	0	0	hsa-miR-4458	-0.89	> 0.99	2007, 2009	Sites in UTR
IGDCC3	NM_004884	immunoglobulin superfamily, DCC subclass, member 3	3	3	0	0	1	0	1	0	hsa-miR-4500	-0.72	> 0.99	2003, 2007, 2009	Sites in UTR
ARID3B	NM_006465	AT rich interactive domain 3B (BRIGHT-like)	5	0	1	4	0	0	0	0	hsa-let-7i	-0.72	> 0.99	2005, 2007, 2009	Sites in UTR
STARD9	NM_020759	StAR-related lipid transfer (START) domain containing 9	1	0	0	1	3	0	2	1	hsa-miR-4458	-0.70	0.79		Sites in UTR
PTAFR	NM_000952	platelet-activating factor receptor	3	2	1	0	0	0	0	0	hsa-miR-4500	-0.69	0.94		Sites in UTR
THrsp	NM_003251	thyroid hormone responsive	1	1	0	0	1	0	1	0	hsa-let-7d	-0.62	0.74	2009	Sites in UTR
SMARCA1	NM_001128429	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1												2005, 2007	
YOD1	NM_018566	YOD1 OTU deubiquinating enzyme 1 homolog (S. cerevisiae)													
FIGN	NM_018086	fidgetin													
TLL4	NM_014640	tubulin tyrosine ligase-like family, member 4													
PRTG	NM_173814	protogenin													
C9orf40	NM_017998	chromosome 9 open reading frame 40													
SERF2	NM_001018108	small EDRK-rich factor 2													
KCTD21	NM_001029859	potassium channel tetramerisation domain containing 21													
PXT1	NM_152990	peroxisomal, testis specific 1													
ARHGEF38	NM_001242729	Rho guanine nucleotide exchange factor (GEF) 38													
DNA2	NM_001080449	DNA replication helicase 2 homolog (yeast)													

miRNA-Target Interaction Types

8mer: Indicates a perfect match of 8 consecutive nucleotides between the miRNA and the target mRNA. Specifically, it involves nucleotides 2–8 of the miRNA (the seed region) and an adenine (A) at position 1 of the target. This is the strongest and most effective type of interaction for inhibiting mRNA.

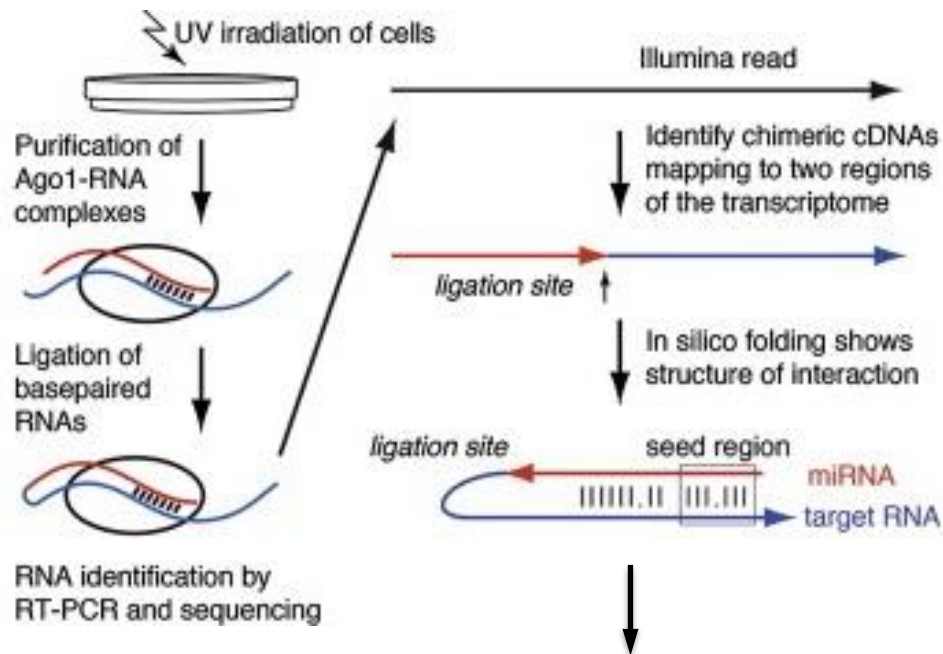
7mer: Indicates a perfect match of 7 consecutive nucleotides. There are two main types, both of which are also highly effective:

7mer-m8: An exact match across nucleotides 2–8 (the seed + position 8).

7mer-A1: An exact match across nucleotides 2–7 (seed only) followed by an adenine (A) at position 1.




Identification of microRNA targets

Experimental identification by crosslinking, ligation, and sequencing of hybrids (CLASH):



Less than 50% of targets showed full seed complementarity, the rest can not be predicted by microRNA/target prediction programmes.

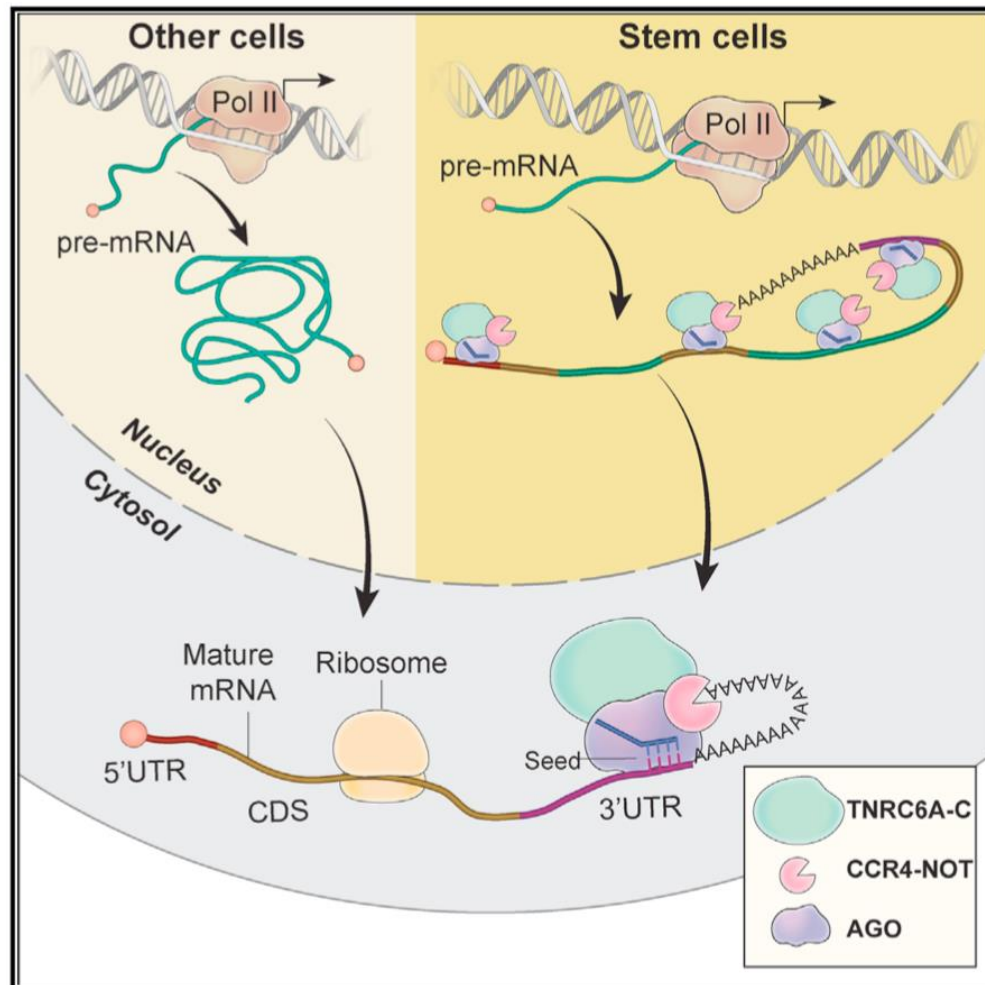
A novel class of microRNA-recognition elements that function only within open reading frames

Kai Zhang^{1,8}, Xiaorong Zhang^{2,8}, Zhiqiang Cai^{1,8}, Jie Zhou^{1,8}, Ran Cao¹, Ya Zhao^{3,4}, Zonggui Chen¹, Dehe Wang¹, Wen Ruan¹, Qian Zhao², Guangqiao Liu², Yuanchao Xue², Yan Qin², Bing Zhou ⁵, Ligang Wu⁴, Timothy Nilsen⁶, Yu Zhou ^{1,7*} and Xiang-Dong Fu ^{1,2,5*}

MicroRNAs (miRNAs) are well known to target 3' untranslated regions (3' UTRs) in mRNAs, thereby silencing gene expression at the post-transcriptional level. Multiple reports have also indicated the ability of miRNAs to target protein-coding sequences (CDS); however, miRNAs have been generally believed to function through similar mechanisms regardless of the locations of their sites of action. Here, we report a class of miRNA-recognition elements (MREs) that function exclusively in CDS regions. Through functional and mechanistic characterization of these 'unusual' MREs, we demonstrate that CDS-targeted miRNAs require extensive base-pairing at the 3' side rather than the 5' seed; cause gene silencing in an Argonaute-dependent but GW182-independent manner; and repress translation by inducing transient ribosome stalling instead of mRNA destabilization. These findings reveal distinct mechanisms and functional consequences of miRNAs that target CDS versus the 3' UTR and suggest that CDS-targeted miRNAs may use a translational quality-control-related mechanism to regulate translation in mammalian cells.

Argonaute-miRNA Complexes Silence Target mRNAs in the Nucleus of Mammalian Stem Cells

Graphical Abstract



Authors

Aishe A. Sarshad, Aster H. Juan, Ana Iris Correa Muler, ..., Astrid D. Haase, Vittorio Sartorelli, Markus Hafner

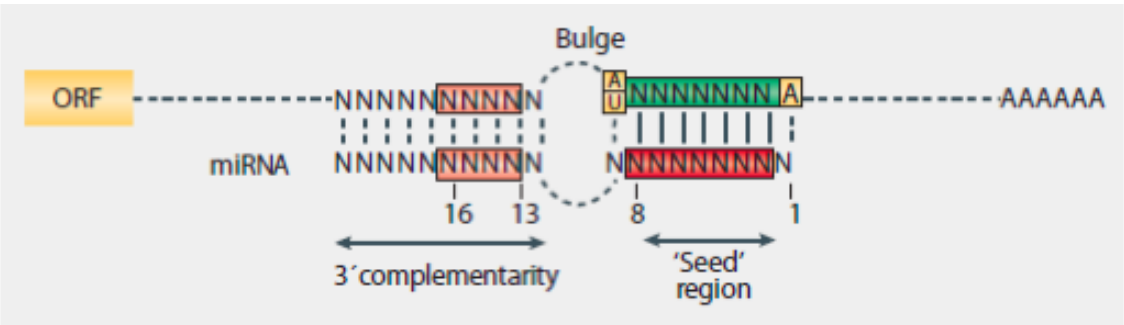
Correspondence

sartorev@mail.nih.gov (V.S.), markus.hafner@nih.gov (M.H.)

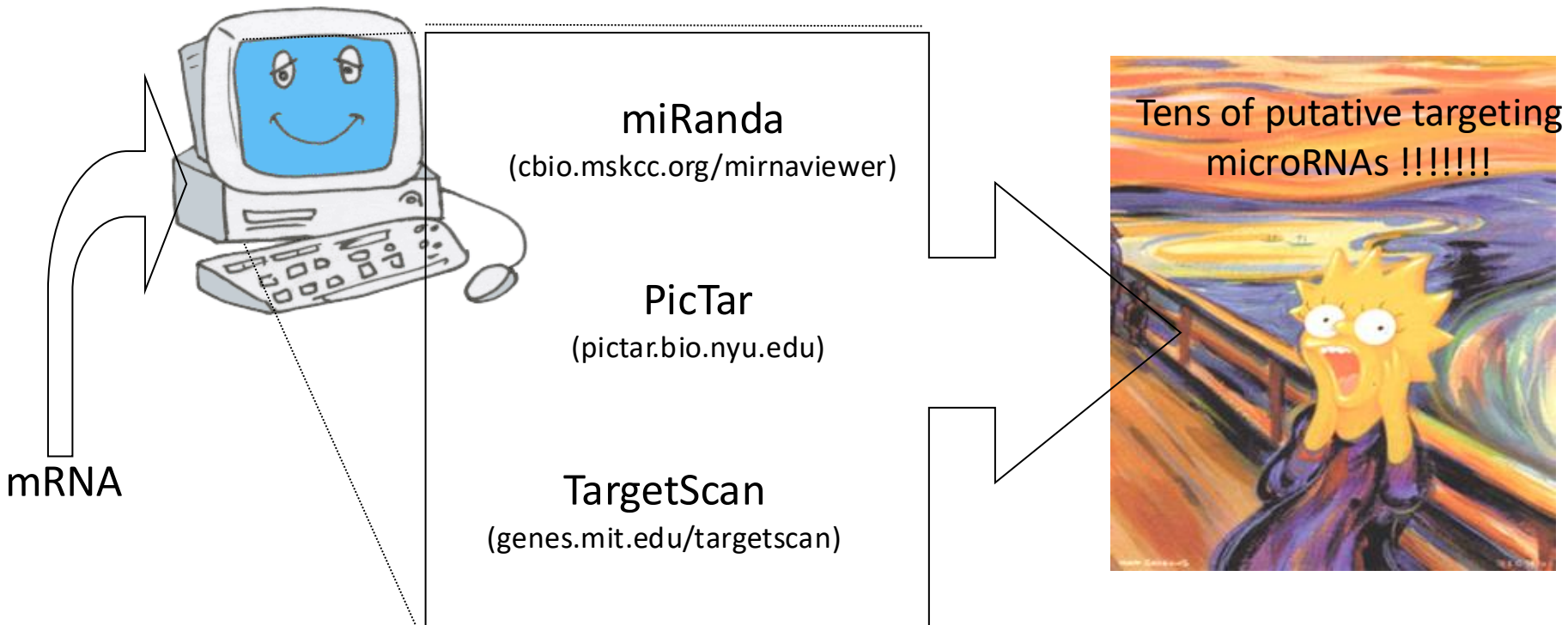
In Brief

In stem cells, half of the AGO proteins are found in the nucleus and allow for the onset of ESC differentiation. Nuclear AGO assembles functional RISC complexes to silence target RNAs in a miRNA- and CCR4-NOT-dependent manner at target sites that include intronic and coding sequences.

Identification of microRNAs targeting specific mRNAs

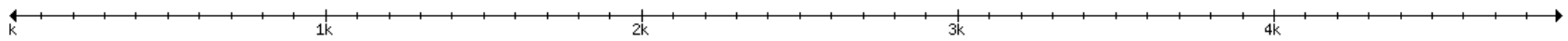


2) Reverse



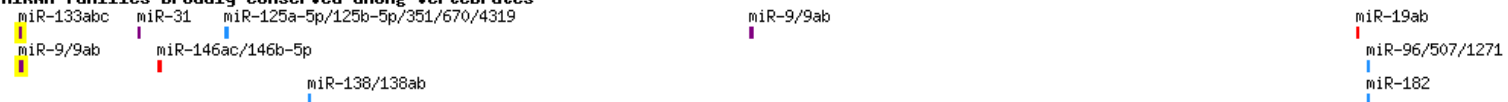
Identification of microRNAs targeting specific mRNAs

Human ELAVL1 3' UTR



Gene
Human ELAVL1 NM_001419 3' UTR length:4910

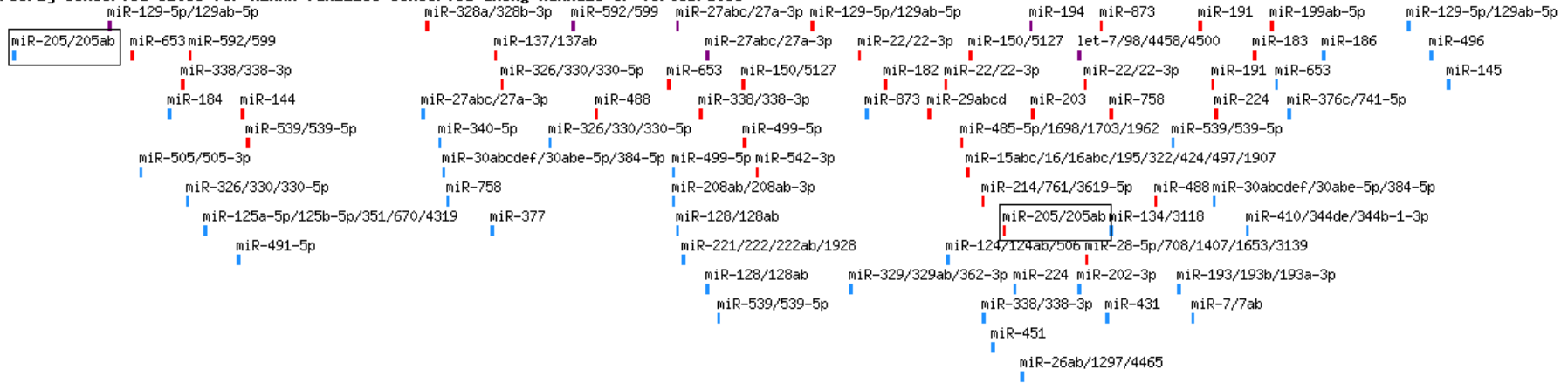
Conserved sites for miRNA families broadly conserved among vertebrates



Conserved sites for miRNA families conserved only among mammals



Poorly conserved sites for miRNA families conserved among mammals or vertebrates



[Hide Conserved sites for miRNA families conserved only among mammals]
 [Hide poorly conserved sites for miRNA families conserved among mammals or vertebrates]
 [Show sites for poorly conserved miRNA families]
 [View SVG image of miRNA sites]
 [View table of miRNA sites]
 [View human genome browser (Feb 09)]

Key:

Sites with higher probability of preferential conservation

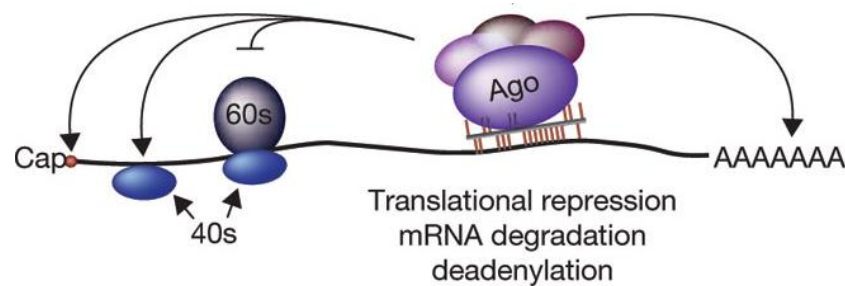
- 8mer
- 7mer-m8
- 7mer-1A
- 3' comp*

Sites with lower probability of preferential conservation

- 8mer
- 7mer-m8
- 7mer-1A
- 3' comp*



Identification of microRNA targets



- **The functions of a given miRNA can be attributed to:**

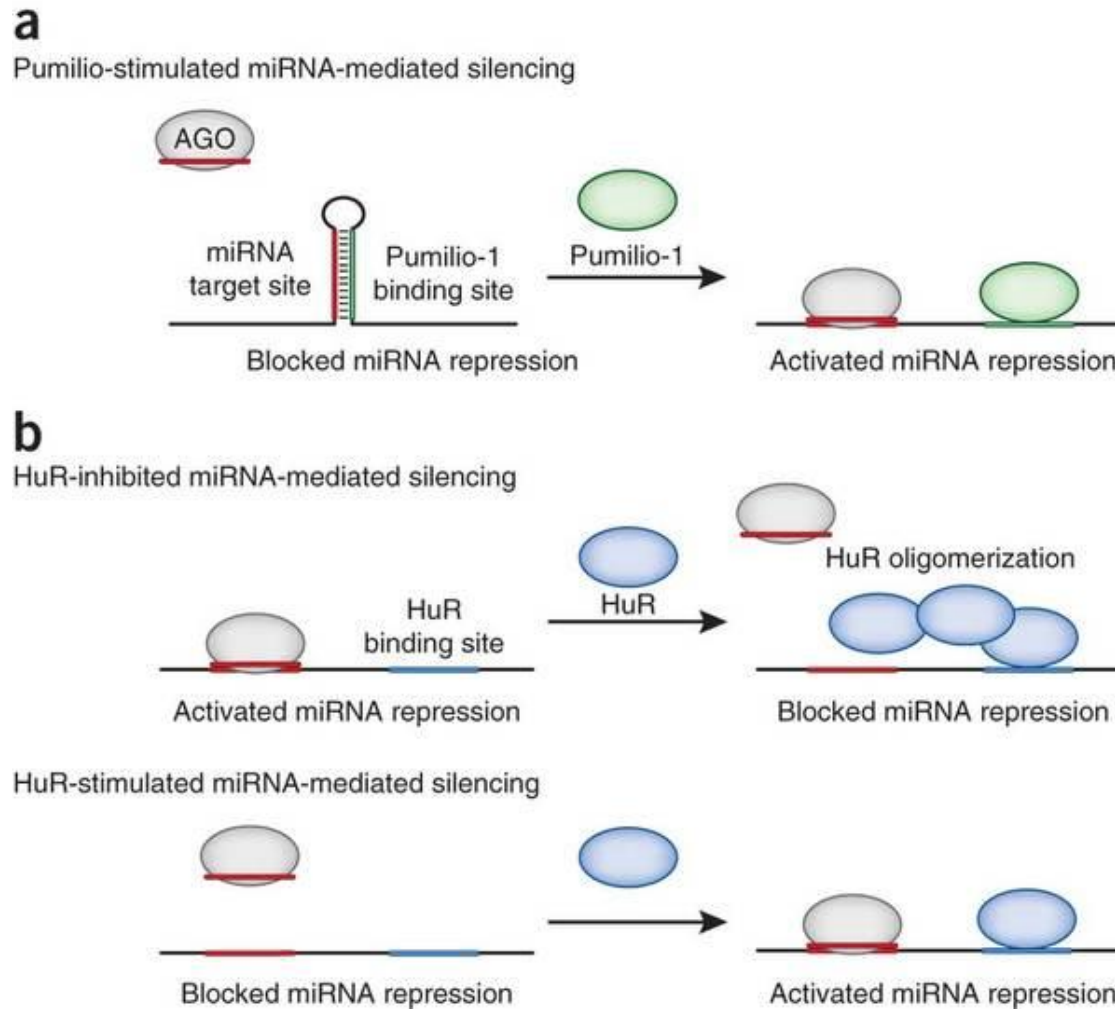
1. Strong regulation of one dominant target (99% of publications)

2. Fine-tuned regulation (less than 2 fold) of many targets simultaneously

simple miRNA:target relationships may dictate some phenotypes and complex networks of gene expression changes may underlie others.

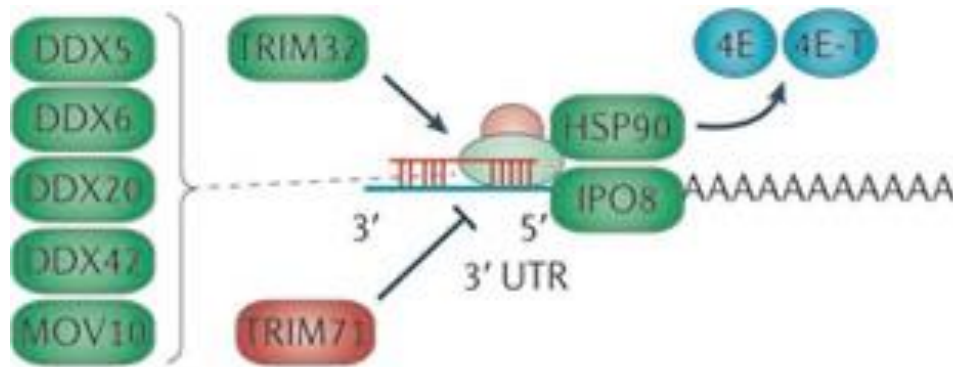
Regulation of microRNA targeting efficiency

- RNA binding proteins: they can shield miRNA target sites from miRNA binding.



Regulation of microRNA targeting efficiency

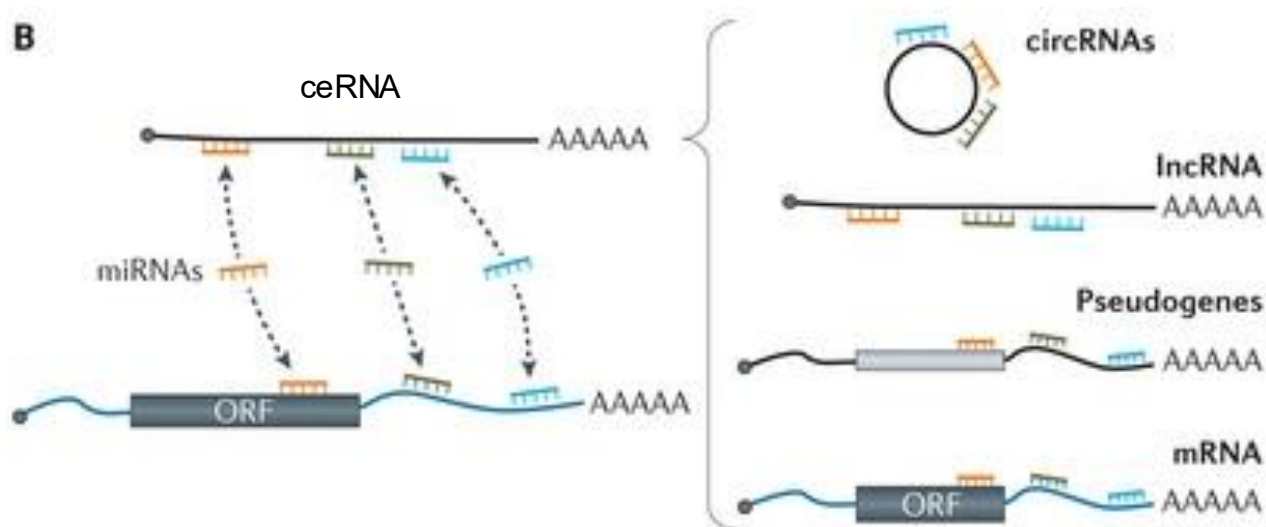
- **RNA binding proteins**: they can shield miRNA target sites from miRNA binding.
- **Factors that associate with the miRISC**: they can positively or negatively affect target binding and regulation.



Nature Reviews | Cancer

Regulation of microRNA targeting efficiency

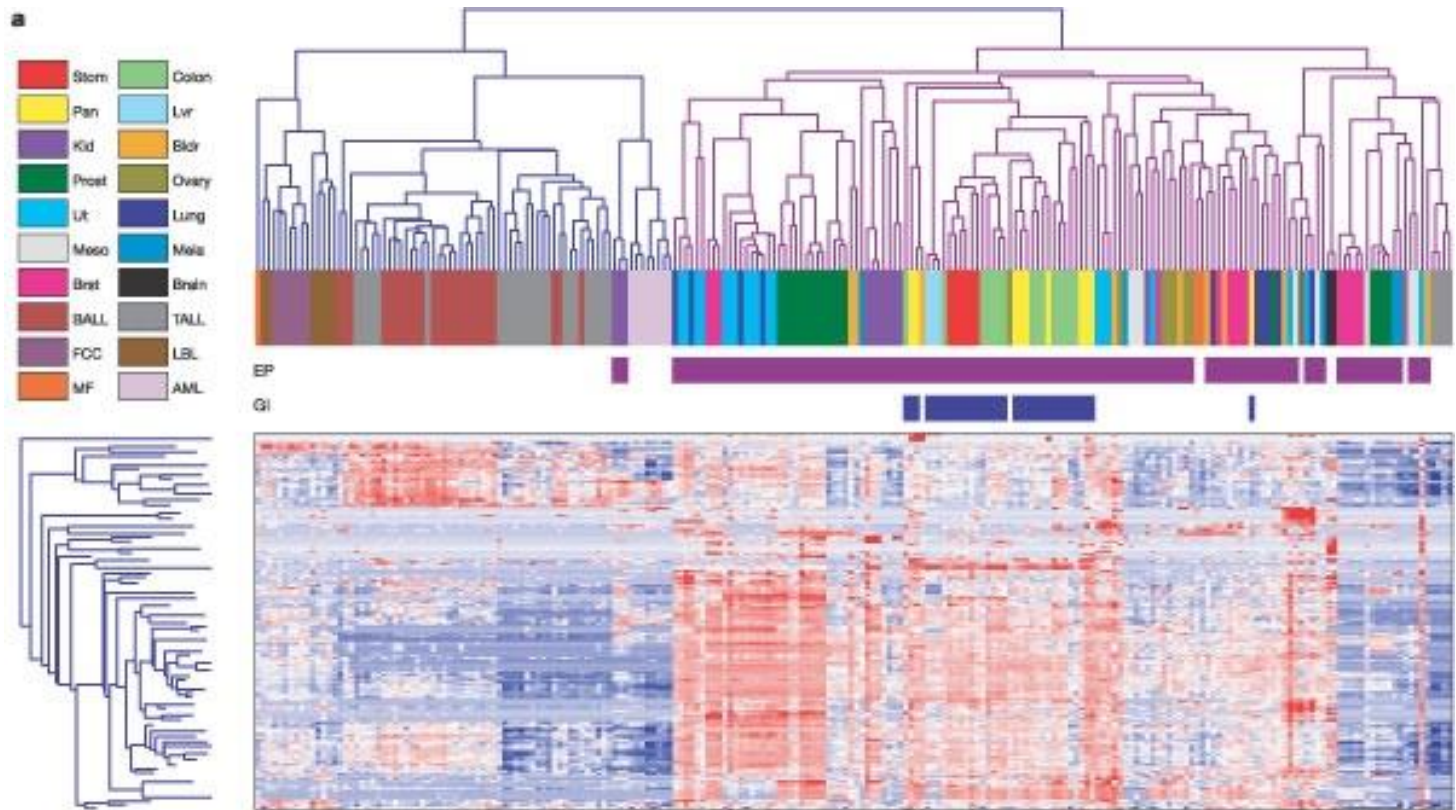
- **RNA binding proteins**: they can shield miRNA target sites from miRNA binding.
- **Factors that associate with the miRISC**: they can positively or negatively affect target binding and regulation.
- **Competing endogenous RNAs (ceRNAs)**: transcripts with target sites for a common miRNA that can compete for miRNA binding.



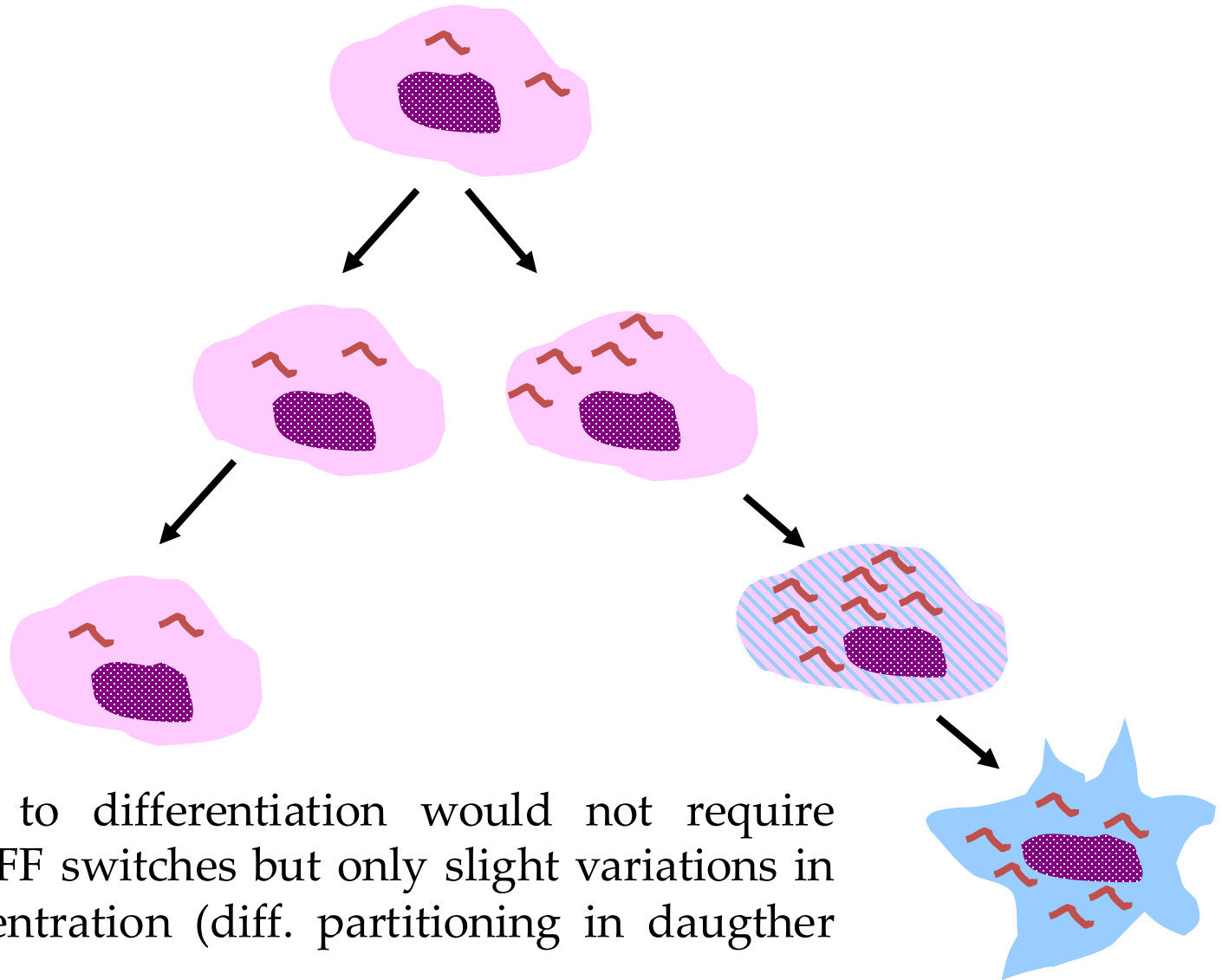
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miRNA expression

- miRNAs show **dynamic temporal and spatial expression patterns**, disruption of which is associated with developmental/physiological abnormalities.

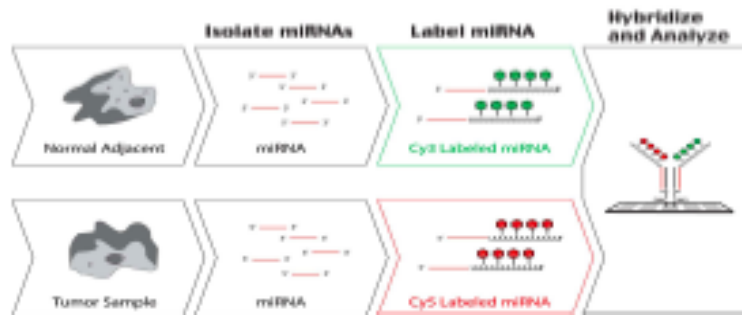


Subtle changes in miRNA levels can lead to drastic and rapid changes in gene expression

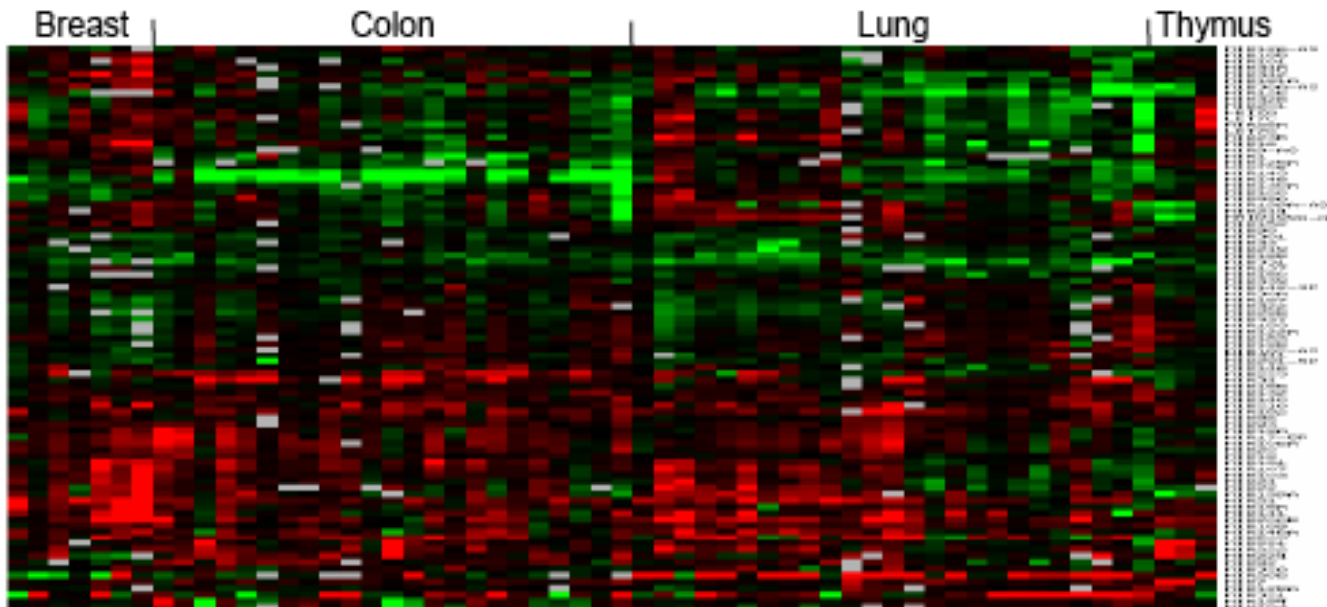


Commitment to differentiation would not require drastic ON/OFF switches but only slight variations in miRNA concentration (diff. partitioning in daughter cells)

miRNAs are differentially expressed in tumor samples



- miRNAs are differentially expressed in tumor samples
- Human tumors are characterized by unique miRNA expression profiles



Oncogenic miRNAs

Name	ncRNA Class	Cancer Types Examined	<i>In Vivo</i> Experimental Techniques Used	Cancer-Related Mechanisms and/or Functions of ncRNA	References
Oncogenic ncRNAs					
miR-10b	miRNA	breast, glioblastoma	antimiRs, CRISPR-Cas9 knockdown in mouse xenografts and allografts; transgenic knockout mouse models	targets several transcripts that encode regulators of cell-cycle progression, migration, invasion, and metastasis	Ma et al., 2010 ; Kim et al., 2016 ; El Fatimy et al., 2017
miR-21	miRNA	lung, B cell lymphoma	transgenic knockout, overexpression mouse models	targets transcripts that encode negative regulators of RAS signaling, leading to increased proliferation and decreased apoptosis	Hatley et al., 2010 ; Medina et al., 2010
miR-31	miRNA	lung, breast	overexpression in mouse xenografts; transgenic knockout, overexpression mouse models	targets transcripts that encode regulators of RAS, WNT, and TGF- β signaling to increase proliferation, stem cell renewal, and metastasis	Edmonds et al., 2016 ; Lv et al., 2017
miR-155	miRNA	lymphoma	transgenic overexpression mouse model, treatment with antimiRs	targets SHIP1 transcript, a negative regulator of AKT, to increase proliferation and survival	O'Connell et al., 2009 ; Babar et al., 2012 ; Cheng et al., 2015
miR-221	miRNA	liver	overexpression, treatment with antimiRs in mouse xenografts	targets transcripts of tumor suppressors and cell cycle inhibitors (e.g., p27, PTEN) to increase proliferation and decrease apoptosis	Pineau et al., 2010 ; Park et al., 2011

Tumor-suppressive miRNAs

let-7	miRNA	lung	antimiRs, mimetics in mouse xenografts or transgenic lung cancer mouse models	targets several transcripts that encode oncogenes, including RAS, leading to decreased cell cycle progression and proliferation	Trang et al., 2010, 2011
miR-15a/16-1	miRNA	prostate, leukemia	knockdown, overexpression in mouse xenografts; transgenic knockout mouse	targets several transcripts that encode cyclins, CDKs, and anti-apoptotic proteins, thereby increasing apoptosis and inhibiting proliferation	Bonci et al., 2008; Klein et al., 2010
miR-34a	miRNA	lung, prostate, breast	overexpression, mimetics, antimiRs in mouse xenografts; mimetics in transgenic lung cancer mouse models	targets several oncogenic transcripts encoding cyclins, CDKs, cell adhesion molecules, RTKs, and other non-RTKs, resulting in decreased proliferation, invasion, and survival	Liu et al., 2011; Trang et al., 2011; Kasinski and Slack, 2012; Adams et al., 2016b
miR-122	miRNA	liver	transgenic knockout mouse models, overexpression in transgenic liver cancer mouse models	targets expression of several genes involved in lipid metabolism, proliferation, and inflammation	Hsu et al., 2012; Tsai et al., 2012
miR-506	miRNA	ovarian	mimetics in mouse xenografts	targets SNAI2 transcript to decrease its expression and inhibit migration, invasion, and EMT	Yang et al., 2013a

Context-dependent miRNAs

miR-26a	miRNA	glioma, leukemia, liver, colorectal	overexpression in transgenic glioma, liver cancer mouse models; transgenic overexpression mouse models	targets both tumor suppressor (PTEN) and cyclin (CCND2/E2) transcripts to either increase or decrease proliferation, depending on the context	Huse et al., 2009 ; Kota et al., 2009 ; Mavrakis et al., 2011 ; Zeitels et al., 2014
miR-29	miRNA	leukemia, glioblastoma	mimetics in mouse xenografts; transgenic overexpression mouse models	promotes proliferation in B cells but can also target and decrease expression of transcripts that enhance lipid synthesis (SCAP, SREBP-1), proliferation (CDK6), and apoptosis (MCL-1)	Garzon et al., 2009 ; Santanam et al., 2010 ; Ru et al., 2016

Circulating miRNAs

Circulating miRNAs are potential cancer biomarkers; their discovery in exosomes led to the hypothesis that they might contribute to intercellular signalling. Uptake of exosomes can be receptor-mediated or receptor-independent. Upon entering a target cell, exosome-delivered miRNAs are thought to regulate target mRNAs.

