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 in the cytoplasm or nucleus. These are:

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

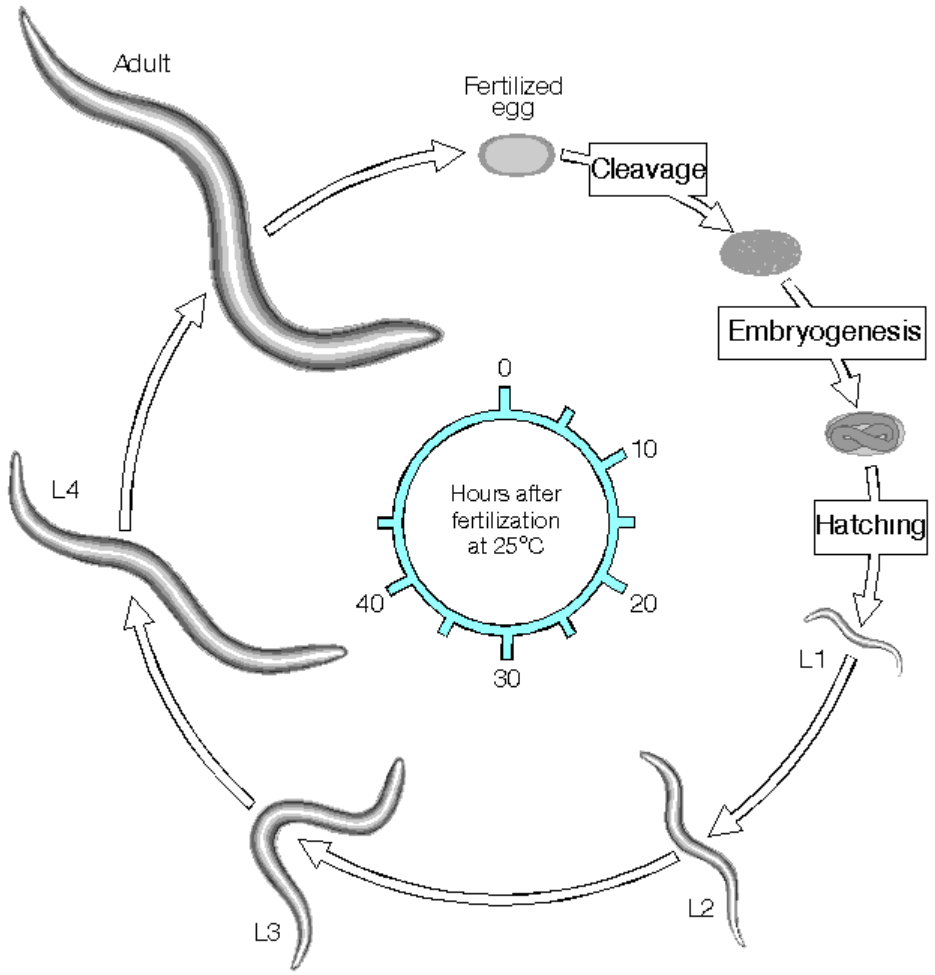
microRNAs: biogenesis and function

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 .



The Nobel Prize in Physiology or Medicine 2024

Ill. Niklas Elmehed © Nobel Prize Outreach

Victor Ambros

Prize share: 1/2

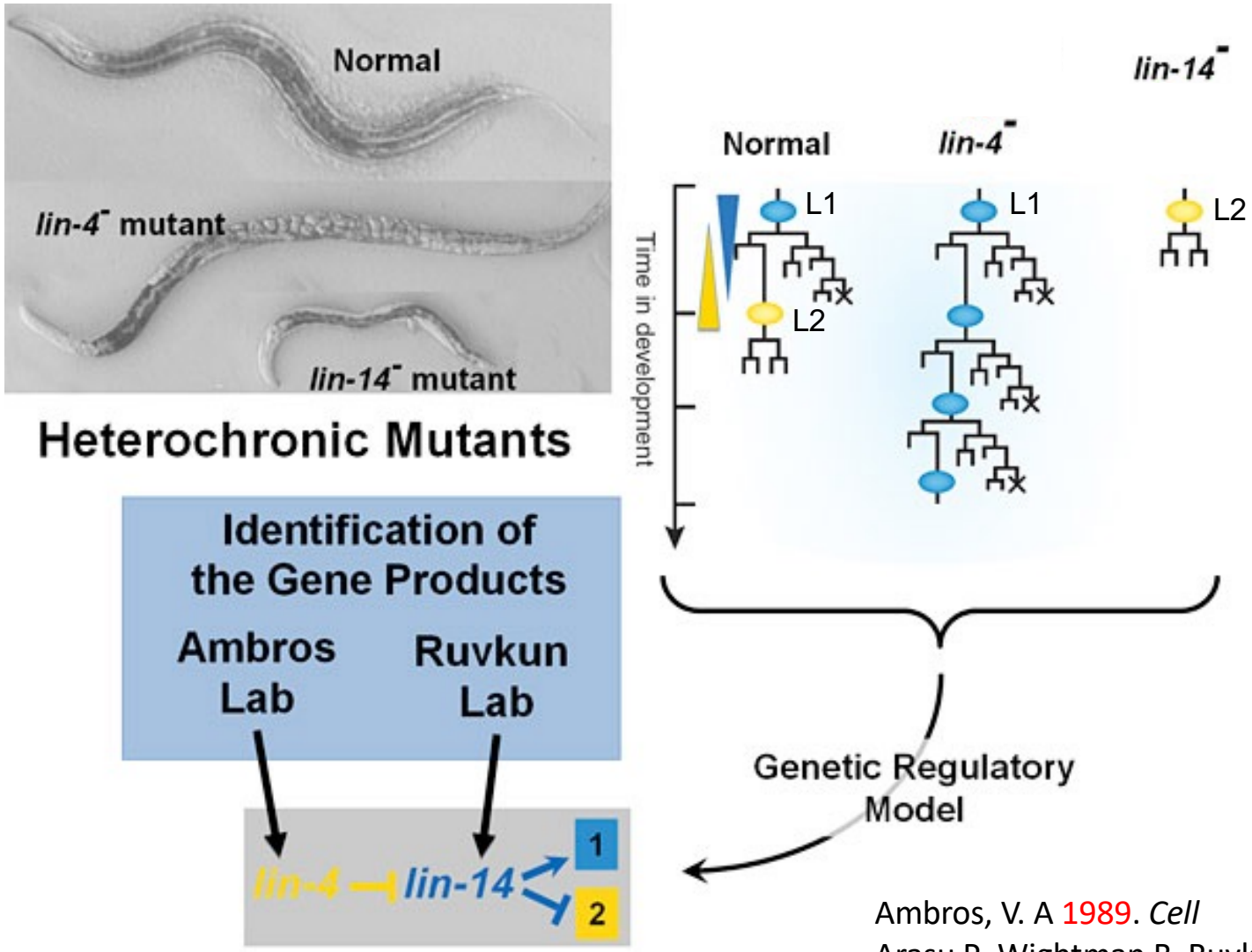
Ill. Niklas Elmehed © Nobel Prize Outreach

Gary Ruvkun

Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2024 was awarded jointly to Victor Ambros and Gary Ruvkun "for the discovery of microRNA and its role in post-transcriptional gene regulation"

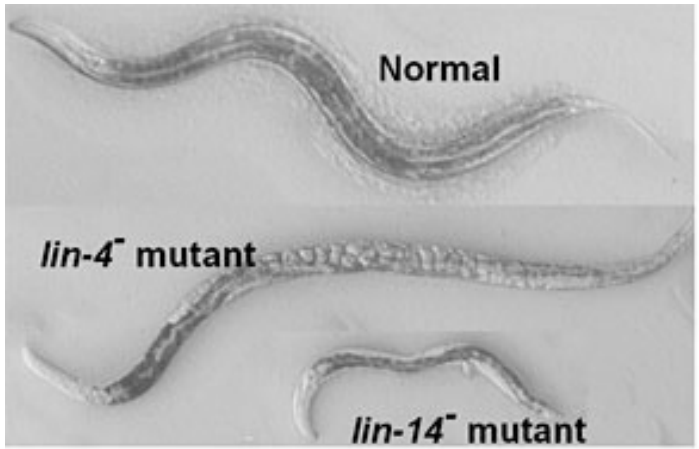
Mutations in *lin-4* disrupt the temporal regulation of larval development, causing L1 specific cell-division patterns to reiterate at later developmental stages. Opposite developmental phenotypes — omission of the L1 cell fates and premature development into the L2 stage — are observed in worms that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



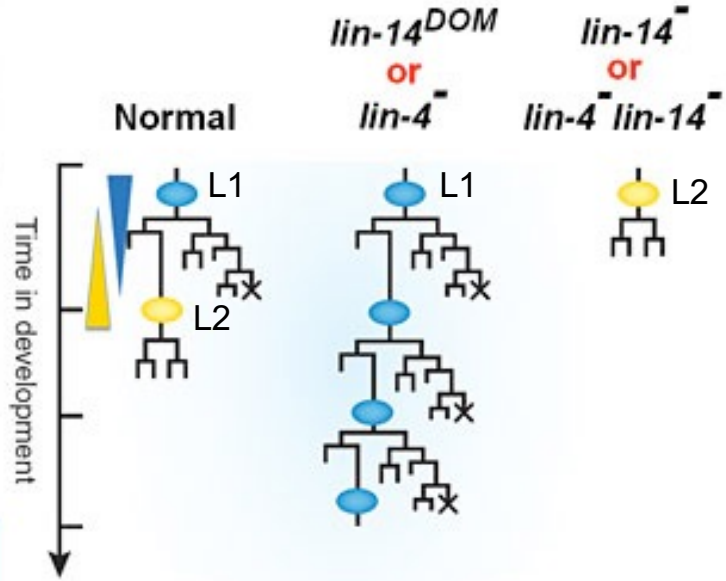
Ambros, V. A 1989. *Cell*

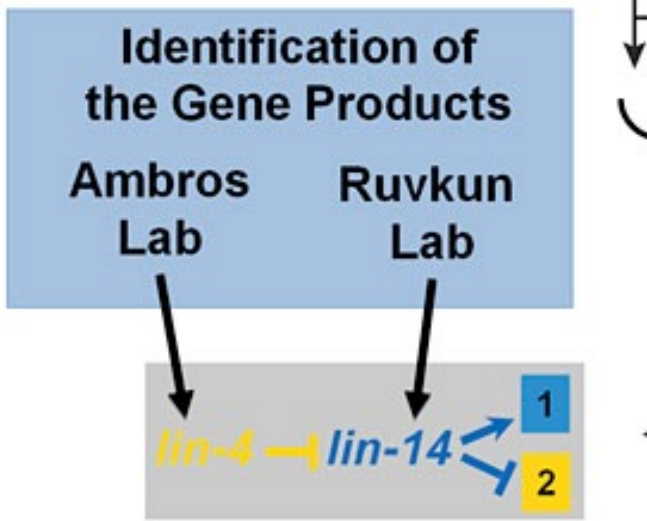
Arasu P, Wightman B, Ruvkun G. 1991. *Gen. Dev.*

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

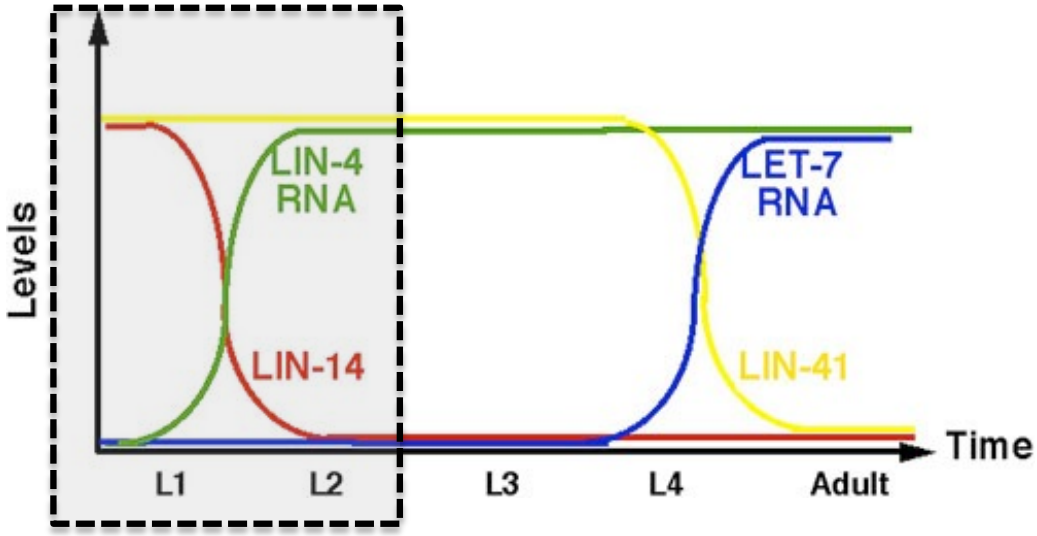




Genetic Regulatory Model

Ambros, V. A 1989. *Cell*
Arasu P, Wightman B, Ruvkun G. 1991. *Gen. Dev.*

EVIDENCE FOR LIN4-LIN14 REGULATORY MODEL



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**

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 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: no protein is produced!

- ✓ the 693 bp of lin-4 sequence was able to rescue the *lin-4 mutant* phenotype

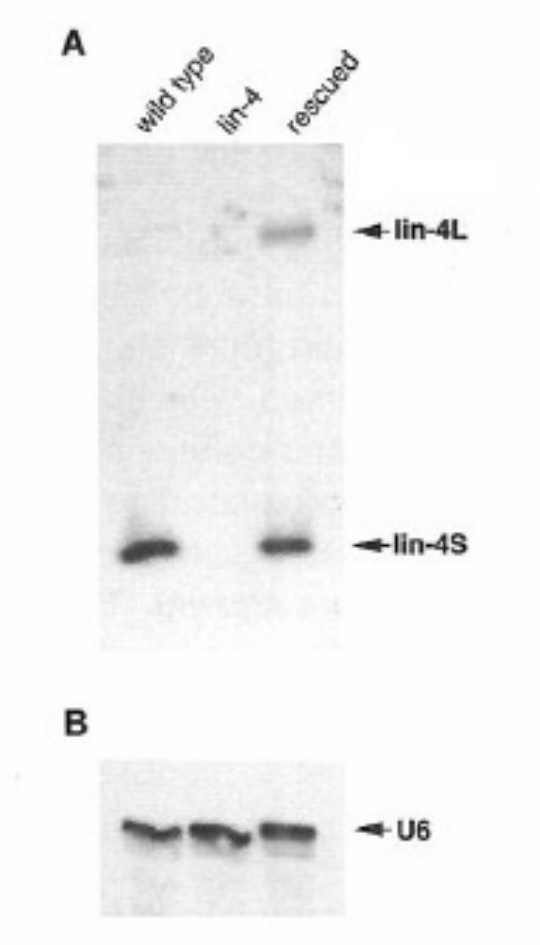
- ✓ the 693 bp sequence does not contain any ORF

Does the 693 bp sequence produce any functional RNA?

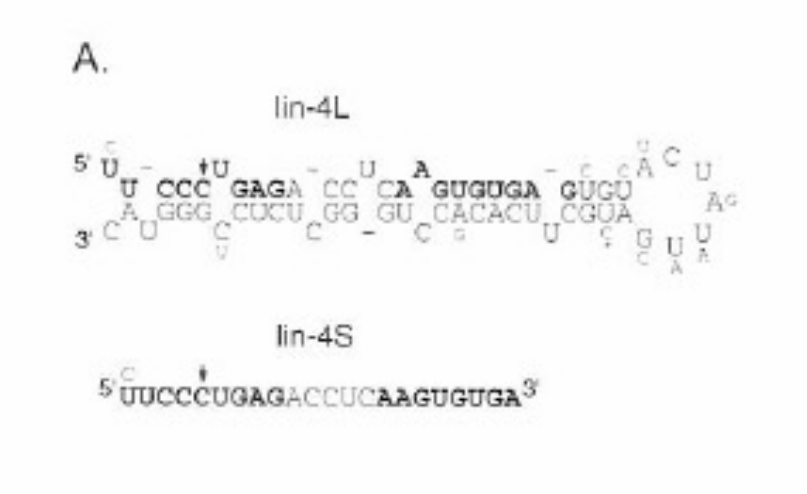
↓

Northern blot using all the labelled 693 nt sequence as probe

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

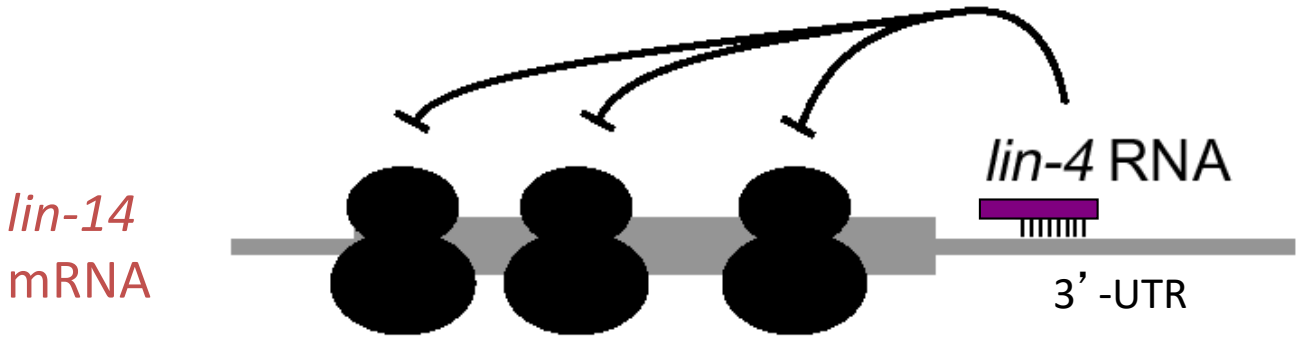


Wightman B, Ha I, Ruvkun G. 1993. *Cell*
Lee RC, Feinbaum RL, Ambros V. 1993.*Cell*

The "small temporal" RNA lin-4

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

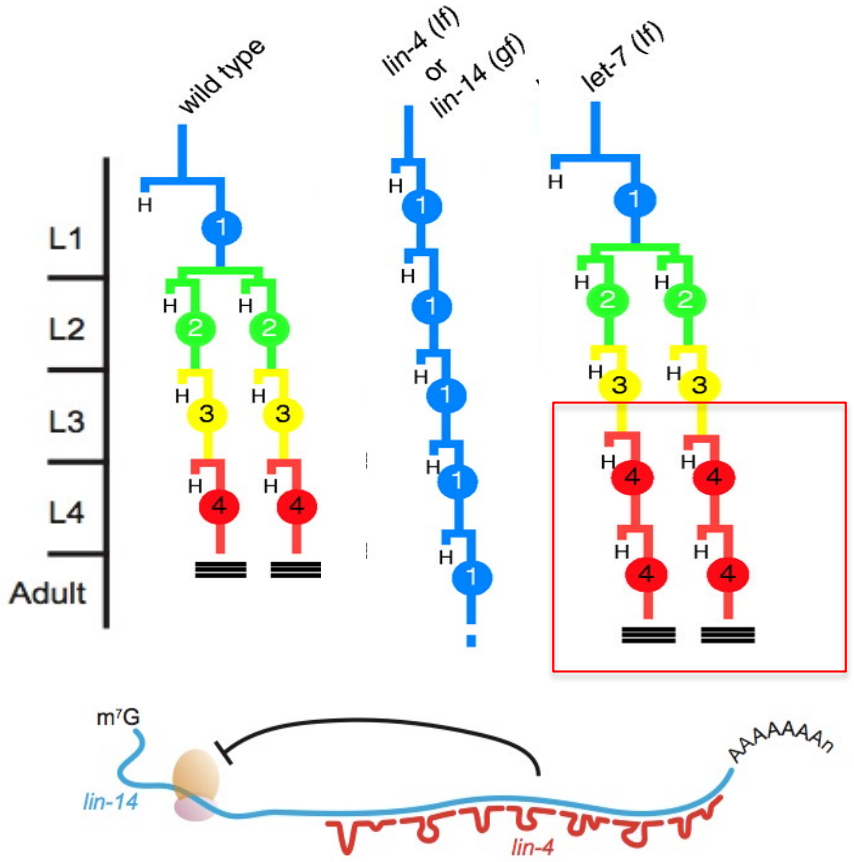
Negative regulation of lin-14 protein is required for L1 to L2 transition



Wightman B, Ha I, Ruvkun G. 1993. *Cell*
Lee RC, Feinbaum RL, Ambros V. 1993.*Cell*

7 year later……stRNA let-7

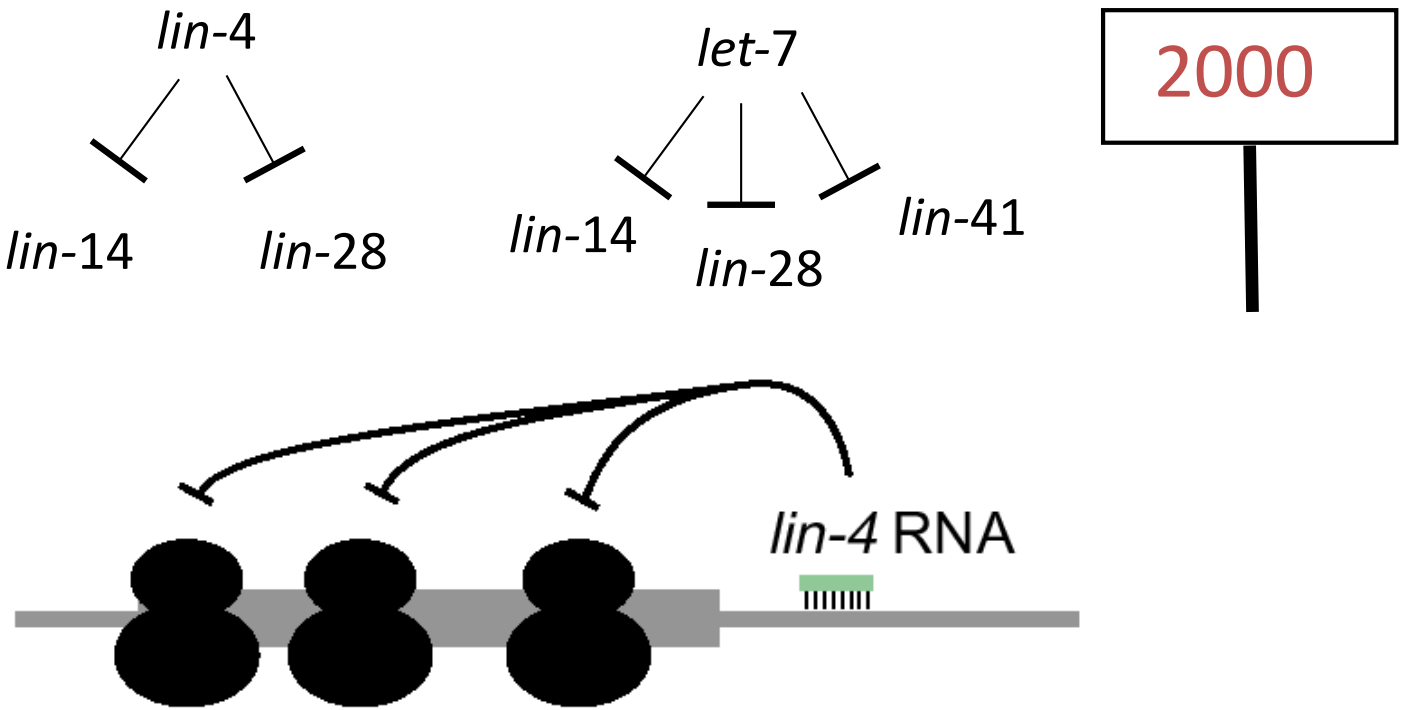
C.elegans



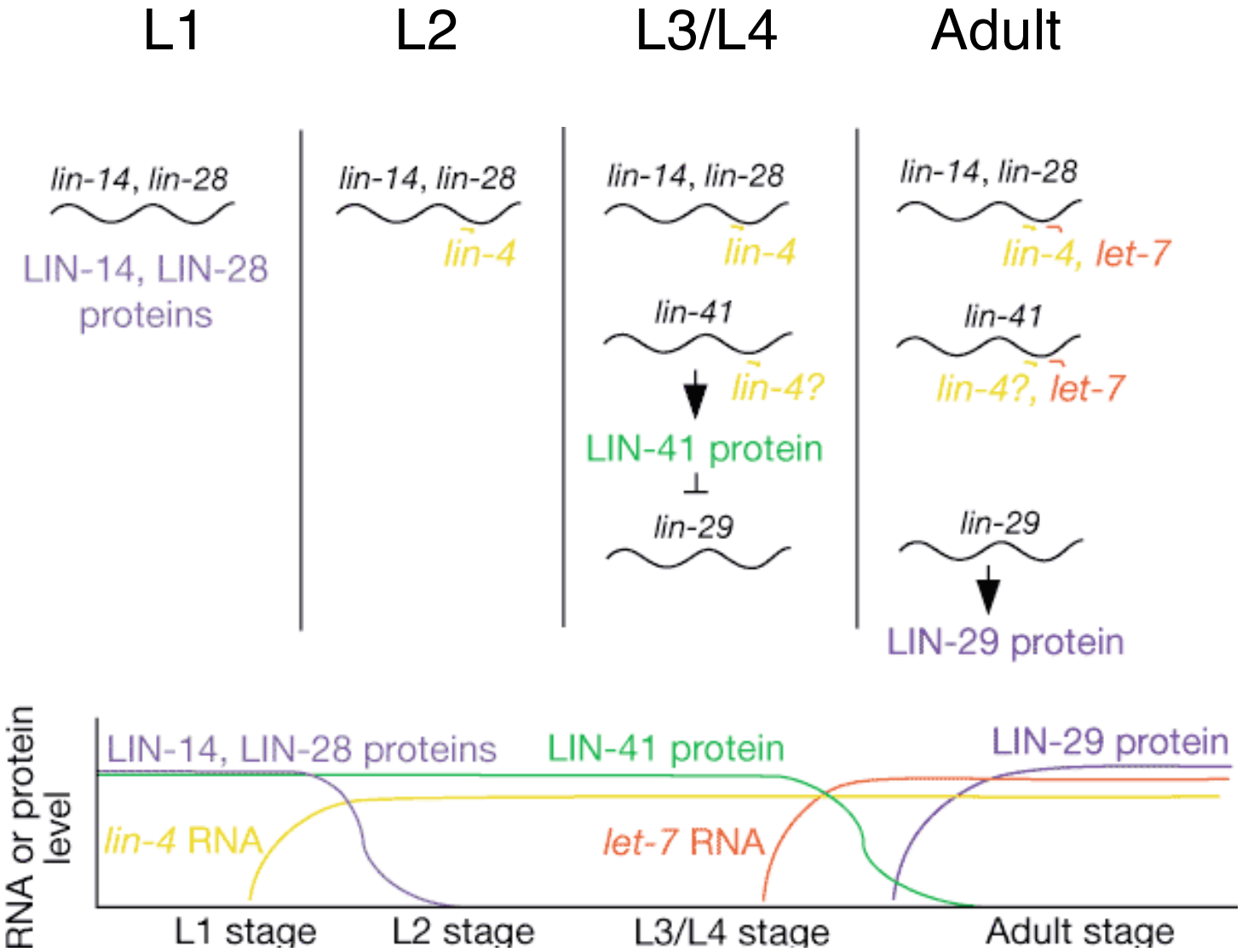
Wightman et al. 1993. *Cell*
Lee et al. 1993.*Cell*
Reinhart et al. 2000. *Nature*

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



stRNAs regulate gene expression during *C.elegans* development



Reinhart BJ et al. 2000. *Nature*

……2000….

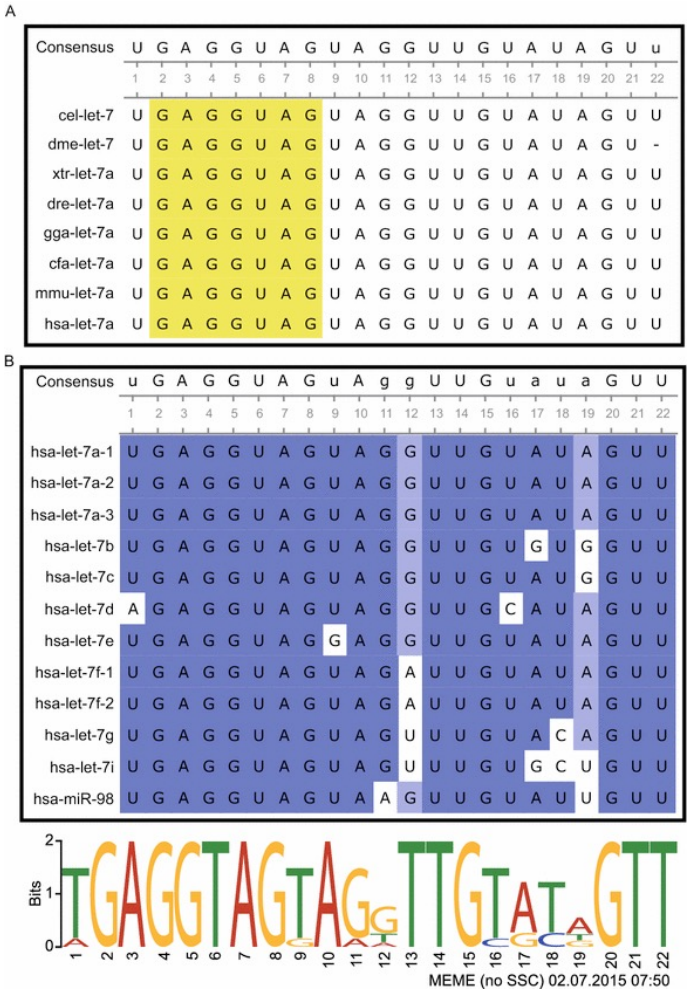
let-7 (stRNA) is conserved in all metazoa

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

Amy E. Pasquinelli*†, Brenda J. Reinhart*†, Frank Slack‡, Mark Q. Martindale§, Mitzi I. Kuroda‖, Betsy Maller‡, David C. Hayward¶, Eldon E. Ball¶, Bernard Degnan#, Peter Müller*, Jürg Spring*, Ashok Srinivasan**, Mark Fishman**, John Finnerty††, Joseph Corbo‡‡, Michael Levine‡‡, Patrick Leahy§§, Eric Davidson§§ & Gary Ruvkun*

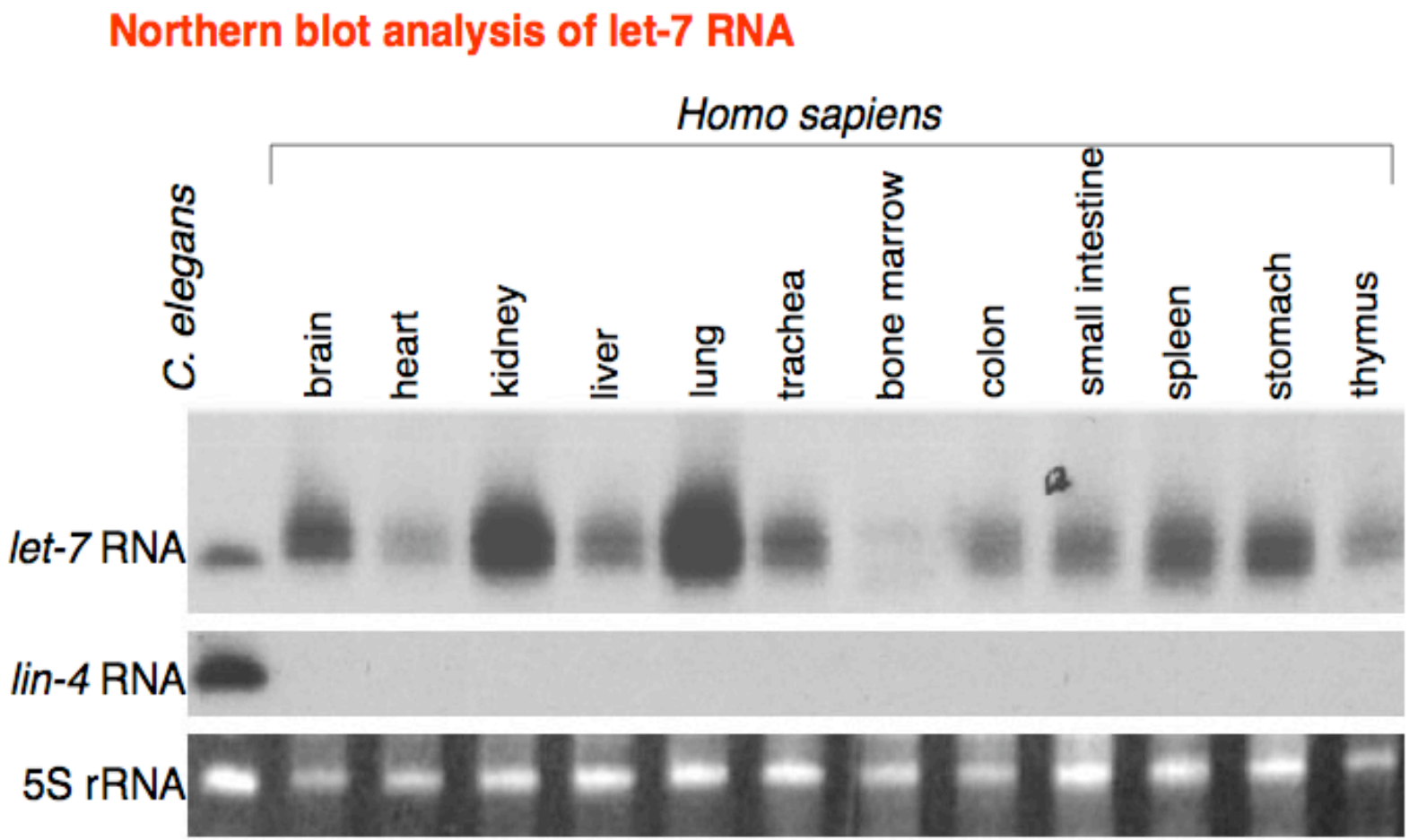
Pasquinelli et al., **2000**. *Nature*

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

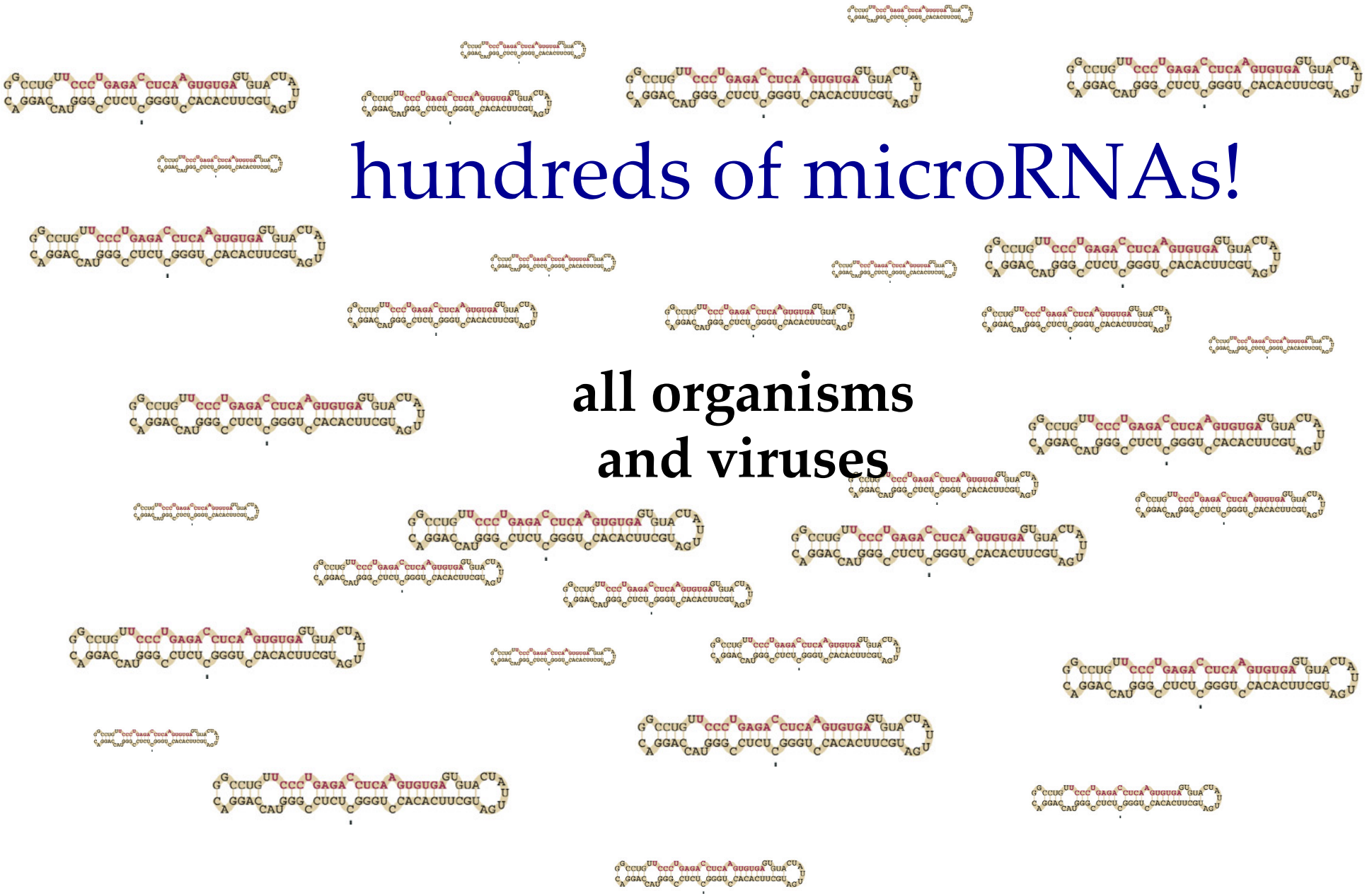


Let-7 expression in human tissues

In mammals, let-7 is expressed during embryogenesis and brain development and remains high in adult tissues



…after 2000



hundreds of microRNAs!

all organisms and viruses

microRNAs

- **complex family of 21-23 nt long small RNAs**
  (~1% of higher eukaryotes genes)

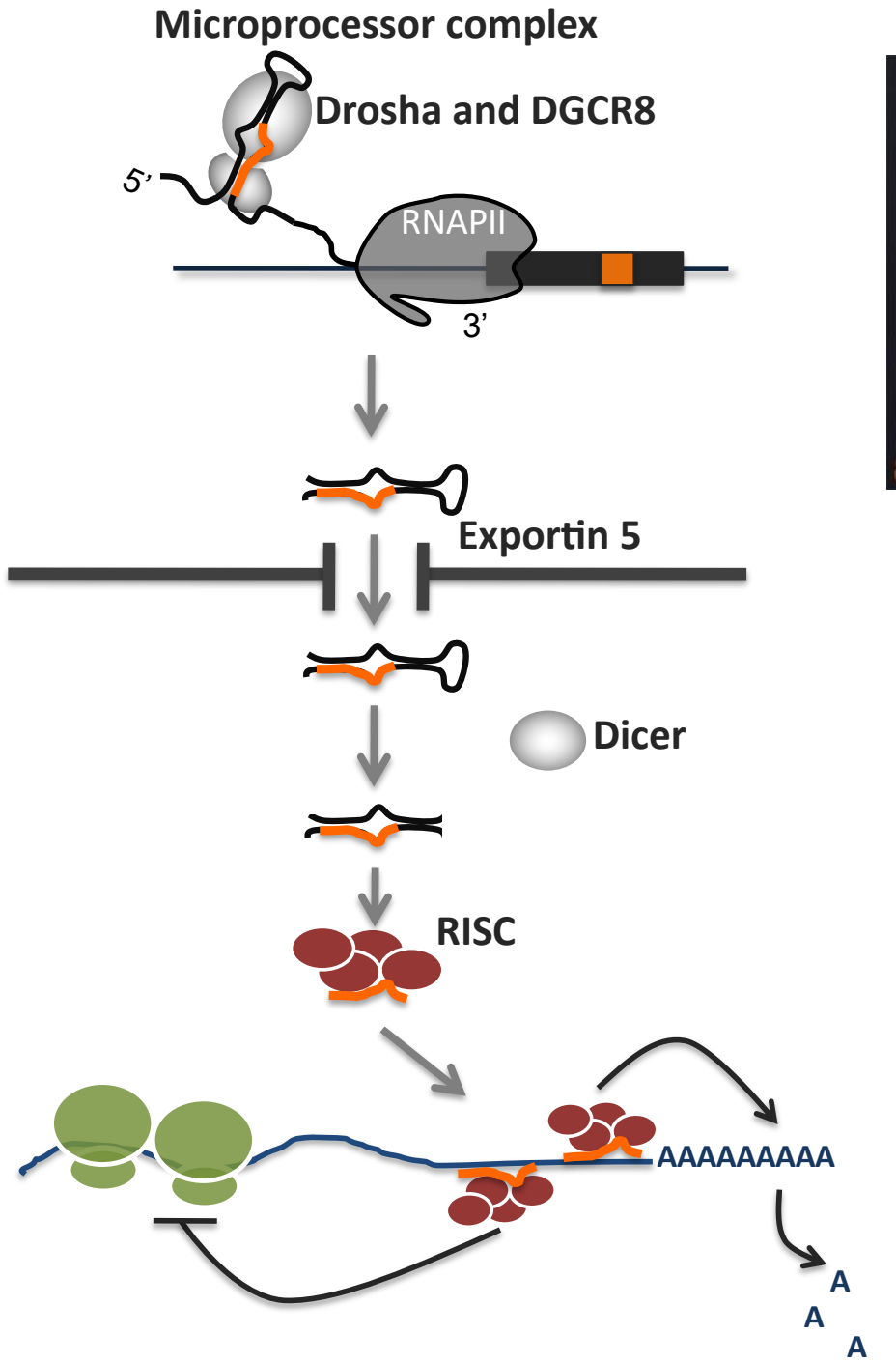
- **present in metazoa and plants**
  469 D.melanogaster
  437 C.elegans
  2654 H.sapiens
  427 A.thaliana

- **and mammalian viruses**
  Herpesviruses (27), EBV (44)…..

they control gene expression by regulating mRNA stability and translation

2. Synthesis

Biosynthesis

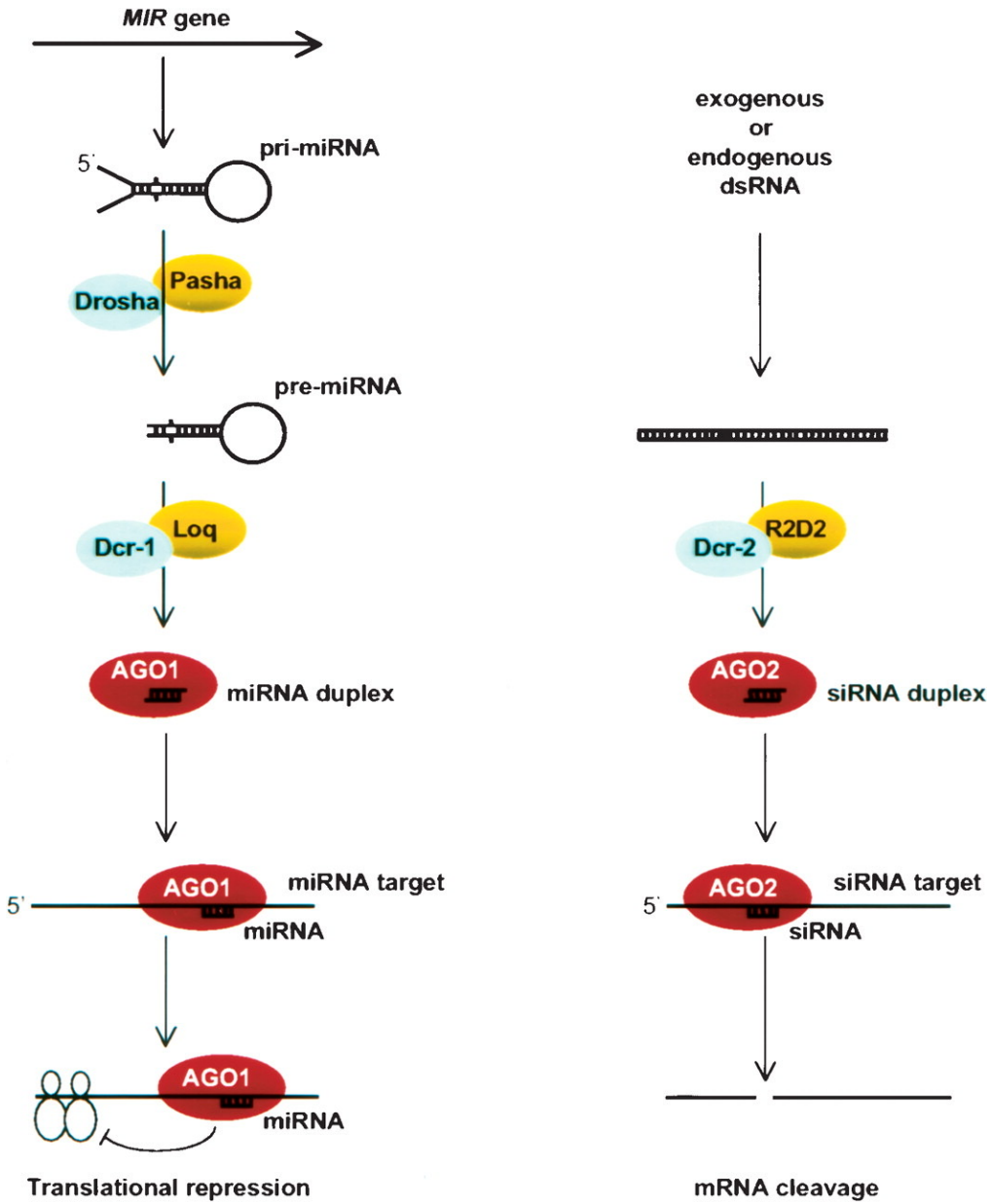


H. sapiens

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

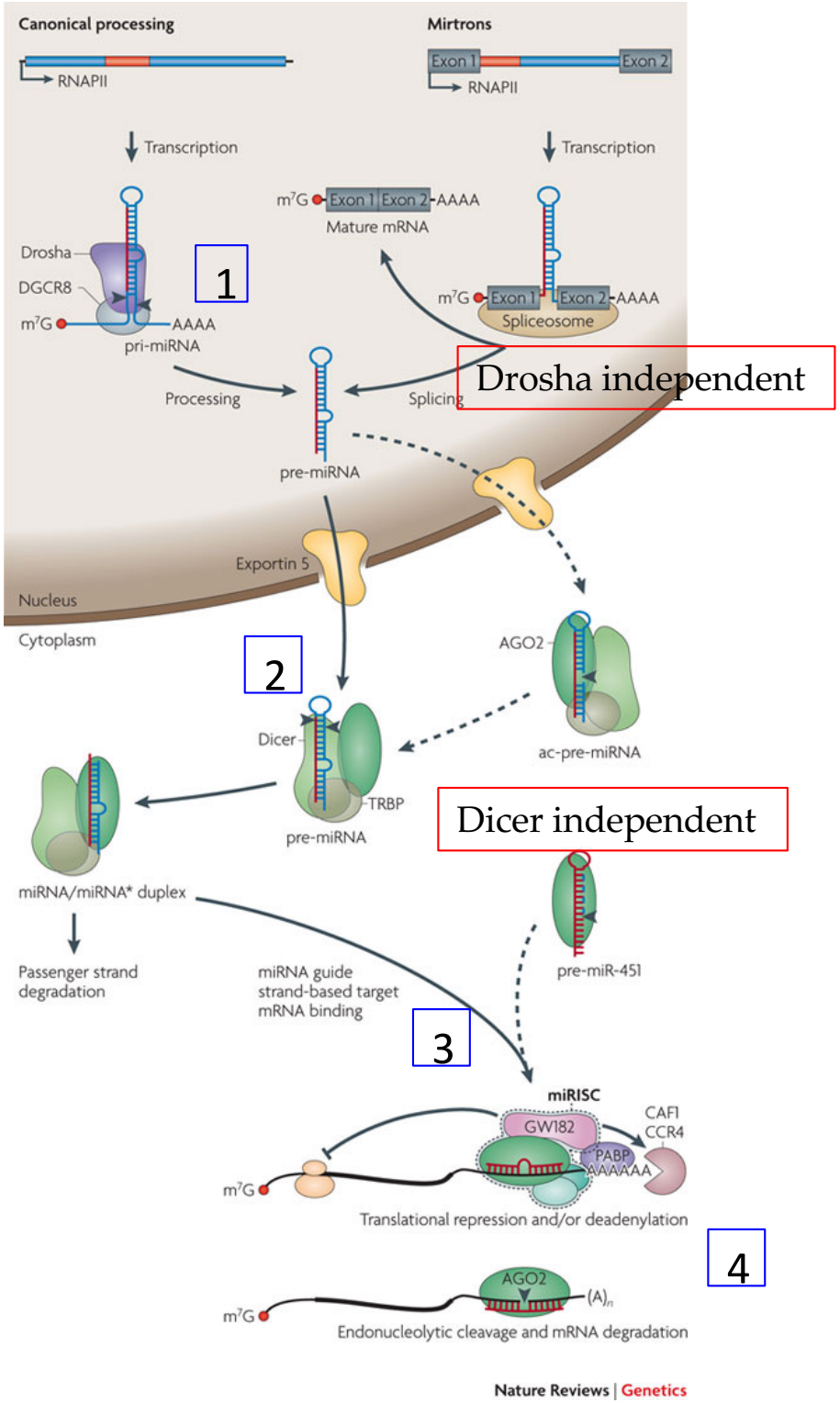
miRNAs and siRNAs

D. melanogaster



Herve Vaucheret Genes Dev. 2006; 20: 759-771

miRNA biogenesis



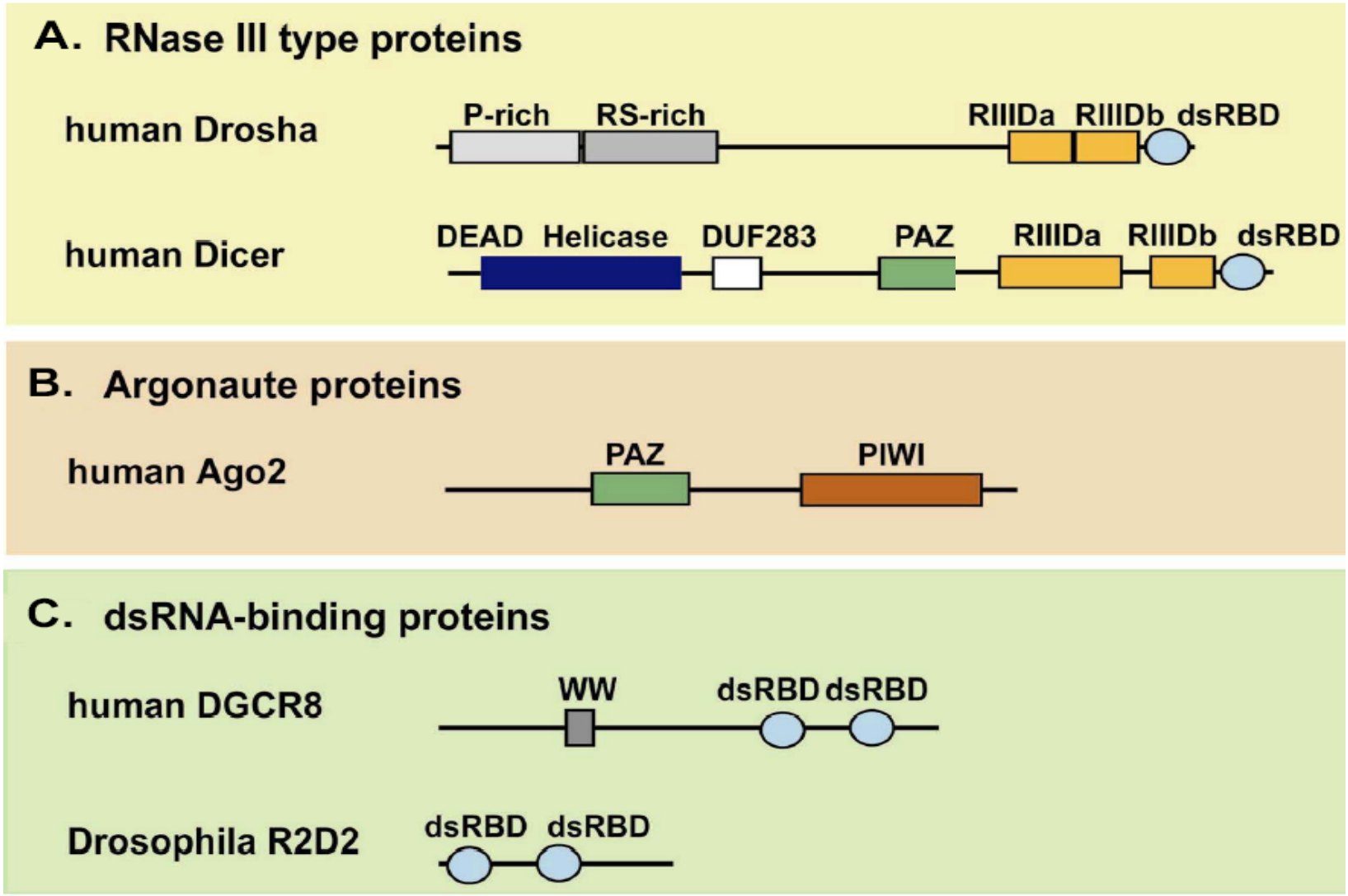
1. The pri-miRNAs fold into hairpins, which act as substrates for two members of the RNase III family of enzymes, Drosha and Dicer.

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.

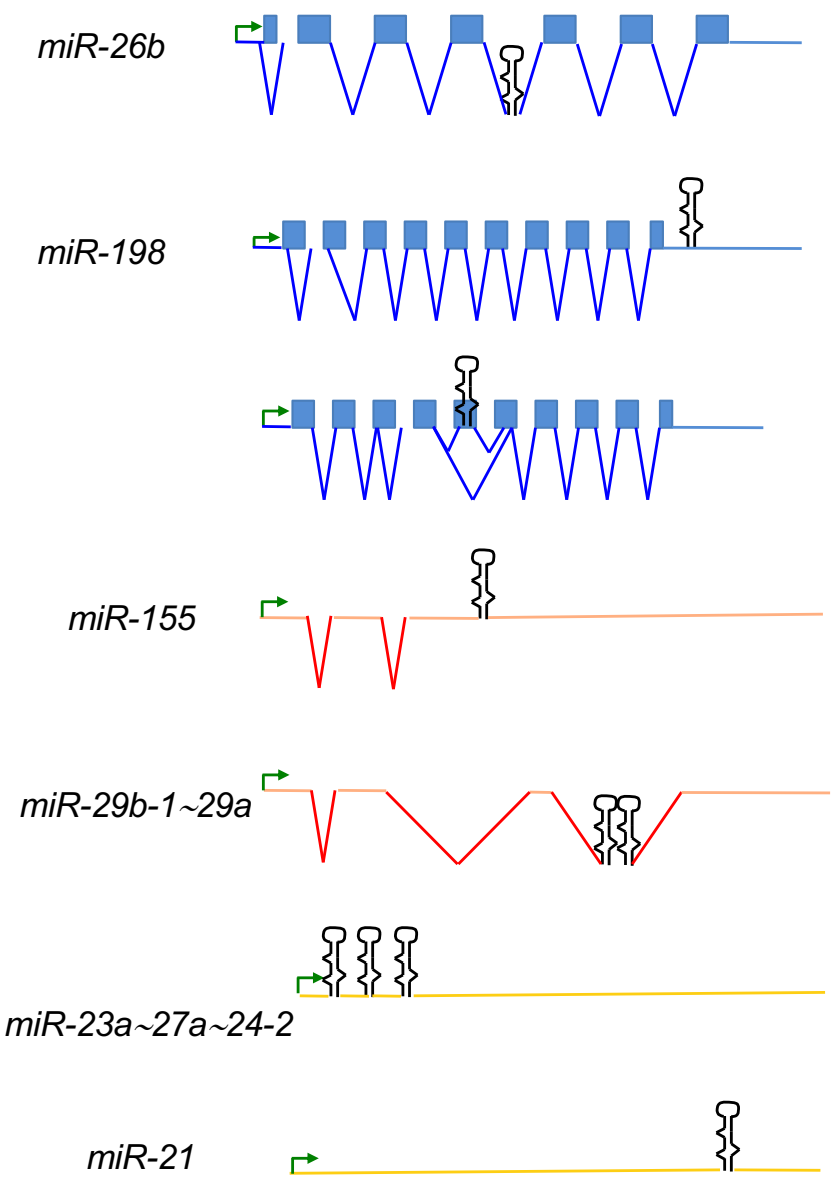
3. One strand of this duplex, representing a mature miRNA, is then incorporated into the miRNA-induced silencing complex (miRISC)

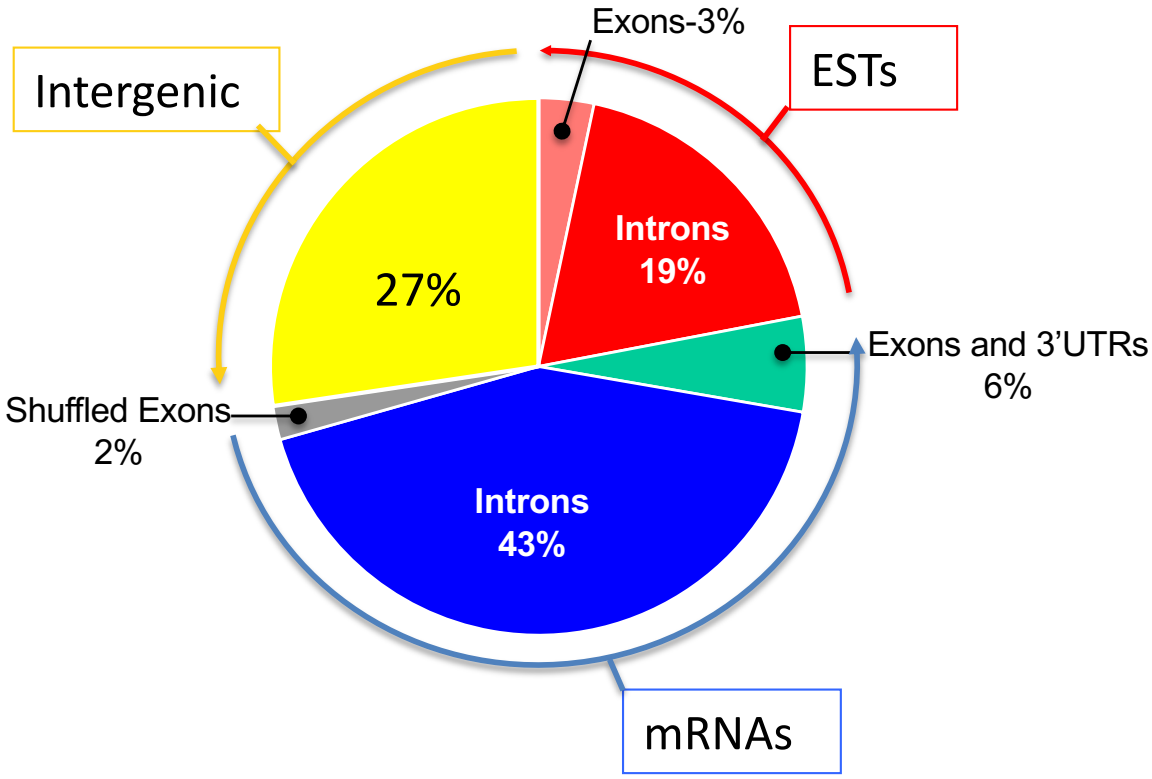
4. As part of miRISC, miRNAs base-pair to target mRNAs and induce their deadenylation and/or translational repression (short complementarity) or cleavage and degradation (perfect complementarity)

Players


A. RNase III type proteins
human Drosha
P-rich
RS-rich
RIIIDa
RIIIDb
dsRBD
human Dicer
DEAD
Helicase
DUF283
PAZ
RIIIDa
RIIIDb
dsRBD
B. Argonaute proteins
human Ago2
PAZ
PIWI
C. dsRNA-binding proteins
human DGCR8
WW
dsRBD
dsRBD
Drosophila R2D2
dsRBD
dsRBD

Genomic organization of human microRNAs

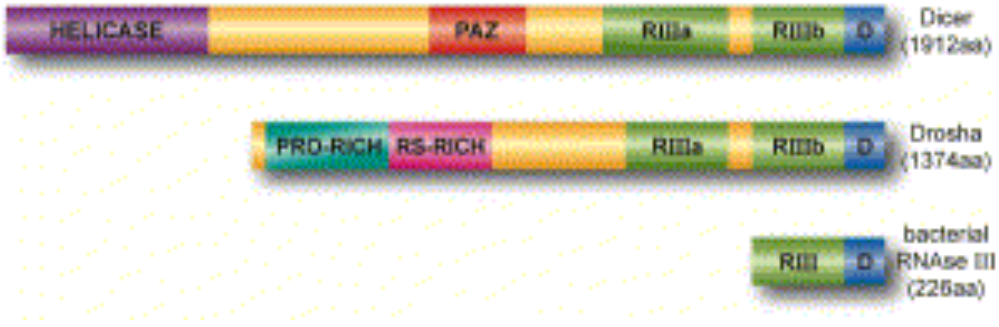




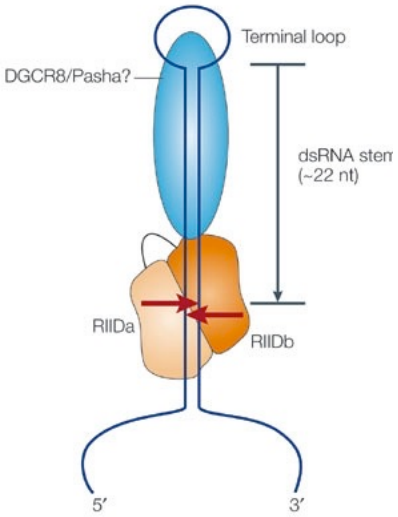
Morlando et al., **NSMB** (2008)

“Drosha” and “Dicer” RNases

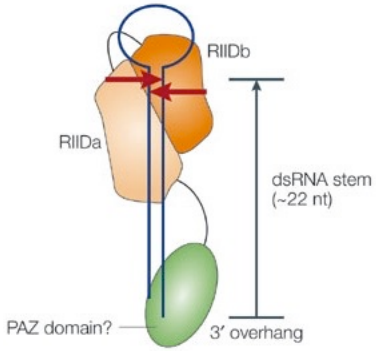
•Two processing events lead to mature miRNA formation in animals. In the first, the nascent miRNA transcripts (pri-miRNA) are processed into ~70-nucleotide precursors (pre-miRNA); in the second event that follows, this precursor is cleaved to generate ~21–25-nucleotide mature miRNAs.



Pri-miRNA

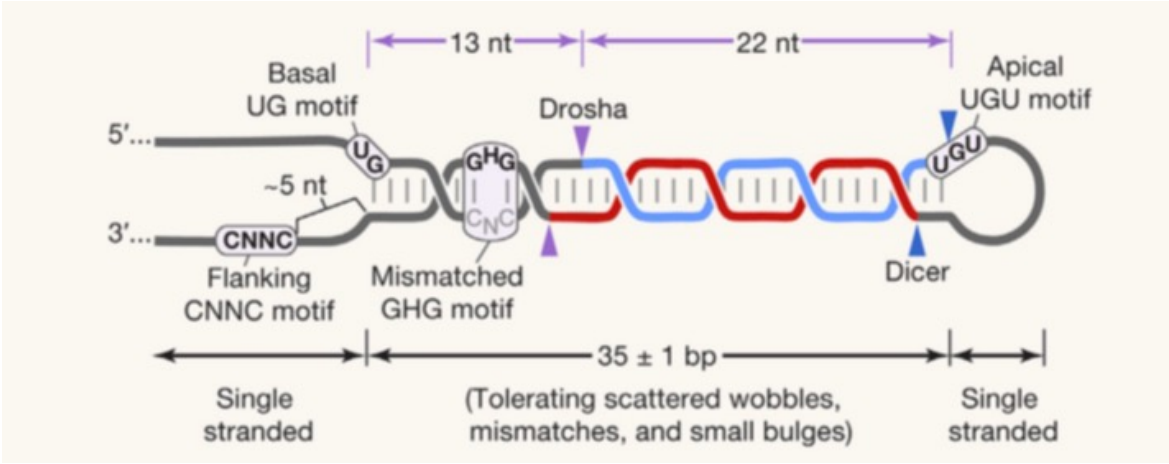


Pre-miRNA



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

Drosha

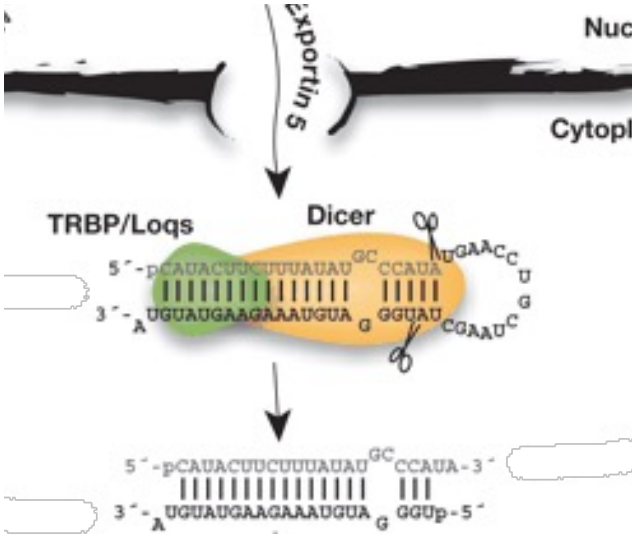


- Drosha is predominantly localized in the nucleus and contains two tandem RNase-III domains, a dsRNA binding domain and an N-terminal segment of unknown function.

- 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).

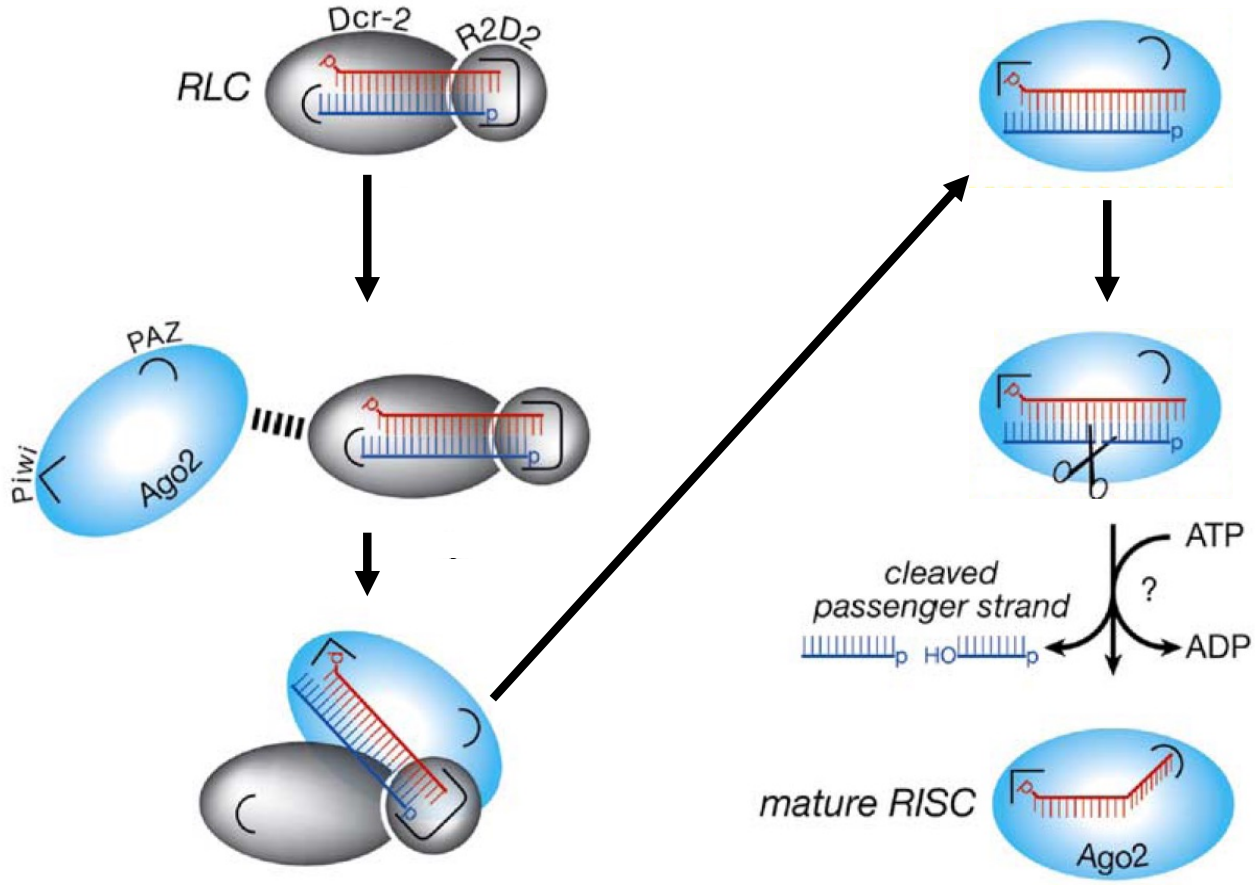
Dicer



- Once inside the cytoplasm, these hairpin precursors are cleaved by Dicer into a small, imperfect dsRNA duplex (miRNA:miRNA*) that contains both the mature miRNA strand and its complementary strand (miRNA*)

- It requires the dsRNA binding protein **TRBP/Loqs** for pre-miRNA cleavage

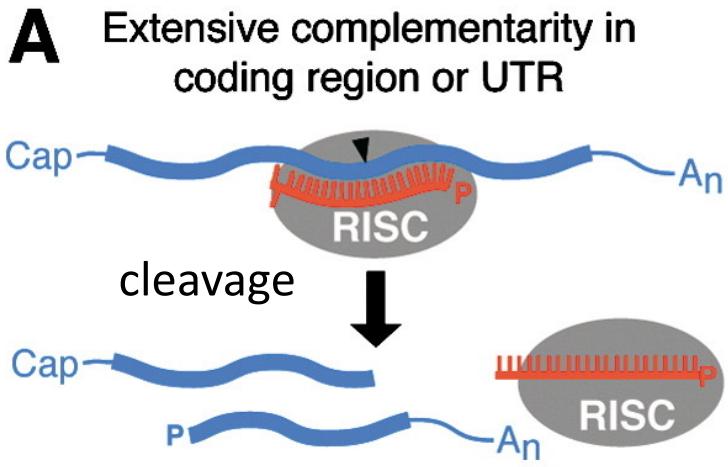
RISC Assembly



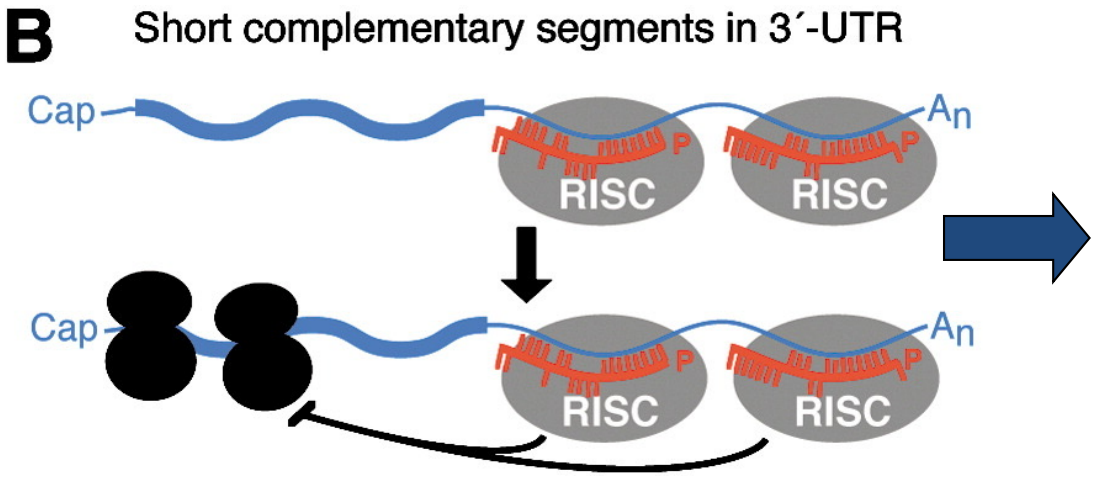
•the less stable 5′ end of the mature miRNA is preferentially assembled into the RISC complex.

3. Mode of action

Post-transcriptional repression by microRNAs



mRNA degradation (plant)

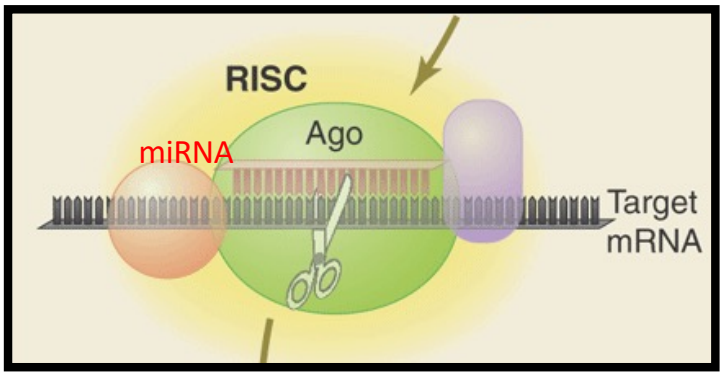


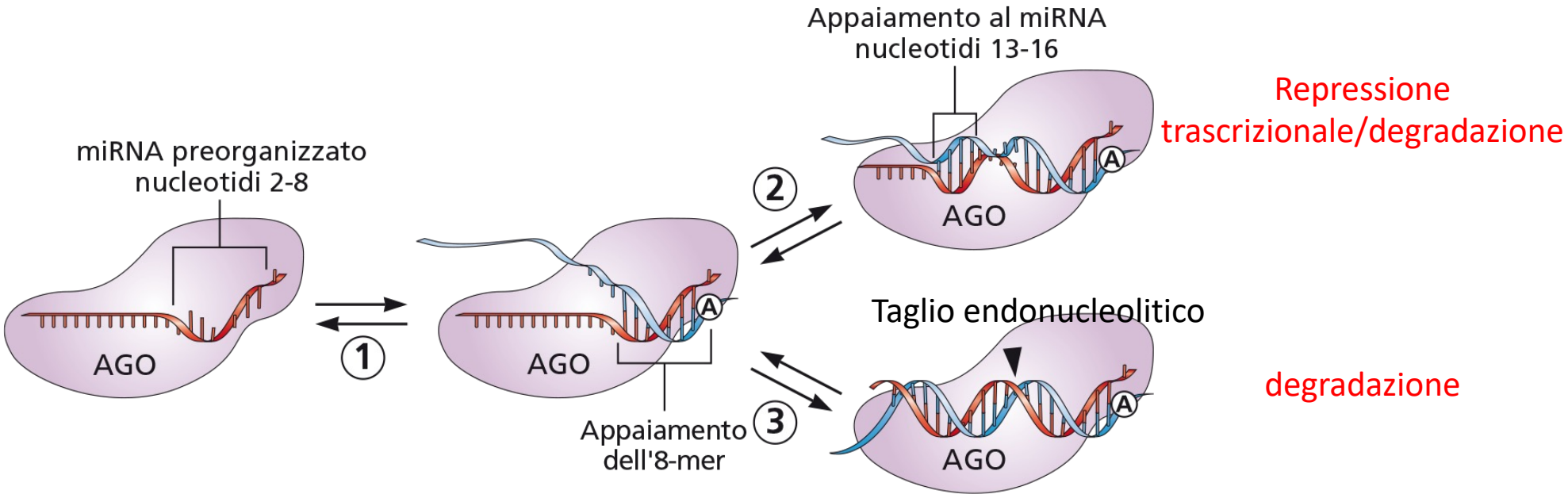
Translational Repression/ mRNA degradation (metazoa)

I microRNA possono indurre la degradazione degli mRNA mediante taglio endonuclolitico

• e' richiesta un perfetta complementarietà tra il microRNA e l'mRNA bersaglio

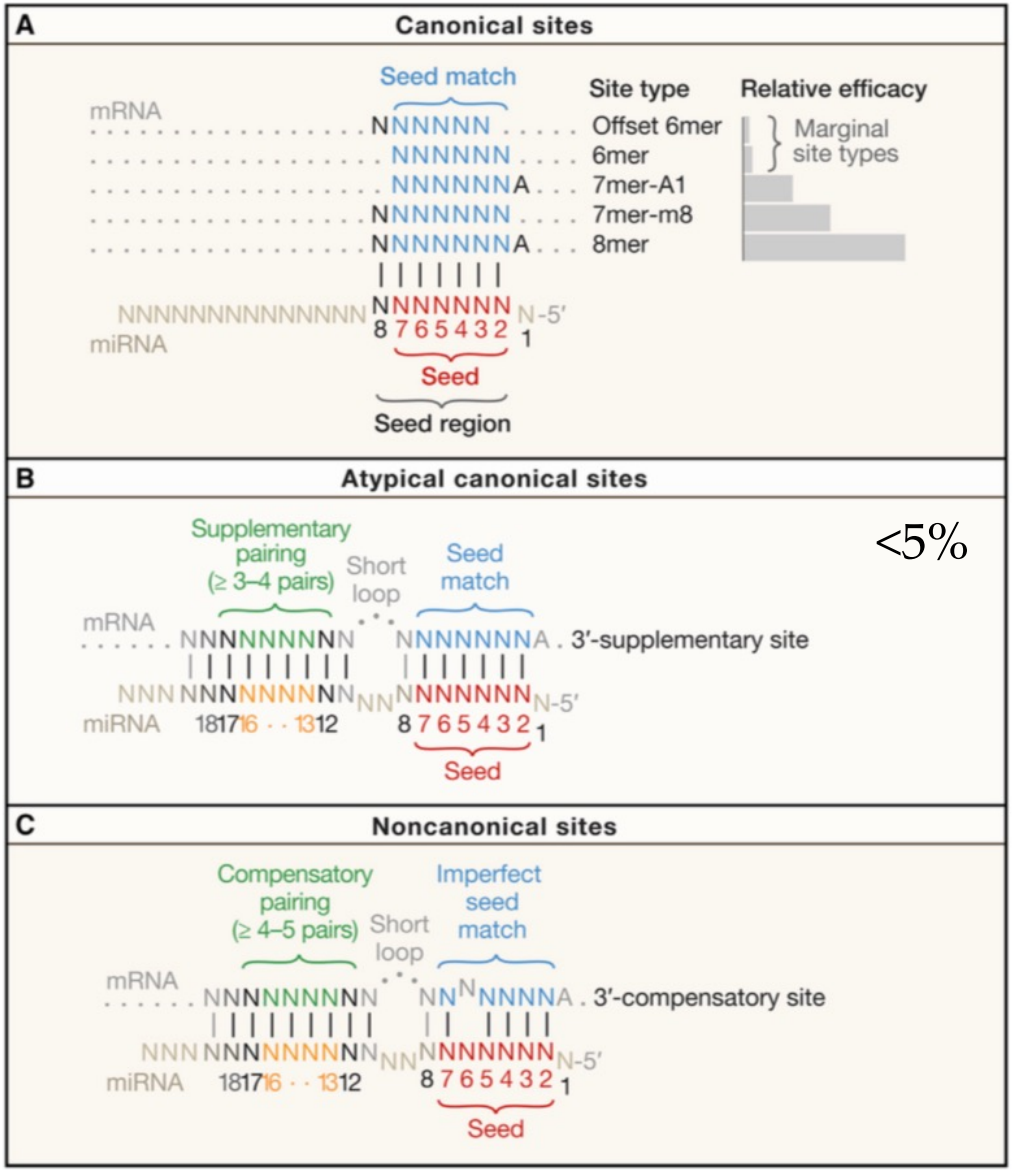
•Quando si verifica tale condizione il doppio filamento viene alloggiato perfettamente nel sito catalitico di Ago2 che taglia il filamento bersaglio.





Post-transcriptional repression by microRNAs

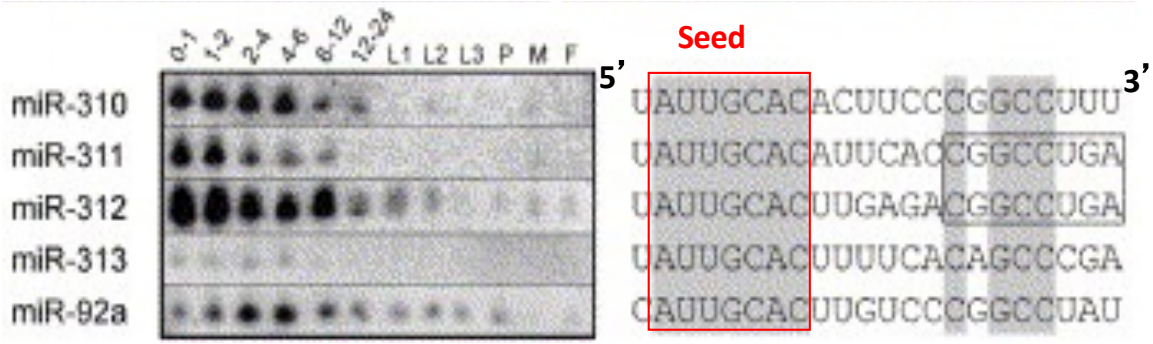
The 5‘-sequence is called **“seed”** region (from the base 2 to base 8 or 9) is particularly important for target site recognition (A). A small fraction of the **canonical sites** (<5%) benefit from pairing to the 3′ region of the miRNA (B). The **noncanonical sites** do not have six contiguous Watson–Crick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



miRNA families

•share the 5′ core sequence and only diverge in their 3′ portions. They might repress common targets but might show distinct expression profiles.

Drosophila



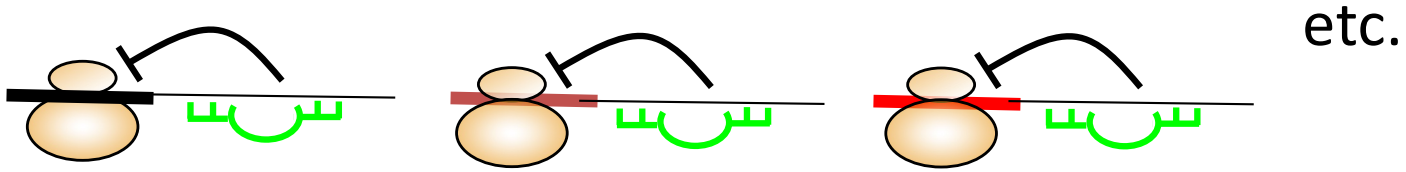
•family members might show distinct phenotypes and target interaction profiles, strongly suggesting that pairing at the 3′ end of miRNAs is biologically significant and utilized in the differential regulation of targets.

Human microRNAs

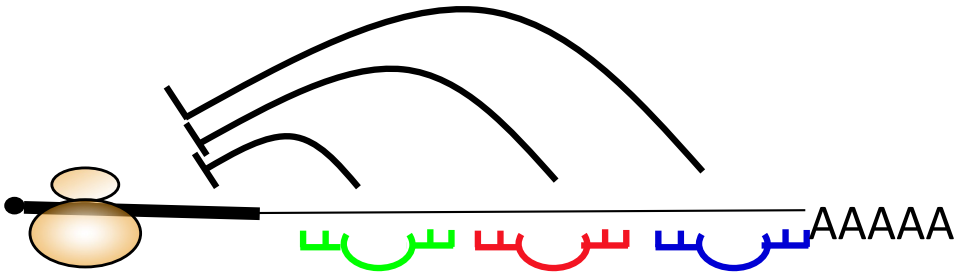
- 2654 distinct microRNAs (20-22 nt) have been identified in humans (data from MiRBase)

- Negatively regulate the translation of mRNAs involved in almost every cellular process.

One microRNA can control hundreds different mRNAs



A single mRNA can be controlled by more than one microRNA



Human microRNAs

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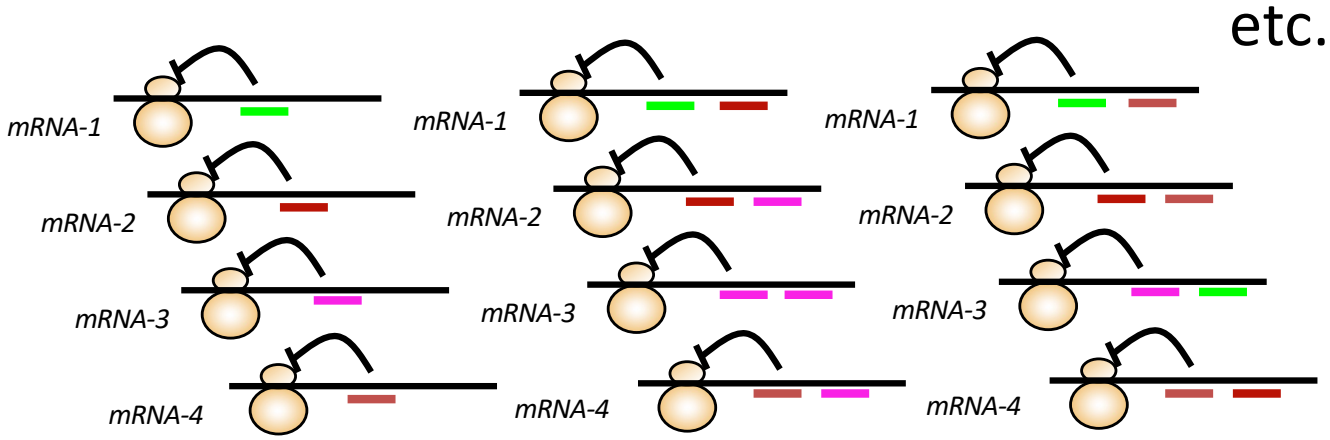
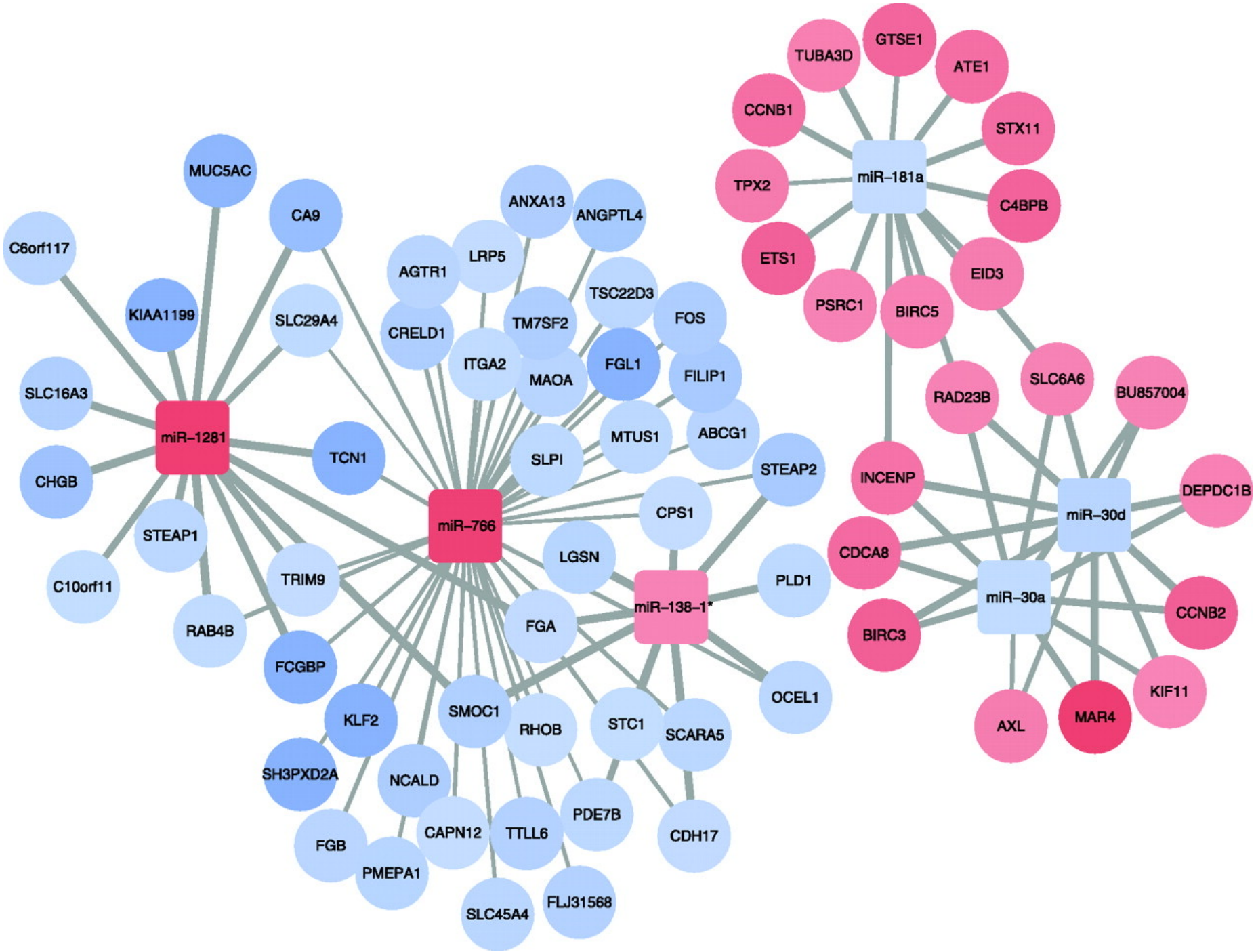
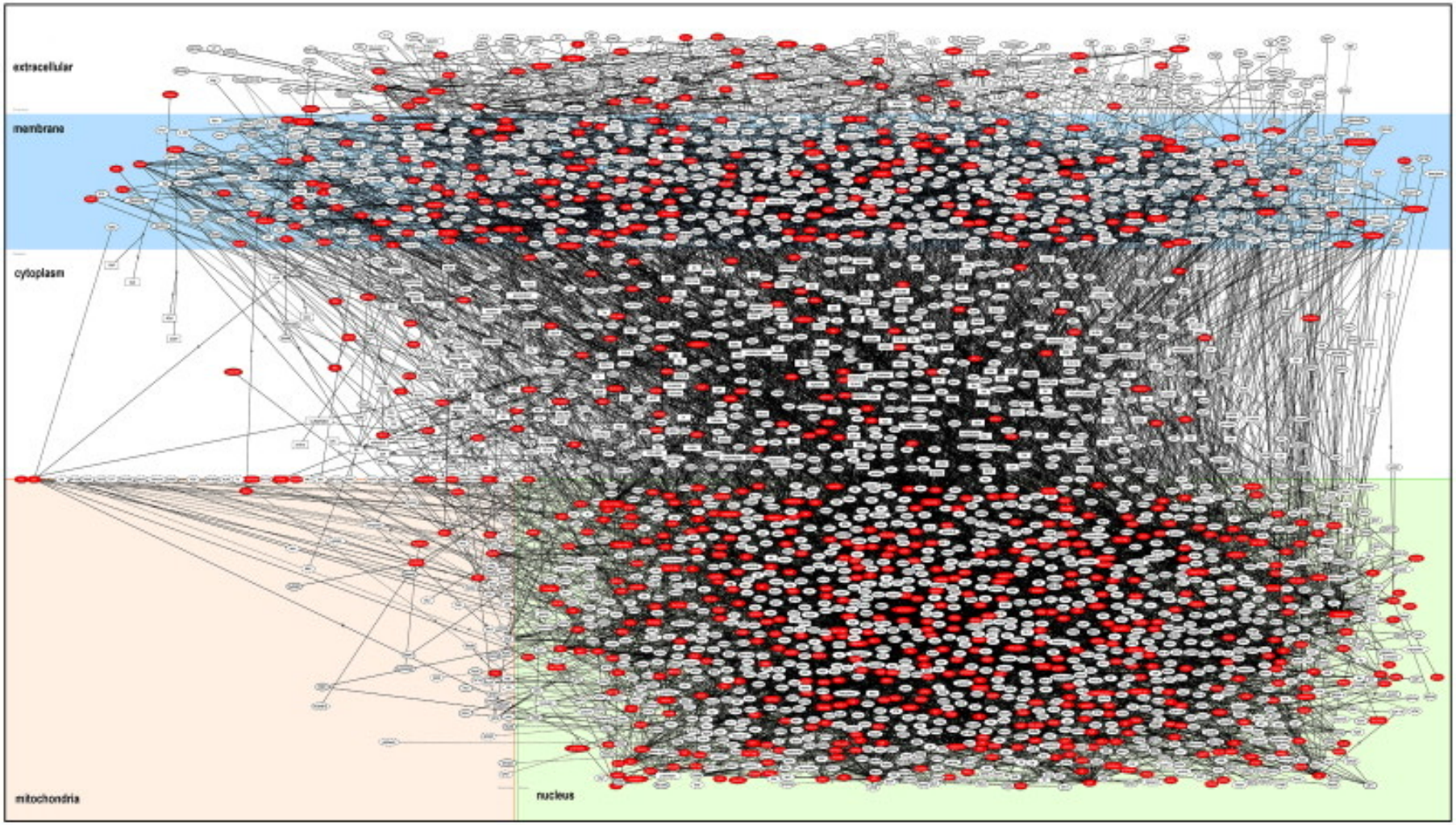
- Negatively regulate the translation of mRNAs involved in almost every cellular process.

- MicroRNAs and their targets form complex regulatory networks




MUC5AC
CA9
C6orf117
KIAA1199
SLC29A4
SLC16A3
miR–1281
TCN1
CHGB
STEAP1
C10orf11
TRIM9
RAB4B
FCGBP
KLF2
SH3PXD2A
FGB
PMEPA1
SLC45A4
FLJ31568
PDE7B
CAPN12
TTLL6
NCALD
CDH17
SMOC1
RHOB
STC1
SCARA5
OCEL1
ANXA13
ANGPTL4
AGTR1
LRP5
CRELD1
TSC22D3
TM7SF2
FOS
ITGA2
MAOA
FGL1
FILIP1
SLPI
MTUS1
ABCG1
STEAP2
miR–766
CPS1
LGSN
FGA
miR–138–1*
PLD1
TUBA3D
GTSE1
ATE1
CCNB1
STX11
TPX2
miR–181a
C4BPB
ETS1
EID3
PSRC1
BIRC5
RAD23B
SLC6A6
BU857004
INCENP
DEPDC1B
miR–30d
CDCA8
miR–30a
CCNB2
BIRC3
KIF11
AXL
MAR4


extracellular
membrane
cytoplasm
mitochondria
nucleus

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:

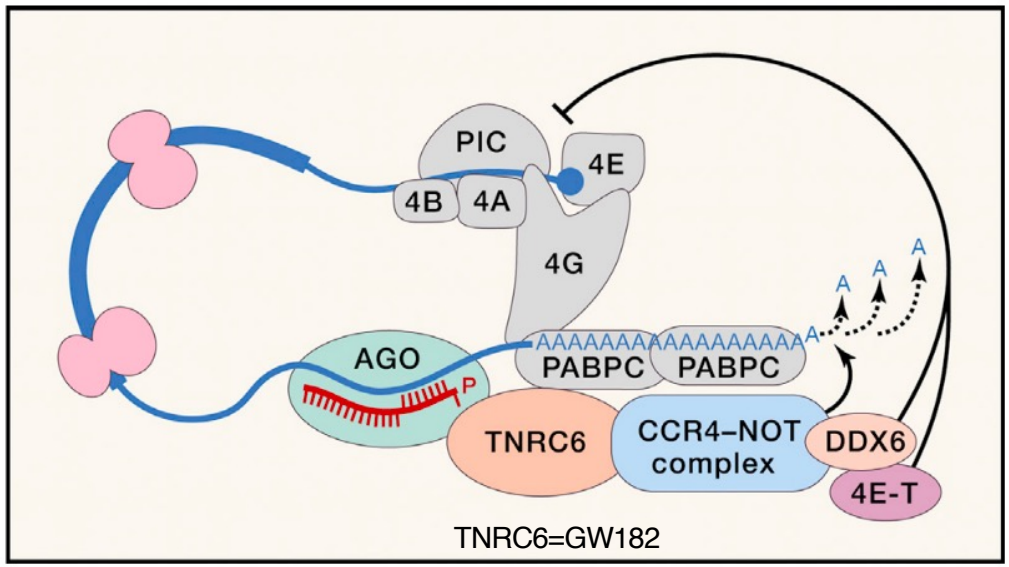
- **Networking and fine-tuning of gene expression**
- **Rapid repression**

Post-transcriptional repression by microRNAs

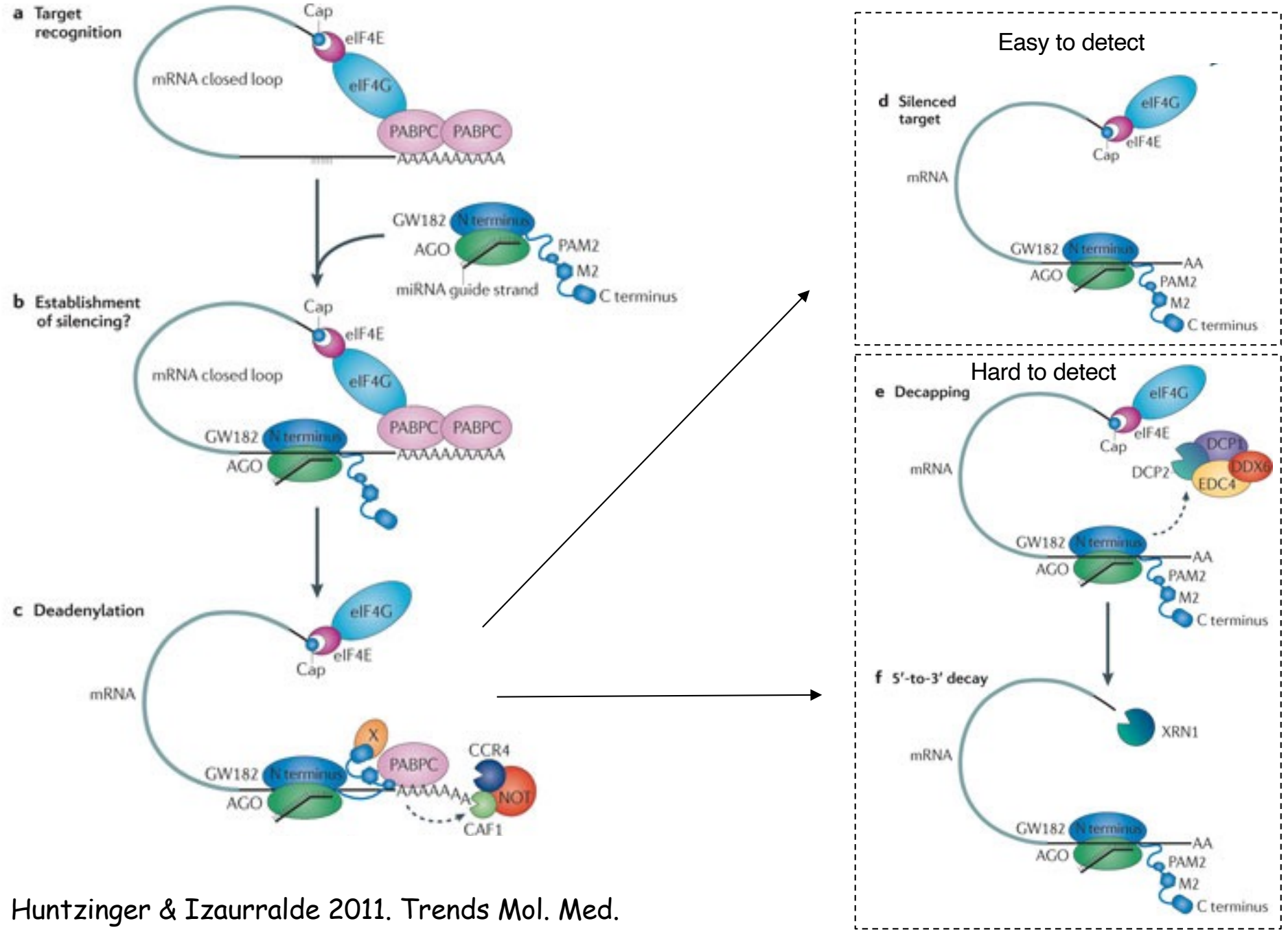
•miRNAs silence gene expression by repressing translation and accelerating target mRNA degradation

•Guided by the miRNA, the silencing complex associates with the mRNA and recruits TNRC6 (GW182), which interacts with PABPC and recruits either the PAN2– PAN3 deadenylase complex or the CCR4–NOT deadenylase complex, either of which shortens the mRNA poly(A) tail.

•TNRC6 can repress translation initiation by recruitment of DDX6 and 4E-T proteins.



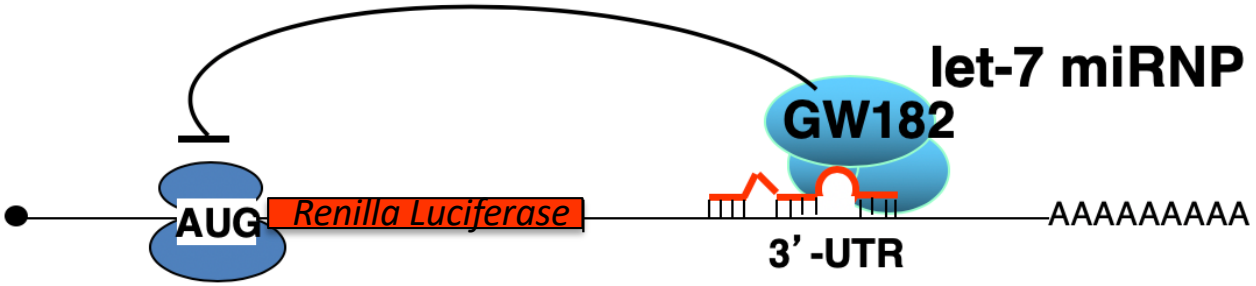
Mechanisms



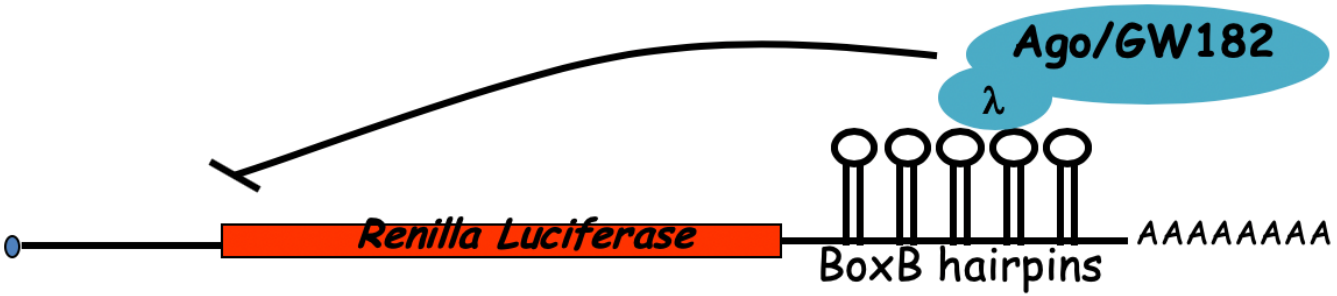
Huntzinger & Izaurralde 2011. Trends Mol. Med.

mRNA reporters to study miRNA-mediated repression

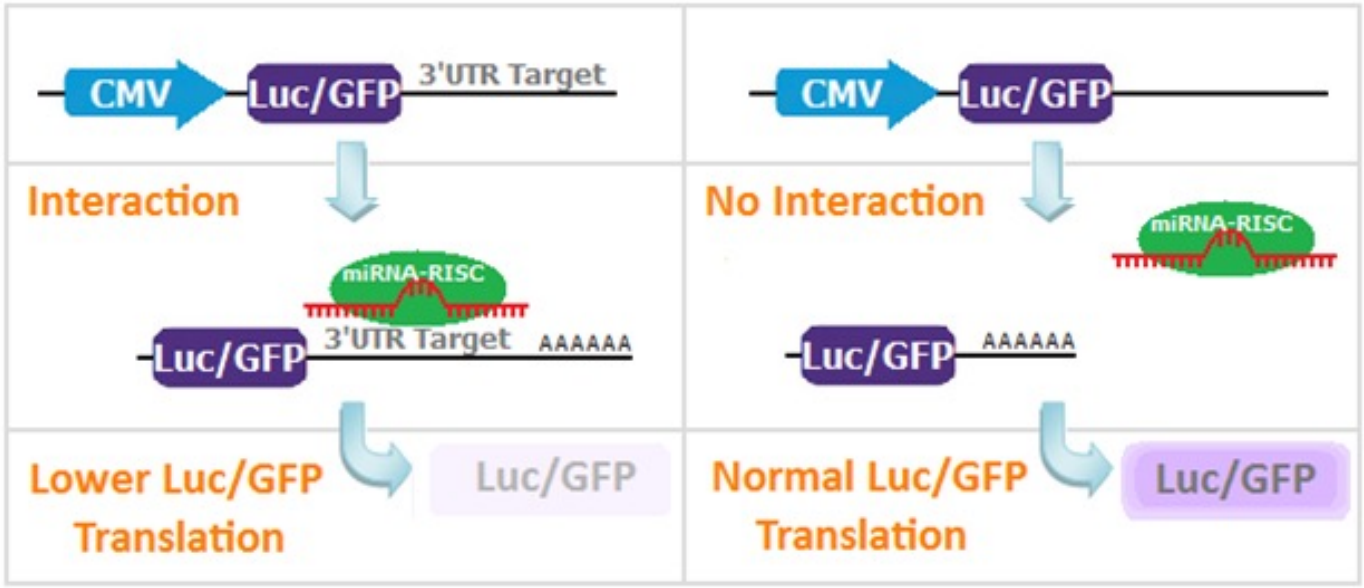
- Reporters responding to the endogenous let-7 miRNP

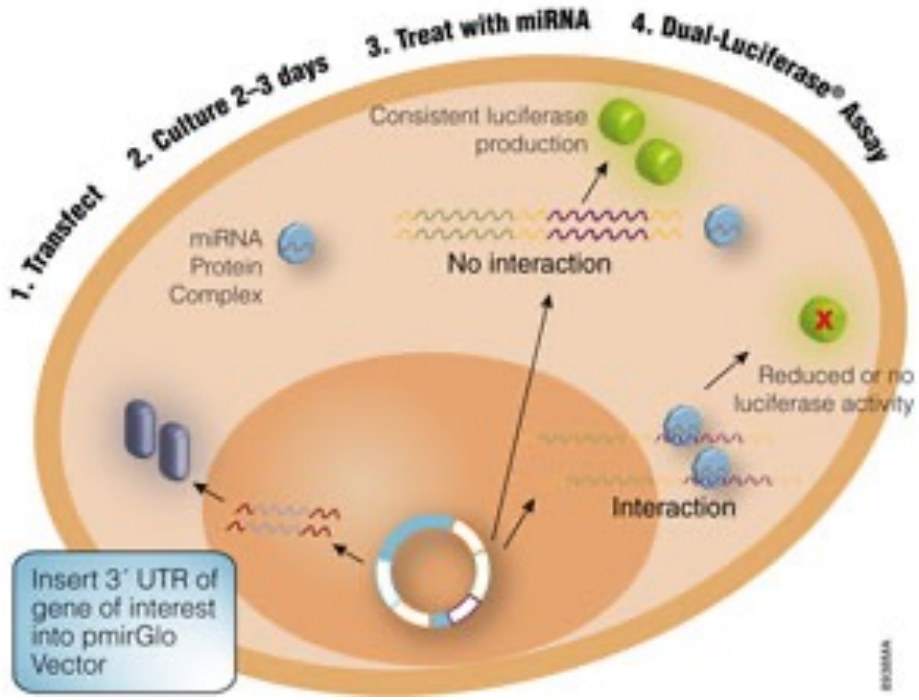


- Reporters responding to the miRNP protein tethering

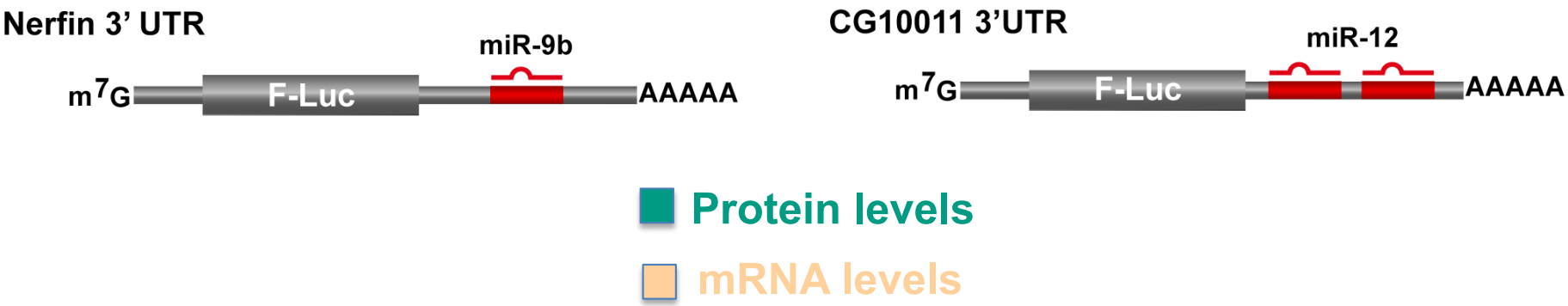


RNA reporters to verify microRNA targets





Silencing by miRNAs involves translational repression and / or mRNA decay



Behm-Ansmant et al.2006. Genes & Dev.

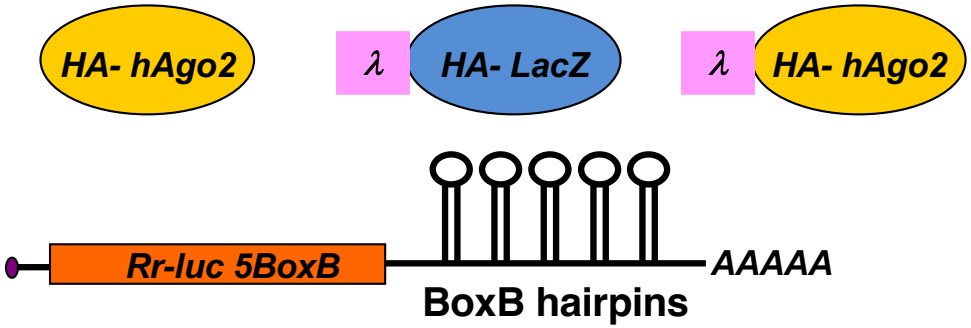
Slicer independent miRNA-mediated decay

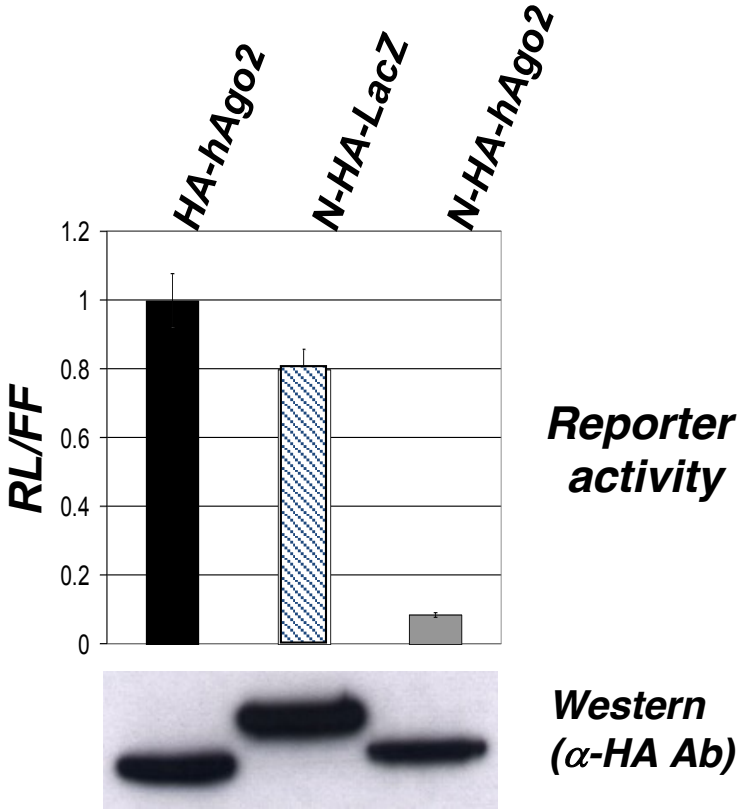
- Introducing a specific miRNA into a cell decreases the levels of transcripts with potential binding sites for the miRNA (Lim et al., 2005; Giraldez et al., 2006).

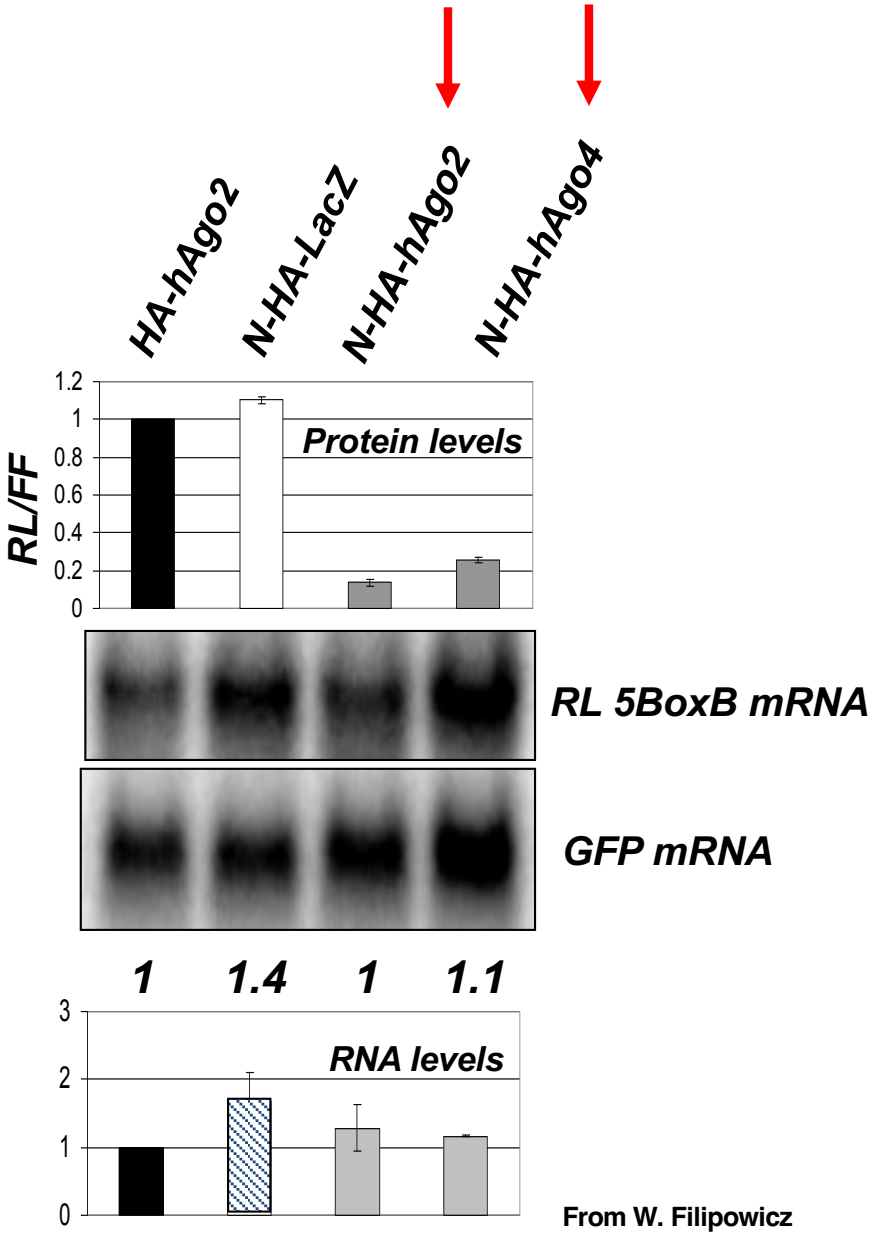
  ✓mRNAs with rapid decay rates may appears to be solely translationally repressed since the mRNA turnover is already fast. In contrast, long-lived mRNAs may be more susceptible to an increase in decay rates by miRNA repression.

  ✓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

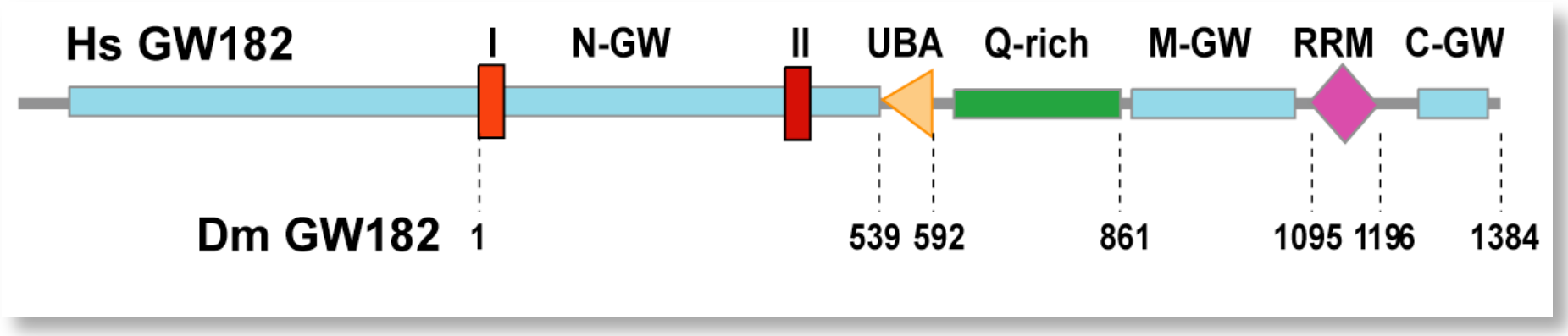






From W. Filipowicz

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



UBA: ubiquitin associated domain

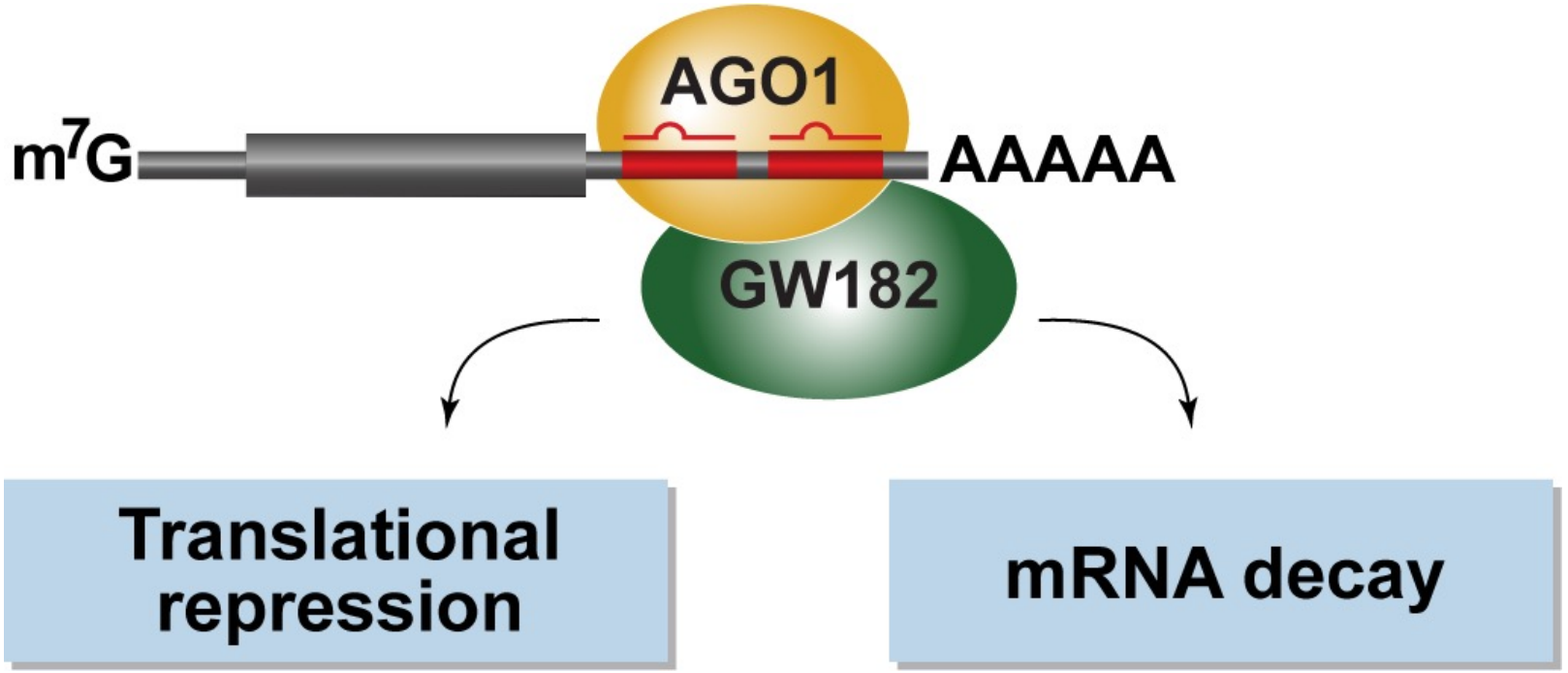
RRM: RNA recognition motif

GW: glycine and tryptophan repeats

I and II: conserved motifs

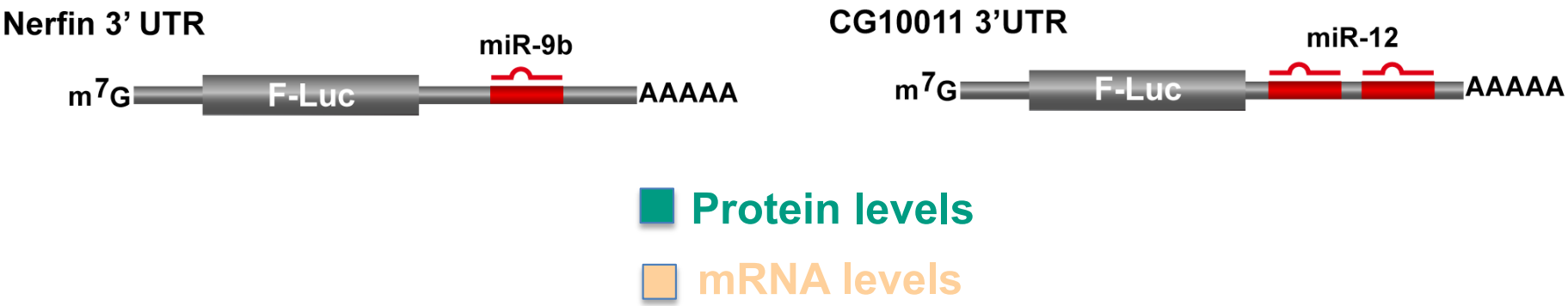
Identified for interaction with Ago

GW182-AGO interaction is essential for silencing

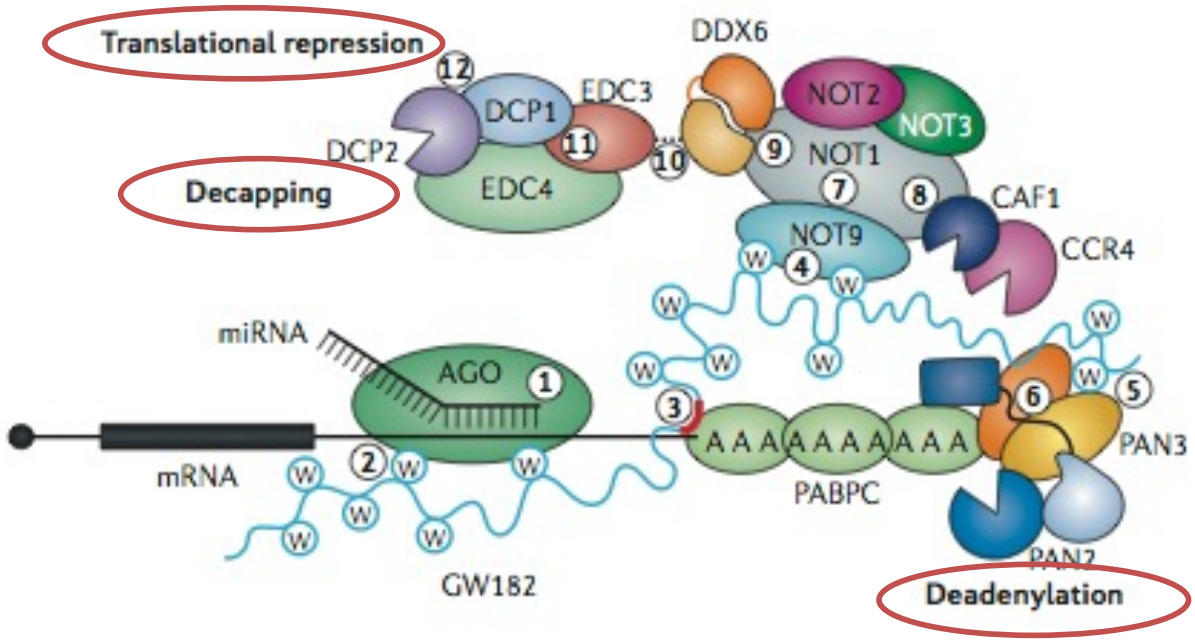


Behm-Ansmant et al. 2006. Genes & Dev. 20:1885

GW182 is required for silencing by miRNAs



Structure-based model of miRNA-mediated silencing

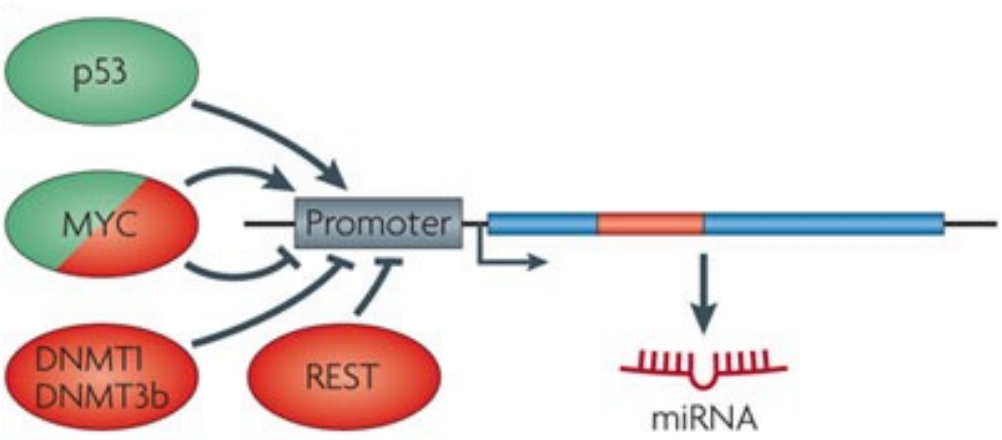


GW182 recruits NOT1 through WG/S/T domains
NOT1 is involved in repression of translation, it recruits DDX6
eiF4G interacts with the DEAD box helicase DDX6
DDX6 represses translation
DDX6 is a decapping activator

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.

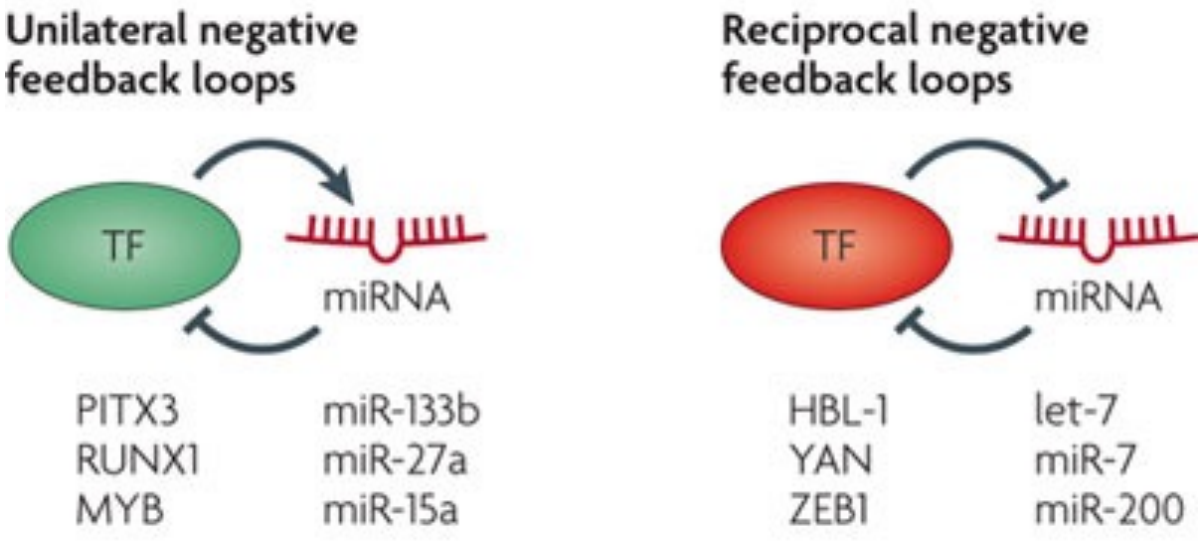
•Many TFs regulate miRNA expression positively or negatively in a tissue-specific or developmental-specific manner



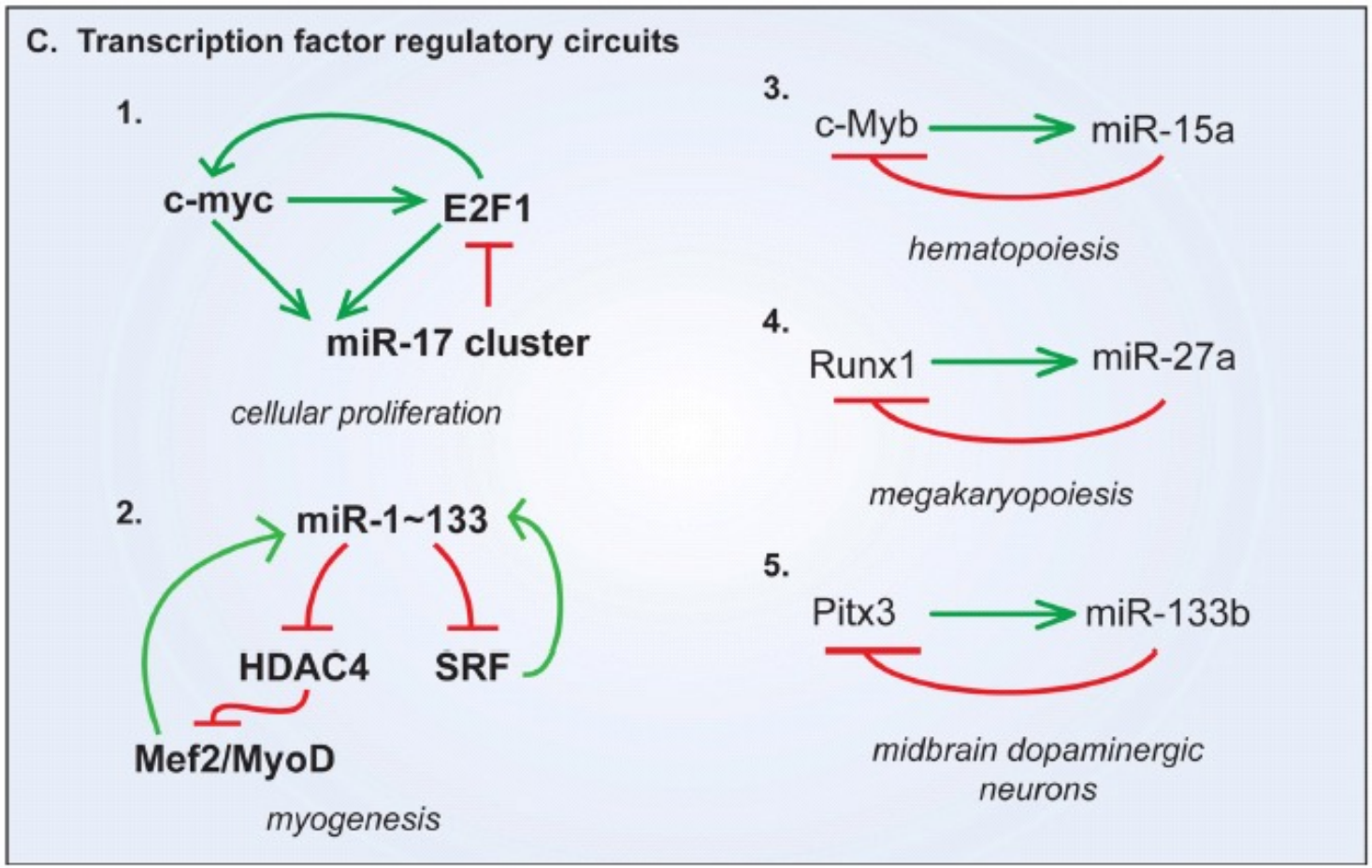
Regulation of miRNA gene transcription

•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.



Regulatory networks between TFs and microRNAs



Regulators of miRNA processing

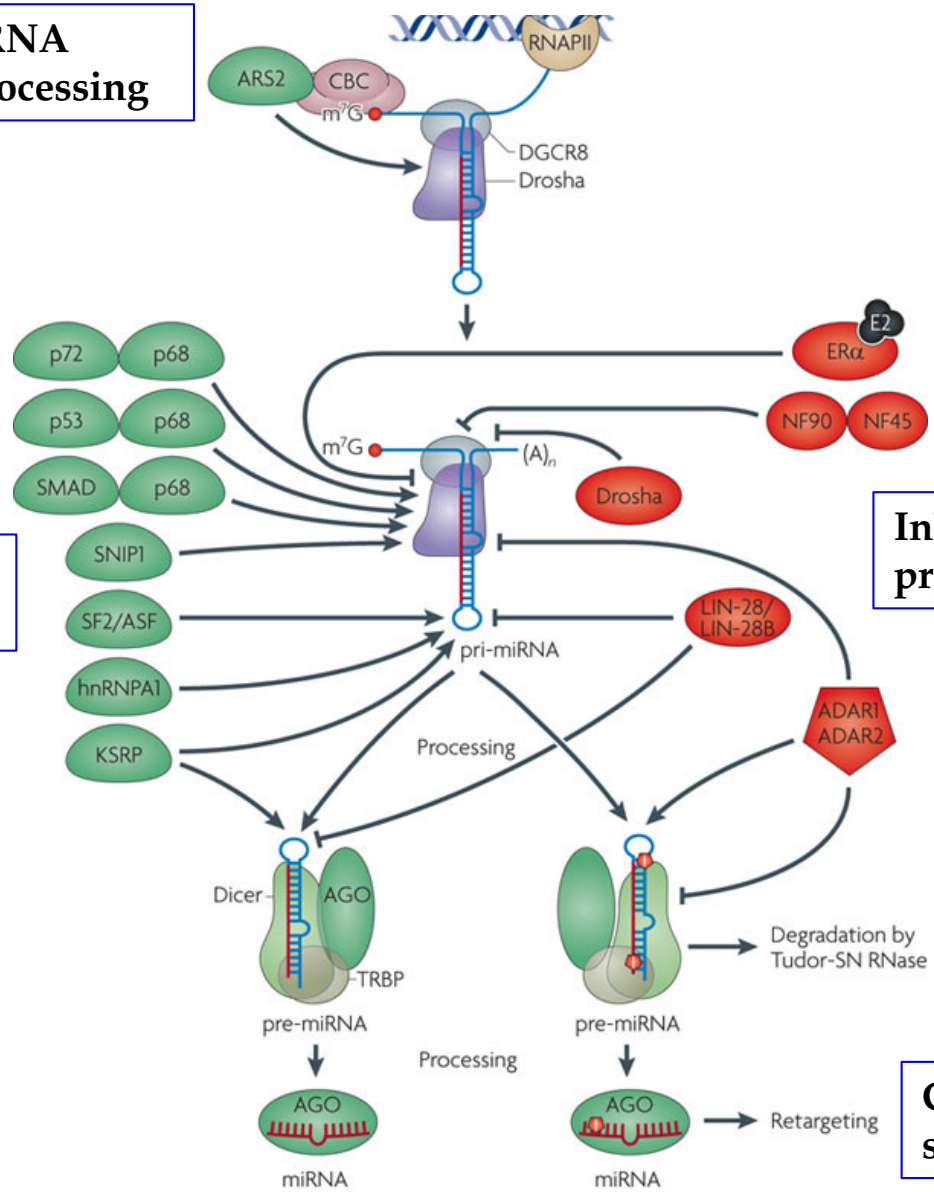
Coupling of pri-miRNA transcription and processing

Stimulation of miRNA processing

Inhibition of miRNA processing

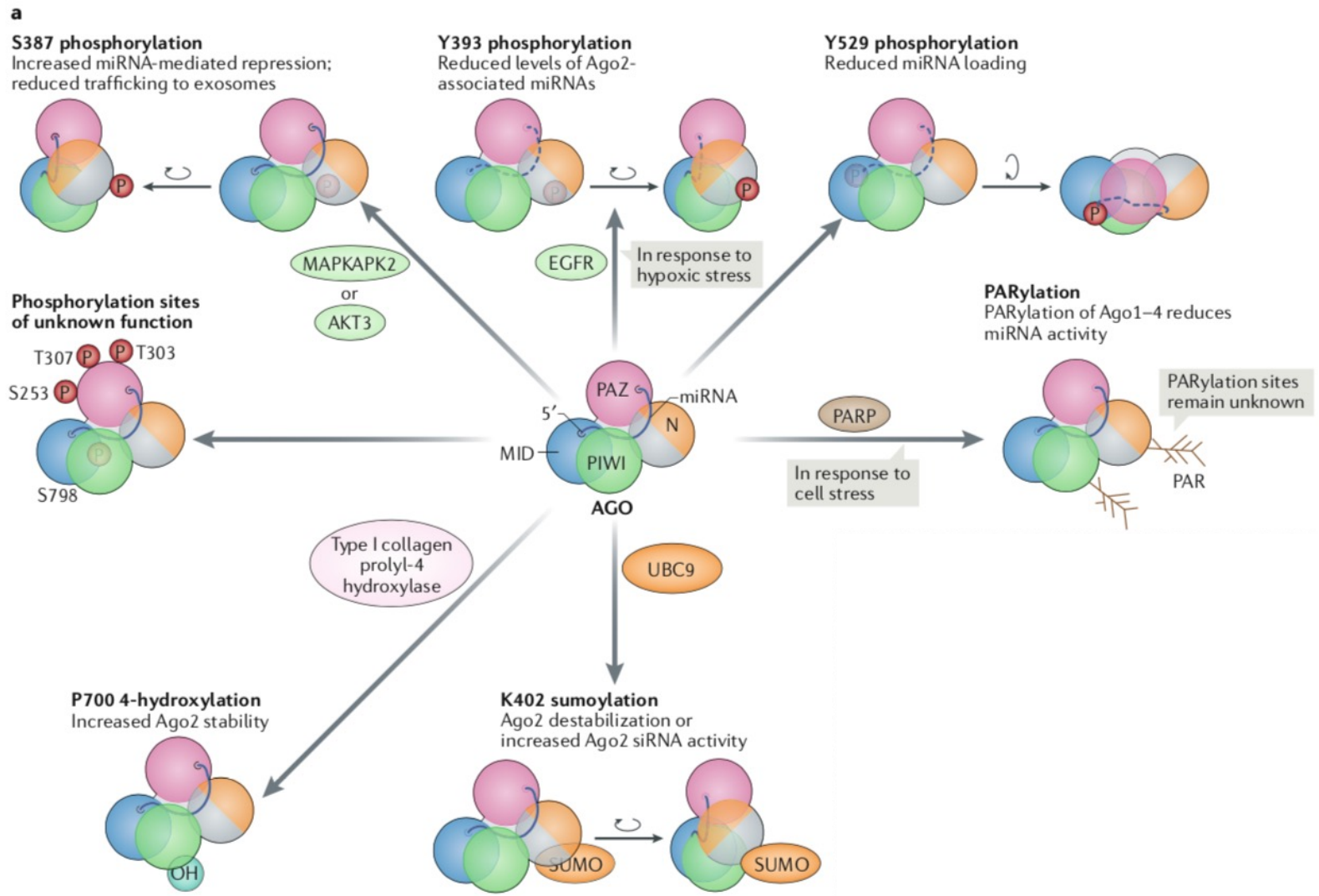
Degradation

Changing miRNA target specificity



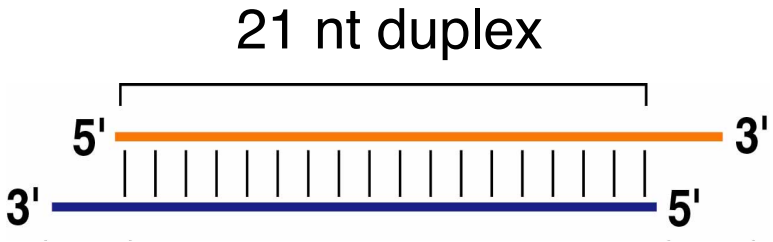
Nature Reviews | Genetics

The miRNA-induced silencing complex is modulated by post-translational modifications of Ago proteins

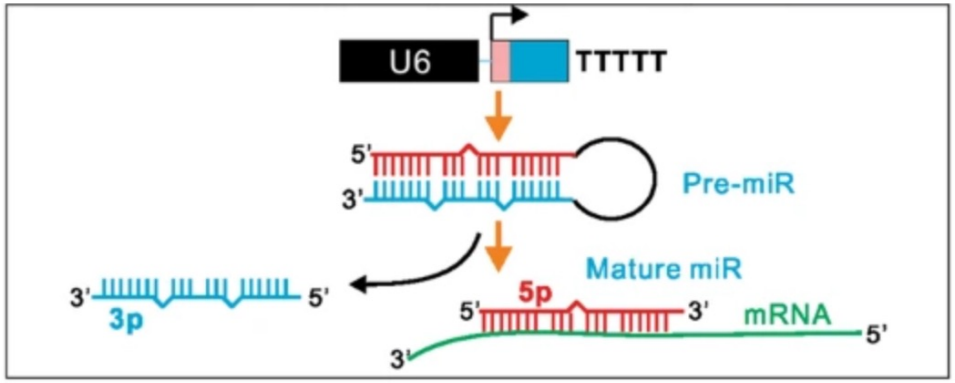


miRNA expression system

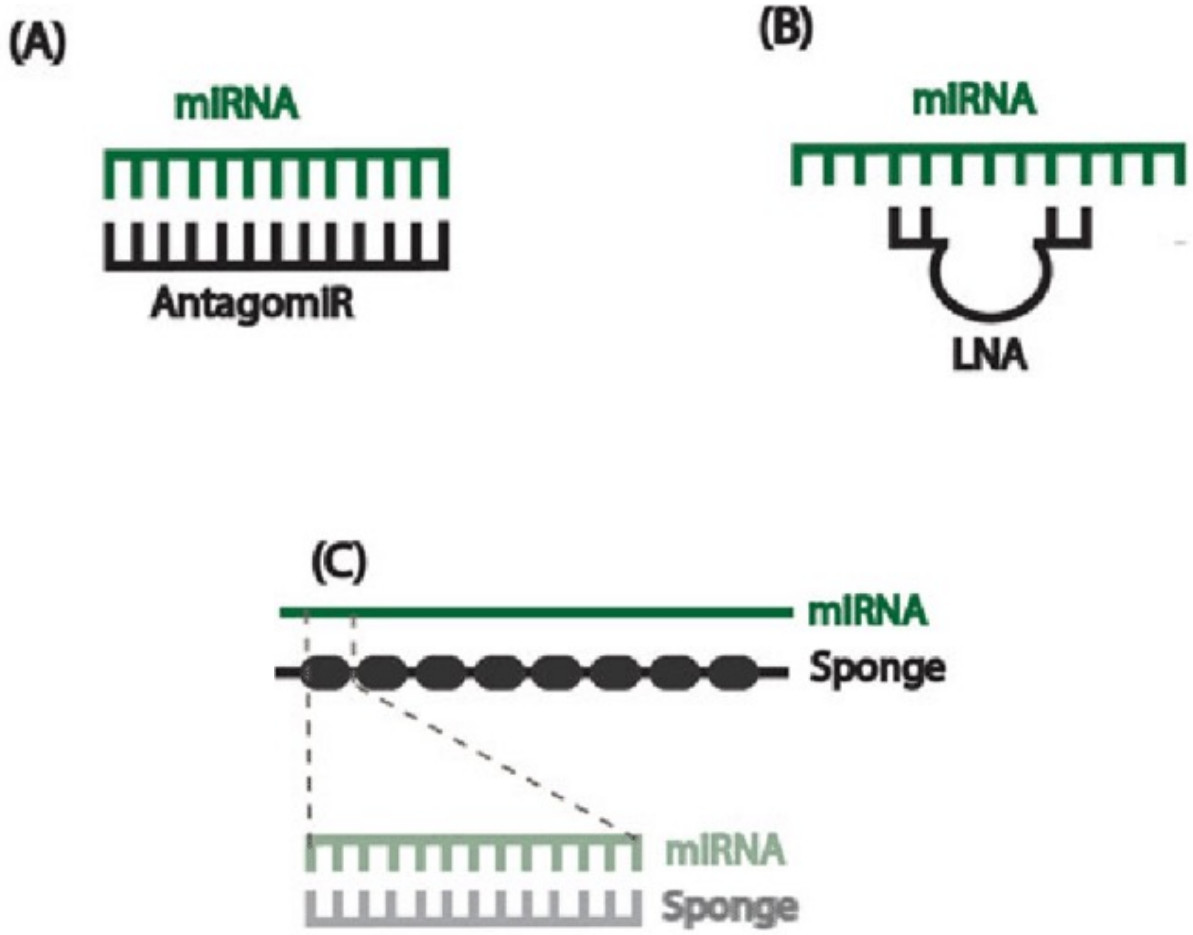
- RNA duplex



- Pol III vector



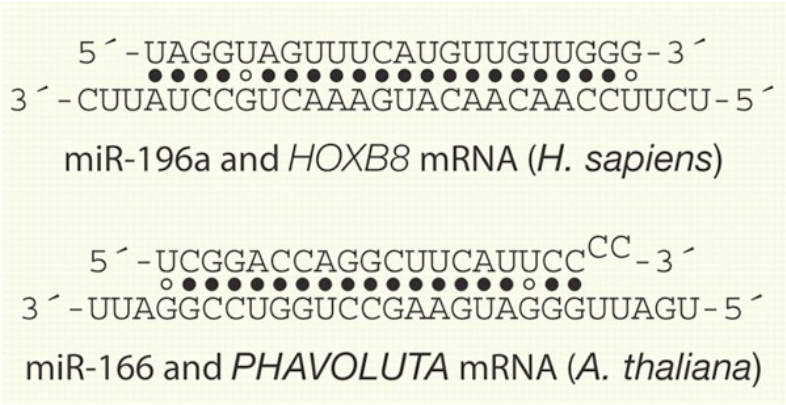
miRNA inhibitors



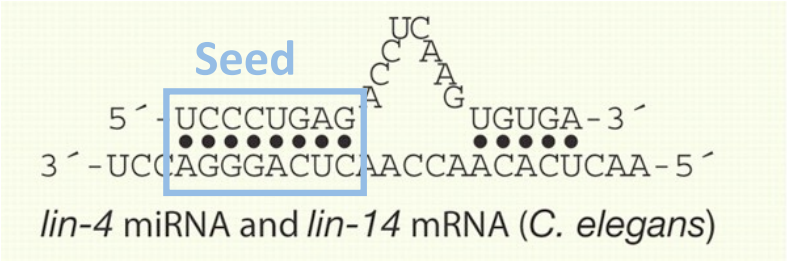
4-Target identification

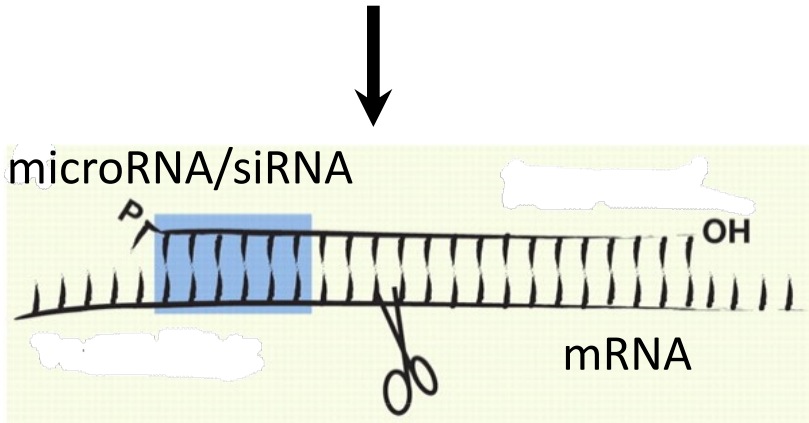
mRNA target recognition

Extensive pairing of a microRNA to a target mRNA

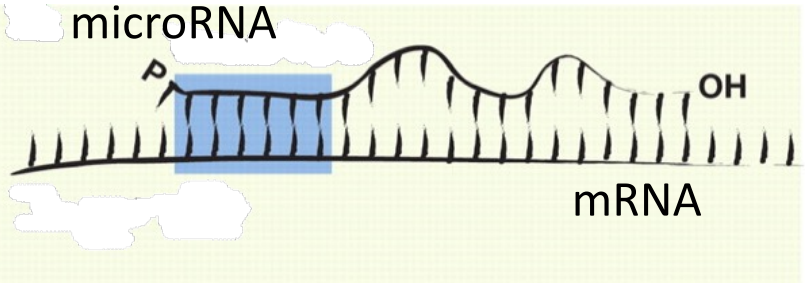


Perfect pairing between the "seed" sequence (nucleotides 1/2 to 7/8 of the microRNA) and target mRNA





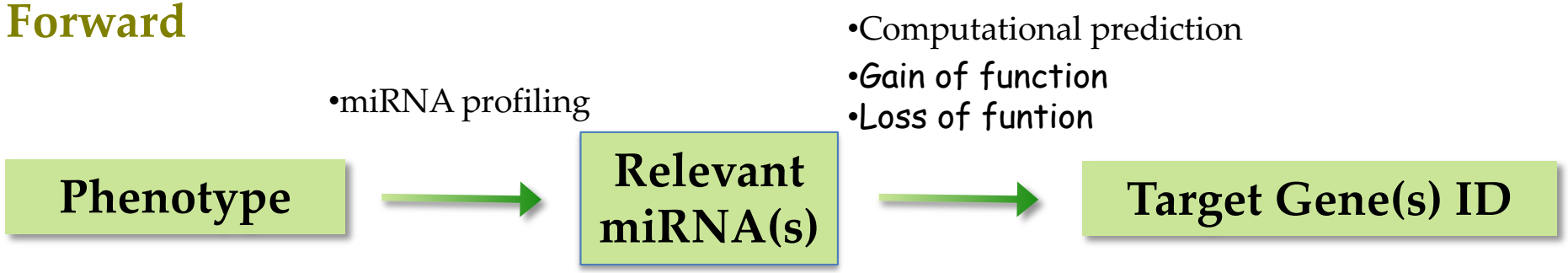
Cleavage



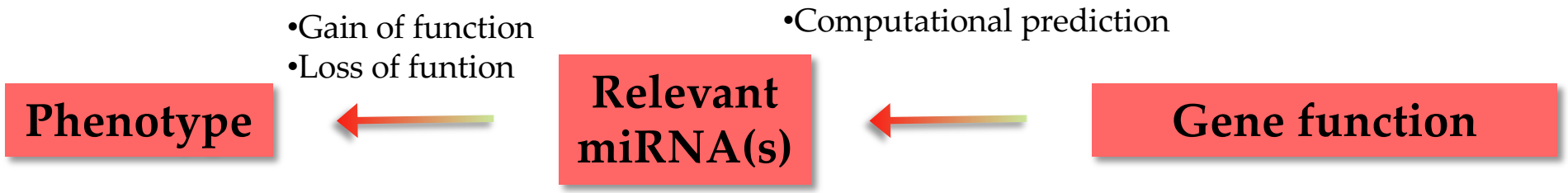
Translational repression

Target identification

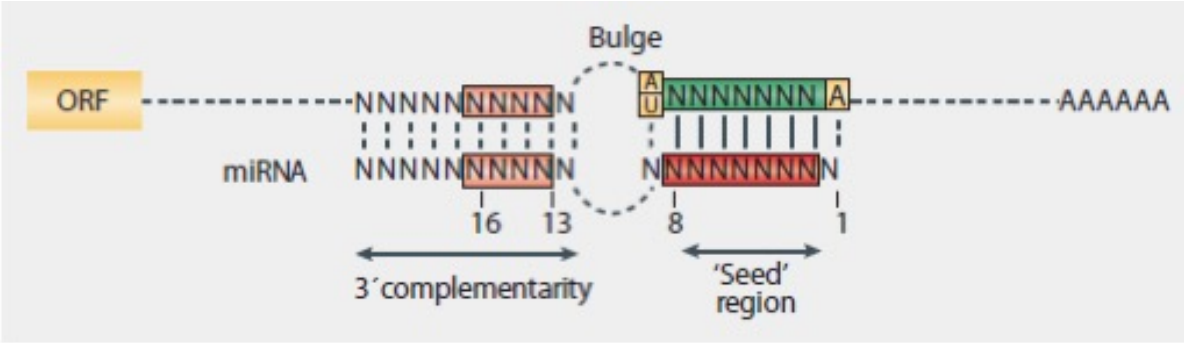
1) Forward



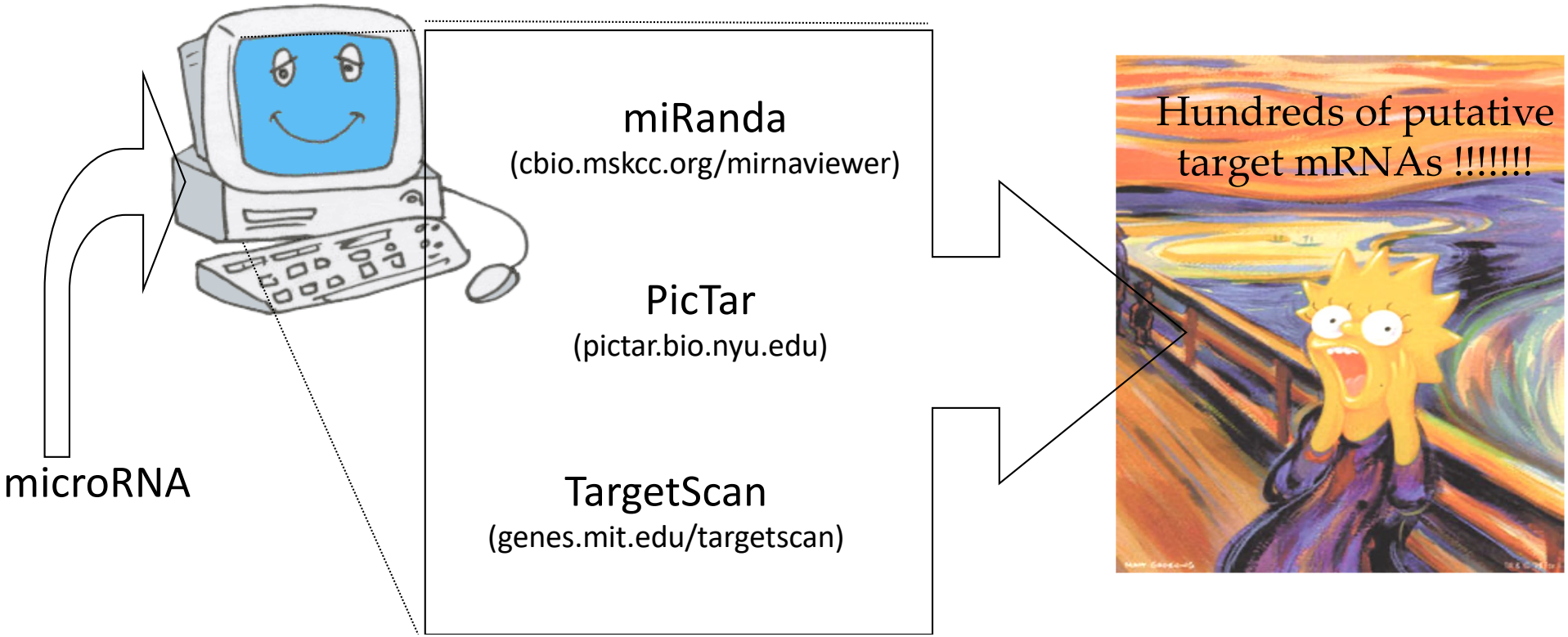
2) Reverse



Identification of microRNA targets

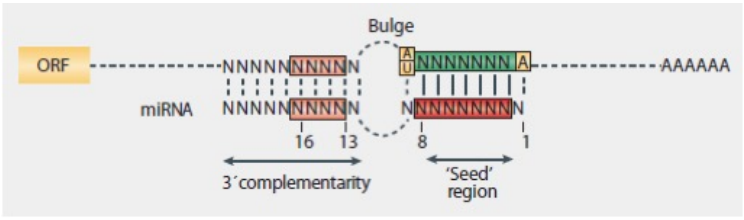


1) Forward



Identification of microRNA targets

miR-223 targets



Human I 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}]

Table sorted by total context score

Ge rved sites are not shown [View top predicted targets, irrespective of site conservation]

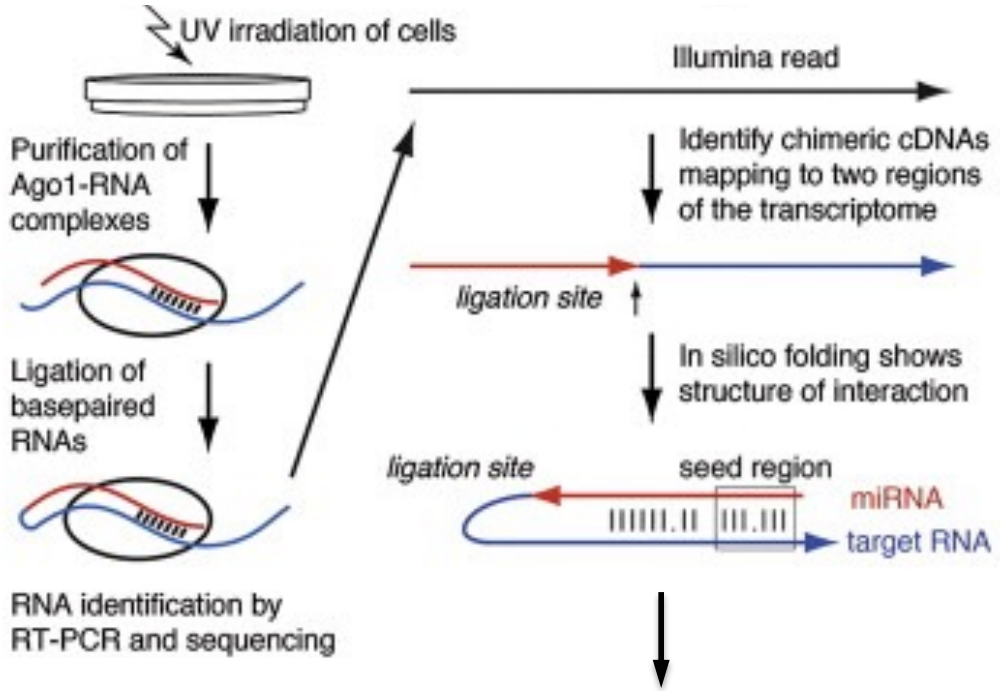
The table shows at most one transcript per gene, selected for having the highest aggregate P_{CT} (or the one with the longest 3' UTR, in case of a tie). [Show all transcripts]

Target gene	Representative transcript	Gene name	Conserved sites				Poorly conserved sites				Repre-sentative 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

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.

Helwack et al, 2013. Cell

2018

nature structural & molecular biology

ARTICLES

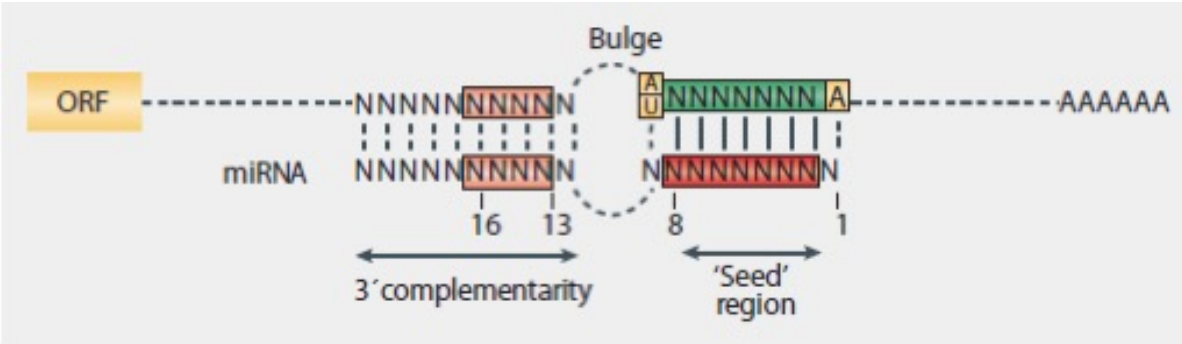
https://doi.org/10.1038/s41594-018-0136-3

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

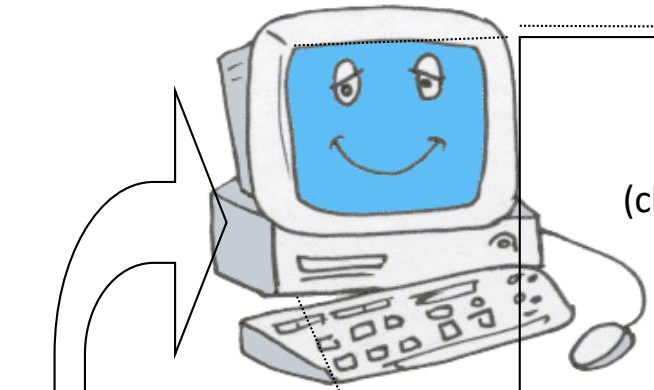
Kai Zhang[1,8], Xiaorong Zhang[2,8], Zhiqiang Cai[1,8], Jie Zhou[1,8], Ran Cao[1], Ya Zhao[3,4], Zonggui Chen[1], Dehe Wang[1], Wen Ruan[1], Qian Zhao[2], Guangqiao Liu[2], Yuanchao Xue[2], Yan Qin[2], Bing Zhou[5], Ligang Wu[4], Timothy Nilsen[6], Yu Zhou[1,7]* and Xiang-Dong Fu[1,2,5]*

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.

Identification of microRNAs targeting specific mRNAs



2) Reverse

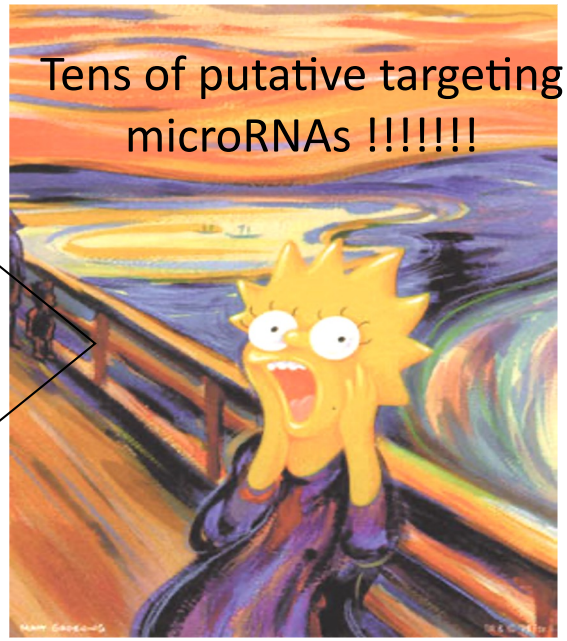


mRNA

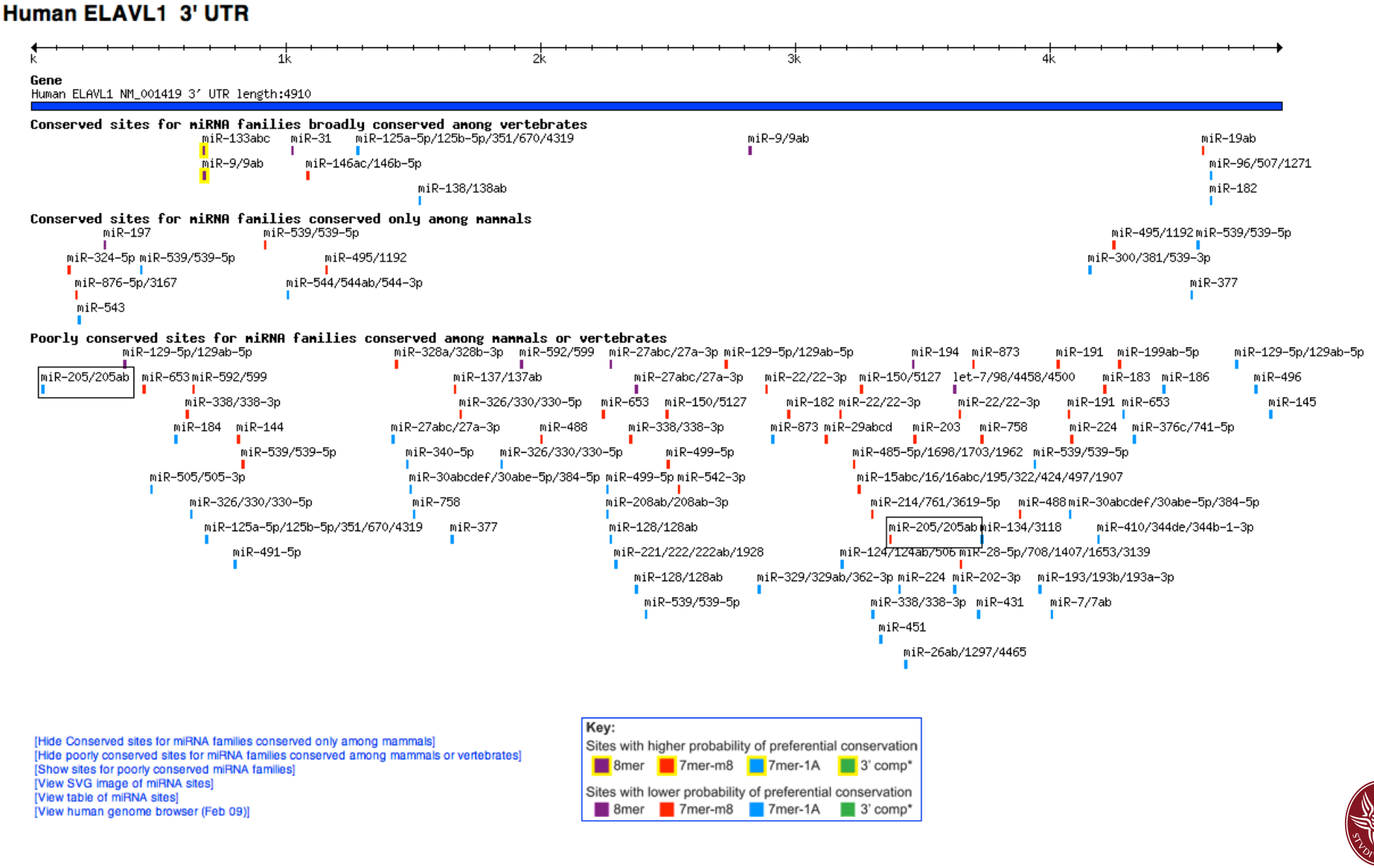
miRanda
(cbio.mskcc.org/mirnaviewer)

PicTar
(pictar.bio.nyu.edu)

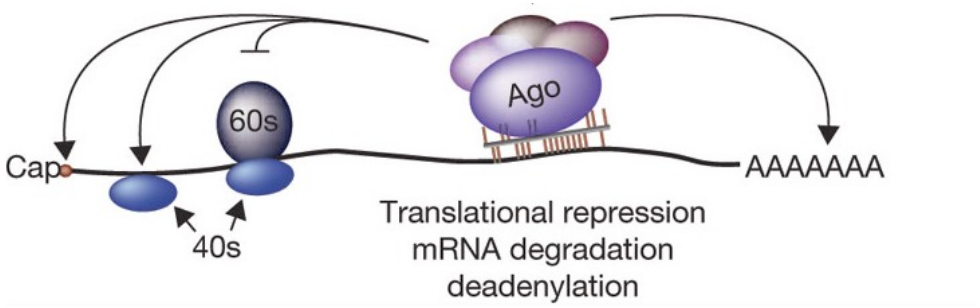
TargetScan
(genes.mit.edu/targetscan)



Identification of microRNAs targeting specific mRNAs



Identification of microRNA targets



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

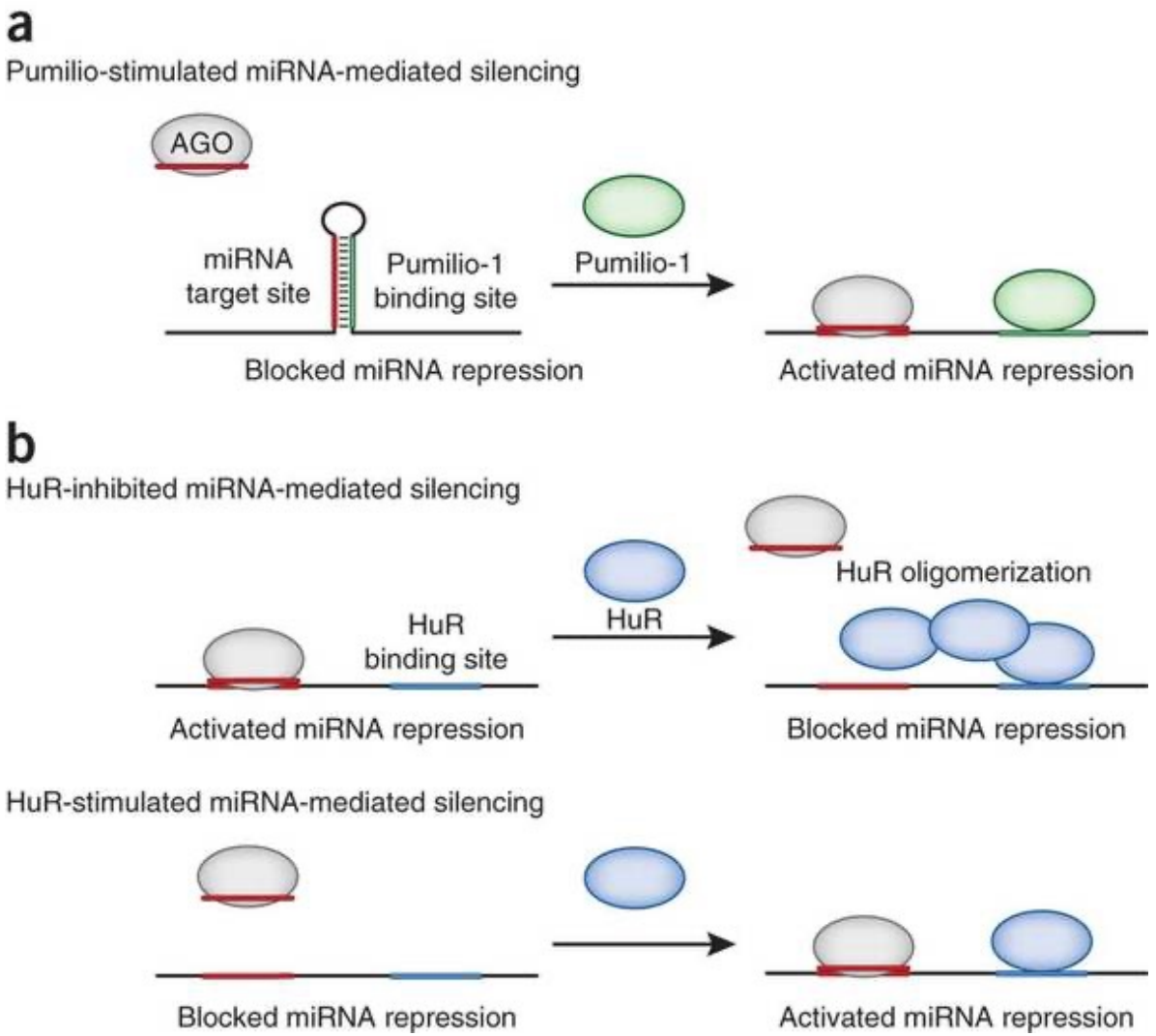
  1. Strong regulation of one dominant target (99% of publications)

  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

- **<u>RNA binding proteins</u>**: they can shield miRNA target sites from miRNA binding.



Fabian & Sonemberg, 2012.