Nobelförsamlingen
The Nobel Assembly at Karolinska Institutet

October 7, 2024

The Nobel Assembly at the Karolinska Institutet

has today decided to award

the 2024 Nobel Prize in Physiology or Medicine

jointly to

Victor Ambros and Gary Ruvkun

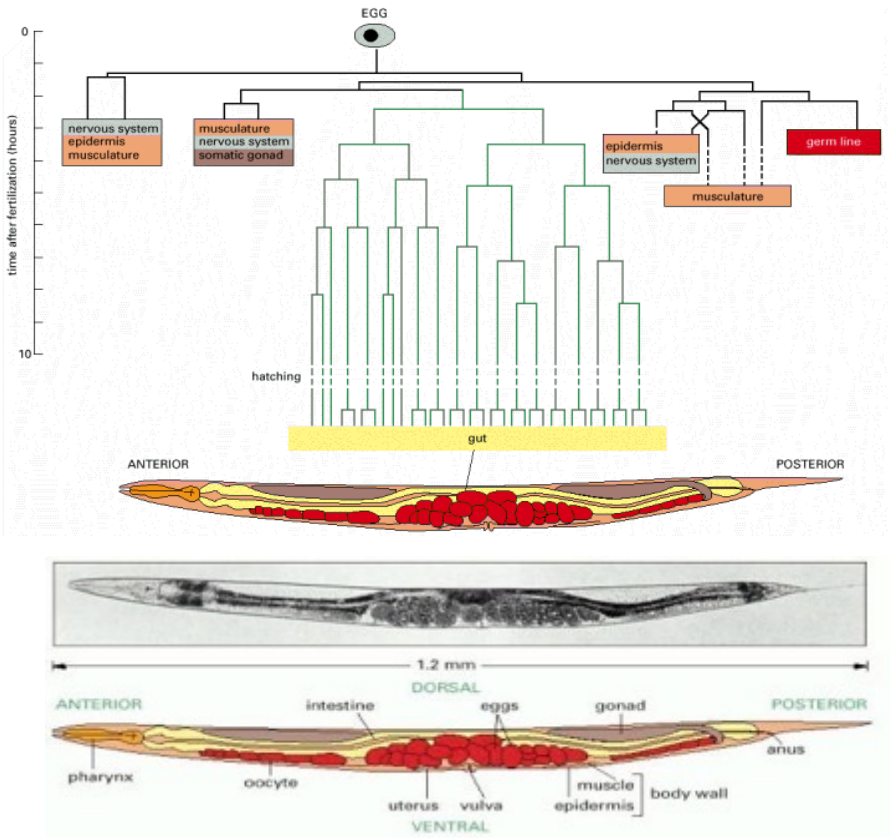
for the discovery of microRNA and its role in post-transcriptional gene regulation

A Nobel laureates' tale

In the late 1980s, Victor Ambros and Gary Ruvkun were postdoctoral fellows in the laboratory of Robert Horvitz (awarded the Nobel Prize in 2002, for apoptosis discovery).

In Horvitz's laboratory, they studied a 1 mm long roundworm, C. elegans: despite its small size, C. elegans possesses many specialized cell types such as nerve and muscle cells, making it a useful model for investigating how tissues develop and mature in multicellular organisms.

Ambros and Ruvkun were interested in genes that control the timing of activation of different genetic programs, ensuring that various cell types develop at the right time.



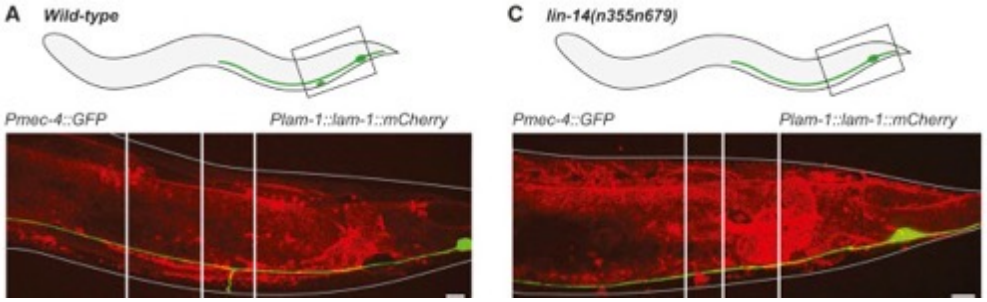
C. elegans is a precious tool in developmental biology:

- it is tiny and grow rapidly
- females are composed of 956 cells
- males are composed of 1031 cells
- the fate of every cell is characterized

They studied two mutant strains of worms, lin-4 and lin-14, that displayed defects in the timing of activation of genetic programs during development: they wanted to identify the mutated genes and understand their function.

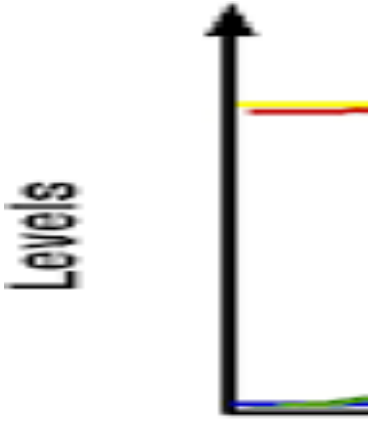
Lin-14

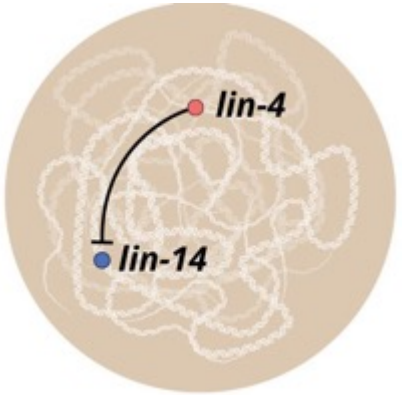
LIN-14 is a transcription factor that plays a key role in development.
The expression of LIN-14 forms a steep temporal gradient during early development, with protein levels decreasing rapidly after the first larval stage, driving development of several cell lineages, including directing cell-fate decisions in precursors of the mechanosensory neurons.



Lin-4

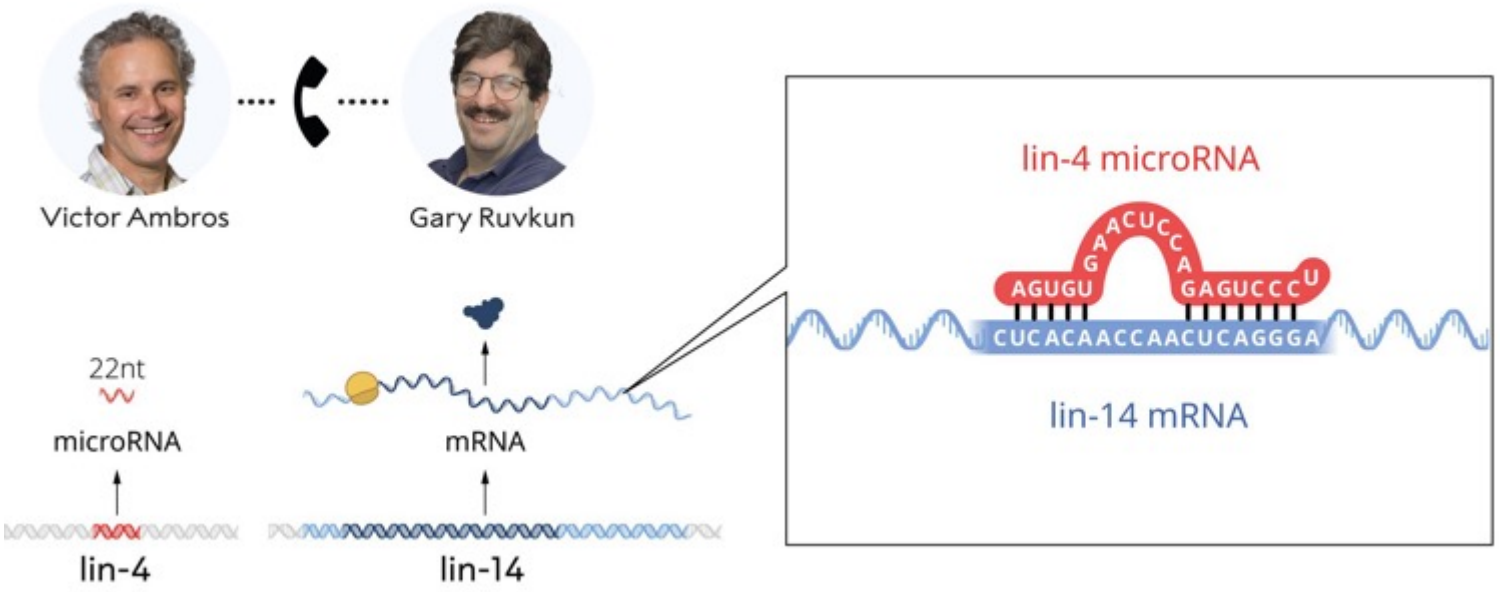
Ambros showed that lin-4 appeared to be a **negative regulator** of lin-14. However, how the lin-14 activity was blocked by lin-4 was unknown.





Ambros discovered that the lin-4 gene encoded a tiny RNA, microRNA, that did not code for a protein.

Ruvkun cloned the lin-14 gene, and the two scientists realized that it is not the production of mRNA from lin-14 that is inhibited by lin-4, but the shutdown of protein production. Moreover, the short lin-4 sequence matched complementary sequences in the critical segment of the lin-14 mRNA.



The lin-4 microRNA turns off lin-14 by binding to the complementary sequences in its mRNA, blocking the production of lin-14 protein

Cell, Vol. 75, 855–862, December 3, 1993, Copyright © 1993 by Cell Press

Posttranscriptional Regulation of the Heterochronic Gene *lin-14* by *lin-4* Mediates Temporal Pattern Formation in C. elegans

Bruce Wightman,*† Ilho Ha,* and Gary Ruvkun

site phenotypes (Ambros and Horvitz, 1987).

Cell, Vol. 75, 843–854, December 3, 1993, Copyright © 1993 by Cell Press

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

Rosalind C. Lee,*† Rhonda L. Feinbaum,*‡ and Victor Ambros†

Ambros and Horvitz, 1987). of-function (*lf*) mutation, *lin-*

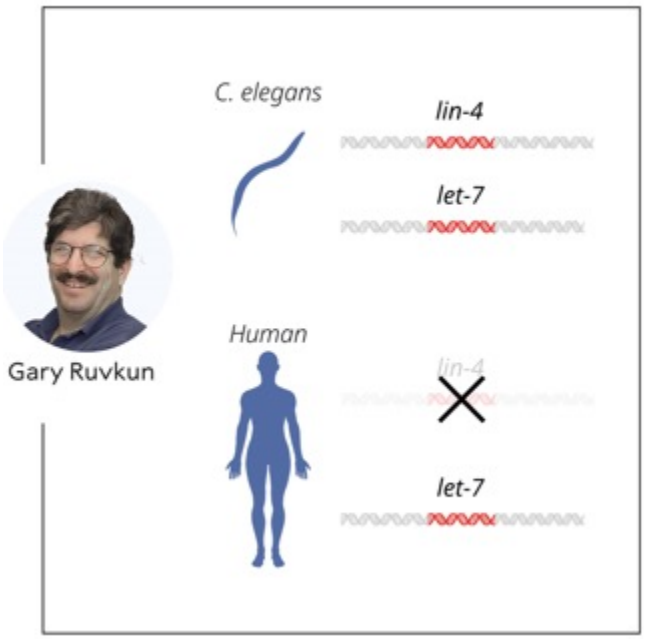
Although the results were interesting, the unusual mechanism of gene regulation was considered a peculiarity of *C. elegans*, irrelevant to humans and other more complex animals.

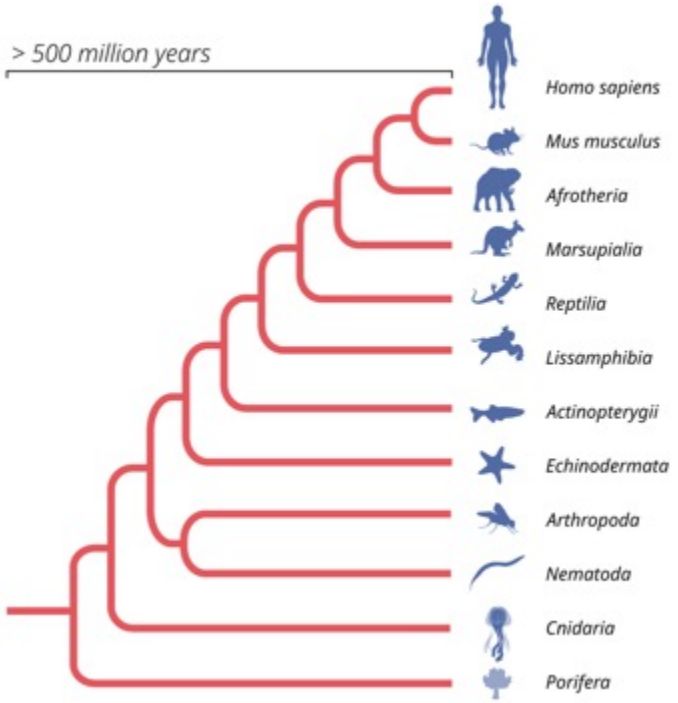
That perception changed in 2000 when Ruvkun's group published their discovery of another microRNA, highly conserved throughout the animal kingdom.

NATURE|VOL 403|24 FEBRUARY 2000|www.nature.com

The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*

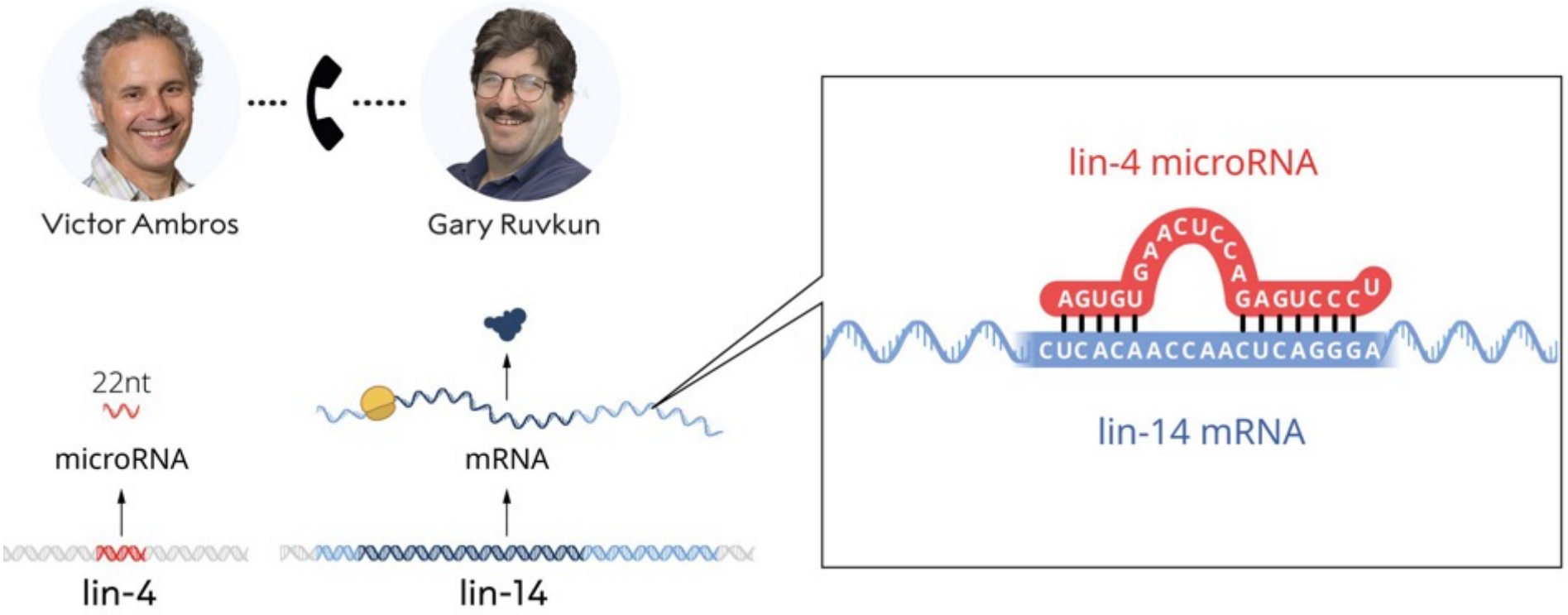
Brenda J. Reinhart*†, Frank J. Slack*†‡, Michael Basson‡§, Amy E. Pasquinelli*, Jill C. Bettinger‡#, Ann E. Rougvie#, H. Robert Horvitz§ & Gary Ruvkun*





Today, we know that

gene regulation by microRNA is universal among multicellular organisms.



Gene regulation by microRNA is universal among multicellular organisms.

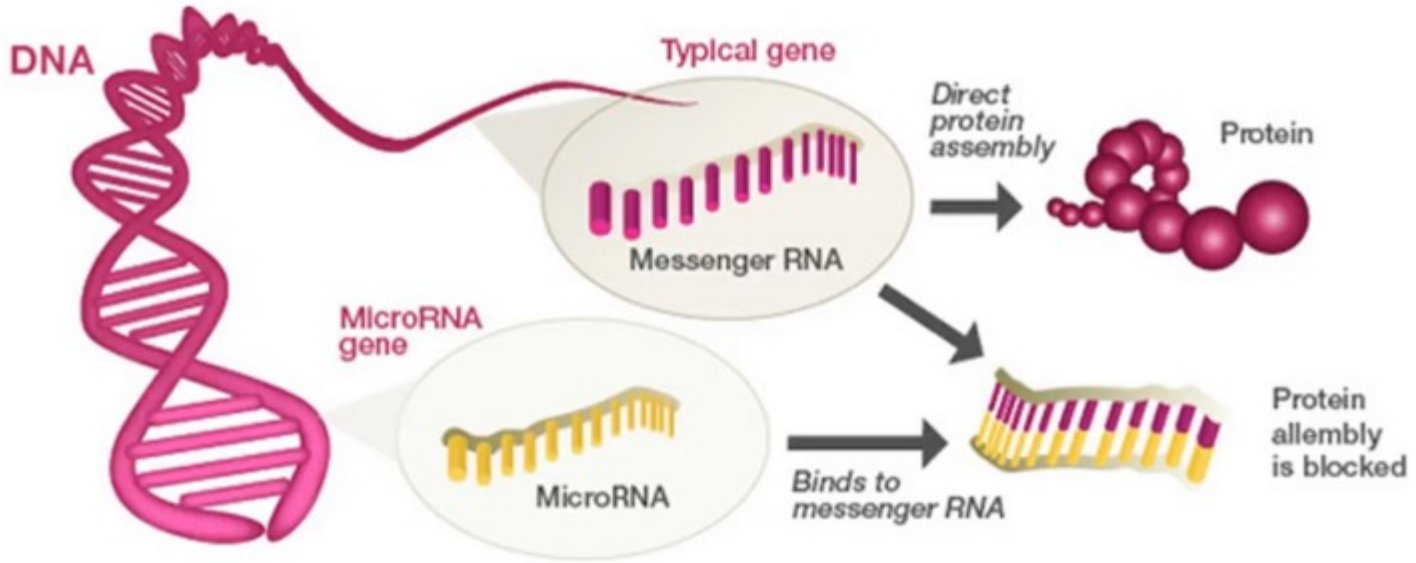
Small RNAs and Gene Silencing

Name	Organism	Length (nt)	Proteins	Source of trigger	Function
miRNA	Plants, algae, animals, viruses, protists	20–25	Drosha (animals only) and Dicer	Pol II transcription (pri-miRNAs)	Regulation of mRNA stability, translation
casiRNA	Plants	24	DCL3	Transposons, repeats	Chromatin modification
tasiRNA	Plants	21	DCL4	miRNA-cleaved RNAs from the *TAS* loci	Post-transcriptional regulation
natsiRNA	Plants	22	DCL1	Bidirectional transcripts induced by stress	Regulation of stress-response genes
		24	DCL2		
		21	DCL1 and DCL2		
Exo-siRNA	Animals, fungi, protists	~21	Dicer	Transgenic, viral or other exogenous dsRNA	Post-transcriptional regulation, antiviral defense
	Plants	21 and 24			
Endo-siRNA	Plants, algae, animals, fungi, protists	~21	Dicer (except secondary siRNAs in *C. elegans*, which are products of RdRP transcription, and are therefore not technically siRNAs)	Structured loci, convergent and bidirectional transcription, mRNAs paired to antisense pseudogene transcripts	Post-transcriptional regulation of transcripts and transposons; transcriptional gene silencing
piRNA	Metazoans excluding *Trichoplax adhaerens*	24–30	Dicer-independent	Long, primary transcripts?	Transposon regulation, unknown functions
piRNA-like (soma)	*Drosophila melanogaster*	24–30	Dicer-independent	In *ago2* mutants in *Drosophila*	Unknown
21U-RNA piRNAs	*Caenorhabditis elegans*	21	Dicer-independent	Individual transcription of each piRNA?	Transposon regulation, unknown functions
26G RNA	*Caenorhabditis elegans*	26	RdRP?	Enriched in sperm	Unknown

adapted from Ghildiyal & Zamore. 2009. Nat Rev Genet. 10:94

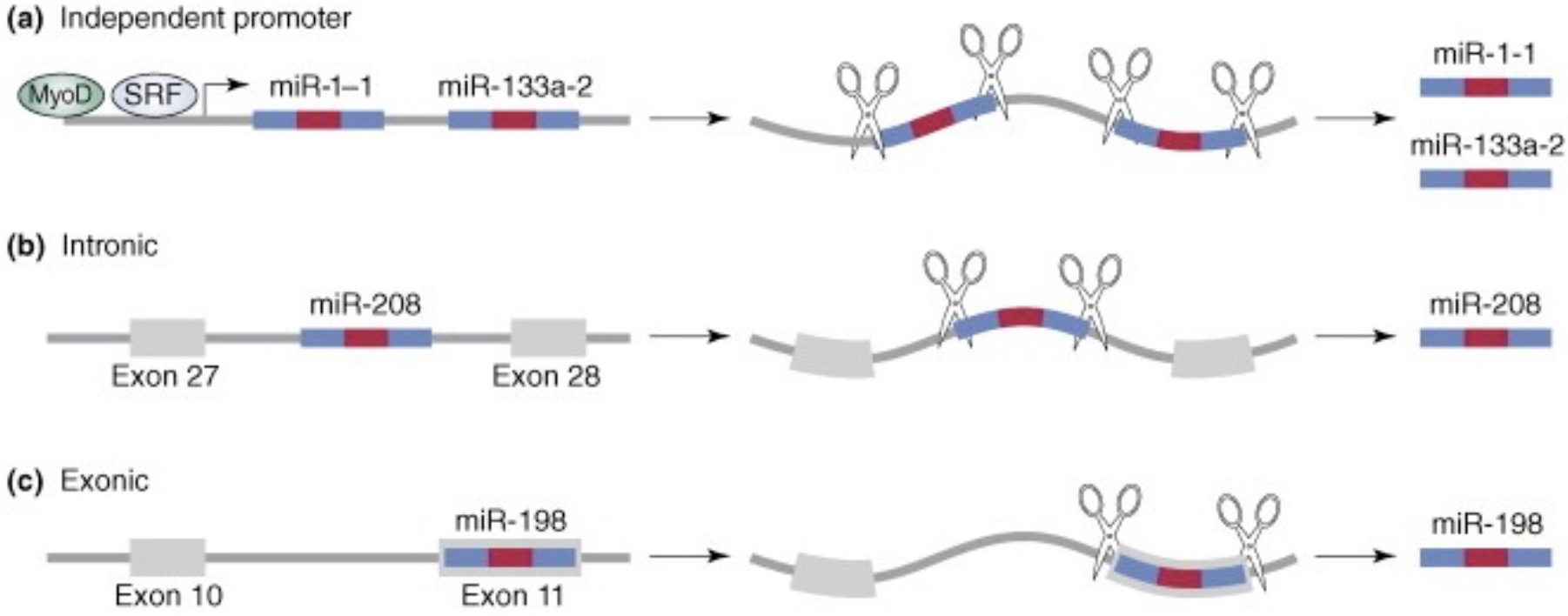
miRNA: microRNAs

What are miRNAs?



- ✓ miRNA genes are encoded in our genome
- ✓ miRNAs are small dsRNA molecules of 21-22 nt
- ✓ Their derive from precursors of 70-100 nt, transcribed by RNA PolII
- ✓ miRNAs interact with the 3′ untranslated region (3′ UTR) of target mRNAs to induce mRNA degradation and translational repression
- ✓ interaction of miRNAs with other regions, including the 5′ UTR, coding sequence, have also been reported
- ✓ Each miRNA is predicted to regulate hundreds of targets
- ✓ miRNAs can be secreted into extracellular fluids and transported to target cells via vesicles, such as exosomes.
- ✓ Extracellular miRNAs function as chemical messengers to mediate cell-cell communication.

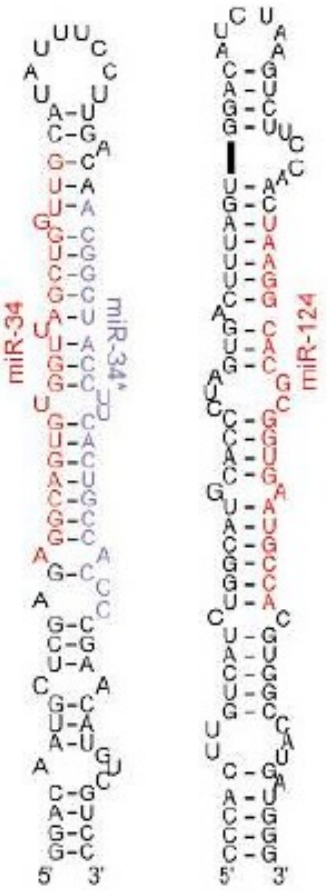
Genomic Organization of miRNA Genes



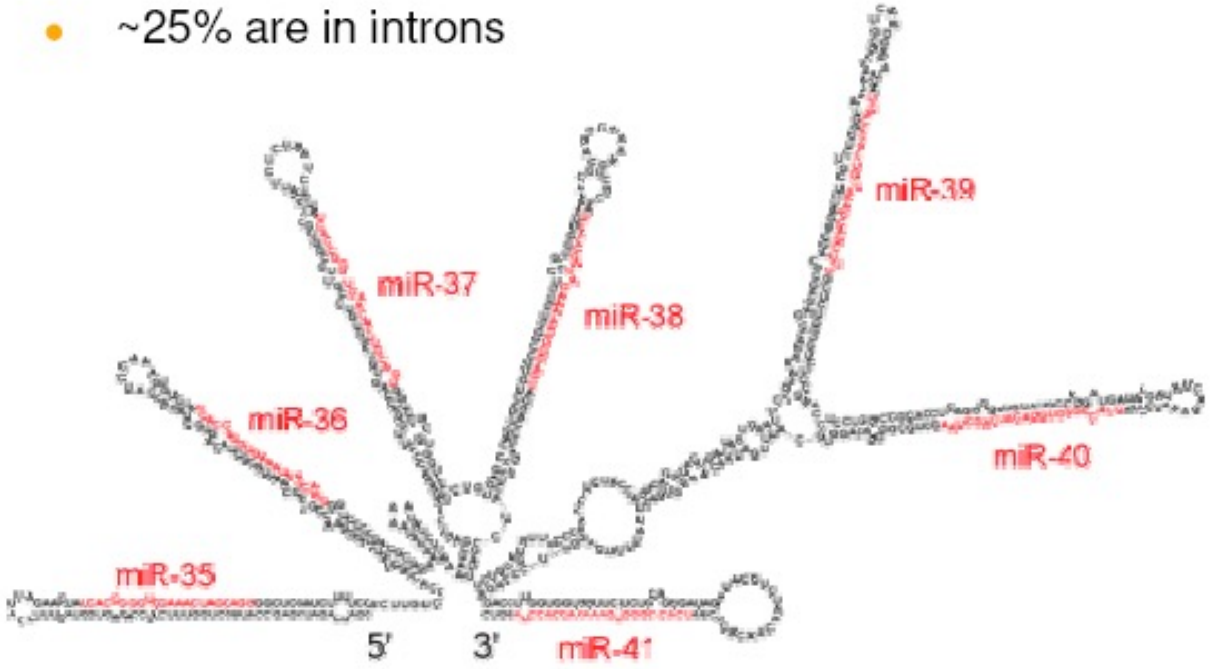
- Intronic miRNAs often in antisense direction, made from own promoter
- Exonic miRNAs - non-coding (or in alternatively spliced exons)

Zhao Y, Srivastava D, TIBS 32:189,2007

miRNA Genes

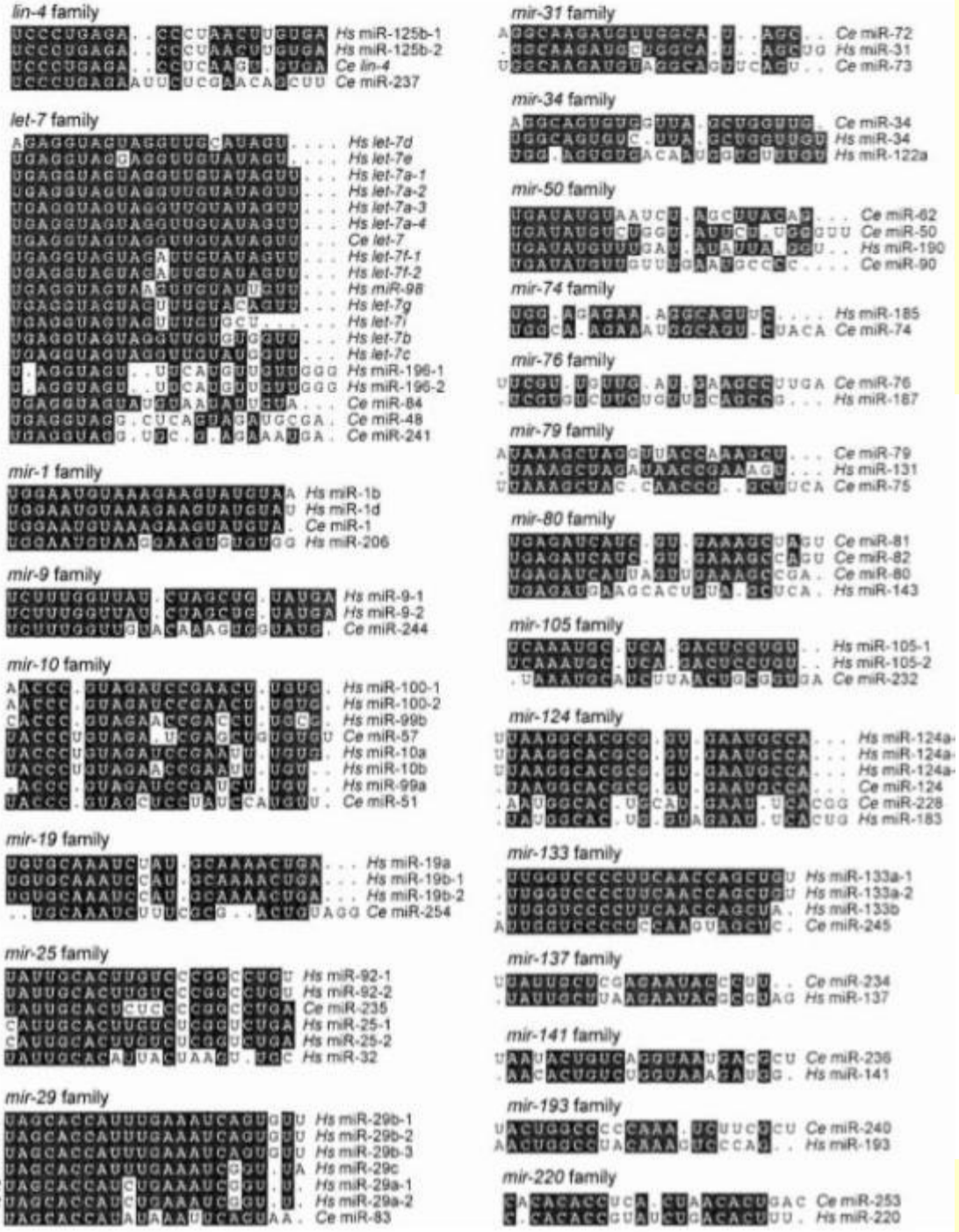


- ~60% of miRNAs are expressed independently
- ~15% of miRNAs are expressed in clusters
- ~25% are in introns



C. elegans

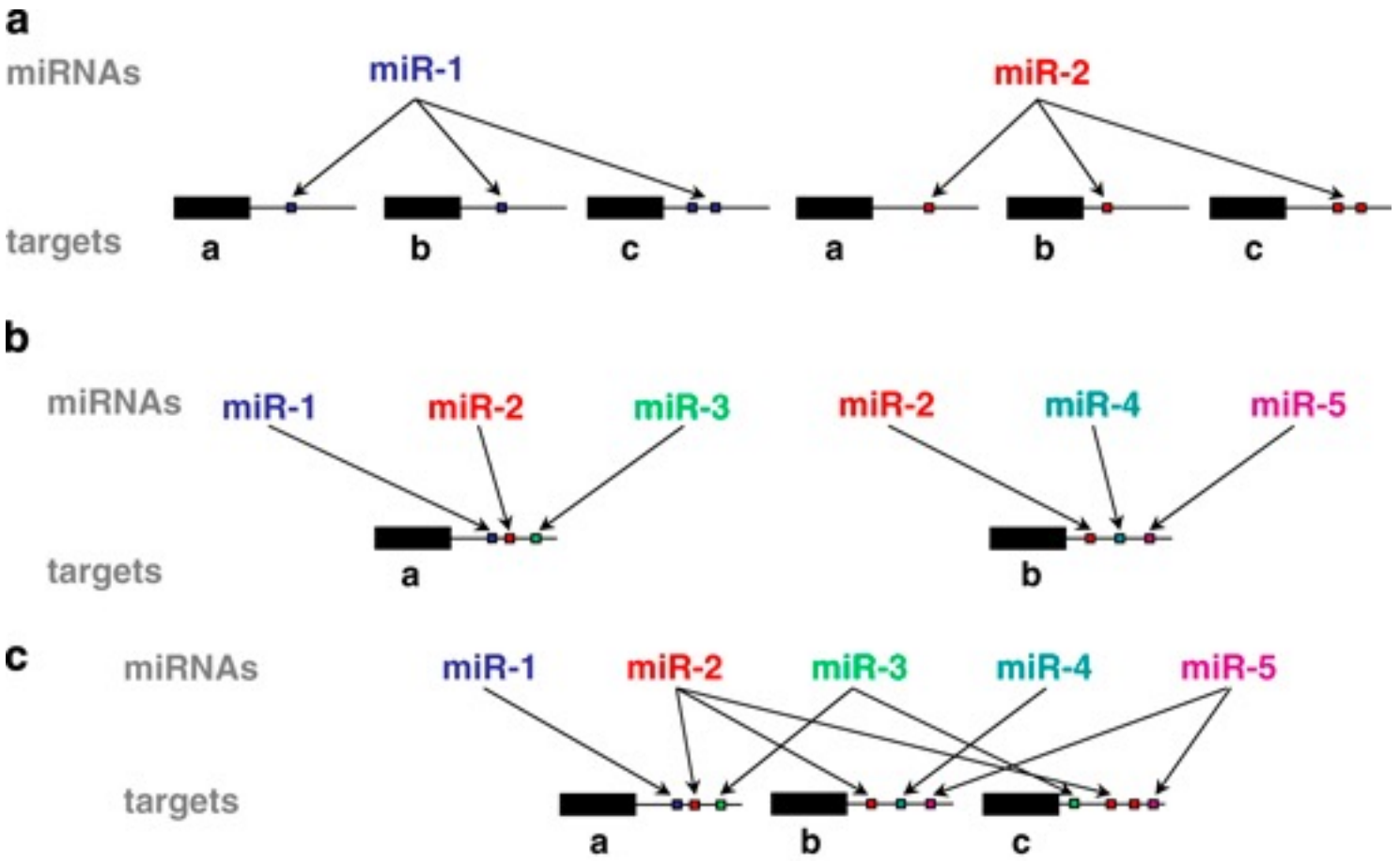
Homology Between *C. elegans* and *Homo sapiens* miRNAs



Lim (2003) Genes & Dev. 17: 991-1008

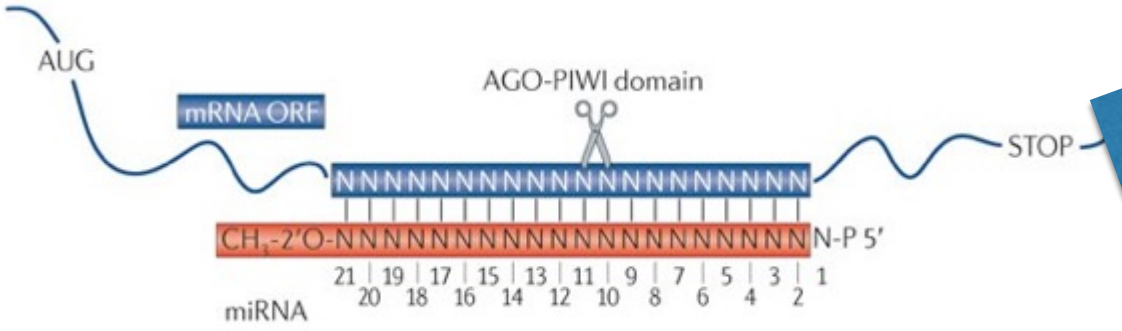
miRNAs target multiple genes and genes are targeted by multiple miRNAs

thereby coordinating and fine-tuning entire networks of genes.

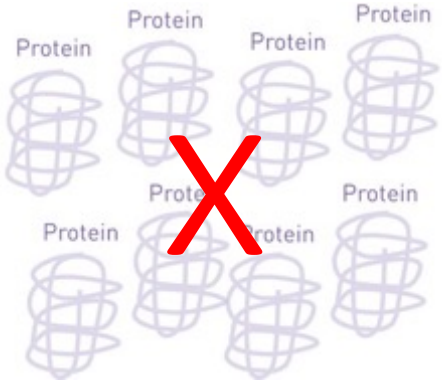


(**a**) miRNAs have multiple targets. (**b**) Many genes have seed matches for multiple miRNAs in their 3′UTRs. (**c**) A complex network of mutual interactions between miRNAs and mRNAs.

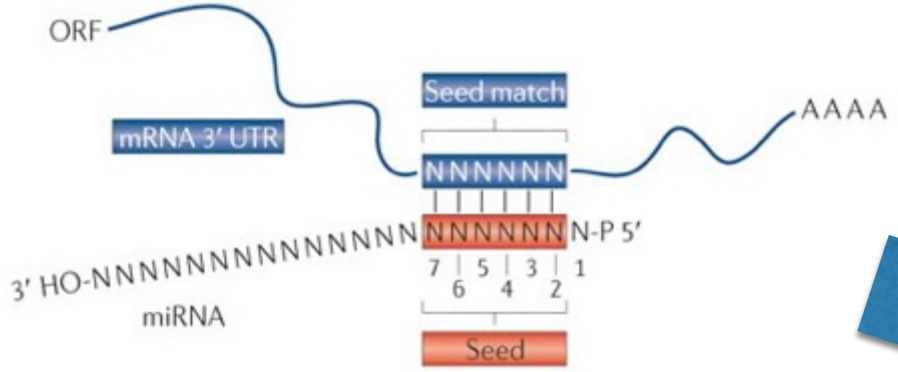
PLANTS



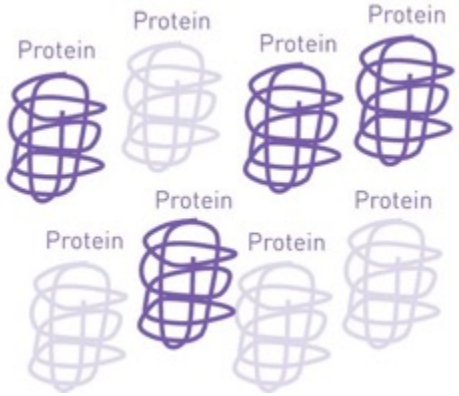
RNA DEGRADATION

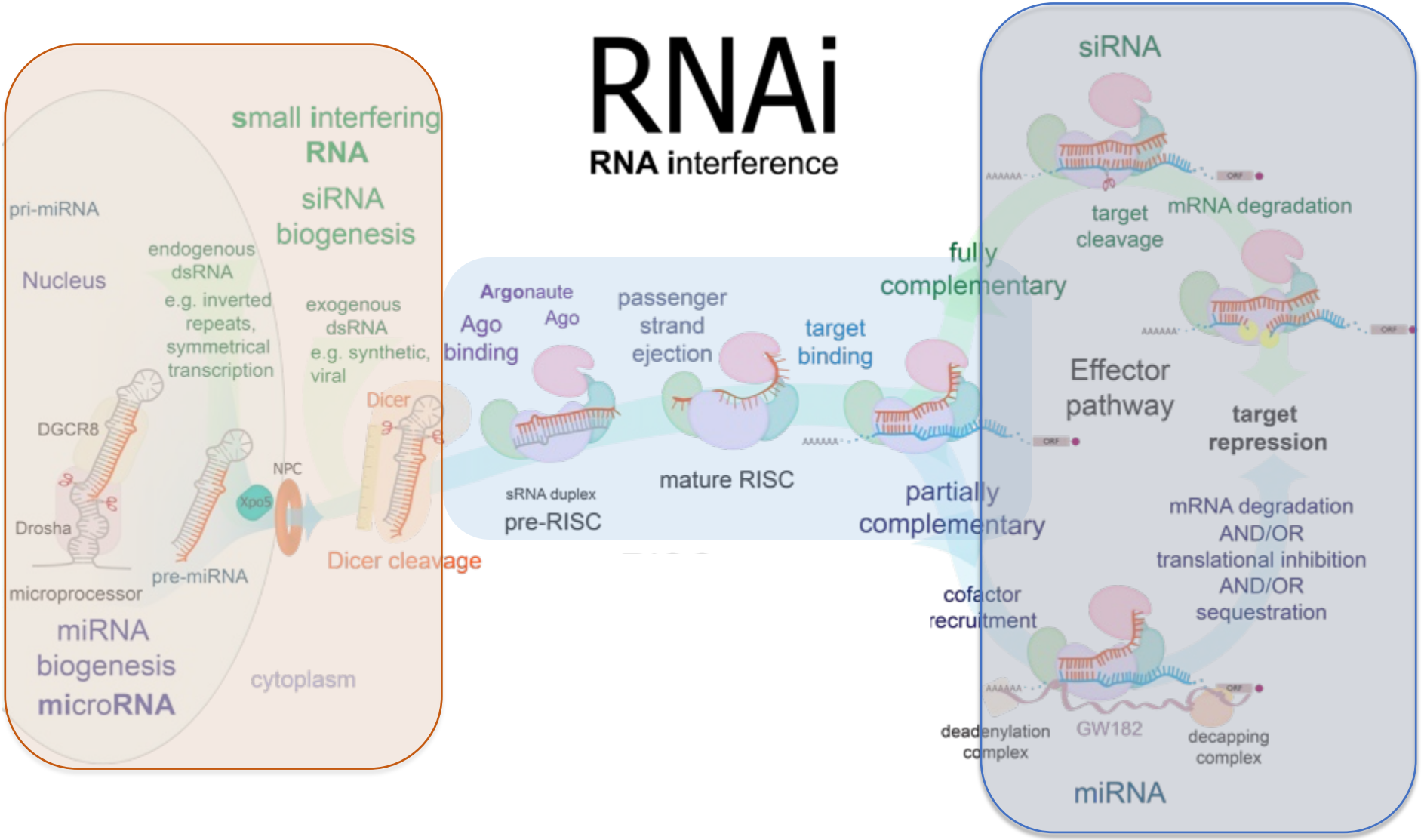


ANIMALS

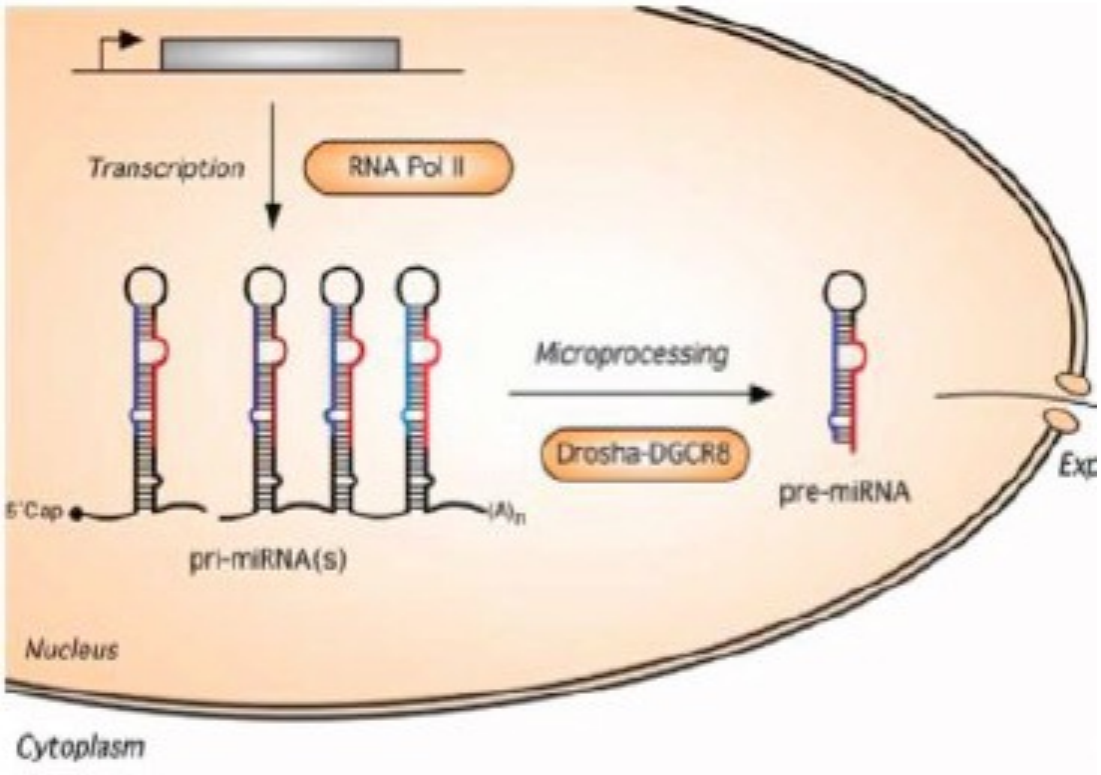


TRANSLATIONAL REPRESSION



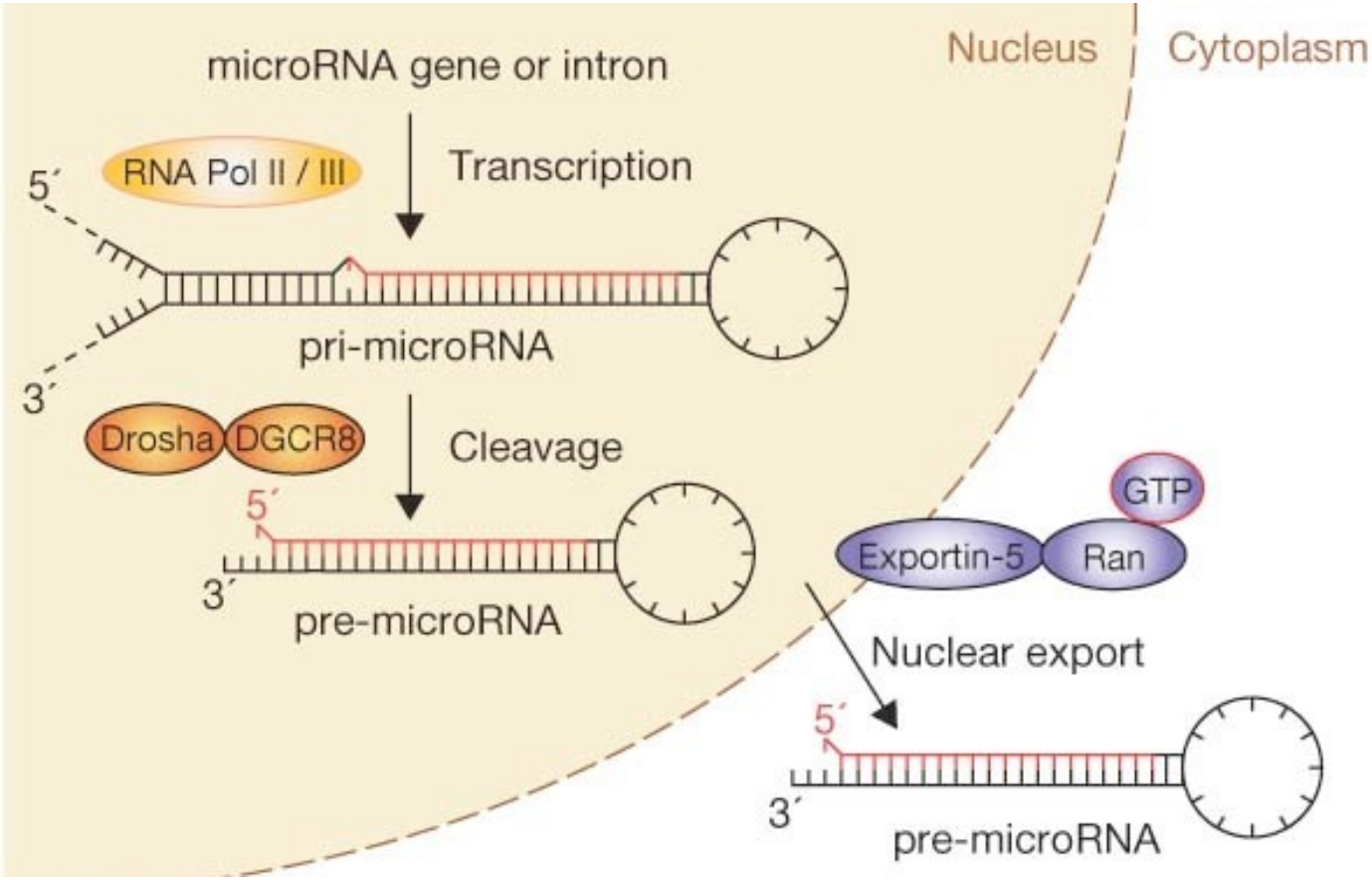

RNAi
RNA interference
small interfering RNA
siRNA biogenesis
pri-miRNA
Nucleus
endogenous dsRNA
e.g. inverted repeats, symmetrical transcription
exogenous dsRNA
e.g. synthetic, viral
DGCR8
Drosha
microprocessor
pre-miRNA
NPC
Xpo5
Dicer
Dicer cleavage
miRNA biogenesis
microRNA
cytoplasm
Argonaute
Ago
Ago binding
passenger strand ejection
target binding
fully complementary
sRNA duplex
pre-RISC
mature RISC
partially complementary
siRNA
target cleavage
mRNA degradation
Effector pathway
target repression
mRNA degradation AND/OR translational inhibition AND/OR sequestration
cofactor recruitment
deadenylation complex
GW182
decapping complex
miRNA

Biogenesis of miRNAs



- miRNAs are encoded by the genome.
- RNA polymeraseII transcribes precursors, called **pri-miRNAs (primary miRNAs)**.
- Primary miRNAs are approx 70 nt long, with 3'overhangs 1-4 nt long, stems 25-30 bp long and small hairpins.
- They bear a 5' CAP and a poli A tail.
- The dsRNA-specific ribonuclease **DROSHA** digests the pri-miRNA in the nucleus into single Hairpins, the **pre-miRNAs**.

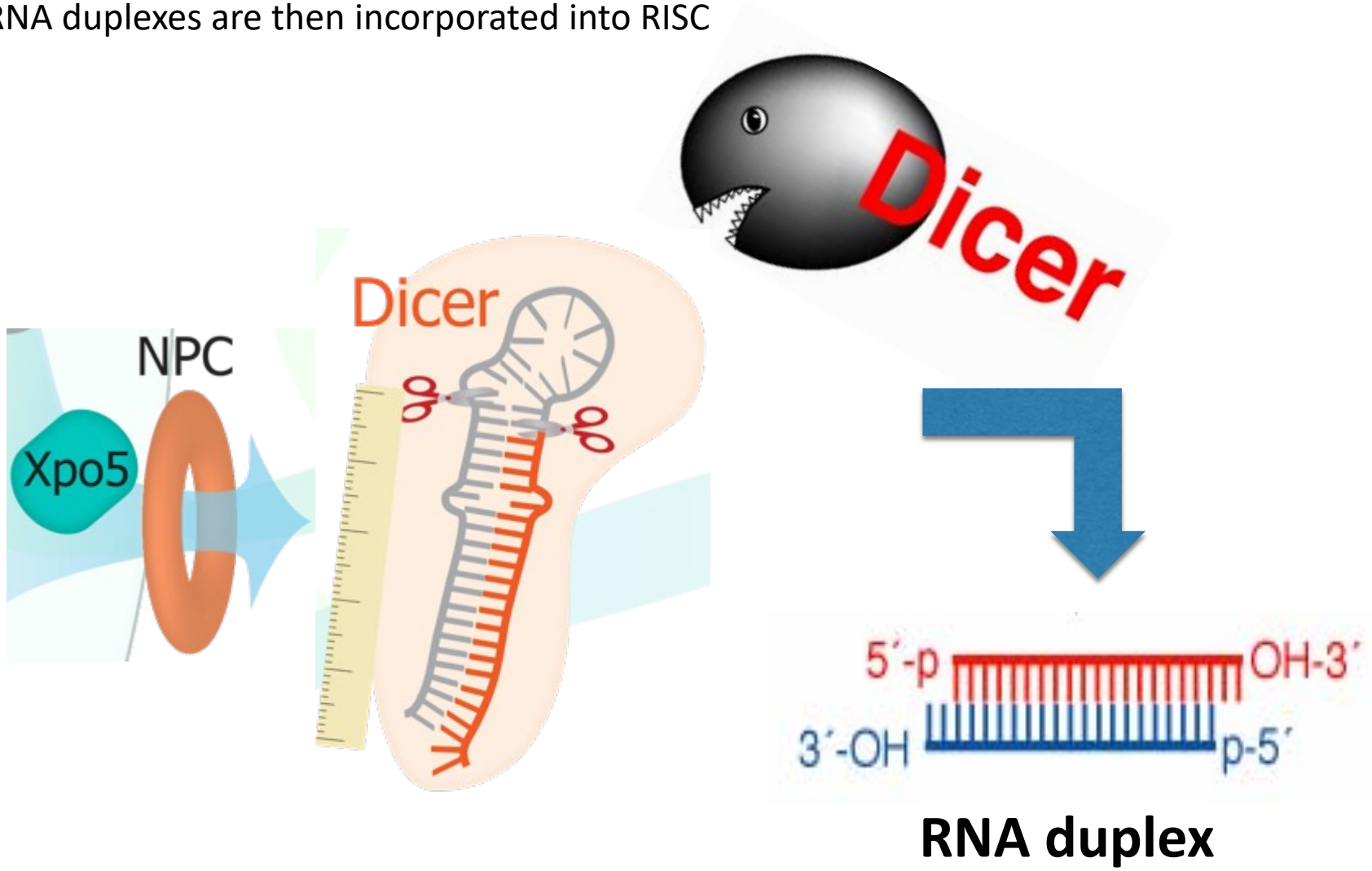
Biogenesis of miRNAs



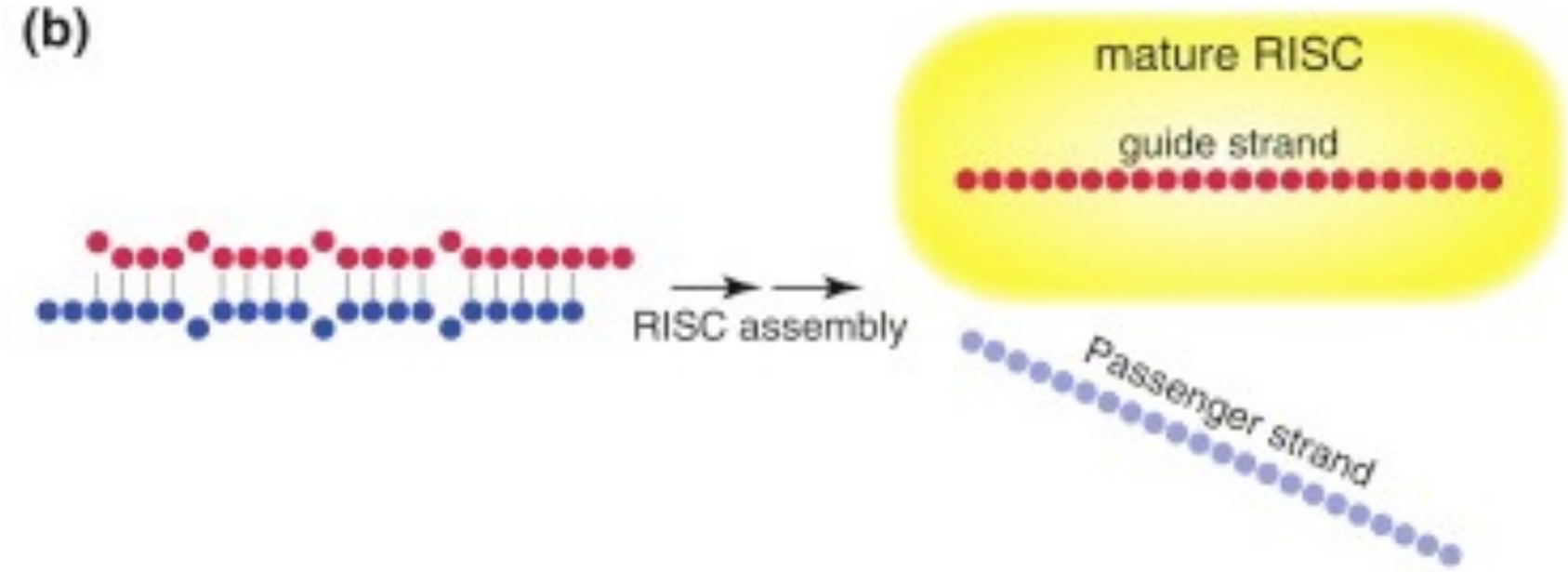
NUCLEAR EXPORT- EXPORTIN-5 (Exp5) translocates the pre-miRNAs from the nucleus to the cytoplasm.
Exp5 directly binds the pri-miRNA correctly processed.

Dicer cleavage

Once the pre-miRNA is in the cytoplasm, the RNAseIII DICER cuts it into:
22 nt RNA duplexes with 3' symmentrical overhangs, containing 5' phosphate groups.
RNA duplexes are then incorporated into RISC

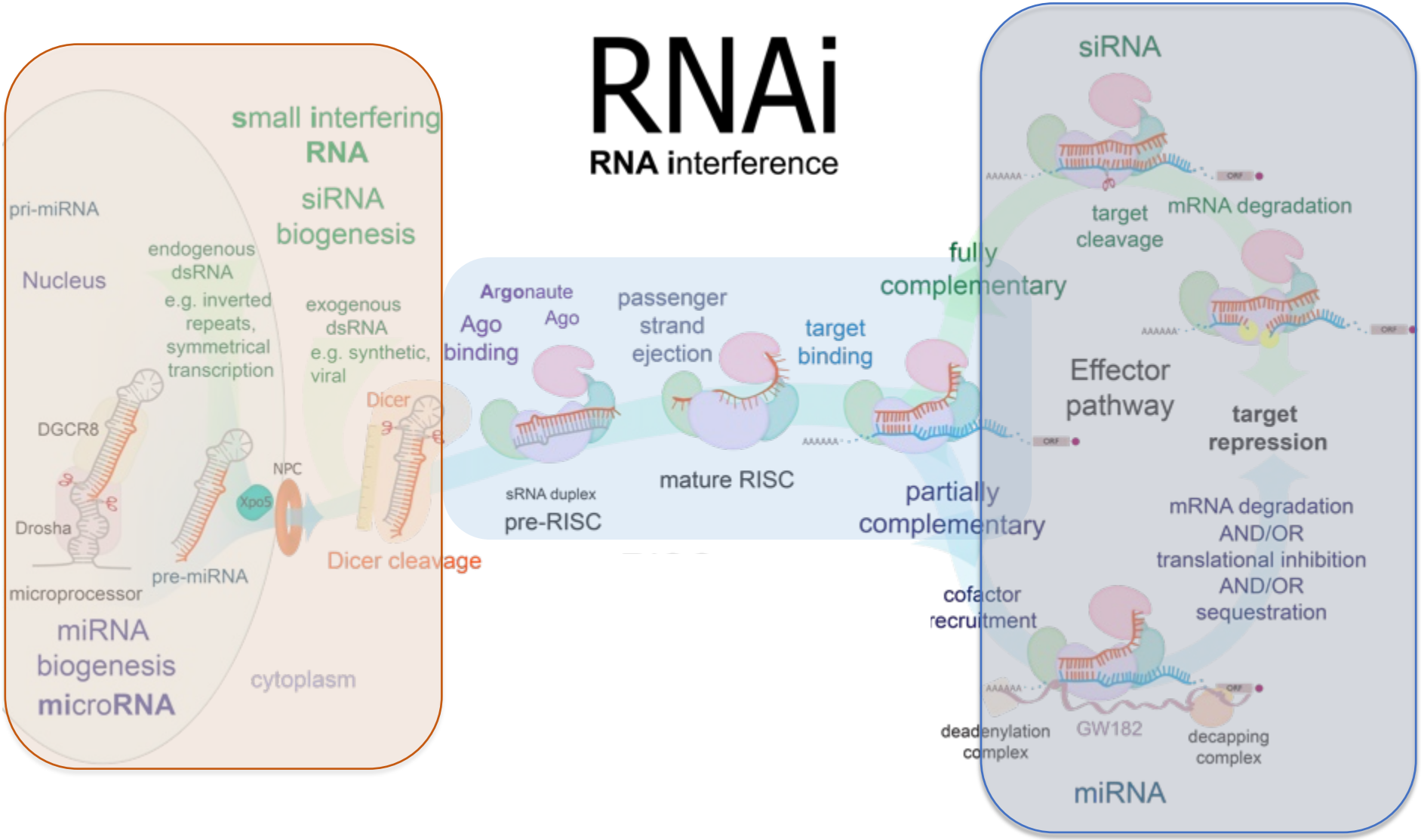


Nomenclature for small RNA strands



Guide and passenger strands

In the RNA duplex, the guide strand is the miRNA sequence responsible for silencing, that will be targeted to its mRNA, whereas the passenger strand is discarded (by the RISC complex).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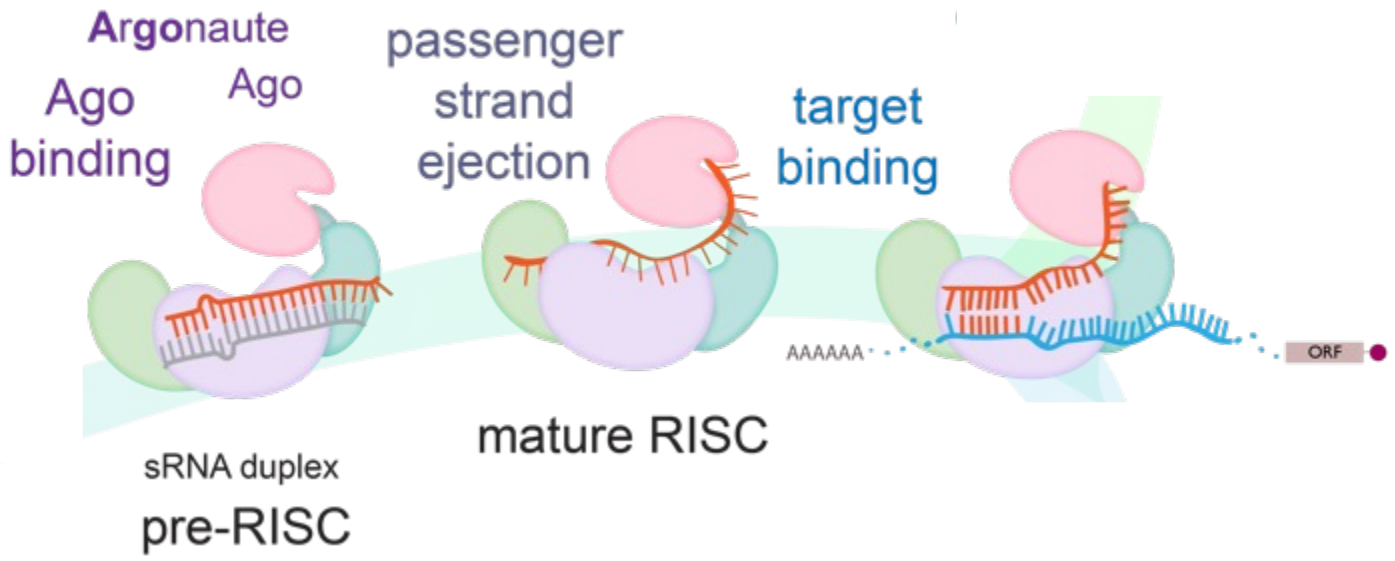
cytoplasm
Argonaute
Ago
Ago binding
passenger strand ejection
target binding
fully complementary
sRNA duplex
pre-RISC
mature RISC
partially complementary
siRNA
target cleavage
mRNA degradation
Effector pathway
target repression
mRNA degradation AND/OR translational inhibition AND/OR sequestration
cofactor recruitment
deadenylation complex
GW182
decapping complex
miRNA

RISC ASSEMBLY



The RNA duplex is recognized by a large (~500 kDa) RNA multiprotein complex, which is the actual molecular machinery responsible for RNAi

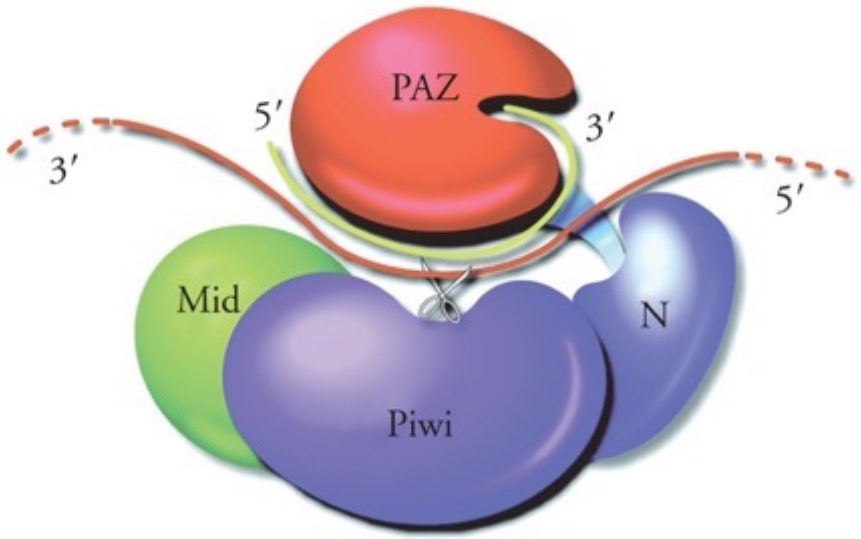
The RISC complex has two main functions:

1- degrades the passenger strands

2- leads the guide strands to its target mRNA

The main effector of RISC is the protein <u>ARGONAUTE</u>

Argonaute

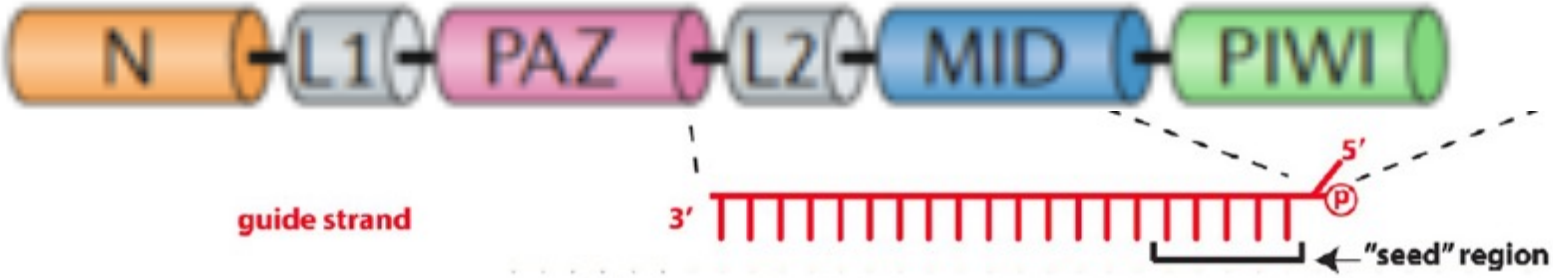


Number of Argonaute family genes in different species

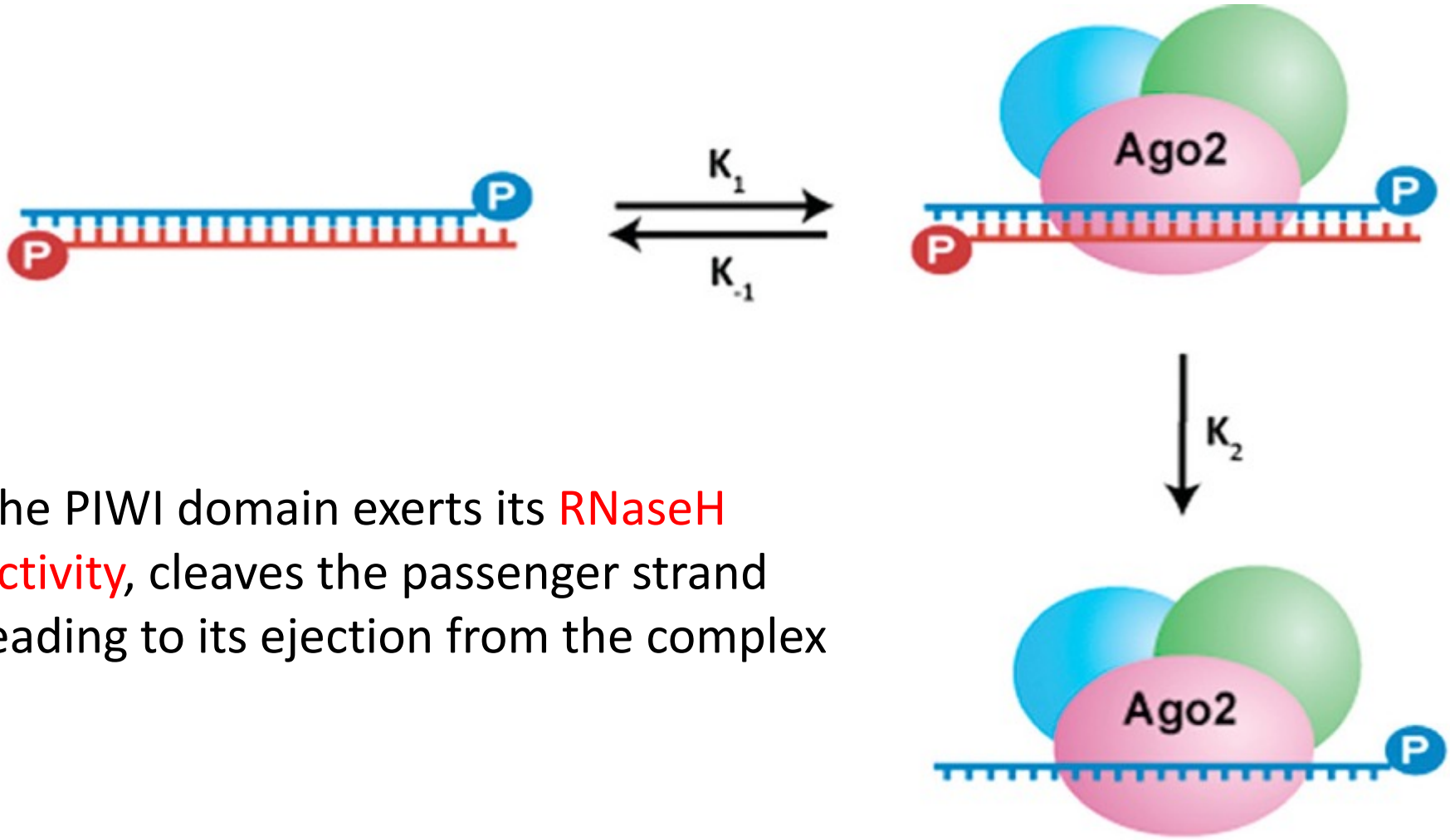
Species	Number of genes
Homo sapiens	8
Rattus norvegicus	8
Mus musculus	8
Drosophila melanogaster	5
Caenorhabditis elegans	27
Arabidopsis thaliana	10
Schizosaccharomyces pombe	1
Neurospora crassa	2

The PAZ Domain has 3’ RNA binding activity

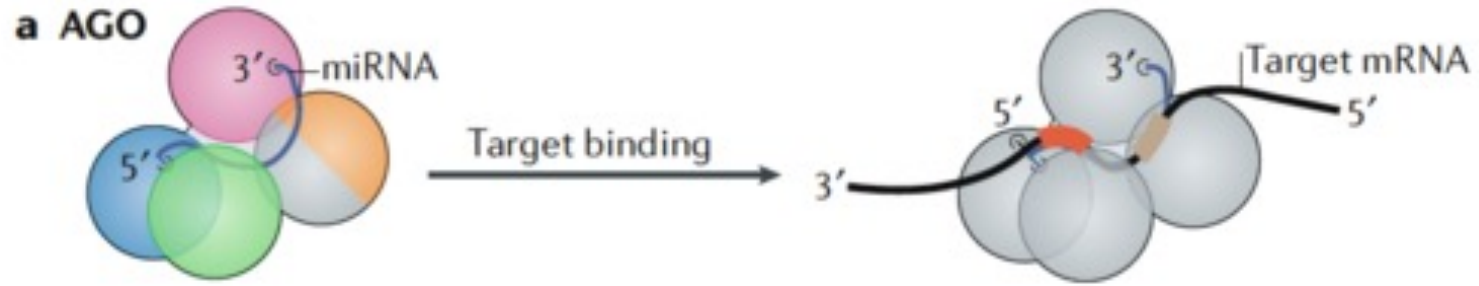
- The PIWI Domain binds the 5’-P RNA end
- It has Rnase H activity



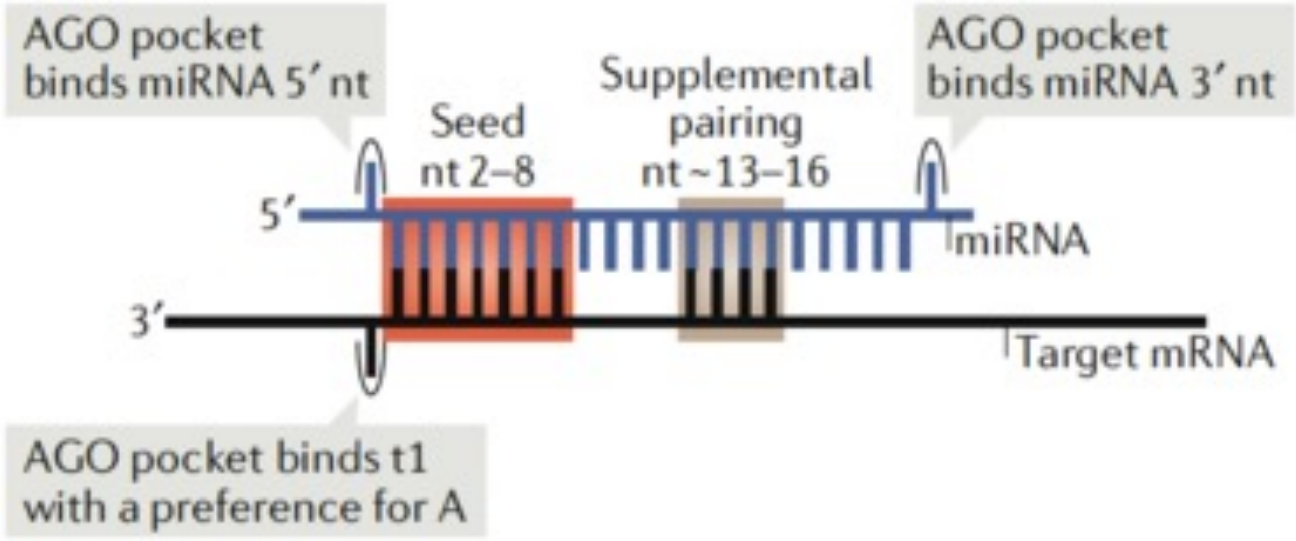
Le proteine AGO:
due domini caratteristici:
1. dominio **PAZ** lega il 3’-OH del piccolo RNA
2. dominio **Piwi** riconosce il terminale 5’-P del piccolo RNA e ha attività endonucleasica



The PIWI domain exerts its RNaseH activity, cleaves the passenger strand leading to its ejection from the complex



The guide strand, protected by the RISC complex from degradation by exonucleases, is targeted onto its mRNA

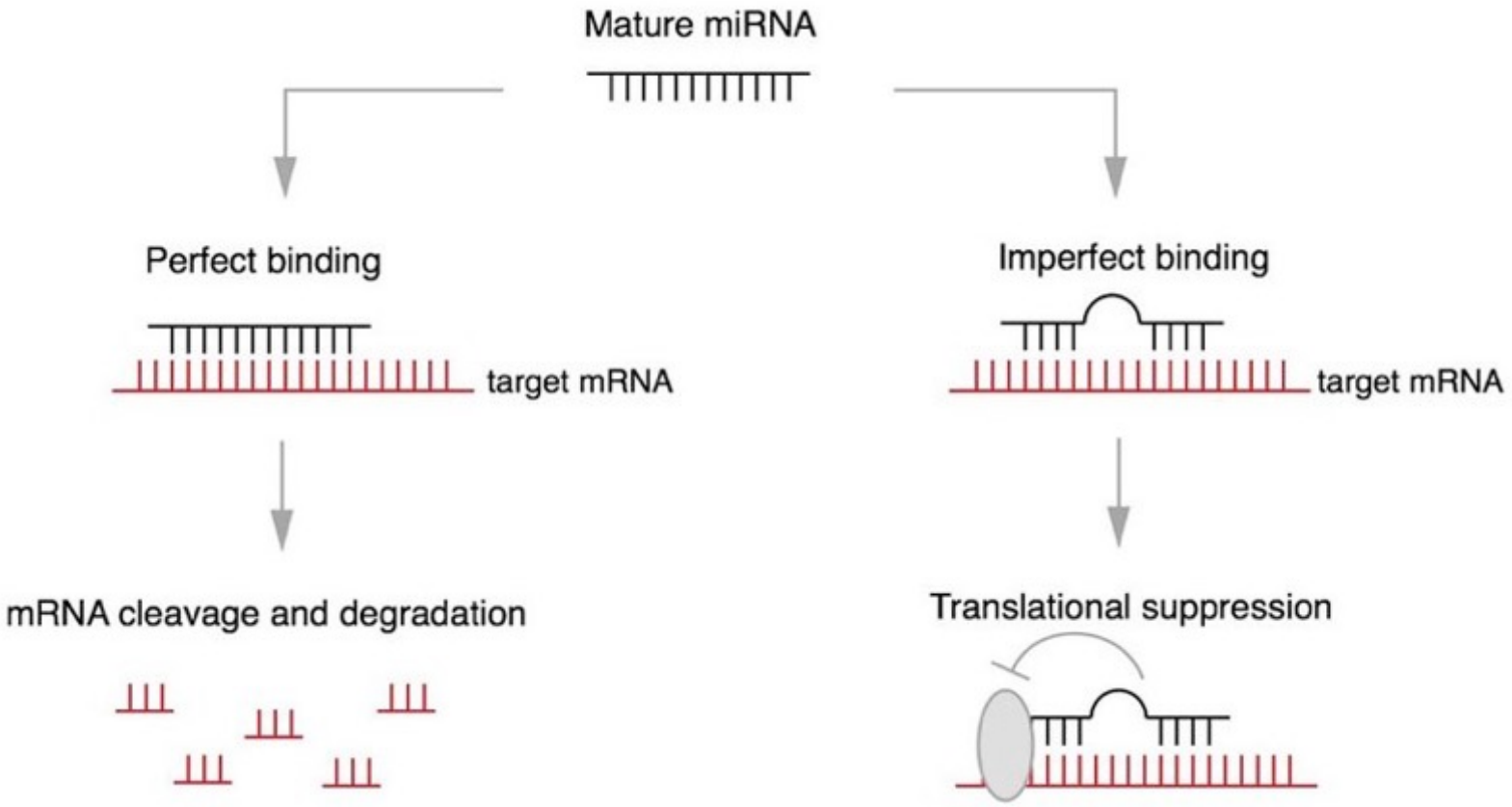


Come funzionano i miRNA?

La funzione primaria dei miRNA e' quella di abbassare il livello proteico degli mRNA target

I miRNAs svolgono la propria funzione attraverso due meccanismi:

- PERFETTA COMPLEMENTARIETA' → DEGRADAZIONE DEL mRNA (piante)
- IMPERFETTA COMPLEMENTARIETA' → REPRESSIONE TRADUZIONALE (animali)



CellPress

Review

The Functions of MicroRNAs: mRNA Decay and Translational Repression

Hiro-oki Iwakawa[1,2] and Yukihide Tomari[1,2,*]

MicroRNAs (miRNAs) are a class of endogenous small noncoding RNAs, which regulate complementary mRNAs by inducing translational repression and mRNA decay. Although this dual repression system seems to operate in both animals and plants, genetic and biochemical studies suggest that the mechanism underlying the miRNA-mediated silencing is different in the two kingdoms. Here, we review the recent progress in our understanding of how miRNAs mediate translational repression and mRNA decay, and discuss the contributions of the two silencing modes to the overall silencing effect in both kingdoms.

Trends

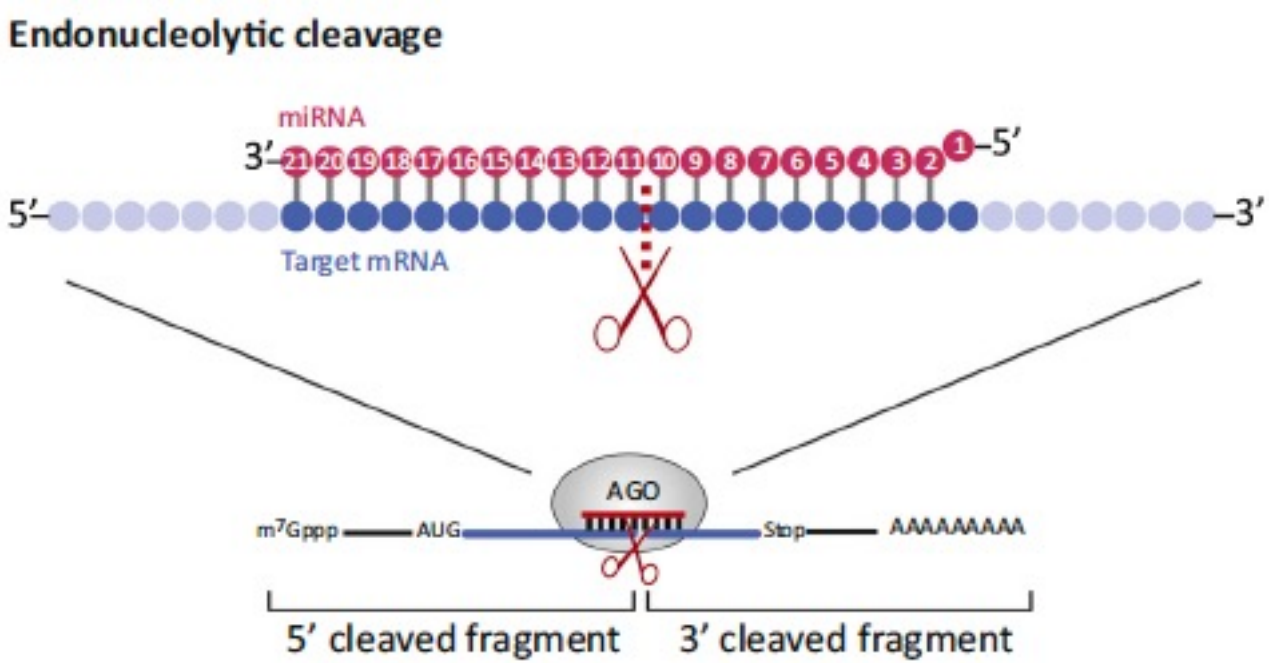
Animal miRNAs promote mRNA decay by recruiting deadenylases and decapping factors onto the target mRNAs through GW182/TNRC6.

Plant miRNAs do not promote deadenylation but cleave nearly perfectly complementary targets. The 3′ end of the 5′ fragment is uridylated, and both the 5′ and 3′ fragments are decayed by the 5′-to-3′ exoribonuclease.

Animal miRNAs repress translation initiation by promoting dissociation of eIF4F through GW182-mediated displacement of PABP, recruitment of translational inhibitors via GW182, and displacement of the ATP-dependent RNA helicase eIF4A from the translation initiation complex eIF4F.

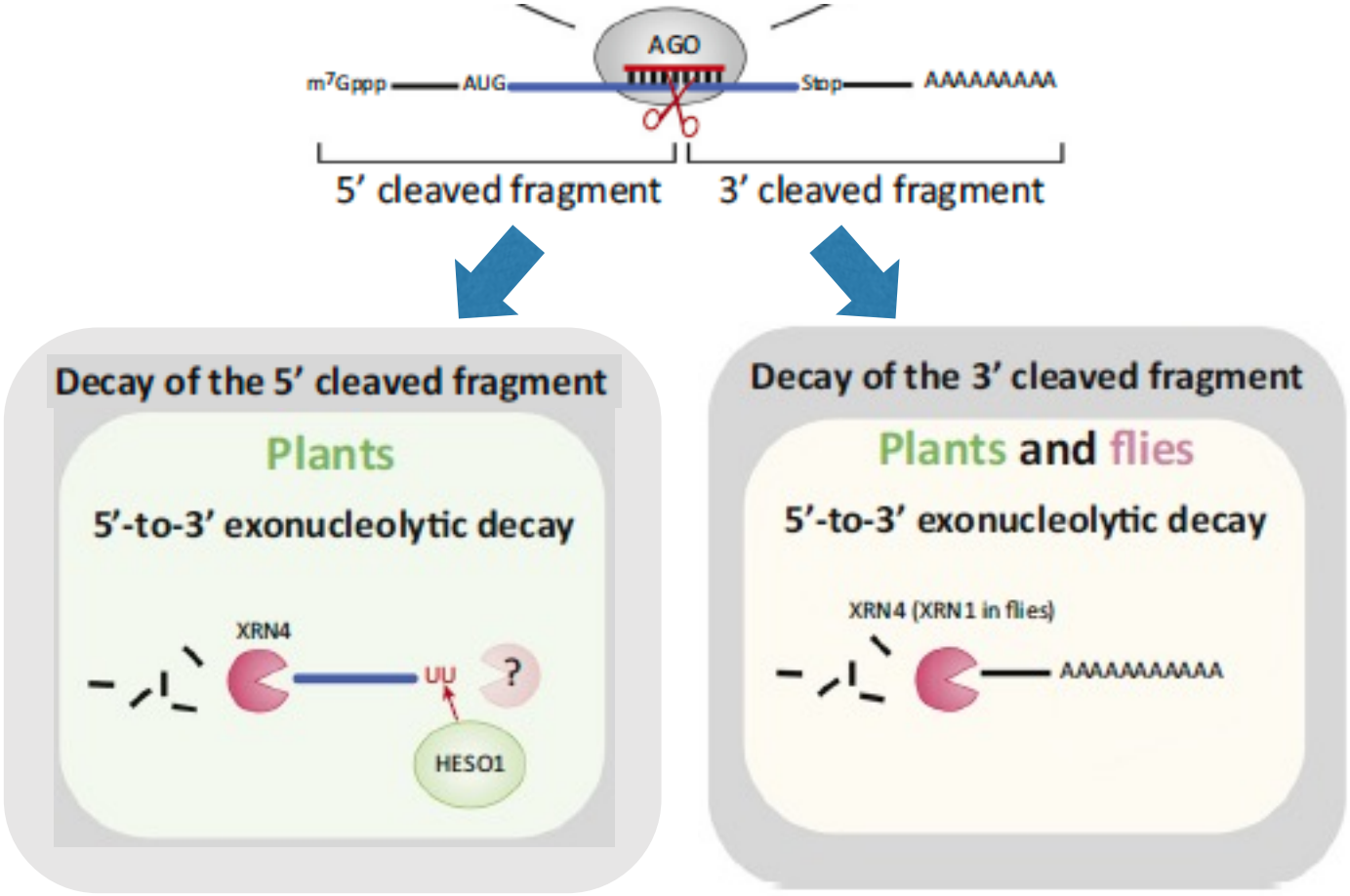
Plant miRNAs repress translation via various organelle-bound factors. Although the mechanism is unclear, *in vitro* studies suggest that AGO1–RISC can block translation initiation and ribosome movement.

miRNA-Mediated mRNA Decay in Plants



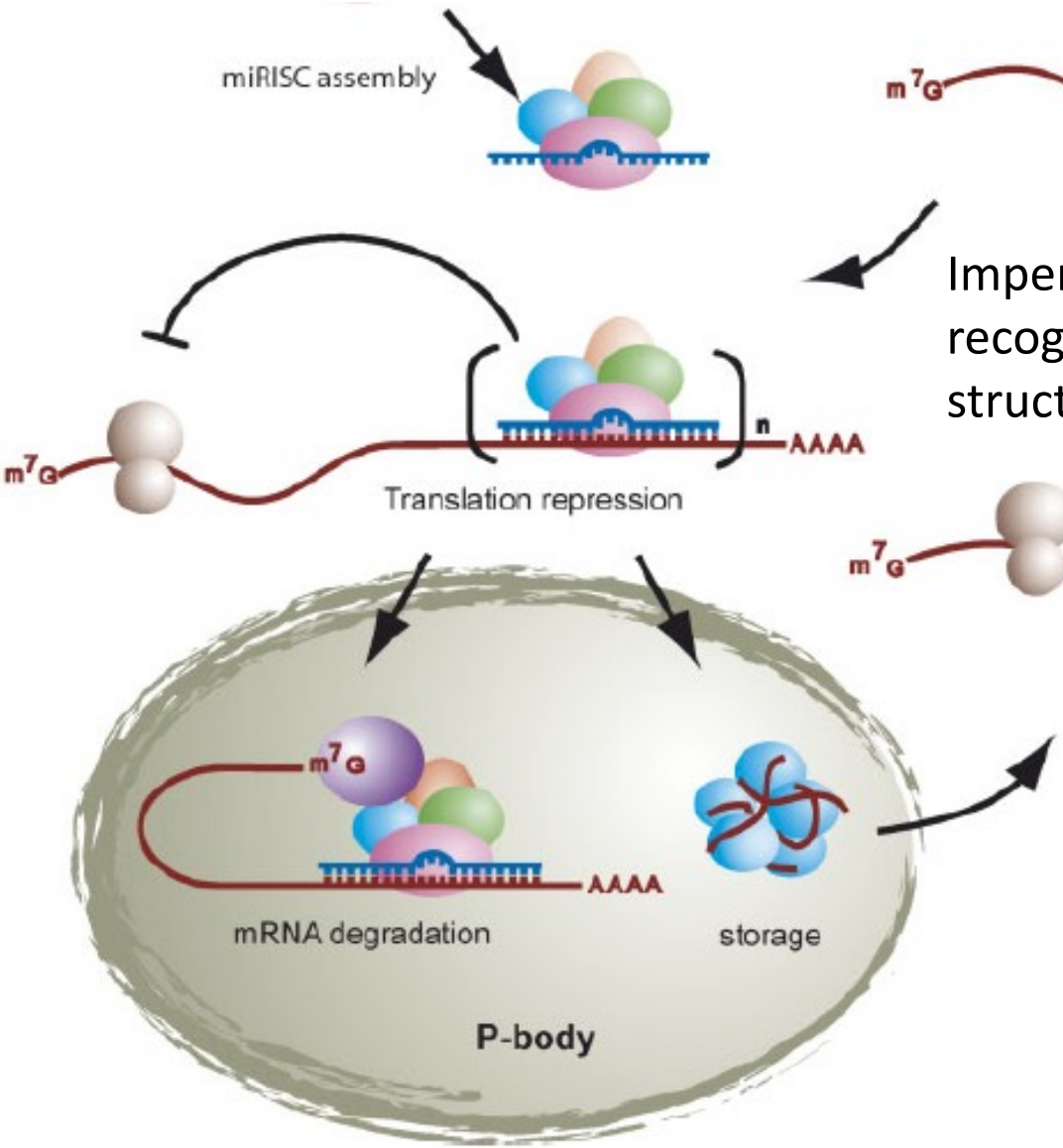
Plant miRNAs bind to nearly fully complementary target sites mainly located in the open reading frame (ORF) and induce endonucleolytic cleavage of the target mRNA.

miRNA-Mediated mRNA Decay in Plants



The 5′ cleavage fragment is degraded by XRN4 in the 5′-to-3′ direction in plants.
The 3′ cleaved fragments are degraded by XRN4 in the 5′-to-3′ direction.

Translational repression in animals



Imperfect base-pairing during target recognition by miRNA results in a bulge structure.

Translation-repressed mRNA and miRISC are concentrated in P-bodies for storage or mRNA decay. Under certain conditions or stimuli, stored mRNAs can be released and re-enter translation pathways.

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miRNA is incorporated into miRISC, recognizes its target mRNA at the 3'UTR, and triggers gene silencing by at least three possible mechanisms:

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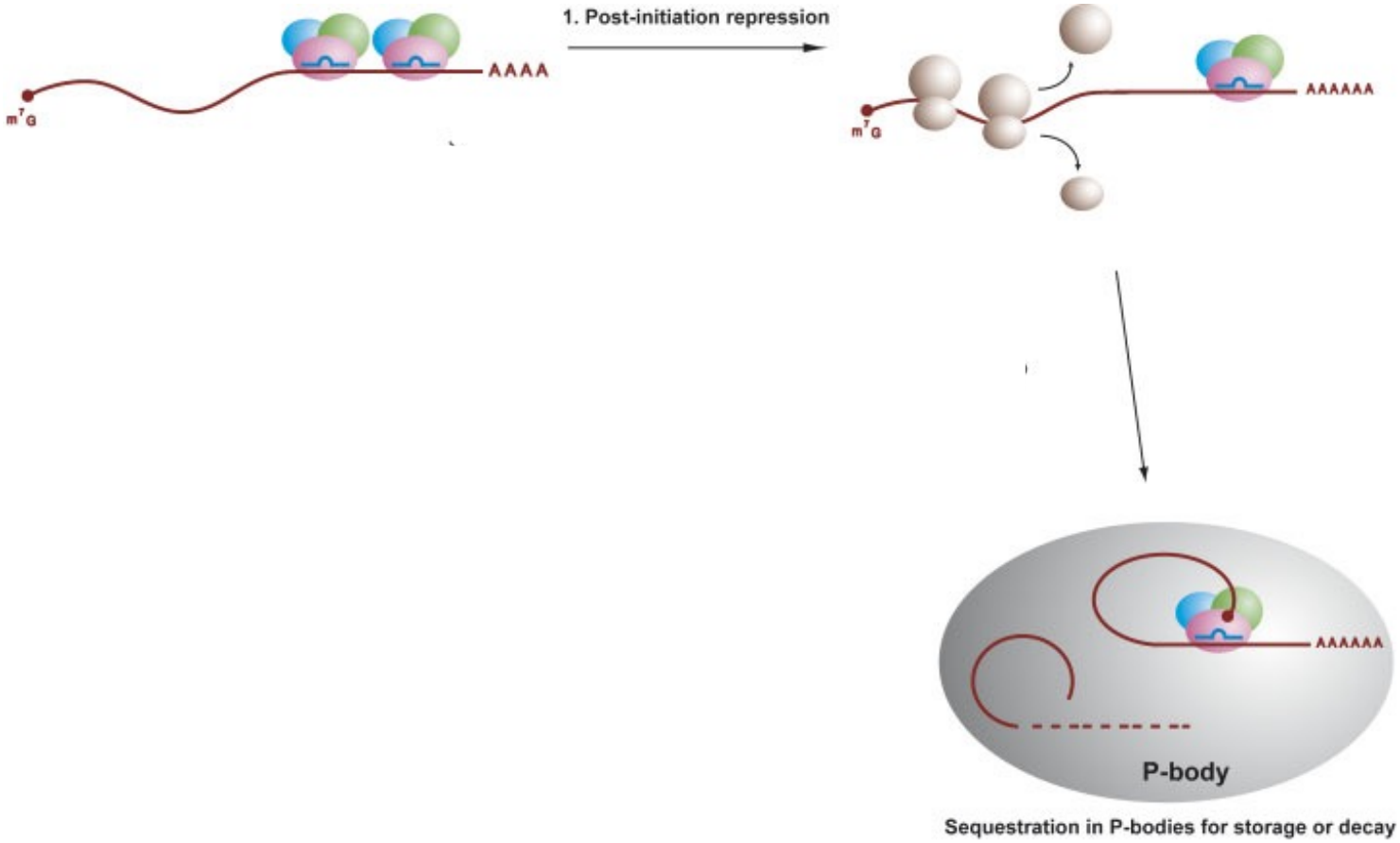

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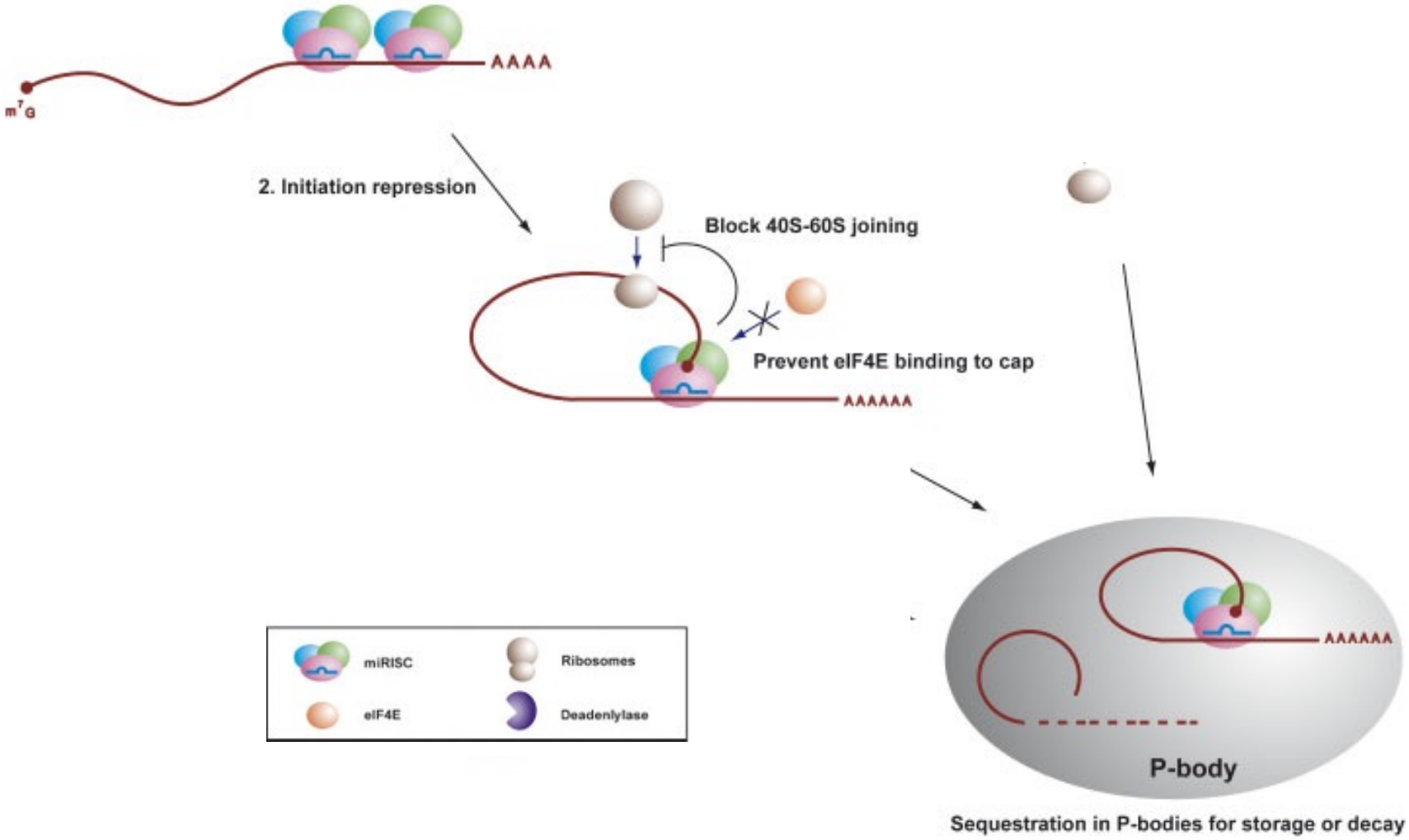

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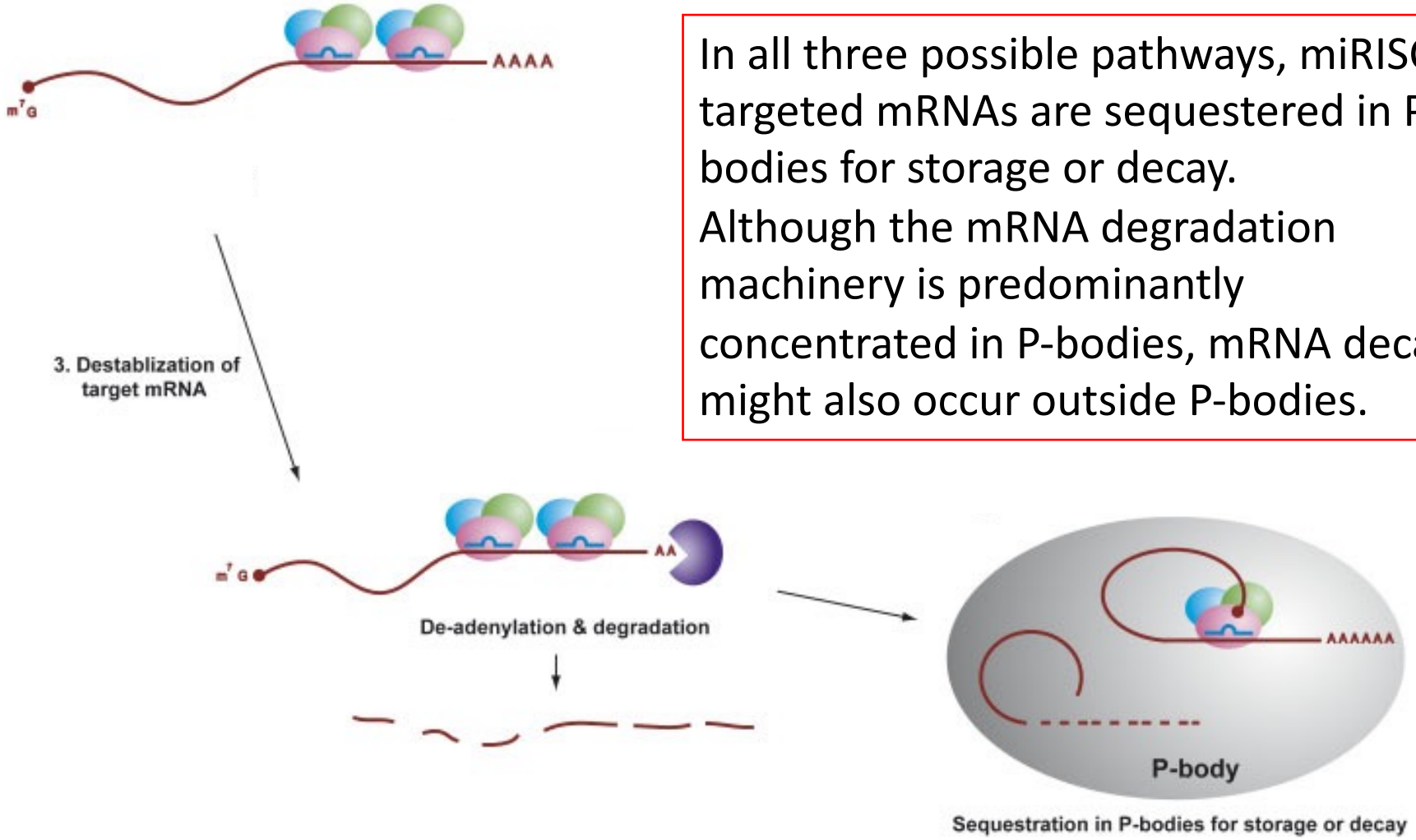
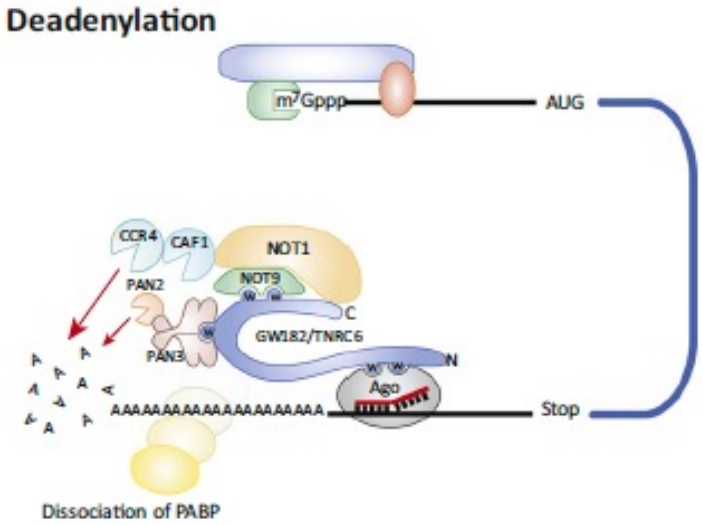
Recognition by miRNA destabilizes the target by inducing its **deadenylation** and decay.



In all three possible pathways, miRISC-targeted mRNAs are sequestered in P-bodies for storage or decay.
Although the mRNA degradation machinery is predominantly concentrated in P-bodies, mRNA decay might also occur outside P-bodies.

miRNA-Mediated mRNA Destabilization in Animals.

There are 3 main steps in miRNA-mediated mRNA decay in animals.



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miRNAs induce poly(A) shortening by recruiting deadenylase complexes to the target mRNAs. Dissociation of poly(A)-binding protein (PABP) is promoted, raising the efficiency of deadenylation.

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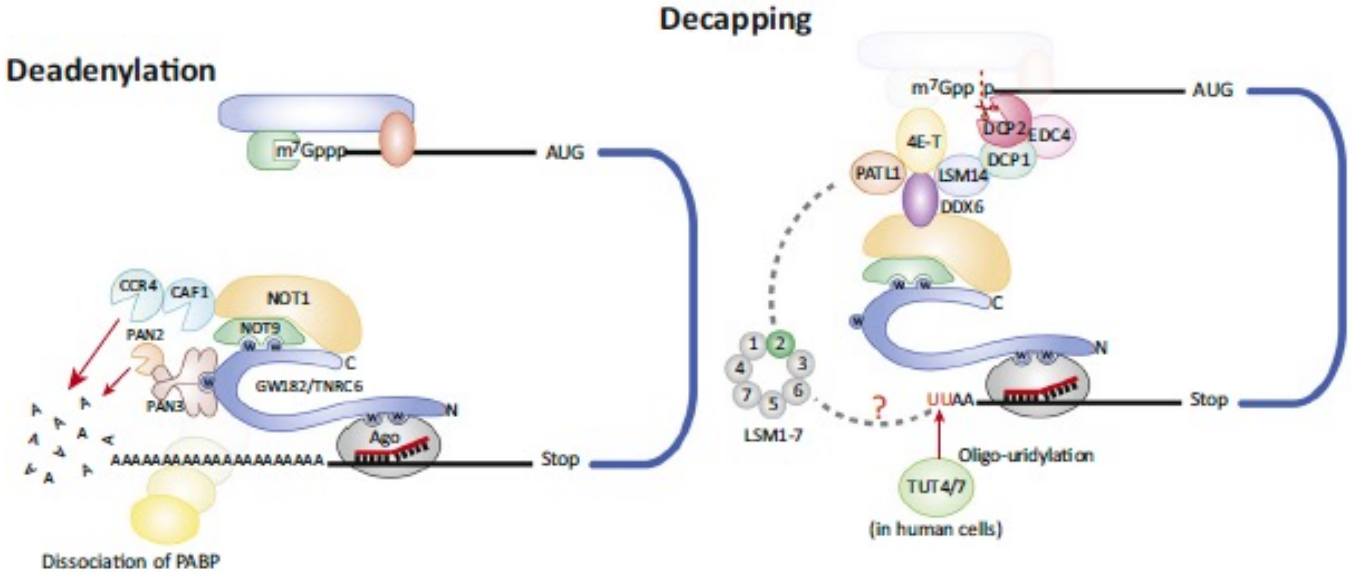

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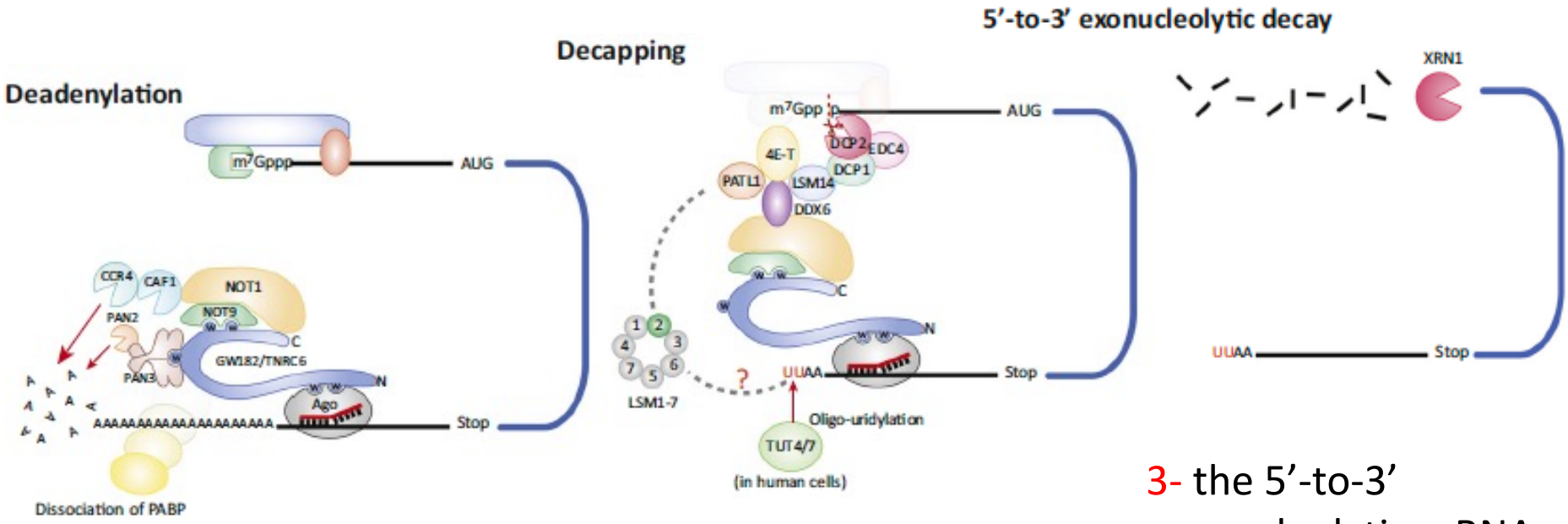

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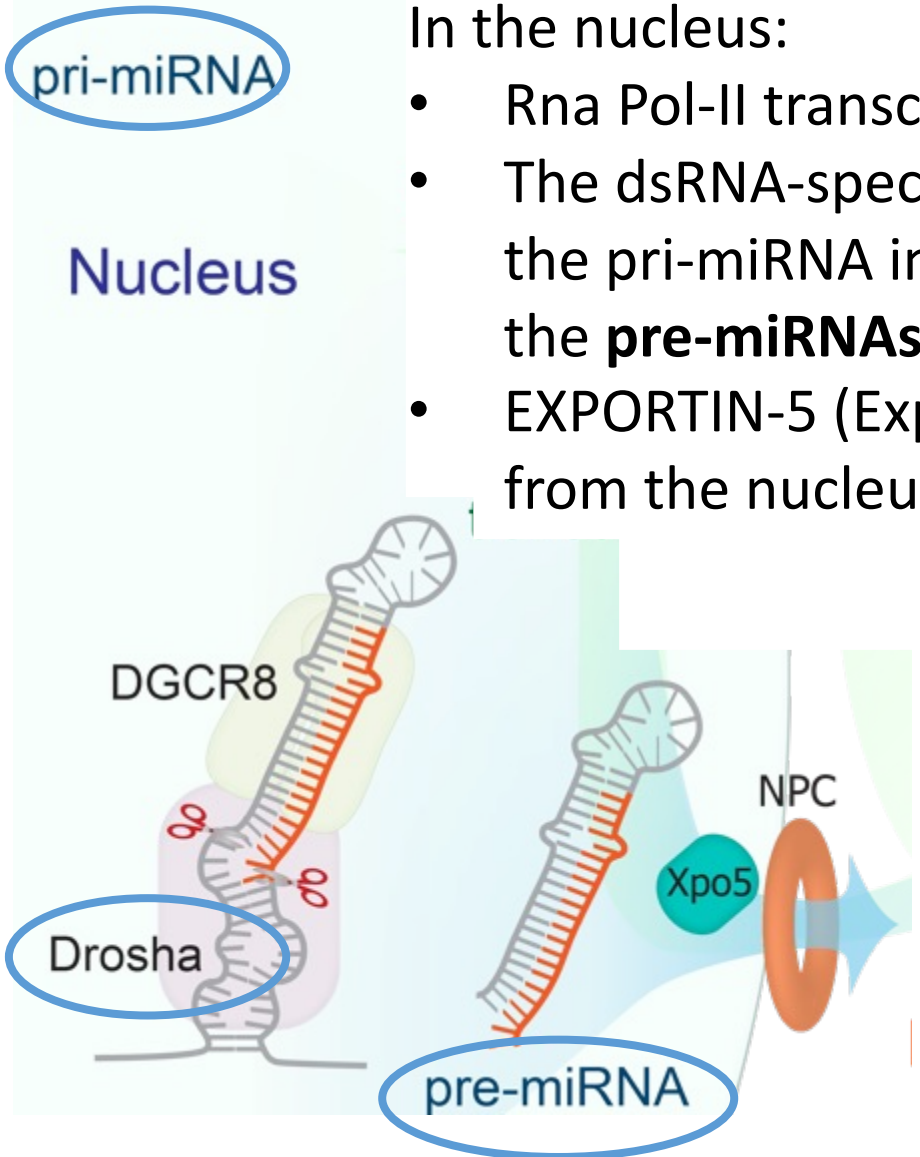
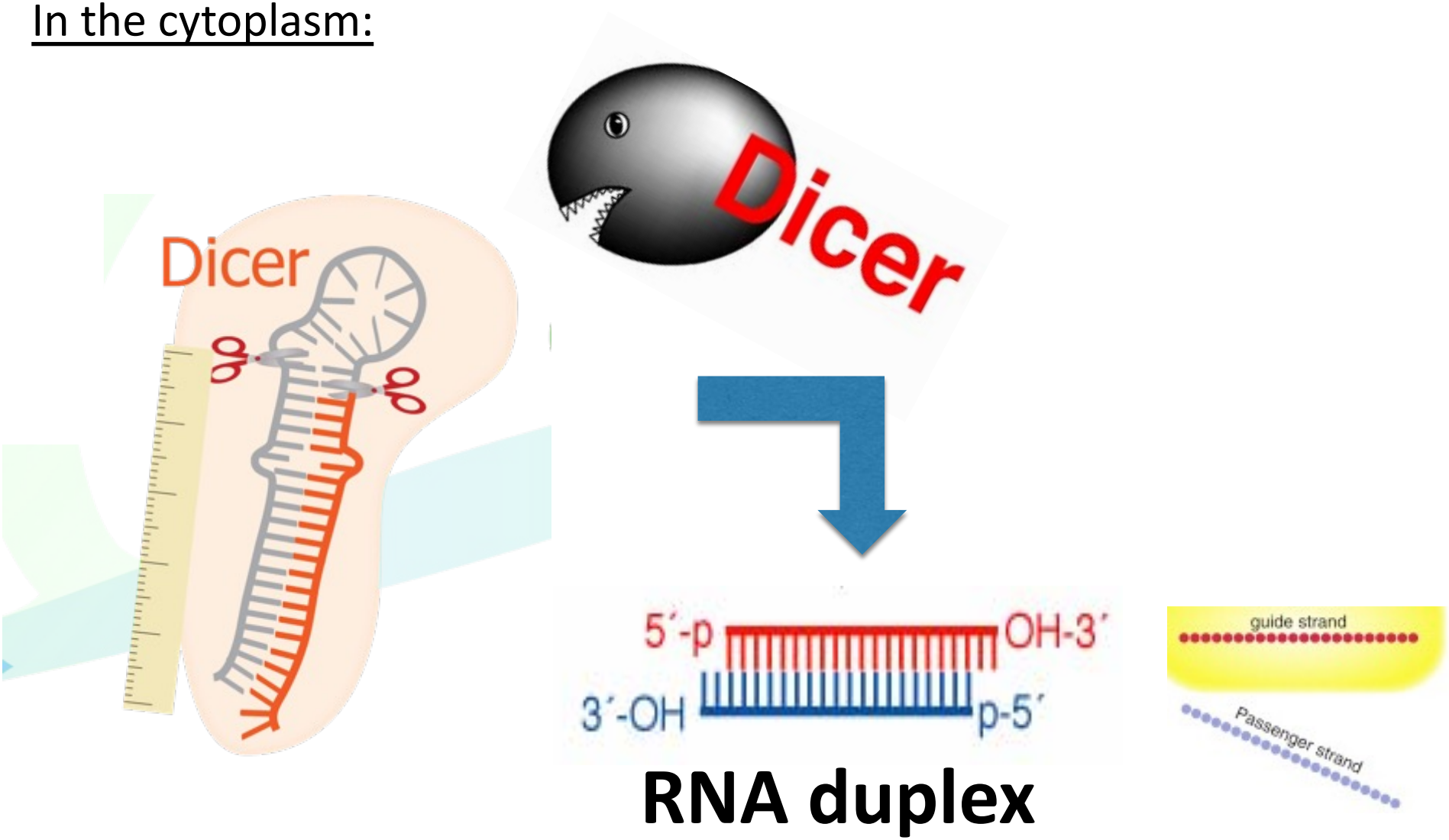
microRNA Biogenesis



In the nucleus:

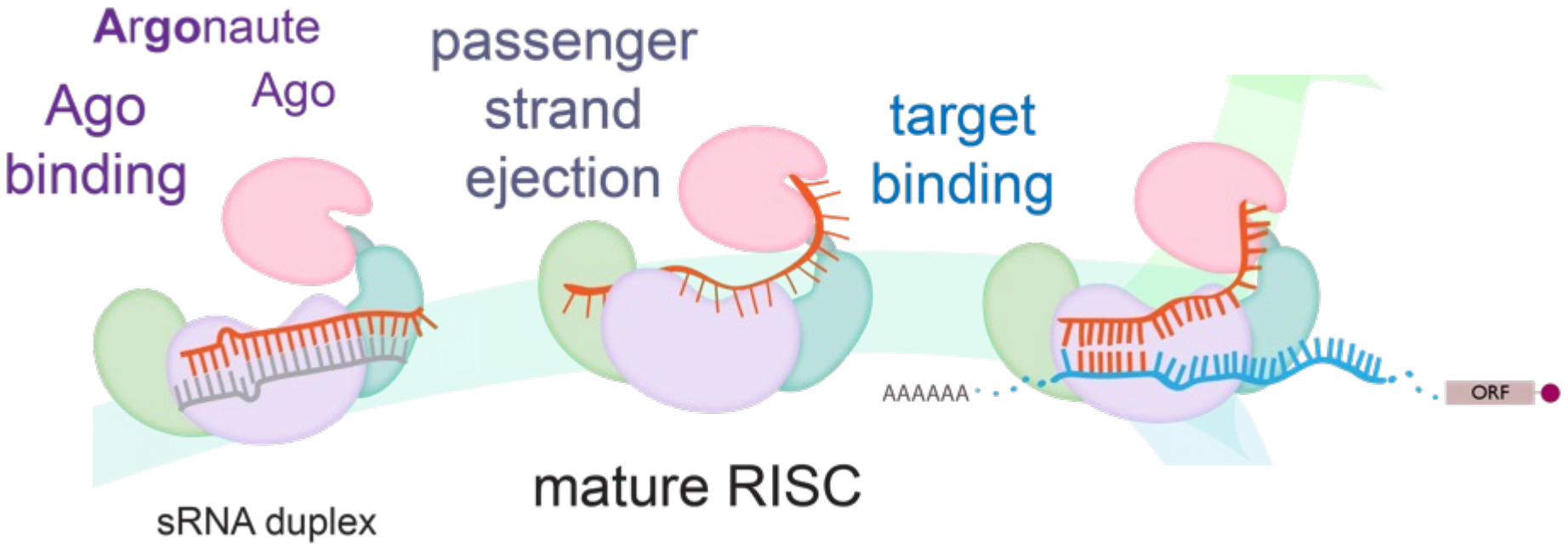
- Rna Pol-II transcribes pri-miRNAs (cap-poliA)
- The dsRNA-specific ribonuclease **DROSHA** digests the pri-miRNA in the nucleus into single Hairpins, the **pre-miRNAs**.
- EXPORTIN-5 (Exp5) translocates the pre-miRNAs from the nucleus to the cytoplasm.

In the cytoplasm:



RNA duplex

THE RISC COMPLEX



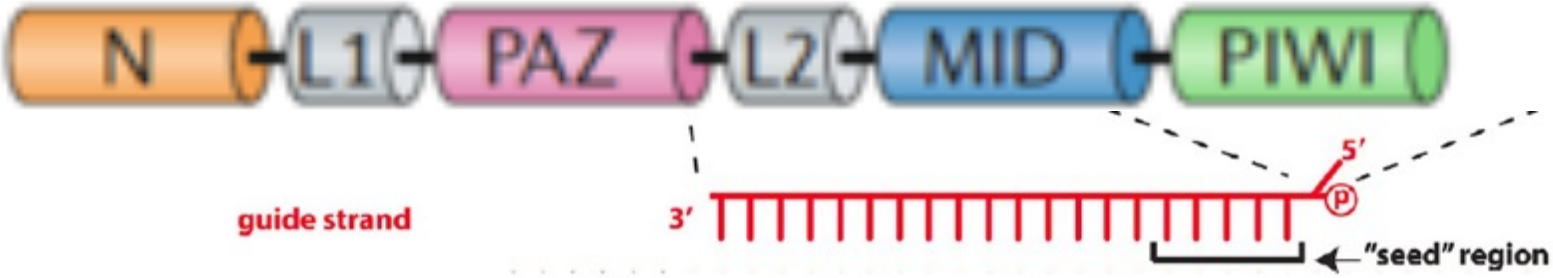
ARGONAUTE has two main functions:

1- degrades the passenger strands

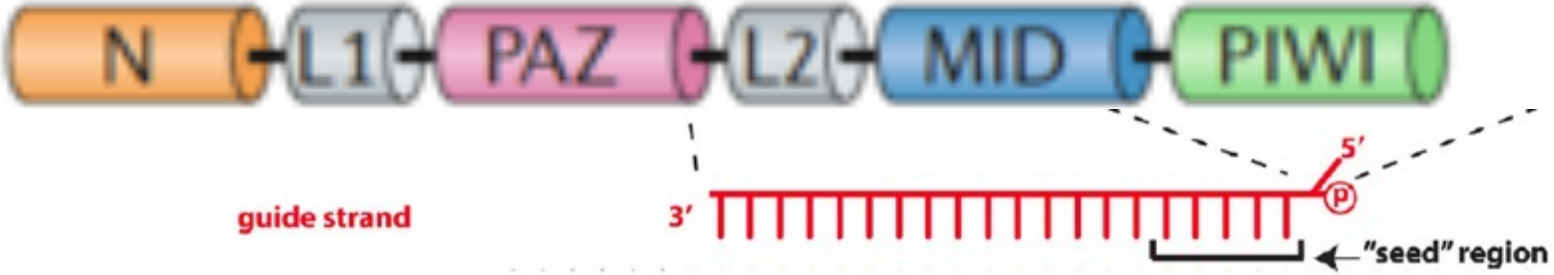
2- leads the guide strand to its target mRNA

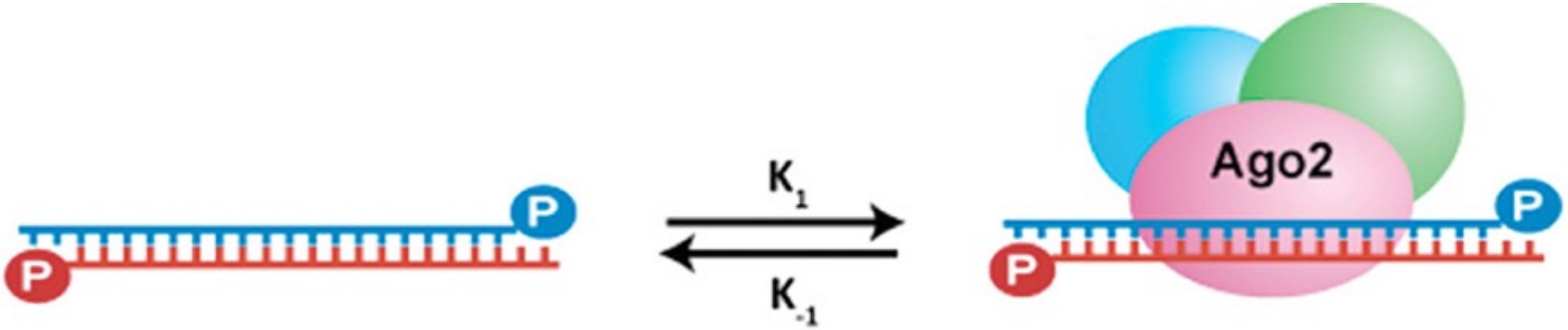
The PAZ Domain has 3’ RNA binding activity

- The PIWI Domain binds the 5’-P RNA end
- It has Rnase H activity

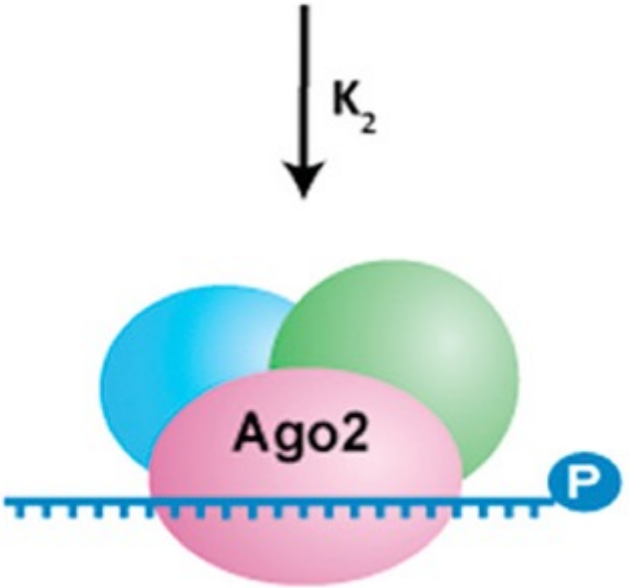


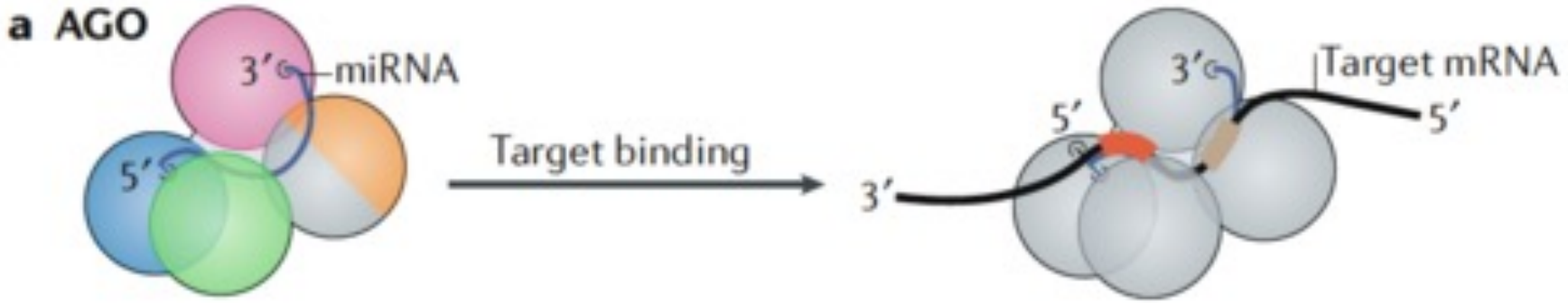
Le proteine AGO:
due domini caratteristici:
1. dominio **PAZ** lega il 3’-OH del piccolo RNA
2. dominio **Piwi** riconosce il terminale 5’-P del piccolo RNA e ha attività endonucleasica





The PIWI domain exerts its RNaseH activity, cleaves the passenger strand leading to its ejection from the complex





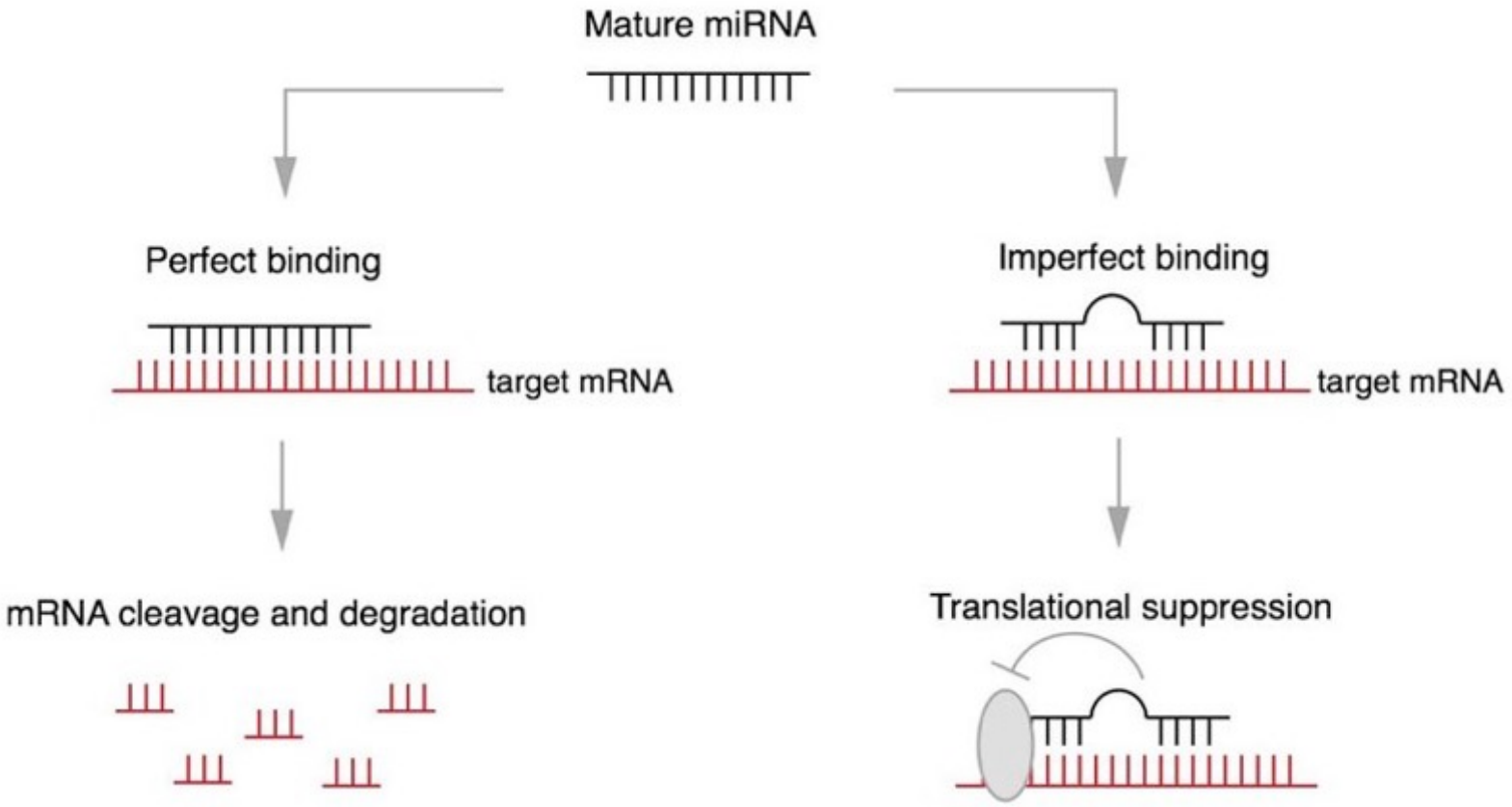
The guide strand, protected by the RISC complex from degradation by exonucleases, is targeted onto its mRNA

Come funzionano i miRNA?

La funzione primaria dei miRNA e' quella di abbassare il livello proteico degli mRNA target

I miRNAs svolgono la propria funzione attraverso due meccanismi:

- PERFETTA COMPLEMENTARIETA' → DEGRADAZIONE DEL mRNA (piante)
- IMPERFETTA COMPLEMENTARIETA' → REPRESSIONE TRADUZIONALE (animali)



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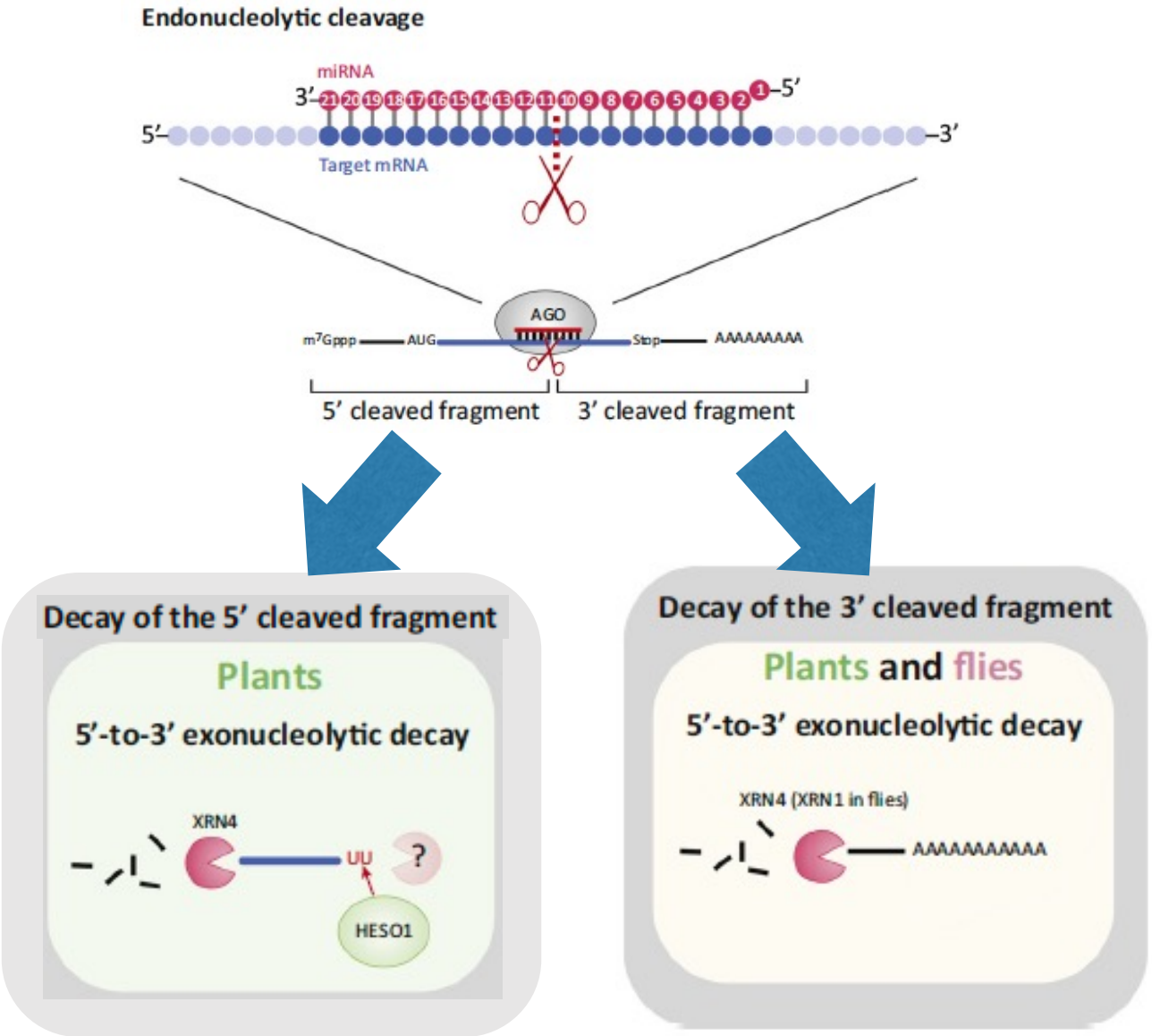

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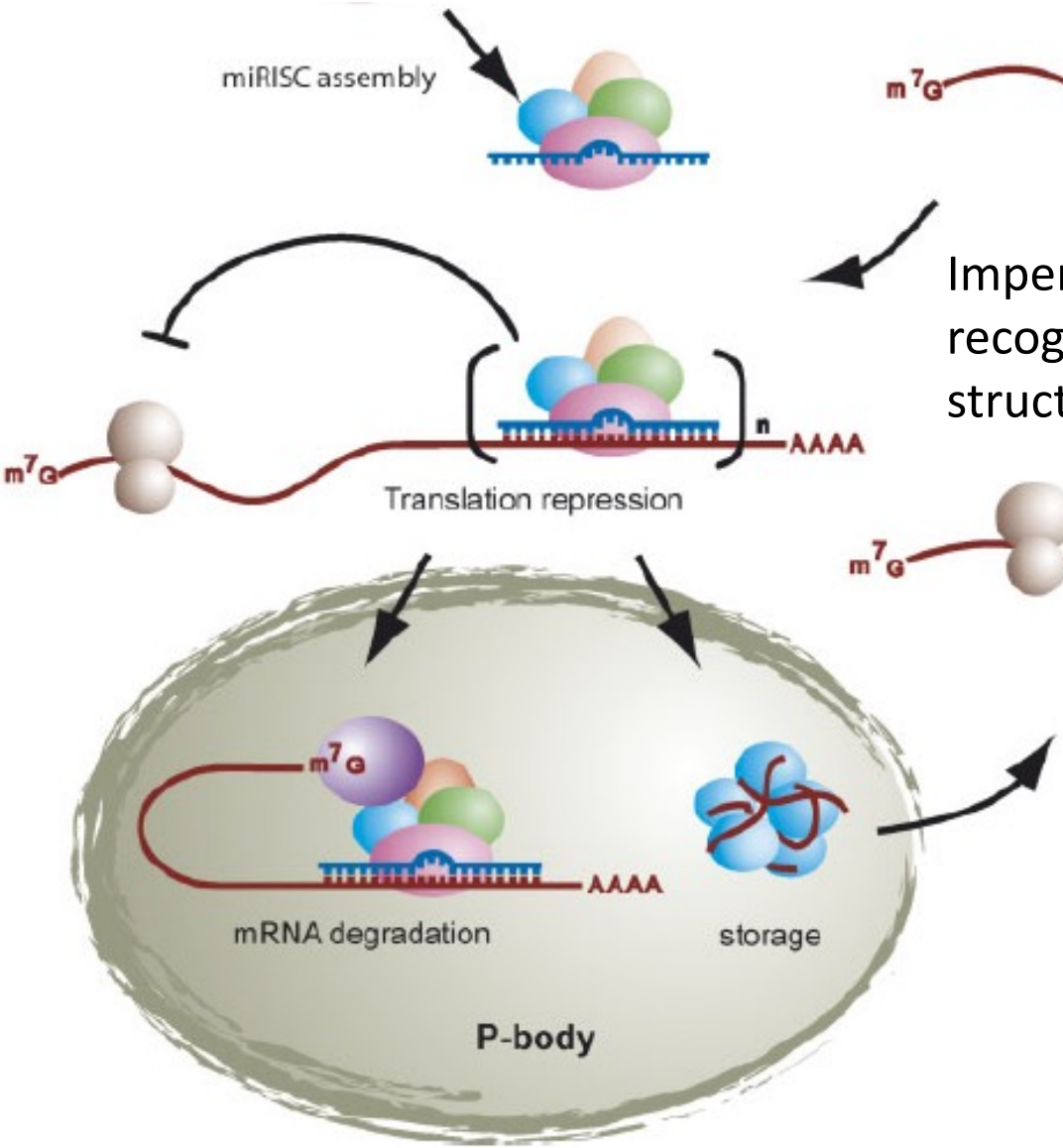

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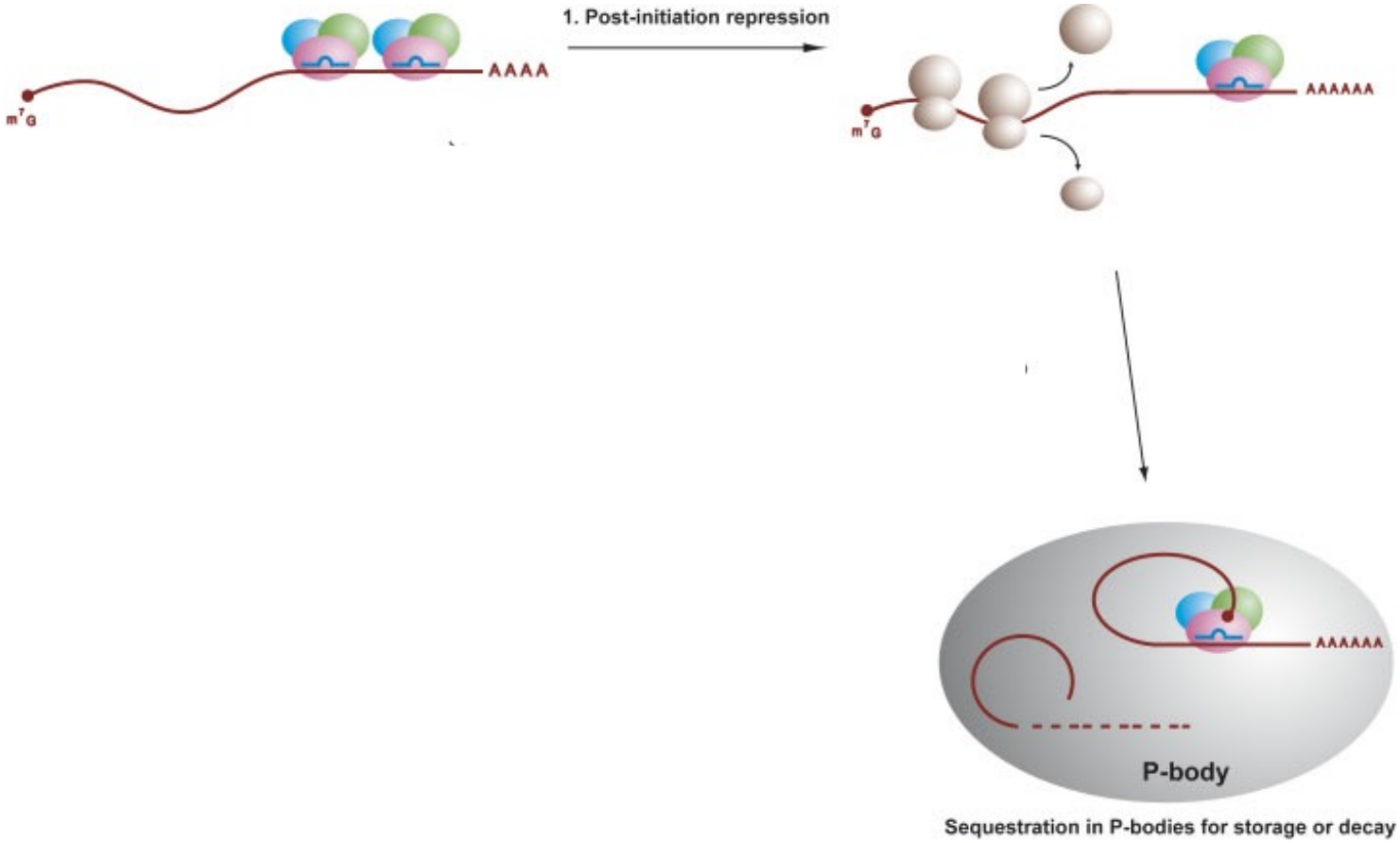

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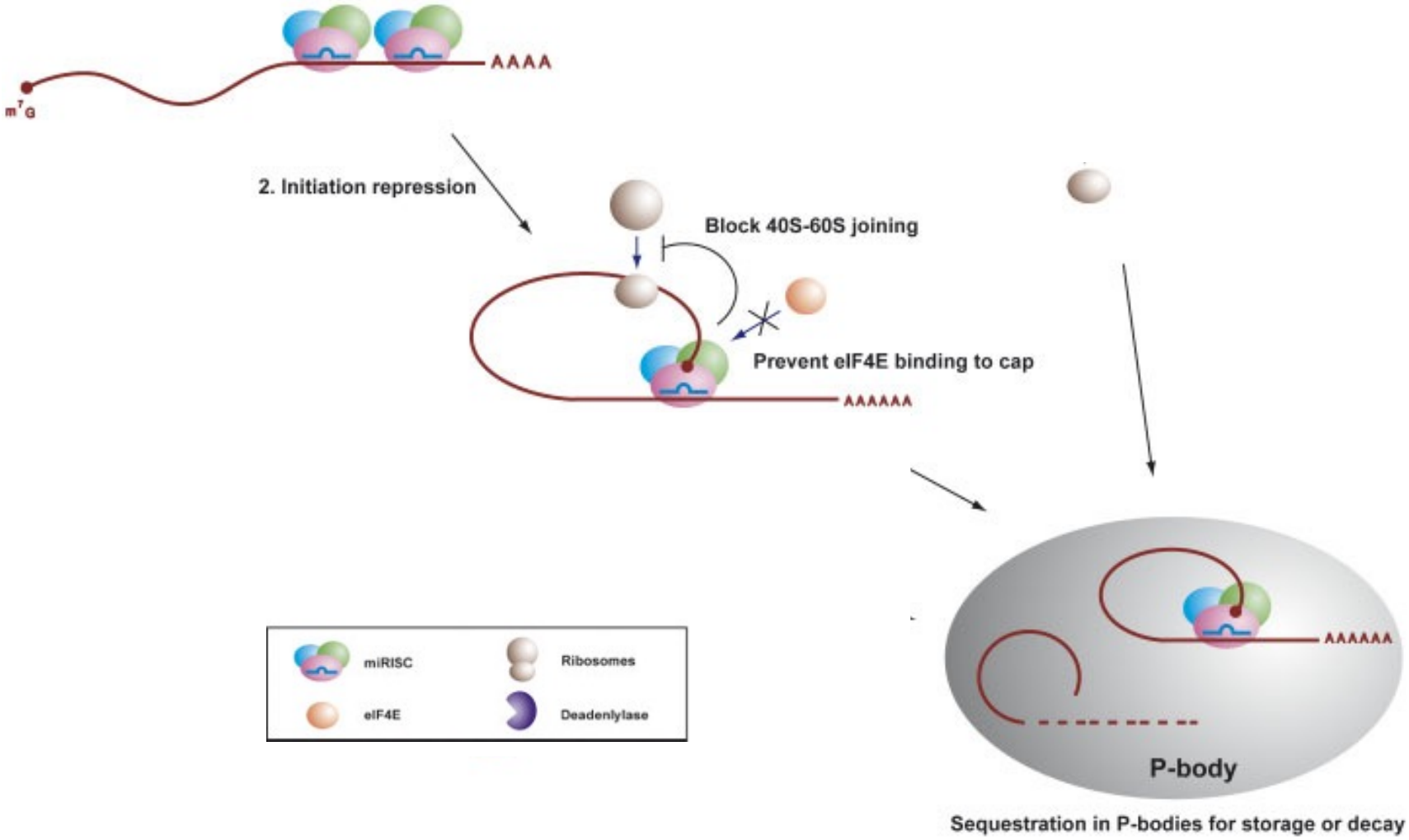

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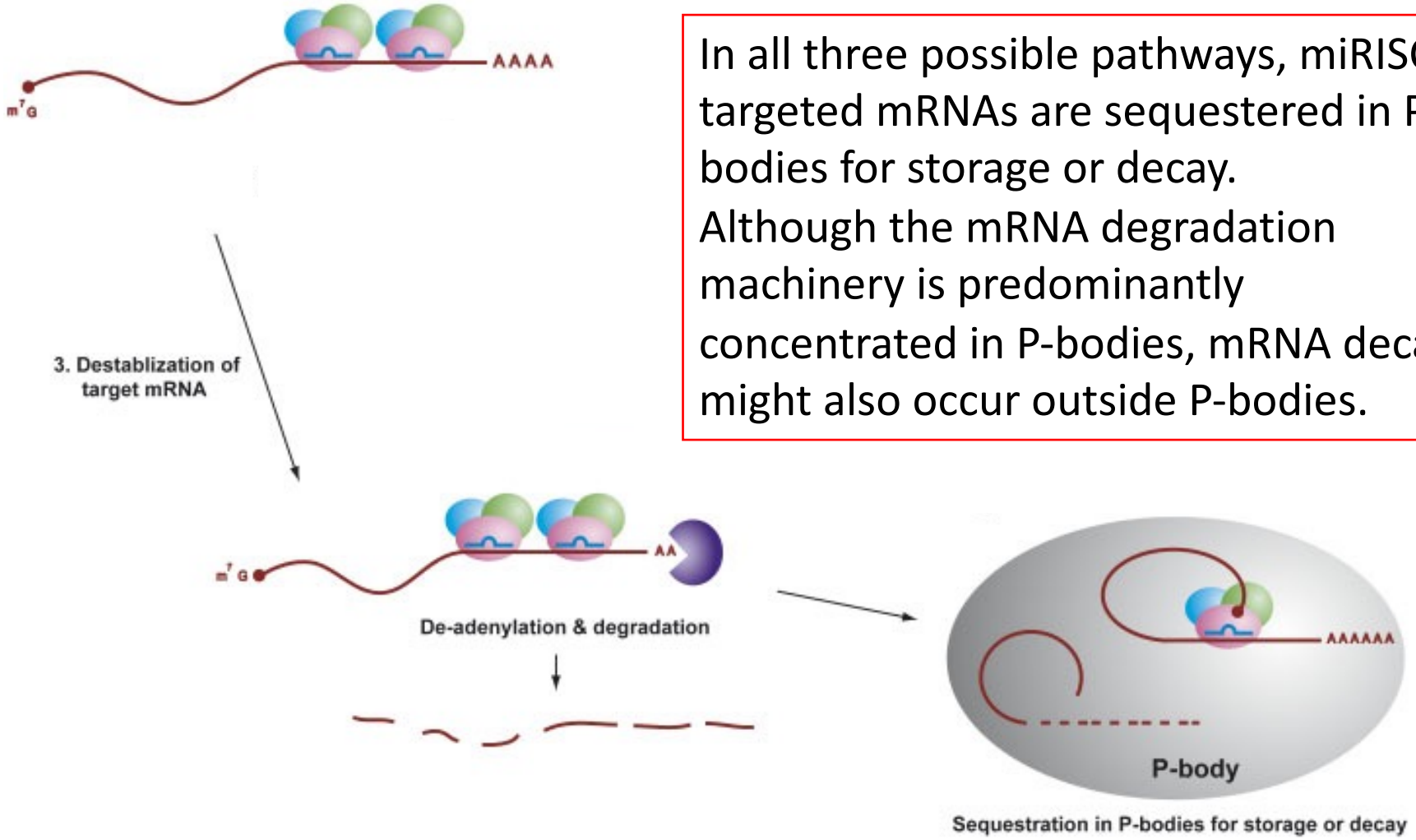

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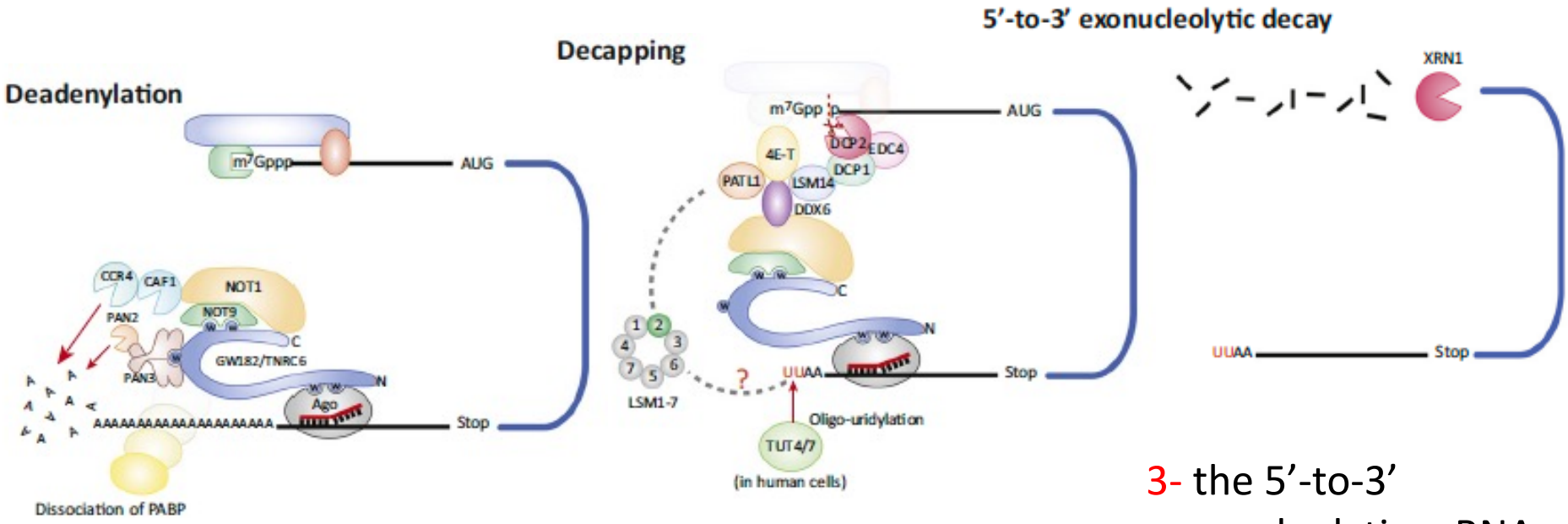

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miRNAs are involved in all aspects of cardiovascular function

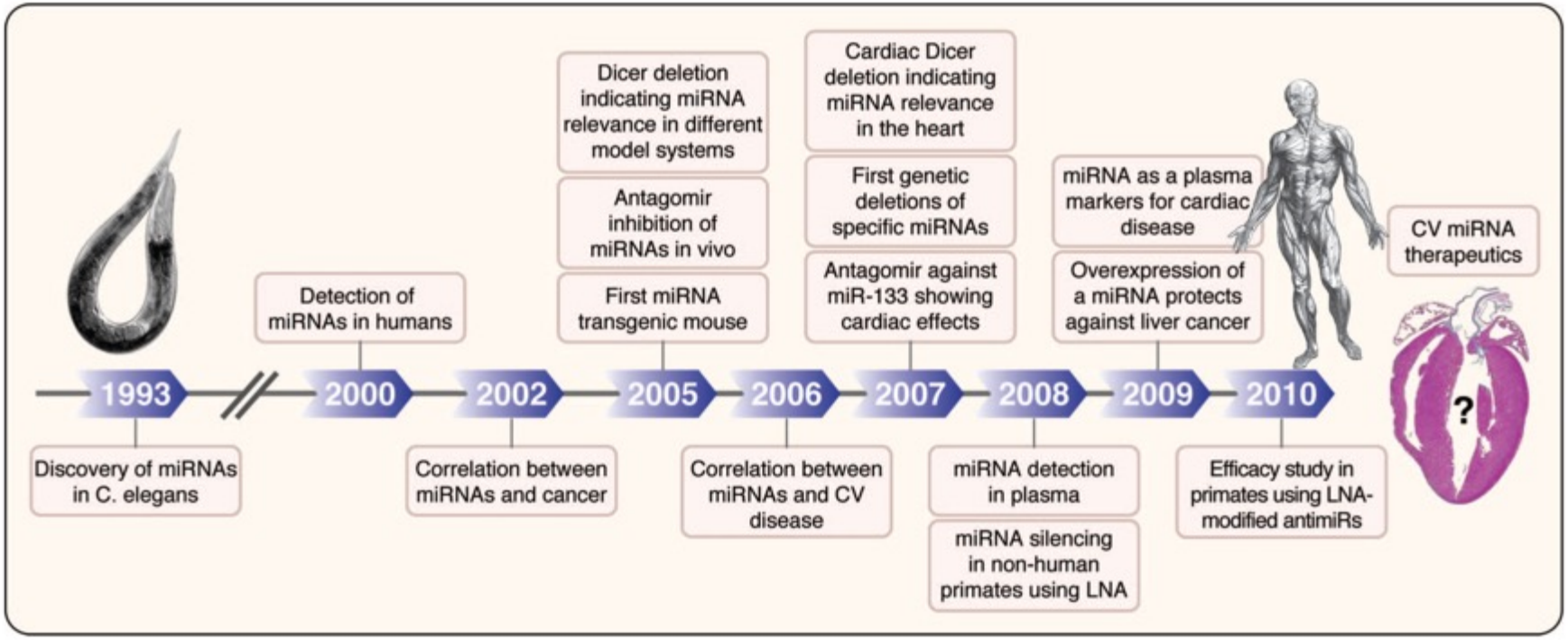
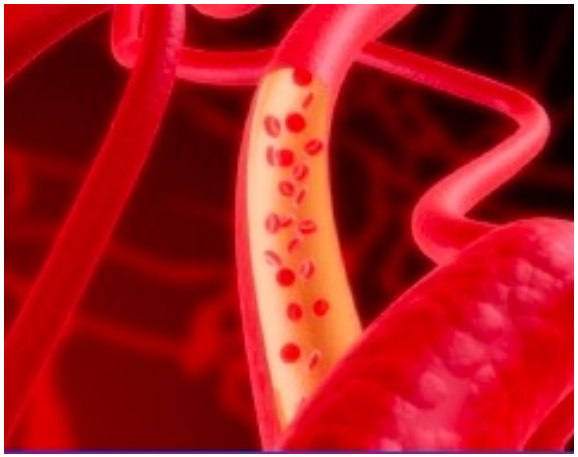


Figure 1. Breakthrough discoveries in miRNA biology. Time line indicating seminal discoveries in miRNA biology with a special focus on the cardiovascular field.

Circ Res. 2011;108:219-234

Non solo miRNA cellulari…. ma anche circolanti



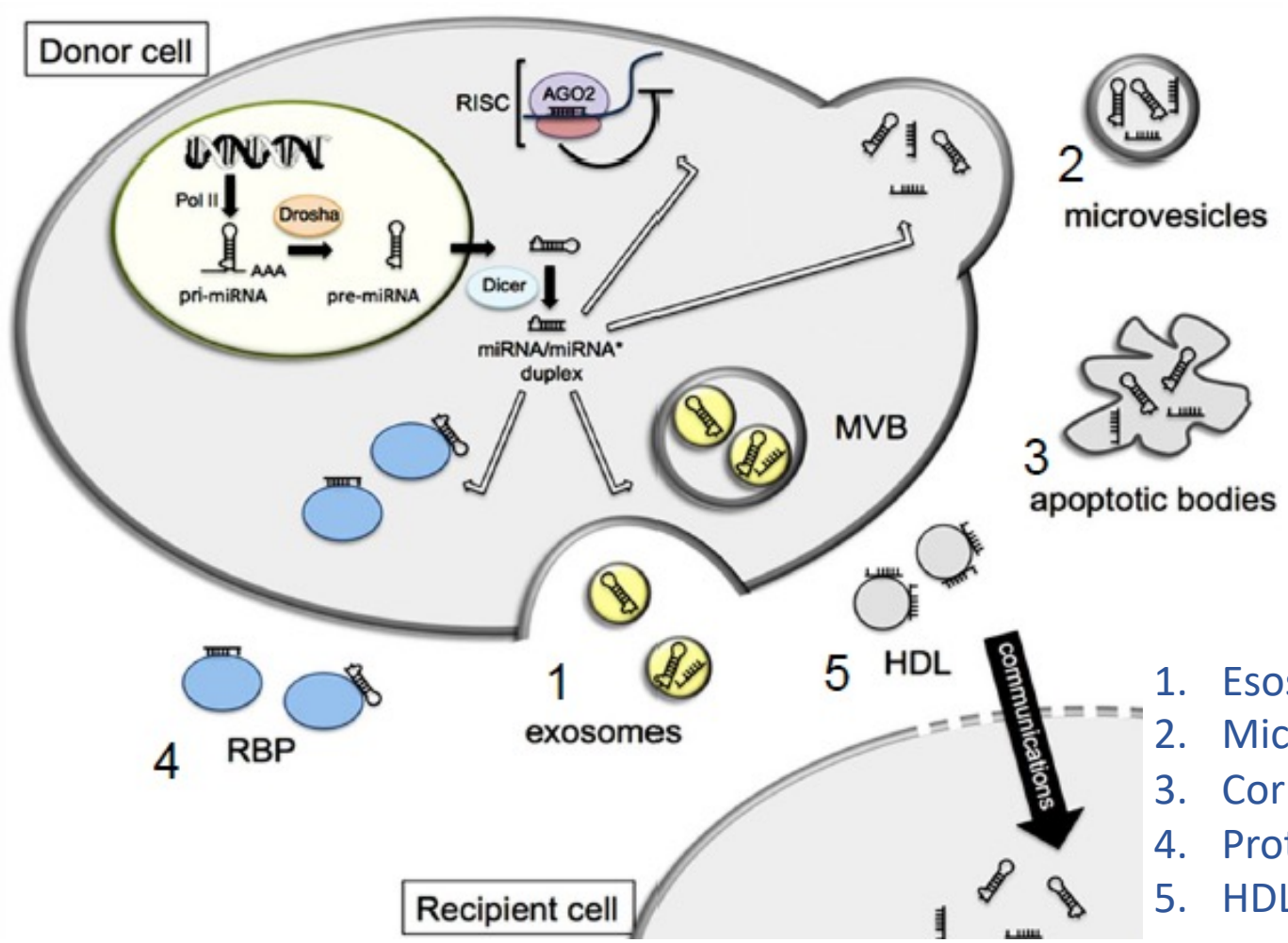
I miRNA possono essere trasportati al di fuori delle cellule e possono essere usati come indici per diagnosi e terapia di alcune patologie.

I miRNA circolanti rappresentano una nuova forma di comunicazione intercellulare attraverso il trasferimento di informazioni genetiche.

I miRNA si trovano:

- Nel siero
- Nel plasma
- Nel latte materno
- Nella saliva
- Nelle lacrime
- Nell'urina

Per essere protetti dalla degradazione delle esonucleasi presenti abbondantemente nei fluidi biologici, sono “impacchettati” secondo 5 meccanismi:



1. Esosomi
2. Microvescicole
3. Corpi apoptotici
4. Proteine che legano l’RNA
5. HDL

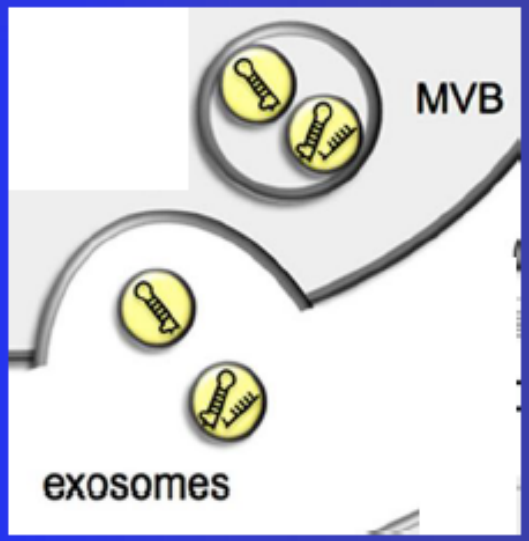
Carriers dei miRNA circolanti

1. ESOSOMI:

Micro-vescicole extracellulari piccole (40-120 nm) che si originano da corpi multi-vescicolari (MVBs) e sono rilasciate tramite esocitosi di questi MVBs.

Prodotti da molti tipi di cellule:

- Epiteliali
- Ematopoietiche
- Endoteliali
- Tumorali



Gli esosomi sono stati identificati in molti fluidi circolanti:

- Plasma
- Urine
- Latte
- Saliva
- Sperma

Processi di selezione devono avere luogo per caricare i miRNA negli esosomi: probabilmente esistono meccanismi cellulari che attivamente concentrano specifiche specie di miRNA negli esosomi

MiRNA circolanti come marcatori diagnostici

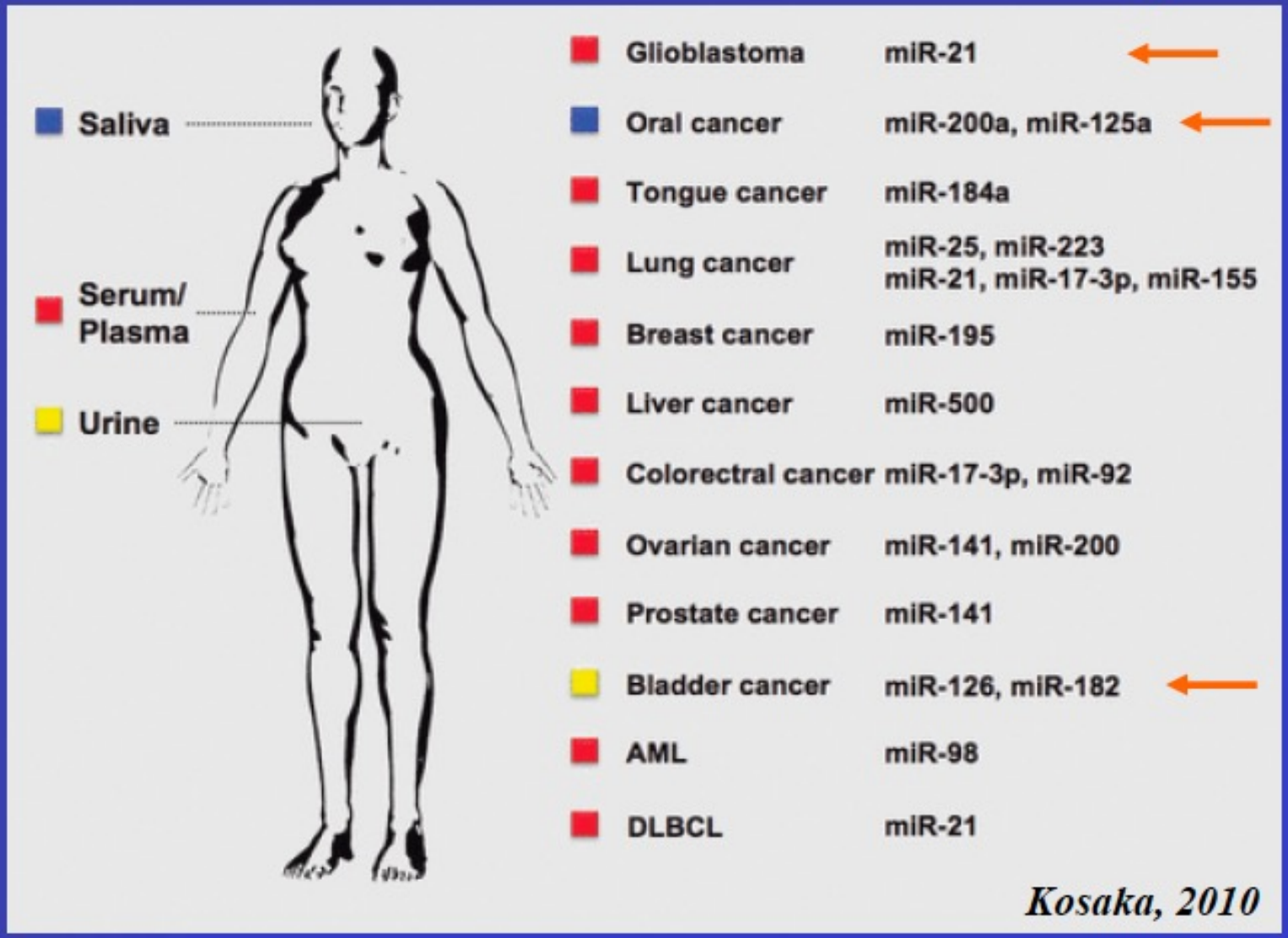
Sulla base della scoperta che i microRNA circolano nel sangue, è stata fatta l'ipotesi che essi abbiano, un ruolo di biomarcatori associati allo sviluppo di patologie

miRNAs nei fluidi del corpo umano sono
marcatori diagnostici tumorali
non invasivi

Molti tipi di miRNA circolanti sono stati riportati in molti tipi di cancro.
In alcuni casi, miRNA circolanti in siero, saliva ed urina sono buoni candidati per un futuro utilizzo

Tuttavia alcuni tipi di cancro non possono essere diagnosticati conoscendo i biomarcatori sierici.

MiRNA circolanti come marcatori diagnostici



AML:

acute myeloid leukemia

DLBCL: diffuse large B-cell lymphoma

Qui sono riassunte ricerche recenti che mostrano l'esistenza di miRNA circolanti nel fluidi di pazienti con il cancro

MiRNA circolanti come marcatori diagnostici - 1

Type of cancer	Biomarker candidate	Reference
Diffuse large B-cell lymphoma (DLBCL)	Expression levels of miR-155, miR-210 and miR-21 were higher in DLBCL patient than control sera	Lawriw, 2008
	High miR-21 expression was associated with relapse-free survival	
Prostate cancer	Serum levels of miR-141 can distinguish patients with prostate cancer from healthy controls	Mitchell, 2008
Ovarian cancer	The levels of the 8 specific miRNAs were similar between cellular and exosomal miRNAs. Exosomal miRNA from ovarian cancer patients exhibited similar profiles, which were significantly distinct from profiles observed in benign disease	Taylor, 2008
	miR-21, -92, -93, -126 and -29a were significantly overexpressed in the serum from cancer patients compared to controls	Resnick, 2009
Non small cell lung cancer	Eleven serum miRNAs were found to be altered more than 5-fold between longer-survival and shorter-survival groups, and levels of four miRNAs were significantly associated with overall survival	Hu, 2010
Acute myeloid leukemia (AML) Acute lymphoblastic leukemia (ALL)	miR-92a decreased in the plasmas of acute leukemia patients	Tanaka 2009
Breast cancer	Increased miR-195 levels in patients were reflected in tumors, and circulating levels of miR-195 and let-7a decreased in cancer patients postoperatively, to levels comparable with control subjects	Heneghan, 2010
	miR-155 was differentially expressed in the serum of women with hormone-sensitive compared to women with hormone-insensitive breast cancer	Zhu, 2009
Gastric cancer	The plasma concentrations of miR-17-5p, miR-21, miR-106a, and miR-106b were significantly higher in patients than controls, whereas let-7a was lower in patients	Tsujiura, 2010
Pancreatic cancer	Circulating miR-210 levels are elevated in pancreatic cancer patients	Ho, 2010
Pancreatic ductal adenocarcinoma	The combined analyses of four miRNAs (miR-21, miR-210, miR-155, and miR-196a) in plasma can discriminate patients from normal healthy individuals	Wang, 2009
Squamous cell carcinoma (SCC) of tongue	Plasma miR-184 levels were significantly higher in tongue SCC patients in comparison with normal individuals, and the levels were significantly reduced after surgical removal of the primary tumors	Wong, 2008
Colorectal cancer	Both miR-17-3p and miR-92 were significantly elevated in the patients, and the plasma levels of these	Ng, 2009

MiRNA circolanti come marcatori diagnostici - 2

Patologia	Biomarker candidato	Ref.
Diffuse large B-cell lymphoma (DLBCL)	I livelli di espressione nel siero di miR-155, miR-210 and miR-21 sono più alti nei pazienti rispetto ai controlli	Lawriw, 2008
	L'elevata espressione di miR-21 è associata con la sopravvivvenza priva di ricidive	
Prostate cancer	I livelli sierici di miR-141 possono distinguere i pazienti con il cancro alla prostata dai controlli sani	Mitchell, 2008
Ovarian cancer	miR-21, -92, -93, -126 and -29a sono sovraespressi nel siero delle pazienti rispetto ai controlli	Resnick, 2009

MiRNA circolanti come marcatori diagnostici - 3

Patologia	Biomarker candidato	Ref.
Non small cell lung cancer	11 miRNA sierici sono stati trovati alterati di oltre 5 volte tra il gruppo dei longer-survival rispetto a quello dei shorter-survival, e i livelli di 4 miRNA sono associati con la sopravvivenza globale	Hu, 2010
Acute myeloid leukemia (AML) e Acute lymphoblastic leukemia (ALL)	miR-92a decresce nel plasma dei pazienti affetti da leucemia cronica	Tanaka 2009
Breast cancer	Nel tumore si è visto l'aumento dei livelli di miR-195 e i livelli dello stasso mir e di let-7a nel sangue diminuisce nelle pazienti dopo l'operazione fino a livelli simili ai controlli sani	Henegha, 2010
	Il miR-155 è differenzialmente espresso nel siero di donne con cancro al seno ormone-responsivo rispetto a quelle ormone-non-resp	Zhu, 2009

MiRNA circolanti come marcatori diagnostici - 4

Patologia	Biomarker candidato	Ref.
Gastric cancer	Le concentrazioni plasmatiche di miR-17-5p, miR-21, miR-106a e miR-106b sono significativamente più elevate nei pazienti rispetto ai controlli, mentre let-7a è più basso nei pazienti	Tsujiura, 2010
Pancreatic cancer	I livelli di miR-210 circolante sono elevati nei pazienti	Ho, 2010
Pancreatic ductal adenocarcinoma	L'analisi combinata di 4 miRNA (miR-21, miR-210, miR-155 e miR-196a) nel plasma può discriminare i pazienti dai controlli sani	Wang, 2009
Squamous cell carcinoma (SCC) of tongue	I livelli plasmatici di miR-184: -sono significativamente elevati nella lingua dei pazienti SCC rispetto ai normali e -sono significativamente ridotti dopo la rimozione chirurgica del tumore primario	Wong, 2008

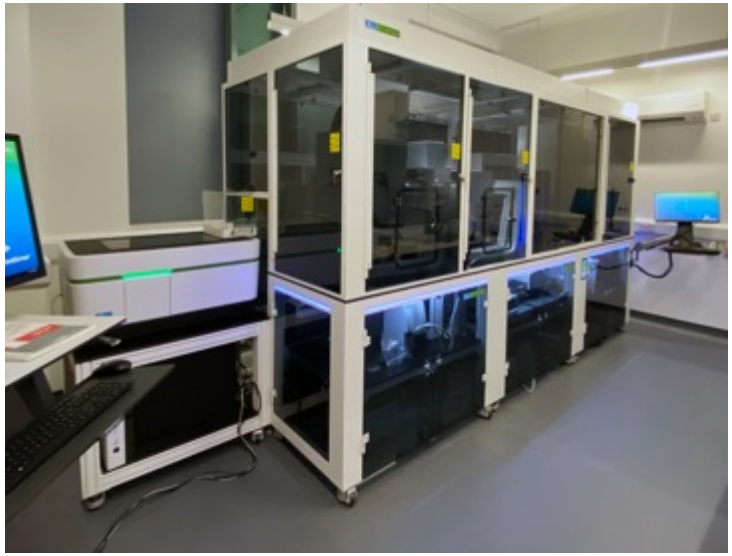
MicroRNA therapeutics: towards a new era for the management of cancer and other diseases

Rajesha Rupaimoole and Frank J. Slack

Abstract | In just over two decades since the discovery of the first microRNA (miRNA), the field of miRNA biology has expanded considerably. Insights into the roles of miRNAs in development and disease, particularly in cancer, have made miRNAs attractive tools and targets for novel therapeutic approaches. Functional studies have confirmed that miRNA dysregulation is causal in many cases of cancer, with miRNAs acting as tumour suppressors or oncogenes (oncomiRs), and miRNA mimics and molecules targeted at miRNAs (antimiRs) have shown promise in preclinical development. Several miRNA-targeted therapeutics have reached clinical development, including a mimic of the tumour suppressor miRNA miR-34, which reached phase I clinical trials for treating cancer, and antimiRs targeted at miR-122, which reached phase II trials for treating hepatitis. In this article, we describe recent advances in our understanding of miRNAs in cancer and in other diseases and provide an overview of current miRNA therapeutics in the clinic. We also discuss the challenge of identifying the most efficacious therapeutic candidates and provide a perspective on achieving safe and targeted delivery of miRNA therapeutics.

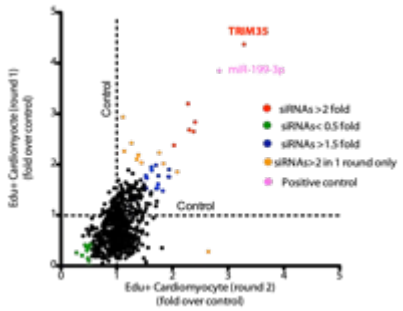
miRNA therapeutics

- Many, but not too many! 1917 miRNA precursors, 2654 mature miRNAs in humans (***miRBase 22, March 2018***)
- Pleiotropic. Each miRNA targets tens or hundreds of transcripts. Ideal to target complex functions
- Can be screened for function
- Small. Can be delivered as synthetic molecules. Can be dosed.

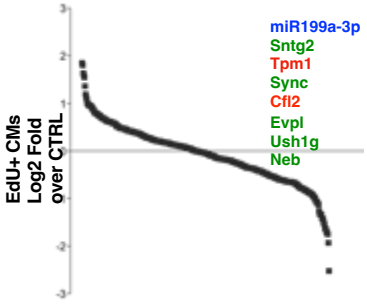


HTS Facility @ School of Cardiovascular Medicine & Sciences, King's College London

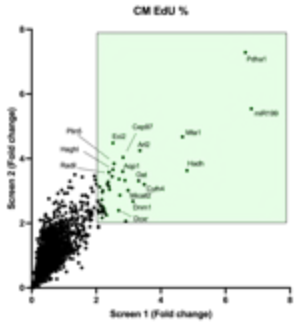
Screenings for cardiomyocyte proliferation



All genes in the UPS (586 siRNAs)

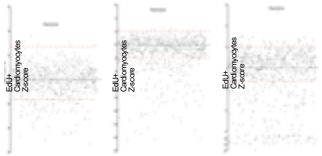


Genes coding for cytoskeleton and sarcomere (400 siRNAs)



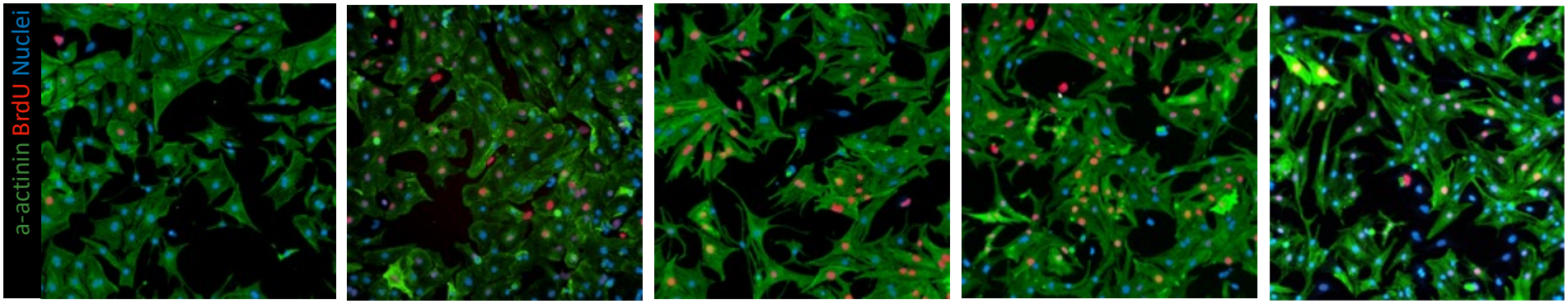
Genes coding for metabolic, sarcomeric and cytoskeletal proteins (1760 siRNAs)

YAP and TRIM35 binding proteins (with M. Mayr)



FDA/EMA approved drugs (1200 drugs)

Control | miR-199a-3p | siRNA TRIM35 | siRNA TXNIP | siRNA Dyrk1a



Where do we go from here?

The NEW ENGLAND JOURNAL of MEDICINE

CLINICAL IMPLICATIONS OF BASIC RESEARCH

New Cells in Old Hearts

Pontus Boström, M.D., Ph.D., and Jonas Frisén, M.D., Ph.D.

Unlike salamanders and zebra fish, in which large parts of the heart are readily regenerated after injury, the mammalian myocardium has limited regenerative capacity. The loss of cardiomyocytes after a myocardial infarction in humans typically results in scar formation, loss of contractile capacity, and reduced cardiac function. One can envisage two conceptually different therapeutic strategies to restore the human myocardium: transplantation of contractile cells, perhaps derived from stem cells in cell culture, or promotion of a latent endogenous regenerative capacity in the heart. Most efforts have been directed toward cell-transplantation strategies.[1] Two recent studies[2,3] described the dynamics of cardiomyocyte renewal and identified ways to promote their regeneration in the adult mouse heart, suggesting that stimulating endogenous cardiac-repair mechanisms may be rational and realistic.

Several studies have demonstrated the continuous generation of cardiomyocytes in the adult mammalian heart, but the estimates of the extent of this process have varied substantially, and it has been unclear whether these new cells derive from resident cardiac stem or progenitor cells or from proliferating cardiomyocytes. Senyo and colleagues[2] used very sophisticated technology to establish the renewal rate and origin of cardiomyocytes in adult mice. By detecting the nonradioactive stable nitrogen isotope ^{15}N in the DNA of cells undergoing mitosis by means of mass spectrometry in tissue sections, they concluded that 0.8% of cardiomyocytes are replaced annually in young adult mice and that this rate of replacement declines during aging.[2] This corresponds closely to estimates of the turnover dynamics in the human heart.[4] By combining the cell-proliferation analysis with genetic fate map-

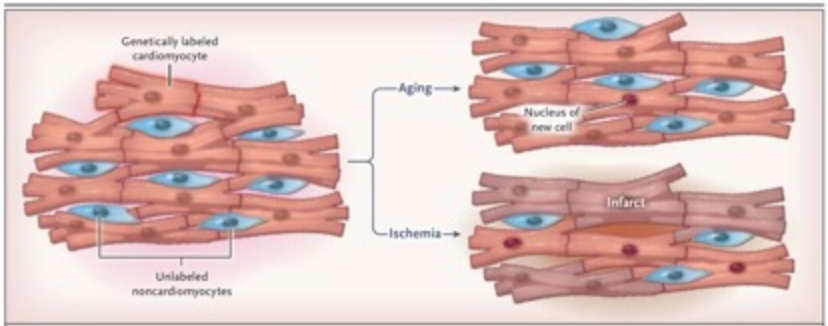


Figure 1. Cardiomyocyte Division.
Senyo and colleagues[2] introduced a stable and heritable genetic marker specifically in cardiomyocytes in the adult mouse heart and simultaneously assessed mitotic cell division by means of incorporation of a labeled DNA building block (new cells are shown with red nuclei). They established that new cardiomyocytes carry the cardiomyocyte lineage marker and thus derive from proliferating cardiomyocytes during normal aging. They also found that the generation of cardiomyocytes increased after myocardial ischemia in mice, mainly through increased cardiomyocyte proliferation but potentially also through nonmitotic differentiation of progenitor cells.

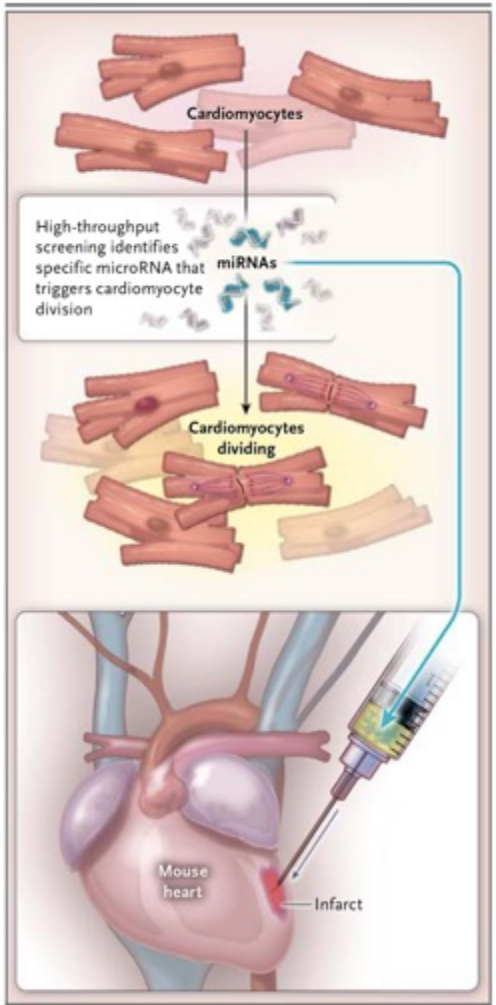


Figure 2. Heart Mending with MicroRNAs (miRNAs).
Eulalio and colleagues[3] performed a cell-culture screen for miRNAs that promote the proliferation of rodent cardiomyocytes. Two of the identified miRNAs were delivered to the heart in mice after myocardial ischemia, resulting in an increase in new cardiomyocytes and improved cardiac function.

- Target cell identification
- Mechanism of action
- Formulation (miRNA delivery as naked RNAs)
- Chemical modification to increase stability
- Delivery in larger animals
- Efficacy in larger animals
- Safety
- Effect in human cells

Patent RM2011A000685
Priority date" 23.11.2011