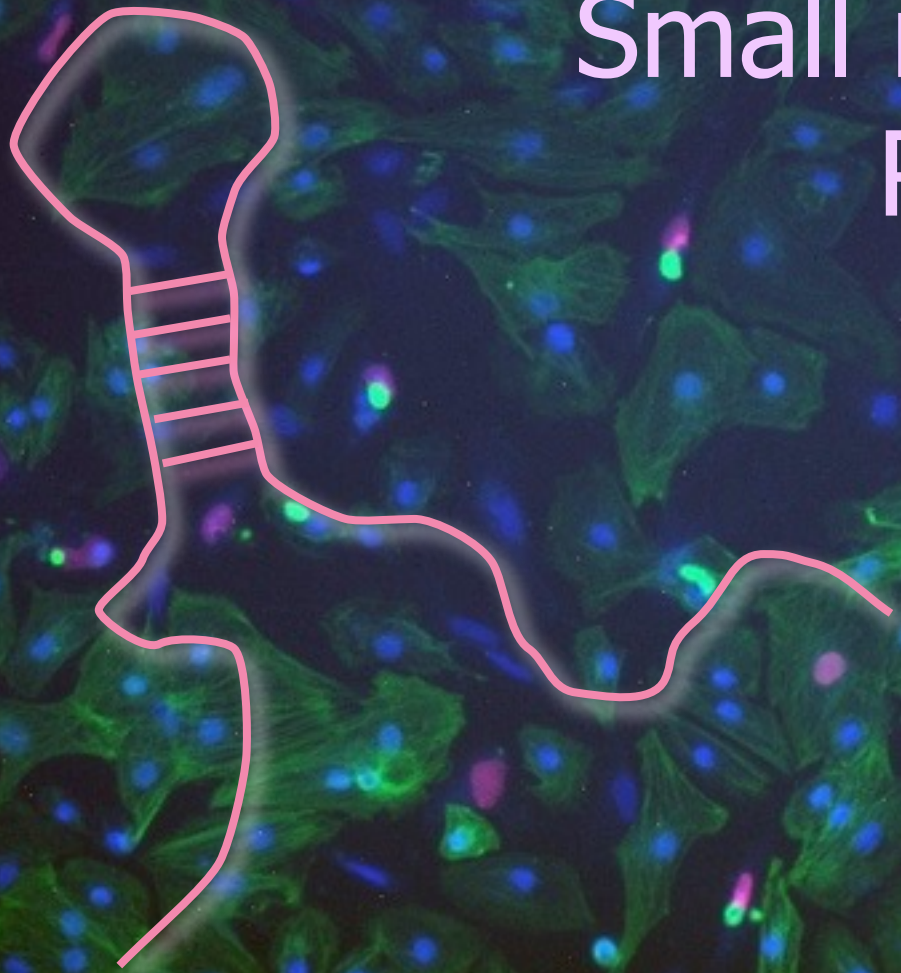
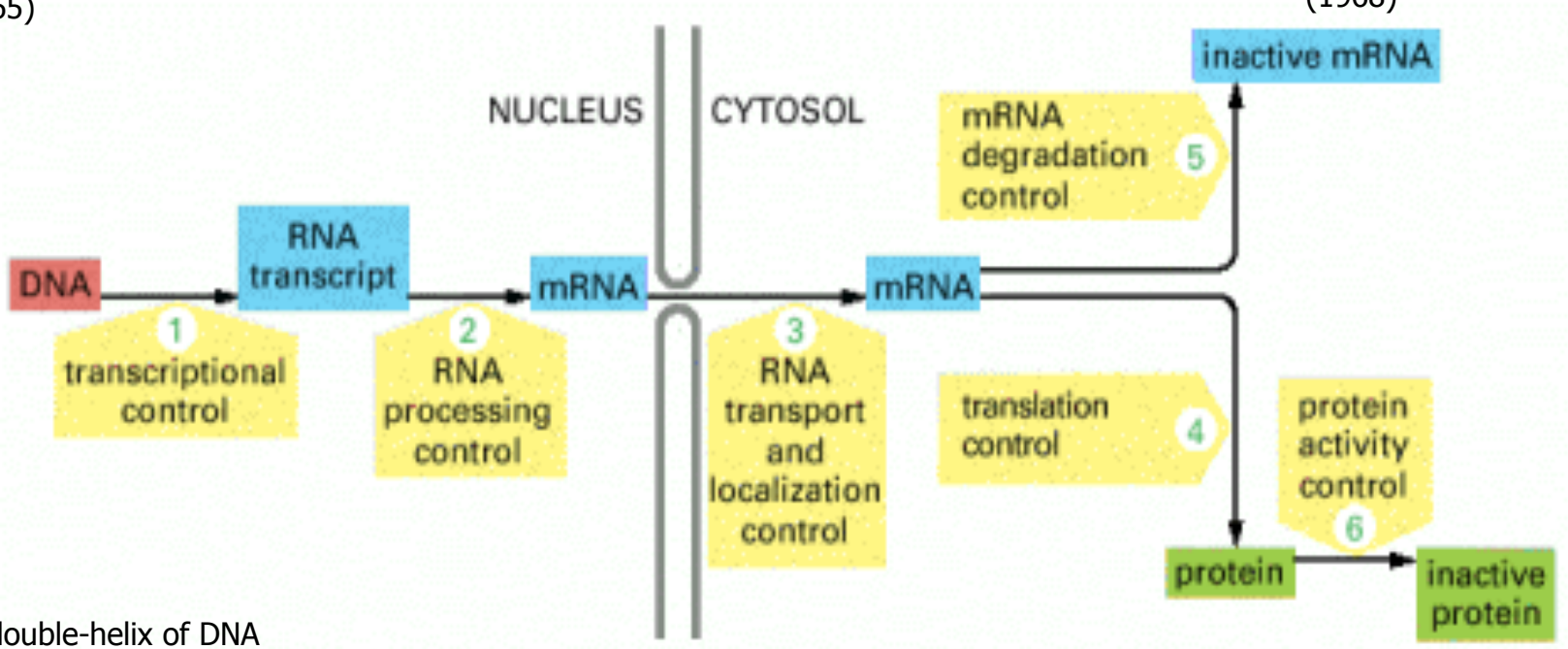
Small regulatory RNAs



RNA in the CONTROL of GENE EXPRESSION - a Nobel story

The gene is transcribed into a specific RNA species, mRNA
F. Jacob and J. Monod; Nobel Prize in Physiology or Medicine (1965)

The genetic code
M. Nirenberg and G. Khorana; Nobel Prize in Physiology or Medicine (1968)

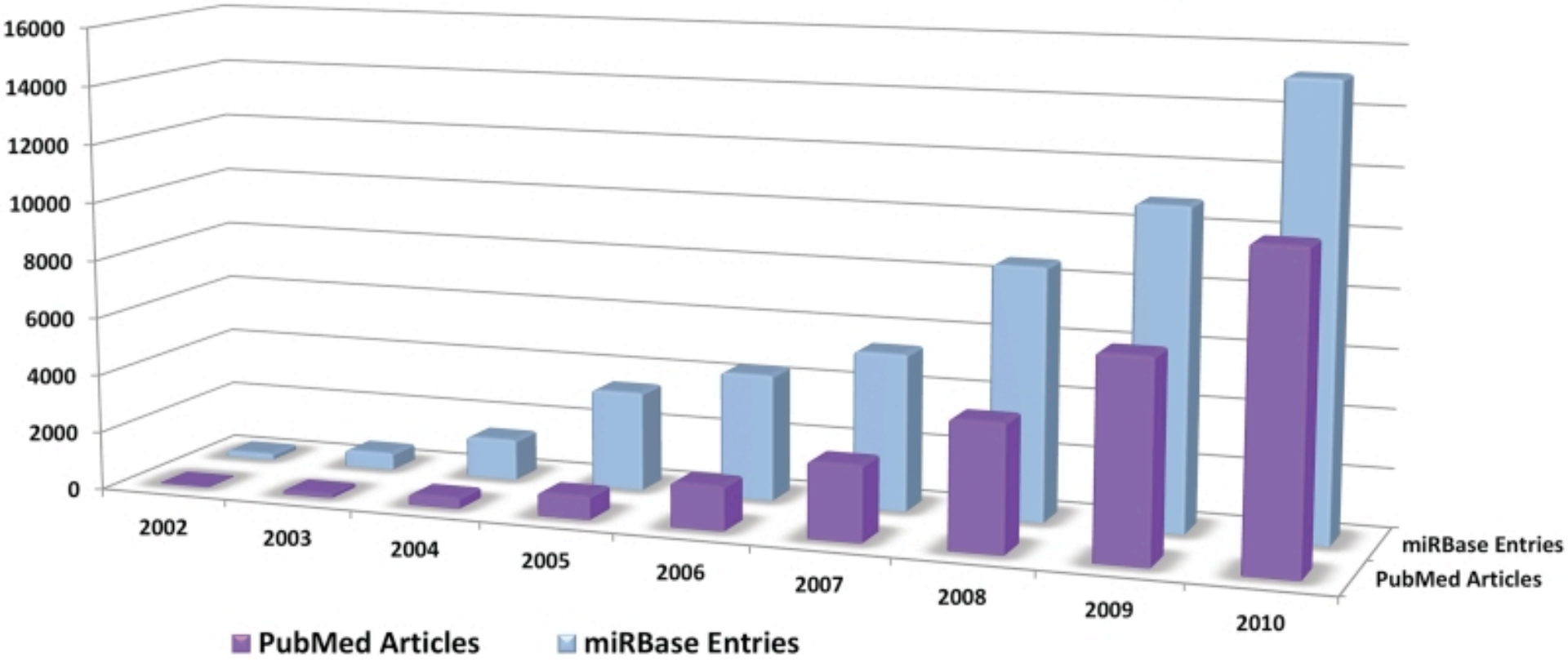


The double-helix of DNA identified as the genetic material
F.Crick, J. Watson and M. Wilkins; Nobel Prize in Physiology or Medicine (1962)

The split gene
P. Sharp and R. Roberts;
Nobel Prize (1993)

RNA can act as a catalyst
S. Altman and T. Cech;
Nobel Prize in Chemistry (1989)

Available miRNA-related Pubmed Articles and miRBase entries per Year



1993	2000	2001	2002	2003	2004
Ambros laboratory discovers the first microRNA lin-4 in *C. elegans*[1]	Ruvkun laboratory discovers let-7 microRNA in *C. elegans*[11]	• Zamore laboratory shows that Dicer makes microRNA[12] • Bartel, Tuschl and Ambros laboratories discover large classes of small RNAs with regulatory roles and name them microRNAs[13–15] • Bartel lab discovers microRNA in plants[16,17]	Croce laboratory shows that miRNAs are implicated in cancer because miR-15 and miR-16 are deleted or downregulated in most chronic lymphocytic leukemias[18]	• miRNA maturation shown to begin in the nucleus[19] • Stoffel laboratory silences miRNAs *in vivo* using chemically engineered oligonucleotides, termed 'antagomirs'[20]	Animal viruses shown to use microRNA[21]

History

- lin-4, first miRNA to be described in C. elegans; important in development of the worm from larva to adult.

- let-7, was also described in C. elegans (Reinhard BJ et al, 2000) as critical to stop the stem-cell-like divisions of seam cells and induce their fully differentiated state. Reduced let-7 expression is associated with human cancers and cancer stem cells, thus suggesting that let-7 in humans also promotes terminal differentiation and is a tumor suppressor.

- 1998-Fire and Mello, experiments in C. elegans, first to show that dsRNA is much more potent at inhibiting gene expression than antisense RNA. Set the stage for understanding the role of miRNAs in development and gene regulation. (Nobel Prize in Physiology and Medicine, 2007).

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

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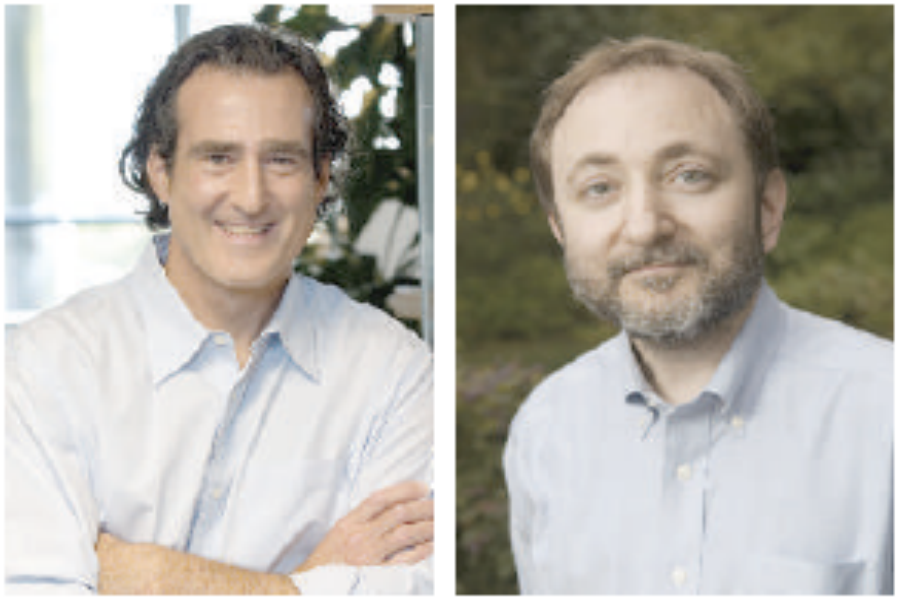
† *Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA*
‡ *Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA*



Silence is golden: Craig Mello (left) and Andrew Fire.

Nobel prize 2006 Physiology and Medicine to Craig Mello and Andrew Fire for their report on RNAi.

Key breakthrough:
dsRNA is the actual trigger of specific mRNA degradation, with the sequence of dsRNA determining which mRNA is degraded

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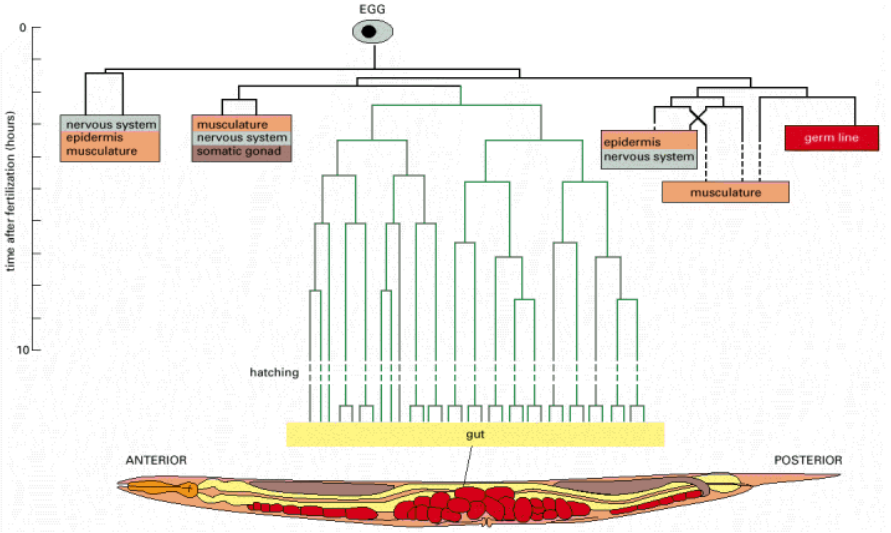
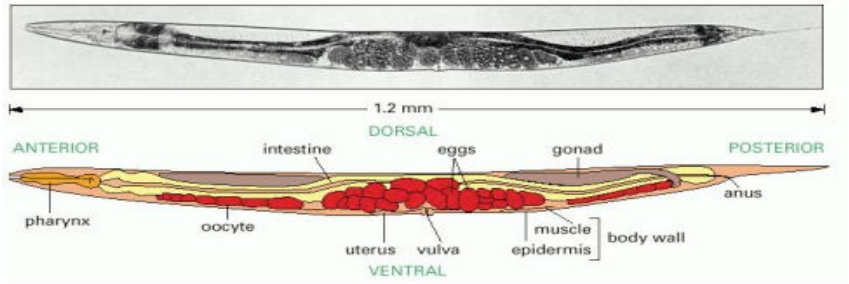
‡ *Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA*

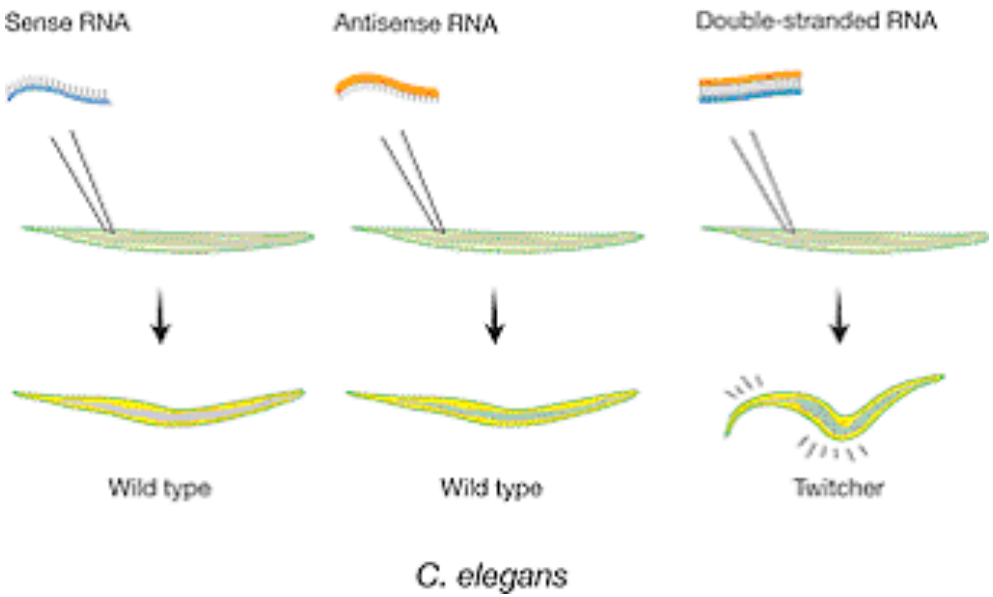
Experimental introduction of RNA into cells can be used in certain biological systems to interfere with the function of an endogenous gene[1,2]. Such effects have been proposed to result from a simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA transcripts. RNA interference has been used in the nematode *Caenorhabditis elegans* to manipulate gene expression[3,4]. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stochiometric interference with endogenous mRNA and suggesting that there could be a catalytic or amplification component in the interference process.





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

Conclusions of Fire&Mello's study:



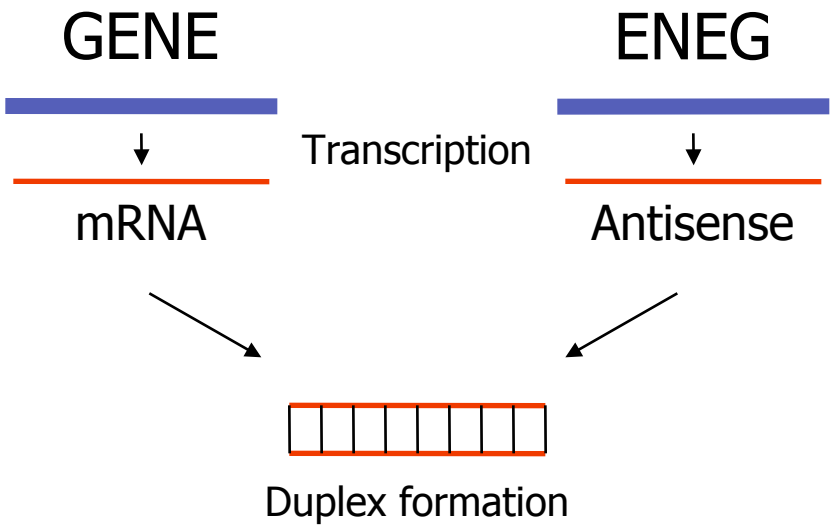
*Phenotypic effect after injection of single-stranded or double-stranded unc-22 RNA into the gonad of C. elegans. The **unc-22** gene encodes a myofilament protein. Decrease in unc-22 activity is known to produce severe twitching movements. Injected double-stranded RNA, but not single-stranded RNA, induced the twitching phenotype in the progeny.*

1) silencing was triggered efficiently by injected **dsRNA**, but weakly or not at all by sense or antisense single-stranded RNAs.
2) silencing was **specific** for an mRNA homologous to the dsRNA; other mRNAs were unaffected
3) the dsRNA had to correspond to the mature mRNA sequence; neither intron nor promoter sequences triggered a response. This indicated a **post-transcriptional**, presumably **cytoplasmic** mechanism
4) the targeted mRNA disappeared suggesting that it was **degraded**
5) only a few dsRNA molecules per cell were sufficient to accomplish full silencing. This indicated that the dsRNA was amplified and/or acted **catalytically** rather than stoichiometrically
6) the dsRNA effect could spread between tissues and even to the progeny, suggesting a **transmission** of the effect between cells

Short history of post-transcriptional gene silencing

- **1962, Singer, Jones, Nirenberg**
  Translation of mRNA can be blocked by complementary (**antisense**) RNA
- **1990, Jorgensen**
  Introduction of transgenes homologous to endogenous genes often results in plants with both genes suppressed (**co-suppression**)
- **1995, Guo and Kemphues**
  Injection of either antisense or sense RNAs in the germline of C.elegans is equally effective at silencing homologous target genes
- **1998, Mello and Fire**
  Combination of sense and antisense RNA (=**dsRNA**) is 10 times more effective than ssRNA

Antisense RNA



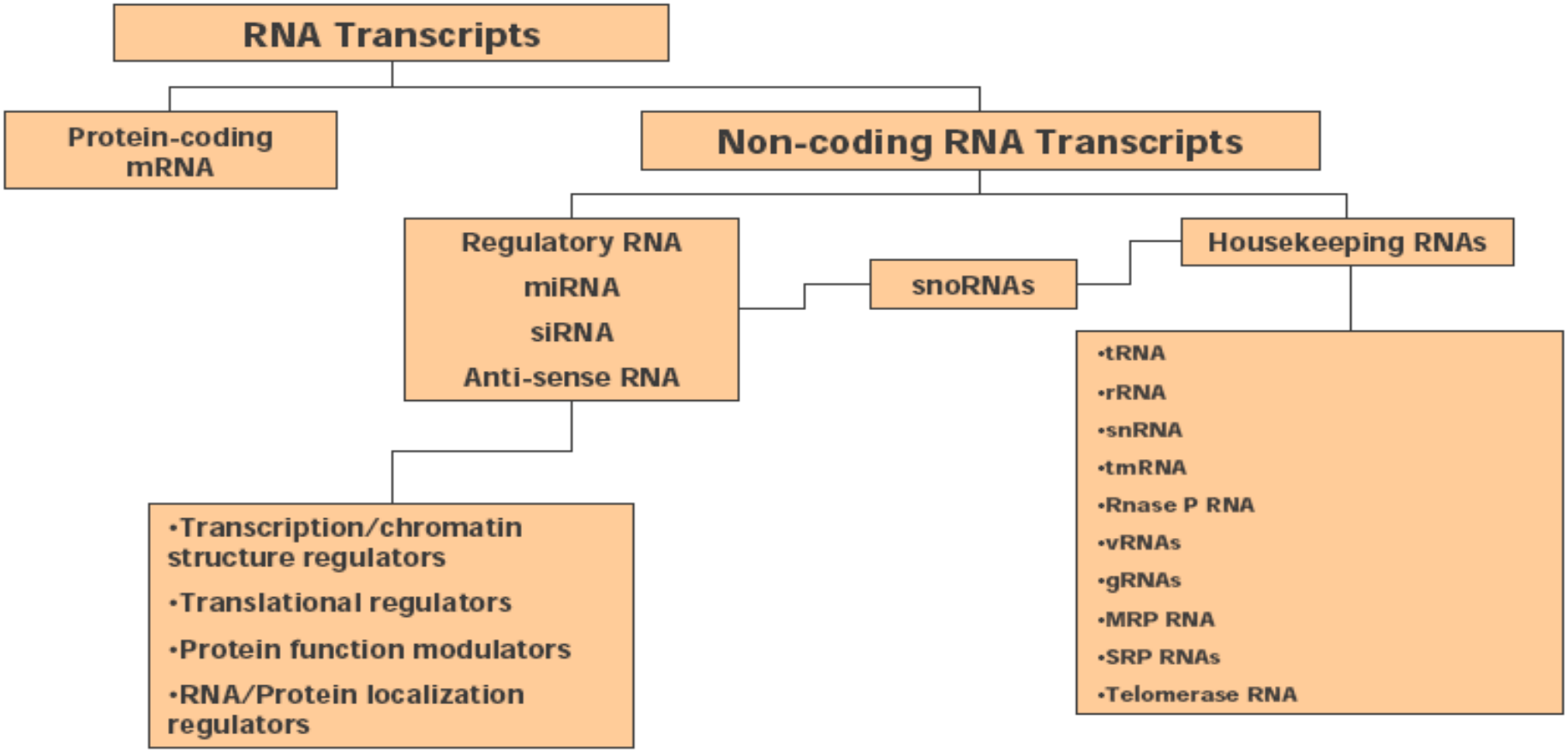
Right: Flower of a tobacco plant carrying a transgene whose transcript is antisense to one of the mRNAs needed for normal flower pigmentation. Left: Flower of another transgenic plant that failed to have its normal pigmentation altered.
(van der Krol, et. al., from Nature 333:866, 1988.)

When the antisense RNA binds to the complementary mRNA, it forms a double-stranded RNA (dsRNA) complex that is similar to double-stranded DNA. The dsRNA complex does not allow normal translation to occur.

The exact mechanism by which translation is blocked is unknown. Several theories include:

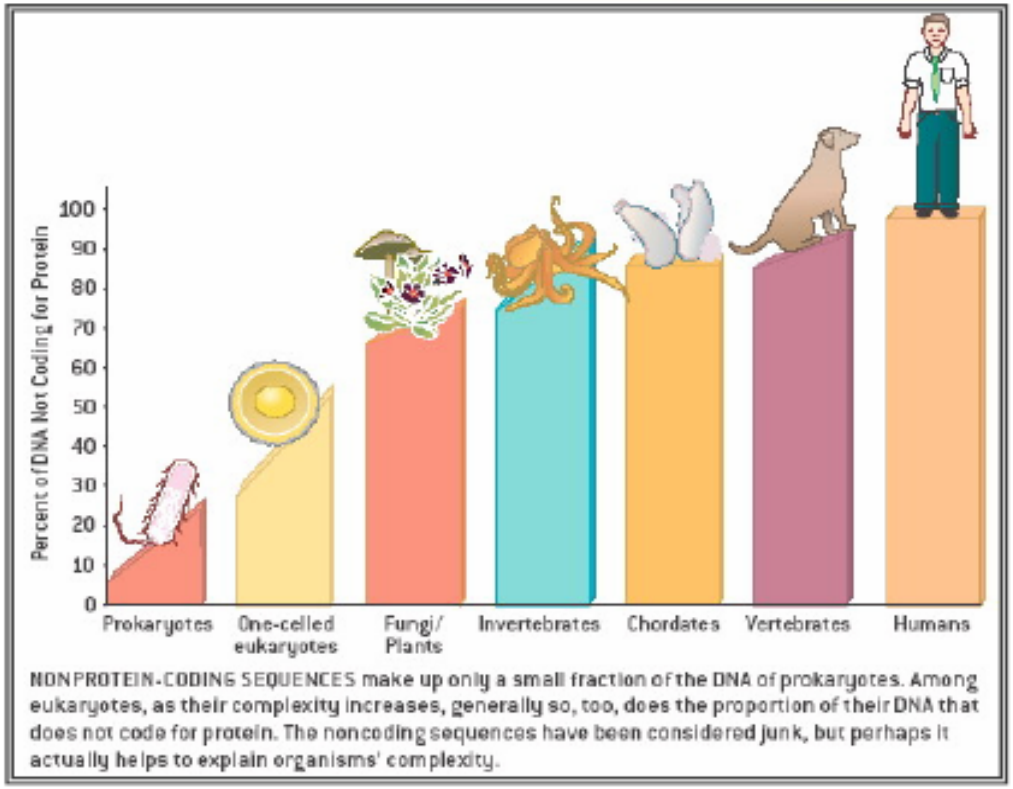
- that the dsRNA prevents ribosomes from binding to the sense RNA and translating (Kimball, Nov 2002)
- that the dsRNA cannot be transported from the nucleus to the cytosol, where translation occurs (Tritton, 1998)
- that dsRNA is susceptible to endoribonucleases that would otherwise not affect single stranded RNA, but degrade the dsRNA (Kimball, Nov 2002)

Non-Coding RNA: Formerly known as *"JUNK"*



NC-RNAs compose majority of transcription in complex genomes

Non-Coding RNA: A Key to Eukaryotic Complexity?



NONPROTEIN-CODING SEQUENCES make up only a small fraction of the DNA of prokaryotes. Among eukaryotes, as their complexity increases, generally so, too, does the proportion of their DNA that does not code for protein. The noncoding sequences have been considered junk, but perhaps it actually helps to explain organisms' complexity.

Organism	Percent of Transcriptional Output	
	Protein Coding RNA	Non Coding RNA
E.*coli*	84	16
S. *cerevisiae*	71	29
C.*elegans*	27	73
D. *melanogoster*	13	87
H. *sapiens*	2	98

Data suggesting role in diverse mechanisms:

- **RNAi**
- **Gene co-suppression**
- **Imprinting/DNA Methylation**

Possible roles in:

- **Cancer**
- **Neurological Disorders**
- **Host-pathogen interactions**

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

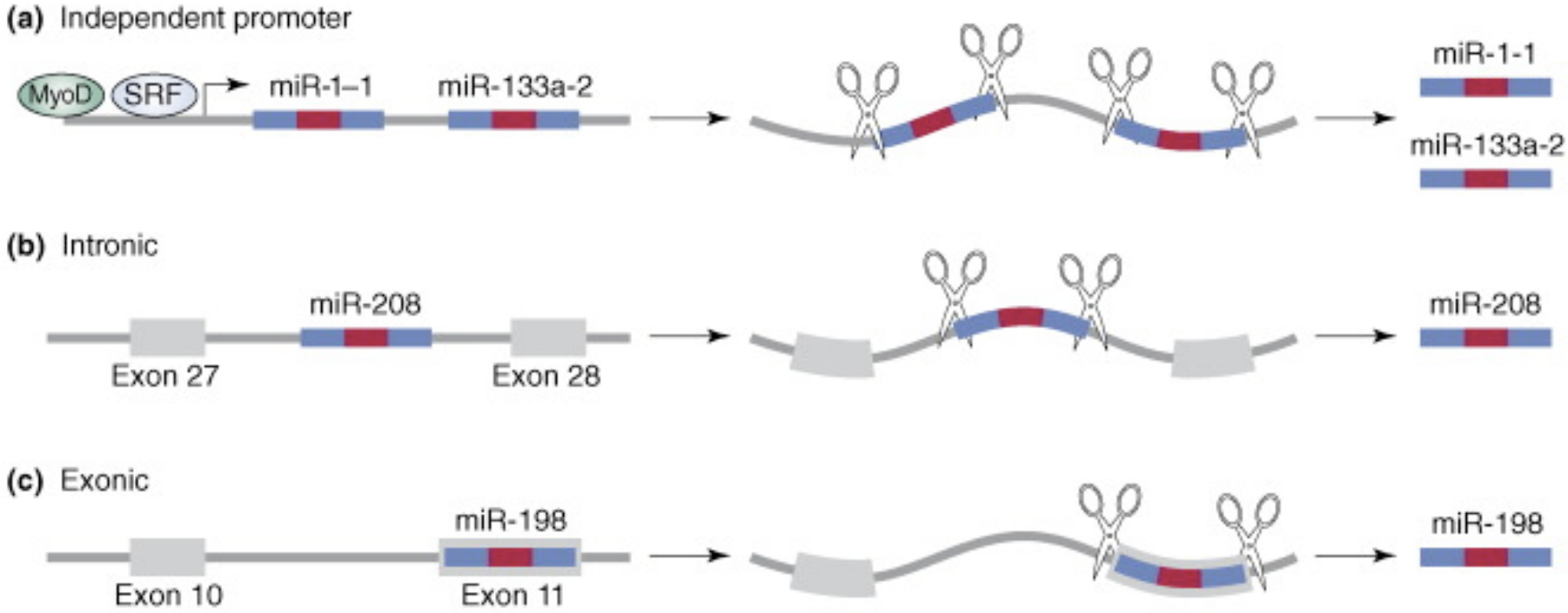
adapted from Ghildiyal & Zamore. 2009. Nat Rev Genet. 10:94

miRNA: microRNAs

microRNAs

- The number of confidently identified miRNA genes is continuously growing (2588 in humans)
- They are small RNA molecules of 21-22 nt
- Their derive from precursors of 70-100 nt
- They silence the expression of partially complementary RNA targets in animal cells, and of fully complementary RNA targets in plants, by binding the 3'UTR region.
- Each miRNA is predicted to regulate hundreds of targets

Genomic Organization of miRNA Genes

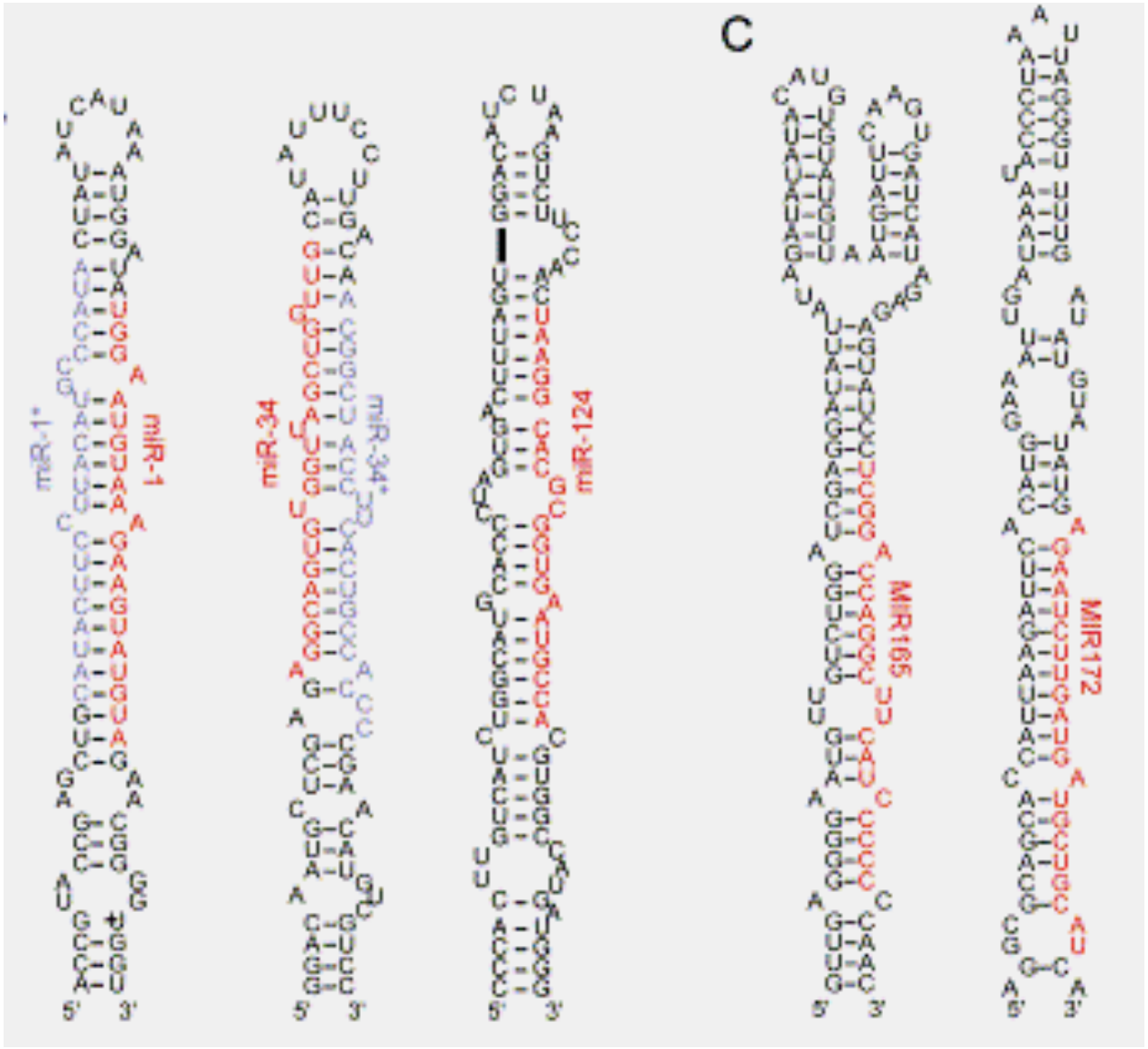


- Intronic miRNAs often in antisense direction, made from own promoter

- Exonic miRNAs - non-coding (or in alternatively spliced exons)

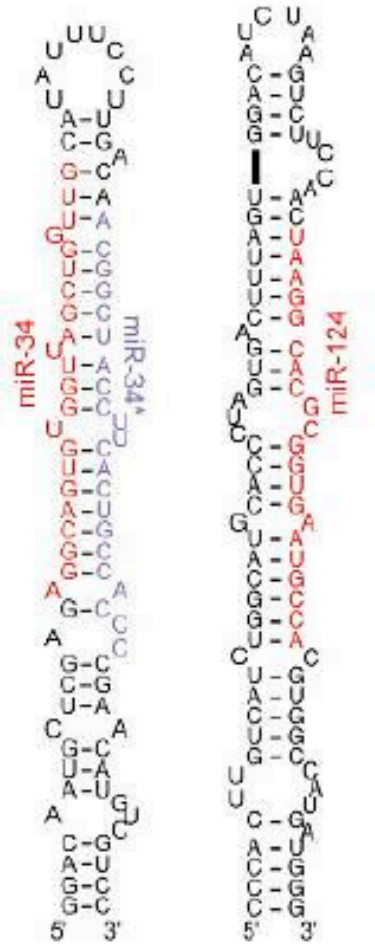
Zhao Y, Srivastava D, TIBS 32:189,2007

Precursor miRNA Products Form Stem Loop Structures

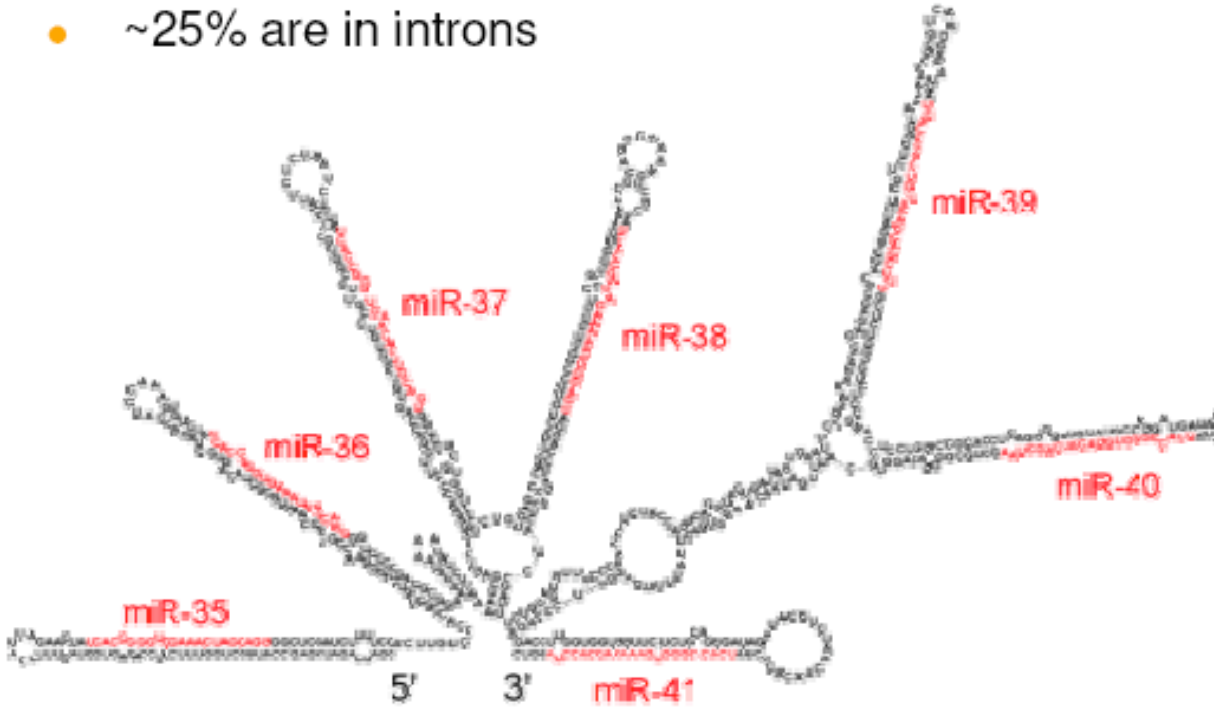


Genomic organization of miRNAs

miRNA Genes

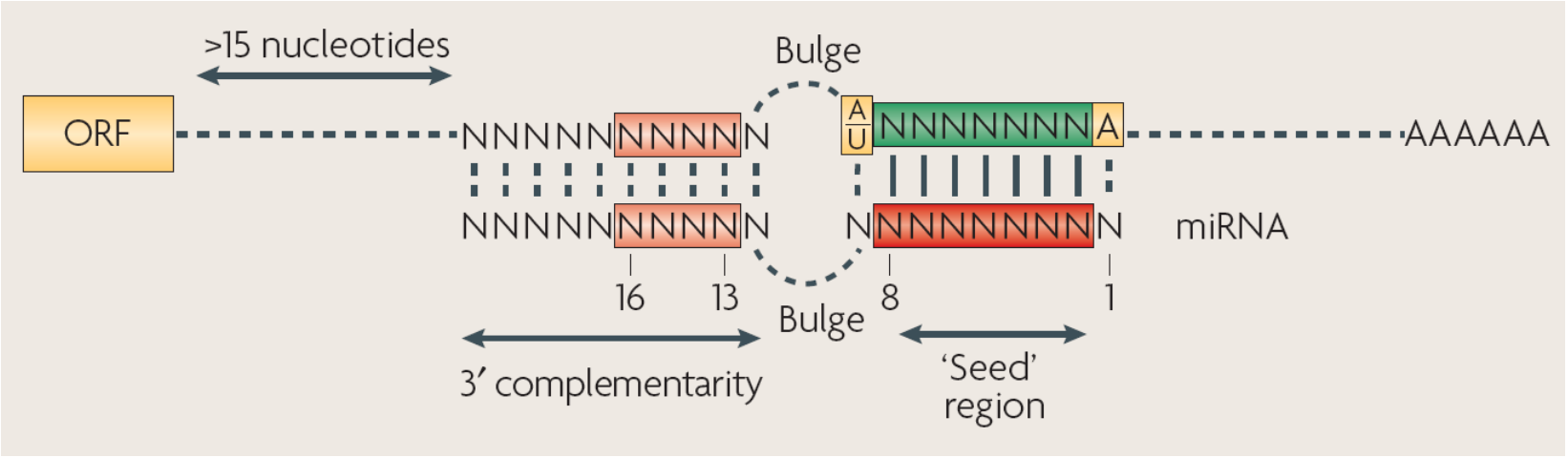


- ~60% of miRNAs are expressed independently
- ~15% of miRNAs are expressed in clusters
- ~25% are in introns



C. elegans

miRNA-mRNA interaction

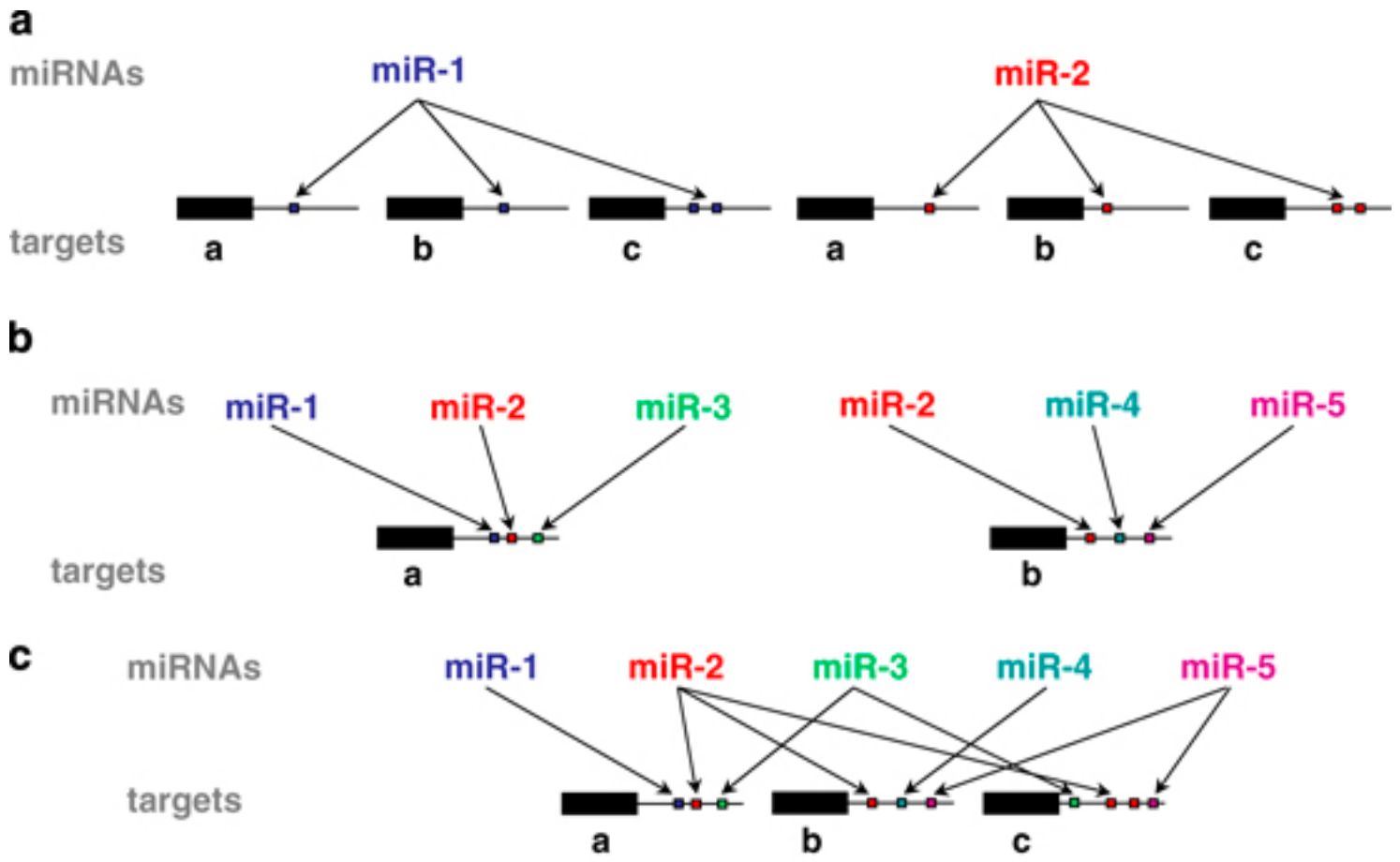


Filipowicz et al. 2008. Nat Rev Genet. 9:102

miRNA-target base pairing rules:

- perfect and contiguous base pairing of miRNA nucleotides 2 to 8
- bulges or mismatches must be present in the central region of the miRNA-mRNA duplex
- Other factors that can improve site efficacy include AU-rich content, and a position that is not too far away from the poly-A tail or the termination codon

miRNAs target multiple genes and genes are targeted by multiple miRNAs



(**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.

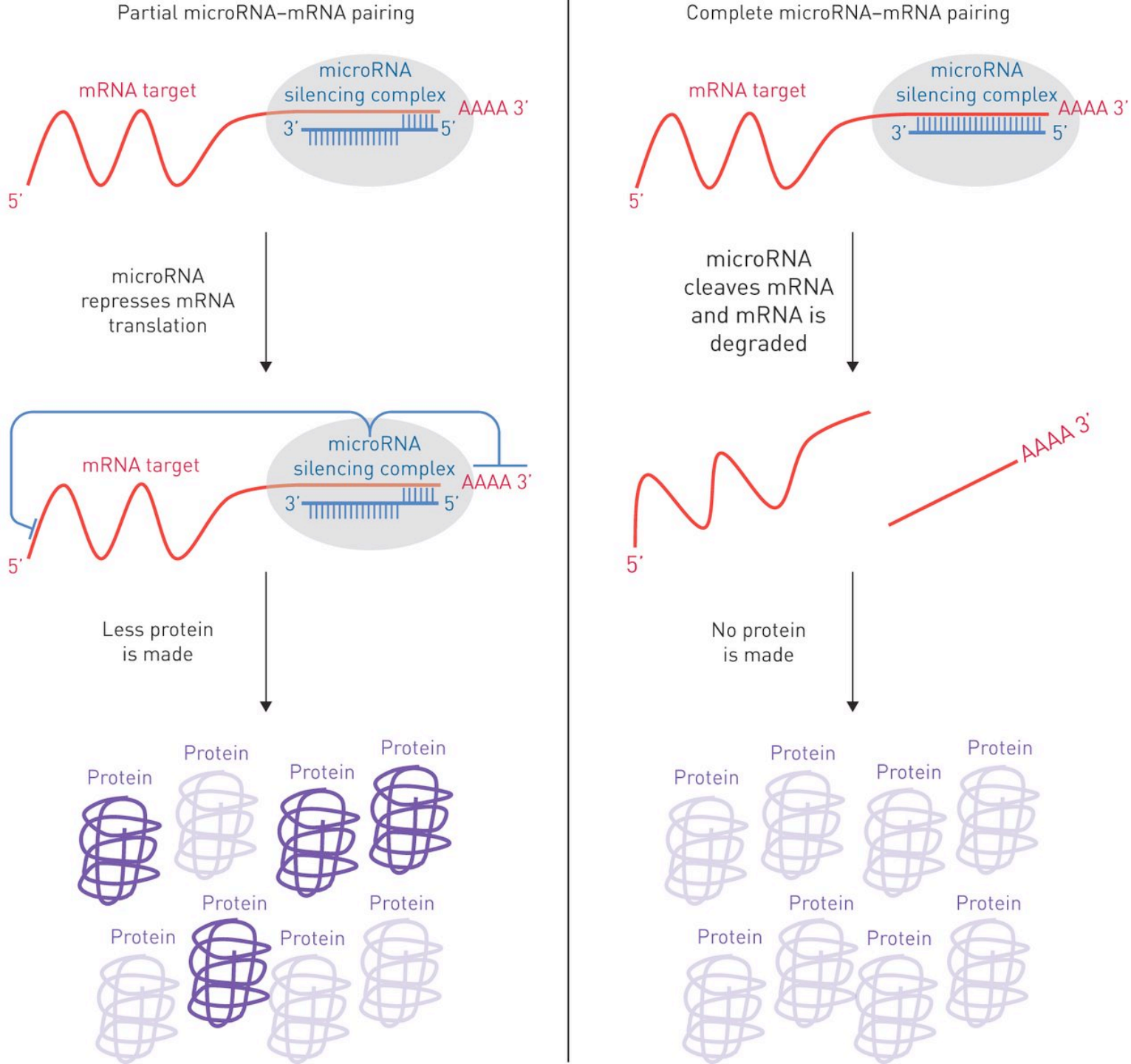
miRNA Modes of Function

- The basic function of miRNAs is the reduction of protein levels of targeted genes

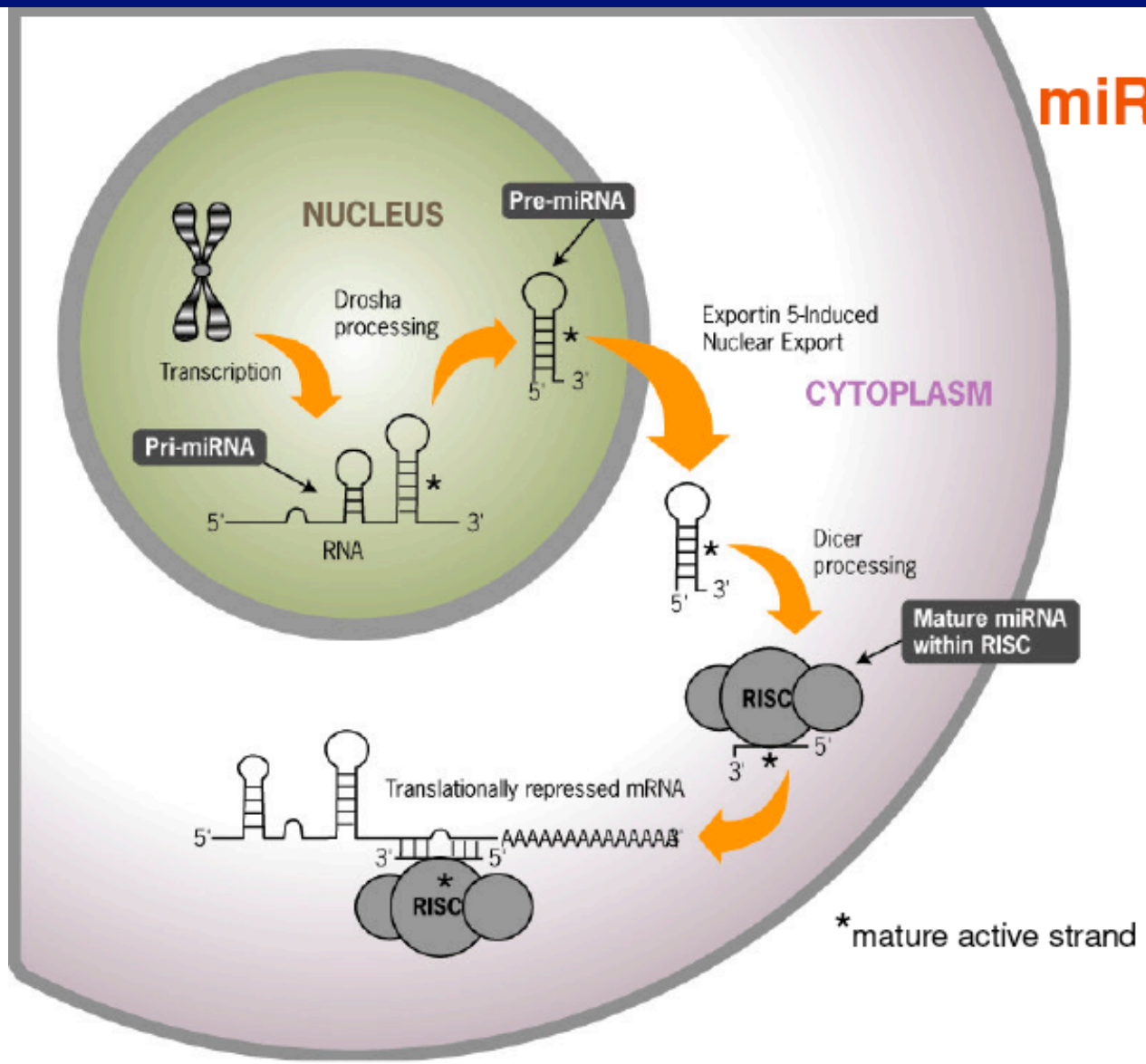
miRNAs work through two main mechanisms:

1. **mRNA CLEAVAGE**, by PERFECT COMPLEMENTARITY (plant and animal miRNAs)
2. **TRANSLATIONAL REPRESSION** by IMPERFECT COMPLEMENTARITY (animal miRNAs)

- Each miRNA appears to regulate the translation of multiple genes, and many genes appear to be regulated by multiple miRNAs.


Partial microRNA–mRNA pairing
mRNA target
microRNA silencing complex
AAAA 3′
3′
5′
5′
microRNA represses mRNA translation
mRNA target
microRNA silencing complex
AAAA 3′
3′
5′
5′
Less protein is made
Protein
Protein
Protein
Protein
Protein
Protein
Protein
Protein
Complete microRNA–mRNA pairing
mRNA target
microRNA silencing complex
AAAA 3′
3′
5′
5′
microRNA cleaves mRNA and mRNA is degraded
AAAA 3′
5′
No protein is made
Protein
Protein
Protein
Protein
Protein
Protein
Protein
Protein

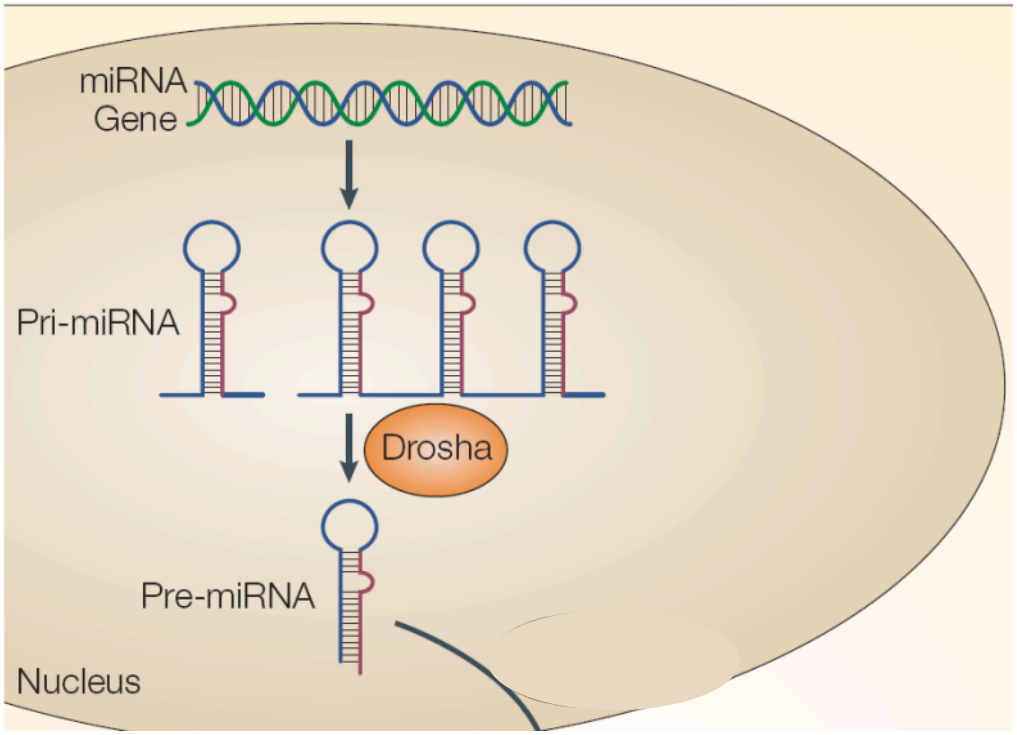
Biogenesis of miRNAs



miRNA Expressio

1. Transcription
2. Hairpin release in the nucleus
3. Export to cytoplasm
4. Dicer processing
5. Strand selection by RISC
6. Translational repression

Biogenesis of miRNAs



MiRNAs are encoded by the genome.

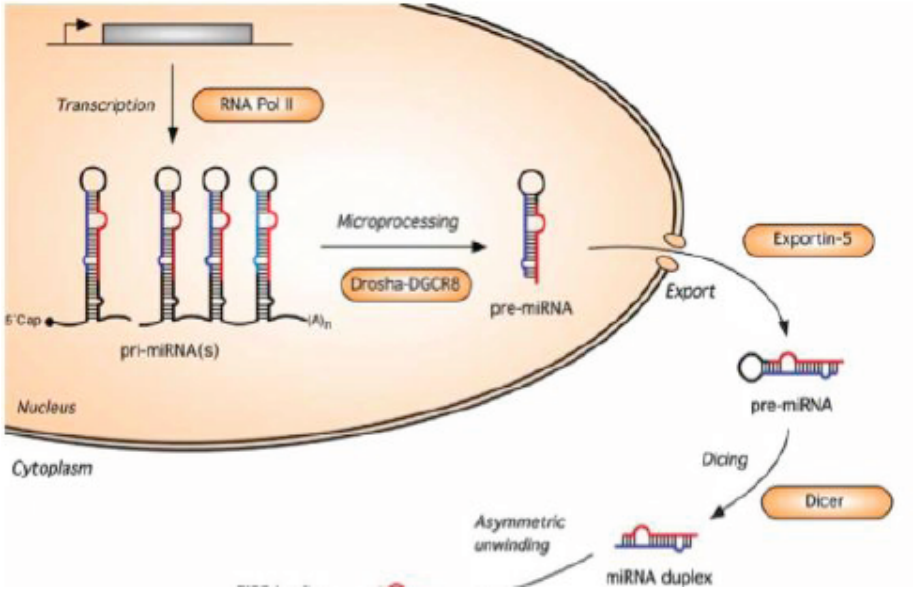
miRNAs derive from precursor transcripts, called pri-miRNA, typically transcribed by RNA polymerase II.

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.

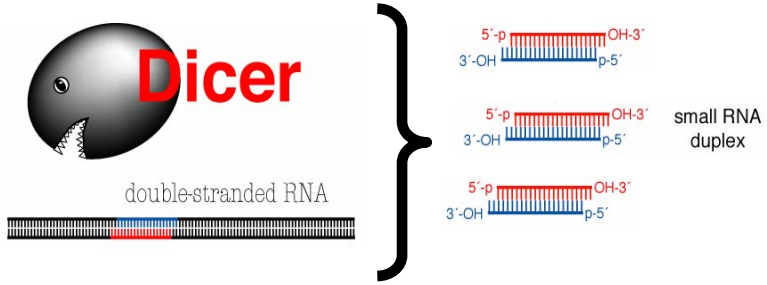
The dsRNA-specific ribonuclease **DROSHA** digests the pri-miRNA in the nucleus into single Hairpins, the **pre-miRNAs**.

Biogenesis of miRNAs

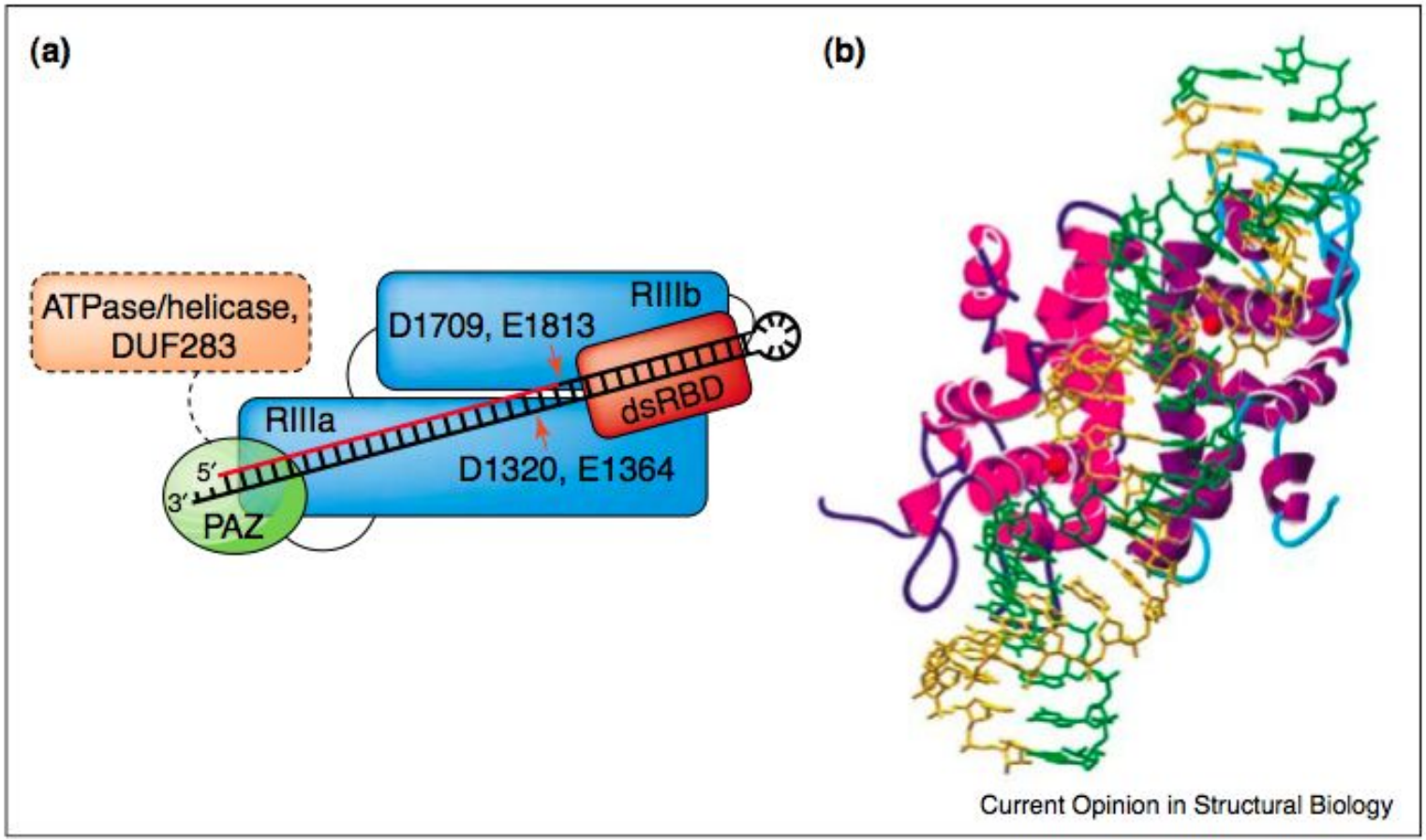


- **NUCLEAR EXPORT-** EXPORTIN-5 (Exp5) translocates the maure miRNAs from the nucleus to the cytoplasm. Exp5 directly binds the pri-miRNA correctly processed.

- **MATURATION-**DICER is a Rnase III family member. When the pre-miRNA is in the cytoplasm, DICER cuts it into:
- 21-25 nt fragment
- 2 nt 3' symmentrical overhangs, containing 5' phosphate groups.

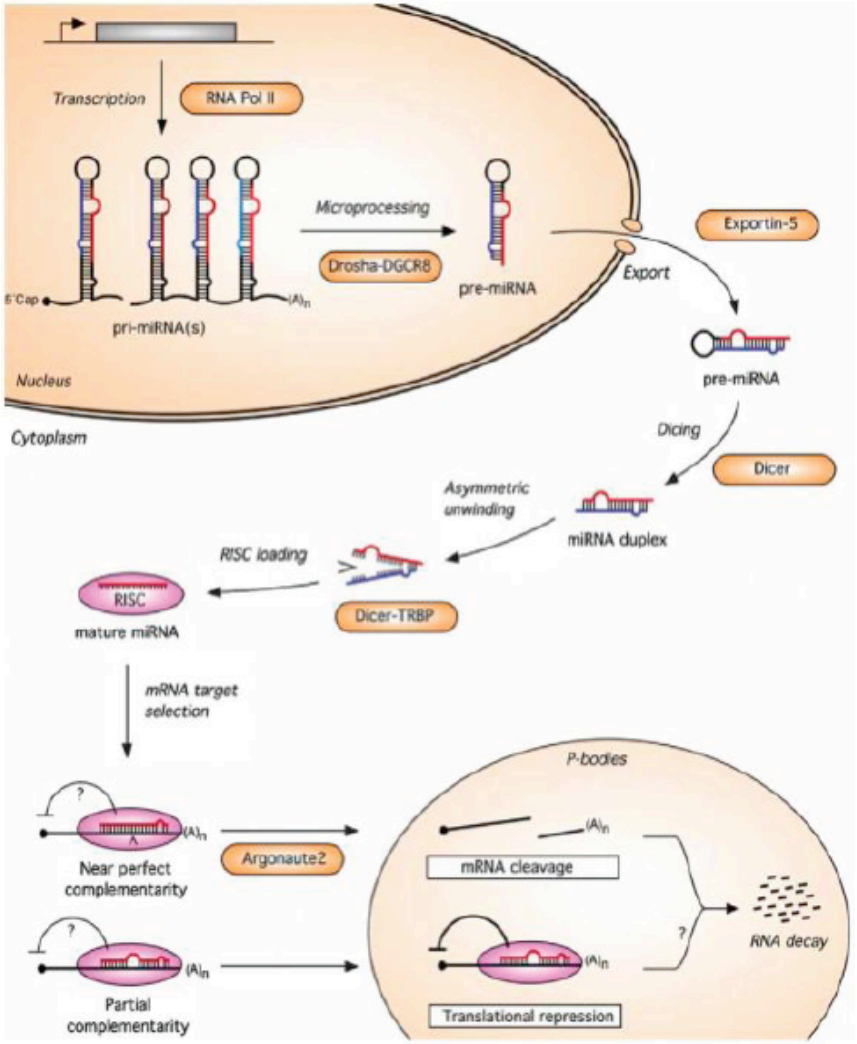


Dicer Structure & Function



Filipowicz, Curr. Op. Structural Biology 15: 331-341 (2005)

Biogenesis of miRNAs



- **STRAND SELECTION BY THE RISC COMPLEX-** The ds-miRNAs generated by DICER must separate their two strands; therefore, the mature ss-miRNA associate the RISC complex. The strand selection is based on the stability of the ds-miRNA ends.

- **RNA-INDUCED SILENCING COMPLEX (RISC)-** Its activities are:

  HELICASE,

  ENDONUCLEASE & ESONUCLEASE

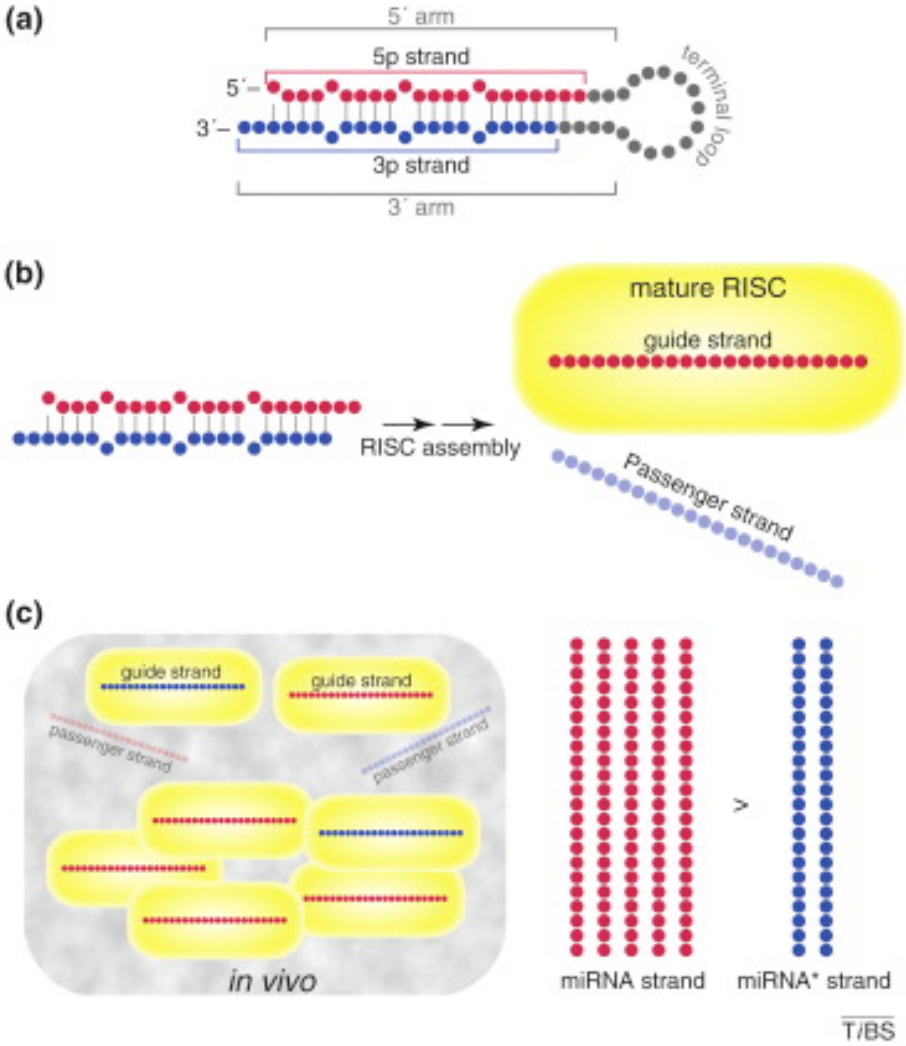
  "HOMOLOGY SEEKING"/RNA BINDING

  Key component of the RISC is the protein **ARGONAUTE**

- **GENE SILENCING**

miRNAs bound to RISC are able to search for targets by its **seed sequence and** guide postrancriptional gene silencing.

Nomenclature for small RNA strands

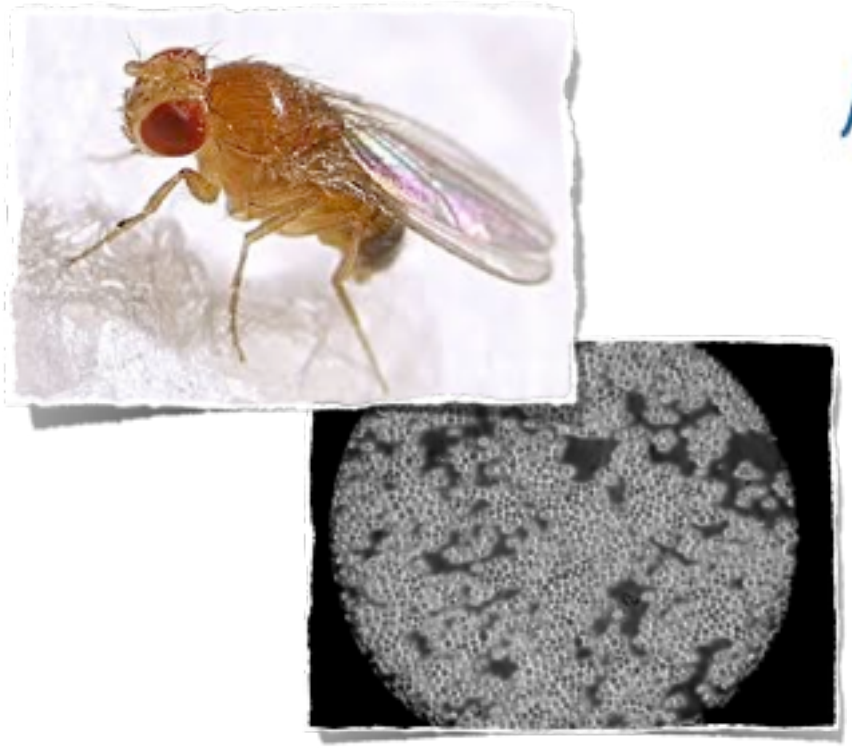


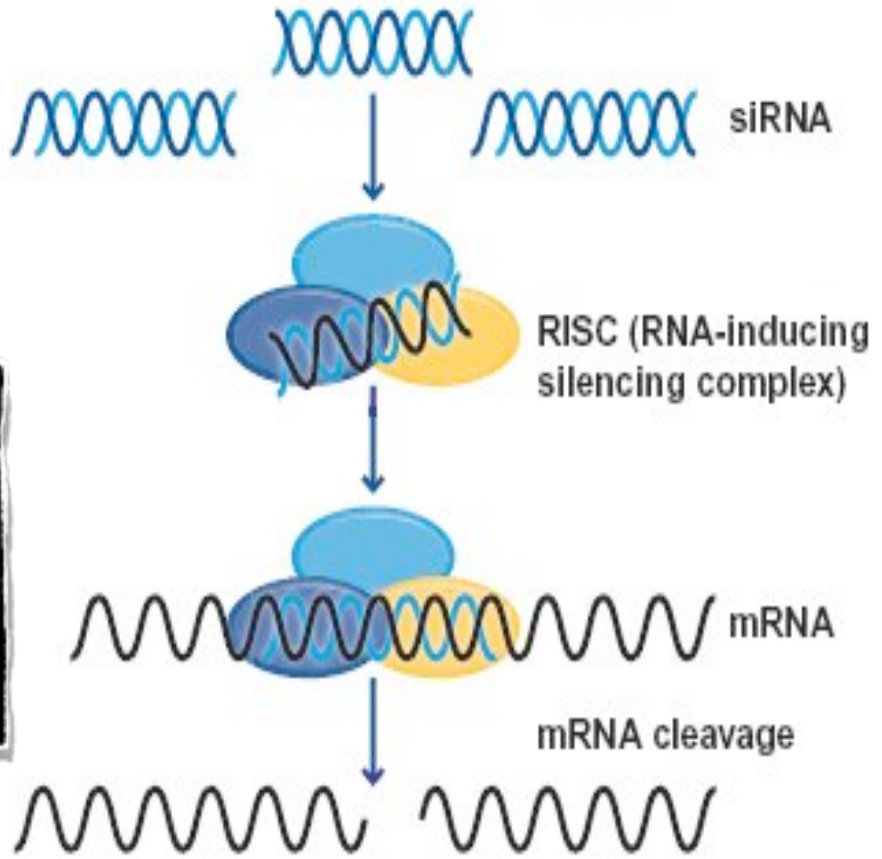
5p and 3p strands: 5p and 3p designate the strands derived from the 5′ arm and 3′ arm of a pre-miRNA, respectively.

Guide and passenger strands: the guide strand is retained in the mature RISC whereas the passenger strand is discarded upon unwinding.

miRNA and miRNA* strands: the miRNA strand is the more abundant (and thereby more frequently cloned) strand overall in vivo, whereas the miRNA* strand is the less abundant strand. Note that a passenger strand is quickly degraded as soon as it is discarded from pre-RISC whereas a guide strand is protected from nucleases in the mature RISC. Consequently, the strand that is more likely to serve as the guide strand tends to accumulate and therefore become the 'miRNA strand'.

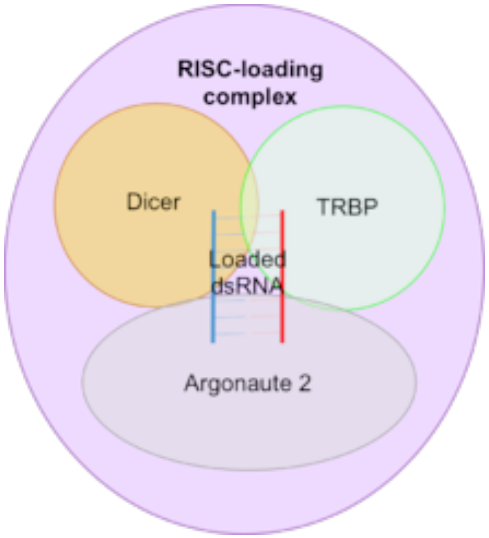
The RISC COMPLEX was discovered in Drosophila cultured cells





The molecular machinery responsible for RNAi involves a large complex, called RISC (RNA-induced silencing complex), which is targeted to the mRNA via the antisense RNA. The mRNA is cleaved and subsequently degraded.

RISC EFFECTOR COMPLEX



RISC is a large (~500 kDa) RNA multiprotein complex

- RISC contains a member of the Argonaute protein family:

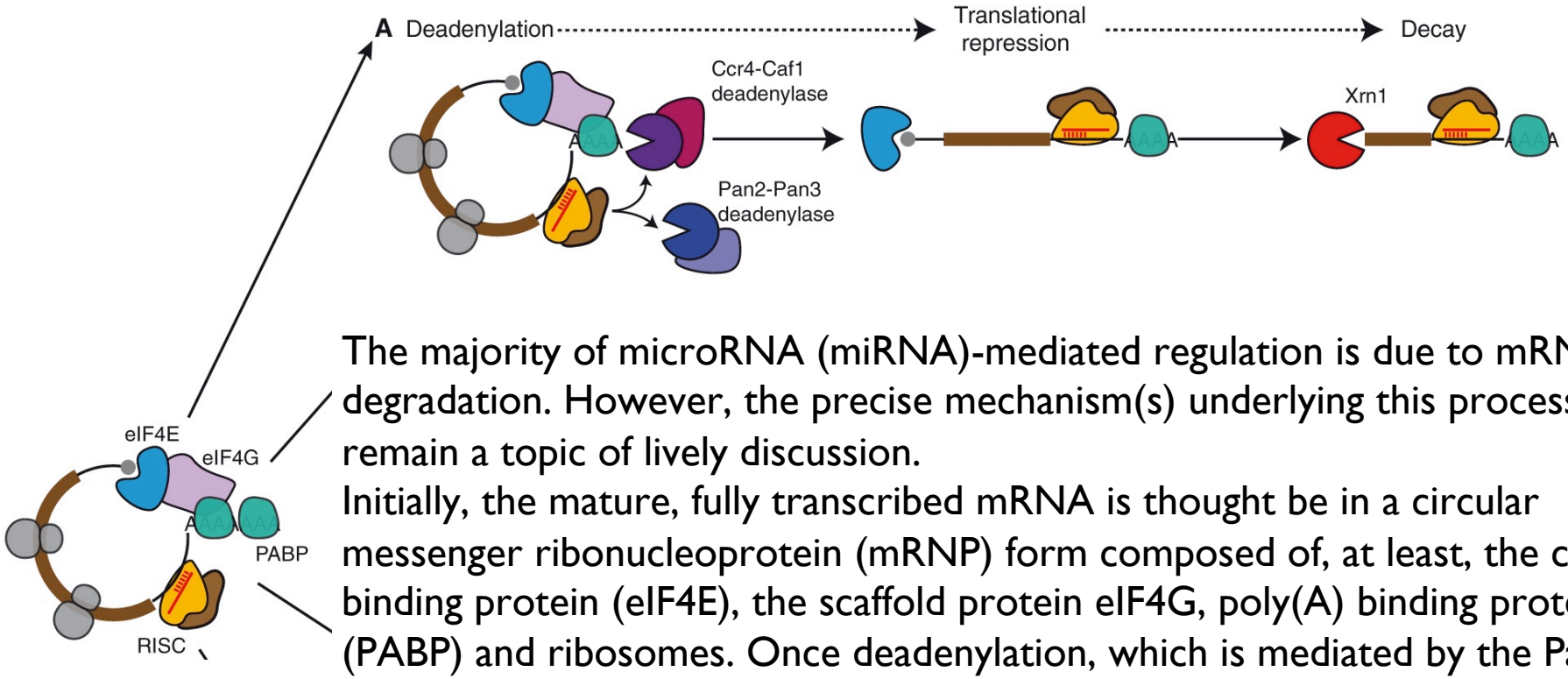
S. pombe: 1 Argonaute
H. sapiens: 8 Argonautes
A. thaliana: 10 Argonautes
C. elegans: 27 Argonautes

AGO proteins are evolutionarily conserved and they are ubiquitously expressed

Number of Argonaute family genes in different species

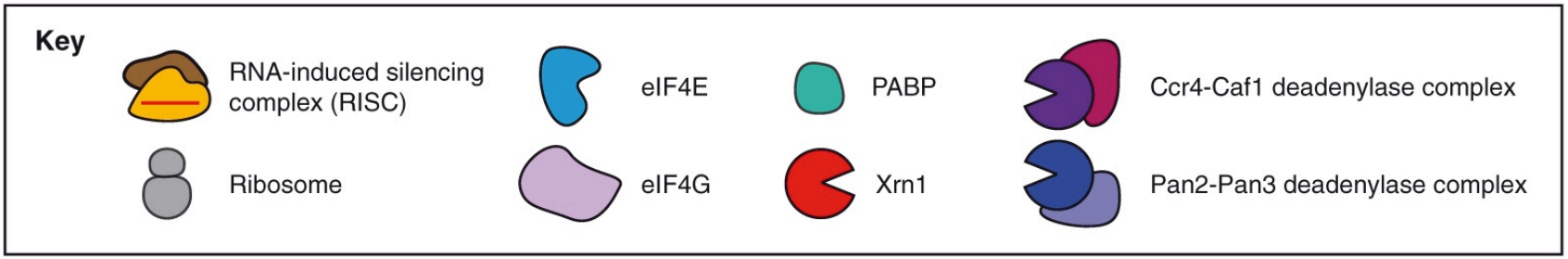
Species	Number of genes
Homo sapiens	8
Rattus norvegicus	8
Mus musculus	8
Drosophila melanogaster	5
Caenorhabditis elegans	27
Arabidopsis thaliana	10
Schizosaccharomyces pombe	1
Neurospora crassa	2

Meccanismo di azione dei miRNA

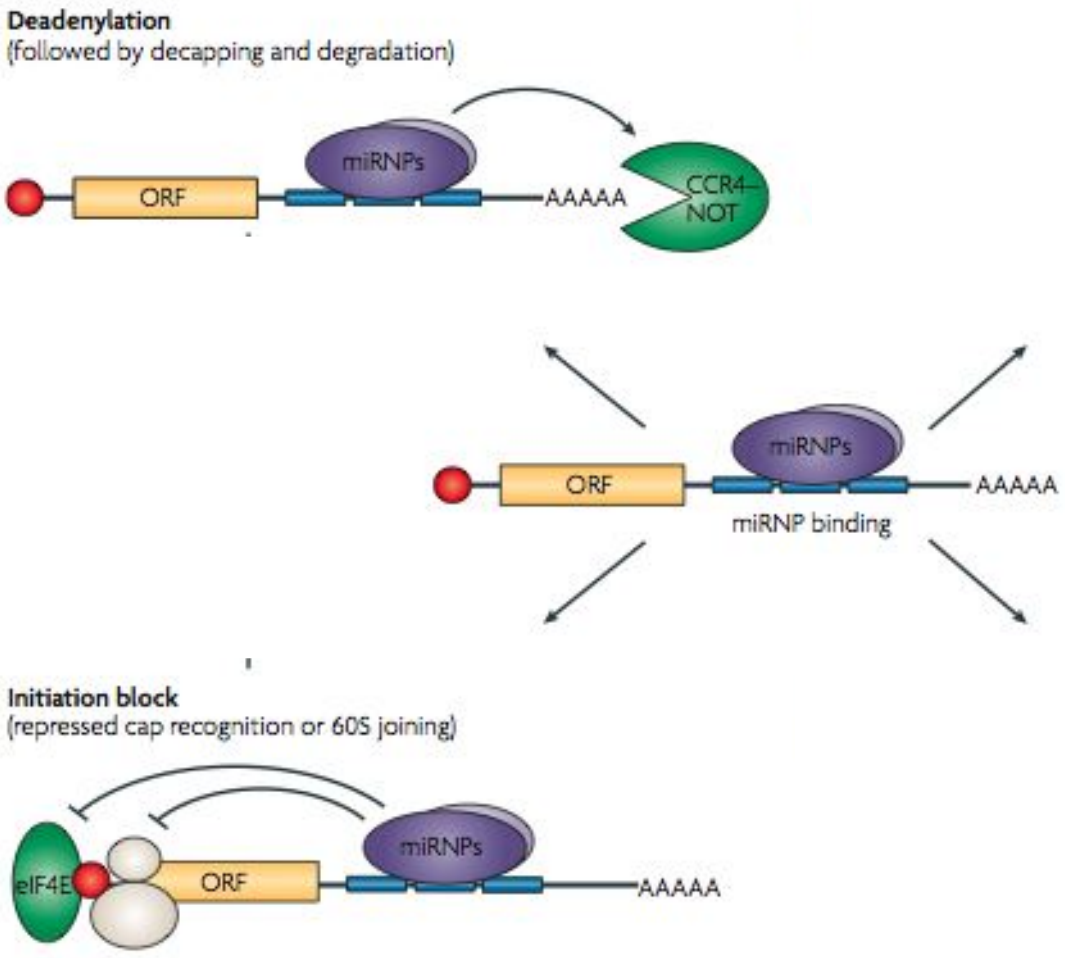


The majority of microRNA (miRNA)-mediated regulation is due to mRNA degradation. However, the precise mechanism(s) underlying this process remain a topic of lively discussion.

Initially, the mature, fully transcribed mRNA is thought be in a circular messenger ribonucleoprotein (mRNP) form composed of, at least, the cap-binding protein (eIF4E), the scaffold protein eIF4G, poly(A) binding protein (PABP) and ribosomes. Once deadenylation, which is mediated by the Pan2-Pan3 and Ccr4-Caf1 deadenylase complexes, has occurred, mRNAs are decapped by the decapping enzyme (not shown) and subsequently degraded by the 5'_ exonuclease Xrn1.

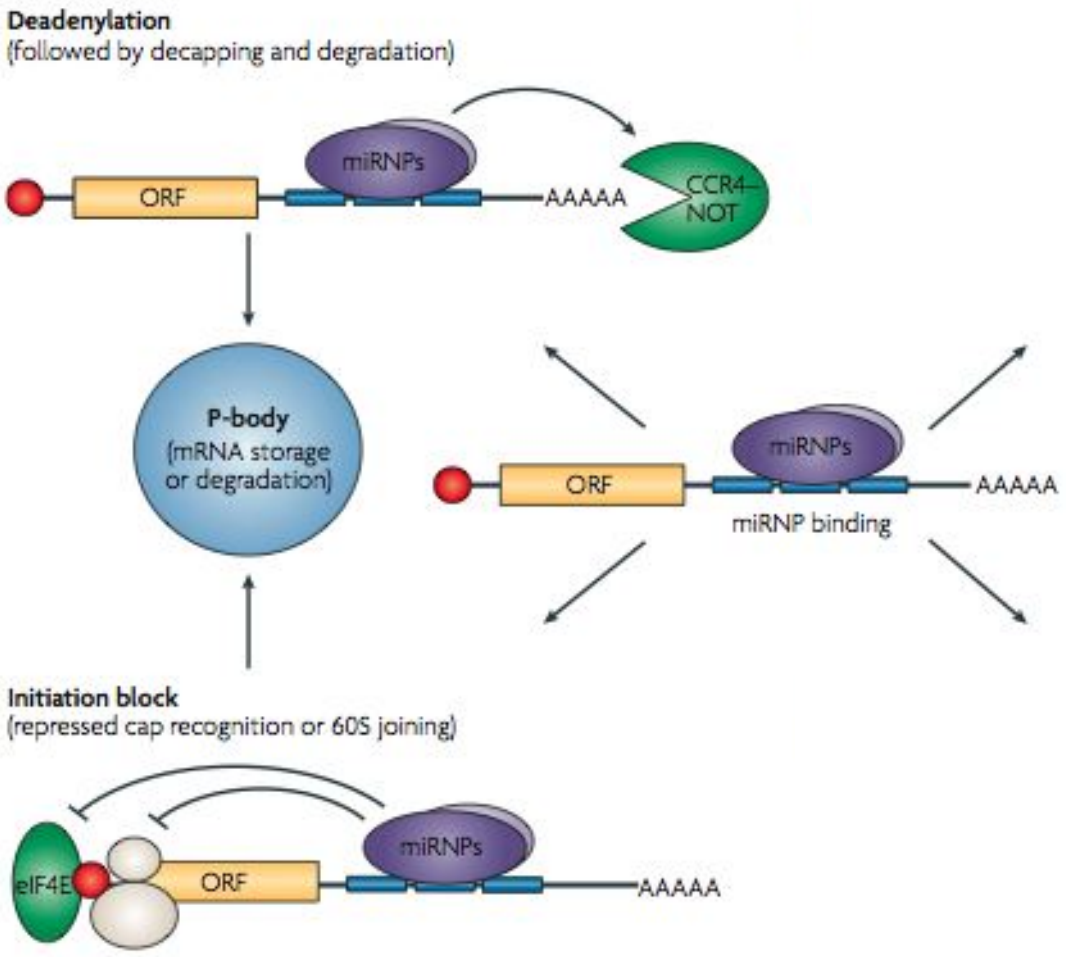


Mechanisms of Translational Regulation by miRNP Complexes



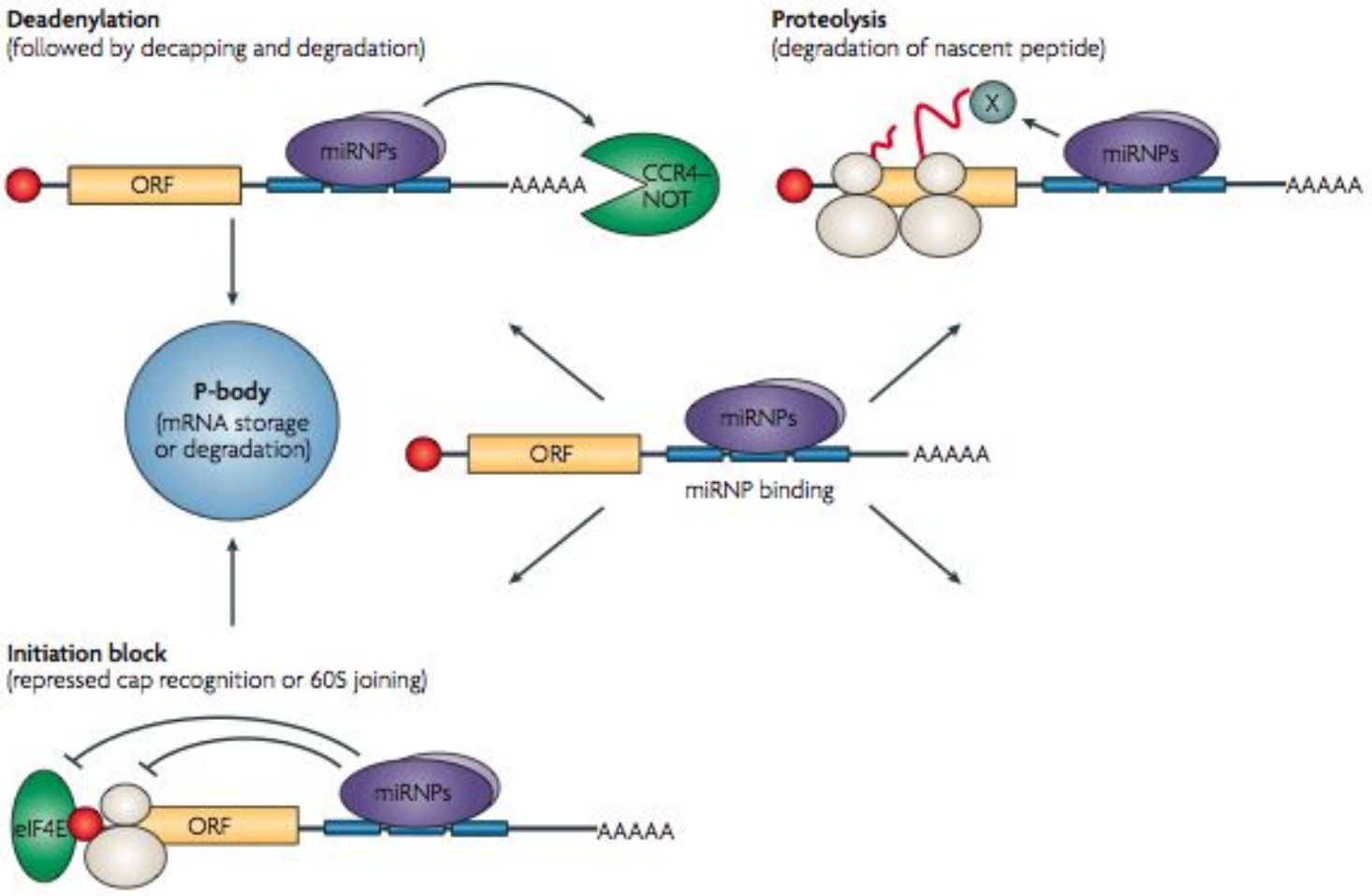
Filipowicz (2008) Nature Review Genetics 9:102-112.

Mechanisms of Translational Regulation by miRNP Complexes



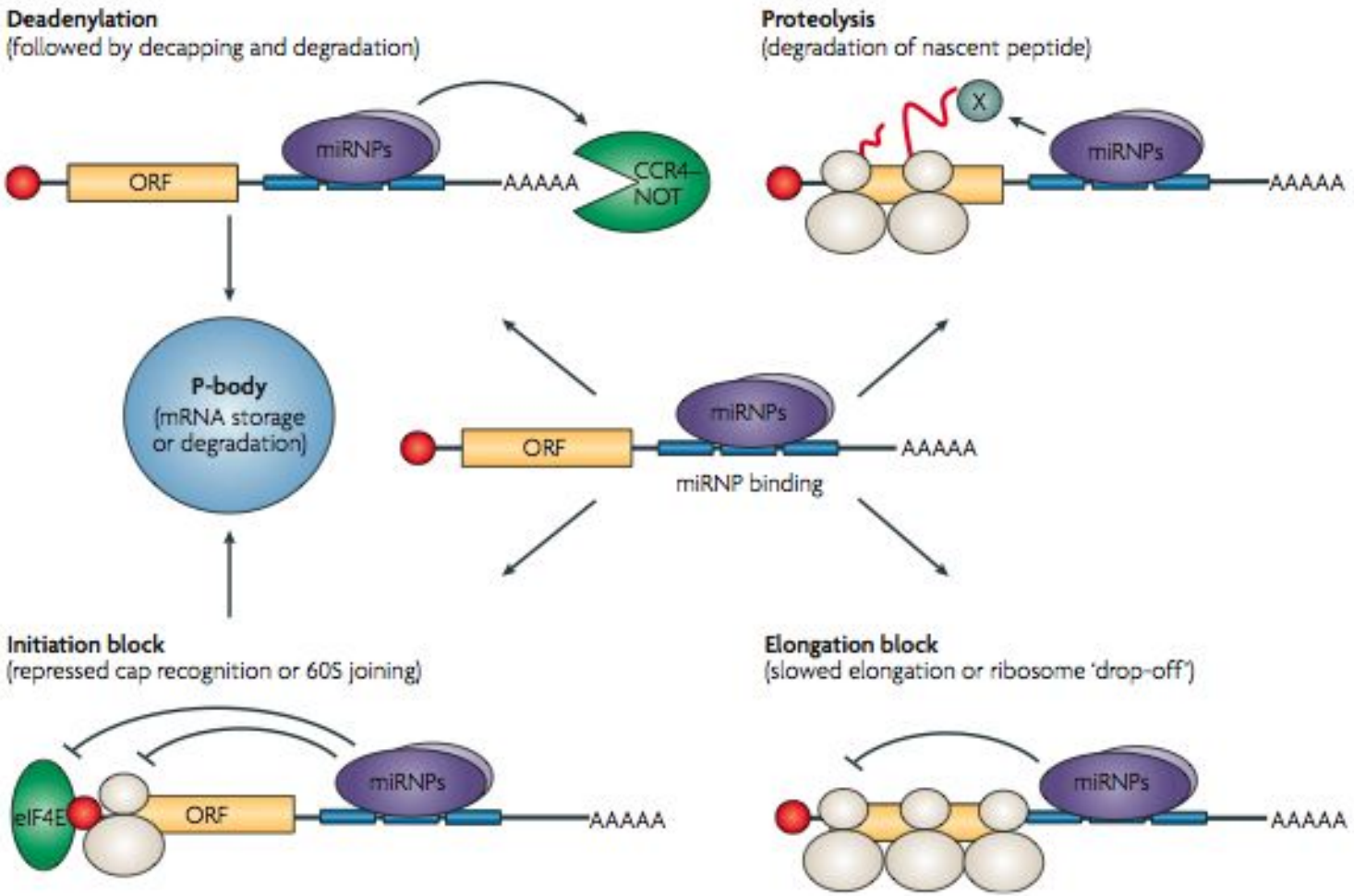
Filipowicz (2008) Nature Review Genetics 9:102-112.

Mechanisms of Translational Regulation by miRNP Complexes



Filipowicz (2008) Nature Review Genetics 9:102-112.

Mechanisms of Translational Regulation by miRNP Complexes



Filipowicz (2008) Nature Review Genetics 9:102-112.

Consegunze dell'azione dei miRNAs

- Taglio diretto dell'mRNA bersaglio (endonucleotidic cleavage), slicer activity e seguente degradazione dell'RNA
- Attivazione di un'attivita' di decapping dell'RNA (mediata da Dcp1 e Dcp2) e seguente degradazione 5'-3'(interazione con Xrn1p e P bodies)
- Riduzione della traduzione dell'mRNA
- Repressione dell'inizio della traduzione, aumentando la quantita' di mRNA non associato a ribosomi bensi associato ai P bodies.

Where does the action take place ?

P bodies:

specific cytoplasmic foci, sites of mRNA decapping and degradation

≥ 35 proteins known, conserved from yeast to man

Also in p bodies:

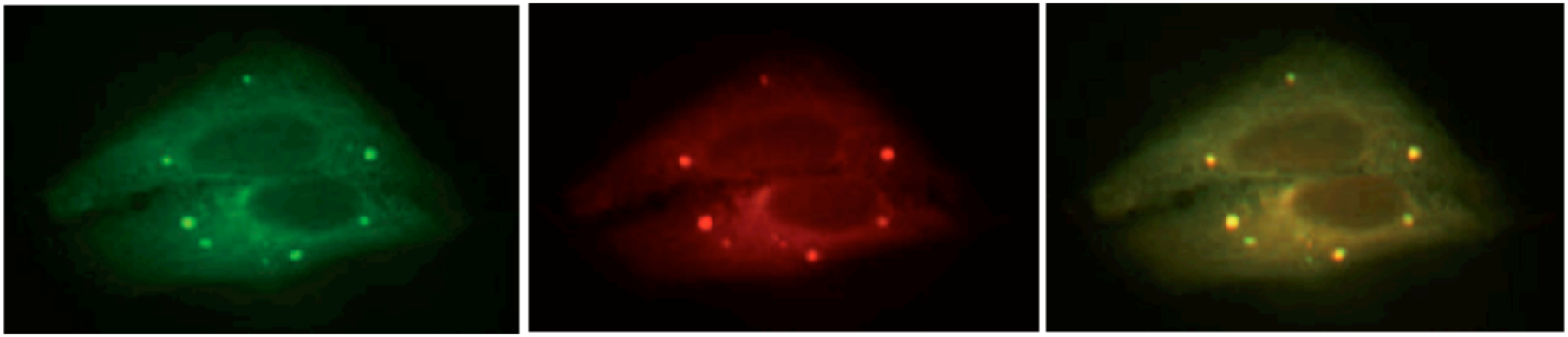
concentration of Argonaute proteins

mRNA targets of miRNAs

Presumable Function:

degradation of mRNAs

storage of mRNAs



Flag-Dcp1a **Myc-Ago2** **Merge**

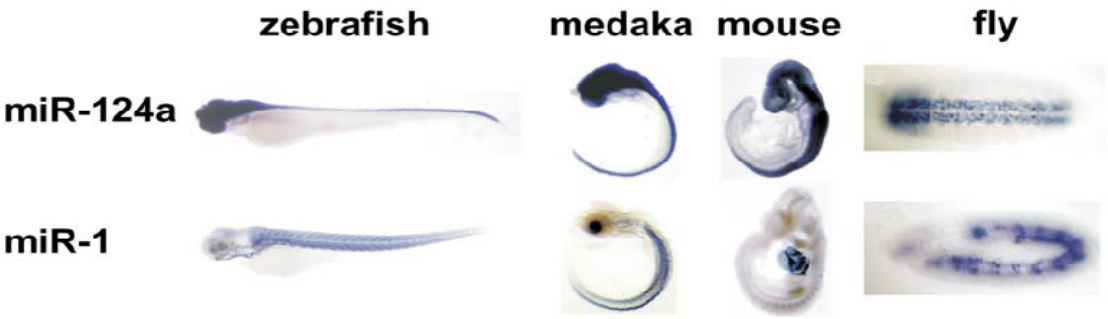
Liu et al., 2005 NatCellBiol 7:719-723

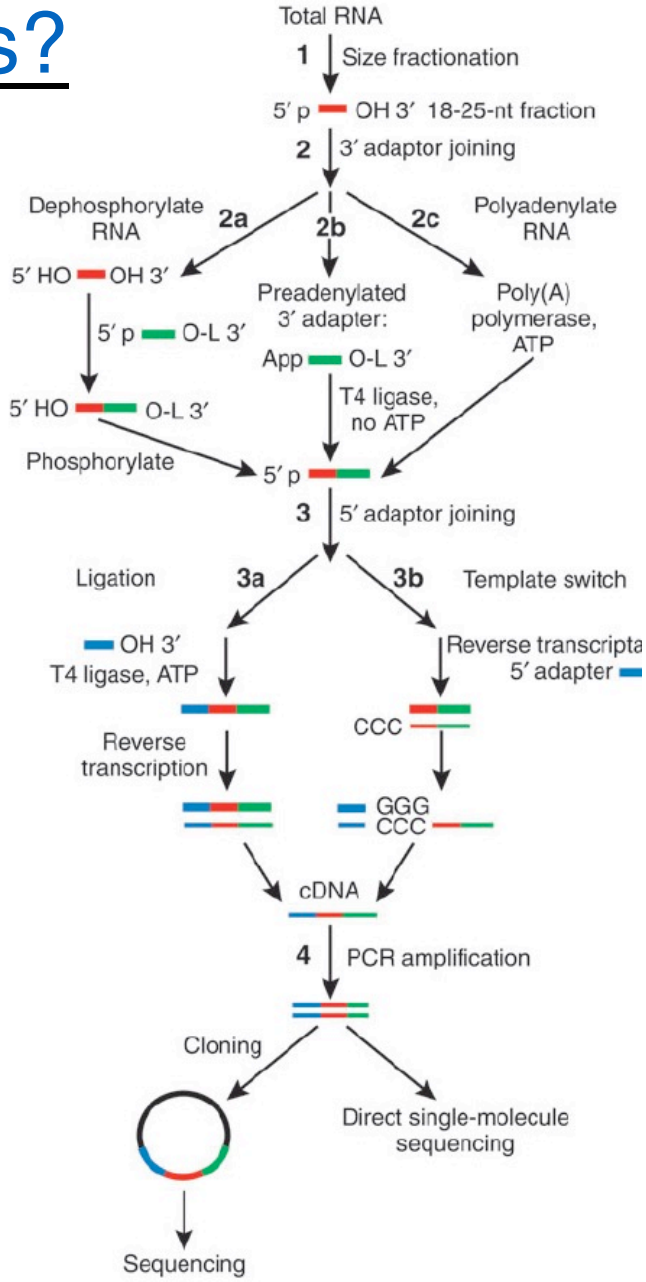
① How do we find microRNA genes?

② Given a microRNA gene, how do we find its targets?

How do we find microRNA genes?

- Biological approach
  - Small-RNA-cloning to identify new small RNAs
- Most microRNA genes are tissue-specific





miR-124a is restricted to the brain and spinal cord in fish and mouse or to the ventral nerve cord in fly

miR-1 is restricted to the muscles and the heart in mouse

miRNA Targets

•In plants, the identification of mRNA targets is straight forward because most miRNAs and their target mRNAs have exact or nearly exact complementarity.

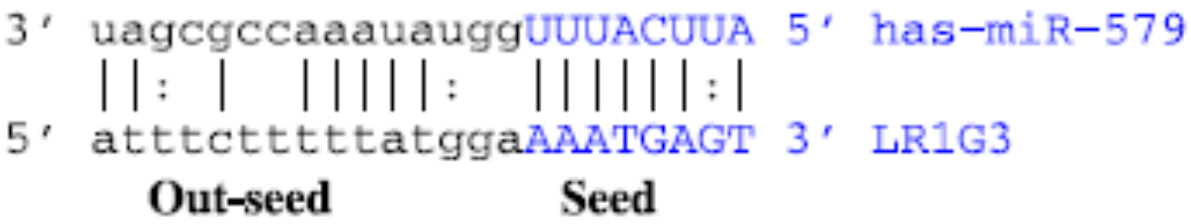
•In animals, the tendency of miRNAs to bind their mRNA targets with imperfect sequence homology poses considerable challenges with target prediction.

• Several computational approaches have been developed to facilitate experimental design and predicting miRNA targets.

•Computational target prediction identifies potential binding sites according to base-pairing rules and across species conservation conditions.

MicroRNA Targets Prediction and Analysis

Target Identification



•The duplex for miRNA hsa-miR-579 and its target LRIG3 is partitioned into two parts, the seed part and the out-seed part

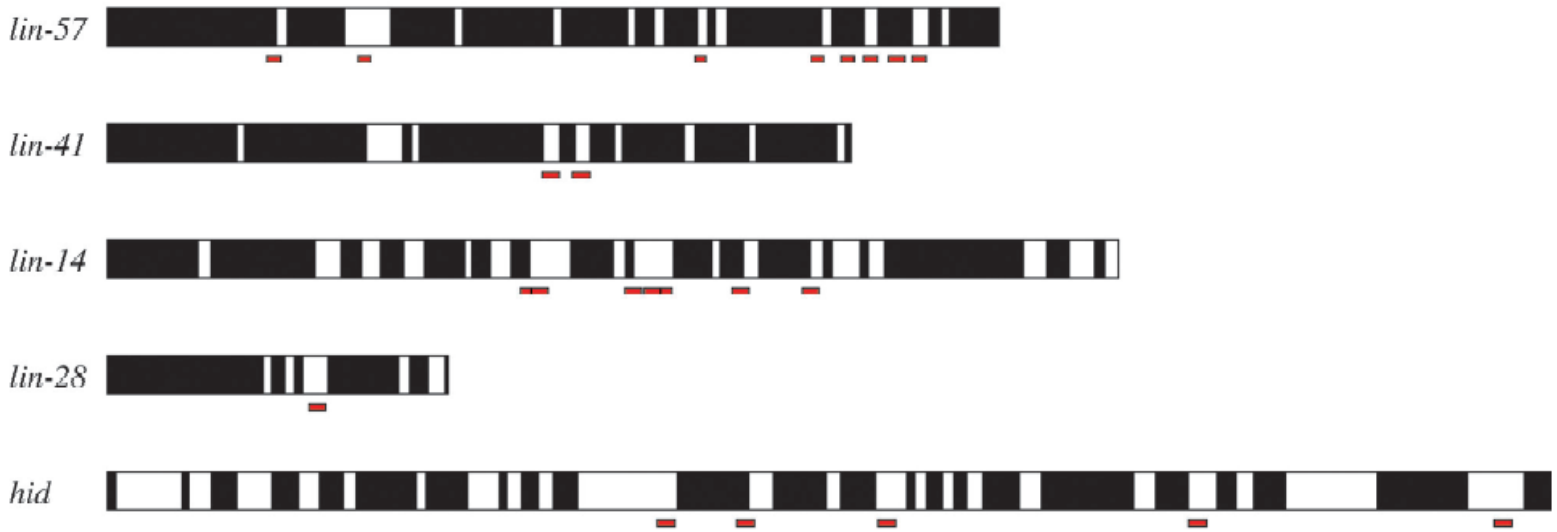
•Six to eight nucleotides at the 5’ end of the mature miRNA sequence are very important in the selection of target site

• Most of the computational tools developed to identify mRNA target sequences depend heavily on complementarity between miRNA seed sequence and the target sequence

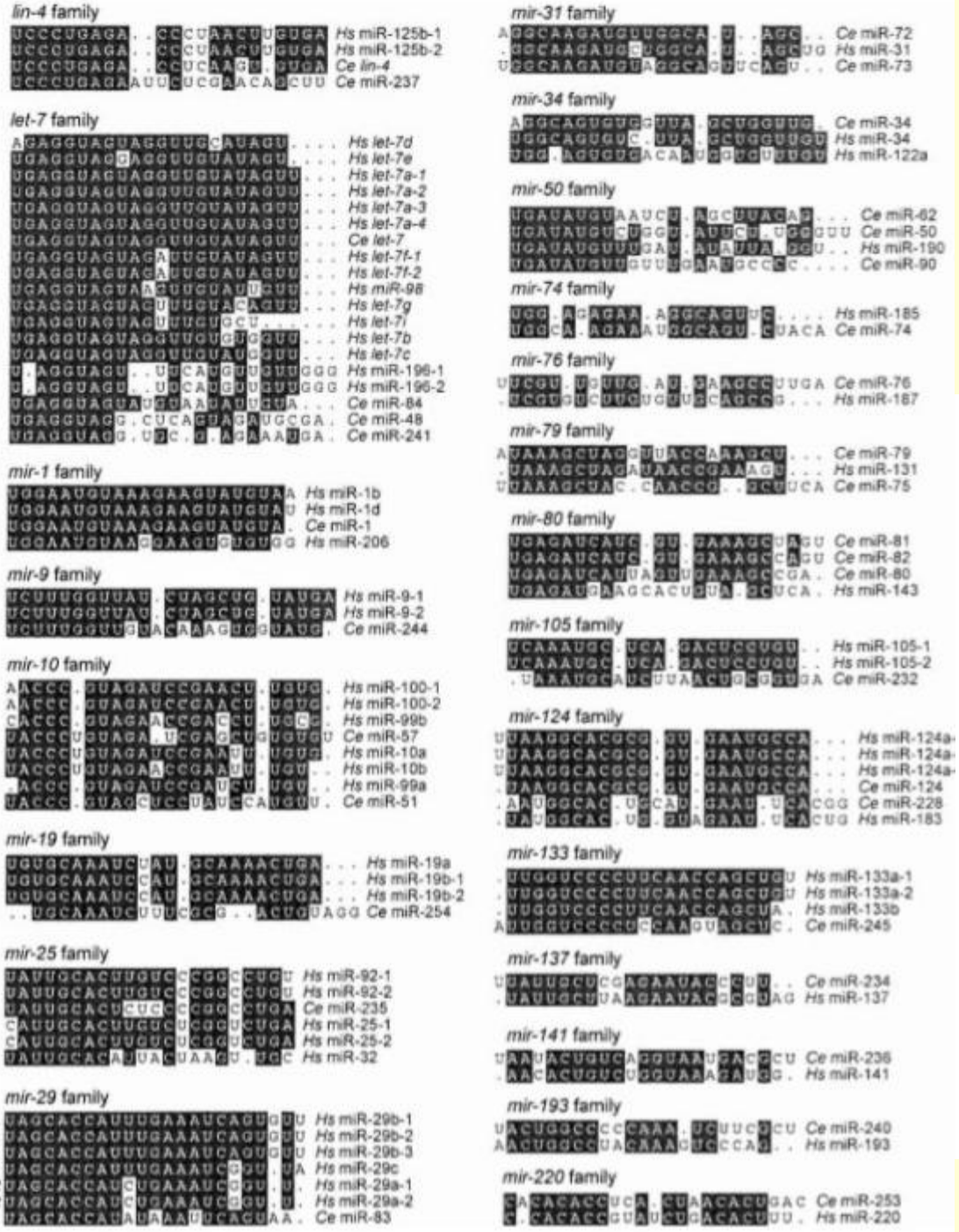
•Most methods mainly use sequence complementarities, thermodynamic stability calculations and evolutionary conservation among species to determine the likelihood of formation of a productive miRNA-mRNA duplex

Other properties of microRNA targets

- MicroRNA targets are conserved across species. (Stark et al. 2003)
- Tend to appear in clusters.



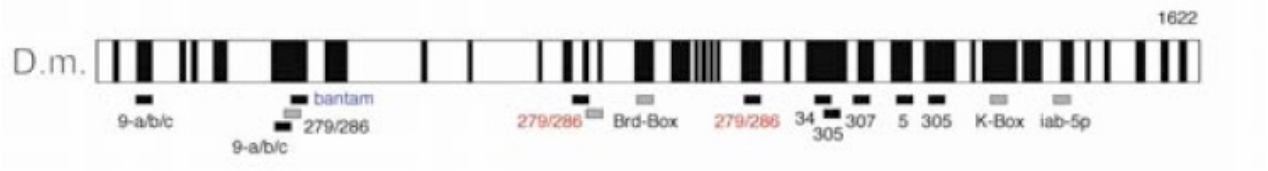
Homology Between *C. elegans* and *Homo sapiens* miRNAs



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

Interesting properties of microRNA targets

- Clusters of microRNA targets
  - Extensive co-occurrence of the sites for different microRNAs in target 3’ UTRs.



MicroRNA Targets Prediction Databases

Scientific Use Case Scenarios

- What are the target genes of a given miRNA?
- What are the miRNAs targeting a given gene and what are their binding sites?
- What is the tissue expression profile of a given miRNA (or set of miRNAs)?
- What is the miRNA expression profile in a given tissue (or set of tissues)?

miRNA Tools

FEBS Journal 276 (2009) 2150–2156

Table 1. Computational tools for miRNA predictions.

Algorithm	Web link	References
MiRseeker		Lai *et al.* [13]
MiRscan	http://genes.mit.edu/mirscan/	Lim *et al.* [9,11]
miRank	MiRank is programmed in MATLAB	Xue *et al.* [27]
proMiR II	http://cbit.snu.ac.kr/~ProMiR2/	Nam *et al.* [20]
mir-abela	http://www.mirz.unibas.ch/cgi/pred_miRNA_genes.cgi	Sewer *et al.* [21]
triplet-SVM	http://bioinfo.au.tsinghua.edu.cn/mirnasvm/	Xue *et al.* [27]
Vmir	http://www.hpi-hamburg.de/fileadmin/downloads/VMir.zip	Grundhoff *et al.*, 2006 [23]
RNA micro	http://www.bioinf.uni-leipzig.de/~jana/software/index.html	Hertel & Stadler [24]
micros	Based on LIBSVM library package [30]	Sheng *et al.* [25]
BayesMiRNAFind	https://bioinfo.wistar.upenn.edu/miRNA/miRNA/login.php	Yousef *et al.* [22]
One-ClassMimaFind	http://wotan.wistar.upenn.edu/OneClassmiRNA/	Yousef *et al.* [26]

Table 2. MicroRNA target prediction tools.

Algorithm	Web link	References
TargetScanS	http://genes.mit.edu/targetscan	Lewis *et al.* [41]
miRanda	http://www.microma.org	John *et al.* [34]
PicTar	http://pictar.bio.nyu.edu	Krek *et al.* [42]
RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid	Rehmsmeier *et al.* [36]
Diana-microT	http://www.diana.pcbi.upenn.edu/cgi-bin/micro_t.cgi	Kiriakidou *et al.* [32]
Target Boost	https://demo1.interagon.com/demo	SaeTrom *et al.* [44]
Rna22	http://cbcsrv.watson.ibm.com/rna22_targets.html	Miranda *et al.* [47]
MicroTar	http://tiger.dbs.nus.edu.sg/microtar/	Thadani and Tammi [46]
NBmiRTar	http://wotan.wistar.upenn.edu/NBmiRTar	Yousef *et al.* [48]
miRecords	http://mirecords.umn.edu/miRecords/	Xiao *et al.* [51]

TargetScan
(Lewis et al., Cell 2003)

- TargetScan predicts biological targets of miRNAs by searching for the presence of conserved 8mer and 7mer sites that match the seed region of each miRNA. As an option, nonconserved sites are also predicted.

- Also identified are sites with mismatches in the seed region that are compensated by conserved 3' pairing.

- In mammals, predictions are ranked based on the predicted efficacy of targeting as calculated using the context scores of the sites. TargetScanHuman considers matches to annotated human UTRs and their orthologs, as defined by UCSC whole-genome alignments.

http://www.targetscan.org/

miRBase

- Aims to provide integrated interfaces to comprehensive miRNA sequence data, annotation and predicted gene targets.

- miRBase takes over functionality from the miRNA Registry and fulfils three main roles: miRBase Sequences, miRBase Targets and miRBase Registry.

http://microrna.sanger.ac.uk/

Sections of miRBase

miRBase contains 3 main sections:

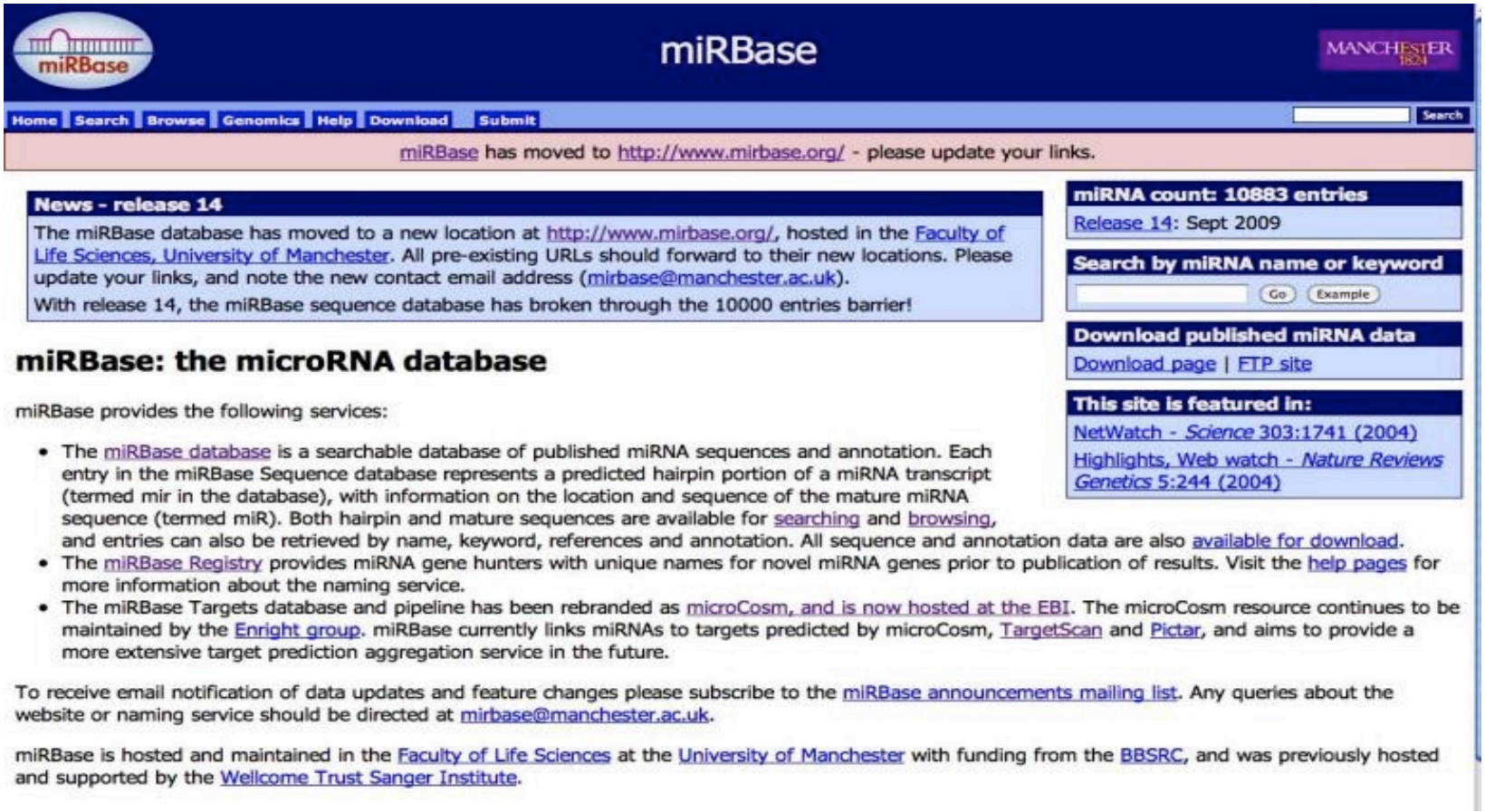
- miRBase Sequences contains all published miRNA sequences, genomic locations and associated annotation.

- miRBase Targets is a newly developed database of predicted miRNA target genes.

- miRBase Registry provides a confidential service assigning official names for novel miRNA genes prior to publication of their discovery

miRBase

http://www.mirbase.org/



Argonaute (miRWalk)

Nucleic Acids Res. 2006 Jan 1;34(Database issue):D115-8

- Curated database

- Argonaute collects latest information from both literature and other databases. In contrast to databases on miRNAs like miRBase::Sequences, NONCODE or RNAdb, Argonaute hosts additional information on the origin of an miRNA, i.e. in which host gene it is encoded, its expression in different tissues and its known or proposed function, its potential target genes including Gene Ontology annotation, as well as miRNA families and proteins known to be involved in miRNA processing.

- Additionally, target genes are linked to an information retrieval system that provides comprehensive information from sequence databases and a simultaneous search of MEDLINE with all synonyms of a given gene. The web interface allows the user to get information for a single or multiple miRNAs, either selected or uploaded through a text file. Argonaute currently has information on miRNAs from human, mouse and rat.

http://www.ma.uni-heidelberg.de/apps/zmf/argonaute/interface

miRNA Target Validation and Considerations in RNAi Experiments

miRNA Target Validation

• Several in vitro methods useful to validate miRNA function:

- RNA interference
- protein analysis

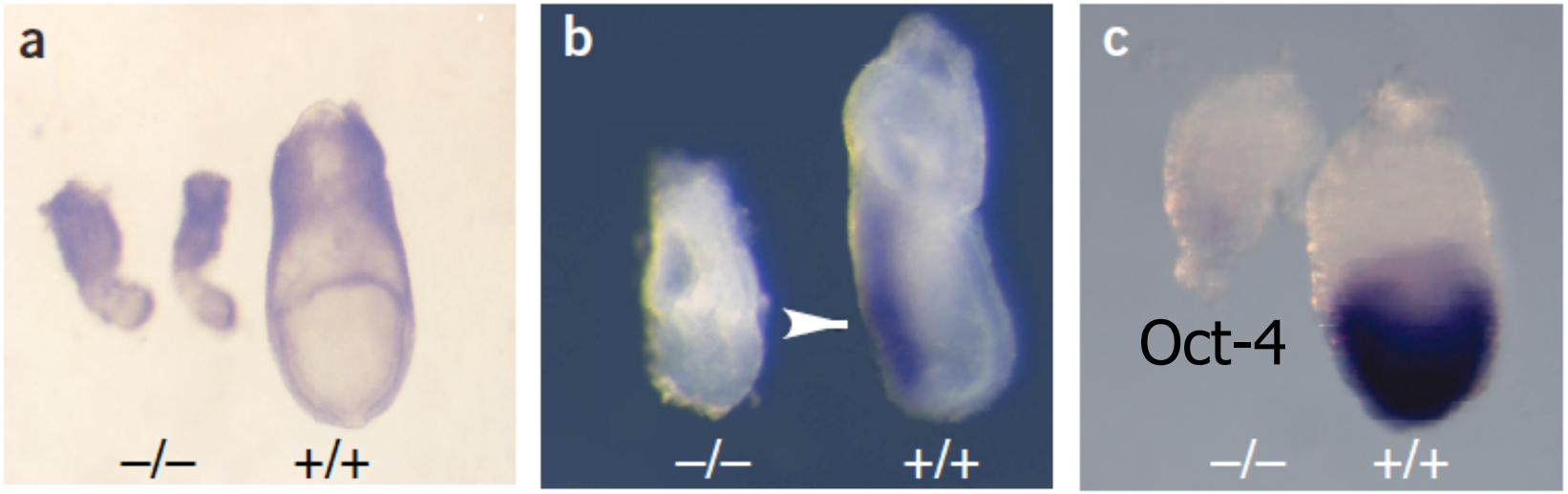
•Over-expression and knockdown functional studies - There are three basic approaches to induce RNA interference:

- synthetic RNAi and siRNA duplexes
- vectors carrying RNAi cassette expressing shRNA or artificial miRNAs
- *in vitro* transcription and dicing of dsRNA to generate pools of siRNA.

The endogenous role of RNAi

Dicer is essential for mouse development

Emily Bernstein[1,2], Sang Yong Kim[1], Michelle A Carmell[1,2], Elizabeth P Murchison[1], Heather Alcorn[3], Mamie Z Li[4], Alea A Mills[1], Stephen J Elledge[4], Kathryn V Anderson[3] & Gregory J Hannon[1]



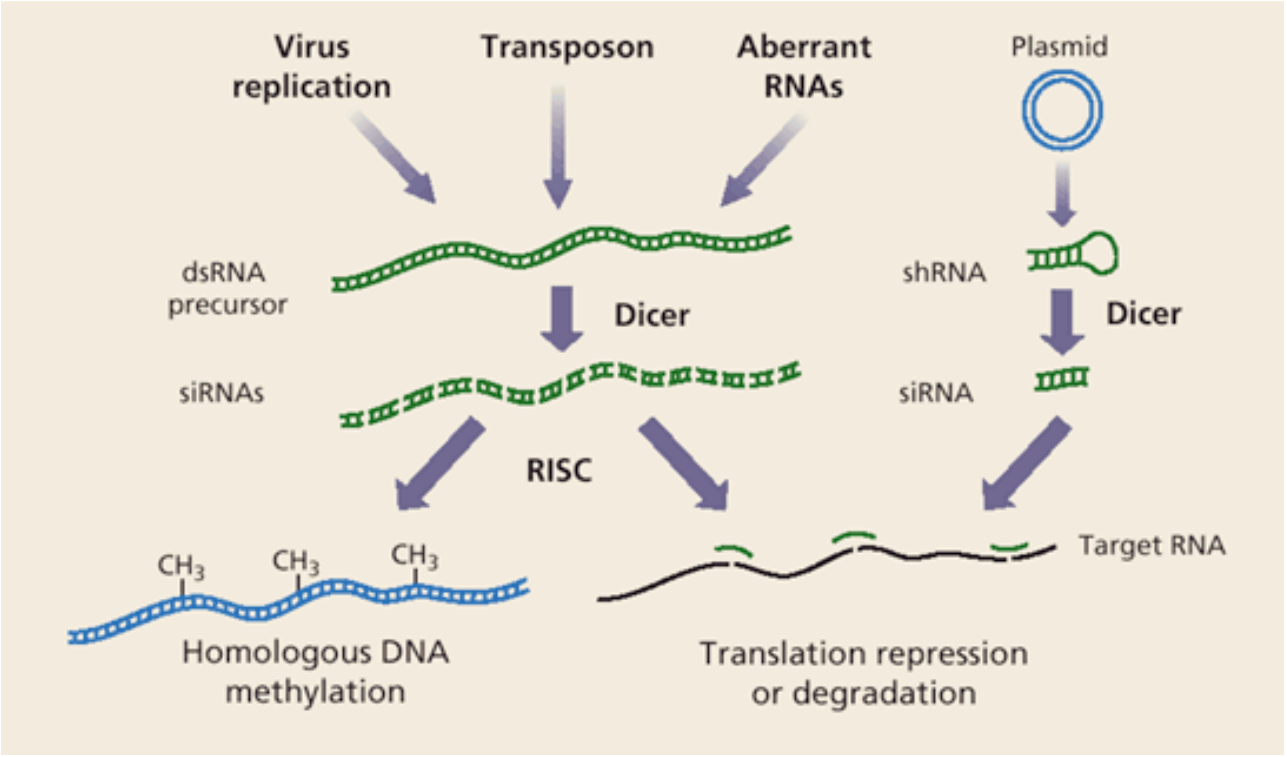
E7.5 embryos - lack of stem cell development

NATURE GENETICS VOLUME 35 | NUMBER 3 | NOVEMBER 2003

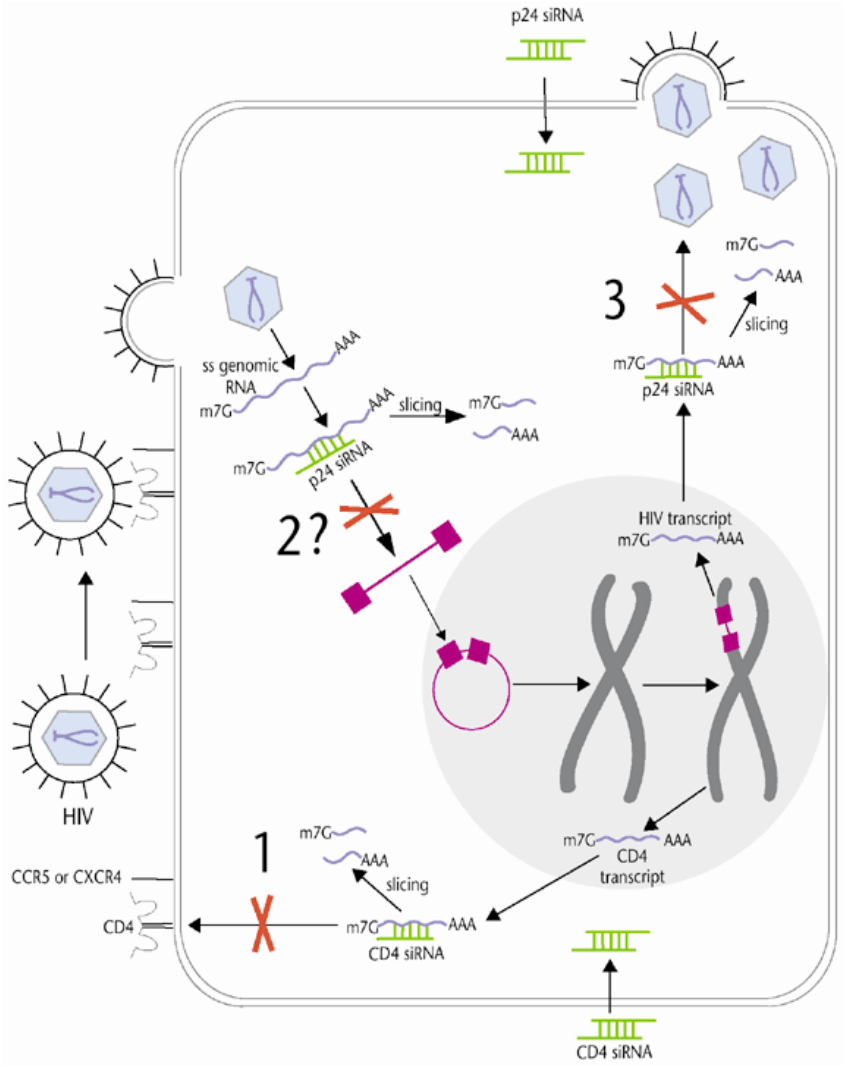
RNA silencing could represent an "immune defense" of the genome

Close to 50% of our genome consists of viral and transposon elements that have invaded the genome in the course of evolution. The RNAi machinery can recognize invading double-stranded viral RNA (or the double-stranded replicative form of the viral RNA) and suppress the infection by degradation of the RNA.

The RNAi system thus shares important features with the vertebrate immune system: it recognizes the invading parasite (dsRNA), raises an initial response and subsequently amplifies the response to eliminate the foreign element.



Silencing viruses by RNAi



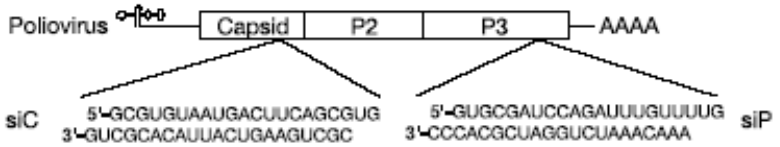
siRNA-directed inhibition of HIV-1 infection
Carl D. Novina *et al.*
Nature Medicine 8, 681 - 686 (2002)

Modulation of HIV-1 replication by RNA interference

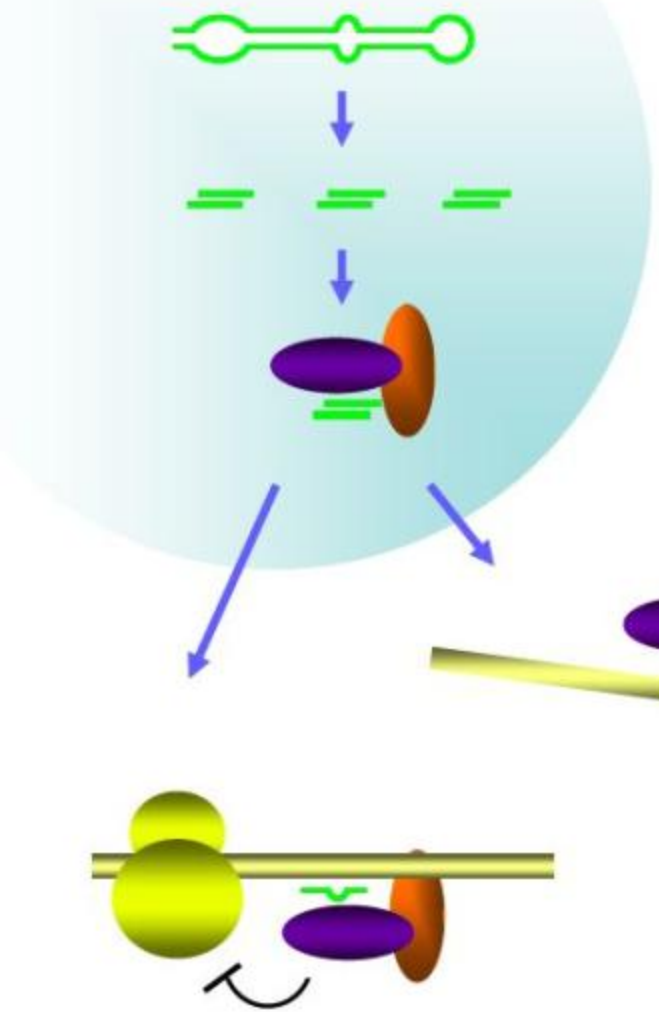
Jean-Marc Jacque, Karine Triques & Mario Stevenson

Short interfering RNA confers intracellular antiviral immunity in human cells

Leonid Gitlin*†, Sveta Karelsky* & Raul Andino*



Thousands of microRNAs act in multiple biological events



Developmental timing

Differentiation

Aging

Apoptosis

Metabolism

Cancer

…

Cell fate/differentiation, Cell cycle…

miRNAs are involved in all aspects of cardiovascular function

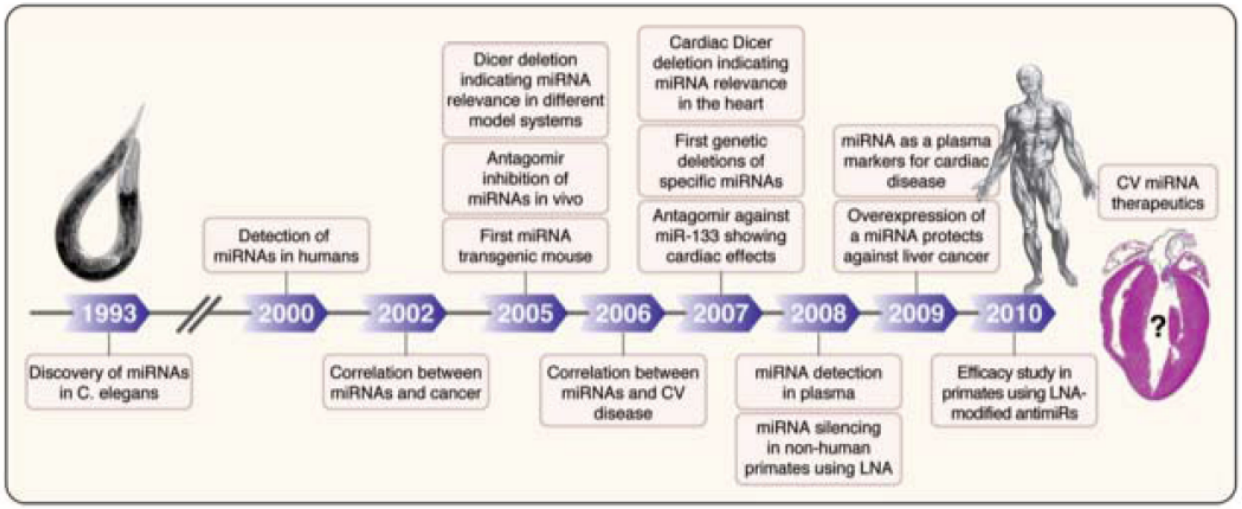


Figure 1. Breakthrough discoveries in miRNA biology. Time line indicating seminal discoveries in miRNA biology with a special focus on the cardiovascular field.

Circ Res. **2011;108:219-234**

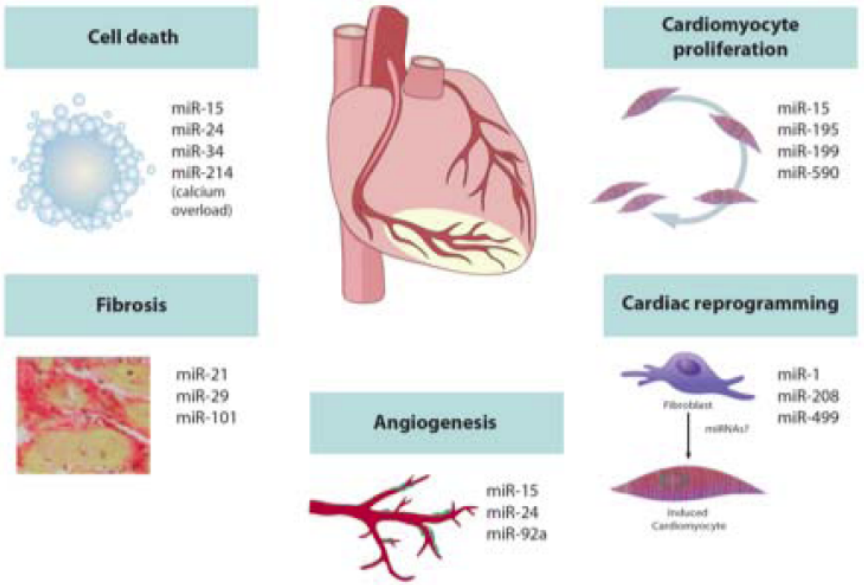
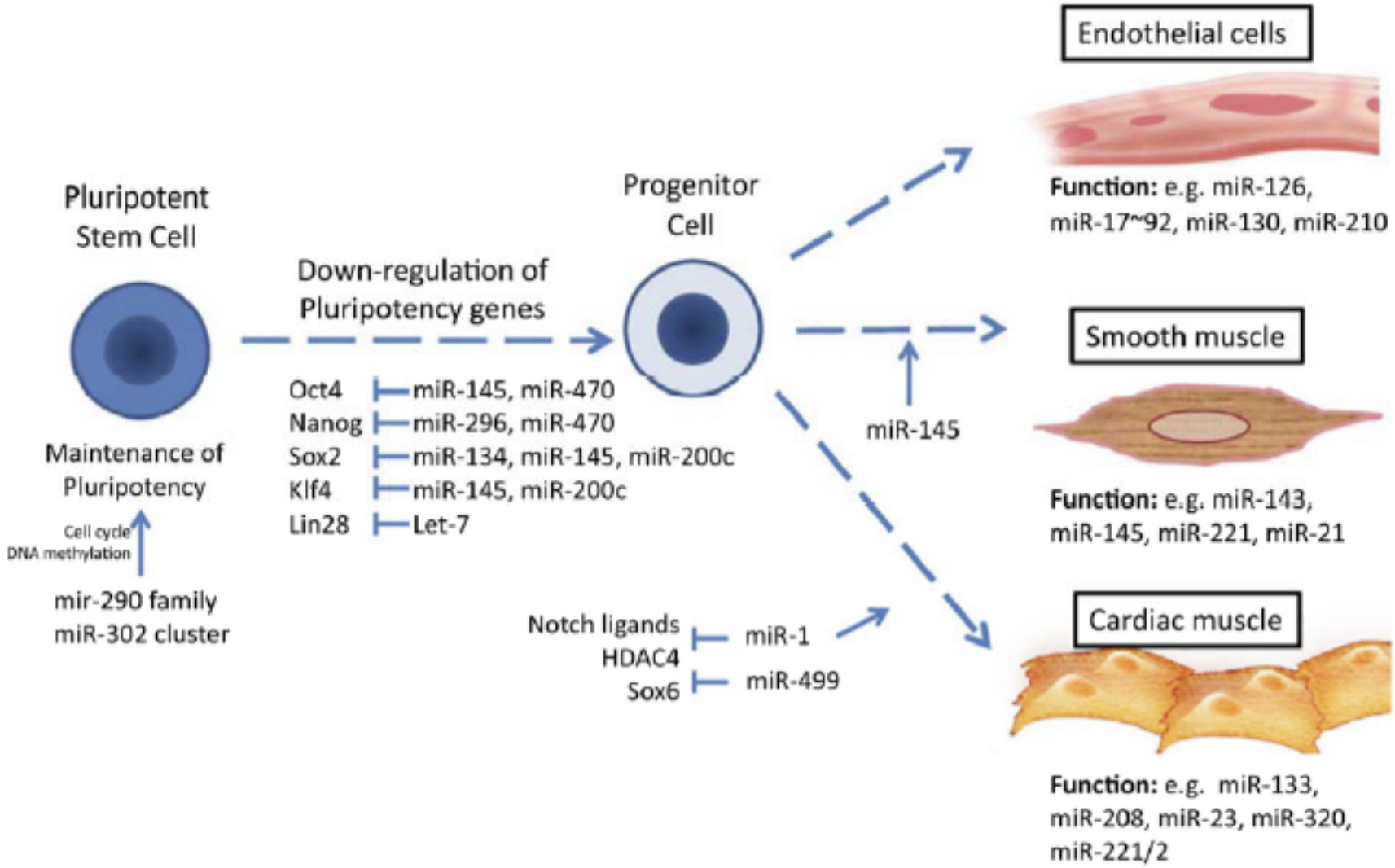


Figure 2. MicroRNAs (MiRNAs) in postinfarction repair.

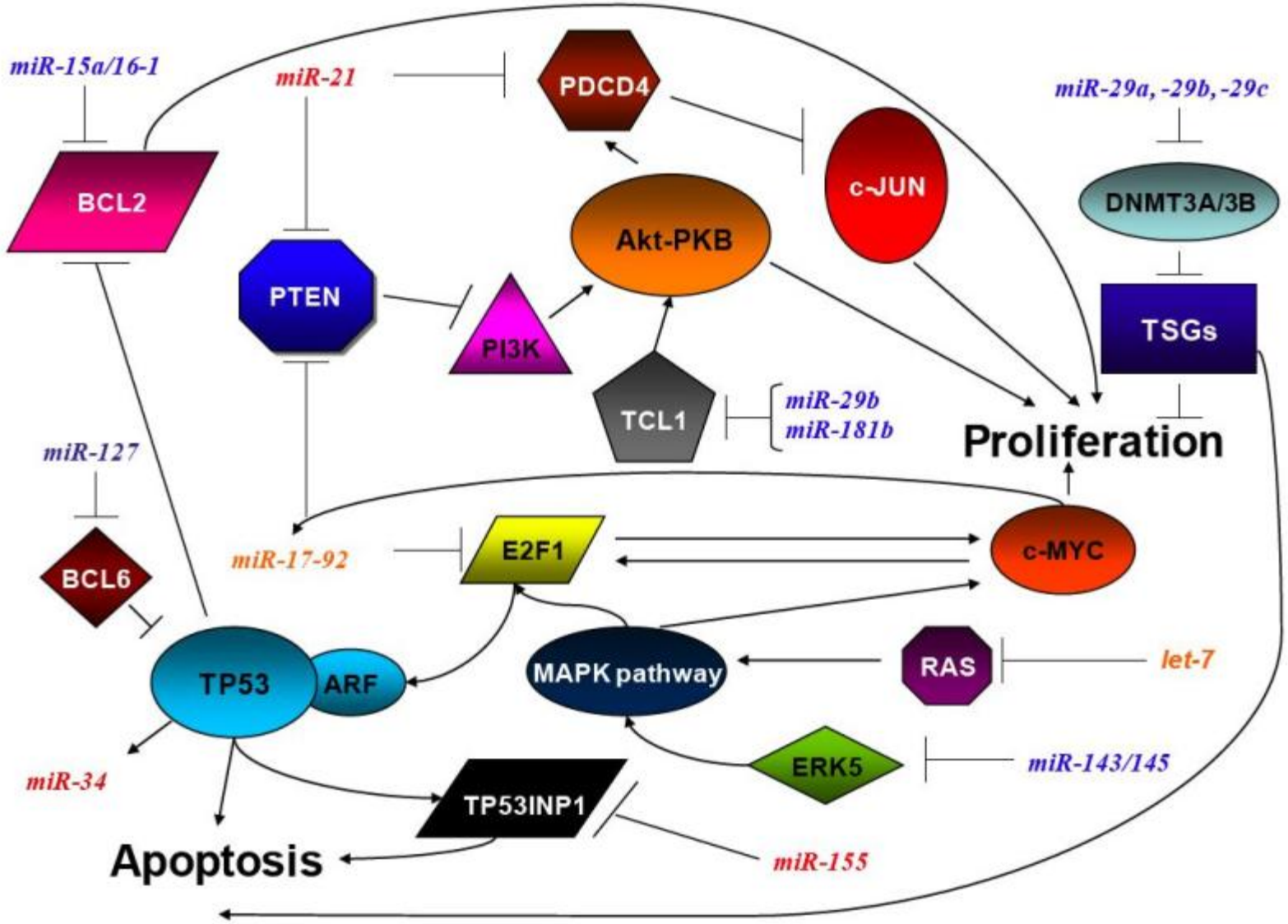
Arterioscler Thromb Vasc Biol. **2013;33:1739-1746**



Control of self-renewal and differentiation by microRNAs

Basic Res Cardiol (2011) 106:5–11

Micro RNAs Regulate Cell Growth and Death



miRNAs Involved in Human Cancer

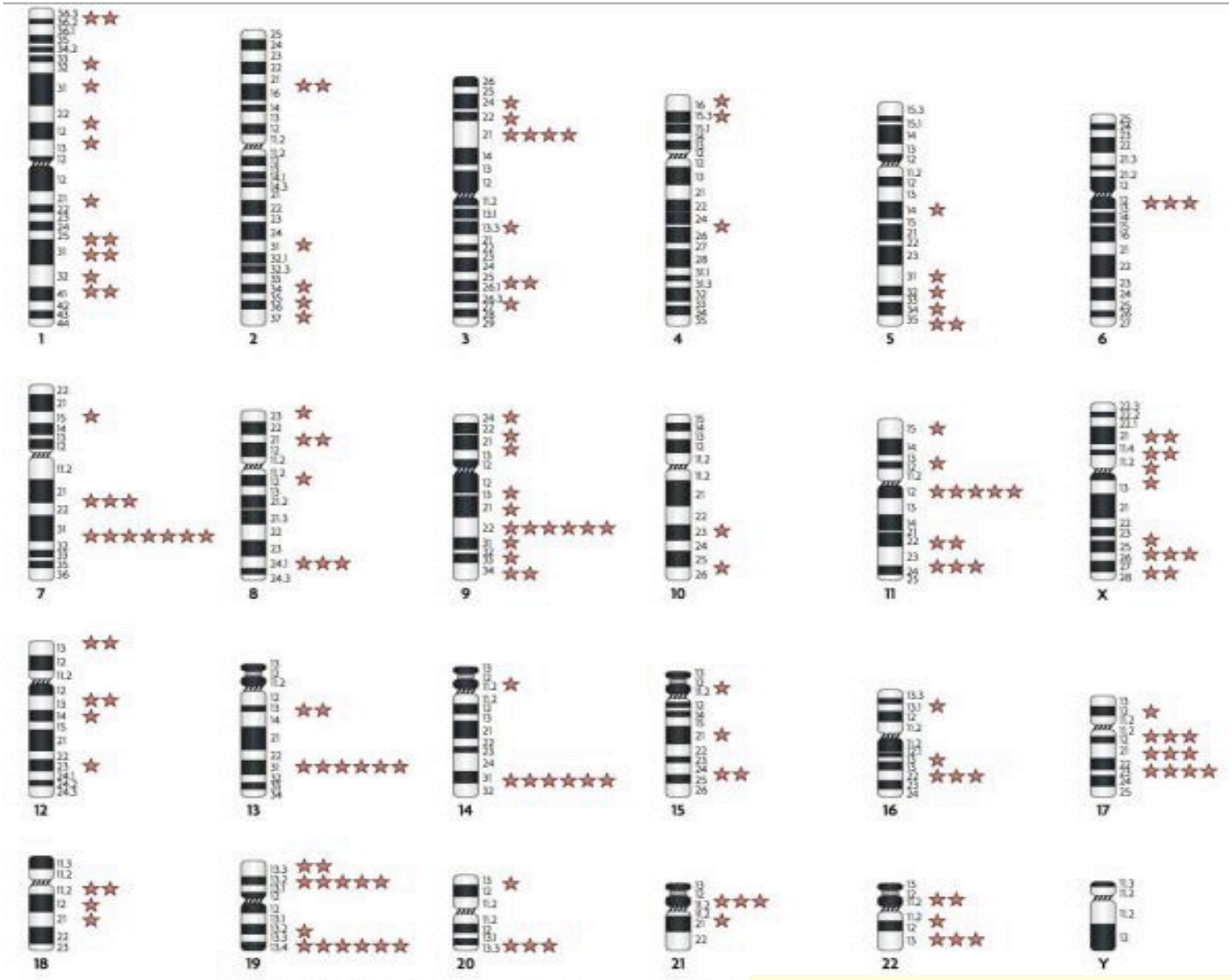


Figure 1 | **MicroRNA genes map to chromosomal regions t**

Croce Nat Rev Genet. 2009 Oct;10(10):704-14

miRNA Dysregulation in Human Cancers

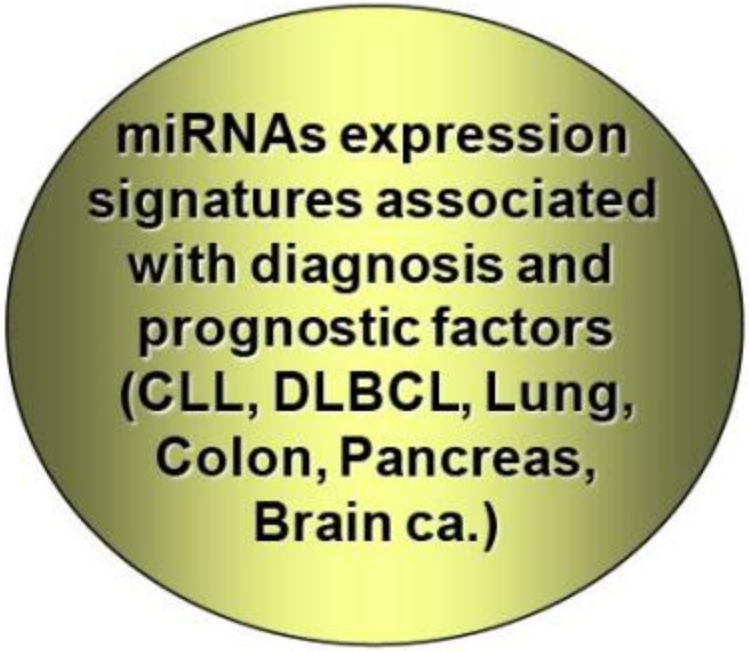
Table 2 | **Consequences of microRNA dysregulation in human cancers**

MicroRNA dysregulation	Targets	Consequences
MicroRNA overexpression	Tumour suppressors	Downregulation of tumour suppressors — for example, PTEN, p22, p57, TIMP3 and PDCD4
MicroRNA loss	Oncogenes	Upregulation of oncogenes — for example, *BCL2*, *MCL1*, *RAS*, *HMGA2*, *MYC* and *MET*
MicroRNA loss	DNA methyltransferases	Downregulation of tumour suppressors — for example, p16, FHIT and WWOX
MicroRNA loss	Chromatin silencers	Downregulation of tumour suppressors

BCL2, B cell leukaemia/lymphoma 2; FHIT, fragile histidine triad protein; *HMGA2*, high mobility group AT-hook 2; *MCL1*, myeloid cell leukaemia sequence 1; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homologue; TIMP3, tissue inhibitor of metalloproteinases 3; WWOX, WW domain-containing oxidoreductase.

Croce Nat Rev Genet. 2009 Oct;10(10):704-14

MIRNA PROFILING AS A NEW DIAGNOSTIC & PROGNOSTIC TOOL FOR CANCER PATIENTS



(Michael et al, Molec Cancer Res 2003; Lu et al, Nature, 2005; Eis et al, PNAS, 2005
Lui et al, Cancer Res 2007, Bloomston et al, JAMA 2007; Mi et al, PNAS, 2007; Garzon et al, Blood in press 2008)

Profiling miRNA expression using custom microarrays