miRNA

Boris Rogelj
Boris.Rogelj@ijs.si

Pregled predavanja

- Nekodirajoče RNA
- Odkritje miRNA in osnovne lastnosti
- Biogeneza miRNA
- Delovanje miRNA
- Detekcija miRNA
- Podatkovne baze miRNA
- Tarče miRNA
- Eksogne miRNA
- miRNA in bolezni
- miRNA in terapija

Raznolikost RNA

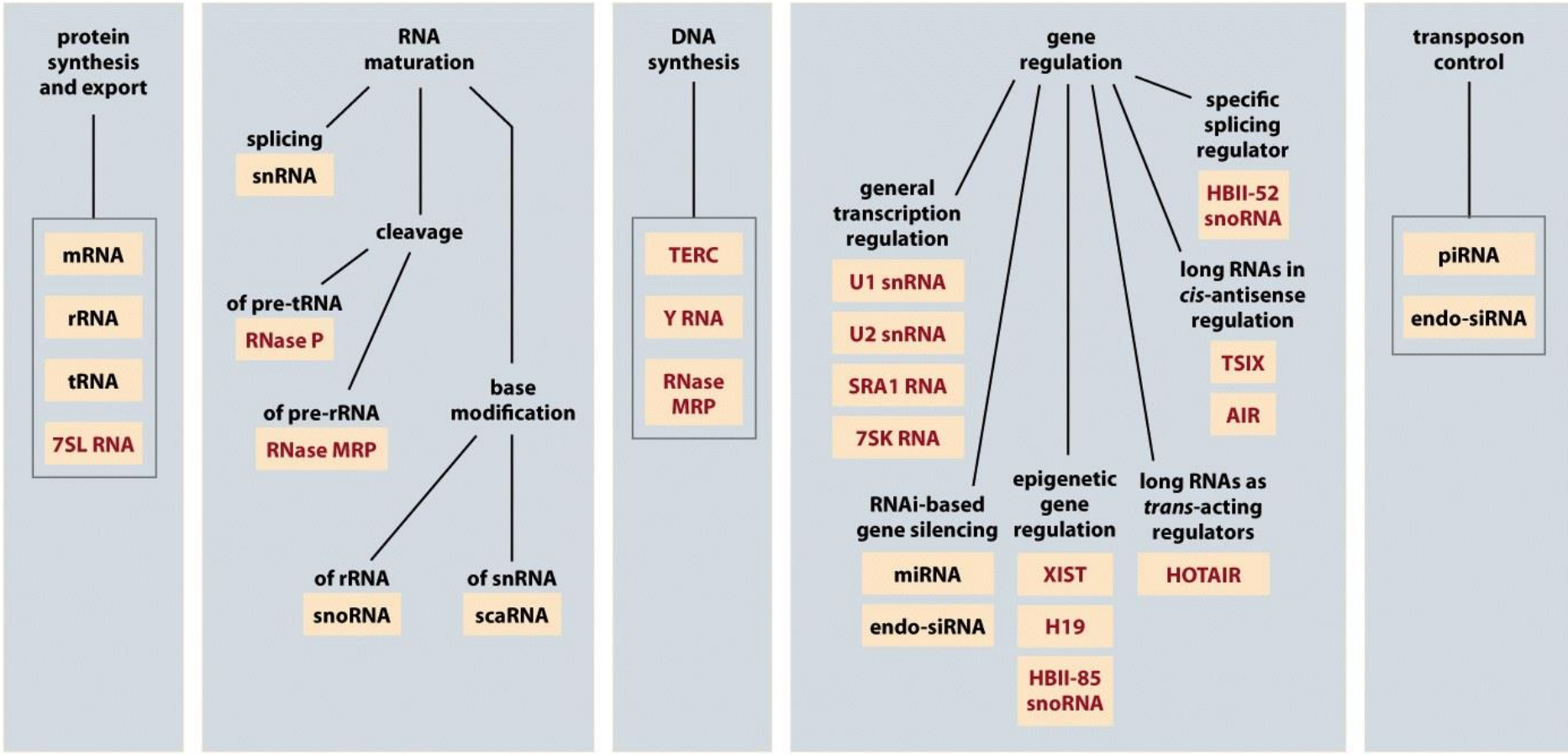


Figure 9.13 Human Molecular Genetics, 4ed. (© Garland Science)

TABLE 9.9 MAJOR CLASSES OF HUMAN NONCODING RNA

RNA class	Subclass or evolutionary/ functional subfamily	No. of different types	Function	Gene organization, biogenesis, etc.
Ribosomal RNA (rRNA), ~120–5000 nucleotides	12S rRNA, 16S rRNA	1 of each	components of mitochondrial ribosomes	cleaved from multigenic transcripts produced by H strand of mtDNA (Figure 9.3)
	5S rRNA, 5.8S rRNA, 18S rRNA, 28S rRNA	1 of each	components of cytoplasmic ribosomes	5S rRNA is encoded by multiple genes in various gene clusters; 5.8S, 18S, and 28S rRNA are cleaved from multigenic transcripts (Figure 1.22); the multigenic 5.8S–18S–28S transcription units are tandemly repeated on each of 13p,14p,15p, 21p, and 22p (= rDNA clusters)
Transfer RNA (tRNA), ~70–80 nucleotides	mitochondrial family	22	decode mitochondrial mRNA to make 13 proteins on mitochondrial ribosomes	single-copy genes. tRNAs are cleaved from multigenic mtDNA transcripts (Figure 9.3)
	cytoplasmic family	49	decode mRNA produced by nuclear genes (Figure 9.13)	700 tRNA genes and pseudogenes dispersed at multiple chromosomal locations with some large gene clusters
Small nuclear RNA (snRNA), ~60–360 nucleotides	spliceosomal family with subclasses Sm and Lsm (Table 9.10)	9	U1, U2, U4, U5, and U6 snRNAs process standard GU–AG introns (Figure 1.19); U4atac, U6atac, U11, and U12 snRNAs process rare AU–AC introns	about 200 spliceosomal snRNA genes are found at multiple locations but there are moderately large clusters of U1 and U2 snRNA genes; most are transcribed by RNA pol II
	non-spliceosomal snRNAs	several	U7 snRNA: 3′ processing of histone mRNA; 7SK RNA: general transcription regulator; Y RNA family: involved in chromosomal DNA replication and regulators of cell proliferation	mostly single-copy functional genes

Table 9.9 part 1 of 3 Human Molecular Genetics, 4ed. (© Garland Science)

RNA class	Subclass or evolutionary/ functional subfamily	No. of different types	Function	Gene organization, biogenesis, etc.
Small nucleolar RNA (snoRNA), ~60–300 nucleotides	C/D box class (Figure 9.15A)	246	maturation of rRNA, mostly nucleotide site-specific 2′-*O*-ribose methylations	usually within introns of protein-coding genes; multiple chromosomal locations, but some genes are found in multiple copies in gene clusters (such as the HBII-52 and HBII-85 clusters—Figure 11.22)
	H/ACA class (Figure 9.15B)	94	maturation of rRNA by modifying uridines at specific positions to give pseudouridine	
Small Cajal body RNA (scaRNA)		25	maturation of certain snRNA classes in Cajal bodies (coiled bodies) in the nucleus	usually within introns of protein-coding genes
RNA ribonucleases, ~260–320 nucleotides		2	RNase P cleaves pre-tRNA in nucleus + mitochondria; RNase MRP cleaves rRNA in nucleolus and is involved in initiating mtDNA replication	single-copy genes
Miscellaneous small cytoplasmic RNAs, ~80–500 nucleotides	BC200	1	neural RNA that regulates dendritic protein biosynthesis; originated from Alu repeat	1 gene, *BCYRN1*, at 2p16
	7SL RNA	3	component of the signal recognition particle (SRP) that mediates insertion of secretory proteins into the lumen of the endoplasmic reticulum	three closely related genes clustered on 14q22
	TERC (telomerase RNA component)	1	component of telomerase, the ribonucleoprotein that synthesizes telomeric DNA, using TERC as a template (Figure 2.13)	single-copy gene at 3q26
	Vault RNA	3	components of cytoplasmic vault RNPs that have been thought to function in drug resistance	*VAULTRC1*, *VAULTRC2*, and *VAULTRC3* are clustered at 5q31 and share ~84% sequence identity
	Y RNA	4	components of the 60 kD Ro ribonucleoprotein, an important target of humoral autoimmune responses	*RNY1*, *RNY3*, *RNY4*, and *RNY5* are clustered at 7q36

TABLE 9.9 (*cont.*) MAJOR CLASSES OF HUMAN NONCODING RNA

RNA class	Subclass or evolutionary/ functional subfamily	No. of different types	Function	Gene organization, biogenesis, etc.
MicroRNA (miRNA), ~22 nucleotides	> 70 families of related miRNAs	~1000	multiple important roles in gene regulation, notably in development, and implicated in some cancers	see Figure 9.17 for examples of genome organization, and Figure 9.16 for how they are synthesized
Piwi-binding RNA (piRNA), ~24–31 nucleotides	89 individual clusters	> 15,000	often derived from repeats; expressed only in germ-line cells, where they limit excess transposon activity	89 large clusters distributed across the genome; individual clusters span from 10 kb to 75 kb with an average of 170 piRNAs per cluster
Endogenous short interfering RNA (endo-siRNA), ~21–22 nucleotides	many	probably more than 10,000[a]	often derived from pseudogenes, inverted repeats, etc.; involved in gene regulation in somatic cells and may also be involved in regulating some types of transposon	clusters at many locations in the genome
Long noncoding regulatory RNA, often > 1 kb	many	> 3000	involved in regulating gene expression; some are involved in monoallelic expression (X-inactivation, imprinting), and/or as antisense regulators (Table 9.11)	usually individual gene copies; transcripts often undergo capping, splicing, and polyadenylation but antisense regulatory RNAs are typically long transcripts that do not undergo splicing

[a]Based on extrapolation of studies in mouse cells.

Table 9.9 part 3 of 3 Human Molecular Genetics, 4ed. (© Garland Science)

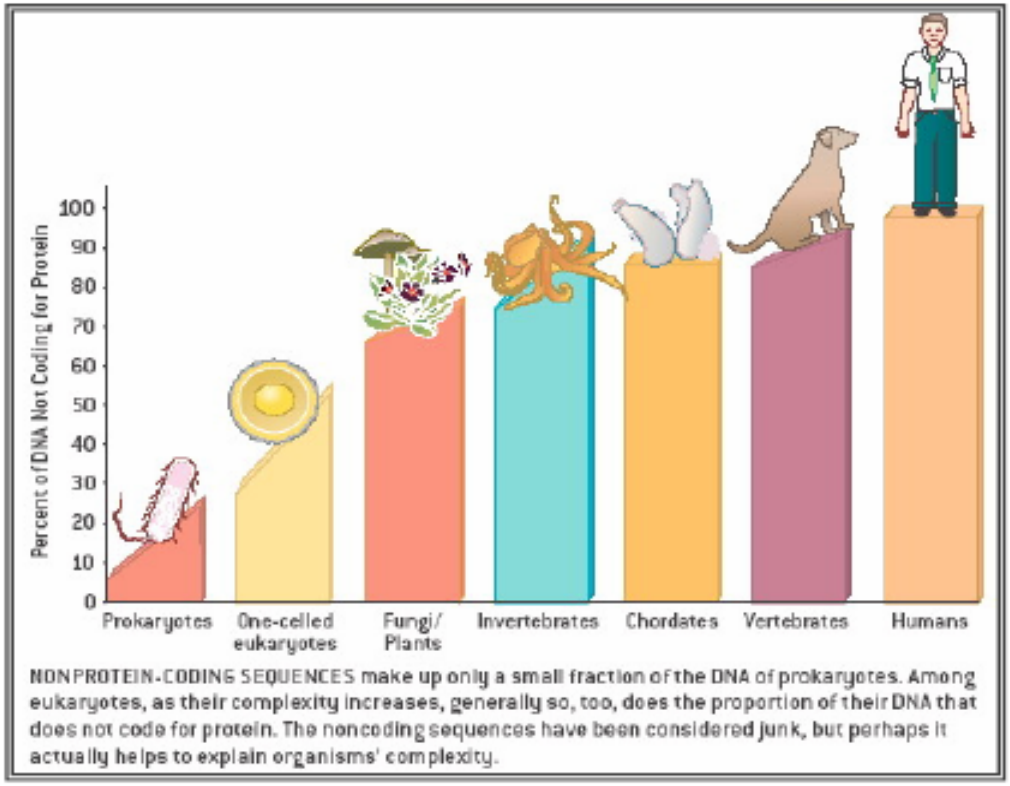
ncRNA podatkovne baze

TABLE 9.10 MAJOR NONCODING RNA DATABASES

Database	Description	URL
NONCODE	integrated database of all ncRNAs except rRNA and tRNA	http://www.noncode.org
Noncoding RNA database	sequences and functions of noncoding transcripts	http://biobases.ibch.poznan.pl/ncRNA/
RNAdb	comprehensive mammalian noncoding RNA database	http://research.imb.uq.edu.au/rnadb/
Rfam	noncoding RNA families and sequence alignments	http://rfam.sanger.ac.uk/
antiCODE	natural antisense transcripts database	http://www.anticode.org
sno/scaRNAbase	small nucleolar RNAs and small Cajal body-specific RNAs	http://gene.fudan.sh.cn/snoRNAbase.nsf
snoRNA-LBME-db	comprehensive human snoRNAs	http://www-snorna.biotoul.fr/
Genomic tRNA Database	comprehensive tRNA sequences	http://lowelab.ucsc.edu/GtRNAdb/
Compilation of tRNA sequences and sequences of tRNA genes	just as its name suggests	http://www.tRNA.uni-bayreuth.de
miRBase	miRNA sequences and target genes	http://microrna.sanger.ac.uk/
piRNAbank	empirically known sequences and other related information on piRNAs reported in various organisms, including human, mouse, rat, and *Drosophila*	http://pirnabank.ibab.ac.in/

Table 9.10 Human Molecular Genetics, 4ed. (© Garland Science)

So ncRNA ključne pri povečanju kompleksnosti organizmov?



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

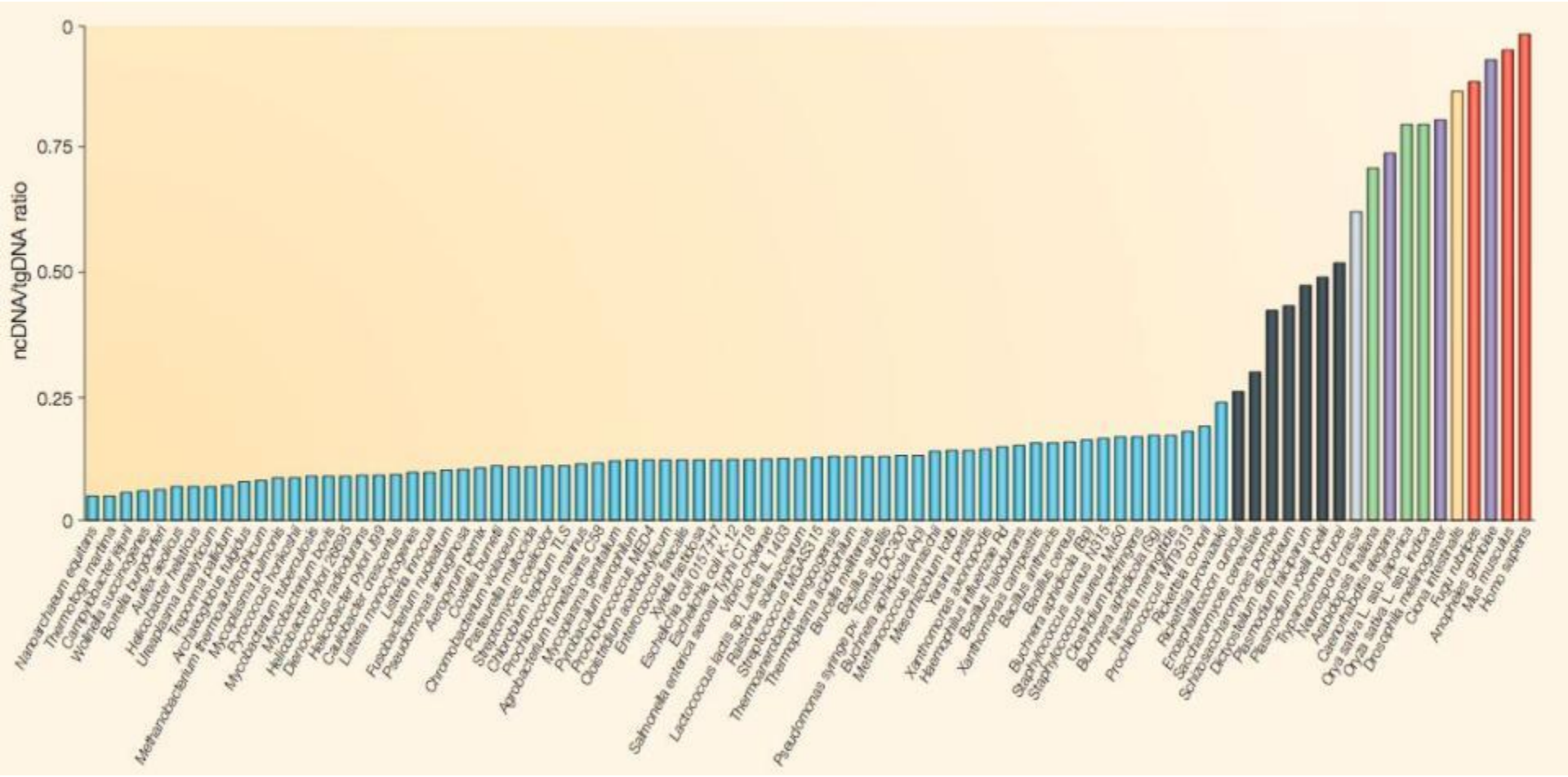
Data suggesting role in diverse mechanisms:

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

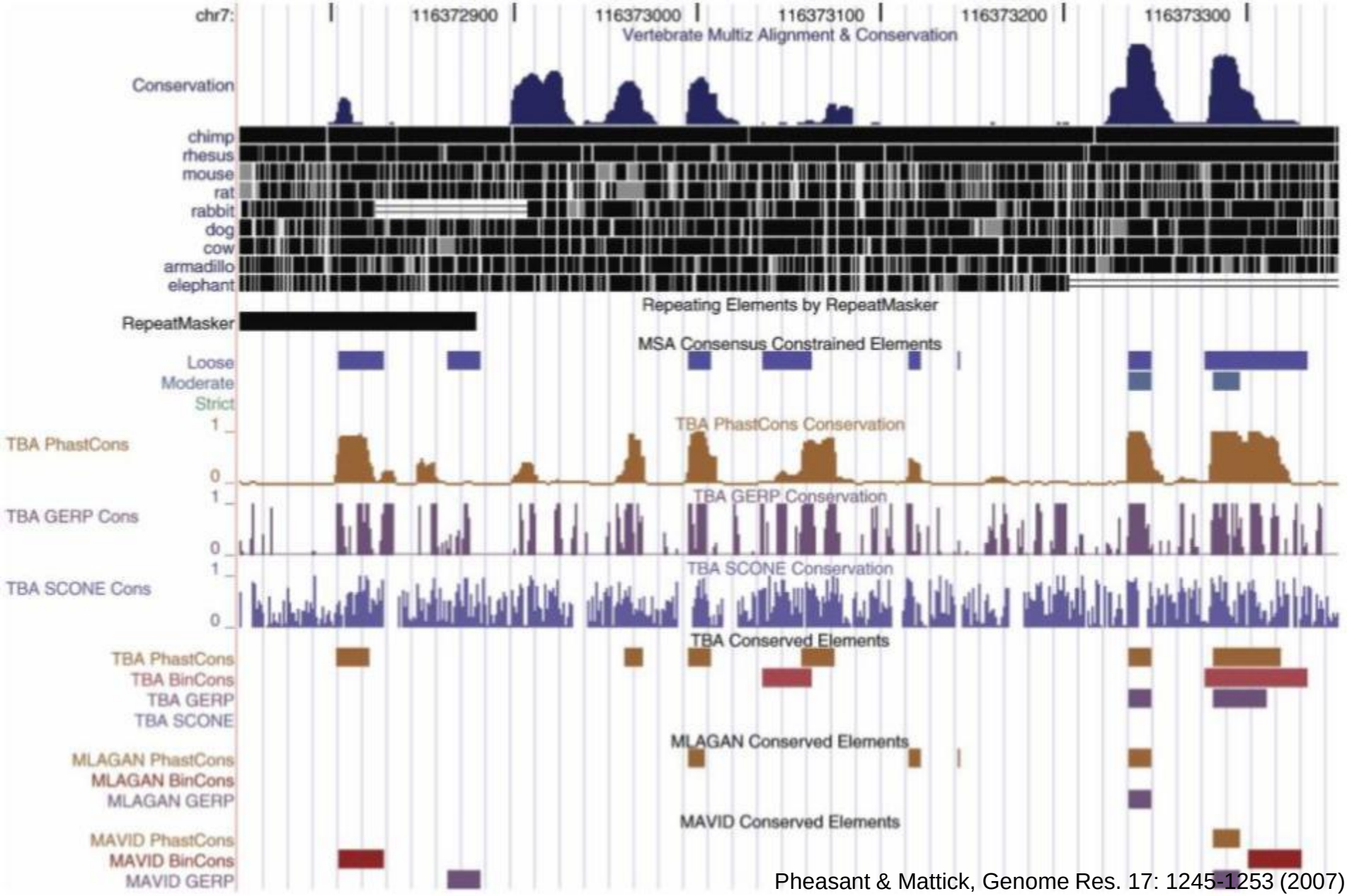
Possible roles in:

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

Evolucijski pogled na razmerje med nekodirajočim in kodirajočim delom genoma



Ohranjene regije v enem intronu CFTR



Pheasant & Mattick, Genome Res. 17: 1245-1253 (2007)

Kaj so miRNA?

Lastnosti miRNA

- Male nekodirajoče RNA
- Dolžina 19-22 nt
- Utiša izražanje komplementarne mRNA
- Uravnavajo izražanje ~30% sesalčjih genov
- Prisotne so pri živalih, rastlinah, glivah.
- Mnoge so ohranjeni med vretenčarji in nevretenčarji.

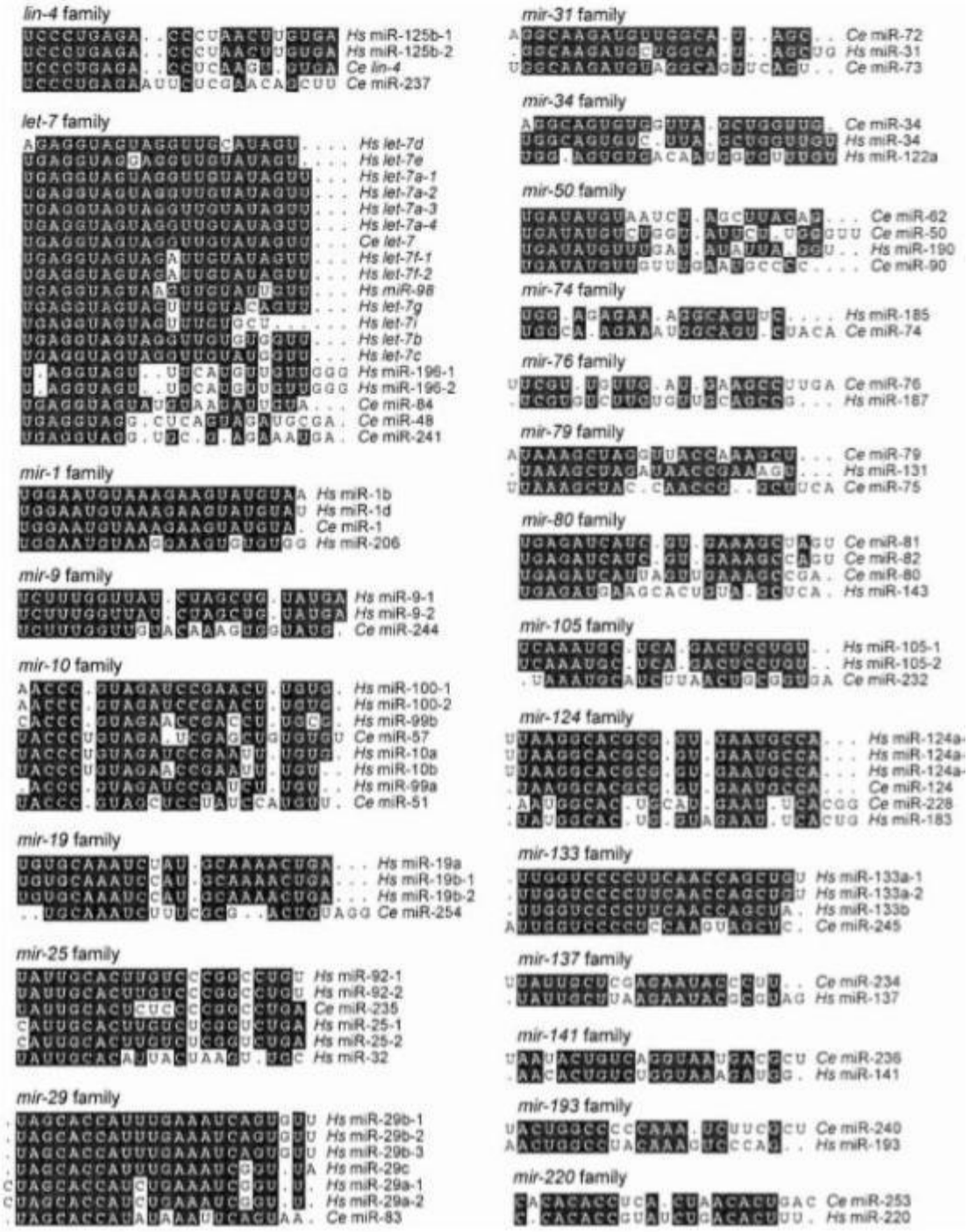
- 1 miRNA -> stotine mRNA

  s spremembami samo ene miRNA lahko spremenimo cel fenotip

- 100+ miRNA -> 1 mRNA

  eno mRNA lahko regulira tudi do več sto miRNA. Odvisno od dolžine 3‘UTR.

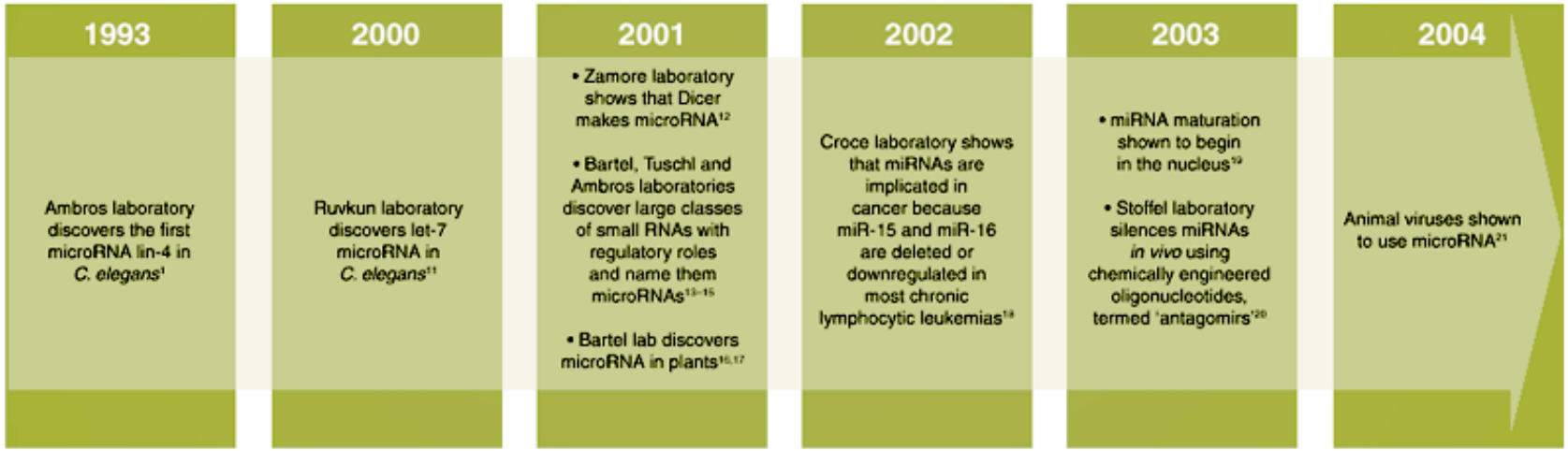
Homologija med miRNA človeka in nematode



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

Zgodovina miRNA

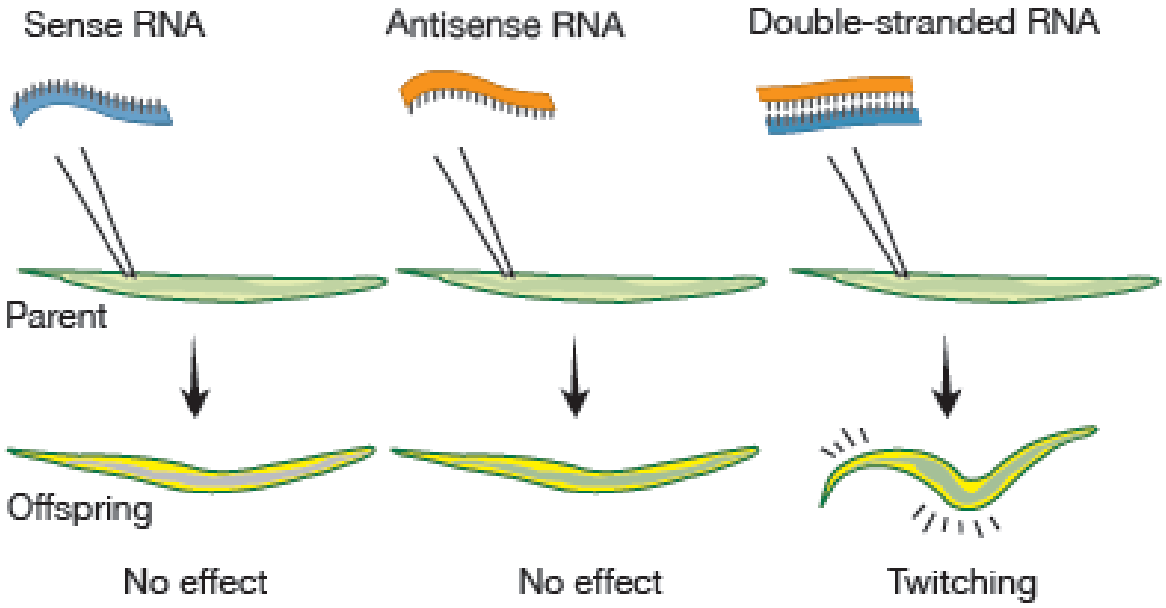
- lin-4 - prva miRNA. Odkrita pri *C. elegans*. Pomembna vloga pri razvoju iz larve v odraslo nematodo.
- Tudi let-7 je bil odkrit pri *C. elegans* (Reinhard BJ et al, 2000). Pomemben pri uravnavanju diferenciacije. Zmanjšano izražanje let-7 je pri človeku povezano s rakom in matičnimi celicami raka. Človeški let-7 je tumorski supresor, ki sodeluje pri končni diferenciaciji celic.
- Fire in Mello (1998) sta prva pokazala, da je v primerjavi s protismisleno RNA („antisense RNA“) dvoverižna RNA (dsRNA) bolj učinkovita pri utišanju izražanja genov. To je bil začetek razumevanja vloge miRNA pri razvoju in regulaciji genov. (Nobelova nagrada za fiziologijo in medicino, 2006).



doi:10.1038/nbt0607-631

RNA interferenca

RNA carrying the code for a muscle protein is injected into the worm *C. elegans*. Single-stranded RNA has no effect. But when double-stranded RNA is injected, the worm starts twitching in a similar way to worms carrying a defective gene for the muscle protein.



Mello in Fire sta injicirala kratko dvoverižno RNA (dsRNA) v *C.elegans* in ugotovila, da pride do utišanja izražanja genov, ki vsebujejo komplementarno zaporedje. To je vodilo do odkritja RNAi – ki je sedaj zelo pomembno orodje v bioloških raziskavah ter biotehnologiji in razvoju terapij.

Nobelova nagrada za medicino

Andrew Z. Fire
Stanford University, USA

&

Craig C. Mello
Univ. of Massachusetts, USA

„za odkritje RNA interference – utišanja genov z dvoverižno RNA"

Watson & Crick

Nobelova nagrada 1962

Roger Kornberg

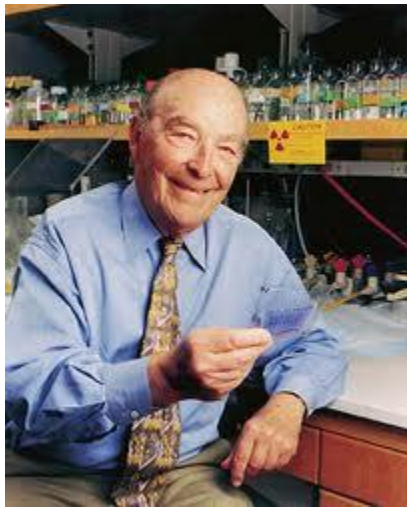
Nobelova nagrada 2006

Fire & Mello

Nobelova nagrada 2006

DNA —transcription→ **RNA** —translation→ **protein**

replication



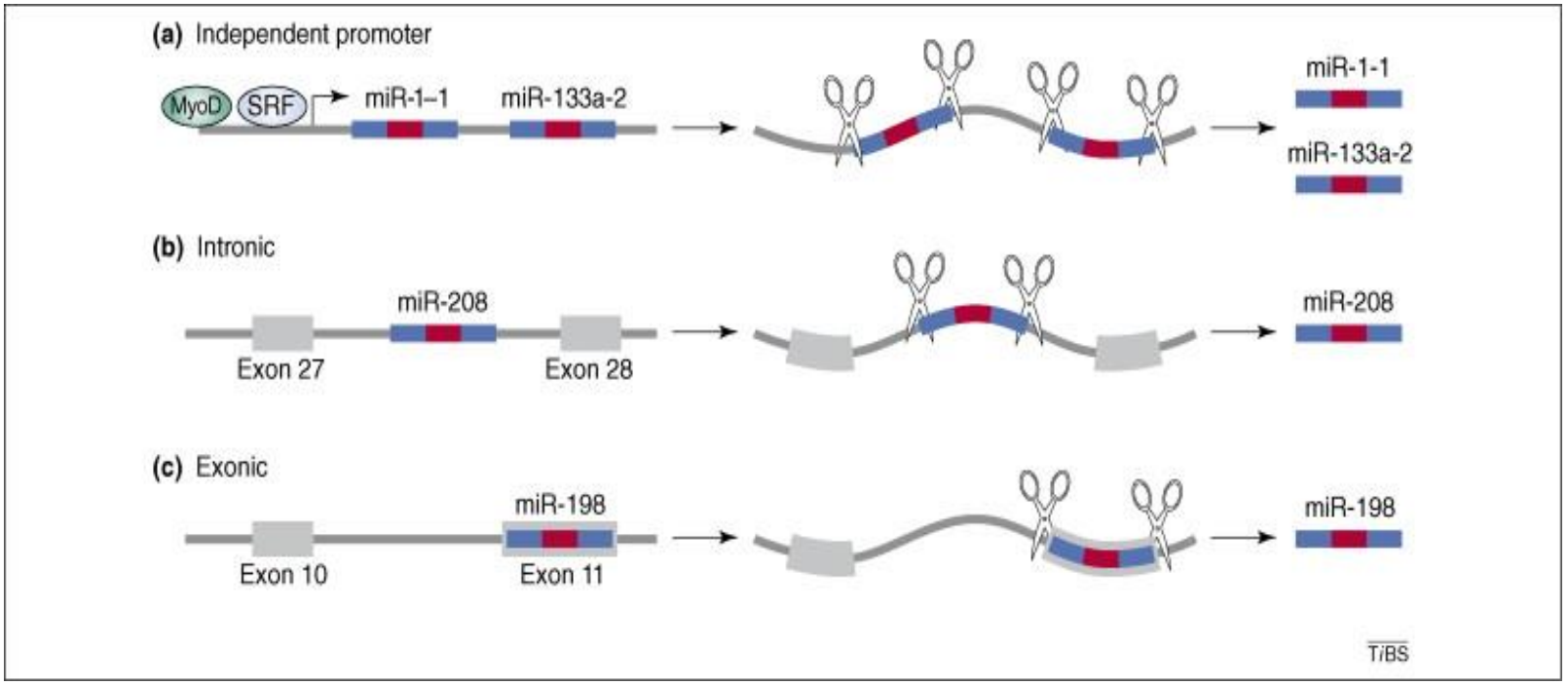
Arthur Kornberg

Nobelova nagrada 1959

"mehanizem biološke sinteze deoksiribonukleinske kisline (DNK)"

RNA interferenca ustavi translacijo mRNA tarčnih genov in vodi do utišanja izražanja teh genov.

Genomski viri miRNA



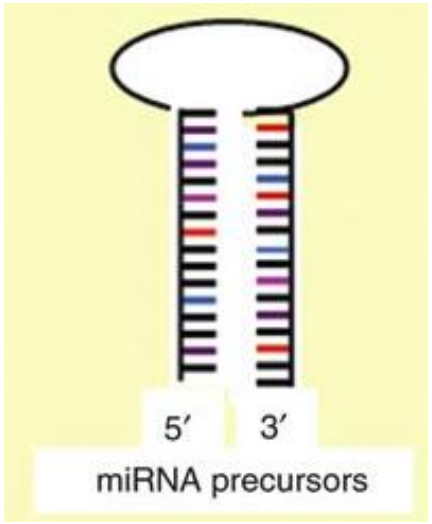
dx.doi.org/10.1016/j.tibs.2007.02.006

a) miRNA gen – lasten promotor. Npr. miR-1–1 in miR-133a-2, ki ju regulirata transkripcijska faktorja SRF in MyoD.

b) Intronski miRNA enako obrnjeni kot pre-mRNA v katerem se nahajajo. Npr. miR-208, je v intronu težke verige srčnega α-miozina – izražanje v srcu.

c) Večina eksonskih miRNA je v 5′ ali 3′ UTR. Npr. miR-198 pri mRNA za protein ‚follistatin-like 1‘

Nomenklatura miRNA (miRBase)



a) **mir**: nezrelo zaporedje (pre-miRNA). Npr. hsa-**mir**-203

b) **miR:** končno miRNA zaporedje. Npr. hsa-**miR**-203

- a/b: paralogni miR, razlika v 1-2 nucleotidih.

  Npr. hsa-miR-9**a**, hsa-miR-9**b**

- 1-2: Identični miR, različni viri.

  Npr. hsa-miR-19b-**1**, hsa-miR-19b-**2**

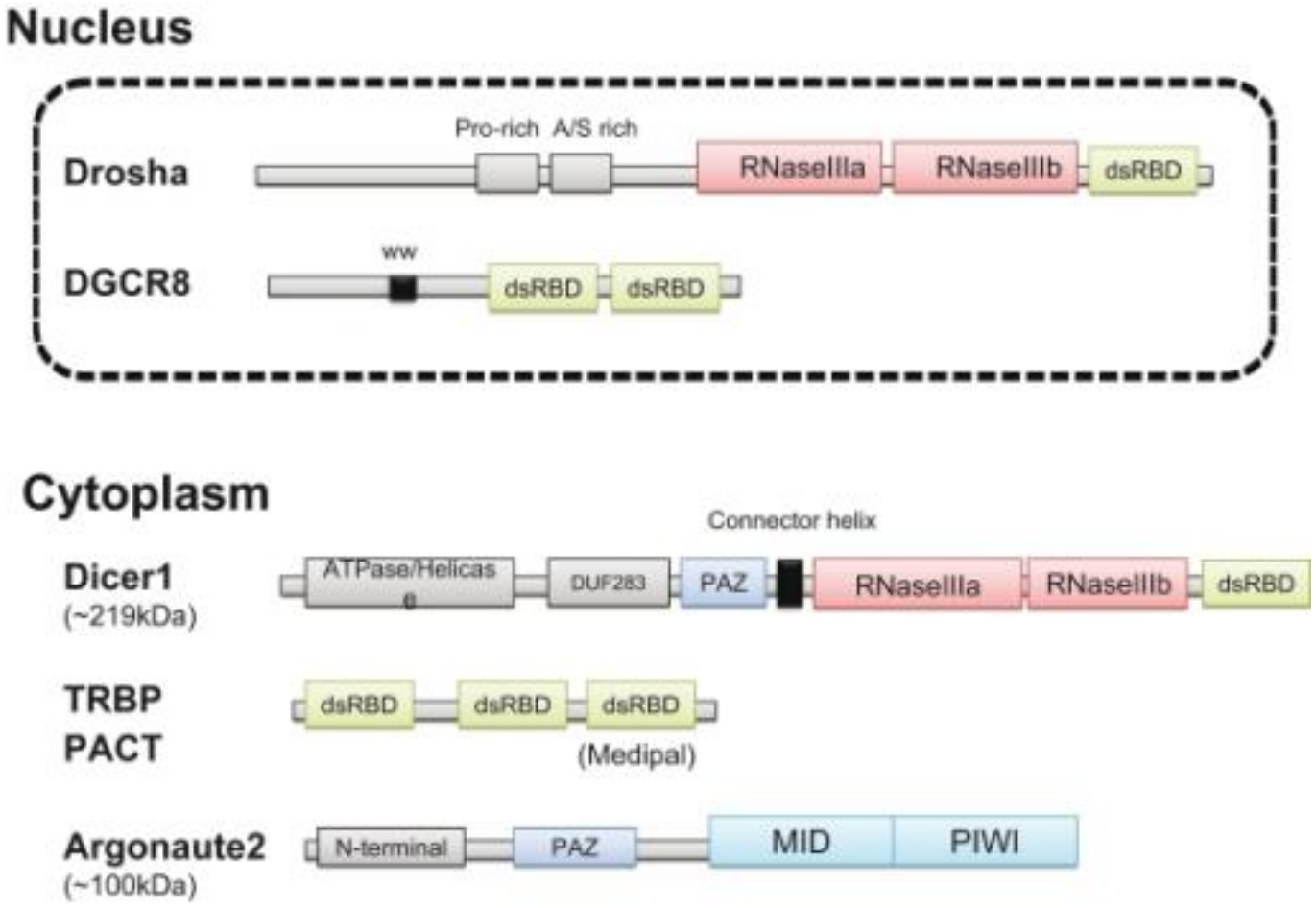
- 5p-3p: končnir miR izvira iz 5’ (ali 3’) konca.

  Npr. hsa-miR-17-**5p**

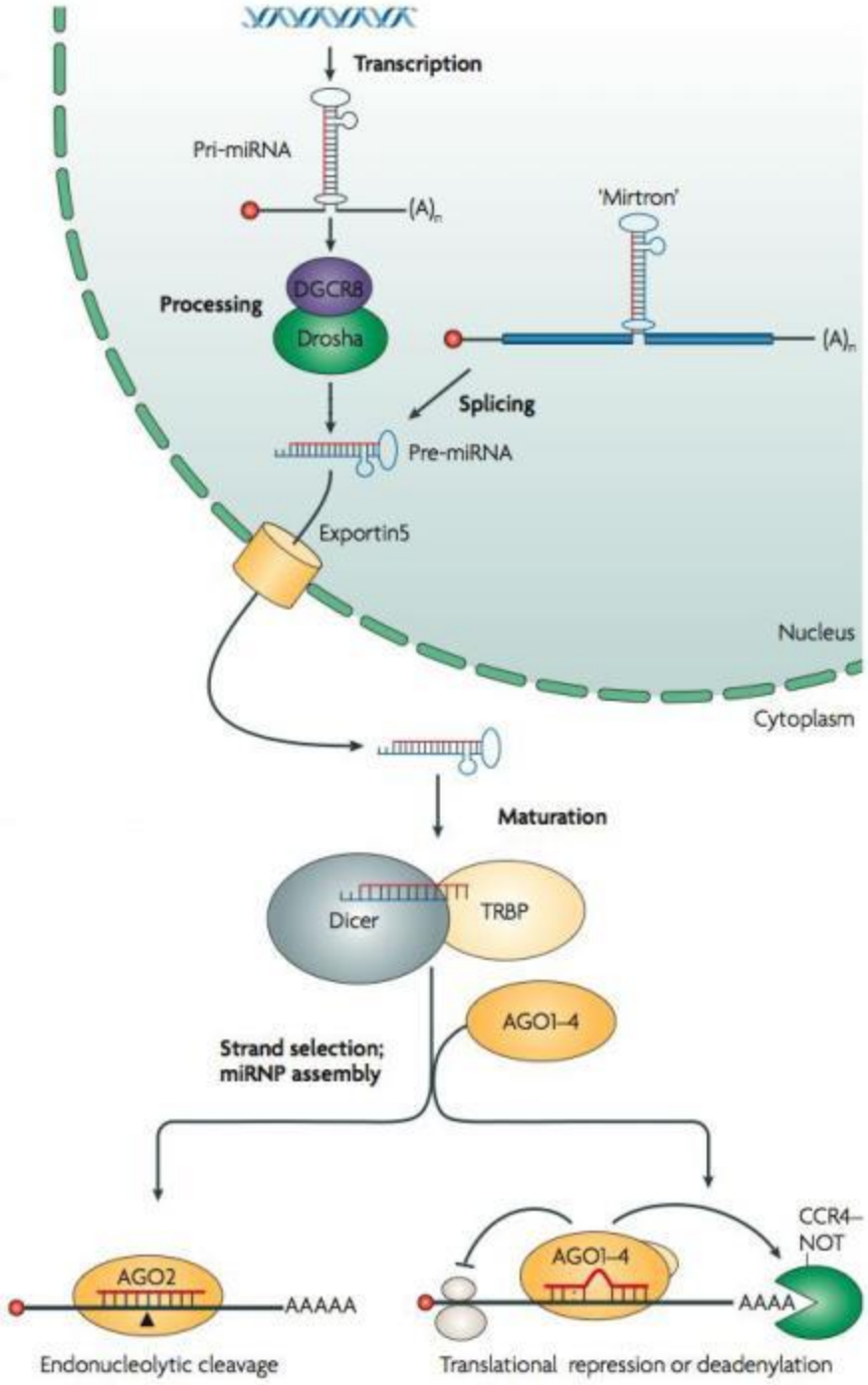
- *: Manj pomembno (sopotniško) zaporedje komplementarno končni miR. Npr. hsa-miR-33a*****

Biogeneza miRNA

Proteini biogeneze miRNA



Biogeneza miRNA



-V jedru pride do transkripcije primarnega zaporedja miRNA (pri-miRNA), ki je dolg nekaj kb. Vsebuje 5’ kapo in poli(A) rep.

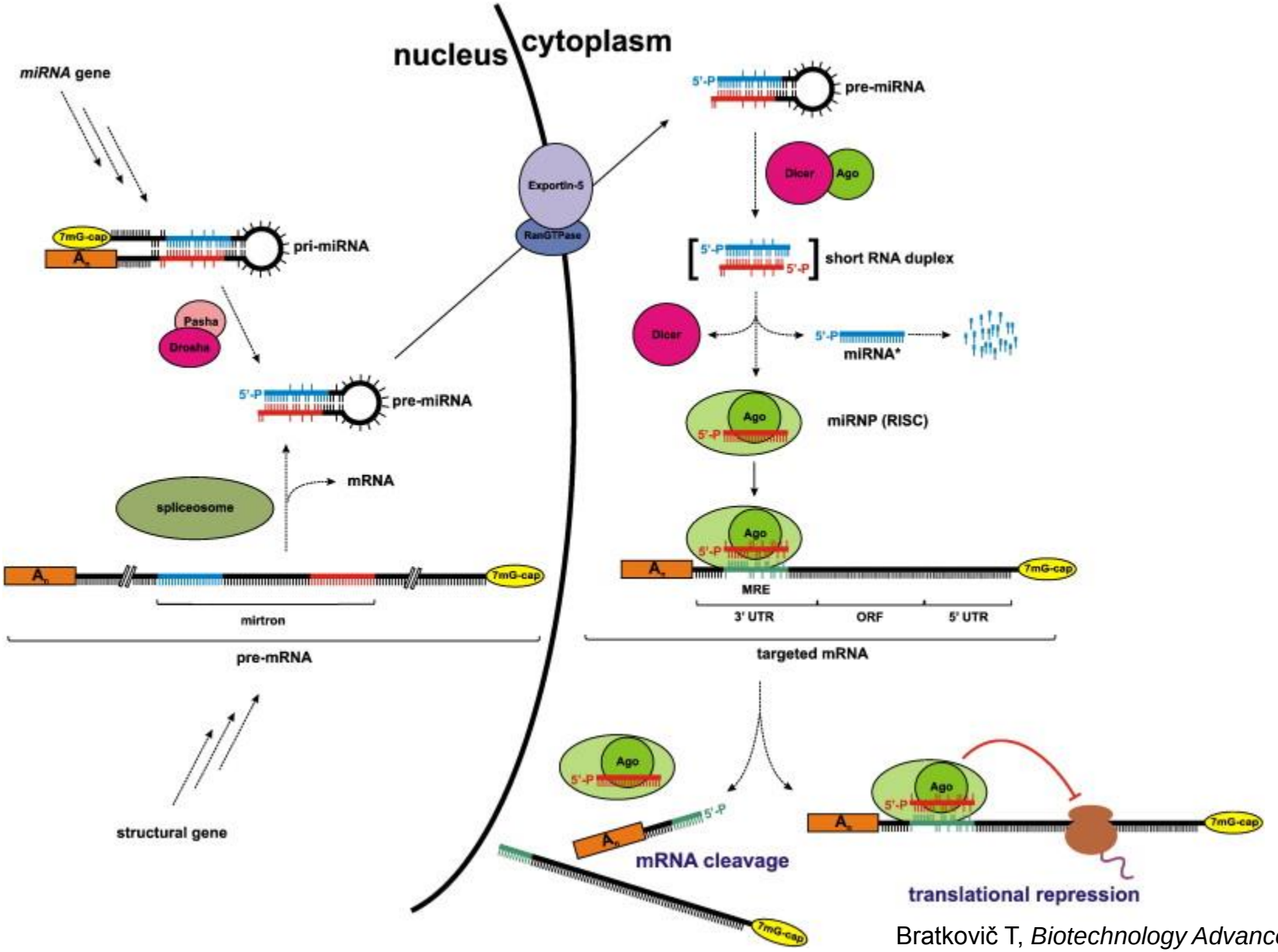
-Encim Drosha reže pri-miRNA v jedru. Dobimo vmesno 70nt dolgo pre-miRNA, ki ima obliko lasnice.

-Prenos pre-miRNA iz jedra v citoplazmo gre skozi jedrne pore s pomočjo proteina Exportin 5.

-V citoplazmi se lasnica cepi z encimom Dicer (RNase III). Dobimo 19-22 nt dolgo dsRNA.

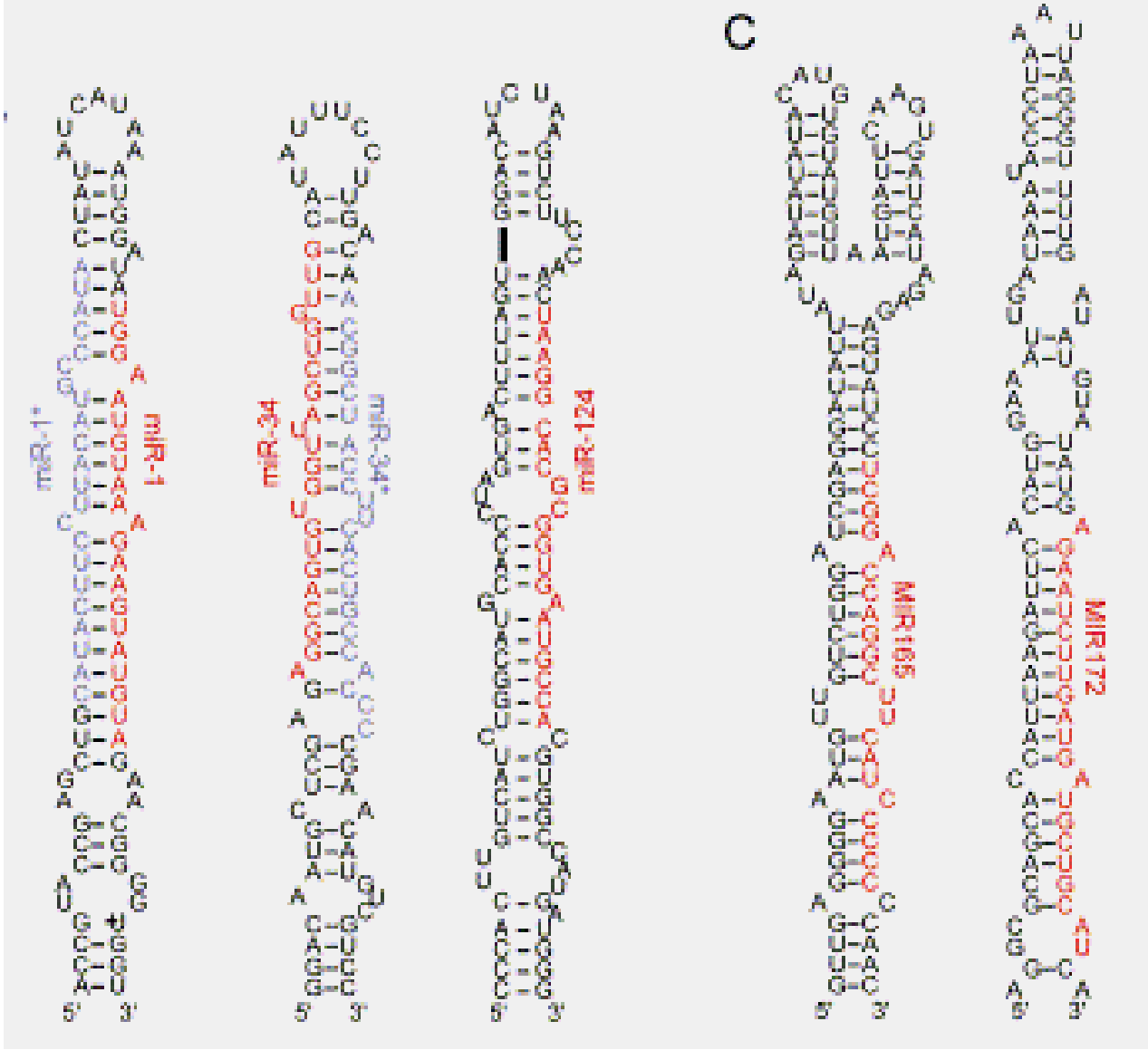
Filipowicz (2008) Nature Review Genetics 9:102-112.

Biogeneza miRNA

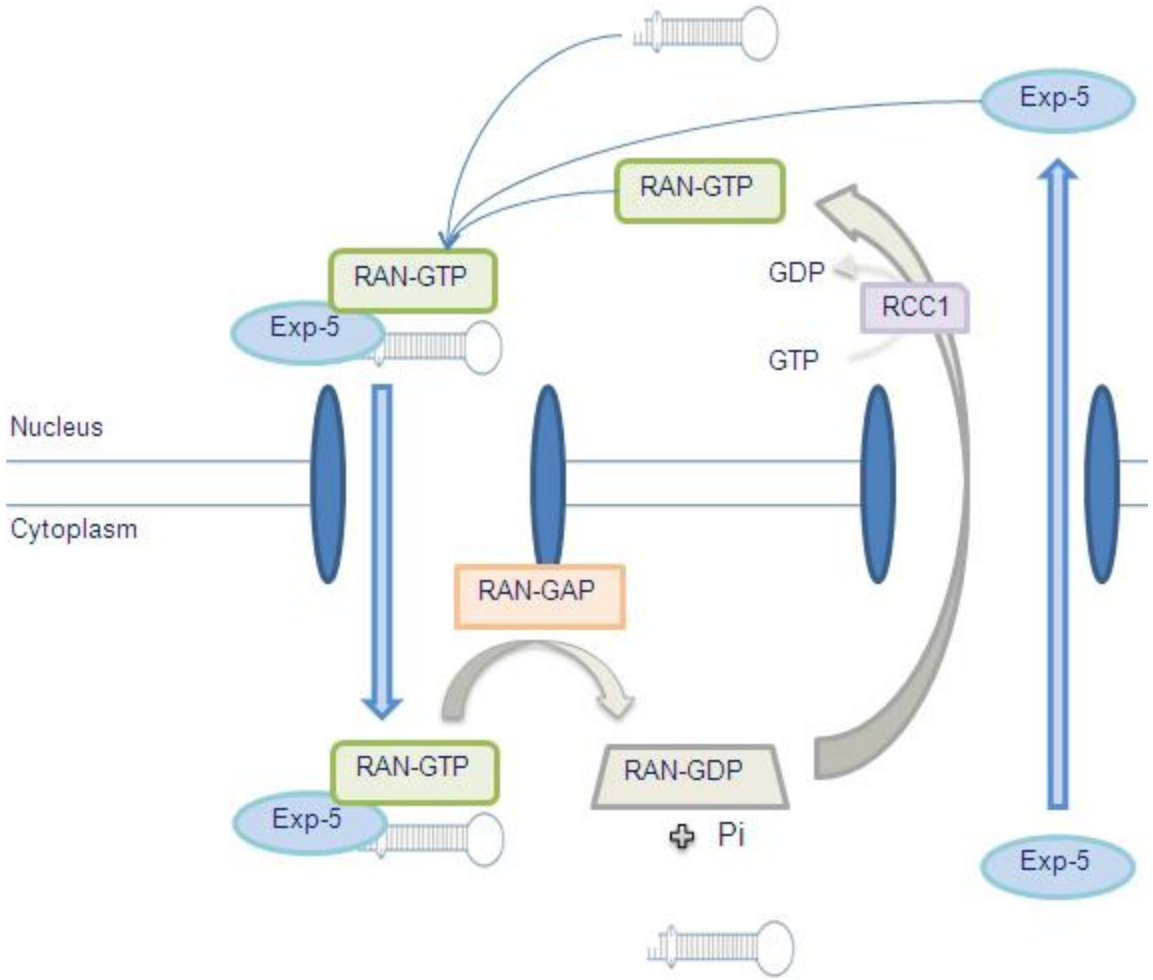


Bratkovič T, *Biotechnology Advances* 2012

Pre-miRNA tvorijo lasnične zanke



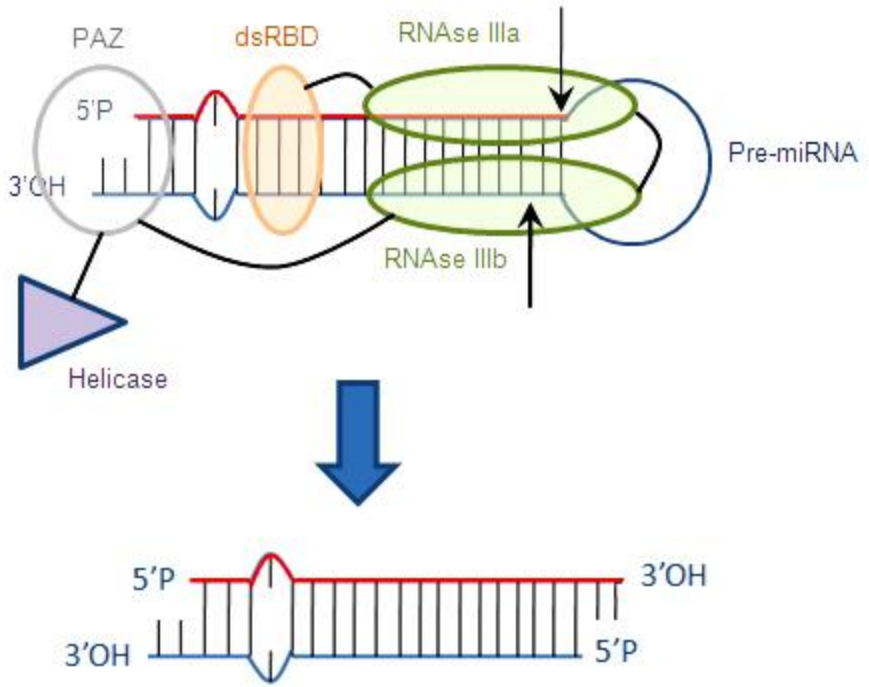
Prenos pre-miRNA v citoplazmo



- Exp-5/RAN-GTP/pre-miRNA kompleks gre v citoplazmo skozi jedrno poro.
- Hidroliza GTP na RAN-GTP z RAN-GAP sprosti pre-miRNA.
- Exp-5 in RAN potujeta nazaj v jedro.

DOI: http://dx.doi.org/10.2741/4068

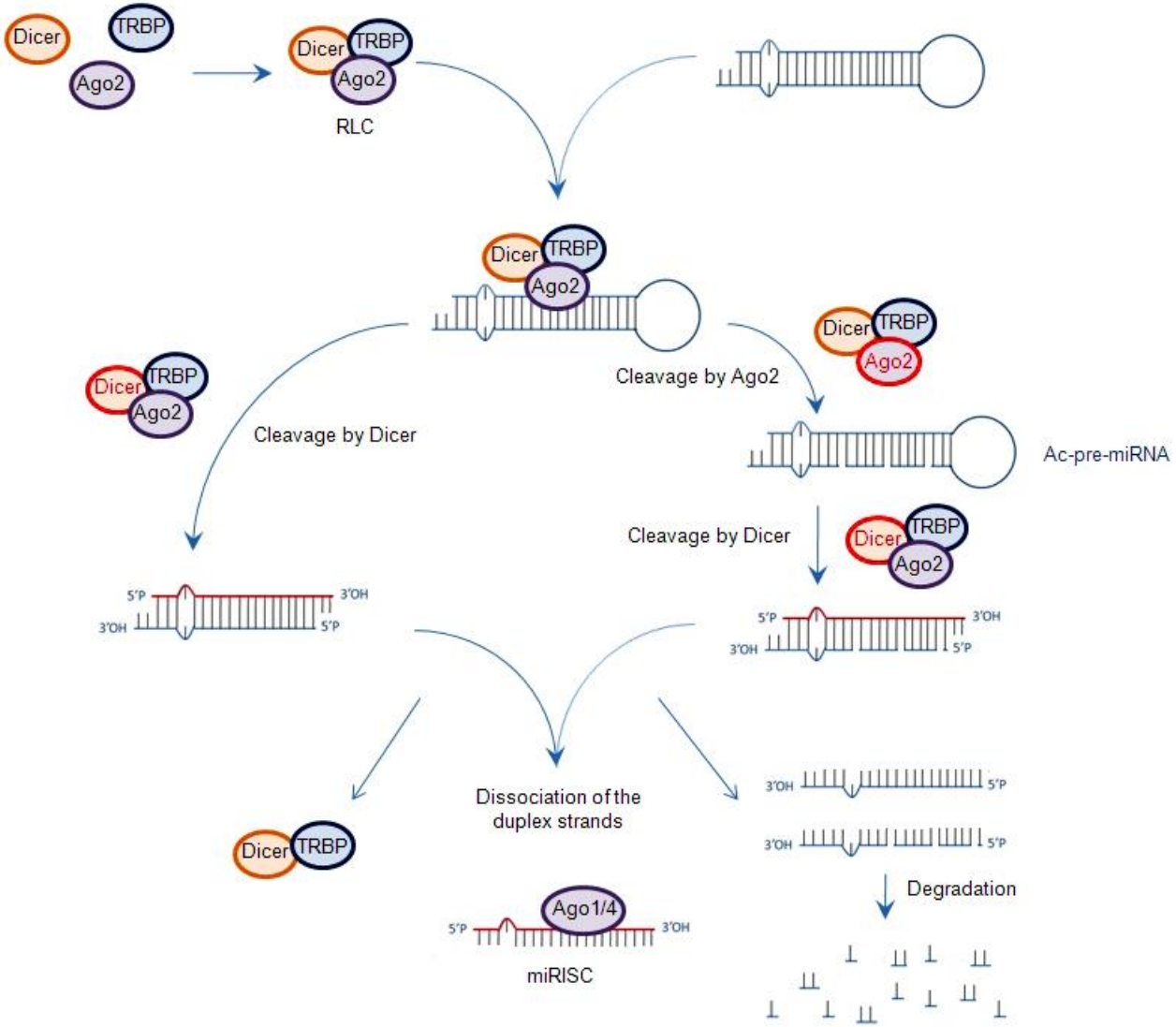
Procesiranje pre-miRNA



- PAZ domena Dicerja prepozna 2 nt dolg podaljšek na 3‘ delu pre-miRNA.
- dsRNA vezavna domena (dsRBD) omogoča natančno lokalizacijo obeh domen RNase III
- RNase III domeni odrežeta zanko.

DOI: http://dx.doi.org/10.2741/4068

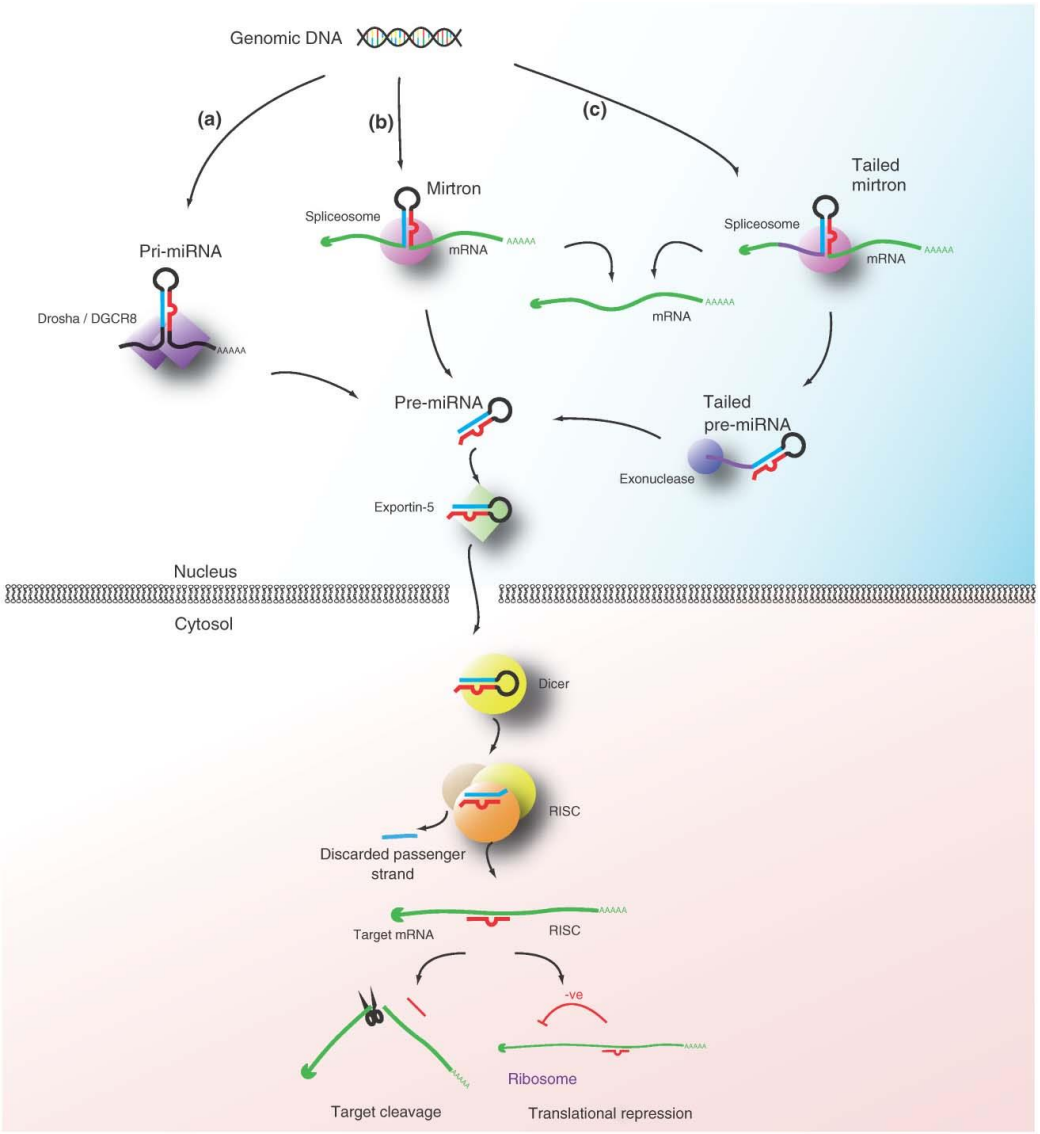
Tvorba mRISC



- RLC vsebuje Dicer, TRBP in Ago2.
- Dve poti tvorbe miRISC.
- Pri levi pride do disociacije dsRNA in vezave vodilne verige na Ago.
- Pri desni Ago2 reže komplementarno (sopotniško) verigo pred rezanjem z Dicerjem. Vmesna oblika je Ac-pre-miRNA (Ago2-clived pre-miRNA).

DOI: http://dx.doi.org/10.2741/4068

Mirtroni



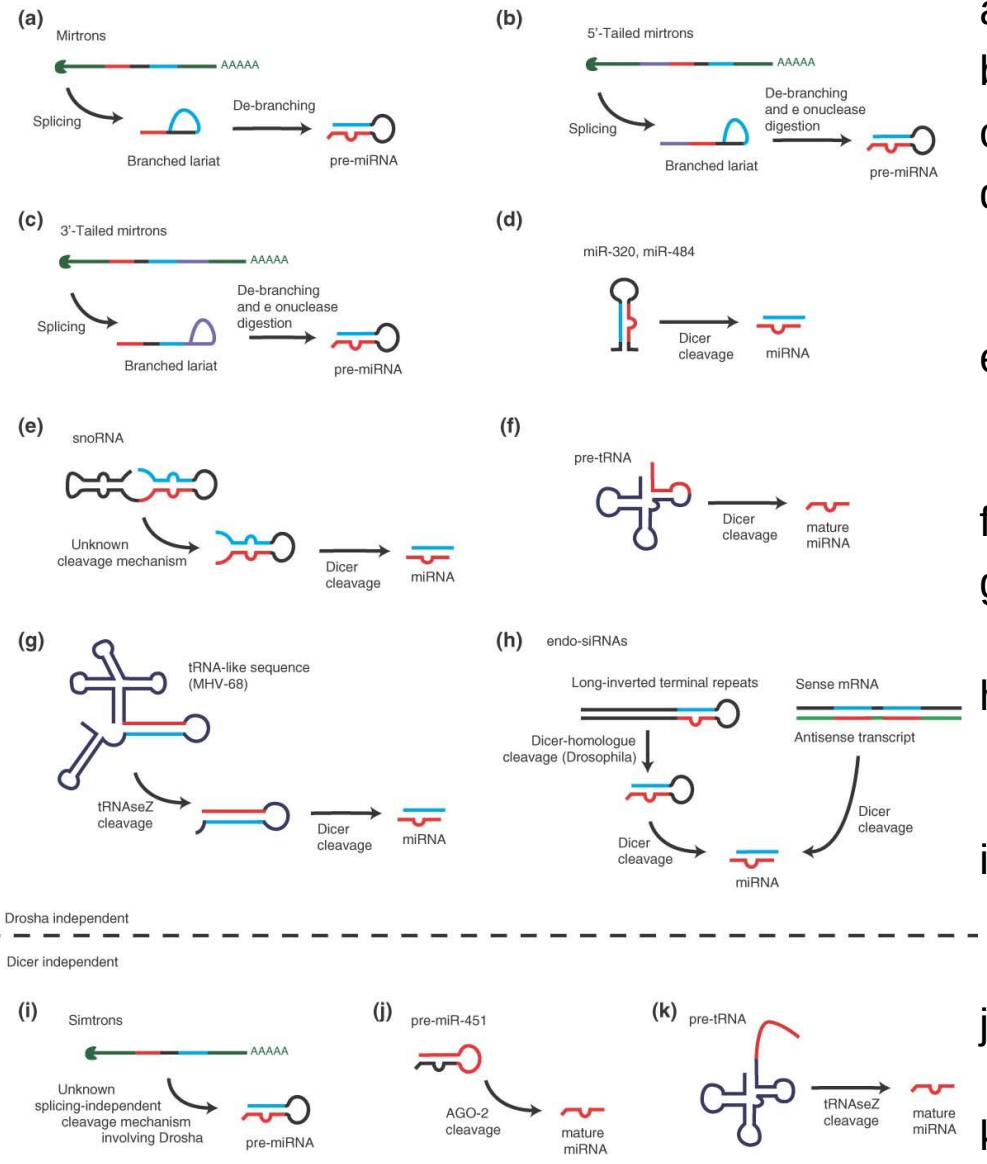
a) Standardna miRNA biogeneza.

b) Mirtronska biosinteza: lasnične zanke pre-miRNA se tvorijo pri izrezovanju intronov iz pre-mRNA in preskočijo Drosho.

c) Mirtroni z repom - izvirajo iz večjih intronov in imajo podaljšan rep na enem koncu (v tem primeru 5‘). Eksonukelaza reže konec. Mirtron nadaljuje v sintezo miRNA po standardni poti.

DOI: 10.1002/wrna.1122

Atipična biogeneza miRNA



a) Mirtroni kratkih intronov.

b) Mirtroni s 5‘ repom.

c) Mirtroni s 3‘ repom.

d) miR-320 in miR-484 pri-miRNA ne vsebujeta zaporedij, ki jih prepoznava Drosha.

e) Neke miRNA izvirajo iz snoRNA. Sekundarne strukture snoRNA so podobne Dicer substratom.

f) miRNA iz 3‘ koncev tRNA.

g) miRNA iz MHV68 virusa - struktura podobna tRNA.

h) Endogene siRNA (endo-siRNA). mRNA trankripti se lahko vežejo na protismiselne transkripte in tvorijo dsRNA.

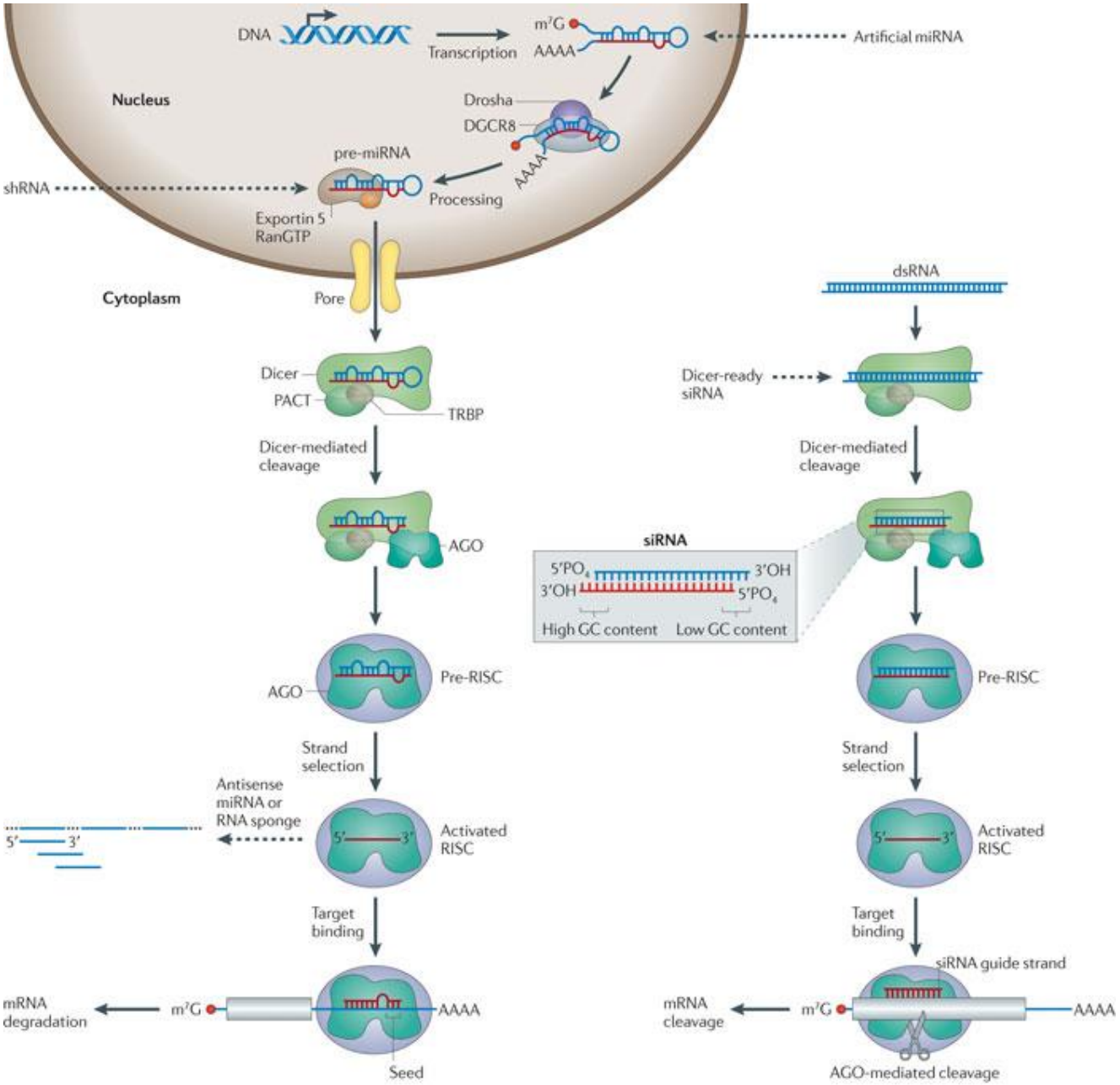
i) Simtroni (splicing independent mirtron-like) – potrebujejo protein Drosha, ne pa izrezovanja, DGCR8 ali Dicerja.

j) Pri-miR-45 je lasnična zanka krajša kot bi jo prepoznal Dicer. AGO2 izreže miRNA.

k) tRNAseZ lahko odreže 3′ konec tRNA prekurzorjev brez Dicerja.

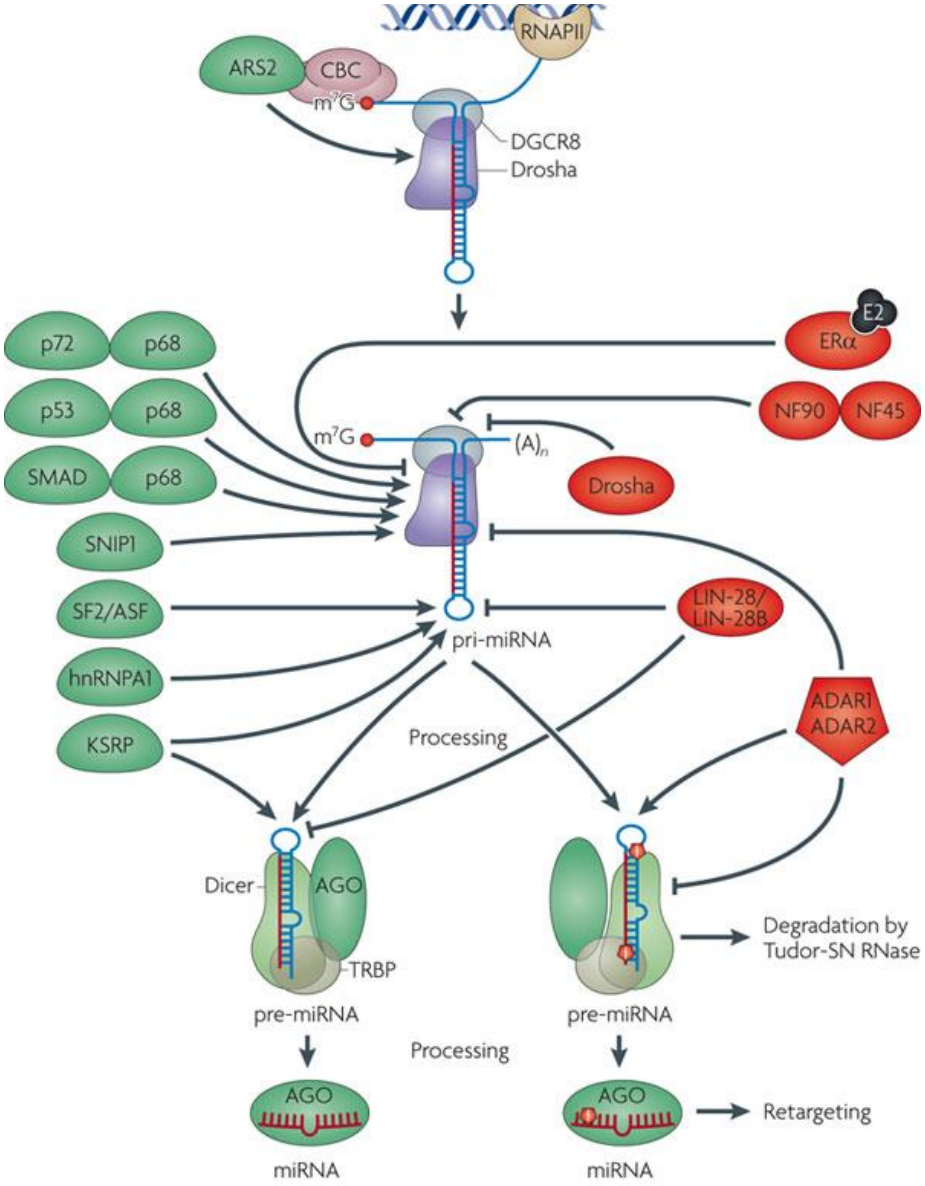
DOI: 10.1002/wrna.1122

siRNA in miRNA



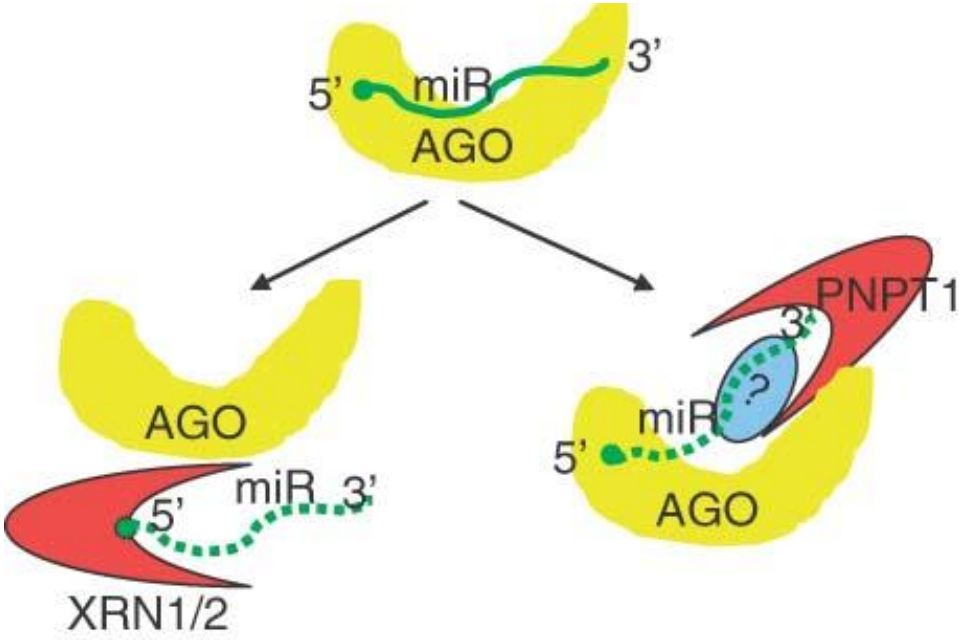
Nature Reviews | Genetics

Regulacija miRNA biogeneze



Nature Reviews | Genetics

Razgradnja miRNA

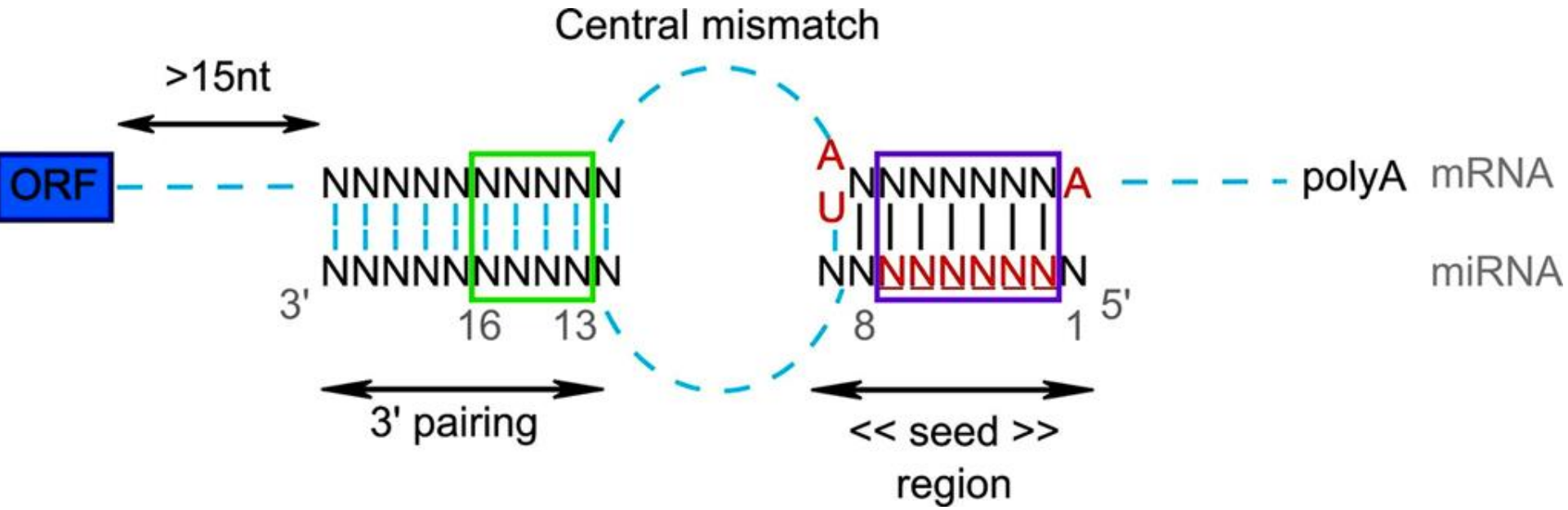


- miRNAs se lahko razgradijo z obeh koncev.
- Eksoribonukleazi XRN1 in XRN2 pomagata pri disocijaciji miRNA z miRISC in režeta miRNA v smeri 5′ → 3′.
- PNPT1 reže miRNA v smeri 3′ → 5′.

DOI: 10.1002/wrna.1114

Delovanje miRNA

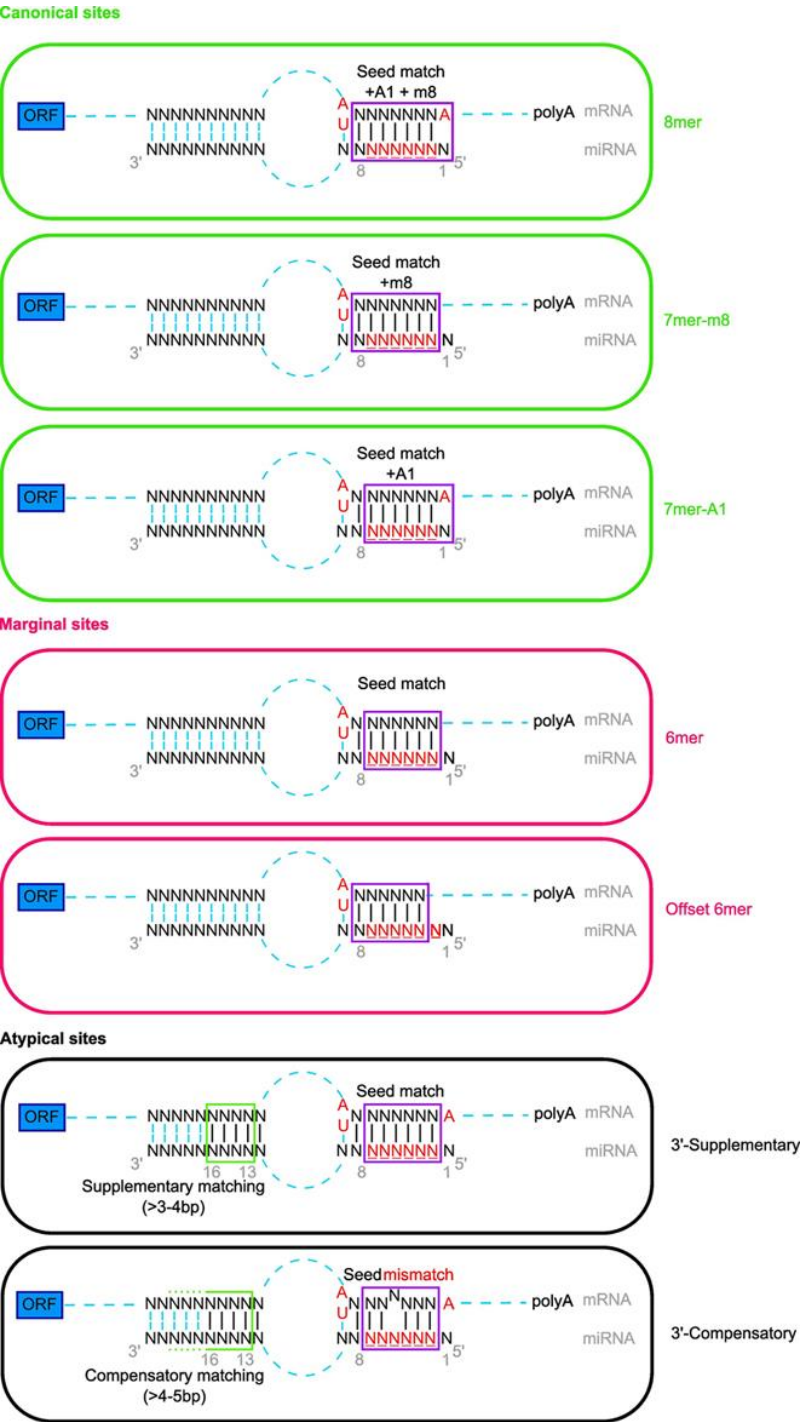
Interakcija med miRNA in tarčno mRNA



- Tarče miRNA se nahajajo predvsem na 3‘UTR mRNA.
- V ‚seed‘ delu (nt 2-7) pride do popolne komplementarnosti.
- Centralni nekomplementarni del onemogoči rezanje z Ago2 (tega ni pri siRNA).
- Za dobro stabilizacijo miRNA/mRNA dupleksa je potrebna komplementarnost nekaj nt v 3‘ koncu miRNA.
- Prisotnost A na poziciji 1 in/ali A ali U na pozicij 9 na mRNA poveča učinek miRNA.

DOI: http://dx.doi.org/10.2741/4068

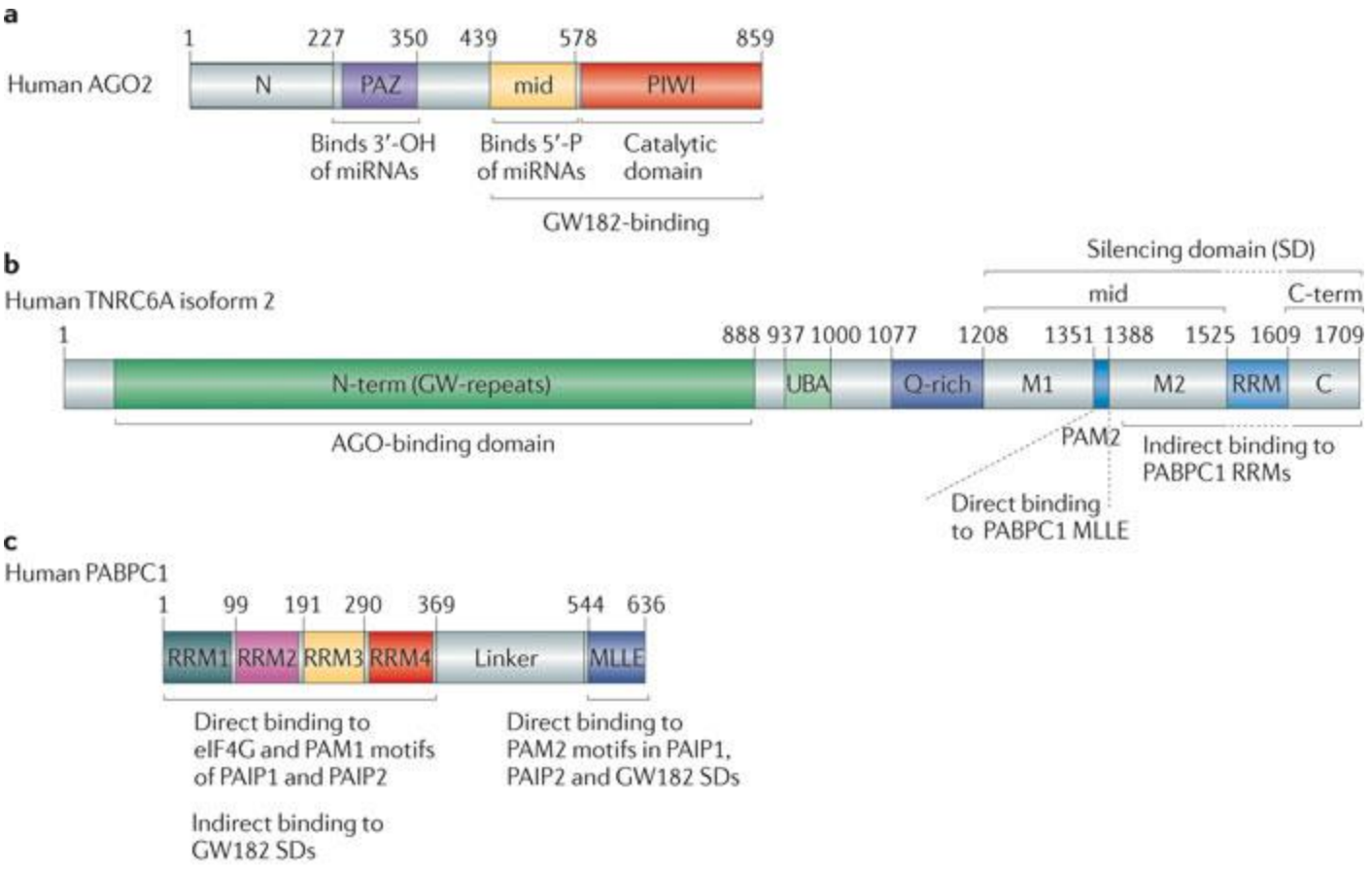
Kategorije tarčnih mest



- Standardna mesta
  - 7-8nt komplementarnost na 5‘ koncu miRNA (6 ‚seed‘ mest in mesta 1 in/ali 8).
- Marginalna mesta
  - 6 nt komplementarnost (‚seed‘ ali mesta 3-8).
- Atipična mesta
  - Komplemetarnost tudi na 3‘ koncu miRNA.

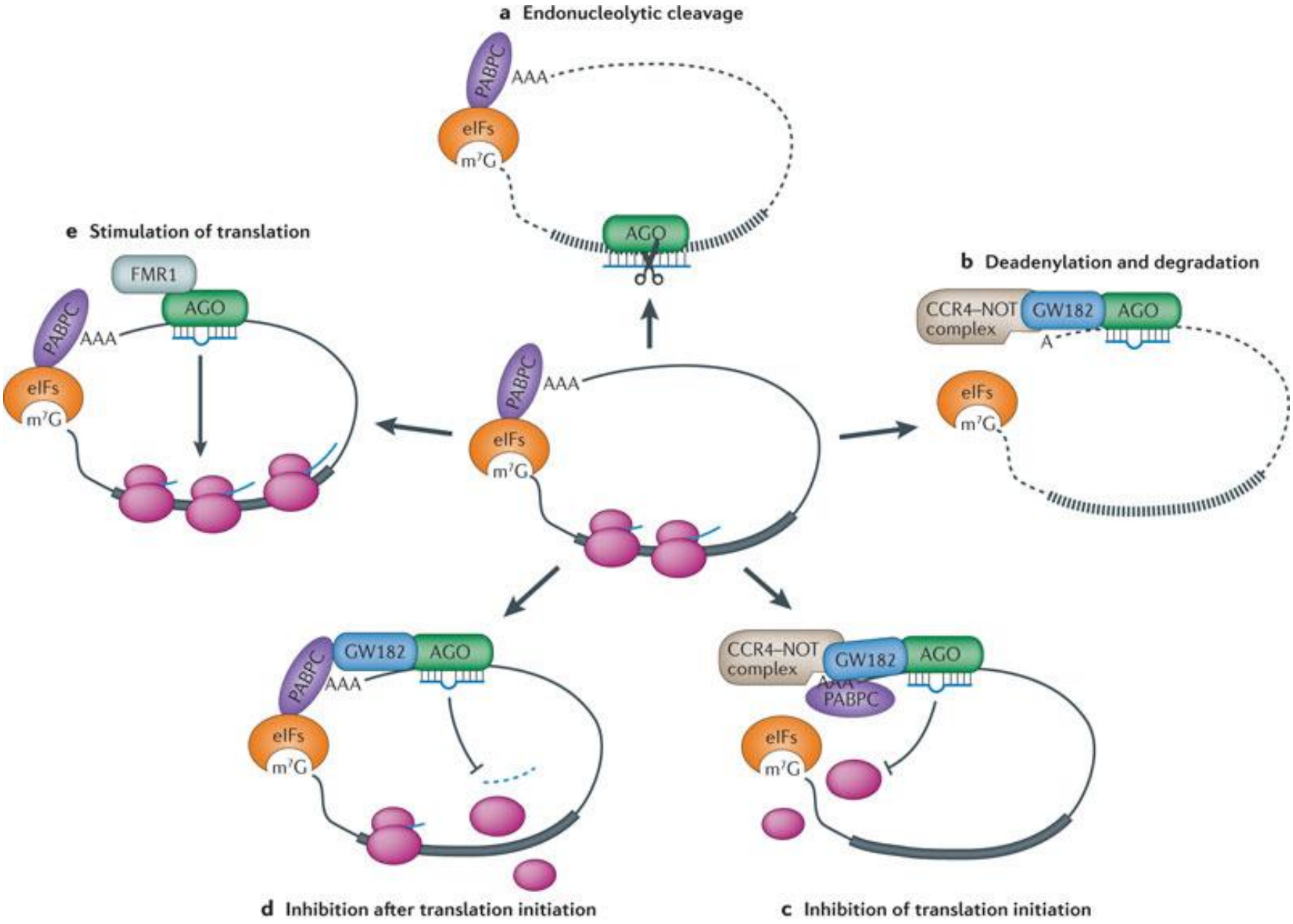
DOI: http://dx.doi.org/10.2741/4068

Proteini mRISC kompleksa

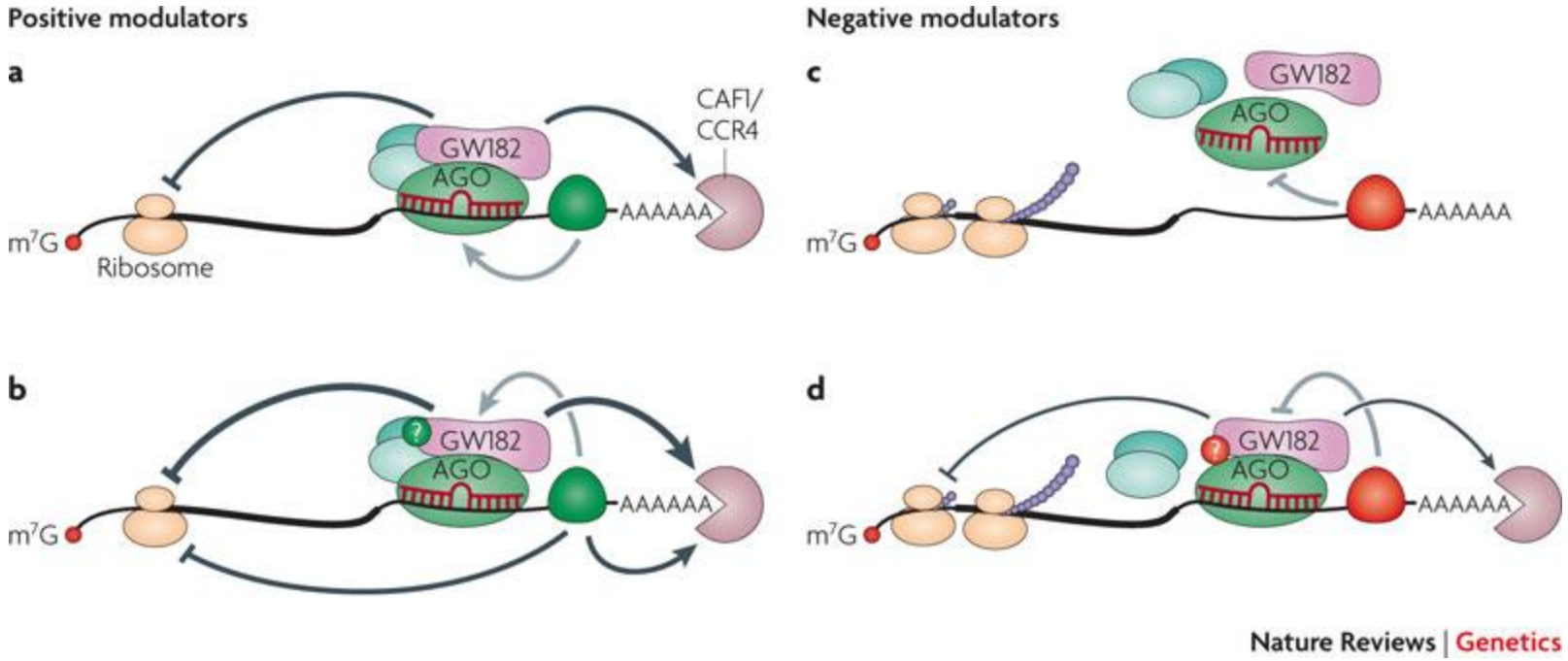


Nature Reviews | Genetics

Načini uravnavanja izražanja z miRNA



RBP uravnavajo aktivnost miRISC



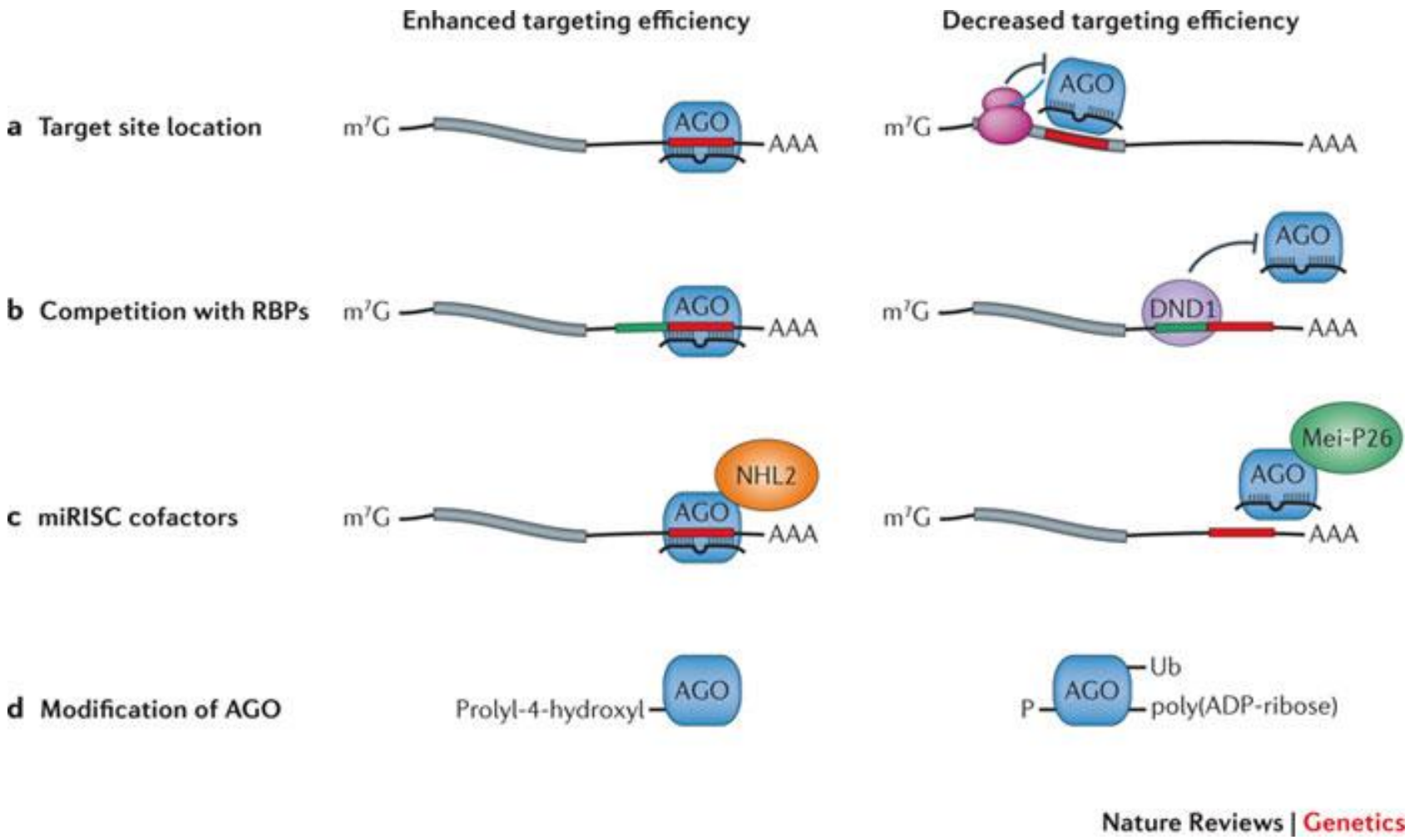
a) RBP lahko povečajo utišanje. Npr. FMRP pomaga in stabilizira vezavo miRISC.

b) Do povečanega utišanja lahko pride tudi s pojačanjem vpliva miRISC.

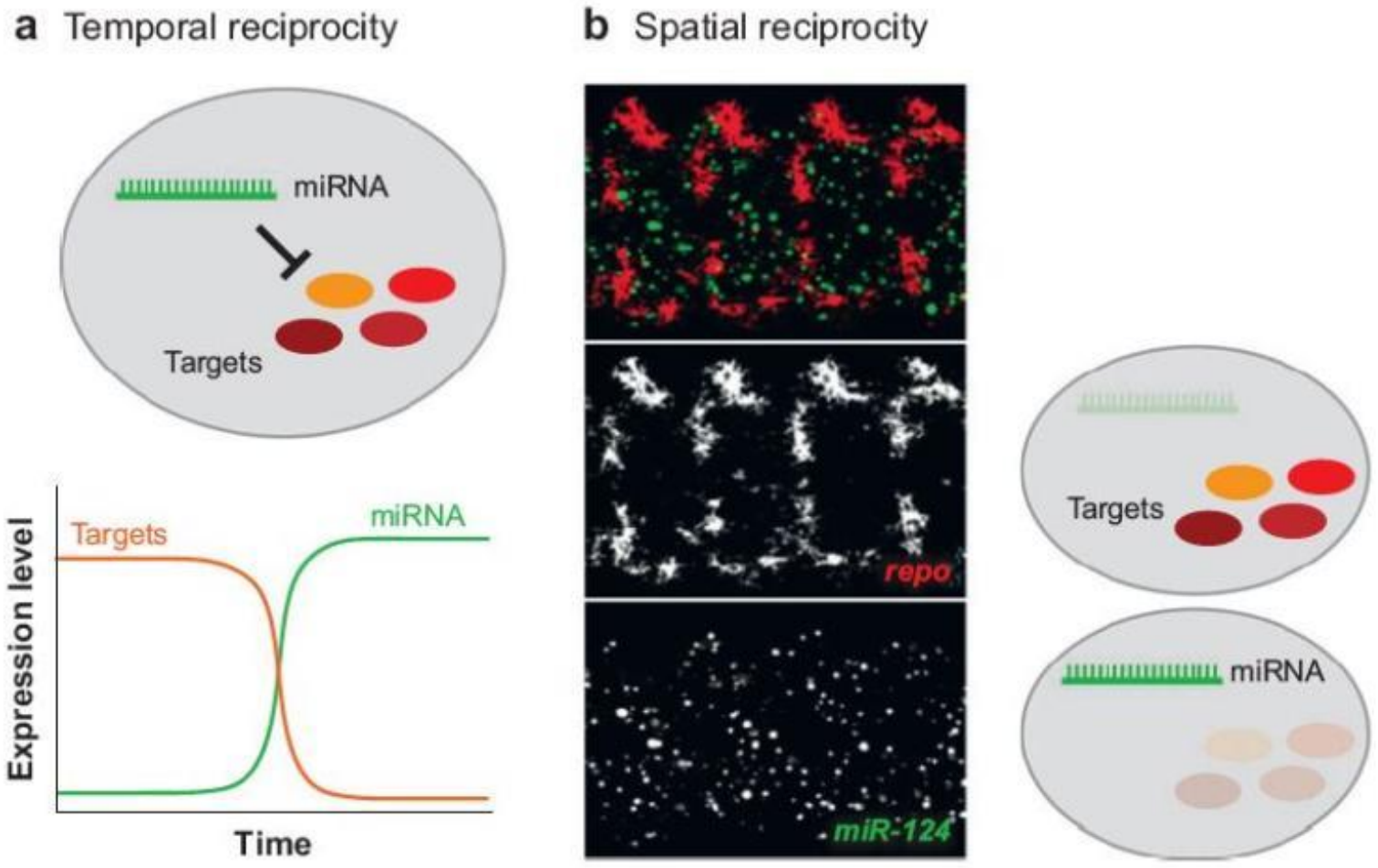
c) RBP lahko zmanjšajo utišanje z inhibicijo vezave miRISC na tračno mesto. Npr. DND1 in HuR.

d) Do zmanjšanja utišanja lahko pride tudi z zmanjšanjem vpliva miRISC.

Drugi dejavniki, ki uravnavajo aktivnost miRISC



Izražanje miRNA ima časovno in prostorsko odvisnost z izražanjem tarče

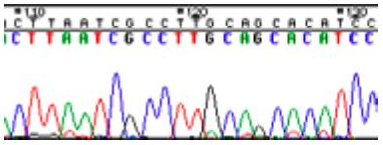


Annu. Rev. Cell Dev. Biol. 2007.23:175-205

Detekcija miRNA

Detekcija miRNA

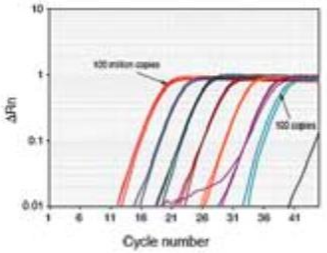
Cloning of miRNAs



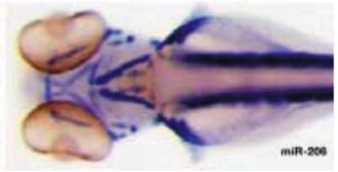
miRNA microarrays

Northern blot analysis

real time RT-PCR analysis



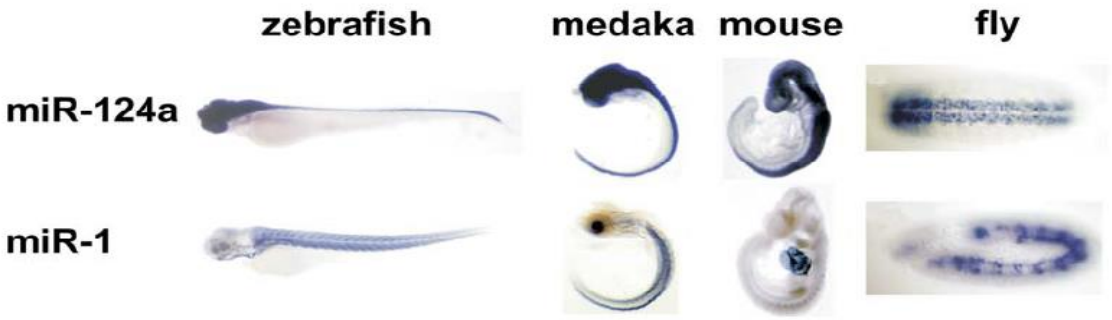
In situ hybridization



Plasterk, 2006 Cell **124**:877-881.

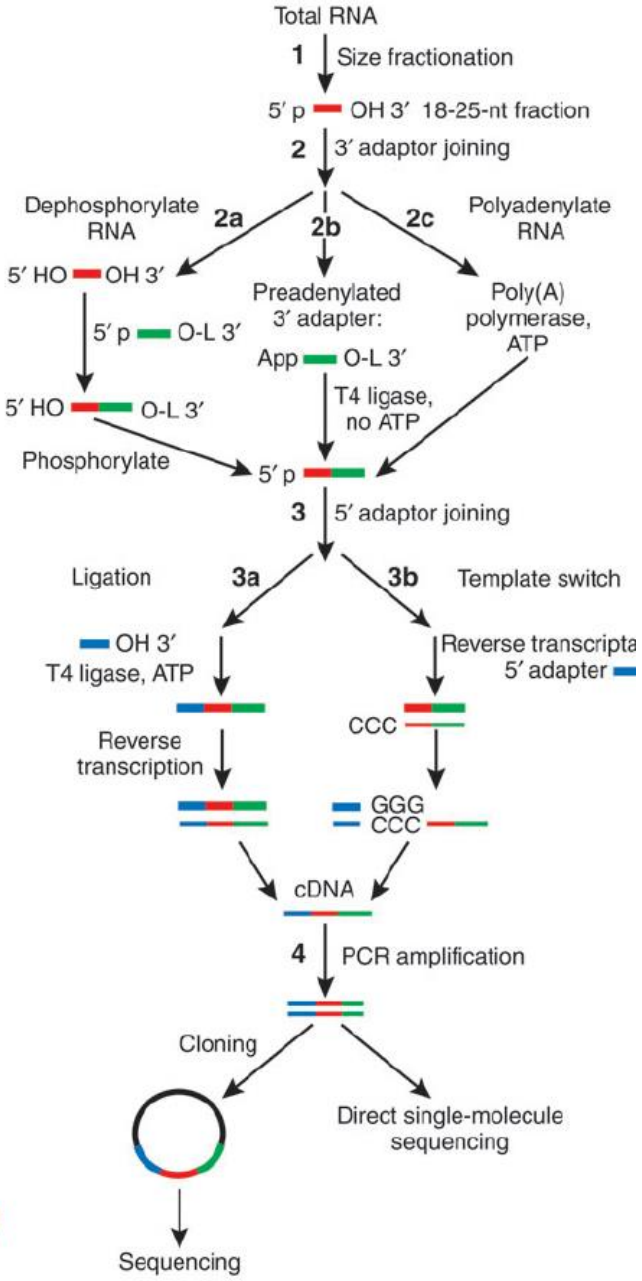
Kako odkrijemo miRNA gene?

- Biološki pristop
  - Kloniranje malih miRNA in identifikacija novih.
- Večina miRNA je tkivno specifična.



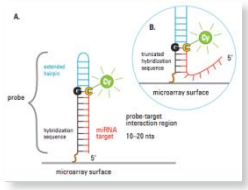
miR-124a se izraža samo v možganih in hrbtenjači.

miR-1 je v mišicah in srcu.



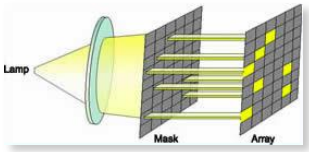
miRNA mikromreže

Agilent



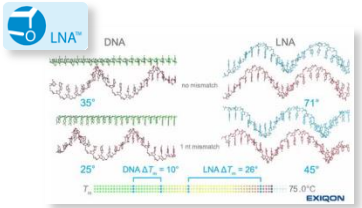
Human, rat, mouse

AFFYMETRIX®



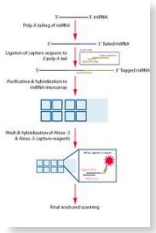
Human, rat, mouse,
dog, chimpanzee, etc

EXIQON



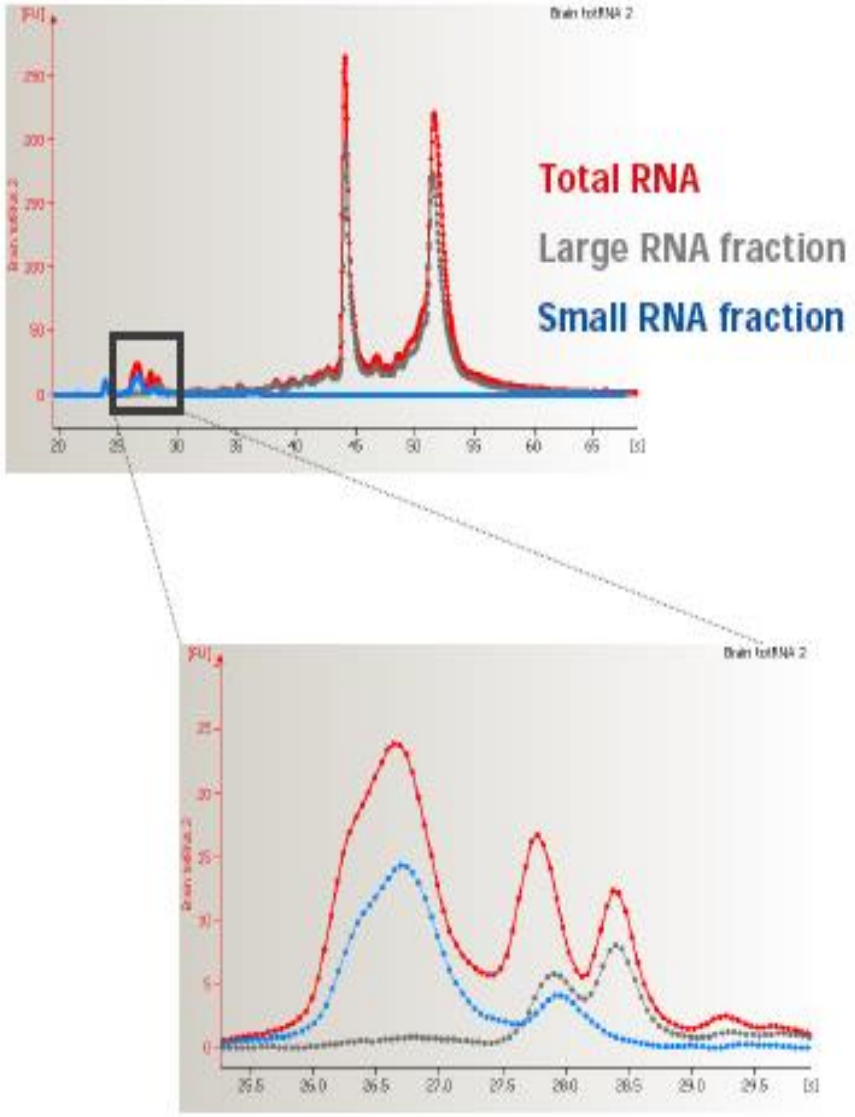
Human, rat, mouse

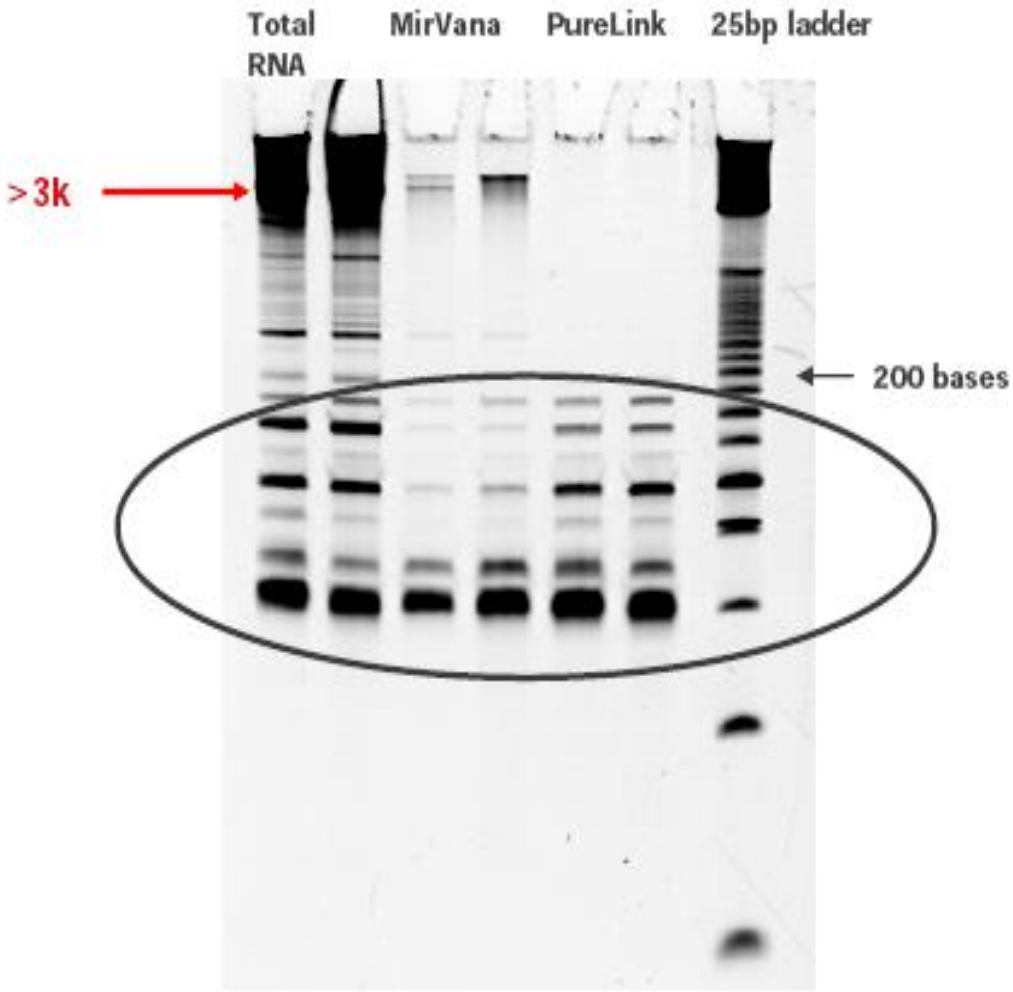
invitrogen™



Human, multispecie

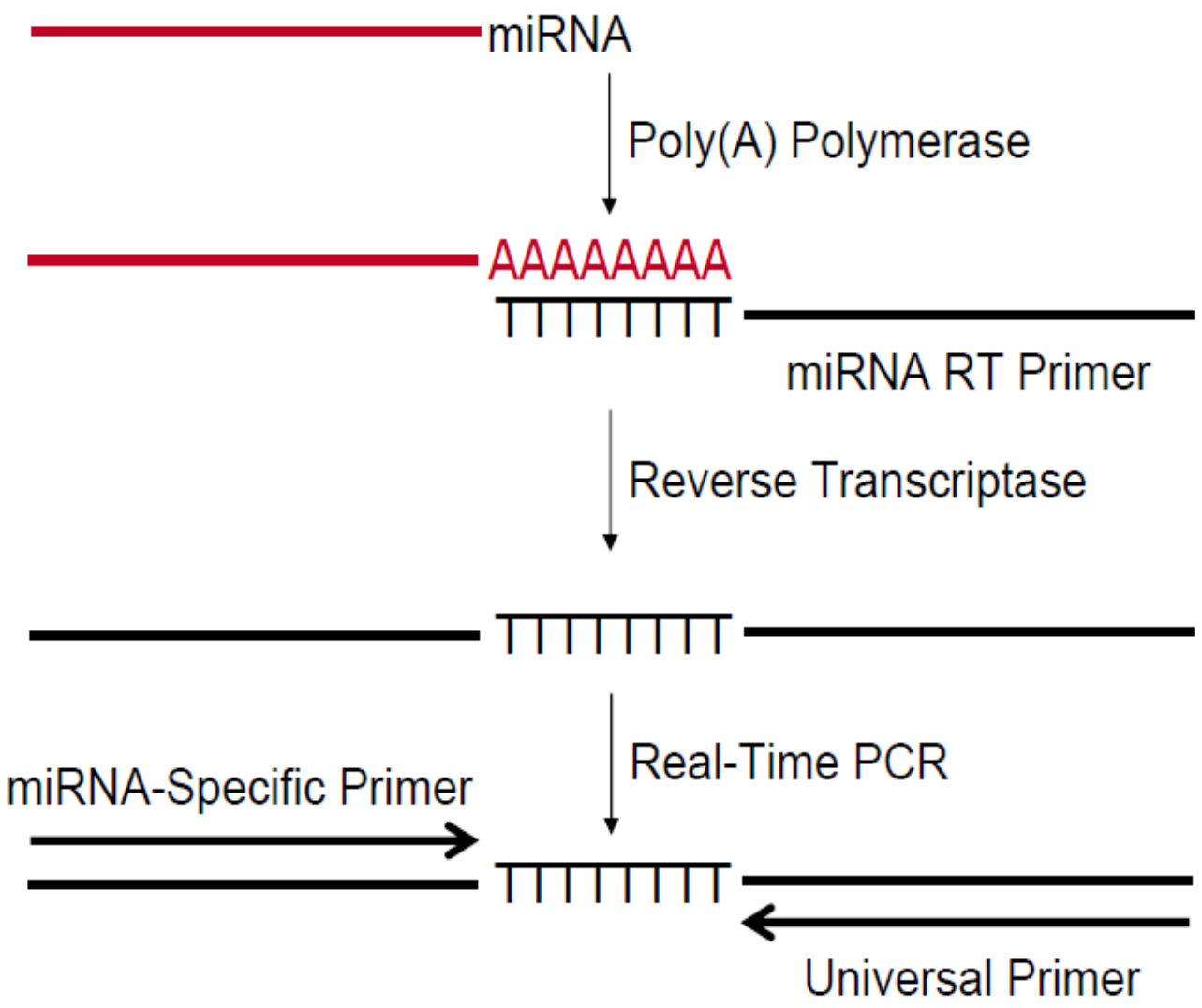
Analiza kvalitete miRNA





miRNA Q-PCR

Same cDNA preparation can assay ANY miRNA



How the miRNA PCR Array Works

Control & Experimental Cells or Tissue

↓

1. Isolate miRNA (MA-01)

↓

2. Reverse Transcription: RT^2 miRNA First Strand Kit (MA-03)

↓

3. Setting Up the PCR Array
Instrument-Specific RT^2 qPCR SYBR Green Master Mix & cDNA Cocktail Aliquot across PCR Array

↓

4. Real-Time PCR
(40 cycles, 100-120 min)

↓

5. Data Analysis: Determine Fold Changes ($\Delta\Delta C_t$ Method)

Podatkovne baze miRNA

miRBase

miRBase vsebuje dva dela:

- miRBase Sequences – je baza vseh objavljenih miRNA, njihovih genomskih lokacij in drugih podatkov.

- miRBase Registry – zaupni portal, ki dodeli imena novim miRNA genom pred objavo njihovega odkritja.

miRBase

Latest miRBase blog posts

High confidence microRNAs By sam (March 4, 2014)

I promised a while ago to provide more information about the miRBase website features associated with the new "high confidence" microRNA set, as described in our 2014 NAR paper. First, some background. Here is a figure showing the growth of the number of sequences deposited in miRBase, and the number of papers in Pubmed that [...]

Website down time, Feb 4th, 8-10am GMT By sam (January 30, 2014)

Due to some essential network maintenance, the miRBase website is at risk of short periods of down time between 8 and 10am GMT on Tuesday 4th Feb. We apologise for any inconvenience.

miRNA count: 24521 entries

Release 20: June 2013

Search by miRNA name or keyword

Go Example

Download published miRNA data

Download page | FTP site

This site is featured in:

NetWatch - *Science* 303:1741 (2004)

Highlights, Web watch - *Nature Reviews Genetics* 5:244 (2004)

miRBase: the microRNA database

miRBase provides the following services:

- The miRBase database is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for searching and browsing, and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also available for download.
- The miRBase Registry provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the help pages for more information about the naming service.

To receive email notification of data updates and feature changes please subscribe to the miRBase announcements mailing list. Any queries about the website or naming service should be directed at mirbase@manchester.ac.uk.

miRBase is hosted and maintained in the Faculty of Life Sciences at the University of Manchester with funding from the BBSRC, and was previously hosted and supported by the Wellcome Trust Sanger Institute.

References

If you make use of the data presented here, please cite the following articles in addition to the primary data sources:

miRBase: annotating high confidence microRNAs using deep sequencing data.
Kozomara A, Griffiths-Jones S.
NAR 2014 42:D68-D73

miRBase: integrating microRNA annotation and deep-sequencing data.
Kozomara A, Griffiths-Jones S.
NAR 2011 39:D152-D157

http://www.mirbase.org/

Pregled po vrstah

2013

- Mammalia
  - Carnivora
    - Canis familiaris (323 precursors, 289 mature) [CanFam2]
  - Laurasiatheria
    - Equus caballus (341 precursors, 360 mature) [EquCab2]
  - Metatheria
    - Monodelphis domestica (156 precursors, 145 mature) [MonDom5]
    - Macropus eugenii (3 precursors, 3 mature)
    - Sarcophilus harrisii (67 precursors, 65 mature) [DEVIL7_0]
  - Primates
    - Atelidae
      - Ateles geoffroyi (60 precursors, 54 mature)
      - Lagothrix lagotricha (48 precursors, 45 mature)
    - Cebidae
      - Saguinus labiatus (42 precursors, 40 mature)
    - Cercopithecidae
      - Macaca mulatta (535 precursors, 544 mature) [MMUL1.0]
      - Macaca nemestrina (74 precursors, 70 mature)
      - Pygathrix bieti (11 precursors, 9 mature)
    - Hominidae
      - Gorilla gorilla (322 precursors, 317 mature) [GorGor3]
      - Homo sapiens (1600 precursors, 2042 mature) [GRCh37.p5]
      - Pan paniscus (88 precursors, 83 mature)
      - Pongo pygmaeus (633 precursors, 651 mature) [PPYG2]
      - Pan troglodytes (655 precursors, 580 mature) [PanTro2.1.4]
      - Symphalangus syndactylus (11 precursors, 10 mature)
    - Lemuridae
      - Lemur catta (16 precursors, 15 mature)
  - Prototheria
    - Ornithorhynchus anatinus (337 precursors, 530 mature) [OANA5]
  - Rodentia
    - Cricetulus griseus (200 precursors, 307 mature)
    - Mus musculus (855 precursors, 1281 mature) [GRCm38]
    - Rattus norvegicus (446 precursors, 723 mature) [RGSC3.4]
  - Ruminantia
    - Bos taurus (766 precursors, 755 mature) [UMD3.1]
    - Ovis aries (55 precursors, 103 mature)
  - Suina
    - Sus scrofa (271 precursors, 306 mature) [Sscrofa9]

2014

- Vertebrata
  - Anolis carolinensis (282 precursors, 416 mature) [AnoCar2.0]
  - Agnathostomata
    - Petromyzon marinus (244 precursors, 302 mature) [Petromyzon_marinus-7.0]
  - Amphibia
    - Xenopus laevis (22 precursors, 21 mature)
    - Xenopus tropicalis (189 precursors, 175 mature) [JGI4.2]
  - Aves
    - Gallus gallus (734 precursors, 996 mature) [Gallus-gallus-4.0]
    - Taeniopygia guttata (246 precursors, 334 mature) [taeGlu3.2.4]
  - Mammalia
    - Carnivora
      - Canis familiaris (324 precursors, 291 mature) [CanFam3.1]
    - Laurasiatheria
      - Artibeus jamaicensis (19 precursors, 19 mature)
      - Equus caballus (341 precursors, 360 mature) [EquCab2.0]
    - Metatheria
      - Monodelphis domestica (460 precursors, 767 mature) [MonDom5]
      - Macropus eugenii (3 precursors, 3 mature)
      - Sarcophilus harrisii (66 precursors, 64 mature) [DEVIL7_0]
    - Primates
      - Atelidae
        - Ateles geoffroyi (60 precursors, 54 mature)
        - Lagothrix lagotricha (48 precursors, 45 mature)
      - Cebidae
        - Saguinus labiatus (42 precursors, 40 mature)
      - Cercopithecidae
        - Macaca mulatta (615 precursors, 912 mature) [MMUL1.0]
        - Macaca nemestrina (74 precursors, 70 mature)
        - Pygathrix bieti (11 precursors, 9 mature)
      - Hominidae
        - Gorilla gorilla (322 precursors, 317 mature) [GorGor3]
        - Homo sapiens (1872 precursors, 2578 mature) [GRCh37.p5]
        - Pan paniscus (88 precursors, 83 mature)
        - Pongo pygmaeus (634 precursors, 653 mature) [PPYG2]
        - Pan troglodytes (656 precursors, 582 mature) [PanTro2.1.4]
        - Symphalangus syndactylus (11 precursors, 10 mature)
      - Lemuridae
        - Lemur catta (16 precursors, 15 mature)

Človeške miRNA

Homo sapiens miRNAs (1600 sequences)

ID	Accession	RPM	Chromosome	Start	End	Strand	Fetch
hsa-let-7a-1	MI0000060	1.41e+05	chr9	96938239	96938318	+	
hsa-let-7a-2	MI0000061	1.25e+05	chr11	122017230	122017301	-	
hsa-let-7a-3	MI0000062	1.64e+05	chr22	46508629	46508702	+	
hsa-let-7b	MI0000063	8.31e+04	chr22	46509566	46509648	+	
hsa-let-7c	MI0000064	1.17e+05	chr21	17912148	17912231	+	
hsa-let-7d	MI0000065	1.13e+04	chr9	96941116	96941202	+	
hsa-let-7e	MI0000066	5.33e+04	chr19	52196039	52196117	+	
hsa-let-7f-1	MI0000067	1.15e+05	chr9	96938629	96938715	+	
hsa-let-7f-2	MI0000068	1.23e+05	chrX	53584153	53584235	-	
hsa-let-7g	MI0000433	1.18e+05	chr3	52302294	52302377	-	
hsa-let-7i	MI0000434	1.45e+04	chr12	62997466	62997549	+	
hsa-mir-1-1	MI0000651	2.09e+03	chr20	61151513	61151583	+	
hsa-mir-1-2	MI0000437	2e+03	chr18	19408965	19409049	-	
hsa-mir-7-1	MI0000263	2.28e+03	chr9	86584663	86584772	-	
hsa-mir-7-2	MI0000264	1.67e+03	chr15	89155056	89155165	+	
hsa-mir-7-3	MI0000265	2.26e+03	chr19	4770682	4770791	+	
hsa-mir-9-1	MI0000466	3.01e+03	chr1	156390133	156390221	-	
hsa-mir-9-2	MI0000467	3e+03	chr5	87962671	87962757	-	
hsa-mir-9-3	MI0000468	1.4e+03	chr15	89911248	89911337	+	
hsa-mir-10a	MI0000266	5.69e+03	chr17	46657200	46657309	-	
hsa-mir-10b	MI0000267	1.01e+04	chr2	177015031	177015140	+	
hsa-mir-15a	MI0000069	8.13e+03	chr13	50623255	50623337	-	

Stem-loop sequence hsa-let-7a-1

Accession	MI0000060
Previous IDs	hsa-let-7a-1L
Symbol	HGNC:MIRLET7A1
Description	Homo sapiens let-7a-1 stem-loop
Gene family	MIPF0000002; let-7

Community annotation

This text is a summary paragraph taken from the Wikipedia entry entitled let-7 microRNA precursor. miRBase and Rfam are facilitating community annotation of microRNA families and entries in Wikipedia. Read more ...

The Let-7 microRNA precursor was identified from a study of developmental timing in C. elegans, and was later shown to be part of a much larger class of non-coding RNAs termed microRNAs. miR-98 microRNA precursor from human is a let-7 family member. Let-7 miRNAs have now been predicted or experimentally confirmed in a wide range of species (MIPF000002). miRNAs are initially transcribed in long transcripts (up to several hundred nucleotides) called primary miRNAs (pri-miRNAs), which are processed in the nucleus by Drosha and Pasha to hairpin structures of about ~70 nucleotide. These precursors (pre-miRNAs) are exported to the cytoplasm by exportin5, where they are subsequently processed by the enzyme Dicer to a ~22 nucleotide mature miRNA. The involvement of Dicer in miRNA processing demonstrates a relationship with the phenomenon of RNA interference.

Show Wikipedia entry | View @ Wikipedia | Edit Wikipedia entry

Stem-loop

```
     u   gu               uuagggucacac
uggga gag  aguagguuguauaguu           c
||||| |||  ||||||||||||||||           c
auccu uuc  ucaucuaacauaucaa           a
     -   ug               uagagggucacc
```

Get sequence

Deep sequencing

8488240 reads, 46 experiments

Comments

let-7a-3p cloned in [6] has a 1 nt 3' extension (U), which is incompatible with the genome sequence.

Genome context

Coordinates (GRCh37.p5)	*Overlapping transcripts*	
chr9: 96938239-96938318 [+]	sense	ENST00000362295; MIRLET7A1-201; exon 1

sequencing	
Comments	let-7a-3p cloned in [6] has a 1 nt 3' extension (U), which is incompatible with the genome sequence.
Genome context	*Coordinates (GRCh37.p5)* chr9: 96938239-96938318 [+] *Overlapping transcripts* sense ENST00000362295; MIRLET7A1-201; exon 1
Clustered miRNAs	*< 10kb from hsa-let-7a-1* hsa-let-7a-1 chr9: 96938239-96938318 [+] hsa-let-7f-1 chr9: 96938629-96938715 [+] hsa-let-7d chr9: 96941116-96941202 [+]
Database links	EMBL: AJ421724 ENTREZGENE: 406881; MIRLET7A1 HGNC: 31476; MIRLET7A1 RFAM: RF00027; let-7

Mature sequence hsa-let-7a-5p

Accession	MIMAT0000062
Previous IDs	hsa-let-7a
Sequence	6 - ugagguaguagguuguauaguu - 27 Get sequence
Deep sequencing	25466940 reads, 49 experiments
Evidence	experimental; cloned [1-3,5-8], Northern [1], Solexa [9]
Validated targets	TARBASE: hsa-let-7a-5p
Predicted targets	DIANA-MICROT: hsa-let-7a-5p MICRORNA.ORG: hsa-let-7a-5p MIRDB: hsa-let-7a-5p RNA22-HSA: hsa-let-7a-5p TARGETMINER: hsa-let-7a-5p TARGETSCAN-VERT: hsa-let-7a PICTAR-VERT: hsa-let-7a

PICTAR-VERT: hsa-let-7a

Mature sequence hsa-let-7a-3p

Accession	MIMAT0004481
Previous IDs	hsa-let-7a*
Sequence	57 - cuauacaaucuacugucuuuc - 77 Get sequence
Deep sequencing	2013 reads, 29 experiments
Evidence	experimental; cloned [6]
Predicted targets	DIANA-MICROT: hsa-let-7a-3p MICRORNA.ORG: hsa-let-7a-3p MIRDB: hsa-let-7a-3p RNA22-HSA: hsa-let-7a-3p TARGETMINER: hsa-let-7a-3p PICTAR-VERT: hsa-let-7a

References

1	PMID:11679670 "Identification of novel genes coding for small expressed RNAs" Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T Science. 294:853-858(2001).
2	PMID:15183728 "Human embryonic stem cells express a unique set of microRNAs" Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, Lee JY, Cha KY, Chung HM, Yoon HS, Moon SY, Kim VN, Kim KS Dev Biol. 270:488-498(2004).
3	PMID:12554860 "Numerous microRNPs in neuronal cells containing novel microRNAs" Dostie J, Mourelatos Z, Yang M, Sharma A, Dreyfuss G RNA. 9:180-186(2003).
4	PMID:14573789 "Reduced accumulation of specific microRNAs in colorectal neoplasia" Michael MZ, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ Mol Cancer Res. 1:882-891(2003).
5	PMID:15325244 "Altered expression profiles of microRNAs during TPA-induced differentiation of HL-60 cells"

microRNAViewer

http://people.csail.mit.edu/akiezun/miRviewer

microRNAviewer

microRNAviewer presents a global view of homologous miRNA genes in many species. microRNAviewer exhibits a comprehensive set of miRNA genes both from miRbase and candidate homologs identified using miRNAminer. microRNAviewer table shows conservation of miRNA genes, grouped by name, in addition to other information (see Help).

The table shows conservation of miRNA genes, grouped by name. Click on group name to see conservation for each miRNA and genomic location. Point cursor on miRNA to see summary of origin information. Symbols • indicate miRNAs present in miRbase (other miRNAs are newly discovered by miRNAminer.) Grey box indicates that the miRNA was not identified in this genome, under stringent parameters. Zoom out to see the full table by pressing Ctr- (or Mac-) a few times. Initial loading of table might take a few moments (for best performance use Firefox or Chrome).

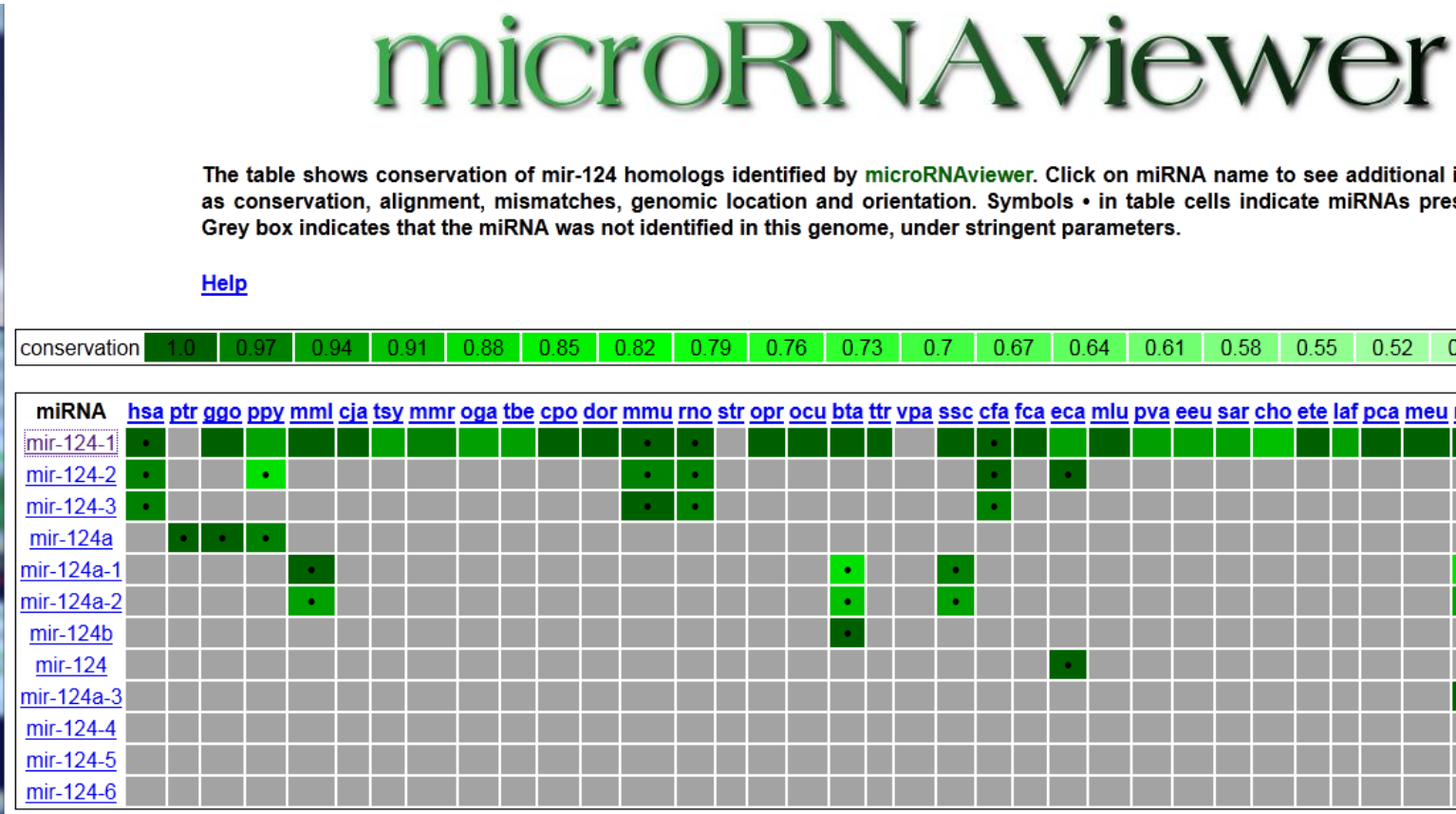
If you use microRNAviewer, please cite (note that the name of the tool has changed):
Adam Kiezun, Shay Artzi, Shira Modai, Naama Volk, Ofer Isakov and Noam Shomron, miRviewer: A multispecies microRNA homologous viewer In *BMC Research Notes 2012, 5:92*

Questions:mirnaminer@gmail.com
microRNAviewer is developed by Adam Kiezun (Broad Institute of Harvard and MIT) , Shay Artzi (IBM) , and Noam Shomron (Tel Aviv) .
Last update Feb 28, 2012

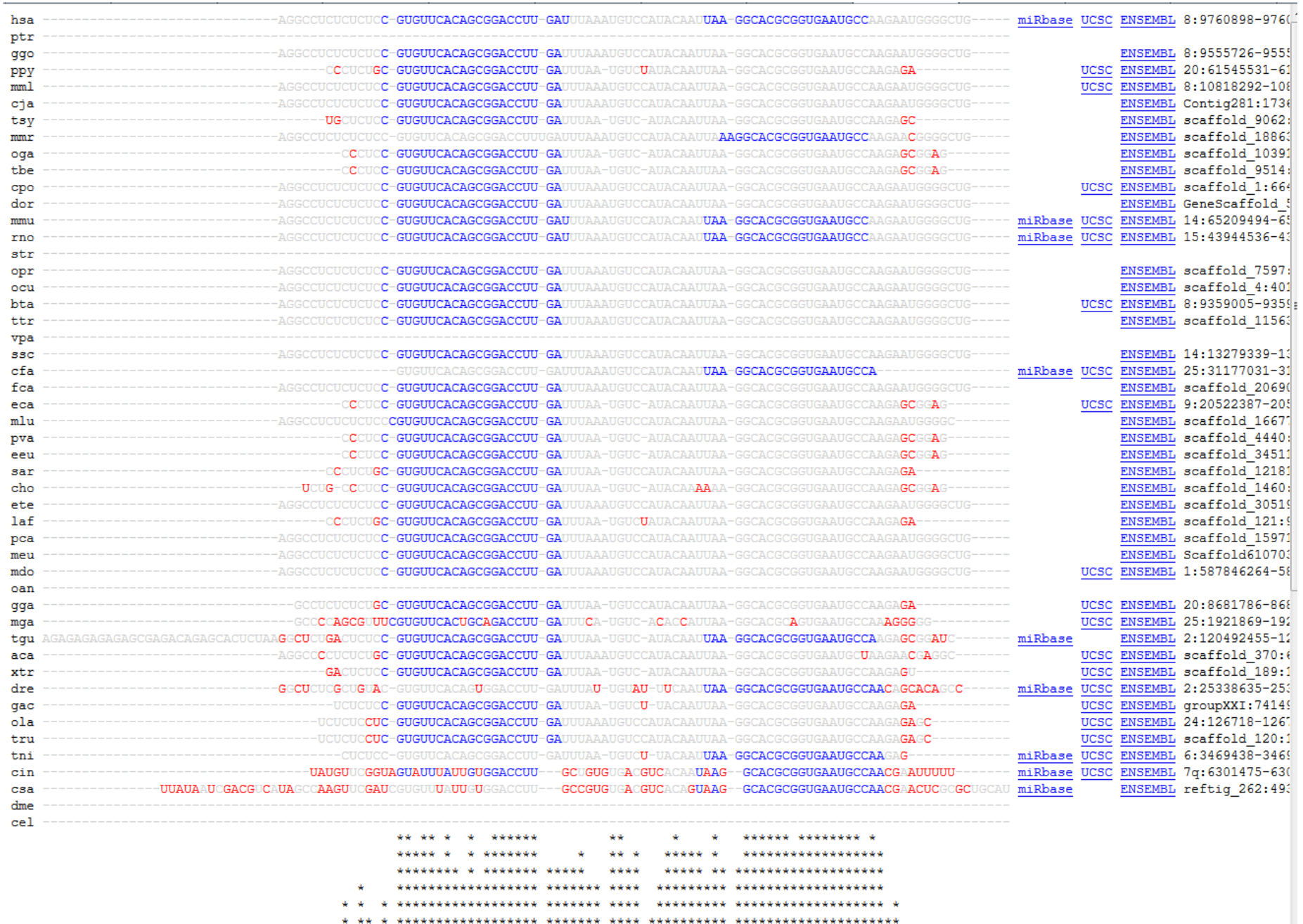
Help

miRNA	hsa	ptr	ggo	ppy	mml	cja	tsy	mmr	oga	tbe	cpo	dor	mmu	rno	str	opr	ocu	bta	ttr	vpa	ssc	cfa	fca	eca	mlu	pv
Known/New	792/48	426/15	68/508	442/163	350/132	0/381	0/194	0/248	0/250	0/205	0/272	0/224	471/40	324/57	0/228	0/210	0/259	502/47	0/286	0/182	158/111	231/90	0/223	251/28	0/221	0/2
let-7 (28)	•	•		•	•								•	•				•			•	•		•		
mir-34 (10)	•	•	•	•	•								•	•				•			•	•		•		
mir-133 (10)	•	•	•	•	•								•	•				•			•	•		•		
mir-92 (10)	•	•	•	•	•								•	•				•			•	•		•		
mir-216 (8)	•	•	•	•	•								•	•				•			•	•		•		
mir-7 (9)	•	•	•	•	•								•	•				•			•	•		•		
mir-124 (12)	•	•	•	•	•								•	•				•			•	•		•		
mir-101 (7)	•	•	•	•	•								•	•				•			•	•		•		
mir-135 (11)	•	•	•	•	•								•	•				•			•	•		•		
mir-15 (6)	•	•	•	•	•								•	•				•			•	•		•		

microRNAViewer: družina mir-124



microRNAViewer: poravnava mir-124-1



Predikcija tarč miRNA

Algoritmi za predikcijo tarč

Table 1. Tools for Predicting Metazoan miRNA Targets

Tool[a]	Clades[b]	Criteria for Prediction and Ranking	Website URL	Recent Reference
Site Conservation Considered				
TargetScan	m	Stringent seed pairing, site number, site type, site context (which includes factors that influence site accessibility); option of ranking by likelihood of preferential conservation rather than site context	http://targetscan.org	Friedman et al., 2008
TargetScan	f,w	Stringent seed pairing, site number, site type	http://targetscan.org	Ruby et al., 2007; Ruby et al., 2006
EMBL	f	Stringent seed pairing, site number, overall predicted pairing stability	http://russell.embl-heidelberg.de	Stark et al., 2005
PicTar	m,f,w	Stringent seed pairing for at least one of the sites for the miRNA, site number, overall predicted pairing stability	http://pictar.mdc-berlin.de	Lall et al., 2006
EIMMo	m,f,w	Stringent seed pairing, site number, likelihood of preferential conservation	http://www.mirz.unibas.ch/EIMMo2	Gaidatzis et al., 2007
Miranda	m,f,w,+	Moderately stringent seed pairing, site number, pairing to most of the miRNA	http://www.microrna.org	Betel et al., 2008
miRBase Targets	m,f,w,+	Moderately stringent seed pairing, site number, overall pairing	http://microrna.sanger.ac.uk	Griffiths-Jones et al., 2008
PITA Top	m,f,w	Moderately stringent seed pairing, site number, overall predicted pairing stability, predicted site accessibility	http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html	Kertesz et al., 2007
mirWIP	w	Moderately stringent seed pairing, site number, overall predicted pairing stability, predicted site accessibility	http://146.189.76.171/query	Hammell et al., 2008
Site Conservation Not Considered				
TargetScan	m	Stringent seed pairing, site number, site type, site context (which includes factors that influence site accessibility)	http://targetscan.org	Grimson et al., 2007
PITA All	m,f,w	Moderately stringent seed pairing, site number, overall predicted pairing stability, predicted site accessibility	http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html	Kertesz et al., 2007
RNA22	m,f,w	Moderately stringent seed pairing, matches to sequence patterns generated from miRNA set, overall predicted pairing and predicted pairing stability	http://cbcsrv.watson.ibm.com/rna22.html	Miranda et al., 2006

[a]Tools are listed according to criteria for prediction and ranking, which for those tools assessed with recent proteomics results generally correspond to their overall performance (Baek et al., 2008).
[b]Letters indicate predictions provided for the mammalian/vertebrate (m), fly (f), worm (w), or additional (+) clades.

Bartel D. *Cell* 2009.

- parjenje baz med **miR ‚seed‘ regijo in tarčno mRNA.**
- **Ohranjenost trače med vrstam.**
- Število vezavnih mest na 3’UTR določenega gena.
- Prosta energija vezave miR s tarčo.
- Dostopnost vezavnega mesta.
- Sekundarne strukture miRNA in tarče.

Orodja za predikcijo tarč

- Targetscan
- MicroCosm
- DIANA
- miRWalk

TargetScan (Lewis et al., Cell 2003)

- TargetScan predpostavi biološke tarče miRNA z iskanjem ohranjenih 8 in 7 – nt dolgih mest, ki ustrezajo ‘seed’ regiji vsake miRNA. Dodatna opcija je napoved neohranjenih mest.

- Določijo se neujemanja v ‘seed’ regiji, ki se kompenzirajo z ohranjenim 3’ parjenjem.

- Pri sesalcih so napovedi rangirane po predvideni učinkovitosti iskanja komplementarnega zaporedja. TargetScanHuman upošteva tiste napovedi, ki ustrezajo anotiranim človeškim UTR in njihovim ortologom, kot je določeno z UCSC poravnavami celih genomov.

http://www.targetscan.org/

TargetScanHuman

Prediction of microRNA targets

Release 6.2: June 2012

Search for predicted microRNA targets in mammals

[Go to TargetScanMouse]
[Go to TargetScanWorm]
[Go to TargetScanFly]
[Go to TargetScanFish]

1. Select a species Human

AND

2. Enter a human Entrez Gene symbol (e.g. "LIN28A")

AND/OR

3. Do one of the following:

- Select a broadly conserved* microRNA family Broadly conserved microRNA families
- Select a conserved* microRNA family Conserved microRNA families
- Select a poorly conserved microRNA family Poorly conserved microRNA families Note that these families also include small RNAs that have been misclassified as miRNAs.
- Enter a microRNA name (e.g. "mmu-miR-1")

Submit Reset

* broadly conserved = conserved across most vertebrates, usually to zebrafish
conserved = conserved across most mammals, but usually not beyond placental mammals

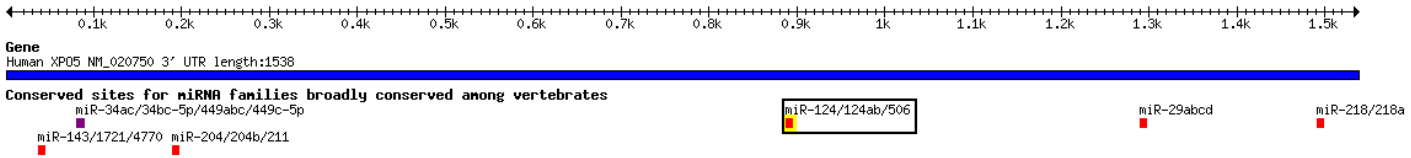
---

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 (ref. 1). 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 (ref. 2). In mammals, predictions are ranked based on the predicted efficacy of targeting as calculated using the context+ scores of the sites (ref. 3, 4). As an option, predictions are also ranked by their probability of conserved targeting (P_{CT}, ref. 2). TargetScanHuman considers matches to annotated human UTRs and their orthologs, as defined by UCSC whole-genome alignments. Conserved targeting has also been detected within open reading frames (ORFs). A listing of these ORF sites can be found at the bottom of Supplemental Table 2 of reference 1.

This search page of TargetScan Release 6.2 retrieves predicted regulatory targets of mammalian microRNAs. Many targets are the same as those presented in previous versions of the TargetScan site (Releases 2.0, 2.1, 3.0, 3.1, 4.0 - 4.2, 5.0 - 5.2, and 6.0). Compared to previous releases, Release 6 extends context score contributions to include seed-pairing stability and target-site abundance (ref. 4), includes all 3' UTRs from RefSeq (rather than just the longest UTR from each gene), and includes more miRNA families.

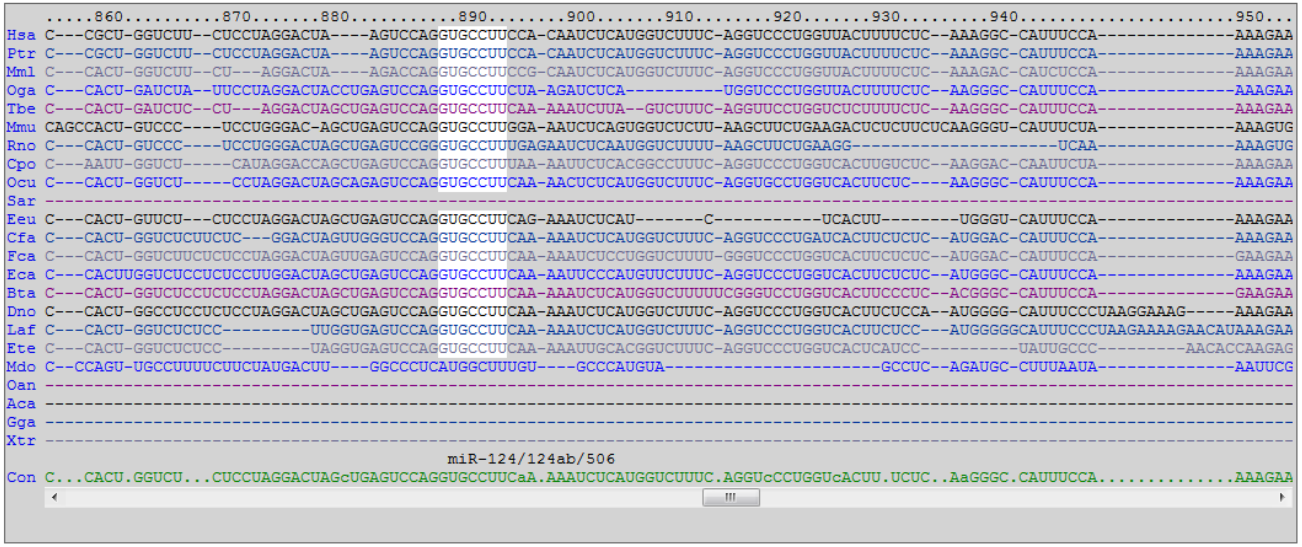
Frequently Asked Questions (FAQs)

Human XPO5 3' UTR



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

Key:
Sites with higher probability of preferential conservation
8mer 7mer-m8 7mer-1A 3' comp*
Sites with lower probability of preferential conservation
8mer 7mer-m8 7mer-1A 3' comp*



[Species key]

Conserved

	predicted consequential pairing of target region (top) and miRNA (bottom)	seed match	site-type contribution	3' pairing contribution	local AU contribution	position contribution	TA contribution	SPS contribution	context+ score	context+ score percentile	conserved branch length	P_{CT}
Position 888-894 of XPO5 3' UTR hsa-miR-124	5' ...UAGGACUAAGUCCAGGUGCCUUC... 3' CCGUAAGUGGCGCACGGAAU	7mer-m8	-0.120	0.003	0.059	0.030	0.012	-0.032	-0.05	25	2.180	0.89
Position 888-894 of XPO5 3' UTR hsa-miR-506	5' ...UAGGACUAAGUCCAGGUGCCUUC... 3' AGAUGAGUCUUCCCACGGAAU	7mer-m8	-0.120	0.003	0.059	0.030	0.012	-0.032	-0.05	25	2.180	0.89

Context+ score and features that contribute to the context+ score are evaluated as in Garcia et al., 2011.
Conserved branch lengths and P_{CT} are evaluated as in Friedman et al., 2008.

MicroCosm

http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/

EMBL-EBI | Enter Text Here | Find | Terms of Use | Privacy | Cookies

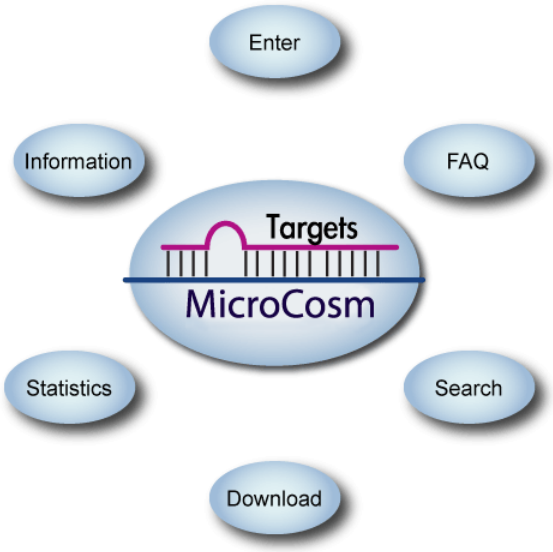
Databases | Tools | Research | Training | Industry | About Us | Help | Site Index

- EMBL-EBI
- Enright Lab
- Services
  - MicroCosm Targets
  - MapMi
  - Sylamer
- MicroCosm Targets
  - Home

EBI > Enright Group > MicroCosm

MicroCosm Targets Version 5

Email microcosm@ebi.ac.uk with queries or problems.



miRBase Targets Release Version v5

MicroCosm Targets (formerly miRBase Targets) is a web resource developed by the Enright Lab at the EMBL-EBI containing computationally predicted targets for microRNAs across many species. The miRNA sequences are obtained from the miRBase Sequence database and most genomic sequence from EnsEMBL. We aim to provide the most up-to-date and accurate predictions of miRNA targets and hence this resource will be updated regularly to incorporate new miRNAs or EnsEMBL sequences. For more information about the computational protocol used for these analyses, please see the information page.

Now: 4°C | Mon: 13°C | Tue: 11°C | Wed: 6°C | Thu: 5°C | Fri: 3°C

Search MicroCosm

- EMBL-EBI
- Enright Lab
- Services
  - MicroCosm Targets
  - MapMi
  - Sylamer
- MicroCosm Targets
  - Home
  - Genomes
  - miRNAs

Select a Genome: H. sapiens

Enter miRNA id:

Enter Gene Name: xpo5

Enter EnsEMBL identifier:

Select a GO class: Function

Enter GO Term:

by phrase

AND OR

Keyword Search:

Search

EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK +44 (0)1223 49 44 44

Target Listing

Highlighted rows in the table indicate genes with published known targets

All miRNA hits for *Homo sapiens* where search terms are

4 hits found.

Gene Name	Transcript	Description	GO Terms	Score	Energy	P-value	Length	Total Sites	No. Cons Species	No. miRNAs	
XPO5	ENST00000265351	Exportin-5 (Exp5) (Ran-binding protein 21). [Source:Uniprot/SWISSPROT;Acc:Q9HAV4]		195	-314	0.000654142	1531	12	3	12 [+]	View
XPO5	ENST00000372258	Exportin-5 (Exp5) (Ran-binding protein 21). [Source:Uniprot/SWISSPROT;Acc:Q9HAV4]		168	-201	0.00123643	1000	10	1	11 [+]	View
XPO5	ENST00000372250	Exportin-5 (Exp5) (Ran-binding protein 21). [Source:Uniprot/SWISSPROT;Acc:Q9HAV4]		268	-406	0.00285349	1000	16	1	17 [+]	View
XPO5	ENST00000372252	Exportin-5 (Exp5) (Ran-binding protein 21). [Source:Uniprot/SWISSPROT;Acc:Q9HAV4]		236	-362	0.00422521	1000	14	1	15 [+]	View

EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK +44 (0)1223 49 44 44

Target Detailed View

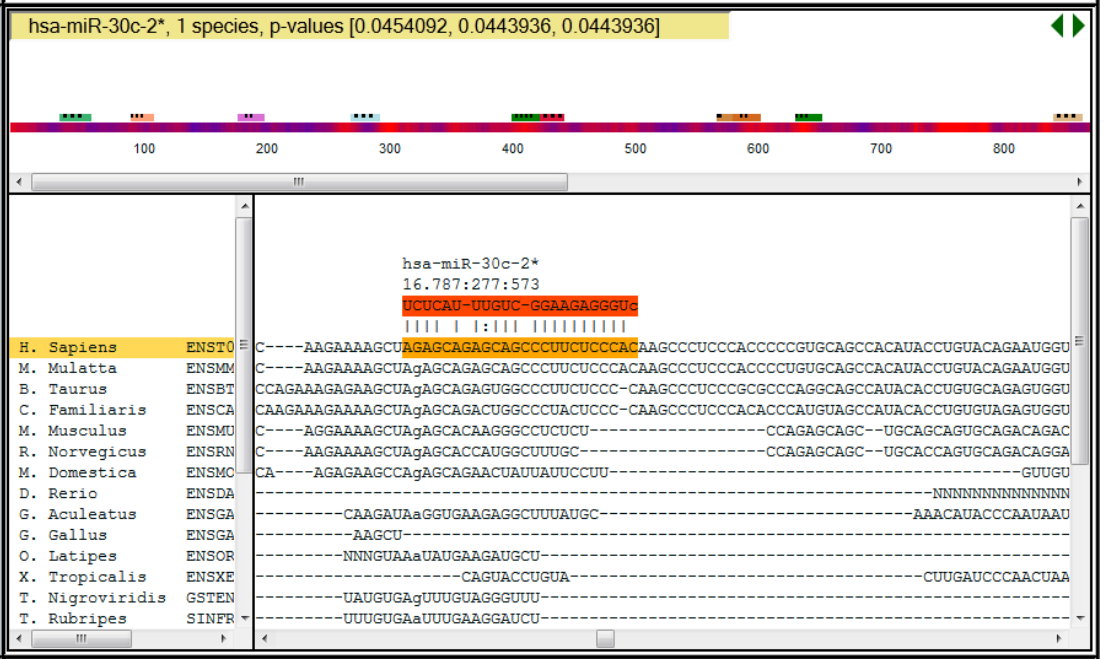
- EMBL-EBI
- Enright Lab
- Services
  - MicroCosm Targets
  - MapMi
  - Sylamer
- MicroCosm Targets
  - Home
  - Genomes
  - miRNAs

Microcosm > Genomes > Targets > Detailed view

Hit information for ENST00000265351

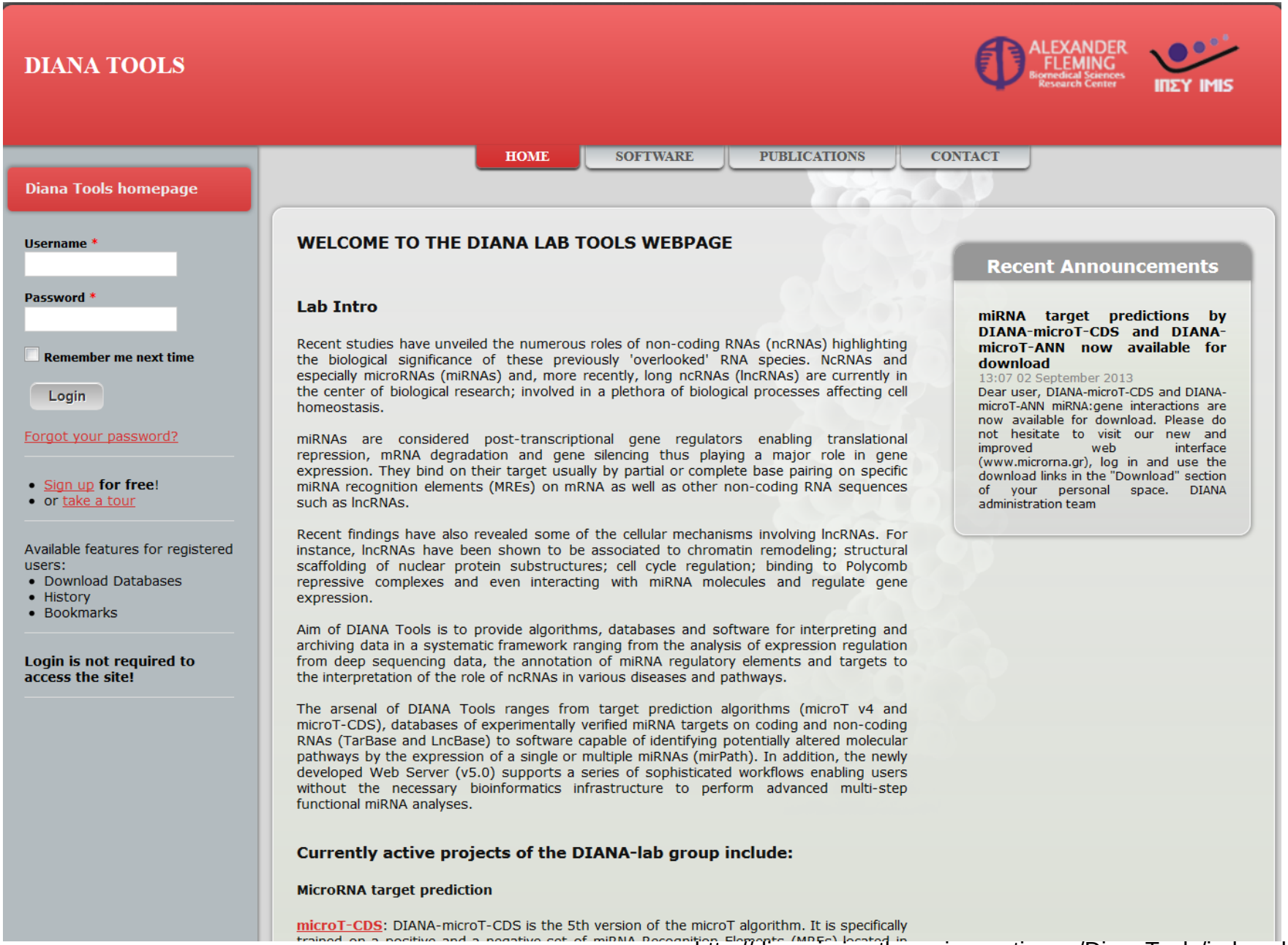
Gene Name	XPO5
Transcript	ENST00000265351
Gene	ENSG00000124571
Description	Exportin-5 (Exp5) (Ran-binding protein 21). [Source:Uniprot/SWISSPROT;Acc:Q9HAV4]

Alignment View [HTML] [Java]



Rfam ID	Score	Energy	Base P	Poisson P	Org P	Start	End	Alignment
mmu-miR-705	17.2324	-31.93	7.157160e-02	6.907040e-02	1.121660e-03	431	450	CGGGUGGGGUGGAGGGUGG GACCACTCCAACTCCCACT
mmu-miR-673-5p	16.787	-38.74	3.947170e-02	3.870280e-02	4.744280e-03	639	660	GAGGUUCCUGGUCUCGACACU CTCCAAGGACCAGGGCTGGGA
hsa-miR-30c-2*	16.787	-29.73	4.540920e-02	4.439360e-02	4.439360e-02	277	300	UCUCAU-UUGUC-GGAAGAGGGU AGAGCAGAGCAGCCCTTCTCCCA
hsa-miR-7	16.7157	-27.01	4.157490e-02	4.072250e-02	7.231070e-03	849	871	GUUGUUUUAGUGAUCAGAAGG TAGCAAAGCCGCTGGTCTTCT

DIANA TOOLS



http://diana.imis.athena-innovation.gr/DianaTools/index.php /

Software » TarBase

Username *

Password *

Remember me next time

Login

Forgot your password?

- Sign up **for free**!
- or take a tour

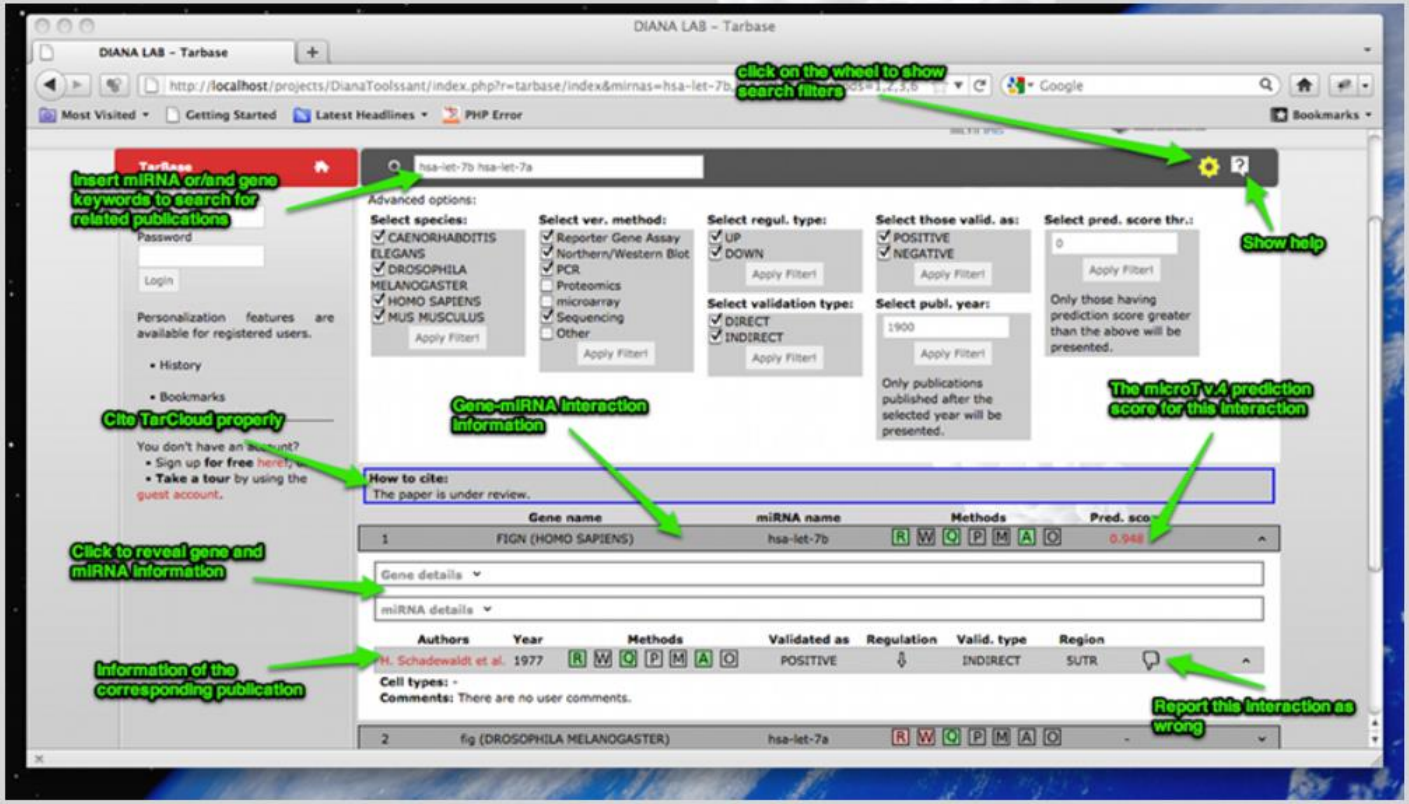
Available features for registered users:
- Download Databases
- History
- Bookmarks

Login is not required to access the site!

Please cite:
Vergoulis, T. I. Vlachos, P. Alexiou, G. Georgakilas, M. Maragkakis, M. Reczko, S. Gerangelos, N. Koziris, T. Dalamagas, AG Hatzigeorgiou; Tarbase 6.0: Capturing the Exponential Growth of miRNA Targets with Experimental Support. Nucl. Acids Res. (2012) 40 (D1): D222-D229. doi: 10.1093/nar/gkr1161

Adv. options:

DIANA-TarBase v1.0 was the first available database of experimentally validated targets. The sixth version (TarBase v6.0) is the largest currently available manually curated target database, indexing more than 65,000 miRNA-gene interactions. The database includes targets for 21 species, derived from specific, as well as high throughput experiments, such as microarrays, proteomics, HITS-CLIP and PAR-CLIP.
It is seamlessly interconnected with other DIANA-Lab tools, such as DIANA-microT, enabling it to extend each validated interaction with in silico predicted information. It is also equipped with powerful searching and filtering capabilities. TarBase v6.0 users can directly enrich the database by submitting data derived from their publications.
TarBase v6.0 dataset is freely available for download. For a license request please go here.



miRWalk

http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/index.html

Index

1. What is miRWalk database and how does it work?

miRWalk is a comprehensive database of human, mouse and rat microRNAs (miRNAs) on their Predicted and Validated Targets associated with genes, pathways, diseases, organs, cell lines and transcription factors. The miRWalk consists of two modules.

The Predicted Target module hosts miRNA-target interactions information on the complete sequence of all known genes of human, mouse and rat. In addition, the results are presented togather with results from 8 established miRNA-target prediction programs for the comparison of the results with different algorithms. Furthermore it provides predicted miRNA binding sites on gene associated with 449 human biological pathways and 2356 OMIM disorders.

The Validated Targets module hosts new and unique features on experimentally validated miRNA interaction information associated to genes, pathways, diseases, organs, cell lines and OMIM disorders. Moreover, it offers information on proteins known to be involved in miRNA processing. The miRWalk is the only database provides the possible miRNA binding sites on the compelete sequence (promoter, 5' UTR, CDS and 3' UTR) of known genes and 3 complete mitochondrial genomes.

miRWalk Algorithm The miRWalk algorithm is based on a computational approach which identifies the longest consecutive complementary between miRNA and gene sequences. Based on Watson-Crick complementary, It starts walking on the complete gene sequence and mitochondrial genomes starting with a heptamer seed of miRNA and identifies possible miRNA binding sites up to possible matching on the complete sequence of all known genes, returns the longest seed match, then it assigns the miRNA binding sites to four regions of protein coding genes i.e. Promoter, 5' UTR, CDS, 3' UTR and mitochondrial genes. In addition, the probability distribution of random matches of a subsequence (5' end miRNA sequence) in the analysed sequence is calculated by using Poisson distribution as shown by Rehmsmeier et al. 2004. Then miRWalk compares its identified miRNA binding sites with the results of 8 established miRNA-target prediction programs i.e. DIANA-microT, miRanda, miRDB, PicTar, PITA, RNA22, RNAhybrid and TargetScan/TargetScanS. Finally miRWalk incorporates all the predicted miRNA binding sites produced by the miRWalk algorithm and the 8 established programs into a relational database (miRWalk). Thereafter, It made an extensive search in the PubMed database to retrieve all the available information on human, mouse and rat miRNAs linked to genes, pathways, diseases, organs, cell lines, OMIM disorders, and proteins known to be involved in miRNA processing. This information is complied and stored as validated information on miRNA in miRWalk database. miRWalk data is generated by executing automated Perl and BioPerl scripts scripts on the server of bwGRID Cluster Heidelberg (High Performance Cluster). The below figure shows miRWalk algorithm and analysis pipeline.

Holistic view of validated disease-miRNA interactions

Select disease

- Adenoviridae Infections
- Adrenal Cortex Neoplasms
- Adrenocortical Carcinoma
- Airway Remodeling
- Albuminuria
- Alcoholism
- Alzheimer Disease
- Amyotrophic Lateral Sclerosis
- Anemia
- Anemia Hemolytic
- Anemia Macrocytic
- Anemia Sickle Cell

Searching, Please wait ...

Disease Target Results

Bottom Paging View

Disease Name	MicroRNA Name	StemLoopName	miR_Chr.	Pubmed ID
Alzheimer Disease	hsa-miR-101	hsa-mir-101-2	9	20395292
Alzheimer Disease	rno-miR-30a	rno-mir-30a	9	23358924
Alzheimer Disease	rno-miR-30a*	rno-mir-30a	9	23358924
Alzheimer Disease	mmu-miR-328	mmu-mir-328	8	18986979
Alzheimer Disease	hsa-miR-29a*	hsa-mir-29a	7	20202123
Alzheimer Disease	hsa-miR-29a	hsa-mir-29a	7	20202123
Alzheimer Disease	hsa-miR-29b	hsa-mir-29b-1	7	20202123
Alzheimer Disease	hsa-miR-29a*	hsa-mir-29a	7	19462468
Alzheimer Disease	hsa-miR-29a	hsa-mir-29a	7	19462468
Alzheimer Disease	hsa-miR-29b-1*	hsa-mir-29b-1	7	19462468
Alzheimer Disease	hsa-miR-29b	hsa-mir-29b-1	7	19462468
Alzheimer Disease	mmu-miR-29b*	mmu-mir-29b-1	6	19462468
Alzheimer Disease	mmu-miR-29b	mmu-mir-29b-1	6	19462468
Alzheimer Disease	mmu-miR-29a*	mmu-mir-29a	6	19462468
Alzheimer Disease	mmu-miR-29a	mmu-mir-29a	6	19462468
Alzheimer Disease	hsa-miR-30a	hsa-mir-30a	6	23358924
Alzheimer Disease	hsa-miR-30a*	hsa-mir-30a	6	23358924
Alzheimer Disease	mmu-miR-29b	mmu-mir-29b-1	6	20202123
Alzheimer Disease	mmu-miR-29a*	mmu-mir-29a	6	20202123
Alzheimer Disease	mmu-miR-29a	mmu-mir-29a	6	20202123
Alzheimer Disease	hsa-miR-146a	hsa-mir-146a	5	18801740
Alzheimer Disease	hsa-miR-146a*	hsa-mir-146a	5	18801740
Alzheimer Disease	rno-miR-34a	rno-mir-34a	5	19936094
Alzheimer Disease	hsa-miR-146a	hsa-mir-146a	5	20937840
Alzheimer Disease	hsa-miR-146a*	hsa-mir-146a	5	20937840
Alzheimer Disease	rno-miR-29a*	rno-mir-29a	4	20202123
Alzheimer Disease	rno-miR-29a	rno-mir-29a	4	20202123
Alzheimer Disease	rno-miR-29b	rno-mir-29b-1	4	20202123
Alzheimer Disease	mmu-miR-34a	mmu-mir-34a	4	19936094
Alzheimer Disease	rno-miR-29a*	rno-mir-29a	4	19462468
Alzheimer Disease	rno-miR-29a	rno-mir-29a	4	19462468
Alzheimer Disease	rno-miR-29b-1*	rno-mir-29b-1	4	19462468
Alzheimer Disease	rno-miR-29b	rno-mir-29b-1	4	19462468
Alzheimer Disease	rno-miR-298	rno-mir-298	3	18986979
Alzheimer Disease	hsa-miR-298	hsa-mir-298	20	18986979
Alzheimer Disease	mmu-miR-298	mmu-mir-298	2	18986979
Alzheimer Disease	rno-miR-328	rno-mir-328	19	18986979

Validacija miRNA tarč

Validacija miRNA Tarč

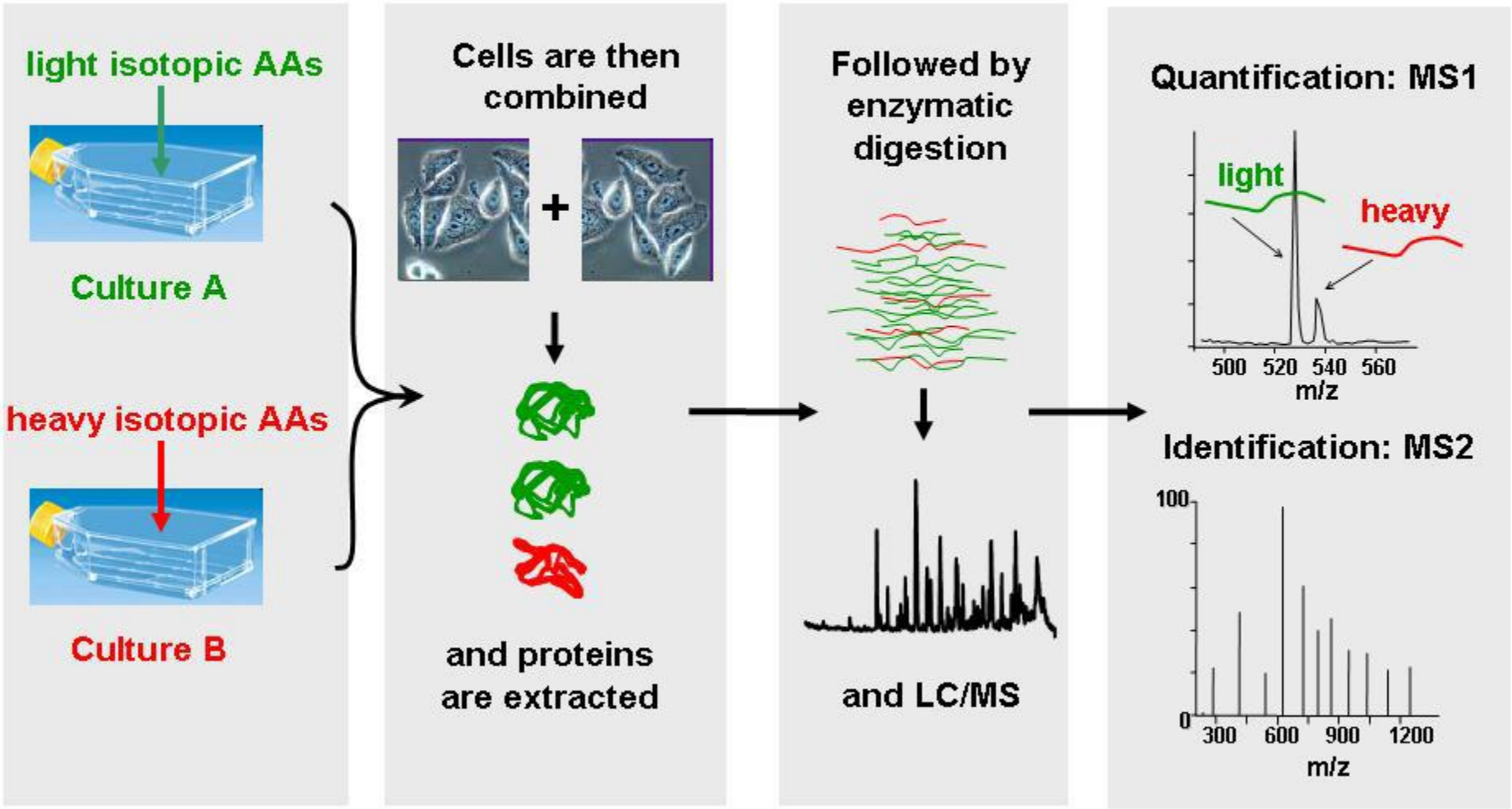
- in vitro metode s katerimi se validira funkcija miRNA:
  - RNA interferenca (siRNA ali shRNA)
  - Analiza izražanja proteinov z metodo SILAC

•SILAC – Če prav analiza izražanja mRNA lahko prikaže spremembe glede na miRNA, ne prikaže pa posledice inhibicije translacije.

- Izražanje proteinov močno korelira s spremembami v izražanju miRNA.
- Uprablja se kombinacija SILAC (stable isotopic labeling by amino acids in cell culture) metode z masno spektrometrijo.
- SILAC je zasnovana na vključitvi ‘lahkih’ ali ‘težkih’ amino kislin v proteine. Omogoča ugotavljanje razlik med celičnimi populavijami. Npr. primerjava normalnih celic in celic z enim utišanim miRNA.

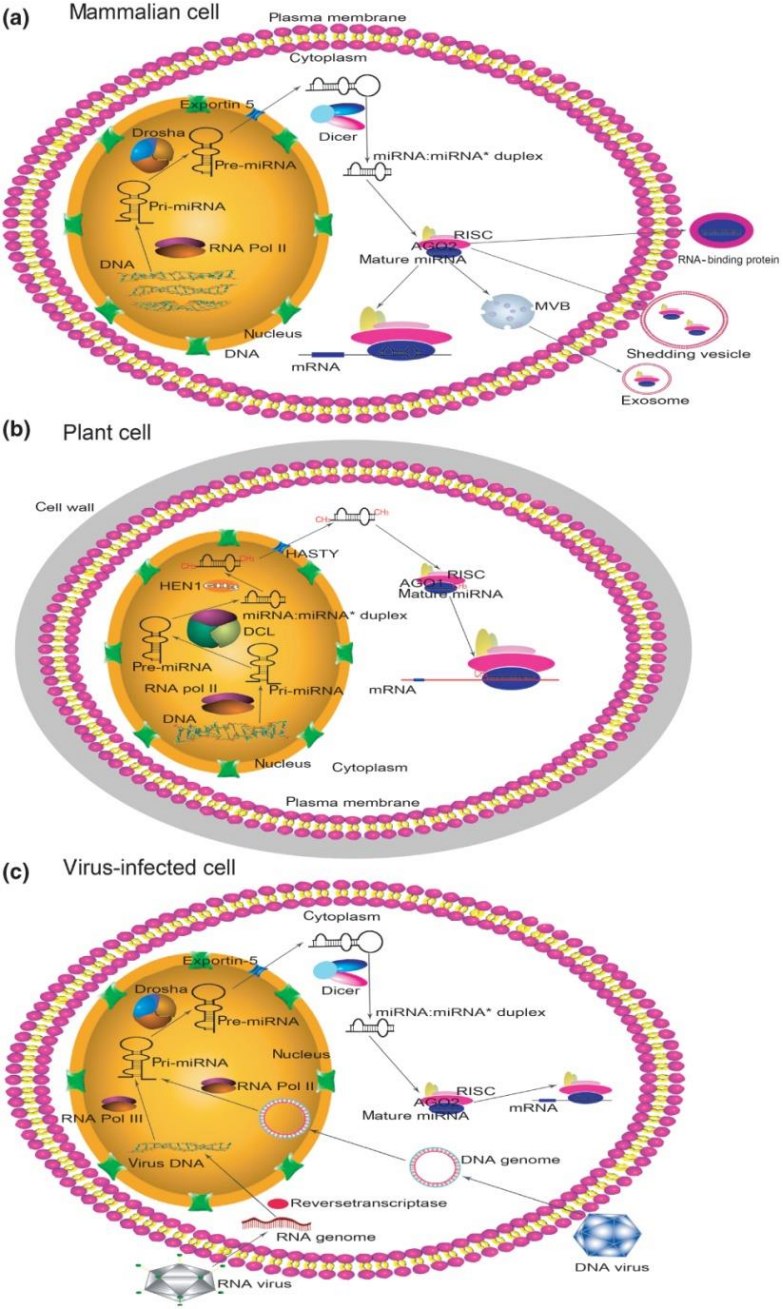
SILAC

Typically 2 – 4 plex:
(SILAC: Stable Isotope Labeling with Amino Acids in Cell Culture)

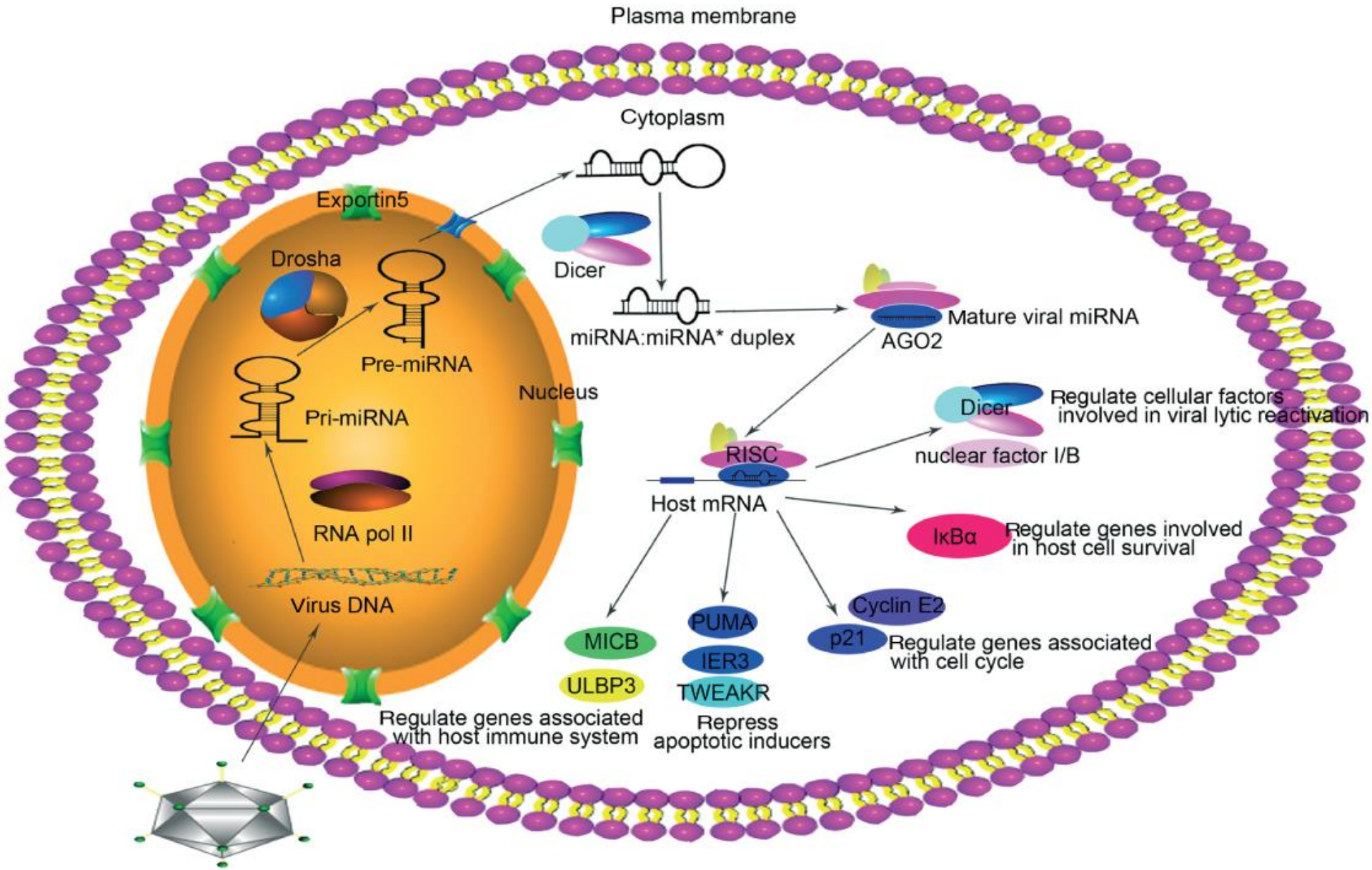


Eksogene miRNA

Biogeneza sesalčjih, rastlinskih in virusnih miRNA

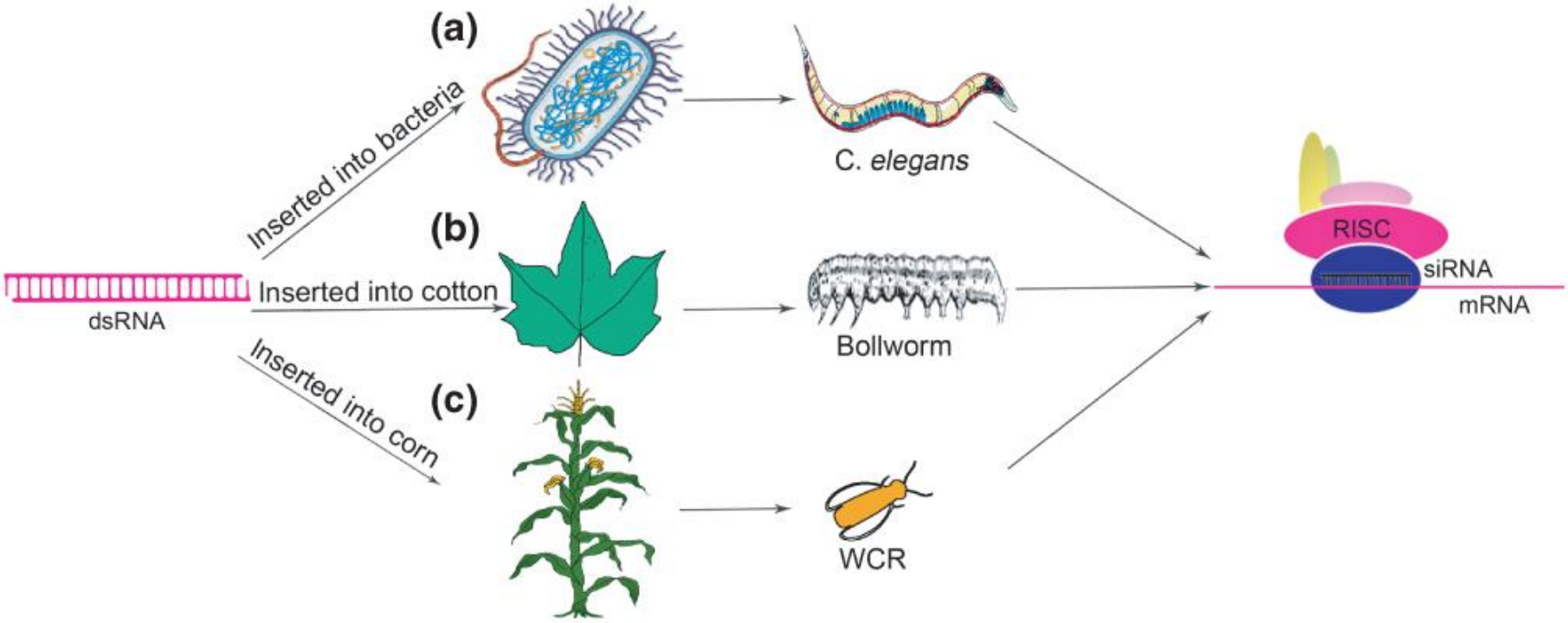


Virusne miRNA uravnavajo izražanje sesalčjih genov



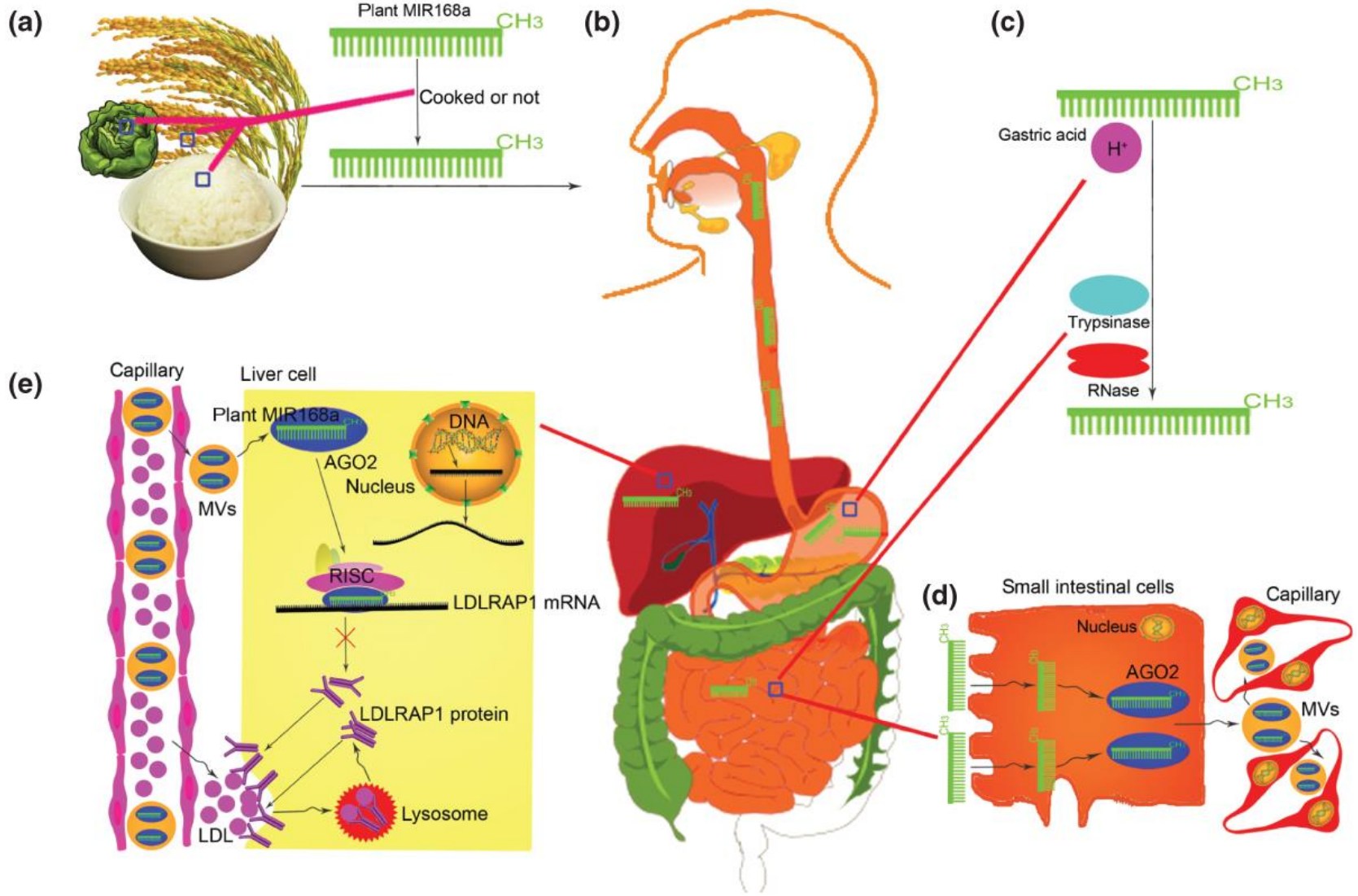
DOI: 10.1002/wrna.1127

RNAi vnos s hrano



DOI: 10.1002/wrna.1127

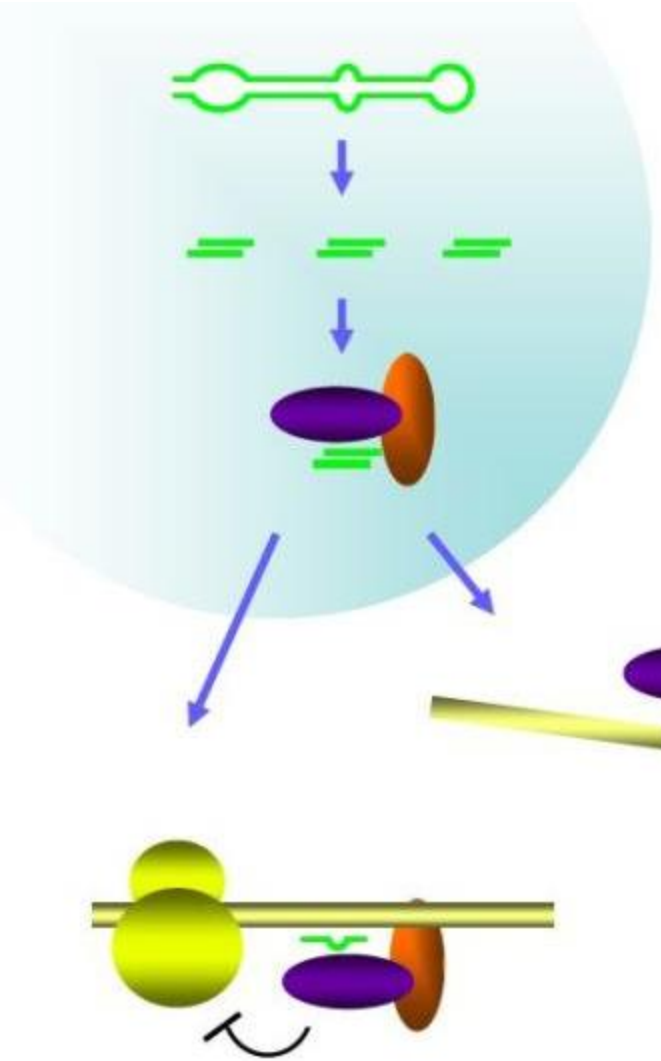
Rastlinske miRNA uravnavajo izražanje človeških genov



DOI: 10.1002/wrna.1127

miRNA in bolezni

miRNA vplivajo na vse!



Developmental timing

Differentiation

Aging

Apoptosis

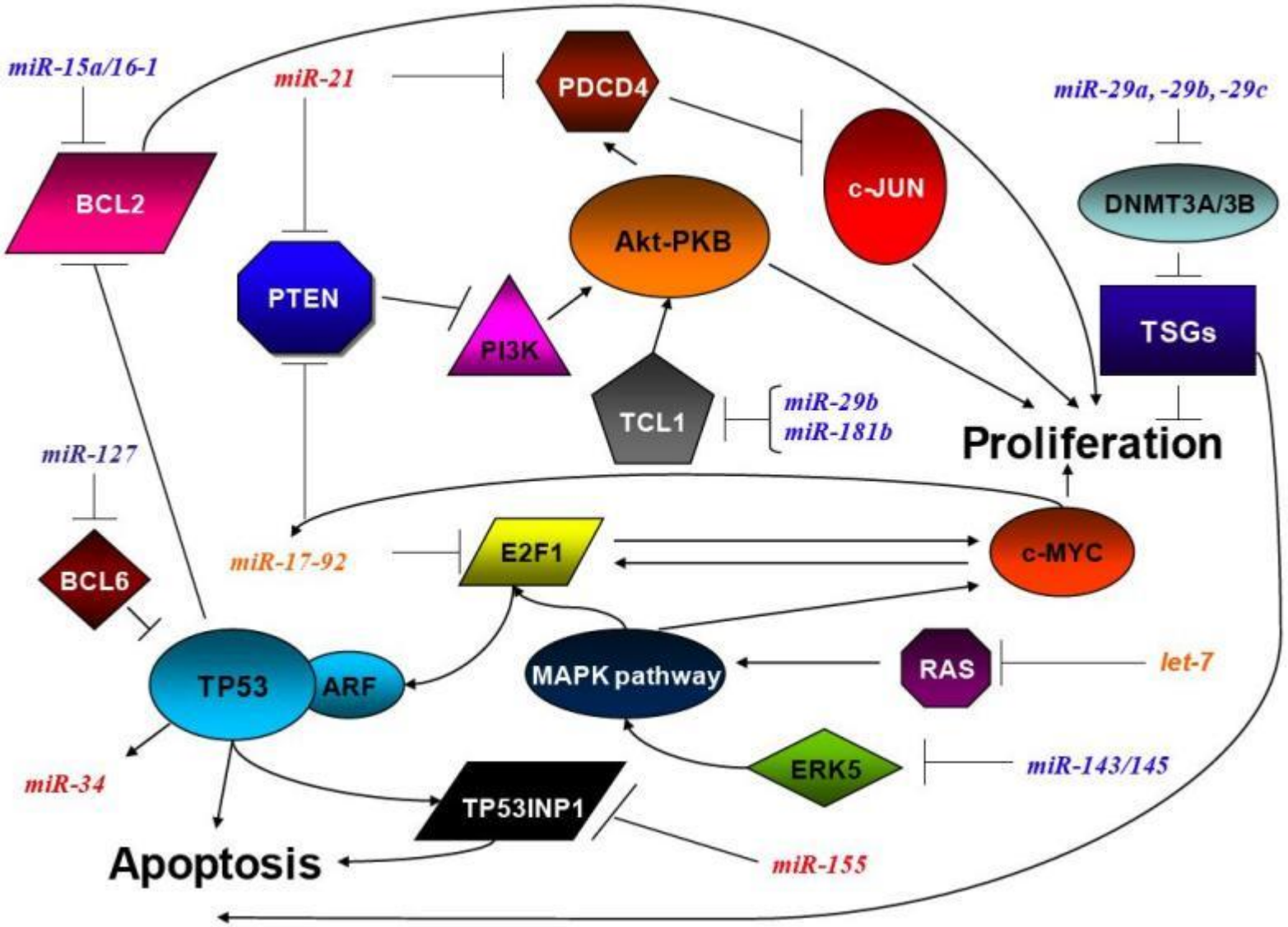
Metabolism

Cancer

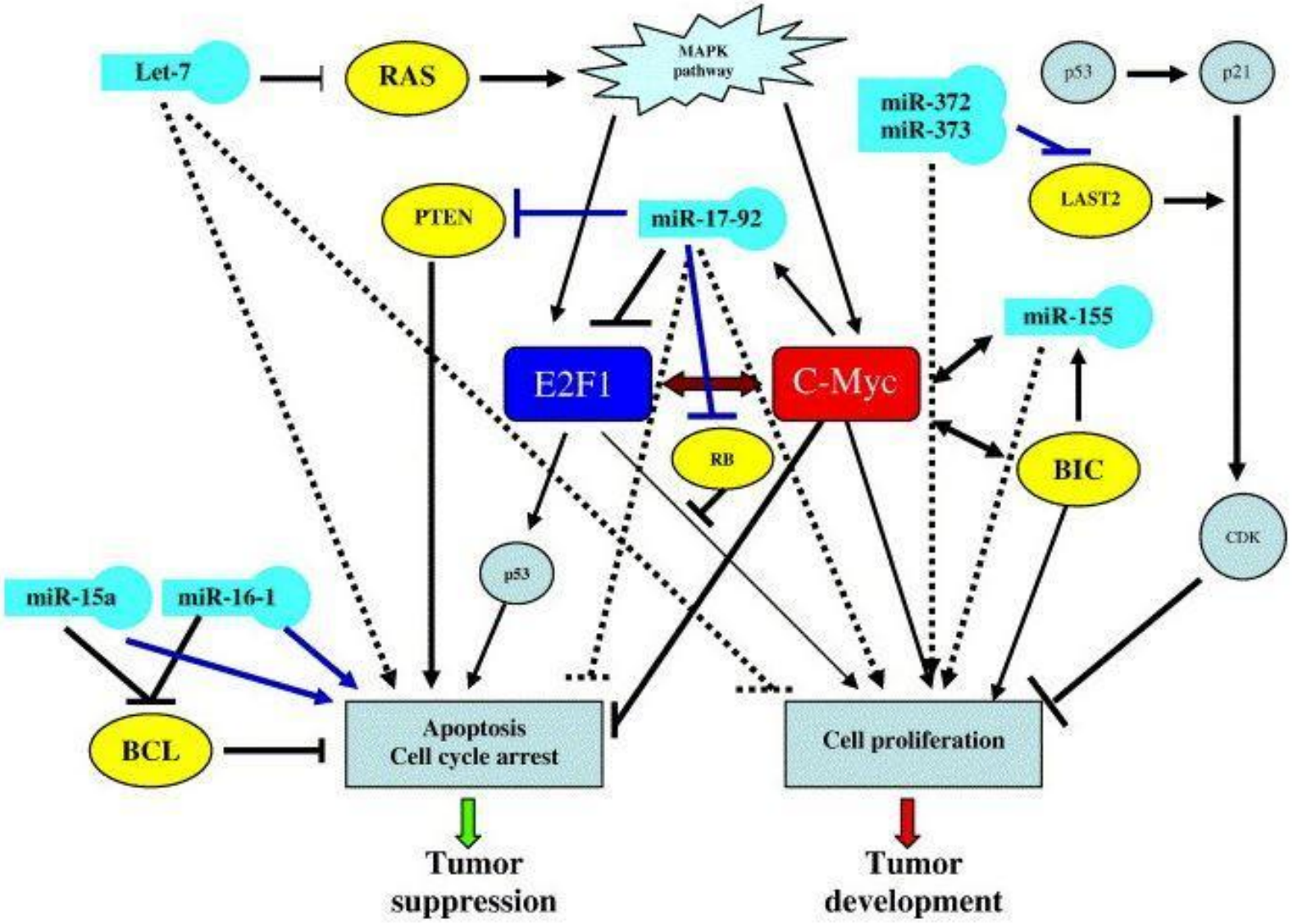
…

Cell fate/differentiation, Cell cycle…

miRNA uravnavajo celično proliferacijo in smrt



Onkogeni ali tumor-supresorske miRNA



Zhang et al Dev Biol. 2007 Feb 1;302(1):1-12

Onkogeni ali tumor-supresorske miRNA

Table 1 | **MicroRNAs that function as oncogenes or tumour suppressor genes in human cancers**

MicroRNA	Dysregulation	Function	Validated targets	Oncogene (ONC) or tumour suppressor (TS)	Refs
miR-15a and *miR-16-1*	Loss in CLL, prostate cancer and multiple myeloma	Induces apoptosis and inhibits tumorigenesis	BCL2, WT1 RAB9B and MAGE83	TS	15,20,23, 30,52,69
let-7 (*a*, *b*, *c*, *d*, *e*, *f*, *g* and *i*)	Loss in lung and breast cancer and in various solid and haematopoietic malignancies	Induces apoptosis and inhibits tumorigenesis	RAS, MYC and HMGA2	TS	22,26, 42,70
miR-29 (*a*, *b* and *c*)	Loss in aggressive CLL, AML (11q23), MDS lung and breast cancers and cholangocarcinoma	Induces apoptosis and inhibits tumorigenicity. Reactivates silenced tumour suppressor genes	TCL1, MCL1 and DNMTs	TS	30,64, 71,72
miR-34	Loss in pancreatic, colon, breast and liver cancers	Induces apoptosis	CDK4, CDK6, cyclin E2, EZF3 and MET	TS	56–58
miR-145	Loss in breast cancer	Inhibits proliferation and induces apoptosis of breast cancer cells	ERG	TS	31
miR-221 and *miR-222*	Loss in erythroblastic leukaemia	Inhibits proliferation in erythroblasts	KIT	TS	30
miR-221 and *miR-222*	Overexpression in aggressive CLL, thyroid carcinoma and hepatocellular carcinoma	Promotes cell proliferation and inhibits apoptosis in various solid malignancies	p27, p57, PTEN and TIMP3	ONC	43,51,73
miR-155	Upregulated in aggressive CLL, Burkitt's lymphoma and lung, breast and colon cancers	Induces cell proliferation and leukaemia or lymphoma in mice	MAF and SHIP1	ONC	32–34, 36,37
miR-17–92 cluster	Upregulated in lymphomas and in breast, lung, colon, stomach and pancreatic cancers	Induces proliferation	E2F1, BIM and PTEN	ONC	19,34,35, 40,41
miR-21	Upregulated in glioblastomas, AML (11q23), aggressive CLL and breast, colon, pancreatic, lung, prostate, liver and stomach cancers	Inhibits apoptosis and increases tumorigenicity	PTEN, PDCD4, TPM1 and TIMP3	ONC	31,37–39, 44–50
miR-372 and *miR-373*	Upregulated in testicular tumours	Promotes tumorigenicity in cooperation with RAS	LATS2	ONC	74

AML, acute myeloid leukaemia; BCL2, B cell leukaemia/lymphoma 2; BIM, Bcl2-interacting mediator of cell death; CLL, chronic lymphocytic leukaemia; DNMT, DNA methyltransferase; HMGA2, high mobility group AT-hook 2; LATS2, large tumour suppressor homologue 2; MCL1, myeloid cell leukaemia sequence 1; MDS, myelodysplastic syndrome; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homologue; SHIP1, SH2 domain-containing inositol-5′-phosphatase 1; TCL1, T cell lymphoma breakpoint 1; TIMP3, tissue inhibitor of metalloproteinases 3; TPM1, tropomyosin 1; WT1, Wilms tumour 1.

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

Onkomirji

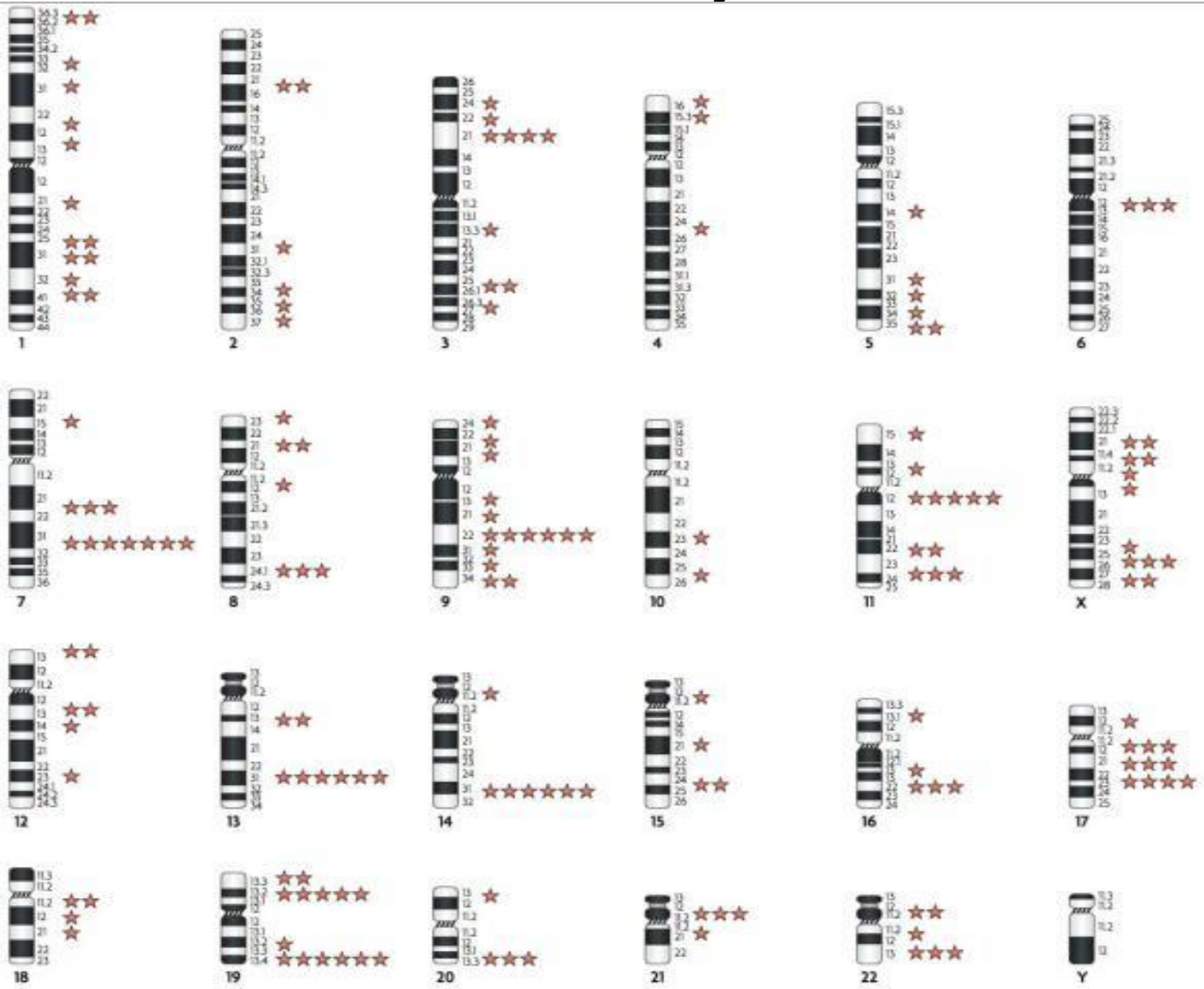


Figure 1 | **MicroRNA genes map to chromosomal regions that are involved in alterations in human cancer.**

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

Patološka regulacija miRNA pri rakih

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.

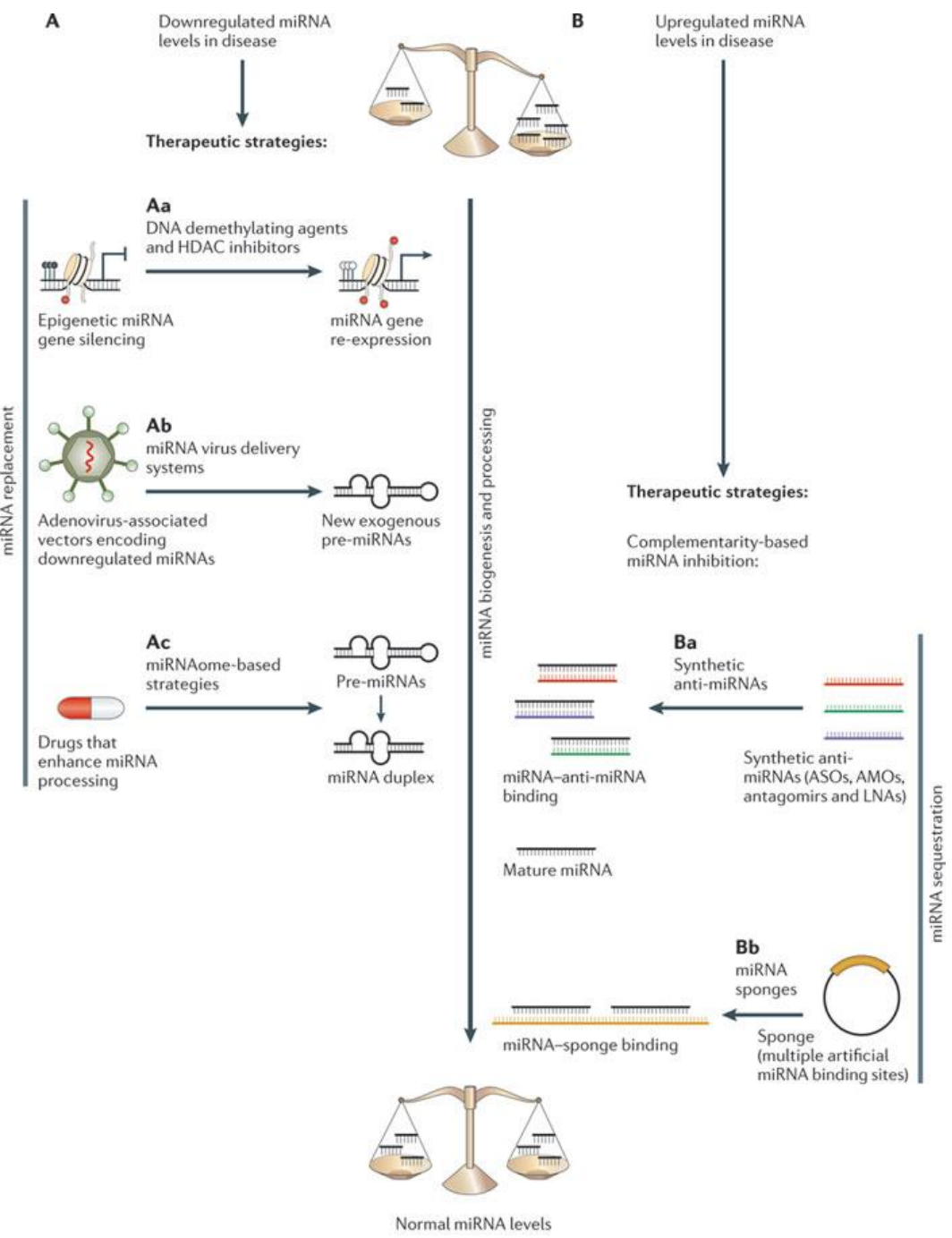
Croce Nat Rev Genet. 2009 Oct;10(10):704-14

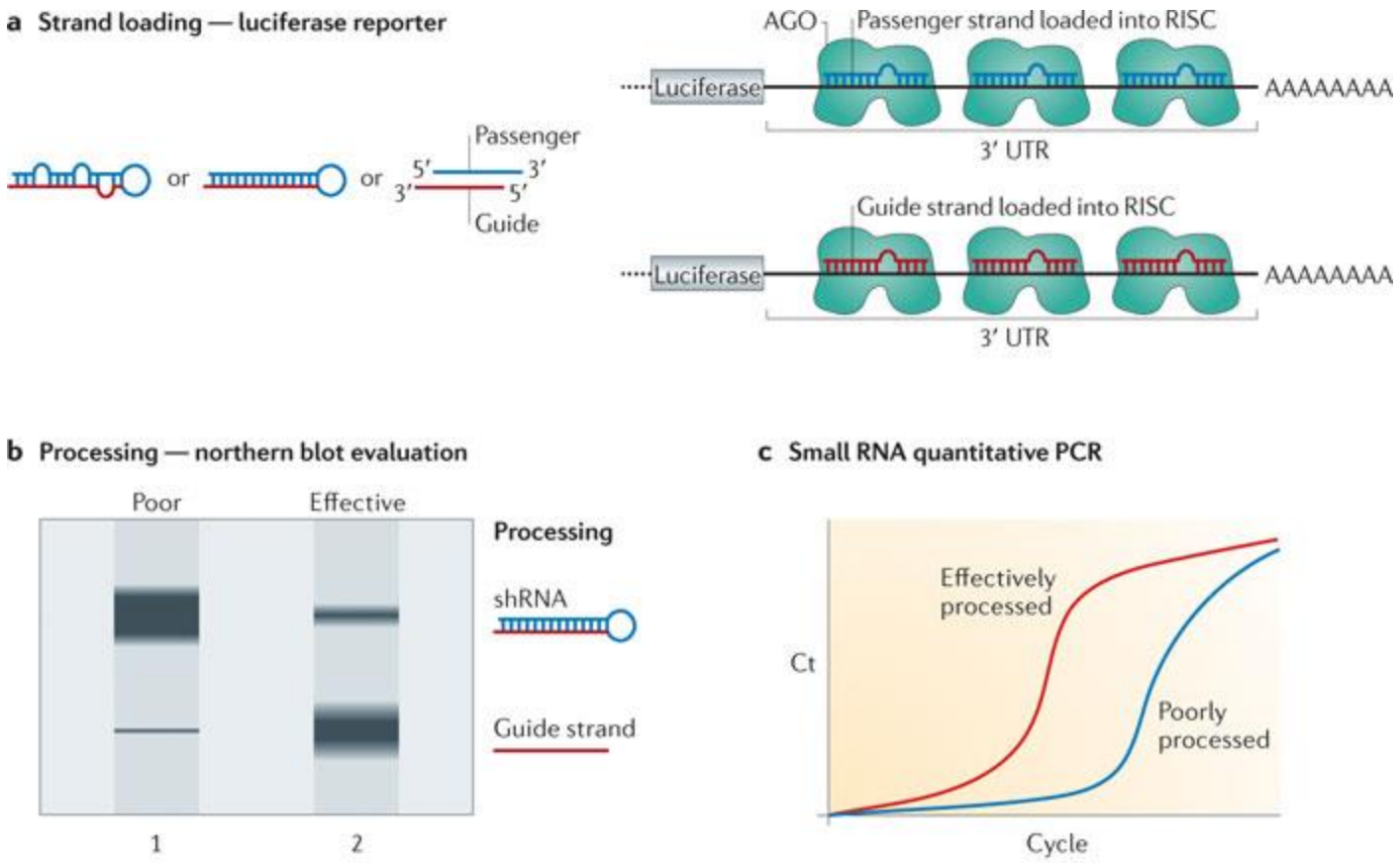
miRNA pri nevroloških boleznih

Table 1. Neurological disorders with reported miRNA abnormalities

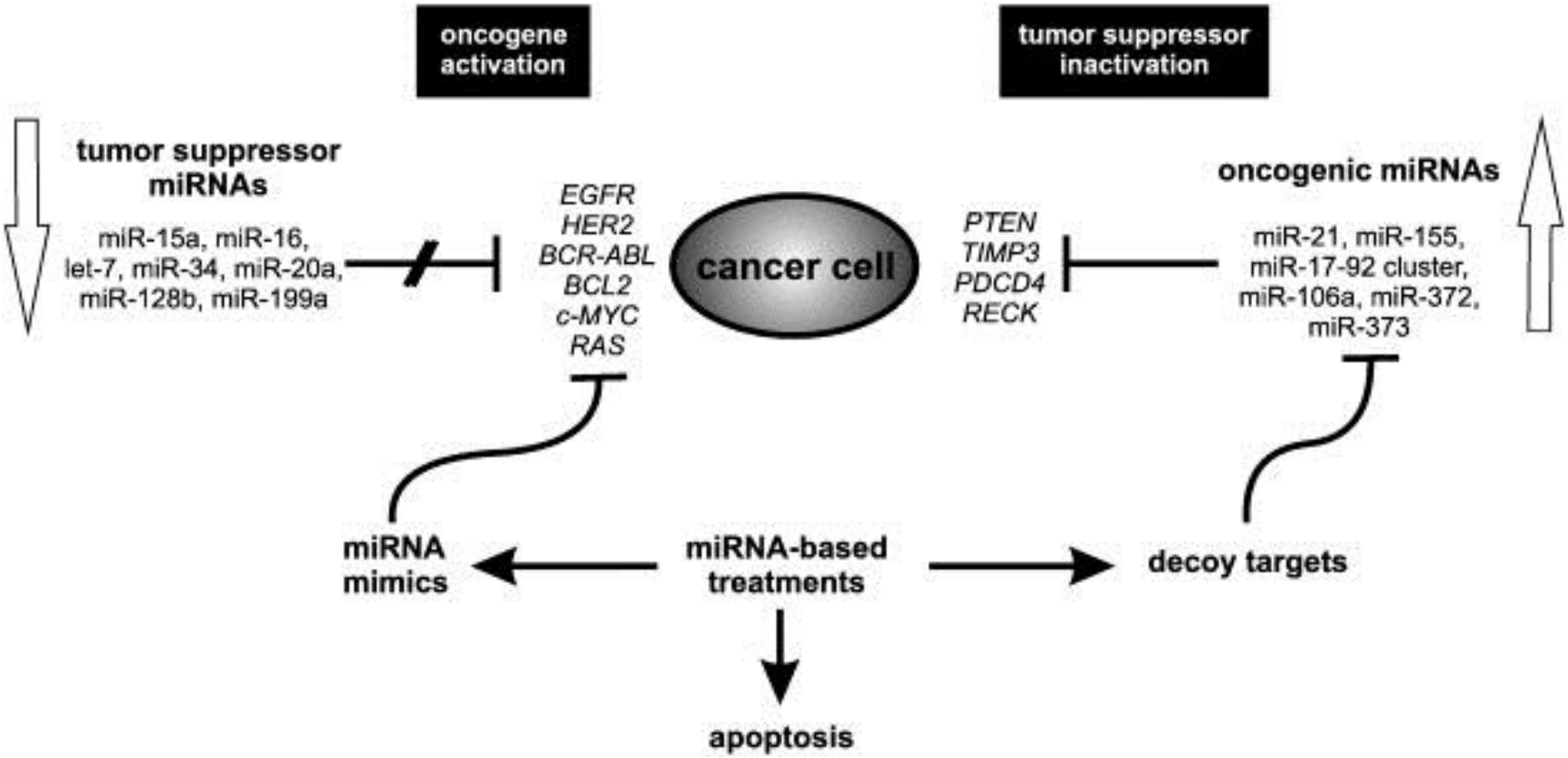
Disease	miRNA abnormality	Functional effect	Ref.
Alzheimer's Disease	Decreased expression of miR-29a/b-1 cluster	miR-29a and miR-29b-1 regulate BACE-1 expression. Loss leads to abnormally high BACE-1 levels	4
Alzheimer's Disease	Decreased expression of miR-107	miR-107 regulates BACE-1 expression. Loss leads to abnormally high BACE-1 levels. Loss seen in early stages of disease	6
Alzheimer's Disease	Decreased expression of miR-106b	miR-106b regulates APP expression	5
Huntington's Disease	Decreased expression of miR-9/9*	miR-9/9* is a bidirectional miRNA with one strand, miR-9, regulating the transcriptional regulator REST, and the other strand, miR-9*, regulating CoREST. In turn REST and CoREST negatively regulate miR-9/9* such that double negative feedback loop is seen	7
Parkinson's Disease	Decreased expression of miR-133b	miR-133b regulates maturation and activity of midbrain dopaminergic neurons in subsequent murine models through a negative feedback loop with transcription factor Pitx3	8
Parkinson's Disease	Polymorphism in fibroblast growth factor 20 target site of miR-433	Parkinson's disease-associated polymorphism leads to reduced miR-433 repression of target and subsequent downstream increase in α-synuclein expression	12
Schizophrenia	Decreased expression of miR-26b, miR-92, miR-24 and miR-30e	Unknown mechanisms. Upto 15 miRNAs decreased and 1 increased with microarrays. Confirmed 4 to be significant with qPCR	9
Autism	Dysregulated expression of 9 miRNAs	Unknown mechanisms. Upto 28 miRNAs dysregulated in initial analysis. Confirmed 9 to be significant with further analysis	10
Tourette's Syndrome	Polymorphism in Slit and Trk-like 1 target site of miR-189	Tourette's-associated polymorphism leads to enhanced miR-189 repression of target site	11
TDP43-frontal temporal dementia	Polymorphism in progranulin target site of miR-659	Common polymorphism leads to enhanced miR-659 repression of target and is associated with 3-fold increase in susceptibility to disease	13
Aggressive behaviour	Polymorphism in serotonin 1B receptor target site of miR-96	Common polymorphism leads to enhanced miR-96 repression of target and is associated with increased aggression	14

miRNA in terapija


A
Downregulated miRNA levels in disease
Therapeutic strategies:
Aa
DNA demethylating agents and HDAC inhibitors
Epigenetic miRNA gene silencing
miRNA gene re-expression
Ab
miRNA virus delivery systems
Adenovirus-associated vectors encoding downregulated miRNAs
New exogenous pre-miRNAs
Ac
miRNAome-based strategies
Drugs that enhance miRNA processing
Pre-miRNAs
miRNA duplex
miRNA replacement
miRNA biogenesis and processing
B
Upregulated miRNA levels in disease
Therapeutic strategies:
Complementarity-based miRNA inhibition:
Ba
Synthetic anti-miRNAs
Synthetic anti-miRNAs (ASOs, AMOs, antagomirs and LNAs)
miRNA–anti-miRNA binding
Mature miRNA
Bb
miRNA sponges
Sponge (multiple artificial miRNA binding sites)
miRNA–sponge binding
miRNA sequestration
Normal miRNA levels


a Strand loading — luciferase reporter
or
or
5′
3′
3′
5′
Passenger
Guide
AGO
Passenger strand loaded into RISC
Luciferase
AAAAAAAA
3′ UTR
Guide strand loaded into RISC
Luciferase
AAAAAAAA
3′ UTR
b Processing — northern blot evaluation
Poor
Effective
Processing
shRNA
Guide strand
1
2
c Small RNA quantitative PCR
Effectively processed
Poorly processed
Ct
Cycle

Terapevtski pristopi uravnavanja miRNA pri rakih



Clinical setting	Drug	Indication(s)	Target(s)	Sponsor	Status
Ocular and retinal disorders	TD101	Pachyonychia congenita	Keratin 6A N171K mutant	Pachyonychia Congenita Project	Completed, Phase I
	QPI-1007	Non-arteritic anterior ischaemic optic neuropathy	Caspase 2	Quark Pharm., Inc.	Active, Phase I
	AGN211745	Age-related macular degeneration; choroidal neovascularization	VEGFR1	Sirna Therapeutics, Inc.	Completed, Phase I, II
	PF-655	Diabetic macular oedema (DME); age-related macular degeneration (AMD)	RTP801	Quark Pharm., Inc.	Active, Phase I
	SYL040012	Glaucoma	β2 adrenergic receptor	Sylentis	Active, Phase I, II
	Bevasiranib	Diabetic macular oedema	VEGF	Opko Health, Inc.	Completed, Phase II
	Bevasiranib	Macular degeneration	VEGF	Opko Health, Inc.	Completed, Phase II
Cancer	CEQ508	Familial adenomatous polyposis	β-catenin	MDRNA, Inc.	Active, Phase I
	ALN-PLK1	Liver tumours	PLK1	Alnyam Pharm.	Active, Phase I
	FANG	Solid tumours	Furin	Gradalis, Inc.	Active, Phase II
	CALAA-01	Solid tumours	RRM2	Calando Pharm.	Active, Phase I
	SPC2996	Chronic myeloid leukemia	BCL-2	Santaris Pharm.	Ongoing, Phase I, II
	ALN-VSP02	Solid tumours	VEGF, kinesin spindle protein	Alnylam Pharm.	Active, Phase I
	NCT00672542	Metastatic melanoma	LMP2, LMP7, and MECL1	Duke University	Active, Phase I
	Atu027	Advanced, recurrent or metastatic solid malignancies	PKN3	Silence Therapeutics	Active, Phase I
Kidney disorders	QPI-1002/I5NP	Acute kidney injury	p53	Quark Pharm., Inc.	Terminated, Phase I
	QPI-1002/I5NP	Delayed graft function kidney transplant	p53	Quark Pharm., Inc.	Active, Phase I, II
	QPI-1002/I5NP	Kidney injury acute renal failure	p53	Quark Pharm., Inc.	Completed, Phase I
LDL lowering	TKM-ApoB	Hypercholesterolaemia	APOB	Tekmira Pharm. Corp.	Terminated, Phase I
	PRO-040,201	Hypercholesterolaemia	APOB	Tekmira Pharm. Corp.	Terminated, Phase I
Antiviral	SPC3649	Hepatitis C virus	miR-122	Santaris Pharm	Active, Phase II
	pHIV7-shI-TAR-CCR5RZ	HIV	HIV Tat protein, HIV TAR RNA, human CCR5	City of Hope Medical Center/Benitec	Active, Phase 0
	ALN-RSV01	RSV in volunteers	RSV nucleocapsid	Alnylam Pharm.	Completed, Phase II
	ALN-RSV01	RSV in lung transplant patients	RSV nucleocapsid	Alnylam Pharm.	Completed, Phase I
	ALN-RSV01	RSV in lung transplant patients	RSV nucleocapsid	Alnylam Pharm.	Active, Phase II

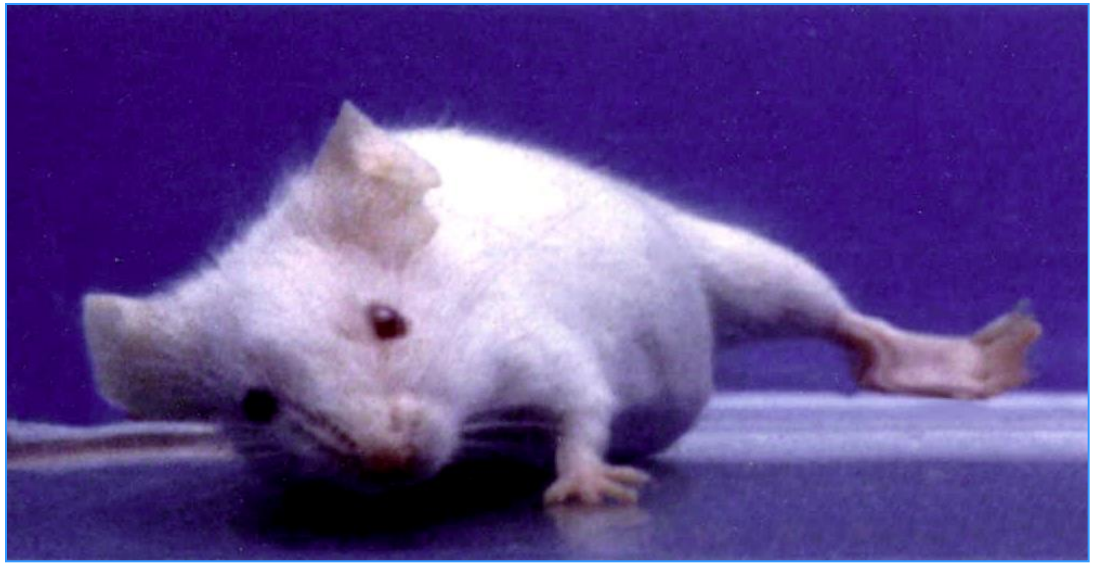
APOB, apolipoprotein B; BCL-2, B-cell CLL/lymphoma 2; CCR5, C-C chemokine receptor type 5; LDL, low-density lipoprotein; LMP2, also known as proteasome subunit beta type 9 (PSMB9); LMP7, also known as proteasome subunit beta type 8 (PSMB8); MECL1, also known as proteasome subunit beta type 10 (PSMB10); Pharm., Pharmaceuticals; PKN3, protein kinase N3; PLK1, polo-like kinase 1; RRM2, ribonucleoside-diphosphate reductase subunit M2; RSV, respiratory syncytial virus; RTP801, also known as DNA damage-inducible transcript 4 protein (DDIT4); VEGF, vascular endothelial growth factor. *From ClinicalTrials.gov.

Vnos terapevtskih miRNA

Species/ formulation	Packaging capacity	Applications and considerations	Refs*
Viral vector			
Adenovirus	Up to ~35 kb, usually <10 kb	dsDNA vector with large packaging capacity, transient expression, highly immunogenic	76,77
Adeno-associated virus (AAV)	~4.5 kb	ssDNA vector, small packaging capacity, mildly immunogenic, lasting expression in nondividing cells, capsid pseudotyping/engineering facilitates specific cell-targeting	82,91, 103,108
Lentivirus	Up to 13.5 kb (larger inserts will decrease titre)	RNA vector, integration competent and incompetent forms available, less immunogenic than adenovirus or AAV, envelope pseudotyping facilitates cell targeting, clinical production more difficult than for adenovirus or AAV	83-88, 140,155
Herpes simplex virus	150 kb	DNA vector, episomal, lasting expression, immunogenic	119
Bacterial vector species‡			
Escherichia coli, S. Typhymurium§		Delivery of short hairpin RNA or small interfering RNA to gut tissue	73-75
Non-viral formulations‖			
Nanoparticle		Self-assembling, may target specific receptors, requires technical expertise to prepare	59
Stable nucleic acid lipid particle (SNALP)		Stable for systemic delivery, broad cell-type delivery	51
Aptamer		Targeting of specific receptors, requires sophisticated screening to develop	53
Cholesterol		Stable for systemic delivery, broad cell-type delivery	46

*Representative references. ‡Bacterial minicells can carry plasmids, short interfering RNAs or drugs. §*Salmonella enterica* subsp. *enterica* serovar Typhimurium. ‖The nucleic acids in non-viral carriers can be any size from small oligonucleotides to large artificial chromosomes.

Uporaba RNAi pri poliQ bolezni Spinocerebelarna ataksija tip 1 (SCA1)

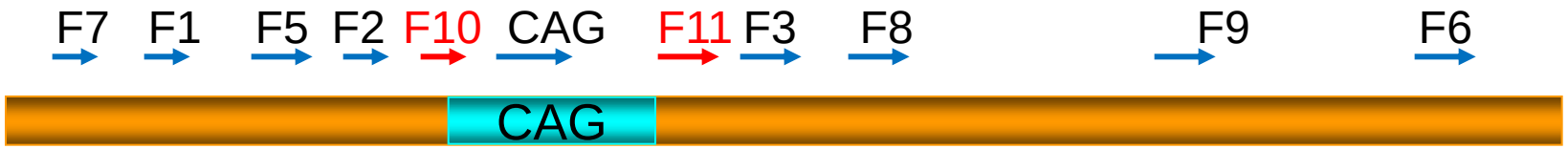


SCA1 transgena miška (Burright et al., Cell, 1995)

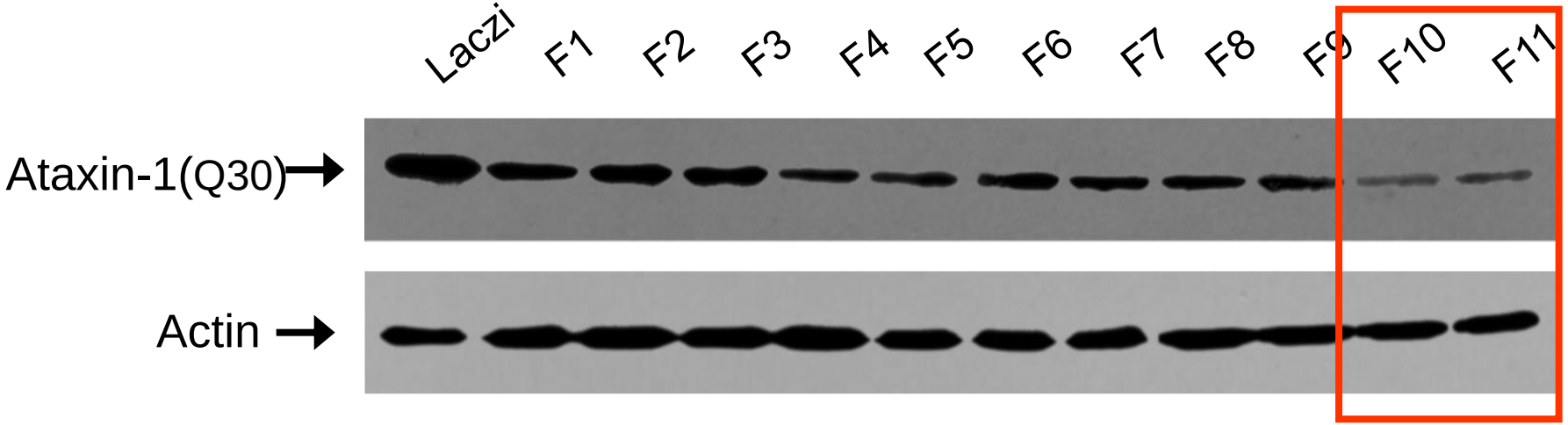
V Purkinjevih celicah v malih možganih izraža človeški gen ataxin-1 z 82 CAG ponovitvami.

Miška kaže napredujočo ataksijo (nekoordiniranost gibov). V jedrih Purkinje celic so agregati mutiranega ataxina-1.

Izbira RNAi proti mutiranemu ataxin-1



Človeški SCA1 ORF, ki kodira za ataxin-1

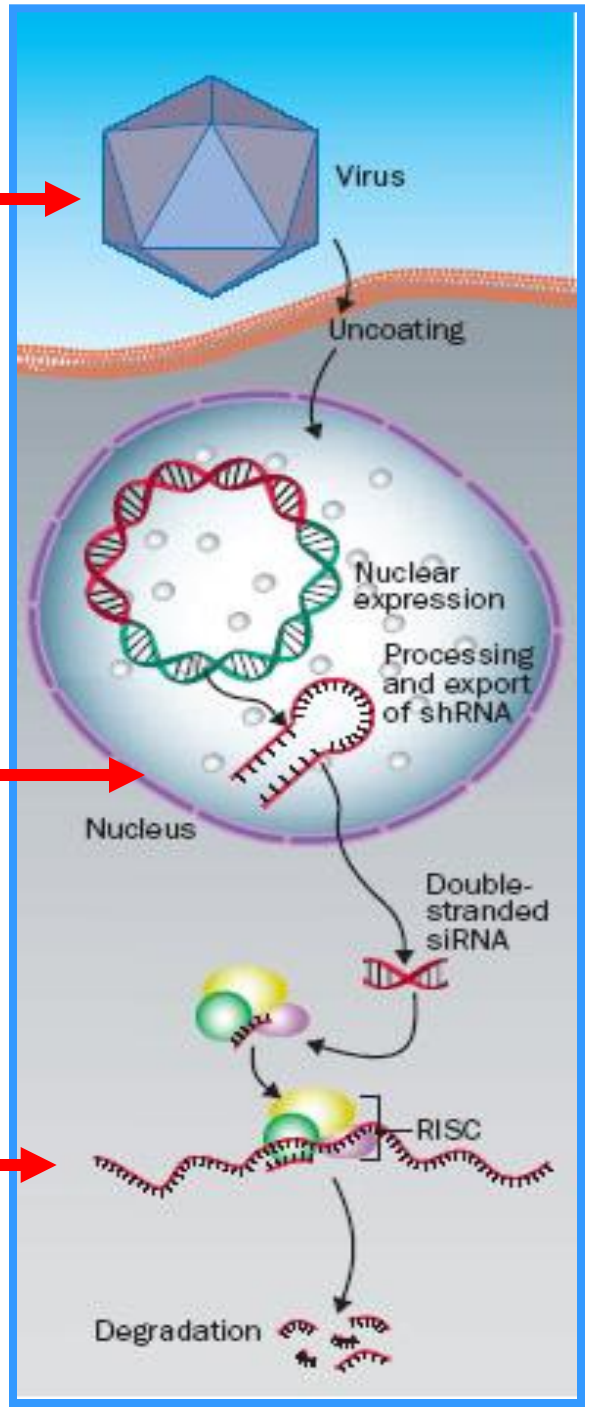


Xia et al, Nature Medicine, 2004

Rekombinantni virus izraža RNA v obliki kratkih lasnic (shRNA)

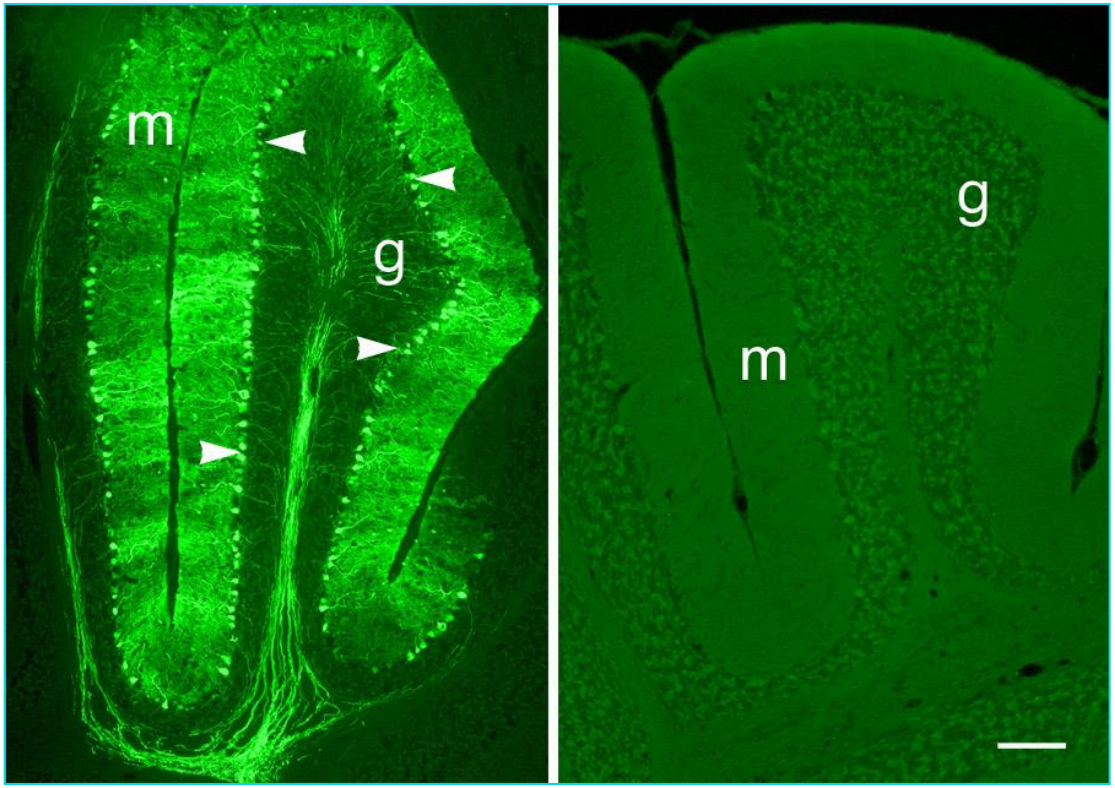
shRNA se veže na ataxin 1 mRNA

Razgradnja ataxin 1 mRNA



The Lancet Neurol 3(3):145-149, 2004

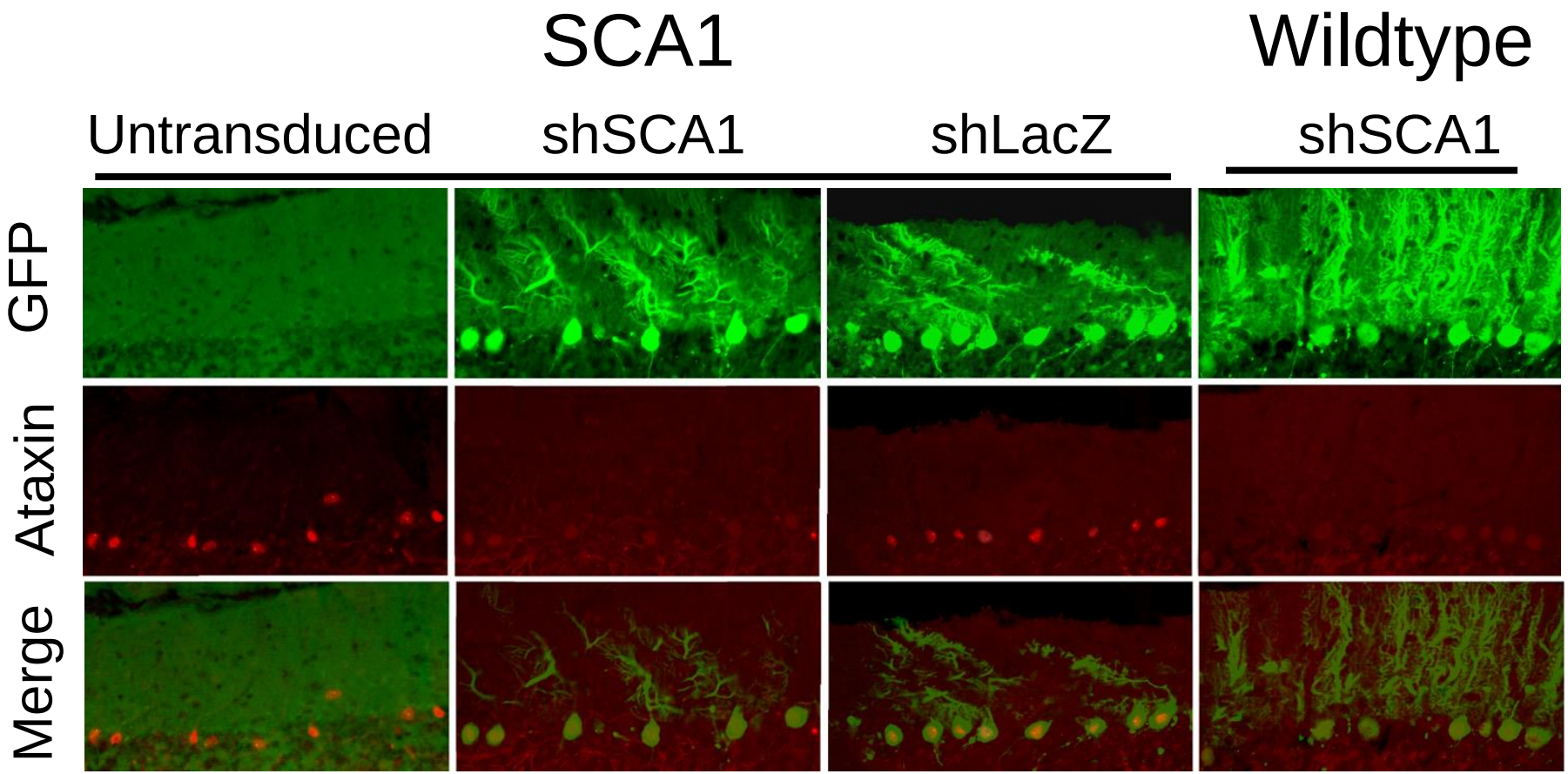
Testiranje dostave *in vivo*



Rekombinantni AAV1 pride v večino Purkinjevih celic.

Xia et al., (2004) Nat Med 10:816

ShSCA1.F10 zniža izražanje ataxin-1(Q82) *in vivo*



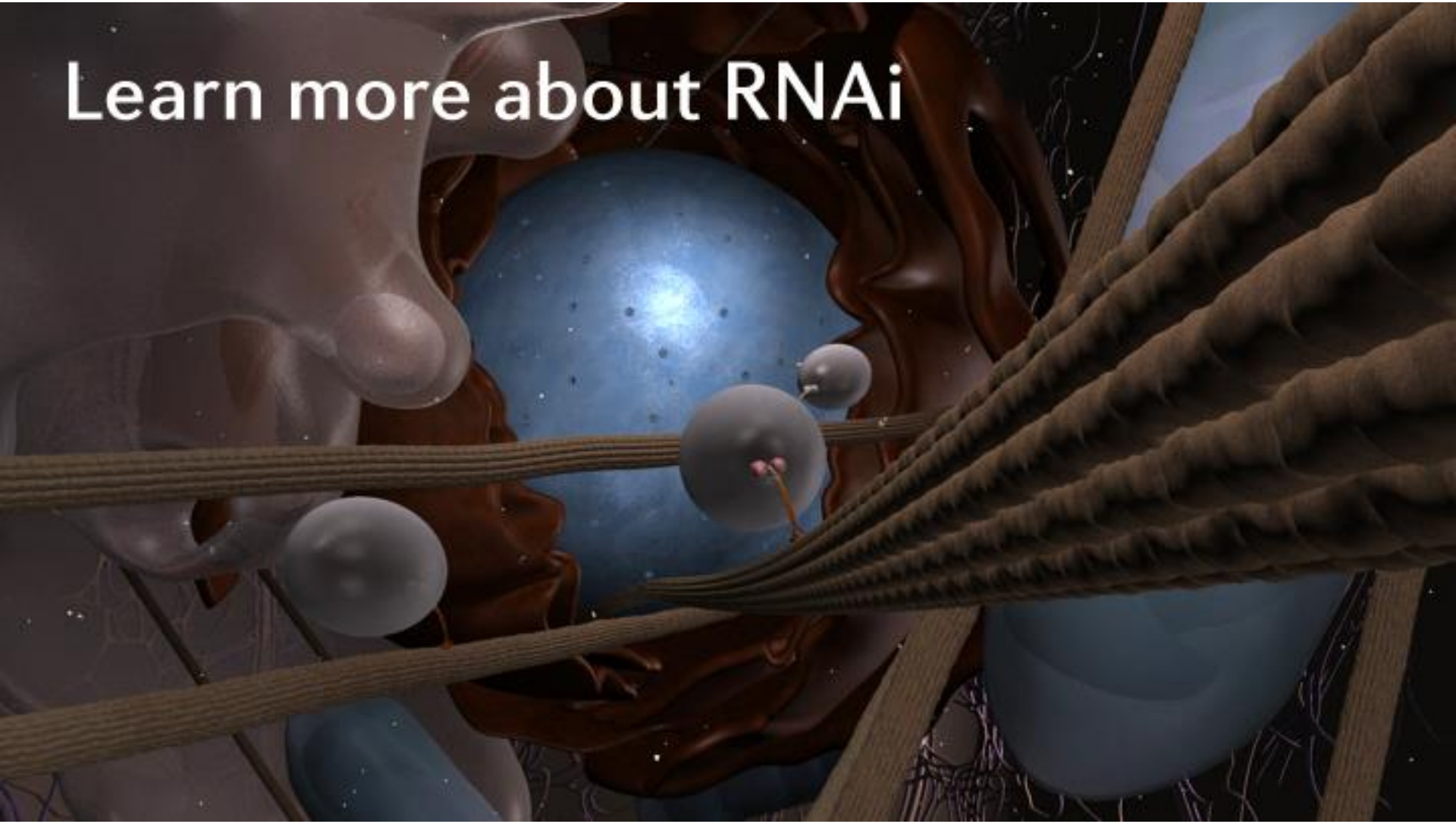
Xia et al., (2004) Nat Med 10:816

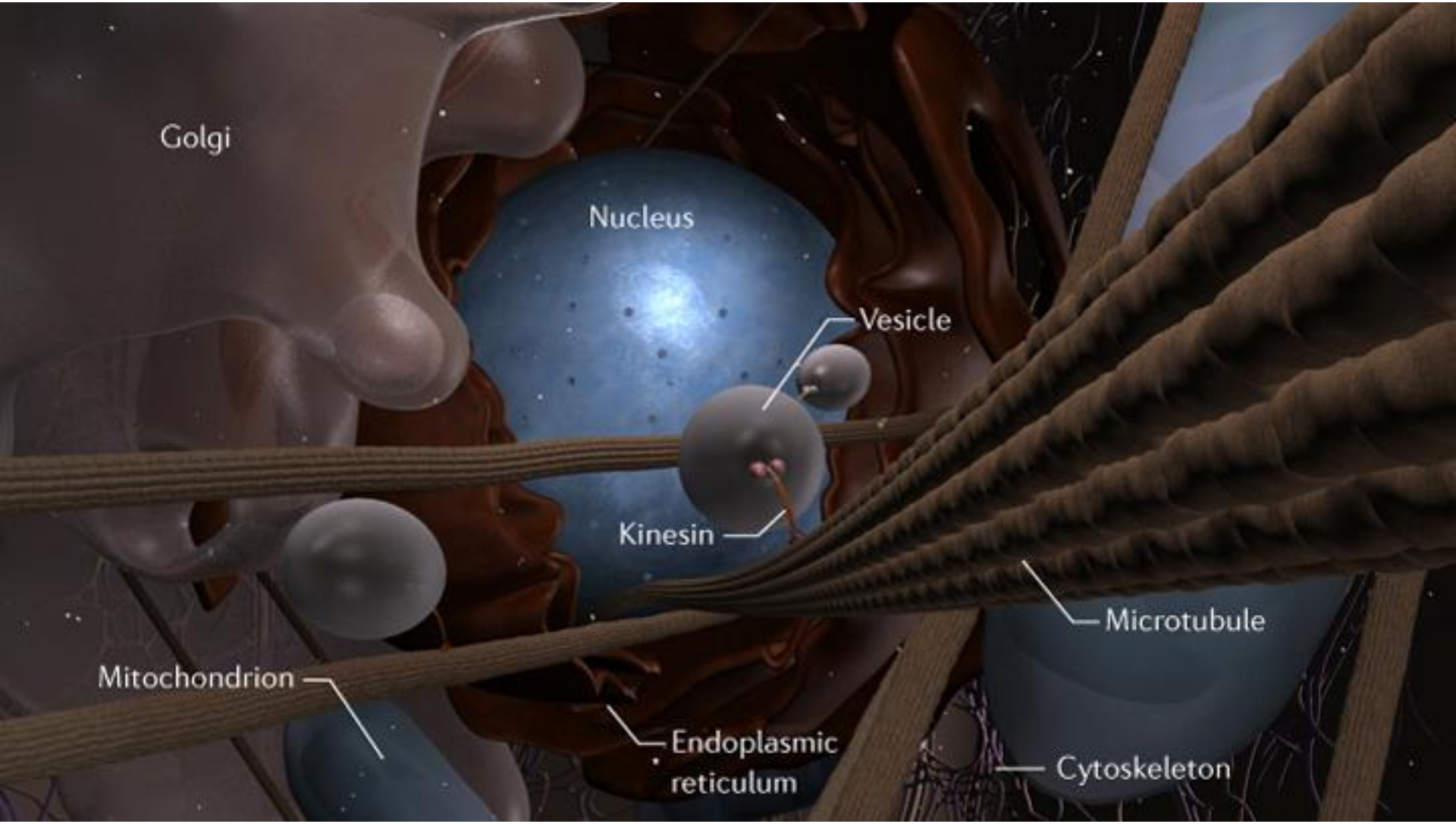
Povzetek rezultatov pri miški SCA1

- Utišanje izražanja proteina Ataxin-1 z uporabo RNAi.
- Zmanjšanje inkluzij, atrofije dendritov in ataksije.
- Miške dobro prenesejo virusno dostavo shRNA.
- Predklinične raziskave je potrebno prenesti v klinične raziskave.

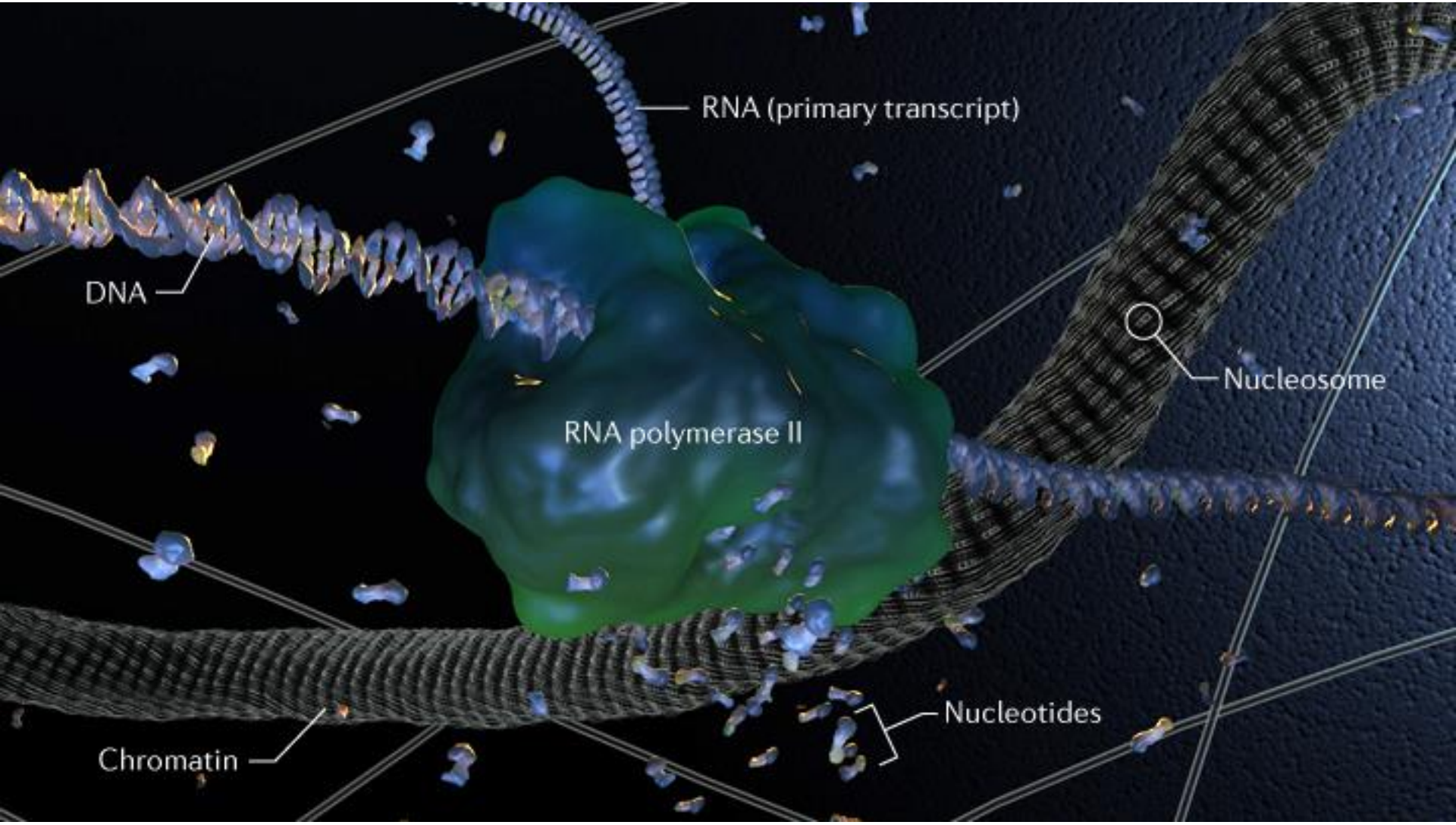
RNAi animacija

- Vir: Nature Reviews Genetics

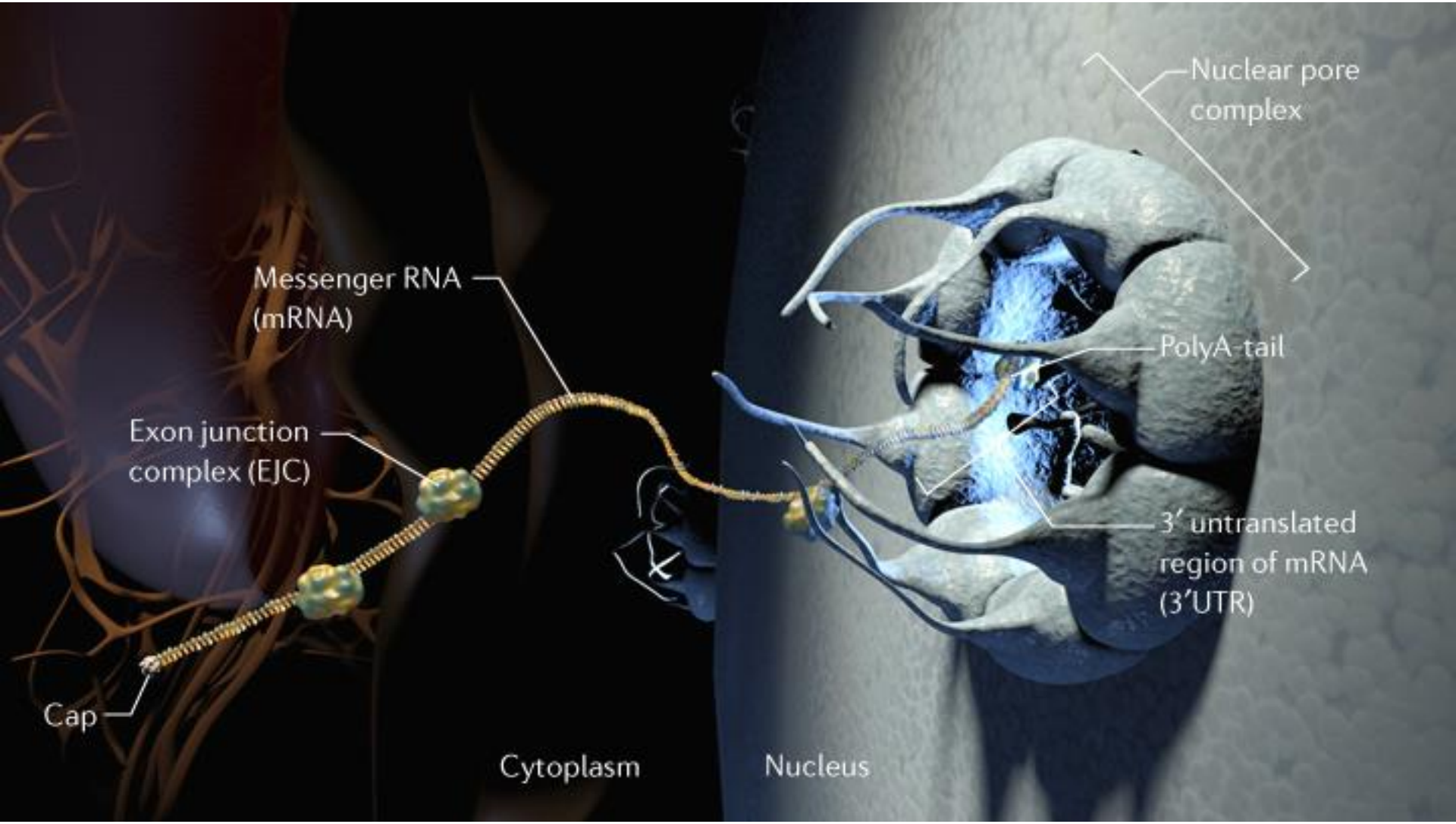

Learn more about RNAi



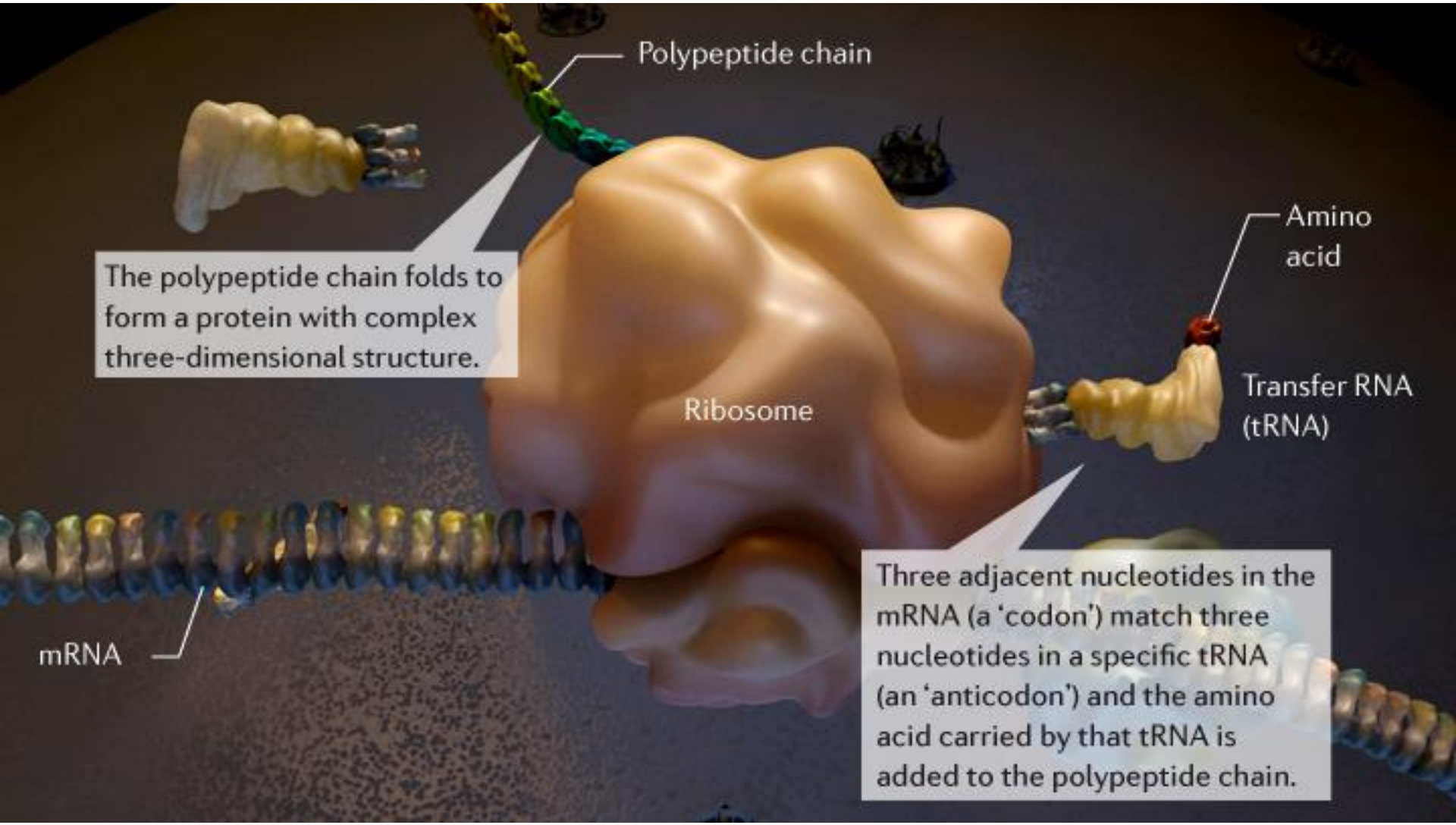
- RNA interferenca — RNAi — je celični proces za utišanje specifičnih genov. Proces onemogoči sintezo proteinov. V RNAi so lahko vpletene različne vrste malih RNA. Pri najbolj znanem procesu RNAi male RNA poiščejo svojo tarčno mRNA v citoplazmi.



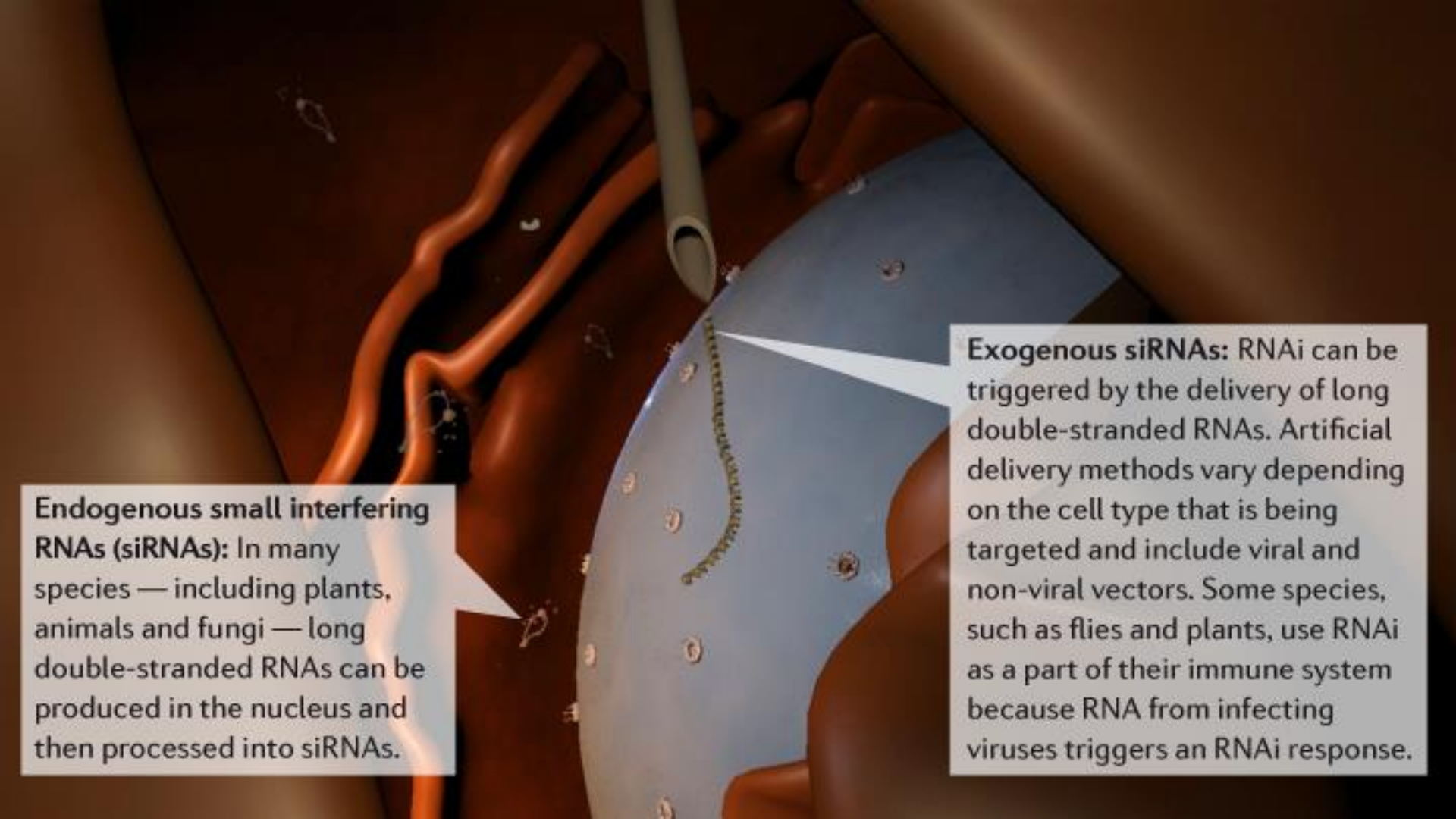
- V evkartiontskih celicah se geni prepišejo z RNA polimerazo II (RNAPII). Pri procesiranju primarnega transkripta v zrelo mRNA se izrežejo introni in doda kapa na 5‘ konec RNA.



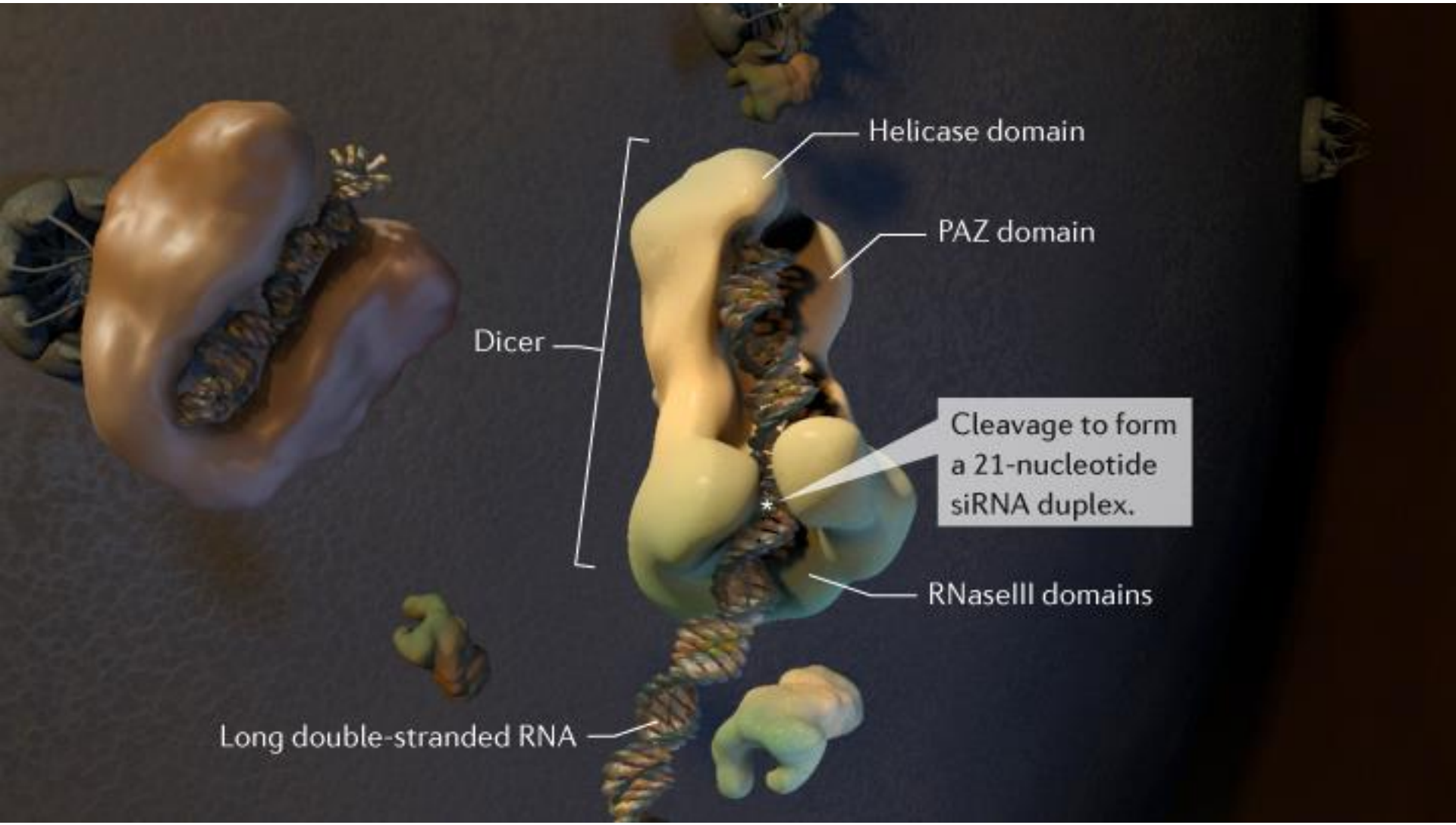
- mRNA potuje iz jedra v citoplazmo skozi jedrno poro. Jedrna pora je eden največjih proteinskih kompleksov v celici in je sestavljena iz več kopij približno 30 proteinov. Filamenti na citoplazemski strani pore usmerjajo mRNA proti ribosomu.



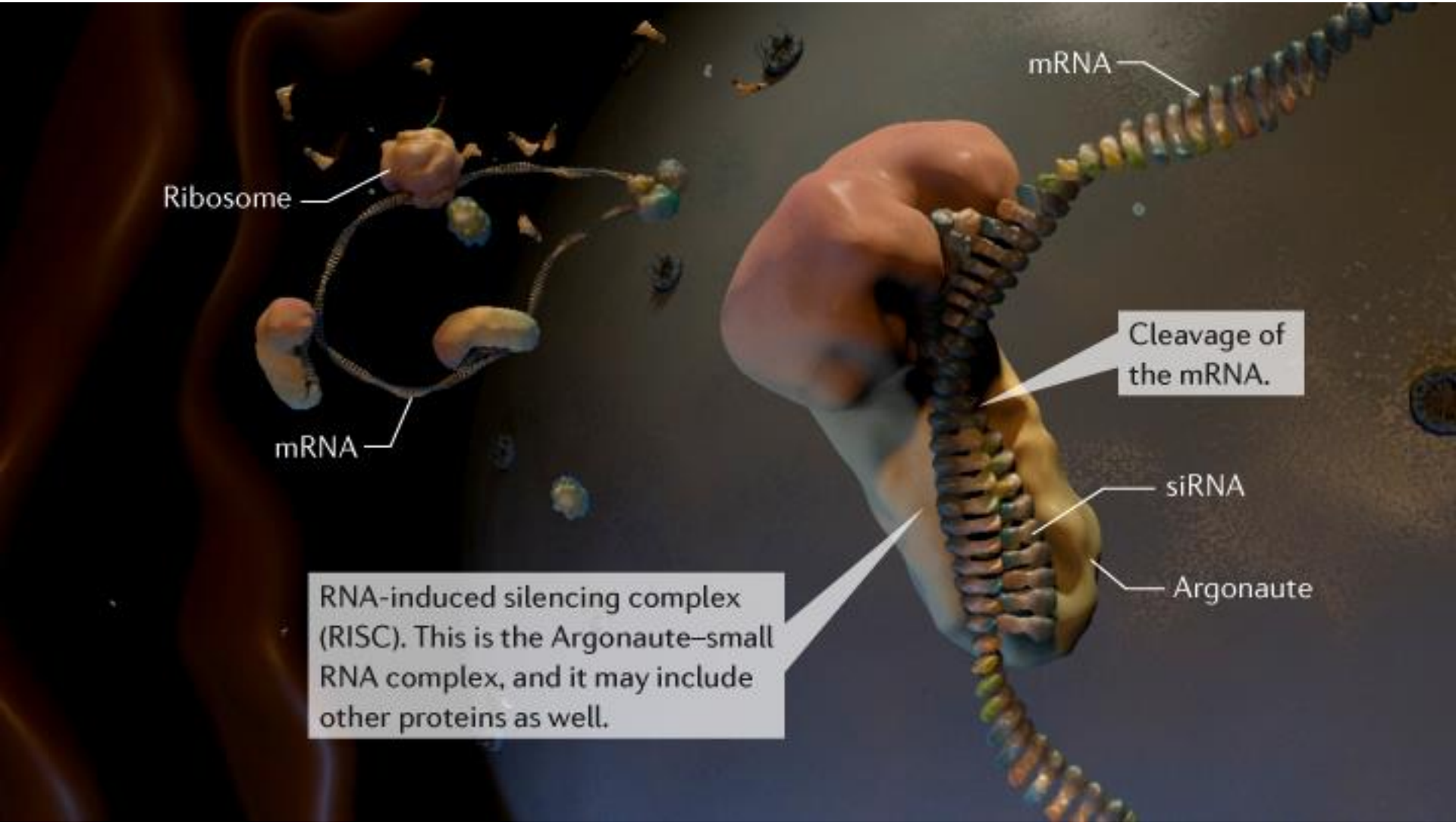
- Translacija poteka na ribosomih, ki so sestavljeni iz proteinov in RNA – rRNA. Polipeptidna veriga se lahko delno zvije med translacijo, glavno mesto, kjer se zvijajo proteini pa je endoplazemski retikulum. Mnogi ribosomi so vezani na ER. RNAi mora delovati na mRNA, da se prepreči sinteza proteinov.



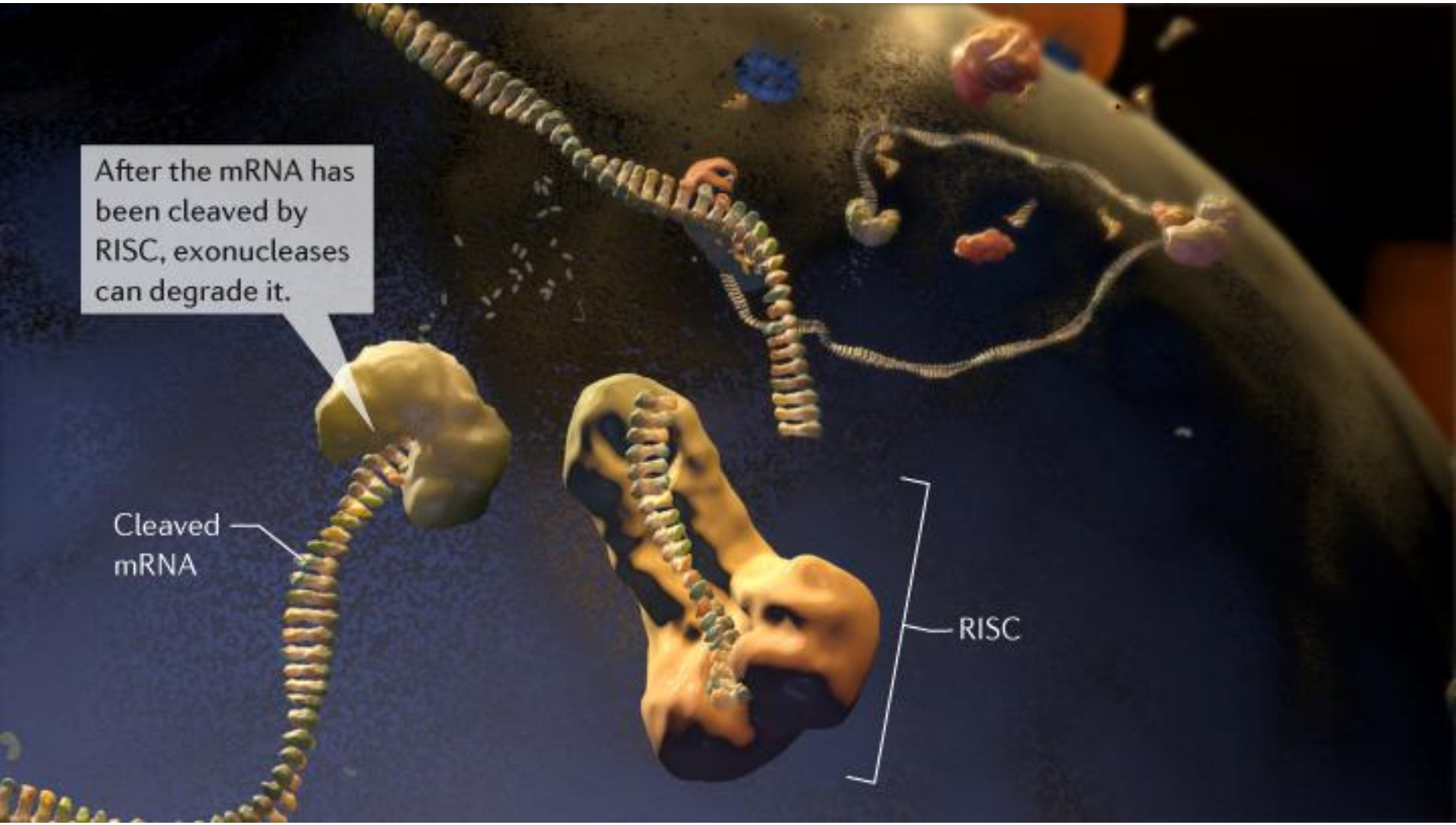
- Utišanje genov z RNAi se široko uporablja v laboratorijih za ugotavljanje funkcij genov, najbolj pogosto z RNA lasnico. Danes so na voljo specifične siRNA za utišanje skoraj vseh genov v človeških kulturah ali modelnih organizmih. Z siRNA naj bi popravili nepravilno izražanje genov pri človeku. Pri terapijah z RNAi je zelo pomembno odkriti način, kako učinkovito dostaviti molekulo na ustrezno mesto – specifično tkivo.



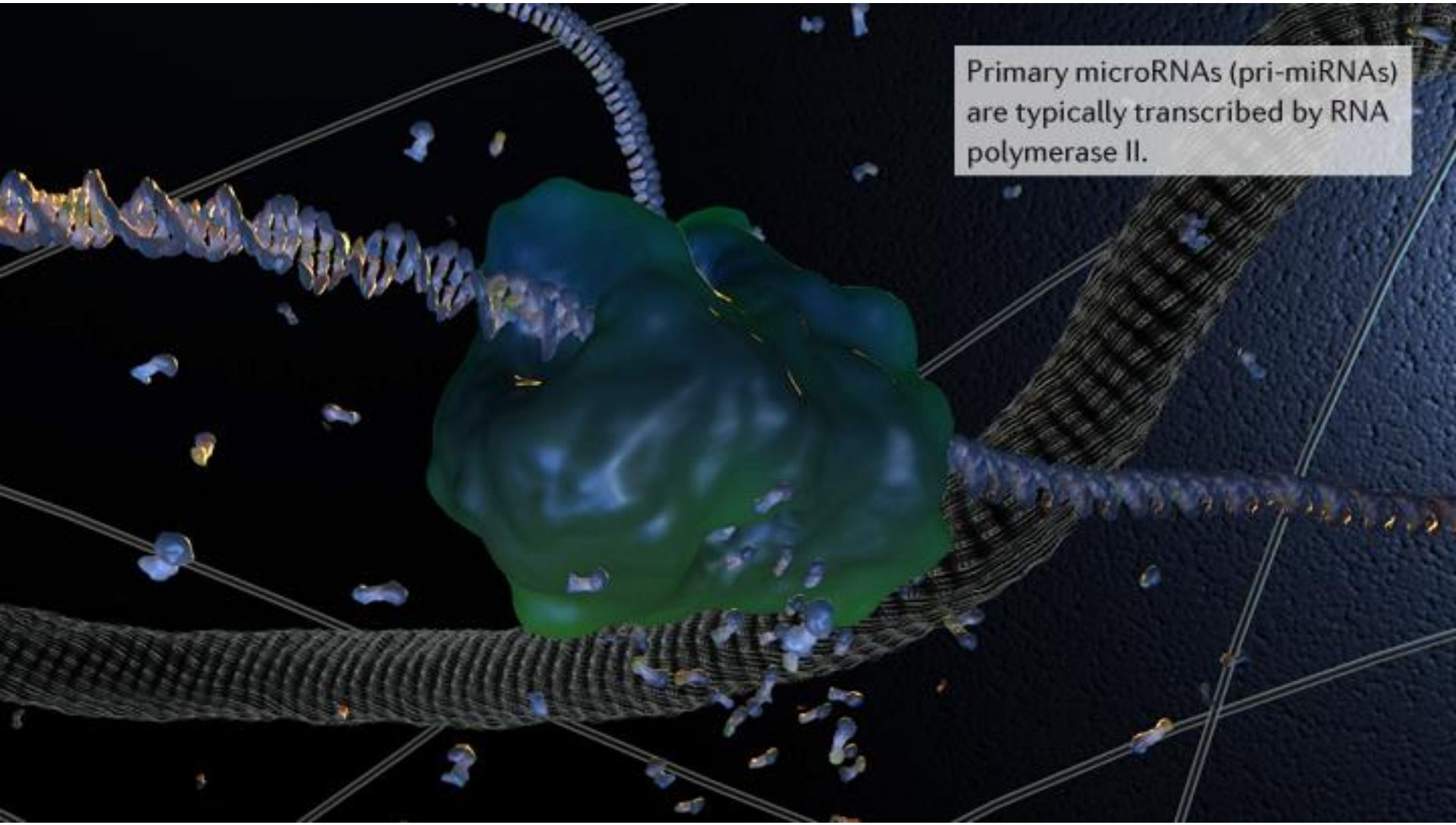
- Dicer je ribonukleaza iz družine proteinov Rnaza III, ki cepi dvoverižno RNA. Pri cepitvi z Dicerjem večinom nastanejo dvoverižne siRNA, dolge 21 nukleotidov. Na 3‘ koncu imajo 2 nukleotida dolg štreleči konec, fosfat na 5‘ koncu in 3‘ hidroksilno skupino. (model na sliki je približek človeškega Dicerja, mesta niso popolnoma pravilna)



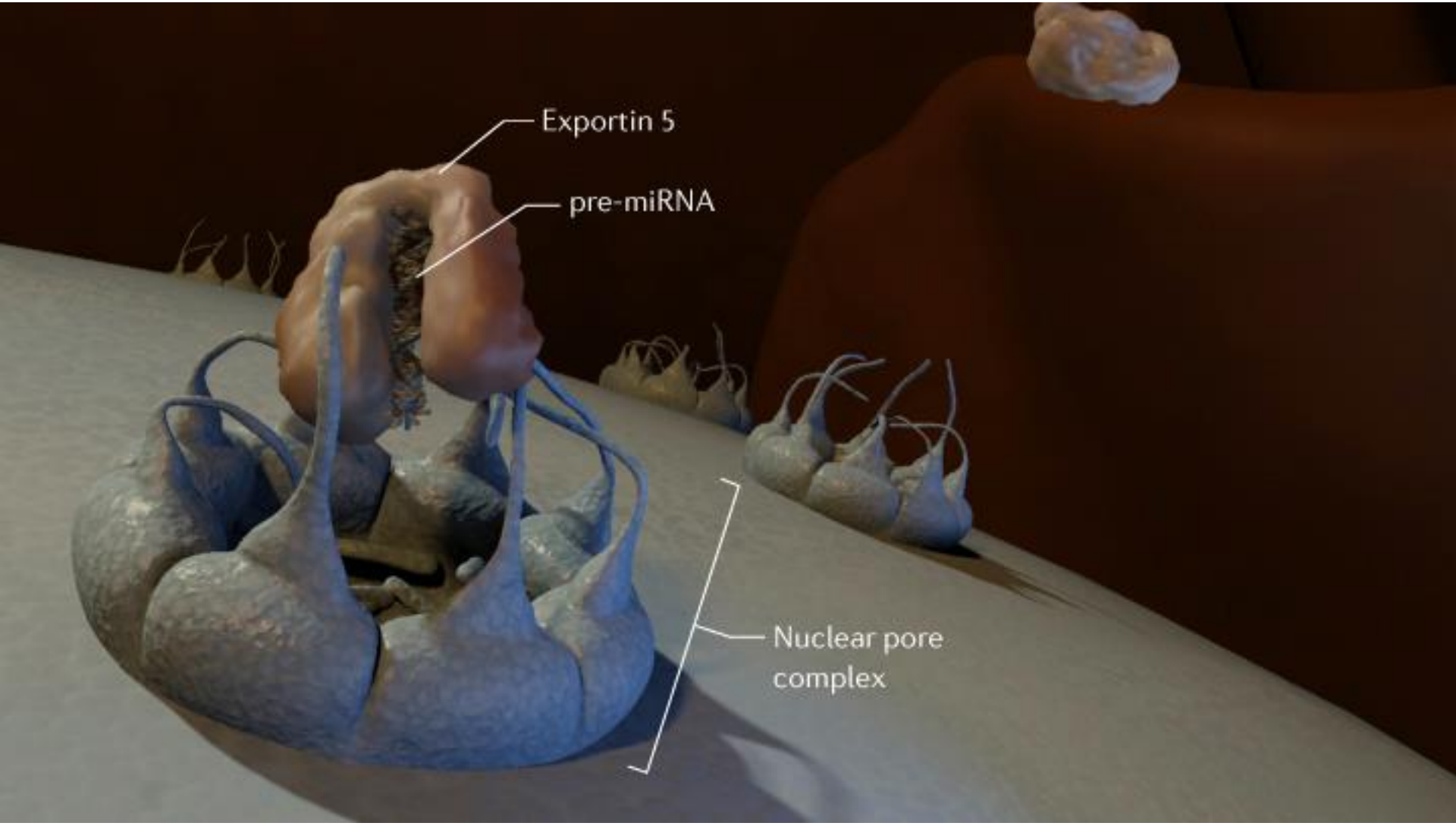
- Argonaute katalizira cepitv mRNA blizu sredine regije, kjer se nanjo veže siRNA. Različni Argonaut proteini so pri različnih vrstah, npr. več kot 25 Argonautov je pri črvu C. elegans, 5 pri Drosophili in 4 pri človeku.



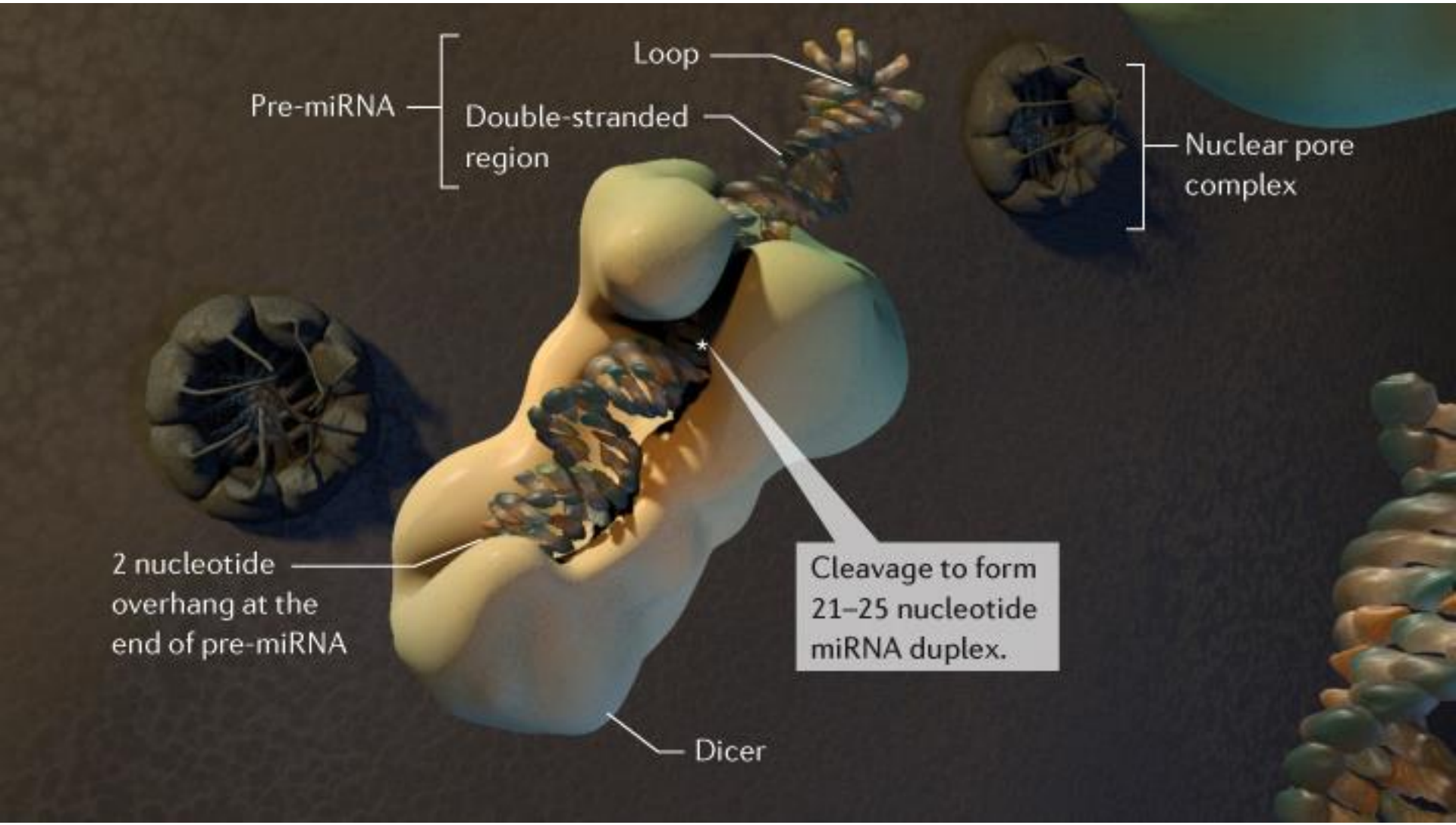
- siRNA preprči sintezo proteinov s tem, da sproži razgradnjo mRNA. Danes se večinom uporabljajo v laboratorijih za utišanje genov, ki jih raziskujejo. V prihodnosti pa naj bi jih uporabljaji kot zdravilo za zmanjšanje produkcije proteinov, ki ne delajo pravilno.



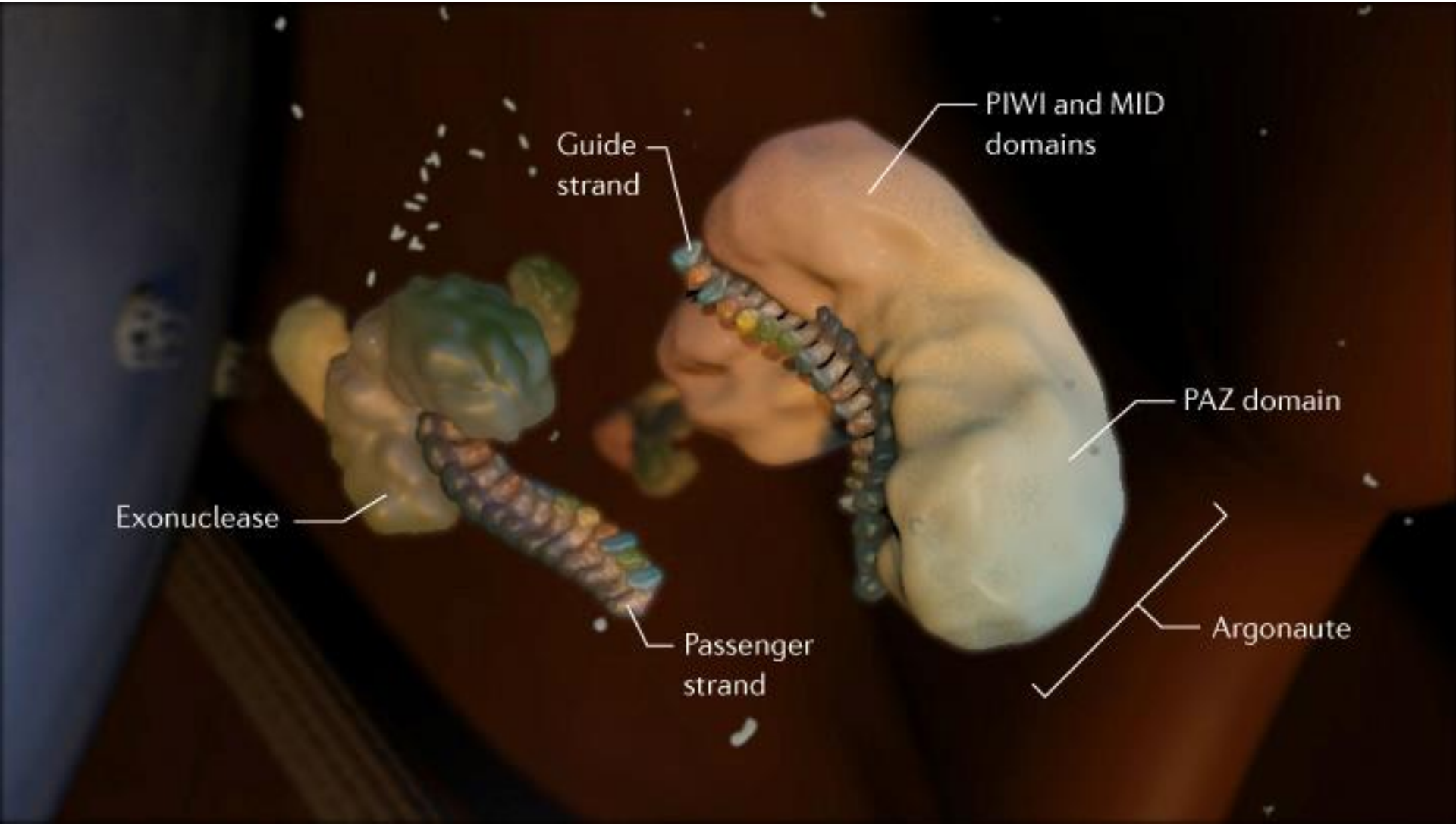
- Pri živalih se pri-miRNA cepi z endonukleazo RNAza III, ki se imenuje Drosha in nastanejo 60–70 nukleotidov dolgi prekurzorji miRNA (pre-miRNA). Drosha je protein, ki se veže na dvoverižno RNA. Pri mušicah je imenovan Pasha, pri rastlinah pa protein DCL1 opravlja funkcije Droshe in Dicerja.



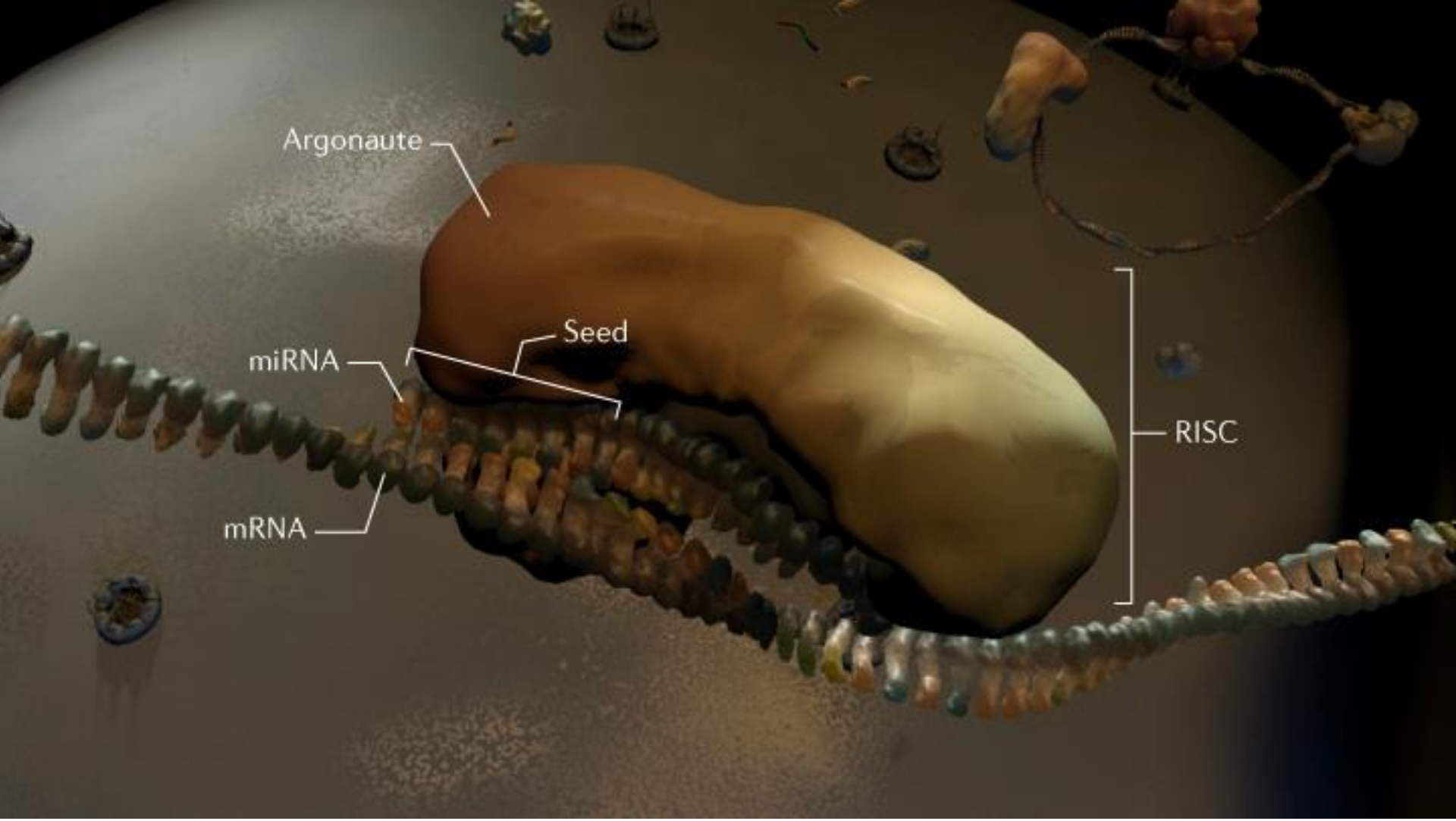
- pre-miRNA je v obliki lasnice z dvoverižno regijo in zanko, na 3‘ koncu ima 2 nt dolg štrleči konec, na 5‘ koncu pa fosfatno skupino. Pri živalih se veže na eksportin 5, s pomočjo katerega potuje skozi jedrno poro v citoplazmo.



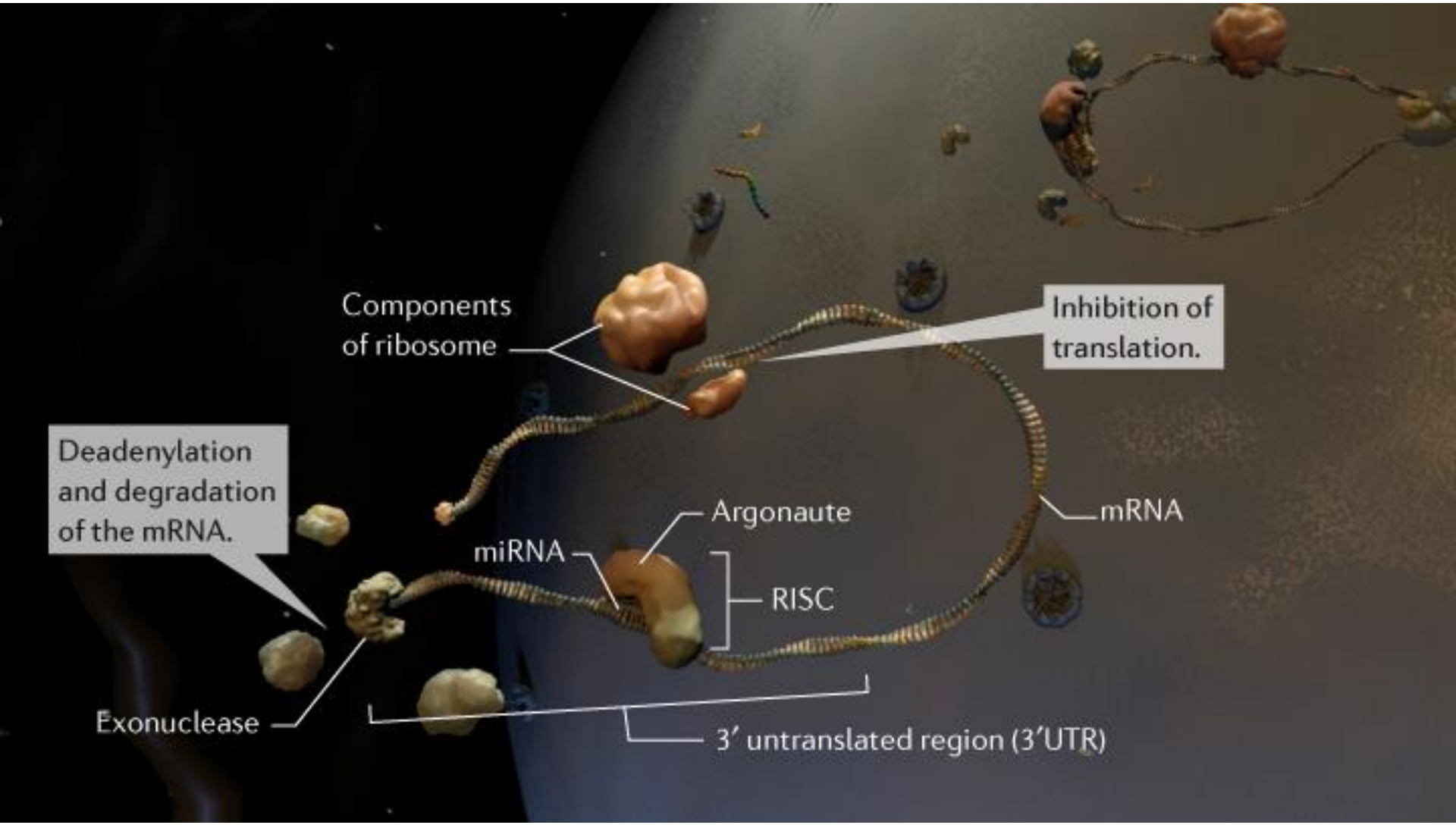
- Pri živalih Dicer v citoplazmi cepi pre-miRNA tako, da nastanejo male dvoverižne RNA. Te verige so označene kot miRNA and miRNA*. Dicer deluje v kompleksu z drugimi proteini.



- Ena veriga je izbrana, da ostane vezana na protein Argonaut, ki vodi protein do tarčne mRNA. Katera veriga se izbere za vezavo na Argonaut je odvisno od več faktorjev (npr. termodinamična stabilnost koncev verig v RNA dupleksu).

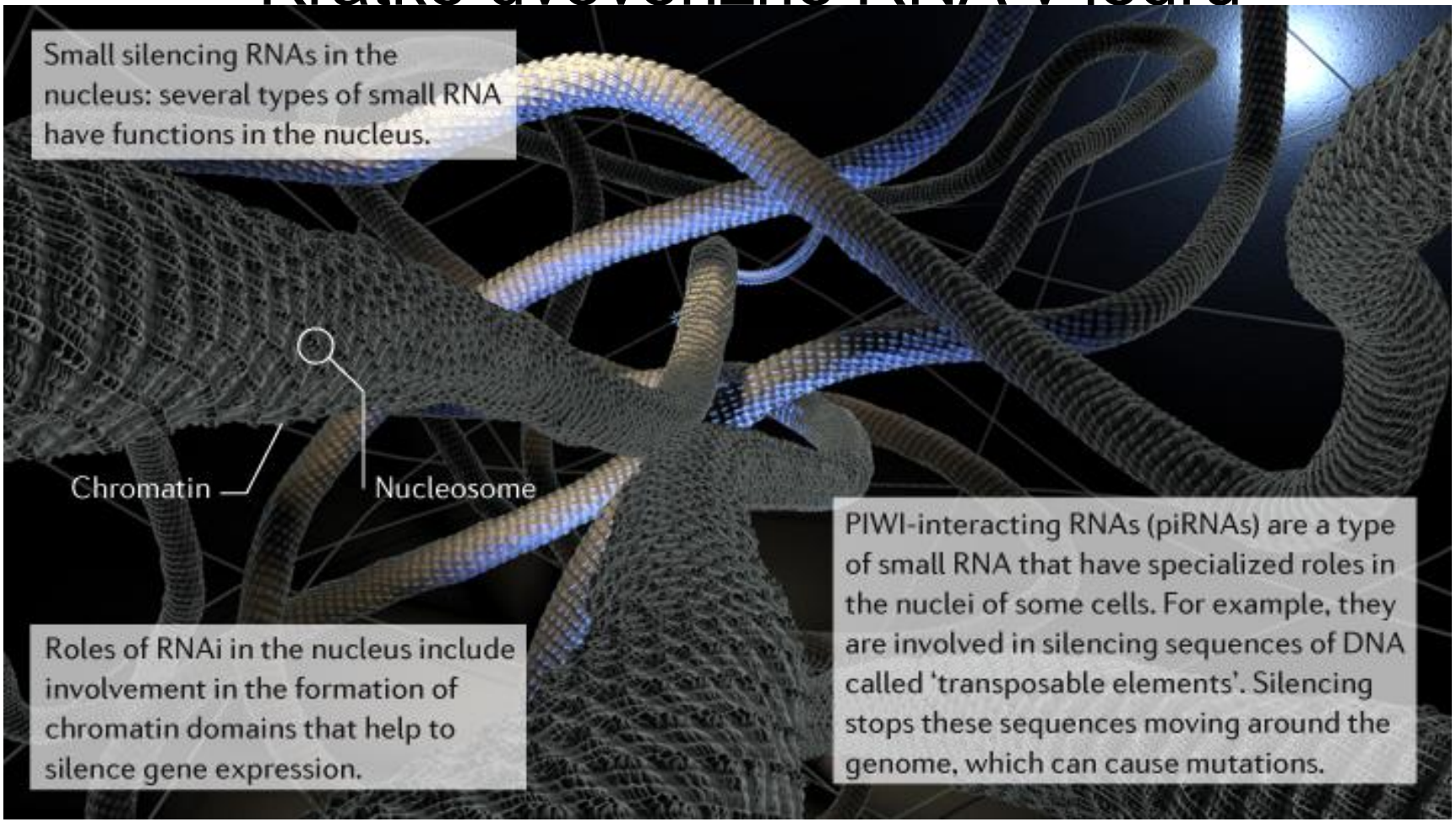


- Pri mušicah in sesalcih se večina miRNA pari s tarčno mRNA le z delom zaporedja. Pogosto je to regija na 5′ koncu, imenovana 'seed‘ regija. To pomeni, da se miRNA lahko veže na več mRNA. Večina miRNA vezavnih mest na mRNA je v 3‘UTR, včasih pa so tudi v kodirajoči regiji ali v 5‘UTR. Pri rastlinah so sprva domnevali, da se vsa miRNA veže na tarčo, sedaj pa so pokazali, da se pari lahko tudi manjši del miRNA.



- Kako miRNA povzročijo utišanje še ni popolnoma znano. Najverjetneje se inhibira translacija, odstani poliA rep, prekine kapa-rep interakcija in razgradi mRNA z eksonukleazo.

Kratke dvoverižne RNA v jedru



- piRNA – RNA, ki interagirajo s PIWI: te RNA se vežejo na proteine Argonat, imenovane PIWI. piRNA so dolge 25-30 nukleotidov in so jih našli pri večini metazoa.
- Endogene siRNA so pri rastlinah in glivah pomembne pri formiranju heterokromatina. Pri živalih vloga siRNA v jedru še ni dobro raziskana.
- Tudi vloga miRNA v jedru ni še poznana.

- http://www.nature.com/nrg/multimedia/rnai/animation/index.html

Literatura

- Bratkovič, Biotech Adv 2012, miRNA pregledni
- Croce, Nat Rev Gen 2009, miRNA in rak
- Curtis, WIRES RNA 2012, mirtroni
- Davidson, Nat Rev Gen 2011, RNAi terapije
- Krol, Nat Rev Gen 2010, miRNA pregledni
- Liang, Wires RNA 2012, Vsi smo pod kotrolo zelja in riža
- Xia, Nature medicine 2004, Sca1 miška
- Zhang, WIRES RNA 2012, razgradnja miRNA