miRNA, lncRNA e circRNA

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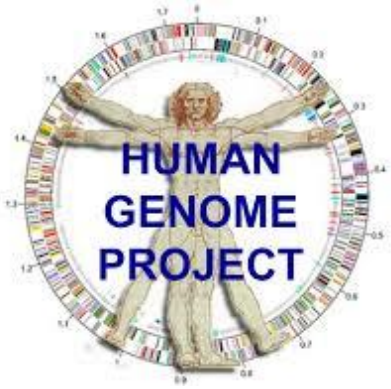
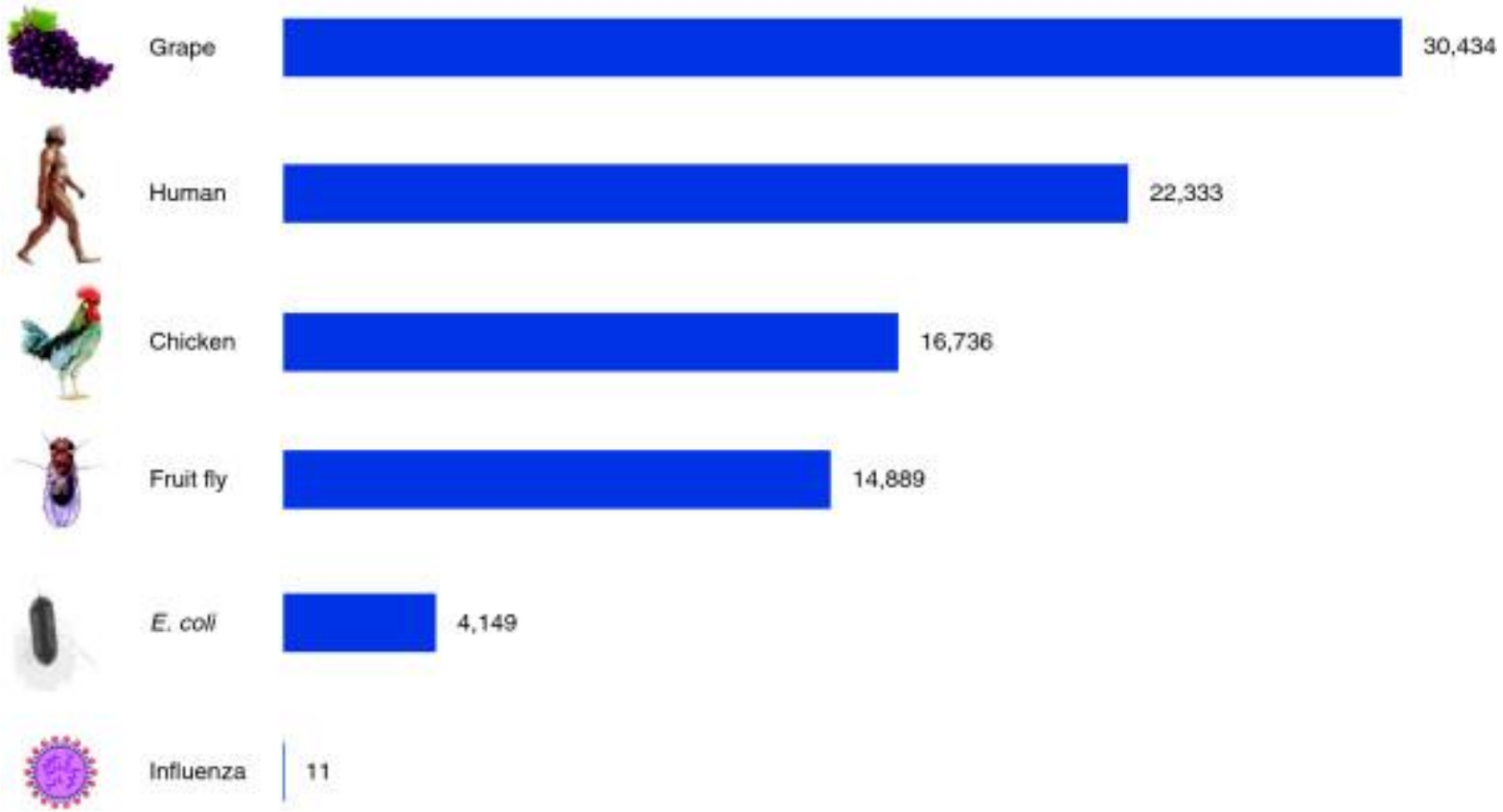
a.a. 2025-2026

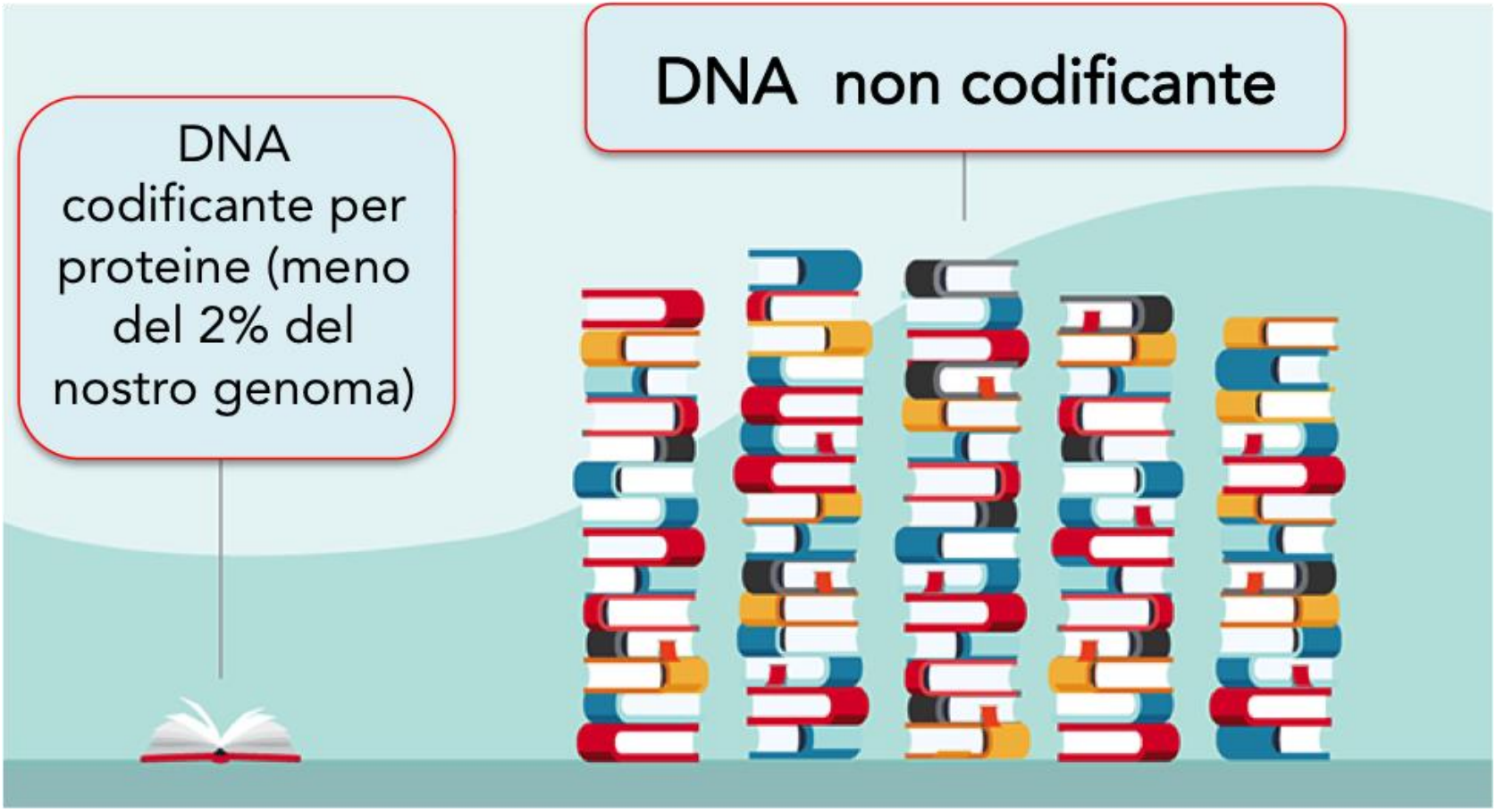


Il progetto 'Genoma Umano', completato nell'aprile del 2003, ha dimostrato che il numero dei geni umani codificanti per proteine è molto piu' basso dell'atteso

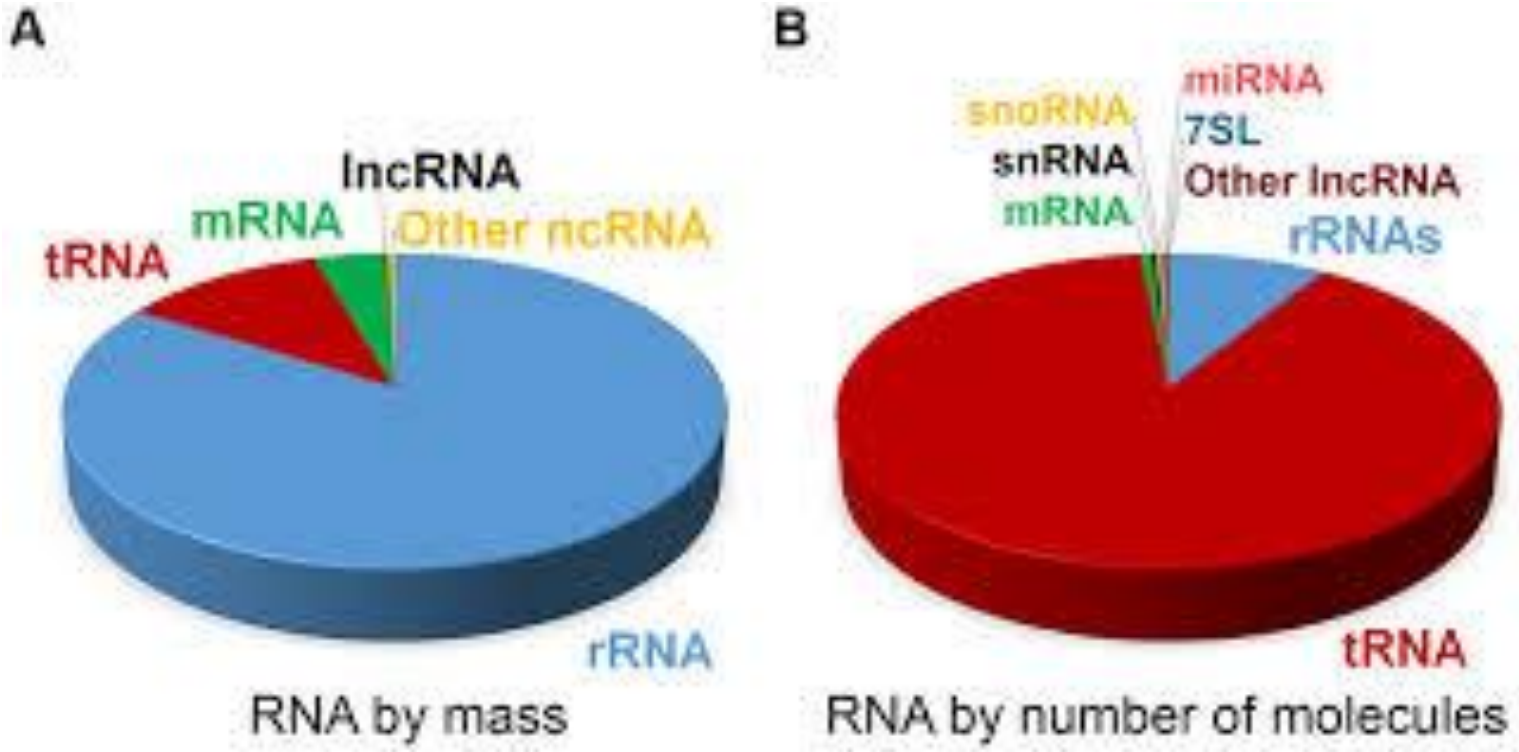
Dall'analisi comparative del genoma di diversi organismi è risultato che noi stiamo tra il pollo e l'uva!



Il genoma dei mammiferi è costituito prevalentemente da DNA non codificante



Il trascrittoma dei mammiferi è costituito prevalentemente da RNA non codificanti (ncRNA)



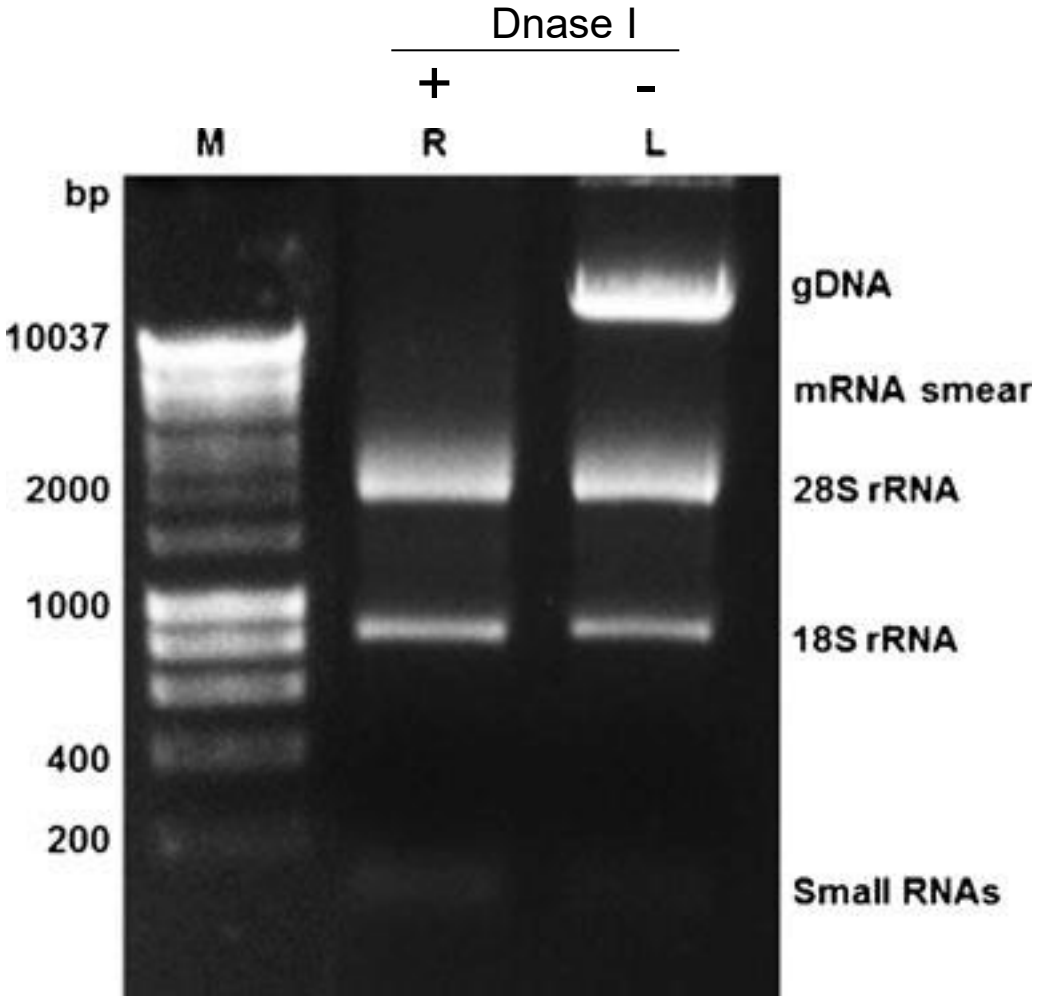
Tipologie e abbondanza dei ncRNA

Type	Percent of total RNA by mass	Molecules per cell	Average size (kb)	Total weight picograms/cell	Notes	Reference
rRNAs	80 to 90	3–10 × 10^6 (ribosomes)	6.9	10 to 30		Blobel and Potter (1967), Wolf and Schlessinger (1977), Duncan and Hershey (1983)
tRNA	10 to 15	3–10 × 10^7	<0.1	1.5 to 5	About 10 tRNA molecules /ribosome	Waldron and Lacroute (1975)
mRNA	3 to 7	3–10 × 10^5	1.7	0.25 to 0.9		Hastie and Bishop (1976), Carter et al. (2005)
hnRNA (pre-mRNA)	0.06 to 0.2	1–10 × 10^3	10*	0.004 to 0.03	Estimated at 2–4% of mRNA by weight	Mortazavi et al. (2008), Menet et al. (2012)
Circular RNA	0.002 to 0.03	3–20 × 10^3	~0.5	0.0007 to 0.005	Estimated at 0.1–0.2% of mRNA**	Salzman et al. (2012), Guo et al. (2014)
snRNA	0.02 to 0.3	1–5 × 10^5	0.1–0.2	0.008 to 0.04		Kiss and Filipowicz (1992), Castle et al. (2010)
snoRNA	0.04 to 0.2	2–3 × 10^5	0.2	0.02 to 0.03		Kiss and Filipowicz (1992), Cooper (2000), Castle et al. (2010)
miRNA	0.003 to 0.02	1–3 × 10^5	0.02	0.001 to 0.003	About 10^5 molecules per 10 pg total RNA	Bissels et al. (2009)
7SL	0.01 to 0.2	3–20 × 10^4	0.3	0.005 to 0.03	About 1–2 SRP molecules/100 ribosomes	Raue et al. (2007), Castle et al. (2010)
Xist	0.0003 to 0.02	0.1–2 × 10^3	2.8	0.0001 to 0.003		Buzin et al. (1994), Castle et al. (2010)
Other lncRNA	0.03 to 0.2	3–50 × 10^3	1	0.002 to 0.03	Estimated at 1–4% of mRNA by weight	Mortazavi et al. (2008), Ramsköld et al. (2009), Menet et al. (2012)

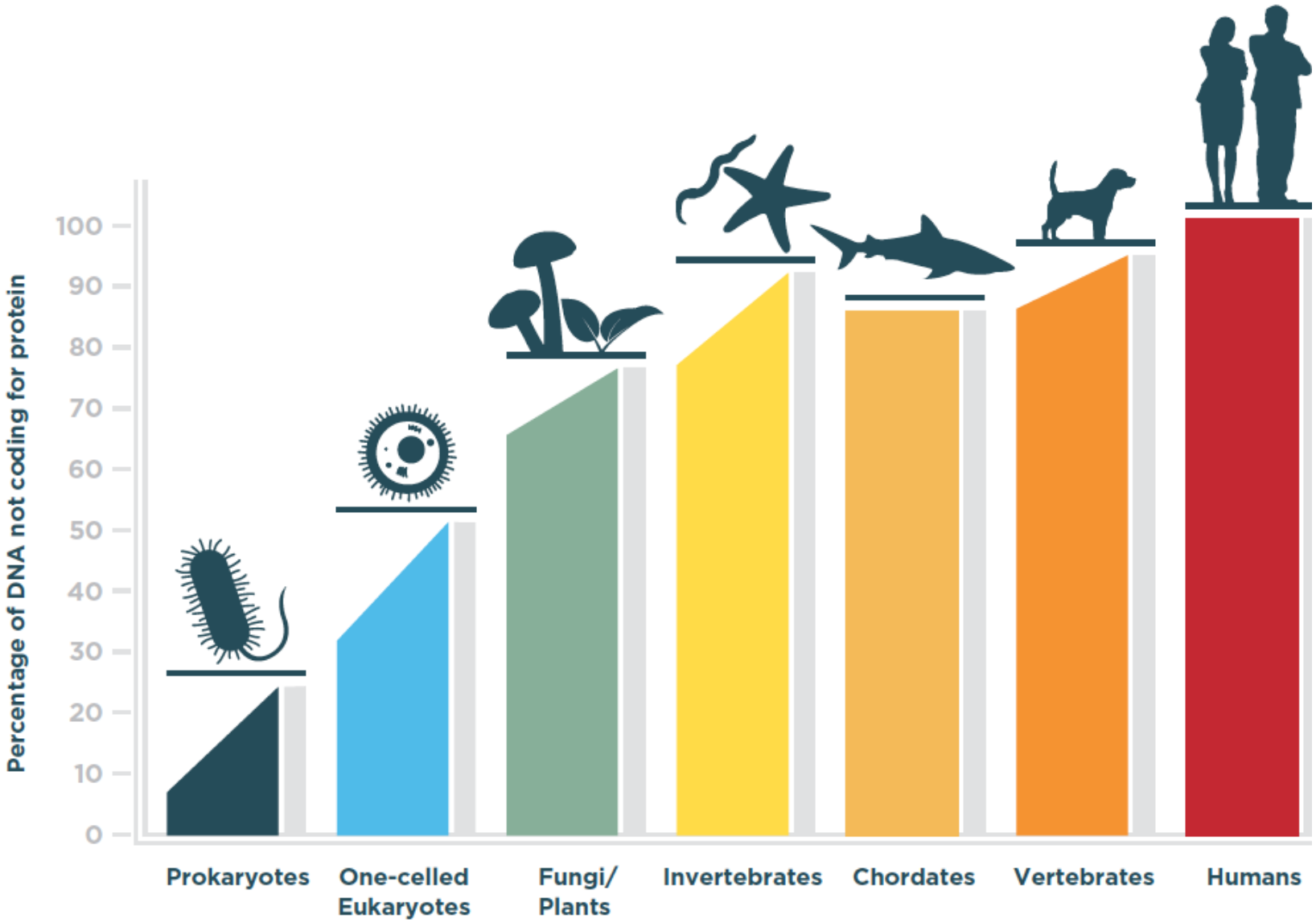
The size for the average unspliced pre-mRNA is 17 kb; however, most pre-mRNAs are partially spliced at any given time, and the average size of hnRNA is estimated at 10 kb (Salditt-Georgieff et al., 1976).
***Based on the finding that 1–2% of all mRNA species generate circular RNA, which is present at 10% of the level of the parental mRNA.*

A. F. Palazzo and E. S. Lee, “Non-coding RNA: what is functional and what is junk?,” *Front. Genet.*, vol. 6, no. 61, p. 326, Jan. 2015.

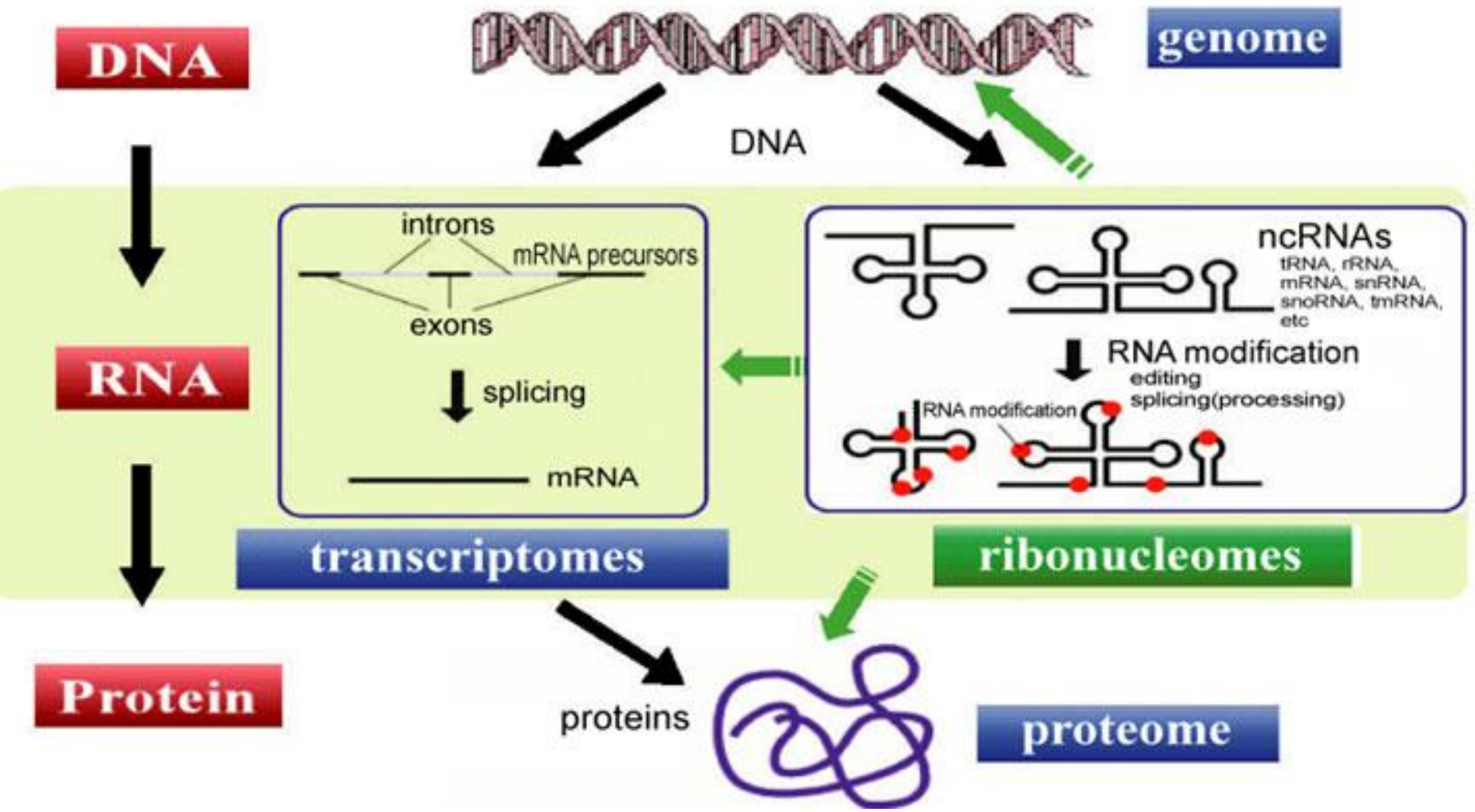
Analisi dell'RNA cellulare mediante elettroforesi su gel di agarosio



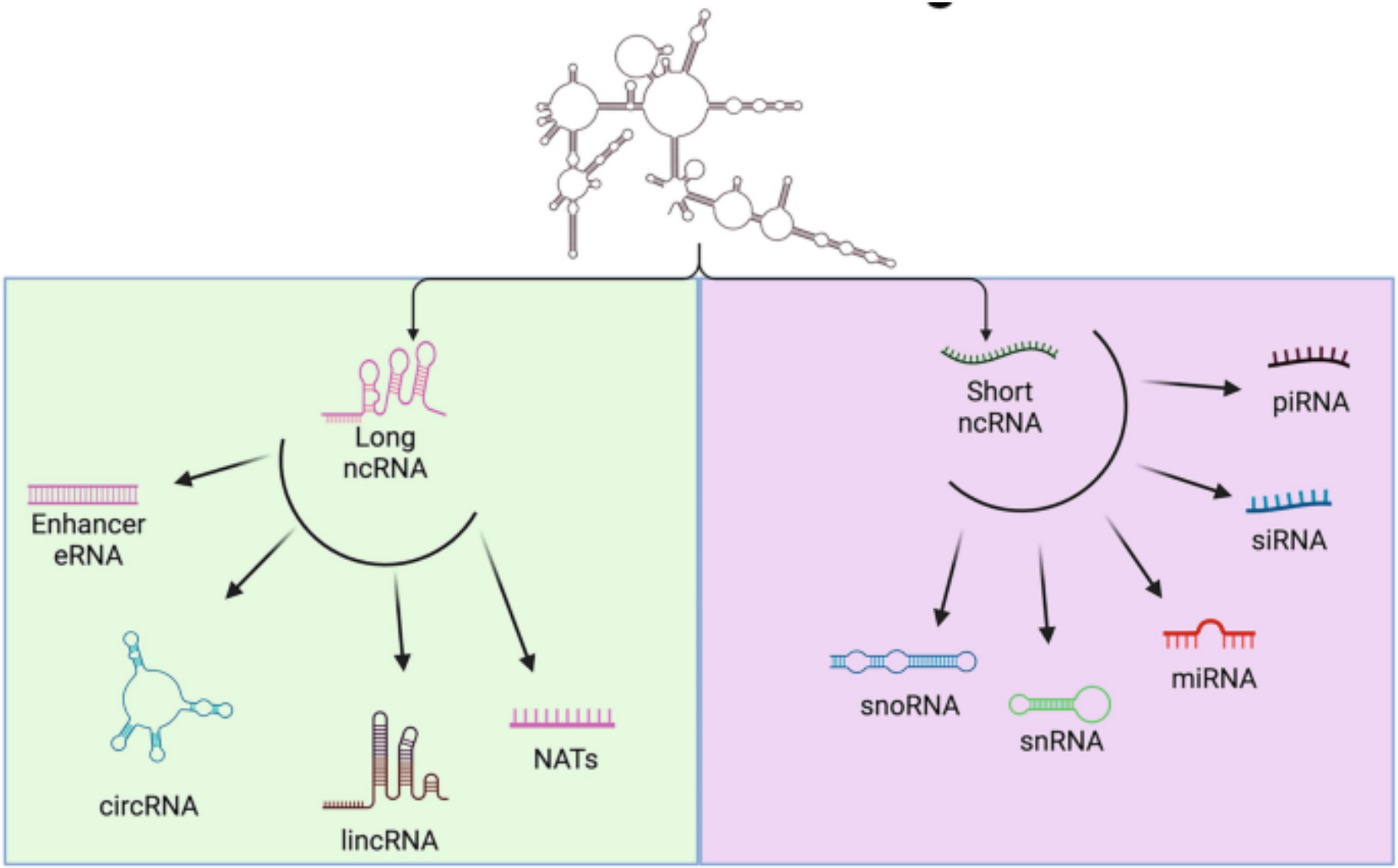
Nel genoma la quantita' relativa del DNA non codificante aumenta con la complessita' evolutiva



Ruolo dei ncRNA nel controllo epigenetico dell'espressione genica

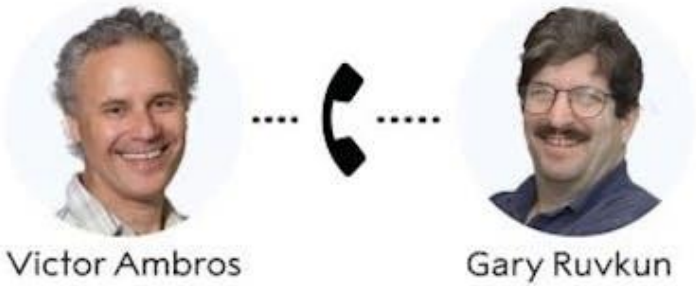


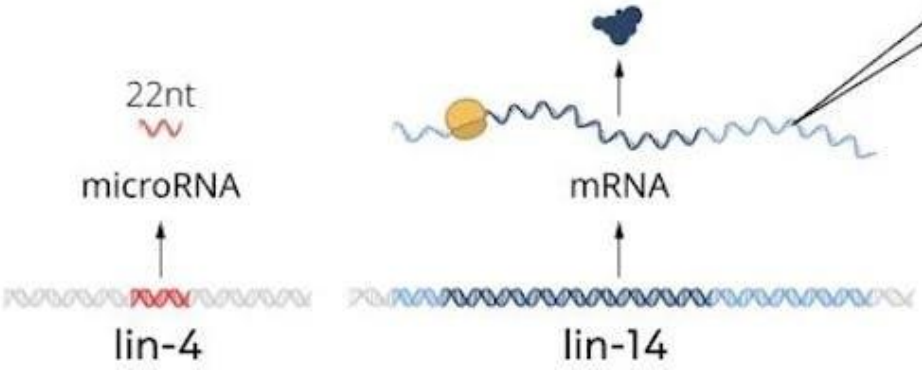
Non coding RNA

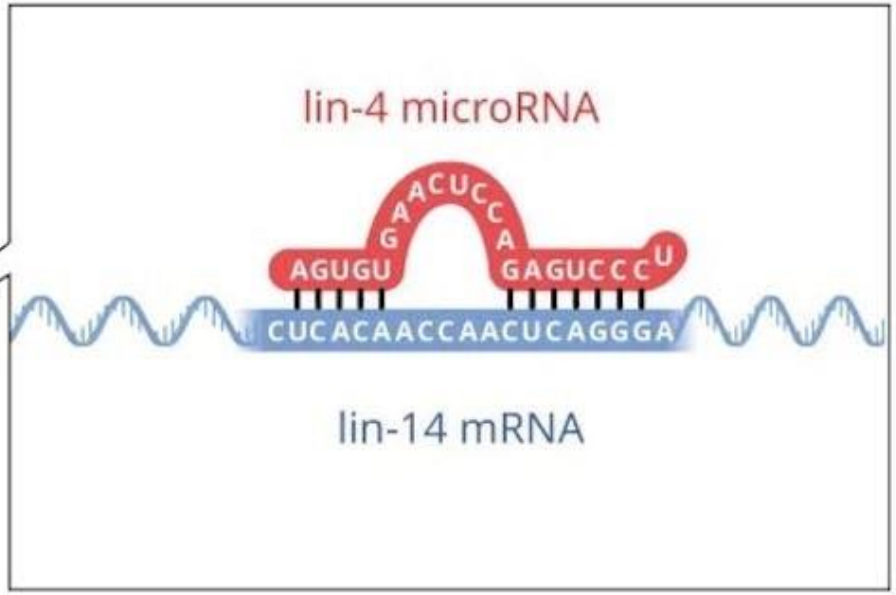


microRNA

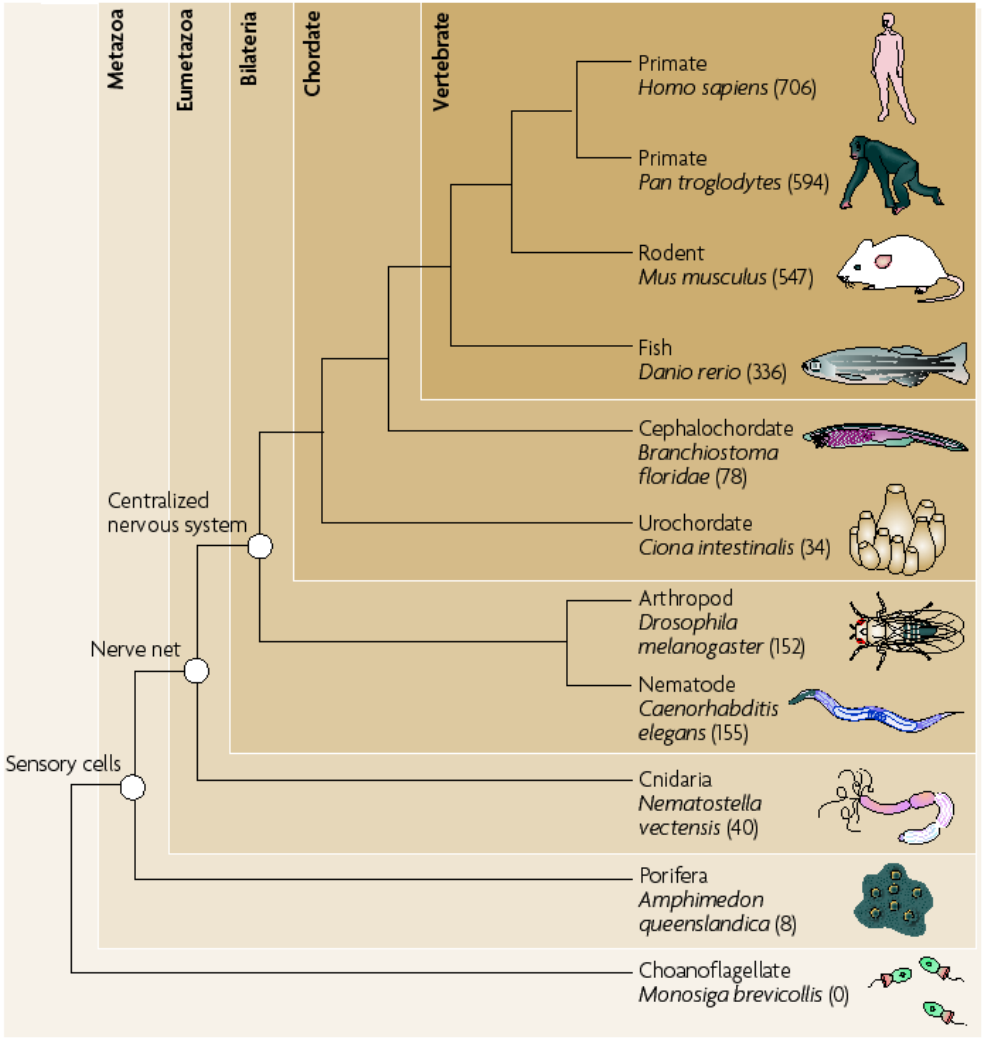

WORLD NEWS
VICTOR AMBROS & GARY RUVKUN
AWARDED 2024 NOBEL PRIZE IN MEDICINE
For Discovery Of MiRNA


Victor Ambros
Gary Ruvkun


22nt
microRNA
mRNA
lin-4
lin-14

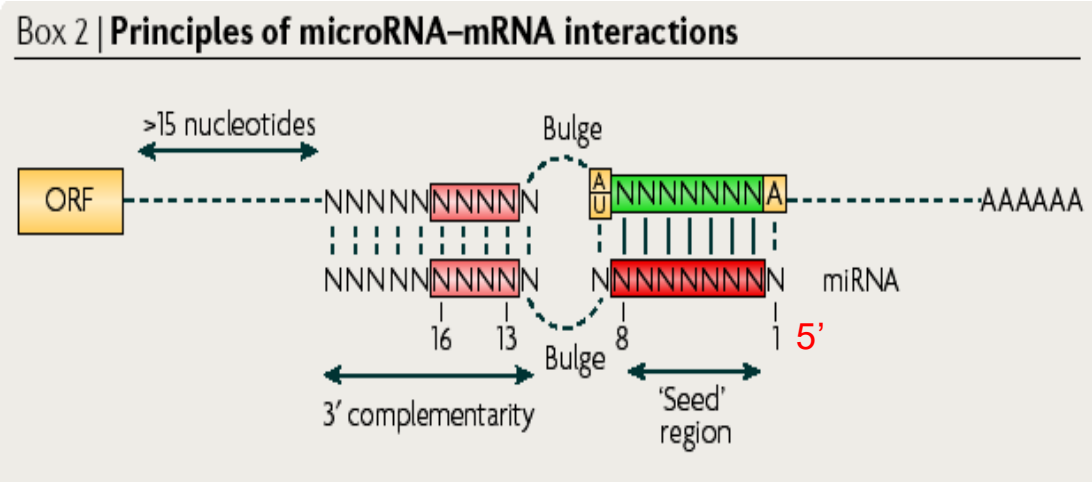

lin-4 microRNA
AGUGU
GAACUCCA
GAGUCCCU
CUCACAACCAACUCAGGGA
lin-14 mRNA

Il numero dei miRNA espressi da una data specie aumenta con la complessita' morfologica ed funzionale



Kosik. MicroRNAs tell an evo–devo story. Nature Rev Neurosc (2009)10: pp. 1-6

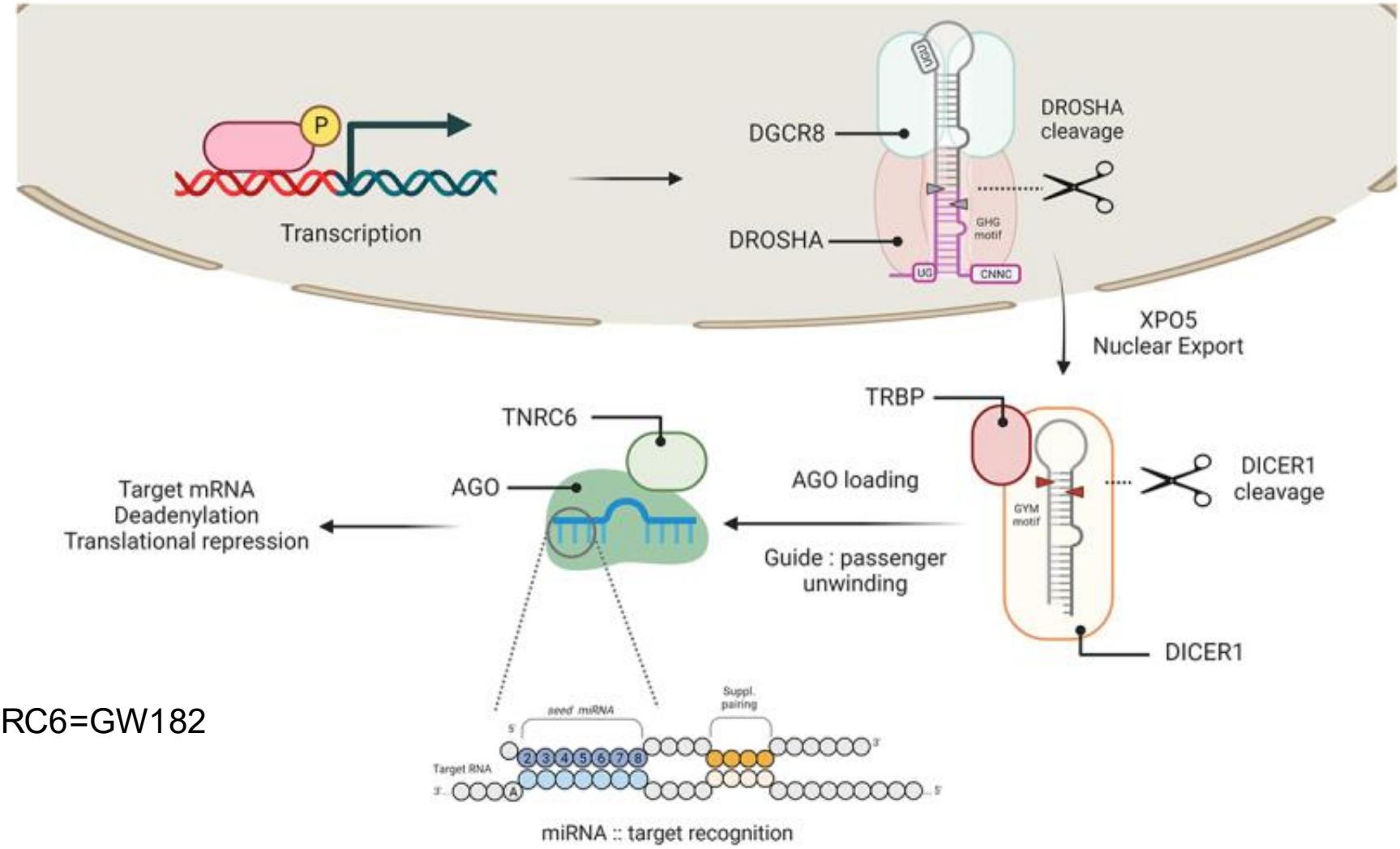
Interazione miRNA : mRNA target



- i microRNA (miRNA) sono piccoli ncRNA , lunghi 19-23 nt
- interagiscono con l'RNA target per complementarieta' di sequenza di 7 o piu' nucleotide presenti nell'estremita' 5' (sequenza *seed*). Altre regioni di complementarieta' con l'RNA target possono essere presenti nella regione al 3' del miRNA.
- Nei mammiferi i miRNA piu' comunemente riconoscono sequenze presenti nella 3'UTR dell'mRNA, talvolta nella 5'UTR o ORF.

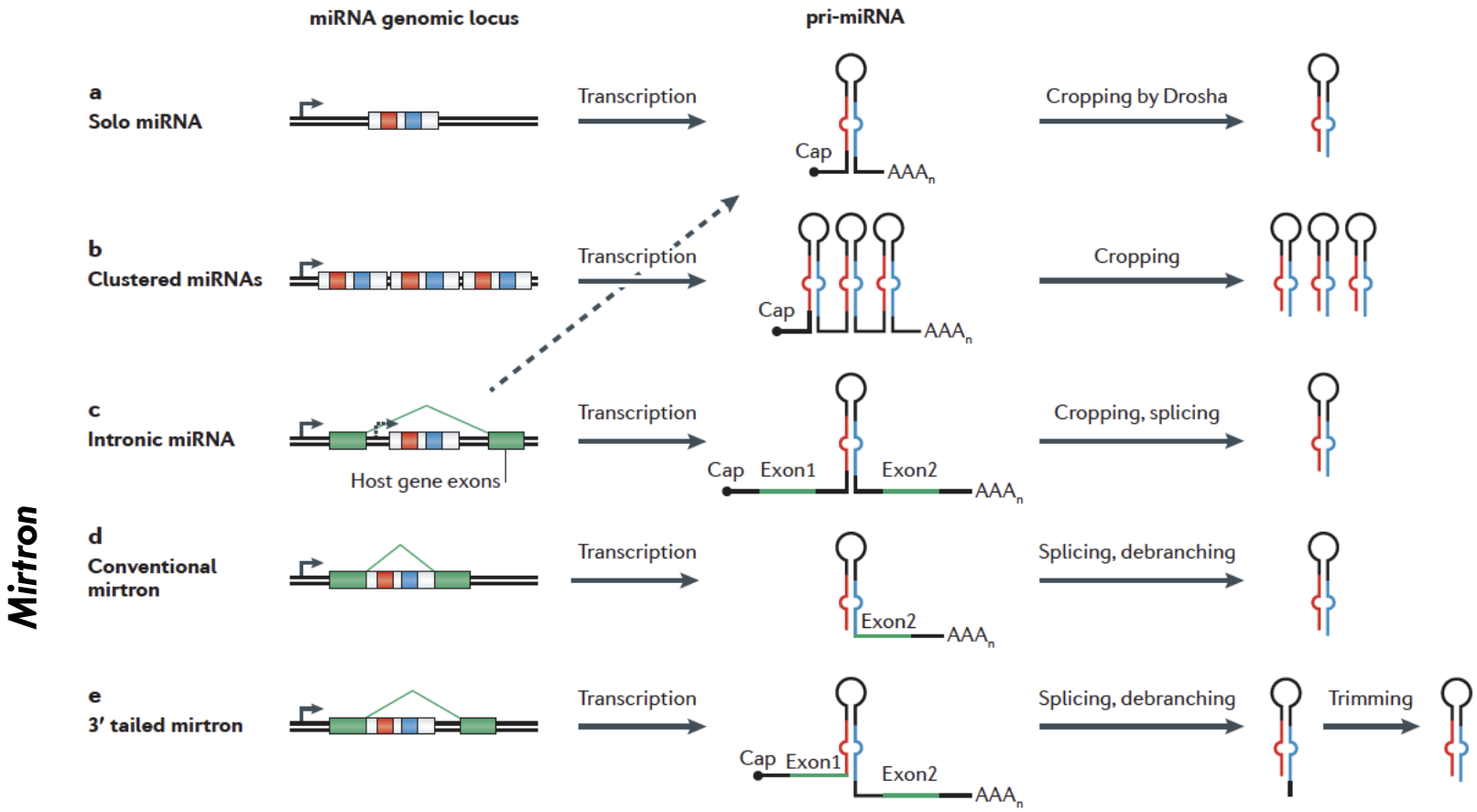
- I miRNA funzionano come molecole guida **nel silenziamento dell'RNA** messaggero.

Biogenesi dei miRNA



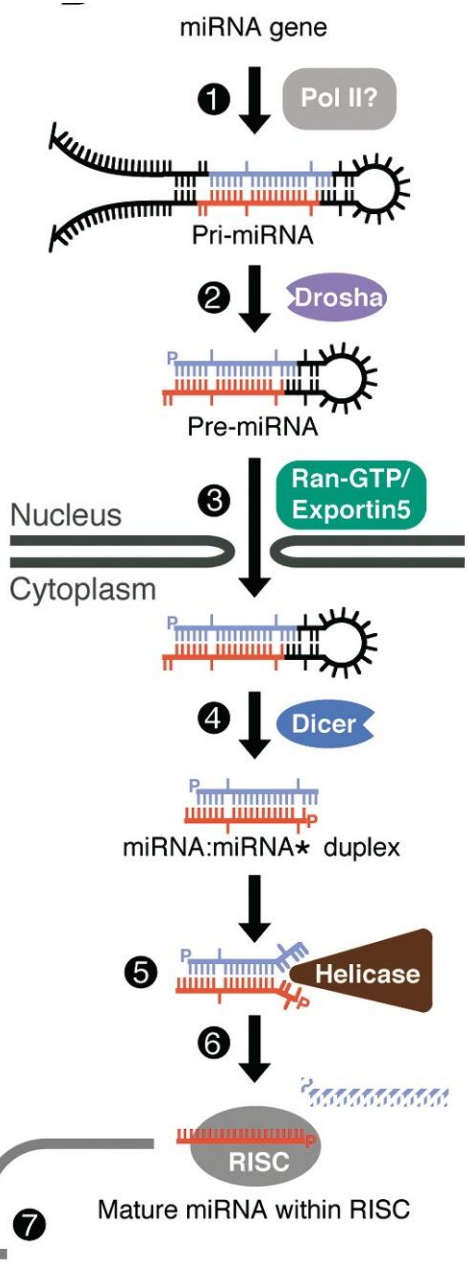
TNRC6=GW182

Geni dei miRNA



I geni per miRNA sono localizzati in regioni intergeniche (27%), come unità trascrizionali autonome; in introni di geni codificanti (43 %) e non codificanti (19%), in questi casi sono chiamati **Mirtron;** a livello di esoni (11%). In questi ultimi 2casi sono trascritti a partire dallo stesso promotore del gene ospite o a partire da promotori criptici indipendenti.Nel caso dei Mirtron il processamento è indipendente da Drosha. Nel caso di miRNA a localizzazione esonica, il taglio determina un'inevitabile destabilizzazione del trascritto ospite.

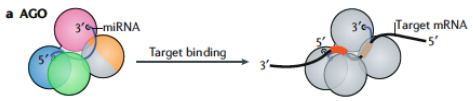
….BIOGENESI dei miRNA



trascrizione da parte di Pol II

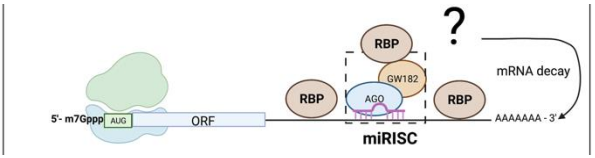
taglio da parte del Microprocessore

taglio da parte di Dicer



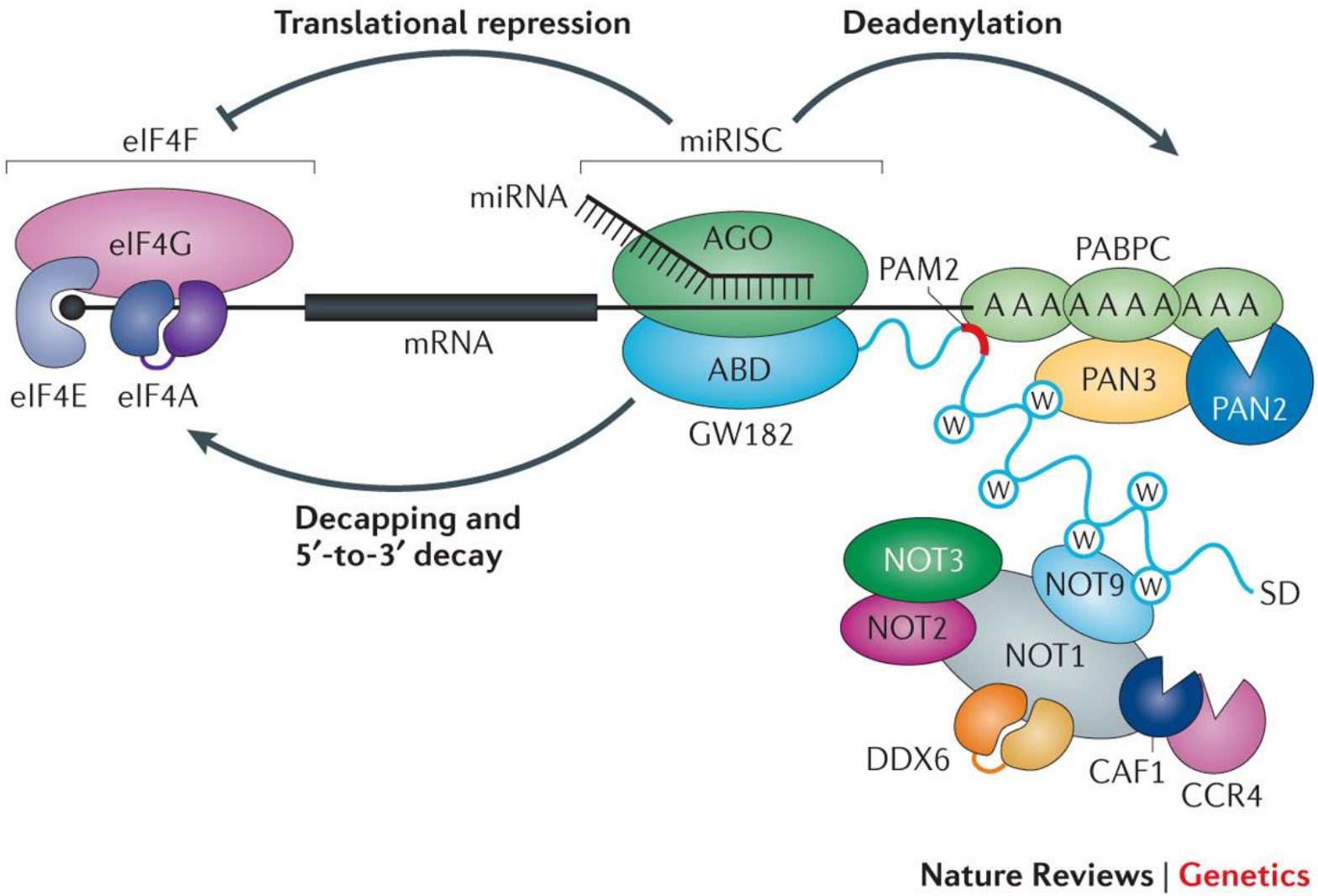
caricamento del duplex su Ago

caricamento di uno dei 2 filamenti nel complesso RISC



miRISC: miRNA-induced silencing complex

Silenziamento genico mediato dai miRNA



Banche dati miRNA

miRBase

Il database miRBase (www.mirbase.org/) è una banca dati in cui sono raccolte le sequenze dei miRNA e dei loro precursori, identificati in 271 organismi diversi. E'consultabile online (*open access*) ed è aggiornata costantemente.

Ora v.22!

miRBase: from microRNA sequences to function

Ana Kozomara, Maria Birgaoanu, Sam Griffiths-Jones

Nucleic Acids Research, Volume 47, Issue D1, 08 January 2019, Pages D155–D162, https://doi.org/10.1093/nar/gky1141
Published: 13 November 2018 **Article history** ▾

Nomenclatura miRNA
esempio miR-124-1-3p umano

hsa-miR-124-1-3p

specie di origine

famiglia dei microRNA

gene

elica di origine

mir-124-1-3p uaaggcacgcggugaaugccaa

hsa-mir-124-1-5p

stem-loop

```
    a       uc    cc          a    ga          uaaau
5'   ggccuc   ucu   guguucac gcg   ccuugauu       g
     ||||||   |||   |||||||| |||   ||||||||       u
3'   ucgggg   aga   cguaagug cgc   ggaauuaa       c
     g        ua    ac          g    ac           cauac
```

hsa-mir-124-1-3p

mir-124-5p è anche indicato mir-124*

hsa-mir-124

miRNA maturo 5’- uaaggcacgcggugaaugccaa - 3’

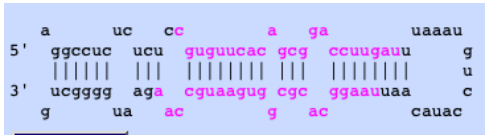
È prodotto da 3 geni:

MI0000443	hsa-mir-124-1
MI0000444	hsa-mir-124-2
MI0000445	hsa-mir-124-3

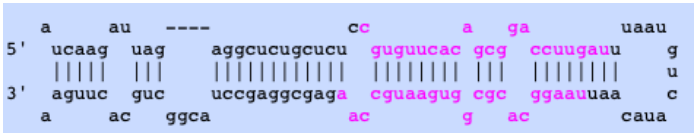
da ciascun gene deriva un trascritto primario diverso ma uno stesso miR-124 maturo

Sequenze stem-loop

hsa-mir-124-1



hsa-mir-124-2



hsa-mir-124-3

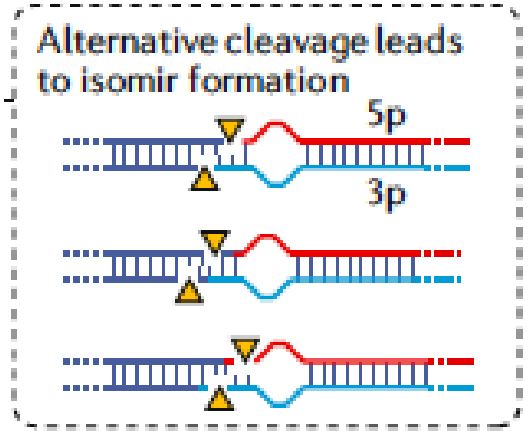
hsa-mir-124 o hsa-mir-124-3p

Iso-miRNA

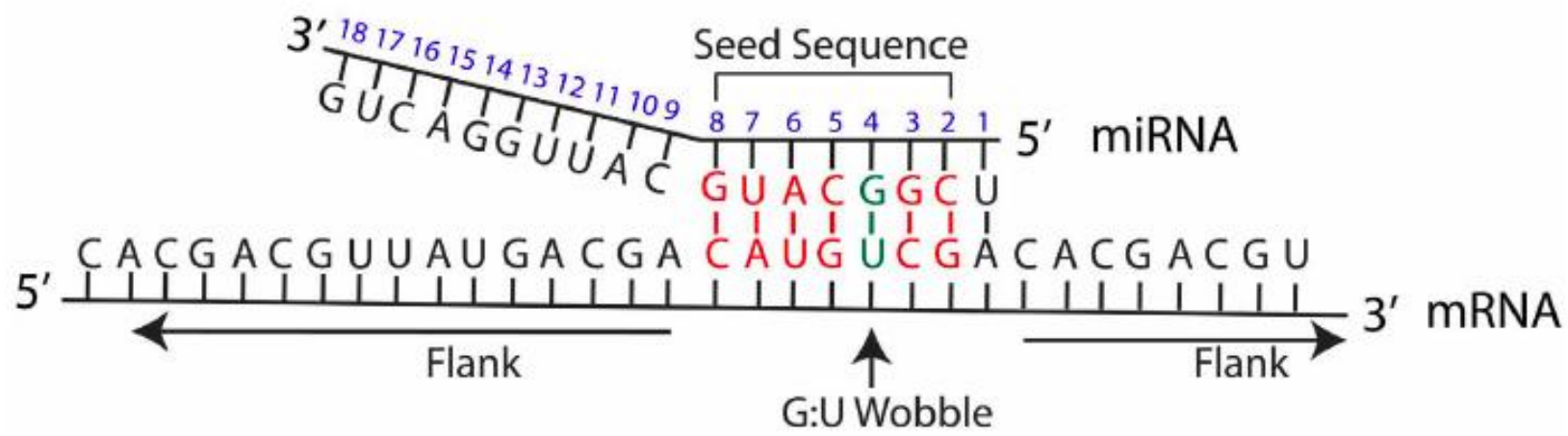
Sono miRNA appartenenti alla stessa famiglia (es. famiglia hsa-mir-34) che differiscono per singoli nucleotidi e condividono la regione *seed* .

```
hsa-mir-34a/hsa-miR-34a-5p    UGGCAGUGUCUUAGCUGGUUGU
hsa-mir-34b/hsa-miR-34b-5p   UAGGCAGUGUCAUUAGCUGAUUG
hsa-mir-34c/hsa-miR-34c-5p    AGGCAGUGUAGUUAGCUGAUUGC
```

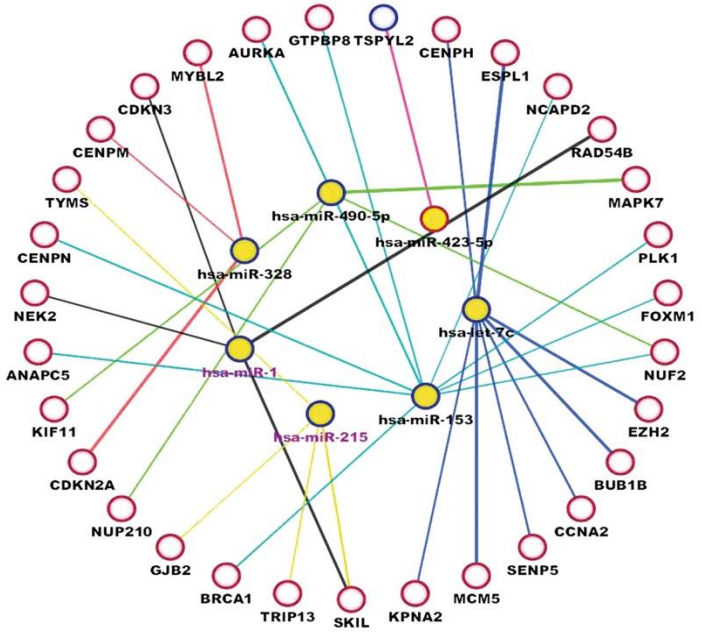
Spesso l'eterogeneita' è alle estremita' 5' o 3' ed è generata nel processo di maturazione da parte di Drosha



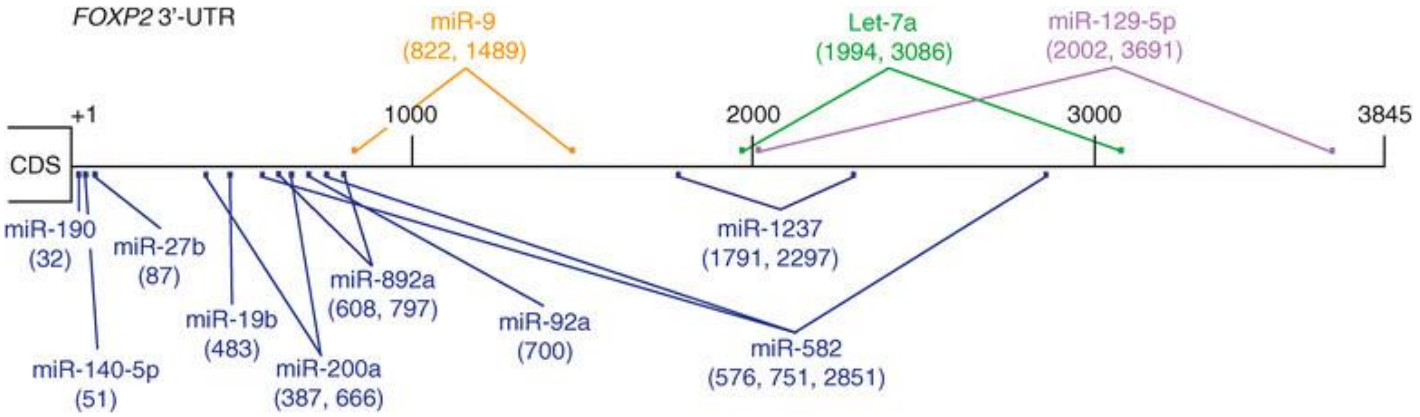
I miRNA regolano ca il 50% dei geni cellulari e potenzialmente ca il 75% di tutti i geni



Un singolo miRNA ha decine/centinaia di RNA target



un singolo mRNA è regolato da diversi miRNA



Predizione computazionale dei potenziali mRNA target di un dato miRNA

Gli stessi algoritmi predicono per un dato mRNA i miRNA che lo potrebbero regolare e viceversa

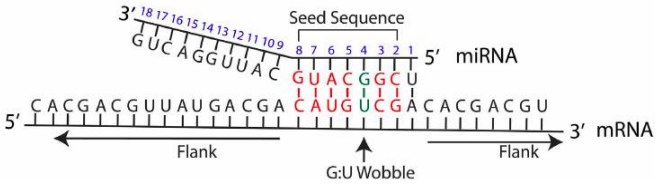
Diversi algoritmi di predizione sono stati elaborati e sono ora disponibili sul web.
Tra i piu' noti:

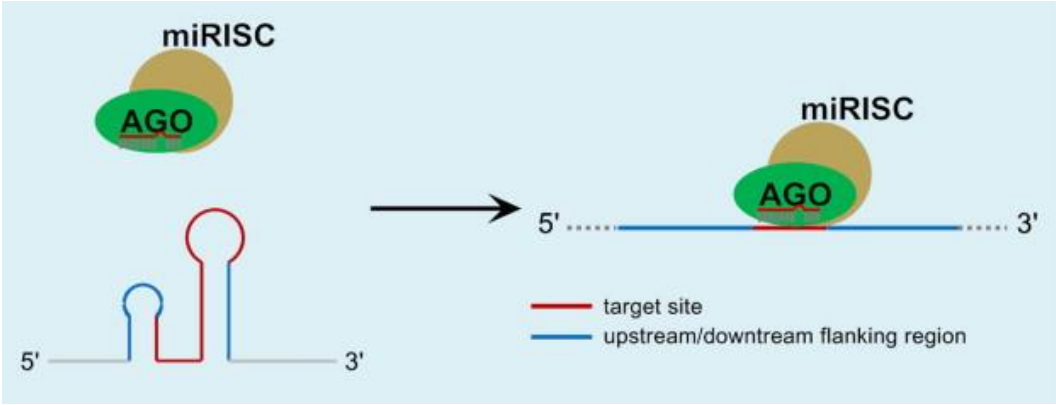
TARGETSCAN	http://www.targetscan.org
miRANDA	http://www.microrna.org/
DIANA-microT-CDS	http://www.microrna.gr/microT-CDS
rna22-GUI	https://cm.jefferson.edu/rna22v1.0/
miRDB	http://mirdb.org/mirdb/index.html

……..

Principali caratteristiche degli algoritmi di predizione di RNA target di un miRNA

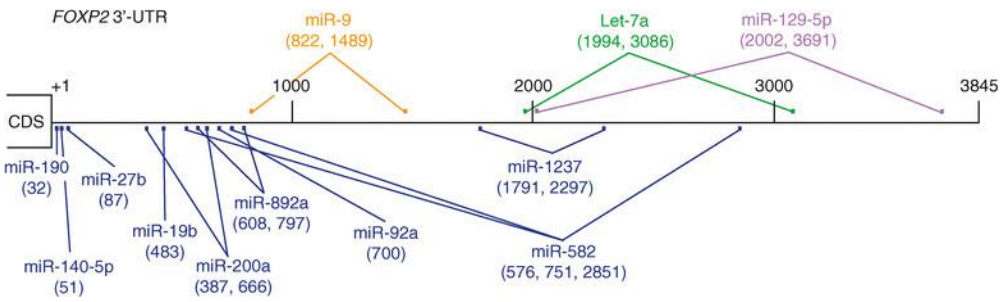
- **Complementarieta' della seq seed (seed match)**
- Conservazione evolutive
- Energia libera di legame (free energy, ΔG di legame)
- Accessibilita' del sito di legame

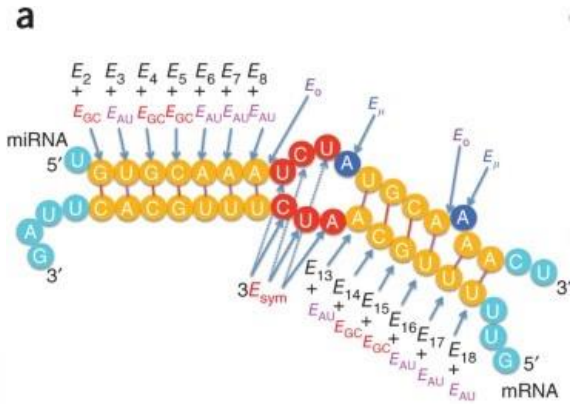


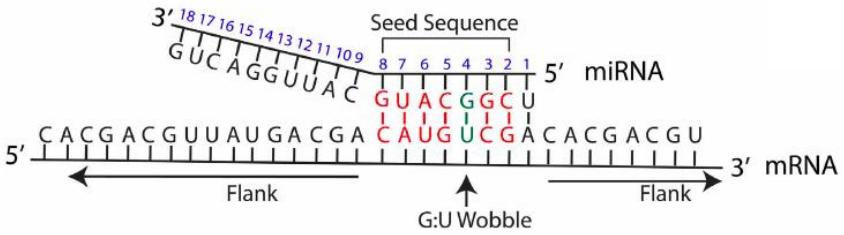


Altre caratteristiche considerate sono:

- Numero delle seq target presenti nella 3'UTR di un mRNA target.
- Contenuto locale di nt AU in prossimita' della regione seed
- su un dato Target
- Appaiamenti GU (*wobble*) nella regione seed
- Appaiamenti compensatori al 3' nt 12-17 del miRNA
- Posizione del sito di legame di un miRNA sulla 3'UTR del mRNA





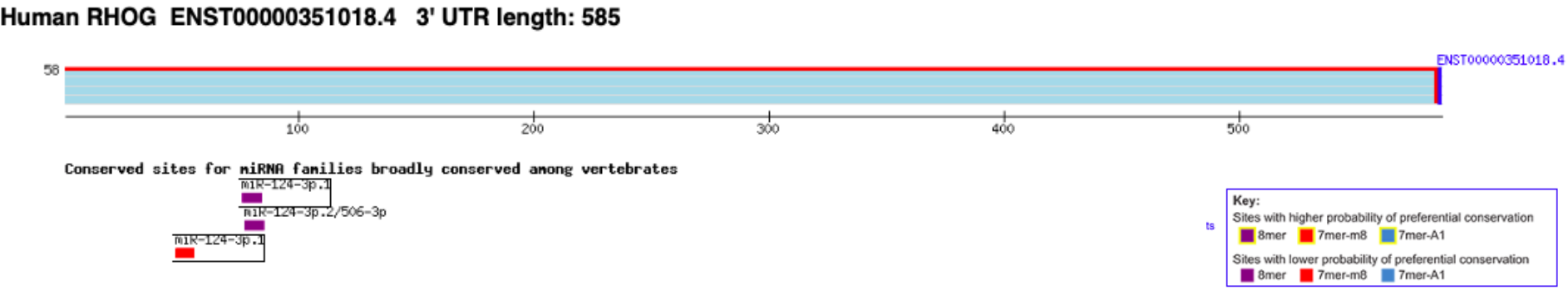


Predizione mediante TARGETSCAN di RNA target per hsa-mir-124-3p

http://www.targetscan.org

Target gene	Representative transcript	Gene name	Number of 3P-seq tags supporting UTR + 5	Link to sites in UTRs	Conserved sites				Poorly conserved sites				6mer sites	Representative miRNA	Cumulative weighted context++ score	Total context++ score	Aggregate P_{CT}	Previous TargetScan publication(s)
					total	8mer	7mer-m8	7mer-A1	total	8mer	7mer-m8	7mer-A1						
RHOG	ENST00000351018.4	ras homolog family member G	58	Sites in UTR	2	1	1	0	2	0	2	0	1	hsa-miR-124-3p.1	-1.44	-1.44	0.91	2007, 2009, 2011
CTDSP1	ENST00000273062.2	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	1299	Sites in UTR	4	0	4	0	1	0	0	1	3	hsa-miR-124-3p.1	-1.37	-1.37	> 0.99	2007, 2009, 2011
SNAI2	ENST00000020945.1	snail family zinc finger 2	1233	Sites in UTR	3	1	2	0	0	0	0	0	0	hsa-miR-124-3p.1	-1.28	-1.28	> 0.99	2009, 2011
LRRC58	ENST00000295628.3	leucine rich repeat containing 58	940	Sites in UTR	3	3	0	0	0	0	0	0	1	hsa-miR-124-3p.1	-1.16	-1.18	> 0.99	2009, 2011
B4GALT1	ENST00000379731.4	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	2265	Sites in UTR	3	1	2	0	0	0	0	0	3	hsa-miR-124-3p.1	-1.14	-1.21	> 0.99	2007, 2009, 2011
SLC10A7	ENST00000264986.3	solute carrier family 10, member 7	77	Sites in UTR	2	1	1	0	0	0	0	0	0	hsa-miR-124-3p.1	-1.01	-1.01	0.98	2007, 2009, 2011
VAMP3	ENST00000054666.6	vesicle-associated membrane protein 3	515	Sites in UTR	3	1	2	0	0	0	0	0	2	hsa-miR-124-3p.1	-0.98	-1.49	> 0.99	2007, 2009, 2011
MAGT1	ENST00000358075.6	magnesium transporter 1	937	Sites in UTR	2	2	0	0	1	0	0	1	0	hsa-miR-124-3p.1	-0.92	-1.05	> 0.99	2011
SERINC2	ENST00000373709.3	serine incorporator 2	624	Sites in UTR	2	1	1	0	0	0	0	0	0	hsa-miR-124-3p.1	-0.90	-0.90	0.98	2007, 2009, 2011
SERP1	ENST00000239944.2	stress-associated endoplasmic reticulum protein 1	3242	Sites in UTR	2	1	1	0	0	0	0	0	3	hsa-miR-124-3p.1	-0.88	-1.06	> 0.99	2007, 2009, 2011
TRIM45	ENST00000256649.4	tripartite motif containing 45	99	Sites in UTR	1	1	0	0	3	0	3	0	1	hsa-miR-124-3p.1	-0.86	-1.01	0.96	2009, 2011
PDCD6	ENST00000505221.1	programmed cell death 6	4780	Sites in UTR	1	1	0	0	0	0	0	0	1	hsa-miR-124-3p.1	-0.81	-0.87	0.95	2007, 2009, 2011
ITGB1	ENST00000396033.2	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	2098	Sites in UTR	2	0	2	0	0	0	0	0	2	hsa-miR-124-3p.1	-0.81	-0.81	0.99	2007, 2009, 2011
CEBPA	ENST00000498907.2	CCAAT/enhancer binding protein (C/EBP), alpha	12449	Sites in UTR	3	0	3	0	0	0	0	0	1	hsa-miR-124-3p.1	-0.81	-0.81	> 0.99	2011
DNAJC1	ENST00000376980.3	DnaJ (Hsp40) homolog, subfamily C, member 1	5	Sites in UTR	1	1	0	0	0	0	0	0	0	hsa-miR-124-3p.1	-0.80	-0.80	0.94	2007, 2009, 2011
SPOPL	ENST00000280098.4	speckle-type POZ protein-like	216	Sites in UTR	2	0	2	0	1	0	1	0	1	hsa-miR-124-3p.1	-0.80	-0.89	0.95	2007, 2009, 2011
RAB27A	ENST00000396307.2	RAB27A, member RAS oncogene family	181	Sites in UTR	1	1	0	0	2	1	0	1	0	hsa-miR-124-3p.1	-0.79	-0.80	0.87	2011
CHIC1	ENST00000373504.6	cysteine-rich hydrophobic domain 1	16	Sites in UTR	4	0	3	1	0	0	0	0	1	hsa-miR-124-3p.1	-0.76	-0.91	> 0.99	2007, 2009, 2011
PTBP1	ENST00000350092.4	polypyrimidine tract binding protein 1	7007	Sites in UTR	2	1	0	1	1	0	0	1	2	hsa-miR-124-3p.1	-0.75	-0.76	0.96	2007, 2009, 2011
SLITRK6	ENST00000400286.2	SLIT and NTRK-like family, member 6	5	Sites in UTR	2	1	1	0	0	0	0	0	0	hsa-miR-124-3p.1	-0.74	-0.74	0.96	2007, 2009, 2011
PTPN12	ENST00000248594.6	protein tyrosine phosphatase, non-receptor type 12	167	Sites in UTR	3	0	1	2	0	0	0	0	0	hsa-miR-124-3p.1	-0.74	-0.74	> 0.99	2007, 2009, 2011

Predizione mediante TARGETSCAN di potenziali interazioni tra miRNA e l'mRNA di Rhog



Target gene	Representative transcript	Gene name	Number of 3P-seq tags supporting UTR + 5	Link to sites in UTRs	Conserved sites				Poorly conserved sites				6mer sites	Representative miRNA	Cumulative weighted context++ score	Total context++ score	Aggregate P_{CT}	Previous TargetScan publication(s)
					total	8mer	7mer-m8	7mer-A1	total	8mer	7mer-m8	7mer-A1						
RHOG	ENST00000351018.4	ras homolog family member G	58	Sites in UTR	2	1	1	0	2	0	2	0	1	hsa-miR-124-3p.1	-1.44	-1.44	0.91	2007, 2009, 2011
CTDSP1	ENST00000273062.2	[illegible] phosphatase 1	1299	Sites in UTR	4	0	4	0	1	0	0	1	3	hsa-miR-124-3p.1	-1.37	-1.37	> 0.99	[illegible] 2011
SNAI2	ENST00000020945.1	snail family zinc finger 2	1233	Sites in UTR	3	1	2	0	0	0	0	0	0	hsa-miR-124-3p.1	-1.28	-1.28	> 0.99	2009, 2011
LRRC58	ENST00000295628.3	leucine rich repeat containing 58	940	Sites in UTR	3	3	0	0	0	0	0	0	1	hsa-miR-124-3p.1	-1.16	-1.18	> 0.99	2009, 2011
B4GALT1	ENST00000379731.4	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	2265	Sites in UTR	3	1	2	0	0	0	0	0	3	hsa-miR-124-3p.1	-1.14	-1.21	> 0.99	2007, 2009, 2011
SLC10A7	ENST00000264986.3	solute carrier family 10, member 7	77	Sites in UTR	2	1	1	0	0	0	0	0	0	hsa-miR-124-3p.1	-1.01	-1.01	0.98	2007, 2009, 2011
VAMP3	ENST00000054666.6	vesicle-associated membrane protein 3	515	Sites in UTR	3	1	2	0	0	0	0	0	2	hsa-miR-124-3p.1	-0.98	-1.49	> 0.99	2007, 2009, 2011
MAGT1	ENST00000358075.6	magnesium transporter 1	937	Sites in UTR	2	2	0	0	1	0	0	1	0	hsa-miR-124-3p.1	-0.92	-1.05	> 0.99	2011
SERINC2	ENST00000373709.3	serine incorporator 2	624	Sites in UTR	2	1	1	0	0	0	0	0	0	hsa-miR-124-3p.1	-0.90	-0.90	0.98	2007, 2009, 2011
SERP1	ENST00000239944.2	stress-associated endoplasmic reticulum protein 1	3242	Sites in UTR	2	1	1	0	0	0	0	0	3	hsa-miR-124-3p.1	-0.88	-1.06	> 0.99	2007, 2009, 2011
TRIM45	ENST00000256649.4	tripartite motif containing 45	99	Sites in UTR	1	1	0	0	3	0	3	0	1	hsa-miR-124-3p.1	-0.86	-1.01	0.96	2009, 2011
PDCD6	ENST00000505221.1	programmed cell death 6	4780	Sites in UTR	1	1	0	0	0	0	0	0	1	hsa-miR-124-3p.1	-0.81	-0.87	0.95	2007, 2009, 2011
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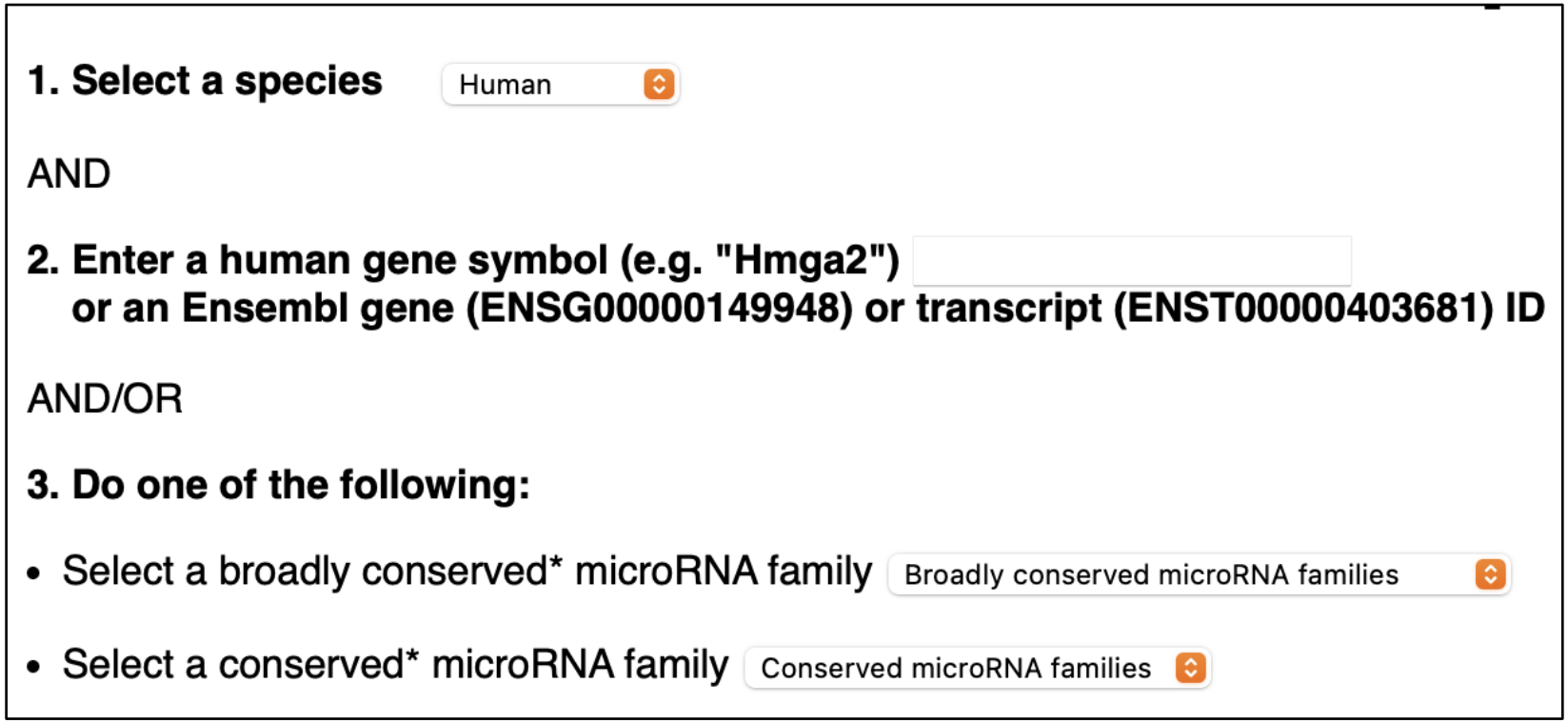
PTBP1	ENST00000350092.4	polypyrimidine tract binding protein 1	7007	Sites in UTR	2	1	0	1	1	0	0	1	2	hsa-miR-124-3p.1	-0.75	-0.76	0.96	2007, 2009, 2011
SLITRK6	ENST00000400286.2	SLIT and NTRK-like family, member 6	5	Sites in UTR	2	1	1	0	0	0	0	0	0	hsa-miR-124-3p.1	-0.74	-0.74	0.96	2007, 2009, 2011
PTPN12	ENST00000248594.6	protein tyrosine phosphatase, non-receptor type 12	167	Sites in UTR	3	0	1	2	0	0	0	0	0	hsa-miR-124-3p.1	-0.74	-0.74	> 0.99	2007, 2009, 2011

Predizione mediante TARGETSCAN di:

1. RNA target per hsa-mir-124-3p
2. potenziali interazioni tra un mRNA (es Cplx2 e miRNA



Database di interazioni miRNA:mRNA validate sperimentalmente

miRTarBase:

The experimentally validated microRNA-target interactions database (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php)

Esempio: mmu-mir-124

					Validation methods									
					Strong evidence			Less strong evidence						
ID	Species (miRNA)	Species (Target)	miRNA	Target	Reporter assay	Western blot	qPCR	Microarray	NGS	pSILAC	Other	CLIP-Seq	Sum	# of papers
MIRT000005	Mus musculus	Mus musculus	mmu-miR-124-3p	Itgb1	✔	✔	✔	✔			✔		5	2
MIRT000360	Homo sapiens	Homo sapiens	hsa-miR-124-3p	SOX9					✔		✔		2	3

Database di meta-analisi
Esempio di miRWalk

miRwalk is an open-source platform providing an intuitive interface that generates predicted and validated miRNA-binding sites of known genes of human, mouse, rat, dog and cow

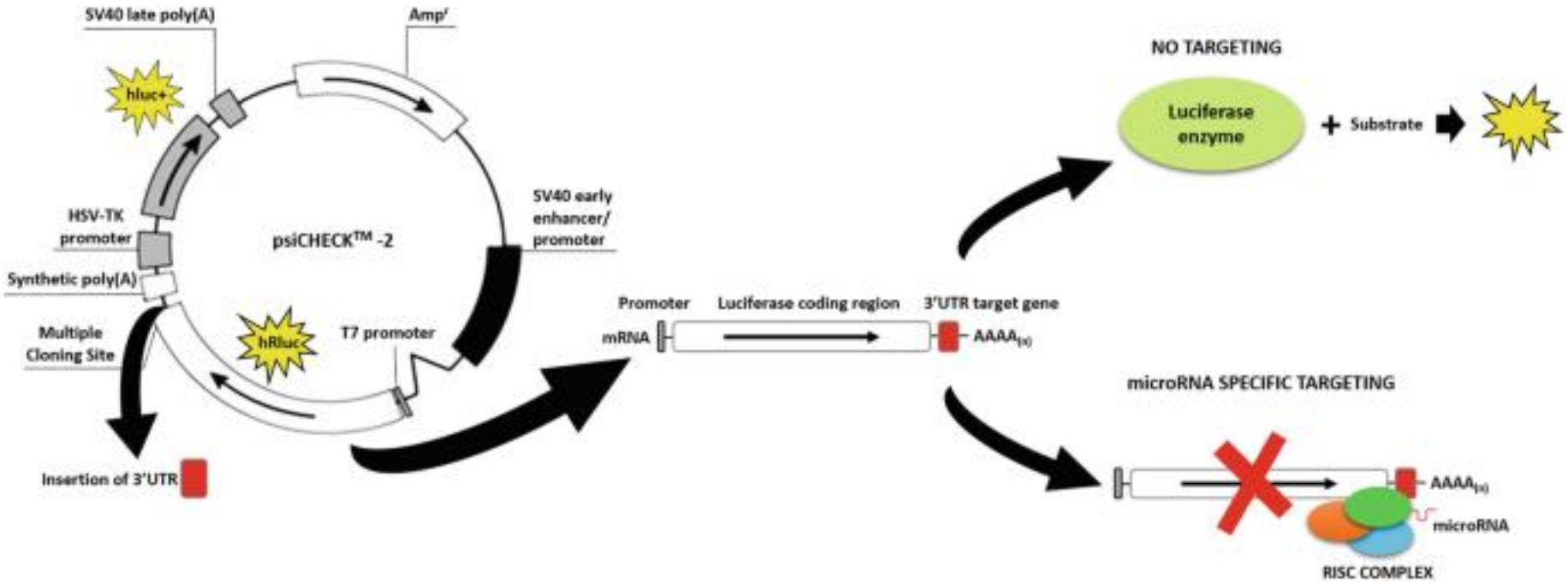
Details

Mirnaid	hsa-miR-124-3p
Mimatid	MIMAT0000422
Sequence	UAAGGCACGCGGUGAAUGCCAA

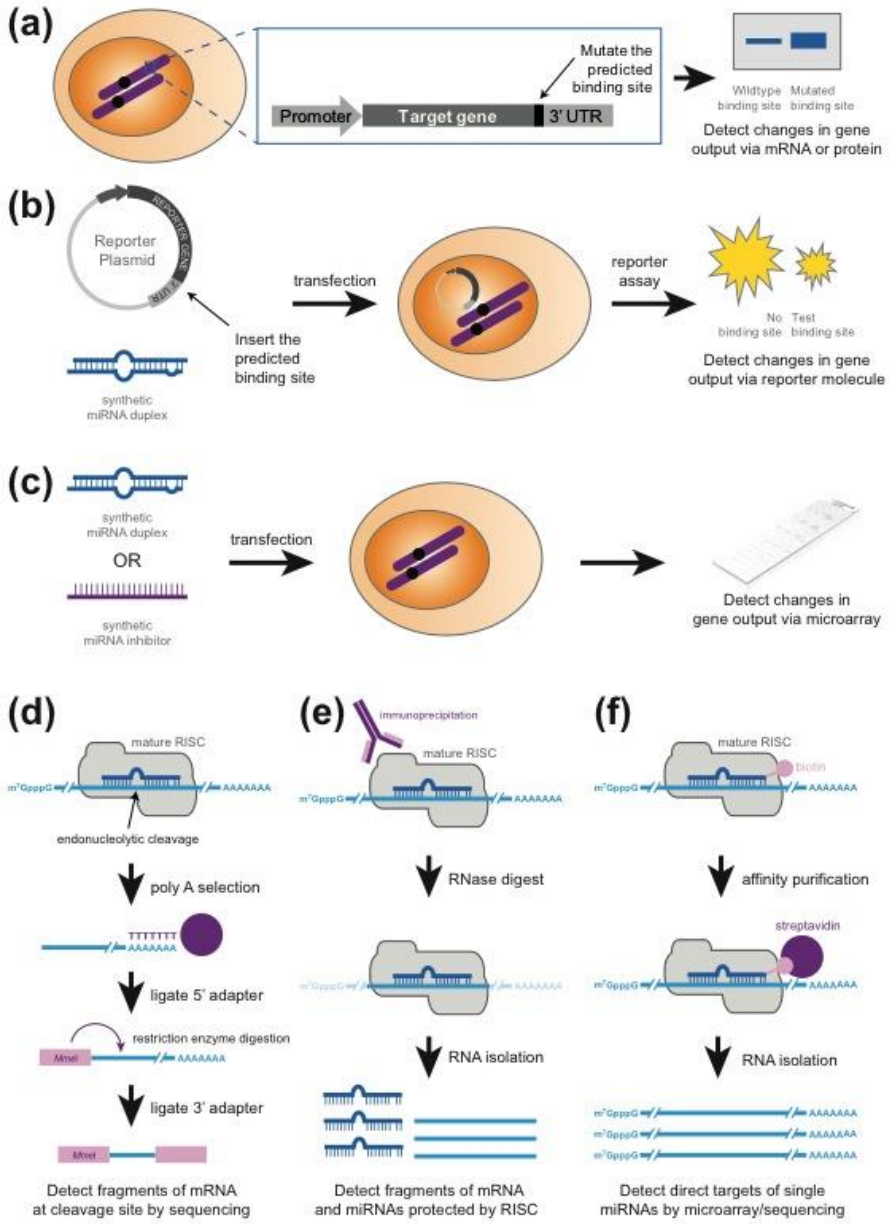
Interactions

Mirna	Refseqid	Genesymbol	Duplex	Score	Position	Binding Site	Au	Me	N Pairings	Targetscan	Mirdb	Mirtarbase
hsa-miR-124-3p	NM_001256424	GUCY1A2	details	1.00	CDS	2003,2027	0.54	-9.339	17	—	—	—
hsa-miR-124-3p	NM_001256420	MAPRE2	details	1.00	3UTR	1363,1387	0.65	-8.813	17	—	—	—

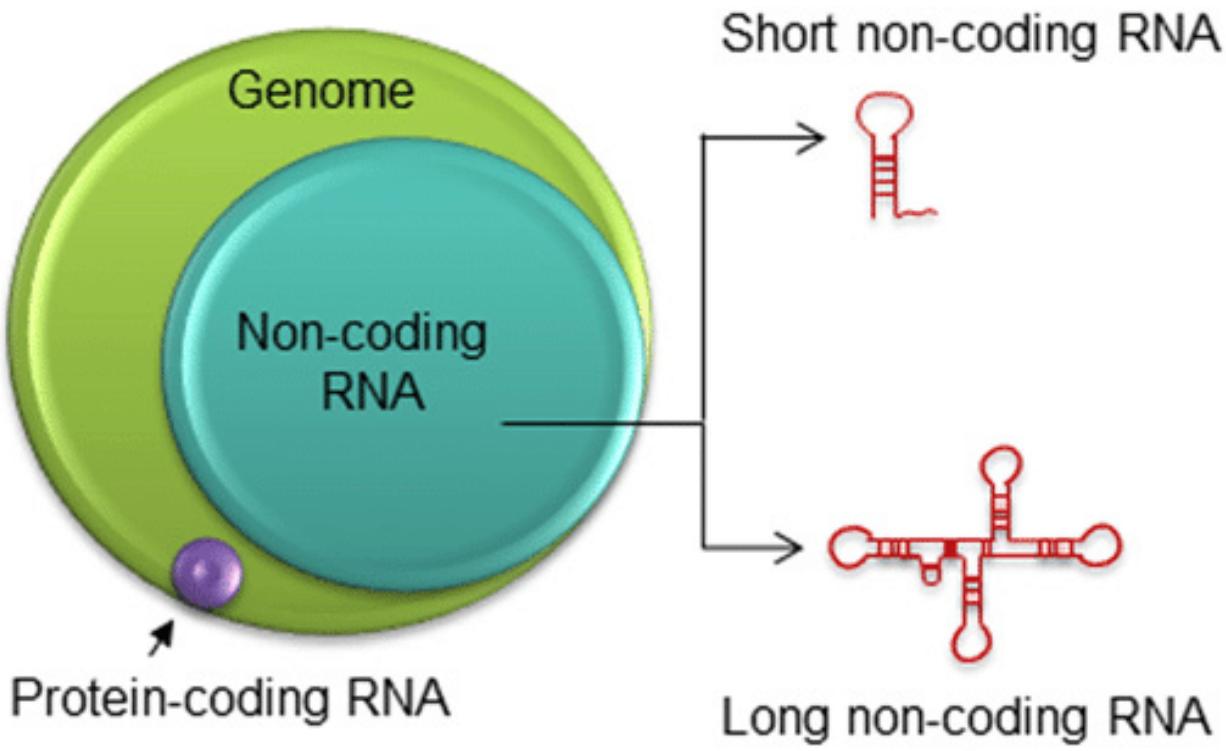
Validazione funzionale interazione miRNA-mRNA target



…Validazione funzionale interazione miRNA-mRNA target



Long non coding RNA (lncRNA)



Tipologie e abbondanza dei ncRNA

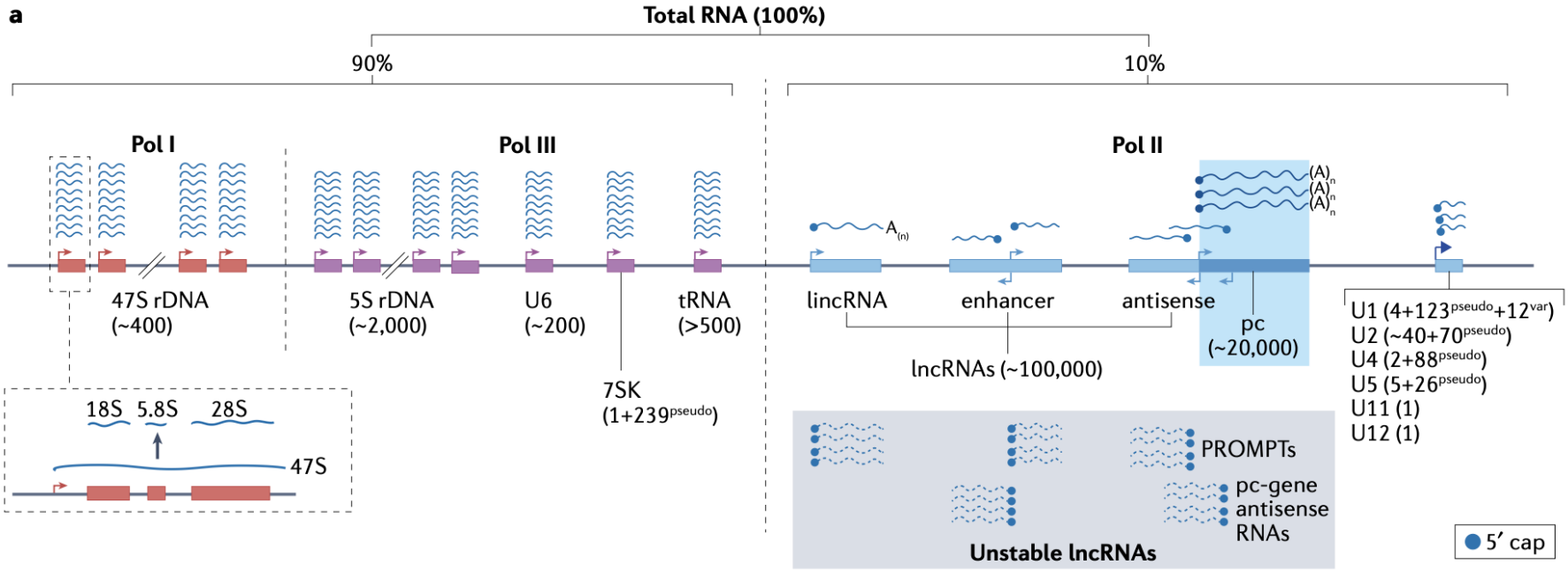
Type	Percent of total RNA by mass	Molecules per cell	Average size (kb)	Total weight picograms/cell	Notes	Reference
rRNAs	80 to 90	3–10 × 10^6 (ribosomes)	6.9	10 to 30		Blobel and Potter (1967), Wolf and Schlessinger (1977), Duncan and Hershey (1983)
tRNA	10 to 15	3–10 × 10^7	<0.1	1.5 to 5	About 10 tRNA molecules /ribosome	Waldron and Lacroute (1975)
mRNA	3 to 7	3–10 × 10^5	1.7	0.25 to 0.9		Hastie and Bishop (1976), Carter et al. (2005)
hnRNA (pre-mRNA)	0.06 to 0.2	1–10 × 10^3	10*	0.004 to 0.03	Estimated at 2–4% of mRNA by weight	Mortazavi et al. (2008), Menet et al. (2012)
Circular RNA	0.002 to 0.03	3–20 × 10^3	~0.5	0.0007 to 0.005	Estimated at 0.1–0.2% of mRNA**	Salzman et al. (2012), Guo et al. (2014)
snRNA	0.02 to 0.3	1–5 × 10^5	0.1–0.2	0.008 to 0.04		Kiss and Filipowicz (1992), Castle et al. (2010)
snoRNA	0.04 to 0.2	2–3 × 10^5	0.2	0.02 to 0.03		Kiss and Filipowicz (1992), Cooper (2000), Castle et al. (2010)
miRNA	0.003 to 0.02	1–3 × 10^5	0.02	0.001 to 0.003	About 10^5 molecules per 10 pg total RNA	Bissels et al. (2009)
7SL	0.01 to 0.2	3–20 × 10^4	0.3	0.005 to 0.03	About 1–2 SRP molecules/100 ribosomes	Raue et al. (2007), Castle et al. (2010)
Xist	0.0003 to 0.02	0.1–2 × 10^3	2.8	0.0001 to 0.003		Buzin et al. (1994), Castle et al. (2010)
Other lncRNA	0.03 to 0.2	3–50 × 10^3	1	0.002 to 0.03	Estimated at 1–4% of mRNA by weight	Mortazavi et al. (2008), Ramsköld et al. (2009), Menet et al. (2012)

The size for the average unspliced pre-mRNA is 17 kb; however, most pre-mRNAs are partially spliced at any given time, and the average size of hnRNA is estimated at 10 kb (Salditt-Georgieff et al., 1976).

***Based on the finding that 1–2% of all mRNA species generate circular RNA, which is present at 10% of the level of the parental mRNA.*

A. F. Palazzo and E. S. Lee, “Non-coding RNA: what is functional and what is junk?,” *Front. Genet.*, vol. 6, no. 61, p. 326, Jan. 2015.

Unità trascrizionali nei mammiferi



pc: protein coding genes

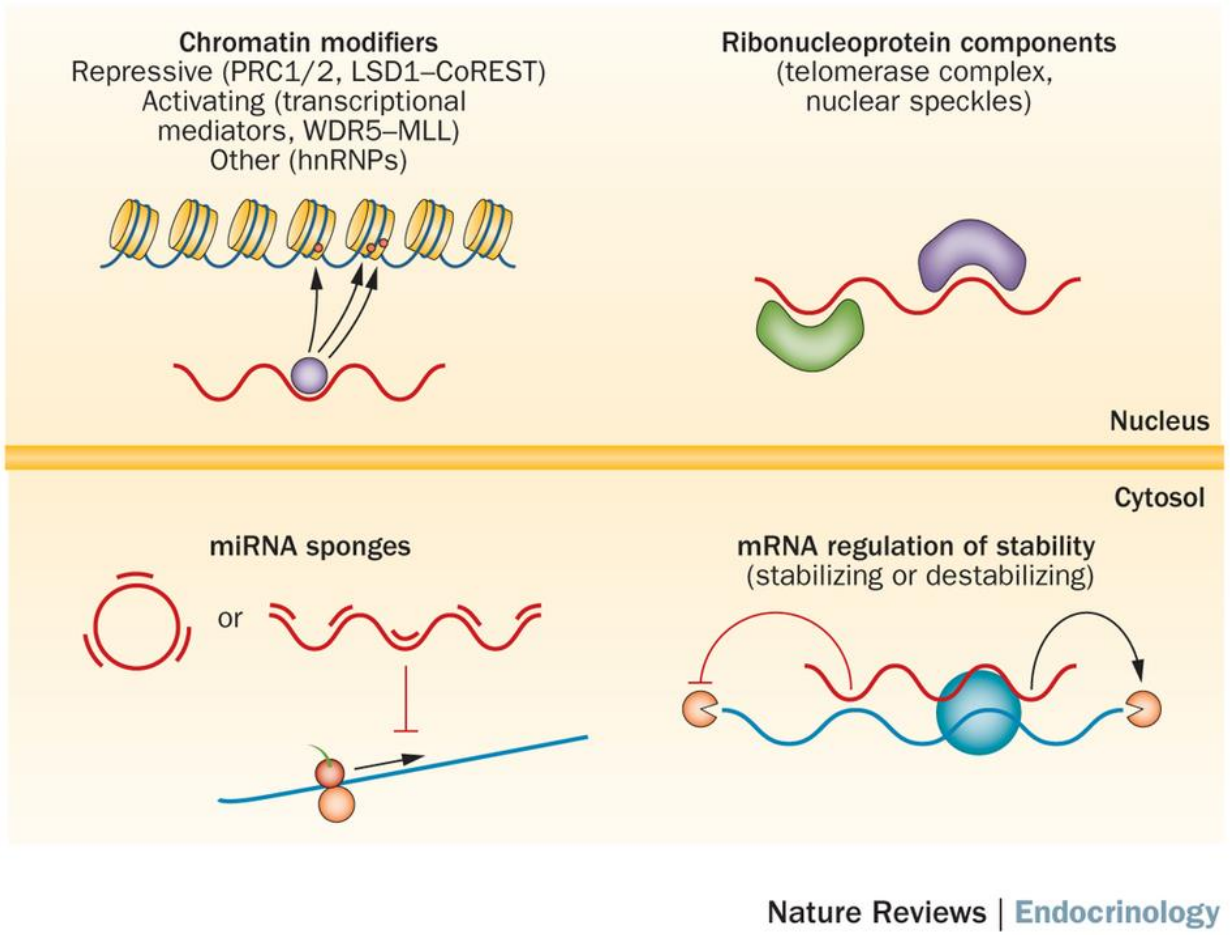
Nojima, T., Proudfoot, N.J. Mechanisms of lncRNA biogenesis as revealed by nascent transcriptomics. *Nat Rev Mol Cell Biol* **23**, 389–406 (2022). https://doi.org/10.1038/s41580-021-00447-6

Long non coding RNA (lncRNA)

- I lnc RNA sono trascritti di dimensione > di 200nt
- Poco abbondanti ed instabili
- La > parte dei lncRNA sono trascritti da RNA pol II, quindi contengono un 5'CAP e una coda di poly A
- A differenza dei piccoli ncRNA (siRNA, miRNA, piRNA) non sono evolutivamente conservati
- Sono regolatori dell'espressione genica, con meccanismi tuttora poco conosciuti.

- Per alcuni lncRNA è stato identificato il meccanismo funzionale

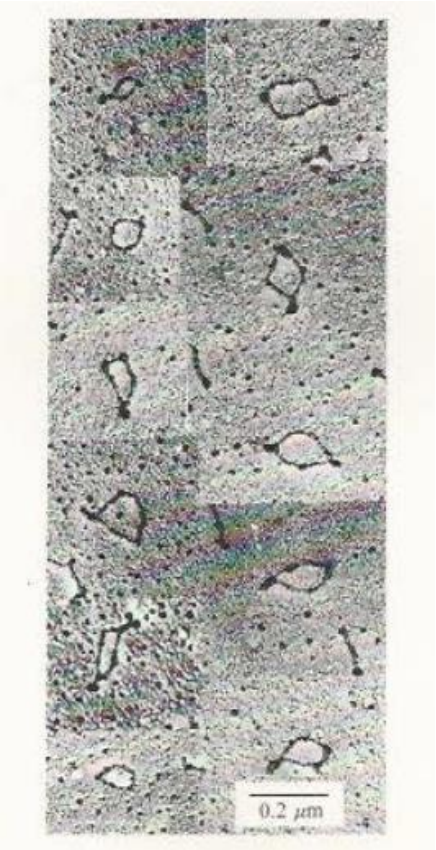
Funzioni note dei lncRNA



I lncRNA stanno emergendo quali importanti componenti di networks regolativi, interagendo con fattori di trascrizione e regolatori epigenetici nel nucleo, miRNA e RBP nel citoplasma.

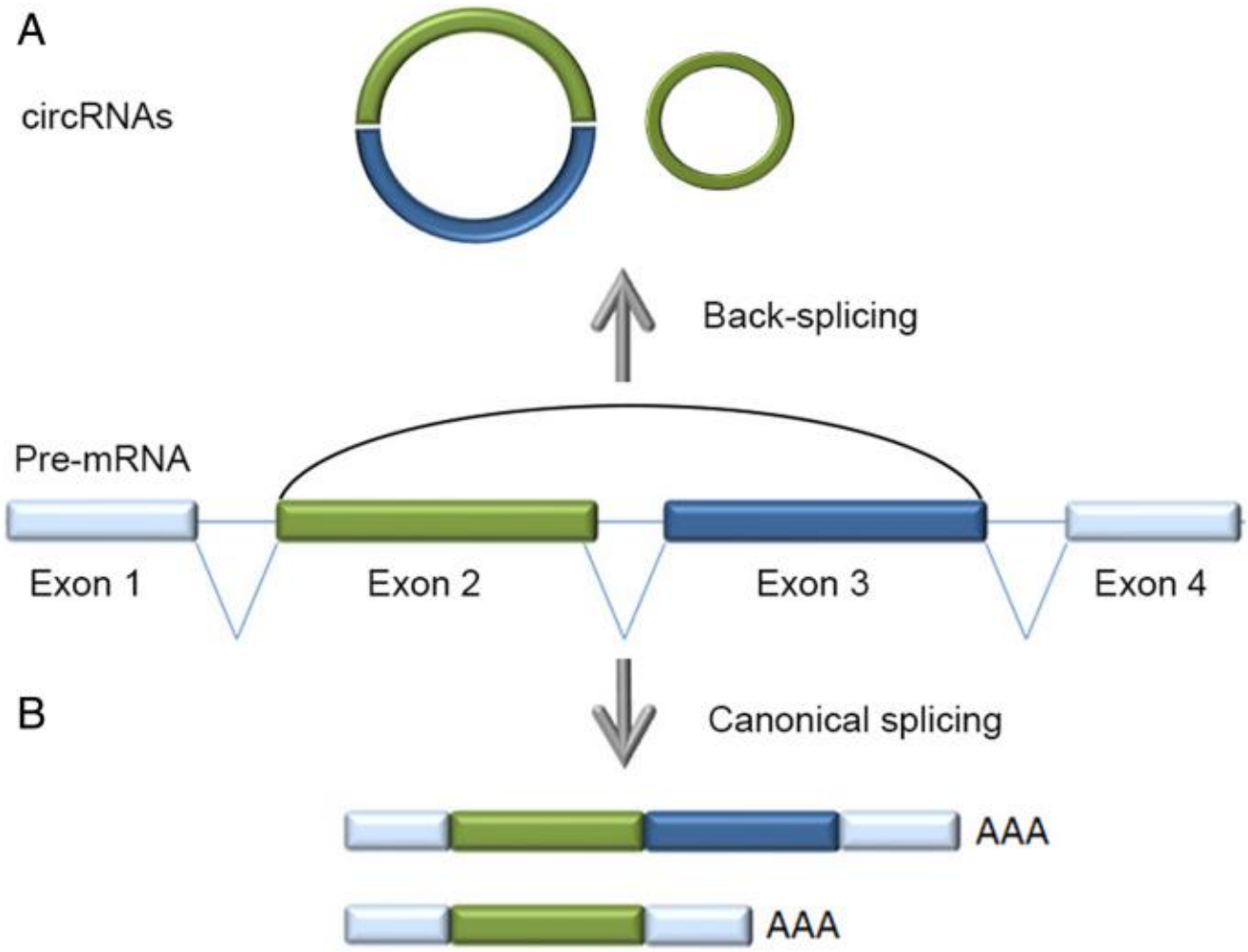
RNA circolari

- 1979- 2011 gli RNA circolari (circRNA) prima del 2011 erano considerati uno 'splicing noise', eccetto che per pochi esempi.
- Dal 2012 grazie allo sviluppo di tecniche di sequenziamento profondo sono stati identificare migliaia di circRNAs come trascritti abbondanti del genoma di vertebrati ed invertebrati.
- In alcuni casi il trascritto circolare è l'isoforma piu' abbondante.
- circRNA sono altamente conservati negli eucarioti
- Sono altamente stabili.
- Hanno un'espressione cellula e tessuto specifica
- Queste caratteristiche suggeriscono importanti funzioni biologiche.



Hsu, M.-T. & Coca-prados, M., 1979 plant viroids

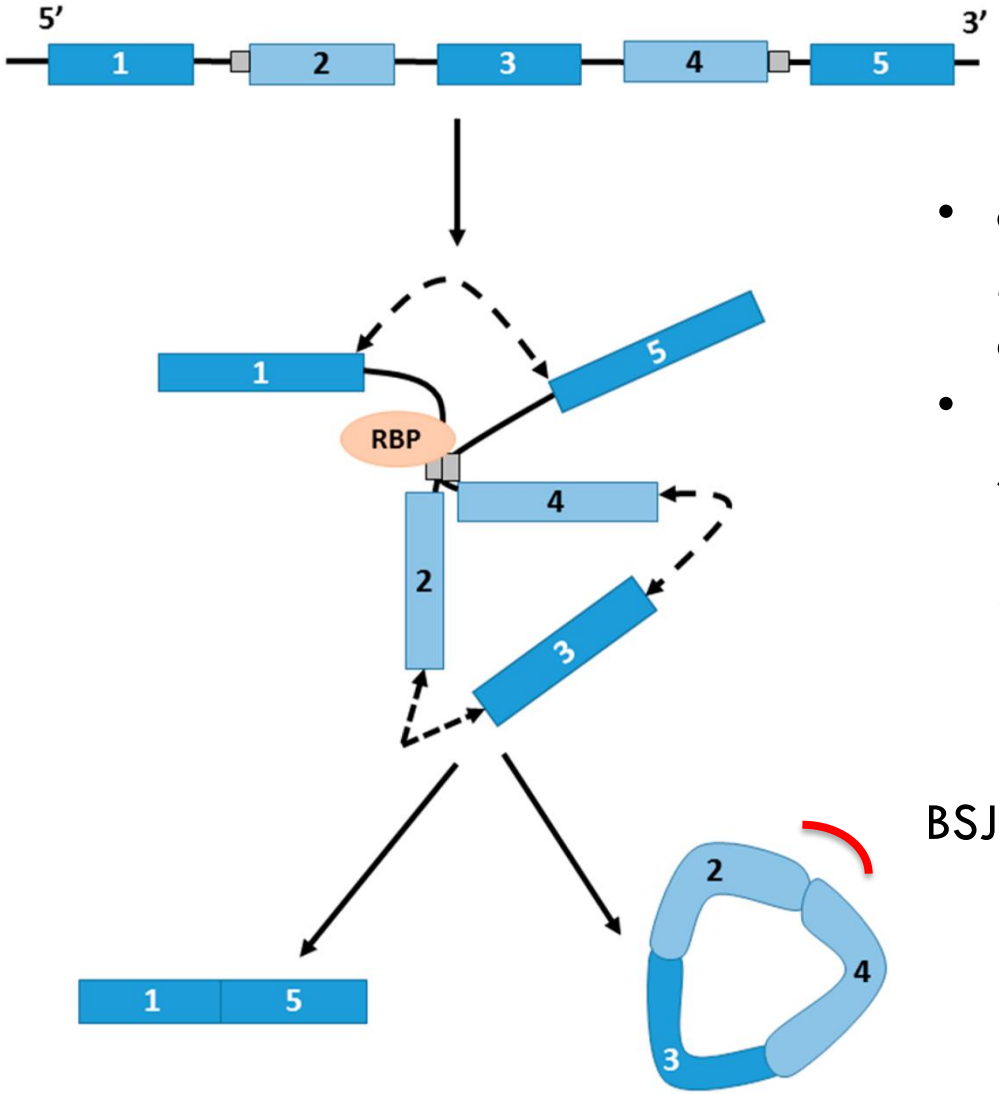
I circRNA sono prodotti di splicing alternativo



Regulazione della biogenesi dei circRNA

- *circRNA formation makes use of the canonical splicing machinery, including proteins that recognize splice sites in introns*
- *inverted repeat sequences in introns (30-50nt) favor circRNA biogenesis*
- *RBPs that interact with specific sequences in introns (es RBP muscleblind-MBL) favor circRNA biogenesis*

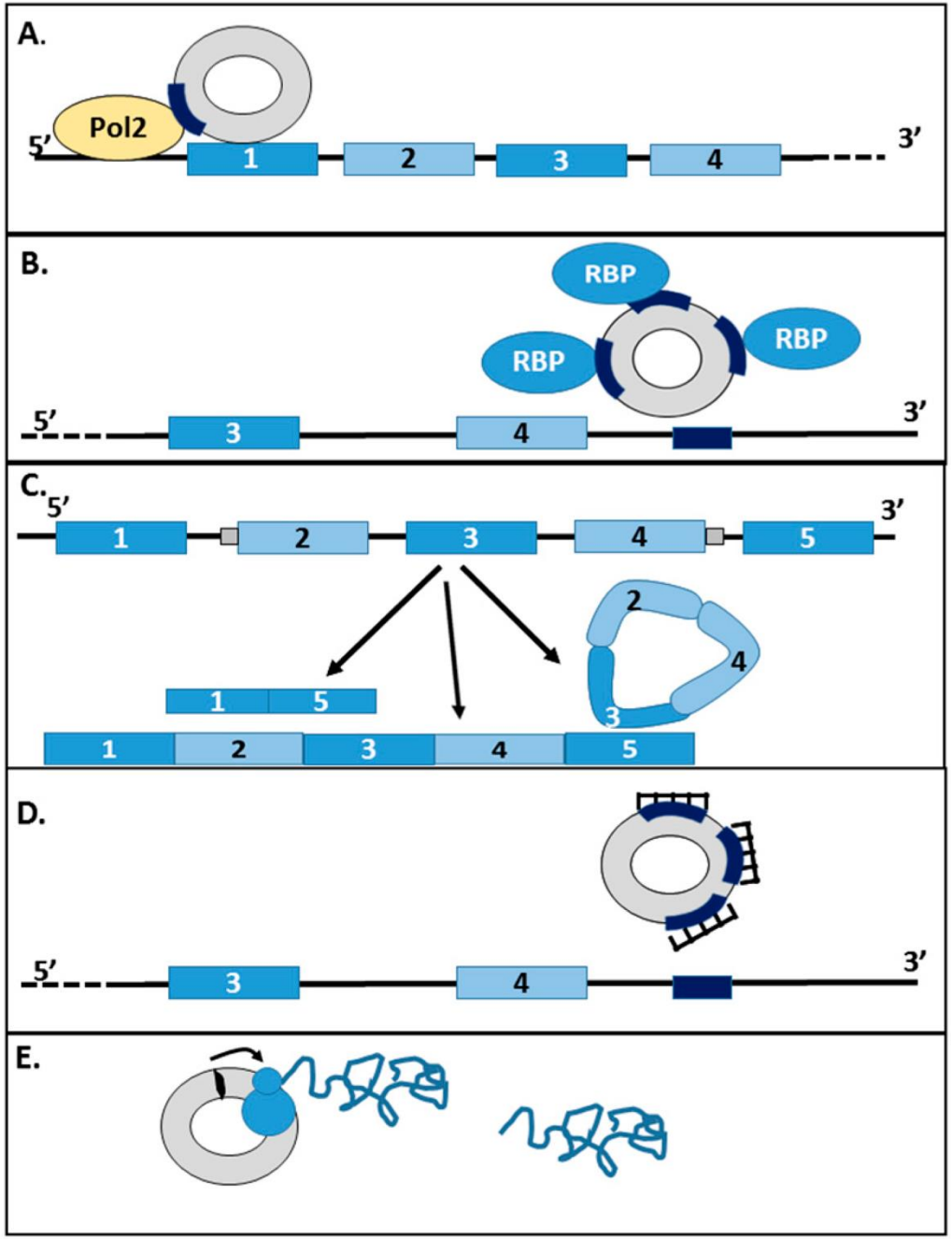
circRNA sono generati da una reazione di 'Back-splicing'



- *downstream splice donor (5'SS) is joined to upstream splice acceptor (3'SS)*
- *this process is called Back splicing (BS) and generates a BS junction (specific of circRNAs) (BSJ)*

BSJ (back splicing junction)

Funzioni biologiche dei circRNA



A. *In the nucleus circRNAs can interact with promoter regions and RNApolII polymerase II (Pol2) to repress or enhance transcription*

B. *circRNAs can bind RBPs that regulate mRNA processing altering the splicing patterns of the genes targets of RBPs, or change mRNA stability*

C. *the production of circular RNAs reduces the amount of linear transcript produced*

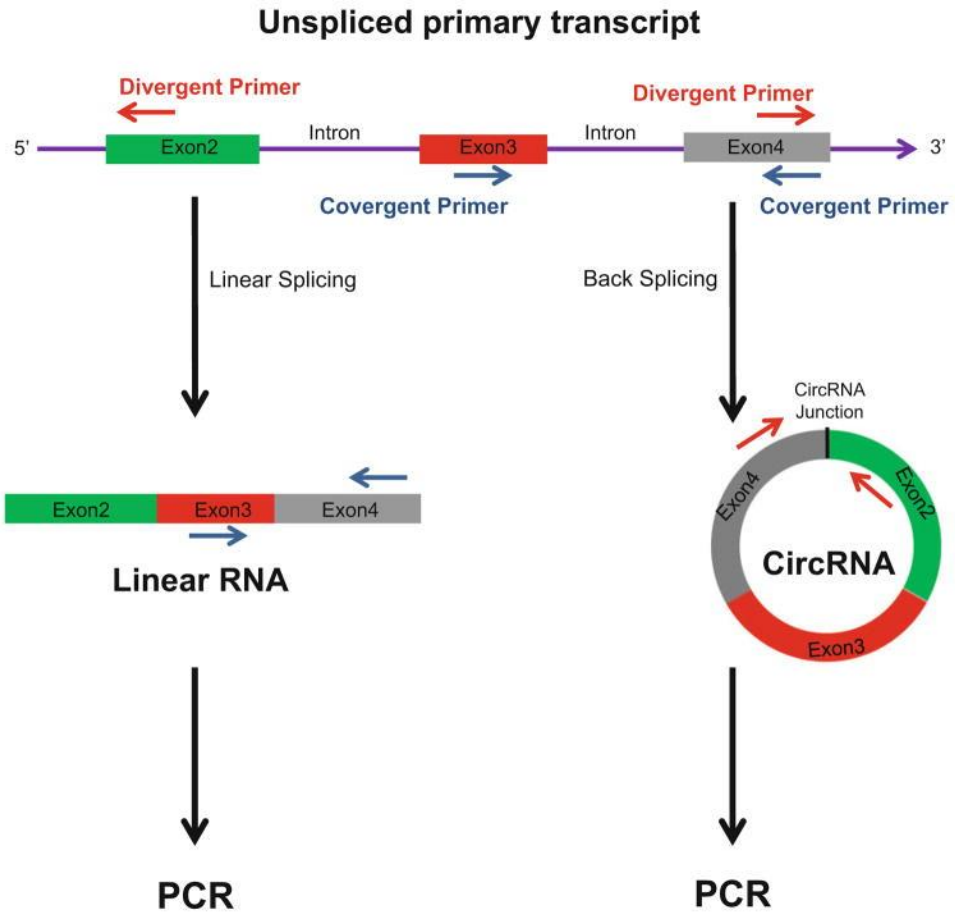
D. *circRNAs can act as miRNA sponges, sequestering them away from their binding sites in target genes*

E. circular RNAs can be translated

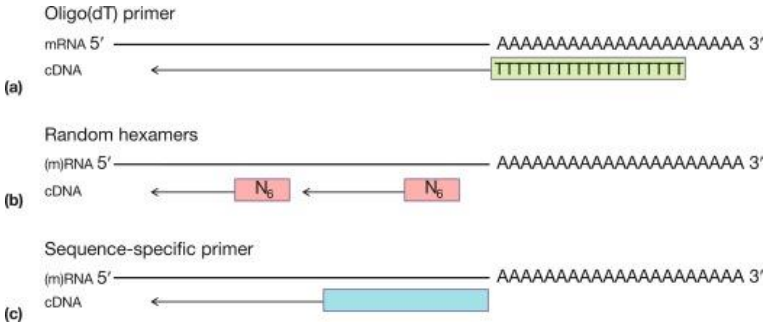
Haque, S. & Harries, L.W.,

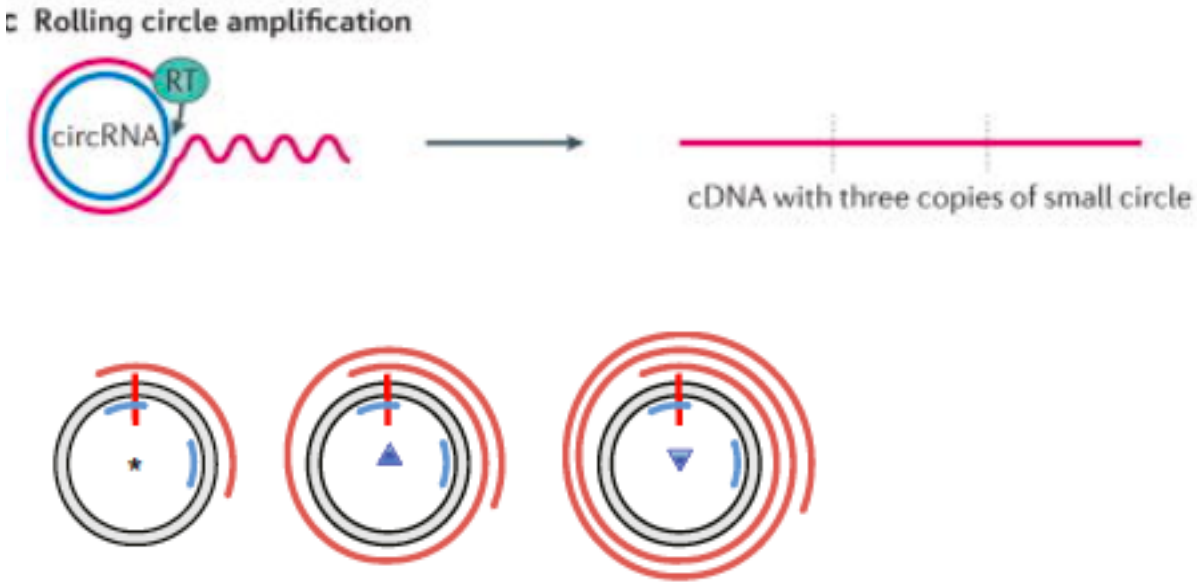
Identificazione circRNA

Reverse Transcription (RT)-qPCR

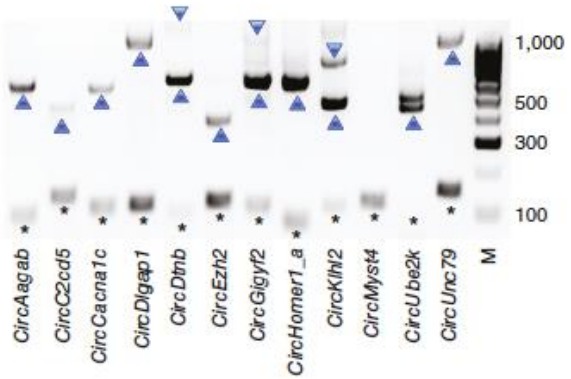


RT-PCR-rolling circles

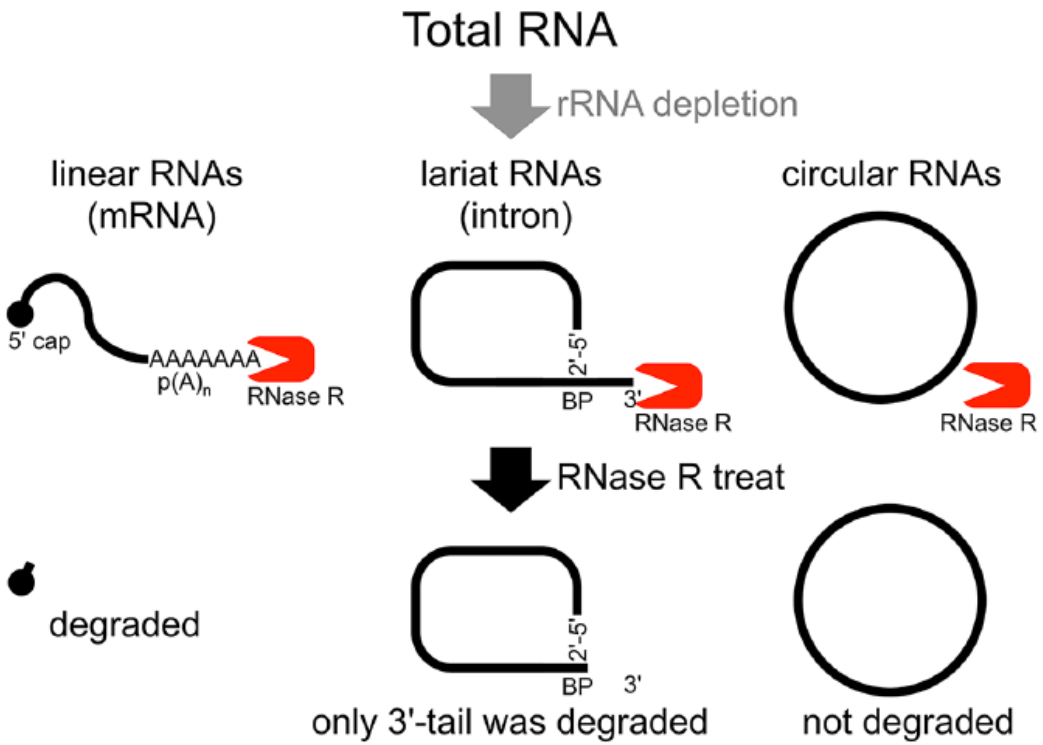




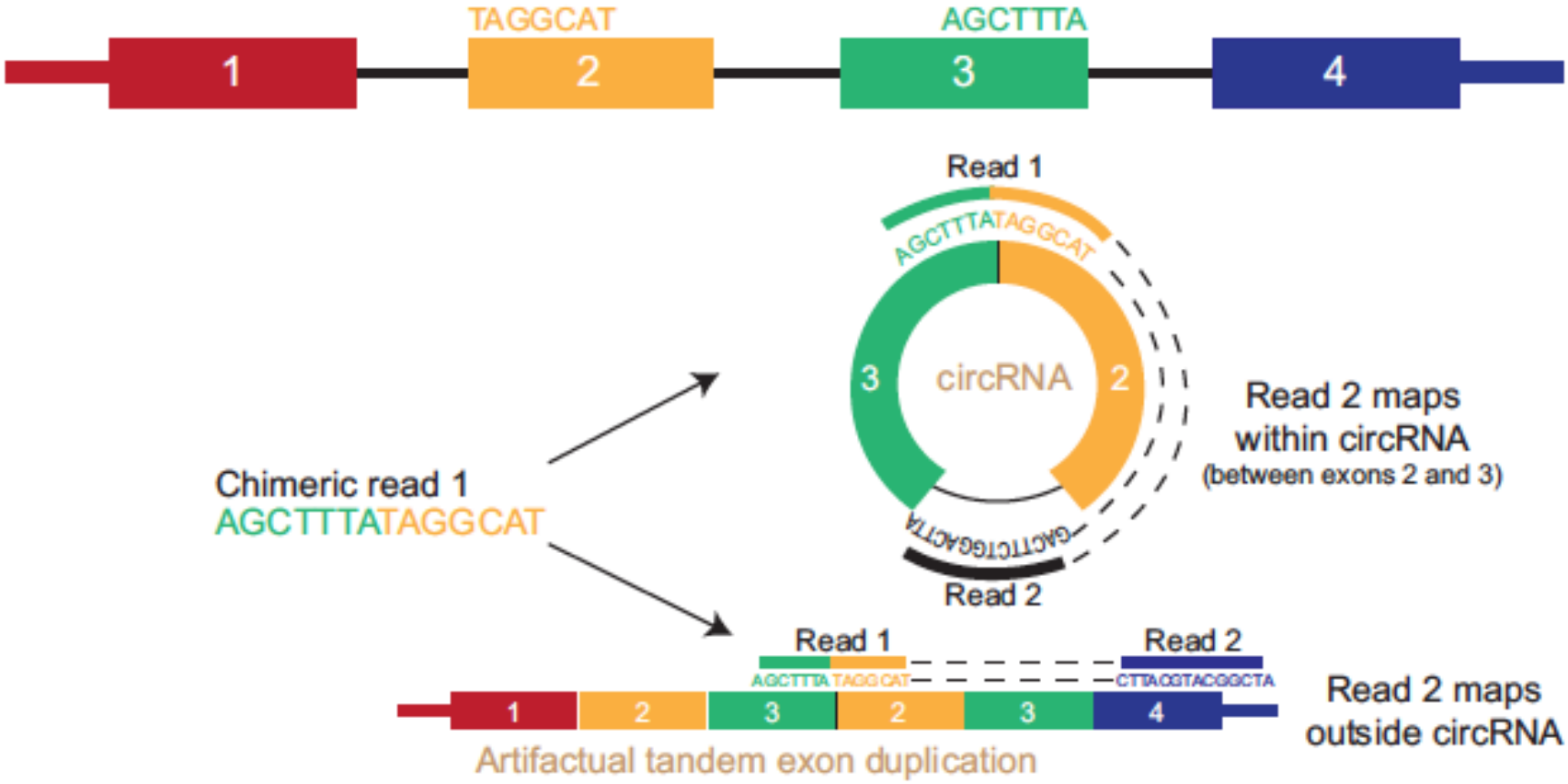
RT-PCR of circRNAs



I circRNA sono resistenti all' RNase R



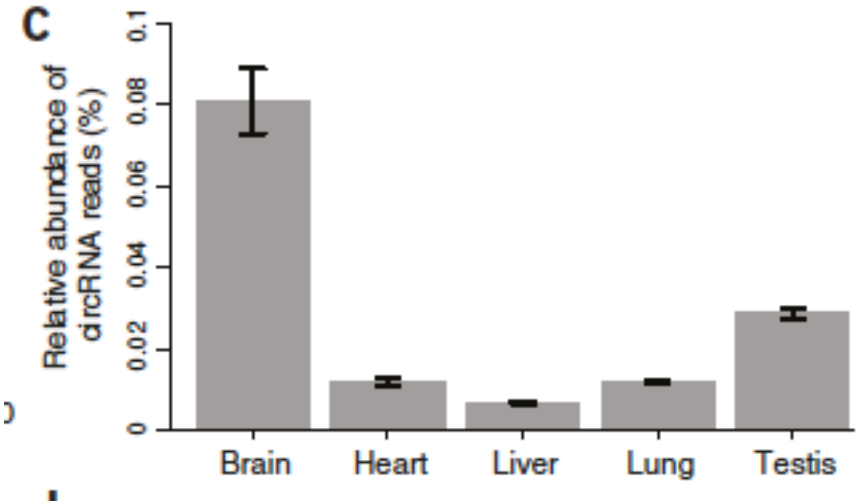
…Identificazione bioinformatica dei circRNA



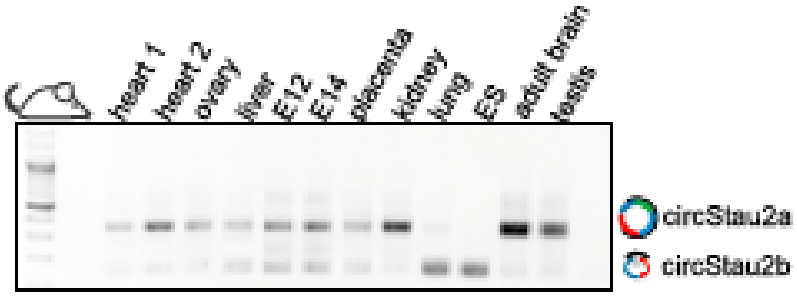
- *circRNAs sequences are found in the fraction of sequencing reads unmapped with the genome (excluded from mRNA seq analysis).*
- *hallmark of circRNAs in RNA-seq data is a chimeric read in which the 3' end of the read maps upstream of the 5' end with respect to the direction of transcription (start in exon 3 and end upstream in exon 2)*

circRNAs sono molto abbondanti nel cervello

- circRNAs are more enriched in neuronal tissues compared with other tissues
- 20% of the neuronal protein-coding genes in brain produce circRNAs

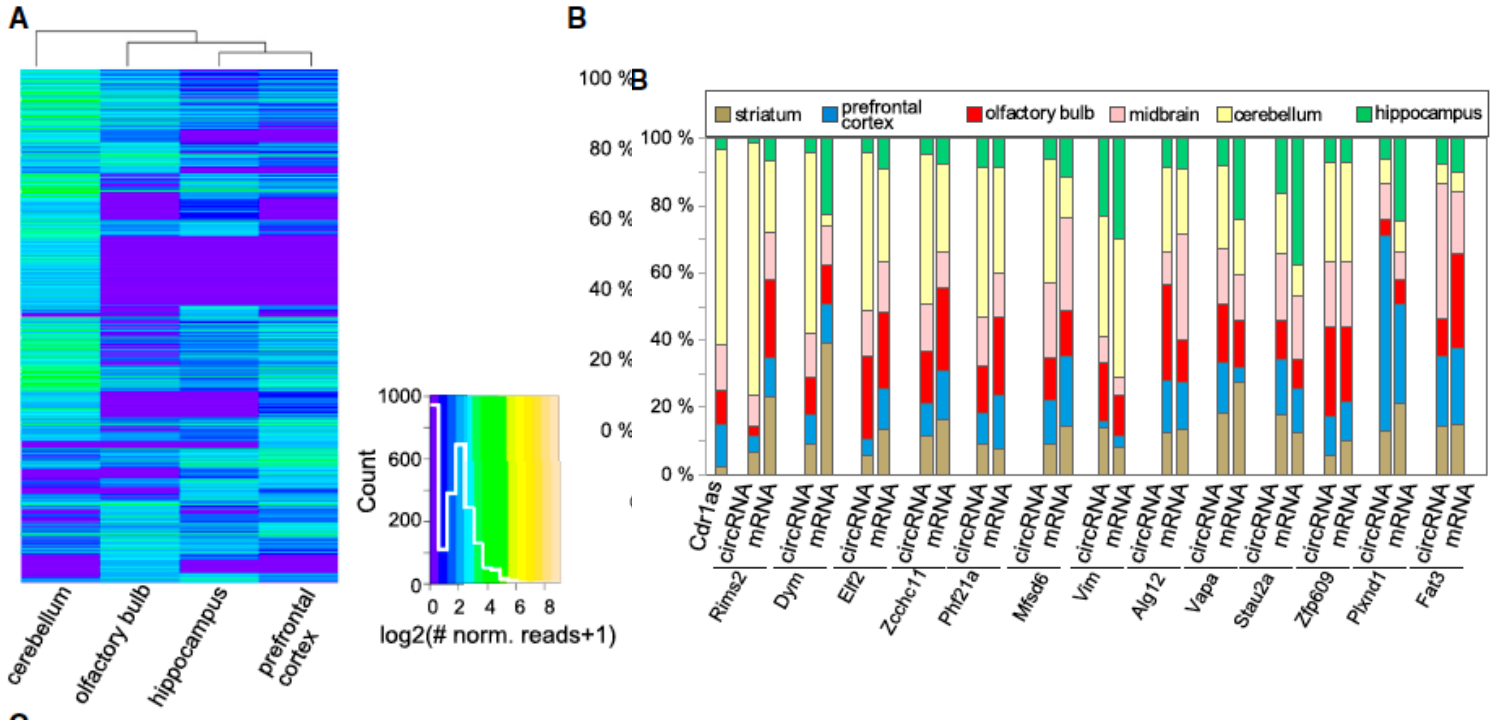


circStau2 isoforms expression in different tissues

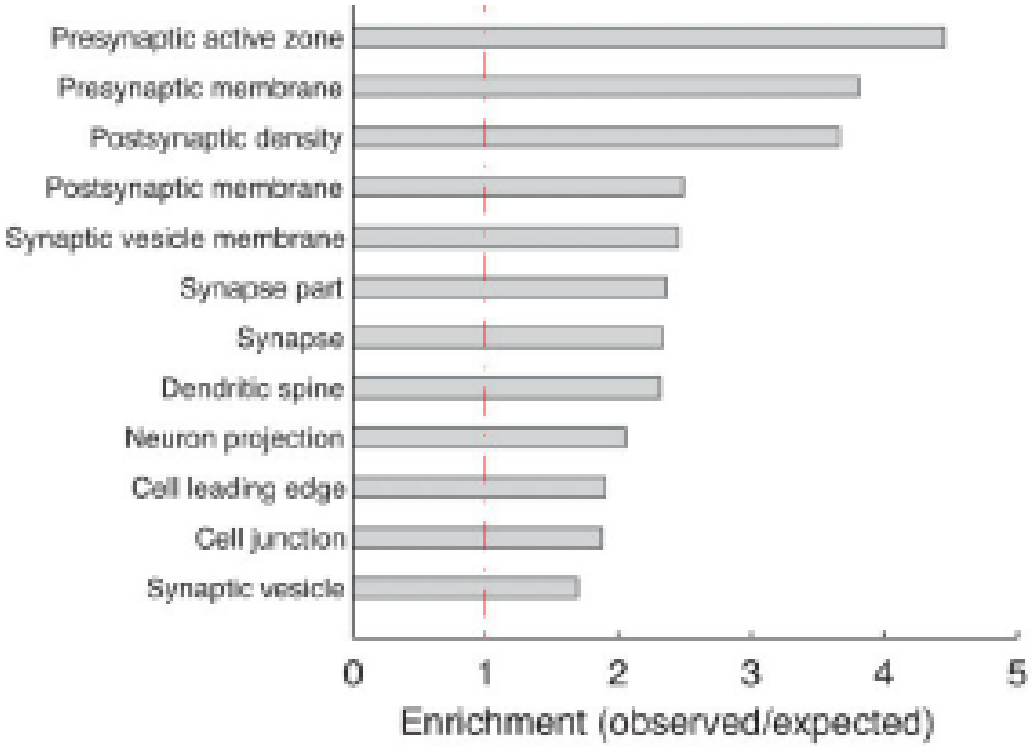


You, X. et al., 2015; Rybak-Wolf, A. et al., 2015; Haque, S. & Harries, L.W., 2017

…e mostrano espressione specifica nelle diverse regioni cerebrali

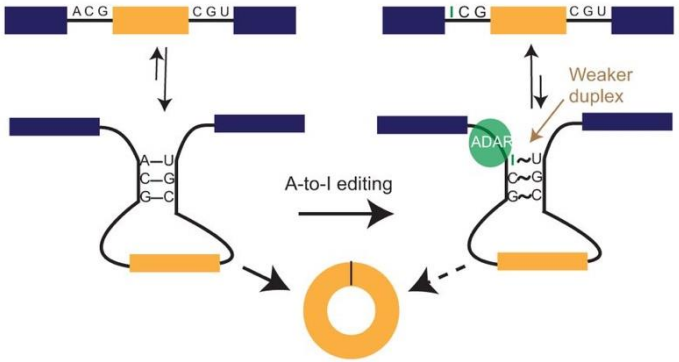


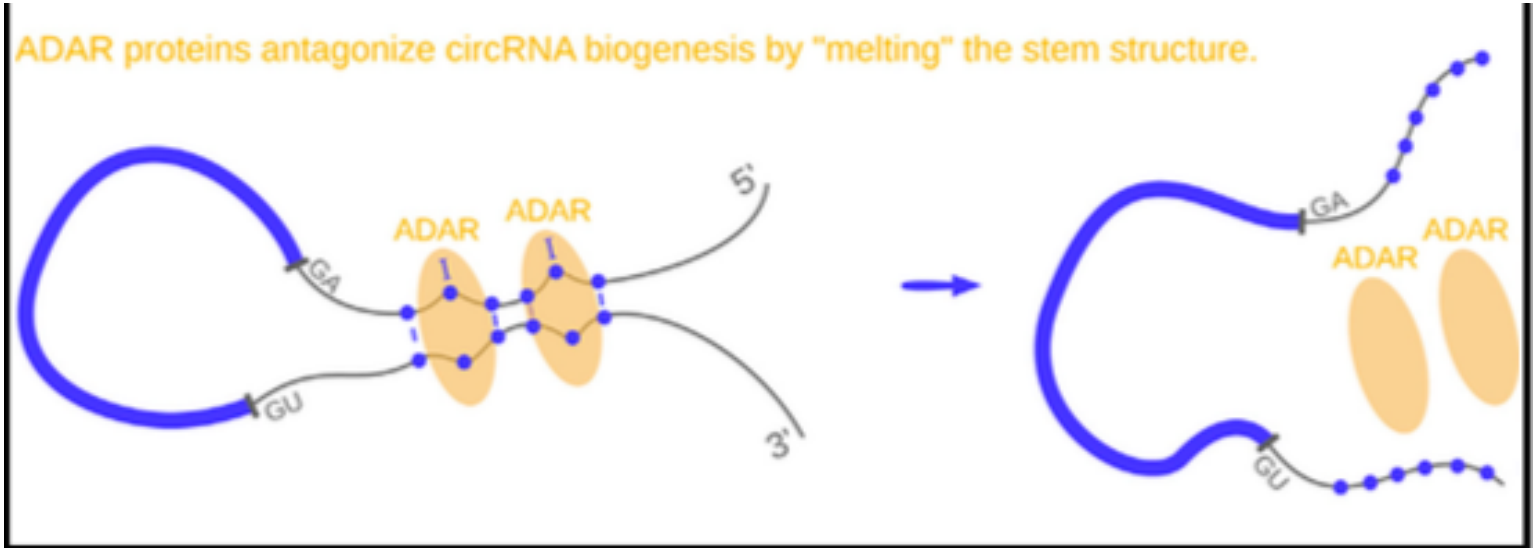
I circRNA derivano da geni neuro-specifici e coinvolti in funzioni sinaptiche



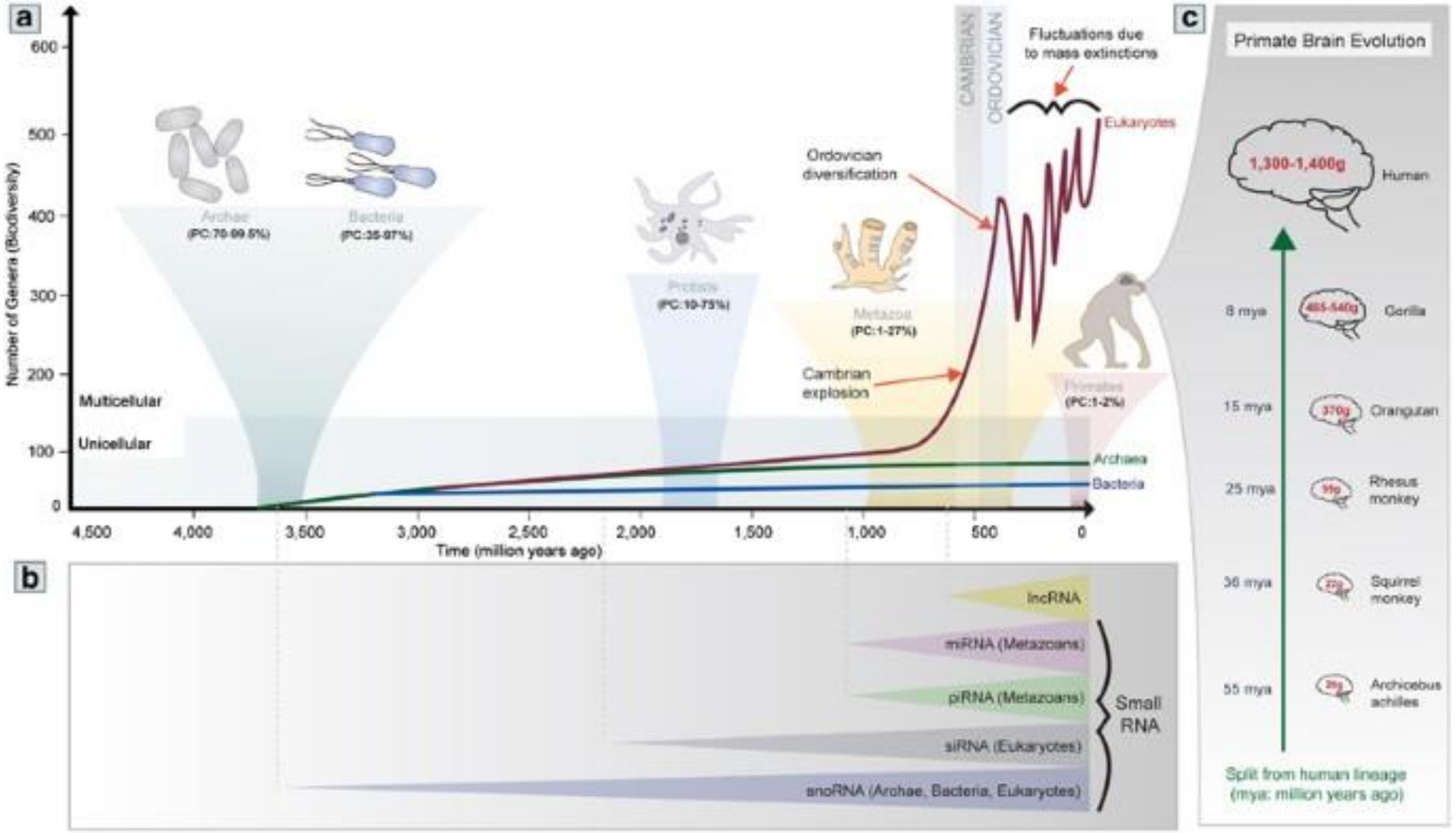
Gene Ontology analysis of circRNA host genes

Nell'uomo la biogenesi dei circRNA è regolata dall'Editing dell'RNA





ncRNA ed evoluzione



Ruolo dei ncRNA nelle patologie umane