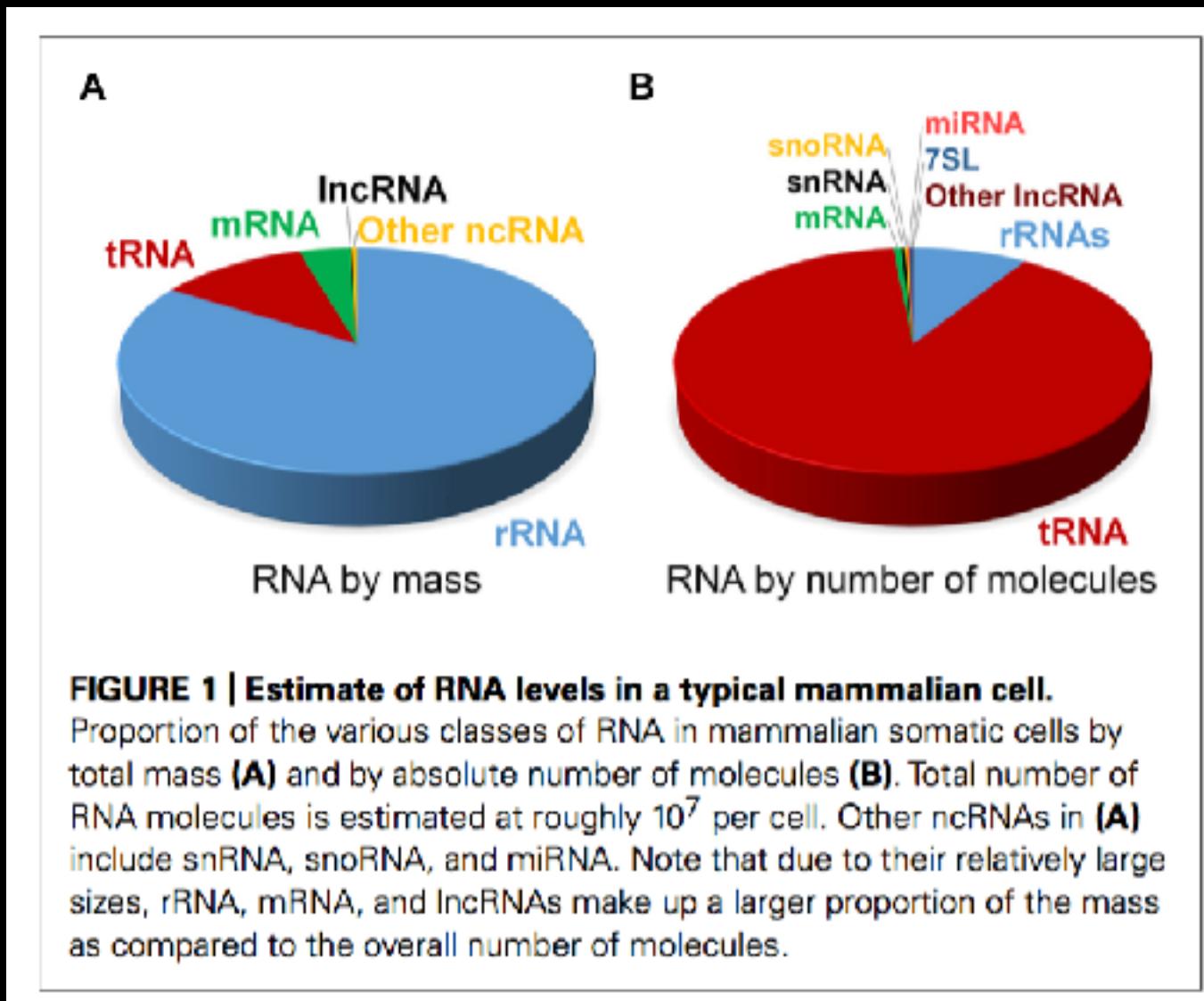


ncRNAs

Quantidade relativa de RNAs



Regulação por ncRNAs

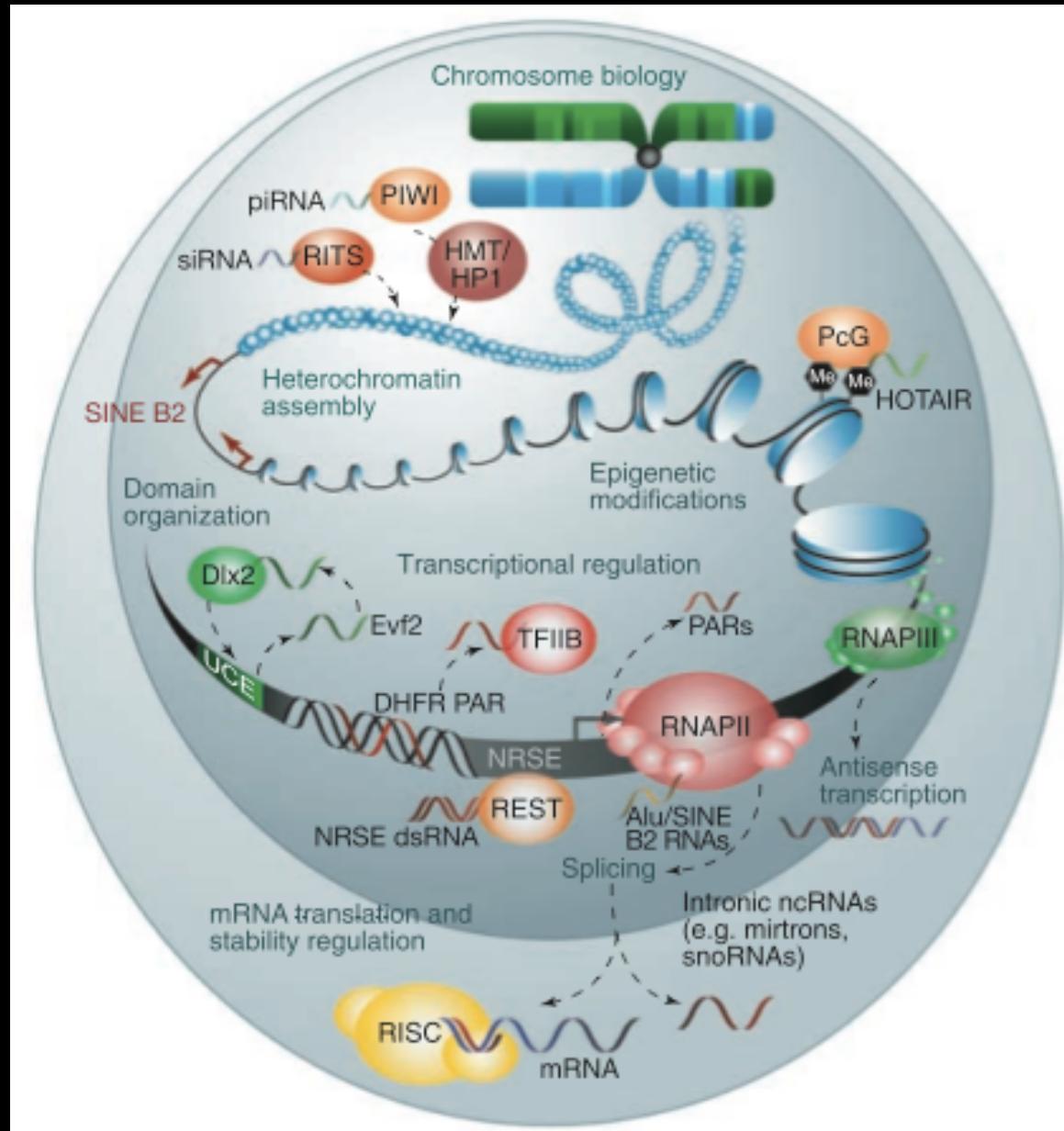


Table 1 | Estimates of total RNA content in mammalian cells.

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

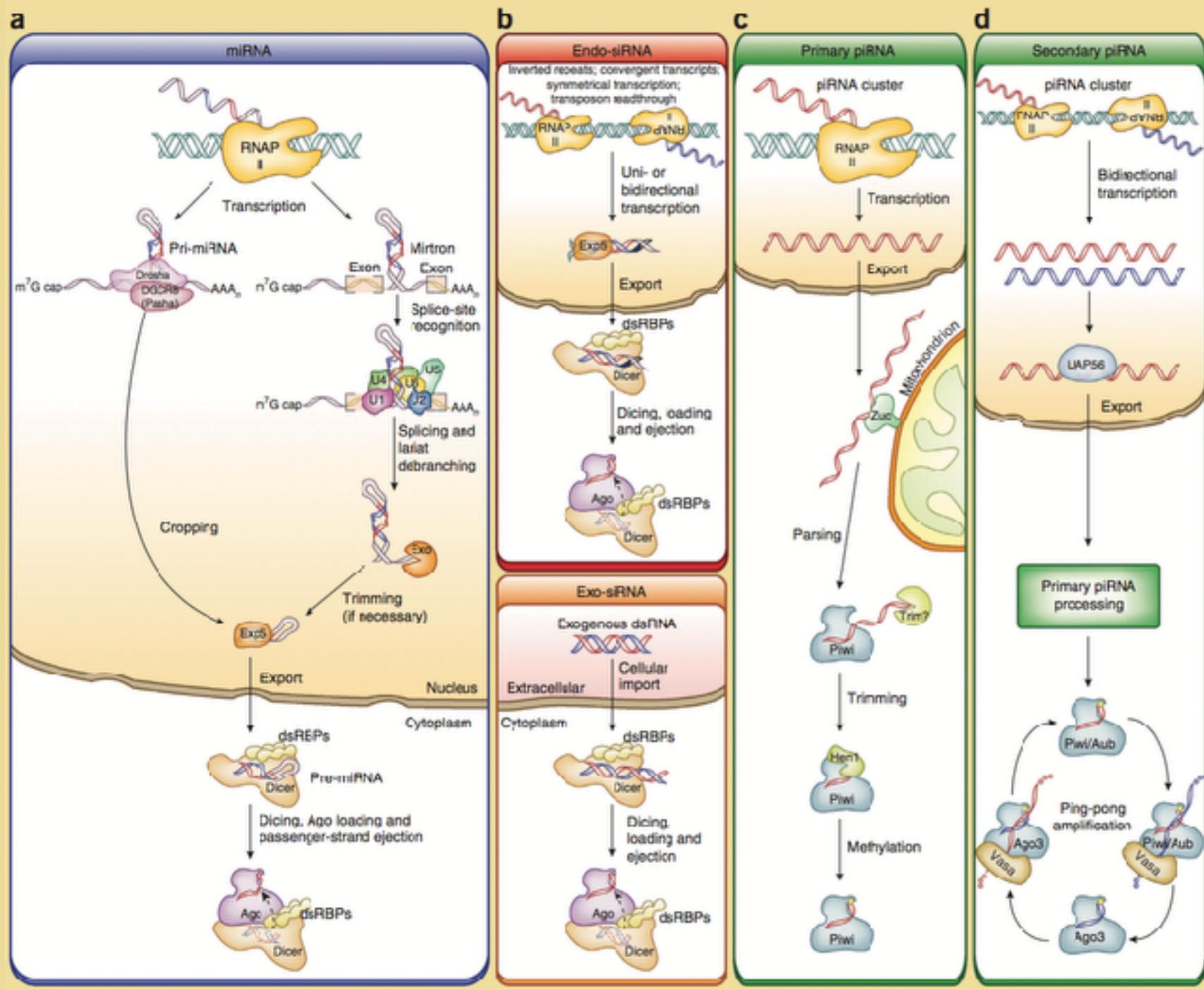
**Based on the finding that 1–2% of all mRNA species generate circular RNA, which is present at 10% of the level of the parental mRNA.

RNAs não codificantes (ncRNAs)

- Constitutivos
 - rRNA
 - tRNA
 - snRNA, snoRNA, scRNA
- Reguladores
 - miRNA
 - siRNA
 - piRNA
 - lncRNA
 - ceRNA (competidores endógenos)
 - circRNA
- Relacionados a transcrição
 - PASRs, PALRs, TASRs (sRNAs e tRNAs associados a promotor e terminador)
 - eRNAs (RNAs associados a *enhancers*; bidirecional, com cap e sem poli(A))
 - Xist (determina inativação de cromossômo X em mulheres)
- Função desconhecida
 - RNA *vault* (*)

Pequeños ncRNAs

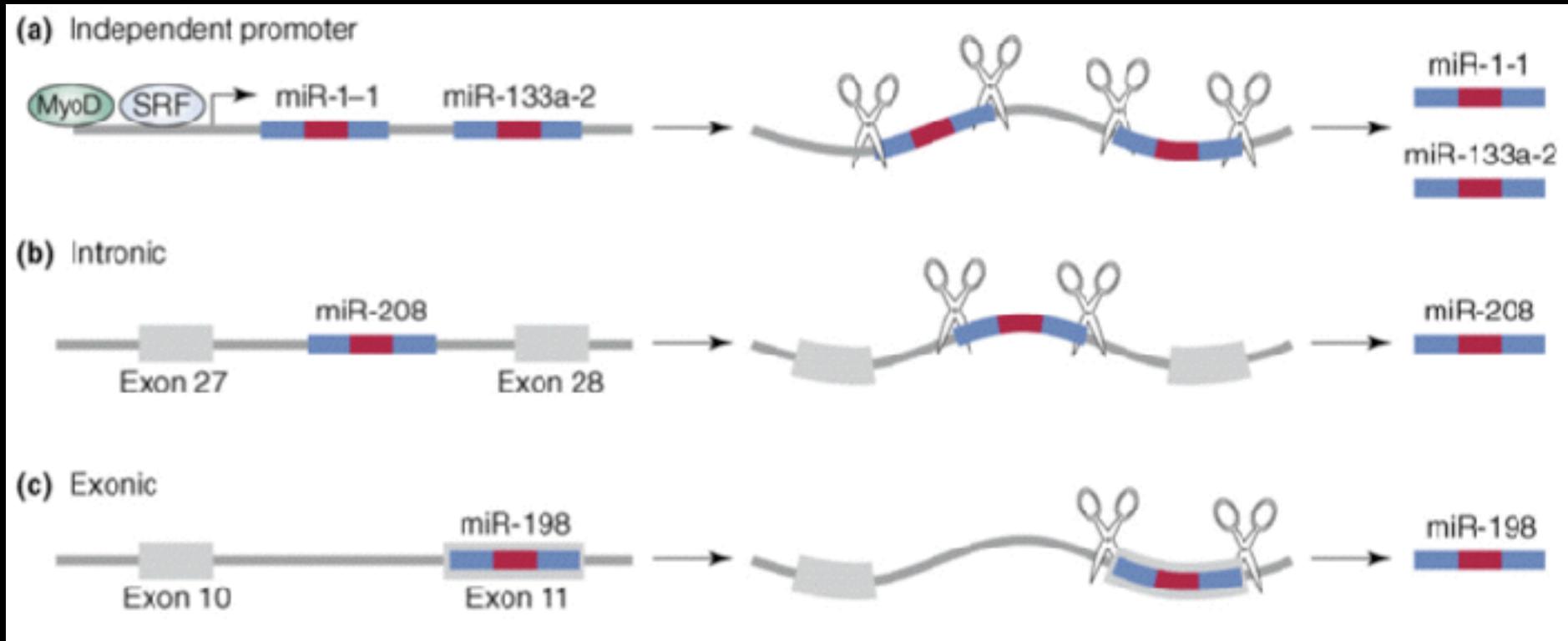
Box 1 Overview of eukaryotic small-RNA biogenesis



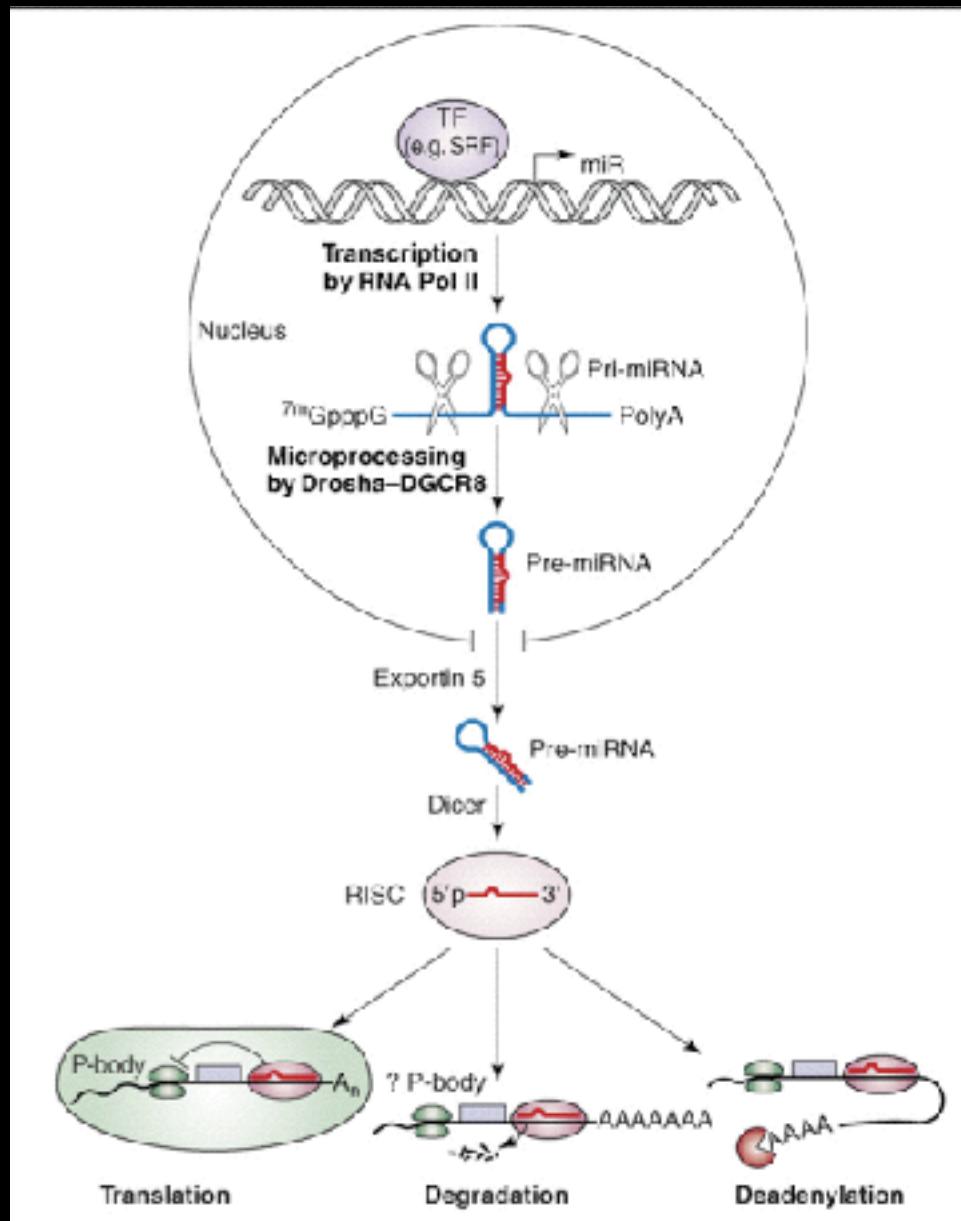
miRNAs: características

- Pequenos RNAs (~22 nt) fita simples
- Pareamento de bases imperfeito com UTR 3'
- Pode afetar centenas de mRNAs
- Regulação complexa
- Ação: regulação gênica pós-transcricional
 - Ação geralmente repressora: bloqueio de tradução seguido de degradação
- Ação: regulação transcricional

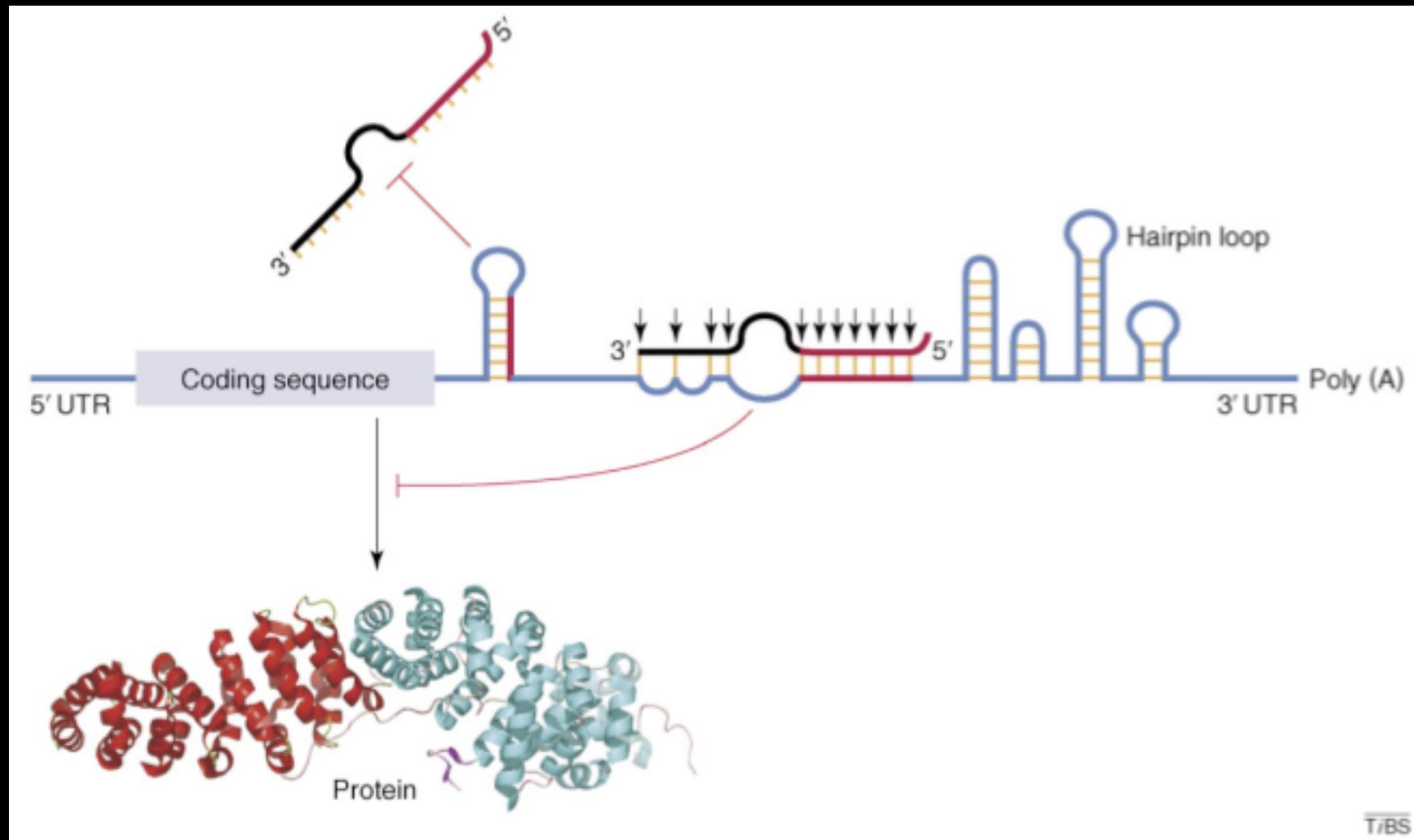
miRNAs: organização gênica



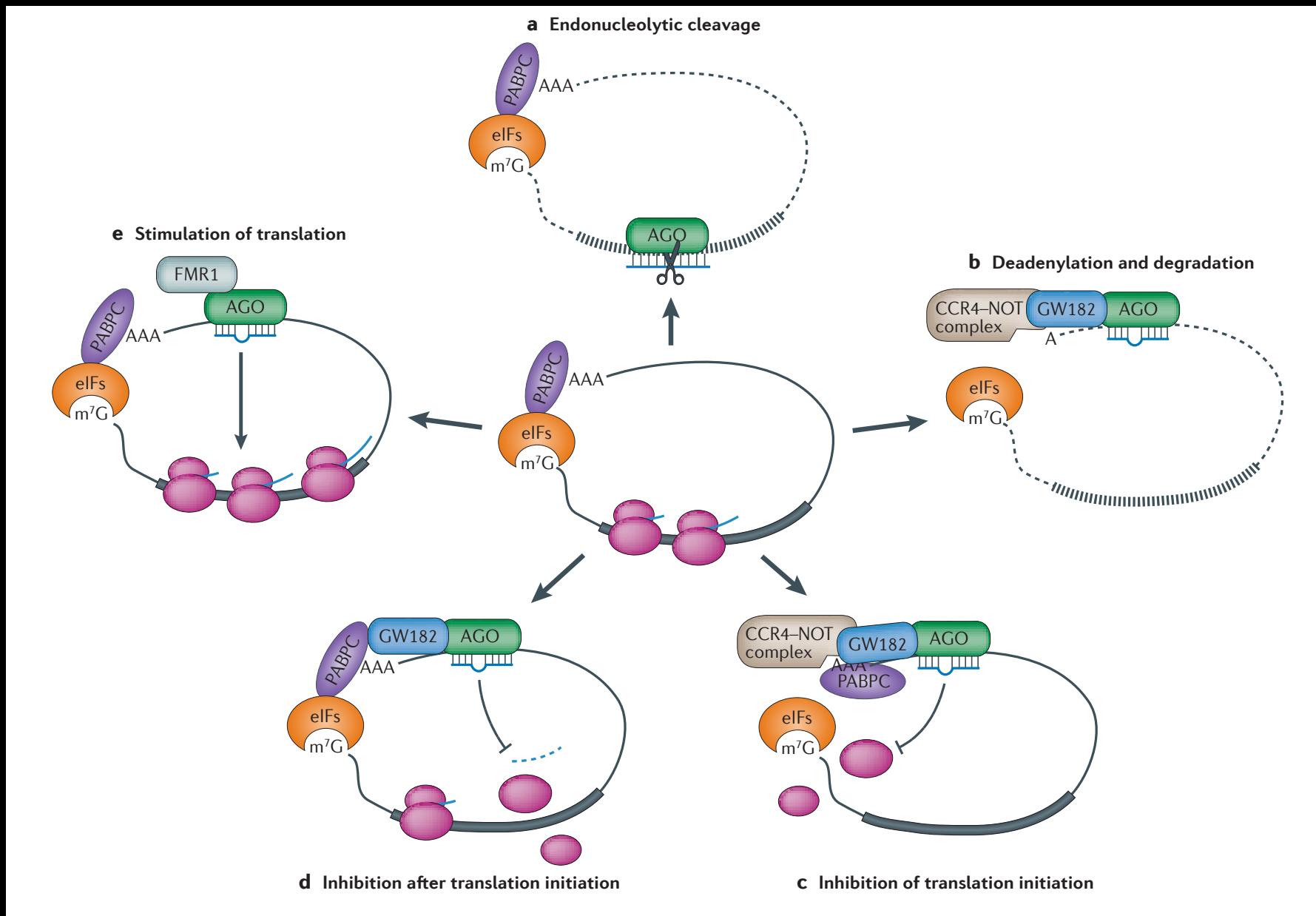
miRNAs: mecanismos



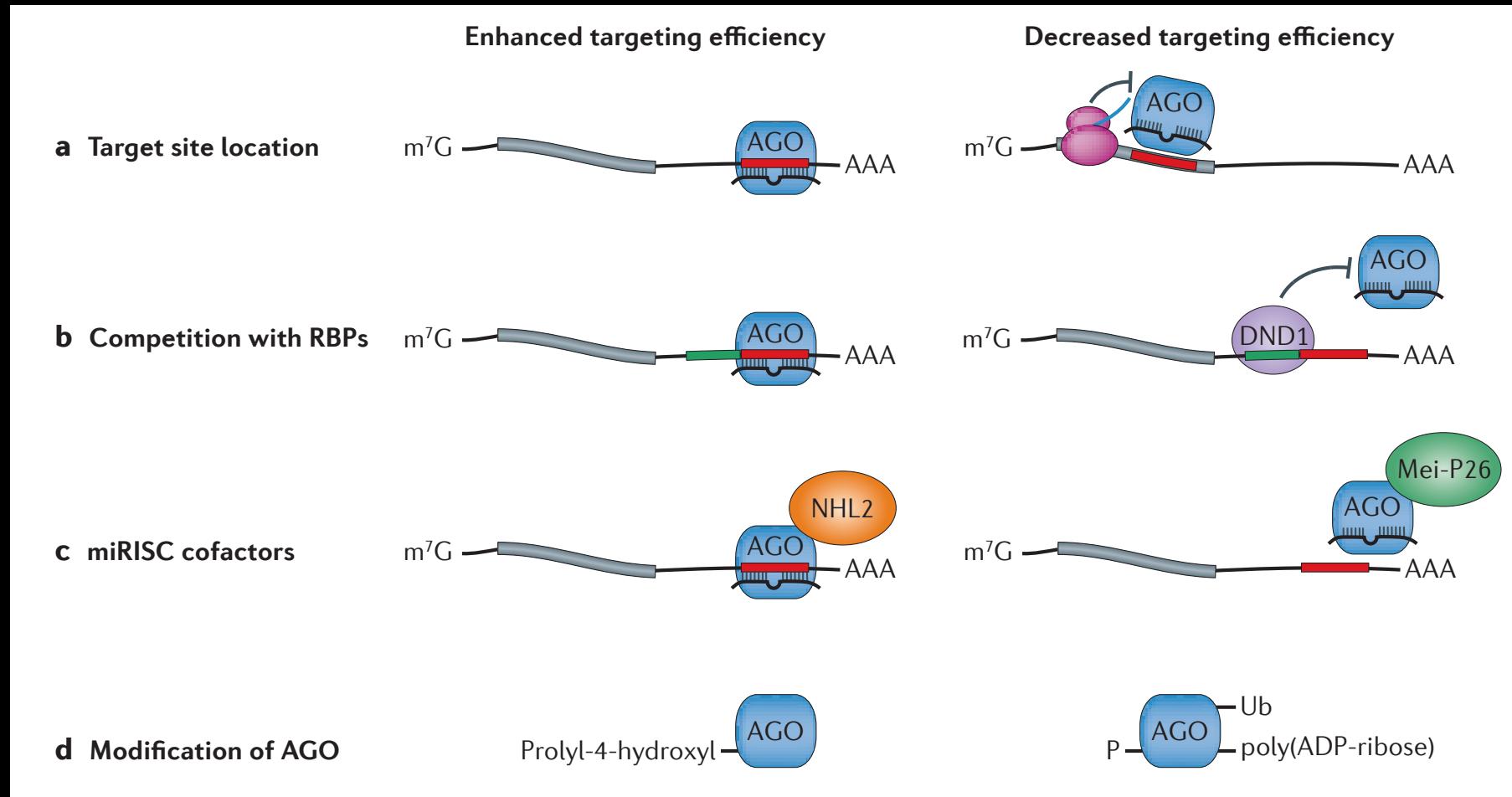
Interação miRNA e mRNA alvo



Mecanismos de ação dos miRNAs



Eficiência da ação dos miRNAs



miRNAs e circRNAs

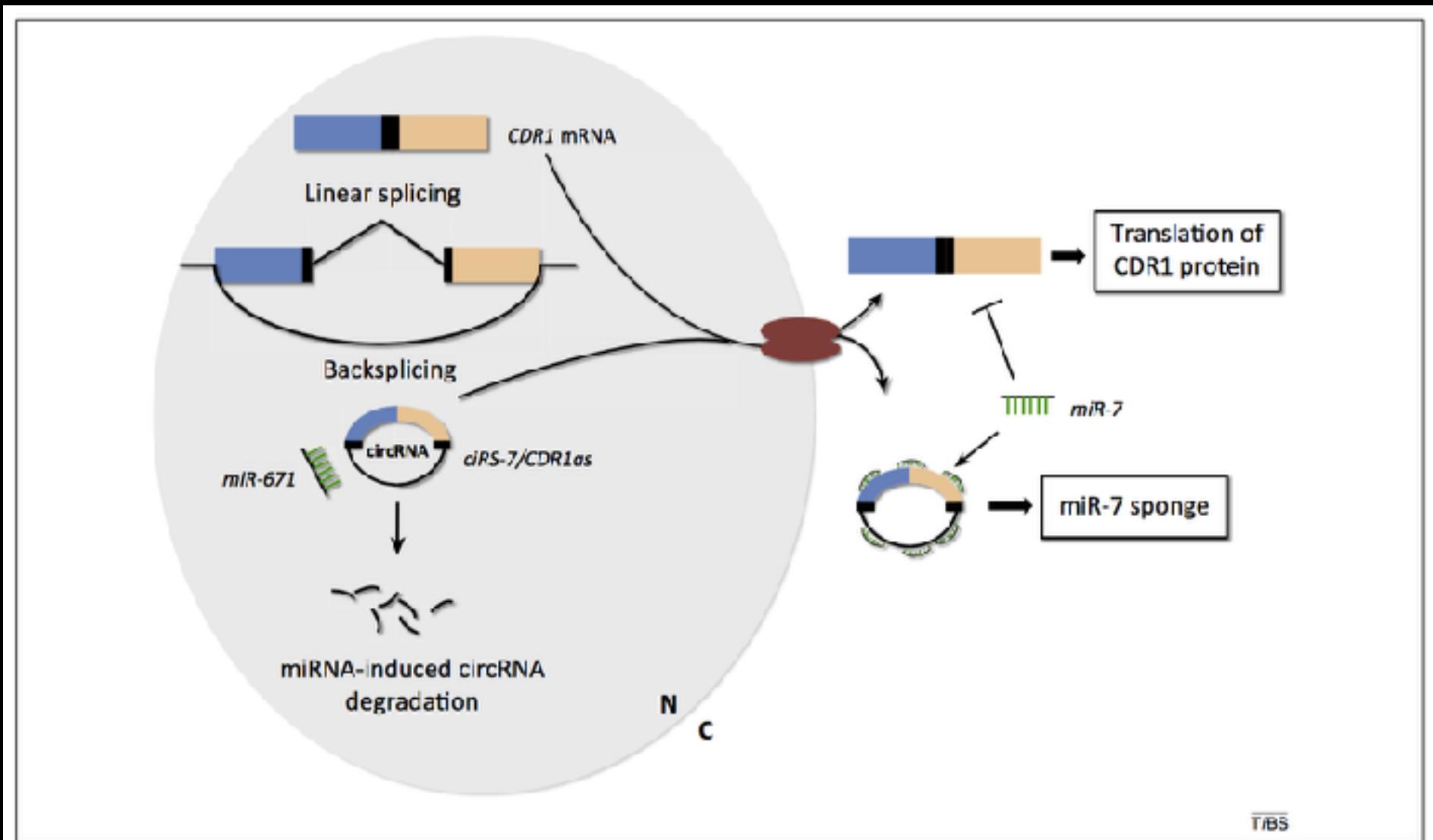


Figure 2. Circular RNAs (circRNAs; as products of backsplicing or through other origins) can be targets of miRNAs or act as sponges. One example of this dual mechanism is the c_iRS-7/CDR1as transcript, which originates from cerebellar degeneration-related protein 1 (CDR1) backsplicing and is a target of miR-671-induced endonucleolytic cleavage in the nucleus. Following export to the cytoplasm, and given the high number of canonical miR-7 binding sites, c_iRS-7 can also function by competing with CDR1 mRNA for miR-7 interaction, thereby alleviating post-transcriptional repression on CDR1. The reduced complementarity between c_iRS-7 and miR-7 probably allows the dsRNA to be densely bound, but not sliced, by miR-7.

miRNAs e ceRNAs: competição

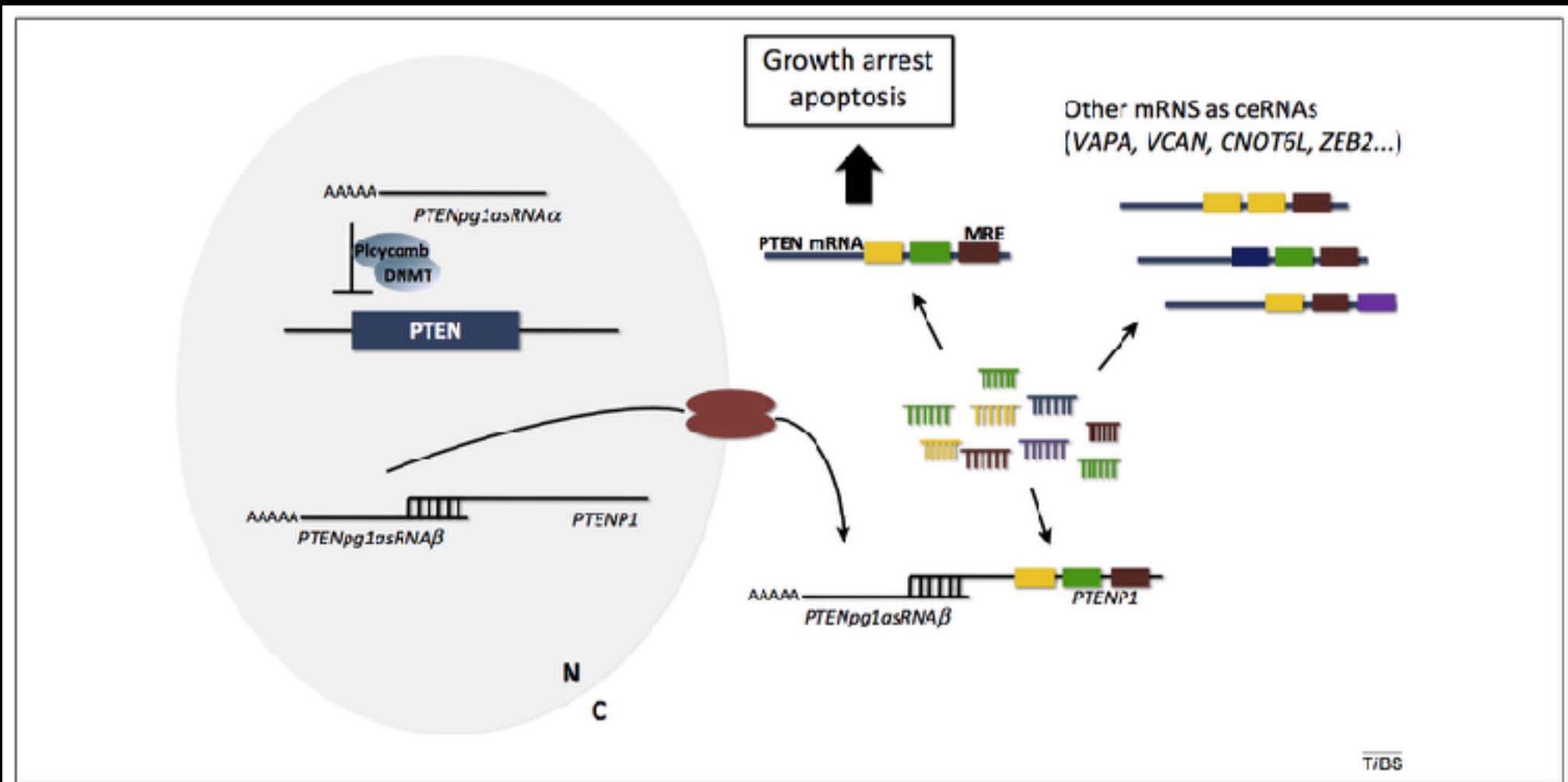


Figure 3. Ternary regulation between related transcripts. In the case of Phosphatase and Tensin Homolog (*PTEN*) tumor suppressor gene, interplay occurs with related noncoding RNAs, which includes the pseudogene *PTENpg1* and at least two more antisense transcripts. The pseudogene *PTENpg1* acts as a functional sponge in the cytoplasm by competing for the binding of miRNAs that would otherwise target the protein-coding *PTEN* mRNA. *PTENpg1* is not polyadenylated, and its stabilization and export to the cytoplasm is enhanced through complementary binding to one of its antisense transcripts, *PTENpg1asRNA β* . Additionally, another *PTENpg1* antisense transcript (*PTENpg1asRNA α*) acts a trans repressor of *PTEN* transcription by recruiting epigenetic repressor complexes comprising Polycomb and DNA cytosine-5-methyltransferase (DNMT) to the *PTEN* promoter. The complexity of the regulation is illustrated by the fact that several other unrelated transcripts (both coding and noncoding) seem to compete with *PTEN* and *PTENpg1* for the binding of a variety of miRNAs. Abbreviations: CNOT6L, CCR4-NOT Transcription Complex, Subunit 6-like; VAPA, Vesicle-associated Membrane Protein-associated Protein A; VCAN, Versican; ZEB2, zinc finger E-box binding homeobox 2.

mRNAs e UTR 3': esponjas de miRs

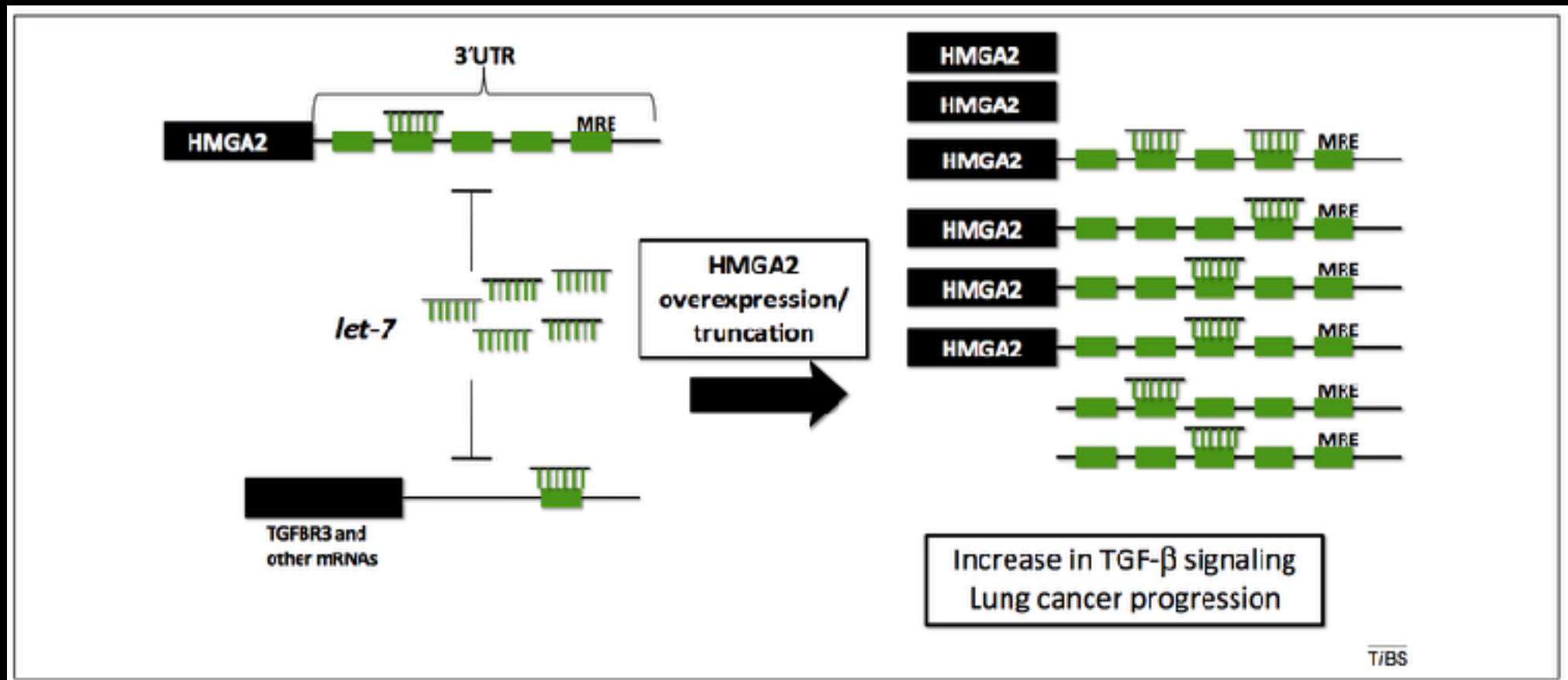


Figure 4. 3' untranslated regions (3'UTRs) can act as sponges in the context of full-length genes or as truncated species. The chromatin-modifying gene high mobility group A1-hock 2 (HMGA2) displays several *let-7* binding site along its 3'UTR and represents a major target for *let-7* in a variety of cancers. Recent evidence points to the ability of HMGA2 mRNA to serve as a *bona fide* *let-7* decoy and competitor in the presence of other described *let-7* targets, such as transforming growth factor, beta receptor III (*TGFBR3*) mRNA. In some cases, truncation of the gene and independent expression of the 3'UTR sequence might uncouple encoding of HMGA2 protein from the miRNA competition activity.

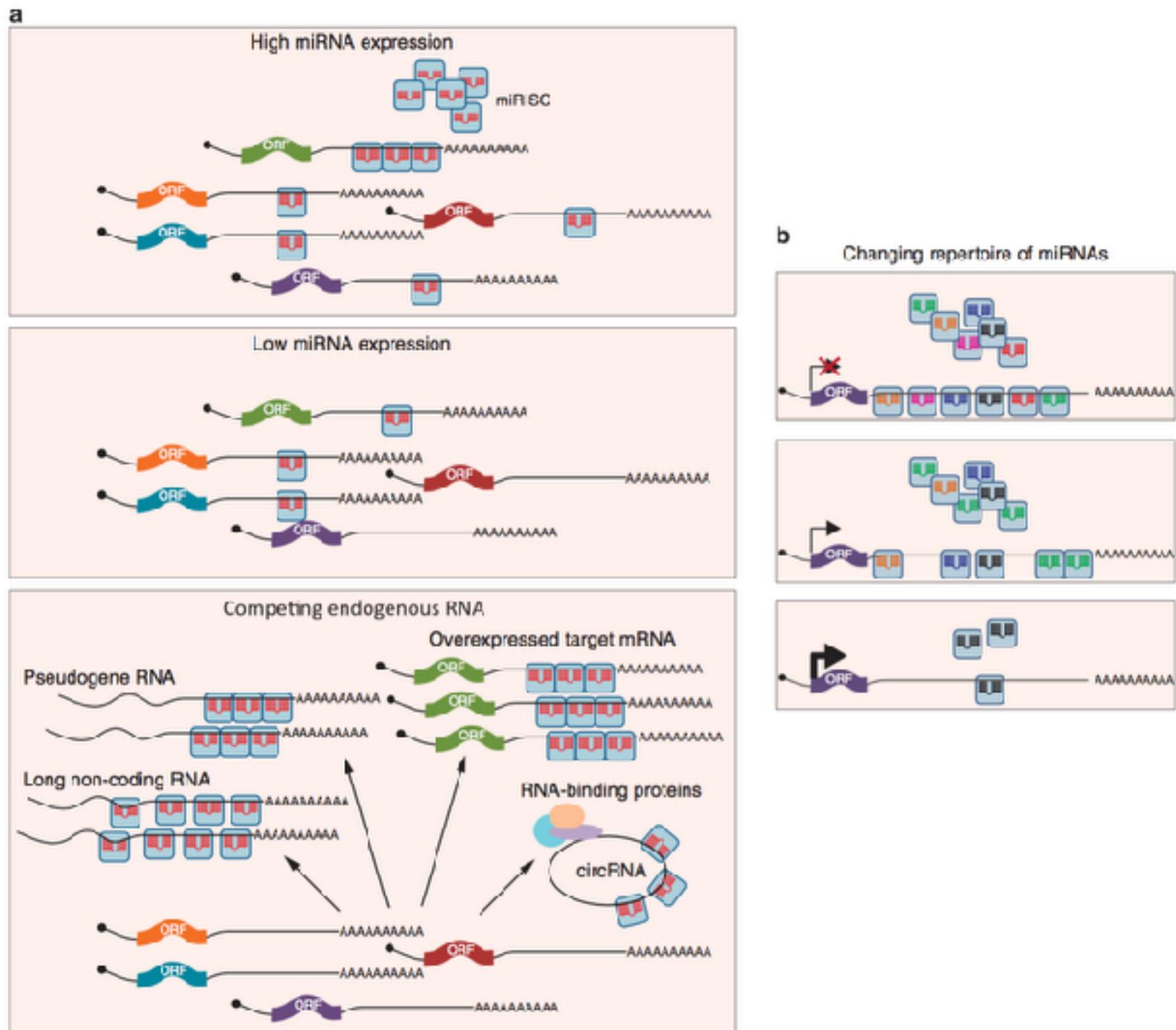
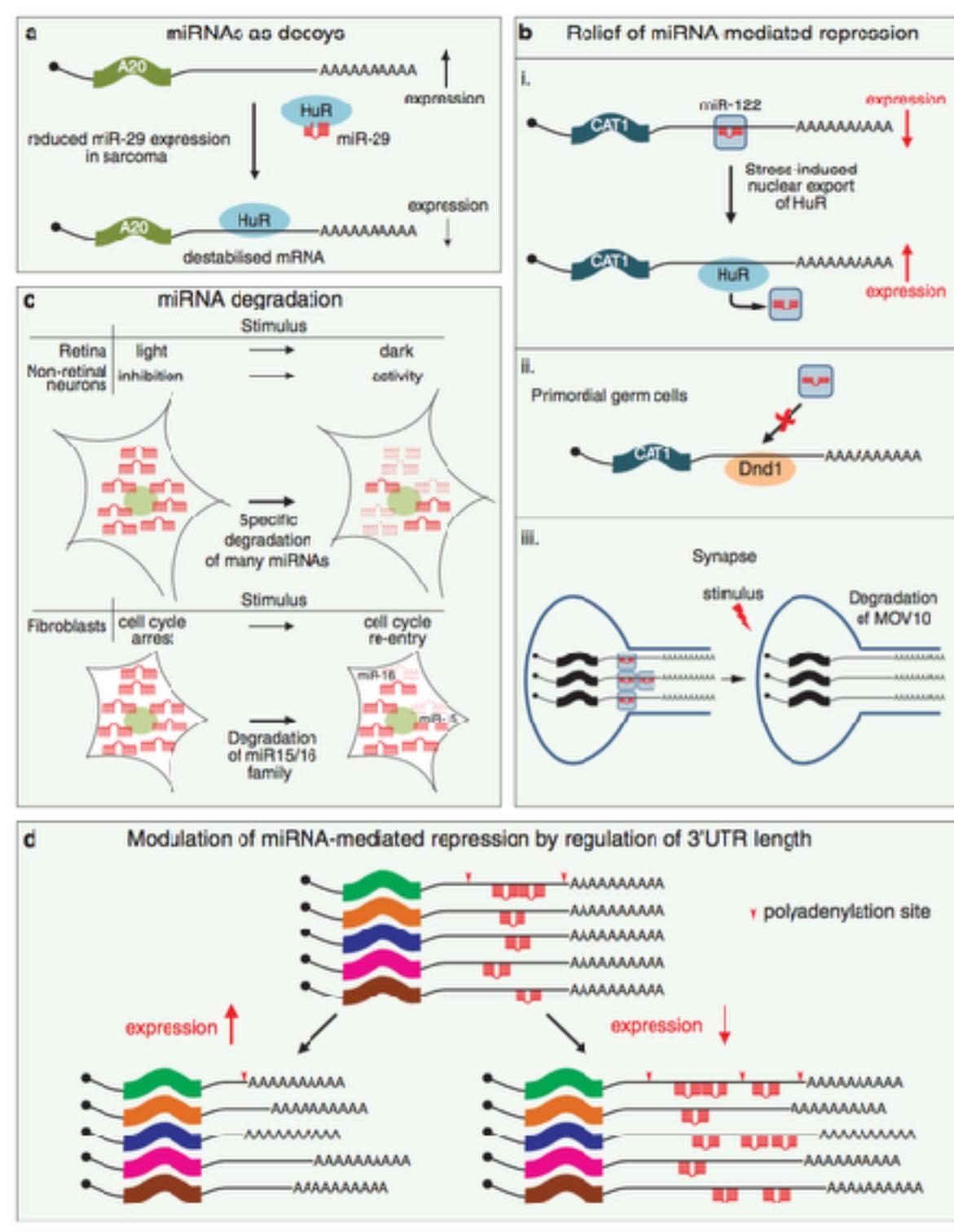
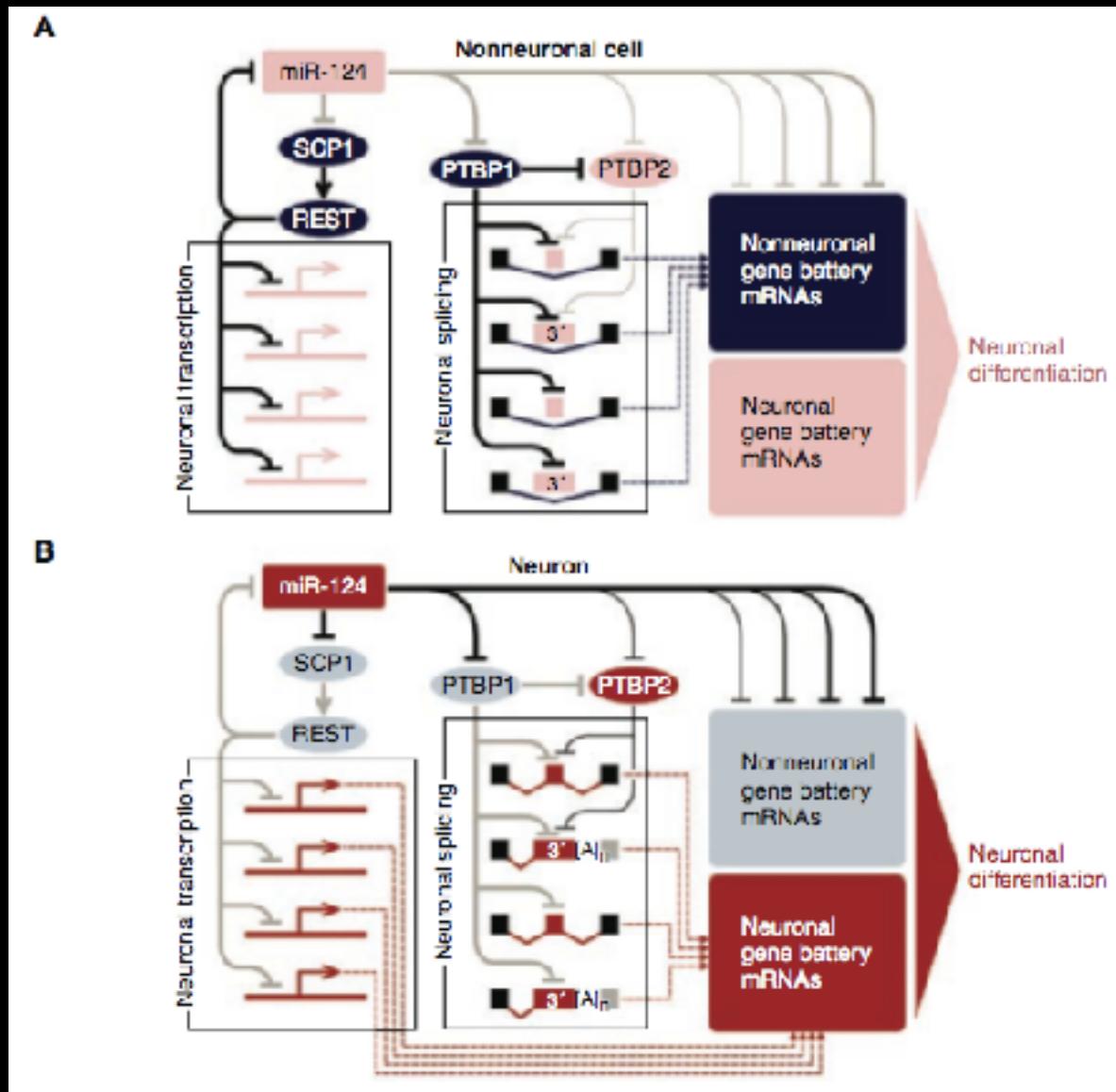


Figure 1 (a) A single mRNA can target hundreds of miRNAs and coordinate regulate them. The extent of regulation of a particular mRNA will depend on the expression levels of the miRNA that targets it as well as competing RNA. (b) One mRNA can have target sites for multiple miRNA. The repertoire of miRNAs in a given cell type, or under certain cellular conditions may result in differential regulation of the same target mRNA.



Rede regulatória do miR-124



Fatores de transcrição e miRNAs

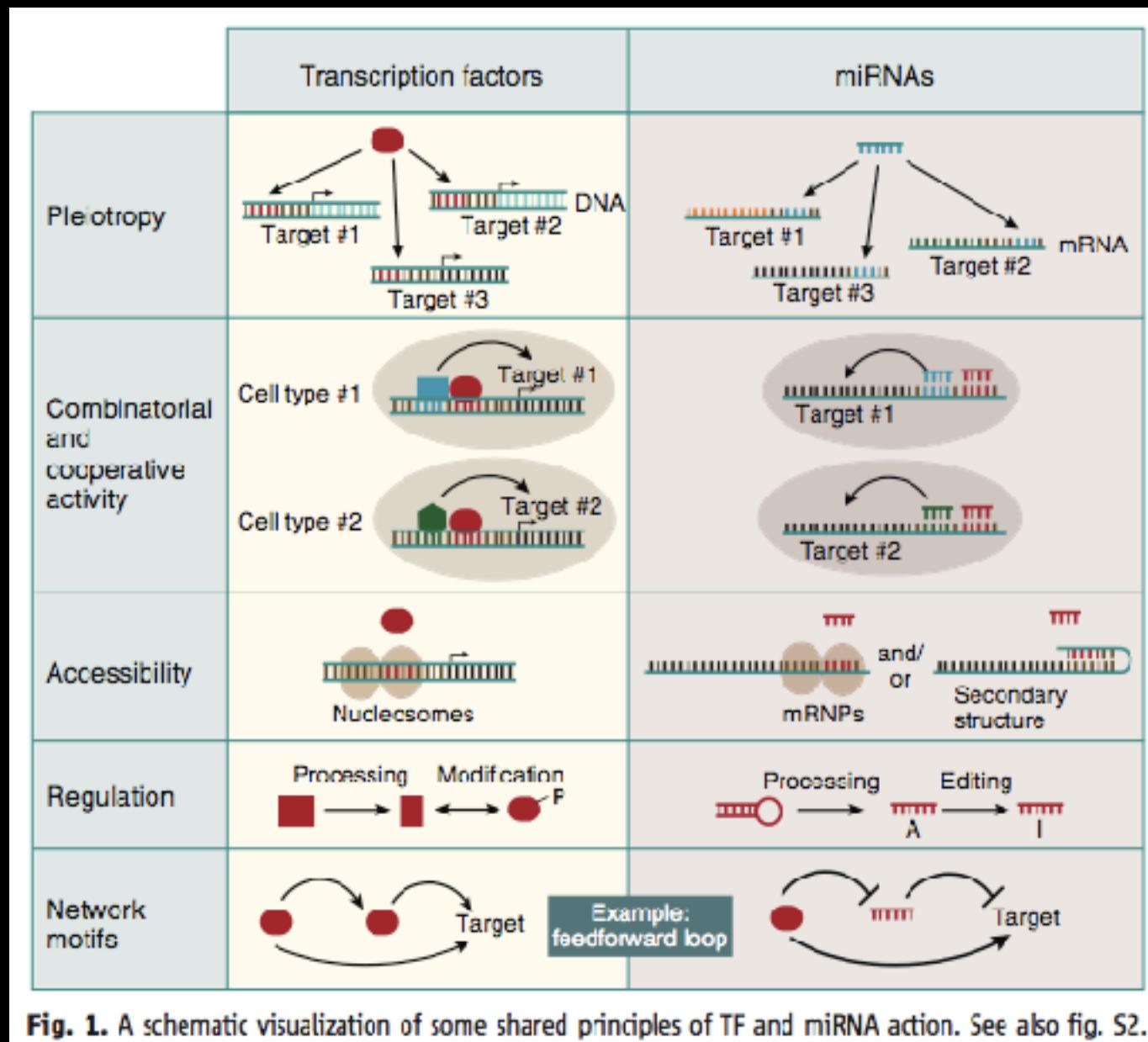
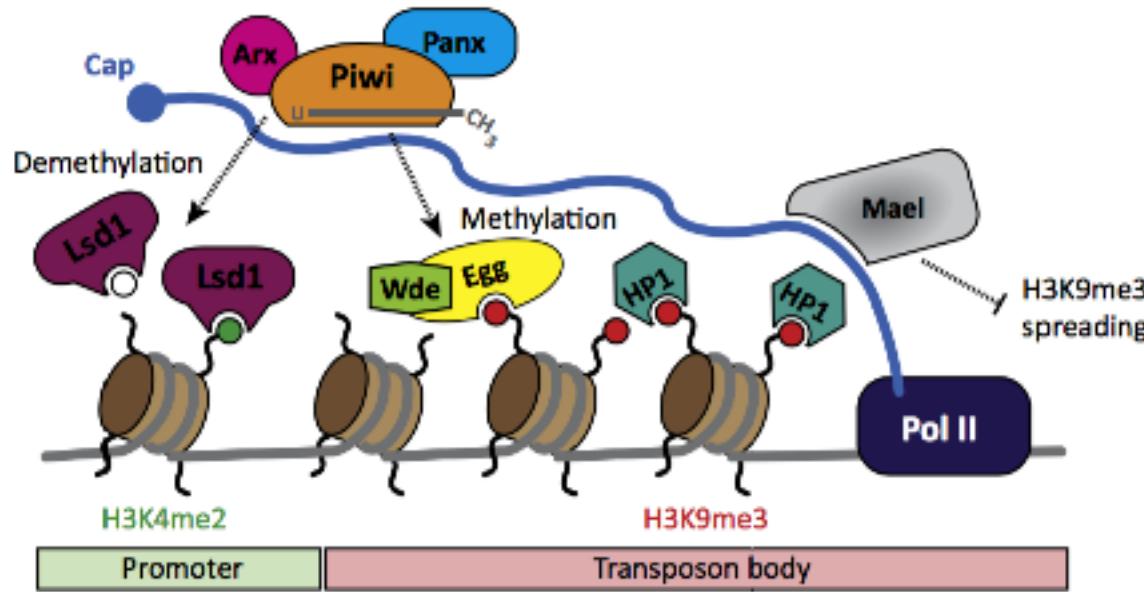


Fig. 1. A schematic visualization of some shared principles of TF and miRNA action. See also fig. S2.

Silenciamento gênico por piRNAs



Trends in Biochemical Sciences

Figure 4. PIWI-interacting RNA (piRNA)-Mediated Transcriptional Silencing. In the *Drosophila* ovary, piRNA-Piwi/Asterix (Arx) complexes scan for, and detect, nascent transposon transcription. Upon target engagement, Piwi likely undergoes conformational changes that lead to the recruitment of Panoramix (Panx). This piRNA-protein (comprising Piwi, Arx, and Panx,) complex induces co-transcriptional repression through recruitment of general silencing machinery components. As a consequence, transposon bodies receive repressive histone 3 lysine 9 trimethylation (H3K9me3) marks, a modification produced by Eggless (Egg) and its cofactor Windel (Wde). Subsequent recruitment of HP1 to H3K9me3 leads to heterochromatin formation. In addition, Lysine-specific demethylase 1 (Lsd1) likely removes active histone 3 lysine 4 dimethylation (H3K4me2) marks from transposon promoter regions, leading to efficient suppression of transposons at the transcriptional level. Maelstrom (Mael), a putative single-stranded RNA-binding protein, is required for transcriptional silencing and blocks H3K9me3 spread.

Grandes ncRNAs

lncRNAs

- Características
 - RNA não codificante longo (> 200 nt)
- Classificações
 - *Cis*-antisenso, sobreposto, bidirecional, intrônico
 - Multigênicos, *trans*-spliced, macroRNAs

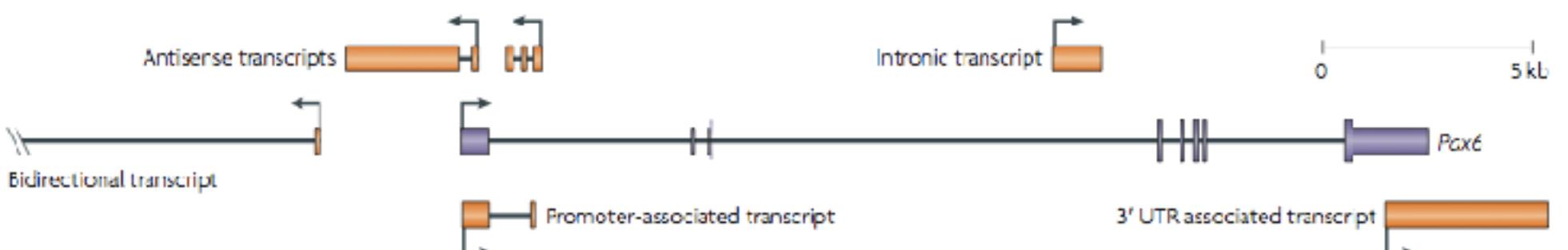


Figure 1 | Genomic organization of coding and non-coding transcripts. Schematic diagram illustrating the complexity of the interleaved networks of long non-coding transcripts (orange) that are associated with paired box gene 6 (Pax6; purple).

Tipos de ncRNAs e lncRNA

Table 2 | Estimate number of human ncRNAs from various sources.

Source	Number of types	Percent of human genome	Estimated average size (kb)	Reference
Mammelien lincRNome	53647	2.3	1.4	Managadze et al. (2013)
LNCipedia (as of March 2014)*	21487	0.67	1	Volders et al. (2013)
FPKM > 1 lincRNAs	35585	1.1**	NA	Hangauer et al. (2013)
Gencode v7 catalog of human ncRNAs	9277	0.29**	Median of 0.6***	Derrien et al. (2012)
LncRNAs – Gencode v21 (as of 2014 November)	15877	0.50**	NA	www.gencodegenes.org/ stats.html
Jia et al. (2010)	5446	0.17**	NA	Jia et al. (2010)
Cabili – low confidence	8195	0.26	1	Cabili et al. (2011)
Cabili – high confidence	4273	0.26	1	Cabili et al. (2011)
Small ncRNAs – Gencode v21 (as of 2014 November)****	9534	0.045	0.15	www.gencodegenes.org/ stats.html
eRNAs	43011	0.34	0.25	Andersson et al. (2014)

*Splice variants were excluded

**Assumes an average length of 1 kb.

***Median size of 592 nucleotides but with a significant fraction at higher sizes.

****Contains snRNAs, snoRNAs, rRNAs, mitochondrial tRNAs and rRNAs, miRNAs, and "miscellaneous RNAs."

lncRNAs

- Função e mecanismos
 - Modificação da cromatina
 - LncRNAs reconhecidos por complexos de remodelamento
 - Regulação transcricional
 - Promotores e *enhancers* são transcritos
 - LncRNAs recrutam fatores de transcrição (via RBPs)
 - Interação com complexos de iniciação de RNAPII
 - Formação de triplex RNA/DNA no promotor
 - Regulação pós-transcricional
 - RNA antisenso afeta *splicing*

Tipos de lncRNAs

- LncRNAs nucleares
 - LncRNAs e regulação proteica
 - eRNAs (*enhancers*)
 - LncRNAs derivados de vírus
- LncRNAs citoplasmáticos
 - ceRNAs de miRs
 - Complexos de lncRNP

Função de lncRNAs

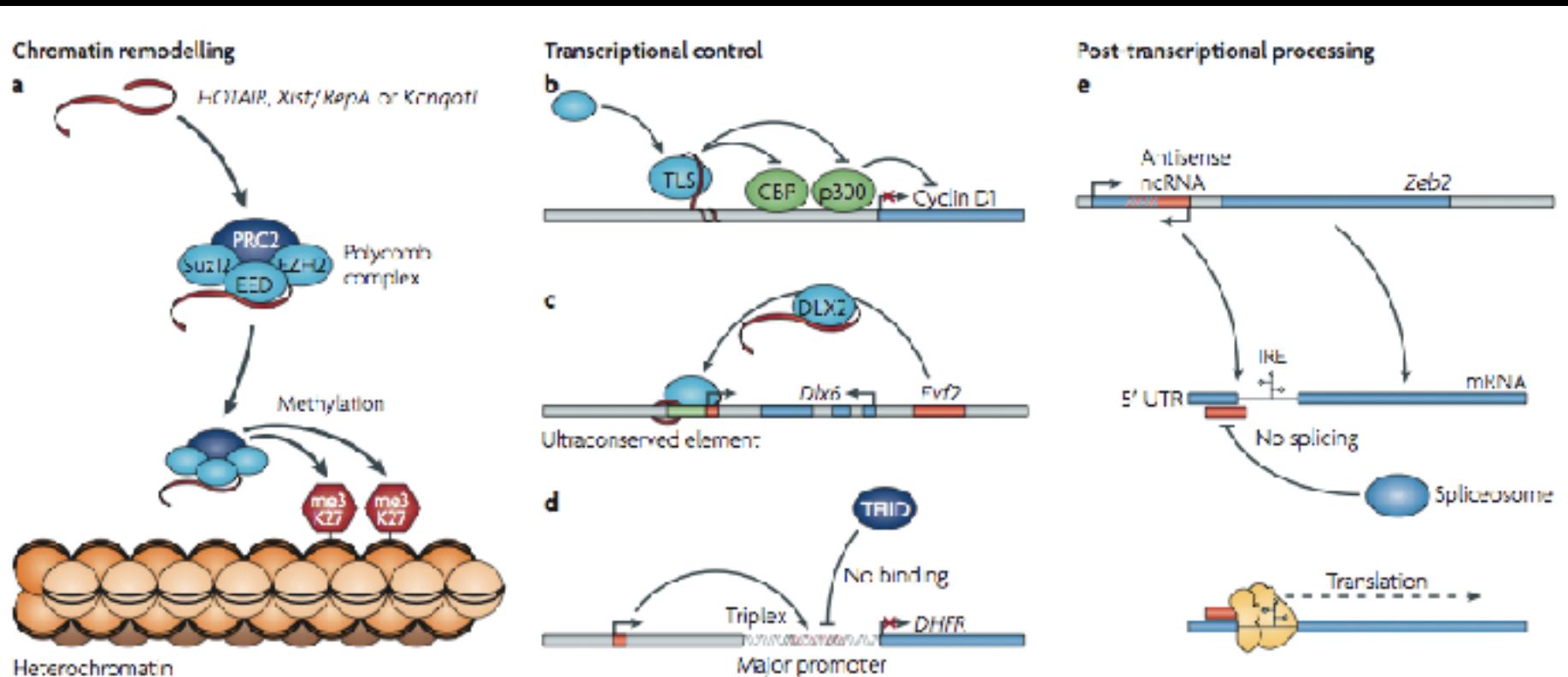


Figure 2 | Functions of long non-coding RNAs (lncRNAs). Illustrative mechanisms by which long ncRNAs regulate local protein-coding gene expression at the level of chromatin remodelling, transcriptional control and post-transcriptional processing. **a** | ncRNAs can recruit chromatin-modifying complexes to specific genomic loci to impart their catalytic activity. In this case, the ncRNAs HOTAIR²¹, Xist and RepA (the small internal non-coding transcript from the Xist locus)²², or Kcnq1 (REF. 24) recruit the Polycomb complex to the HoxD locus, the X chromosome, or the Kcnq1 domain, respectively, where they trimethylate lysine 27 residues (me3K27) of histone H3 to induce heterochromatin formation and repress gene expression. **b** | ncRNAs can regulate the transcriptional process through a range of mechanisms. ncRNAs tethered to the cyclin D1 gene recruit the

RNA binding protein TLS to modulate the histone acetyltransferase activity of CREB binding protein (CBP) and p300 to repress gene transcription²³. **c** | An ultraconserved enhancer is transcribed as a long ncRNA, Evf2, which subsequently acts as a co-activator to the transcription factor DLX2, to regulate the Dlx6 gene transcription²⁰. **d** | A ncRNA transcribed from the DHFR minor promoter in humans can form a triplex at the major promoter to occlude the binding of the general transcription factor TFIID, and thereby silence DHFR gene expression²¹. **e** | An antisense ncRNA can mask the 5' splice site of the zinc finger homeobox mRNA Zeb2 from the spliceosome, resulting in intron retention. The translation machinery can then recognize and bind an internal ribosome entry site (IRE) in the retained intron, resulting in efficient Zeb2 translation and expression²⁵.