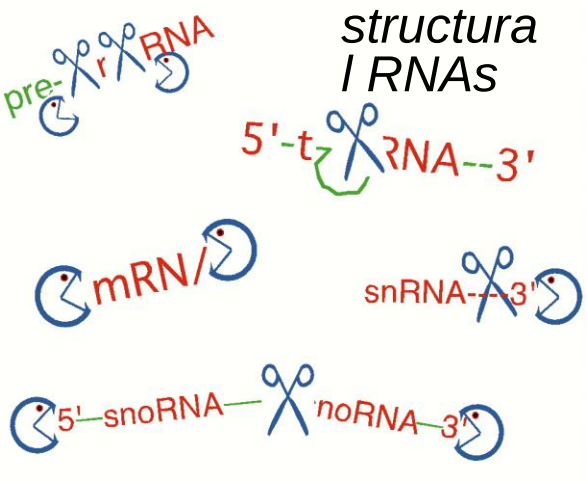
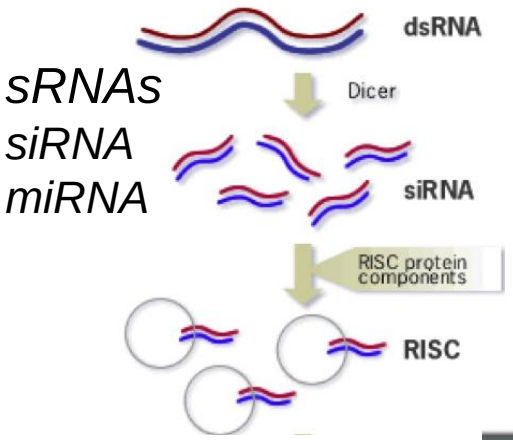
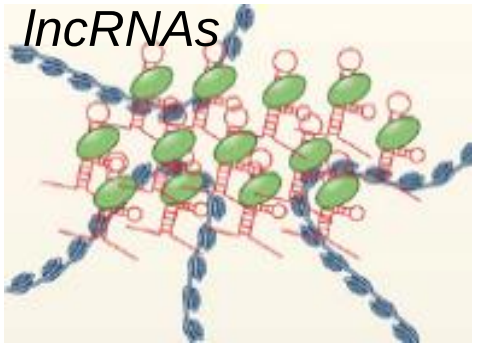
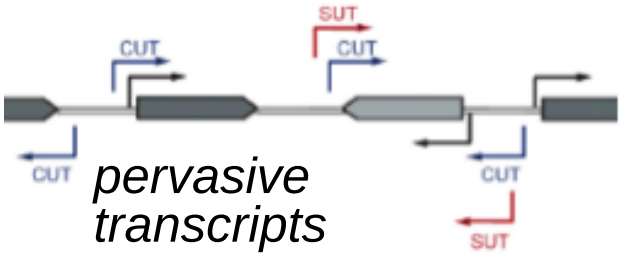
ncRNAs
czyli RNA są różniste, kuliste, w kształcie grzyba i cygara









– Czy może nam pani powiedzieć, na terenie jakiego zakładu się obecnie znajdujemy?

– Tego ni mogę powiedzieć, bo to jest tajemnica państwowa! Mogę tylko powiedzieć, że mam 5 złotych od bombki.

– Czy pani jeszcze może nam powiedzieć, jakie bombki produkujecie?

– Panie, różne, różniste. Tu się robi tak: kuliste, w kształcie grzyba i cygara.

– Cały czas mowa o o bombkach choinkowych.

– Też. A, a wie pan, jaki mamy asortyment? Od A do N...

Kabaret TEY

ncRNA

- **Housekeeping**

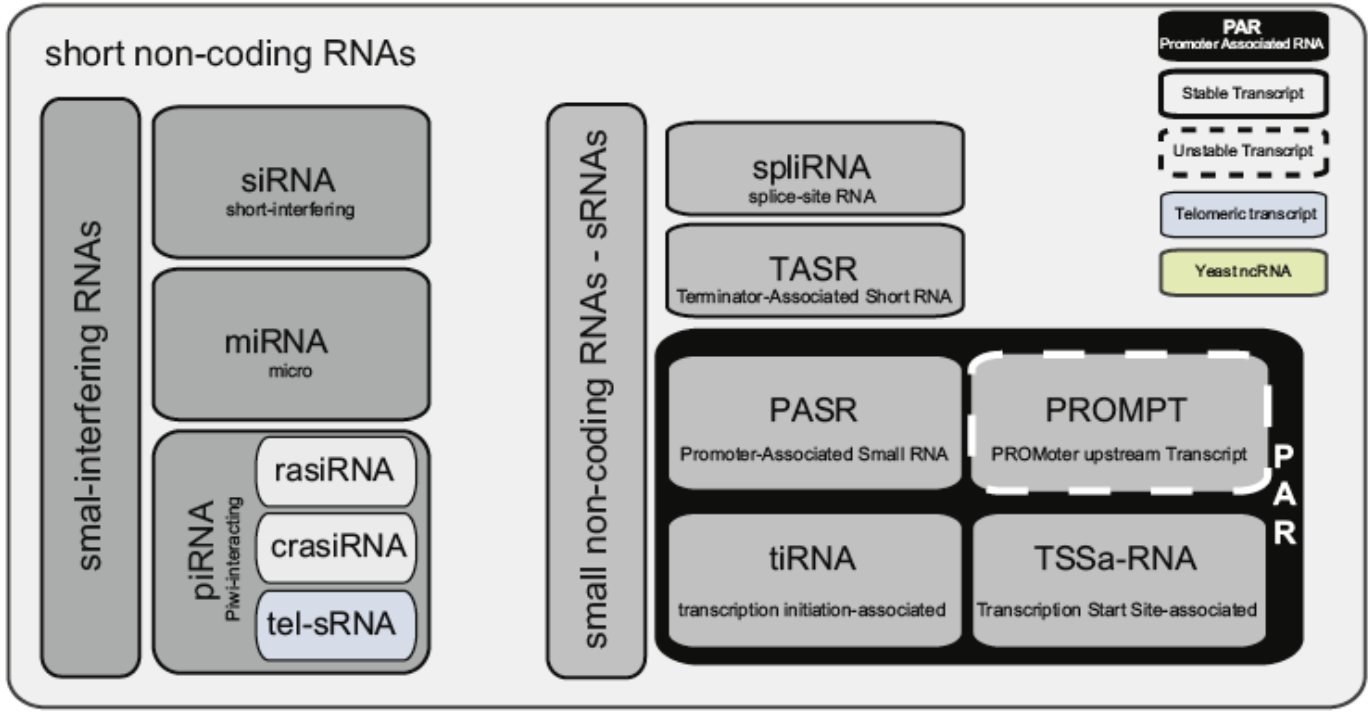
- constitutively expressed
- required for normal function and cell viability

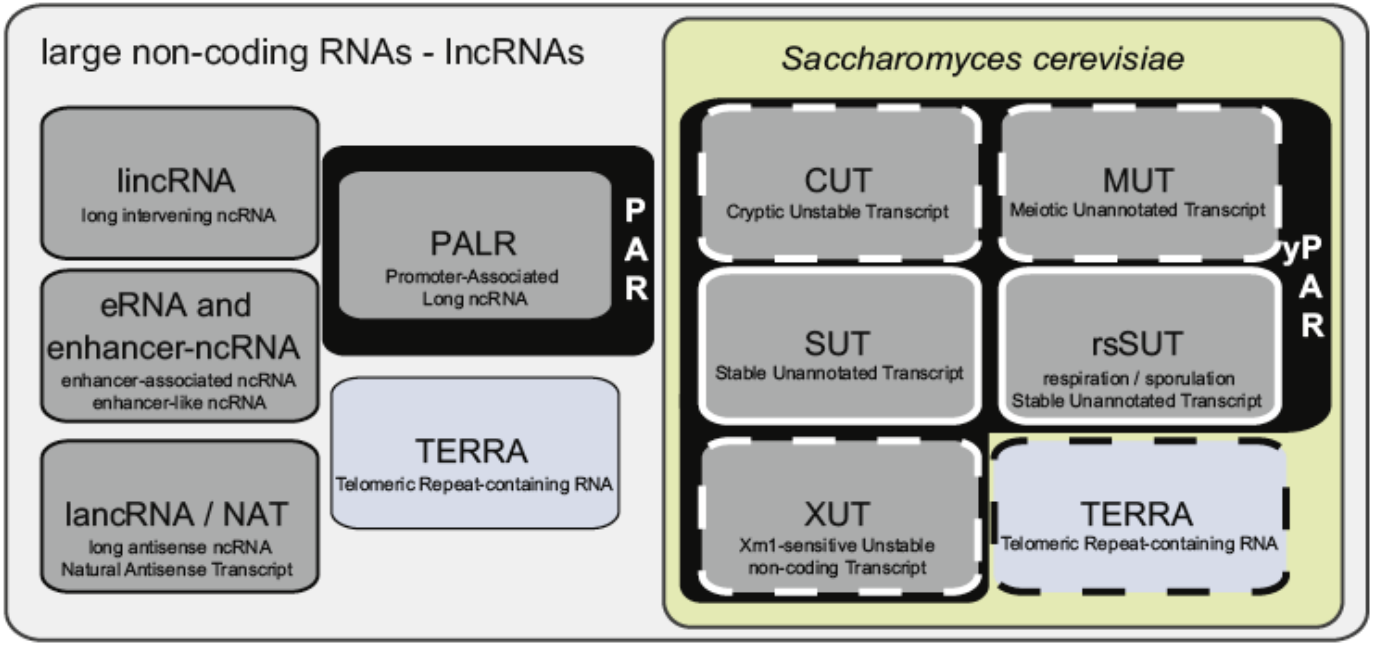
  - **tRNA** and **rRNA** – translation
  - **snRNA** – splicesosome components, pre-mRNA splicing
  - **snoRNA** – rRNA processing and modification, scaRNA (CB specific)
  - RNA components of **RNase P** and **RNase MRP** – endonucleases: tRNA and rRNA processing
  - Signal Recognition Particle **SRP RNA** – protein secretion to ER
  - **tmRNA** tRNA-mRNA hybrid- targeting nascent proteins for degradation
  - **gRNA** – guide RNA in RNA editing
  - **telomerase RNA** – synthesis of telomers

- **Regulatory**

- expressed temporarily (development, response to stimuli)
- affect gene expression at the level of transcription or translation

  - **sRNAs: siRNA** (exo-siRNAs and endo-siRNAs; ta-siRNA; nat-siRNA; lsiRNAs); **miRNA**; **piRNA** – act in TGS or PTGS
  - **lncRNAs** – much less known, usually act in TGS (chromatin level)





ALL ncRNAs ?

Tisseur et al., Biochemie, 2011

INVISIBLE RNAs

Cell, Vol. 121, 725–737, June 3, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.04.030

Cryptic Pol II Transcripts Are Degraded by a Nuclear Quality Control Pathway Involving a New Poly(A) Polymerase

Françoise Wyers,[4,5,7] Mathieu Rougemaille,[5,7] Gwenaël Badis,[1] Jean-Claude Rousselle,[2] Marie-Elisabeth Dufour,[4] Jocelyne Boulay,[5] Béatrice Régnault,[3] Frédéric Devaux,[6] Abdelkader Namane,[2] Bertrand Séraphin,[2,5,*] Domenico Libri,[5,*] and Alain Jacquier[1,*]

3262–3267 | PNAS | February 28, 2006 | vol. 103 | no. 9

Accumulation of unstable promoter-associated transcripts upon loss of the nuclear exosome subunit Rrp6p in *Saccharomyces cerevisiae*

Carrie Anne Davis and Manuel Ares, Jr.*

Vol 457|19 February 2009|doi:10.1038/nature07728

Bidirectional promoters generate pervasive transcription in yeast

Zhenyu Xu[1]*, Wu Wei[1]*, Julien Gagneur[1], Fabiana Perocchi[1], Sandra Clauder-Münster[1], Jurgi Camblong[2], Elisa Guffanti[3], Françoise Stutz[3], Wolfgang Huber[4] & Lars M. Steinmetz[1]

Vol 457|19 February 2009|doi:10.1038/nature07747

Widespread bidirectional promoters are the major source of cryptic transcripts in yeast

Helen Neil[1], Christophe Malabat[1], Yves d'Aubenton-Carafa[2], Zhenyu Xu[3], Lars M. Steinmetz[3] & Alain Jacquier[1]

INVISIBLE RNAs

SCIENCE VOL 322 19 DECEMBER 2008

RNA Exosome Depletion Reveals Transcription Upstream of Active Human Promoters

Pascal Preker,[1] Jesper Nielsen,[2] Susanne Kammler,[1]* Søren Lykke-Andersen,[1] Marianne S. Christensen,[1] Christophe K. Mapendano,[1] Mikkel H. Schierup,[2] Torben Heick Jensen[1]†

SCIENCE VOL 322 19 DECEMBER 2008

Divergent Transcription from Active Promoters

Amy C. Seila,[1]* J. Mauro Calabrese,[1,2]*† Stuart S. Levine,[3] Gene W. Yeo,[4]‡ Peter B. Rahl,[3] Ryan A. Flynn,[1] Richard A. Young,[2,3] Phillip A. Sharp[1,2]§

Vol 457|19 February 2009|doi:10.1038/nature07759

Post-transcriptional processing generates a diversity of 5′-modified long and short RNAs

Affymetrix/Cold Spring Harbor Laboratory ENCODE Transcriptome Project*

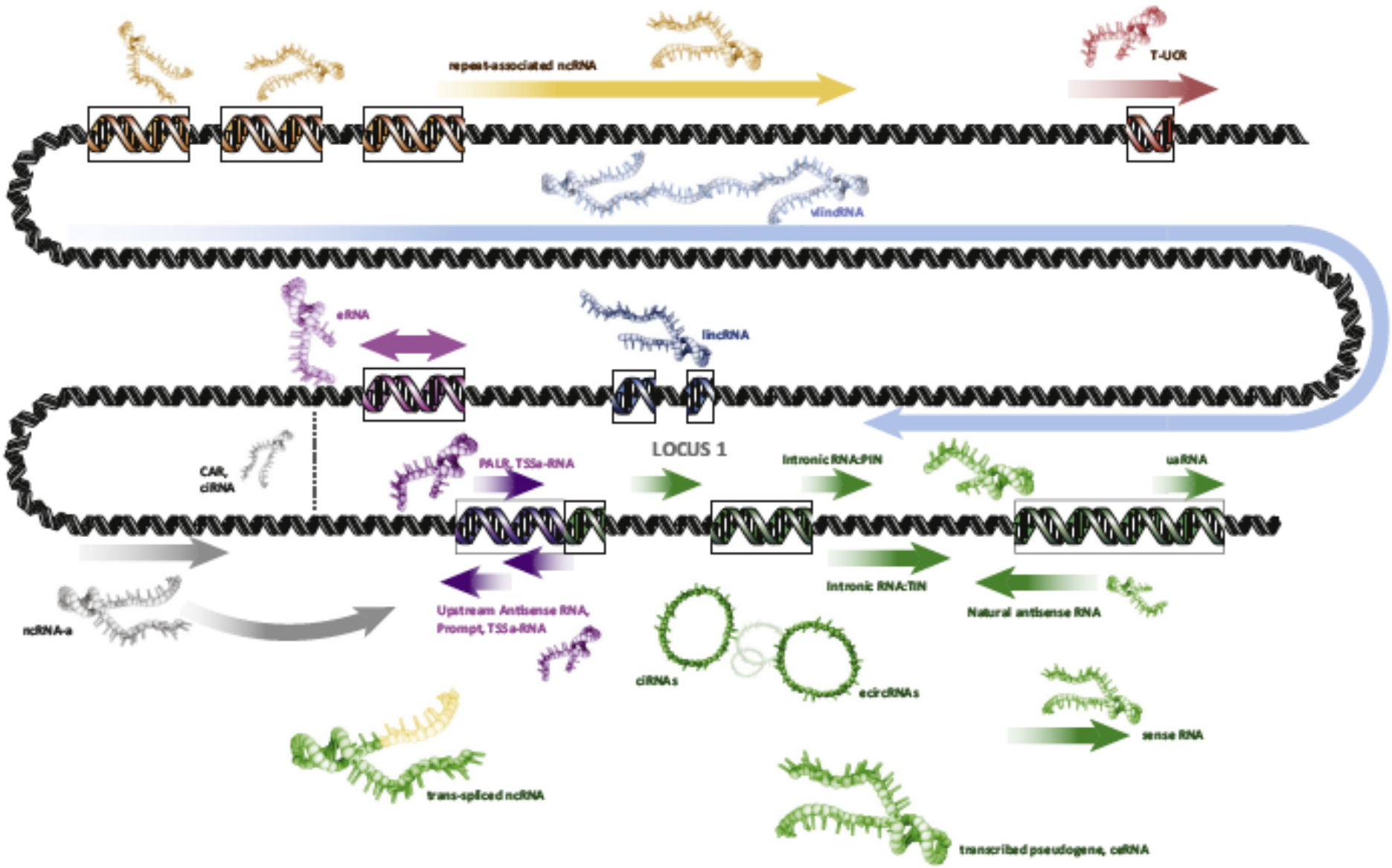
Cold Spring Harbor Laboratory Katalin Fejes-Toth[1,2]*, Vihra Sotirova[1,2], Ravi Sachidanandam[1]†, Gordon Assaf[1,2], Gregory J. Hannon[1,2]; **Affymetrix** Philipp Kapranov[3]*, Sylvain Foissac[3], Aarron T. Willingham[3], Radha Duttagupta[3], Erica Dumais[3] & Thomas R. Gingeras[1,3]

Cell *131*, 1340–1353, December 28, 2007

Genome-Wide High-Resolution Mapping of Exosome Substrates Reveals Hidden Features in the *Arabidopsis* Transcriptome

Julia A. Chekanova,[1,2,8,13] Brian D. Gregory,[3,4,8] Sergei V. Reverdatto,[2] Huaming Chen,[4] Ravi Kumar,[1] Tanya Hooker,[1] Junshi Yazaki,[3,4] Pinghua Li,[2,10] Nikolai Skiba,[5,9] Qian Peng,[3,6] Jose Alonso,[3,11] Vladimir Brukhin,[7,12] Ueli Grossniklaus,[7] Joseph R. Ecker,[3,4,*] and Dmitry A. Belostotsky[1,2,13,*]

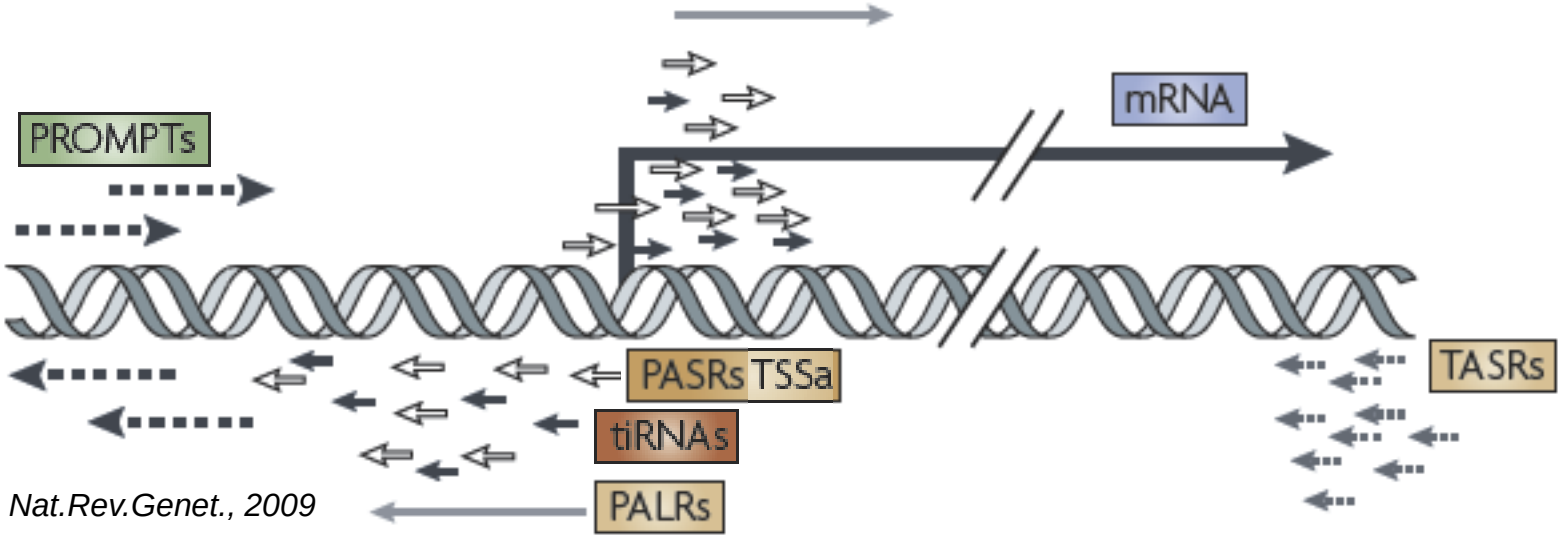
LONG ncRNAs



Laurent at al, TiG 2015

PERVASIVE TRANSCRIPTION OF THE GENOME

All possible types of RNAs, detected by tiling microarrays and "deep sequencing", SAGE and GRO, accompany major coding transcripts



Jacquier, Nat.Rev.Genet., 2009

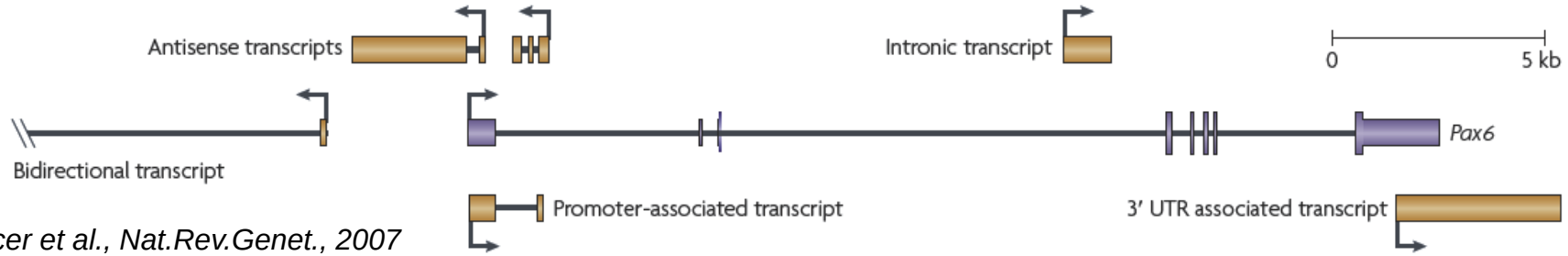
(1) protein-coding mRNA; (2) PROMPT - promoter upstream transcripts (short); (3) PASR- promoter-associated sRNAs (< 200 nts); (4) TSSa transcription start site-associated RNAs (20-90 nts); (5) TASR –terminator associated sRNAs (< 200 nts); (6) PARL - promoter-associated long RNAs (> 200 nts); (7) tiRNAs - tiny transcription-initiation RNAs (18 nts)

SAGE, CAGE, GRO tags

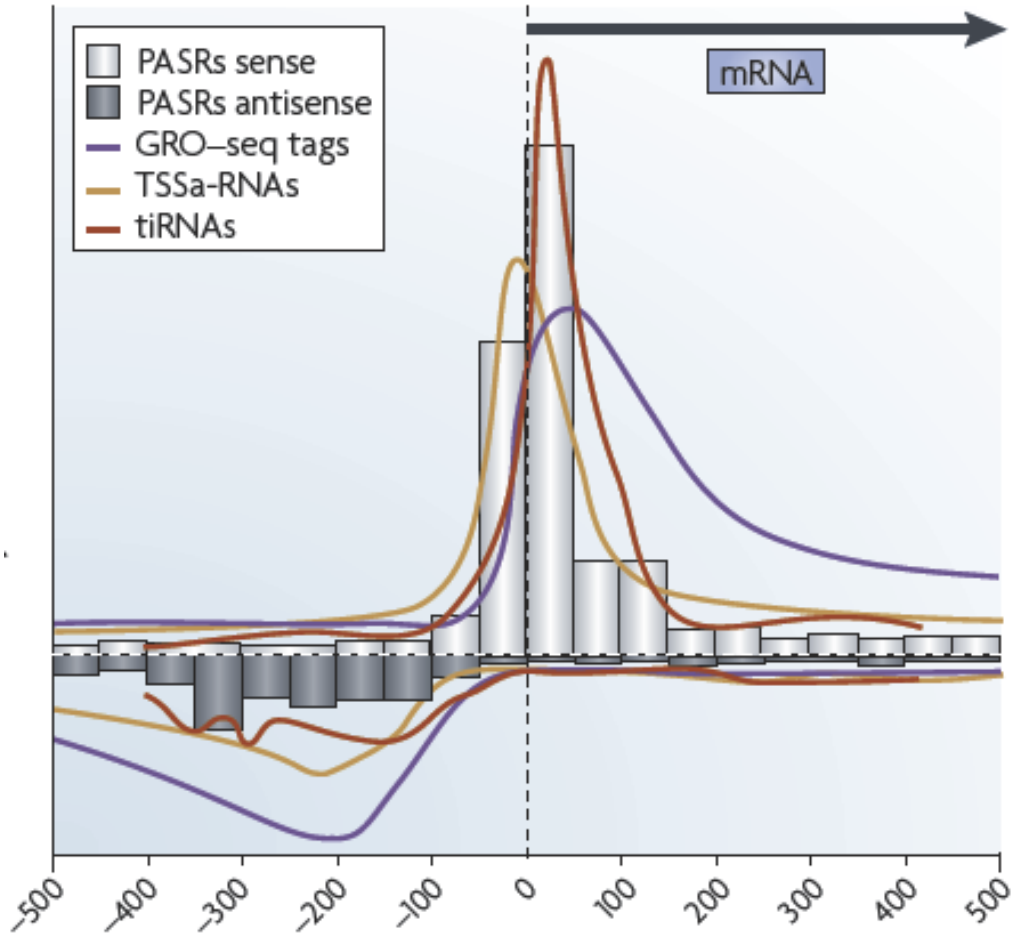
antisense RNAs (can be long)

CUTs, SUTs - cryptic unstable or stable unannotated transcripts (200-600 nts)

PRESENCE of ncRNAs



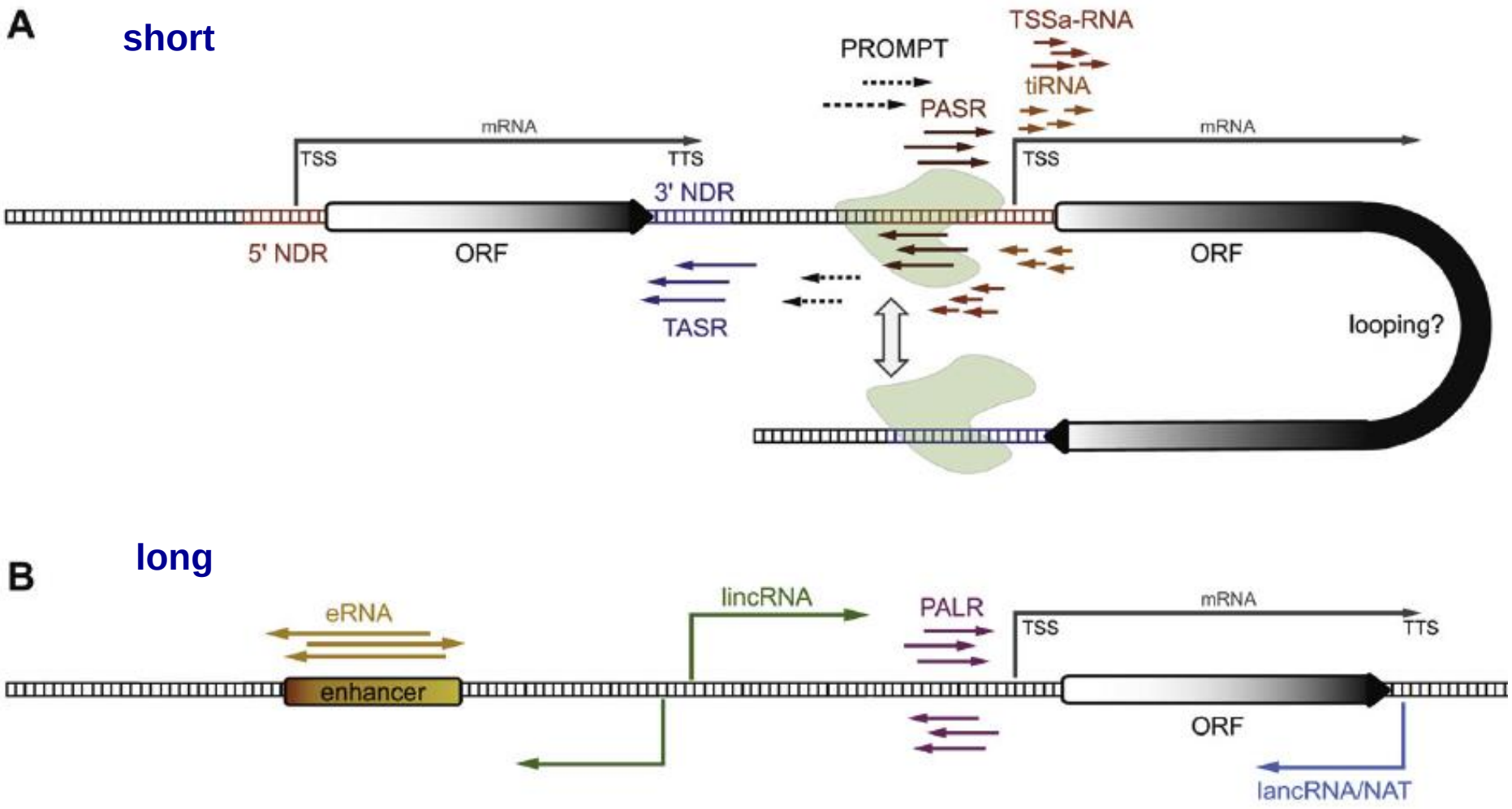
Mercer et al., Nat.Rev.Genet., 2007



DENSITY of small RNAs

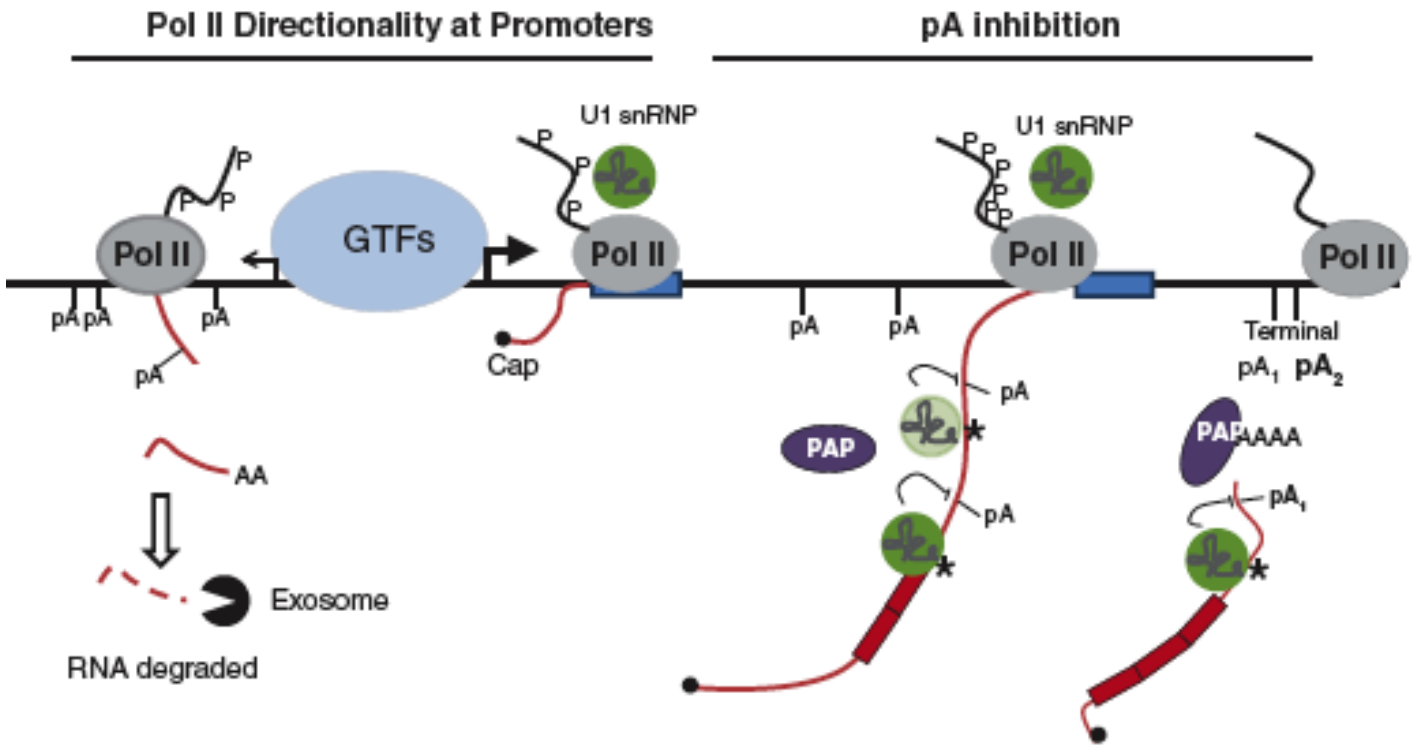
Jacquier, Nat.Rev.Genet., 2009

GENOMIC ORGANIZATION of ncRNA

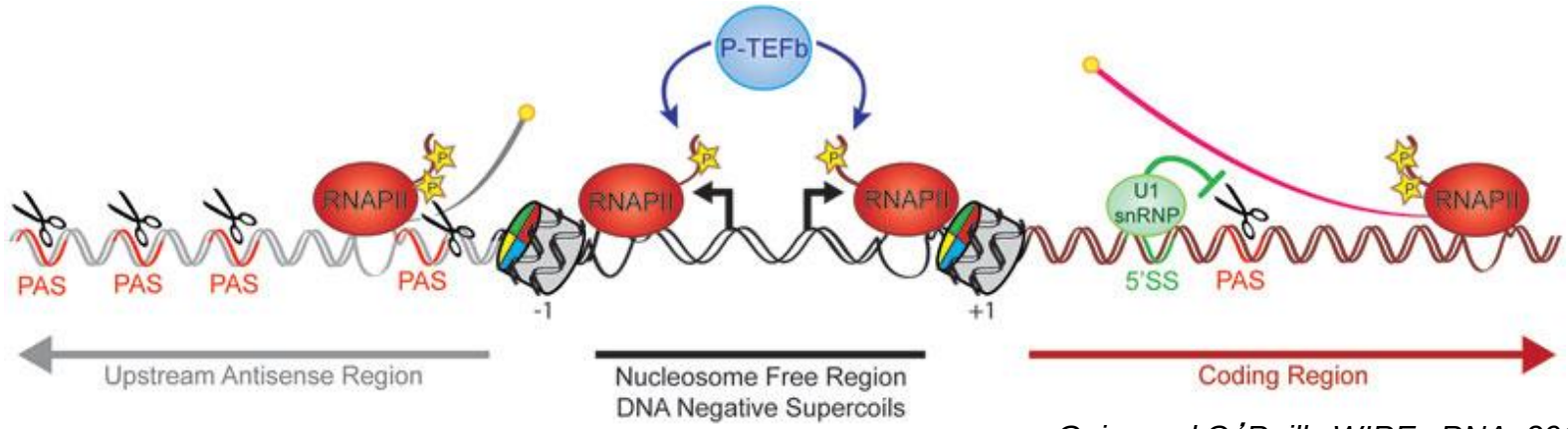


Tisseur et al., Biochemie, 2011

U1 and non-coding transcription (telescripting)

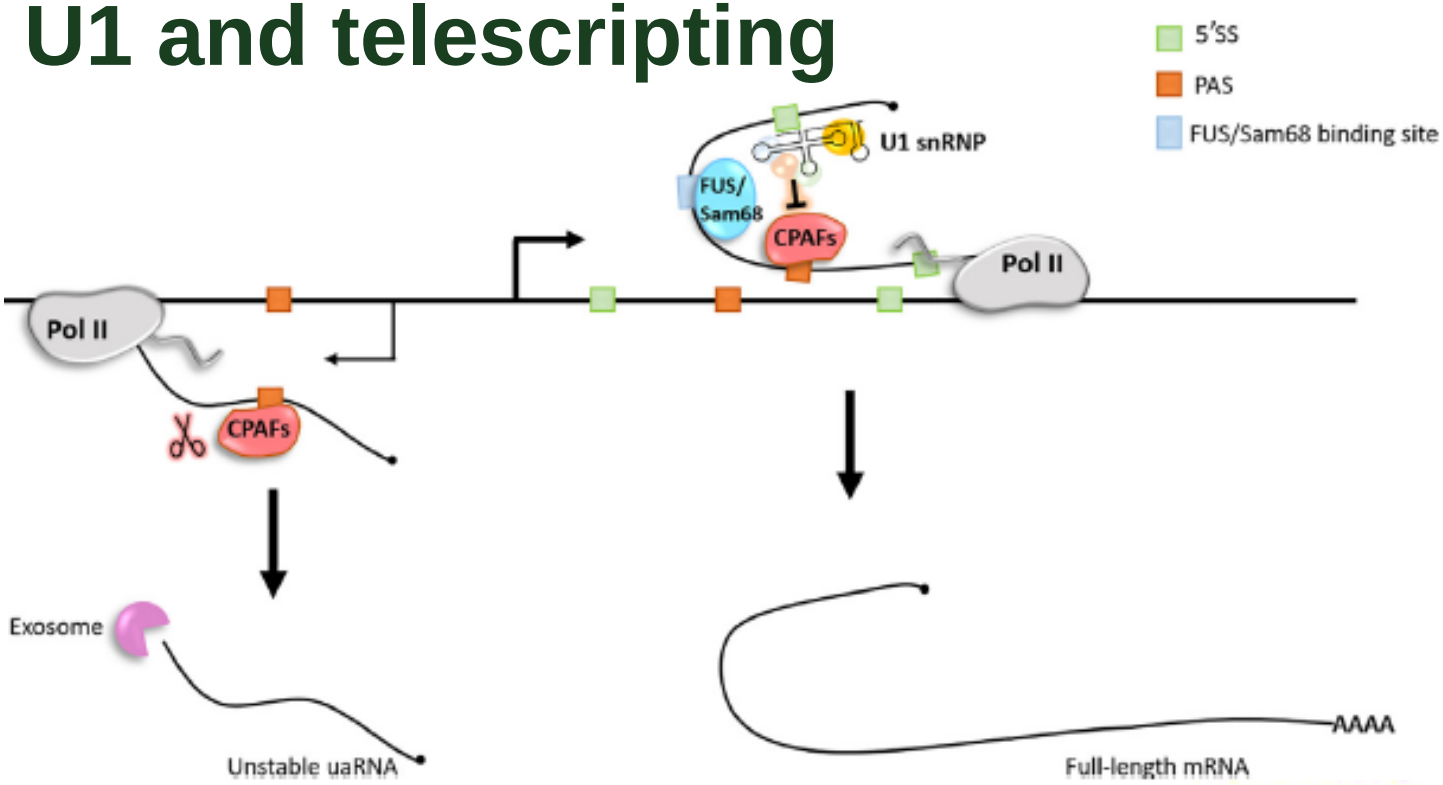


U1 participates in pA site selection and Pol II directionality at promoters



Guiro and O'Reilly WIREs RNA, 2015

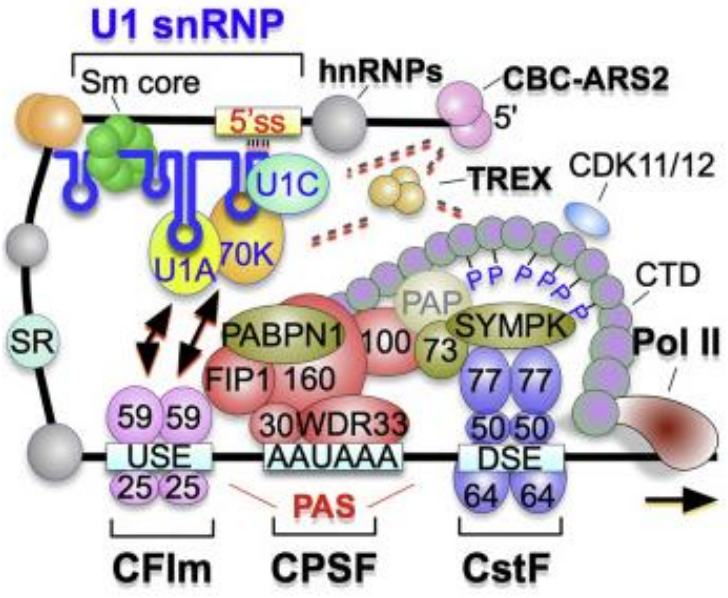
U1 and telescripting



Studniarek, Egloff and Murphy, TiG 2020

U1 controls promoter directionality by suppressing cryptic PAS usage in the sense direction.

In the antisense direction, depletion of 5′SSs favours PAS usage, giving rise to short unstable transcripts degraded by the exosome



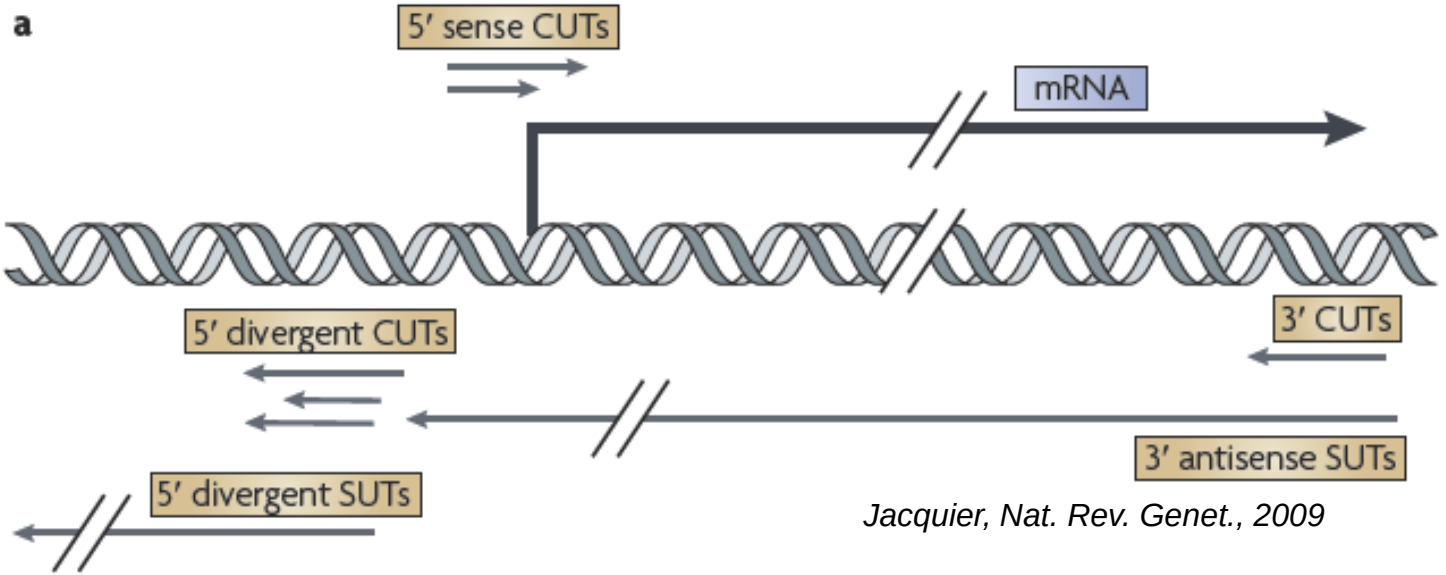
CUTs, SUTs, XUTs, MUTs and ALL THAT JAZZ

CUT = Cryptic Unstable Transcripts
SUT = Stable Unannotated Transcripts
SAT = Ssu72-associated Transcripts
XUT = Xrn1-dependent UnstableTranscripts
MUT = Meiotic Unstable Transcripts

NO LONGER TRANSCRIPTIONAL NOISE

(yeast, mammals, worms, plants - all organisms?)

- **not visible in normal wild-type cells**
- **accumulate in RNA degradation mutants (EXOSOME, XRN family, TRAMP) or various metabolic conditions (aging, nutrient change, cell cycle etc)**
- **originate from widespread bidirectional promoters**
- **„mRNA-like" Pol II transcripts (capped, polyadenylated)**



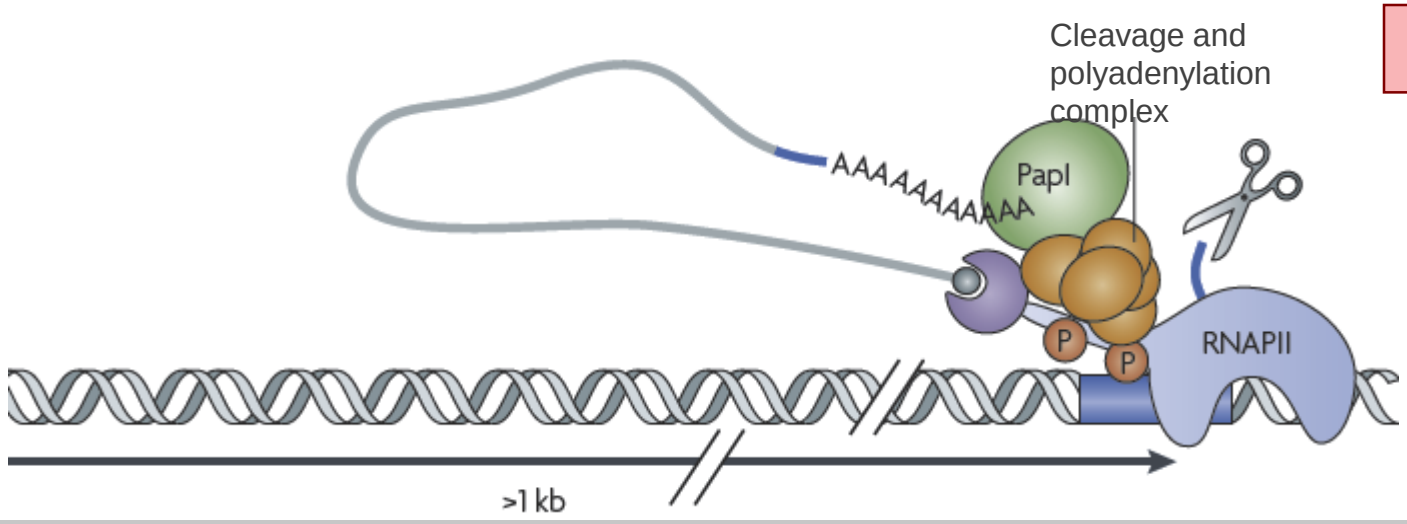
Jacquier, Nat. Rev. Genet., 2009

ncRNA instability and their termination mode

3’ end CLEAVAGE and POLYADENYLATION (CP)

mRNAs, SUTs

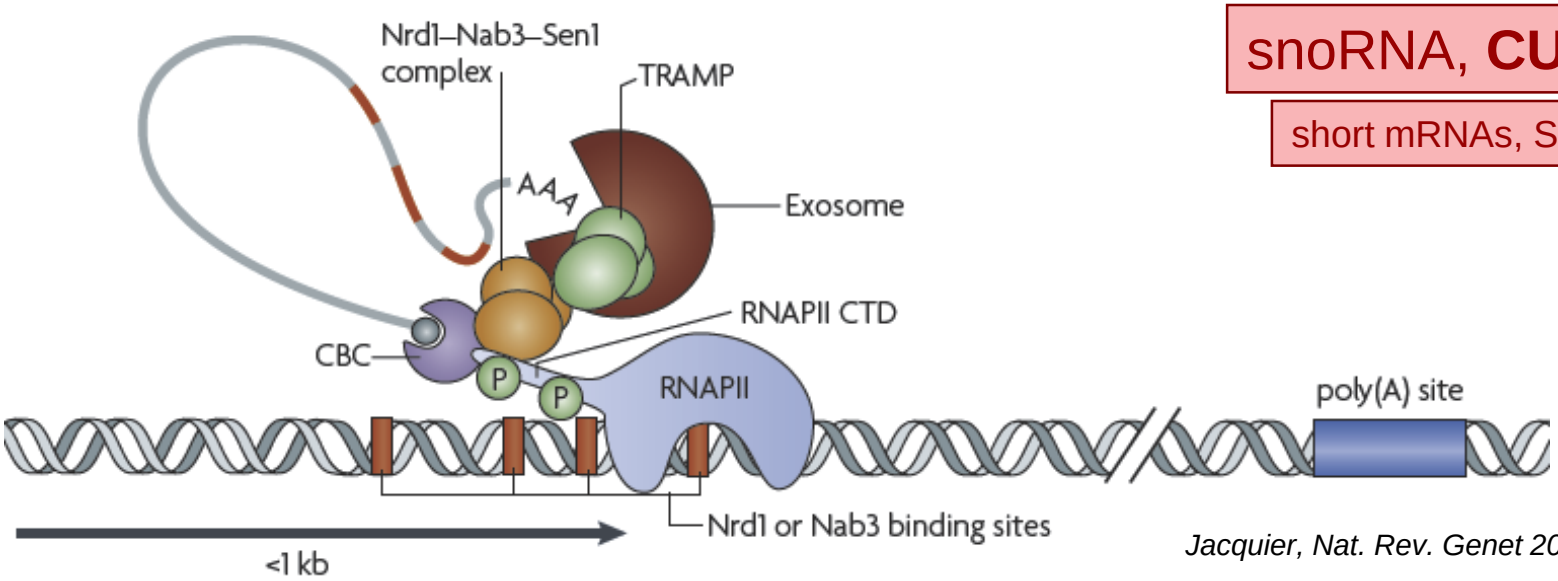
snoRNAs, CUTs



Nrd1/Nab3/Sen1-dependent termination

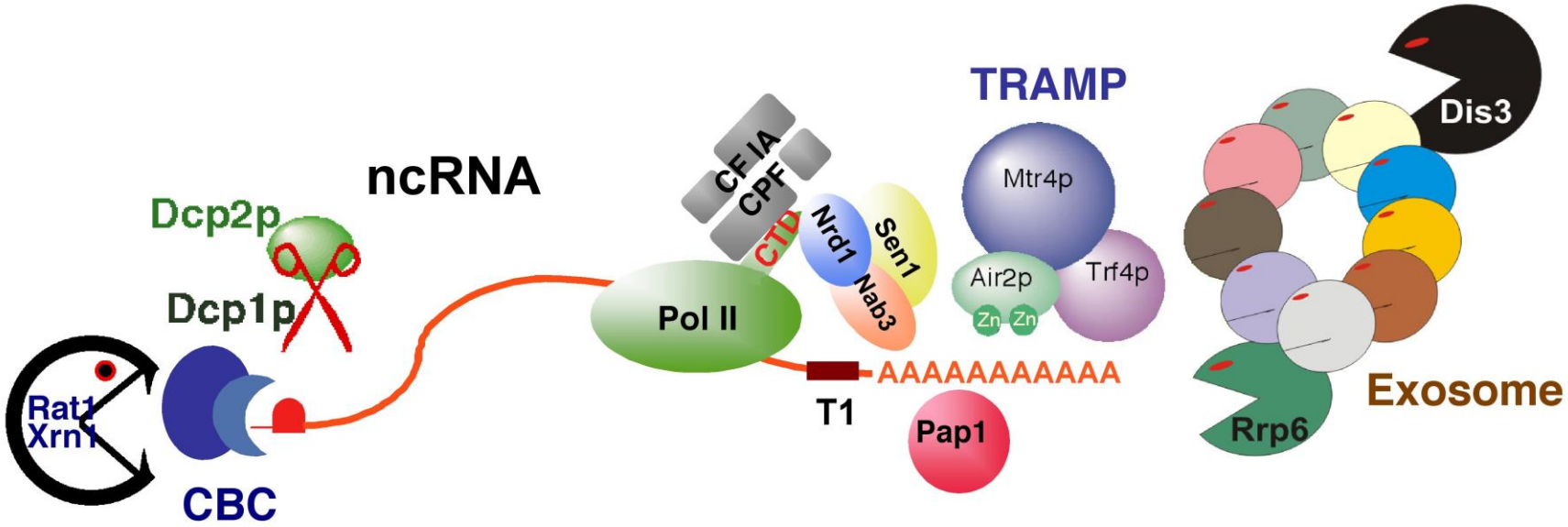
snoRNA, **CUTs**

short mRNAs, SUTs



Jacquier, Nat. Rev. Genet 2009

ncRNA instability and their termination mode

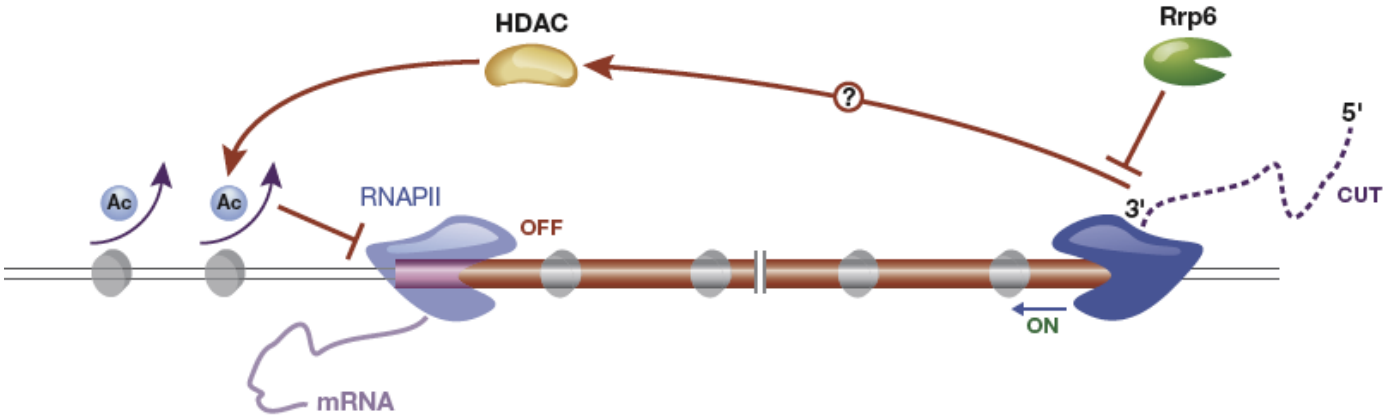


Unstable CUTs (*versus more stable SUTs*)
- are detected in TRAMP or exosome mutants
- are terminated by Nrd1/Nab3-dependent mechanism and polyadenylated by Trf4/TRAMP
- Nrd1/Nab3, TRAMP and exosome complexes interact

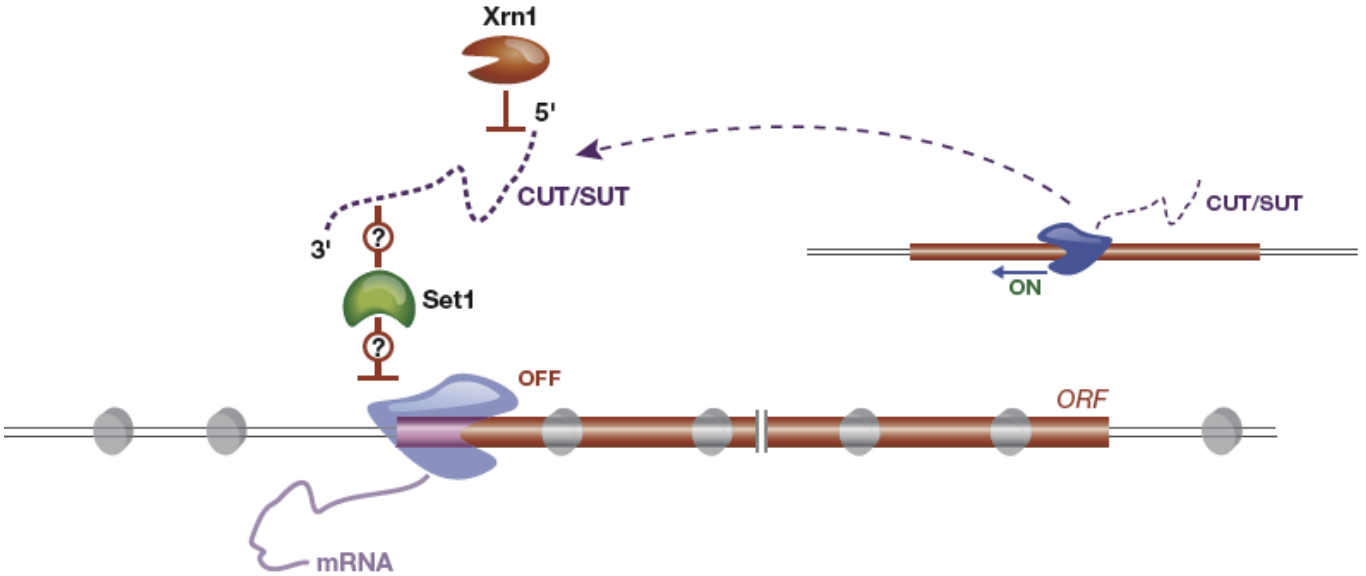
- some CUTs (SRG1, IGS1-R) are polyadenylated by Pap1
- some CUTs are exported to the cytoplasm (XUTs) and degraded by Xrn1
- ncRNP composition is largely unknown

Wyers et al., Cell, 2005; Arigo et al., Mol.Cell, 2006a; Thiebaut et al., Mol.Cell, 2006, 2008; Houseley et al., EMBO J, 2007; Camblong et al., Cell, 2007; Thompson and Parker, Mol.Cell. Biol., 2007; Houseley et al., Mol. Cell, 2008; Vasiljeva et al., Mol.Cell, 2008; Luke et al., Mol. Cell, 2008; Berretta et al., Gene Dev., 2008; Preker et al., Science, 2008; Seila et al., Science, 2008; Xu et al., Nature, 2009; Neil et al., Nature, 2009

CUT ACTION *in-cis* or *in-trans*



CUT transcribed *in-cis*, when stabilized, recruits chromatin modification enzymes (HDAC) to gene promoter



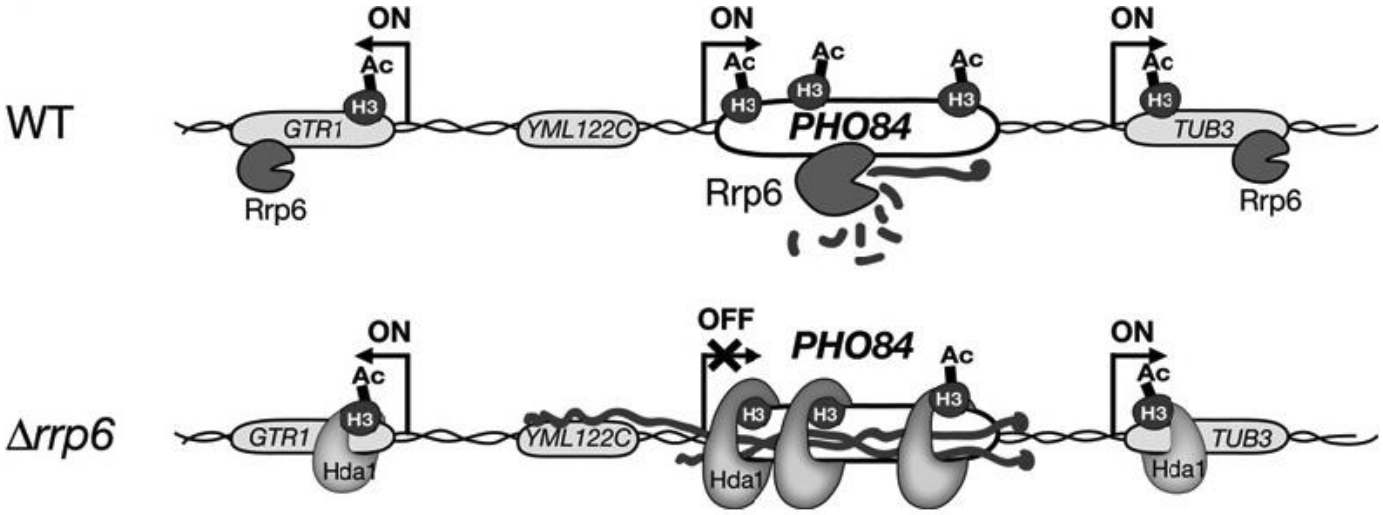
CUT transcribed from a distant locus, when stabilized, recruits chromatin modification enzymes (HTM) to inhibit transcrition

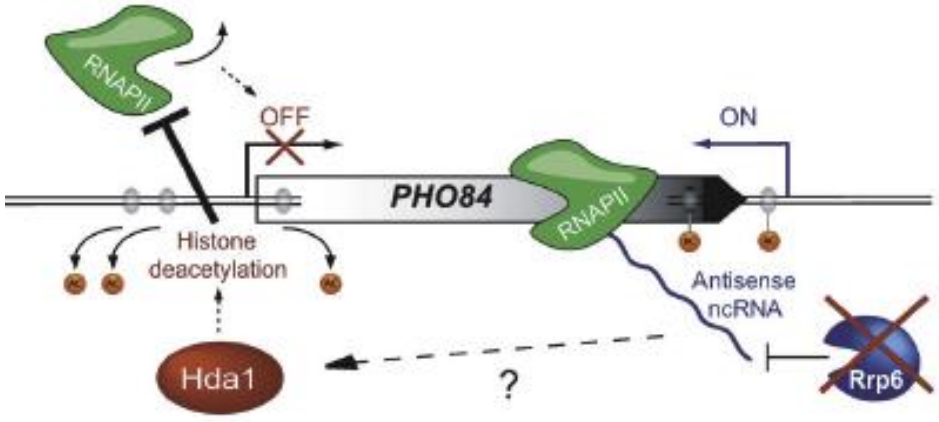
Berretta and Morillon, Embo Rep. 2009

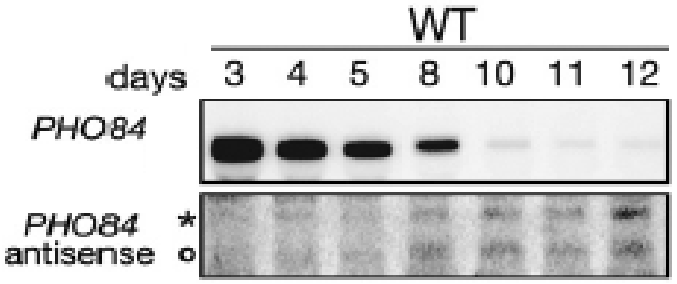
PHYSIOLOGICAL FUNCTIONS of CUTs

Regulation of gene expression via antisense RNA and epigenetic modification: *PHO84* (inorganic phosphate transporter)

Stabilization of as CUT leads to H3K18 deacetylation by Hda1 at *PHO84* promoter





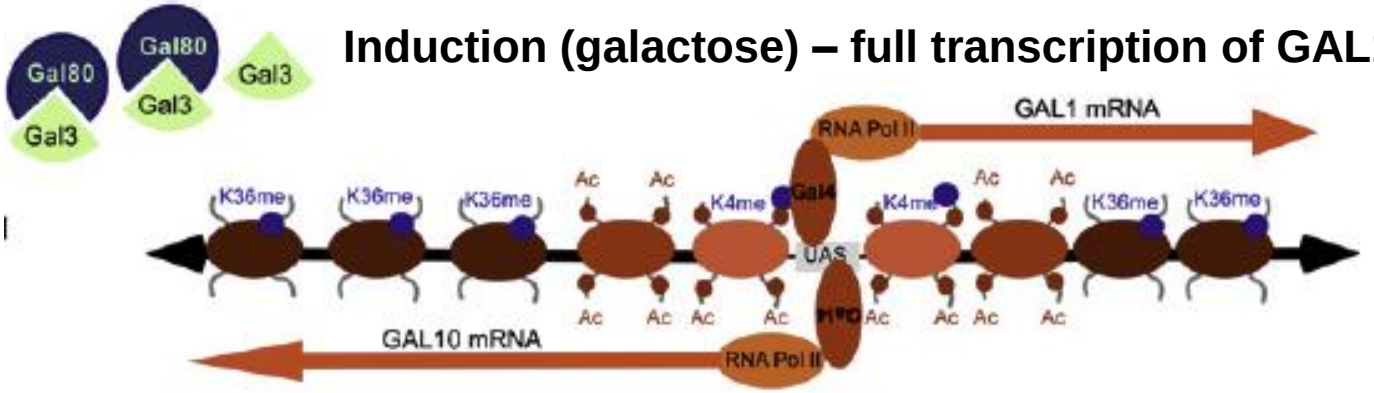


Similar *PHO84* silencing occurs in aging yeast

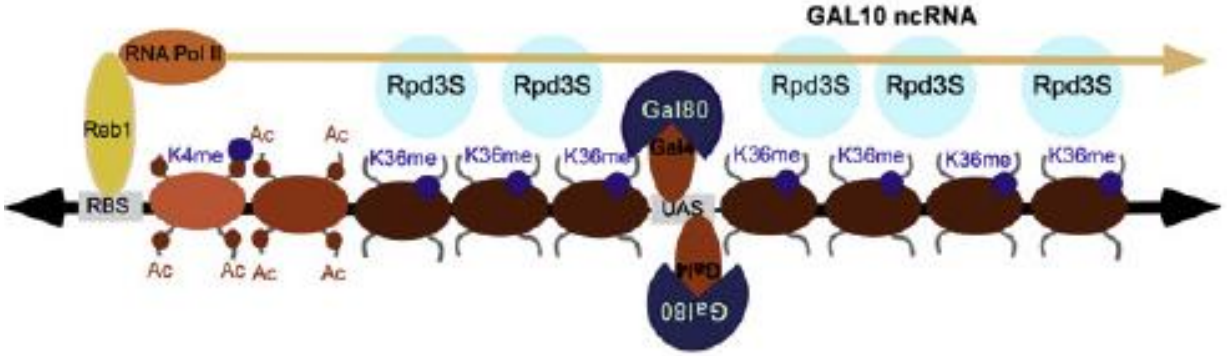
Camblong et al., Cell, 2007; Wery et al., WIREsSMB ’11

PHYSIOLOGICAL FUNCTIONS of CUTs

Regulation of gene expression via antisense RNA and epigenetic modification: *GAL10-GAL1* locus



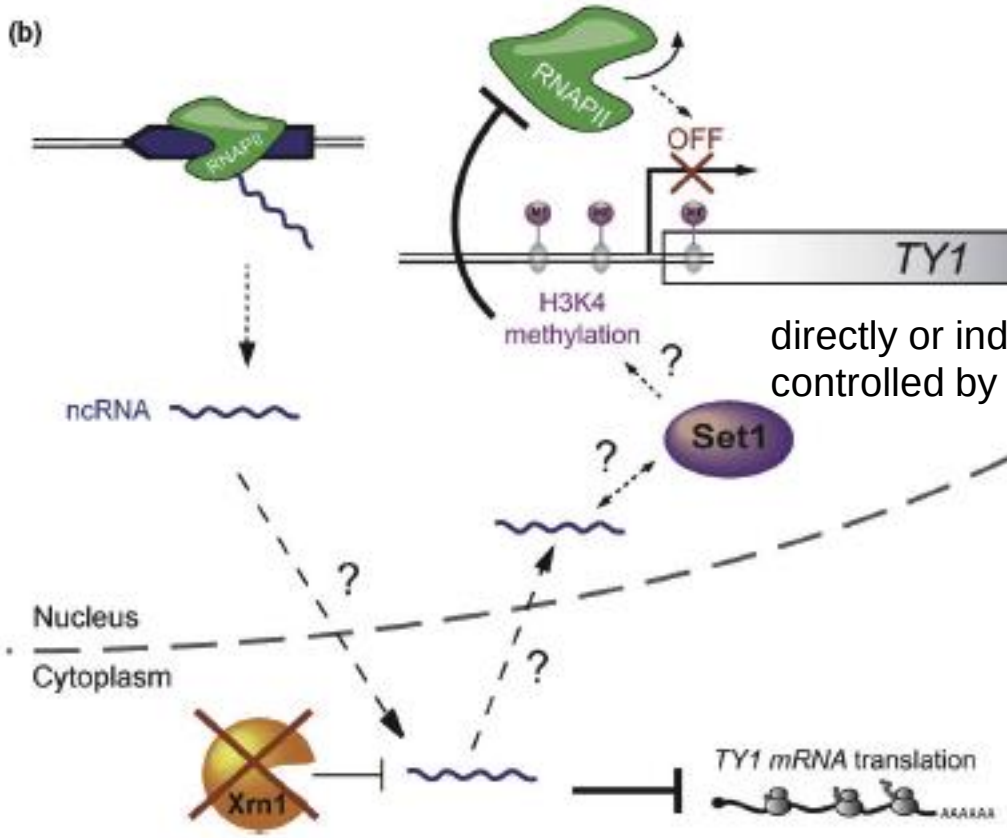
Repression (glucose) – Gal80/4 inhibitor binding at UAS inhibits transcription of GAL1/GAL10 mRNAs and allows Reb1 binding within GAL10 gene. This induces transcription of CUT RNA, which in turn leads to H3K36 histone methylation by HTM Set1 and Set2, histone deacetylation via recruitment of histone deacelylase complex Rpd3S, and further inhibition of mRNA transcription



Houseley et al., Mol.Cell, 2008

PHYSIOLOGICAL FUNCTIONS of XUTs

Transcriptional silencing of the Ty1 transposon



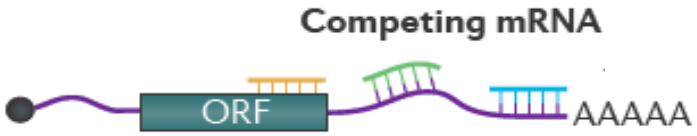
antisense *TY1* XUT

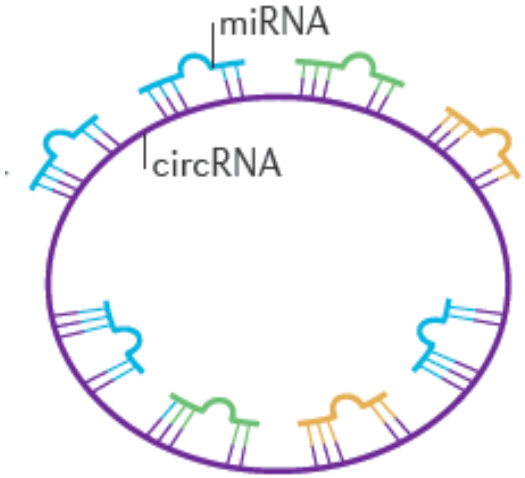
- **polyadenylated Pol II transcript**
- **antisense to TY1 promoter**
- **degraded by cytoplasmic Xrn1**
- **silences TY1 expression by promoting histone deacetylation and trimethylation (by Set1)**
- **can act *in-trans***

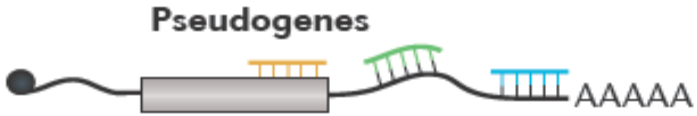
Berretta et al., Gene Dev, 2008; Wery et al., WIREsSMB´11

miRNA sponges

Non-coding or coding competing RNAs that bind and sequester miRNAs and in this way stabilize their mRNA targets

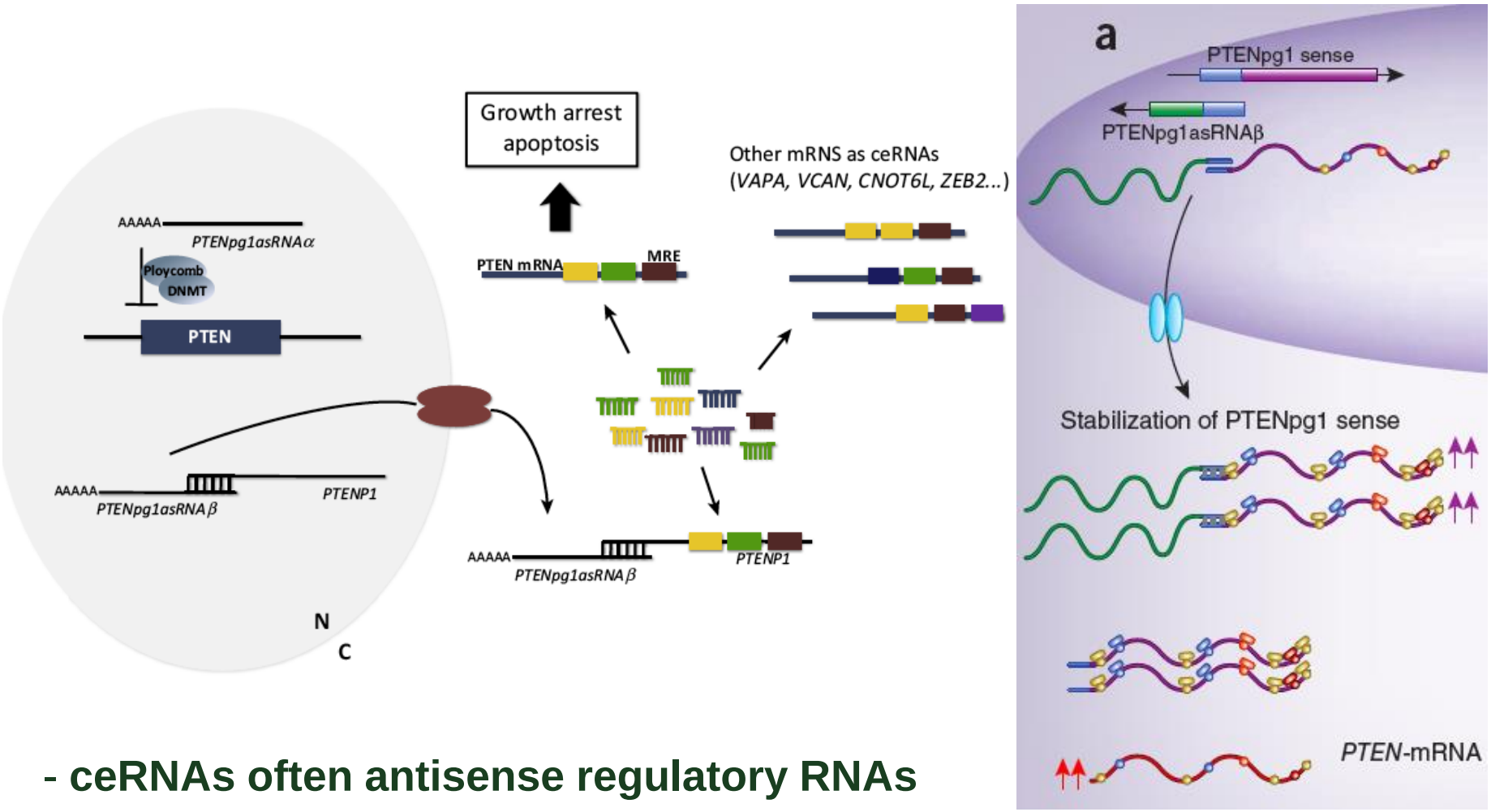






Hausser and Zavolan, NatRevGent, 2015

Competing endogenous RNAs: ceRNAs

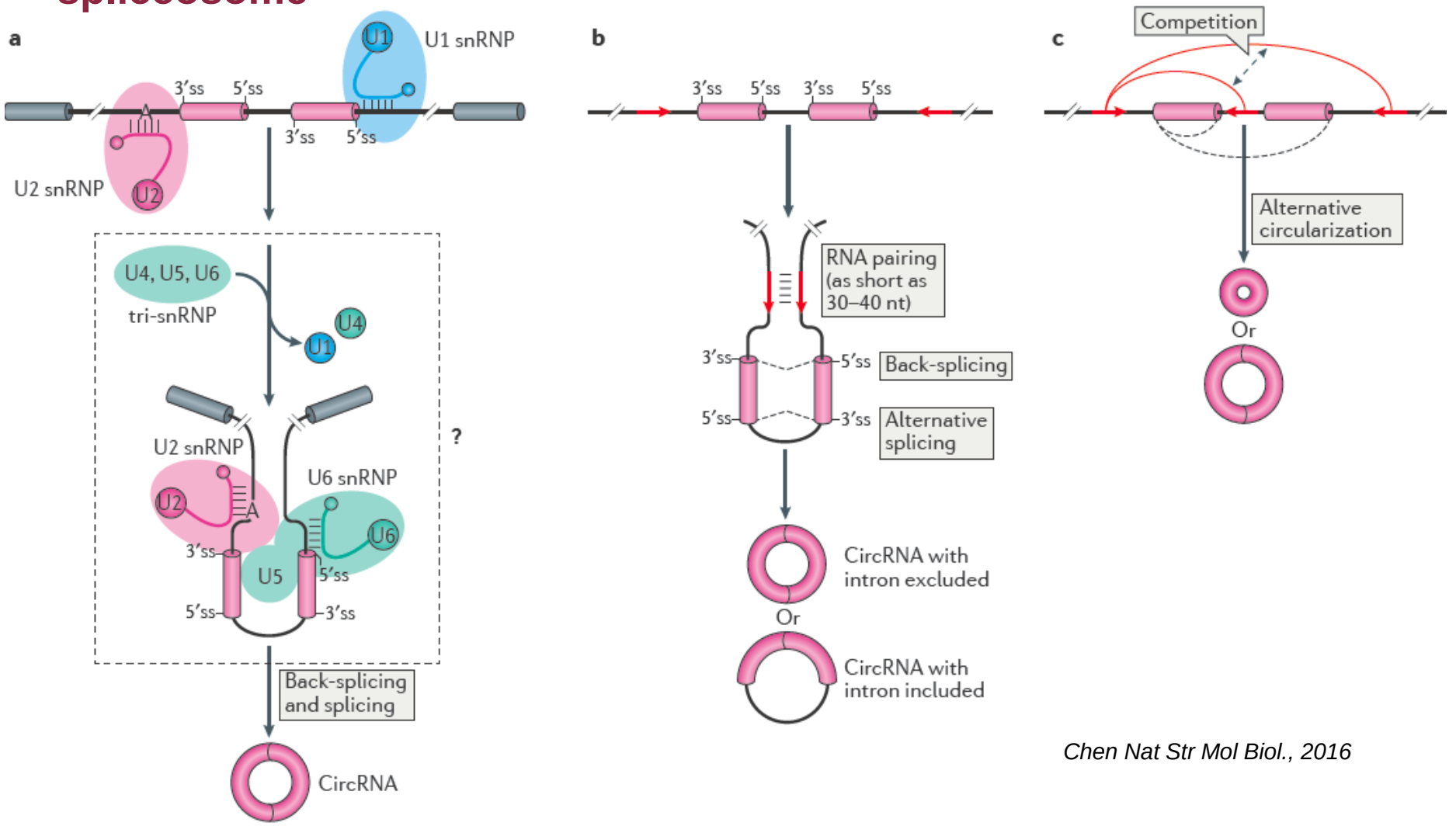


- ceRNAs often antisense regulatory RNAs
- stabilize mRNA by sequestering miRNAs that target mRNA
- implicated in cancer

Guil and Esteller, TiBS 2015

Circular RNAs: circRNAs

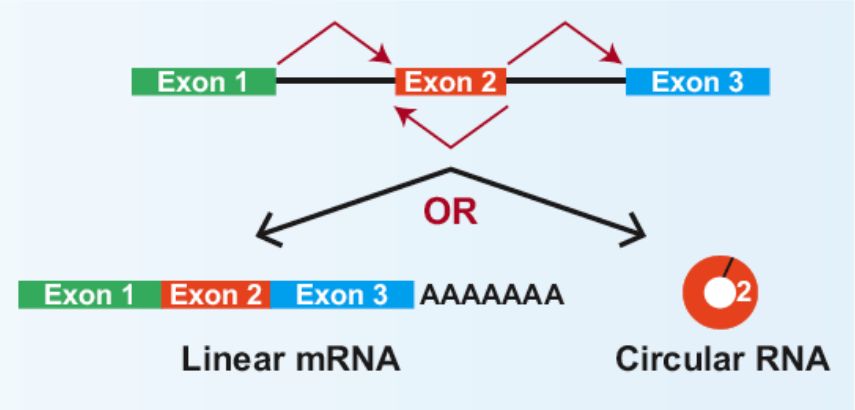
Made of exons, arise by noncanonical back splicing catalysed by the spliceosome



Chen Nat Str Mol Biol., 2016

CircRNA synthesis may be stimulated by some RNA binding proteins (Mbl, QKI) that bind to intronic sequences and stabilize short hairpins

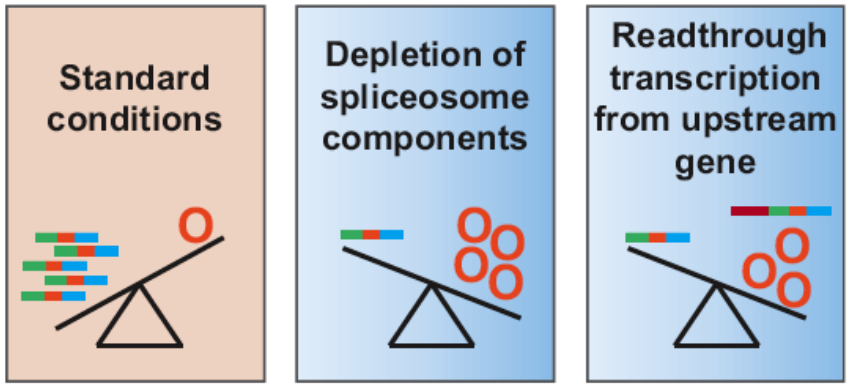
circRNA expression

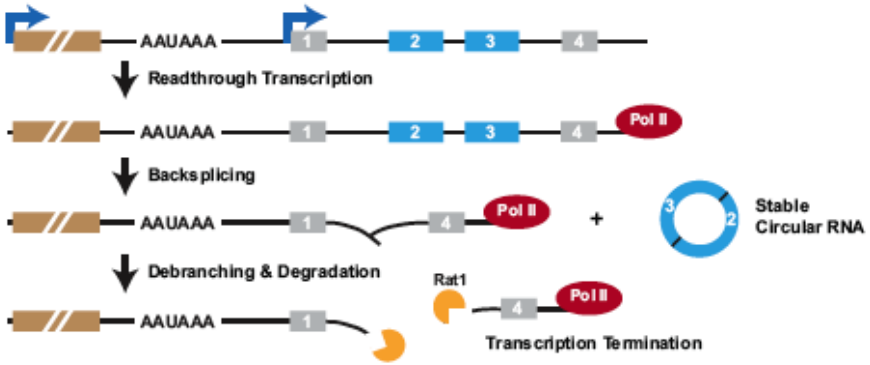


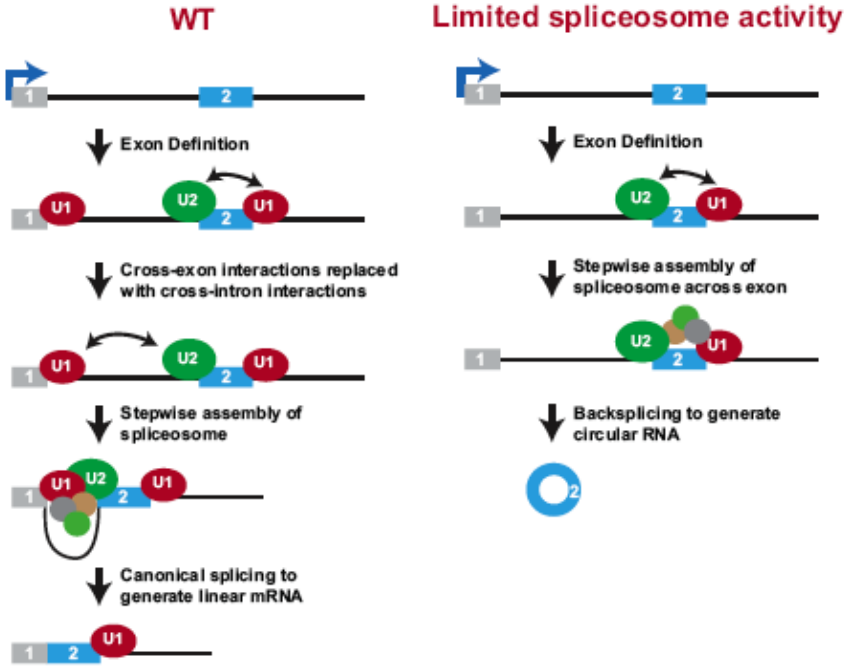
circRNA expression is stimulated by

- inhibition of canonical splicing (depletion of spliceosome components)
- readthrough transcription

The amounts of linear vs. circular RNA can be modulated:



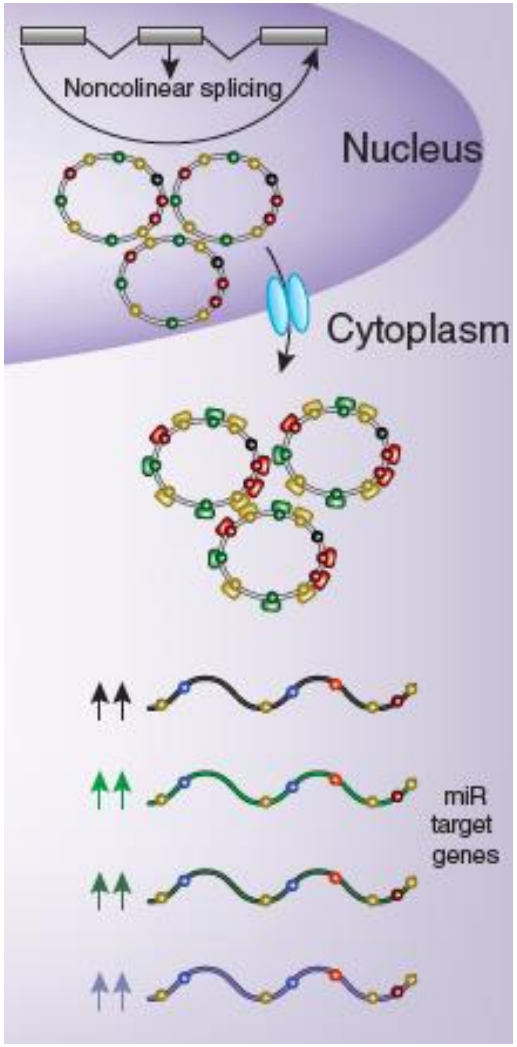


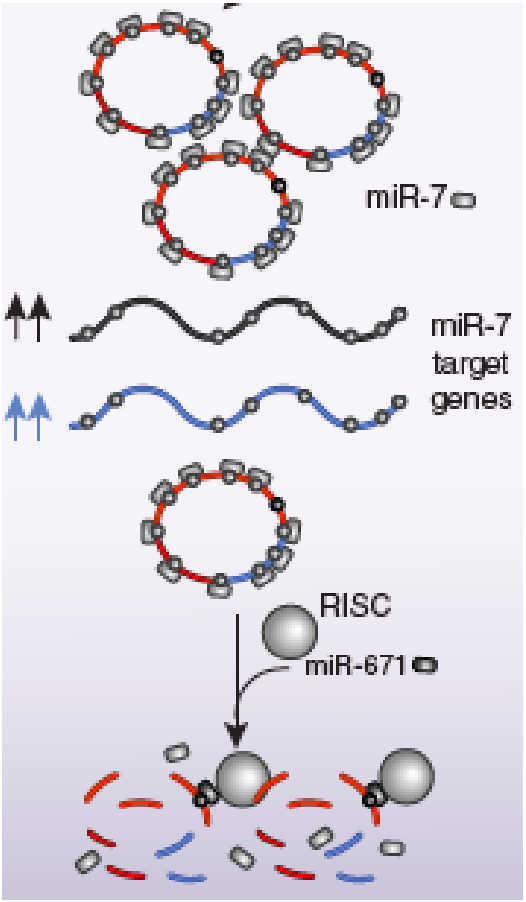


Liang Mol Cell., 2017

circRNAs: functions

Some circRNAs contain miR-responsive elements and sequester miRNAs
Are often regulated via miRNAs and degraded by Ago2 Slicer
CircRNAs with distinct MREs may sequester different miRNAs
CircRNAs may also sequester proteins



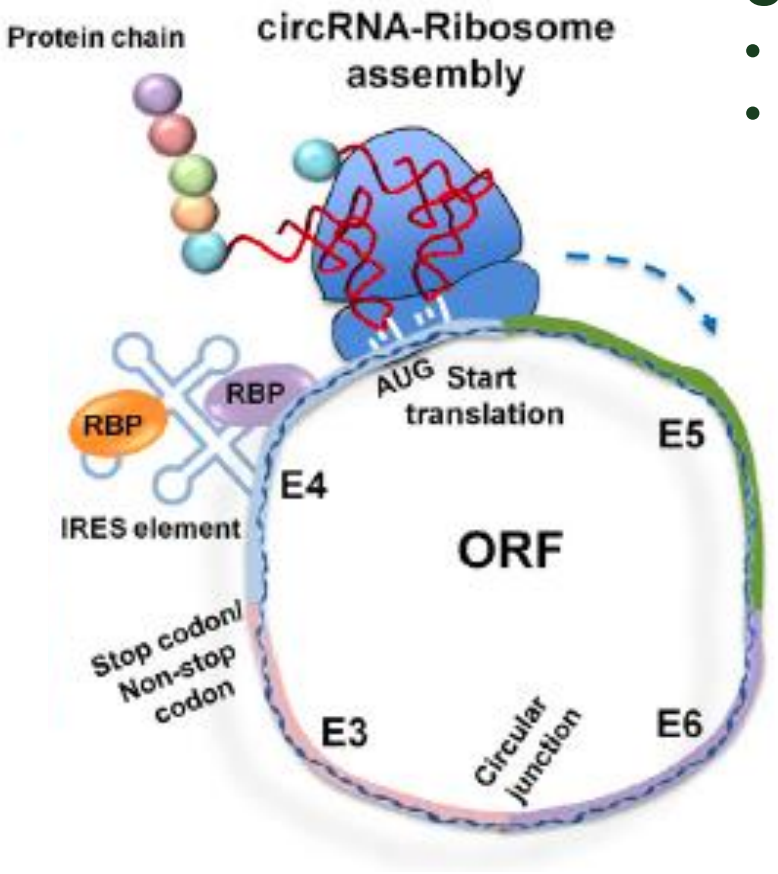


Taulli et al., Nat Str Mol Biol., 2013
Cortes-Lopez and Miura, YJBM, 2016

circRNA	microRNA sponged
CDR1as	miR-7 (+70 sites) miR-671
circRNA-Sry	miR-138 (38 sites)
circRNA-CER	miR-136
circRNA-001569	miR-145
circ-HRCR	miR-223
circ-Foxo3	miR-36, miR-49, miR-433, and 5 other miRNAs.
circHIPK3	miR-124 and 8 other miRNAs.

circRNA	Interacting Proteins
circ-Foxo3	ID-1, E2F1, FAK, HIF1α p21-CDK2

but circRNAs can be translated...



CircRNA translation:

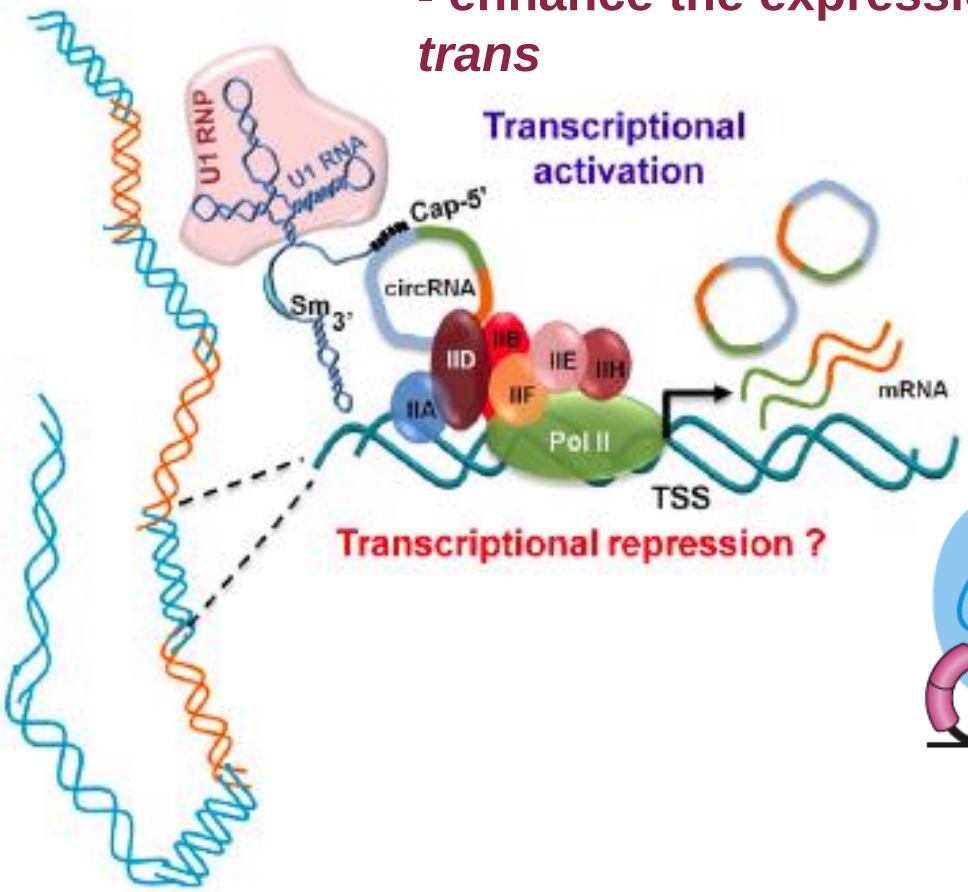
- **in a cap-independent manner (IRES)**
- **often driven by m^{6}A modification**

Model	CircRNA feature	Sequence feature	Peptide/protein
Virus			
Hepatitis delta (δ) virus (HDV)	Circular single-stranded RNA	OFR with a TGA stop codon	Protein of 122 amino acids
Rice yellow mottle virus	Covalently closed circular RNA (220 nt)	Infinite ORF, IRES-dependent sequence	16-kDa highly basic protein
Bacteria			
Escherichia coli	795-nt circular mRNA	Infinite GFP ORF, IRES-independent sequence	GFP
Mammals			
HEK-293 cells	Single exon minigene	IRES-dependent sequence	GFP
HEK-293 cells	Exonic	Poly-A tail-independent translation	NH2-terminal portion of NCX1 protein (70-kDa)
Rabbit reticulocyte lysate	Exonic	Cap-independent translation, IRES-independent sequence, poly-A tail-independent translation	EGF, IGF-1, IGF-2
HeLa cells	Exonic		

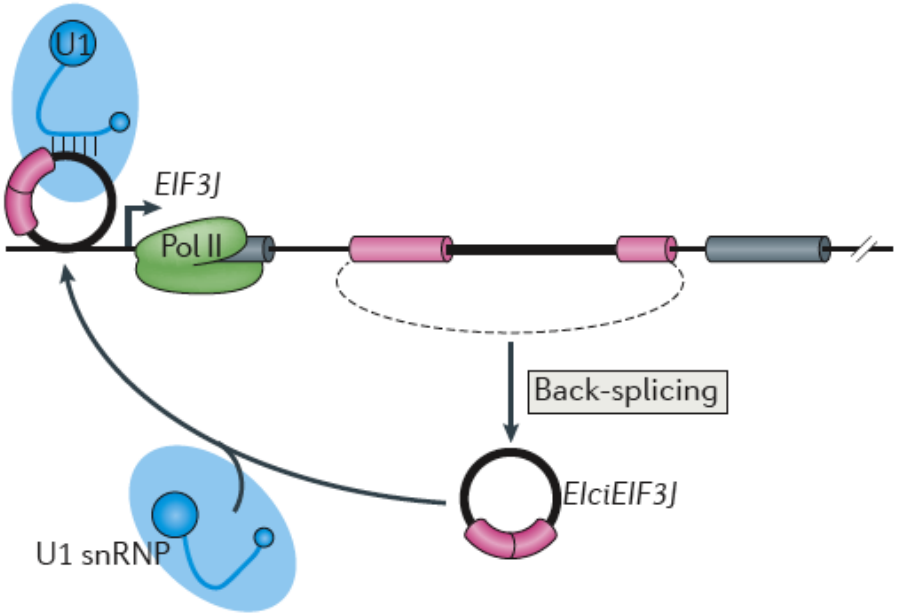
Granados-Riveron and Aquino-Jarquin, BBA., 2016

circRNAs may regulate transcription

exon-intron circRNAs (EIciRNAs)
- associate with U1 snRNP in the nucleus
- enhance the expression of their parental gene *in trans*



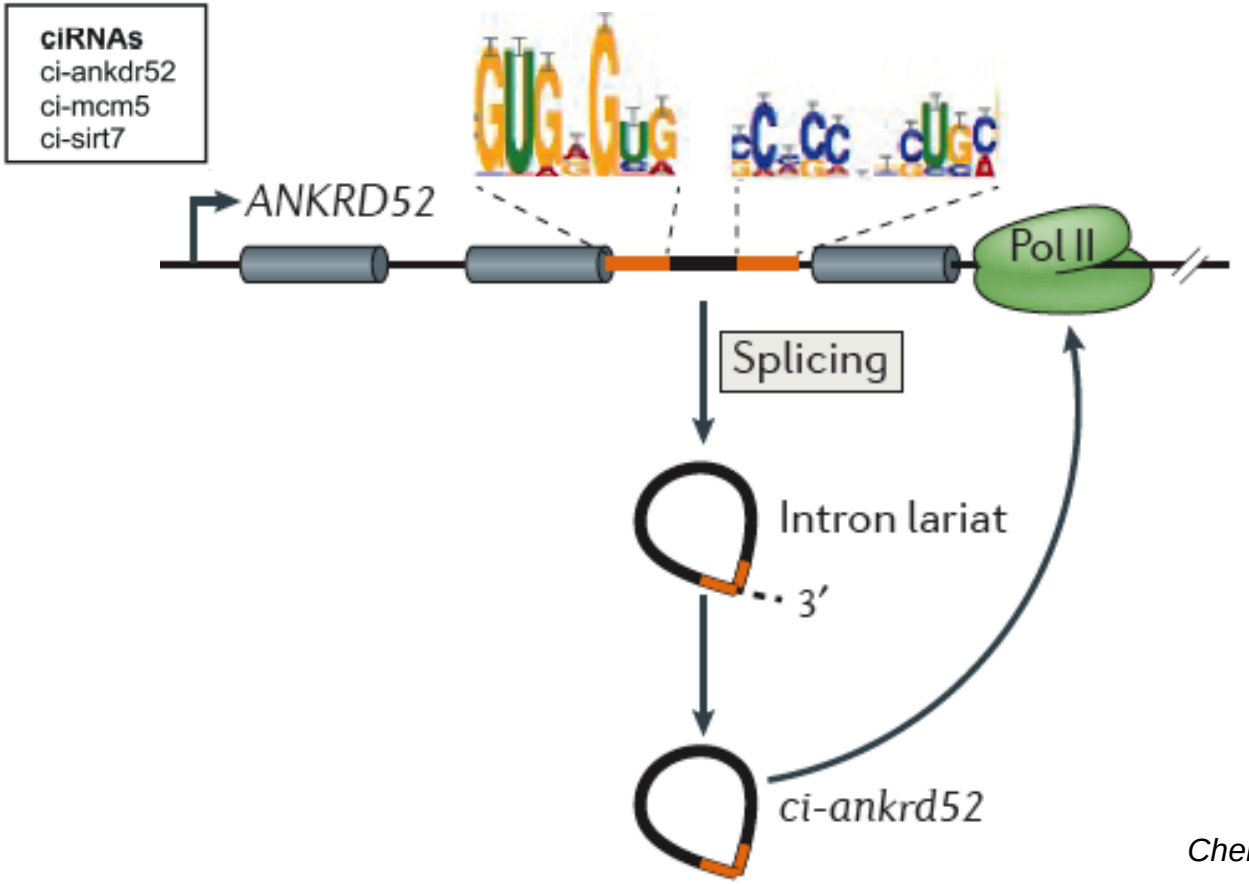
EIciRNAs
circRIF3J
circPAIP2



Li at al., Nat Struct Mol Biol, 2015;
Chen, NatRevMolCellBiol, 2016
Granados-Riveron and Aquino-Jarquin, BBA., 2016

Circular intron-derived ciRNAs regulate transcription

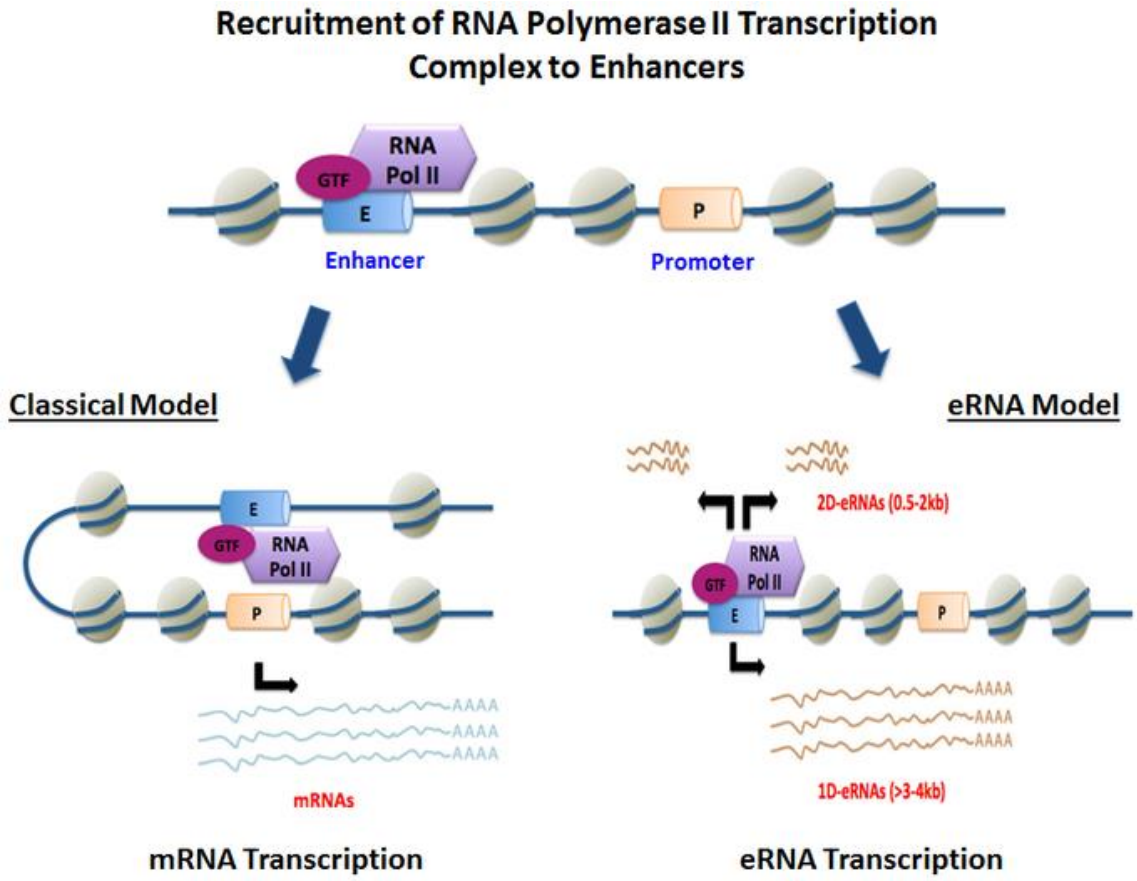
- accumulate in human cells due to lariat debranching defect, in the nucleus
- processing depends on GU-rich motive near 5' splice site and branchpoint
- interact with phosphorylated Pol II and modulate Pol II elongation
- regulate the expression of their parental gene

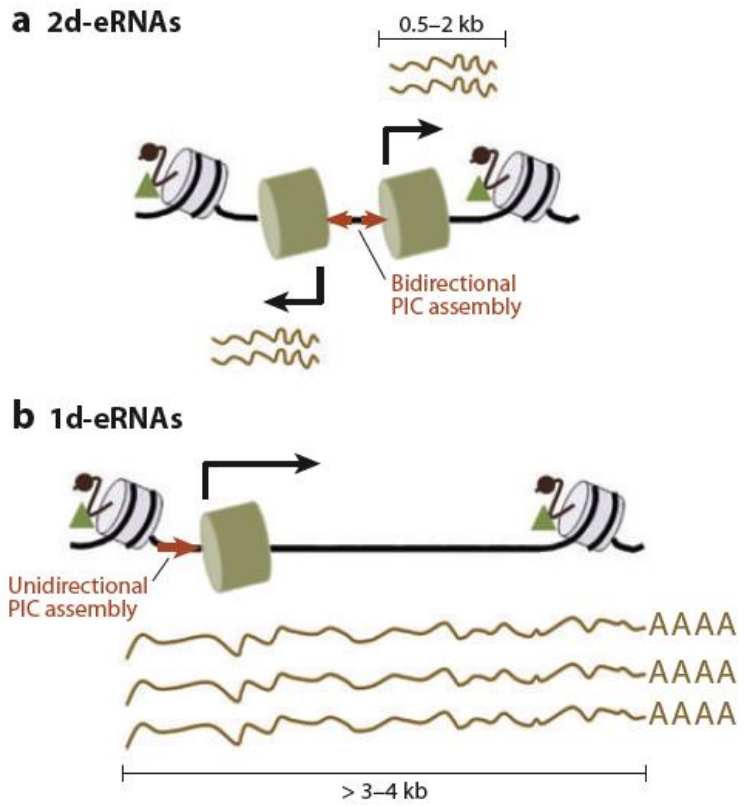


Chen, NatRevMolCellBiol, 2016

Enhancer RNAs: eRNAs

eRNAs: short (not always, up to 2 kb) **ncRNAs transcribed from enhancer regions**

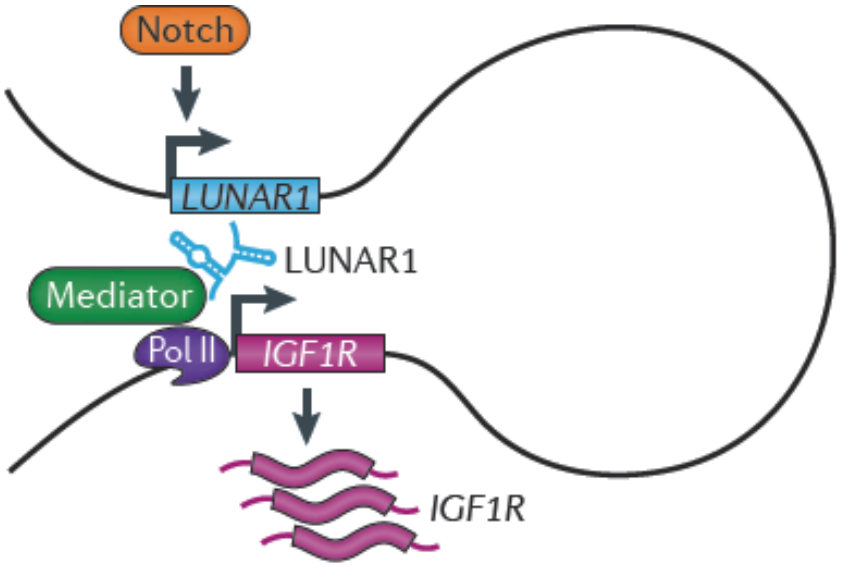




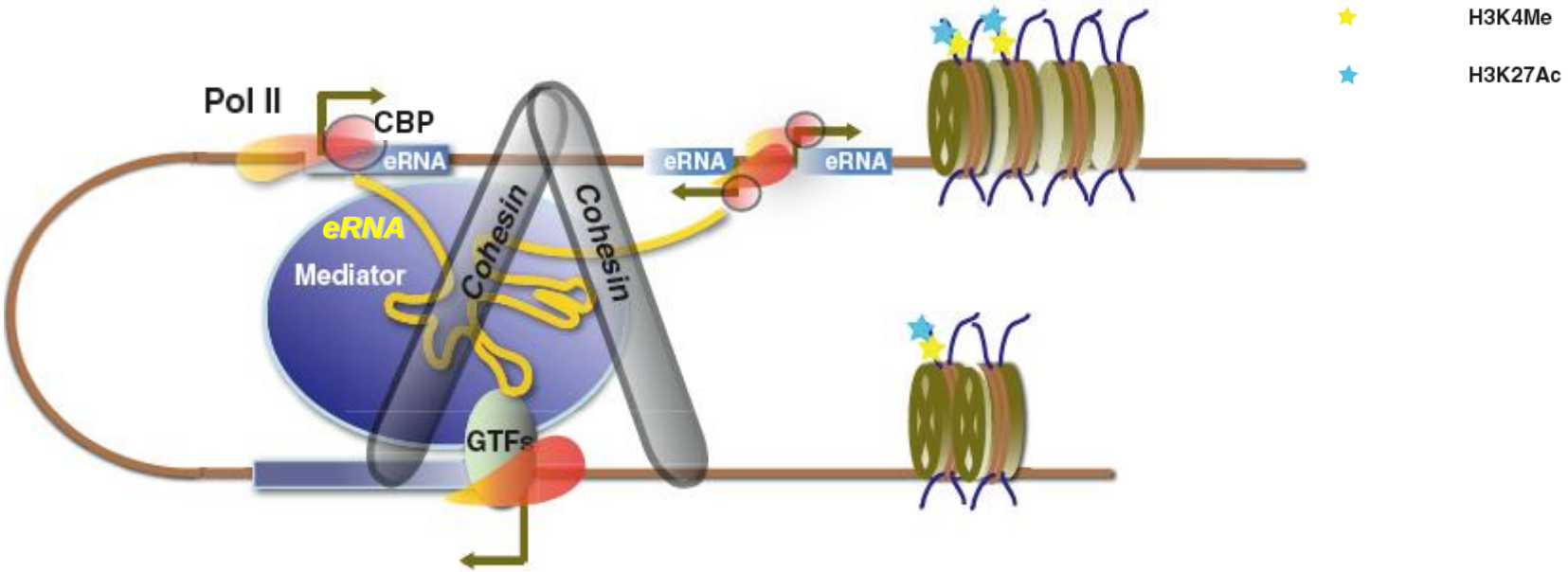
2d-eRNAs: bidirectional, comparatively short, nonpolyadenylated
1d-eRNAs: unidirectional, long, polyadenylated

Natoli and Andrau, Annu Rev Genet., 2012

eRNAs: functions
Chromosome looping

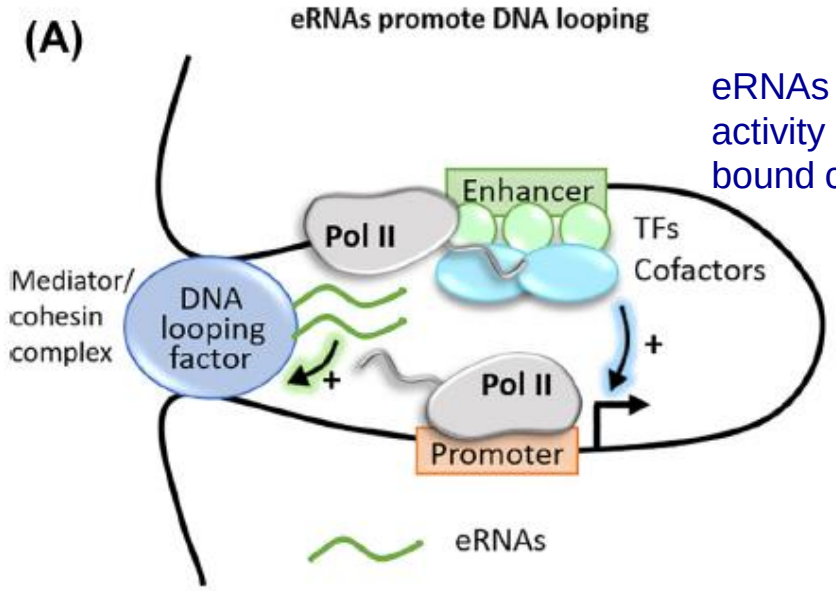


eRNAs interact with DNA looping factors (Mediator, cohesin) to stabilize enhancer-promoter interactions and stimulate PolII activity

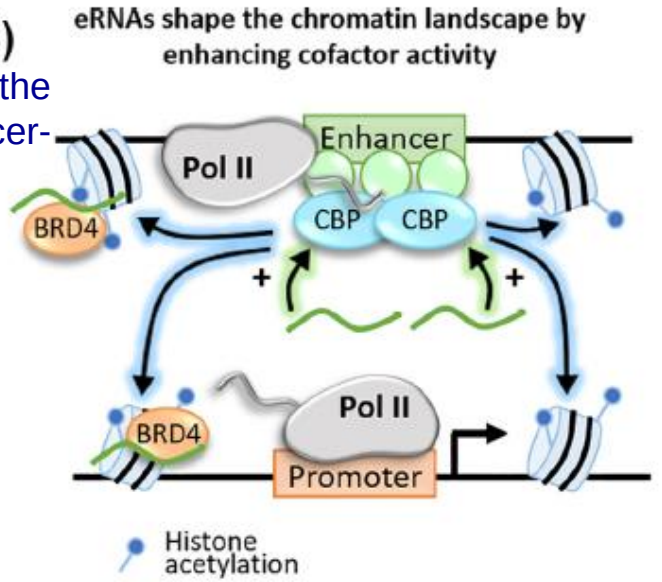


Quinn and Chang, Nat Rev Genet 2015;
Lai and Shiekhattar, Curr Op Gene Dev 2014

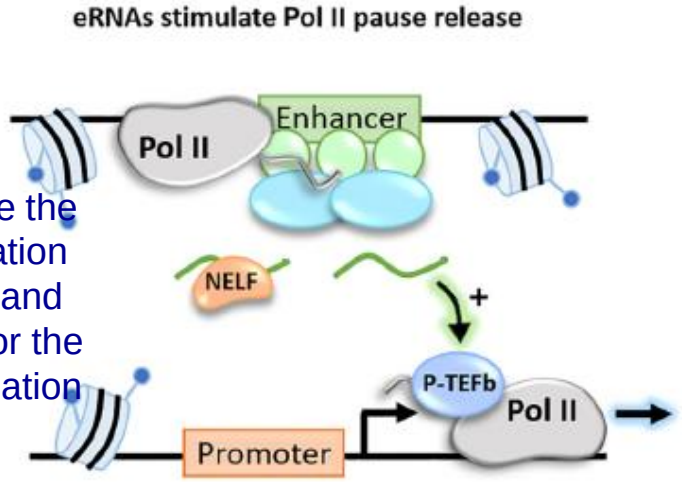
eRNAs: functions

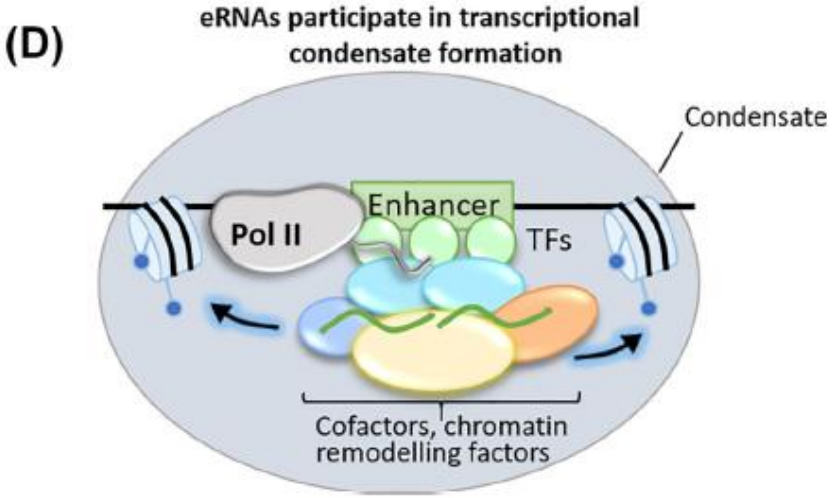


eRNAs enhance the activity of enhancer-bound cofactors



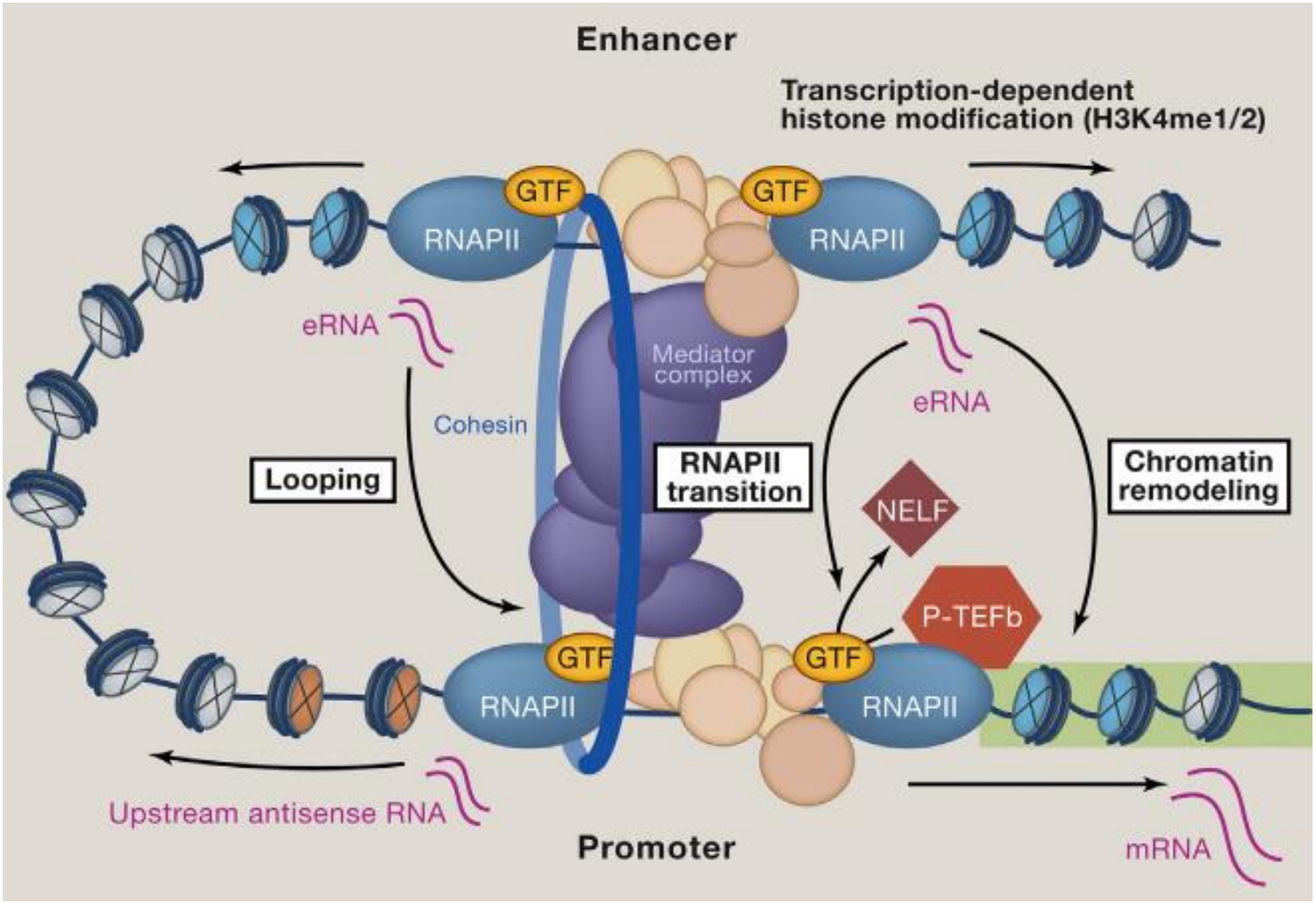
eRNAs activate the positive elongation factor P-TEFb and act as decoy for the negative elongation factor NELF





Studniarek, Egloff and Murphy, TiG 2020

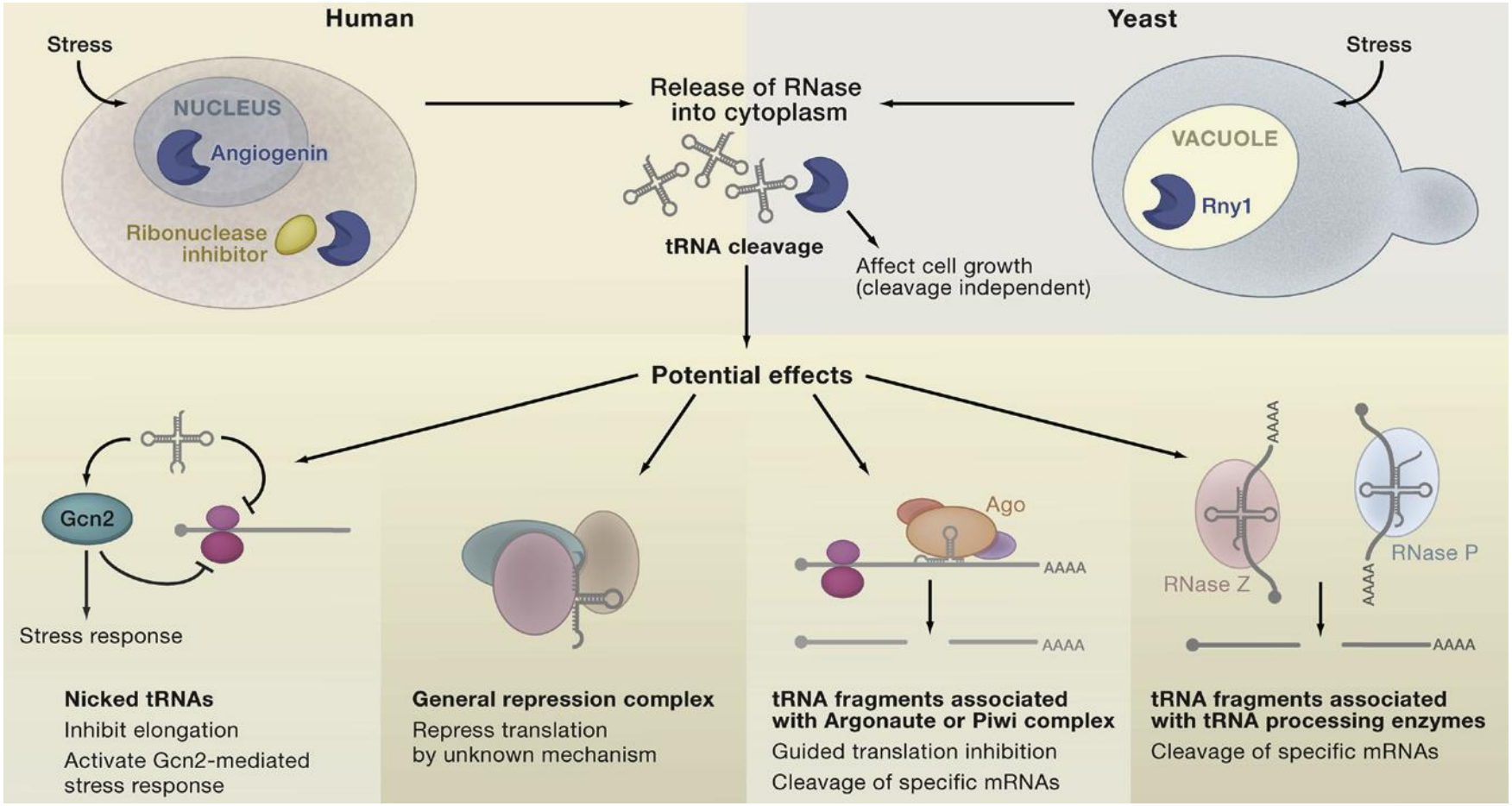
eRNAs: functions



Unusual ncRNAs: tRFs tRNA-derived RNA fragments

Stress-induced enzymatic tRNA cleavage

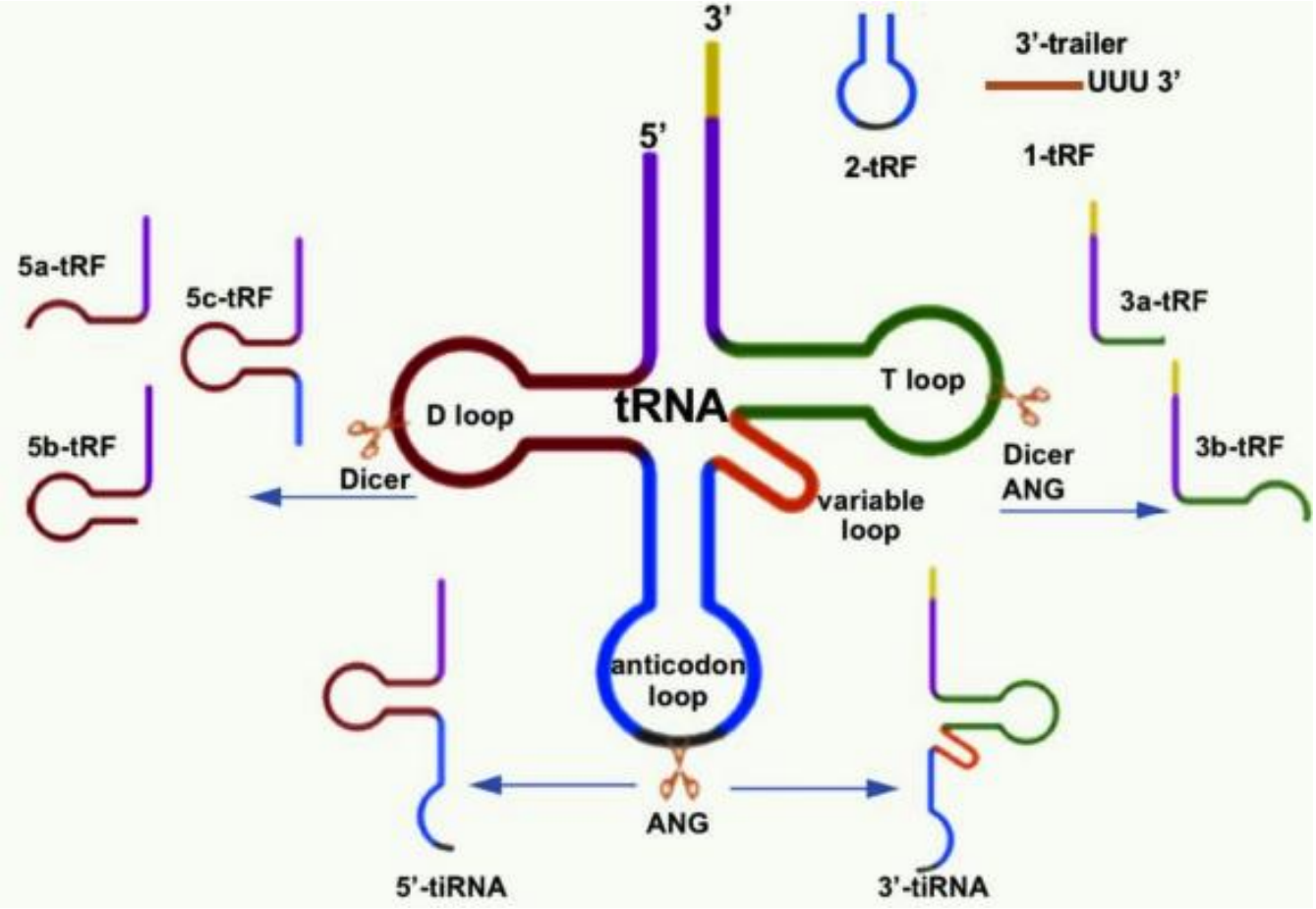
(*S. cerevisiae, D. melanogaster, A. thaliana, A. nidulans*, human cell lines)



Thompson and Parker, Cell, 2009

- **act as miRNAs**
- **regulate translation**
- **regulate cellular stress response**
- **role in disease: cancer, viral infection, metabolic and neurological disease**

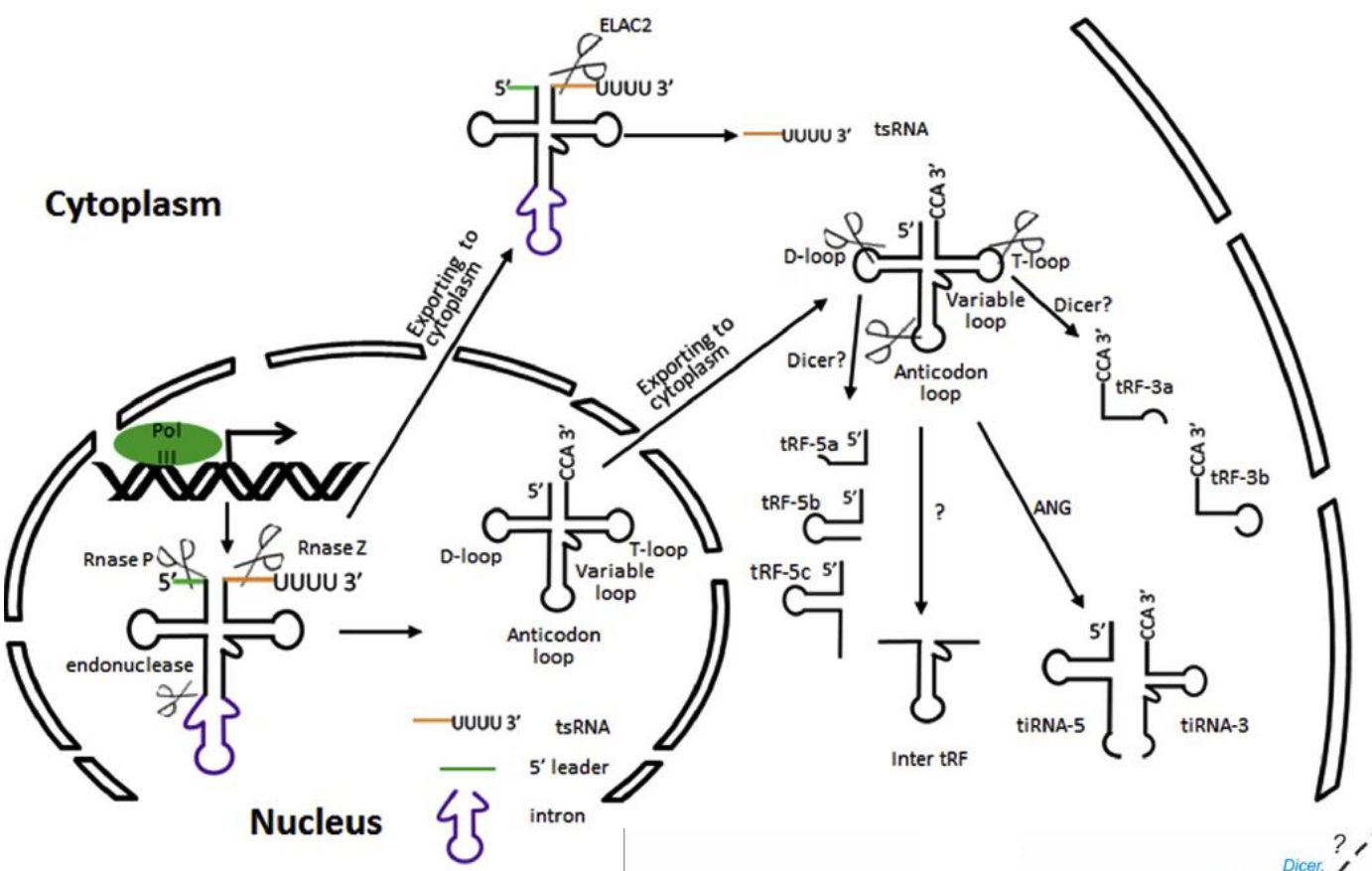
Unusual ncRNAs: tRFs tRNA-derived RNA fragments

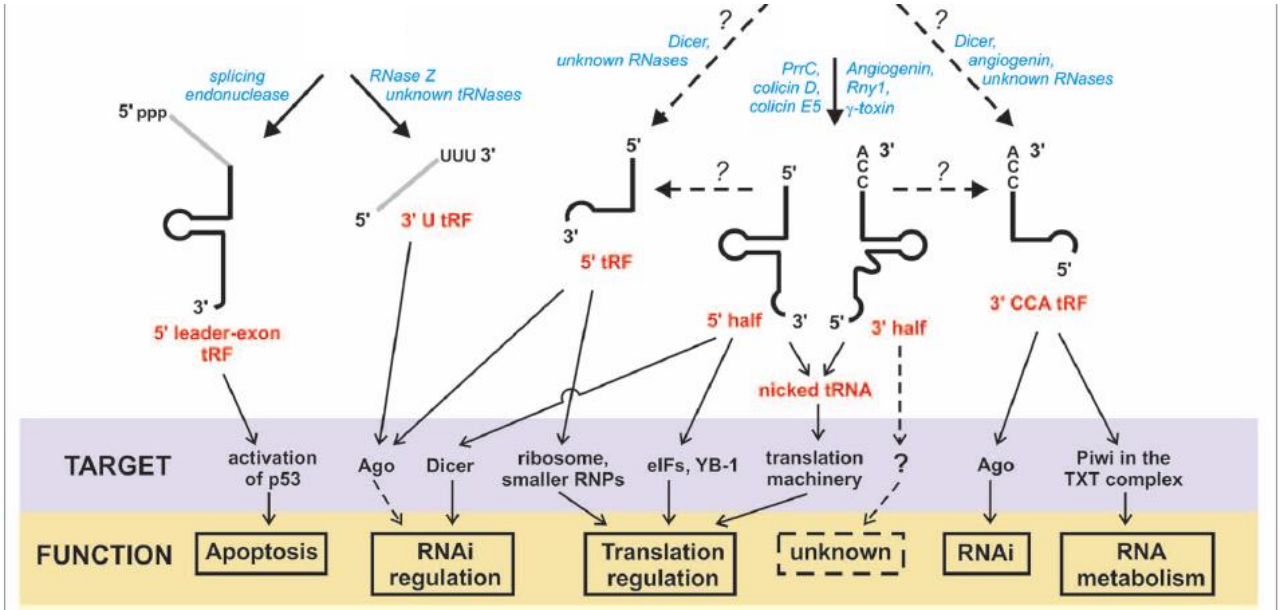


Li et al,
Gene 2018

- **> 17 short abundant tRFs (13-26 nts), generated by RNase Z from mature (5' and 3' ends) and precursor (3' trailer) tRNAs (cytoplasm, prostate cancer).**
- **Abundant Dicer-dependent class I tRFs from mature 3' and 5' ends (HeLa)**
- **Class II tRFs from RNAseZ 3' cleavage to Pol III termination (cytoplas) associate with Ago2-3. Regulation of silencing via association with Ago proteins?**

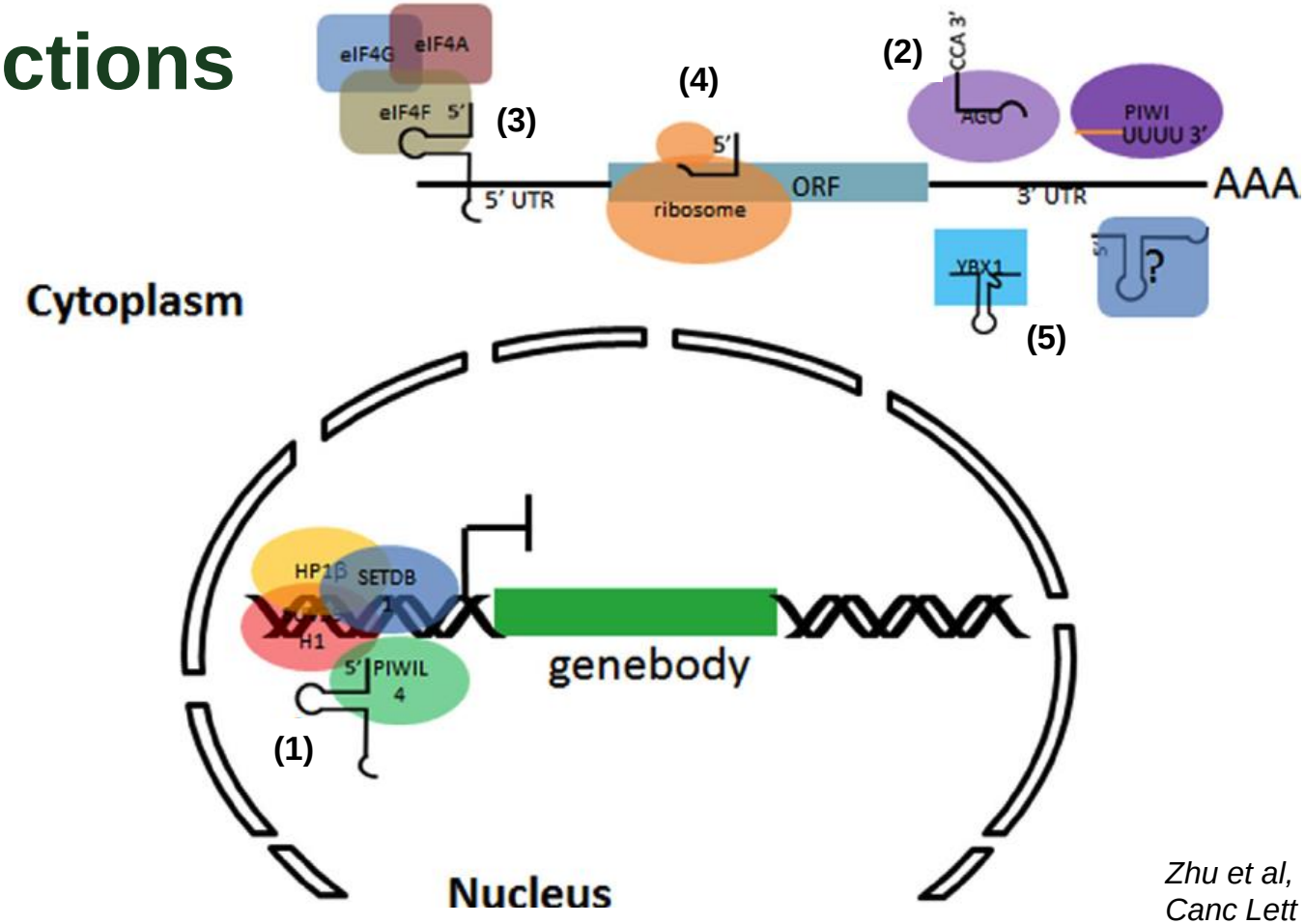
tRFs and tiRNAs





Zhu et al, Canc Lett 2018

tRFs: functions



Zhu et al,
Canc Lett 2018

(1) tiRNAs incorporated with Piwi suppress gene transcription

(2) tRFs associated with AGO/Piwi and suppress target gene expression.

(3) tiRNA inhibits translation by displacing translation initiation factor from mRNA

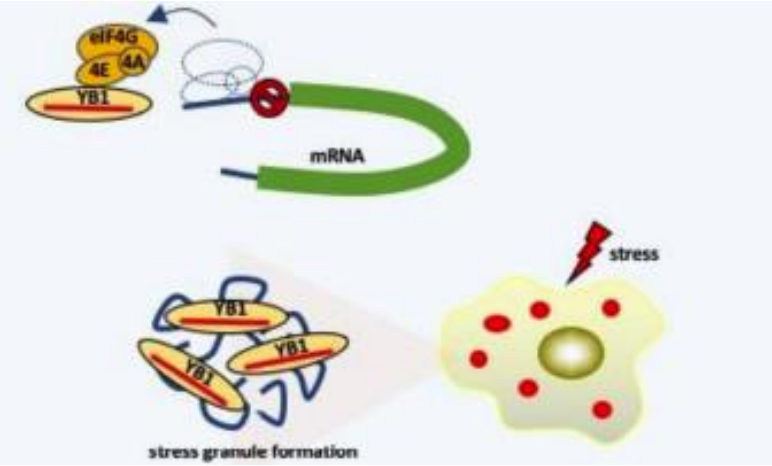
(4) tRFs can suppress translation through affecting ribosome elongation

(5) tRFs can reduce mRNA stability by displacing YBX1 from 3'UTR of mRNA

tRFs: functions

Translational repression by angiogenin-derived 5'-tiRNAs with terminal 5'-oligoG

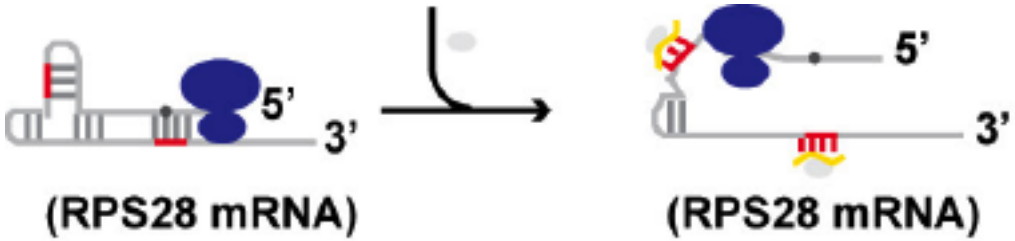
- **represses translation *in vitro* and *in vivo***
- **displaces eIF4G/eIF4A from uncapped transcripts and eIF4F from m^7G cap**
- **triggers formation of stress granules (SGs)**
- **translational repressor YB-1 contributes to tiRNA-mediated repression**



Li et al, Gene 2018

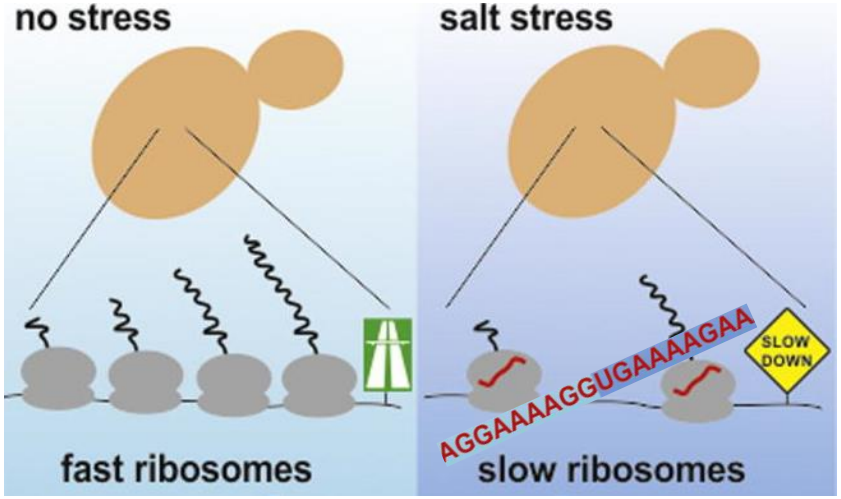
Translational activation by affecting ribosome biogenesis

- **LeuCAG3′ tsRNA binds to *RPS28* and *RPS15* mRNAs and enhances their translation by disrupting secondary structure**
- **RPS28 and RPS15 stimulate biogenesis of 40S ribosome, and so affect cell viability and apoptosis**



Kim et al, Nature 2017

Unusual ncRNAs: stress derived RNA fragments



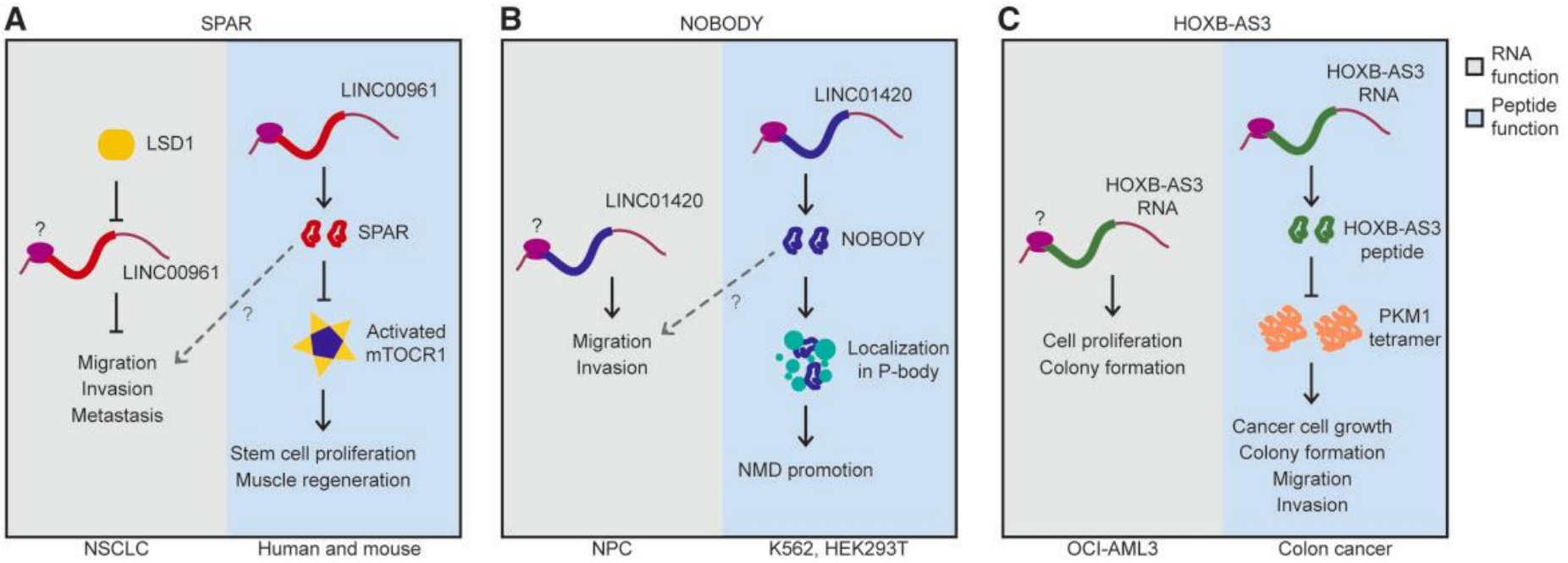
18-mer ncRNA derived from *TRM10* mRNA during salt stress in yeast

- **associates with polysomes**
- **inhibits general translation**

Pircher et al, Mol. Cell 2014
Gebetsberger and Polacek, RNA Biol., 2013

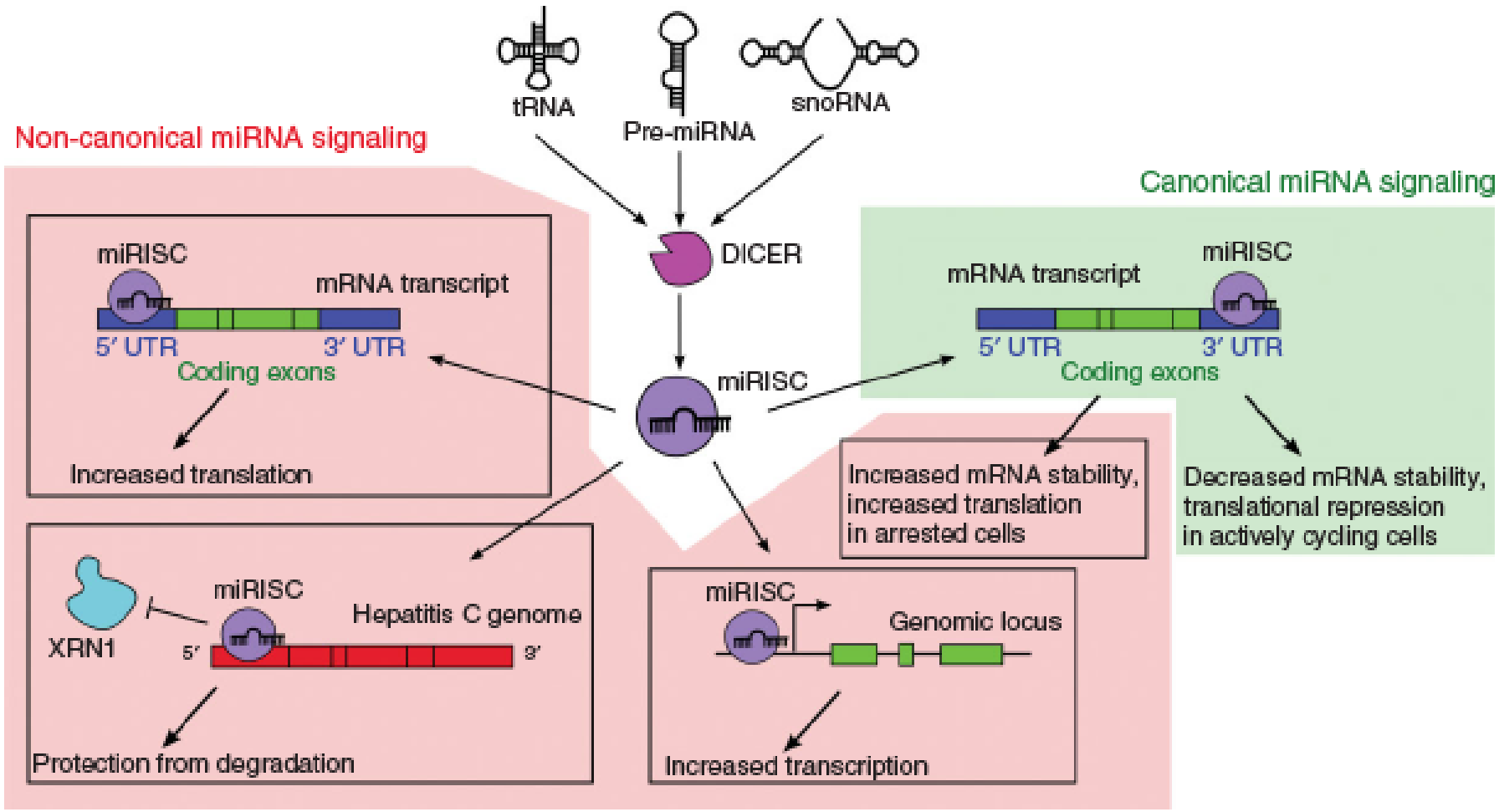
ncRNAs and sPEP (small peptides)

Some ncRNA code for sPEP with a functional potential



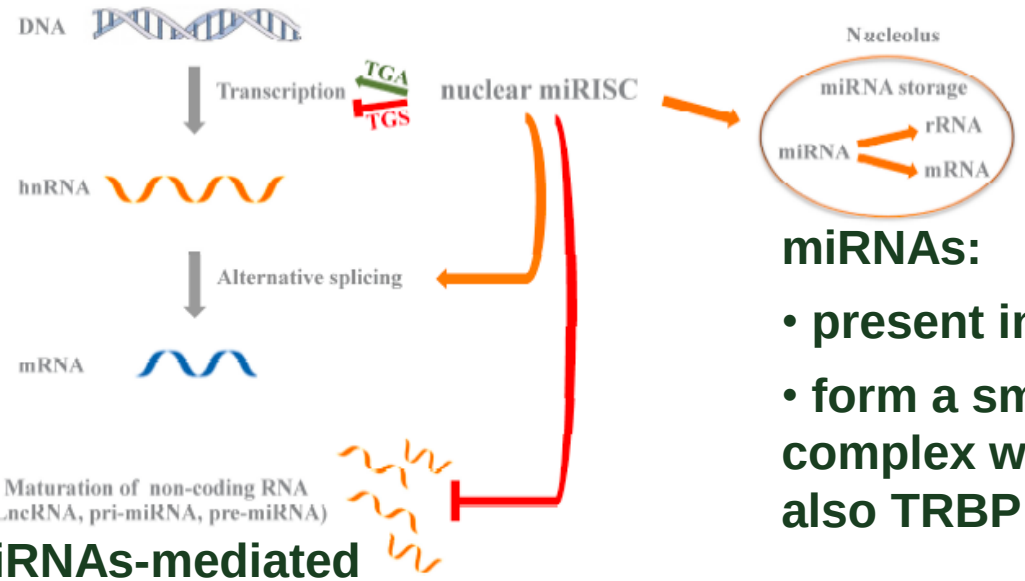
Lauressergues et al, Nature 2015
Choi et al, Brief Bioinfo 2019

Non-canonical miRNAs



Maute et al, WIREs RNA., 2014

NUCLEAR FUNCTIONS of miRNAs

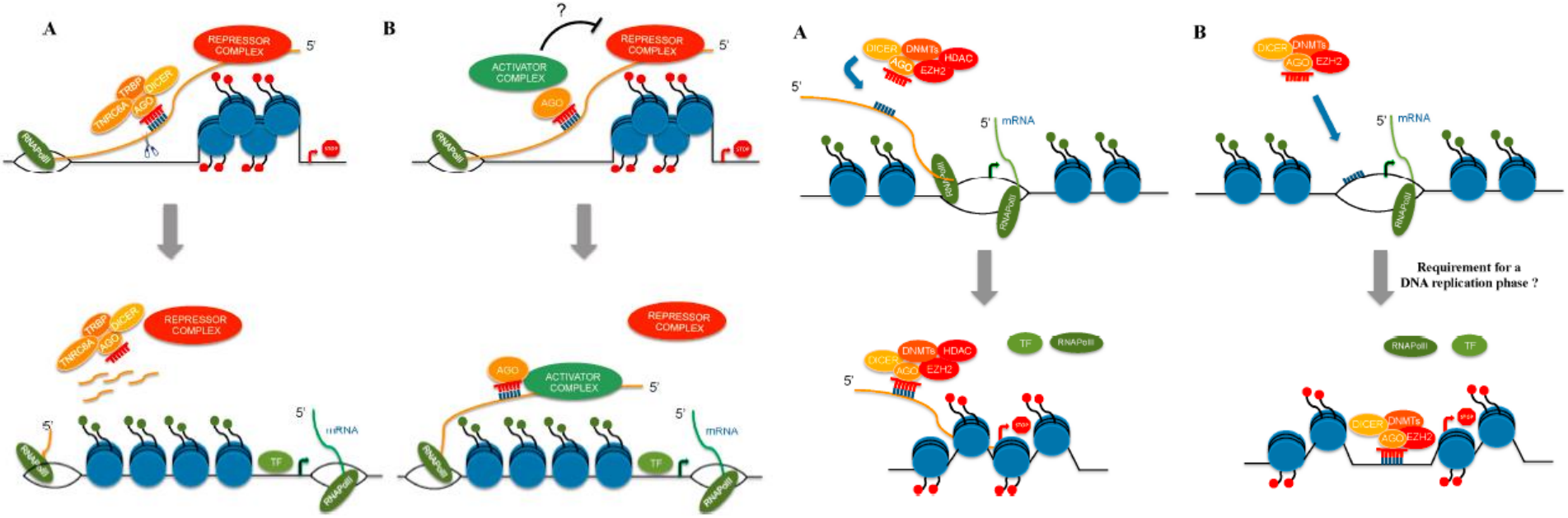


miRNAs:

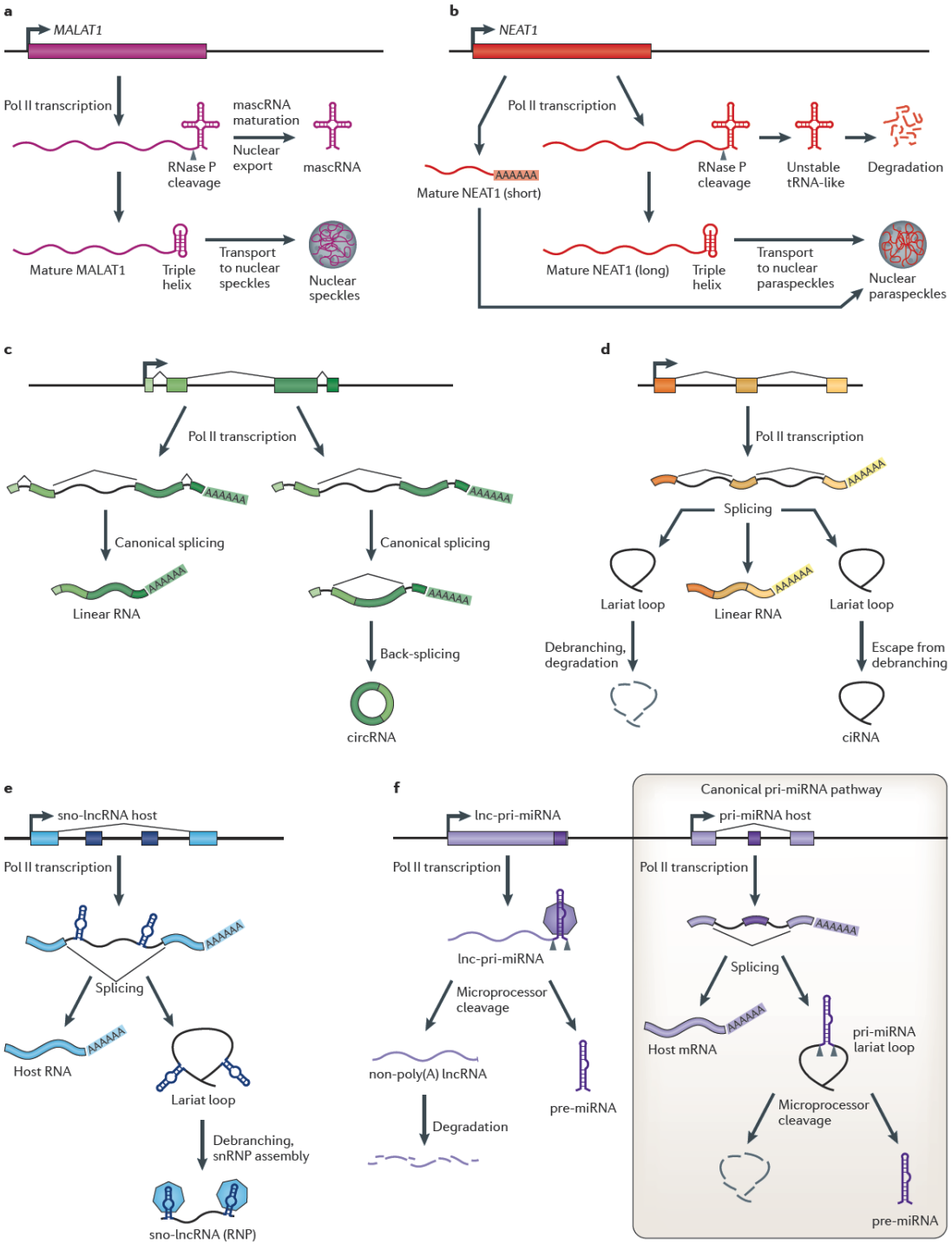
- **present in the nucleus and nucleolus**
- **form a smaller nuclear miRISC complex with AGO2/AGO3, DICER and also TRBP and TNRC6A (TGA)**

Catalanotto et al., IntJMolSci, 2016

miRNAs-mediated Transcriptional Gene Activation (TGA) and Transcriptional Gene Silencing (TGS)

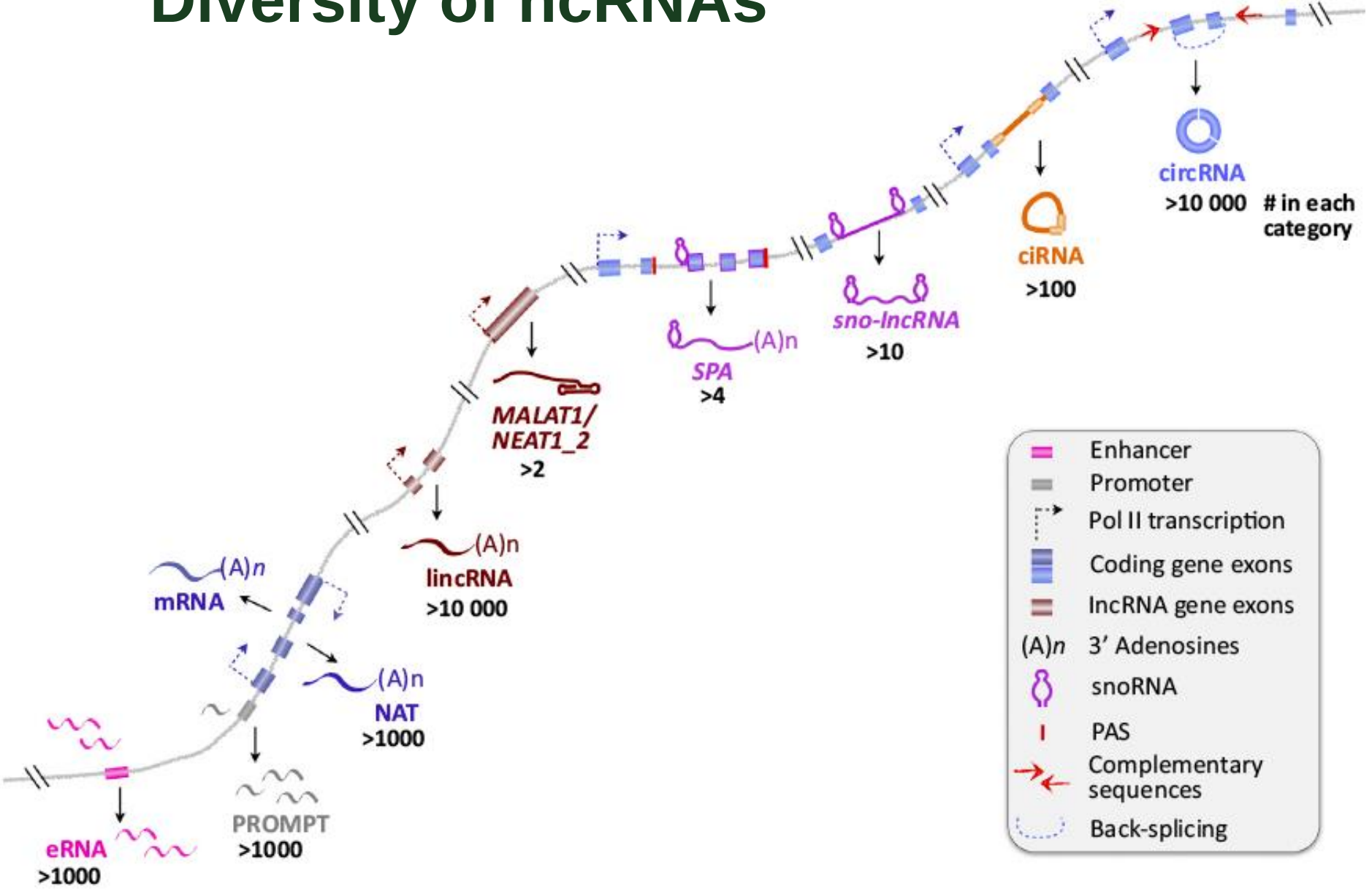


Unusual ways of ncRNAs



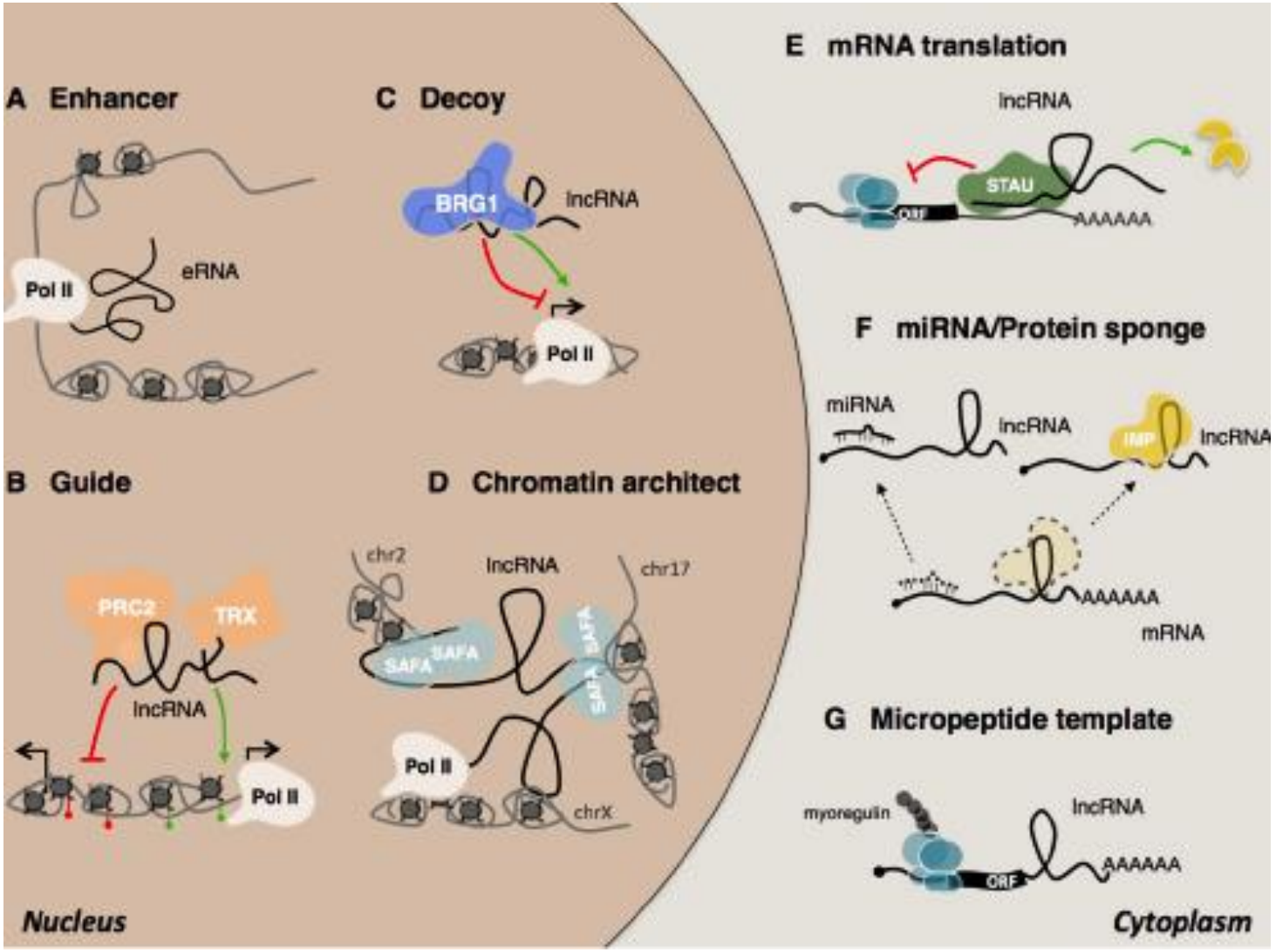
Quinn and Chang, Nat Rev Genet 2015

Diversity of ncRNAs

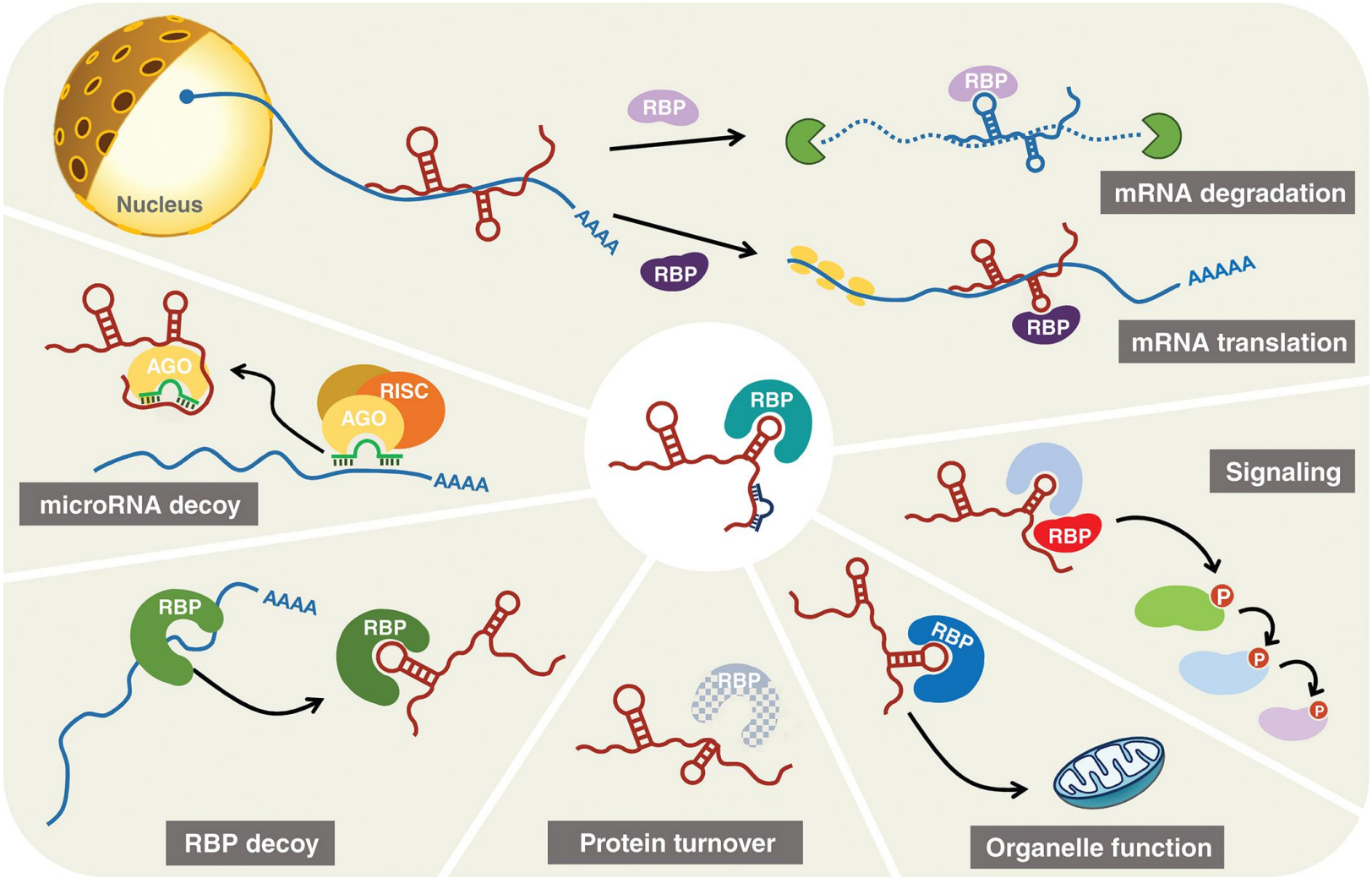


Wu et al, TiG, 2017

Diversity of ncRNA functions



Diversity of ncRNA cytoplasmic functions



Noh at al, WIREs RNA 2018

TAKE-HOME MESSAGE

- **The majority of eukaryotic genomes are transcribed giving rise to a variety of RNAs**
- **At least some of the "invisible" transcripts in some conditions form functional ncRNAs**
- **These usually act in transcriptional silencing *in-cis* or *in-trans* by recruiting modifying enzymes (DNA, histones) to promoters or interacting with DNA (pRNA)**
- **Defects in ncRNA level or activity correlate with several diseases**