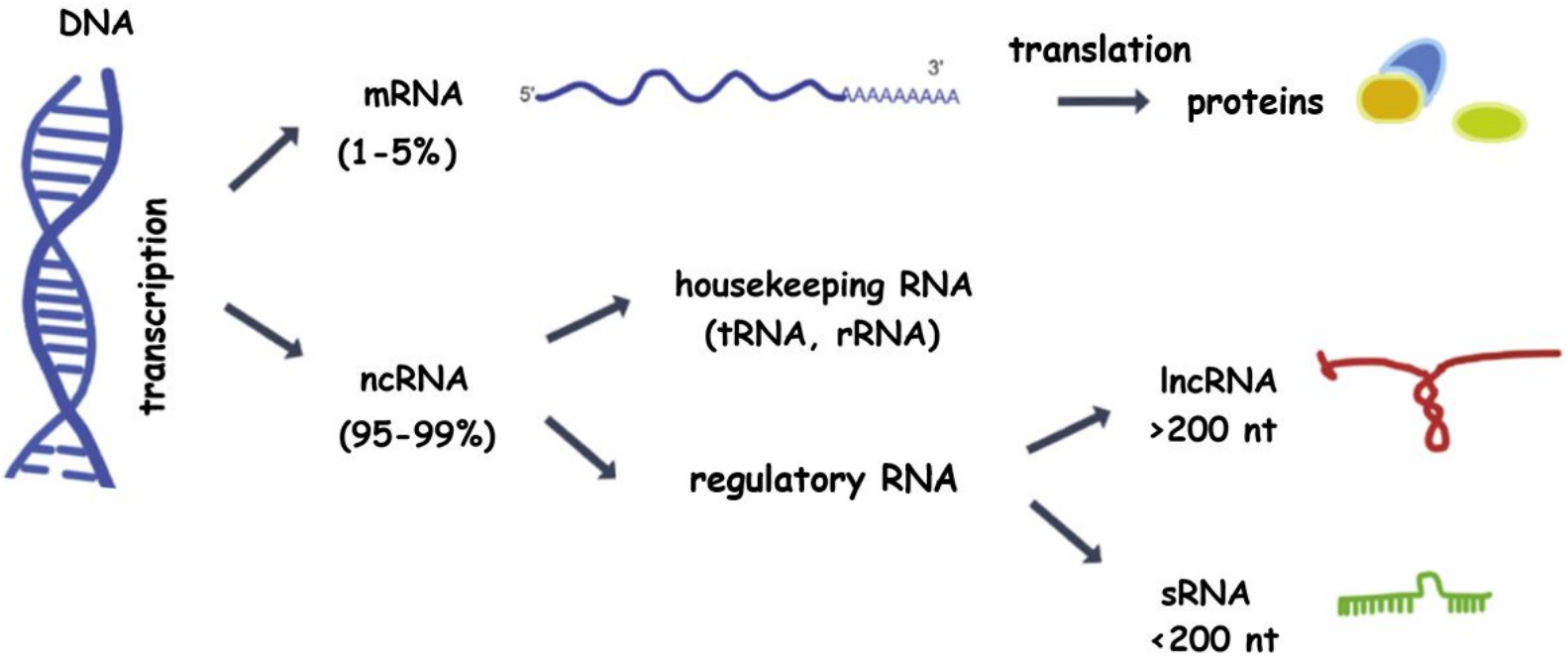
Noncoding RNA
(ncRNA)

Molecular techniques of RNA analysis

Monika Zakrzewska-Płaczek

RNA categories



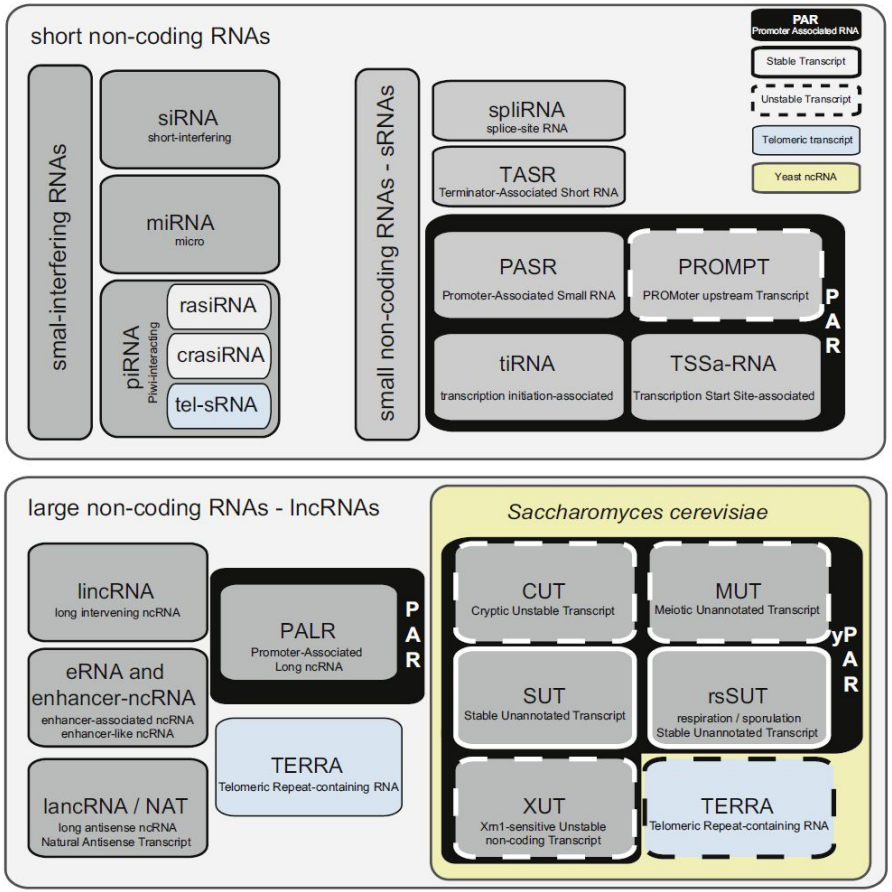
ncRNAs can be divided into 2 groups according to the functions they perform:

Housekeeping RNA Constitutive expression Necessary for normal cell functioning	**Regulatory RNA** Periodic expression e.g. in response to a stimulus, in a specific phase of development, cell cycle, etc. Impact on gene expression at the level of transcription or translation
tRNA i **rRNA** – translation **snRNA** – spliceosome components, pre-mRNA splicing **snoRNA** – rRNA maturation and modification **scaRNA** (CB specific) RNA components of **RNase P** and **RNase MRP** – endonucleases: tRNA and rRNA maturation Signal Recognition Particle **SRP RNA** – protein transport to the ER **tmRNA** (tRNA-mRNA hybrids) - directing nascent proteins for degradation **gRNA** – guide RNA (RNA editing) **telomerase RNA** – telomere synthesis	**sRNA**: siRNA (exo-siRNAs i endo-siRNAs; ta-siRNA; nat-siRNA; lsiRNAs); miRNA; piRNA  → act in transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) **lncRNA** – less known, mostly works in TGS at the chromatin level 

ncRNA types based on molecular size:

- short ncRNA <200nt
- long ncRNA >200nt

Both groups of ncRNAs are very heterogeneous, including many different RNAs with various names and functions



Tisseur M, et al. Biochimie 2011

What are small RNAs (sRNAs, smRNAs)

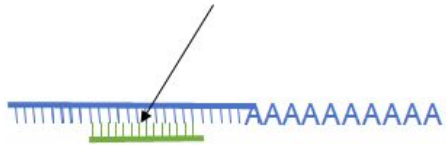
sRNA – 21-30 nt 

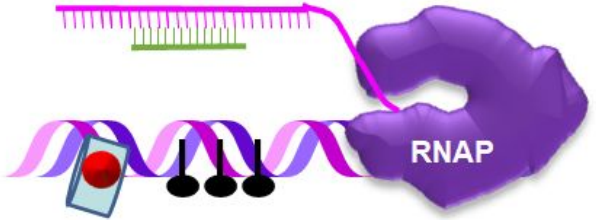
gene silencing, RNA silencing

post-transcriptional gene silencing, PTGS
mRNA degradation, translation inhibition

transcriptional gene silencing, TGS
epigenetic modifications of chromatin

Silencing specificity
is ensured by base complementarity between the silencing sRNA and the target RNA



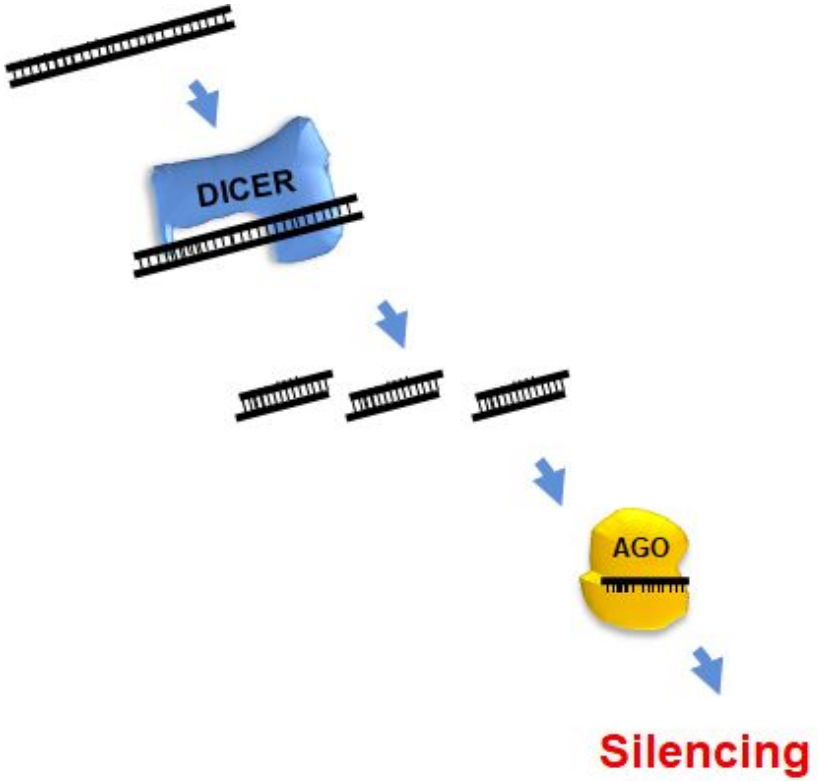


Histone modification, DNA methylation

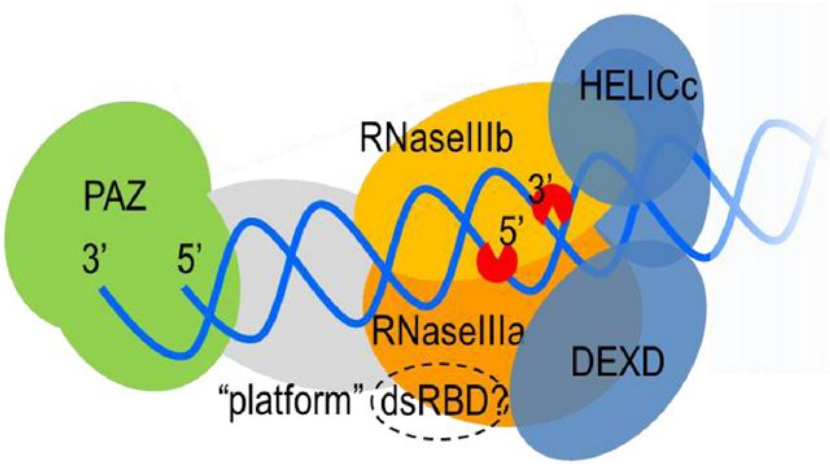
The core of RNA silencing – Dicers and Argonautes

RNA silencing uses a set of core reactions in which **double-stranded RNA (dsRNA)** is processed by **Dicer** and its homologues into **short RNA duplexes**.

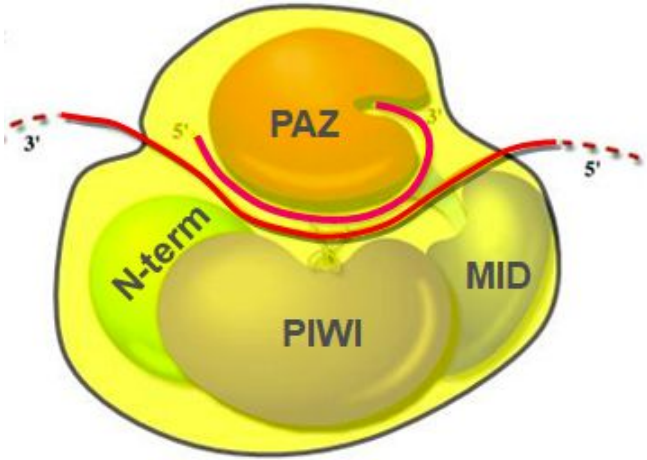
These small RNAs subsequently associate with members of the **ARGONAUTE** family of proteins to confer silencing.



The core of RNA silencing – Dicers and Argonautes



Svobodova E, et al. Pflugers Arch. 2016



In siRNA and miRNA biogenesis, **Dicer** proteins cleave long dsRNA or hairpin RNA into ~ 21 – 25 nt fragments.
Dicer's structure allows it to measure the RNA it is cleaving.

Argonaute (AGO) proteins bind small RNAs and their targets.

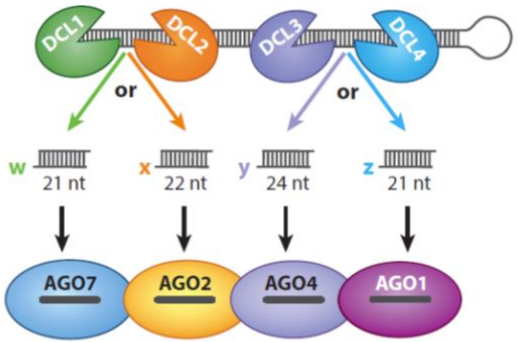
PIWI domain: RNase H-like structure in some AGO proteins → cleavage of RNA associated with sRNA (**slicer activity**)

Dicers and Argonautes in different organisms

	Species	AGO-PIWI-like		Dicer-like	RDRP
		AGO	PIWI		
Plantae	*Arabidopsis thaliana*	10	-	4	6
	Oryza sativa	18	-	5	5
Fungi	*Saccharomyces cerevisiae*	-	-	-	-
	Schizosaccharomyces pombe	1	-	1	1
	Neurospora crassa	1	-	1	3
	Aspergillus nidulans	1	-	1	2
Metazoa	*Caenorhabditis elegans*	5	3	2 (Dicer + Drosha)	4
	Drosophila melanogaster	2	3	3 (2 Dicers + Drosha)	-
	Danio rerio	4	4	2 (Dicer + Drosha)	-
	Homo sapiens	4	4	2 (Dicer + Drosha)	-

Arabidopsis thaliana:

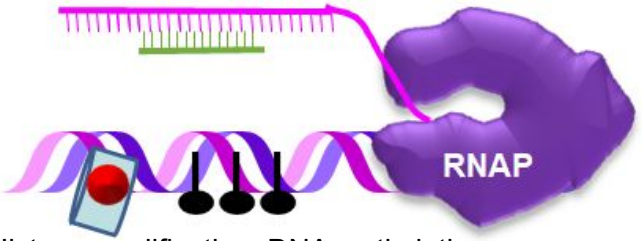
- **DCL1 → 21nt miRNA → AGO1/7/10**
- **DCL2 → 22nt siRNA**
- **DCL3 → 24nt siRNA → AGO4/6**
- **DCL4 → 21nt siRNA (tasiRNA) → AGO1**



PTGS: post-transcriptional gene silencing

miRNA *(microRNA)*	plants, animals, viruses, *Protista*	20–25nt	Drosha (u zwierząt) + Dicer	Transcription by Pol II/Pol III	Regulation of mRNA stability (mRNA cleavage), translation inhibition
mirtrons – *derived from introns of mRNA precursors of protein-coding genes; occur in animals; independent of Drosha*					
siRNA *(small interferring RNA) – most act in cis, except tasiRNA*					
exo-siRNA *(exogenous)*	plants, fungi, animals, *Protista*	21-24nt	Dicer	Transgenic, viral or other exogenous RNA	Post-transcriptional regulation of gene expression, antiviral defense
endo-siRNA *(pochodzenia endogennego)*	plants, fungi, animals, *Protista*	~21nt	Dicer	Bidirectional or convergent transcription, binding of mRNA to pseudogene transcripts of opposite orientation	Post-transcriptional and transcriptional regulation of gene expression, regulation of transposon activity
tasiRNA *(trans-acting siRNA)*	plants	21nt	DCL4	*TAS* RNA cleaved by miRNA	Post-transcriptional regulation
natsiRNA *(natural antisense transcripts-derived siRNA)*	plants	24nt 21nt	DCL2 DCL1	Stress-induced bidirectional transcription	Regulation of stress response genes

TGS: transcriptional gene silencing



Histone modification, DNA methylation

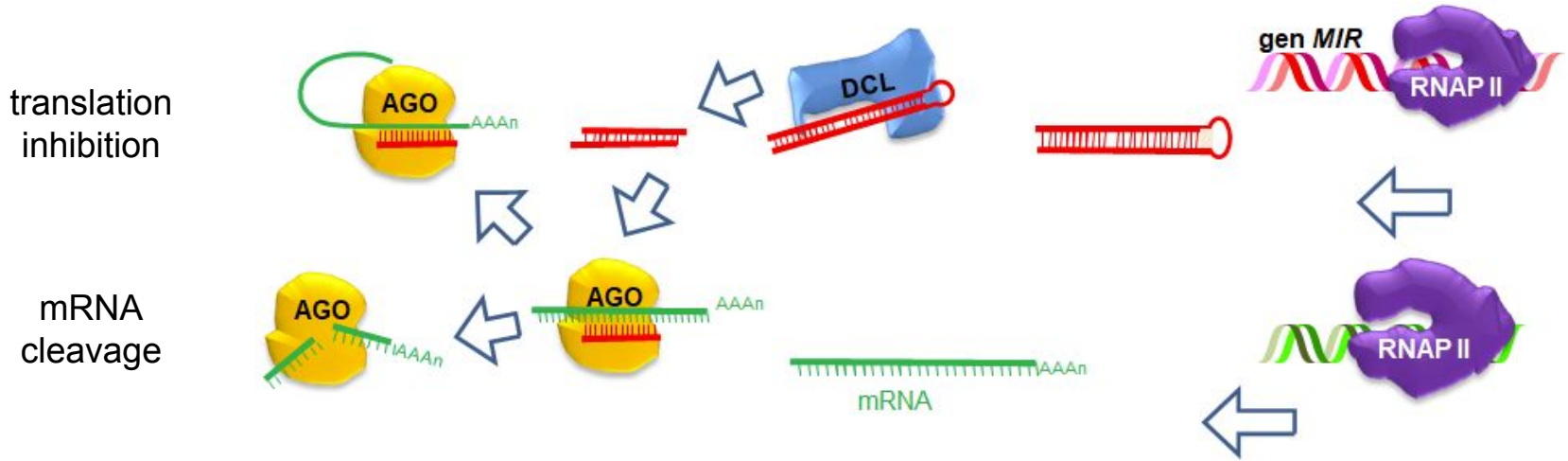
siRNA *(small interferring RNA)*					
endo-siRNA *(endpgenous)*	plants, fungi, animals, *Protista*	~21nt	Dicer	Bidirectional or convergent transcription, binding of mRNA to pseudogene transcripts of opposite orientation	Post-transcriptional and transcriptional regulation of gene expression, regulation of transposon activity
hc-siRNA *(heterochromatic siRNA)*	plants, *S. pombe*	24-26nt	DCL3	Transposons, repetitions	Chromatin modification
piRNA (Piwi-interacting RNA)	*Drosophila*, *C. elegans*, mammals, *Danio rerio*	24–30nt	Dicer -independent	Long primary transcripts (?)	Wyciszanie transpozonów, inne nieznane funkcje

miRNAs in plants

miRNAs are encoded by specific *MIR* genes, but they influence the expression of other genes - they are regulatory molecules acting in trans

miRNAs regulate developmental and physiological processes

miRNAs are believed to have evolved from siRNAs - they are created and mature in a similar (to some extent) way



microRNAs act by cutting mRNA or inhibiting translation

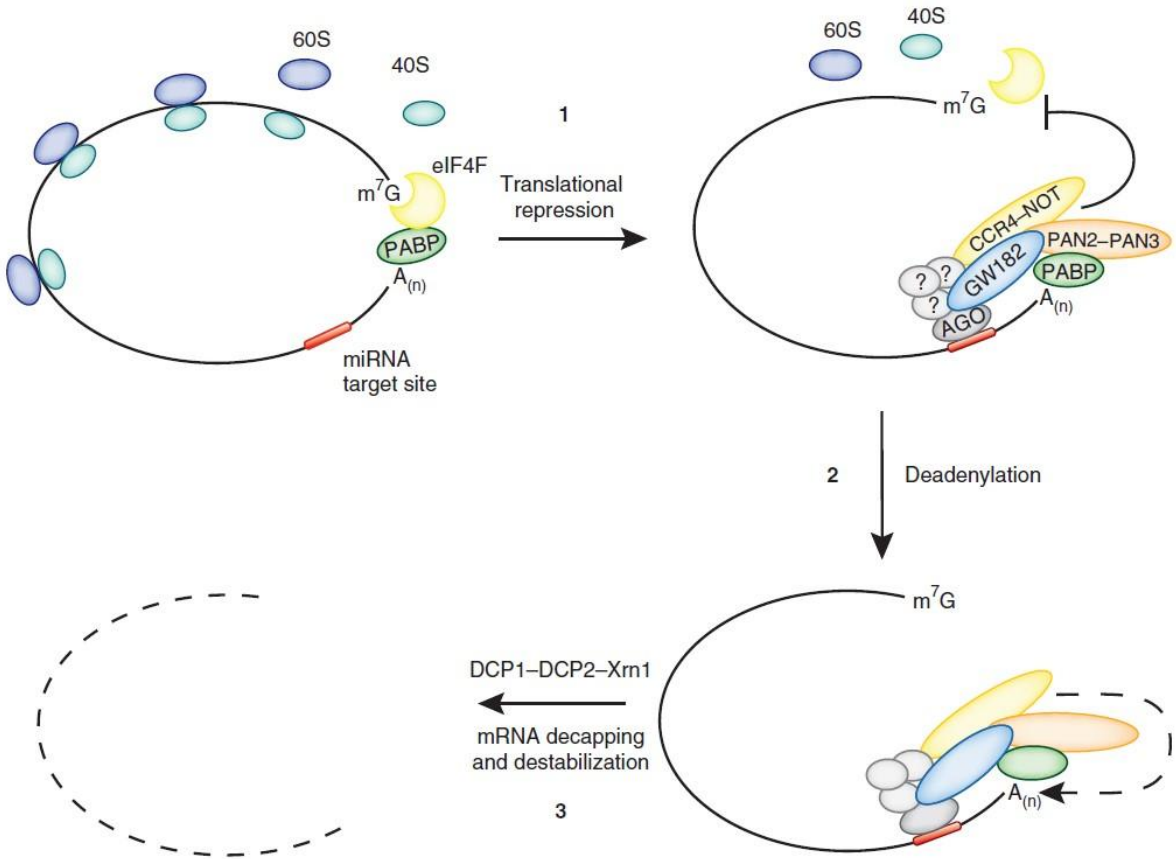
miRNAs in animals

Translational repression:

INITIATION BLOCK
miRISC inhibits translation initiation by interfering with eIF4F-cap recognition and 40S recruitment or by antagonizing 60S subunit joining and preventing 80S ribosomal complex formation.

Interaction of GW182 with PABP might interfere with the closed loop formation mediated by the eIF4G-PABP interaction and this contributes to the repression of translation initiation.

POST-INITIATION BLOCK
miRISC might inhibit ribosome elongation, induce ribosome drop-off or facilitate proteolysis of nascent polypeptides.



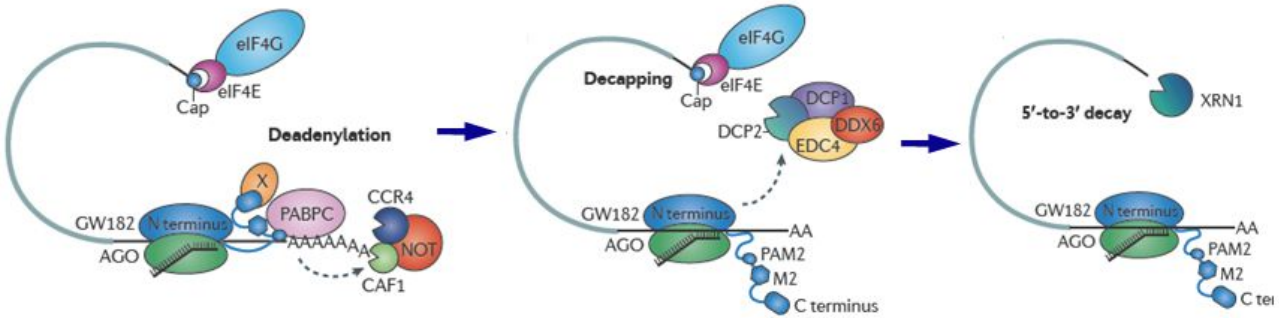
Fabian MR, Sonenberg N. NSMB 2012

Vol 466 | 12 August 2010 | doi:10.1038/nature09267 nature

Mammalian microRNAs predominantly act to decrease target mRNA levels

Huili Guo[1,2], Nicholas T. Ingolia[3,4], Jonathan S. Weissman[3,4] & David P. Bartel[1,2]

Destabilization of target mRNA is the predominant reason for reduced protein output.



Kinetic analysis reveals successive steps leading to miRNA-mediated silencing in mammalian cells

Julien Béthune[1+], Caroline G. Artus-Revel[1] & Witold Filipowicz[1,2++]

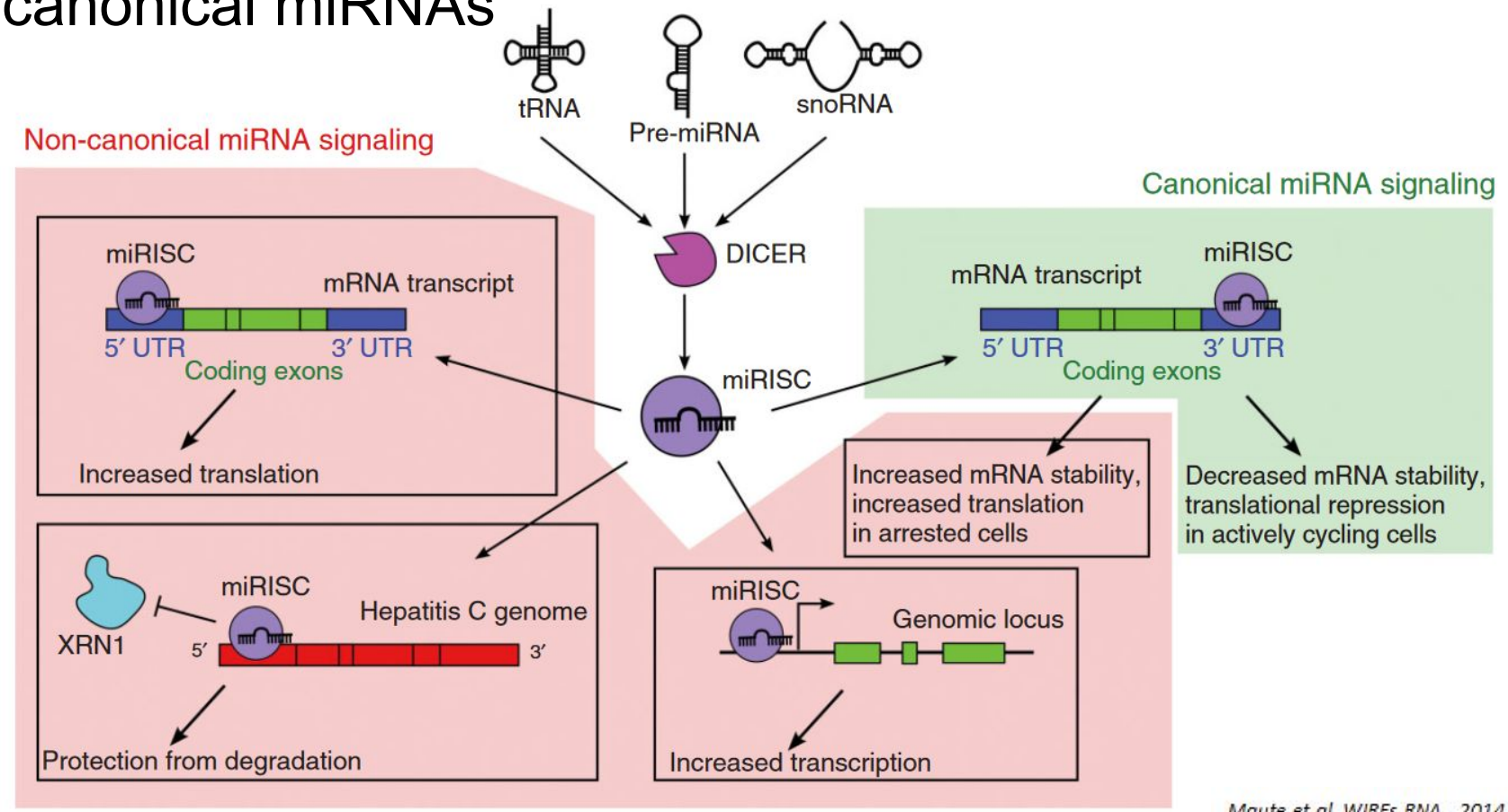
EMBO *reports* VOL 13 | NO 8 | 2012

Step 1 Initial effect of miRNAs: inhibition of translation at the initiation step without mRNA decay.

Step 2 mRNA deadenylation by PAN2–PAN3 and CCR4–NOT complexes recruited by miRISC as a consequence of translation inhibition that makes poly(A) tail more accessible.

Step 3 Stimulated deadenylation potentiates the effect on translational inhibition and leads to decay of target mRNAs through the recruitment of the decapping machinery.

Non-canonical miRNAs



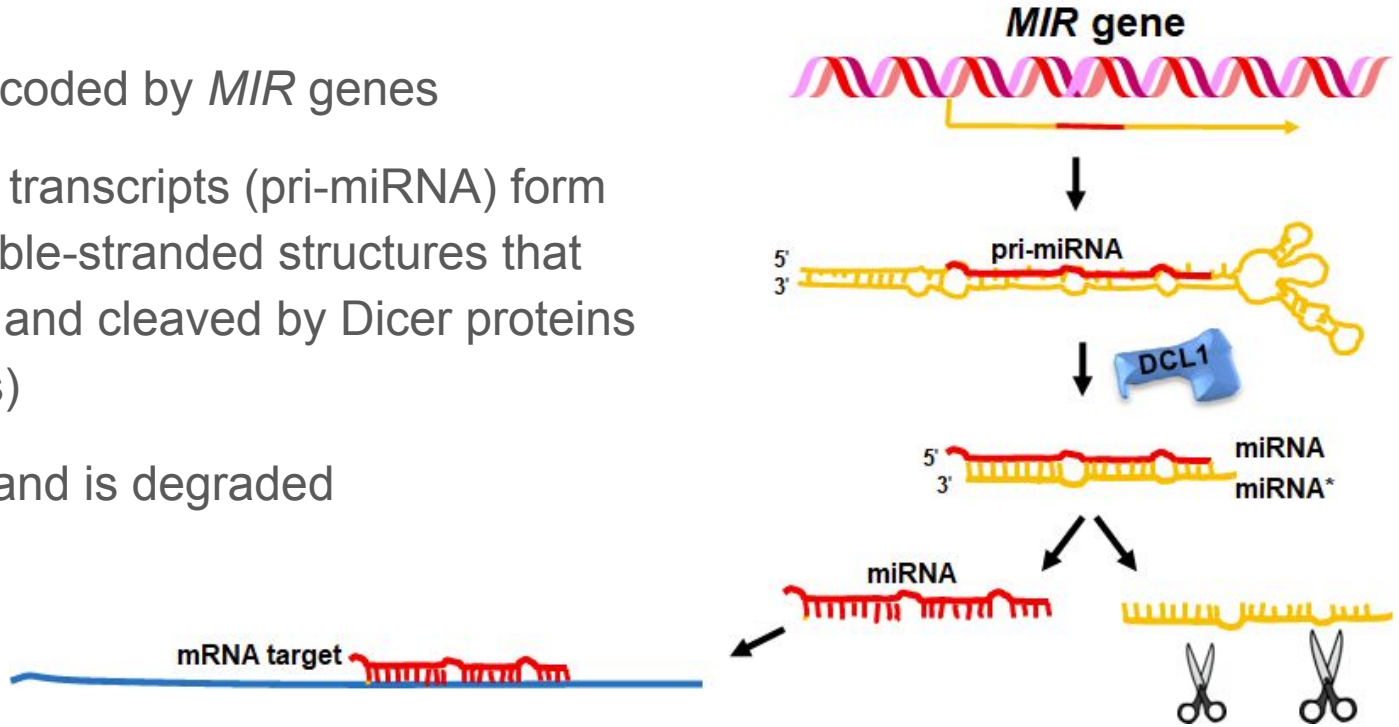
Maute et al, WIREs RNA., 2014

MIR genes: transcription of long pri-miRNA molecules that give rise to miRNAs

miRNAs are encoded by *MIR* genes

primary miRNA transcripts (pri-miRNA) form secondary, double-stranded structures that are recognized and cleaved by Dicer proteins (DCL1 in plants)

the miRNA* strand is degraded

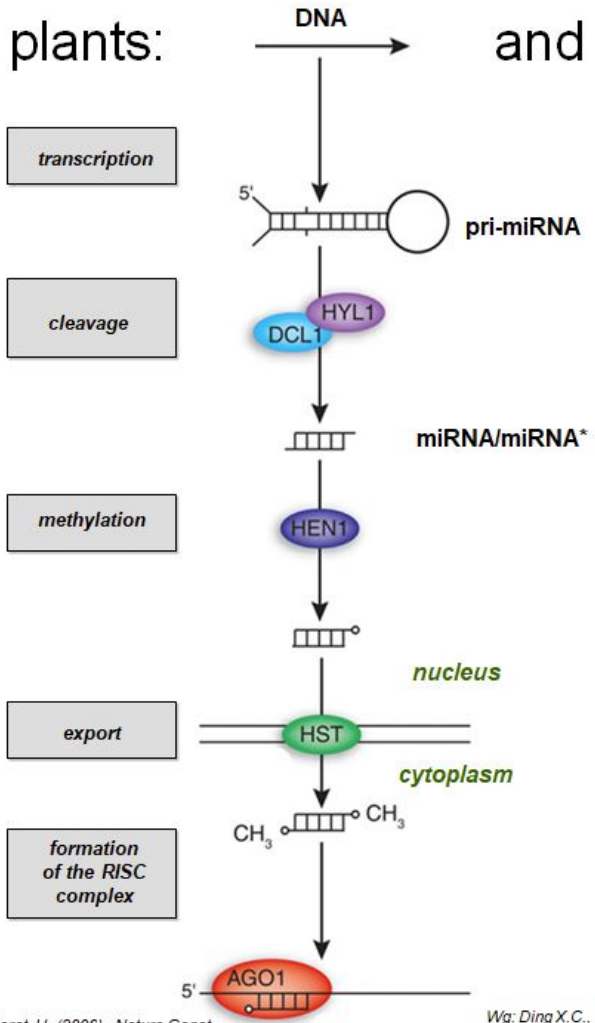


miRNA biogenesis in plants:

HYL1:
dsRNA-binding protein

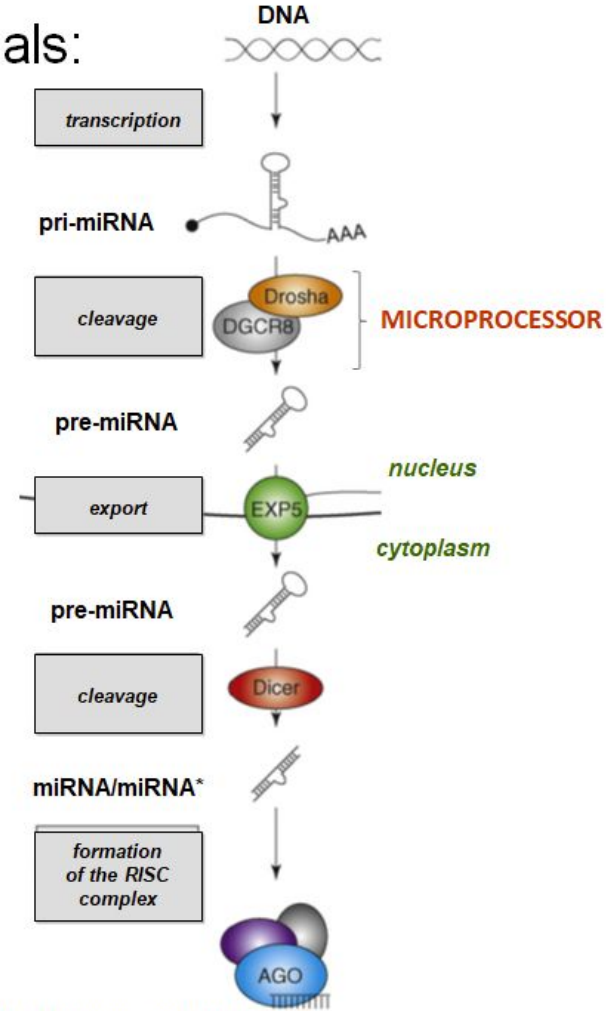
HEN1:
methyltransferase
methylation of miRNA/miRNA*

HST (*HASTY*):
miRNA/miRNA* export
exportin 5 (EXP5) homolog



Mallory A., Vaucheret H. (2006), Nature Genet.

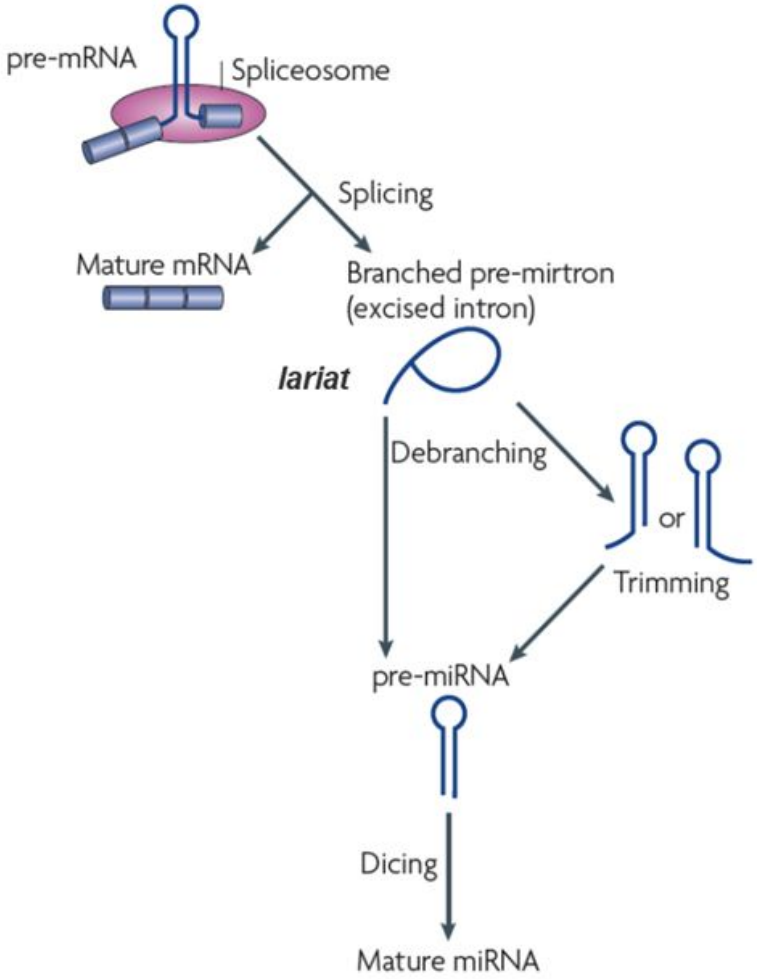
and animals:



Wg: Ding X.C., Weiler J., Grosshans H. (2009), Trends in Biotechnology

Mirtrons

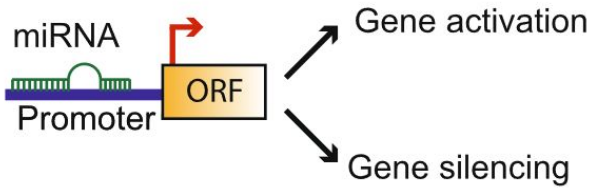
- present in *D. melanogaster*, *C. elegans*, and mammals
- miRNAs can be created from introns cut out from pre-mRNA during mRNA splicing
- independent of Drosha
- cleavage of the lariat structure (debranching) leads to the formation of pre-miRNA
- pre-miRNA → miRNA biogenesis

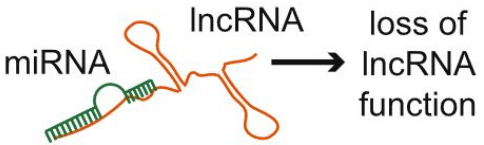


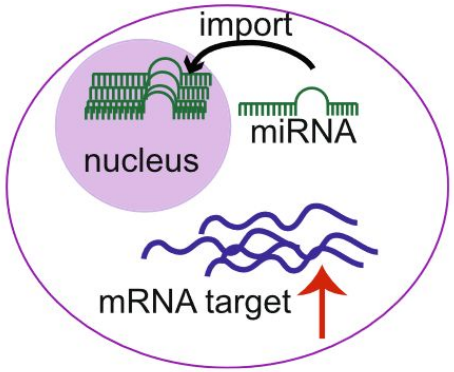
Kim V. N., Han J., Siomi M.C. (2009) Mol Cell Biol

miRNA nuclear functions

miRNAs present in the cell nucleus and nucleolus form a smaller nuclear miRISC complex with AGO2/AGO3, DICER, TRBP and TNRC6A proteins (TGA)







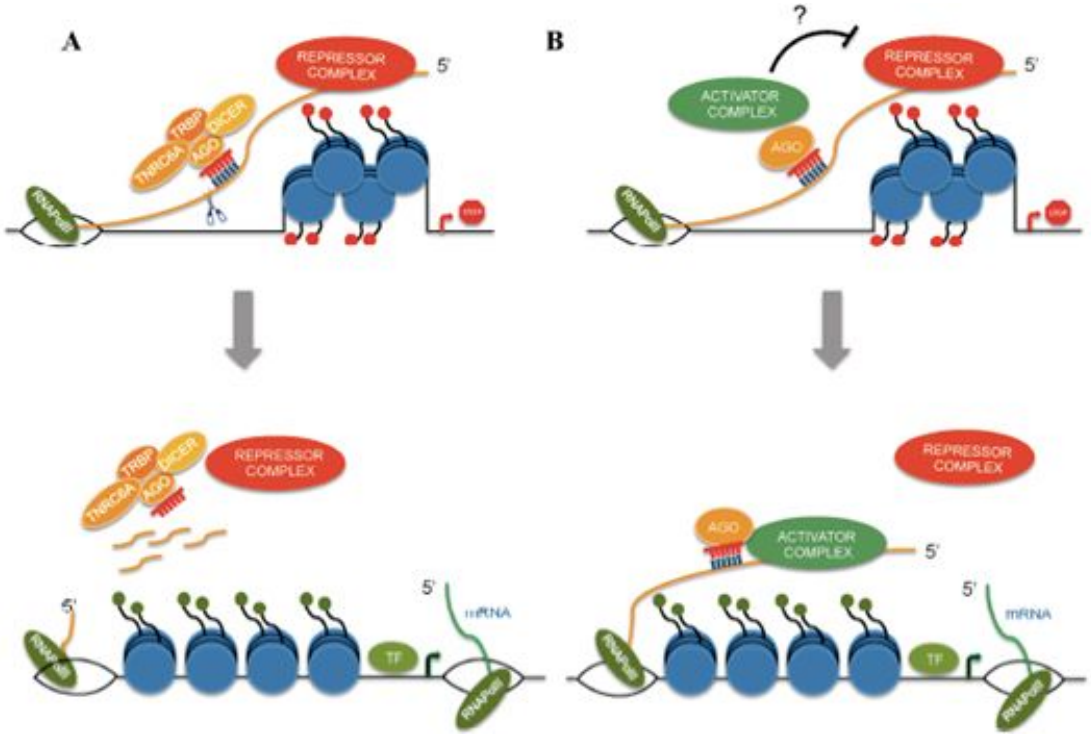
Rasko and Wong, Journal of Hematology & Oncology, 2017

miRNA nuclear functions

TGA - transcriptional gene activation

Long ncRNA can regulate/inhibit gene expression by recruiting a transcriptional repressive complex. miRNA targeting a complementary sequence within the lncRNA would recruit a nuclear RISC and induce cleavage of lncRNA promoting exclusion of repressive complex.

Alternatively, miRNAs induce gene activation by recruiting a complex of transcriptional activators.



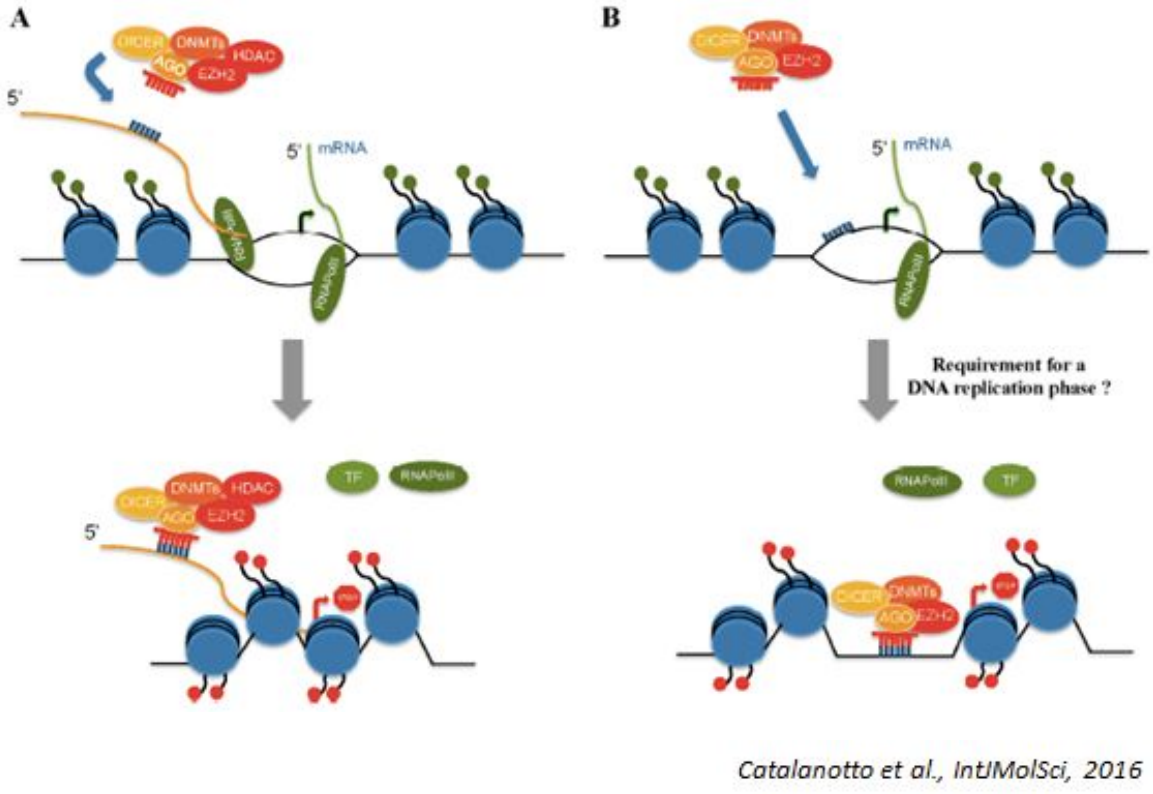
Catalanotto et al., IntJMolSci, 2016

miRNA nuclear functions

TGS - transcriptional gene silencing

miRNA-targeted non-coding promoter associated RNA represents a docking platform for a protein inhibitory complex (RISC, PcG proteins and chromatin modulators) → this enables a protein inhibitor complex to be in close proximity of the targeted region → modification of chromatin structure.

miRNA guided recognition and interaction with promoter regions might occur through a direct inetraction between RNA and ssDNA complementary regions.



Catalanotto et al., IntJMolSci, 2016

siRNA: protecting and maintaining genome stability

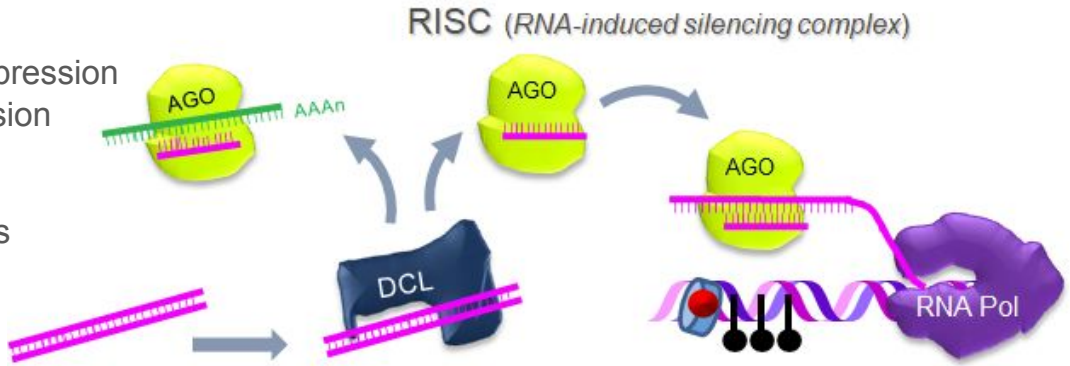
exo-siRNA:

protection of the genome against "foreign" genetic material:
transgenic
viral (VIGS – viral induced gene silencing)

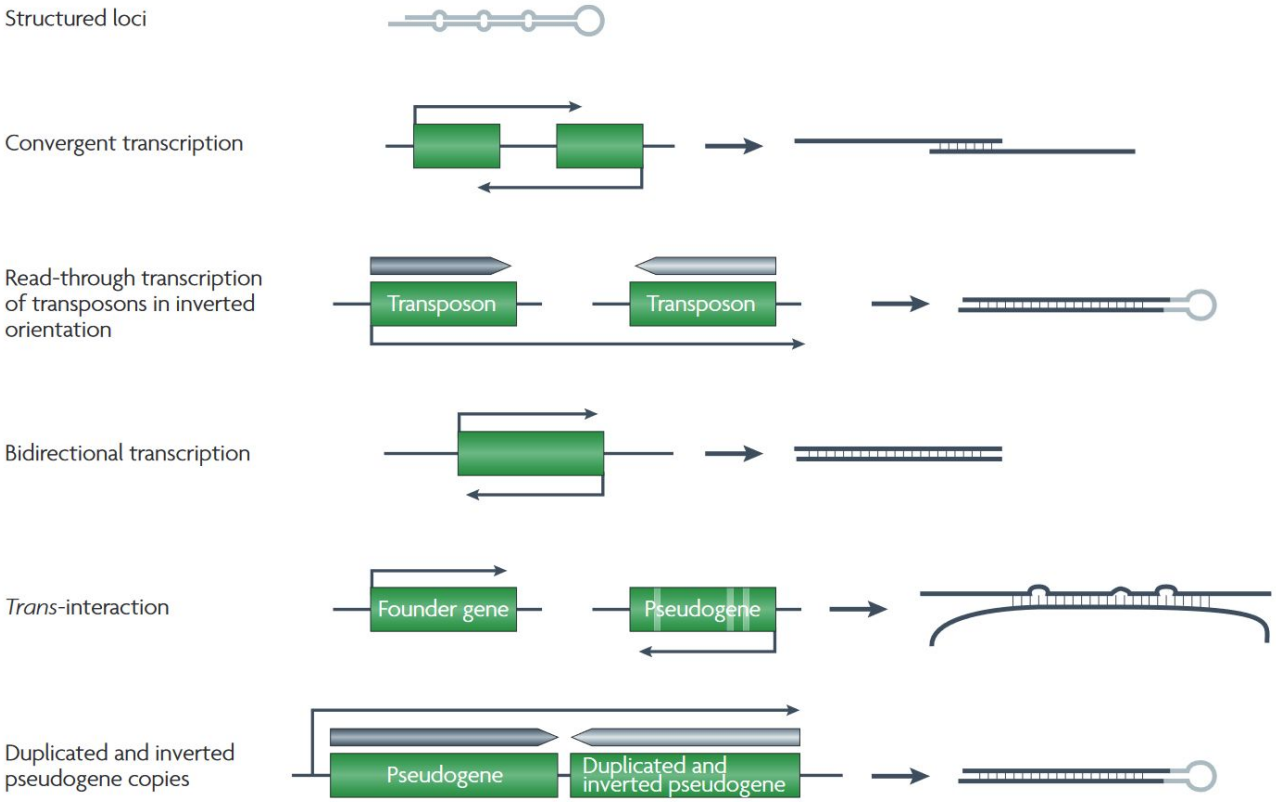
Artificially introduced transgenes are often silenced by siRNA; post-transcriptionally or transcriptionally

Silencing can be triggered by:
very high level of transgene expression
double-stranded RNA derived from transgene expression
abnormal RNAs resulting from transgene expression

endo-siRNA:
silencing of transposons and repeated sequences
keeping some genes in an epigenetically
inactive state



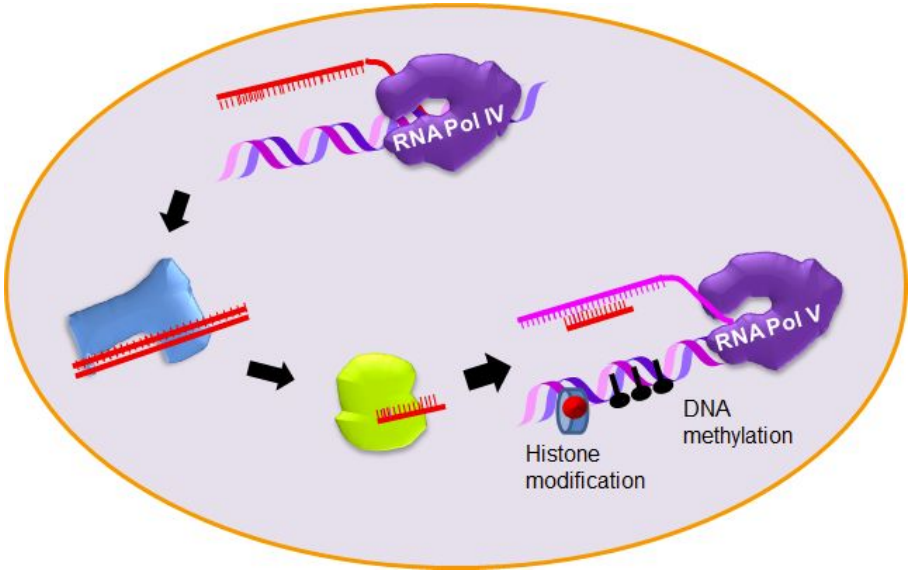
Endogenous siRNA



Ghildiyal & Zamore (2009) Nat Rev Genet

Endogenous siRNA in plants: hc-siRNA

= heterochromatin siRNA



Two plant-specific RNA polymerases are associated with the biogenesis and function of hc-siRNA:

- RNA polymerase IV participates in siRNA biogenesis
- RNA polymerase V noncoding transcripts direct the silencing machinery to appropriate DNA sequences

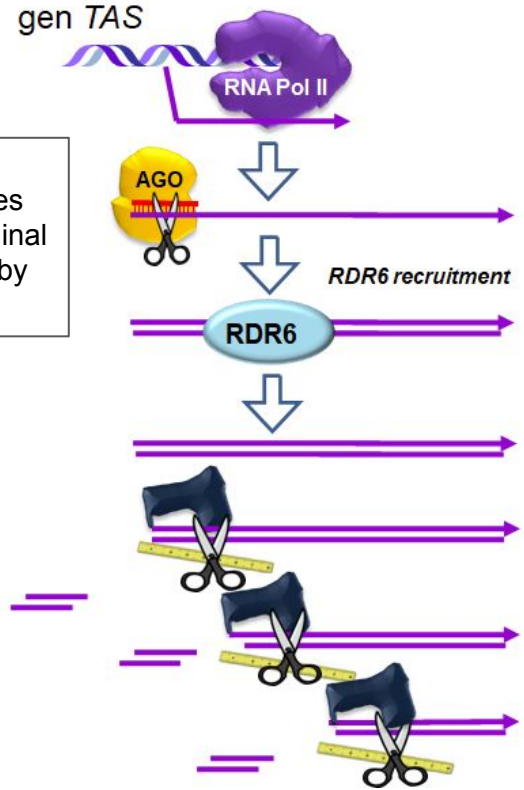
=> RNA-directed DNA Methylation (RdDM)

This type of silencing is often associated with permanently transcriptionally inactive DNA, including centromeric regions and transposons, but also occurs in genes.

tasiRNA: plant endogenous siRNA

(trans-acting siRNA)

- plant specific
- encoded by TAS genes
- processing of the original transcript is initiated by miRNA



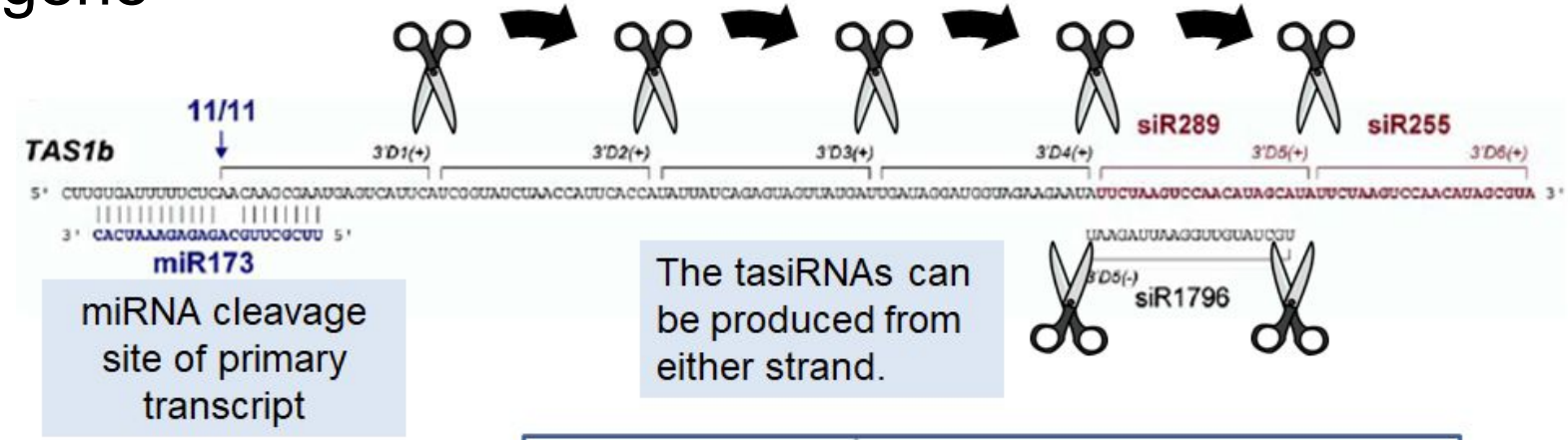
transcription of the TAS locus by RNA polymerase II

miRNA/RISC binding and cleavage

second strand RNA synthesis by RDR6 (RNA-dependent RNA polymerase)

dsRNA is cleaved by DCL4 into a series of shorter dsRNAs, releasing multiple tasiRNA molecules from a single TAS gene

Several “phased” tasiRNAs are derived from each *TAS* gene

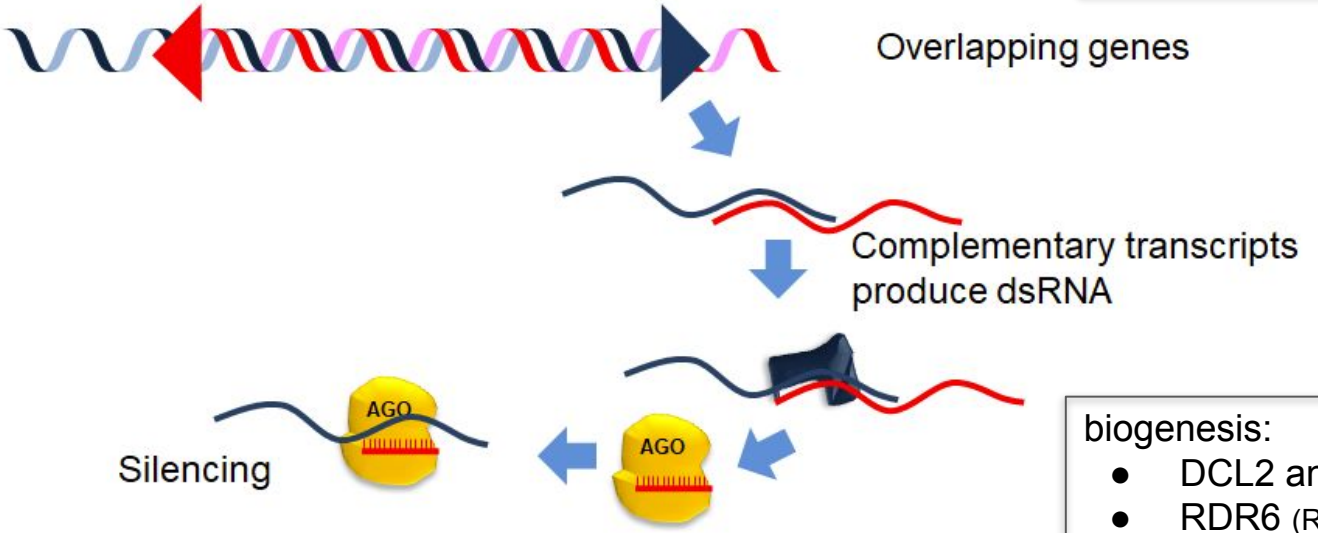


crucial for ensuring the specificity of tasiRNA; DCL4 begins to cleave the precursor accurately at this point and cuts at intervals of 21nt

Reprinted from Allen, E., Xie, Z., Gustafson, A M., and Carrington, J.C. (2005) microRNA-directed phasing during *trans*-acting siRNA biogenesis in plants. Cell 121: 207-221, with permission from Elsevier.

natsiRNAs: plant endogenous siRNAs

Nat-siRNAs – Natural *cis*-acting siRNAs
Derived from overlapping transcripts
Involved in abiotic and biotic stresses



A. thaliana: **lsiRNAs** (long siRNAs; 30-40nt) also arise from NAT transcripts, other proteins participate in biogenesis

biogenesis:

- DCL2 and/or DCL1
- RDR6 (RNA-dependent RNA polymerase)
- SGS3 (RNA-binding protein)
- RNA polymerase IV

Dicer-independent small RNAs: piRNAs

~25-30 nt

2'-O-methylated 3' ends

occur in animals,
identified in *Drosophila* germline

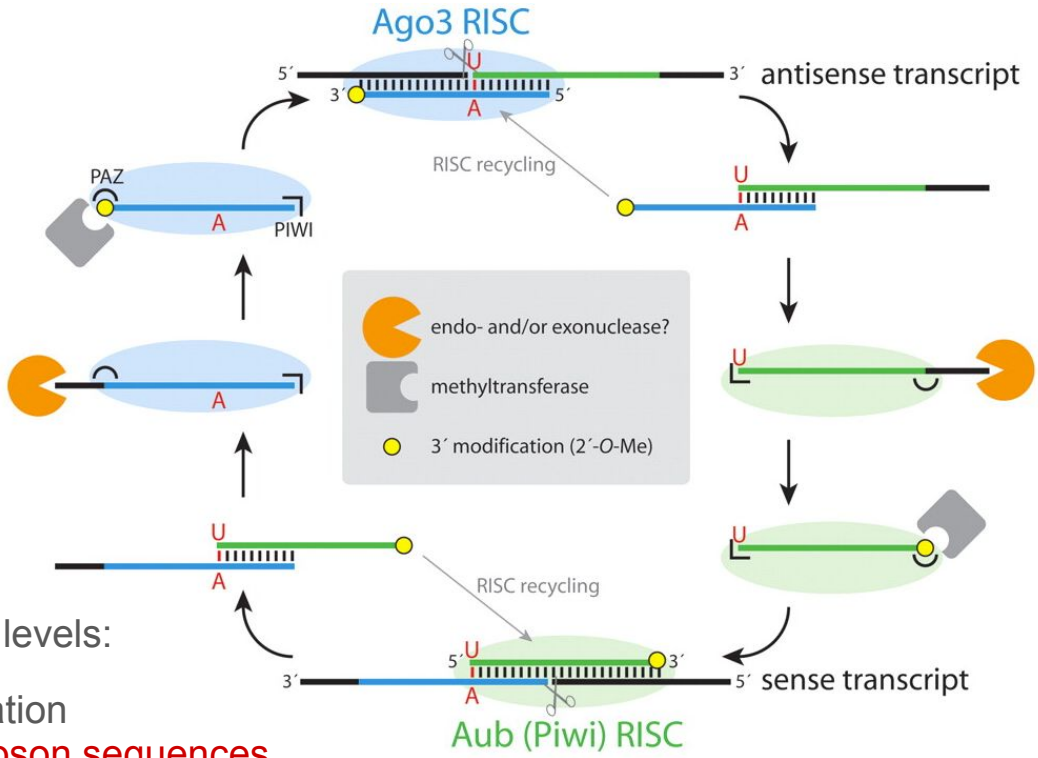
bind to PIWI proteins:

- Piwi, Aubergine, Ago3 – *Drosophila*
- MILI, MIWI, MIWI2 – mouse
- HILI, HIWI1, HIWI2 – human

silencing of transposons and DNA repeats

it is believed that they can operate at different levels:

- posttranscriptionally – transcript degradation
- in mammals, DNA methylation of transposon sequences



Hartig et al., 2007, Genes Dev., modified

Dicer-independent small RNA: priRNA

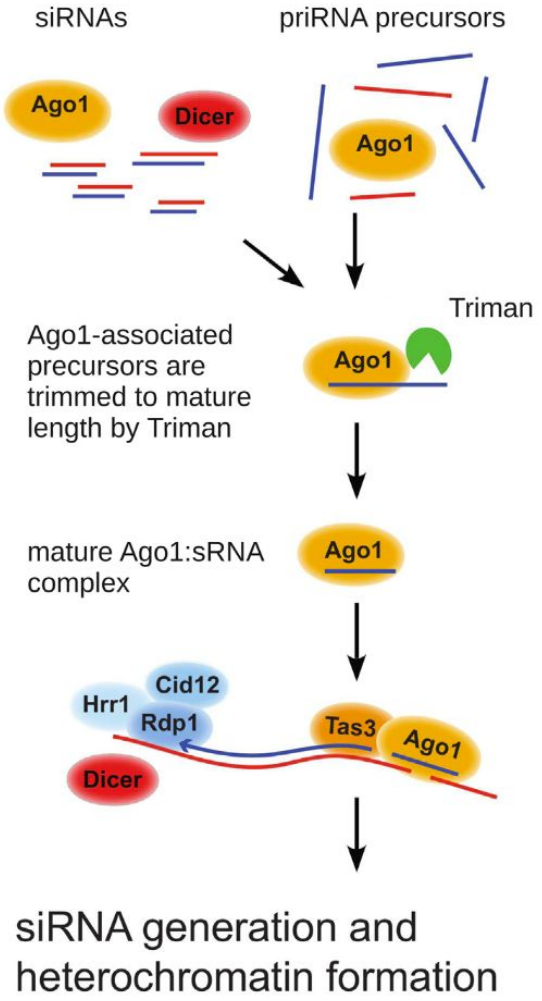
priRNA – primal small RNAs

- identified in *S.pombe*
- formation/maintenance of heterochromatin in centromeric regions
- Triman: 3'-5' exoribonuclease
  – processing of priRNA and siRNA precursors

Molecular Cell
Article

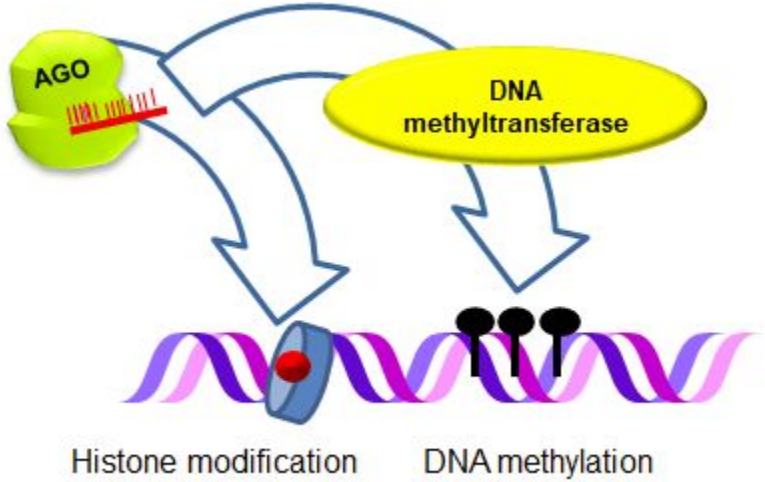
Argonaute and Triman Generate Dicer-Independent priRNAs and Mature siRNAs to Initiate Heterochromatin Formation

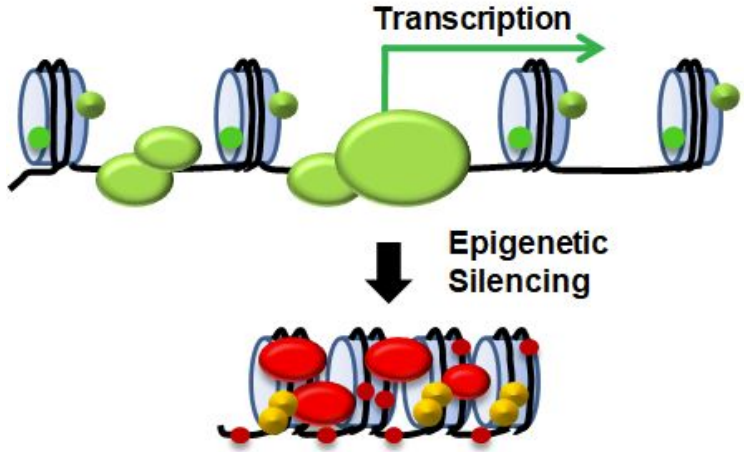
Mirela Marasovic,[1] Manuel Zocco,[1] and Mario Halic[1,*]
[1]Gene Center Munich and Department of Biochemistry, Ludwig-Maximilians-Universität München, 81377 Munich, Germany



Transcriptional gene silencing (TGS)

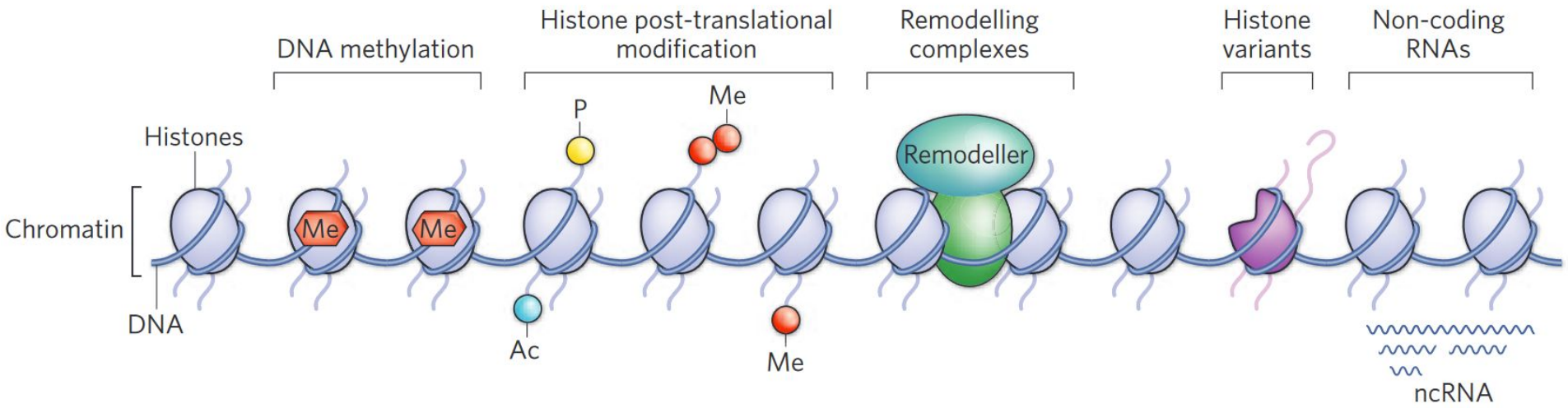
- siRNAs can silence DNA through enzymes that methylate cytosine or modify histone proteins
- Two plant-specific RNA polymerases are involved in the mechanism of transcriptional DNA silencing by siRNA: Pol IV and Pol V





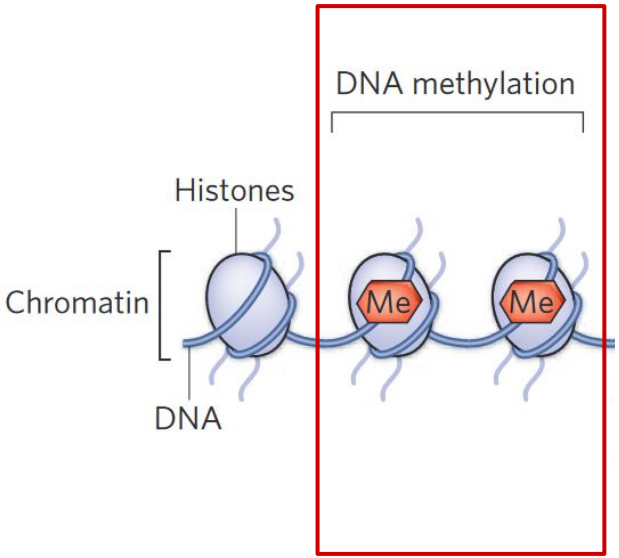
Epigenetic regulation of gene expression

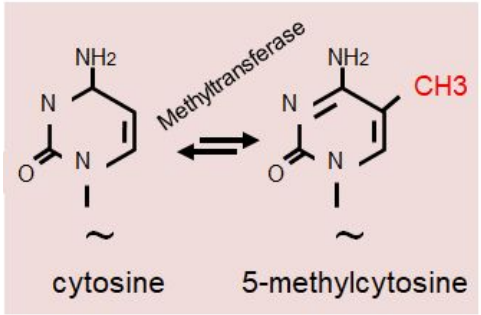
Epigenetic regulation of gene expression



Dulac C., 2010, Nature

DNA methylation



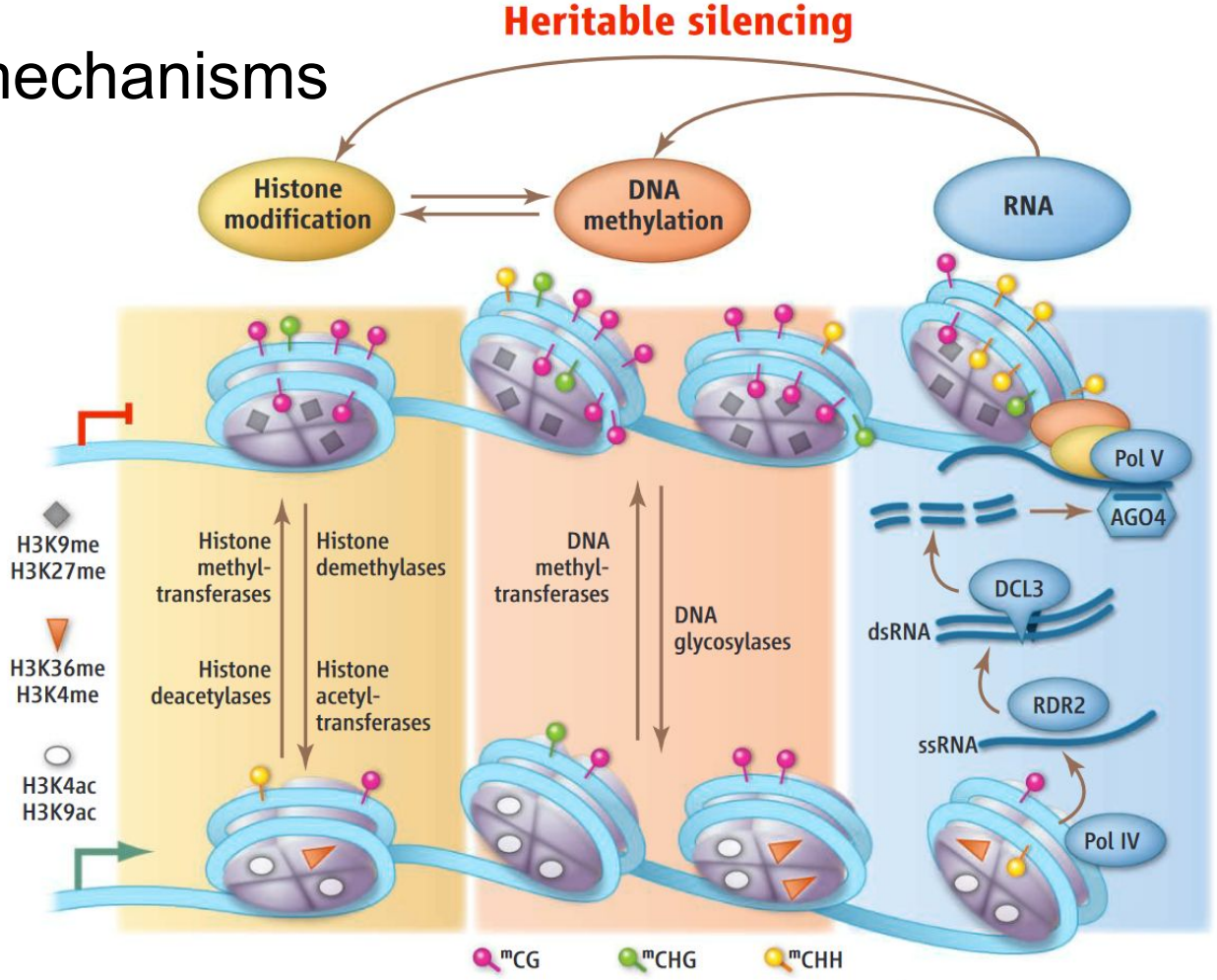


DNA can be covalently modified by cytosine methylation.

The role of DNA methylation: imprinting, X chromosome inactivation, embryonic development, repression of repeated sequences and transposons

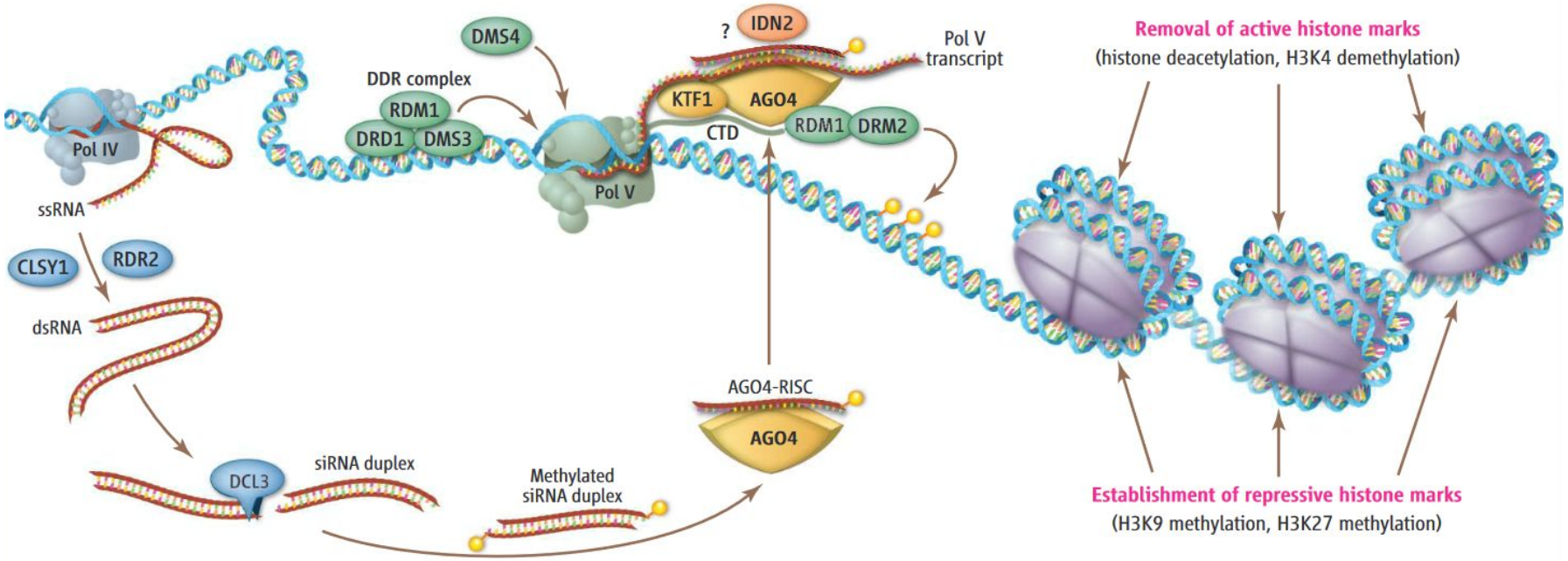
Plant epigenetic mechanisms

Plant epigenetic mechanisms include DNA methylation, histone modification, and RNA-directed DNA methylation (RdDM). RdDM involves two plant-specific RNA polymerases (Pol IV and Pol V), an RNA-dependent RNA polymerase (RDR2), an enzyme that cleaves dsRNA (DCL3), and an Argonaute-family RNA-binding protein (AGO4).



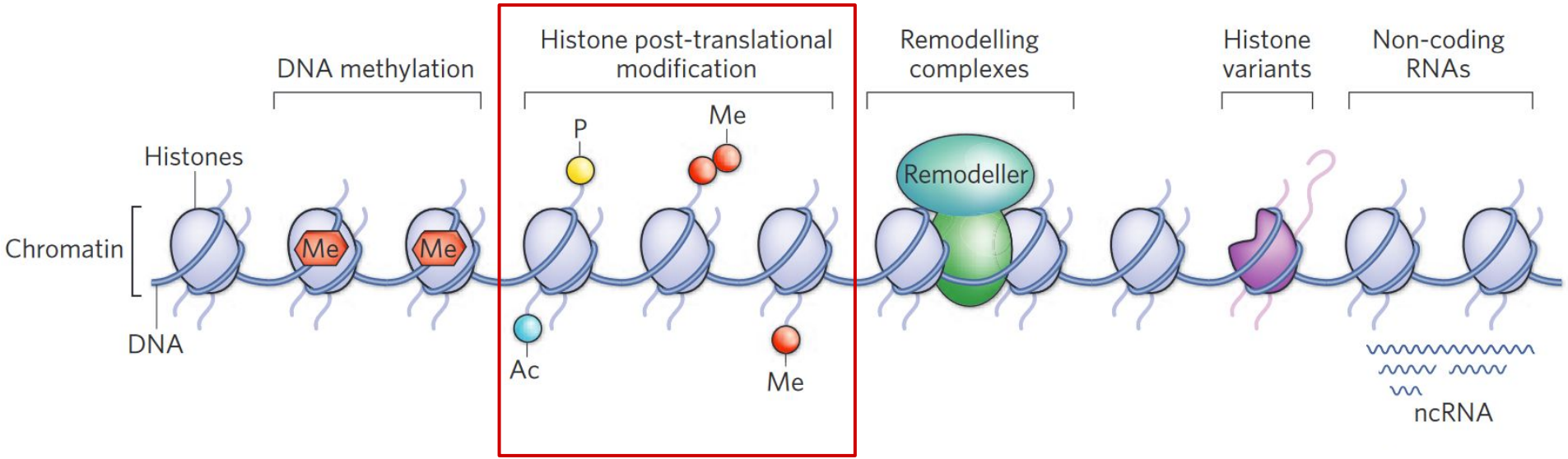
Fedoroff N. V., 2012, Science

The RNA-directed DNA methylation pathway



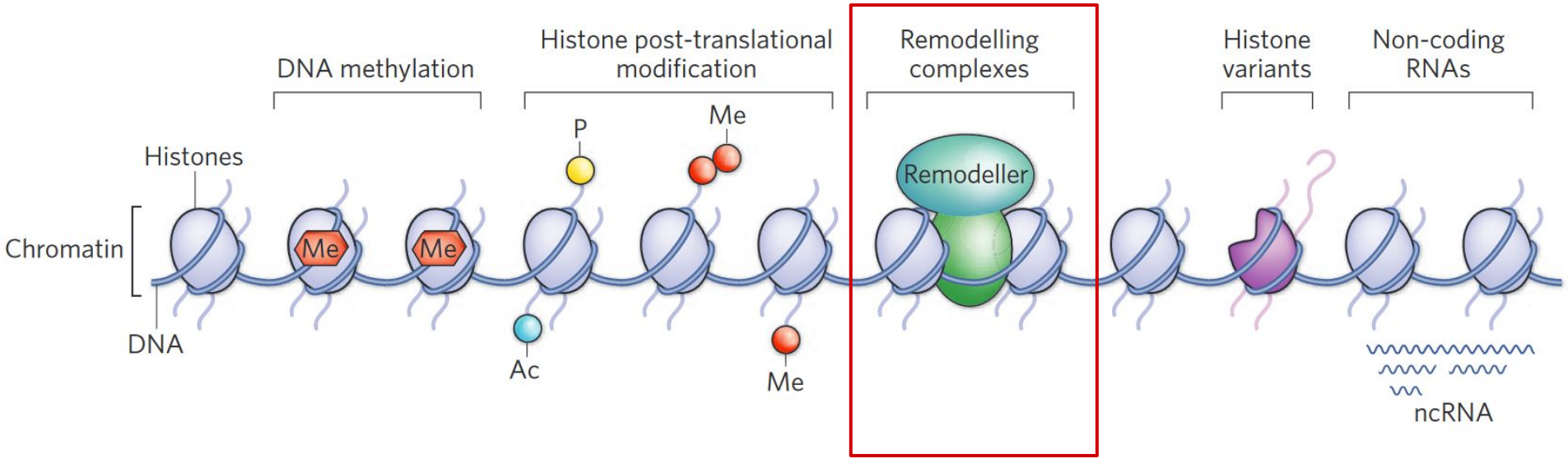
Fedoroff N. V., 2012, Science

Post-translational modifications of histones



Modifications of histone proteins influence changes in chromatin structure. Depending on the site, modifications may contribute to activation or inactivation of transcription.

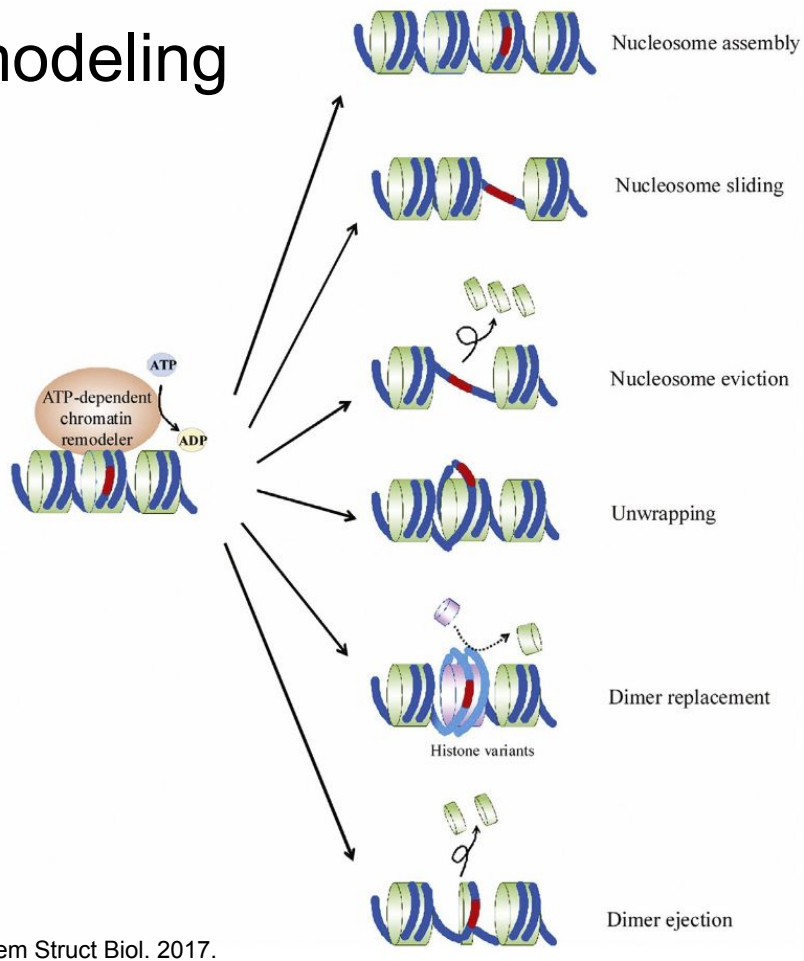
Chromatin remodeling



The activity of chromatin-remodeling complexes depends on ATP, and as a result of their action, the manner of histone-DNA interaction changes. Remodeling complexes are involved in both transcriptional activation and repression.

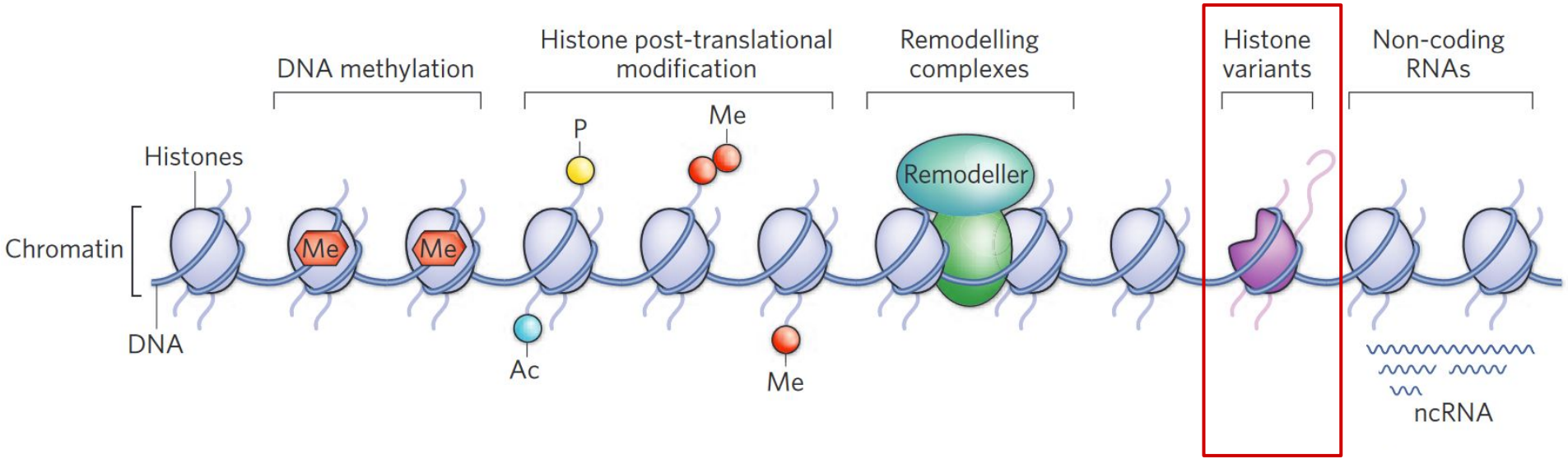
Consequences of chromatin remodeling

Upon ATP addition, chromatin remodelers bound on the chromatin change the nucleosome conformation, which results in the exposure of the binding site (red) for transcriptional regulators (octamer sliding, octama eviction, or localized unwrapping), or the altered composition of nucleosomes (histone replacement or histone ejection)



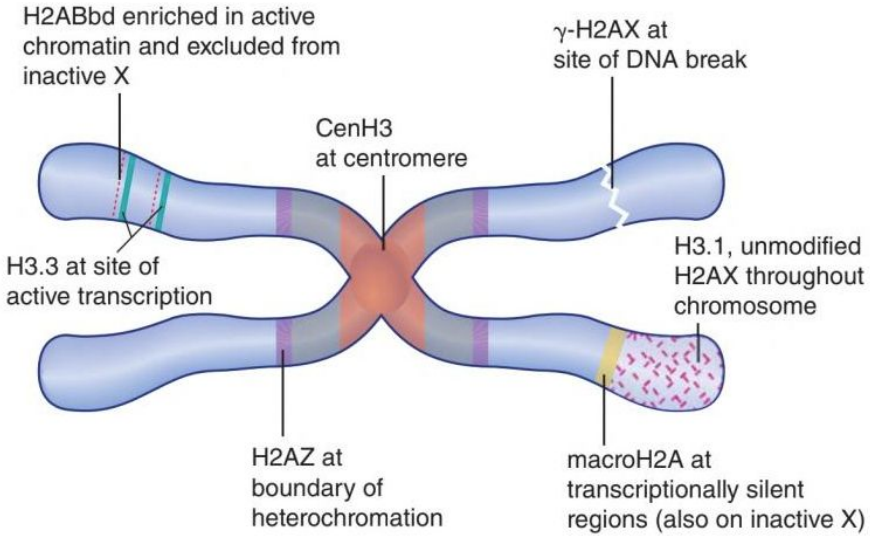
Chen W, et al. Adv Protein Chem Struct Biol. 2017.

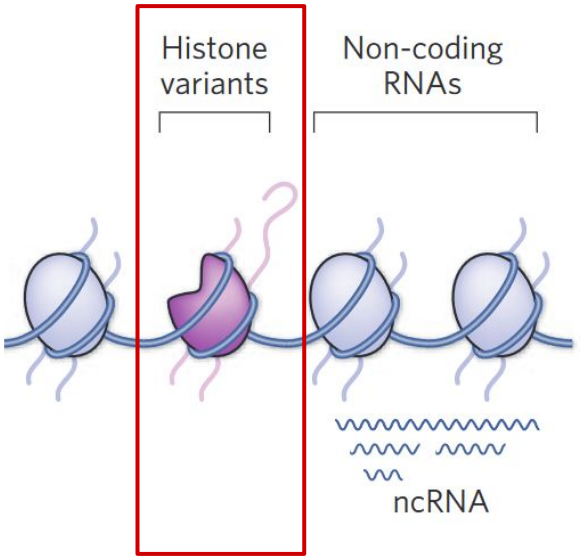
Histone variants



Chromatin remodeling complexes from the INO80/SWR family are responsible for the exchange of various histone variants in the histone octamer.

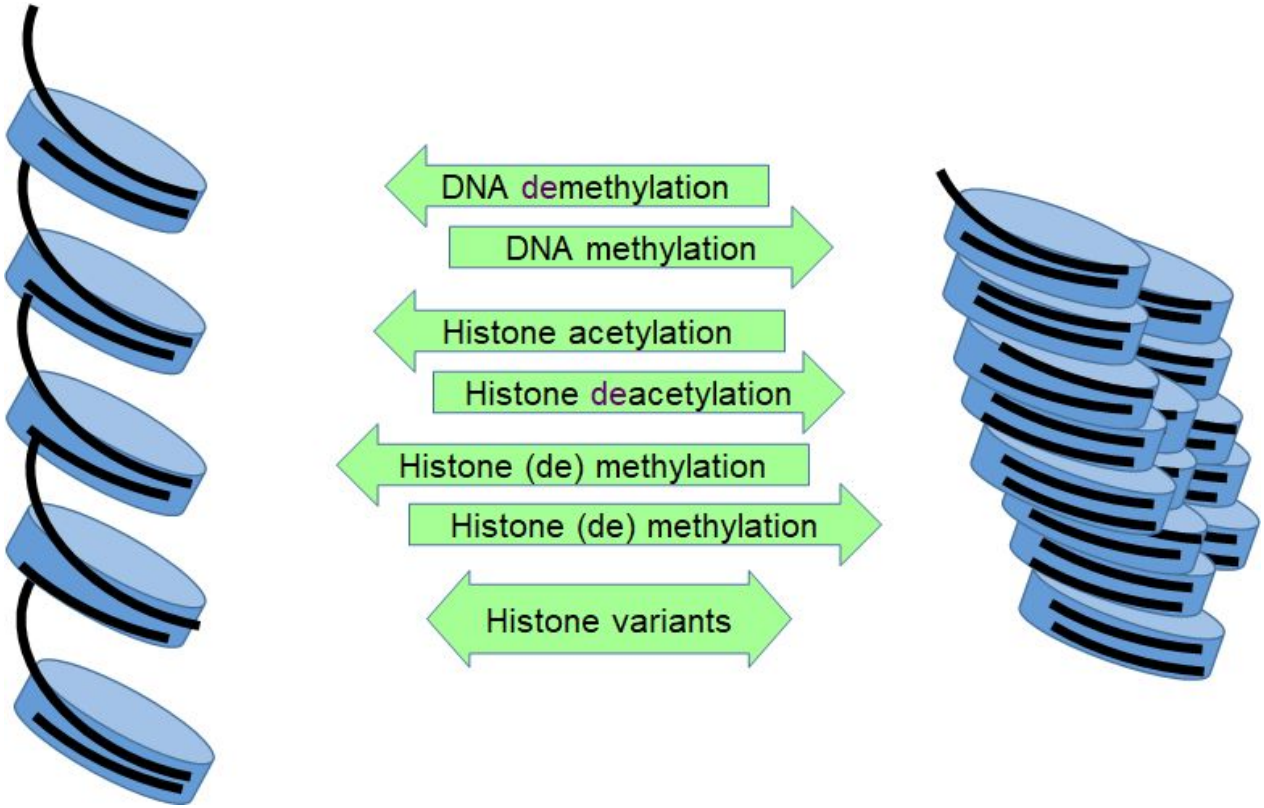
Histone variants - characteristic patterns



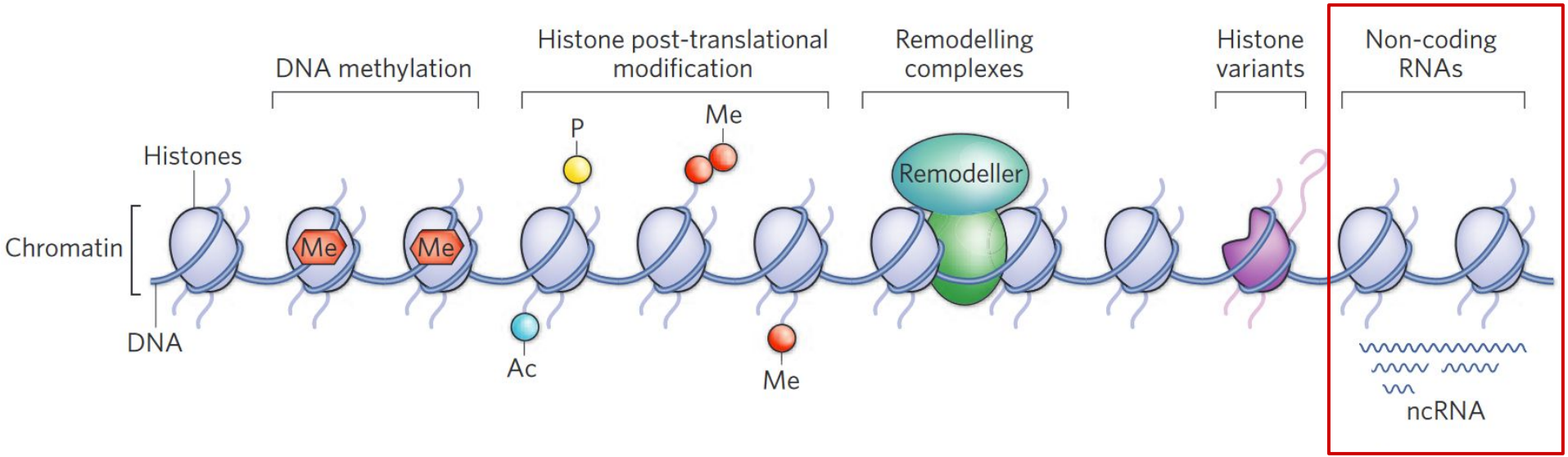


Some histone variants are spread throughout all or most of the chromosome, whereas others show specific distribution patterns. Histone variant distributions can be different on dosage-compensated sex chromosomes (like the mammalian inactive X), in sperm chromatin, or other highly specialized chromatin states.

Epigenetic mechanisms of regulation of gene expression

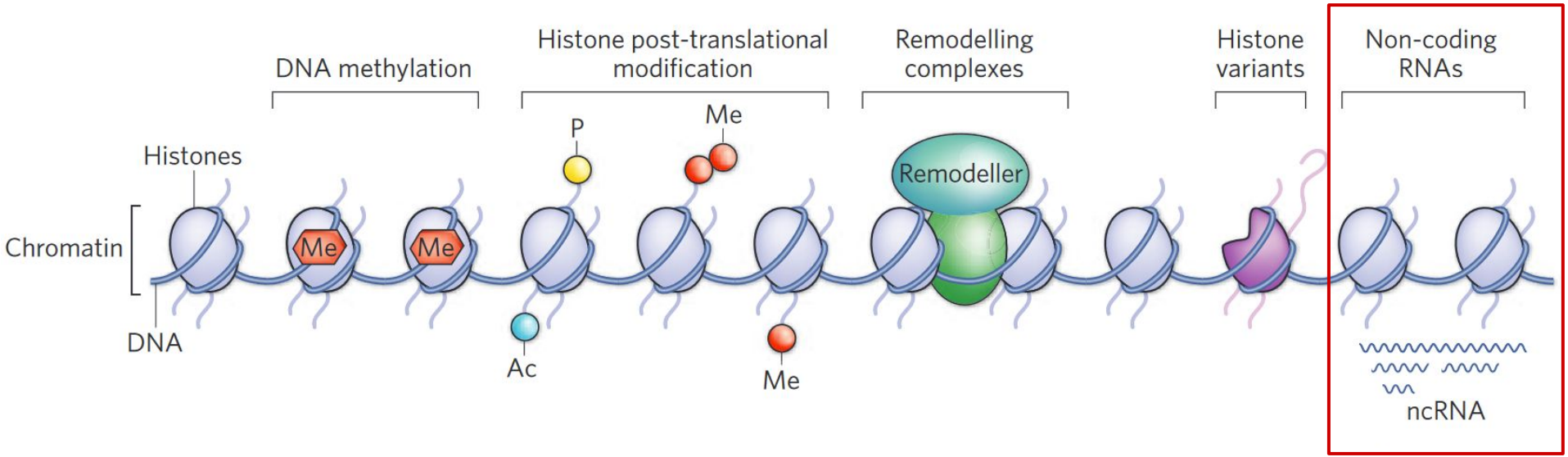


Epigenetic regulation of gene expression by ncRNAs



So far, the only known factor initiating epigenetic inheritance and distinguishing sequences that need to be silenced or activated is RNA

Epigenetic regulation of gene expression by ncRNAs



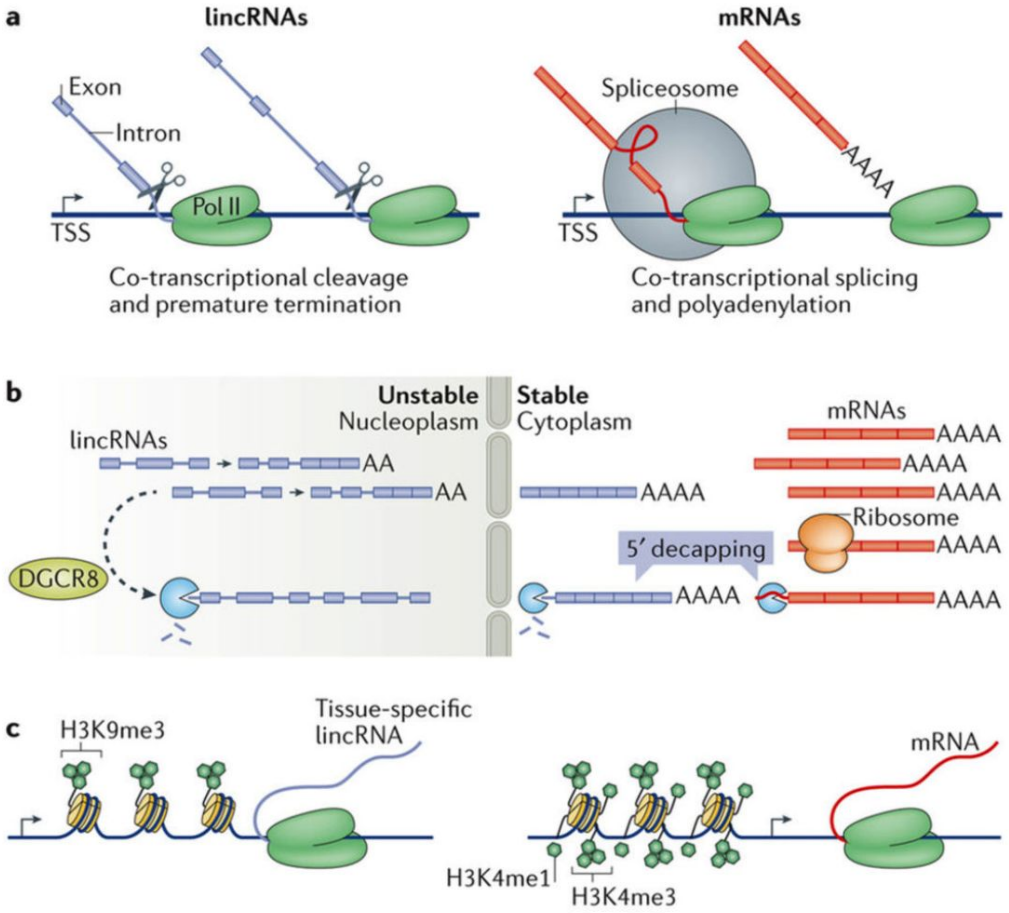
1. transcriptional silencing by siRNA (TGS)

2. regulation of expression by long non-coding RNAs (lncRNAs)

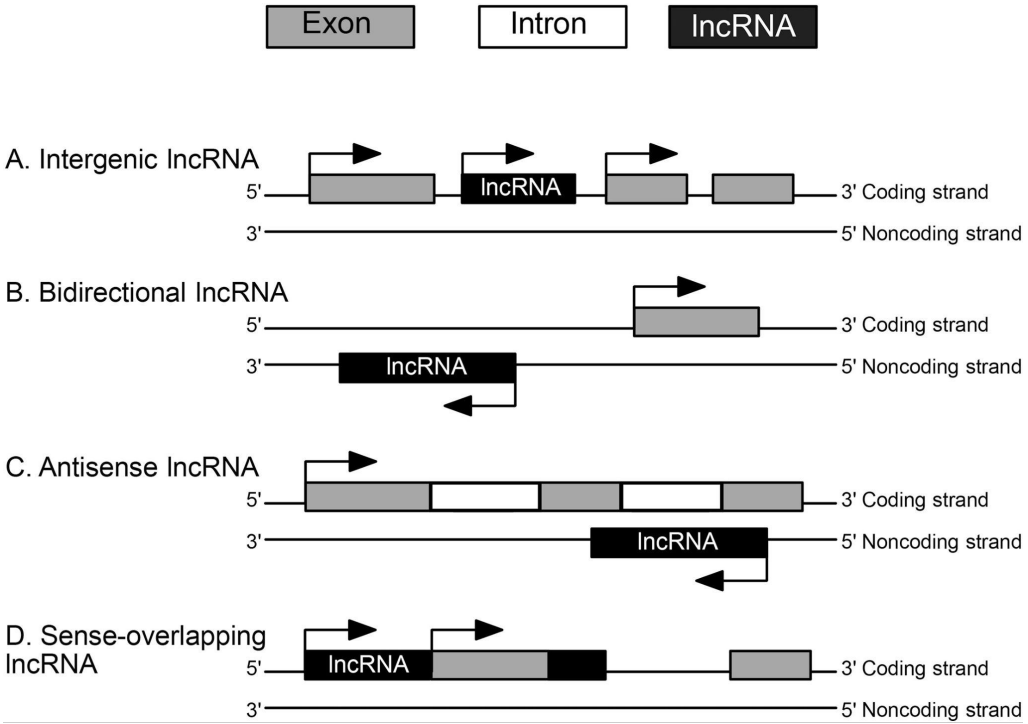
lncRNA vs mRNA

lincRNA (long intergenic non-coding RNA)

- autonomously transcribed ncRNAs, longer than 200 nt, whose sequences do not overlap with protein-coding genes
- in humans, they constitute more than half of lncRNA transcripts

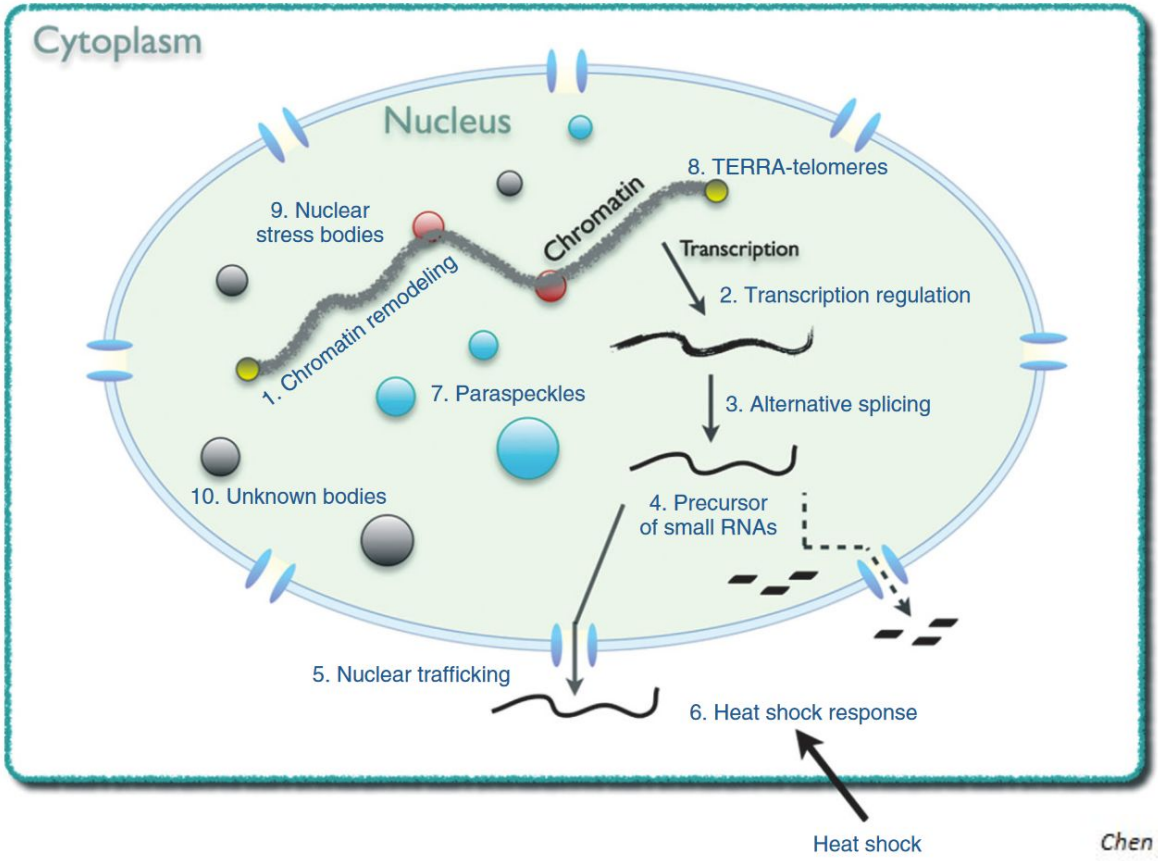


LncRNA classification based on genomic location



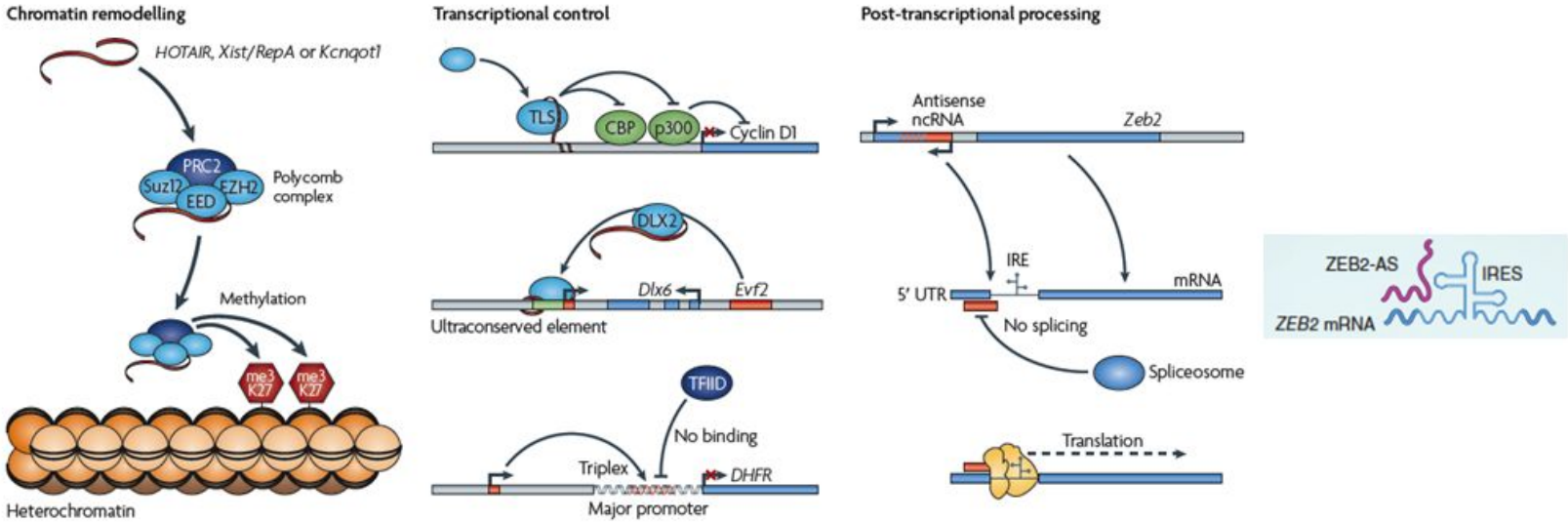
Balas and Johnson, Non-coding RNA Research 2018

lncRNA functions



Chen and Carmichael, WIREsRNA, 2010

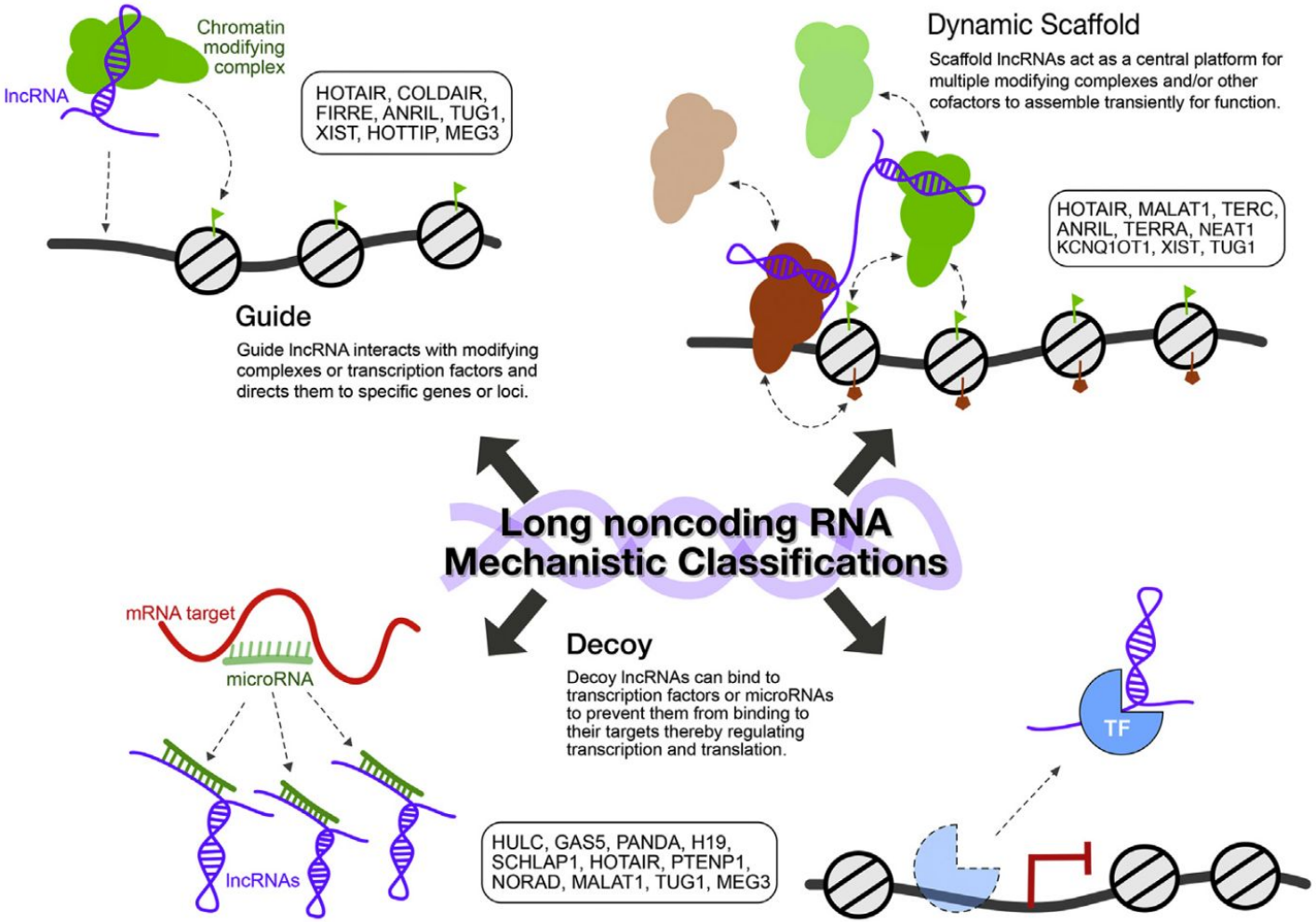
Mechanisms of action of lncRNAs



ncRNAs recruit chromatin modifying complexes → histone modifications (H3meK27) and heterochromatin formation

act as repressors or enchancers of transcription by binding to protein or DNA factors; may act as "baits" that bind transcription factors

they mask the 5' splice site, resulting in intron retention, IRE recognition and translation



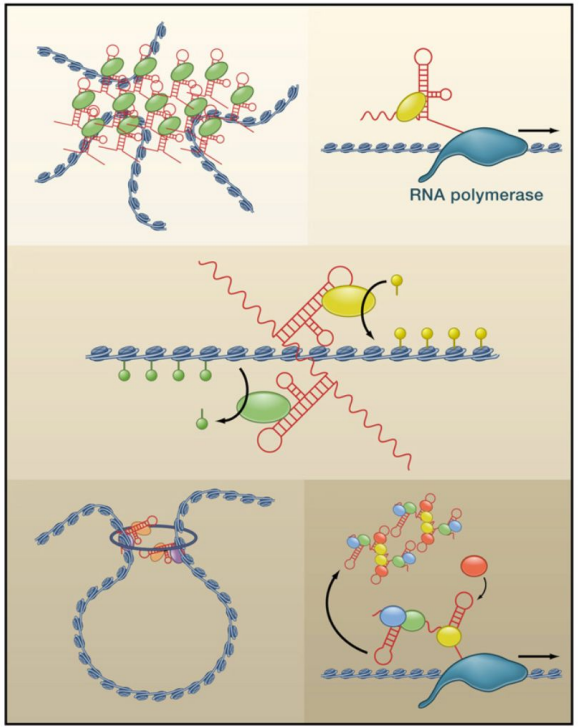
Balas and Johnson, Non-coding RNA Research 2018

Modes of lncRNA Activity

lncRNAs may nucleate chromatin from either the same or different chromosomes and create compartments enriched for chromatin modifiers

one lncRNA may serve as a scaffold for multiple chromatin modifiers that alter different histone marks

higher-order chromatin loops appear to involve lncRNA

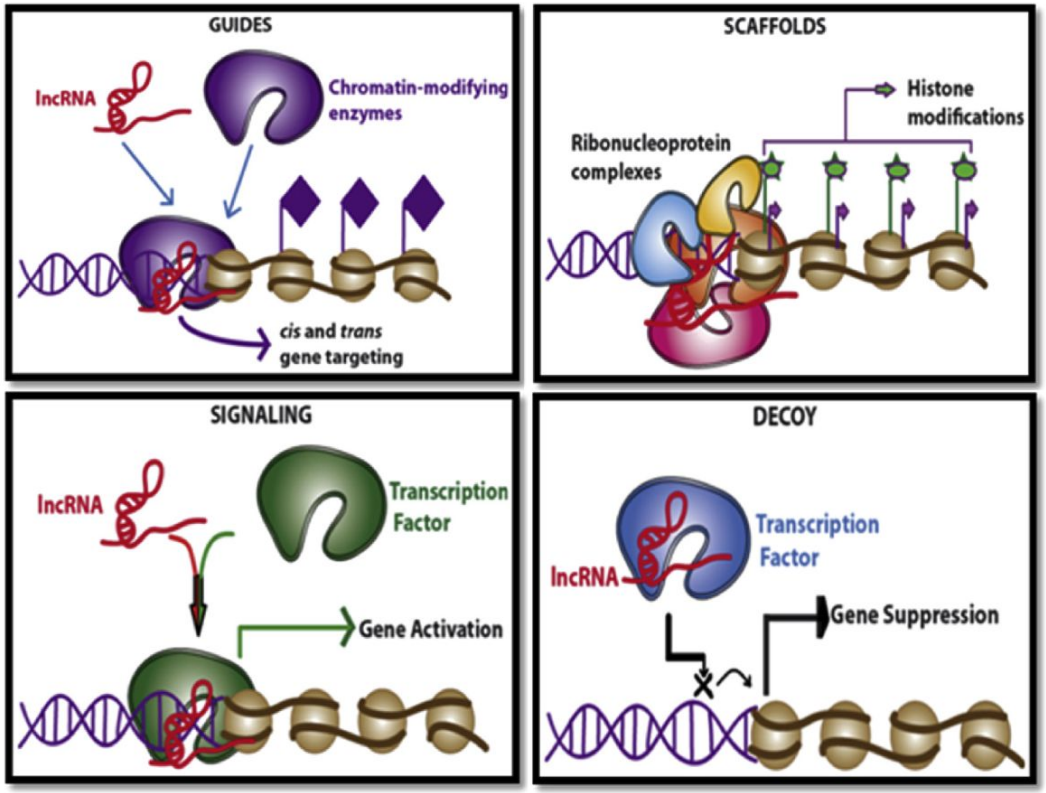


lncRNA may cotranscriptionally recruit chromatin-modifying factors to specific chromosomal loci

lncRNAs generate the dynamic assembly of nuclear structures (e.g. paraspeckles) by serving as scaffolds for proteins

Nagano & Fraser, Cell, 2011

LncRNA-mediated transcriptional regulation



Interaction with and recruitment of chromatin-modifying enzymes (e.g., histone methylases, acetylases, and deacetylases). Modulation of the chromatin state leads to activation or repression of local genes.

Interaction with other RNA-binding factors to form RNP complexes. RNPs can either promote transcription by recruiting key proteins to the target gene promoters or repress gene transcription by binding to existing gene repressors.

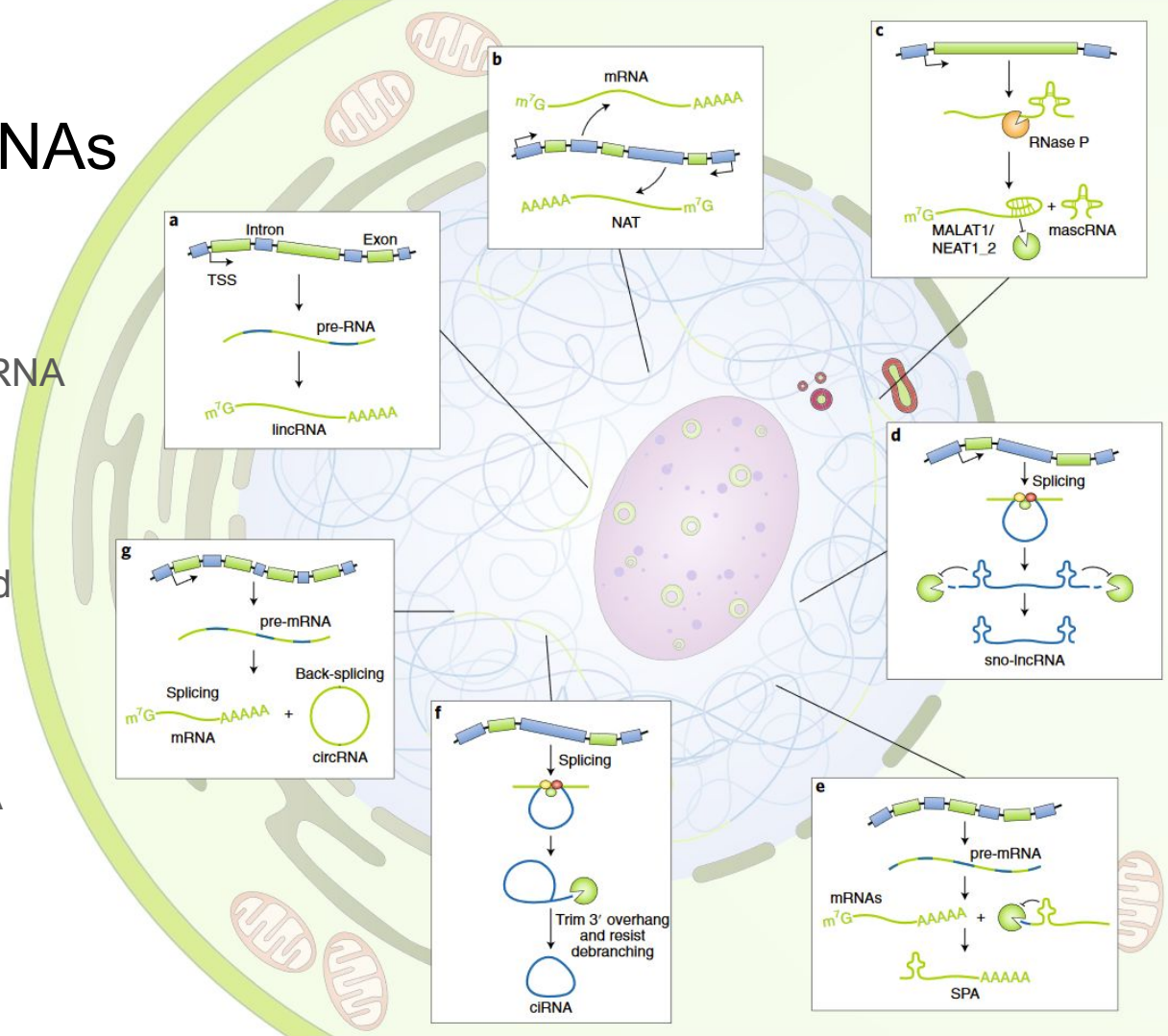
LncRNAs also have enhancer functions and help to change the chromatin architecture and recruit transcriptional machinery proteins to adjacent target gene locus to promote its transcription.

LncRNAs are also involved in the repression of some genes by acting as a decoy for the transcription factor.

Bhat SA, et al. Noncoding RNA Res. 2016

The diversity of lncRNAs
in mammalian cells

a. lincRNA – long intergenic ncRNA
b. NAT – natural antisense transcripts
c. MALAT1 i NEAT1_2
d. sno-lncRNA – snoRNA-ended lncRNA
e. SPA - 5' snoRNA-ended and 3’-polyadenylated lncRNA
f. ciRNA – circular intronic RNA
g. circRNA – circular RNA



Yao et al., Nature Cell Biology 2019

lncRNAs

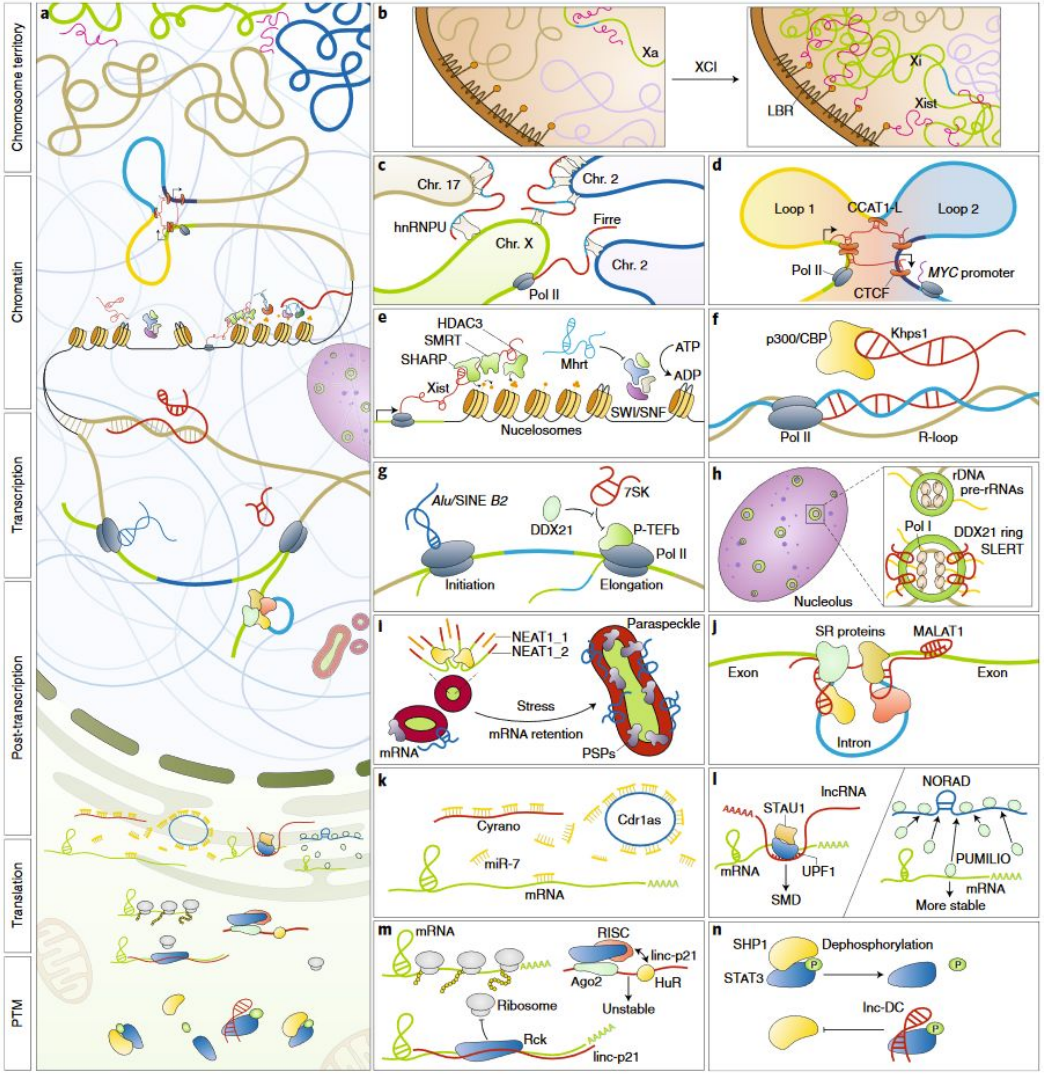
Category	Abbreviation	Specific examples
Classification based on transcript length		
Long noncoding RNA	lncRNA	
Long-intergenic noncoding RNA; large intervening noncoding RNA, long-intervening noncoding RNA	lincRNA	ANRIL [117], H19 [147], HOTAIR [18], HOTTIP [148], lincRNA-p21 [149], XIST [150], Paupar [151]
Very long intergenic noncoding RNA	vlincRNA	HELLP transcript [42], Vlinc_21, vlinc_185, vlinc_377, vlinc_500 [29]
macroRNA		Airn, Gtl2lt, KCNQOT1, Lncat, Nespas (reviewed in [152]), STAiR1 [28]
Promoter-associated long RNA	PALR	
Classification based on association with annotated protein-coding genes		
Intronic ncRNA; stable intronic sequence RNA; totally intronic RNA, partially intronic RNA	sisRNA, TIN, PIN	
Circular intronic RNAs	ciRNAs	
Sense ncRNA		
Natural antisense ncRNA	asRNA, NAT	BACE1-AS [153], aHIF [154], Tsix [155]
Mirror antisense		Globin antisense [67]
Exonic circular RNAs	ecircRNAs	cANRIL [118]
Chimeric RNAs, trans-spliced RNAs, exon juxtaposition		
Stand-alone ncRNAs made from 3′UTRs	uaRNA	
Chromatin-interlinking RNA	ciRNA	
Transcription start site-associated RNAs	TSSa-RNAs	
Classification based on association with other DNA elements of known function		
Enhancer-associated RNA	eRNA	
Promoter-associated long RNA	PALR	
Upstream antisense RNA	uaRNA	
PROMoter uPstream Transcript	PROMPT	
Telomeric repeat-containing RNA	TERRA	
Classification based on protein-coding RNA resemblance		
mRNA-like noncoding RNAs	mlncRNAs	
Long-intergenic noncoding RNA; large intervening noncoding RNA, long-intervening noncoding RNA	lincRNA	ANRIL [117], H19 [147], HOTAIR [18], HOTTIP [148], lincRNA-p21 [149], XIST [150]
Classification based on association with repeats		
C0T-1 repeat RNA		
Long interspersed nuclear element	LINE1/2	
Transcribed endogenous retroviruses		
Expressed Satellite Repeats		
Non-coding RNA driven by promoters within repeats	vlincRNAs, NASTs	Vlinc_21, vlinc_185, vlinc_377, vlinc_500 [29]
Polypurine-repeat-containing RNA	GRC-RNA	
Transcribed pseudogenes		PTENP1 and KRASP1 [86]
Classification based on association with a biochemical pathway or stability		
Nrd1-unterminated transcript	NUT	
miRNA primary transcripts		H19 [166]
piRNA primary transcripts		
Cryptic unstable transcript	CUT	
PROMoter uPstream Transcript	PROMPT	
Xrn1-sensitive unstable transcript	XUT	
Stable Uncharacterized Transcript, Stable Unannotated Transcript	SUT	
Classification based on sequence and structure conservation		
Transcribed-ultraconserved regions	T-UCR	UCR106 [95]
Hypoxia-induced noncoding ultraconserved transcript	HINCUT	
Long-intergenic noncoding RNA; large intervening noncoding RNA, long-intervening noncoding RNA	lincRNA	HOTAIR [18], HOTTIP [148]
RNA-Z regions		
EvoFold regions		

Category	Abbreviation	Specific examples
Classification based on expression in different biological states		
Long stress-induced noncoding transcript	LSINCT	
Hypoxia-induced noncoding ultraconserved transcript	HINCUT	
Non-Annotated Stem Transcript	NAST	
Classification based on association with subcellular structures		
Chromatin-associated RNA	CAR	
Chromatin-interlinking RNA	ciRNA	
Nuclear bodies associated RNAs		
PRC2 associated RNAs		
Classification based on function		
Long noncoding RNAs with enhancer-like function; ncRNA-activating	ncRNA-a	ncRNA-a7 [108]
miRNA primary transcripts		H19 [166]
piRNA primary transcripts		
Competing endogenous RNA	ceRNA	PTENP1 and KRASP1

lnRNA	lnaRNA function
ANRIL Xist HOTAIR COLDAIR Kcnq1ot1	Target PRC1 or PRC1 in cis to mediate histone methylation in **transcriptional gene silencing** for dosage compensation, imprinting and developmental gene expression; ANRIL affects cell senescence
MALAT1	Sequesters SR splicing factors to regulate **alternative splicing**
PANDA	p53-inducible, titrates away NF-YA to favor survival th during **DNA damage**
TERRA	Controls **telomerase** access to telomeres in a cell-cycle manner
pRNA	Targets DNMT3b in cis to the rDNA locus via an RNA:DNA:DNA triplex for DNA methylation and **gene silencing**
SRA	Enhances insulator function of CTCF
Gas5	Binds to glucocorticoid receptor as a decoy and titrates GR away from target genes
lincRNA-p21	Targets hnRNP-K in trans to mediate p53-dependent gene repression
HOTTIP	Bind to and localizes the MLL complex and H3K4me3 via chromosomal looping for gene activation
1/2 SBS	Pairs with mRNAs via Alu repeats and targets them into a NMD pathway
HULC H19 PTENP1	miRNA decoys: HULC induces PRKACB translation, H19 interferes with let-7 activity, PTENP1 depresesses PTEN production
LINK RNAs	Cellular signalling, activate of kinases, promote protein phoshorylation
1/2-sbsRNA TINCR	STAUFEN1-dependent mRNA decay, induce mRNA degradation or stabilication
HOTAIR NRON	**Protein turnover**, stimilate degradation of Snurportin-1 and Ataxin-1 (HOTAIR) or HIV proteins tat (NRON_

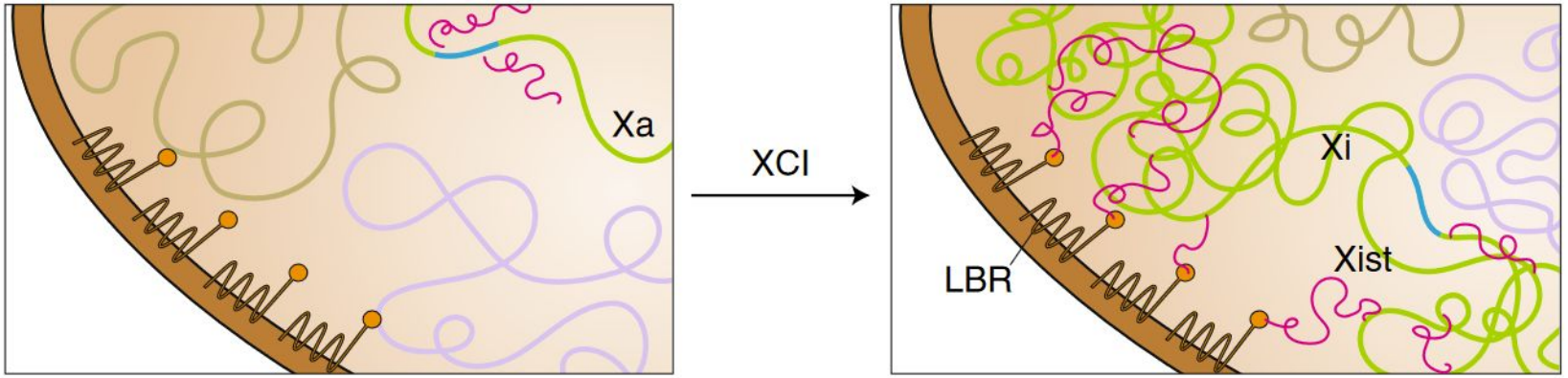
Laurent et al, TiG 2015

Cellular functions of lncRNAs



Yao et al., Nature Cell Biology 2019

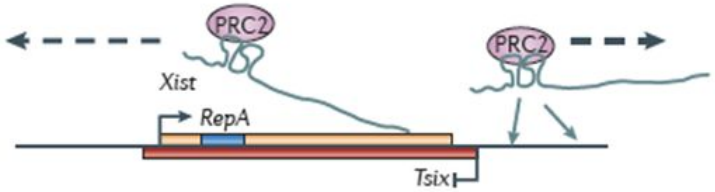
Cellular functions of lncRNAs



Xist modulates inactive X chromosome (Xi) architecture during X chromosome inactivation (XCI) by recruiting Xi to associate with the lamin B receptor (LBR) at the nuclear lamina to silence transcription

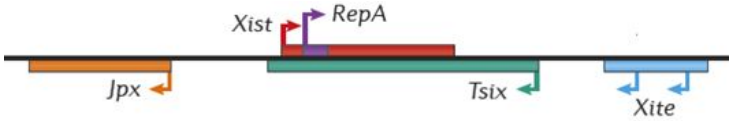
Yao et al., Nature Cell Biology 2019

Xist ncRNA – X chromosome inactivation



Xist (X-inactive specific transcript, 19 kb)
Expression on inactive X
Coats the X chromosome

Tsix (40 kb) expression on active X

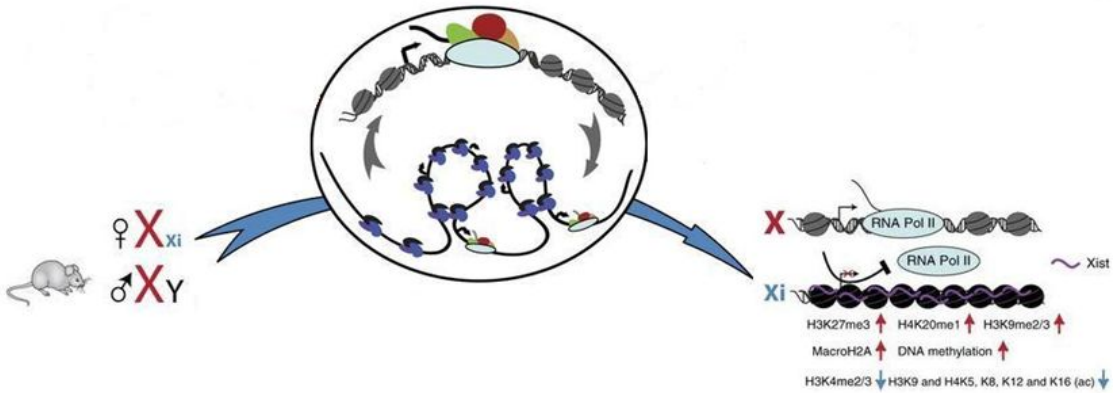


- Dosage compensation – one copy of the chromosome in females is epigenetically silenced
- RepA (repeat element) 1.6kb ncRNA (5' Xist) binds PRC2 complexes (Polycomb)
- Tsix – protects the active X chromosome from silencing; combines X reactivation and stem cell reprogramming
- Tsix and Xite control allele selection and designate the active X chromosome
- Jpx and RepA are positive regulators of Xist

X chromosome inactivation: epigenetic silencing

Xist ncRNA triggers epigenetic changes that provide a "cellular memory" of the inactive state:

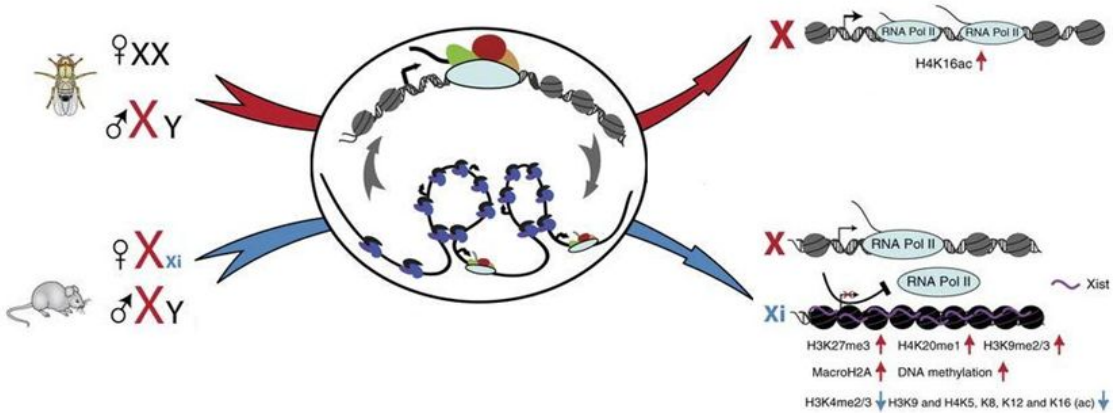
- replacement of histone H2A with macroH2A
- histone H3 methylation: H3K9, H3K27
- histone H4 deacetylation (?)
- DNA methylation /after chromosome inactivation



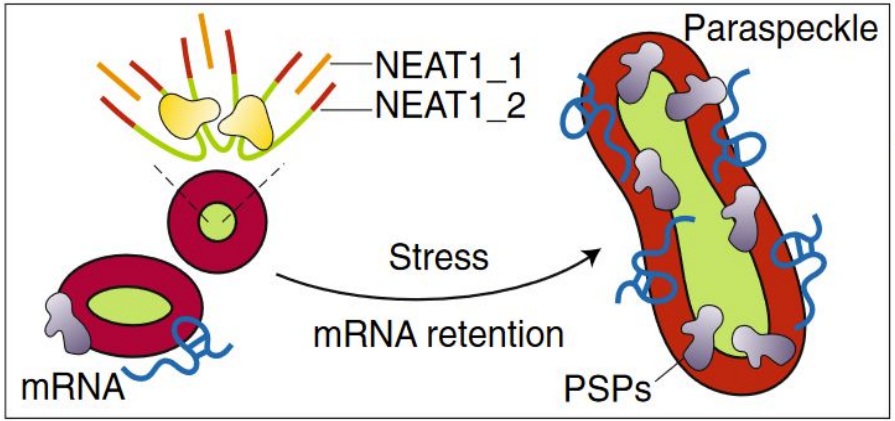
X chromosome inactivation: epigenetic silencing

dosage compensation in *Drosophila melanogaster* → roX

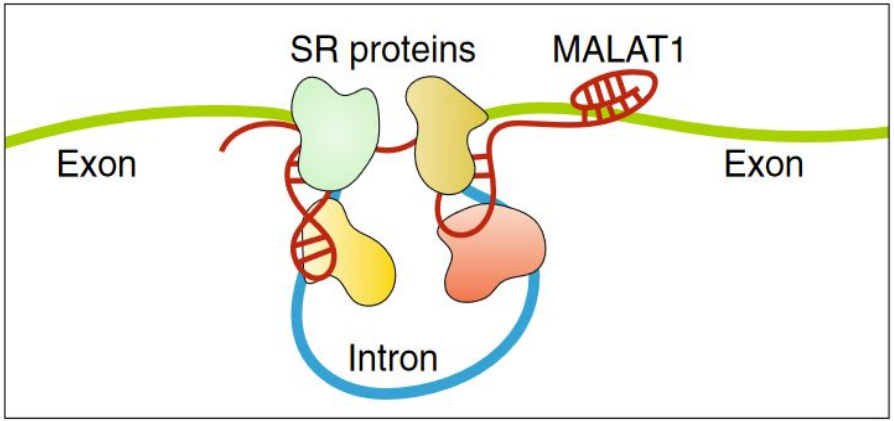
- roX1/roX2 ncRNAs initiate histone modifications → in *Drosophila* males, increased X chromosome activity
- histone acetylation
- H3K9 demethylation



Cellular functions of lncRNAs



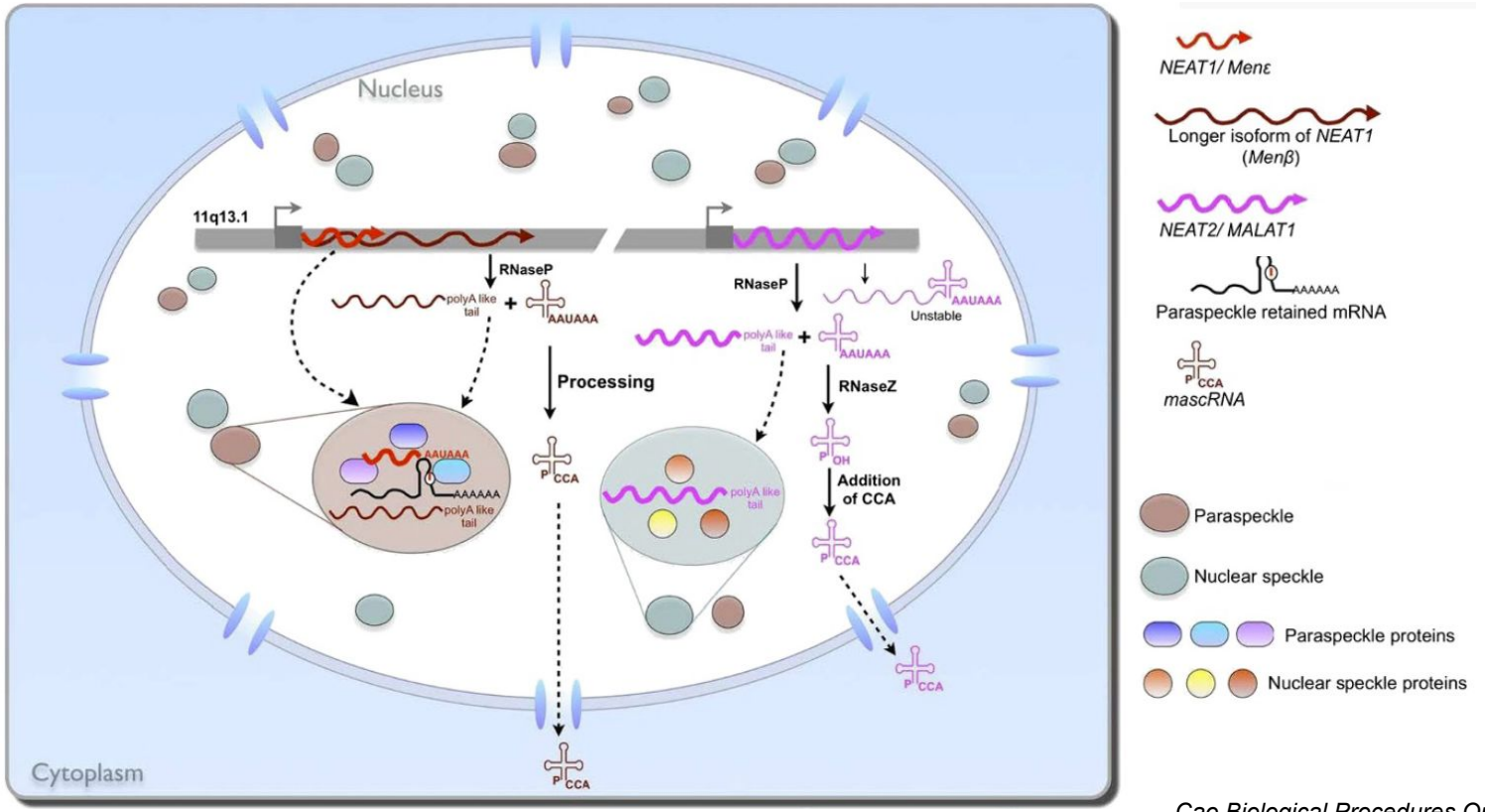
NEAT1 is an architectural lncRNA that nucleates paraspeckles. Upon cellular stress, altered NEAT1 transcription and processing lead to changes of paraspeckles. PSP, paraspeckle proteins.



MALAT1 interacts with SR proteins and alters their phosphorylation to impact pre-mRNA splicing in splicing speckles.

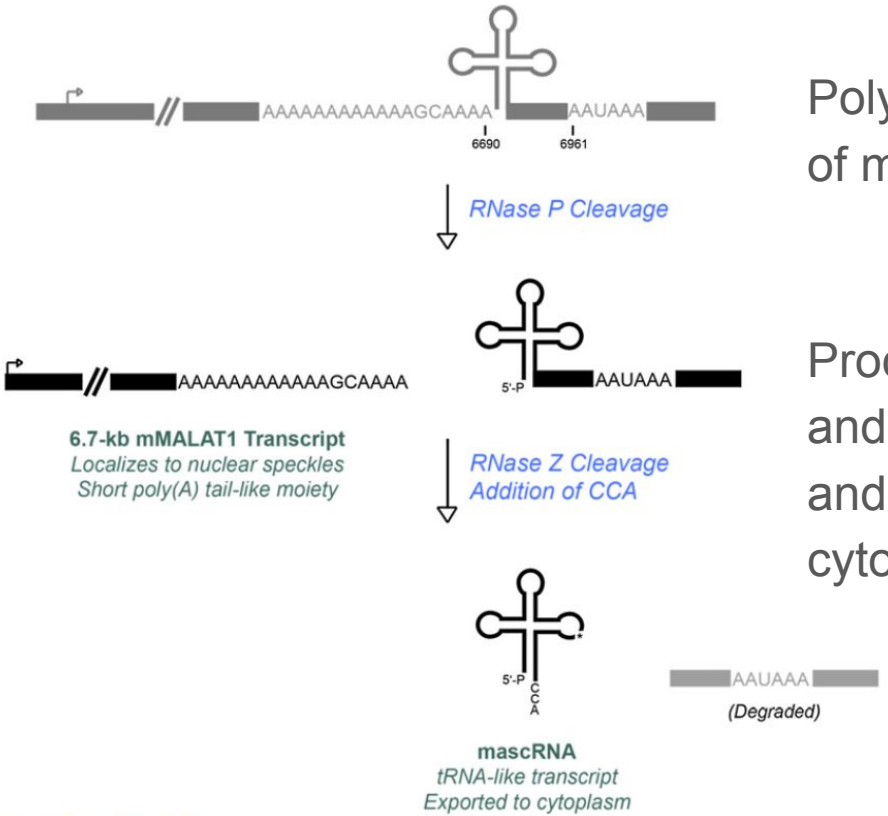
Yao et al., Nature Cell Biology 2019

Long non-coding RNAs in nuclear sub-compartments



Cao Biological Procedures Online 2014

MALAT1/mascRNA



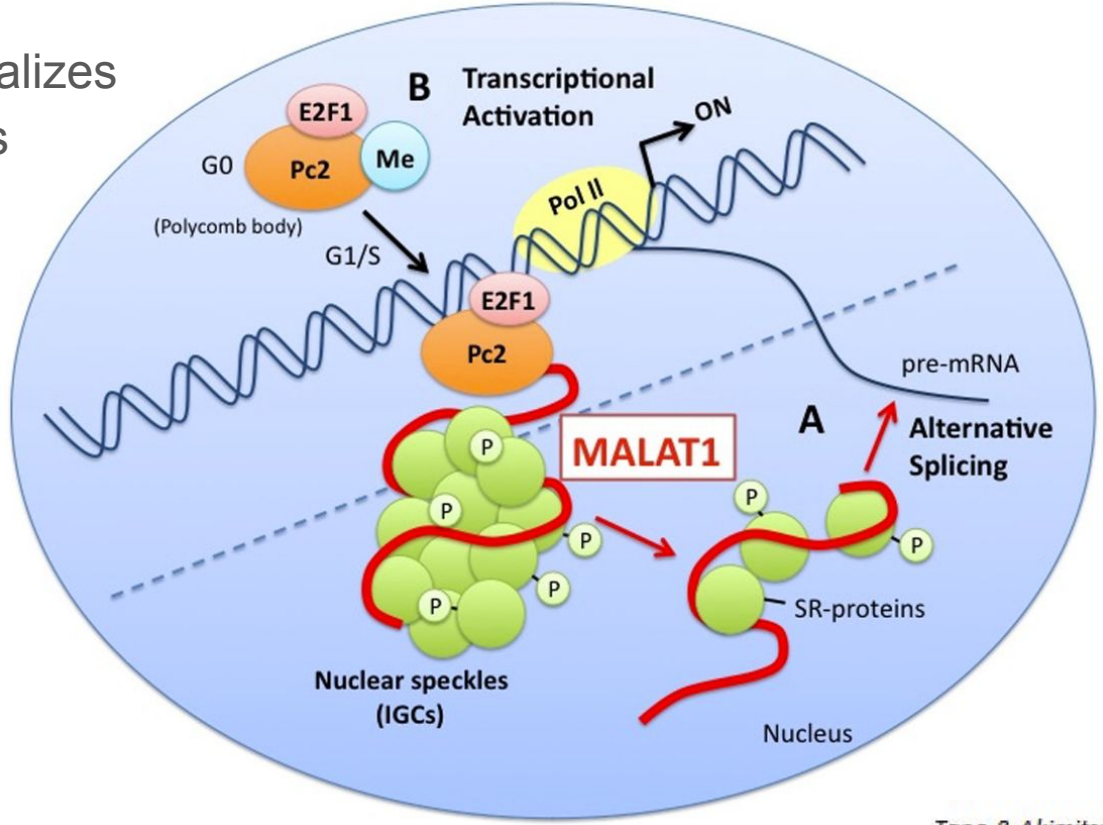
Polyadenylated Pol II transcript, precursor of mature MALAT1, and mascRNA

Processing of the precursor by RNase P (5') and RNase Z (3') releases 6.7 kb of MALAT1 and mascRNA (tRNA-like), exported to the cytoplasm upon addition of CCA

Wilusz & Spector, RNA, 2010

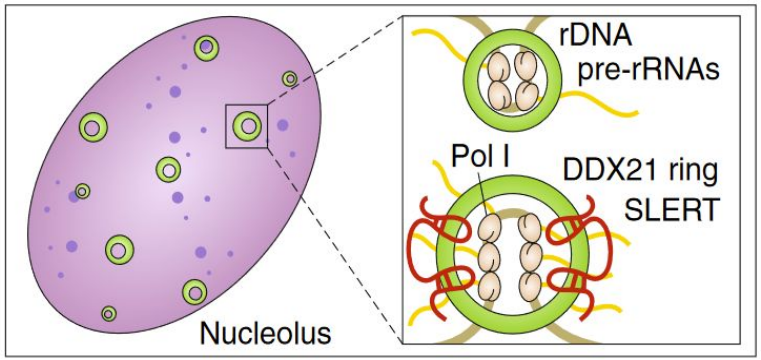
A model for the functions of MALAT1

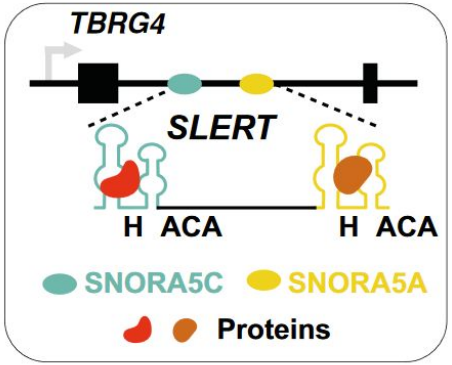
MALAT1 stably localizes to nuclear speckles



Tano & Akimitsu, Frontiers in Genetics, 2012

Cellular functions of lncRNAs

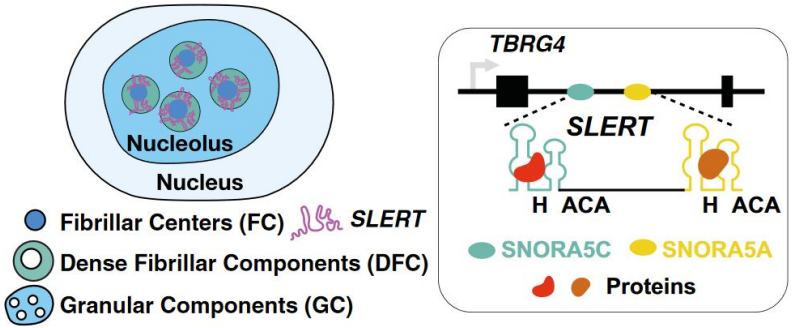


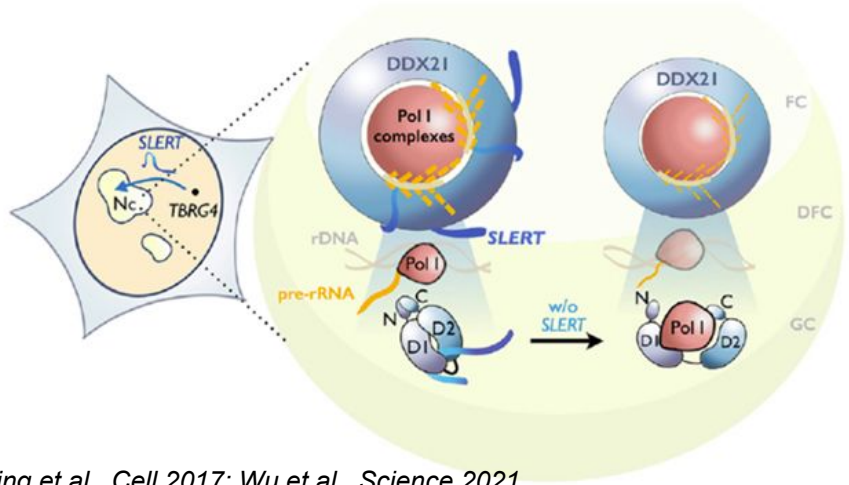


SLERT = ncRNA snoRNA- ended lncRNA enhances pre-rRNA transcription; a member of the family of sno-lncRNAs

SLERT promotes Pol I transcription by binding DDX21 to alter its conformation, thereby releasing its inhibitory effect on Pol I

SLERT – Pol I transcription (human)





snoRNAs at both ends of SLERT are required for biogenesis and nucleolar localization

DDX21 RNA helicase forms ring structures around Pol I complexes → suppression of pre-rRNA transcription

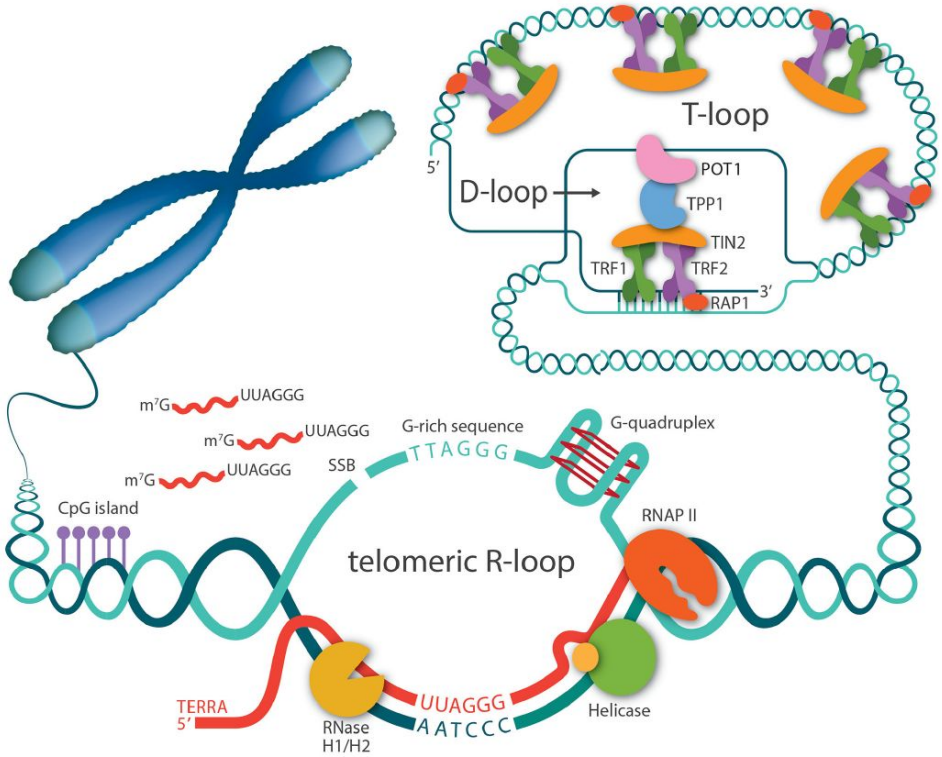
SLERT binds to DDX21 and modulates DDX21 rings to reduce Pol I suppression (so SLERT positively affects rDNA transcription)

SLERT-DDX21 interactions regulate differential rDNA expression

lncRNA SLERT controls phase separation of FC/DFCs to facilitate Pol I transcription

Xing et al., Cell 2017; Wu et al., Science 2021

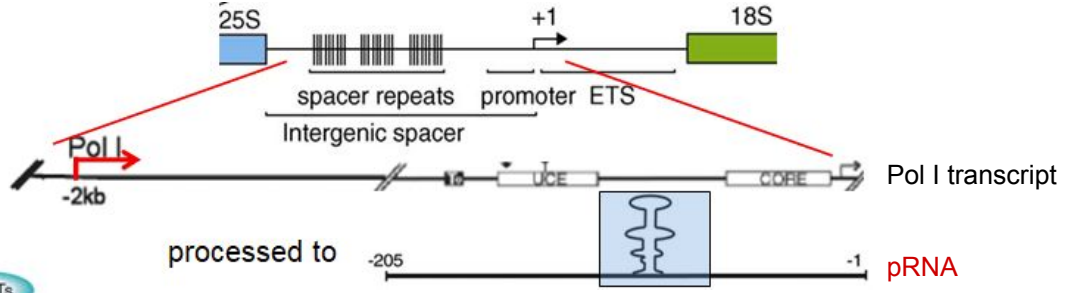
TERRA – telomeric repeat-containing RNA

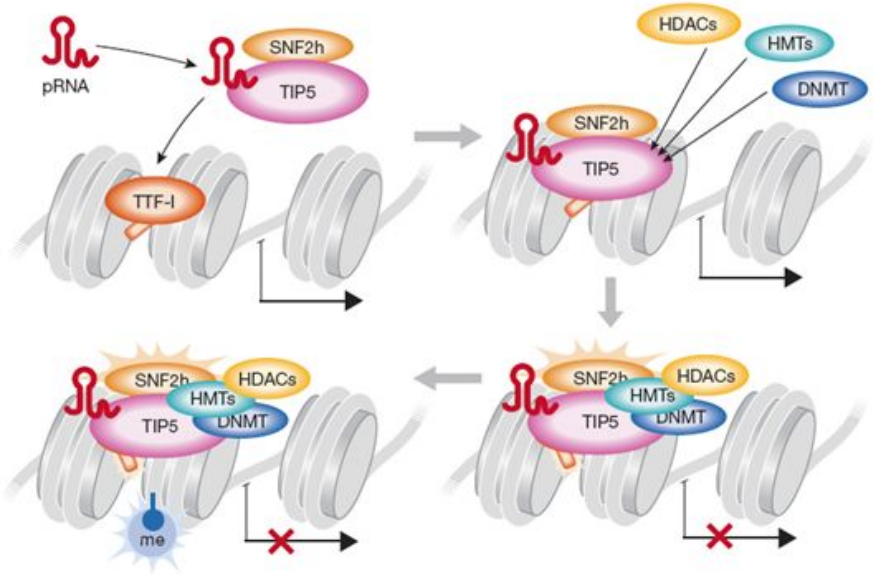


- in yeast and human cells
- polyadenylated Pol II transcript
- in subtelomeric and telomeric regions, component of telomeric heterochromatin
- association with telomeres and telomeric proteins (Trf1, Trf2)
- regulated by RNA surveillance factors (Rat1, Trf4, NMD factors, RNase H)
- regulates telomerase (telomere shortening) by creating RNA-DNA hybrids
- works in chromatin remodeling processes (development and differentiation)
- impact on telomere replication

Gong and Liu, Cancers 2023

rDNA silencing by pRNA and NoRC

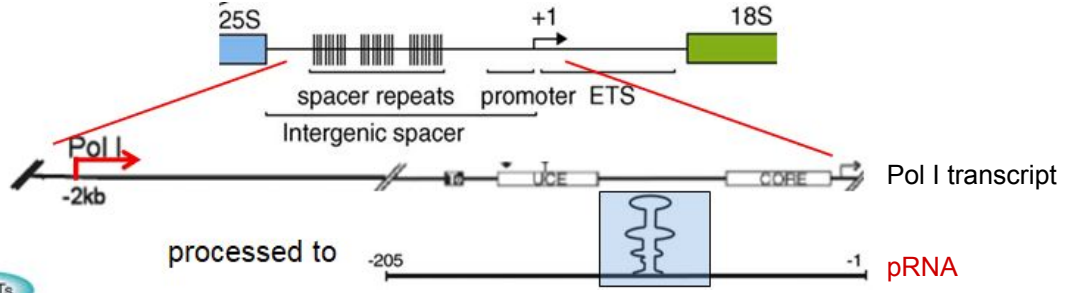


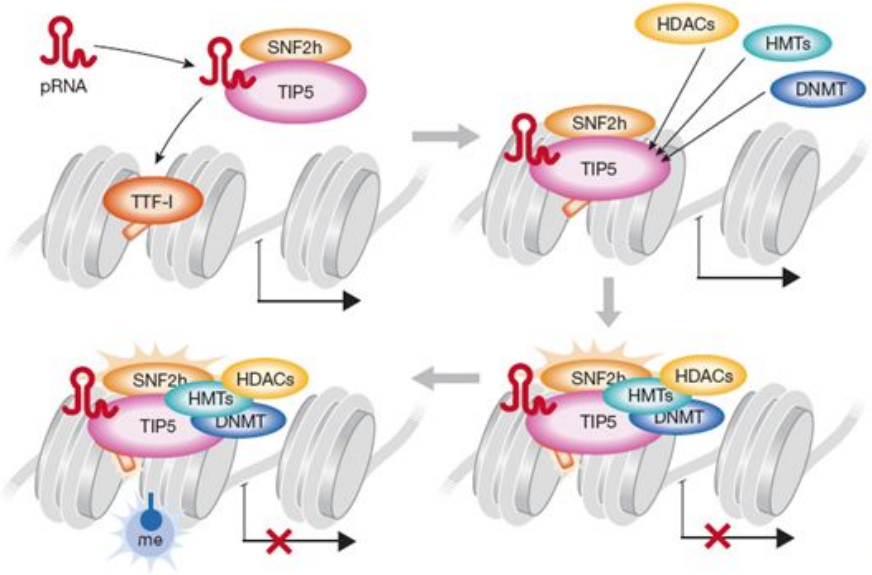


- NoRC mammalian nucleolar remodeling complex
- requires association of TIP5 with pRNA
- NoRC provides enzymes that modify DNA and histones → formation of hetrochromatin
- CpG-133 methylation prevents UBF binding → inhibition of transcription complex formation

Stark and Taliansky, Embo Rep., 2008; Mayer et al., Mol. Cell, 2006; Embo Rep., 2008; Schmitz et al., Gene Dev., 2010

rDNA silencing by pRNA and NoRC



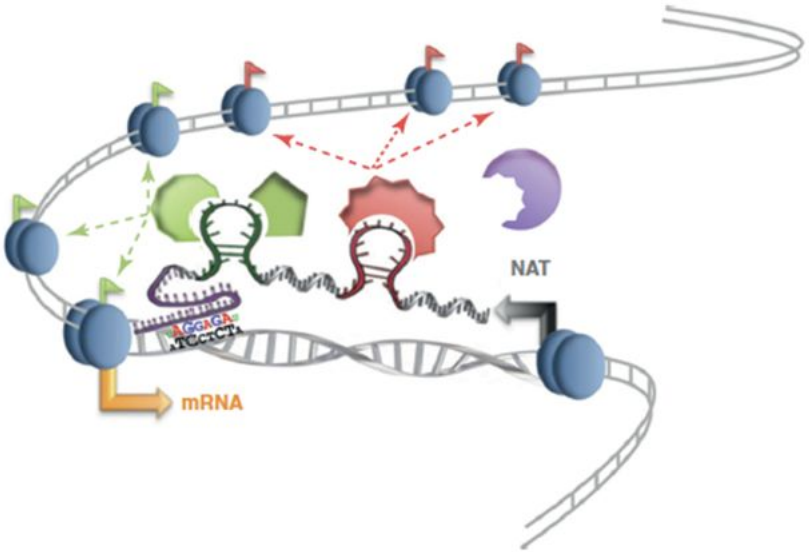


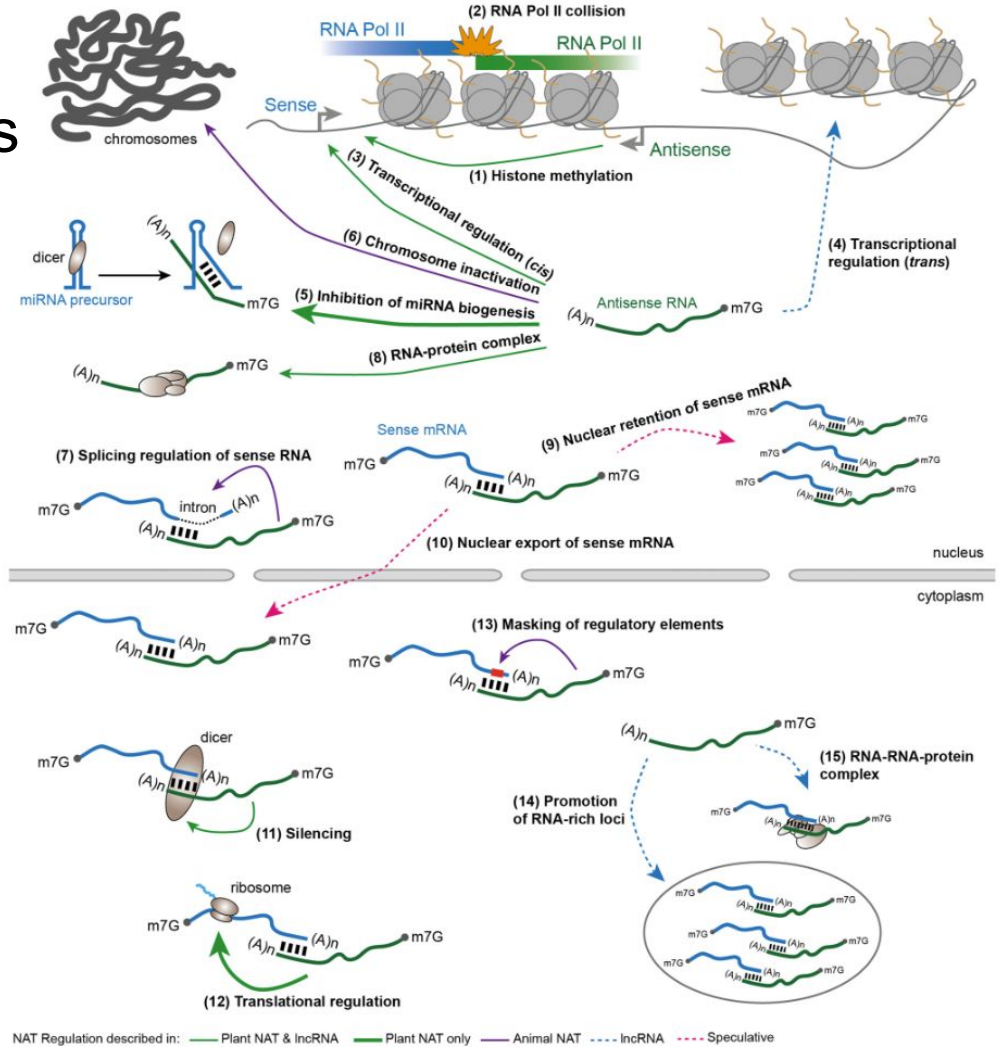
ADDITIONAL SILENCING

- pRNA binds to the rDNA promoter at T0, independently of TTF-I and other proteins, forming an RNA-DNA triplex
- pRNA competes with TTF-I
- rDNA/pRNA triplex recruits DNMT3b methyltransferase → chromatin hypermethylation and rDNA silencing

Stark and Taliansky, Embo Rep., 2008; Mayer et al., Mol. Cell, 2006; Embo Rep., 2008; Schmitz et al., Gene Dev., 2010

Epigenetic regulation by NATs (natural antisense transcripts)

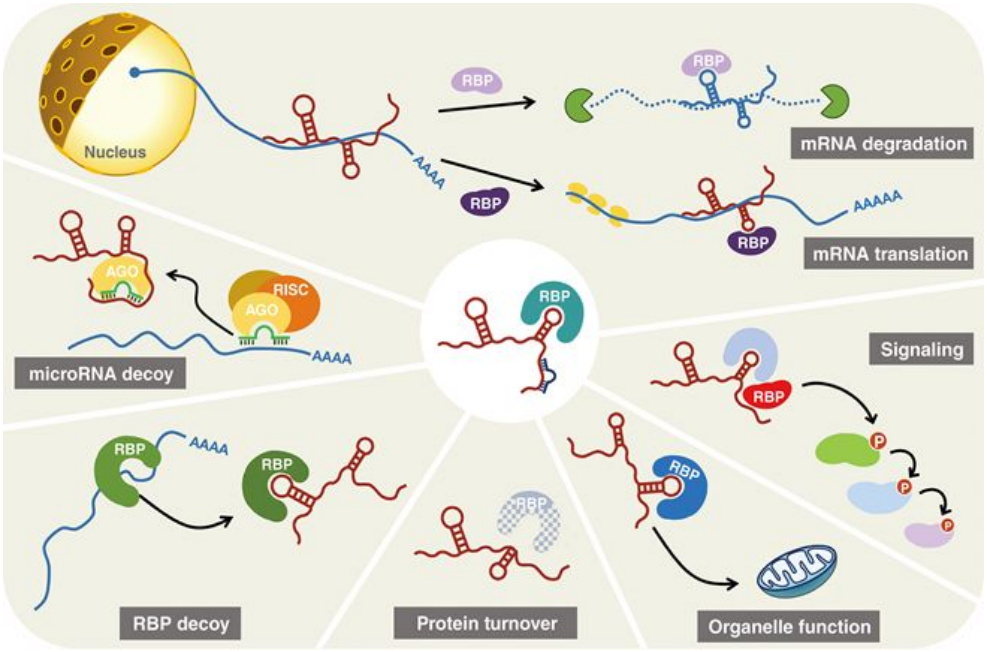


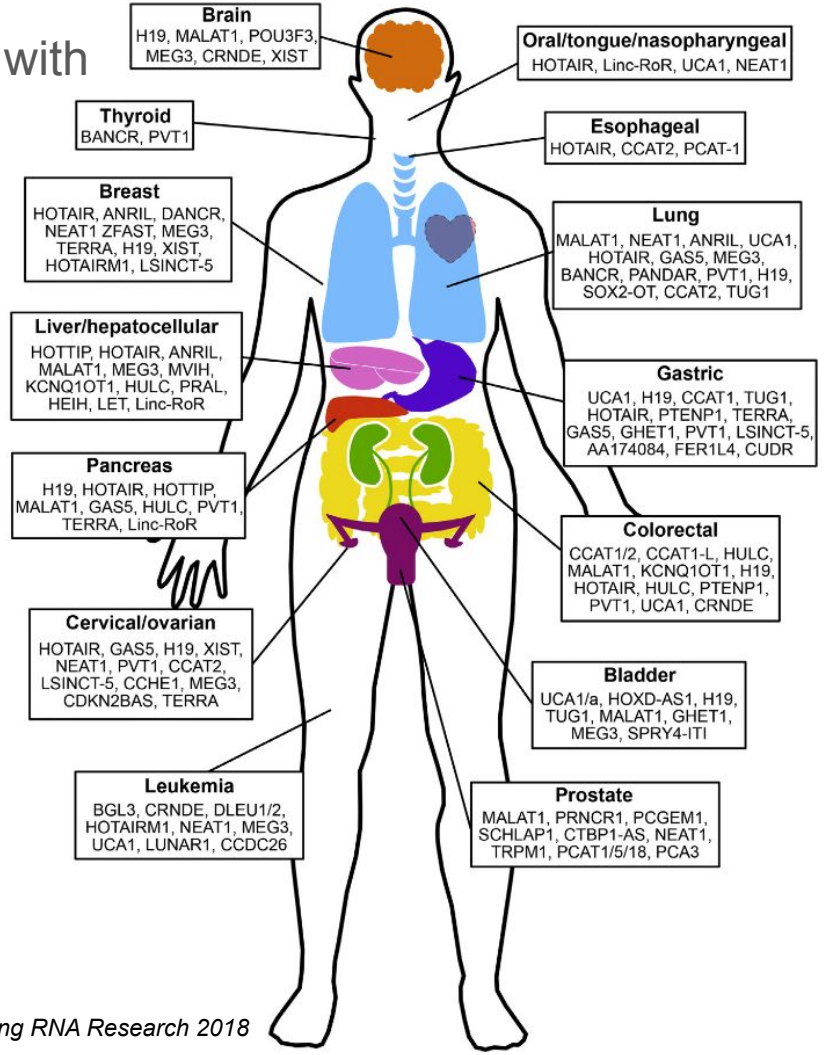


Reis and Poirier, Trends in Plant Science 2021

lncRNA functions

LncRNAs associated with various cancer types





Noh at al, WIREs RNA 2018

Balas and Johnson, Non-coding RNA Research 2018