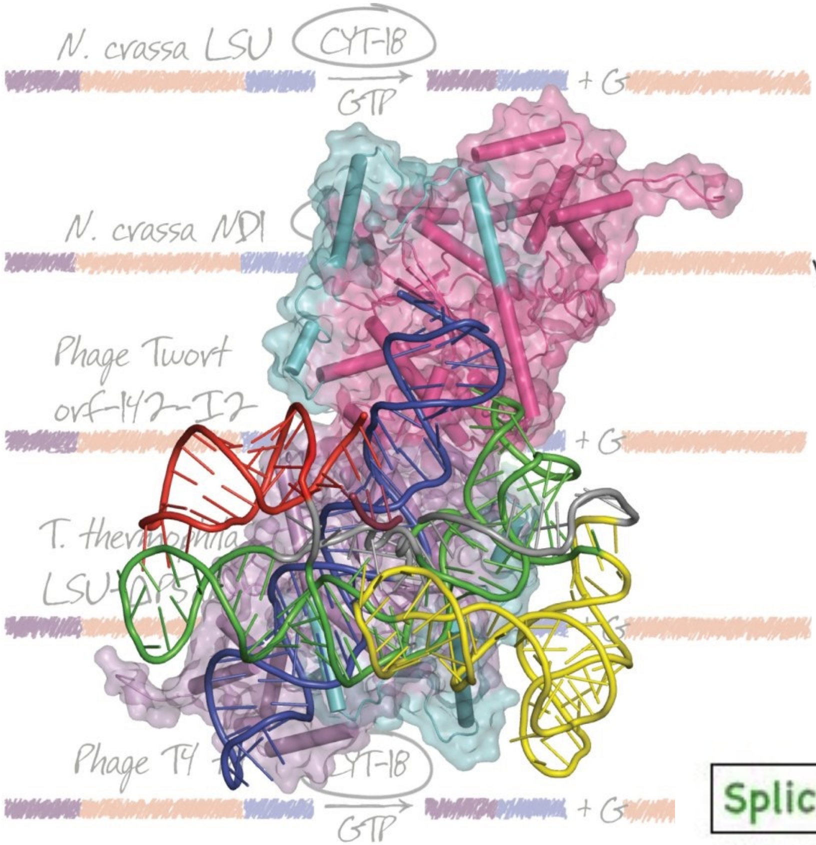
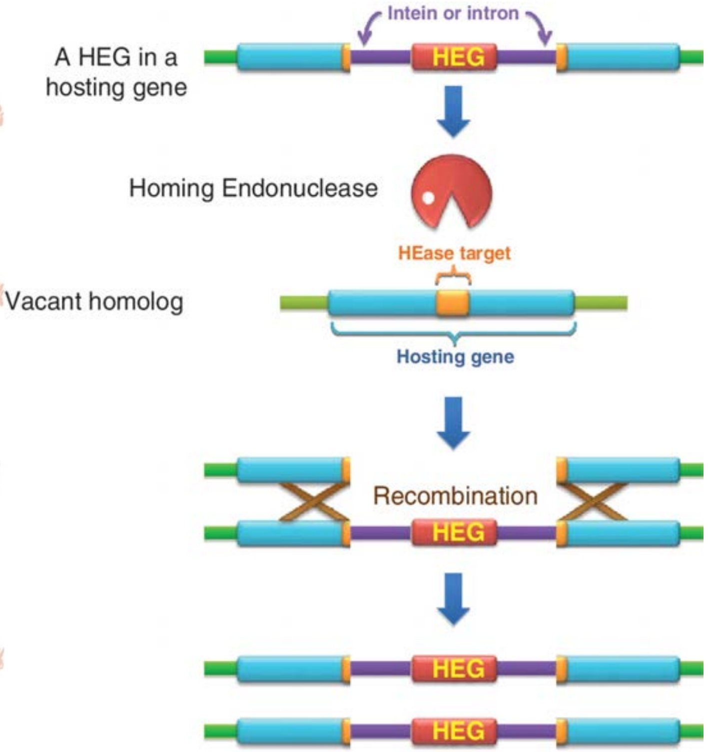
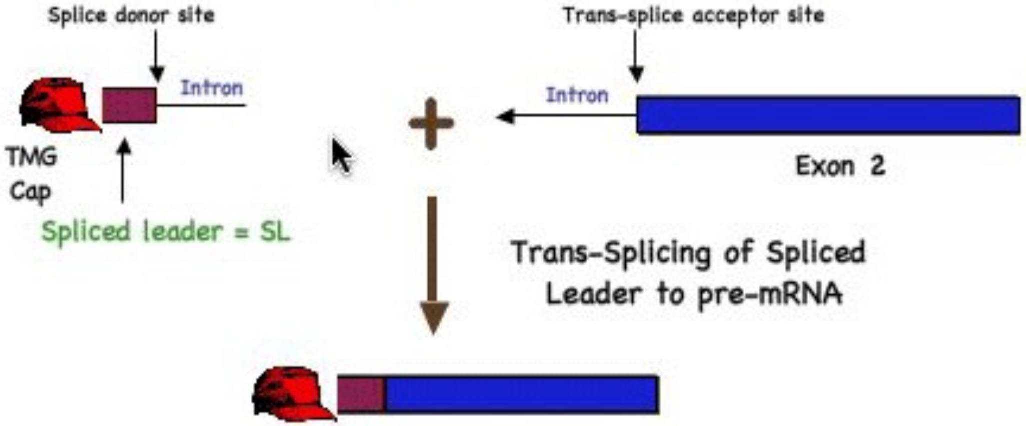
OTHER RNA PROCESSING

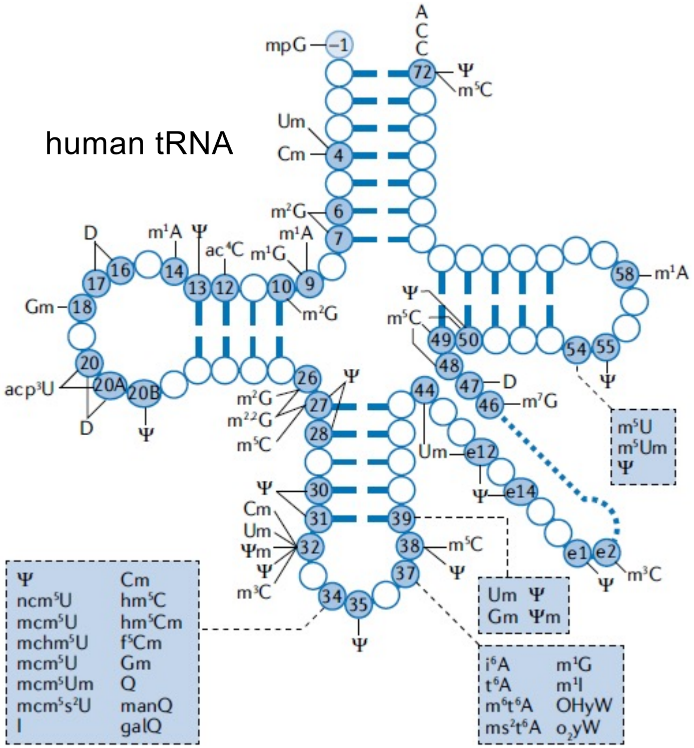


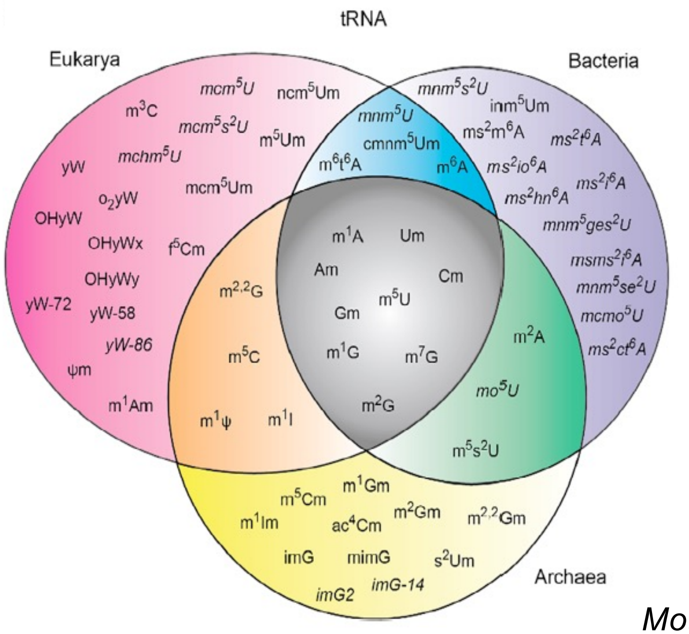


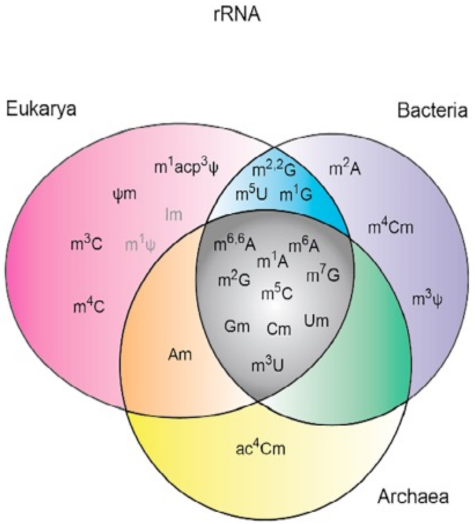


RNA modifications

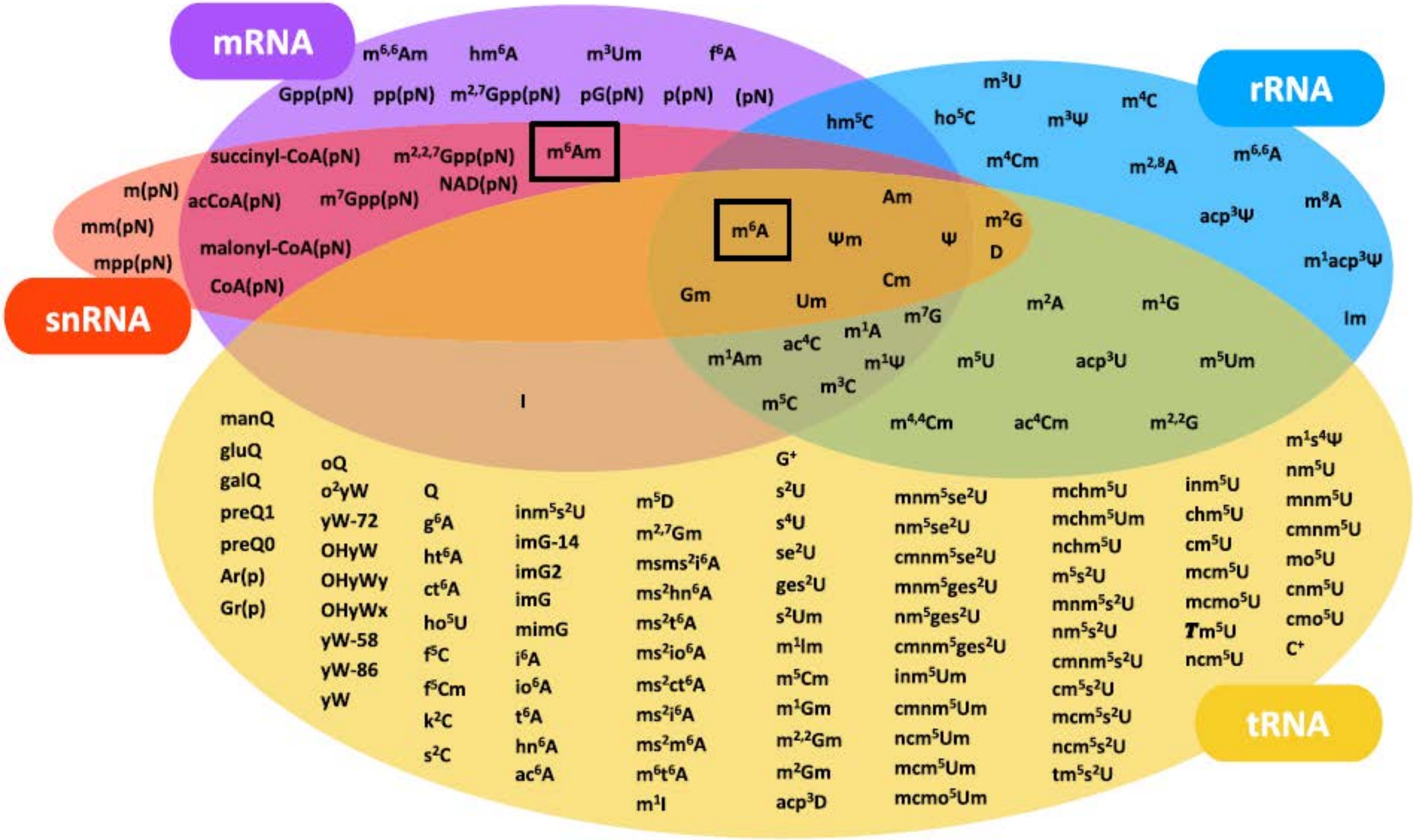
- **Present in all domains of life**
- **150 modifications identified in all types of RNAs**
- **Added co-transcriptionally (mRNA, rRNA) or post-transcriptionally (tRNA, snRNA)**
- **Include methylation, hydroxylation, acetylation, deamination, isomerization, selenylation, reduction, cyclization and conjugation with amino acids and sugars**
- **mainly in tRNAs and rRNAs**
- **80% in tRNAs; 10% -20% of tRNA residues are modified**
- **16 novel modifications in tRNAs identified in XXI**
- **termed “epitranscriptomics” provide gene regulation at the post- transcriptional level**







Motorin and Helm, WIREsRNA 2021
Suzuki Nat Rev Mol Cell Biol 2021



mRNA

m^{6,6}Am hm^{6}A m^{3}Um f^{6}A

Gpp(pN) pp(pN) m^{2,7}Gpp(pN) pG(pN) p(pN) (pN)

rRNA

m^{3}U

hm^{5}C ho^{5}C m^{3}Ψ m^{4}C

m^{4}Cm m^{2,8}A m^{6,6}A

succinyl-CoA(pN) m^{2,2,7}Gpp(pN) m^{6}Am

NAD(pN)

m(pN) acCoA(pN) m^{7}Gpp(pN)

Am m^{2}G acp^{3}Ψ m^{8}A

mm(pN) m^{6}A Ψm Ψ D

malonyl-CoA(pN) m^{1}acp^{3}Ψ

mpp(pN)

CoA(pN) Cm

Gm Um m^{2}A m^{1}G

snRNA

m^{7}G Im

m^{1}A

ac^{4}C

m^{1}Am m^{1}Ψ m^{5}U acp^{3}U m^{5}Um

m^{3}C

I m^{5}C

m^{4,4}Cm ac^{4}Cm m^{2,2}G

manQ
gluQ
galQ
preQ1
preQ0
Ar(p)
Gr(p)

oQ
o^{2}yW
yW-72
OHyW
OHyWy
OHyWx
yW-58
yW-86
yW

Q
g^{6}A
ht^{6}A
ct^{6}A
ho^{5}U
f^{5}C
f^{5}Cm
k^{2}C
s^{2}C

inm^{5}s^{2}U
imG-14
imG2
imG
mimG
i^{6}A
io^{6}A
t^{6}A
hn^{6}A
ac^{6}A

m^{5}D
m^{2,7}Gm
msms^{2}i^{6}A
ms^{2}hn^{6}A
ms^{2}t^{6}A
ms^{2}io^{6}A
ms^{2}ct^{6}A
ms^{2}i^{6}A
ms^{2}m^{6}A
m^{6}t^{6}A
m^{1}I

G^{+}
s^{2}U
s^{4}U
se^{2}U
ges^{2}U
s^{2}Um
m^{1}Im
m^{5}Cm
m^{1}Gm
m^{2,2}Gm
m^{2}Gm
acp^{3}D

mnm^{5}se^{2}U
nm^{5}se^{2}U
cmnm^{5}se^{2}U
mnm^{5}ges^{2}U
nm^{5}ges^{2}U
cmnm^{5}ges^{2}U
inm^{5}Um
cmnm^{5}Um
ncm^{5}Um
mcm^{5}Um
mcmo^{5}Um

mchm^{5}U
mchm^{5}Um
nchm^{5}U
m^{5}s^{2}U
mnm^{5}s^{2}U
nm^{5}s^{2}U
cmnm^{5}s^{2}U
cm^{5}s^{2}U
mcm^{5}s^{2}U
ncm^{5}s^{2}U
tm^{5}s^{2}U

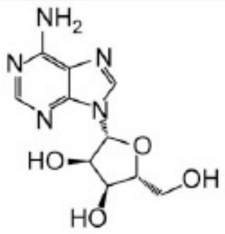
inm^{5}U
chm^{5}U
cm^{5}U
mcm^{5}U
mcmo^{5}U
τm^{5}U
ncm^{5}U

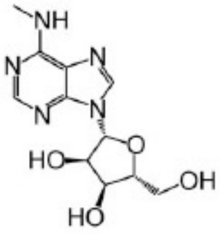
m^{1}s^{4}Ψ
nm^{5}U
mnm^{5}U
cmnm^{5}U
mo^{5}U
cnm^{5}U
cmo^{5}U
C^{+}

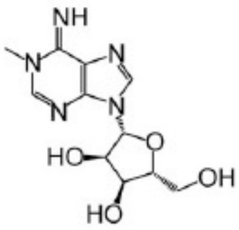
tRNA

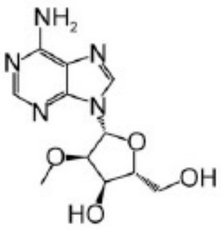
Oerum et al, NAR 2021

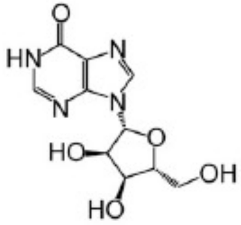
RNA modifications

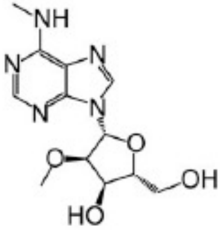

A

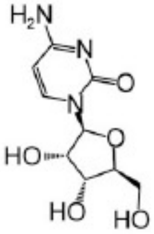

m^6A

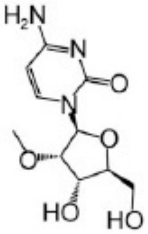

m^1A

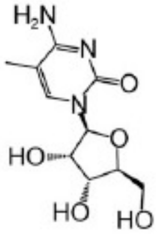

Am

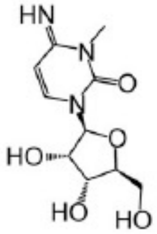

I

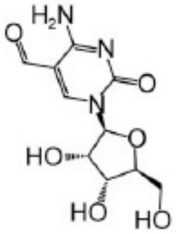

m^6Am

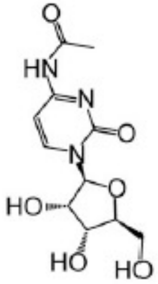

C

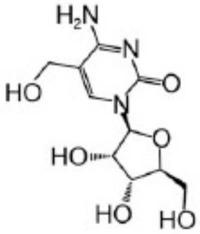

Cm

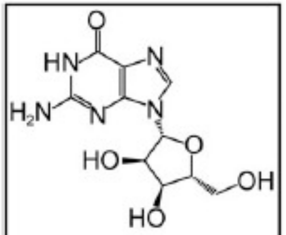

m^5C

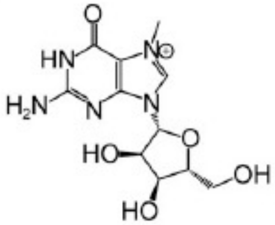

m^3C

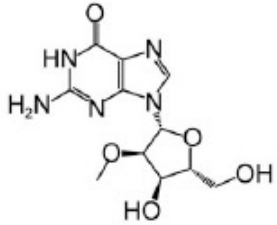

f^5C

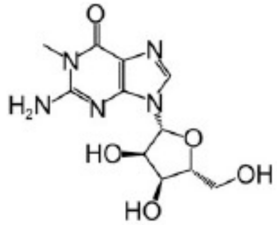

ac^4C

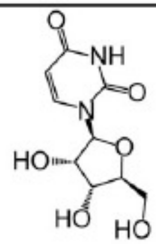

hm^5C

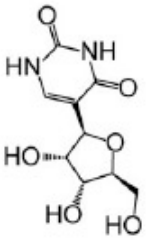

G

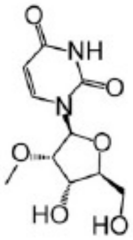

m^7G


Gm


m^1G

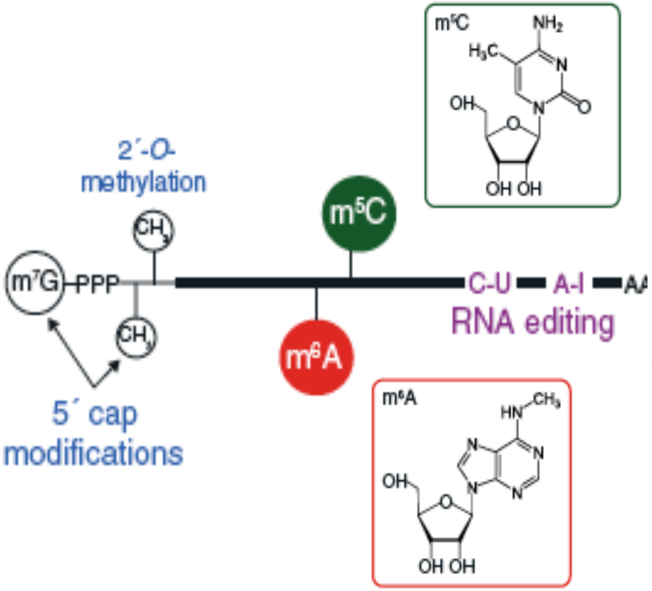

U

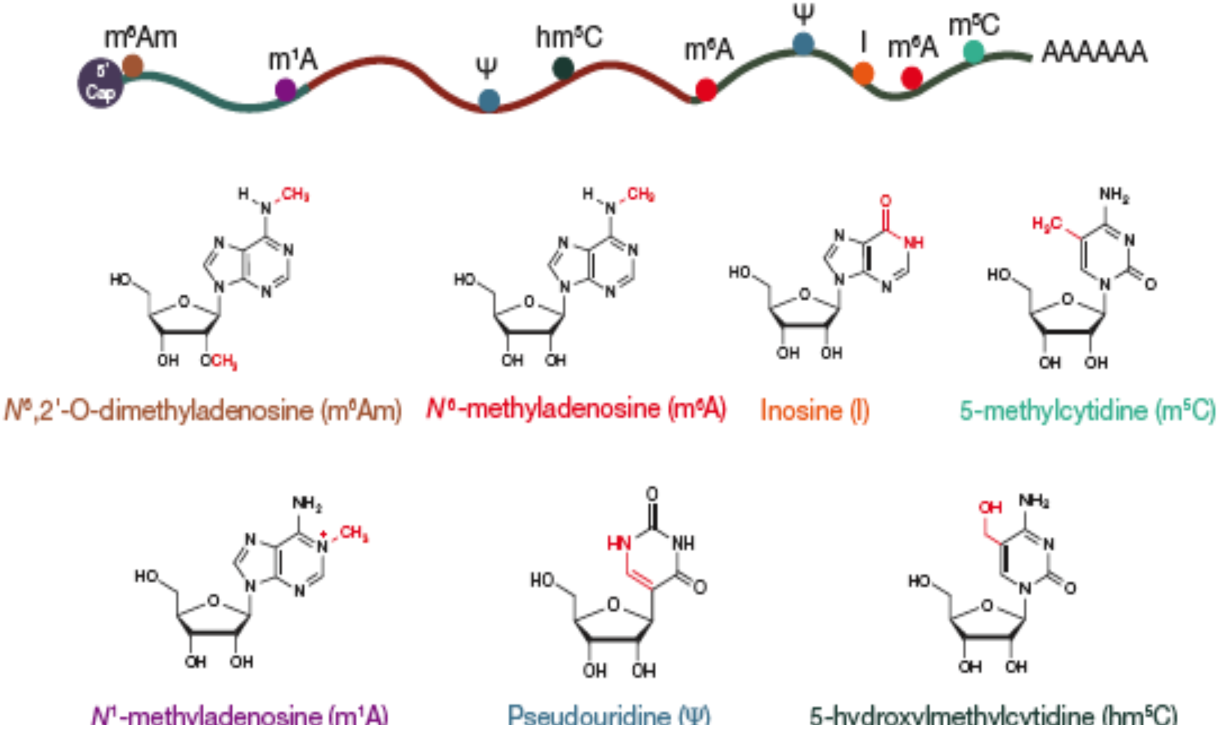

Ψ


Um

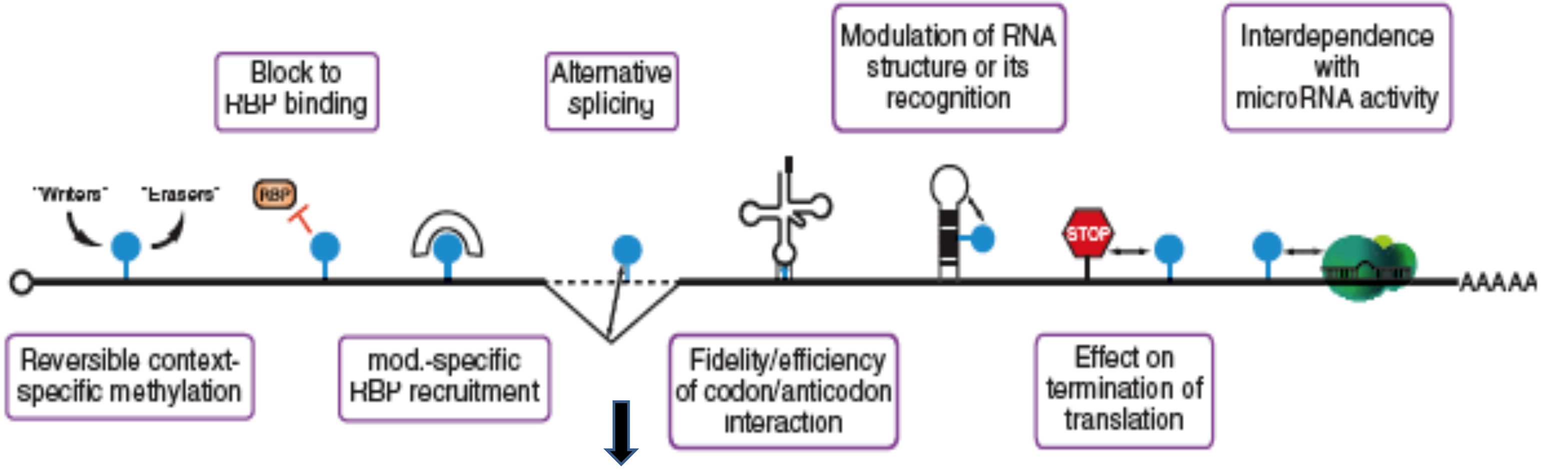
Jones et al., WIREsRNA 2021

RNA modifications





Functions



Block to RBP binding

Alternative splicing

Modulation of RNA structure or its recognition

Interdependence with microRNA activity

Reversible context-specific methylation

mod.-specific RBP recruitment

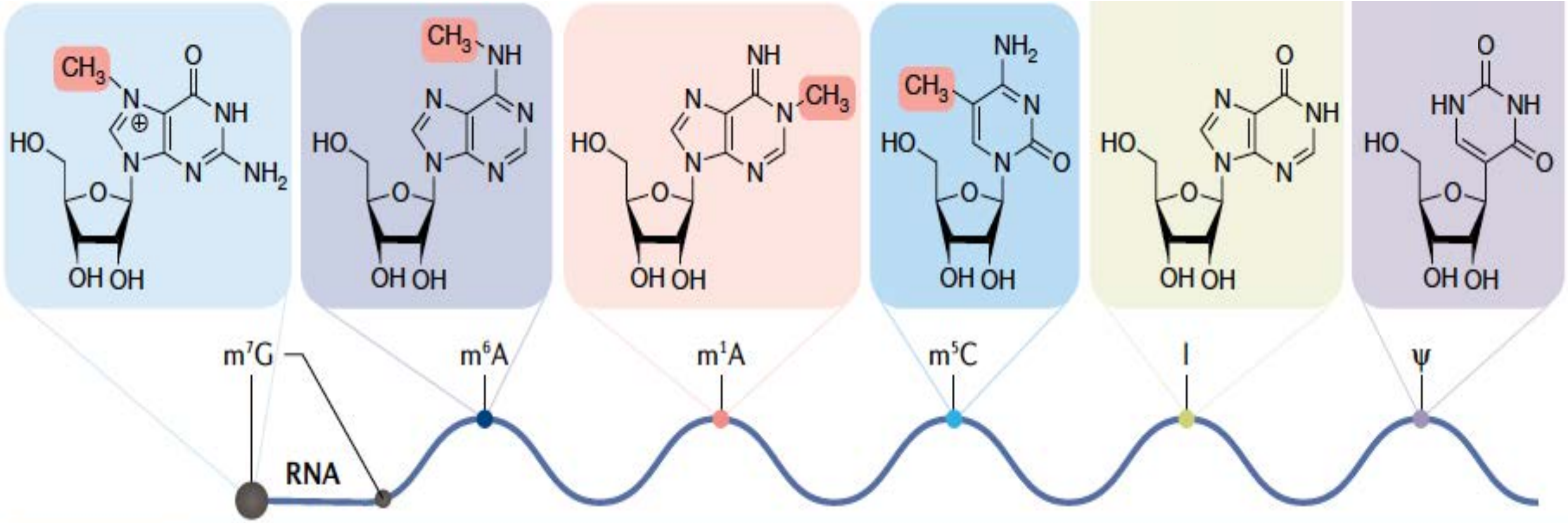
Fidelity/efficiency of codon/anticodon interaction

Effect on termination of translation

mRNA splicing, export, localisation, decay, translation, innate immunity

Sibbrrit et al, WIREsRNA 2013
Jones et al., WIREsRNA 2021

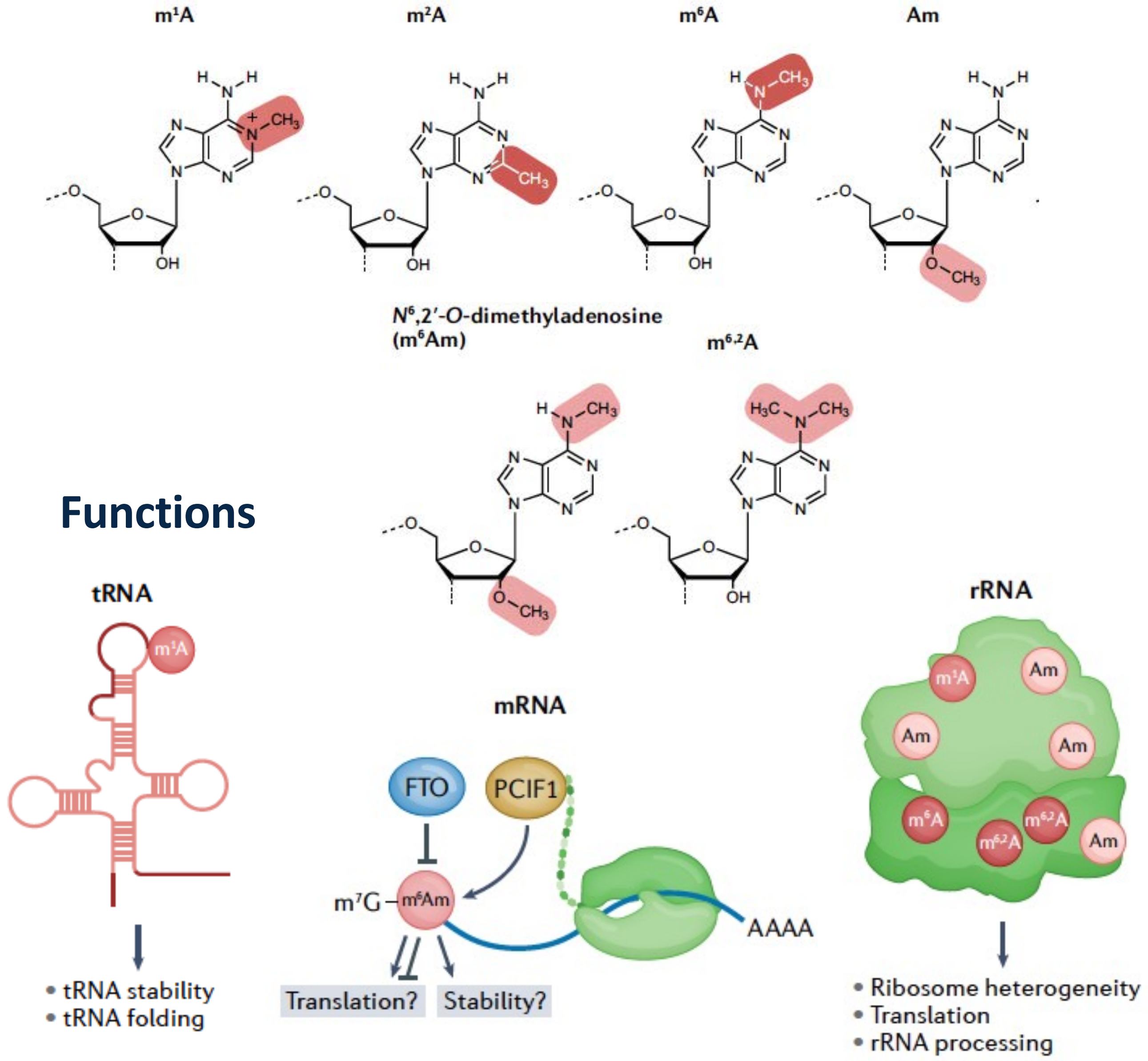
RNA modifications

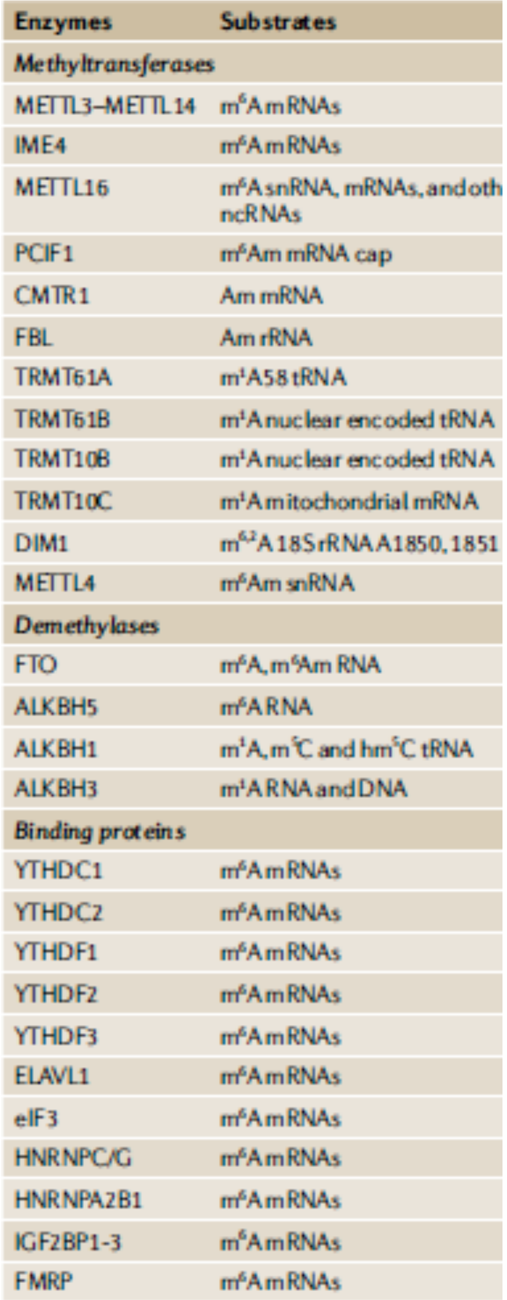


Writers	• METTL1 • WBSCR22 • RNMT	• METTL3–METTL14 • METTL16 • METTL5 • ZCCHC4	• TRMT10 • TRM6–TRM61	• NSUN1 to NSUN7 • DNMT2	• ADAR1 and ADAR2 • ADAT2 and ADAT3	• PUS1 to PUS10 • PUS7L • RPUSD1 to RPUSD4 • DKC1
Readers	Unknown	• YTHDF1 to YTHDF3 • YTHDC1 and YTHDC2	YTHDF2?	ALYREF	Unknown	Unknown
Erasers	Unknown	• ALKBH5 • FTO?	ALKBH1 and ALKBH3	Unknown	Unknown	Unknown

Balbieri and Kouzarides Nat Rev Cancer 2020

RNA modifications: A methylation





Enzymes	Substrates
Methyltransferases	
METTL3–METTL14	m^6A mRNAs
IME4	m^6A mRNAs
METTL16	m^6A snRNA, mRNAs, and oth ncRNAs
PCIF1	m^6Am mRNA cap
CMTR1	Am mRNA
FBL	Am rRNA
TRMT61A	m^1A58 tRNA
TRMT61B	m^1A nuclear encoded tRNA
TRMT10B	m^1A nuclear encoded tRNA
TRMT10C	m^1A mitochondrial mRNA
DIM1	$m^{6,2}A$ 18S rRNA A1850, 1851
METTL4	m^6Am snRNA
Demethylases	
FTO	m^6A, m^6Am RNA
ALKBH5	m^6A RNA
ALKBH1	m^1A, m^5C and hm^5C tRNA
ALKBH3	m^1A RNA and DNA
Binding proteins	
YTHDC1	m^6A mRNAs
YTHDC2	m^6A mRNAs
YTHDF1	m^6A mRNAs
YTHDF2	m^6A mRNAs
YTHDF3	m^6A mRNAs
ELAVL1	m^6A mRNAs
eIF3	m^6A mRNAs
HNRNPC/G	m^6A mRNAs
HNRNPA2B1	m^6A mRNAs
IGF2BP1-3	m^6A mRNAs
FMRP	m^6A mRNAs

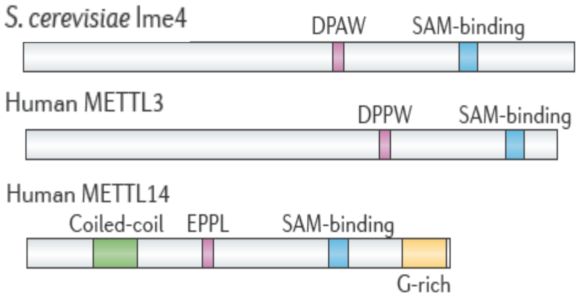
Boulias and Greer, Nat Rev Mol Cell Biol 2022

RNA modifications: mRNA m^{6}A

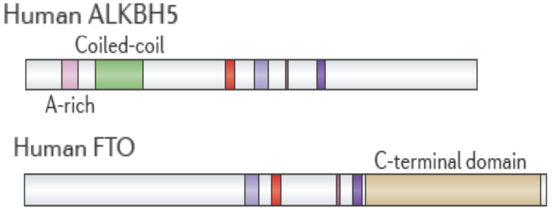
N^6-methyladenosine:

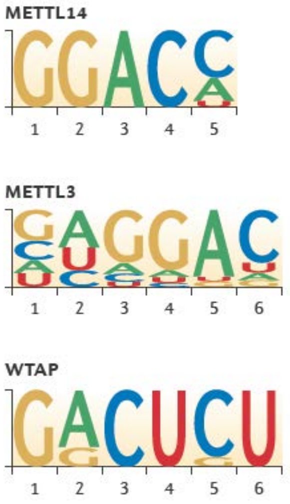
- **in eukaryotic mRNAs and lncRNAs** (discovered in 1970s)
- **reversible, conserved, present in 0.1–0.4% of As in mammals** (~3–5 m^{6}A sites per mRNA)
- **methyltransferases METTL3 or METTL4-METTL14** (with **WTAP), METTL16, IME4**
  in a [G/A/U][G>A]m6AC[U>A>C] context
- **demethylases FTO** and **ALKBH1, 3, 5**
- **readers YTH proteins : YTHDF1-3, YTHDC1-2, eIF3, ELAVL1, FMRP…**

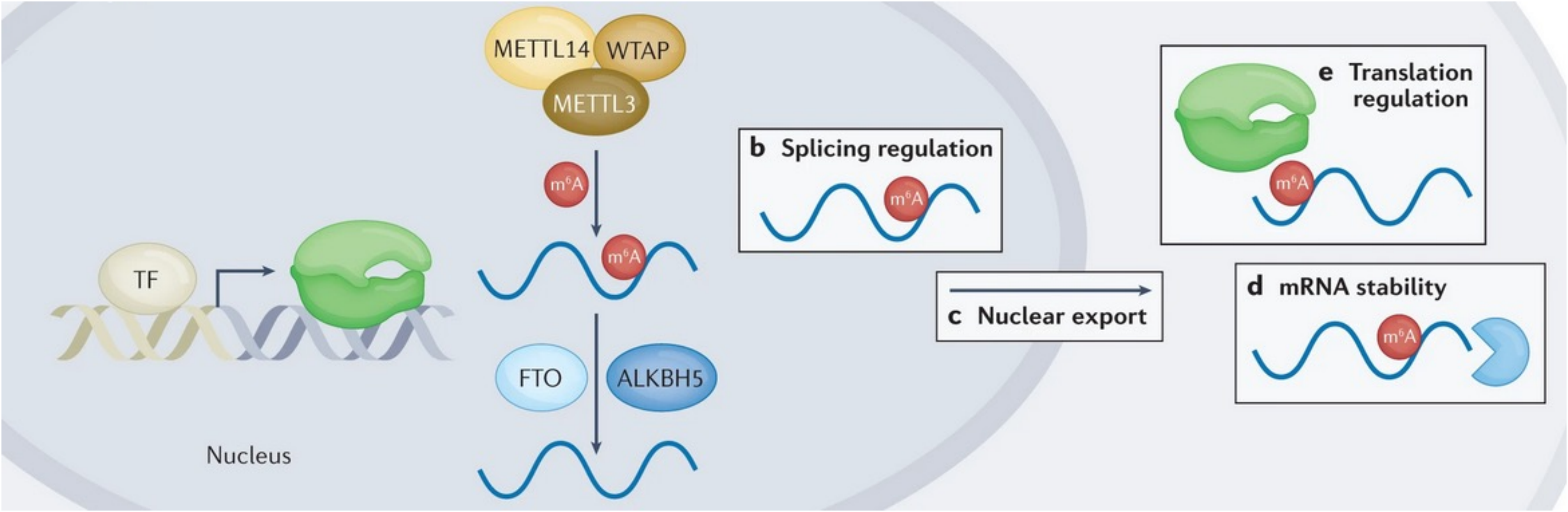
methyltransferases



demethylases

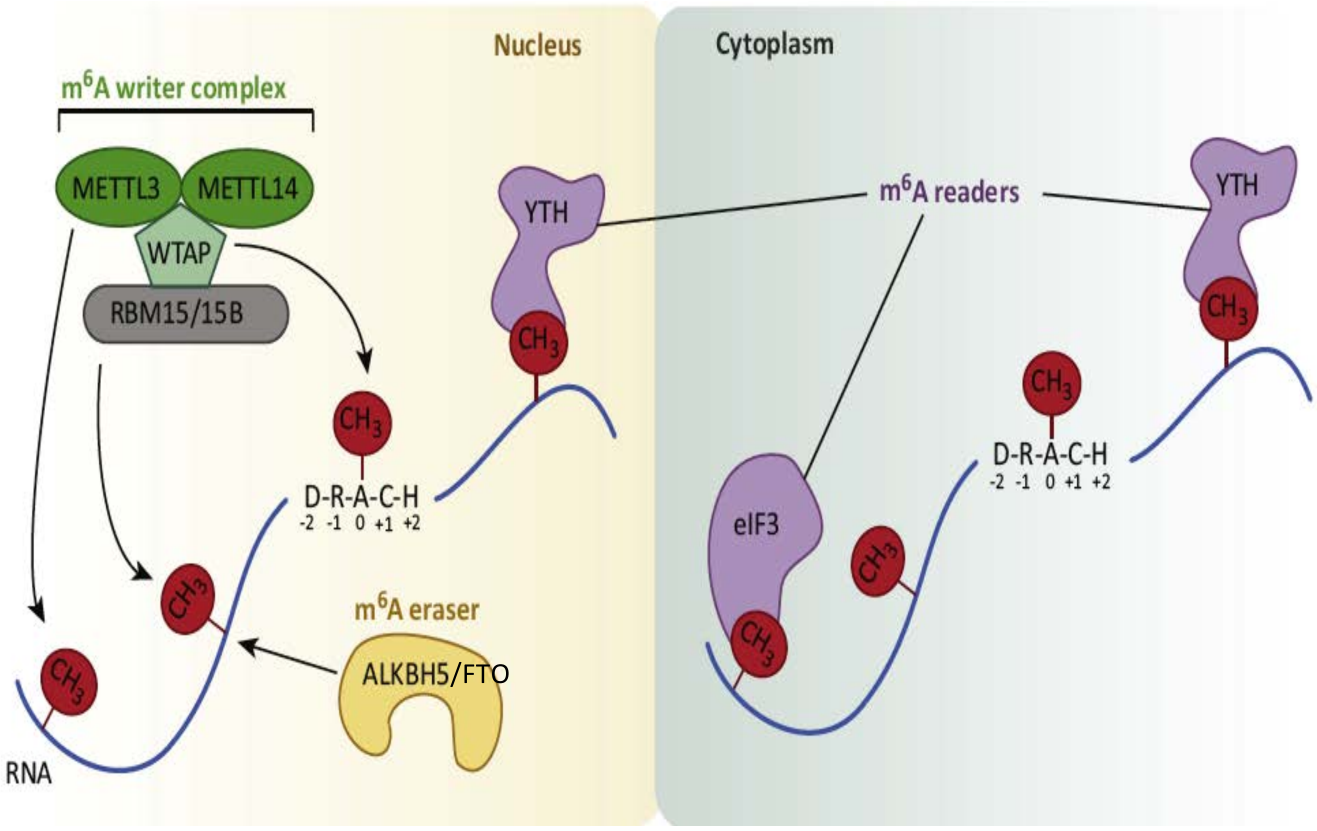


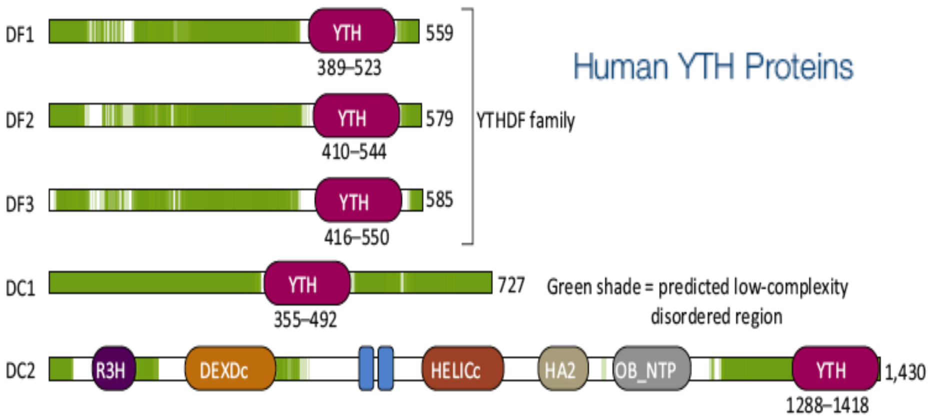




Dominissini at al, Nat.Rev.Genet., 2014; Boulias and Greer, Nat Rev Mol Cell Biol 2022

RNA modifications: mRNA m^{6}A





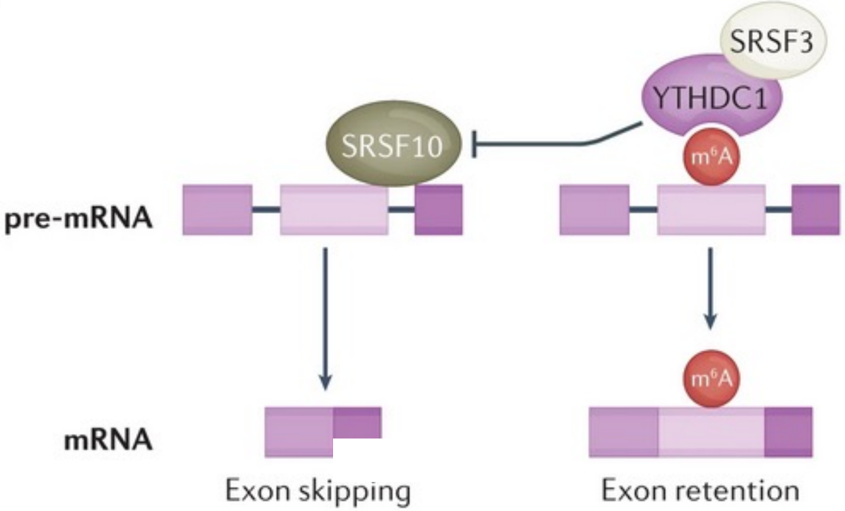
Readers

YTH family recognizes m^{6}A RNA

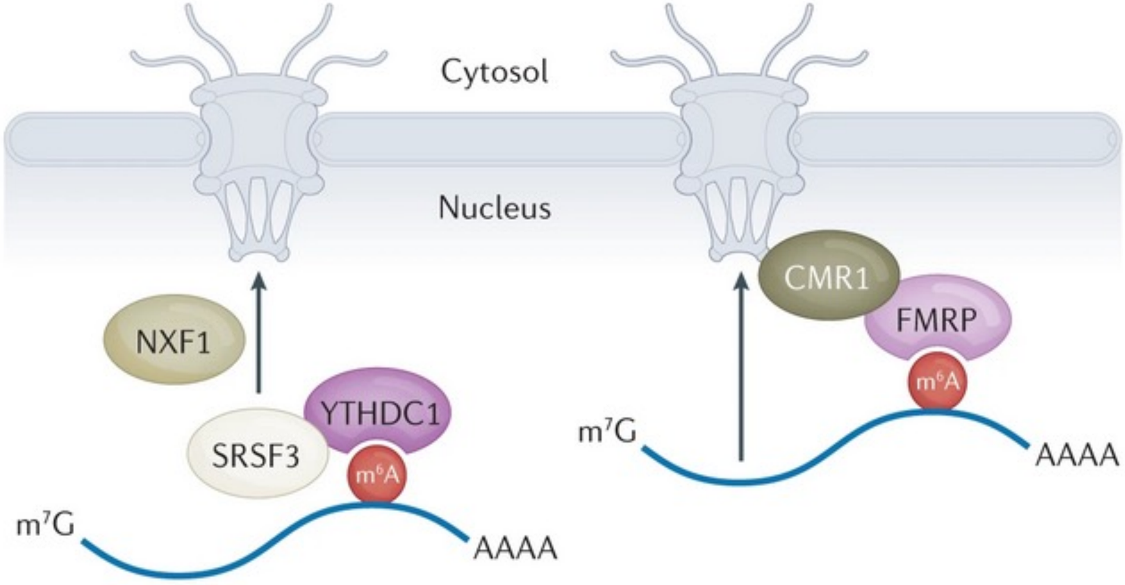
m^{6}A can be also read by hnRNPs or translation factors (eIF3)

Patil at al., TiCB, 2018

RNA modifications: mRNA m^{6}A



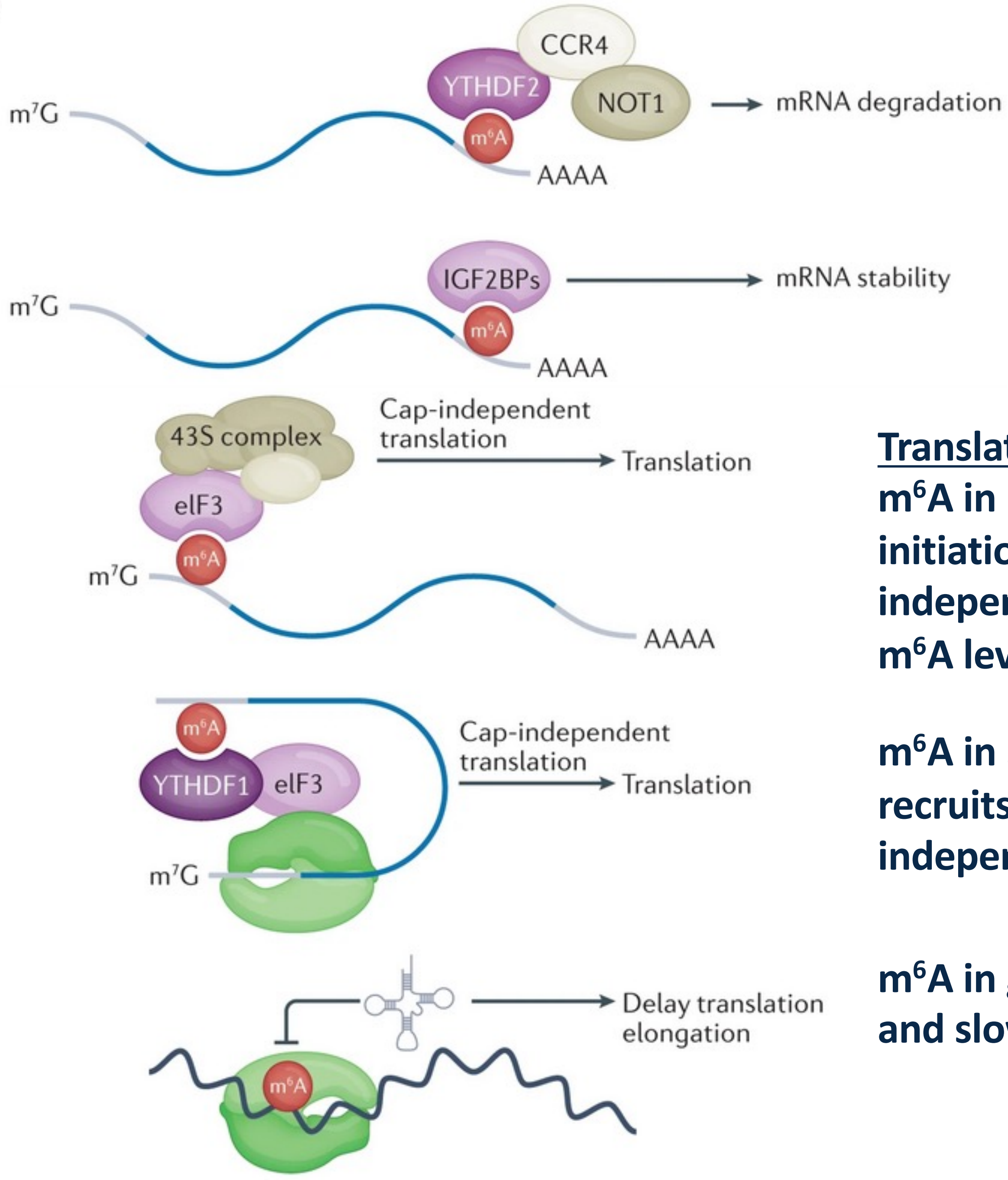
Splicing
YTHDC1 m^{6}A reader facilitates binding of SRSF3 and blocks of SRSF10 (SR proteins), promoting exon inclusion



mRNA export
m^{6}A readers facilitate mRNA export by binding export factors

Boulias and Greer, Nat Rev Mol Cell Biol 2022

RNA modifications: mRNA m^{6}A



mRNA stability

YTHDC2 m^{6}A reader recruits CCR4-NOT complex, stimulating mRNA deadenylation.

IGF2BPs m^{6}A reader promotes mRNA stability.

Translation

m^{6}A in 5'UTR: eIF3 m^{6}A reader recruits 43S initiation complex to promote cap-independent translation.

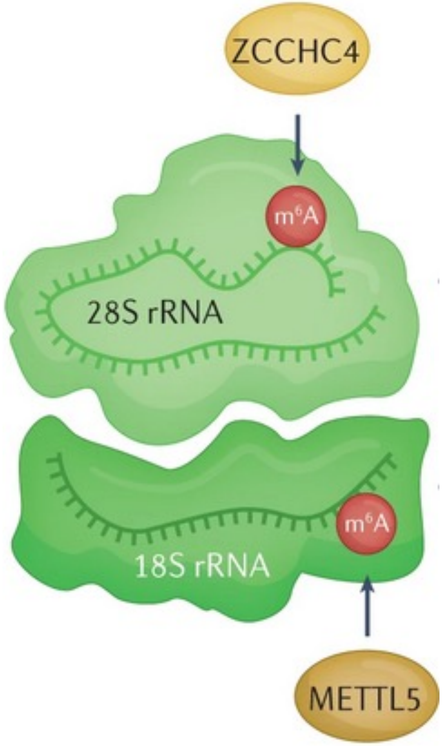
m^{6}A level in 5'UTR is enhanced by stress.

m^{6}A in 3'UTR: YTHDF1/F3 bind eIF3 which recruits 43S and stimulates cap-independent translation.

m^{6}A in gene body inhibits tRNA selection and slows translation elongation.

Boulias and Greer, Nat Rev Mol Cell Biol 2022

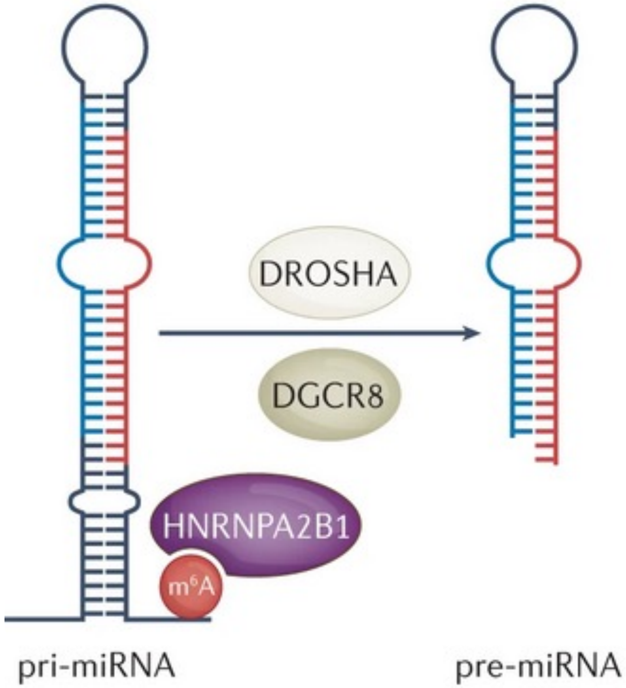
RNA modifications: ncRNA m^{6}A



rRNA, translation regulation

ZCCHC4 methylates A4220 in 28S rRNA resulting in increased translation and inhibition of cell proliferation

METTL5-mediated methylation of A1832 in 18S rRNA results in selectively increased translation of a unique sets of transcripts

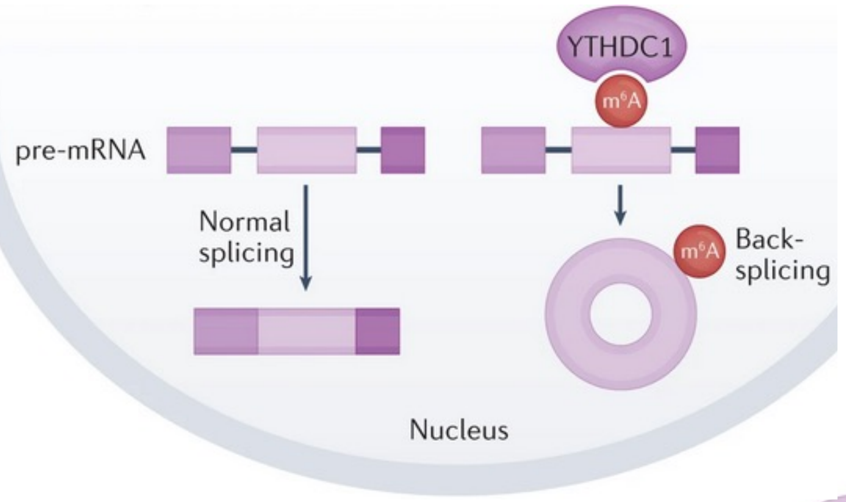


miRNA processing

m^{6}A deposited by METTL3 and recognized by HNRNPA2B1 that recruits Microprocessor is required for pri-miRNA processing by DROSHA/DGCR8

Boulias and Greer, Nat Rev Mol Cell Biol 2022

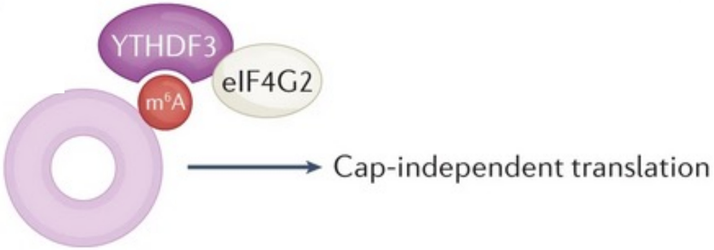
RNA modifications: ncRNA m^{6}A

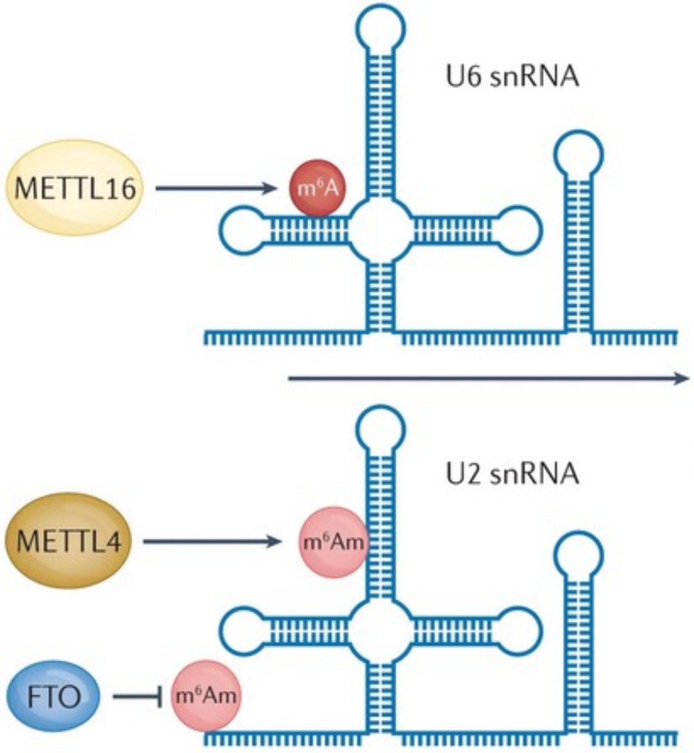


circRNA biogenesis and translation

m^{6}A induces back- splicing of circRNA through a YTHDC1- dependent mechanism.

YTHDF3 binds to m^{6}A- modified circRNAs to enhance cap- independent translation





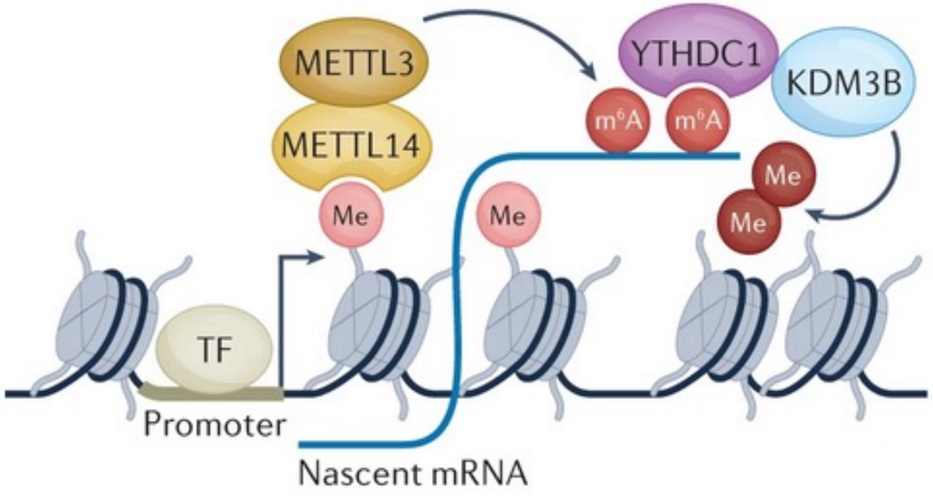
Spliceosome regulation

METTL16-dependent m^{6}A deposition on U6 snRNAs regulates spliceosome assembly or 5′ splice site recognition.

m^{6}Am modification of U2 snRNAs by METTL4 increases splicing whereas FTO demethylation of U2 snRNAs inhibits splicing

Boulias and Greer, Nat Rev Mol Cell Biol 2022

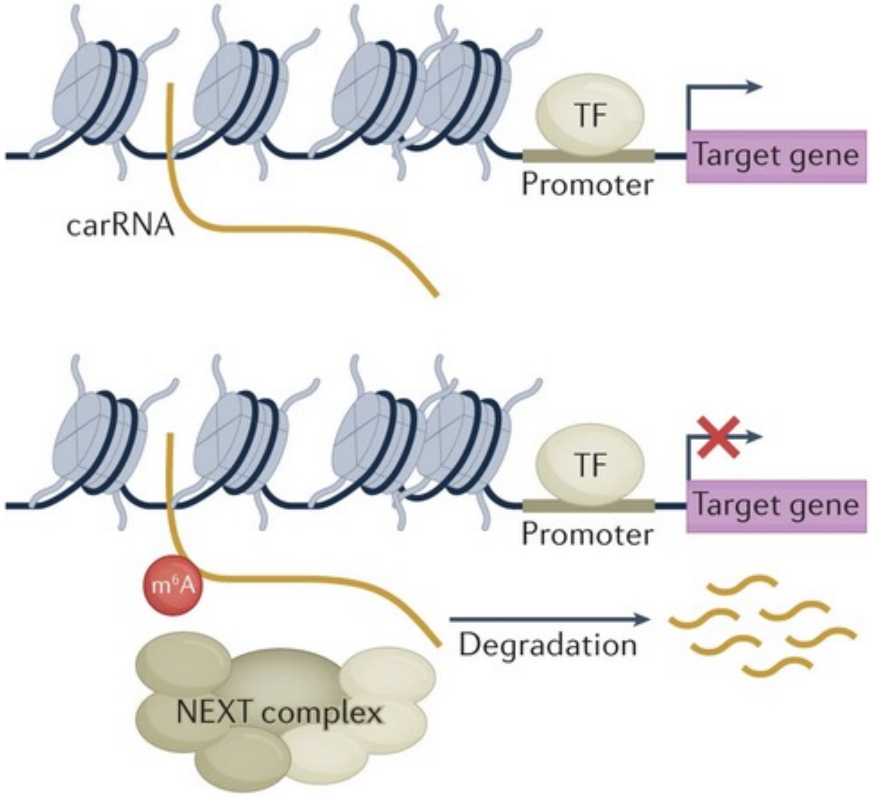
RNA modifications: ncRNA m^6A



Chromatin crosstalk via nascent RNA

YTHDC1 binding to m^6A in nascent transcripts and to KDM3B (H3K9 demethylase) induces histone demethylation and reinforces chromatin accessibility in transcribed regions.

METTL3-METTL14 writer that methylates nascent transcripts is recruited by H3K36-Me.



carRNA suppression

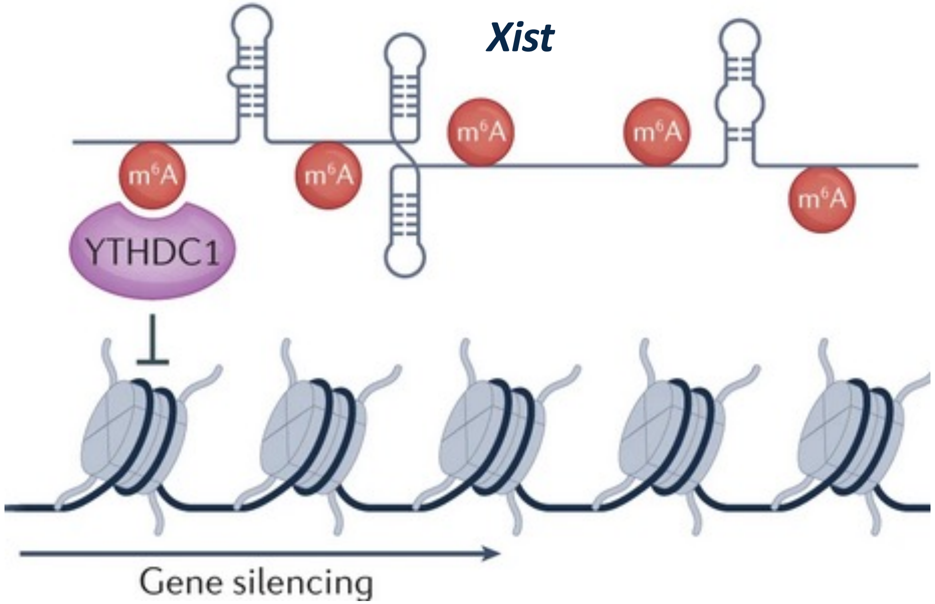
m^6A in causes their degradation via the NEXT complex, resulting in gene repression

carRNAs promote chromatin accessibility

chromosome-associated regulatory RNAs
PROMPTs (promoter associated RNAs)
eRNAs (enhancer RNAs)
repeat RNAs

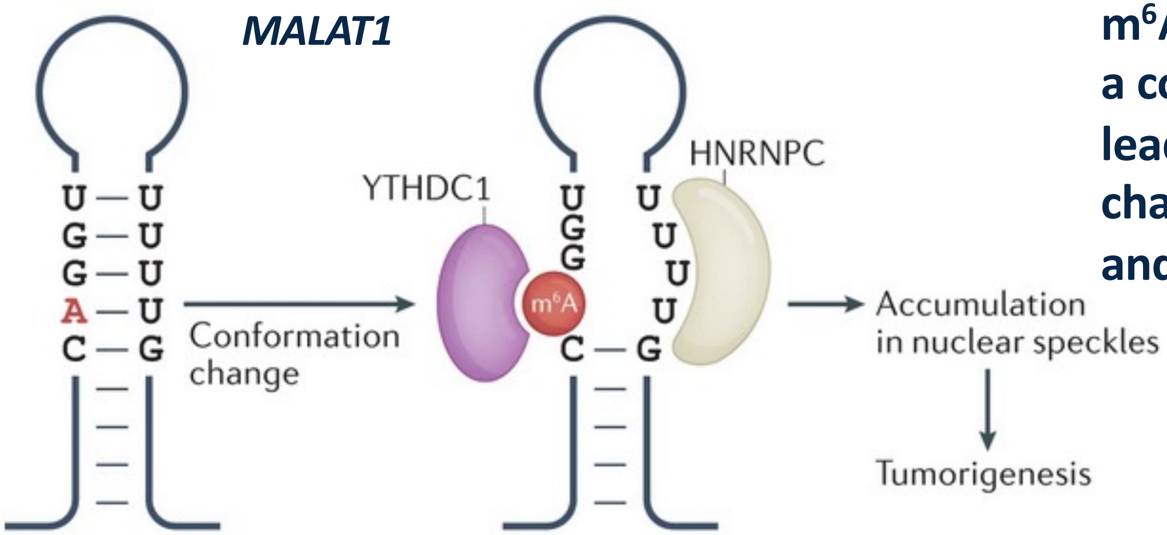
Boulias and Greer, Nat Rev Mol Cell Biol 2022

RNA modifications: ncRNA m^{6}A



lncRNA regulation

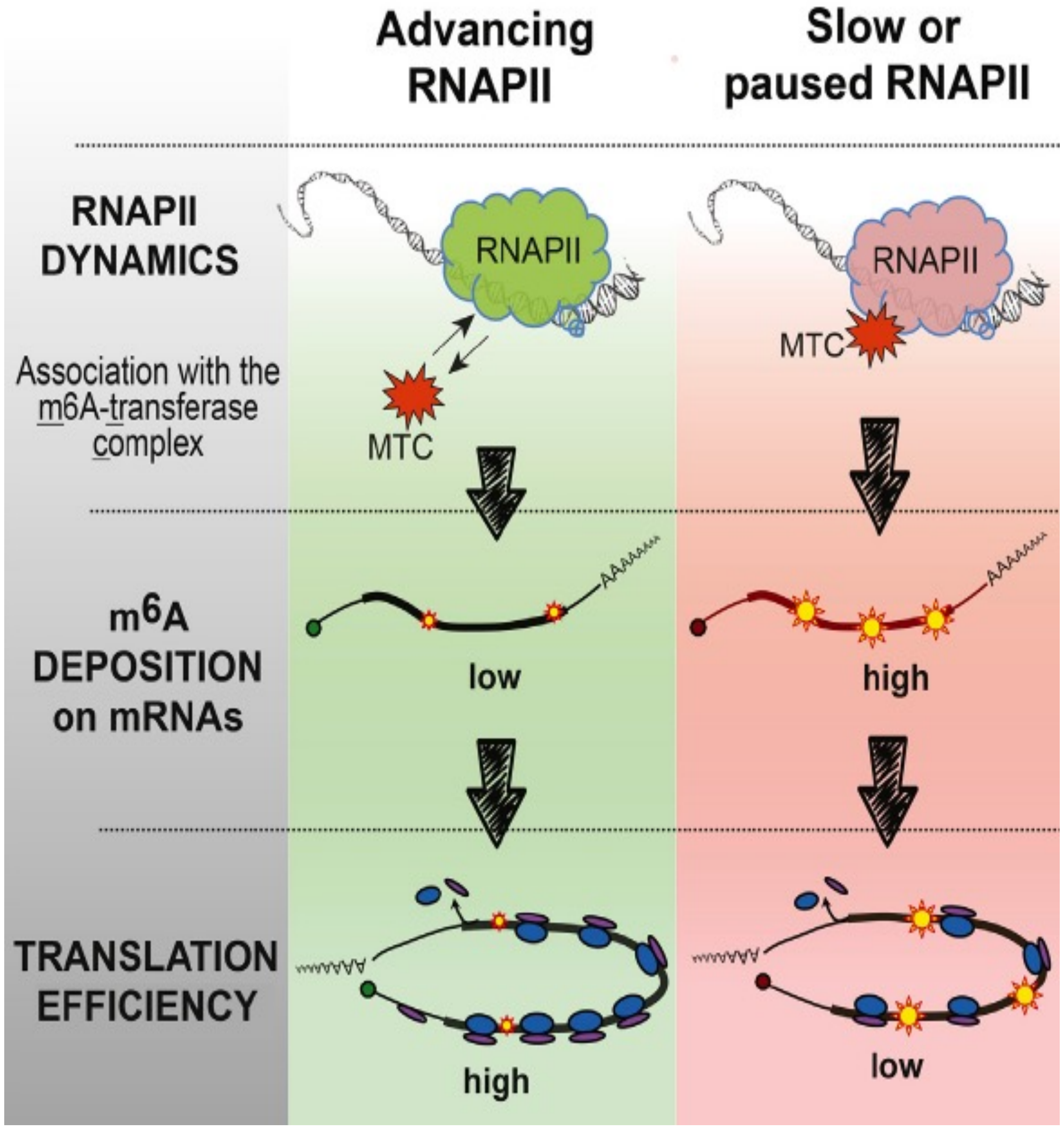
Xist **lncRNA m^{6}As promote** ***Xist*****-mediated gene silencing and X chromosome inactivation in a YTHDC1- dependent manner**



m^{6}A in ***MALAT1*** **lncRNA induces a conformational change, which leads to binding of HNRNPC and changes in nuclear organization and tumorigenesis**

Boulias and Greer, Nat Rev Mol Cell Biol 2022

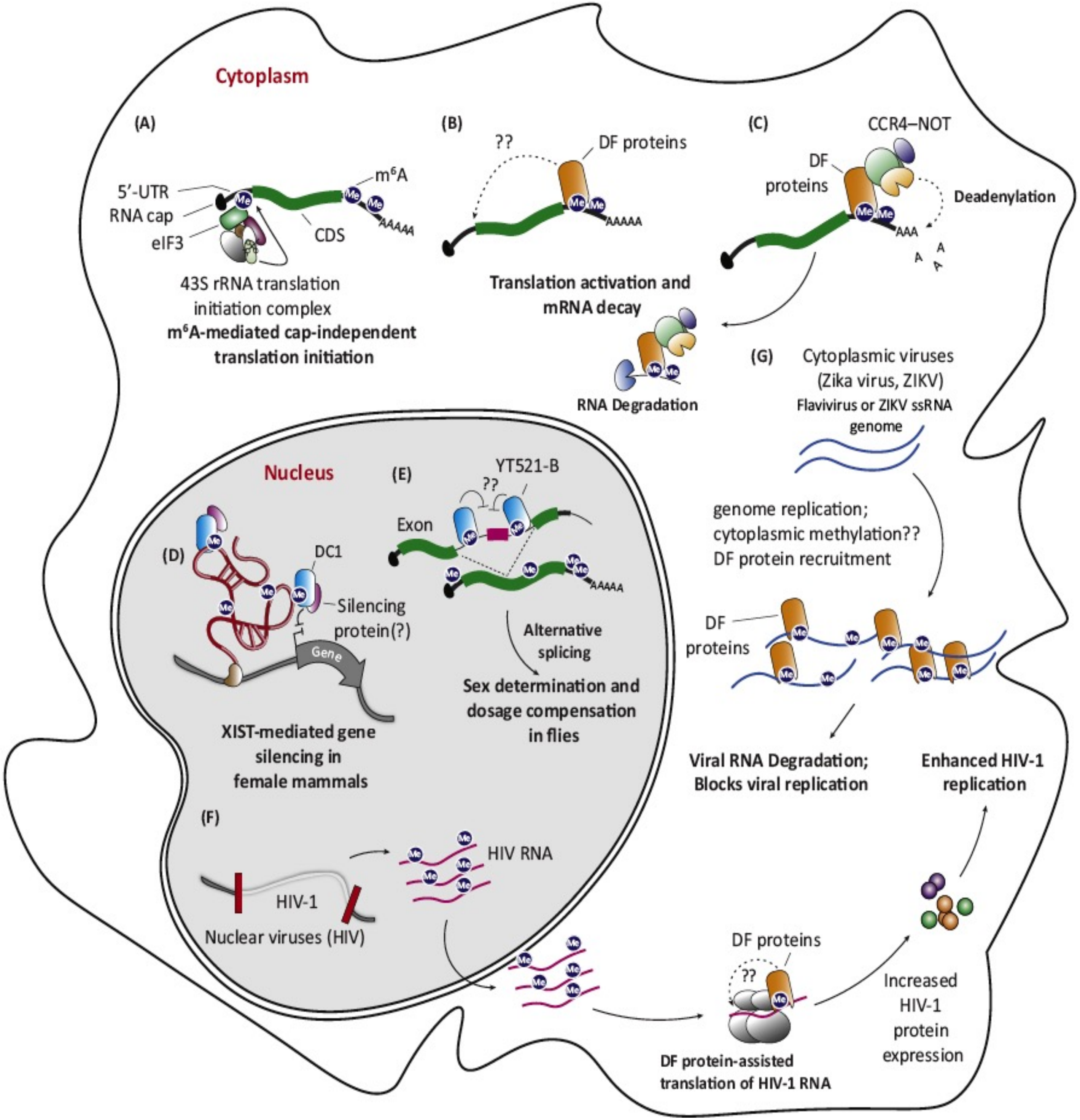
m^6A, Pol II and translation



- mRNA transcription rates correlate with translation
- MTC complex associates with slow Pol II
- slow Pol II results in higher level of m^6A in mRNAs
- high level of m^6A reduces translation rate
- nuclear control of protein abundance

Slobodin et al, Cell, 2017

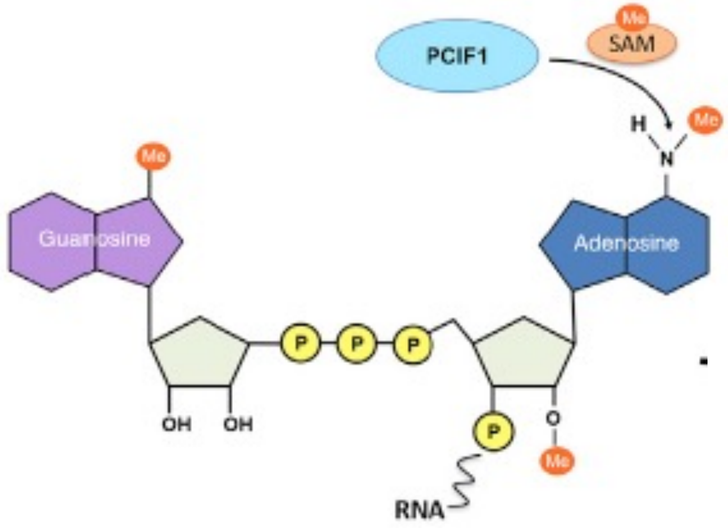
m^{6}A multiple functions

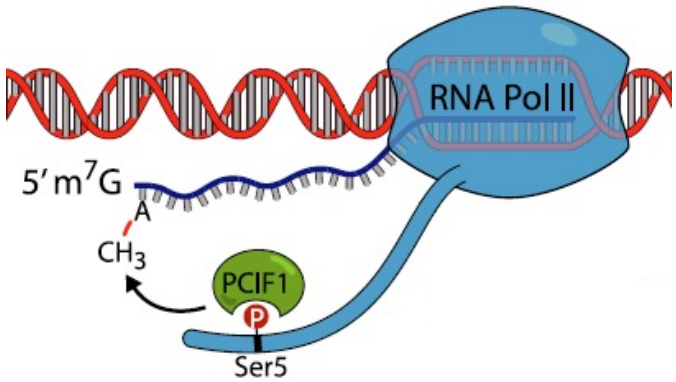


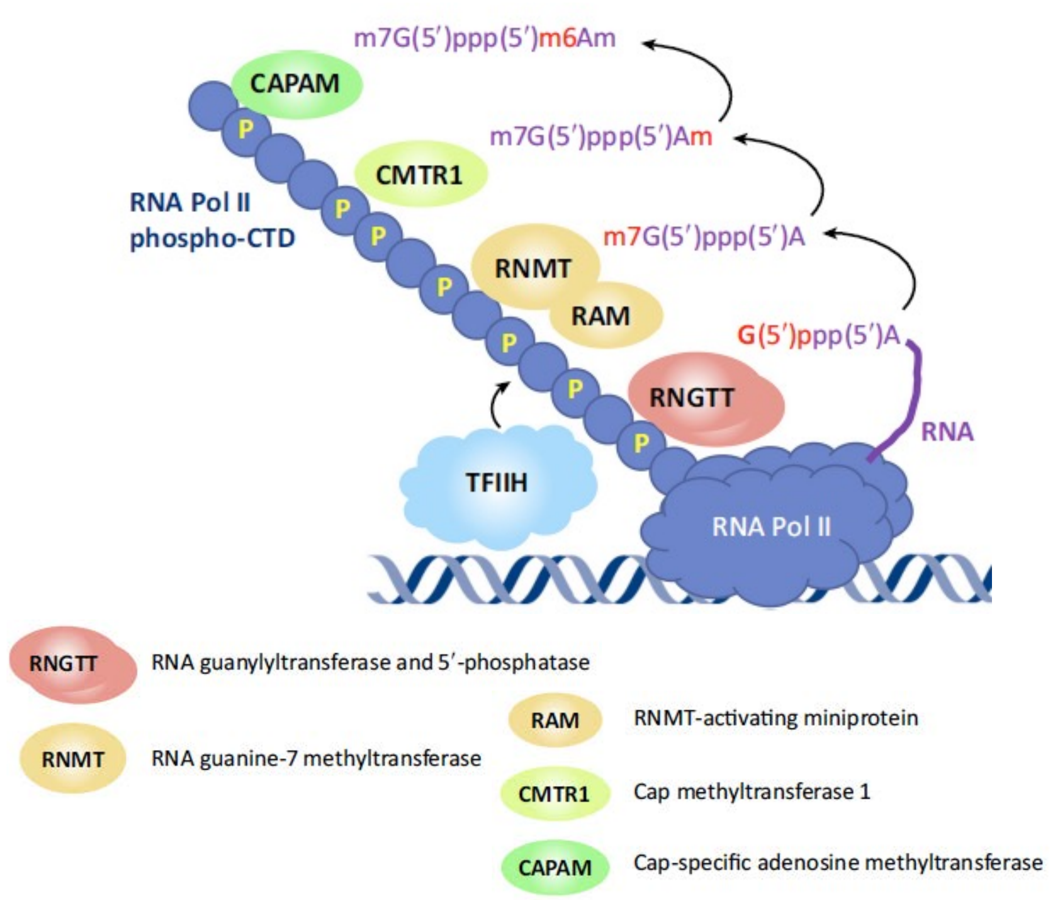
Epitranscriptomics
Meiosis
Sex determination
Cellular differentiation
Development
Pluripotency and Reprogramming
Disease
Cancer

Patil et al, 2018, TiCB

RNA modifications: mRNA m^{6}Am







RNGTT	RNA guanylyltransferase and 5′-phosphatase
RNMT	RNA guanine-7 methyltransferase
RAM	RNMT-activating miniprotein
CMTR1	Cap methyltransferase 1
CAPAM	Cap-specific adenosine methyltransferase

N^6-methyladenosine:

- **in eukaryotic mRNAs, introduced close to m^{7}G cap (at TSS)**
- **methyltransferase PCIF1/CAPAM, demethylase FTO**
- **resistant to Dcp2 decapping**
- **controls mRNA stability, RNAs with m^{6}Am are more stable, but has little effect on translation**

Mauer et al, 2017; Boulias et al, Mol Cell 2019; Pandey et al, Cell Rep 2020; Oerum et al, NAR 2021

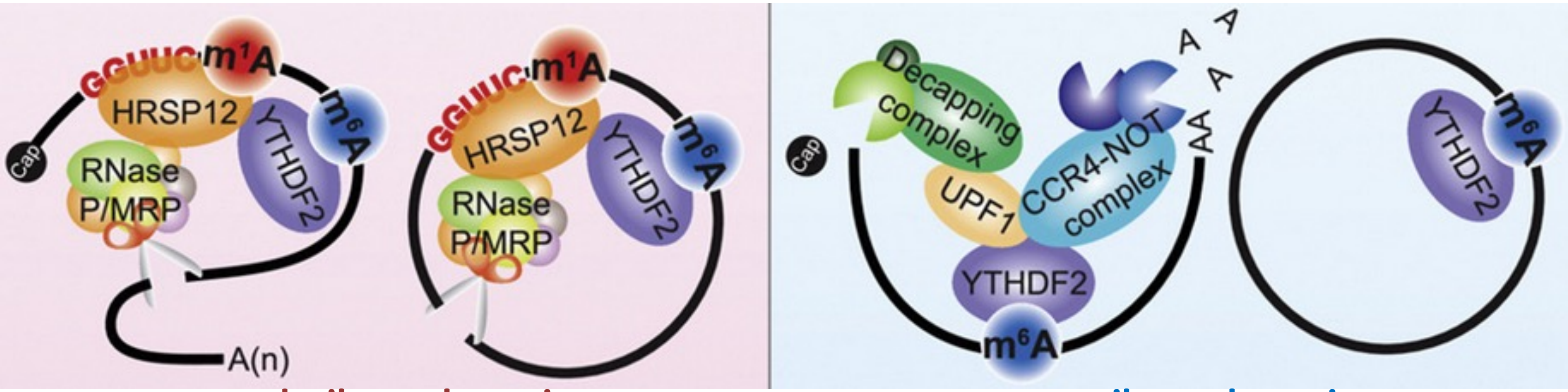
RNA modification: mRNA m^{1}A

m^{1}A introduced by TRMT6/TRMT61A (nuclear) **or TRMT61B, TRMT10C** (mitochondrial)

~~m^{1}A is abundant in mRNAs (20% in humans), prevalent in mitochondrial transcripts, enriched near start codons and promotes translation or differently impacts translation depending on the position in mRNA region~~
incorrect due to unspecific antibodies that recognize m^{7}G cap!
(Dominissini et al, Nature, 2017; Li at at, Mol Cell, 2017)

- **m^{1}A in mRNAs are rare and occur at very low stoichiometry**
- **m^{1}A are present in some mt-RNAs** (confirmed for *mt-ND5*)
- **m^{1}As are avoided in cells, they disrupt W-C basepairing and lead to translational repression**

(Safra et al, Nature, 2017 ; Grozhik et al, Nat Comm, 2019)

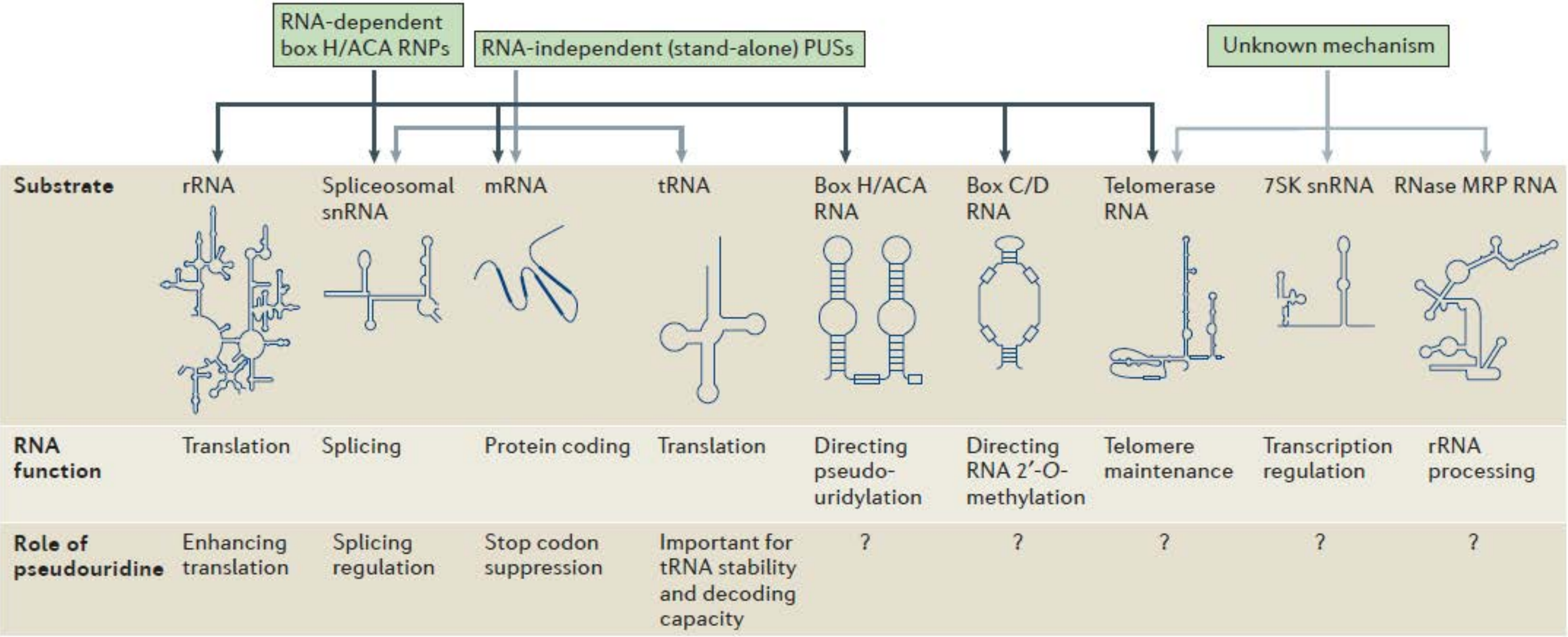


endoribonucleotytic **exoribonucleotytic**

Boo et al, Cell Rep, 2022

m^{1}A are recognized by HRSP12 at GGUUC consensus
m^{1}A and m^{6}A cooperatively promote the interaction between RNA and HSRP12-YTHDF2
m^{1}A facilitates m^{6}A-mediated mRNA degradation via HRSP12-YTHDF2 by RNaseP/MRP
m^{1}A promotes rapid degradation of m^{6}A-containing circRNAs by the same pathway

RNA modifications: pseudoU



RNA-dependent box H/ACA RNPs

RNA-independent (stand-alone) PUSs

Unknown mechanism

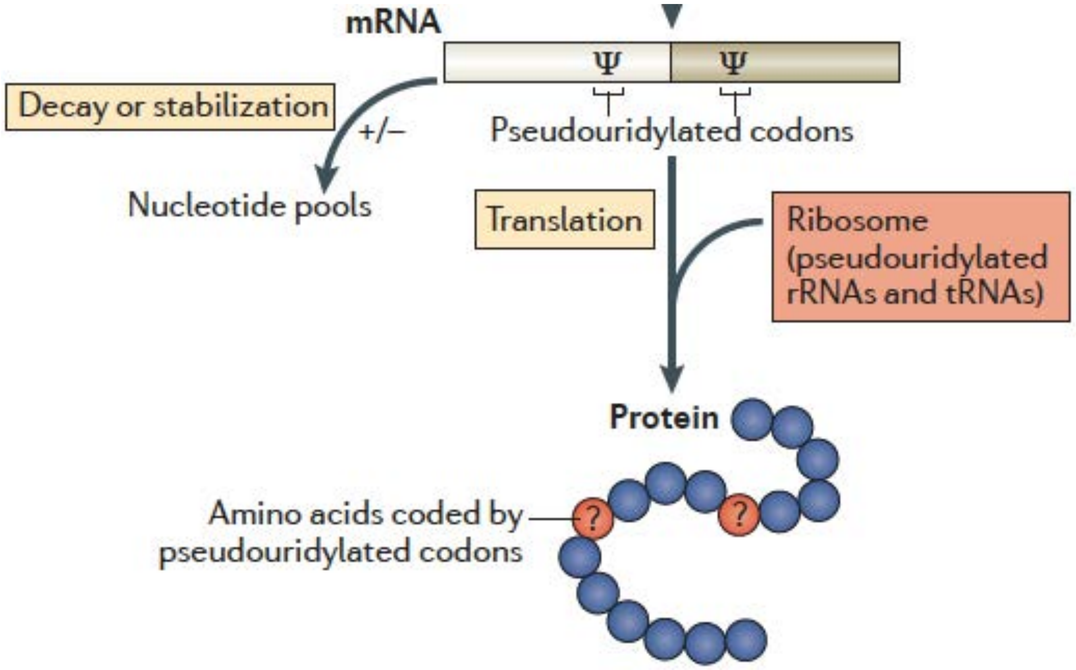
Substrate	rRNA	Spliceosomal snRNA	mRNA	tRNA	Box H/ACA RNA	Box C/D RNA	Telomerase RNA	7SK snRNA	RNase MRP RNA
RNA function	Translation	Splicing	Protein coding	Translation	Directing pseudouridylation	Directing RNA 2′-*O*-methylation	Telomere maintenance	Transcription regulation	rRNA processing
Role of pseudouridine	Enhancing translation	Splicing regulation	Stop codon suppression	Important for tRNA stability and decoding capacity	?	?	?	?	?

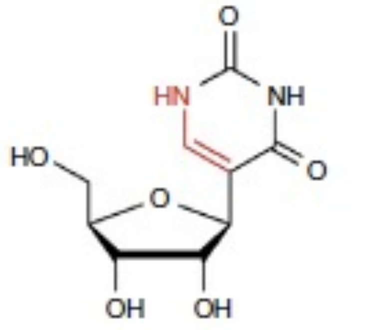
Karijolich et al, Nat Rev Mol Cell Biol, 2015

RNA modifications: mRNA pseudoU

PseudoU (Ψ):

- generated by pseudouridin synthases
- affects translation efficiency
- impacts mRNA structure





Pseudouridine (ψ)

Karijolich et al, NatRevMolCellBiol, 2015

Ψ

- reduces rate of translation elongation and EF-Tu GTPase activation

- increases the levels of amino acid substitution

- suppresses stop codons

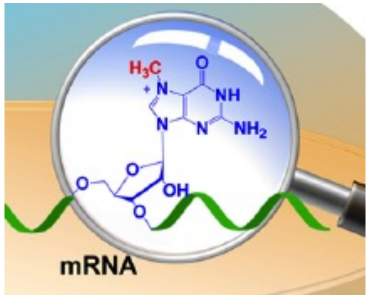
Eyler et al, PNAS, 2019

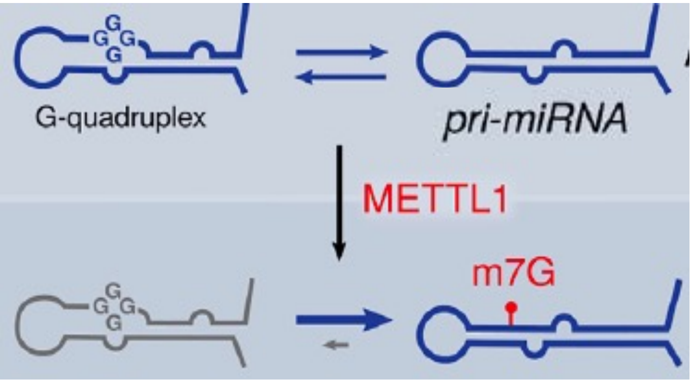
N1mΨ (N1-methyl-pseudouridine)

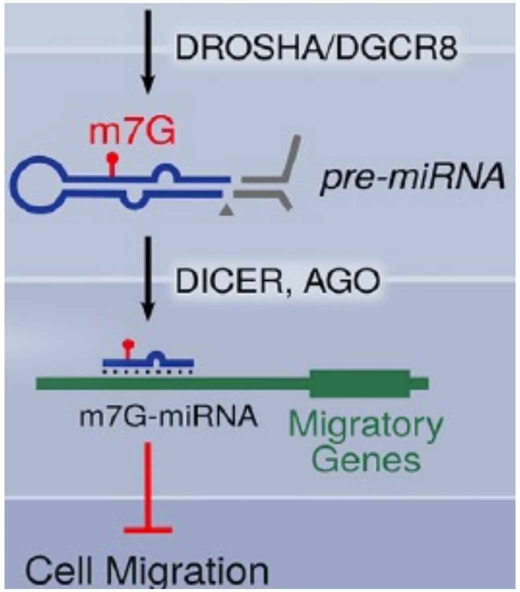
- enhances translation by increasing ribosome density

Svitkin et al, NAR, 2017

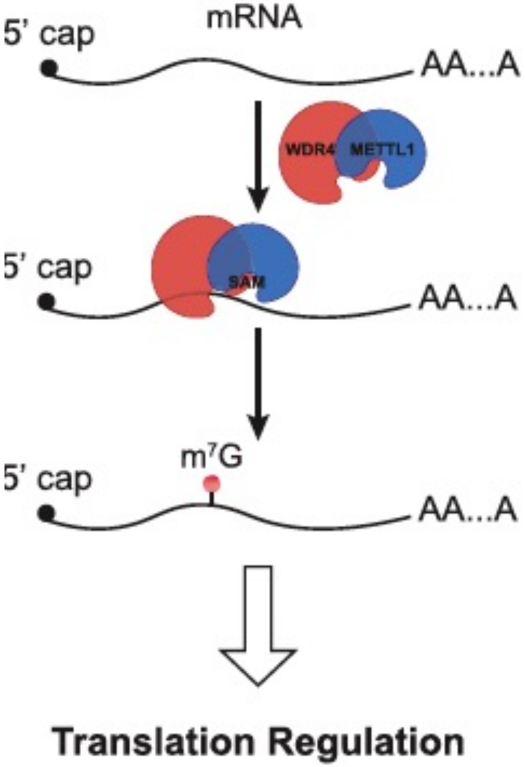
RNA modifications: internal m^{7}G







Pandolfini et al, 2019, MCell

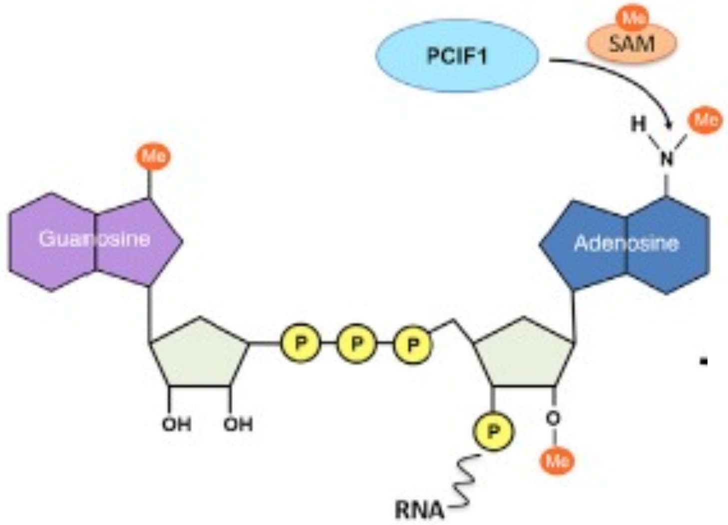


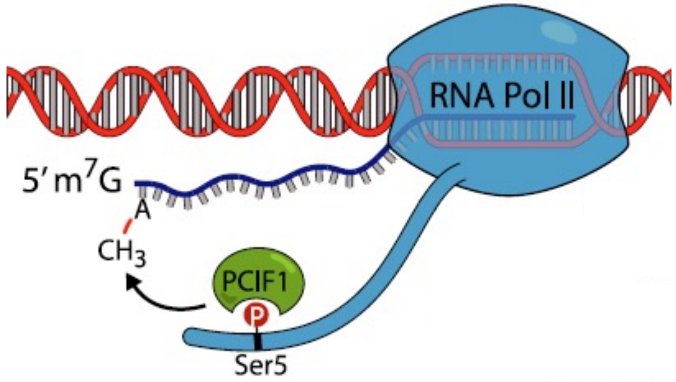
- **specific miRNAs are m^{7}G-modified by METTL1**
- **m^{7}G promotes miRNA processing by antagonizing G4 in pre-miRNAs**
  (G4 inhibit pre-miRNA processing)

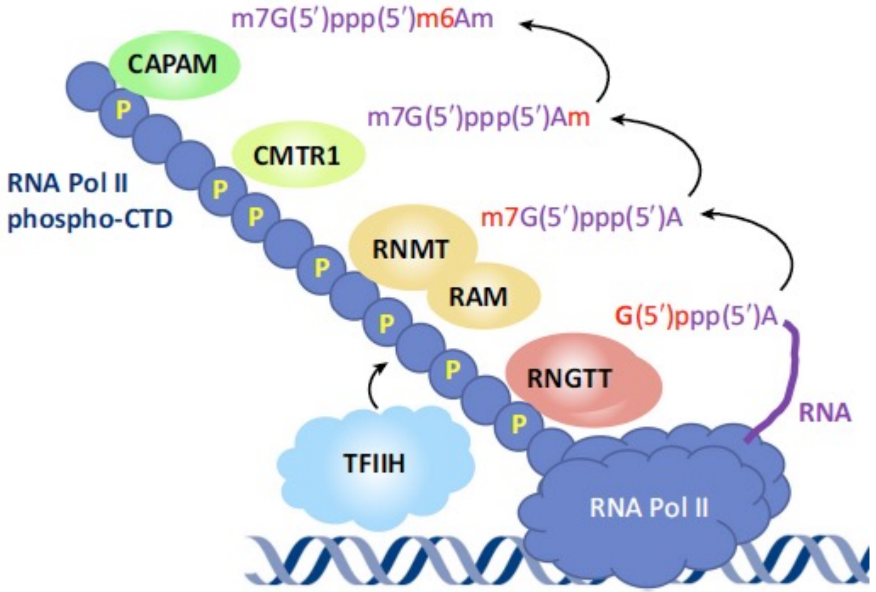
- **human mRNAs contain internal m^{7}Gs**
- **some m^{7}Gs are introduced by METTL1**
- **internal m^{7}G affect translation**

Zhang et al, 2019, MCell

RNA modifications: mRNA m^6Am







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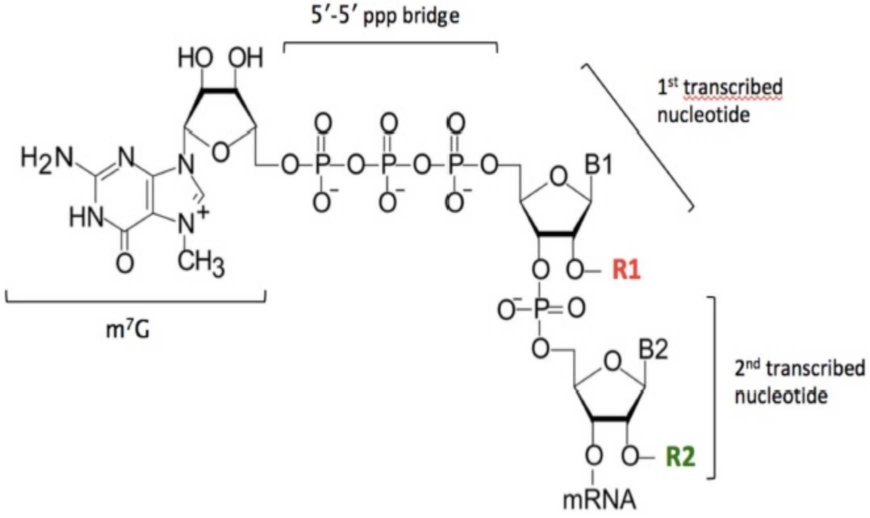
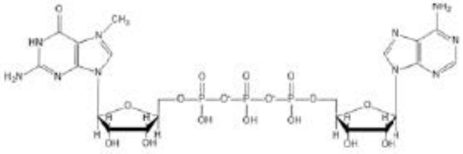
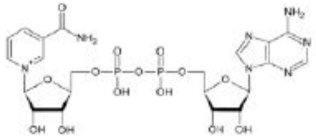
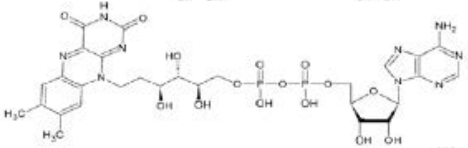
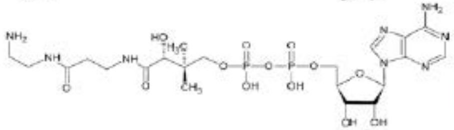
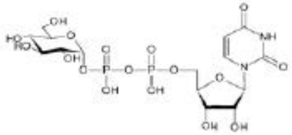
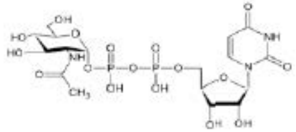
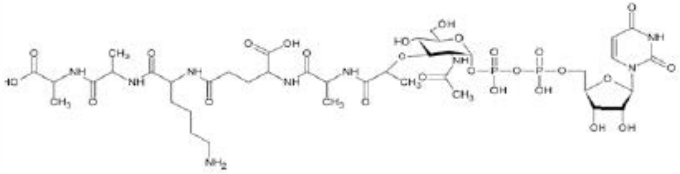
Mauer et al, 2017; Boulias et al, Mol Cell 2019; Pandey et al, Cell Rep 2020; Oerum et al, NAR 2021

RNA modifications: alternative caps

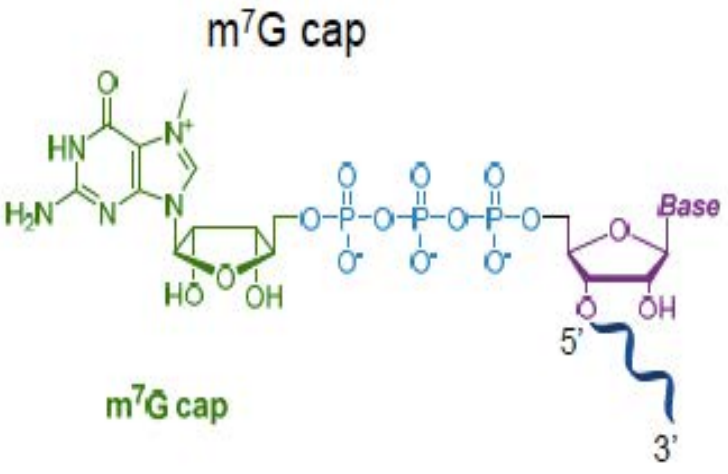


Cap0	Cap1	Cap2
R1 = H	R1 = CH_3	R1 = CH_3
R2 = H	R2 = H	R2 = CH_3

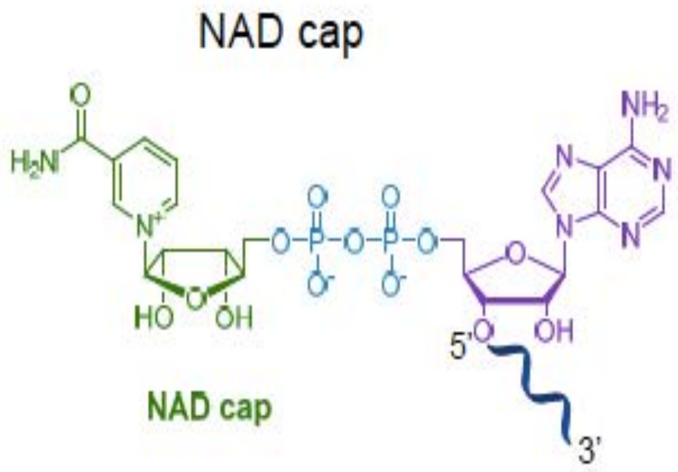
	m^7G 7-methyl-guanosine, attached to 5' end of RNA	**classic cap**
	NAD^+ nicotinamide adenine dinucleotide	**ADP cofactors**
	FAD flavine adenine dinucleotide	
	DP-CoA dephospho coenzymeA	
	UDP-Glc UDP-glucose	**Cell wall synthesis precursors**
	UDP-GlcNAc UDP-N-acetyl glucosamine	
	MurNAc-pentapeptide UDP-N-acetyl muramyle-pentapeptide	

Dimitrova et al, Genes 2019;
Jukius and Yuzenkova, WIREsRNA 2018

NAD$^+$ RNA cap



Nicotinamide Adenine Dinucleotide

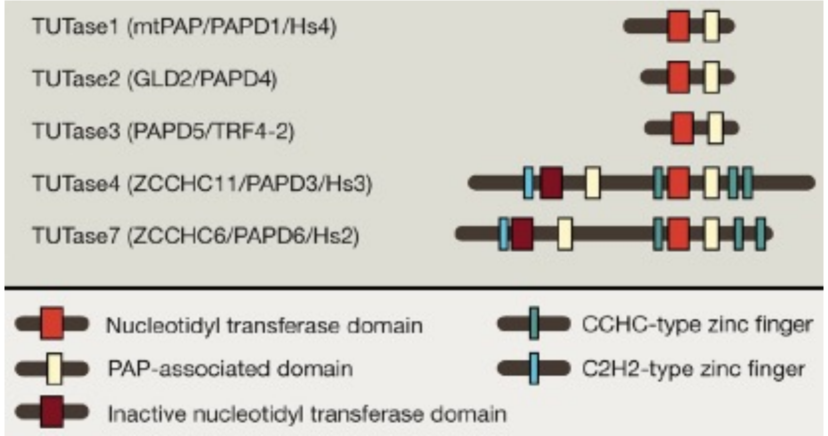


- **Found in bacteria** (2009)**, *S. cerevisiae*** (2017), **mammalian cells** (2017, **plants** (2019)
- **Added cotranscriptionally by RNAP or post-transcriptinally**
- **Targets mRNA for degradation in eukaryotes, stabilizes mRNA in bacteria**
- **Hydrolyzed by specific enzymes, DXO and NUDT families**
- **NAD$^+$ capped RNAs constitute only 1-5% of total RNAs**
- **NAD$^+$ capped RNAs are more abundant in mitochondria**
  (15% in humans, 60% in yeast)

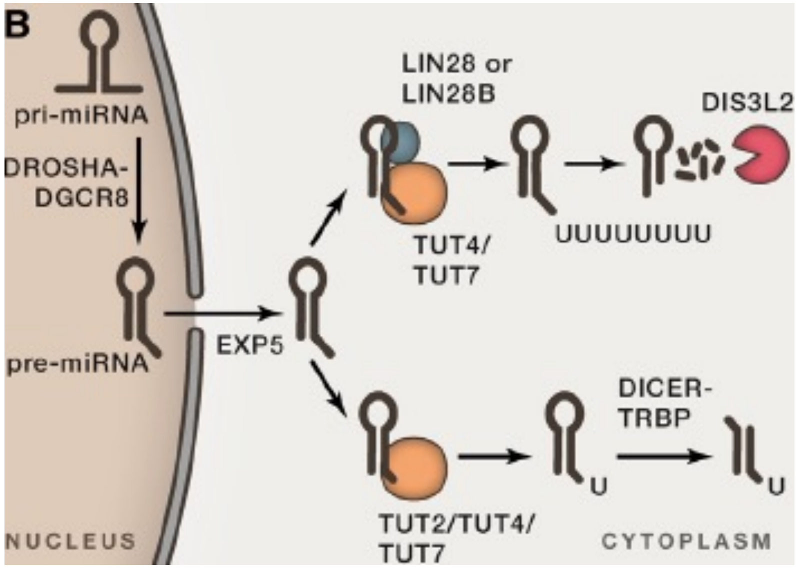
Not an RNA

URIDYLATION

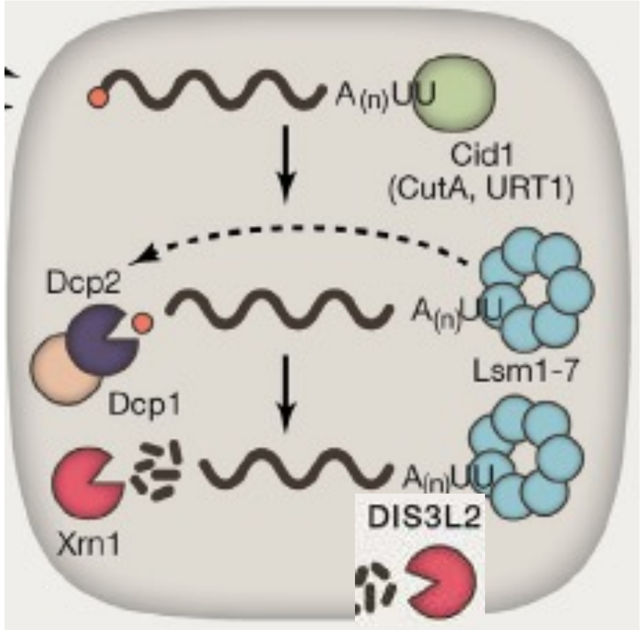
TUTases



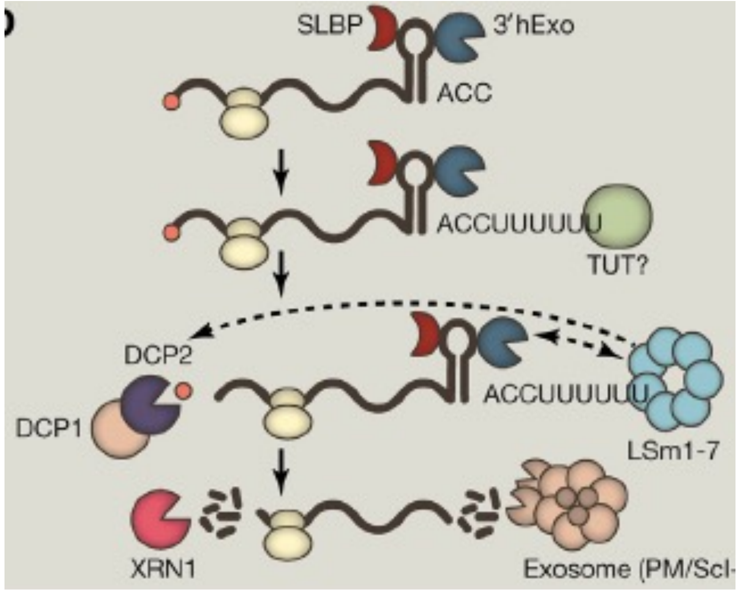
Uridylation of pre-miRNAs and miRNAs



Uridylation-dependent mRNA decay



Degradation of histone mRNAs



Lee et al, Cell, 2014

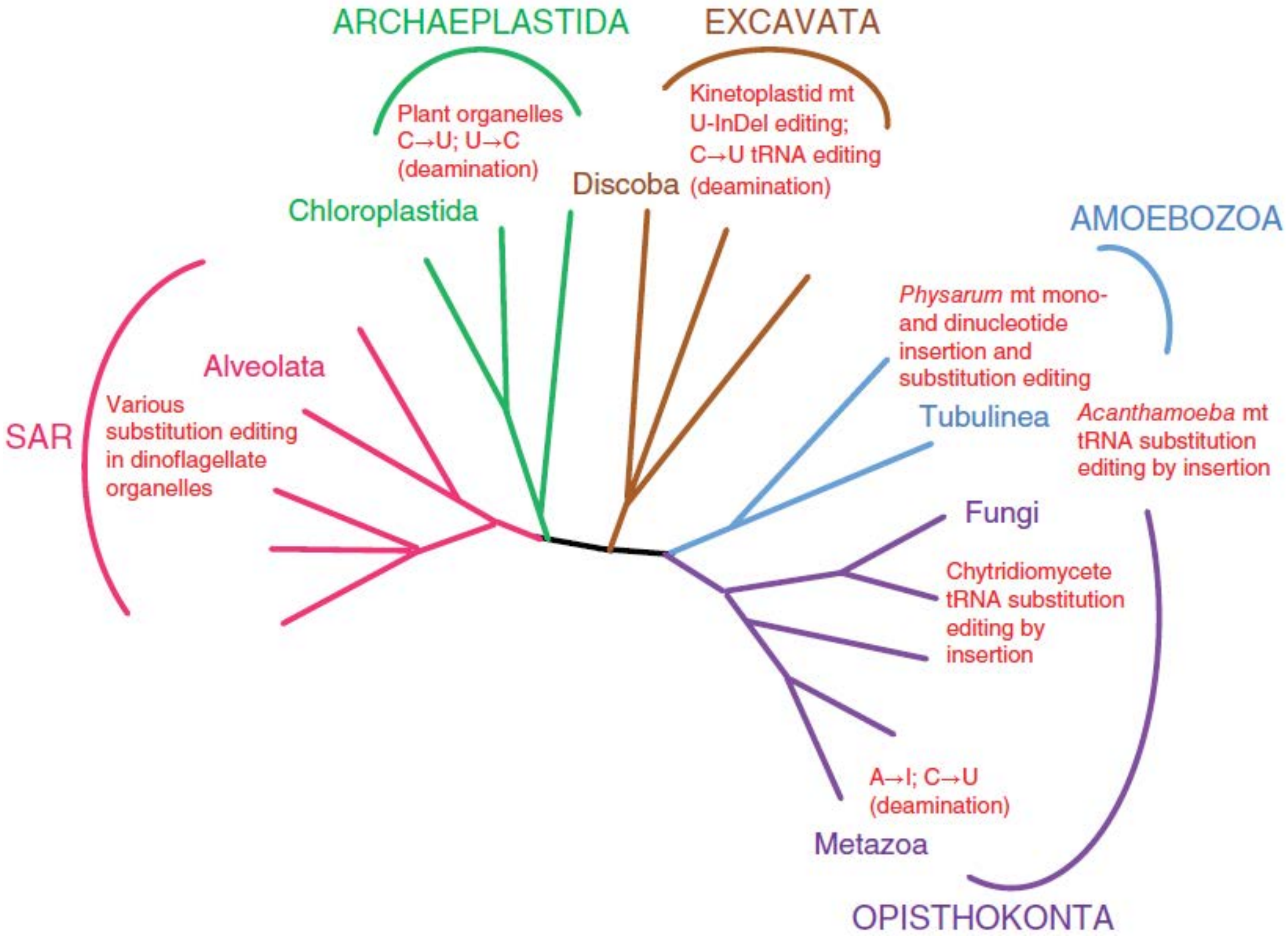
RNA modifications: EDITING

RNA editing: post-transcriptional modification of RNA sequence

- up to 50-60% in Trypanosomes!

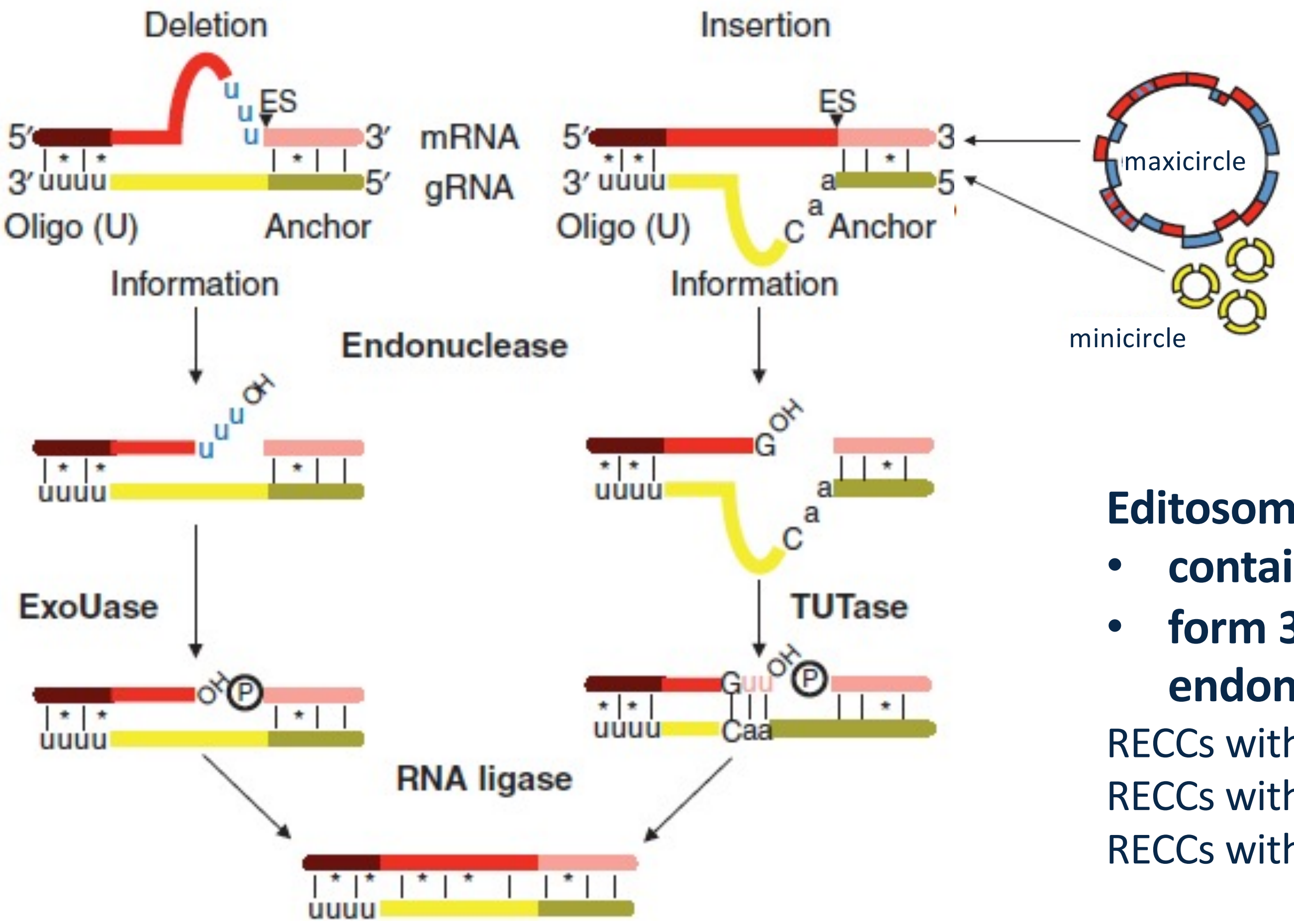
- insertions and deletions

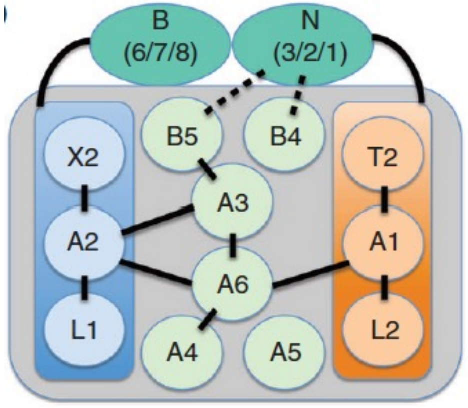
- C to U or A to I exchange



Read et al, WIREsRNA, 2016

RNA modifications: EDITING





Read et al, WIREsRNA, 2016

Editosome

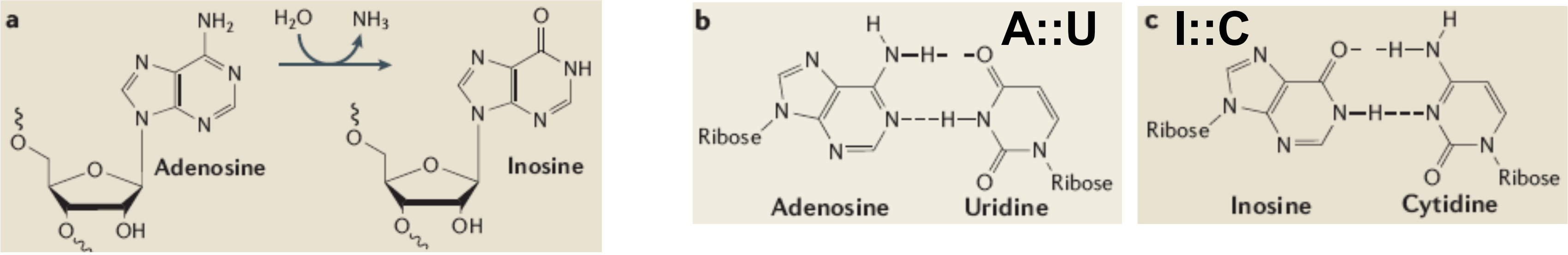
- **contains 12 common KRE proteins**
- **form 3 subcomplexes with KREN endonucleases**

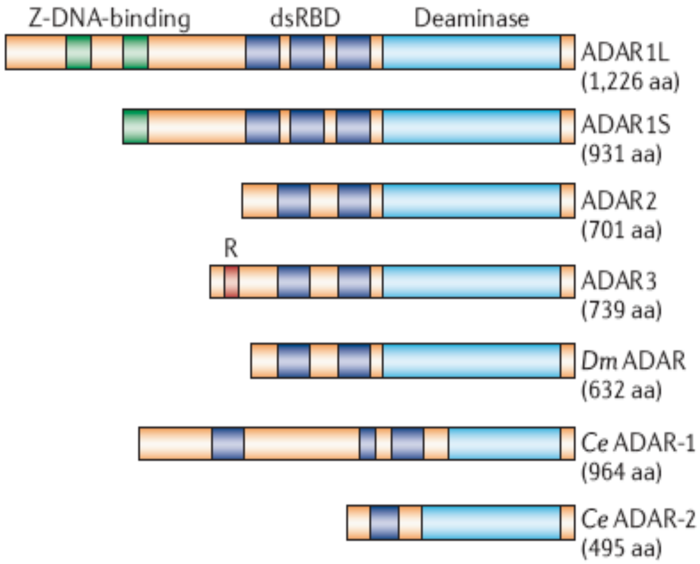
RECCs with KREN1 catalyze U deletion
RECCs with KREN2 catalyze U insertion
RECCs with KREN3 catalyze insertion in *cox2*

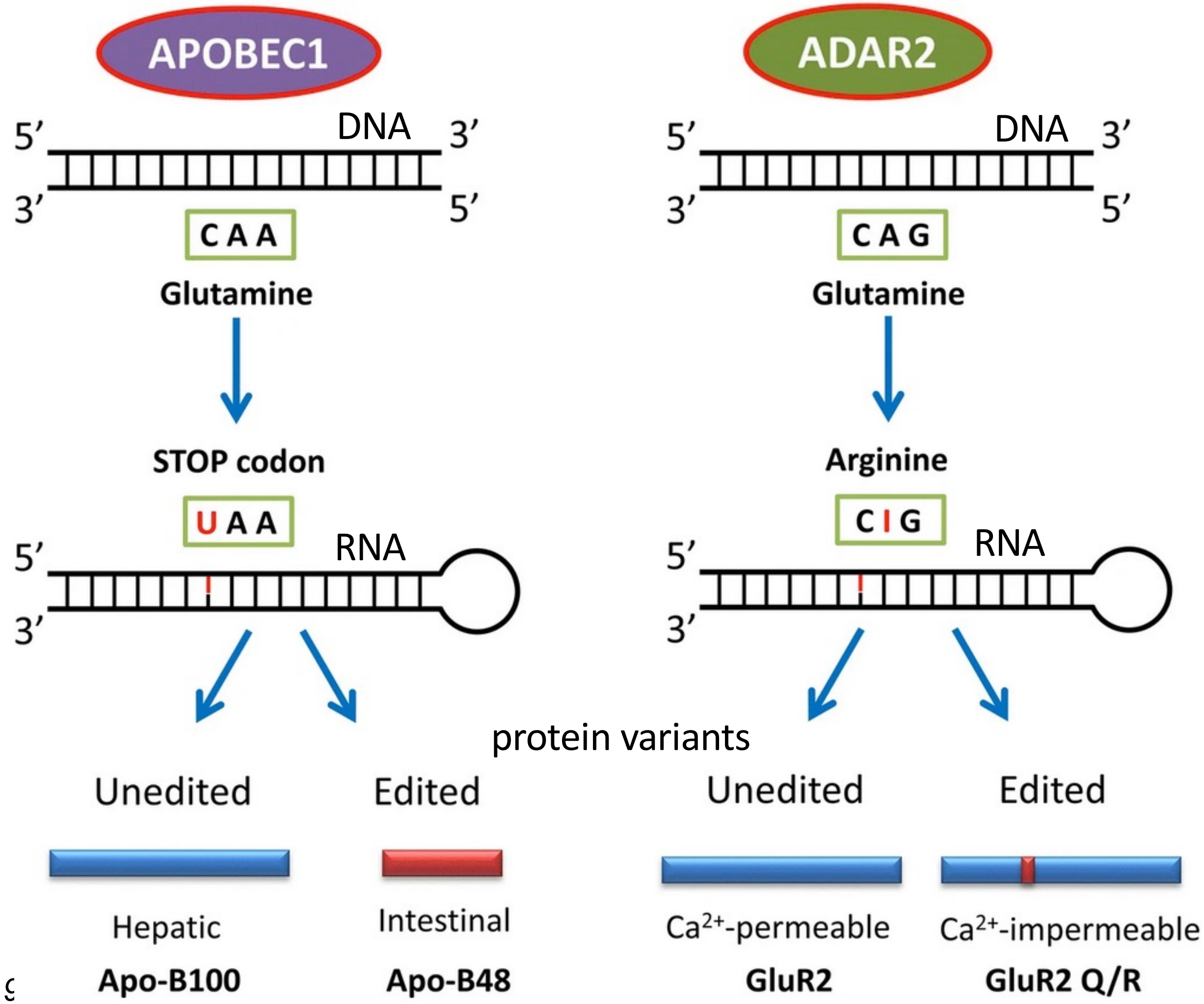
Pre-edited mRNAs are transcribed from mitochondrial maxicircles, gRNAs from minicircle mtDNA. gRNA 5′ anchor base pairs with mRNA and the gRNA center directs the number of Us inserted or deleted. The gRNA 3′ oligo(U) tail stabilizes the gRNA/mRNA interaction.

The gRNA and enzymes are present in the 20S RNP complex (RECC or editosome). Endonuclease catalyzes mRNA cleavage at editing sites, TUTase inserts Us, U-specific exoribonuclease deletes Us. Cleaved mRNAs are resealed by RNA ligases.

RNA EDITING: A-to-I or C-to-U







APOBEC1

C A A

Glutamine

STOP codon

U A A

Unedited — Hepatic — **Apo-B100**

Edited — Intestinal — **Apo-B48**

ADAR2

C A G

Glutamine

Arginine

C I G

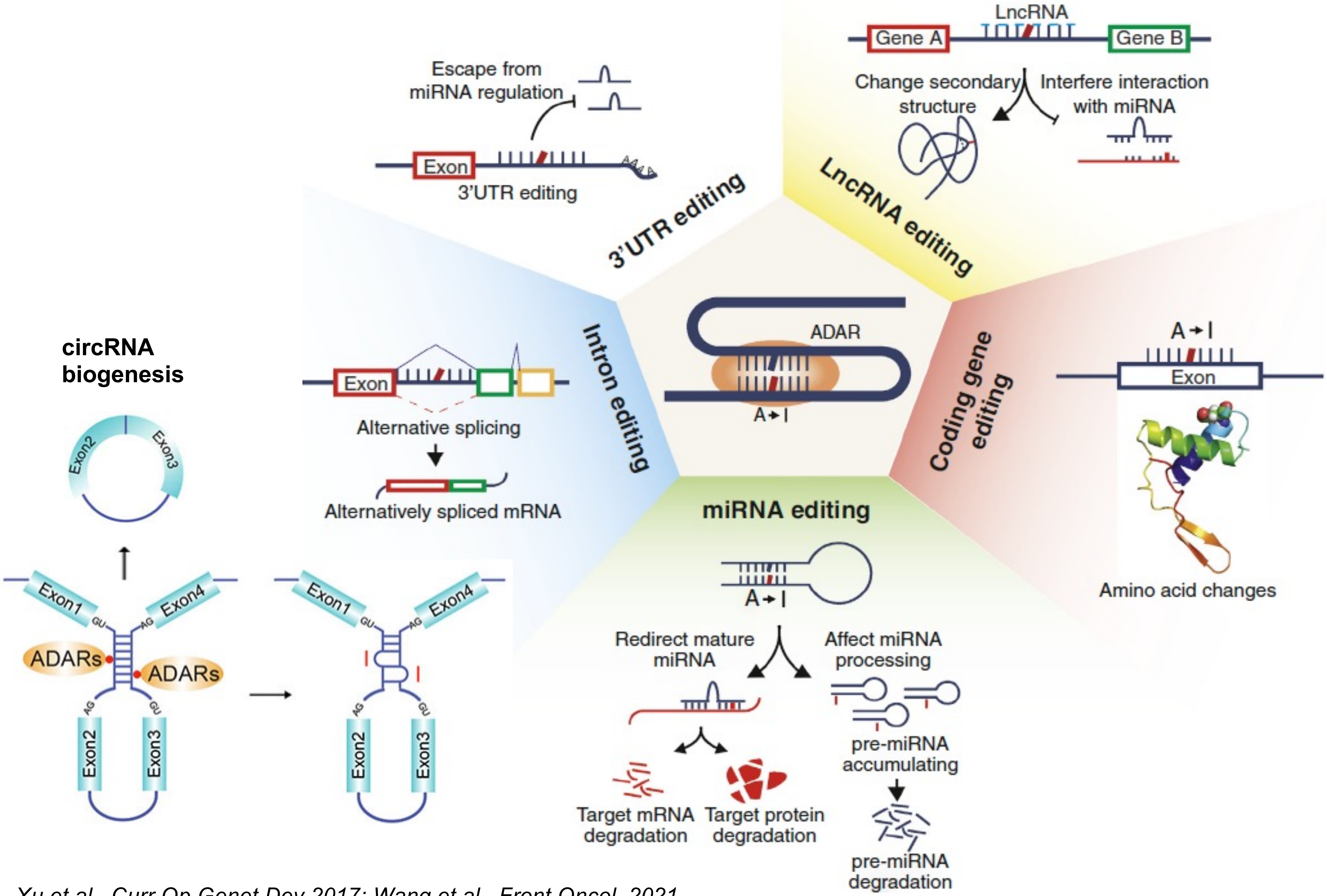
Unedited — Ca^{2+}-permeable — **GluR2**

Edited — Ca^{2+}-impermeable — **GluR2 Q/R**

A-to-I by ADAR
adenosine deaminase
acting on RNA
C-to-U by APOBEC
cytidine deaminase

Nishikura, Nat Rev Mol Cel Biol , 2006
Christofi and Zaravinos, J Transl Medicine, 201

RNA editing: A-to-I by ADAR



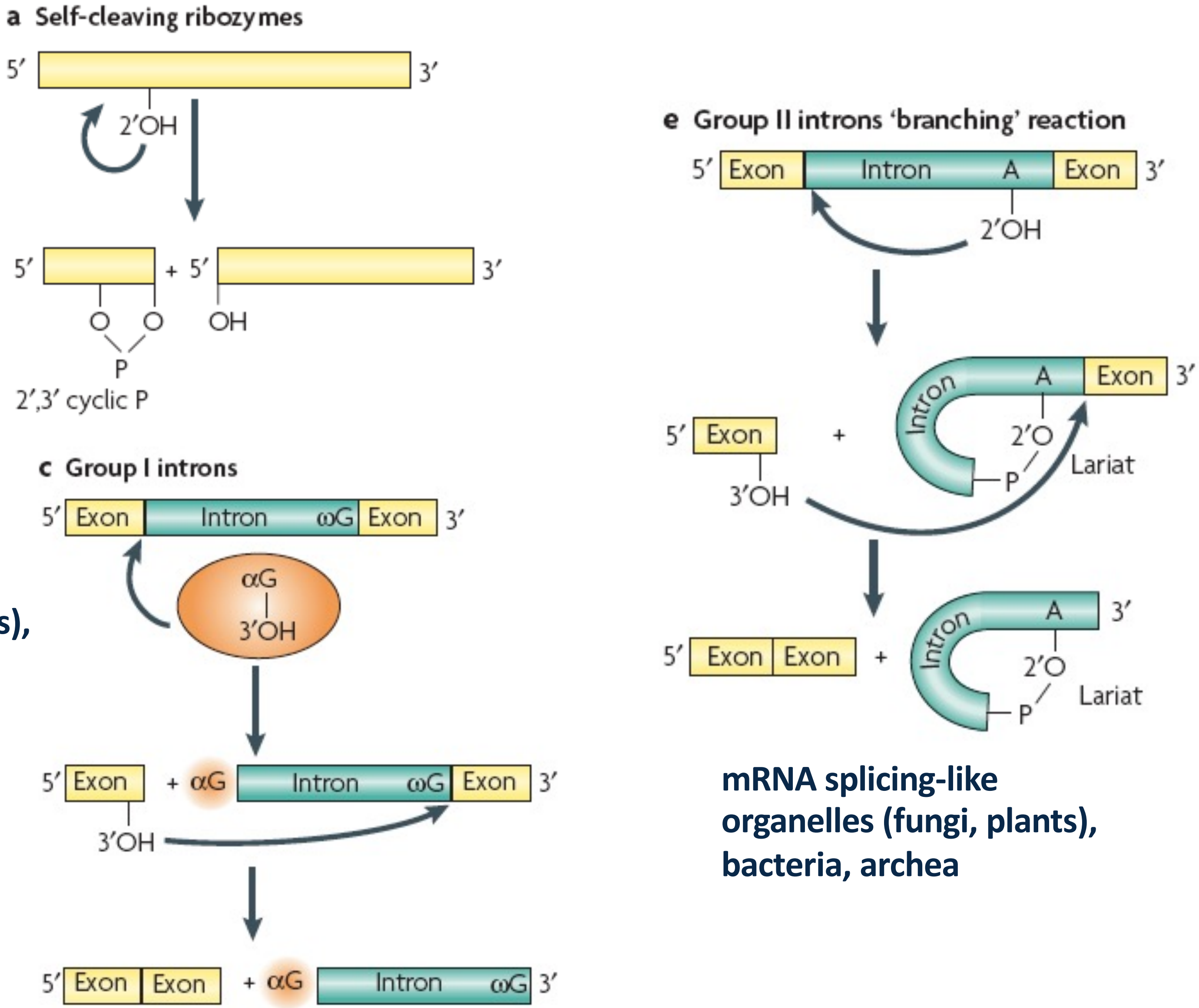
Xu et al., Curr Op Genet Dev 2017; Wang et al., Front Oncol, 2021

Self-splicing RIBOZYMES

Hammerhead,
Hairpin, HDV

viroids, eukaryotes
plant satellite RNA,
viruses

organelles (fungi, plants),
bacteria,
mitochondria (animals)



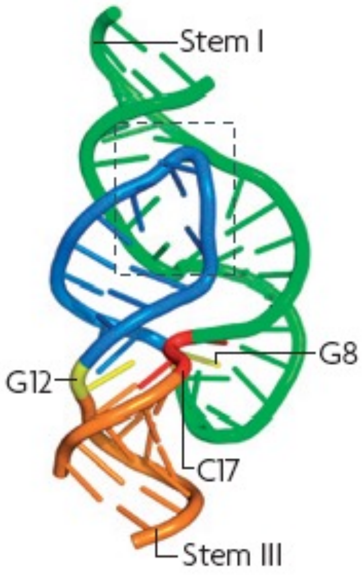
mRNA splicing-like
organelles (fungi, plants),
bacteria, archea

Serganov and Patel, Nat. Rev. Genet., 2007

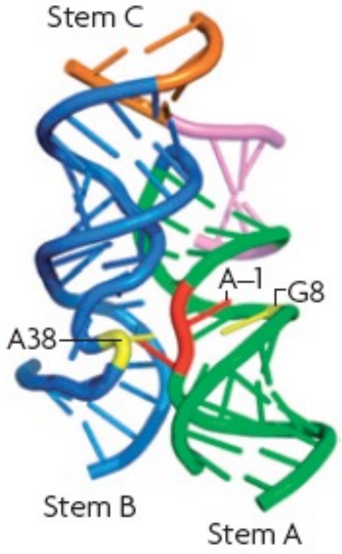
Mechanism: nucleophilic attack of the ribose -OH group (H_2O, Me^{2+}) on the phosphate

Self-splicing RIBOZYMES

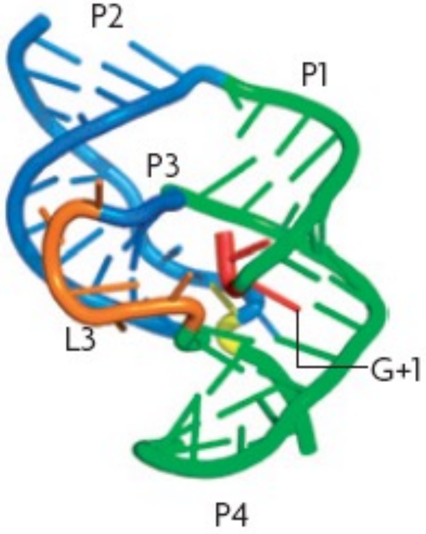
Hammerhead

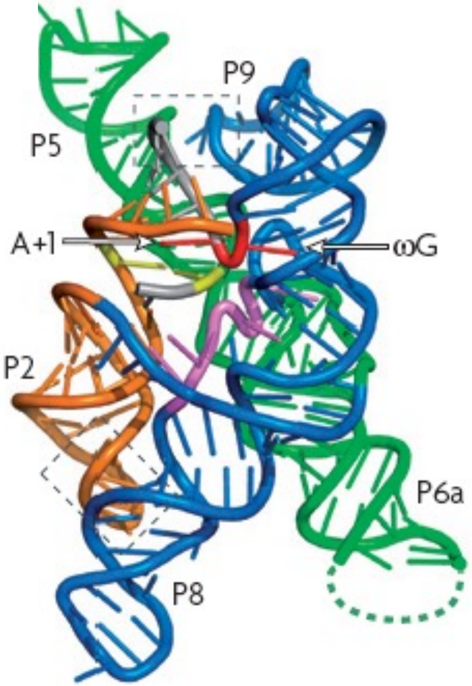


Hairpin

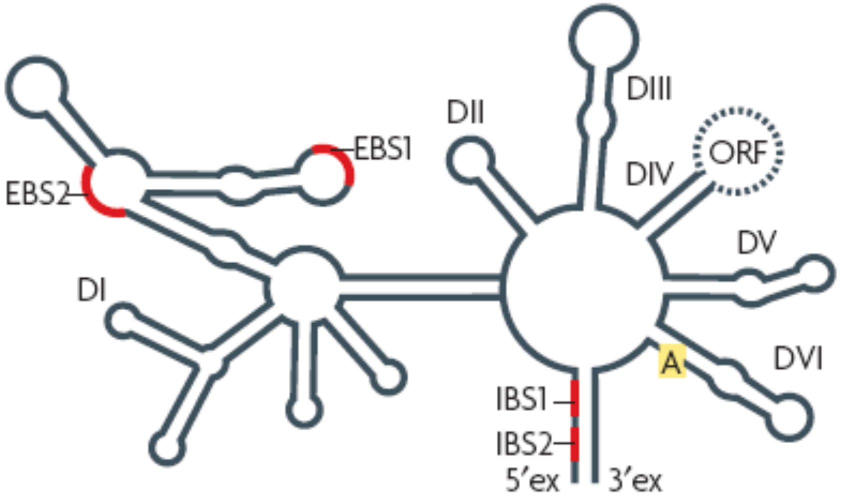


HDV





Group I intron



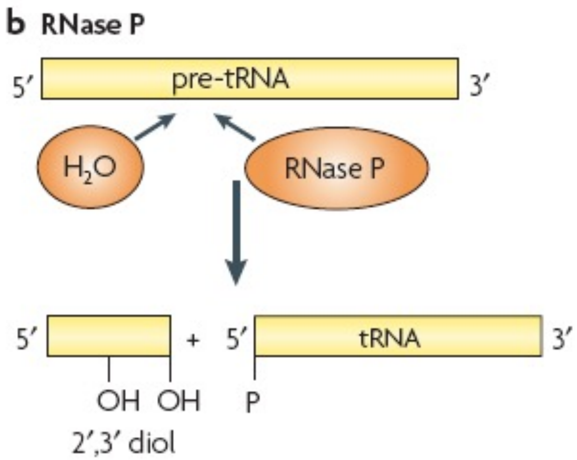
Group II intron

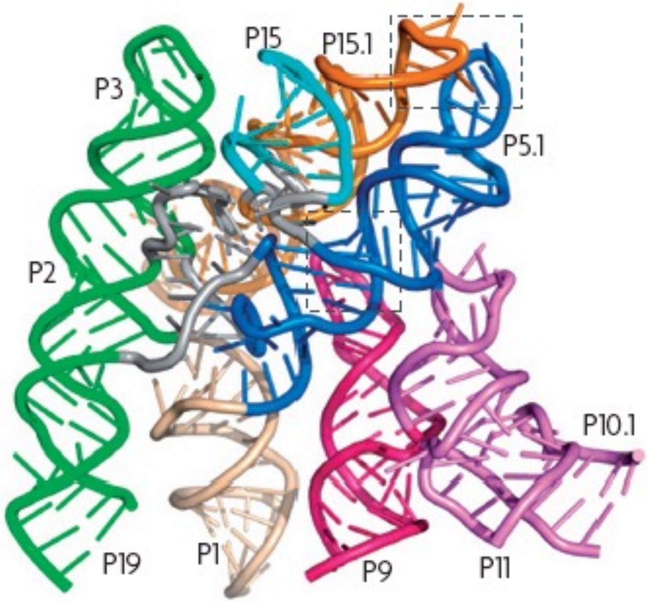
Serganov and Patel, *Nat. Rev. Genet.*, 2007

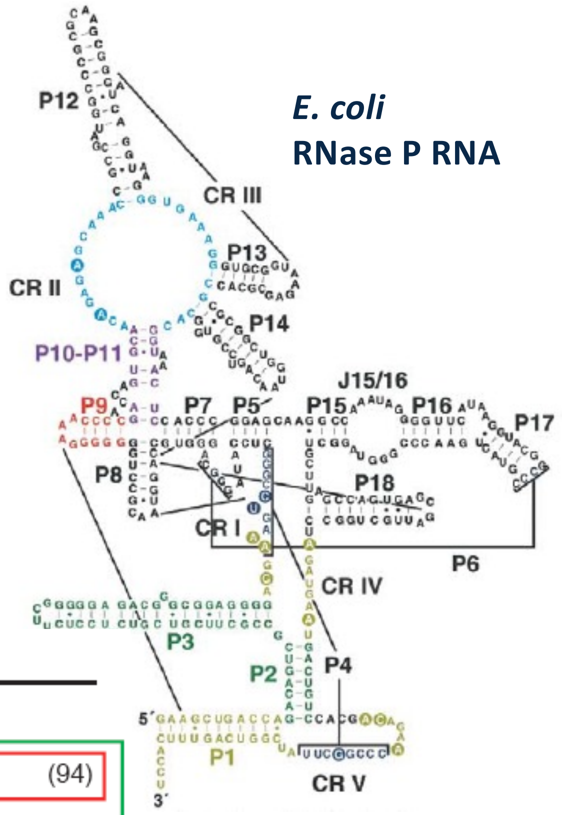
RNase P RNA – a true enzyme

tRNA processing, multiple turnover

Serganov and Patel, Nat Rev Genet, 2007; Evans et al, TiBS, 2006



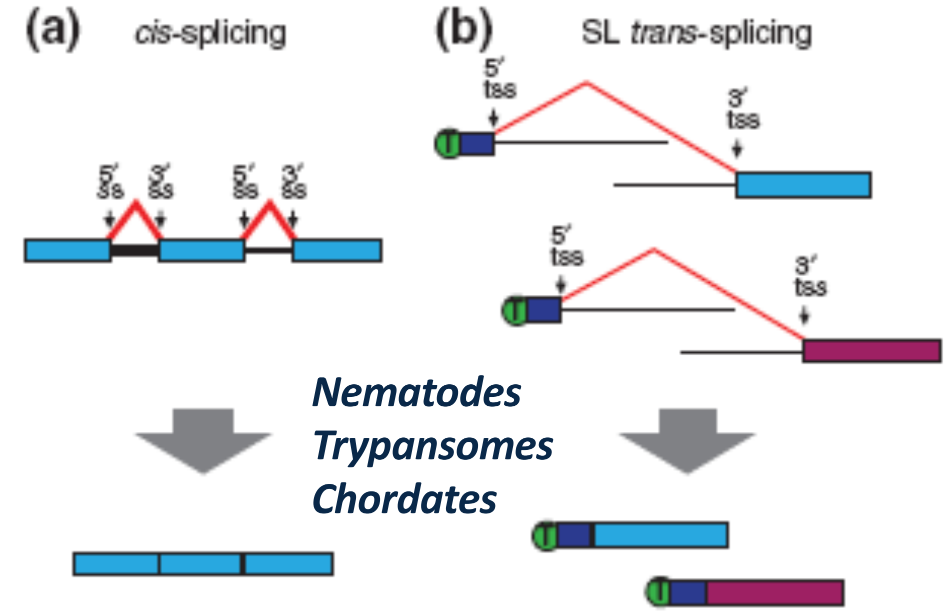




E. coli
RNase P RNA

Bacteria	**Eukarya**		**Archaea**		
Eco	*Sce*	*Hsa*	*Pfu*	*Pho*	*Mth*
RNA (121)	RNA (118)	RNA (109)	RNA (106)	RNA (106)	RNA (94)
RnpA (13.8)					
	Pop5 (19.6)	hPop5 (18.8)	PF1378 (13.8)	PH1481* (14.0)	MTH687 (14.6)
	Rpp1 (32.2)	Rpp30 (29.3)	PF1914 (24.5)	PH1877 (24.7)	MTH688 (27.7)
	Rpr2 (16.3)	Rpp21* (17.6)	PF1613 (14.3)	PH1601* (14.6)	MTH1618 (17.0)
	Pop4 (32.9)	Rpp29* (25.4)	PF1816 (15.0)	PH1771* (15.1)	MTH11 (10.7)
	Pop1 (100.5)	hPop1 (114.7)			
	Pop3 (22.6)	Rpp38 (31.8)			
	Pop7 (15.8)	Rpp20 (15.7)			
	Pop6 (18.2)				
	Pop8 (15.5)				
		Rpp40 (34.6)			
		Rpp25 (20.6)			
		Rpp14 (13.7)			

Trans-splicing

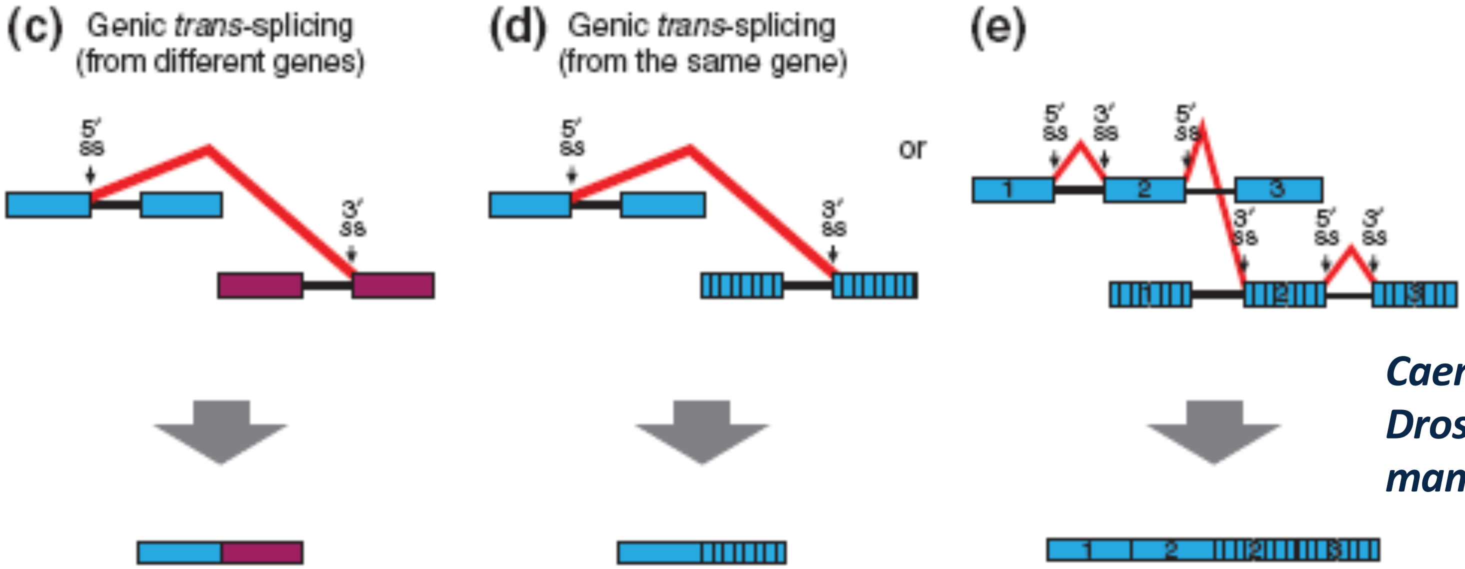


Nematodes
Trypansomes
Chordates

- **SL (spliced leader) *trans*-splicing joins short 5’ exon from the specialized SL RNA**
- **Genic *trans*-splicing joins exons of different pre-mRNA transcripts**
- **Both utilize the basic splicing machinery with SL snRNP**

(U2, U4, U5, U6, <u>no U1</u>)

- **Used by protozoa (Kinetoplastae) to produce variable surface antigens and when changing life-stages**



Caenorhabditis elegans
Drosophila melanogaster
mammals

Lasda and Blumenthal, WIRERNA., 2011

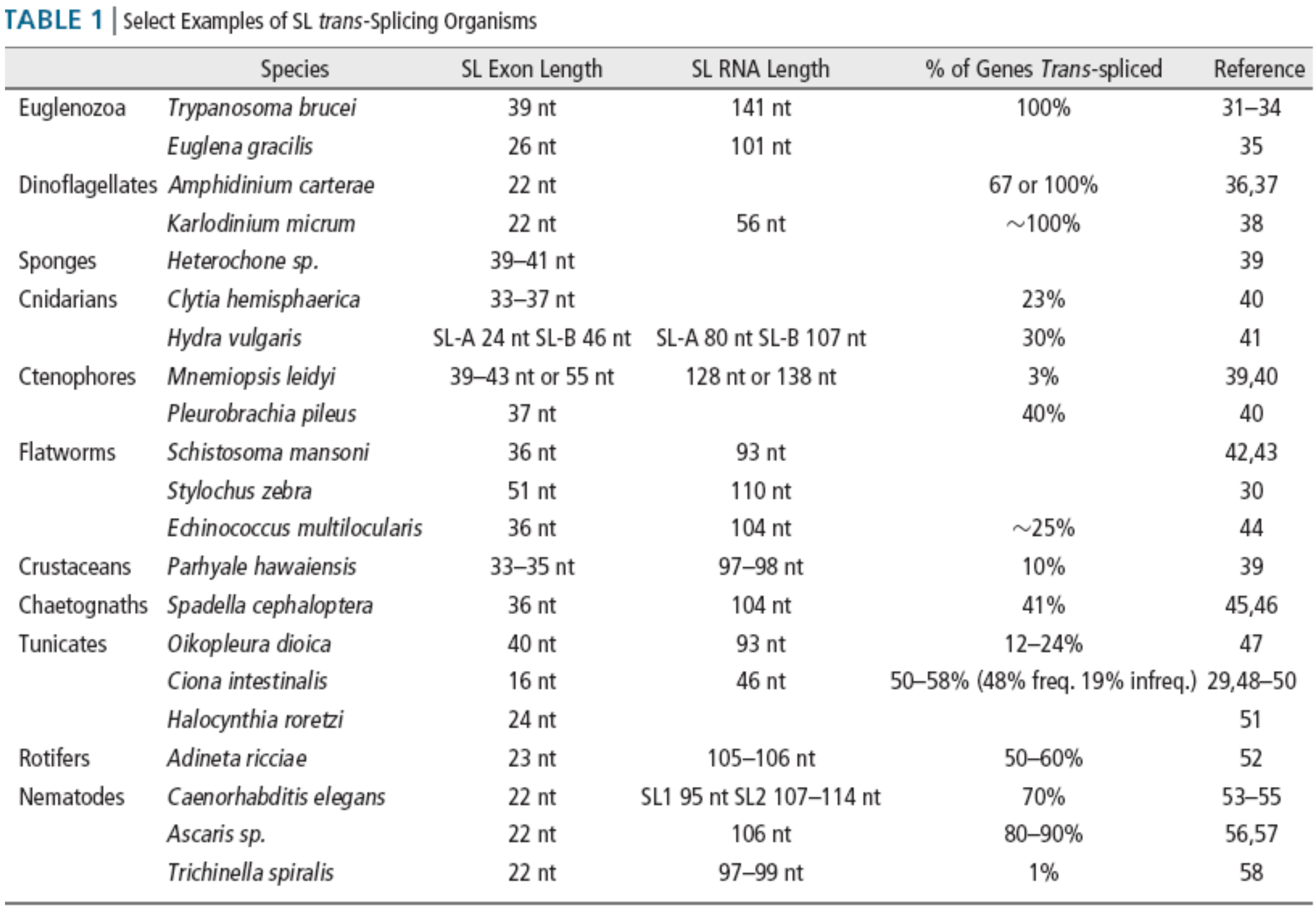


TABLE 1 | Select Examples of SL *trans*-Splicing Organisms

	Species	SL Exon Length	SL RNA Length	% of Genes *Trans*-spliced	Reference
Euglenozoa	*Trypanosoma brucei*	39 nt	141 nt	100%	31–34
	Euglena gracilis	26 nt	101 nt		35
Dinoflagellates	*Amphidinium carterae*	22 nt		67 or 100%	36,37
	Karlodinium micrum	22 nt	56 nt	∼100%	38
Sponges	*Heterochone sp.*	39–41 nt			39
Cnidarians	*Clytia hemisphaerica*	33–37 nt		23%	40
	Hydra vulgaris	SL-A 24 nt SL-B 46 nt	SL-A 80 nt SL-B 107 nt	30%	41
Ctenophores	*Mnemiopsis leidyi*	39–43 nt or 55 nt	128 nt or 138 nt	3%	39,40
	Pleurobrachia pileus	37 nt		40%	40
Flatworms	*Schistosoma mansoni*	36 nt	93 nt		42,43
	Stylochus zebra	51 nt	110 nt		30
	Echinococcus multilocularis	36 nt	104 nt	∼25%	44
Crustaceans	*Parhyale hawaiensis*	33–35 nt	97–98 nt	10%	39
Chaetognaths	*Spadella cephaloptera*	36 nt	104 nt	41%	45,46
Tunicates	*Oikopleura dioica*	40 nt	93 nt	12–24%	47
	Ciona intestinalis	16 nt	46 nt	50–58% (48% freq. 19% infreq.)	29,48–50
	Halocynthia roretzi	24 nt			51
Rotifers	*Adineta ricciae*	23 nt	105–106 nt	50–60%	52
Nematodes	*Caenorhabditis elegans*	22 nt	SL1 95 nt SL2 107–114 nt	70%	53–55
	Ascaris sp.	22 nt	106 nt	80–90%	56,57
	Trichinella spiralis	22 nt	97–99 nt	1%	58

Lasda and Blumenthal, WIRERNA., 2011

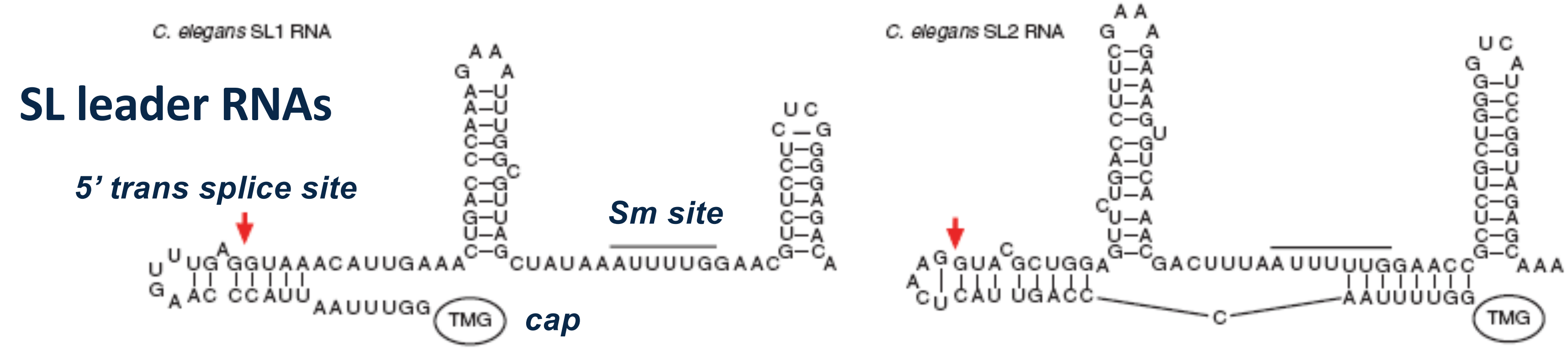
Trans-splicing

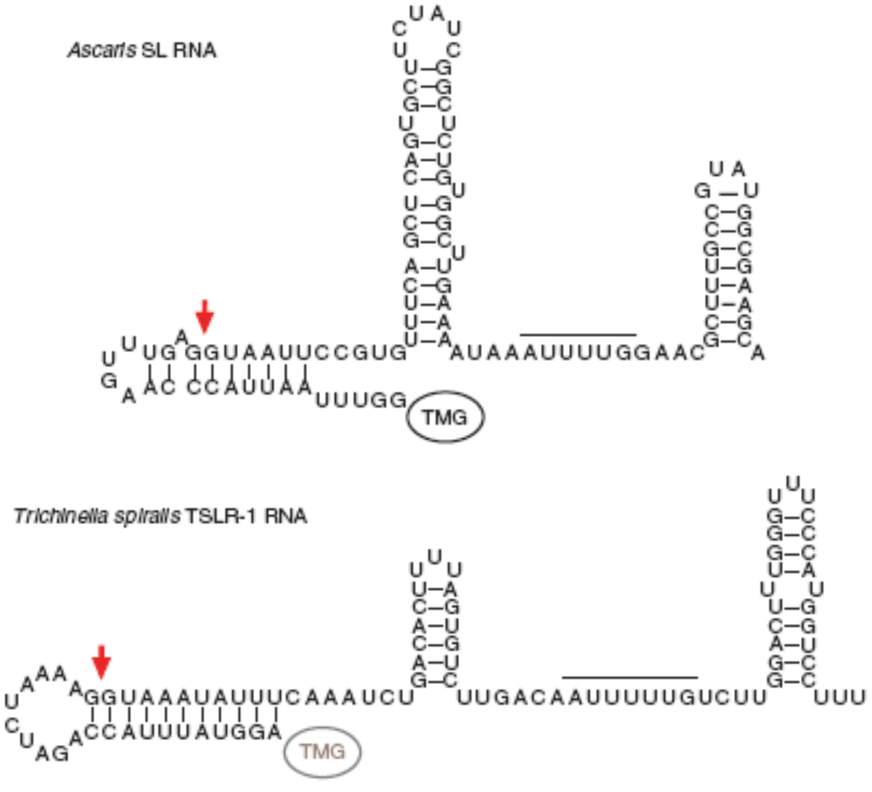
SL leader RNAs

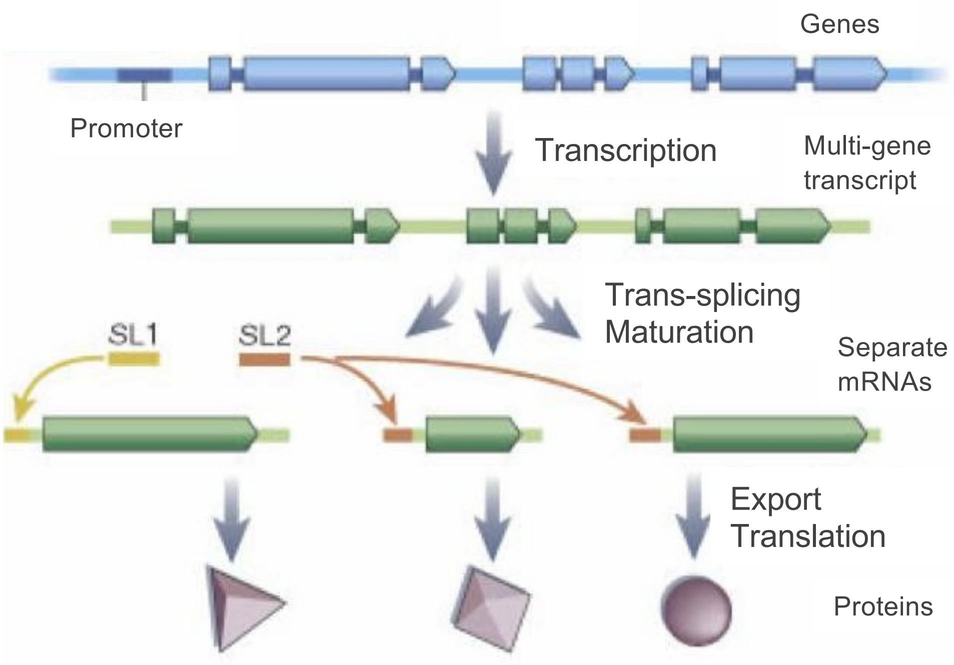
5' trans splice site

Sm site

cap





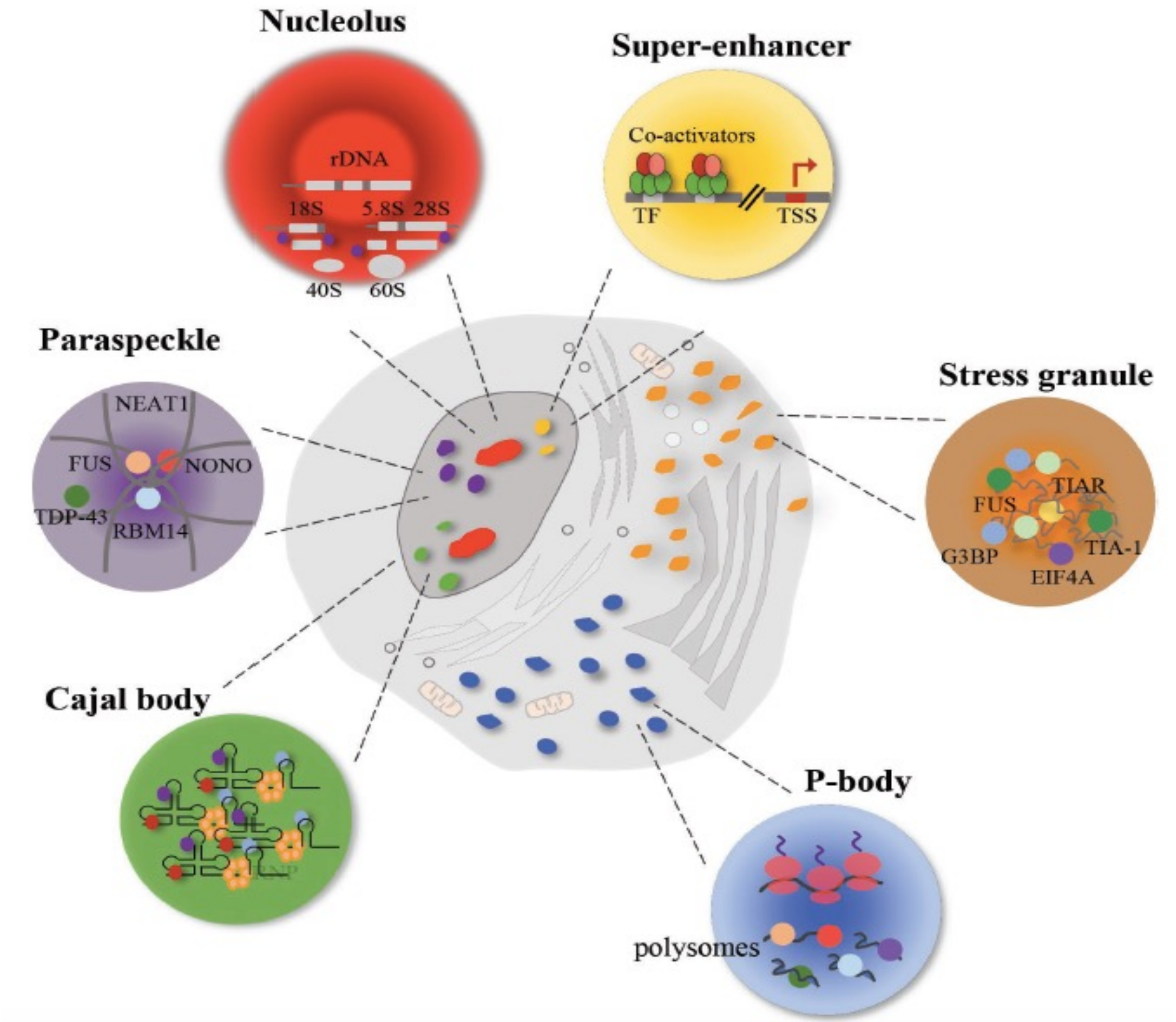


SL RNAs have features of a 5' splice site and snRNA

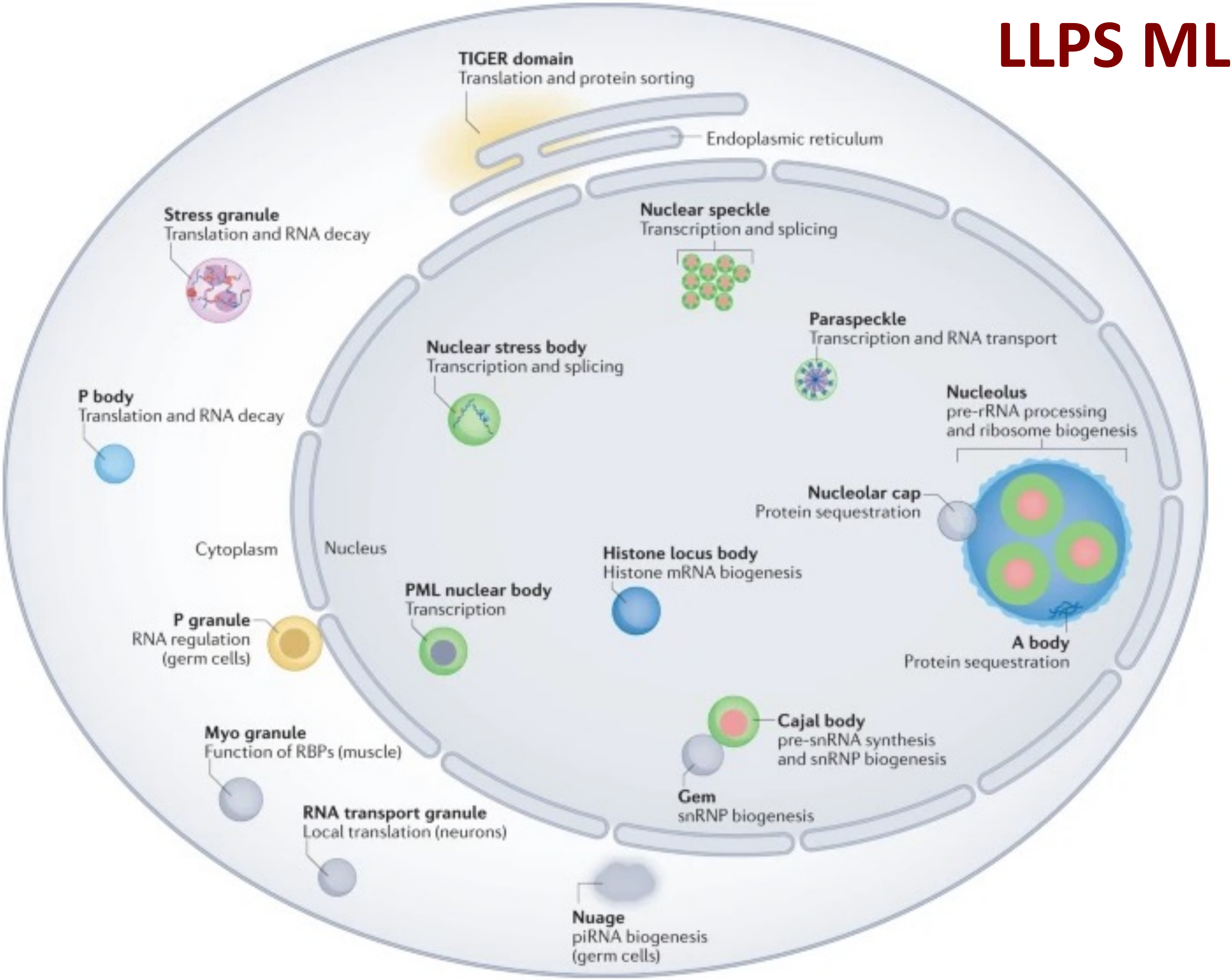
~70% of *C. elegans* genes begin with a 22 nucleotide SL sequence

Lasda and Blumenthal, WIRERNA., 2011; Blumenthal, Trans-splicing and operons, 2005

Subcellular structures, bodies, condensates, LLPS, MLOs

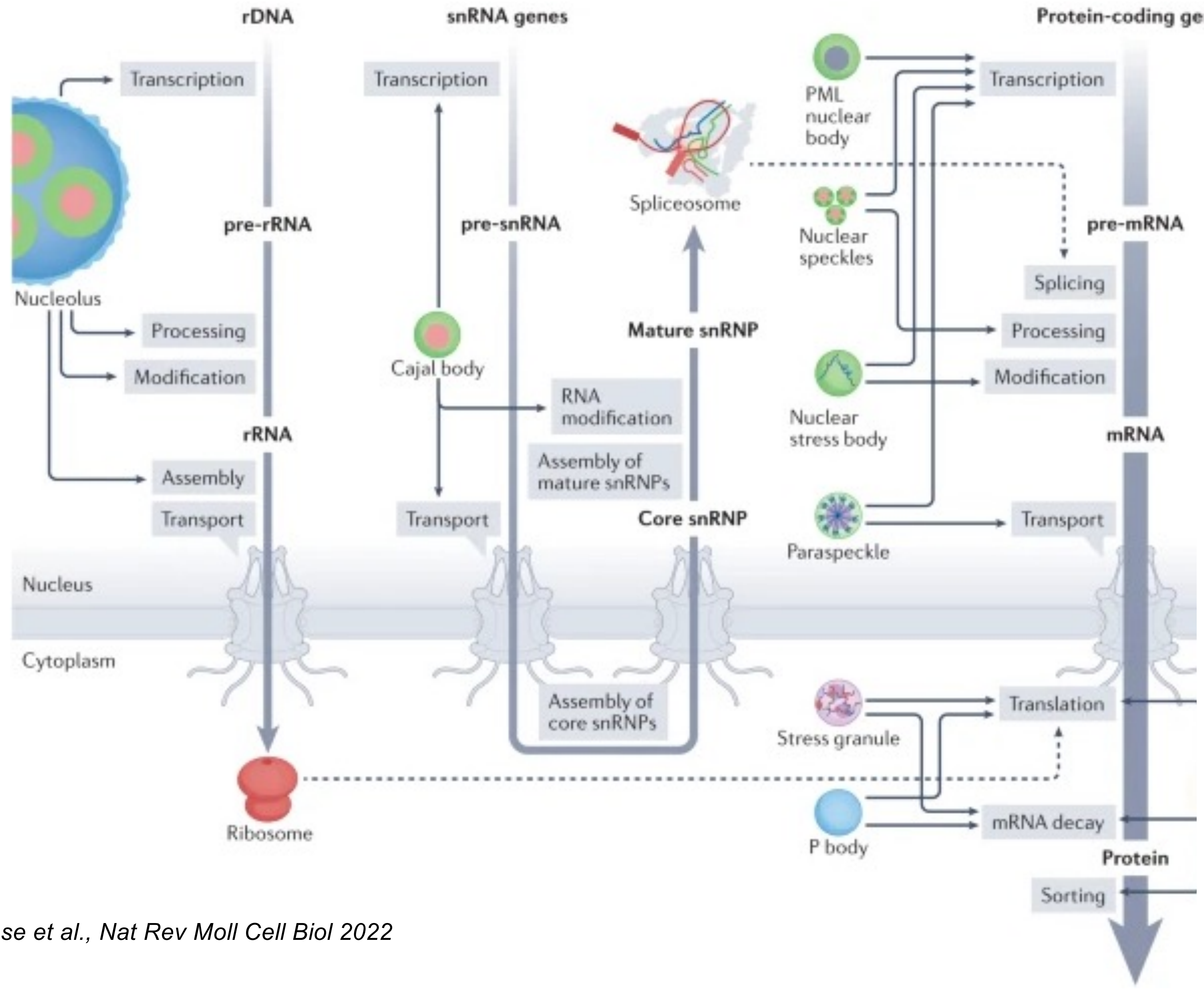


LLPS MLOs



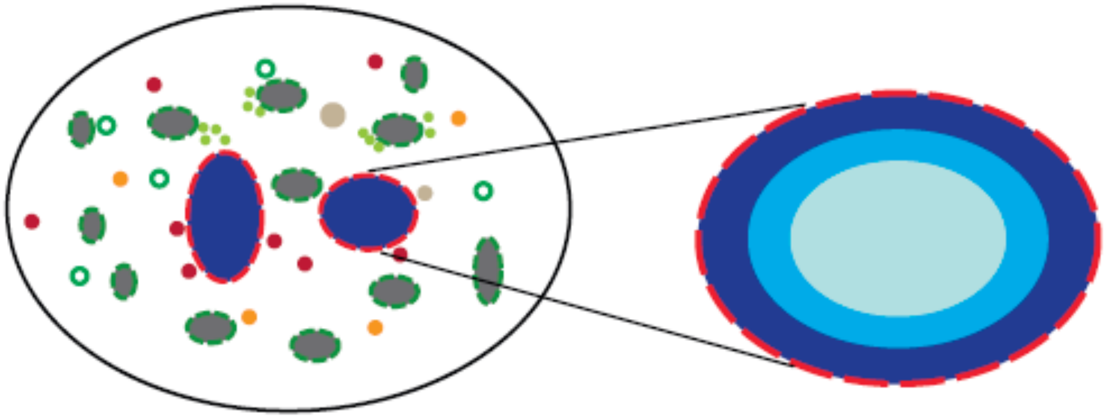
Hirose et al., Nat Rev Moll Cell Biol 2022

Functions of MLOs in gene regulation



Hirose et al., Nat Rev Moll Cell Biol 2022

Nuclear bodies, Membranless organelles (MLOs)

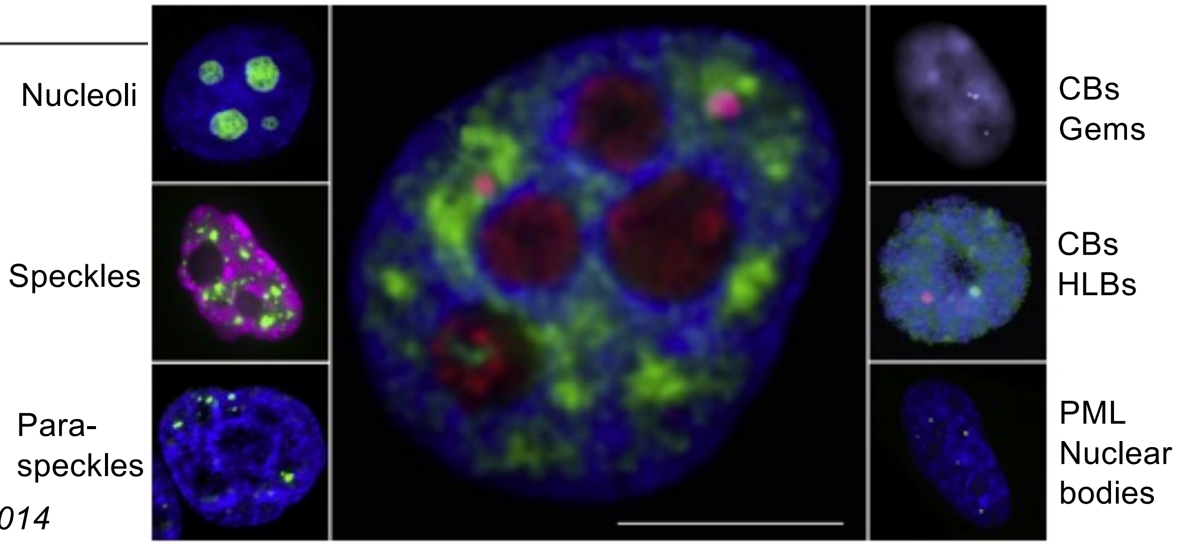


Nuclear bodies

- ● Cajal body
- ● Nuclear speckle
- ● Nucleolus
- ● Paraspeckle
- ● PML body
- ● Polycomb body
- ○ Transcription factory

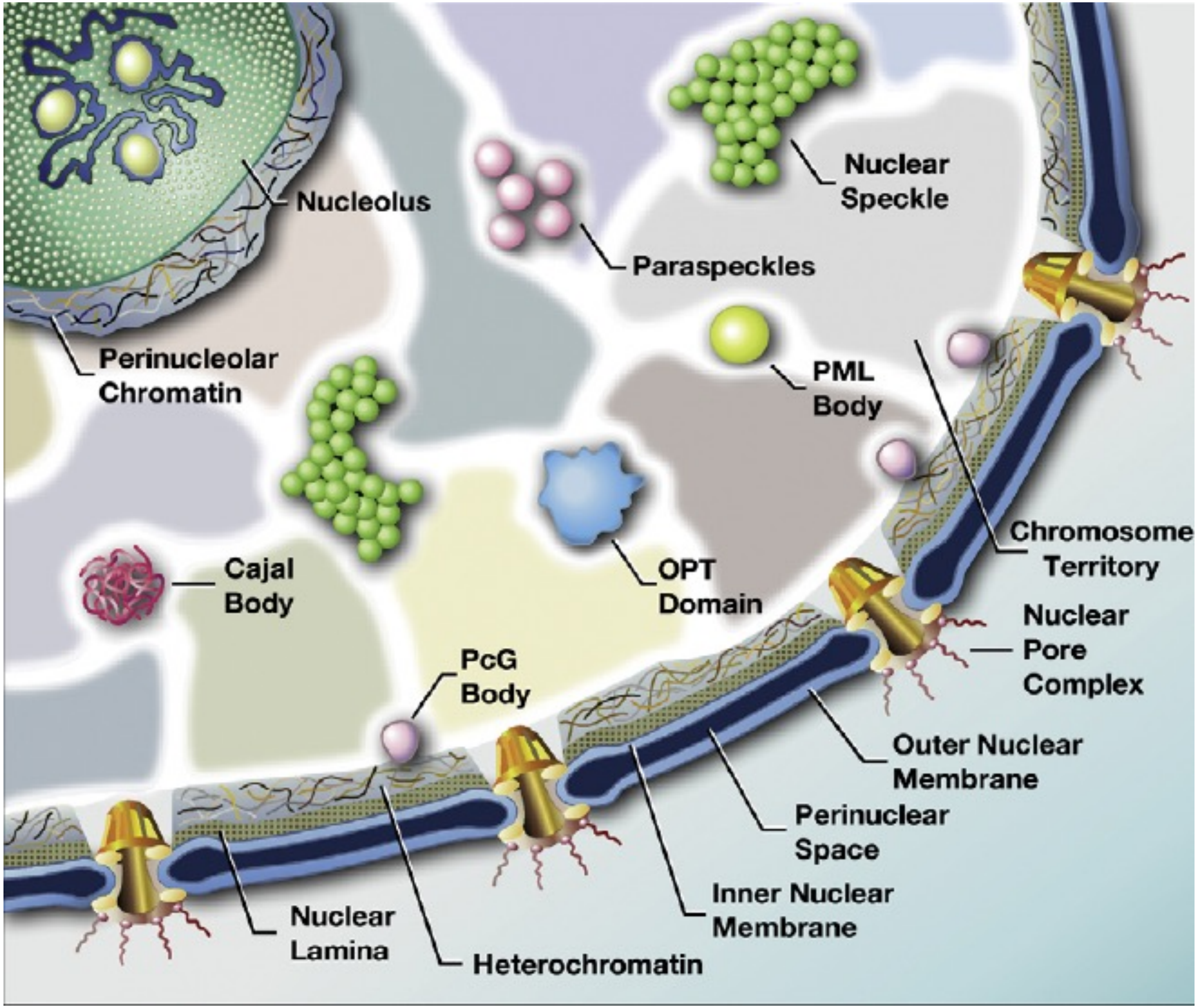
Nucleolus

- ● Fibrillar center
- ○ Dense fibrillar component
- ○ Granular component
- ◌ Perinucleolar heterochromatin



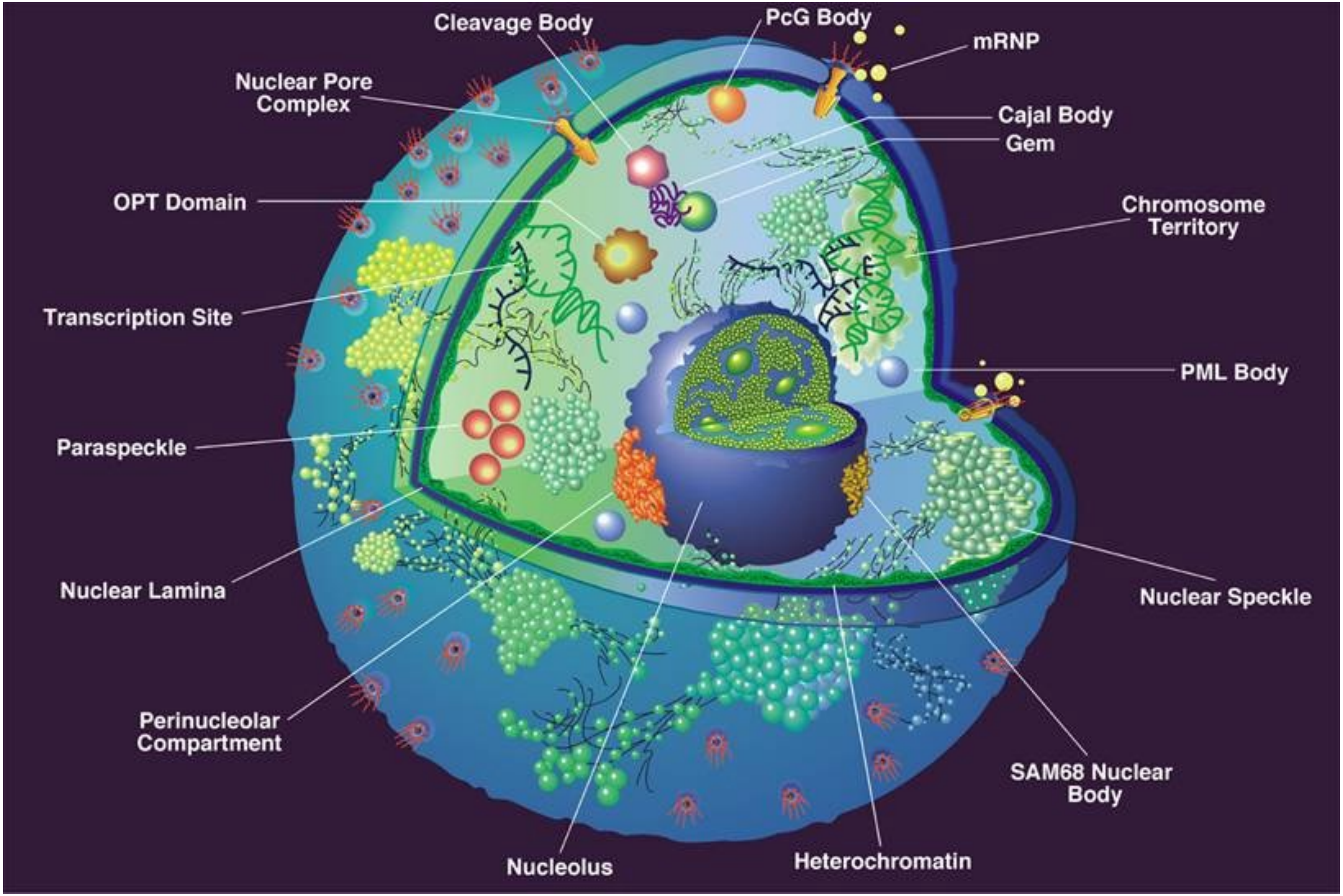
Nemeth and Langst. Trends in Genet, 2011;
Sleeman and Trinkle-Mulcahy, Curr Op Cell Biol, 2014

Nuclear bodies



Zhao at al. Curr. Op. Gen. Dev., 2009

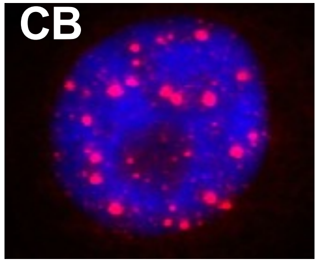
Nuclear bodies

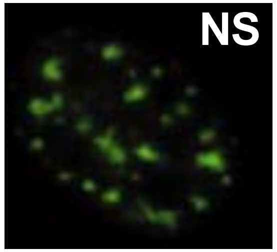


http://bellsouthpwp2.net/b/h/bhagavathula4745/The%20nucleus.htm

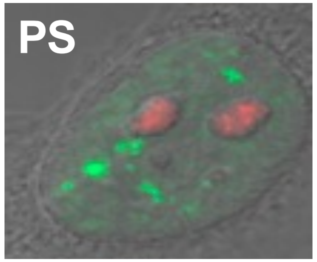
Nuclear bodies

Cajal Bodies (Ramon y Cajal, 1903)
- RNP assembly factory: sn/snoRNA, telomerase RNA processing, modification and assembly
- contain specific scaRNAs (RNA modification)
- associate with telomeres and histone and snRNA gene loci
- different composition (coilin, SMN protein,

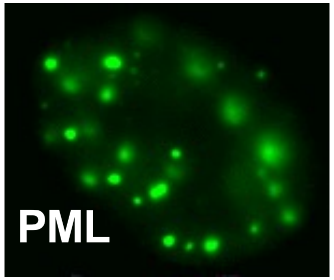




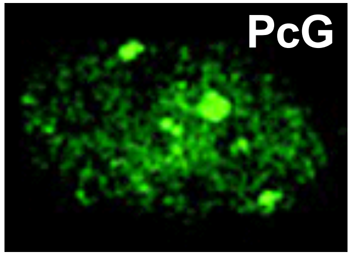
Nuclear Speckles (Interchromatin Granule Clusters, IGCs)
- enriched in splicing related factors (snRNP, SR proteins)
- usually not transcriptionally active but transcriptionally active genes also associate with NS
- role in RNA processing or storage of RNA processing factors



Paraspeckles
- regulate the expression of some genes by nuclear retention of RNA
- contain PSF/SFPQ, P54NRB/NONO and PSPC1 proteins
- organized around ncRNA (eg. NEAT1)



PML Bodies (Promyelocytic Leukemia Nuclear Bodies)
- role in tumor suppression, viral defense, DNA repair, transcriptional regulation
- localize to gene-rich and transcriptionally active regions of chromatin
- composition might be heterogeneous and functionally different
- found also in the cytoplasm

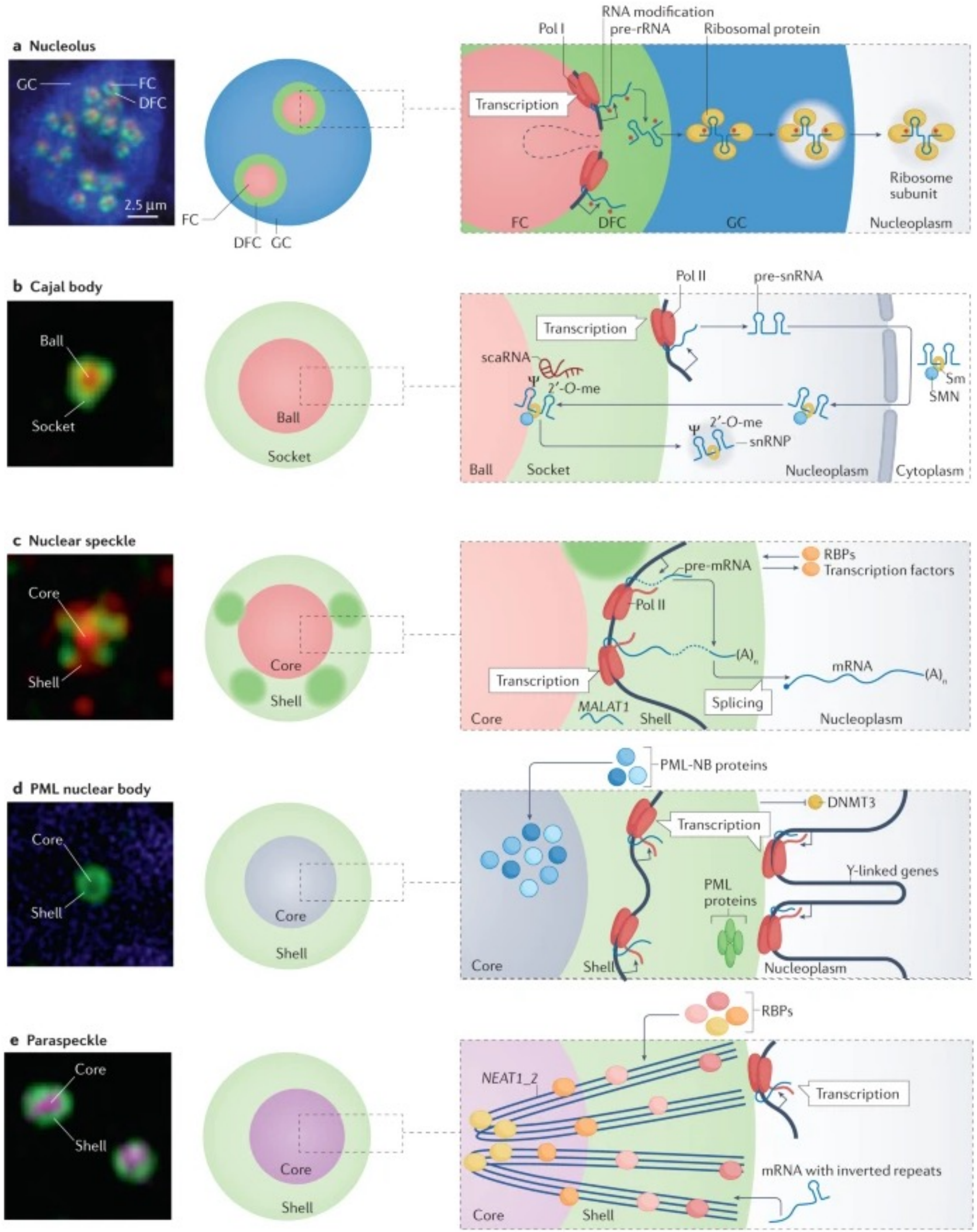


PcG Bodies – Polycomb Bodies
- a subset of RNAi factors (AGO, Dicer, Piwi) localize to PcG Bodies
- located close to heterochromatin, centers of gene repression

OPT Domain appear in G1 phase, represent sites of DNA damage

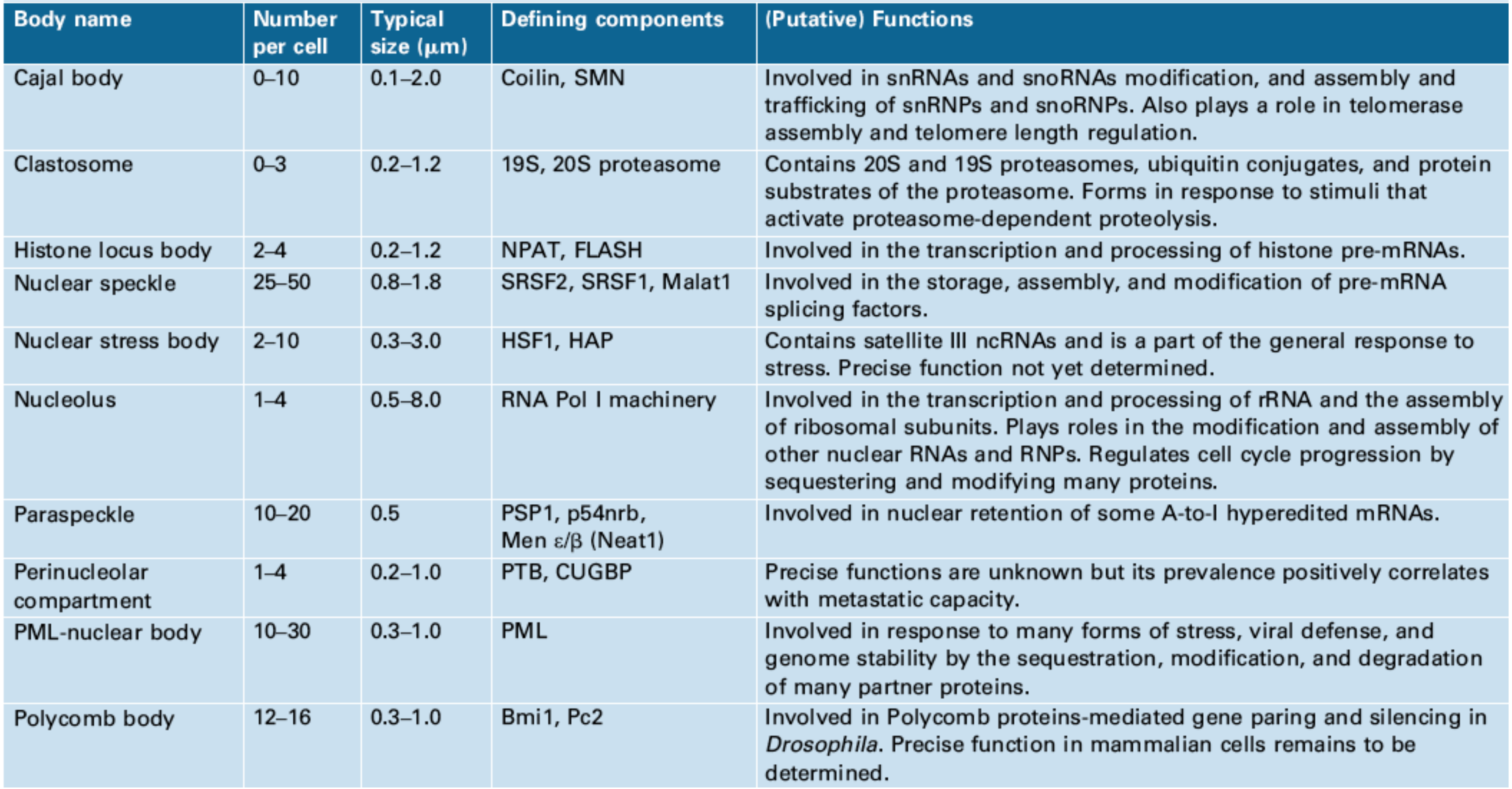
plant Dicing bodies contain DCL1, HYL1 and SE proteins, function in pri-miRNA processing

Nuclear bodies



Hirose et al., Nat Rev Moll Cell Biol 2022

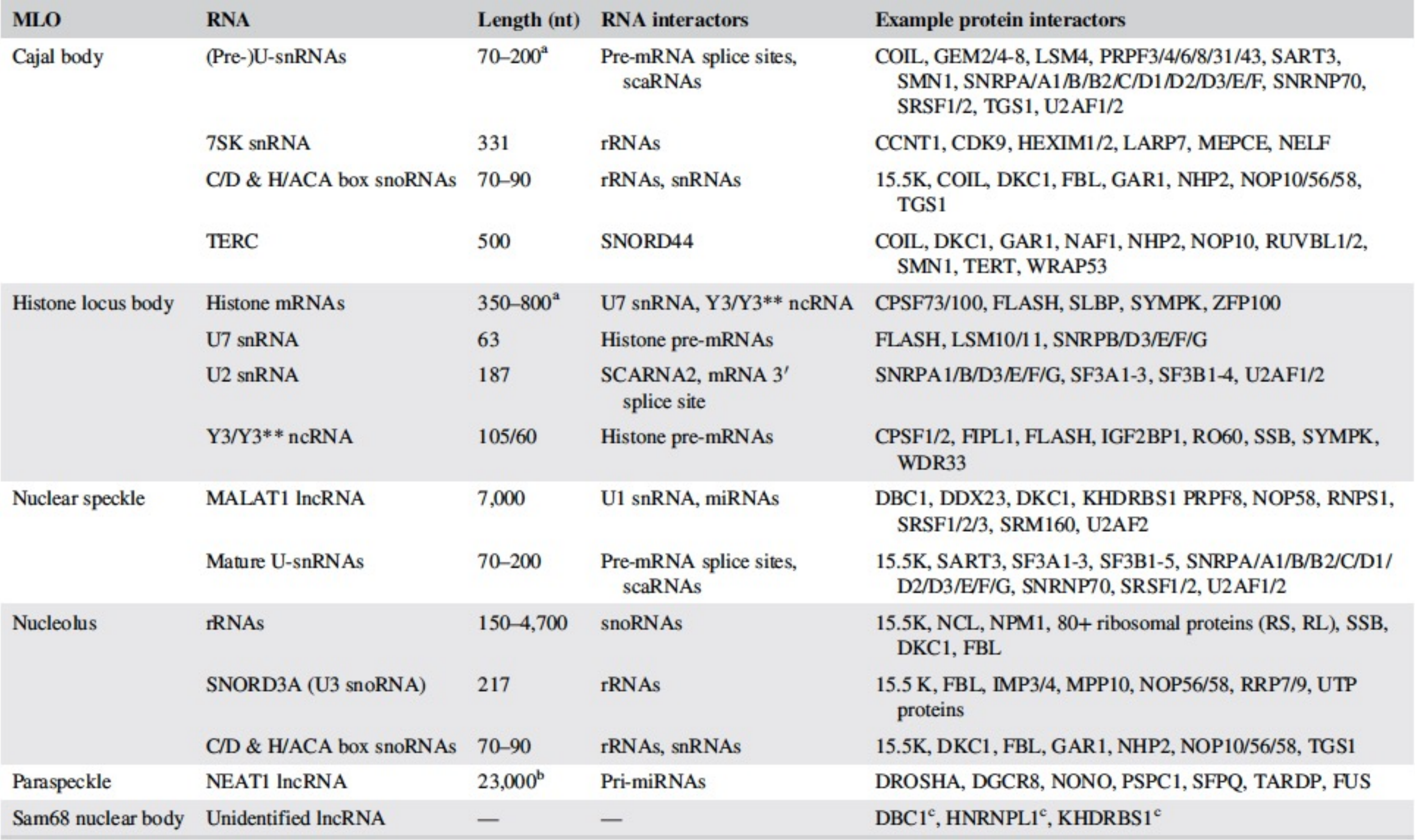
Nuclear bodies



Body name	Number per cell	Typical size (µm)	Defining components	(Putative) Functions
Cajal body	0–10	0.1–2.0	Coilin, SMN	Involved in snRNAs and snoRNAs modification, and assembly and trafficking of snRNPs and snoRNPs. Also plays a role in telomerase assembly and telomere length regulation.
Clastosome	0–3	0.2–1.2	19S, 20S proteasome	Contains 20S and 19S proteasomes, ubiquitin conjugates, and protein substrates of the proteasome. Forms in response to stimuli that activate proteasome-dependent proteolysis.
Histone locus body	2–4	0.2–1.2	NPAT, FLASH	Involved in the transcription and processing of histone pre-mRNAs.
Nuclear speckle	25–50	0.8–1.8	SRSF2, SRSF1, Malat1	Involved in the storage, assembly, and modification of pre-mRNA splicing factors.
Nuclear stress body	2–10	0.3–3.0	HSF1, HAP	Contains satellite III ncRNAs and is a part of the general response to stress. Precise function not yet determined.
Nucleolus	1–4	0.5–8.0	RNA Pol I machinery	Involved in the transcription and processing of rRNA and the assembly of ribosomal subunits. Plays roles in the modification and assembly of other nuclear RNAs and RNPs. Regulates cell cycle progression by sequestering and modifying many proteins.
Paraspeckle	10–20	0.5	PSP1, p54nrb, Men ε/β (Neat1)	Involved in nuclear retention of some A-to-I hyperedited mRNAs.
Perinucleolar compartment	1–4	0.2–1.0	PTB, CUGBP	Precise functions are unknown but its prevalence positively correlates with metastatic capacity.
PML-nuclear body	10–30	0.3–1.0	PML	Involved in response to many forms of stress, viral defense, and genome stability by the sequestration, modification, and degradation of many partner proteins.
Polycomb body	12–16	0.3–1.0	Bmi1, Pc2	Involved in Polycomb proteins-mediated gene paring and silencing in *Drosophila*. Precise function in mammalian cells remains to be determined.

Mao at al TiG., 2011

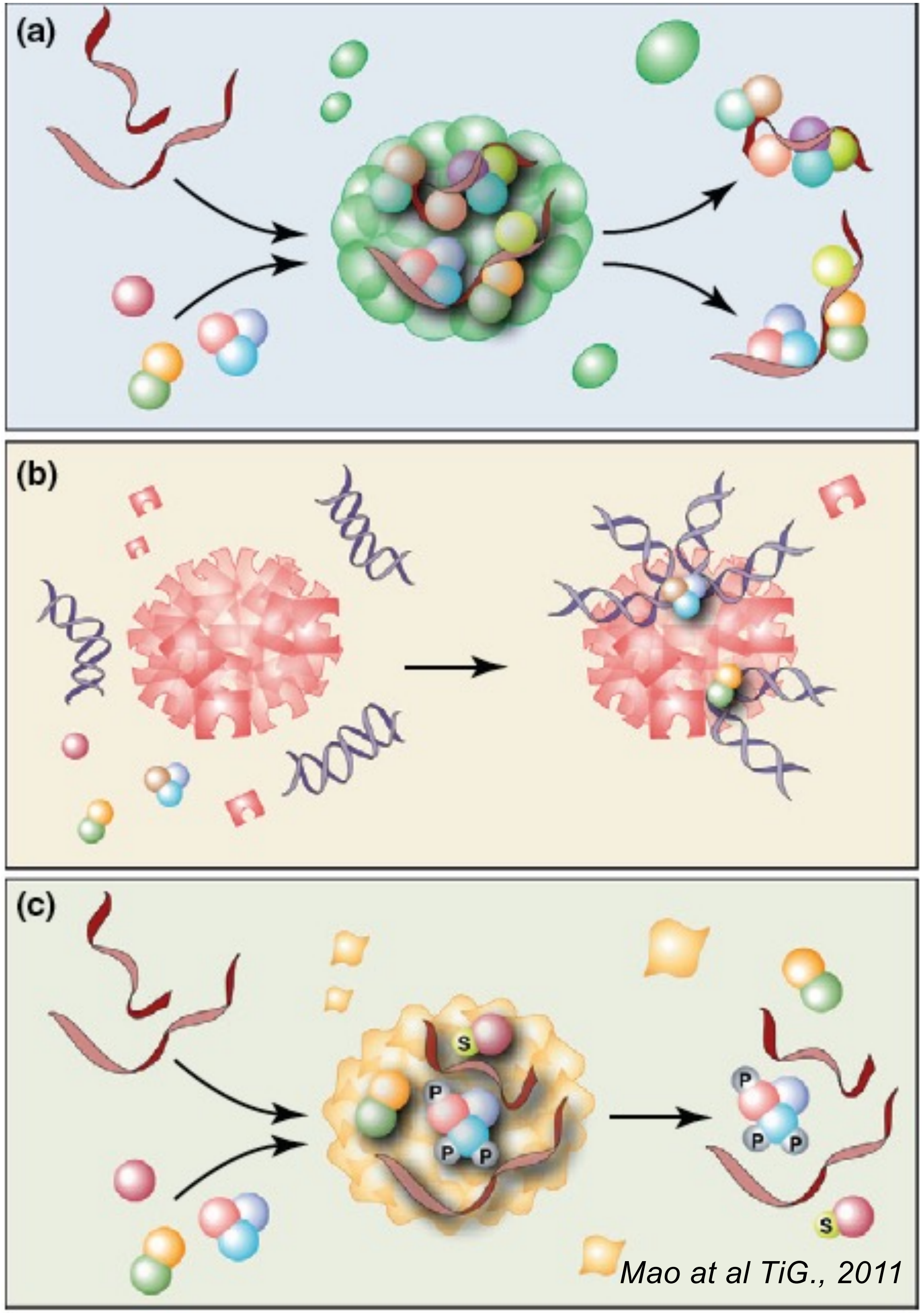
MLO RNAs and their interactors



MLO	RNA	Length (nt)	RNA interactors	Example protein interactors
Cajal body	(Pre-)U-snRNAs	70–200[a]	Pre-mRNA splice sites, scaRNAs	COIL, GEM2/4-8, LSM4, PRPF3/4/6/8/31/43, SART3, SMN1, SNRPA/A1/B/B2/C/D1/D2/D3/E/F, SNRNP70, SRSF1/2, TGS1, U2AF1/2
	7SK snRNA	331	rRNAs	CCNT1, CDK9, HEXIM1/2, LARP7, MEPCE, NELF
	C/D & H/ACA box snoRNAs	70–90	rRNAs, snRNAs	15.5K, COIL, DKC1, FBL, GAR1, NHP2, NOP10/56/58, TGS1
	TERC	500	SNORD44	COIL, DKC1, GAR1, NAF1, NHP2, NOP10, RUVBL1/2, SMN1, TERT, WRAP53
Histone locus body	Histone mRNAs	350–800[a]	U7 snRNA, Y3/Y3** ncRNA	CPSF73/100, FLASH, SLBP, SYMPK, ZFP100
	U7 snRNA	63	Histone pre-mRNAs	FLASH, LSM10/11, SNRPB/D3/E/F/G
	U2 snRNA	187	SCARNA2, mRNA 3′ splice site	SNRPA1/B/D3/E/F/G, SF3A1-3, SF3B1-4, U2AF1/2
	Y3/Y3** ncRNA	105/60	Histone pre-mRNAs	CPSF1/2, FIPL1, FLASH, IGF2BP1, RO60, SSB, SYMPK, WDR33
Nuclear speckle	MALAT1 lncRNA	7,000	U1 snRNA, miRNAs	DBC1, DDX23, DKC1, KHDRBS1 PRPF8, NOP58, RNPS1, SRSF1/2/3, SRM160, U2AF2
	Mature U-snRNAs	70–200	Pre-mRNA splice sites, scaRNAs	15.5K, SART3, SF3A1-3, SF3B1-5, SNRPA/A1/B/B2/C/D1/D2/D3/E/F/G, SNRNP70, SRSF1/2, U2AF1/2
Nucleolus	rRNAs	150–4,700	snoRNAs	15.5K, NCL, NPM1, 80+ ribosomal proteins (RS, RL), SSB, DKC1, FBL
	SNORD3A (U3 snoRNA)	217	rRNAs	15.5 K, FBL, IMP3/4, MPP10, NOP56/58, RRP7/9, UTP proteins
	C/D & H/ACA box snoRNAs	70–90	rRNAs, snRNAs	15.5K, DKC1, FBL, GAR1, NHP2, NOP10/56/58, TGS1
Paraspeckle	NEAT1 lncRNA	23,000[b]	Pri-miRNAs	DROSHA, DGCR8, NONO, PSPC1, SFPQ, TARDP, FUS
Sam68 nuclear body	Unidentified lncRNA	—	—	DBC1[c], HNRNPL1[c], KHDRBS1[c]

Sawyer at al WIREsRNA., 2018

Nuclear bodies



Mao at al TiG., 2011

Reaction sites

Pre-RNA processing in nucleoli

Modification and assembly of snRNAs in CB

Hot spots

Gene activation or repression

Epigenetic reactions

Stabilization of interactions between gene loci

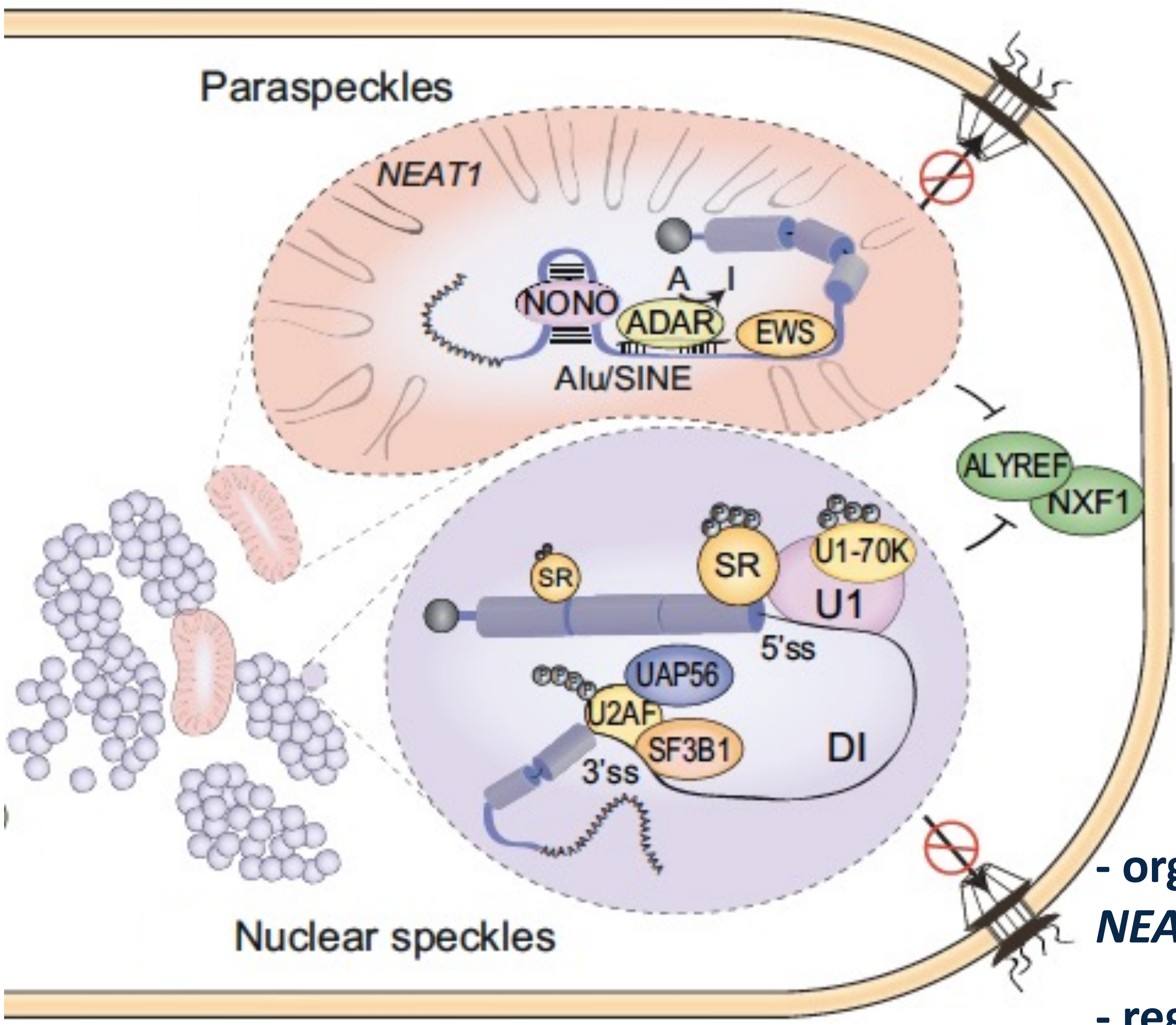
Storage/modification sites of proteins and RNAs

Some A-to-I hyperedited mRNAs are retained in paraspecles

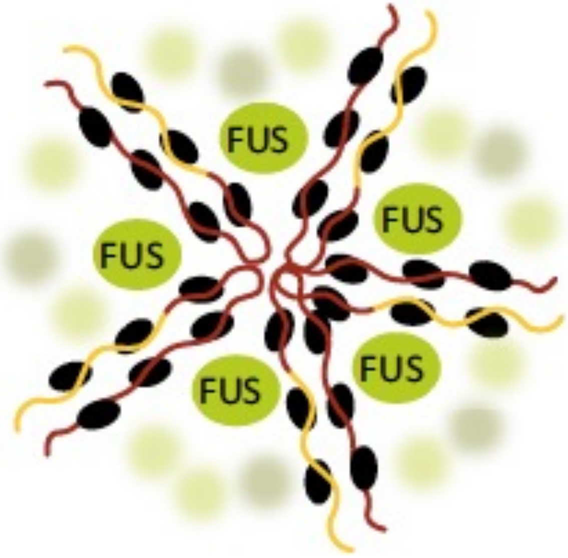
Phosphorylation of SR proteins in nuclear speckles

Sumoylation of nuclear proteins in PcG bodies

Paraspeckles
Nuclear speckles



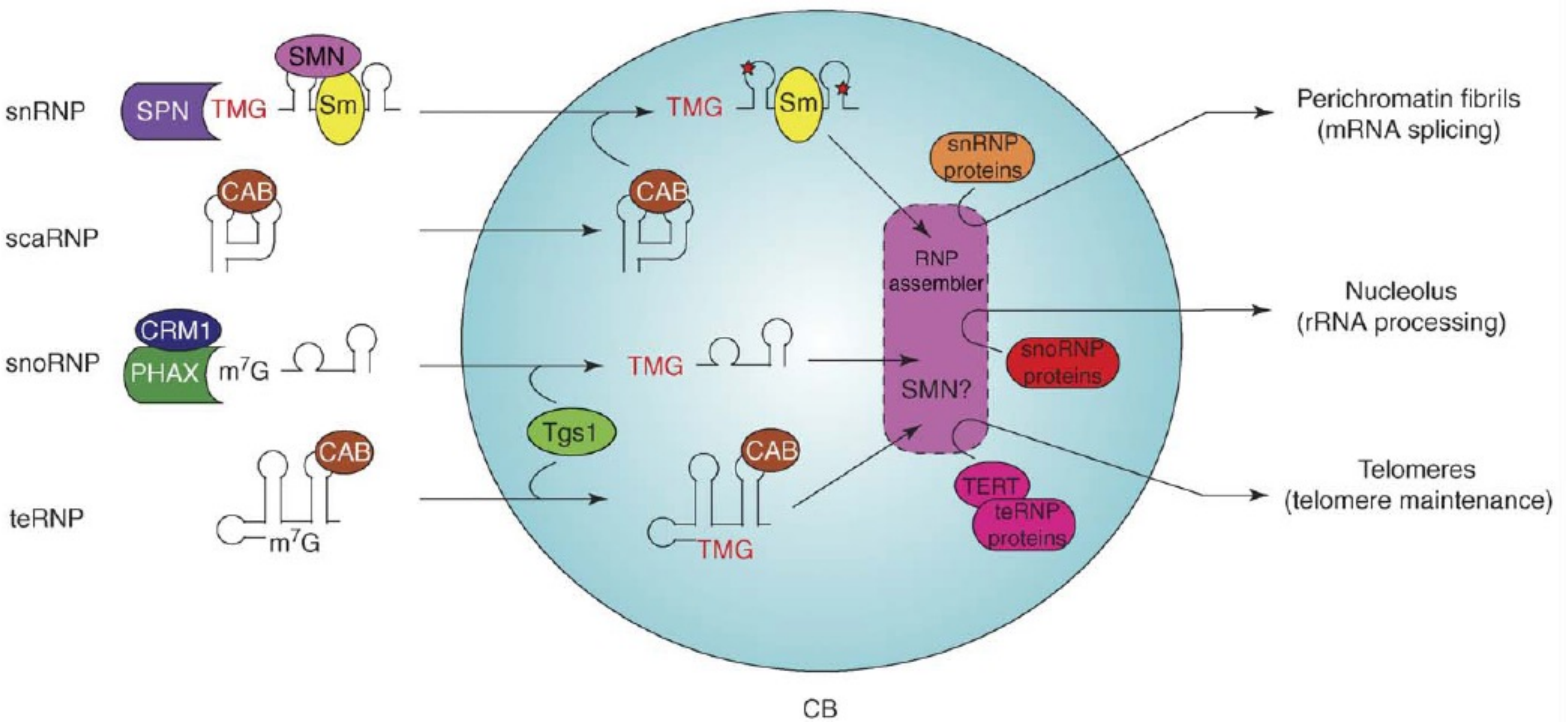
Wegener and Müller-McNicoll, Sem Cell Dev Biol 2018



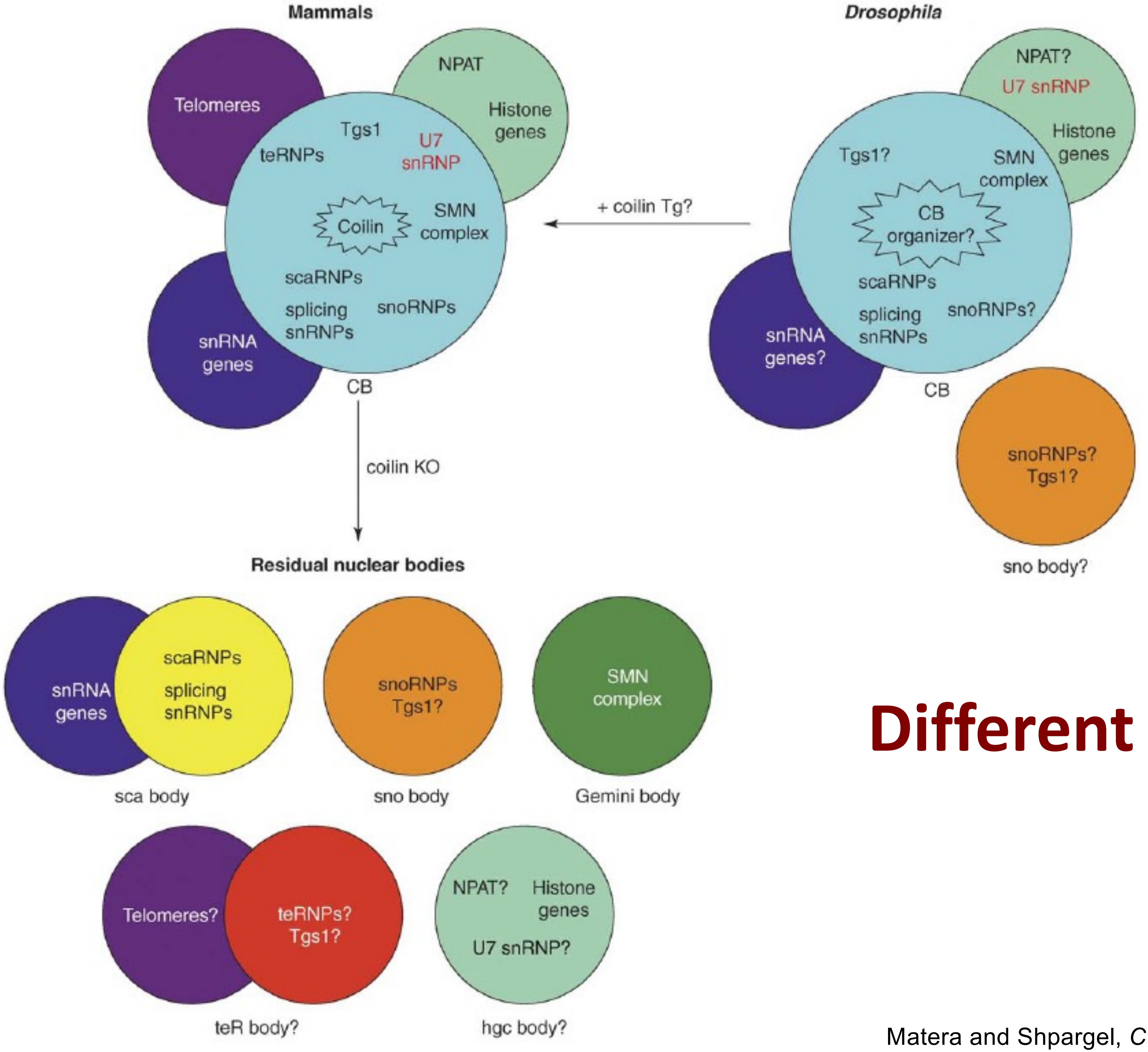
- organized around lncRNAs: *NEAT1* (PS) or *MALAT1* (NS)

- regulate gene expression by mRNA nuclear retention

Cajal body function



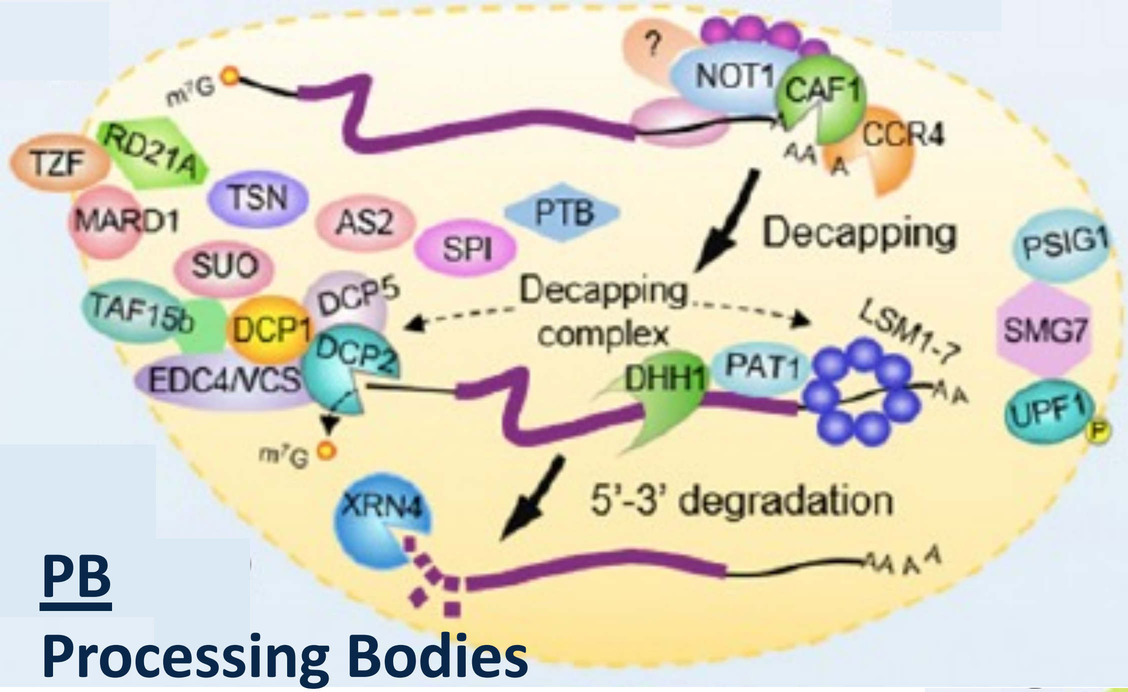
Matera and Shpargel, Curr. Op. Cel. Biol., 2006



Different CBs?

Matera and Shpargel, *Curr. Op. Cel. Biol.*, 2006

Cytoplasmic P-bodies and Stress Granules



Dynamic biomolecular condensates

Formed by phase separation of RNAs and proteins

Role in translational control and proteome buffering upon translational arrest (PB) and stress (SG)

PB
Processing Bodies

mRNA storage
mRNA decay?

PB

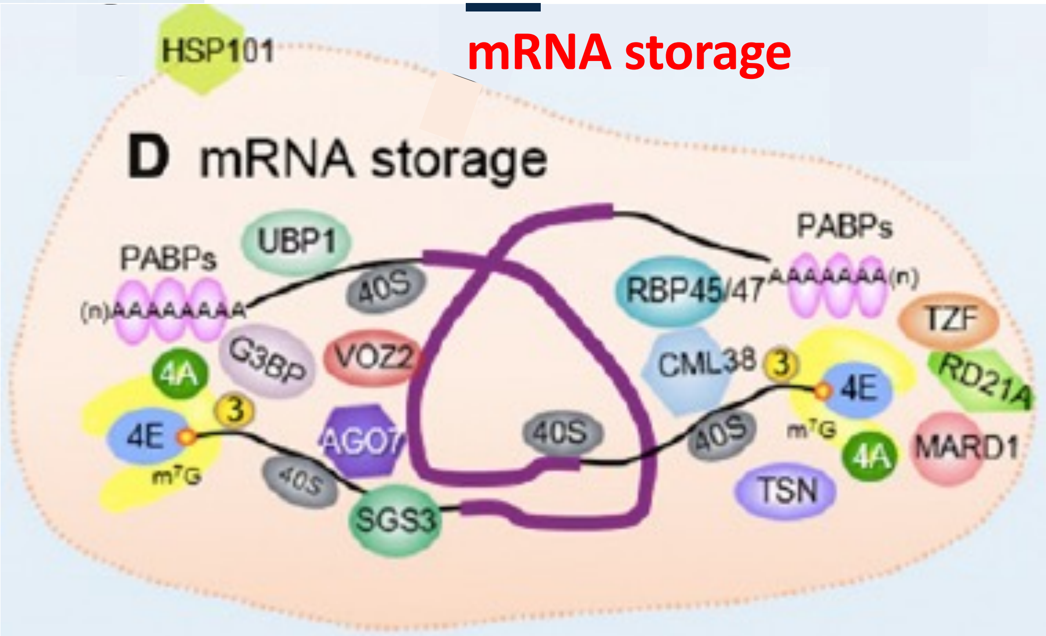
Translationally stalled mRNAs devoid of initiation factors shuttle to PBs

SG

Global translation halts in stress, mRNAs bound to the translational machinery and other SG proteins

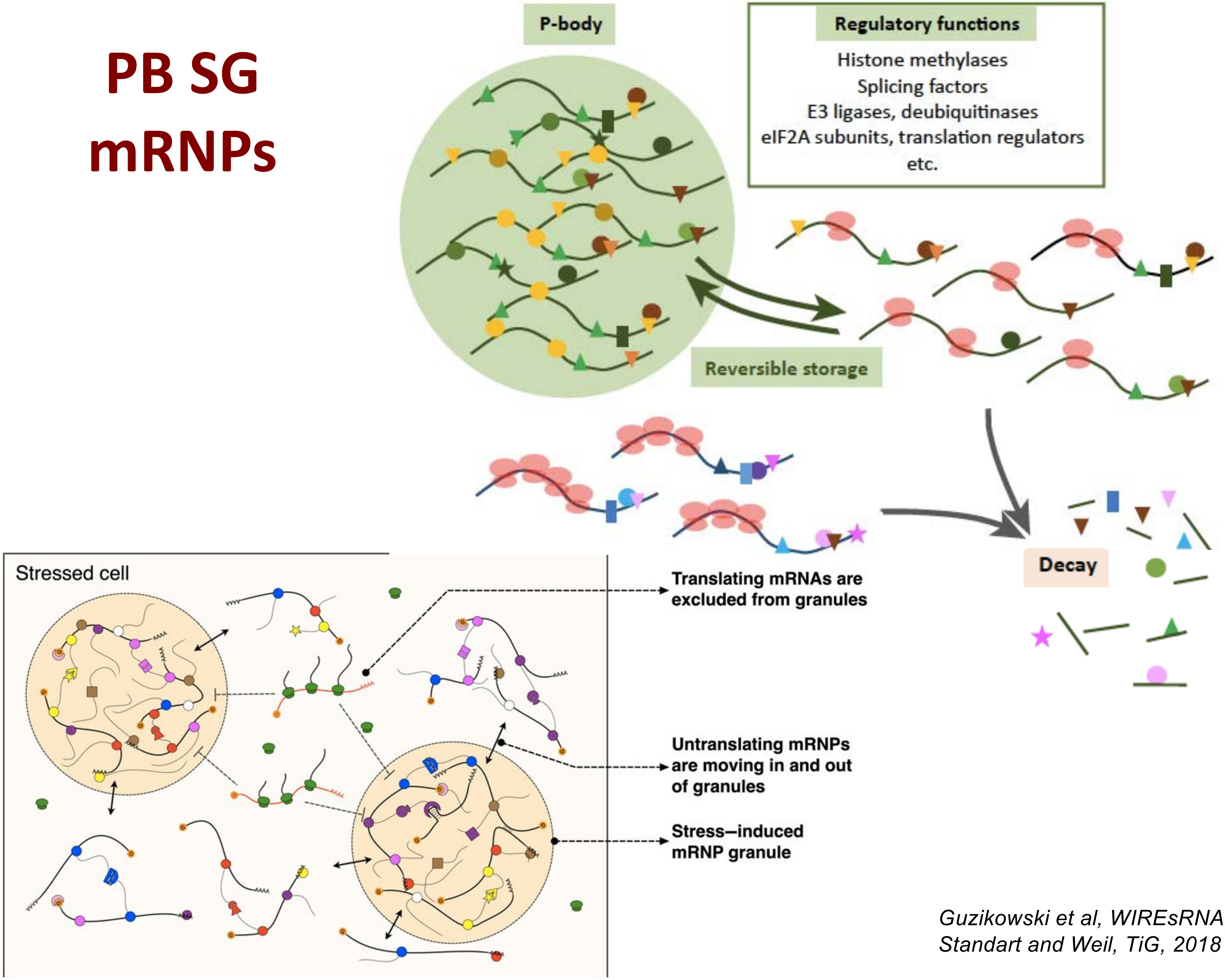
SG Stress Granules

mRNA storage



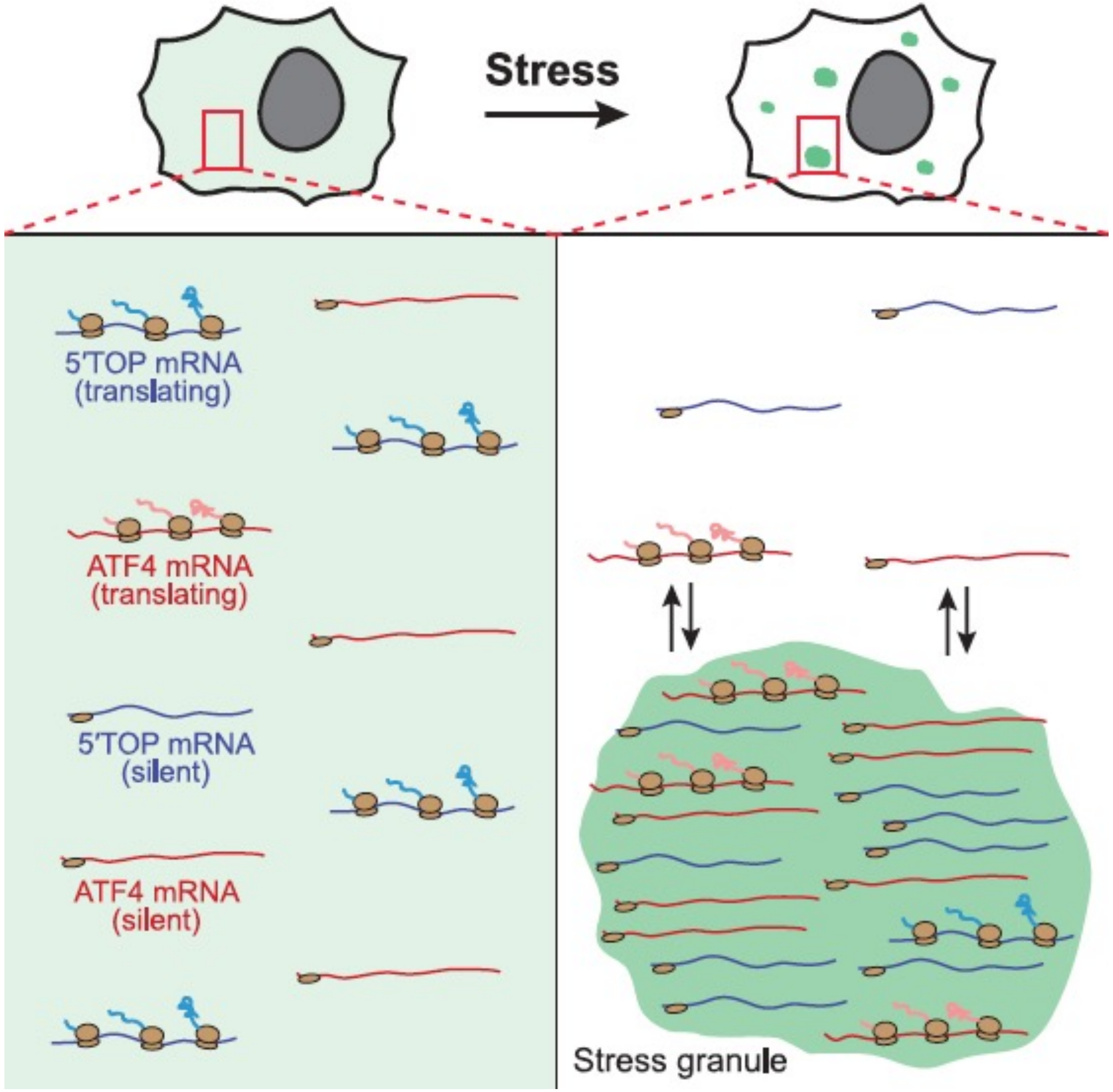
Chantarachot and Bailey-Serres, Plant Phys, 2018

PB SG mRNPs



Guzikowski et al, WIREsRNA, 2019; Standart and Weil, TiG, 2018

Translation in SGs

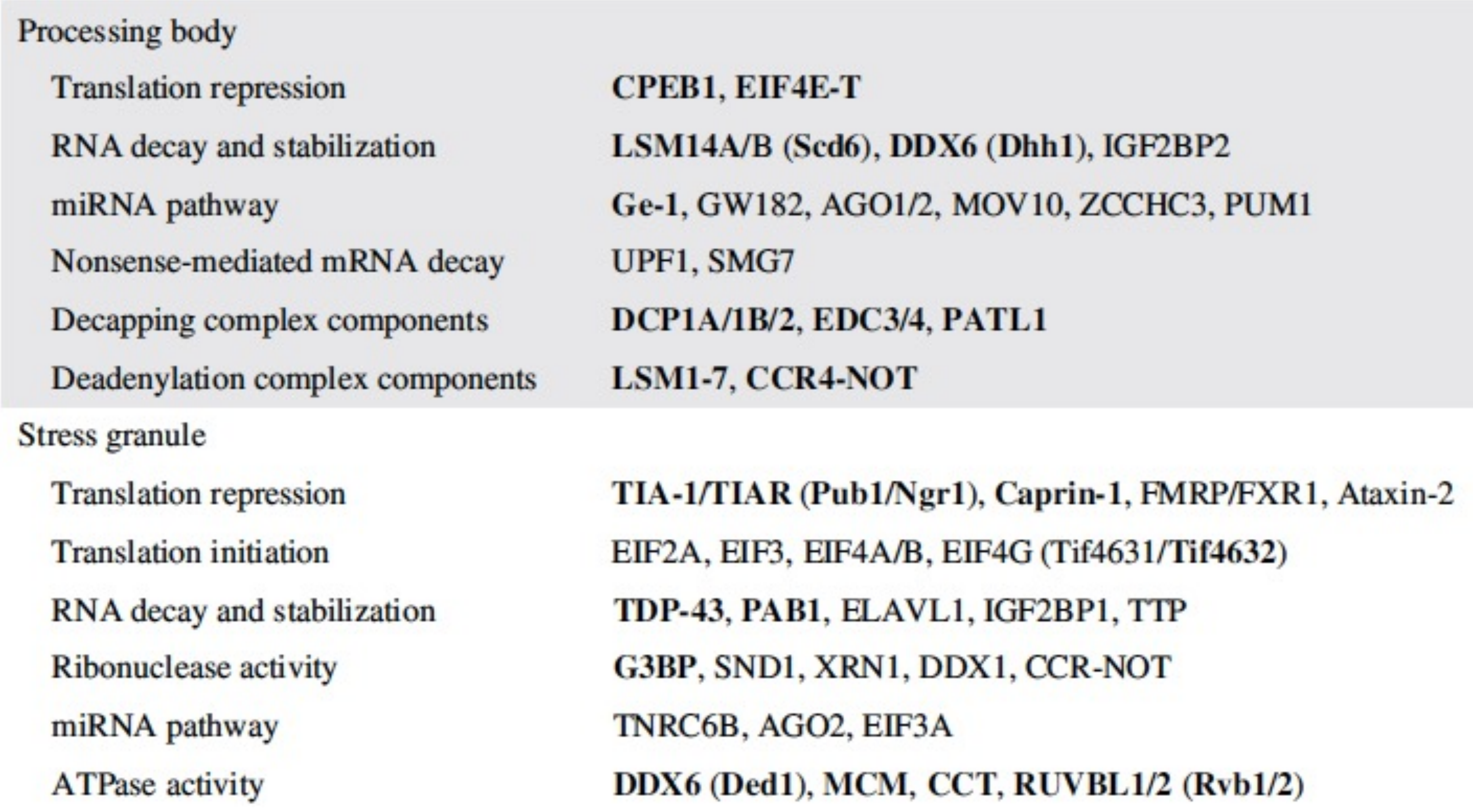


- **nontranslating mRNAs are preferentially recruited to SGs**
- **mRNAs in SGs can undergo translation** (complete cycle)
- **translating mRNAs can enter, leave, or stably localize to SGs**
- **translation in SGs mainly, but not only, occurs on mRNAs enhanced under stress**

(shown using single-molecule mRNA imaging, SunTag)

Mateju et al., Cell, 2020;

Cytoplasmic PBs and SGs

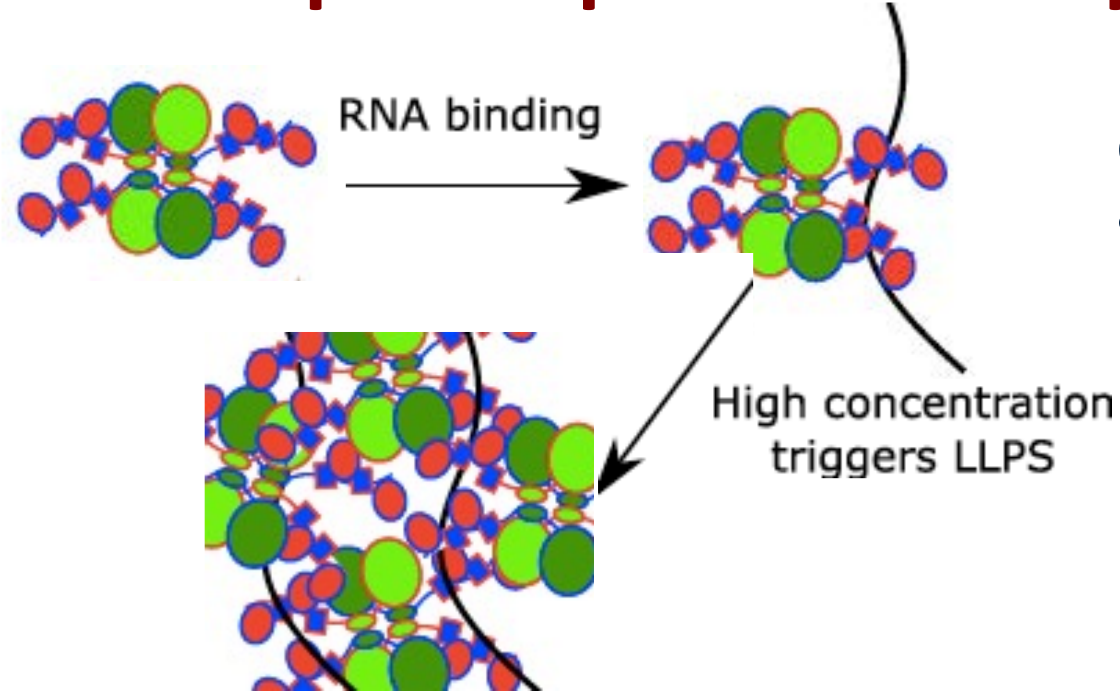


Processing body	
Translation repression	**CPEB1, EIF4E-T**
RNA decay and stabilization	**LSM14A/B (Scd6), DDX6 (Dhh1)**, IGF2BP2
miRNA pathway	**Ge-1**, GW182, AGO1/2, MOV10, ZCCHC3, PUM1
Nonsense-mediated mRNA decay	UPF1, SMG7
Decapping complex components	**DCP1A/1B/2, EDC3/4, PATL1**
Deadenylation complex components	**LSM1-7, CCR4-NOT**
Stress granule	
Translation repression	**TIA-1/TIAR (Pub1/Ngr1), Caprin-1**, FMRP/FXR1, Ataxin-2
Translation initiation	EIF2A, EIF3, EIF4A/B, EIF4G (Tif4631/Tif4632)
RNA decay and stabilization	**TDP-43, PAB1**, ELAVL1, IGF2BP1, TTP
Ribonuclease activity	**G3BP**, SND1, XRN1, DDX1, CCR-NOT
miRNA pathway	TNRC6B, AGO2, EIF3A
ATPase activity	**DDX6 (Ded1), MCM, CCT, RUVBL1/2 (Rvb1/2)**

composition of SG and PB proteome database
(http://rnagranuledb.lunenfeld.ca/)

Guzikowski et al, WIREsRNA, 2019

Phase transition, Droplets, MLOs, Liquid-Liquid Phase Separation (LLPS)

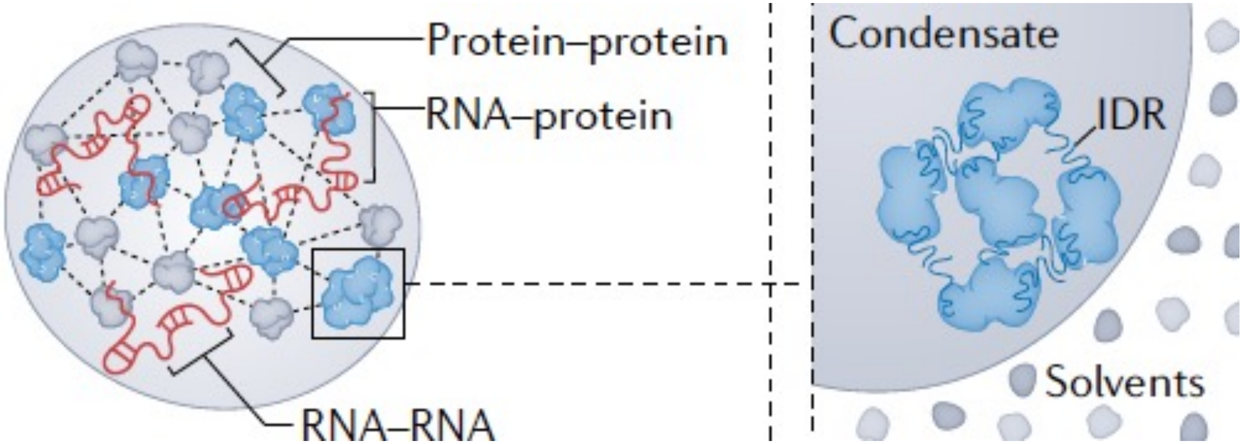


Formed by unstructured disordered protein domains around RNA or DNA

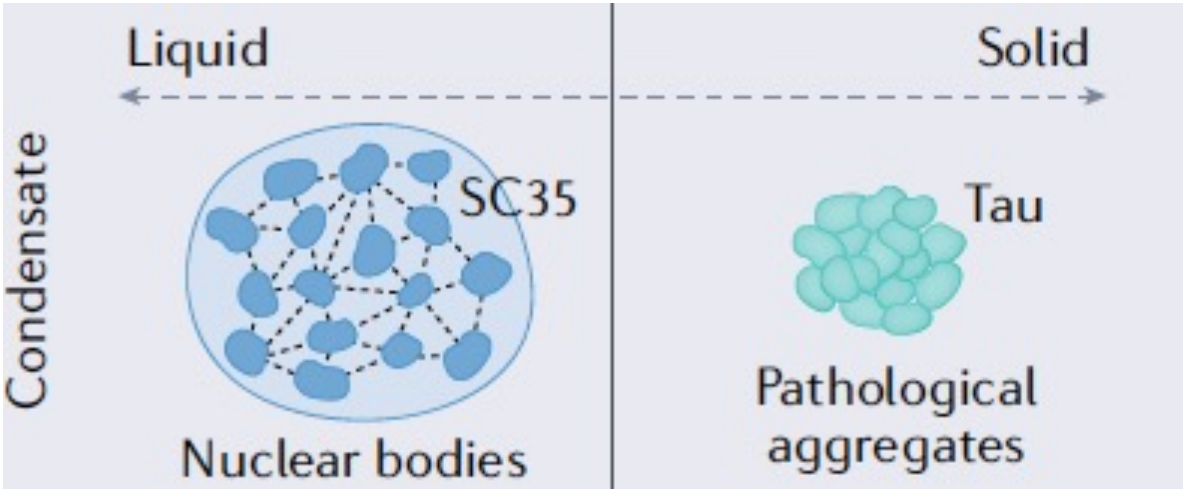
Organize several cellular processes:

- Heterochromatin structure (HP1)
- Transcription (Mediator, Pol II CTD)
- Processing (nucleolus, spliceosome, SR proteins, CBs)
- RNA retention and storage (NS, PS, PB, SG)
- RNA decay (degradosome)
- Protein modification and degradation (autophagosome, proteasome)

Condensates



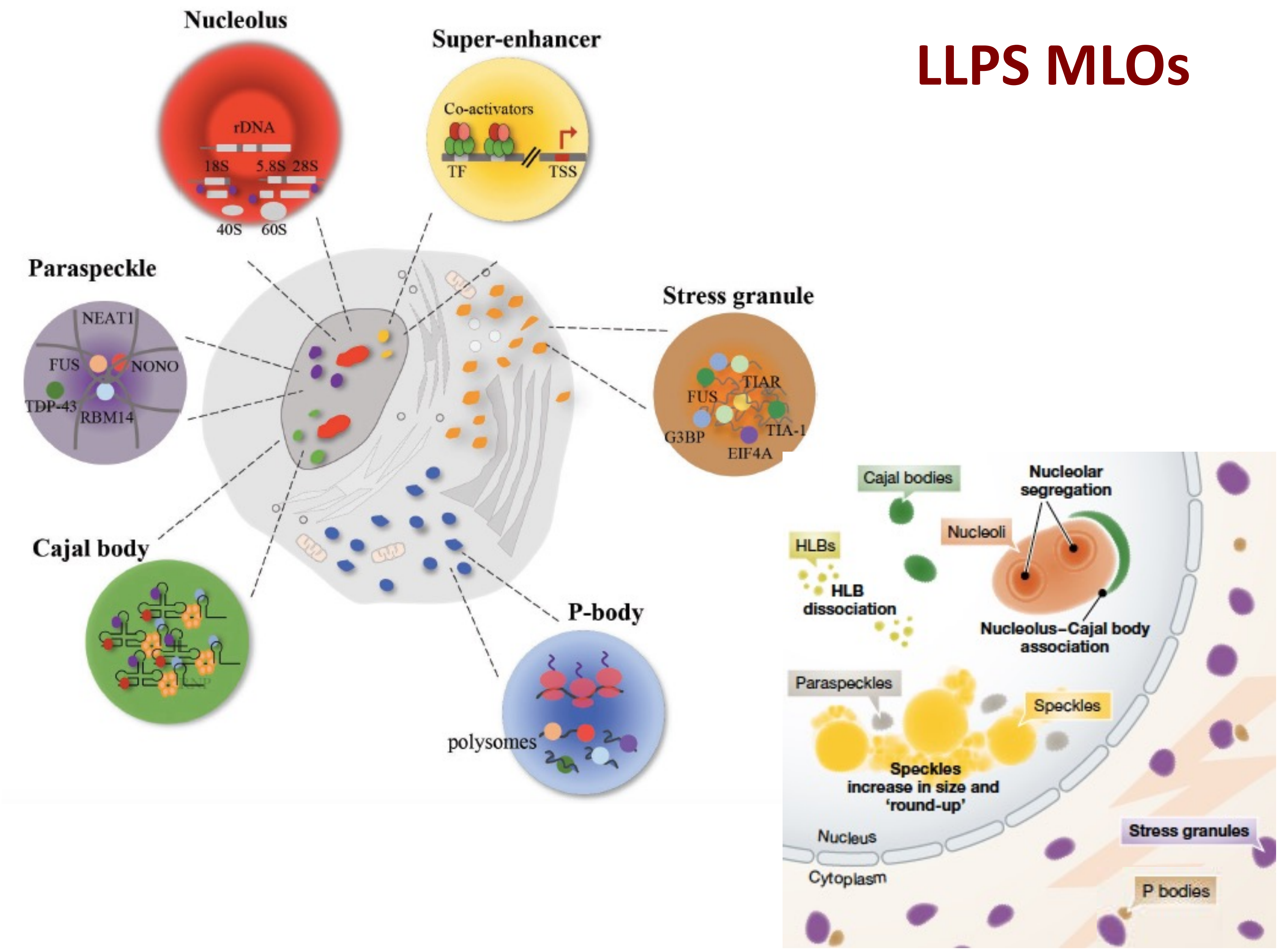
IDR – Intrinsic Disordered Regions



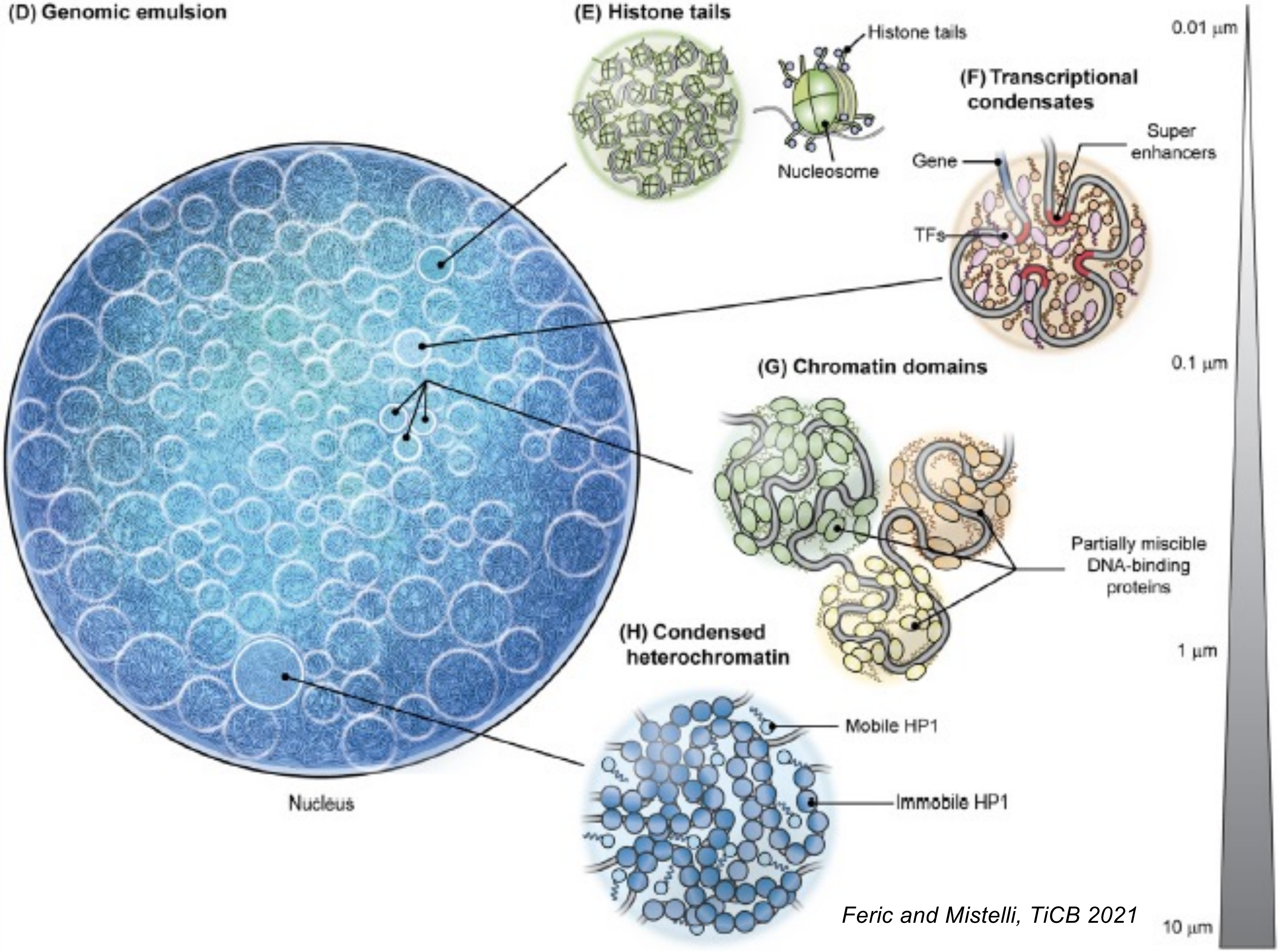
- **transient or stable**
- **specific or unspecific**
- **with RNA, DNA or protein only**
- **may undergo transition from gel to liquid to solid aggregates**

Bhat et al., Nat Rev Moll Cell Biol 2021

LLPS MLOs


Nucleolus
rDNA
18S
5.8S 28S
40S
60S
Super-enhancer
Co-activators
TF
TSS
Paraspeckle
NEAT1
FUS
NONO
TDP-43
RBM14
Stress granule
TIAR
FUS
G3BP
TIA-1
EIF4A
Cajal body
P-body
polysomes
Cajal bodies
Nucleolar segregation
Nucleoli
HLBs
HLB dissociation
Nucleolus–Cajal body association
Paraspeckles
Speckles
Speckles increase in size and 'round-up'
Nucleus
Cytoplasm
Stress granules
P bodies

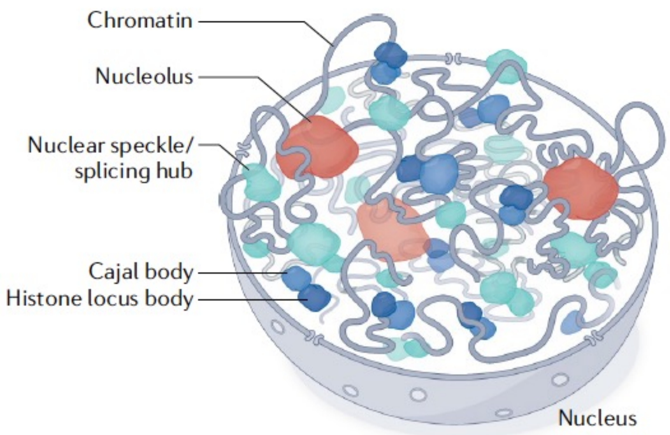
LLPS in the nucleus

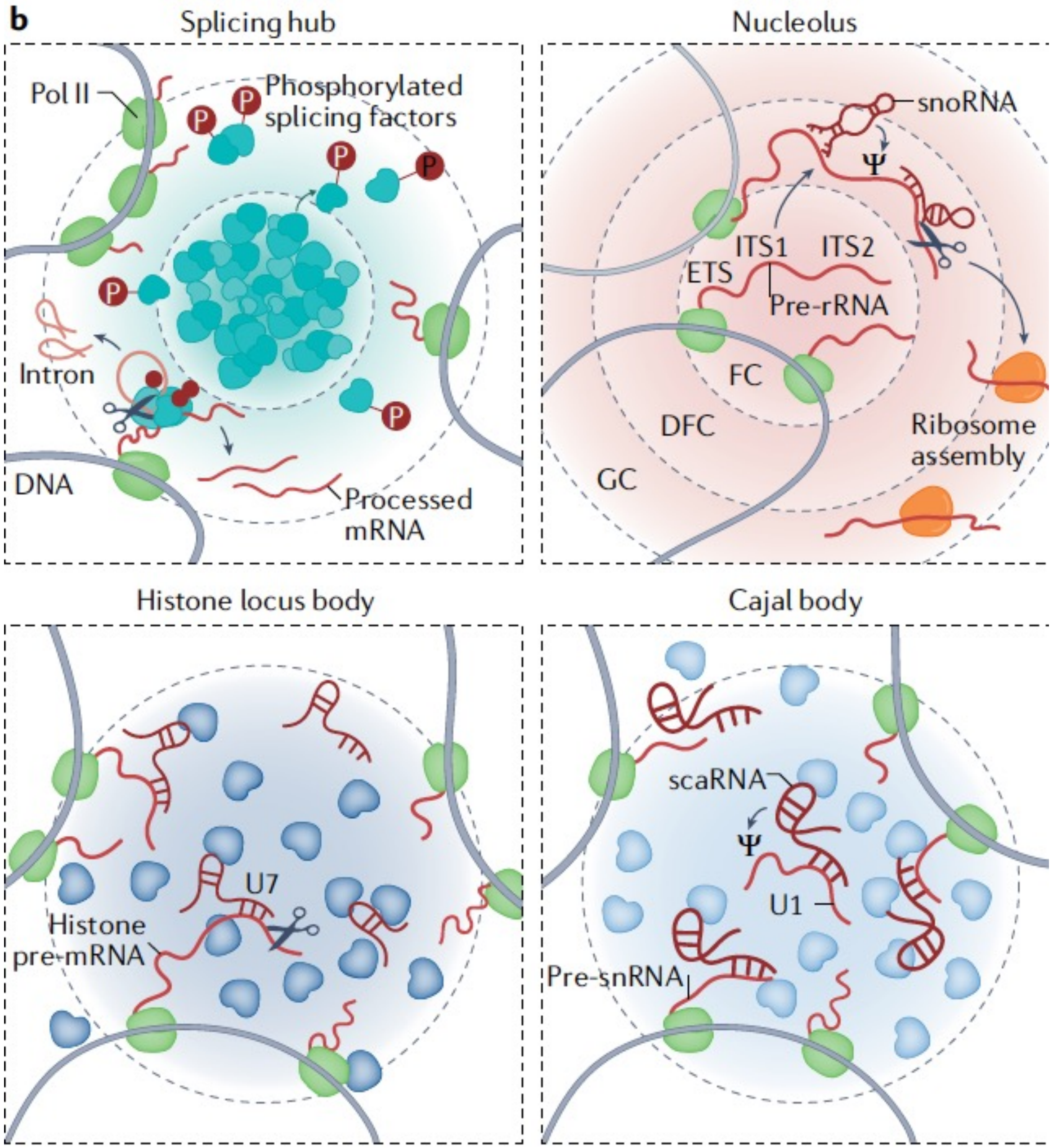


Feric and Mistelli, TiCB 2021

LLPS in the nucleus

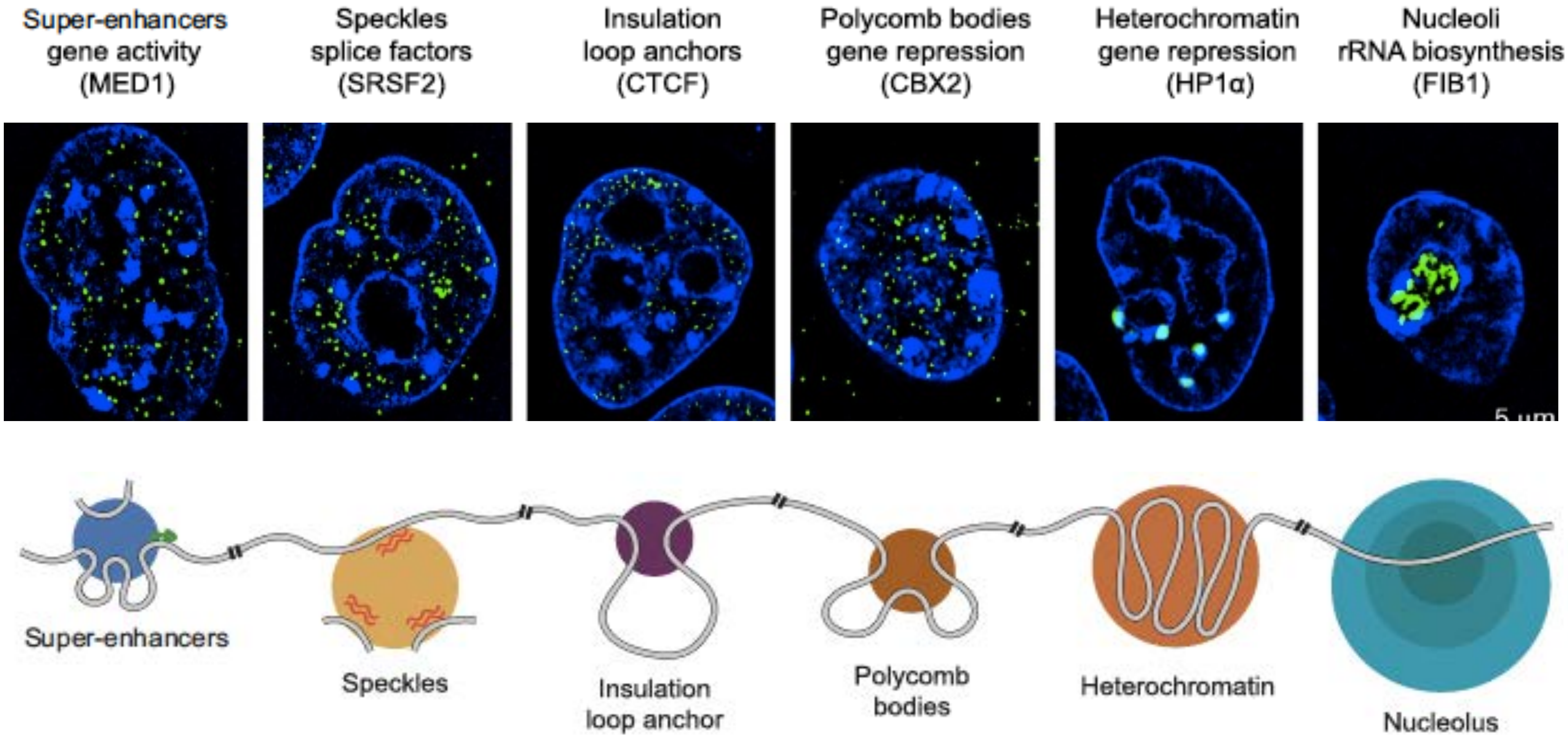
LLPS as RNA processing hubs





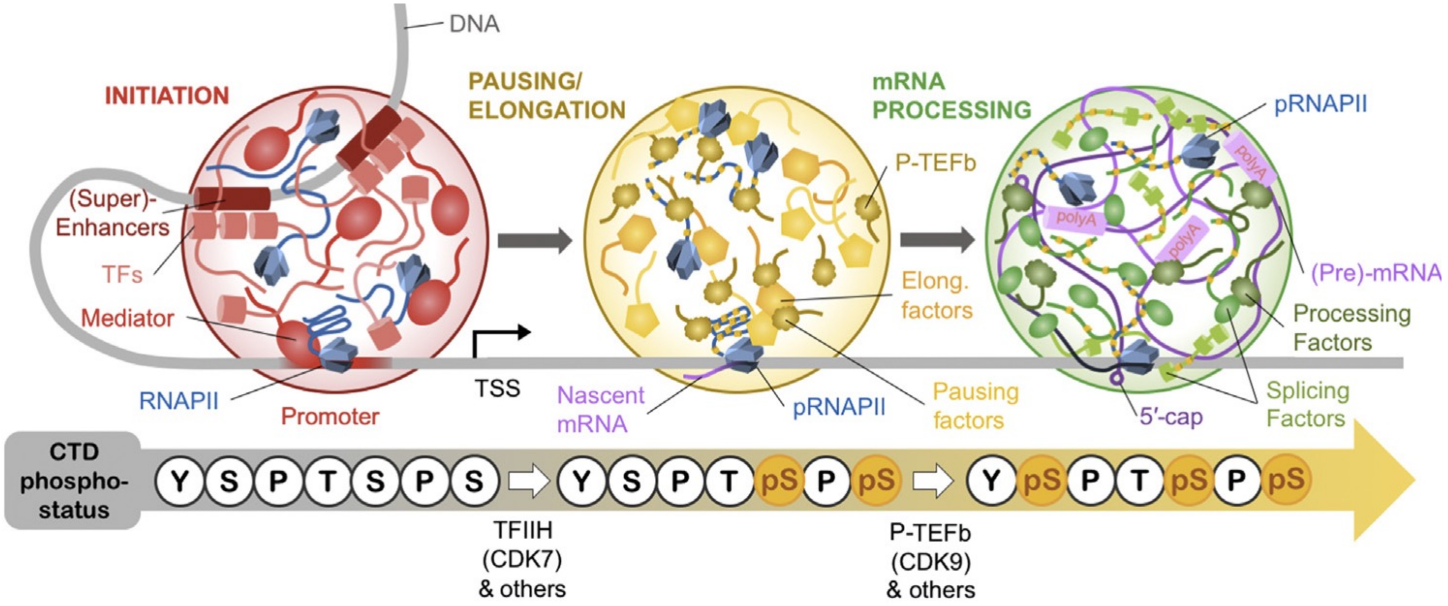
Bhat et al., Nat Rev Moll Cell Biol 2021

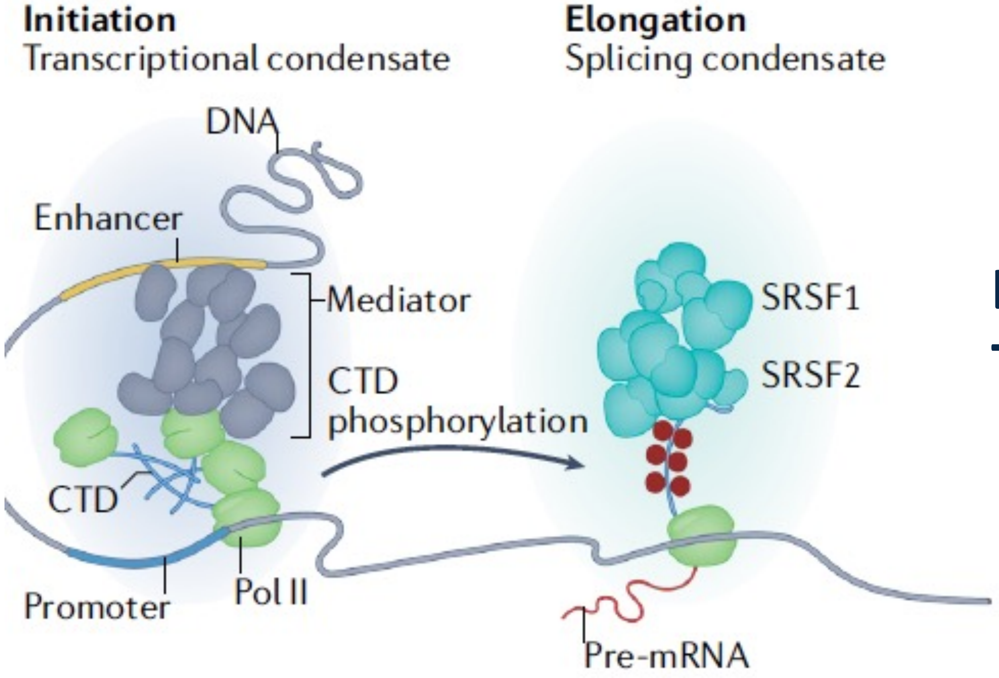
LLPS in the nucleus – Pol II



Nosella et al, Curr Op Cell Biol, 2021

LLPS in the nucleus – Pol II

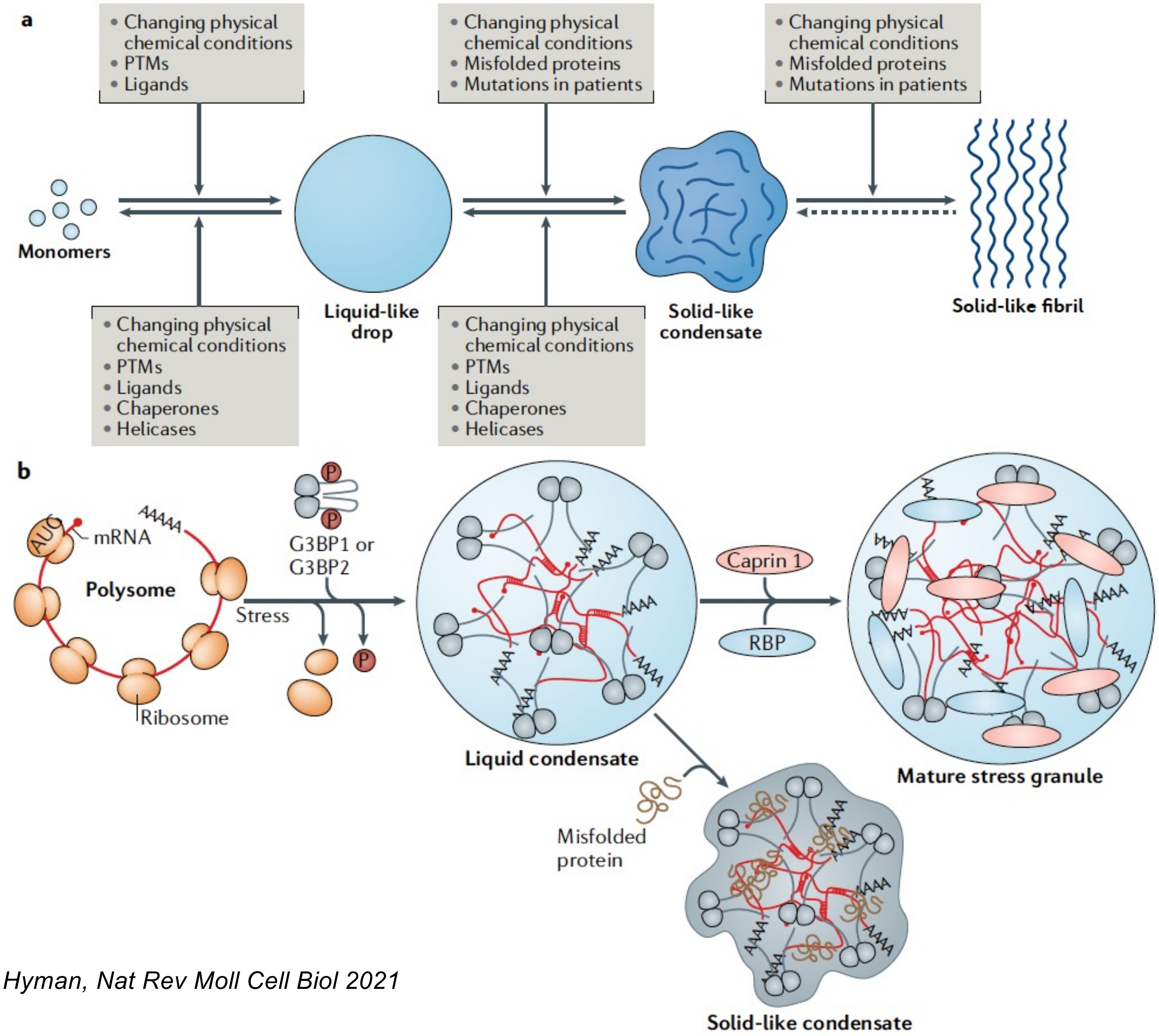




LLPS
Transcription and splicing

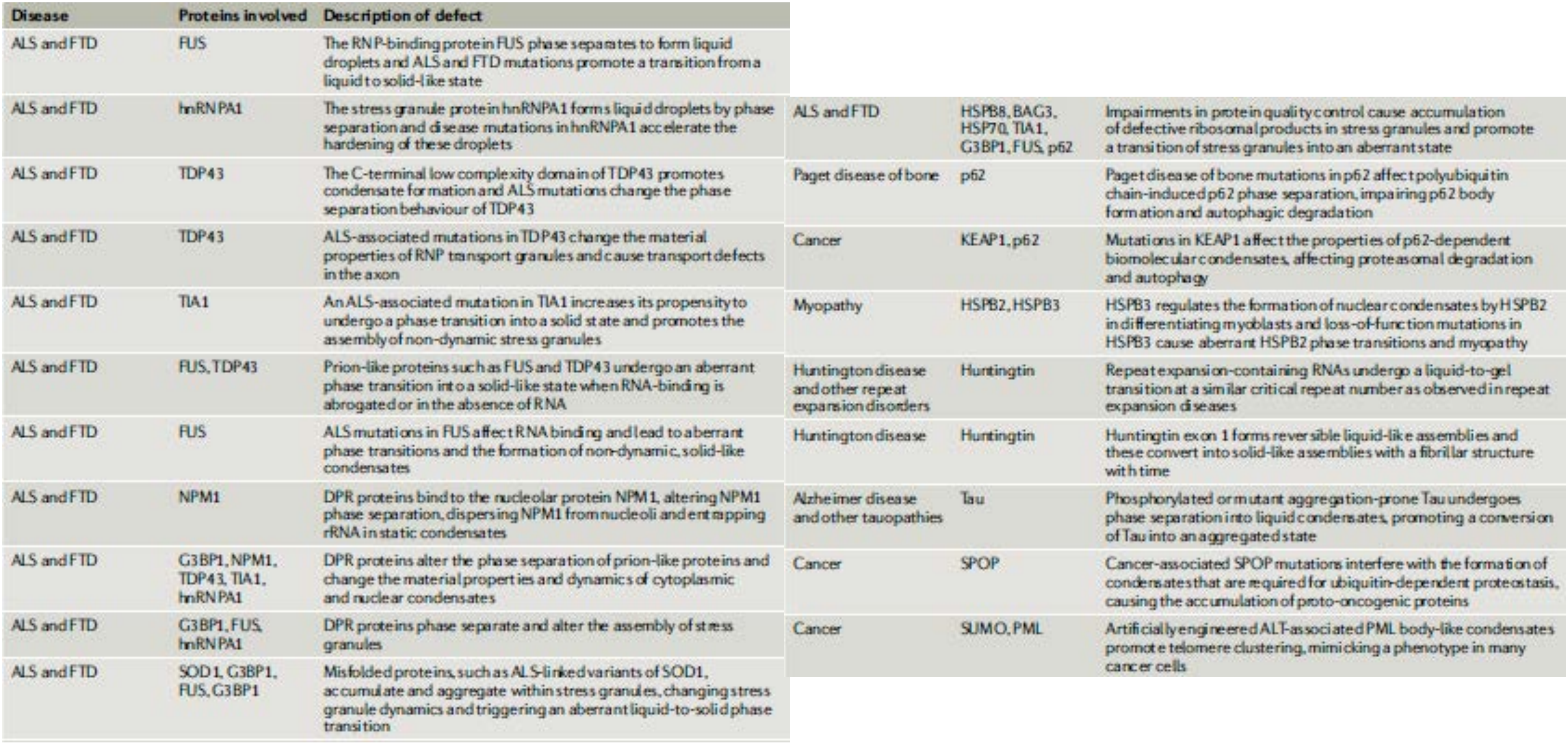
Nosella et al, Curr Op Cell Biol, 2021
Bhat et al., Nat Rev Moll Cell Biol 2021

Aberrant condensates



Alberti and Hyman, Nat Rev Moll Cell Biol 2021

Aberrant condensates in age-related human disease



Disease	Proteins involved	Description of defect
ALS and FTD	FUS	The RNP-binding protein FUS phase separates to form liquid droplets and ALS and FTD mutations promote a transition from a liquid to solid-like state
ALS and FTD	hnRNPA1	The stress granule protein hnRNPA1 forms liquid droplets by phase separation and disease mutations in hnRNPA1 accelerate the hardening of these droplets
ALS and FTD	TDP43	The C-terminal low complexity domain of TDP43 promotes condensate formation and ALS mutations change the phase separation behaviour of TDP43
ALS and FTD	TDP43	ALS-associated mutations in TDP43 change the material properties of RNP transport granules and cause transport defects in the axon
ALS and FTD	TIA1	An ALS-associated mutation in TIA1 increases its propensity to undergo a phase transition into a solid state and promotes the assembly of non-dynamic stress granules
ALS and FTD	FUS, TDP43	Prion-like proteins such as FUS and TDP43 undergo an aberrant phase transition into a solid-like state when RNA-binding is abrogated or in the absence of RNA
ALS and FTD	FUS	ALS mutations in FUS affect RNA binding and lead to aberrant phase transitions and the formation of non-dynamic, solid-like condensates
ALS and FTD	NPM1	DPR proteins bind to the nucleolar protein NPM1, altering NPM1 phase separation, dispersing NPM1 from nucleoli and entrapping rRNA in static condensates
ALS and FTD	G3BP1, NPM1, TDP43, TIA1, hnRNPA1	DPR proteins alter the phase separation of prion-like proteins and change the material properties and dynamics of cytoplasmic and nuclear condensates
ALS and FTD	G3BP1, FUS, hnRNPA1	DPR proteins phase separate and alter the assembly of stress granules
ALS and FTD	SOD1, G3BP1, FUS, G3BP1	Misfolded proteins, such as ALS-linked variants of SOD1, accumulate and aggregate within stress granules, changing stress granule dynamics and triggering an aberrant liquid-to-solid phase transition
ALS and FTD	HSPB8, BAG3, HSP70, TIA1, G3BP1, FUS, p62	Impairments in protein quality control cause accumulation of defective ribosomal products in stress granules and promote a transition of stress granules into an aberrant state
Paget disease of bone	p62	Paget disease of bone mutations in p62 affect polyubiquitin chain-induced p62 phase separation, impairing p62 body formation and autophagic degradation
Cancer	KEAP1, p62	Mutations in KEAP1 affect the properties of p62-dependent biomolecular condensates, affecting proteasomal degradation and autophagy
Myopathy	HSPB2, HSPB3	HSPB3 regulates the formation of nuclear condensates by HSPB2 in differentiating myoblasts and loss-of-function mutations in HSPB3 cause aberrant HSPB2 phase transitions and myopathy
Huntington disease and other repeat expansion disorders	Huntingtin	Repeat expansion-containing RNAs undergo a liquid-to-gel transition at a similar critical repeat number as observed in repeat expansion diseases
Huntington disease	Huntingtin	Huntingtin exon 1 forms reversible liquid-like assemblies and these convert into solid-like assemblies with a fibrillar structure with time
Alzheimer disease and other tauopathies	Tau	Phosphorylated or mutant aggregation-prone Tau undergoes phase separation into liquid condensates, promoting a conversion of Tau into an aggregated state
Cancer	SPOP	Cancer-associated SPOP mutations interfere with the formation of condensates that are required for ubiquitin-dependent proteostasis, causing the accumulation of proto-oncogenic proteins
Cancer	SUMO, PML	Artificially engineered ALT-associated PML body-like condensates promote telomere clustering, mimicking a phenotype in many cancer cells

Alberti and Hyman, Nat Rev Moll Cell Biol 2021

RNA condensates: functional or incidental

	PROs	**CONTRAs**
P-bodies Translational repression RNA decay	PBs contain translationally repressed mRNAs and RNA decay factors	Mutants in PBs are competent for translational repression or RNA decay
Stress Granules Translational repression	SGs enrich translationally repressed mRNAs and stalled translation initiation factors	Only a fraction of repressed mRNAs are in SGs SGs are not required for translational repression. mRNAs in SGs are translated
Nucleolus Ribosome biogenesis	Nucleolus enriches ribosome assembly factors in layers rRNA moves in nucleolus by flow Nucleolar proteins form droplets *in vitro*	Nucleolar morphology varies between species In human nucleoli, rRNAs remain near transcribing rDNA repeats
Transcription condensates	Pol II CTD undergoes LLPS *in vitro* Pol II and TFs are around active transcription sites LLPS correlates with increased transcriptional output	Condensation does not correlate with transcriptional output Low IDR concentrations promote transcription, high concentrations and condensation suppress transcription Not all transcription foci are condensates

Putnam et al, Genes Dev, 2023

RNA condensates: functional or incidental

	PROs	**CONTRAs**
Nuclear speckles Gene expression	Nuclear speckles correlate with enhanced gene expression Splicing-defective mRNAs are retained in nuclear speckles Blocking splicing leads to accumulation of spliceosomes in nuclear speckles	Speckles accumulate inactive splicing factors that leave to be active Speckles are not major sites of transcription and do not contain active Pol II In some species speckle markers are diffuse in the nucleoplasm
Paraspecles Sequestration of RNAs and proteins	*NEAT1* lncRNA is required for export of RNAs with Alu repeats *NEAT1 KD* releases paraspeckle proteins and activates gene expression	Paraspeckle composition diverges across evolution *NEAT1* is mammalian-specific, but Paraspecles also assemble in other species (*Drosophila*, *C. elegans*)
Cajal bodies snRNP assembly	Coilin- Nopp140 complex assembles CBs on snRNA genes Depletion of coilin leads to decrease in snRNPs and splicing	Coilin-null mutants do not assemble CBs but have normal levels of snRNAs and are viable

Putnam et al, Genes Dev, 2023