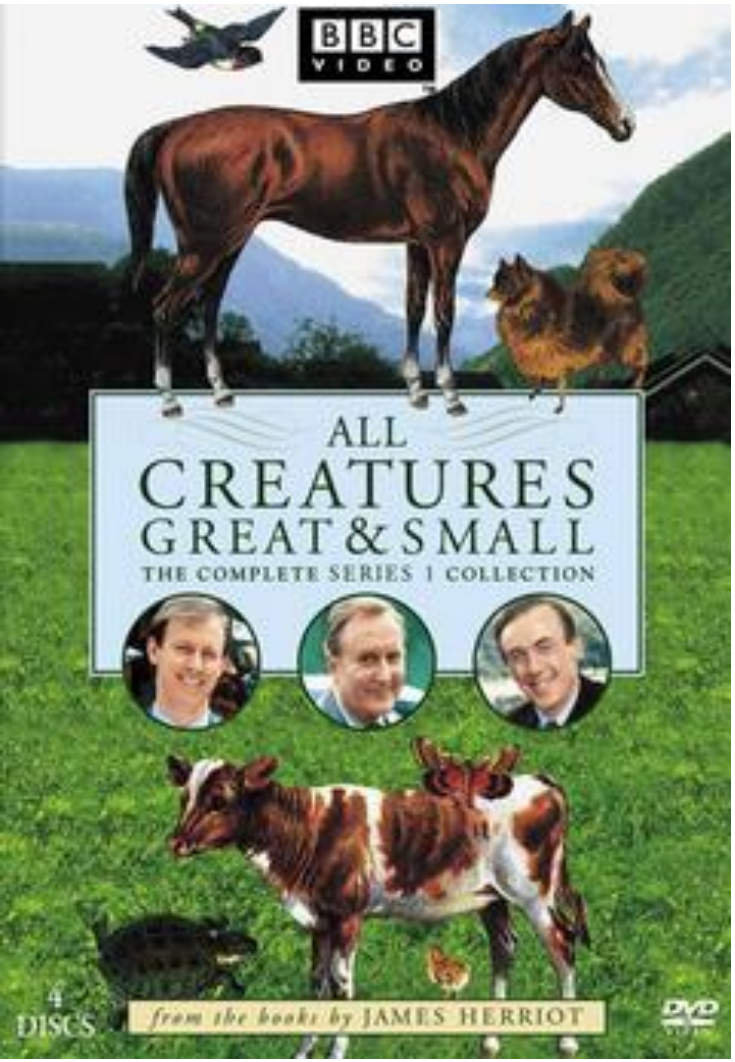
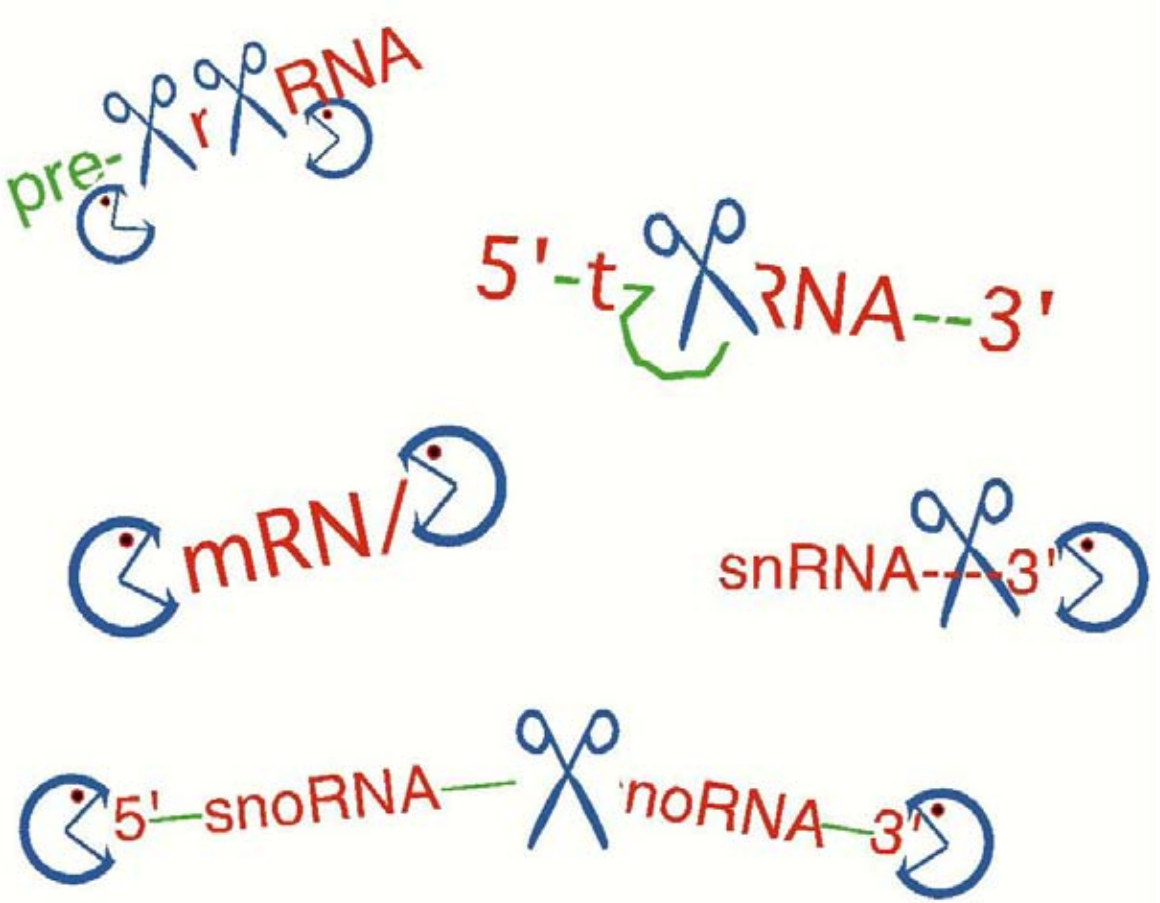
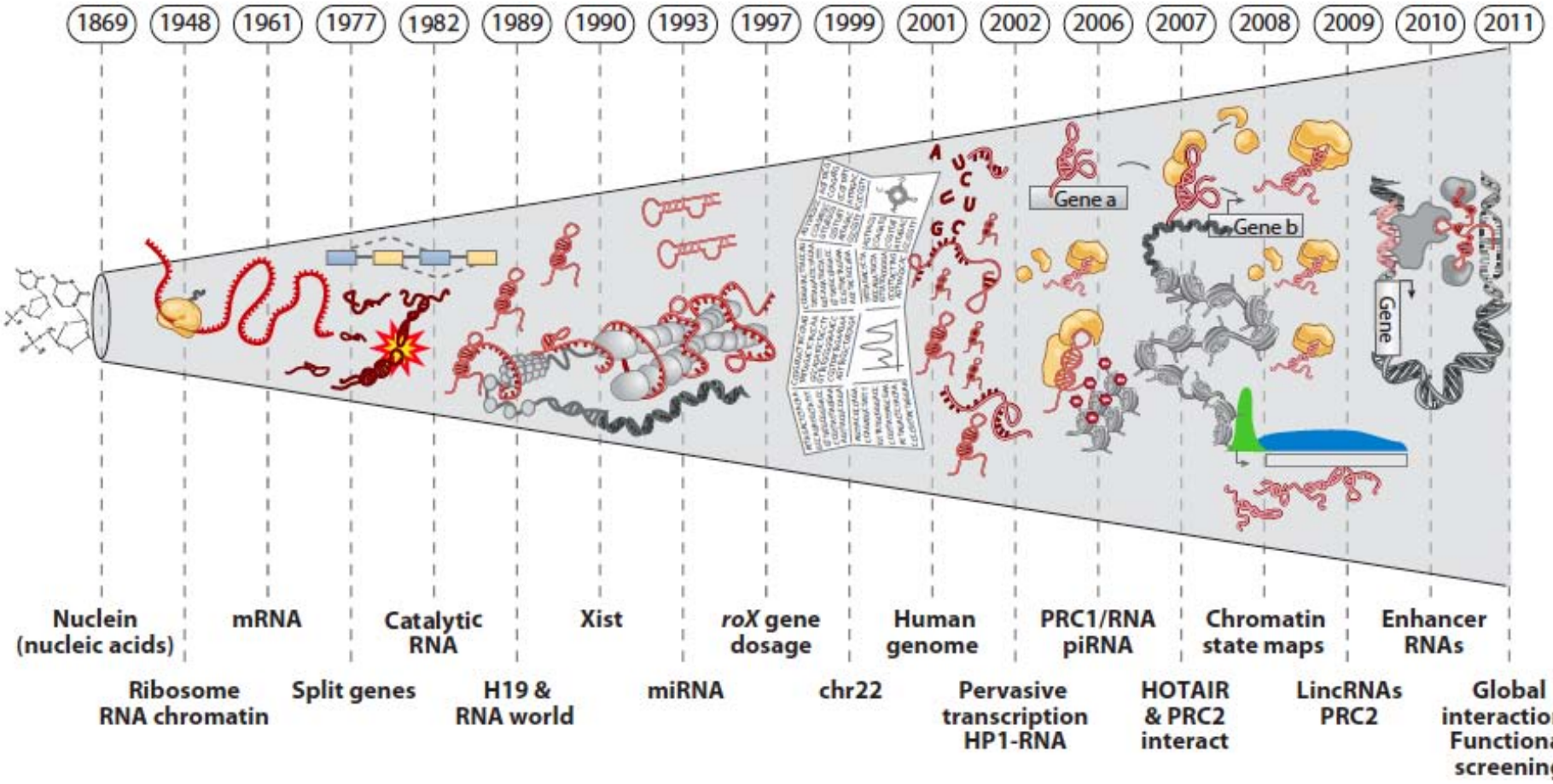
All RNAs great and small





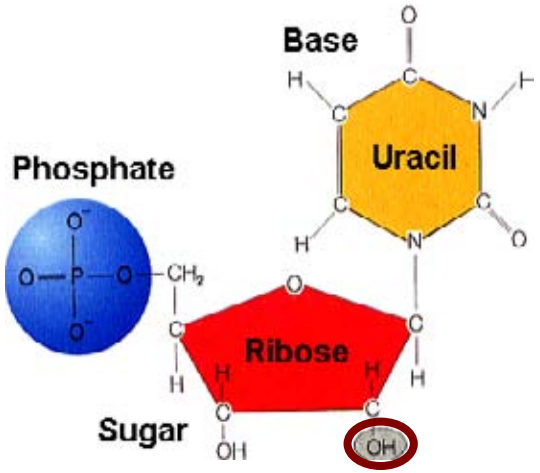
Institute of Genetics and Biotechnology
University of Warsaw

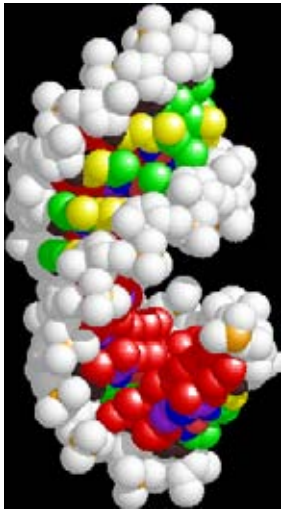
HISTORY OF RNA

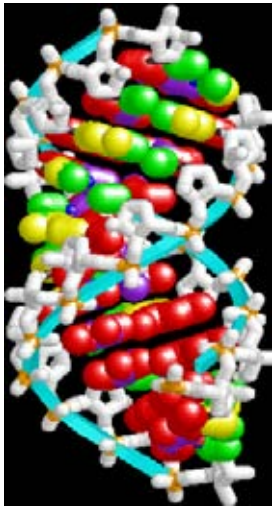


Rinn and Chang, Ann. Rev. Biochem, 2012

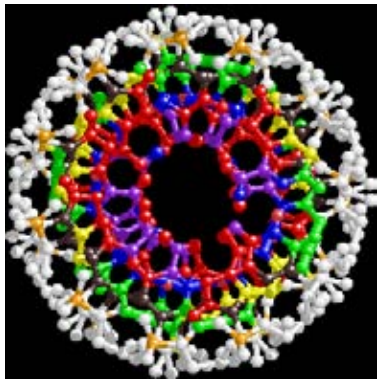
RNA – *aka* My Favorite Molecule







RNA form A helix

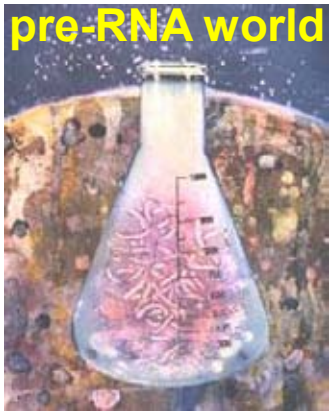


- **narrow inaccessible major groove** (red)
- **shallow minor groove** (green)

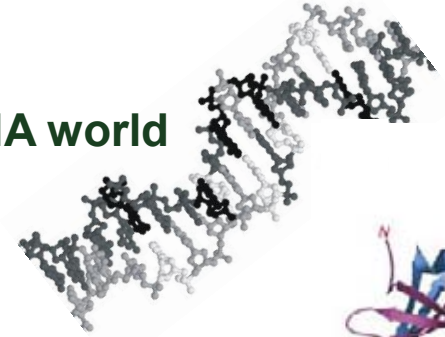
- **versatile and flexible**
- **catalytically active (splicing, translation, modification)**
- **self-sufficient?**
- **labile (regulation of expression)**
- **create complex 3D structures**
- **specific and unspecific interactions with proteins and other RNAs**

„THE RNA WORLD" hypothesis

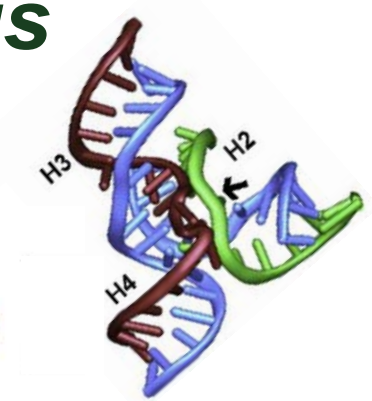
„primordial soup"
„prebiotic soup"



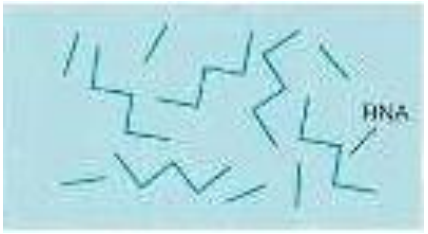
RNA world



RNA+proteins

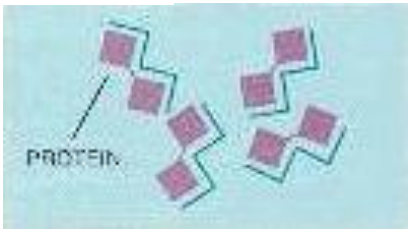


RNA+DNA+ proteins

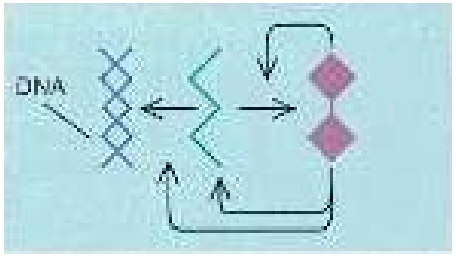


RNA made via condensation from ribose and other organic substances

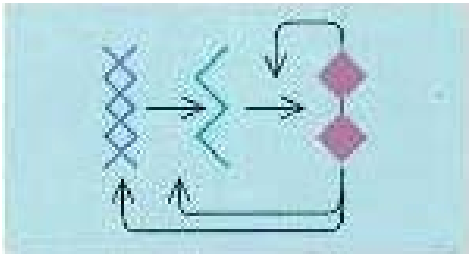
RNA evolution- molecules learns to replicate



RNA starts to join aminoacids and synthesises polypeptides and proteins



Proteins aid RNA to replicate and make proteins. dsRNA evolves into stable DNA.



DNA and proteins take over major roles as genetic information and enzymes

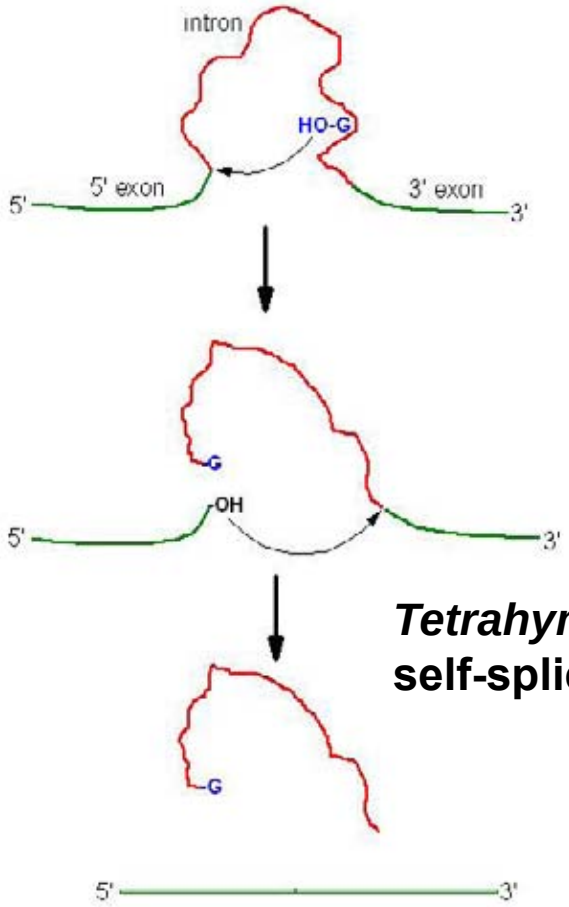
RNA capacity - CATALYTIC RNAs

Nobel 1989

RNA enzymes **– RIBOZYMES**

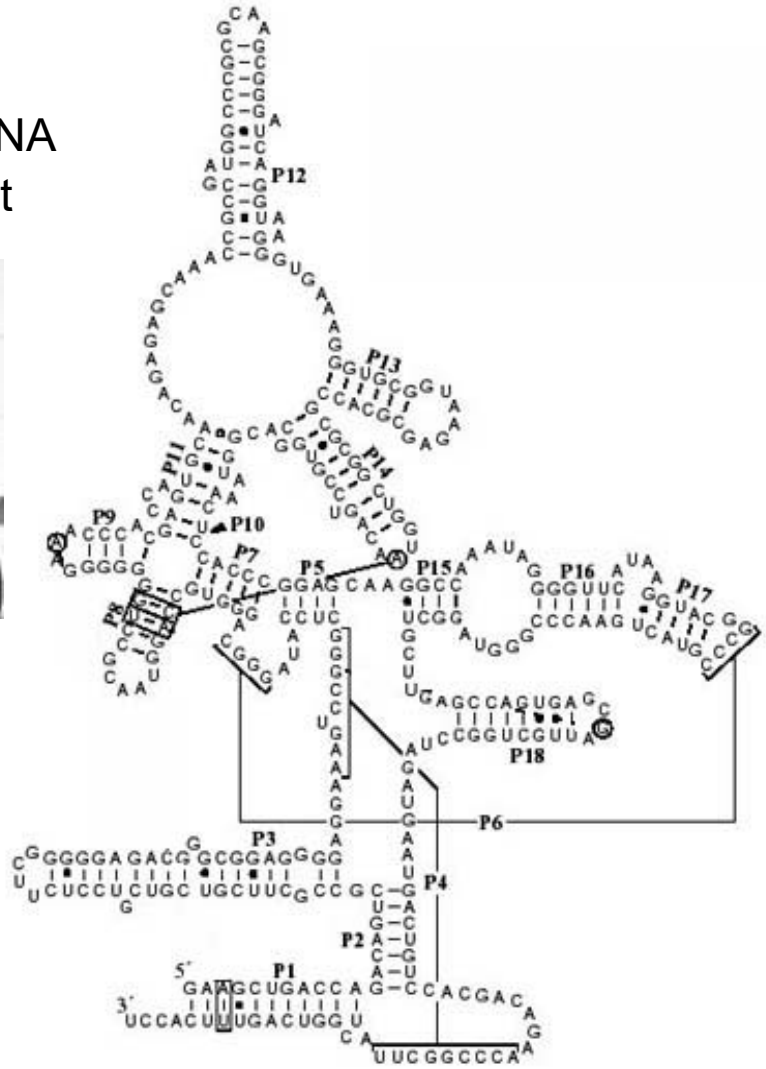
-1981/82 Tom Cech - self-splicing in *Tetrahymena* rRNA

-1982 Sidney Altman - bacterial RNaseP RNA subunit

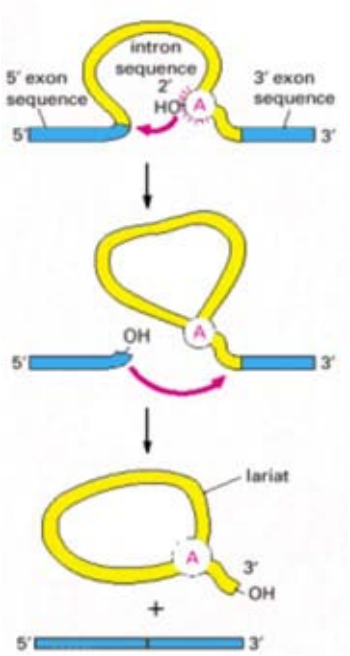


Thomas Cech
Sidney Altman

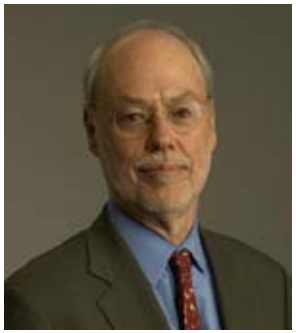
***Tetrahymena* group I self-splicing intron**



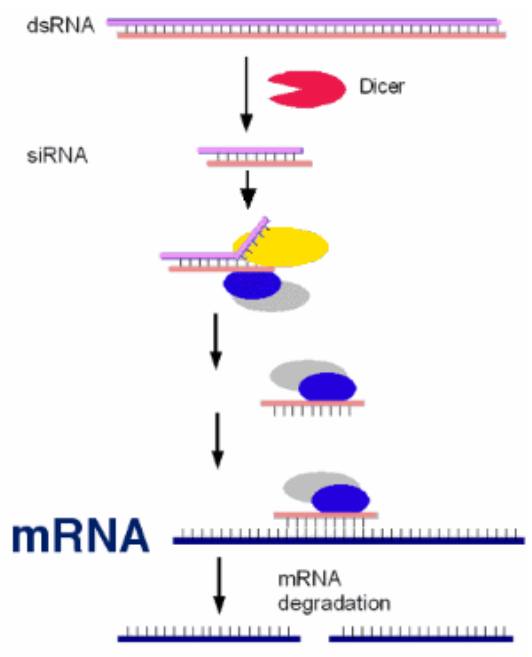
***Escherichia coli* RNaseP RNA**



mRNA SPLICING Nobel 1993



Phil Sharp
Richard Roberts



RNAi Nobel 2006

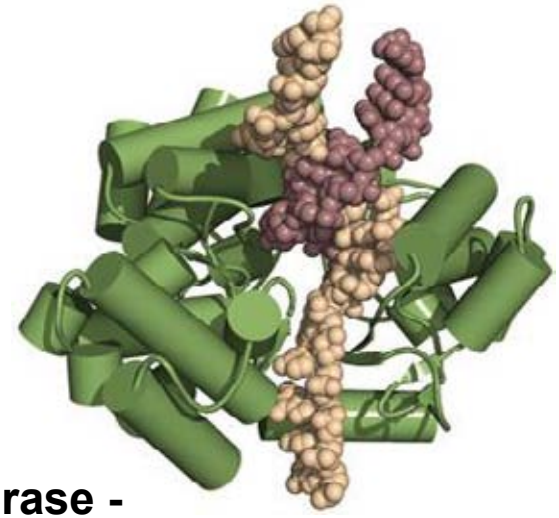
Andrew Fire
Craig Mello

RNAs – STRUCTURE AND FUNCTION

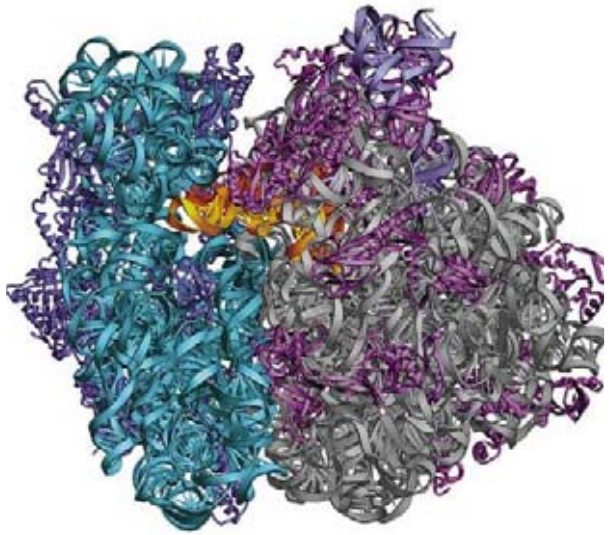
Nobel 2009

Elizabeth Blackburn
Jack Szostak
Carol Greider

Venkatraman Ramakrishnan
Ada Yonath
Thomas Steitz



Telomerase -
maintaing chromosome ends



Crystal structure of the ribosome

RNPs - STRUCTURE/METHODOLOGY

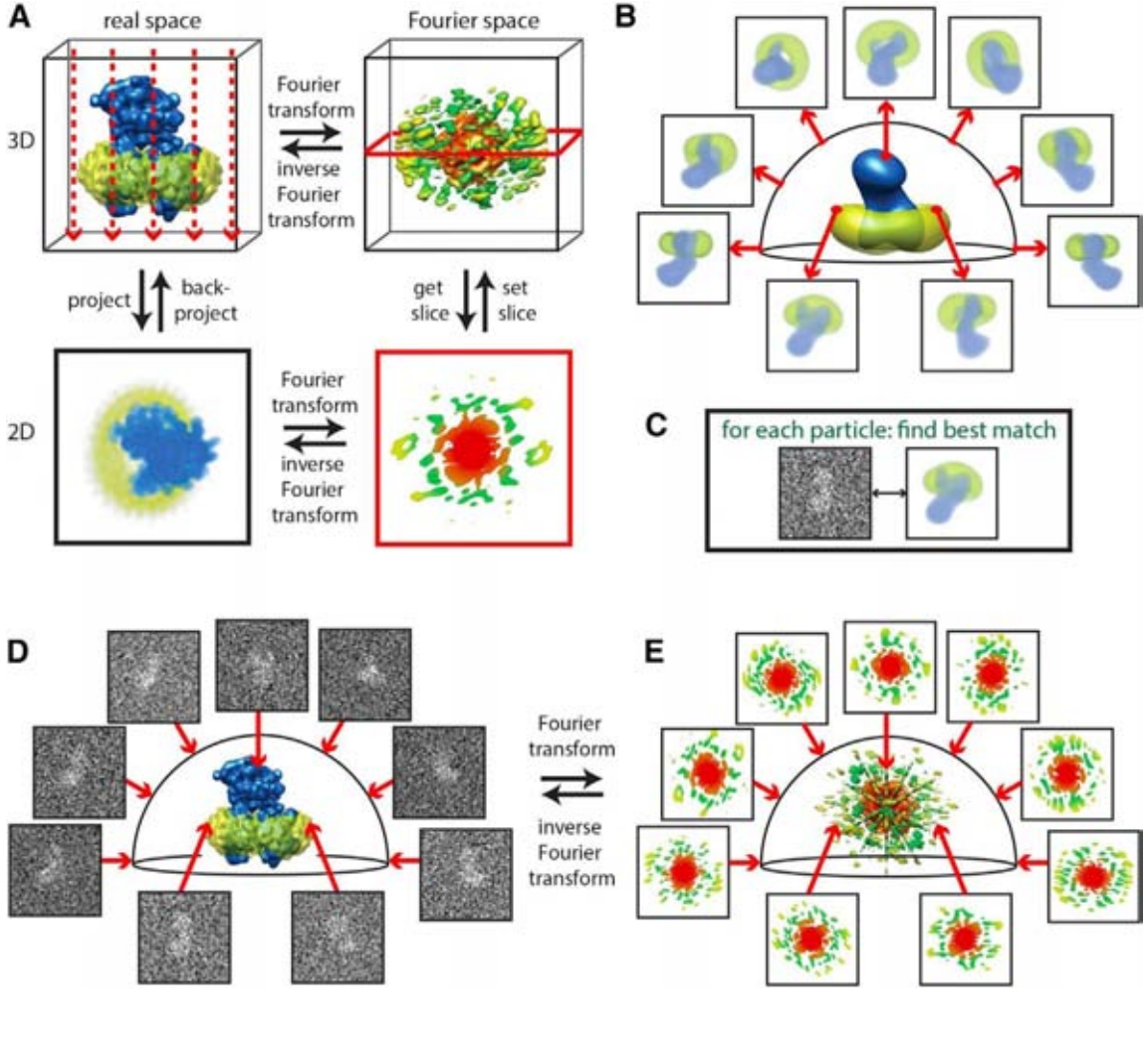
Jacques Dubochet

Joachim Frank

Richard Henderson

Nobel 2017

CRYO-EM

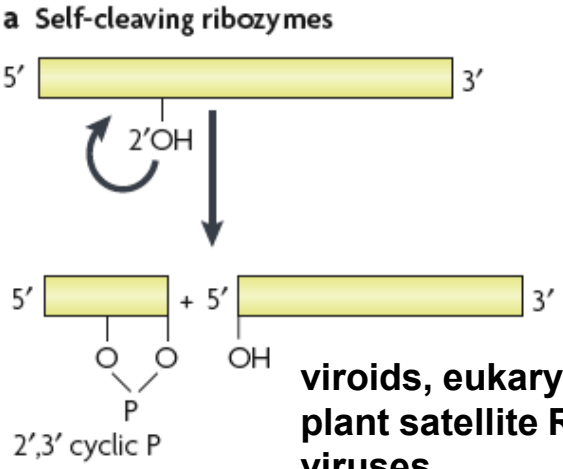


Nogales and Scheres, Mol Cell 2015

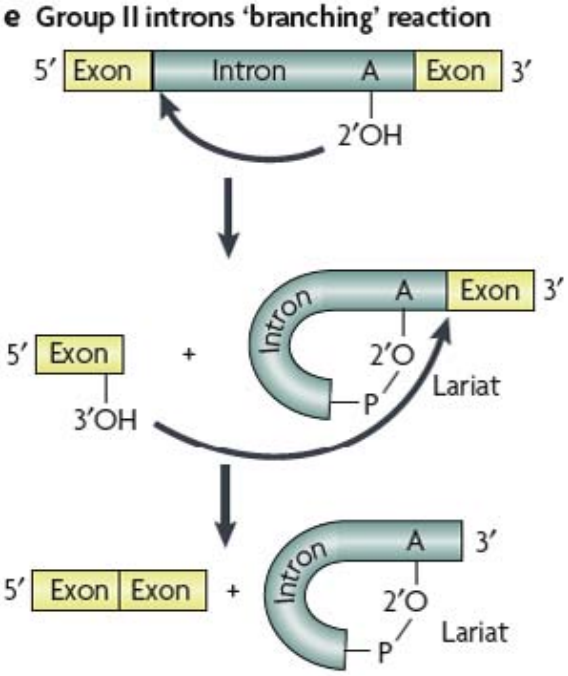
Lecture on crystallography and CryoEM by Marcin Nowotn

RIBOZYMES

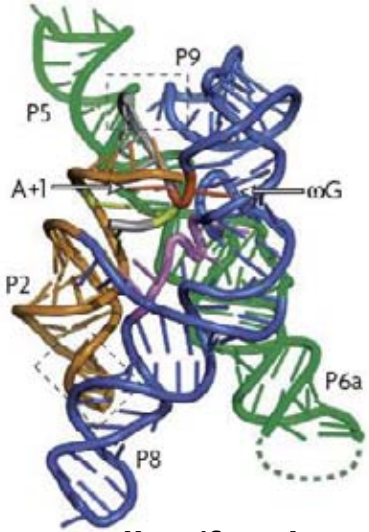
Hammerhead, Hairpin, HDV



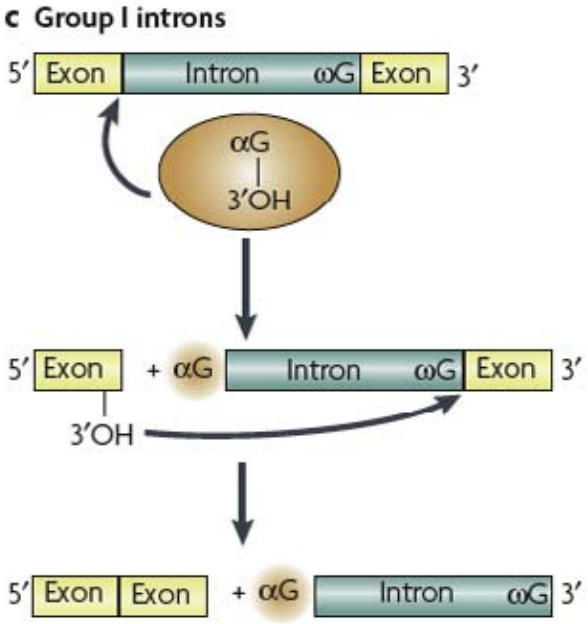
viroids, eukaryotes plant satellite RNA, viruses

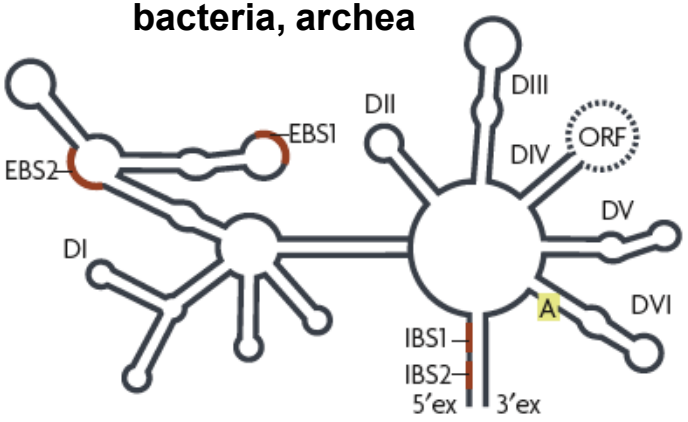


mRNA splicing-like organelles (fungi, plants), bacteria, archea



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





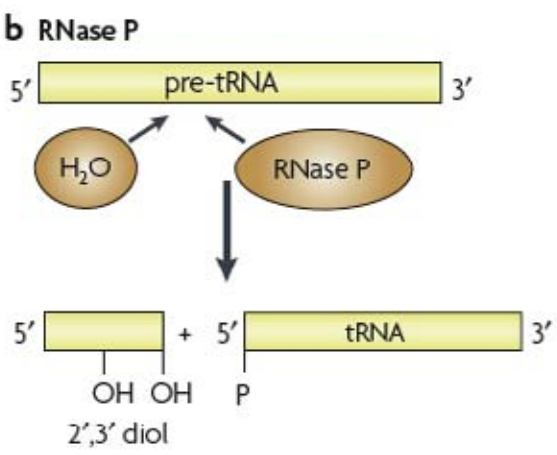
Serganov and Patel, *Nat. Rev. Genet.*, 2007

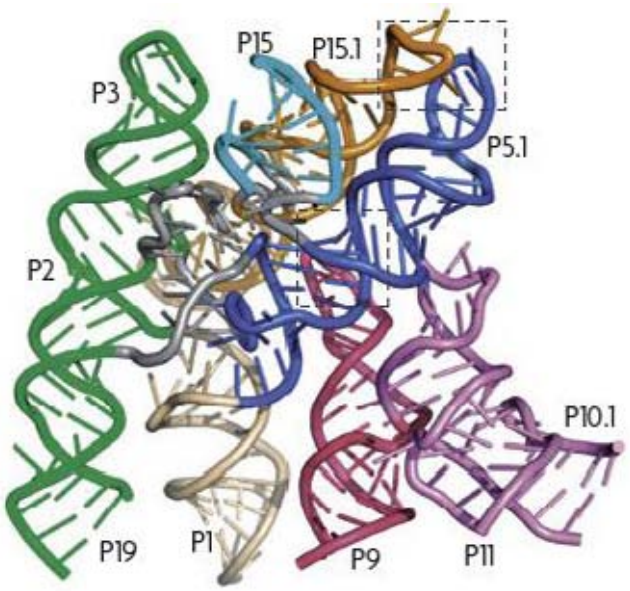
Mechanism: nucleophilic attack of the ribose -OH group (H_2O, Me^{2+}) on the phosphate

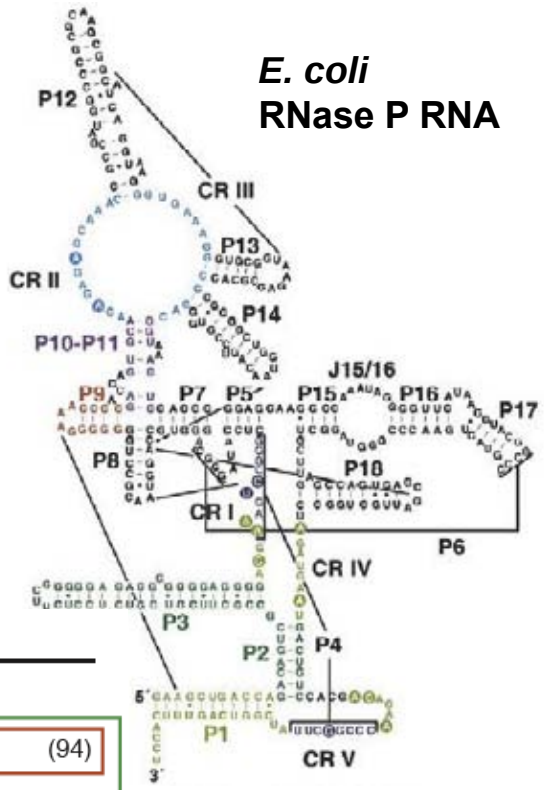
RNase P RNA – a true enzyme

tRNA processing, multiple turnover

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







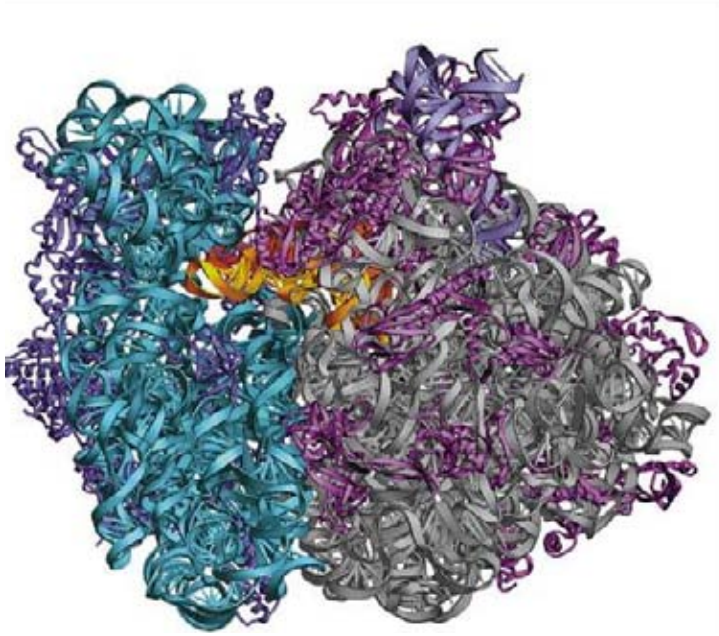
E. coli
RNase P RNA

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)						

MODERN RNA WORLD

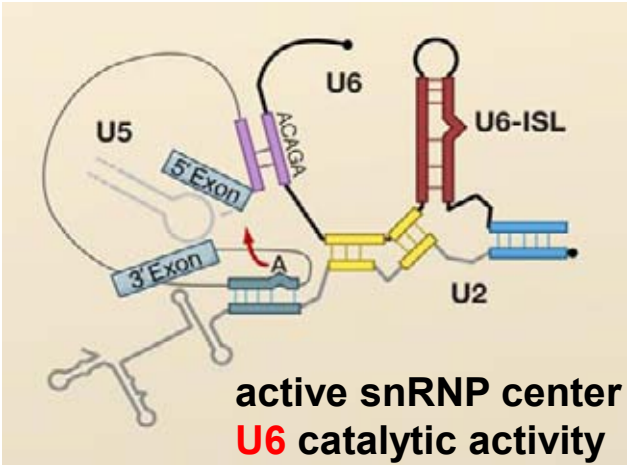
RNA vestiges- catalytic RNAs with active centres made of RNA

RIBOSOME - protein synthesis

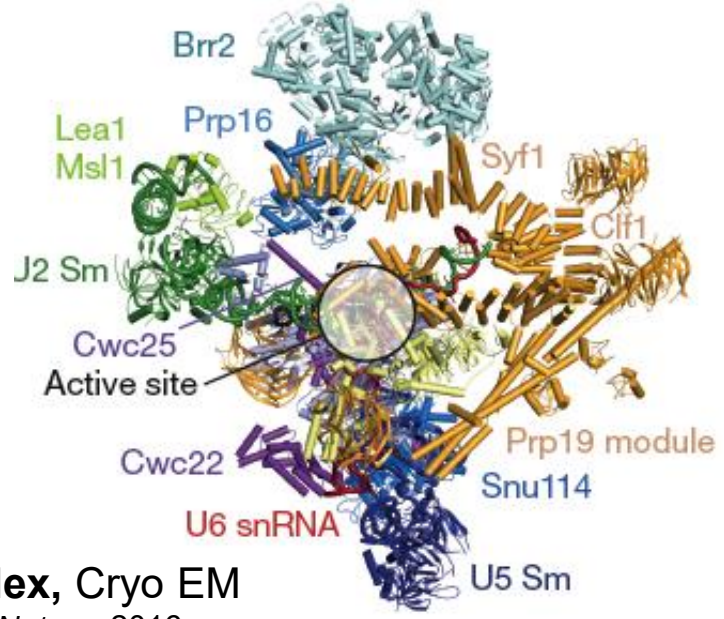


Ribosome, crystal structure
Cryo EM
Ditlev Brodersen, Venki Ramakrishnan

SPLICEOSOME - pre-mRNA splicing

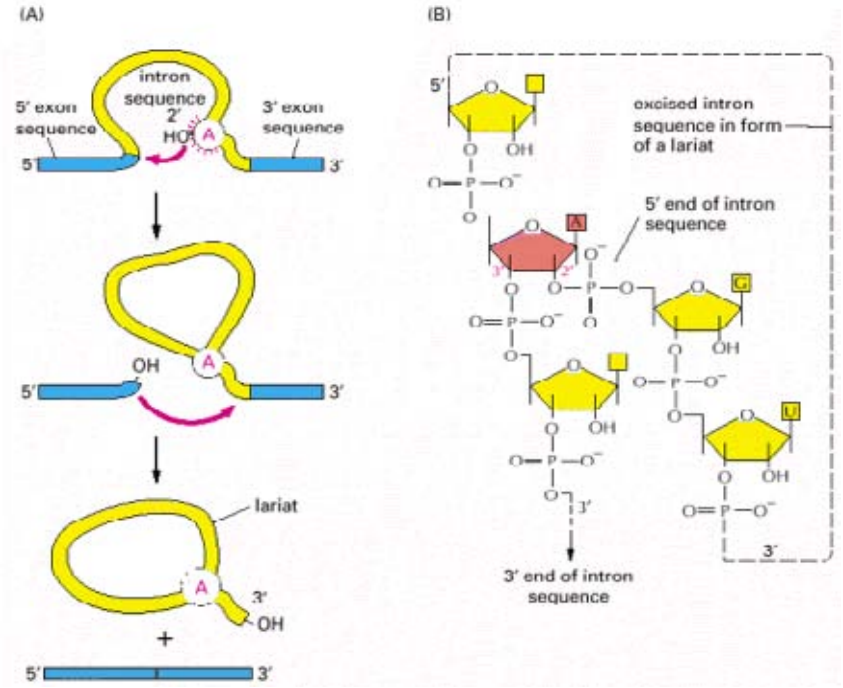


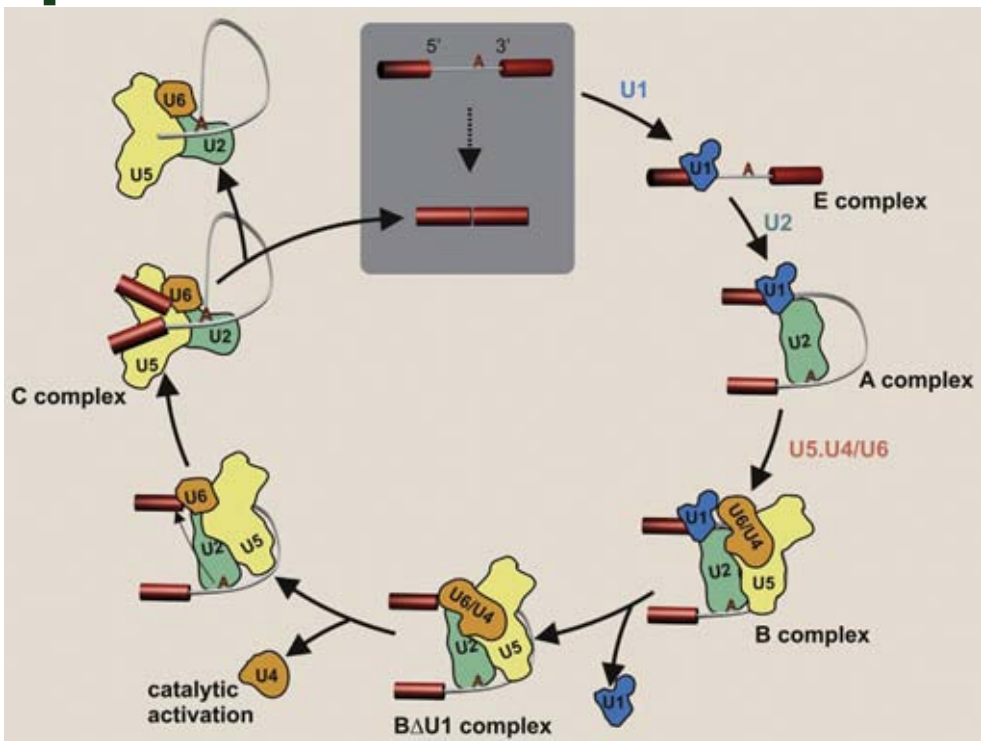
5 snRNAs
U1, U2, U4, U5, U6



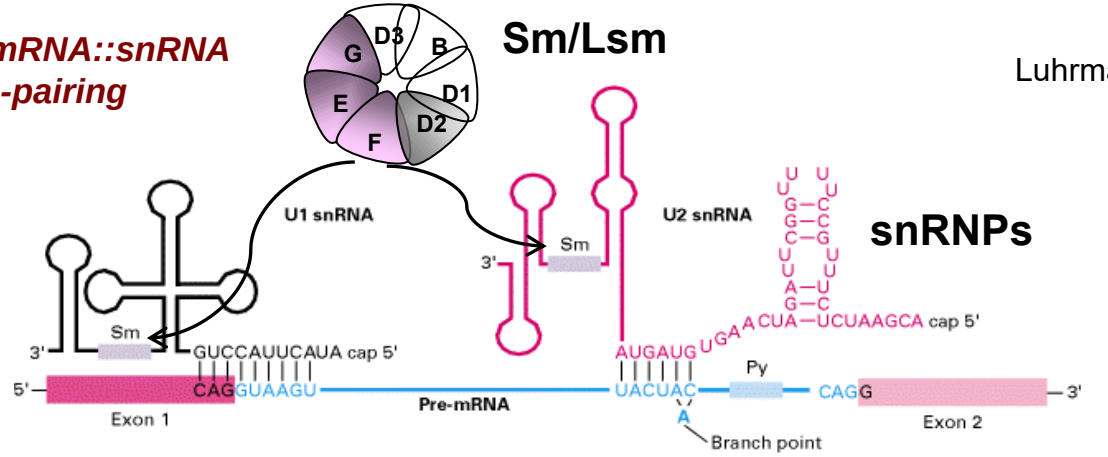
C complex, Cryo EM
Galej *et al, Nature,* 2016

SPLICEOSOME: pre-mRNA SPLICING





pre-mRNA::snRNA base-pairing

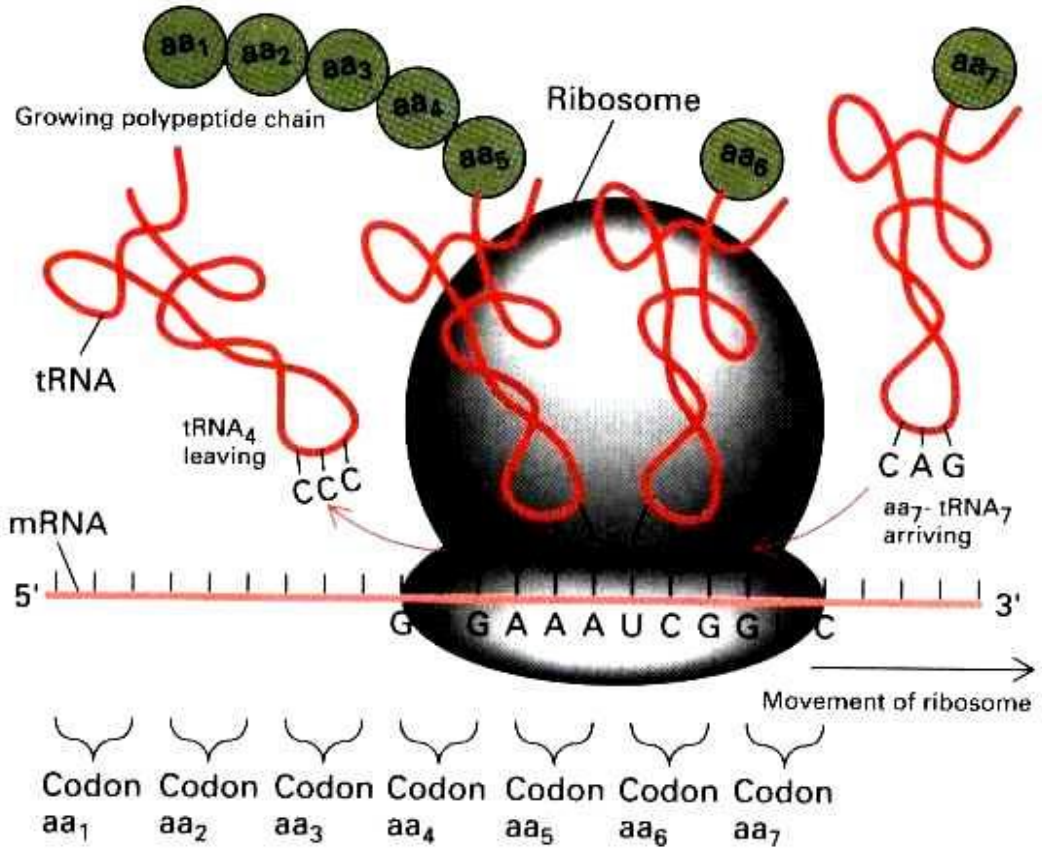


Luhrmann and Stark, *Curr. Op. Str. Biol.*, 2009

SPLICEOSOME -ribonucleoprotein complex (RNP) organised around snRNAs

RIBOSOME: TRANSLATION

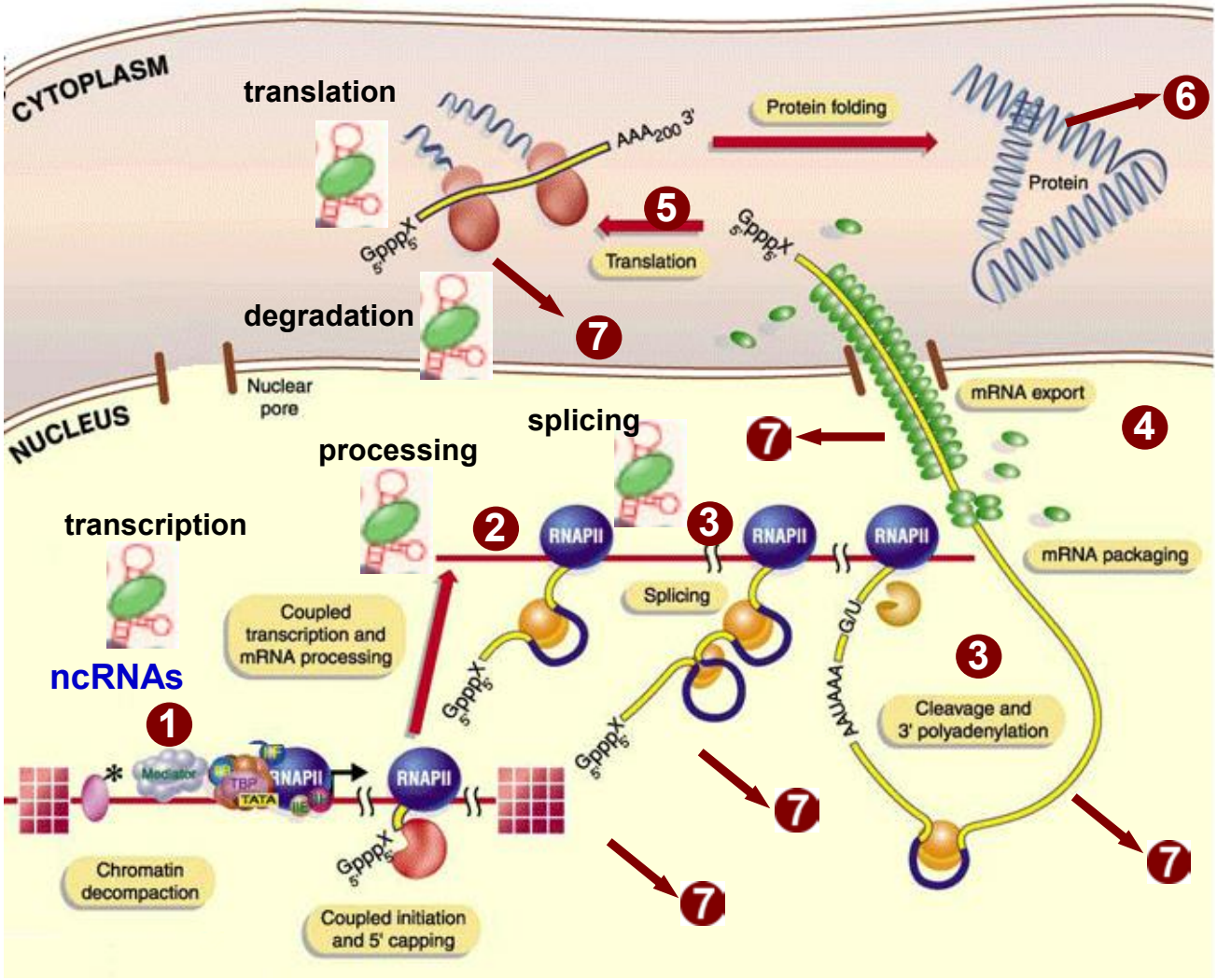
lecture by Marcin Nowotny



- **mRNA** - messenger, informative
- **tRNA** - transfer, transport of aminoacids
- **rRNA** - ribosome, translation machinery

Lecture on translation by Michał Świrski

REGULATION OF GENE EXPRESSION



1) chromatin

2) transcription

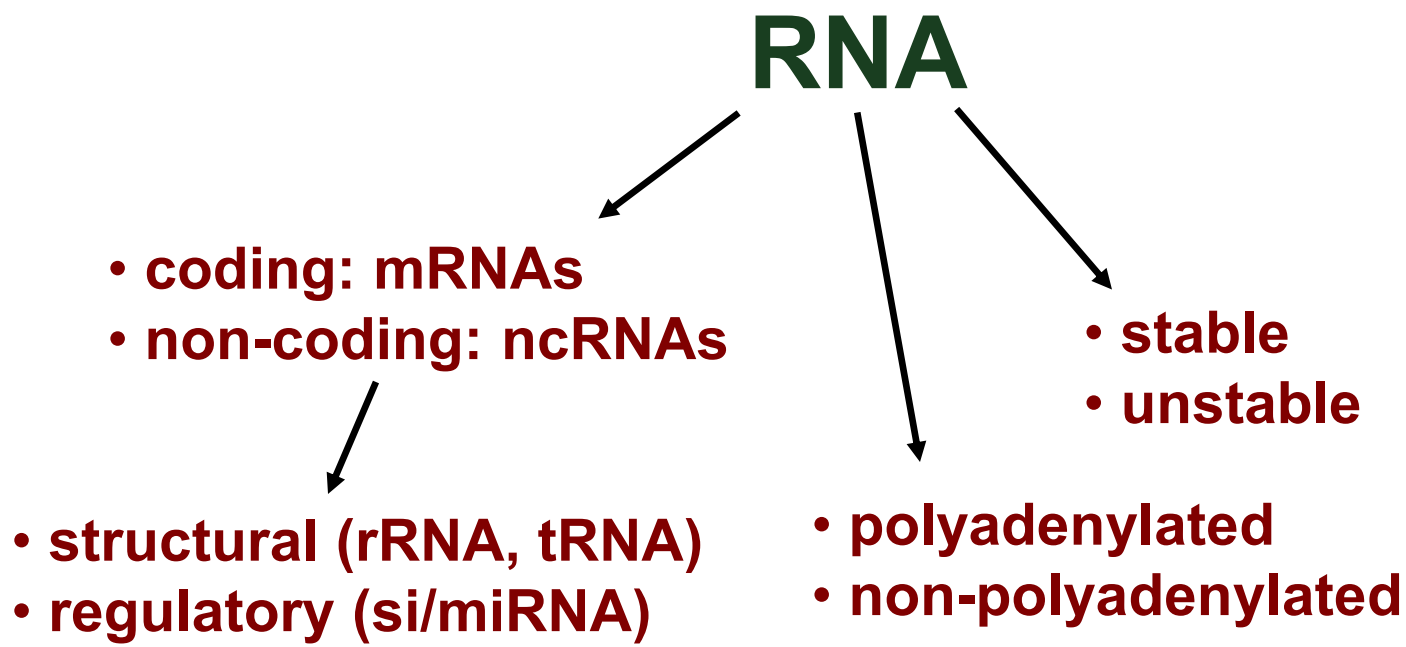
3) RNA processing

4) RNA export

5) translation (mRNA)

6) protein stability

7) RNA degradation



There are no „free" RNAs in the cell

All cellular RNAs exist as ribonucleoprotein particles (RNPs)

All RNA types are synthesised as precursors and undergo processing

RNA transcription, processing and decay are tightly coordinated

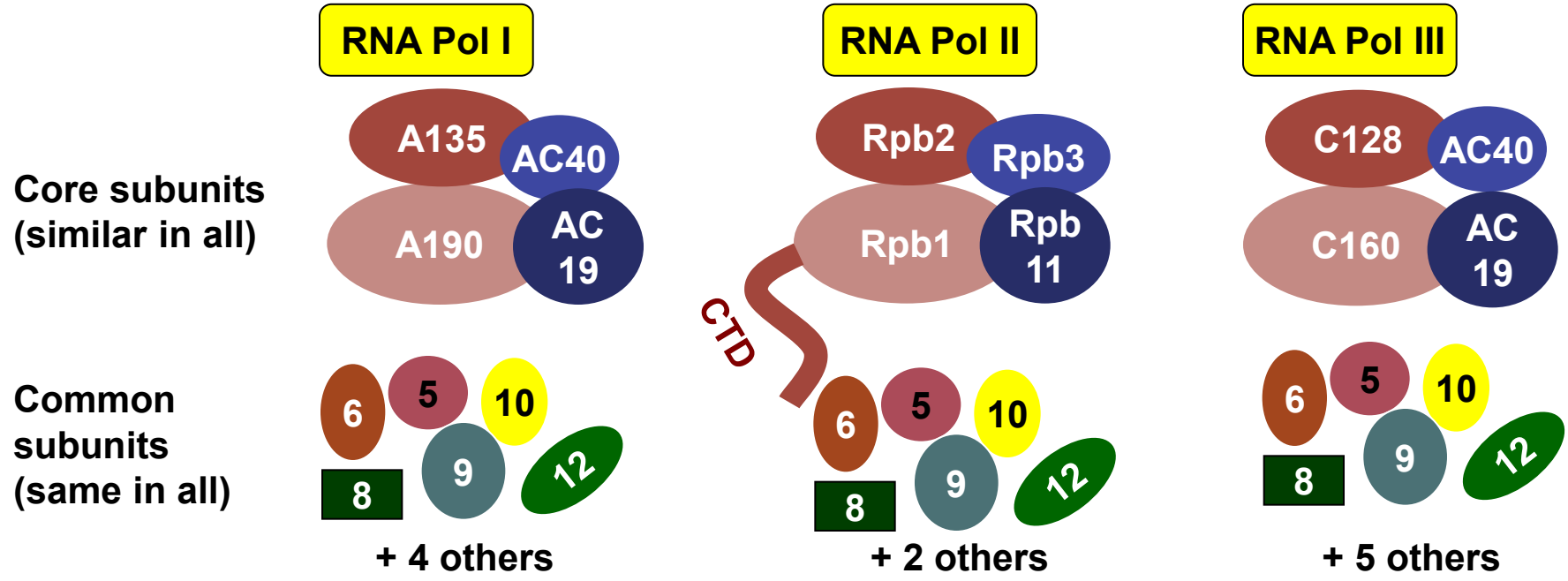
Several RNA processing steps occur co-transcriptionally

Regulation of RNA biogenesis involves alternative processes:

aTSS, aTIS, AS, APA

Lecture on ncRNAs by Monika Zakrzewska-Płaczek

TRANSCRIPTION



Zbigniew Dominski, *lectures* 2008

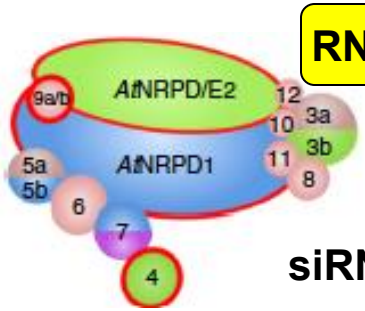
RNAs:

ribosomal RNA
35S precursor contains
18S, 5.8S and 25S rRNAs

mRNA , most snRNAs
(U1, U2, U3, U4, U5,
U11, U12, U4atac),
snoRNAs, microRNAs,
telomerase RNA

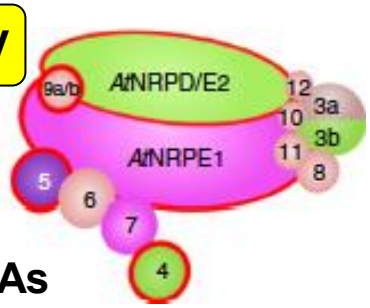
tRNA, 5S rRNA, U6
snRNA, U6atac snRNA,
7SK RNA, 7SL RNA,
RNase P RNA,
RNase MRP RNA

Additional plant Polymerases



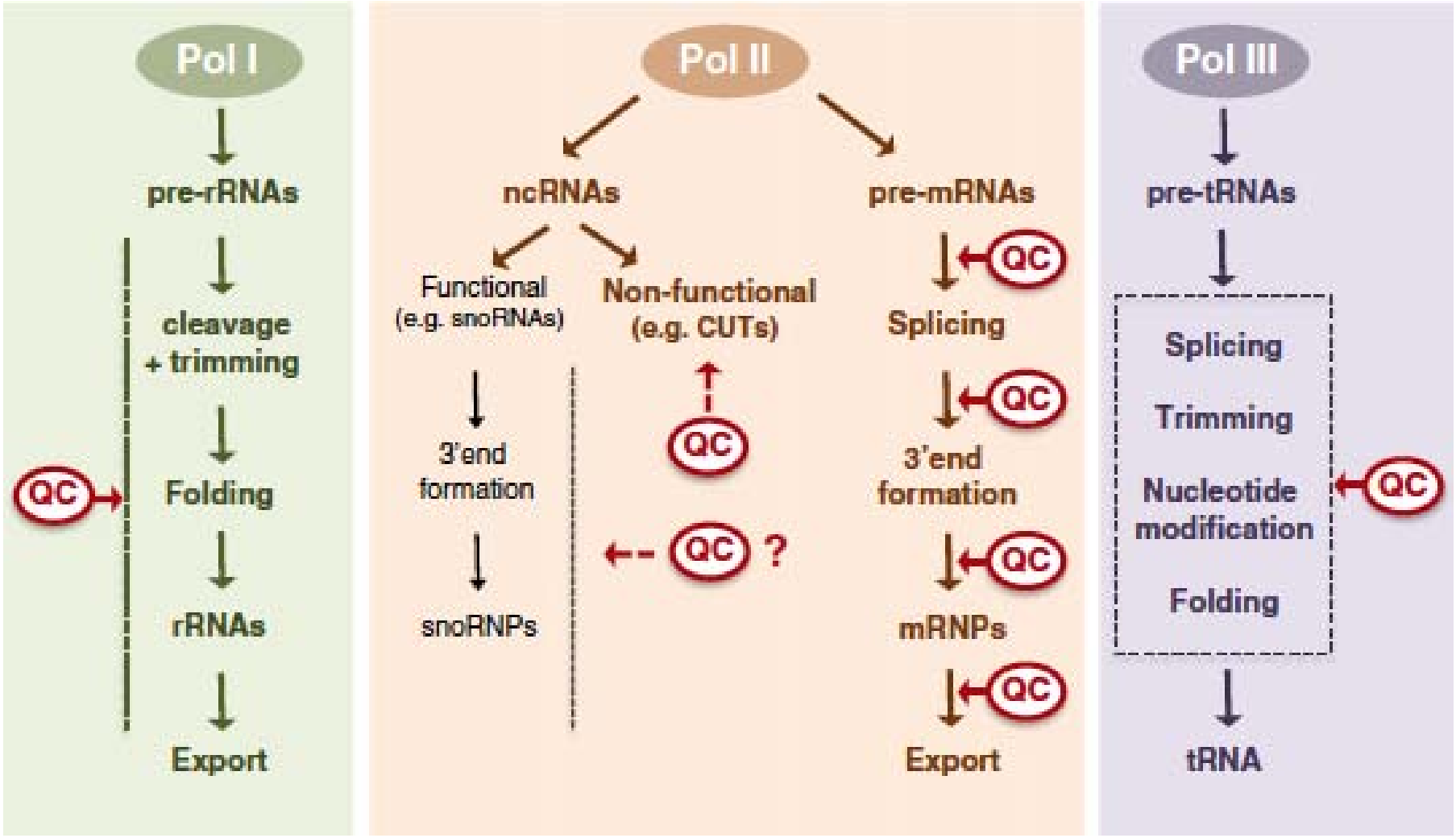
Involved in transcriptional gene silencing

siRNAs



lncRNAs

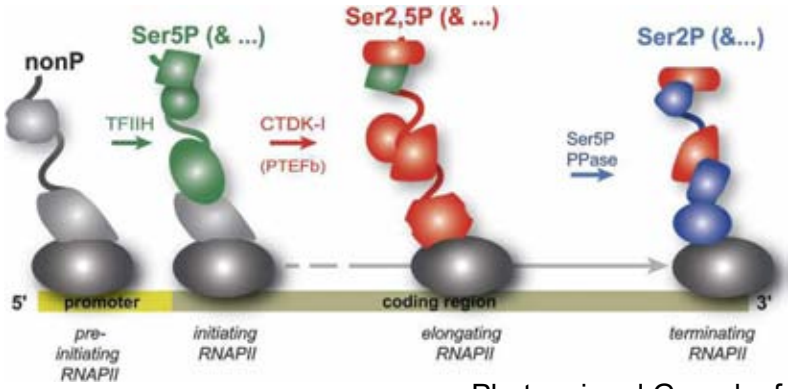
Pol I, II, III - comparison



Porrua and Libri, BBA, 2013

CO-TRANSCRIPTIONAL PROCESSES: Pol II CTD

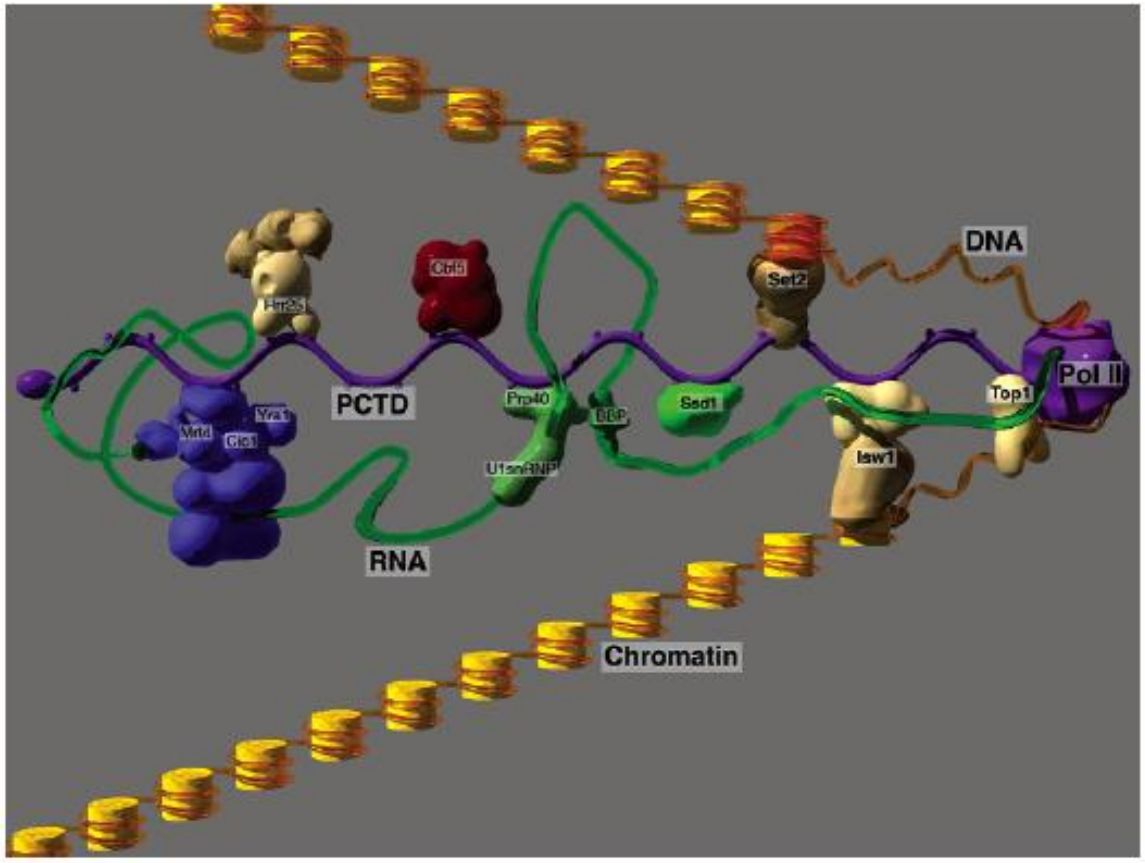
CTD posphorylation status



Phatnani and Greenleaf, *2006*

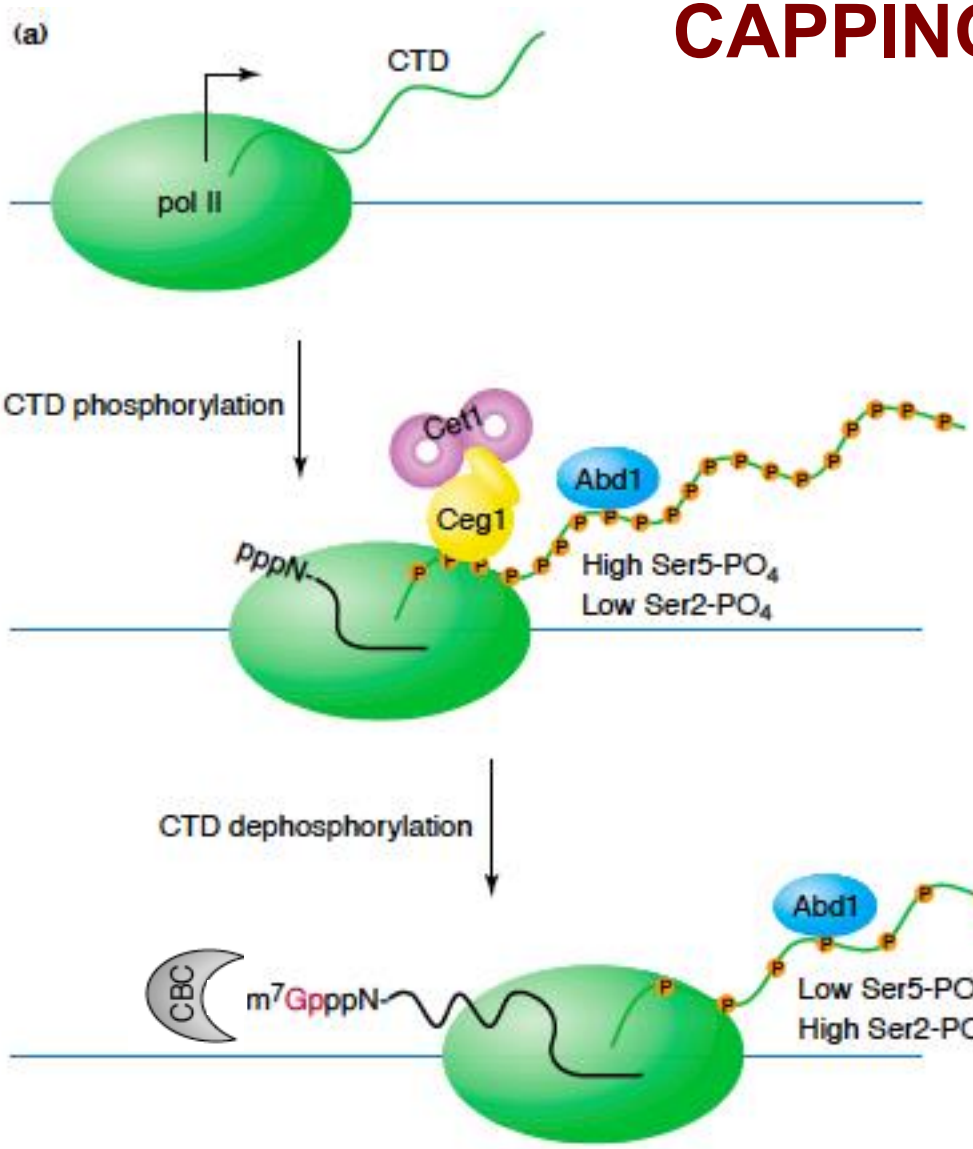
Phospho-CTD Associated Proteins

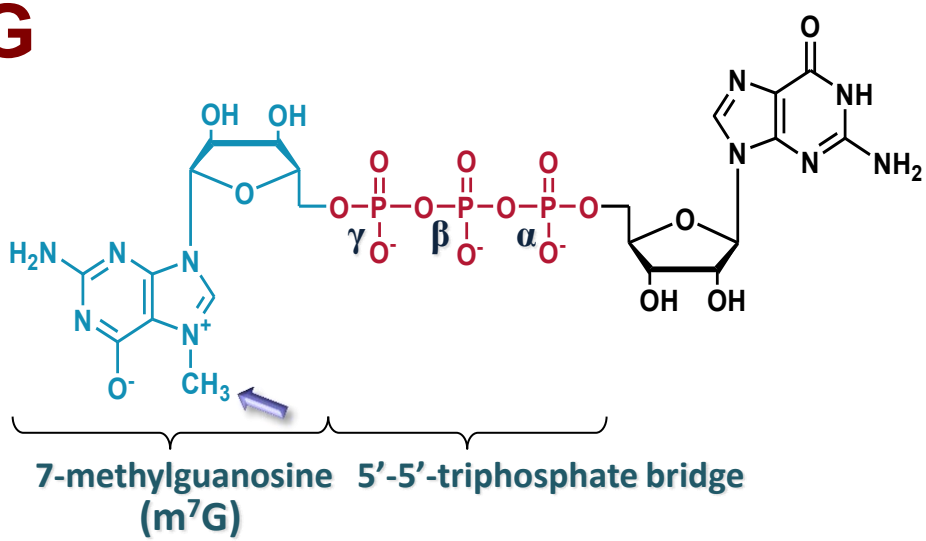
- transcription
- chromatin structure
- RNA processing (splicing, 3' end formation)
- RNA export
- RNA degradation
- snRNA modification
- snoRNP biogenesis
- DNA metabolism
- protein synthesis and degradation



CO-TRANSCRIPTIONAL PROCESSES

CAPPING





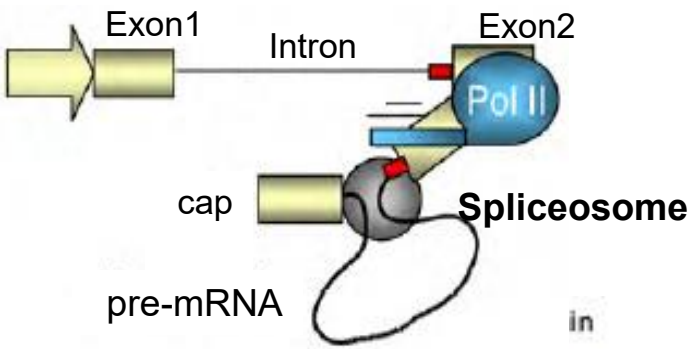
Co-transcriptional capping
- occurs after the synthesis of 10-15 nt of RNA
- CE recruitment to CTD requires high Ser5-P

GT/Ceg1-guanylyltransferase
MT/Abd1-methyltransferase
(promote early elongation)
Cet1-RNA triphopshatase
(inhibits re-initiation)
CBC-cap binding complex

Guo and Lima, Cur. Op.Str.Biol., 2005

CO-TRANSCRIPTIONAL PROCESSES

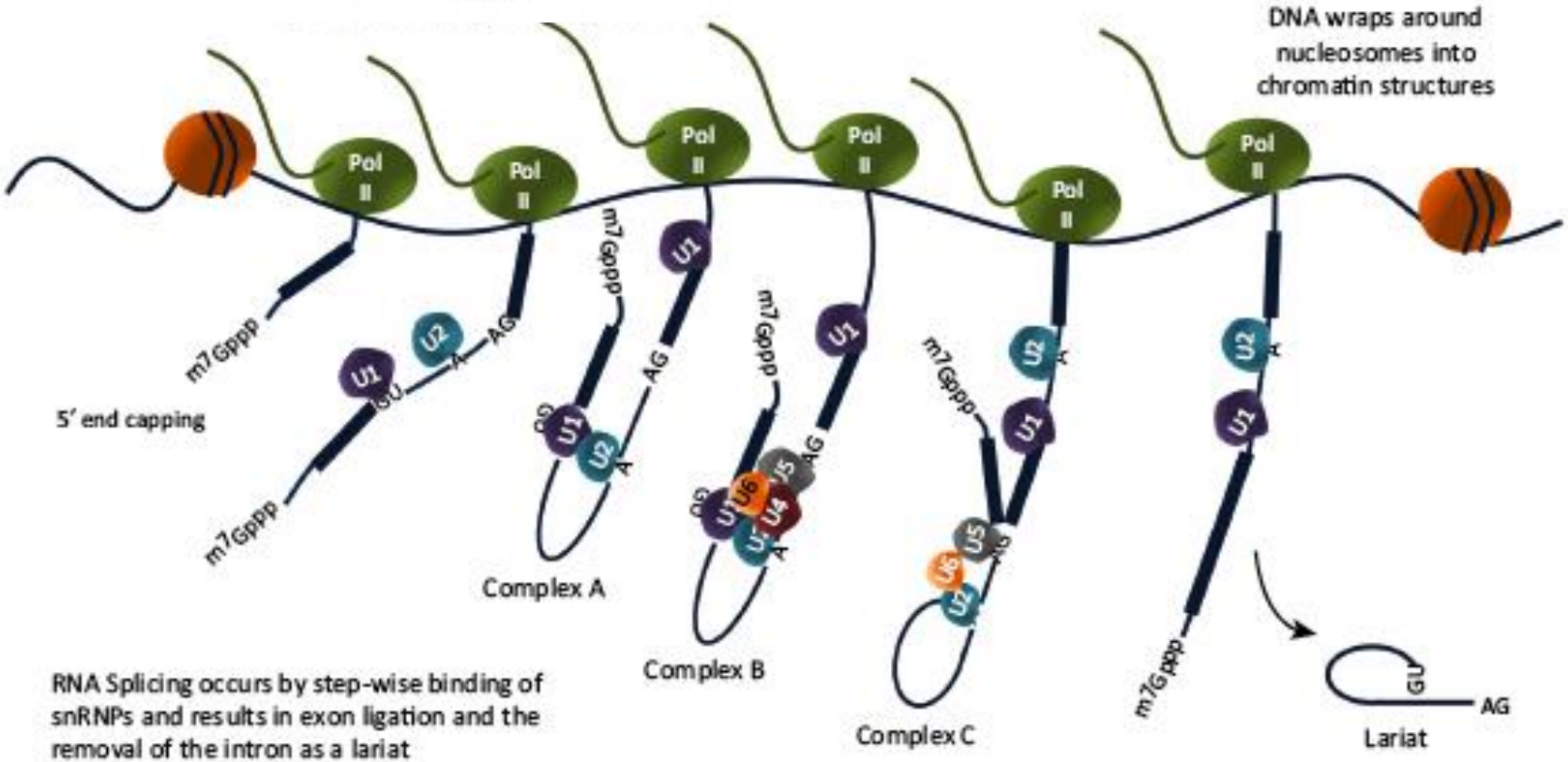
SPLICING



- spliceosome assembly (Ser5-P)
- majority of splicing (up to 70-80%)

Munoz *et al.*, *TiBS*, 2009

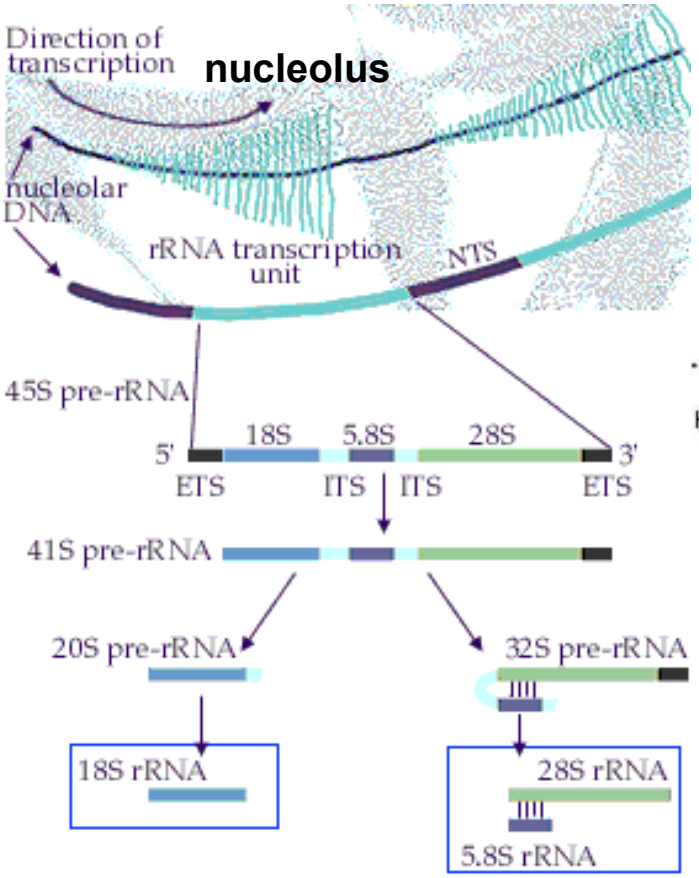
in



Wong *et al.*, *TiG*, 2014

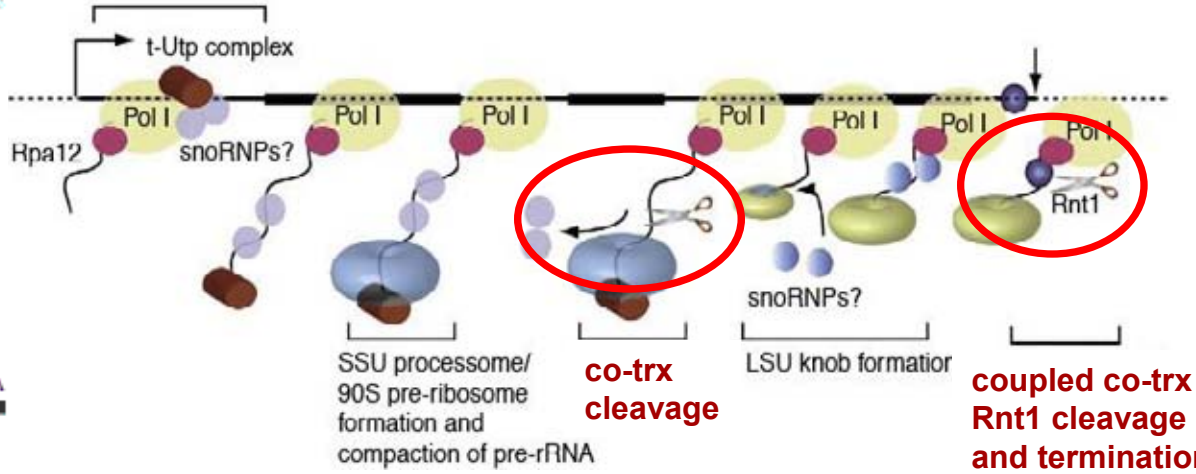
CO-TRANSCRIPTIONAL PROCESSES

PRE-rRNA PROCESSING AND MODIFCATION

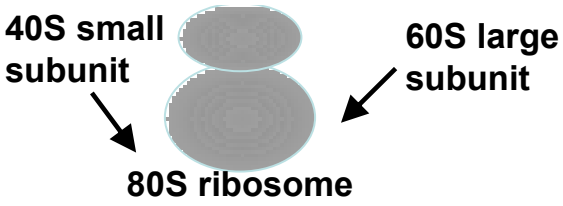


70-80% of cellular transcription is for rRNA by Pol I

50% of Pol II transcription is for RP genes



Granemman and Baserga, Curr.Op.CellBiol., 2005;
Kos and Tollervey, Mol.Cell'10



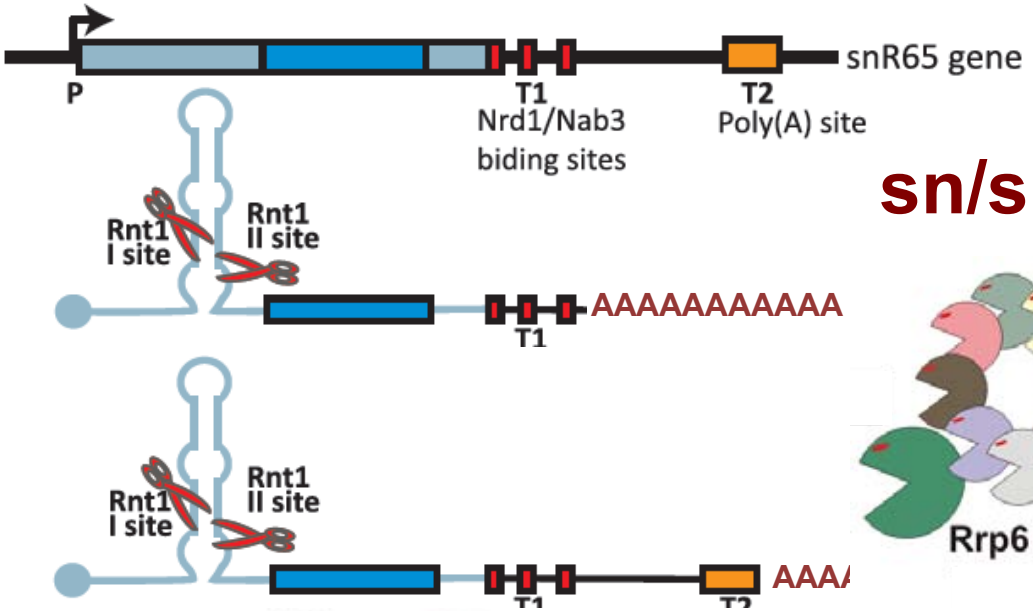
Co-transcriptional:

- association of SSU subcomplexes

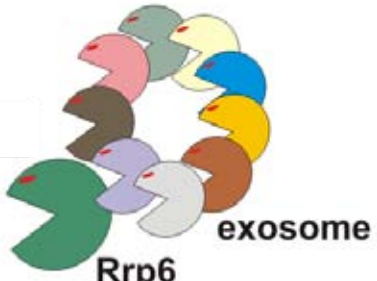
- pre-rRNA cleavages dividing small and large subunits (70%)

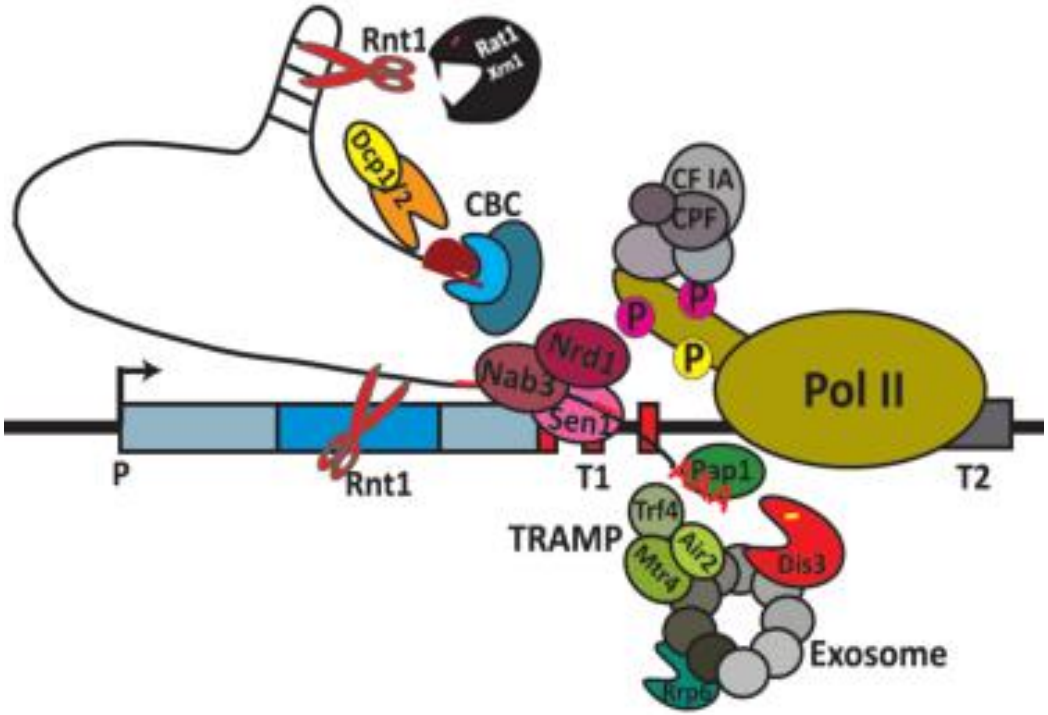
- ribose modification (2'-O-methylation)

CO-TRANSCRIPTIONAL PROCESSES



sn/snoRNA PROCESSING





Exosome: 3' - 5' exo/endo-nuclease

- complex; 10 core components (RNA BP)
- catalytically active hydrolytic Dis3/Rrp44 (RNase II)
- nuclear cofactors- RNA BP Rrp47, nuclease Rrp6 (RNase D), RNA helicase Mtr4
- cytoplasmic cofactors- Ski2-3-8 complex (RNA helicase Ski2), GTPase Ski7
- subtrates- processing and/or degradation of almost all RNAs

TRAMP: nuclear surveillance

Trf4/5	+	Air1/2	+	Mtr4
poly(A) polymerase		RNA binding proteins		RNA DEVH helicase

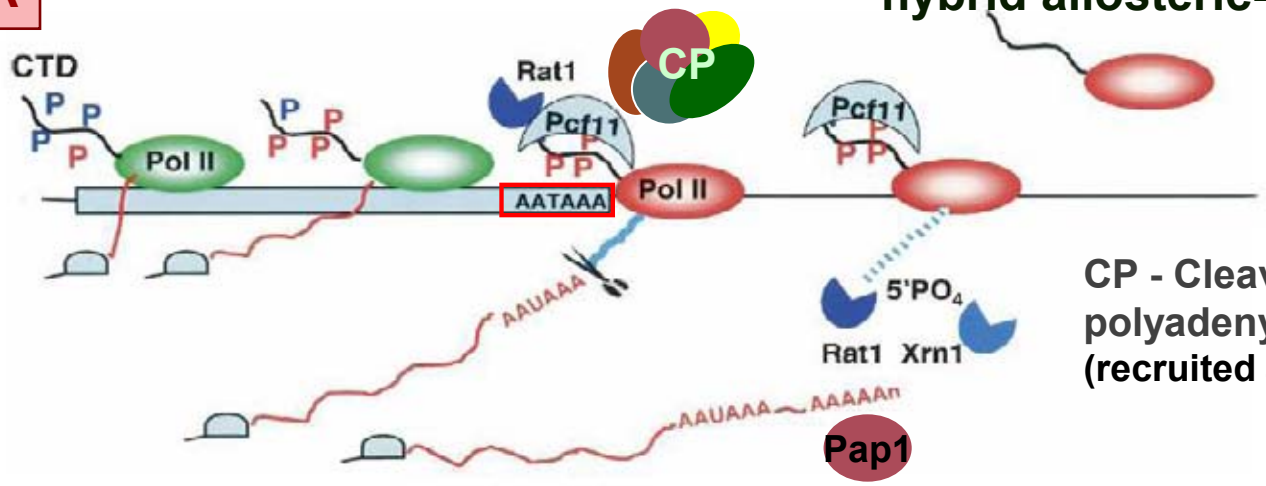
CO-TRANSCRIPTIONAL PROCESSES

POL II TRANSCRIPTION TERMINATION

mRNA

ncRNA

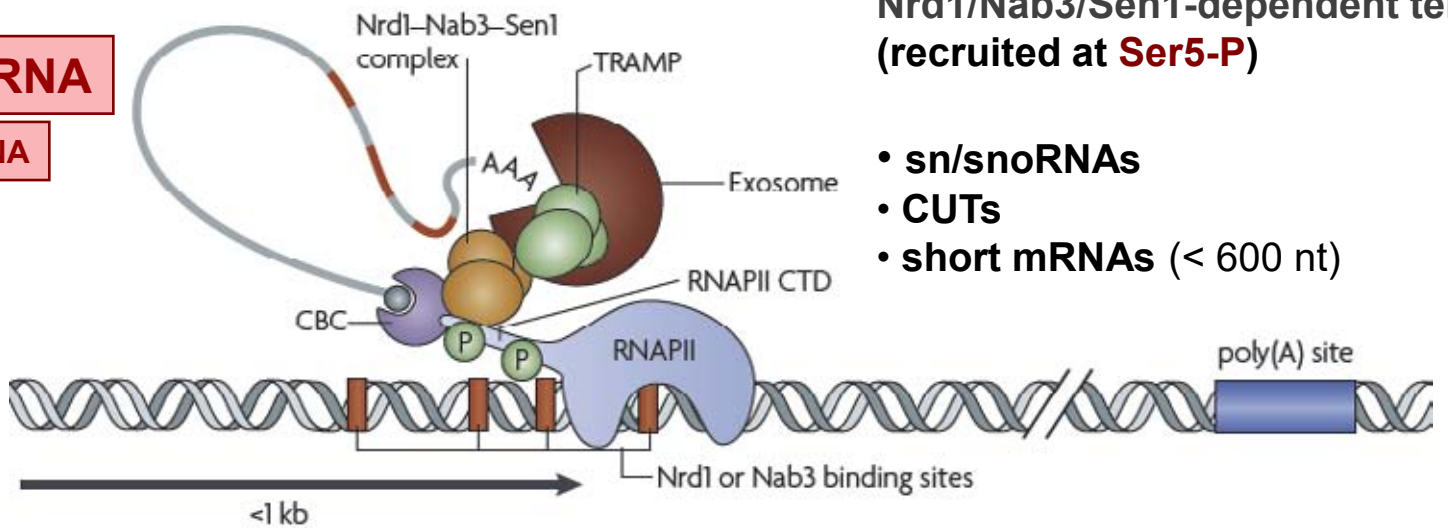
hybrid allosteric- torpedo model



CP - Cleavage and polyadenylation complex (recruited at Ser2-P CTD)

ncRNA

mRNA



Nrd1/Nab3/Sen1-dependent termination (recruited at Ser5-P)

- **sn/snoRNAs**
- **CUTs**
- **short mRNAs** (< 600 nt)

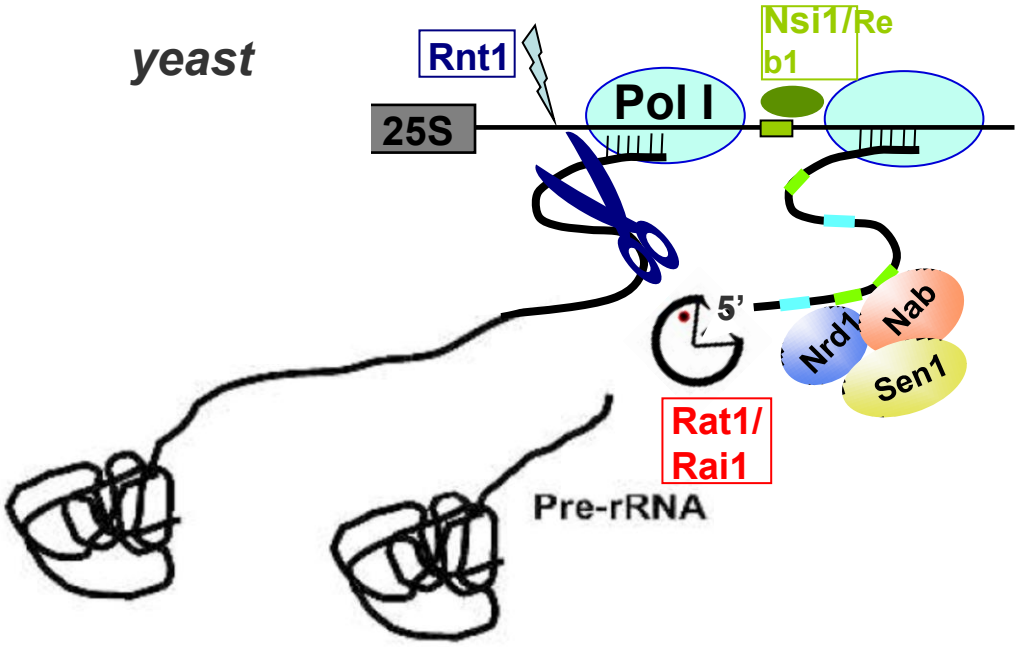
Jacquier, Nat. Rev. Genet 2009 *Luo and Bentley, Gene Dev, 2006*

Lecture on transcription termination by Michał Koper

CO-TRANSCRIPTIONAL PROCESSES

POL I TRANSCRIPTION TERMINATION

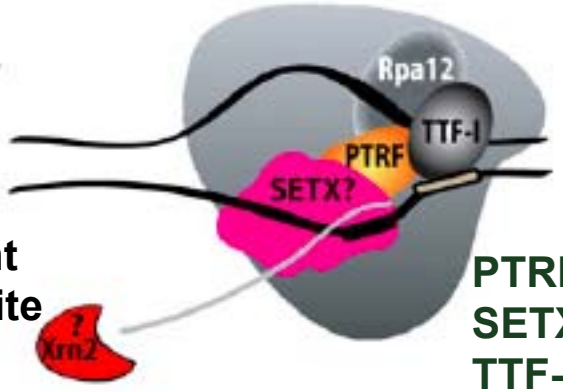
yeast



Pol I termination factors:

- DNA-binding protein Nsi1/Reb1
- Pol I subunit Rpa12
- endonuclease Rnt1
- RFB binding protein Fob1
- 5'-3' exonuclease Rat1/Rai1 *(torpedo mechanism)*
- RNA helicase Sen1
- Nrd1/Nab3 complex (??)

mammalian



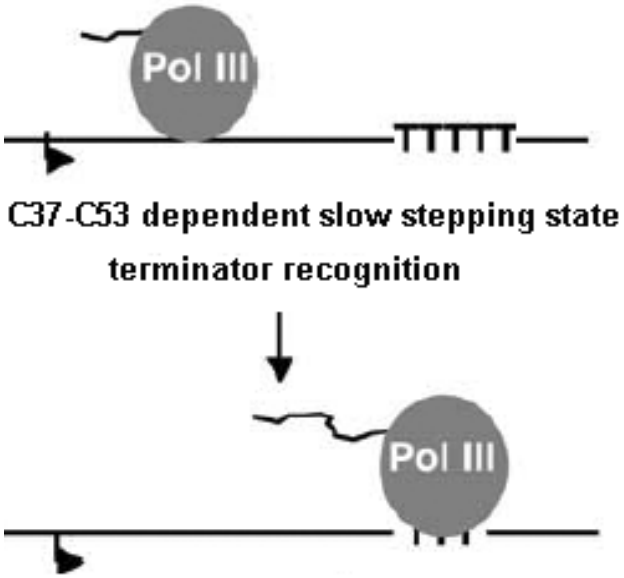
transcript release element
T-stretch + TTF-I pause site

PTRF – release factor
SETX – helicase, Sen1 homolog
TTF-I – transcription termination factor I

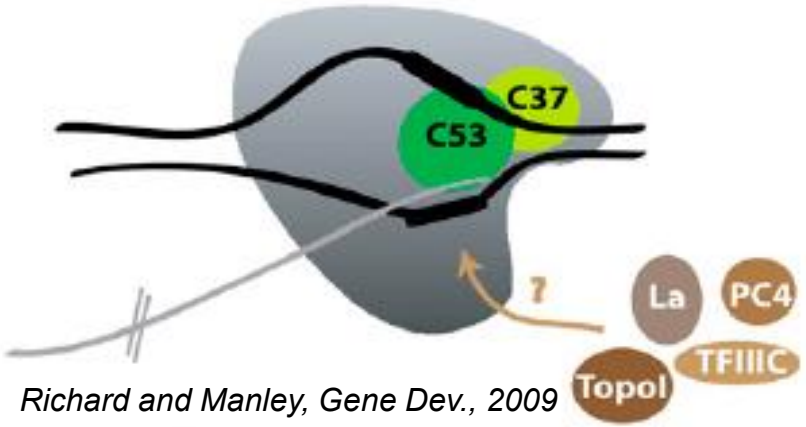
Richard and Manley, GeneDev., 2009

CO-TRANSCRIPTIONAL PROCESSES

POL III TRANSCRIPTION TERMINATION

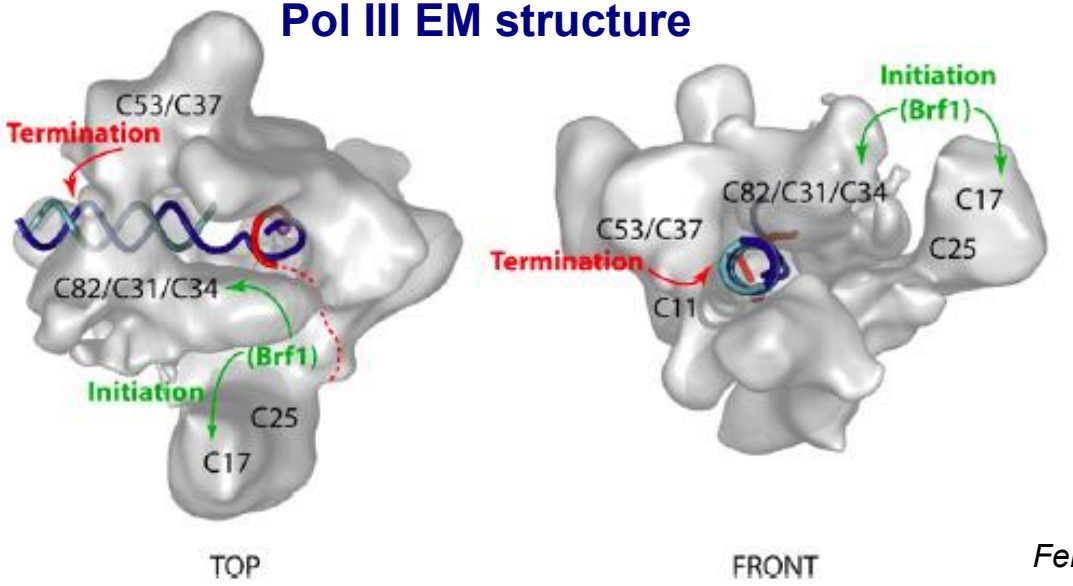


Landrieux et al., EMBO J., 2006



Richard and Manley, Gene Dev., 2009

Pol III EM structure



C1, C2 core subunits

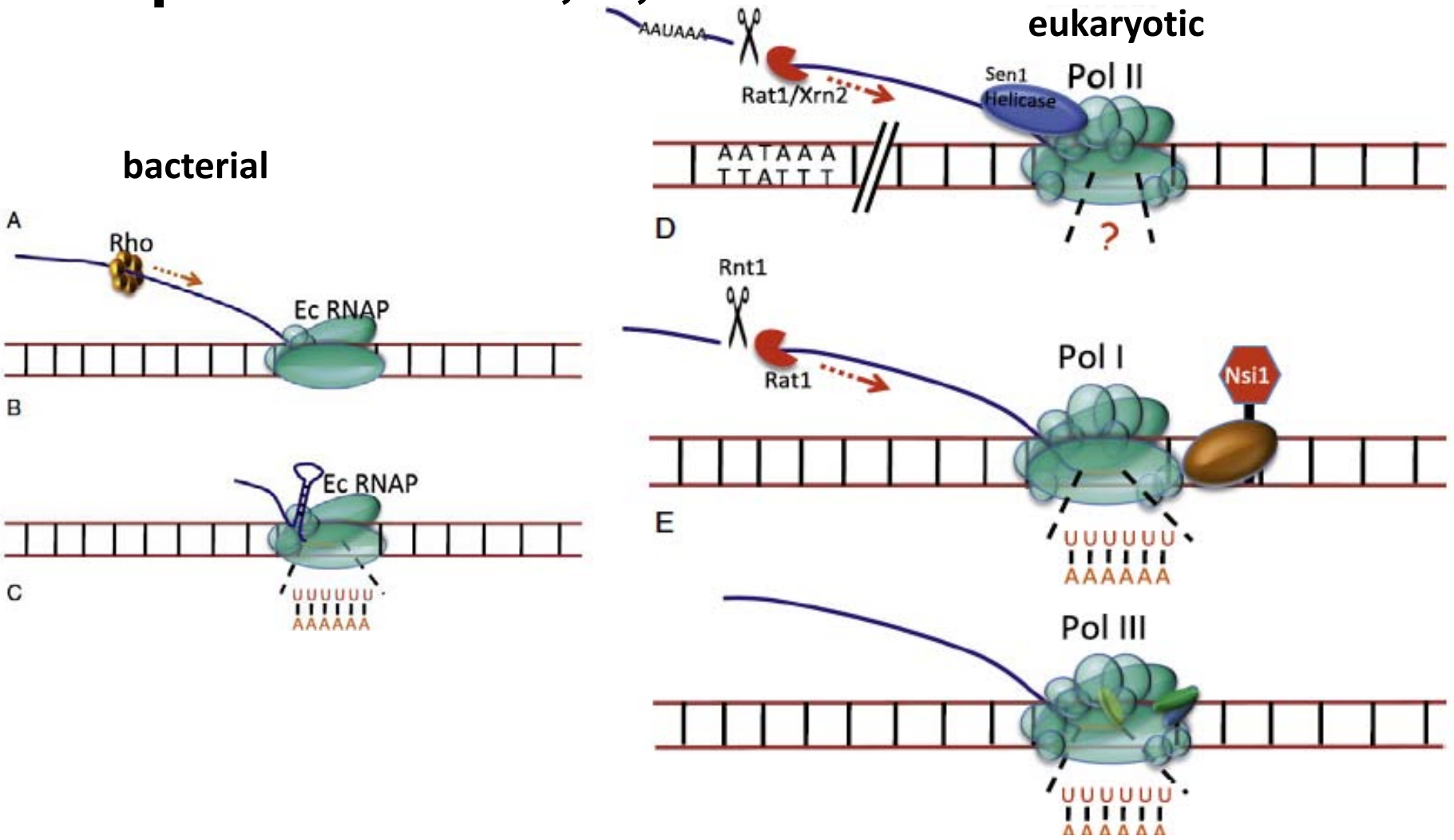
(Pol pausing)

C37-C53 subcomplex is situated across the cleft near RNA exit

C11 (TFIIS) subunit of Pol III has intrinsic RNA cleavage activity important for Pol III termination

Fernadez-Tornero et al., Mol. Cell, 2007

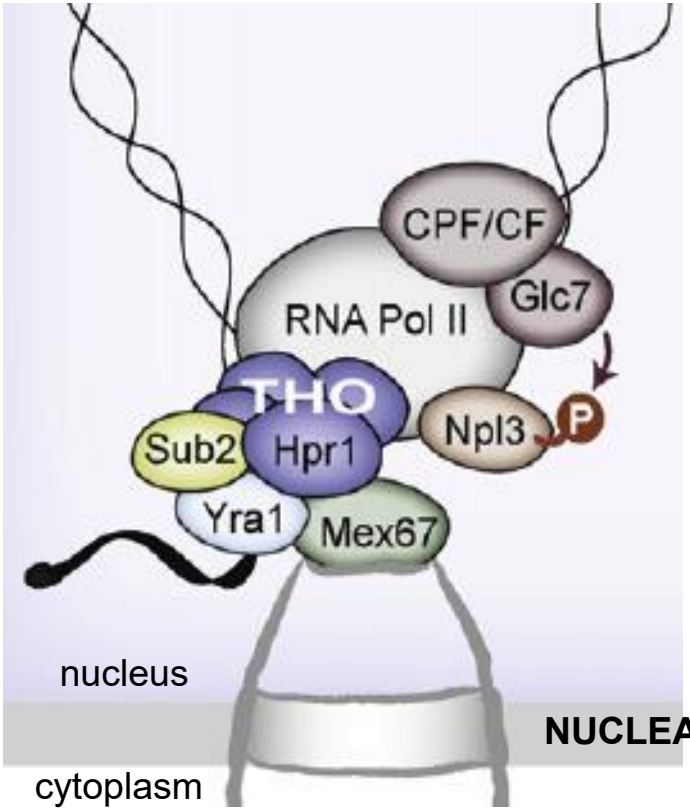
Transcription termination comparison Pol I, II, III

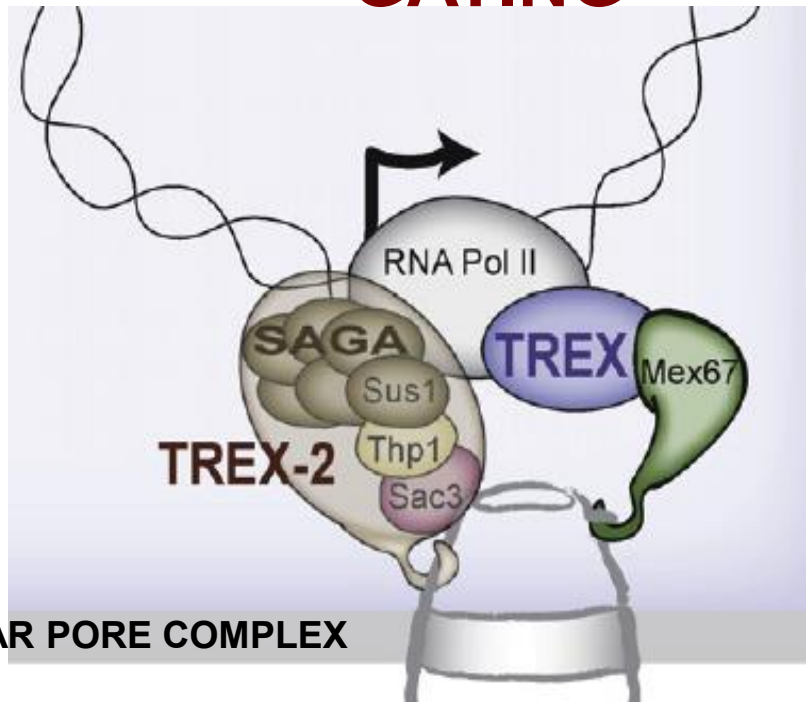


Arimbaasseri et al., BBA, 2013

CO-TRANSCRIPTIONAL PROCESSES

mRNA EXPORT: GENE GATING





Iglesias and Stutz, FEBS Lett, 2008

SAGA histone acetyltransferase complex (including **Spt, Ada, Gcn5**); trx activation

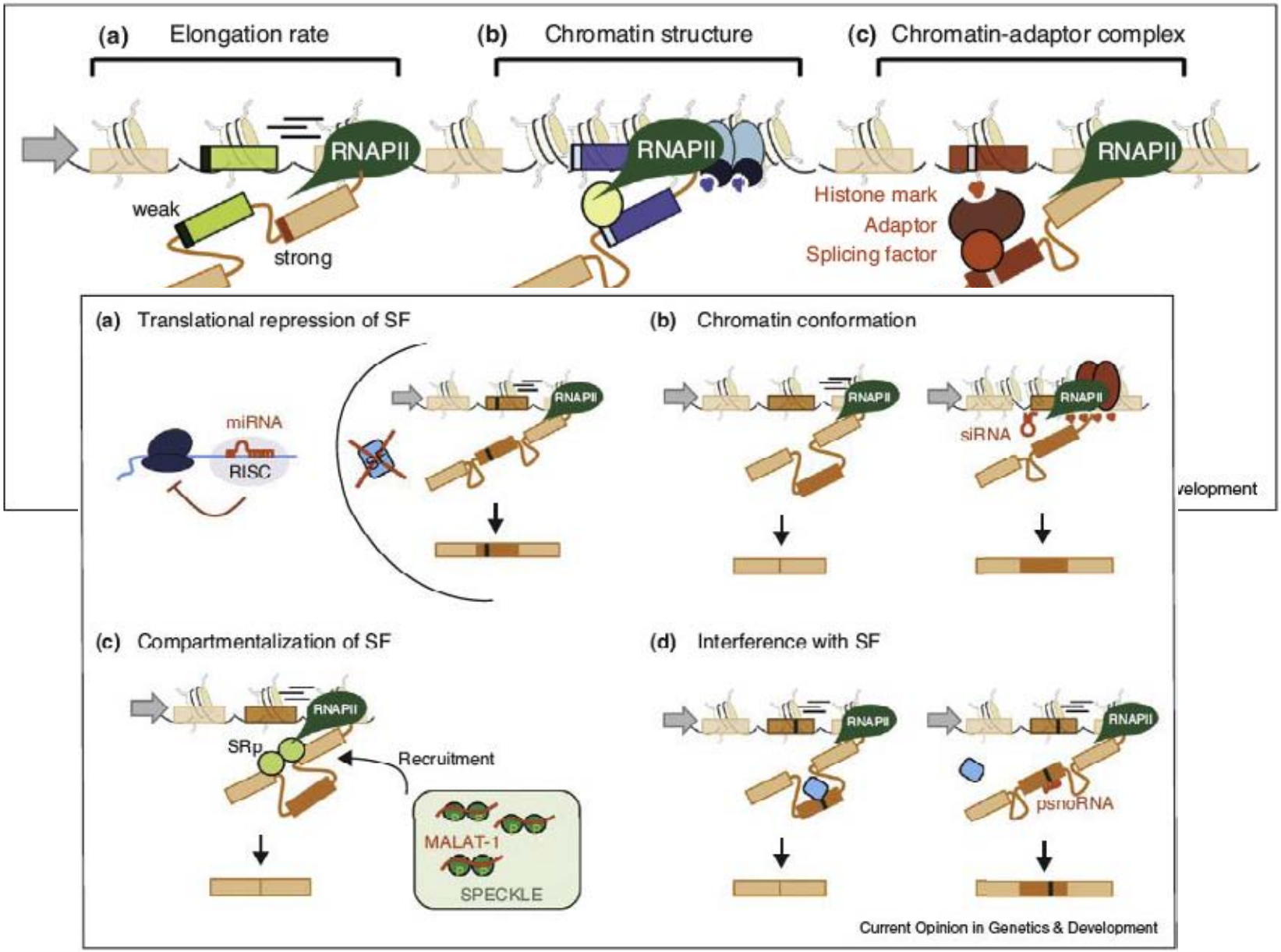
THO mRNP biogenesis and export: **Hpr1, Mft1, Tho2** and **Thp2** (human **THOC1-7**)

TREX transcription-export complex: **THO/Sub2/Yra1,** interacts with NPC via Mex67-Mtr2

TREX-2 transcription-export complex: **Cdc31/Thp1/Sac3** and **Sus1** from **SAGA**

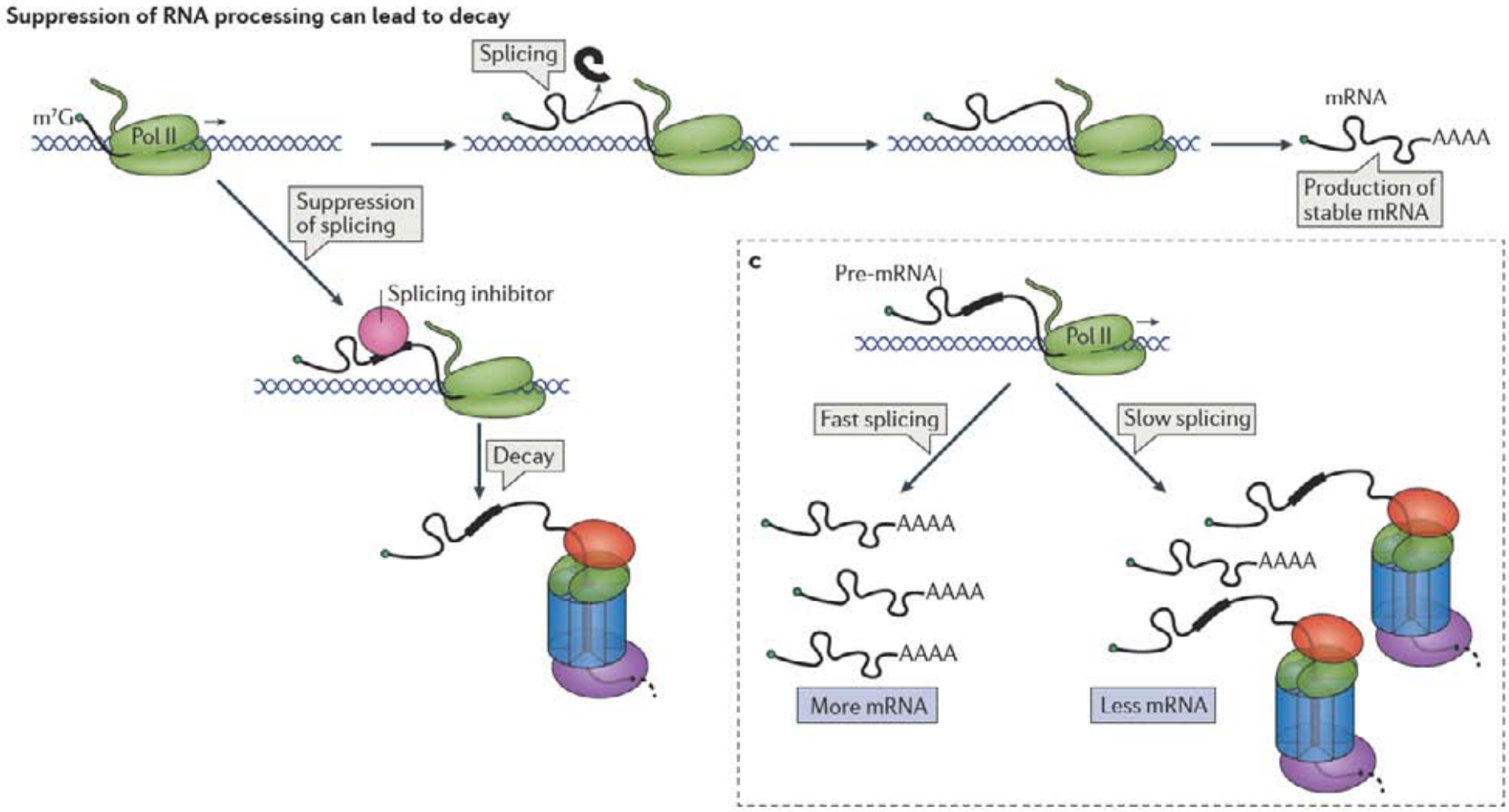
TREX-2 and TREX complexes link transcription (Pol II via THO, initiation complex SAGA via Sus1) to export receptors (Mex67, Yra1) and Nuclear Pore Complex

COORDINATION: ALTERNATIVE SPLICING, CHROMATIN, ncRNAs, SPLICING FACTORS



Luco and Misteli, Curr Op Gene Dev, 2011

COORDINATION: SPLICING AND RNA DECAY

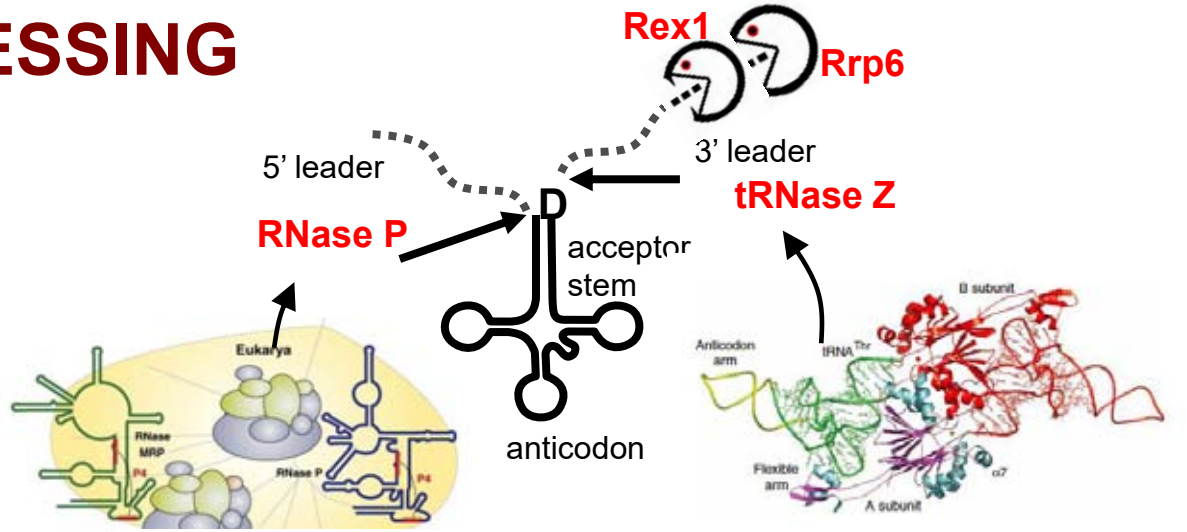


Kilchert et al, Nat Rev Mol Cell biol, 2016

POST-TRANSCRIPTIONAL PROCESSES

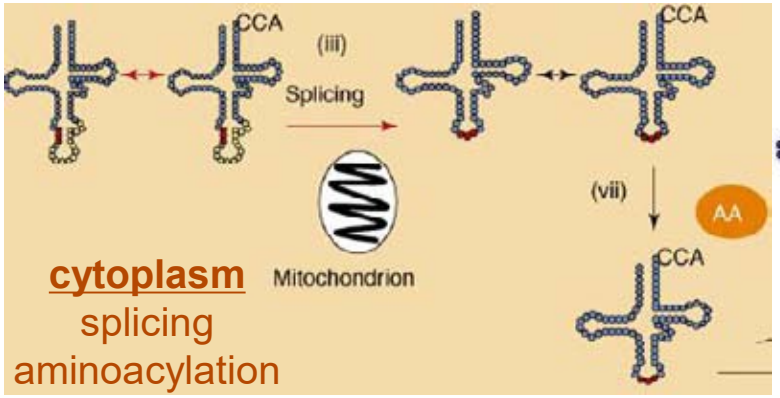
tRNA PROCESSING

tRNA precursors:
- 5' end by RNAse P
- 3' end by tRNase Z
- alternative 3' pathway:
exonucleolytic by Rex1 and R

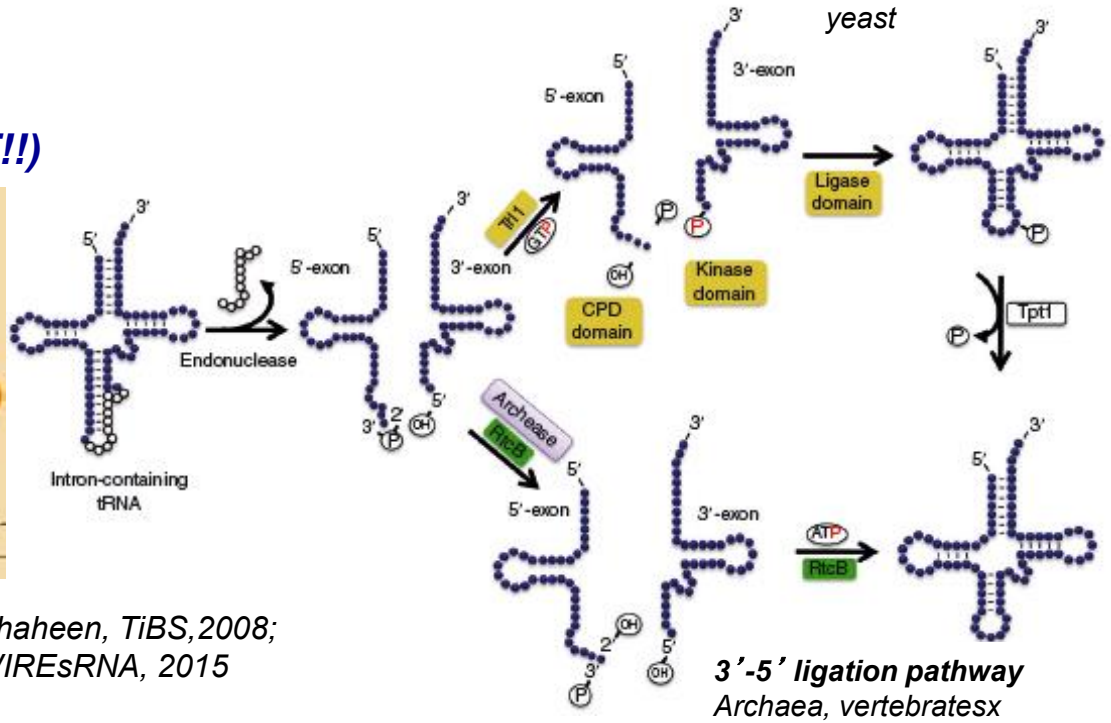


tRNA SPLICING

In the cytoplasm on the mitochondrial membrane *(YEAST!!)*



5'-3' ligation pathway
yeast



Hopper and Shaheen, TiBS,2008;
Lopes et al, WIREsRNA, 2015

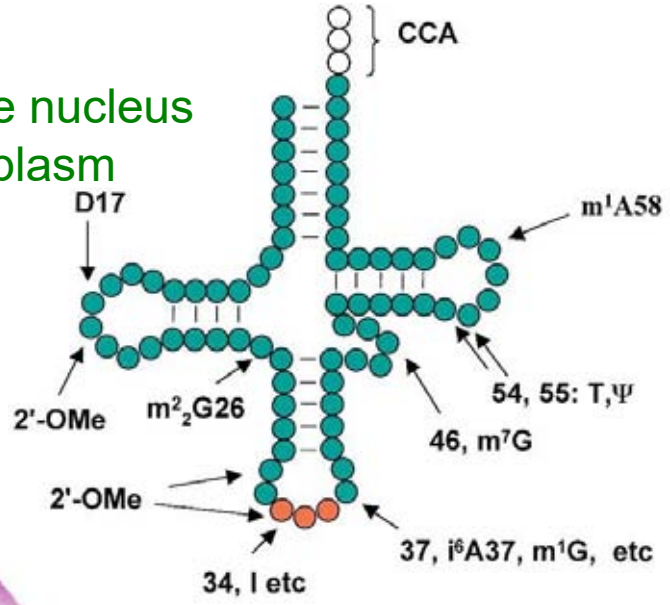
3'-5' ligation pathway
Archaea, vertebratesx

POST-TRANSCRIPTIONAL PROCESSES- tRNA

tRNA MODIFICATION

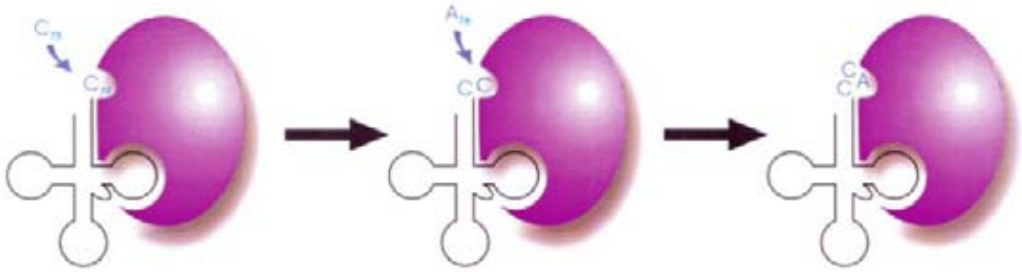
can occur in the nucleus and in the cytoplasm

Functions of modifications:
- **contribute to folding**
- **provide stability**
- **facilitate alternative structures**
- **affect codon recognition** (wobble bp)
- **contribute to translation** (frameshifting)



Hopper and Phizicky, GeneGev. 2003

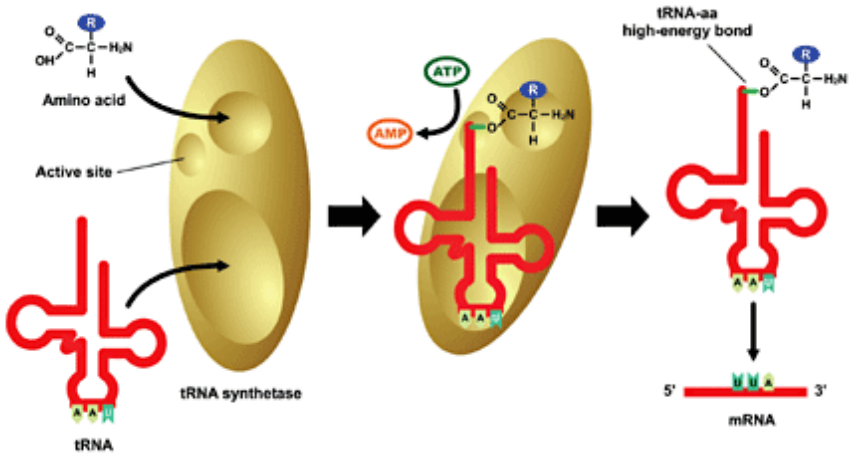
tRNA CCA ADDITION



by tRNA nucleotidyl-transferase

tRNA AMINOACYLATION

by tRNA aminoacyl synthetases
two classes: class I and class II
(aminoacylate 2'-OH or 3'-OH of A)



Schurer et al, Biol.Chem., 2001

RNA DECAY

RNases

Endonucleases

processing (RNase P, RNase III, RNase E):

specific, cleavage results in 3’-OH and 5’-P (monophosphate)

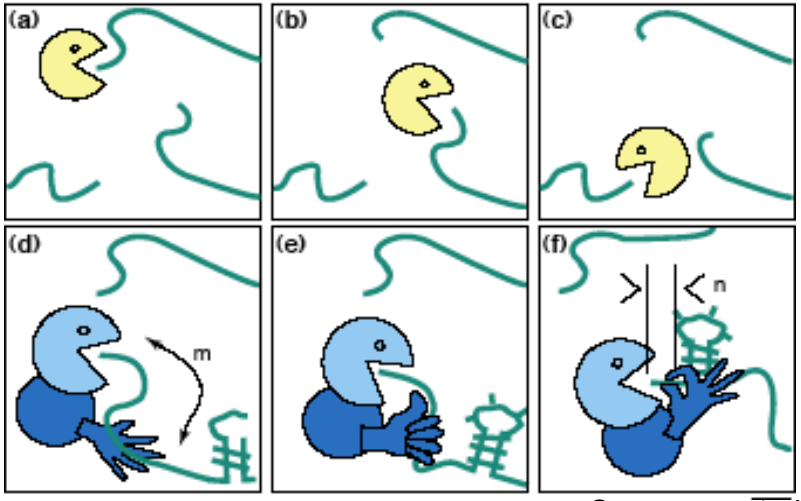
degrading (RNase I, RNAse A):

unspecific, cleavage results in 5’-OH and 3’-P (cyclic phosphate)

Exonucleases

hydrolytic: attacking group H_2O, results in 3’-OH and 5’-P

phosphorolytic: attacking group inorganic phosphate, results in 3’-OH and 5’-PP



distributive

processive

Symmons et al, TiBS, 2002

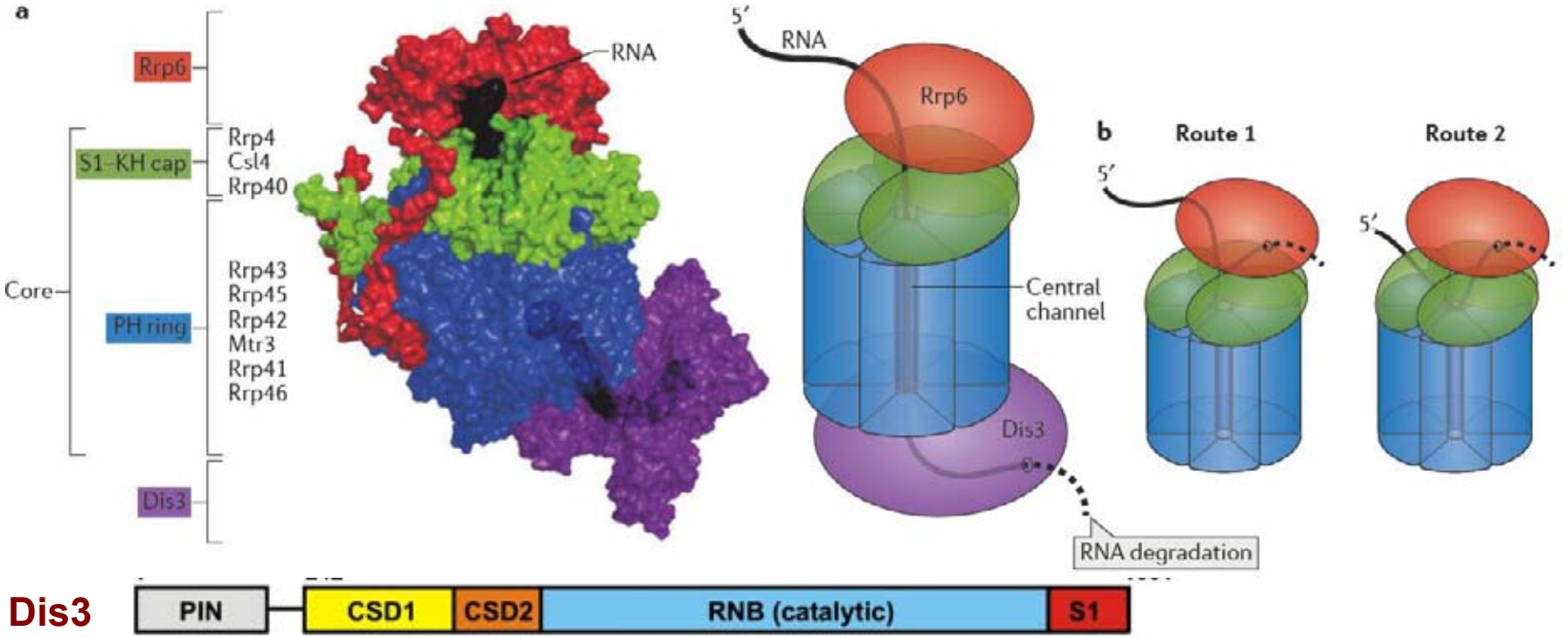
RNA PROCESSING and DECAY machinery: RNases

Protein	Function	Characteristics
Exonucleases 5'→ 3'		
Xrn1	cytoplasmic, mRNA degradation	
Rat1	nuclear, pre-rRNA, sn/snoRNA, pre-mRNA processing and degradation	
Rrp17/hNol12	nuclear, pre-rRNA processing	
Exosome 3'→5'	**multisubunit exo/endo complex**	subunits organized as in bacterial PNPazy
Rrp44/Dis3	catalytic subunit	Exo/PIN domains, distributive, hydrolytic
Rrp4, Rrp40	pre-rRNA, sn/snoRNA processing, mRNA degradation	
Rrp41-43, 45-46	participates in NMD, ARE-dependent, non-stop decay	
Mtr3, Ski4		
Rrp6, Rrp47p	nuclear helicase cofactor	DEAD box
Ski2,3,7,8	cytoplasmic exosome cofactors	helicase, GTPase
Other 3'→5'		
Rex1-4	3'-5' exonucleases, rRNA, snoRNA, tRNA processing	RNase D homolog
DXO	3'-5' exonuclease in addition to decapping	
mtEXO 3'→5'	**mitochondrial degradosome** RNA degradation in yeast	
Suv3/ Dss1	helicase/ 3'-5' exonuclease	DExH box/ RNase II homolog
Deadenylation		
Ccr4/NOT	major deadenylase complex (Ccr, Caf, Pop, Not proteins)	Ccr4- Mg^{2+} dependent endonuclease
Pop2	deadenylation regulator, deadenylase activity	RNase D homolog
Pan2p/Pan3	additional deadenylases (poliA tail length)	RNase D homolog, poly(A) specific nuclease
PARN	mammalian deadenylase	RNase D homolog, poly(A) specific nuclease
Endonucleases		
RNase III		
-Rnt1	pre-rRNA, sn/snoRNA processing, mRNA degradation	dsRNA specific
-Dicer, Drosha	siRNA/miRNA biogenesis, functions in RNAi	PAZ, RNA BD, RNase III domains
Ago2 Slicer	mRNA cleavage in RNAi	
SMG6	mRNA cleavage in NMD	PIN domain
RNase P	5' tRNA end processing	RNP complex
RNase MRP	pre-rRNA processing	RNP complex, similar to RNase P
RNase L	rRNA degradation in apoptosis	oligo 2-5A dependent $(ppp(A2'p)_nA)$
ELAC2/Trz1	3' tRNA endonuclease	PDE motif and Zn^{2+} -binding motif

Eukaryotic auxiliary decay factors

Protein	Function / Characteristics
5'→3' decay: decapping	
Dcp1/Dcp2	Dcp2- pyrophosphatase catalytic activity, Nudix domain, Dcp1- protein binding
Hedls/Ge-1/Edc4	decapping cofactor, WD40 domain
Edc1,2,3	decapping enhancers, stimulate cap binding/catalysis, Edc1-2 (yeast), Edc3 (all eykaryotes)
Dhh1	DexD/H ATPase, decapping activator by translation repression
Lsm1-7	decapping activator, heptameric complex, binds mRNA 3' end-U rich tracts
Pat1	decapping activator by translation repression
TRAMP complex: nuclear RNA surveillance, polyadenylation-dependent degradation	
Trf4/Trf5	nuclear alternative poly(A) polymerases
Mtr4	DEAD box helicase
Air1/Air2	RNA binding proteins, also nuclear exosome cofactor
Nrd1-Nab3-Sen1 complex: PolII termination of small RNAs, TRAMP-depdendent degradation	
Nrd1	Pol II C-terminal domain (CTD) binding, RNA binding
Nab3	RNA binding
Sen1	RNA helicase

EXOSOME: 3’→5’ decay machinery



- 3’→5’ exo/endo nuclease complex;
- 10 core components (RNA BP)
- catalytically active exo hydrolytic Dis3/Rrp44 (RNase II)
- PIN domain with endo activity
- nuclear cofactors- RNA BP Rrp47, nuclease Rrp6 (RNase D), RNA helicase Mtr4
- cytoplasmic cofactors- Ski2-3-8 complex (RNA helicase Ski2), GTPase Ski7
- subtrates- processing and/or degradation of almost all RNAs

Lecture on the exosome by Rafał Tomeck

Dziembowski et al, Mol.Cell, 2008; Nature, 2008Kilchert et al, Nat Rev Mol Cell biol, 2016

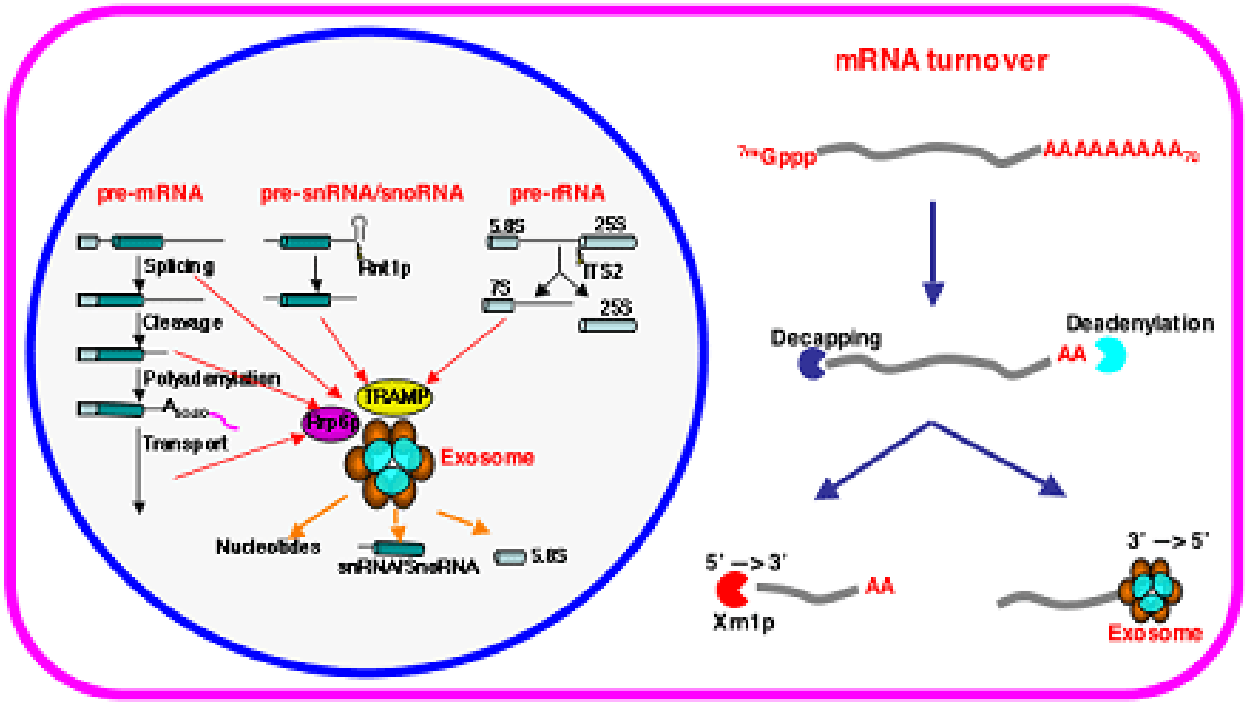
EXOSOME: 3’→5’ decay: FUNCTION

NUCLEAR: Rrp6 and core components have partly separate functions

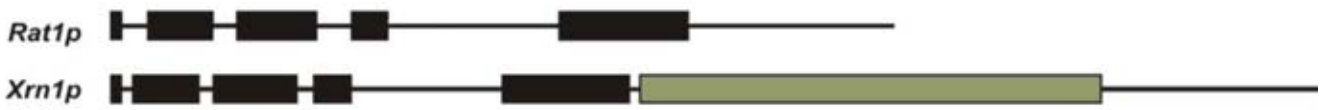
- 3’ end processing of 5.8S rRNA, sn/snoRNAs, tRNAs, SRP RNA
- degradation of pre-mRNAs, tRNAs, sn/snoRNAs
- degradation of other ncRNAs: CUTs, PROMPTS

CYTOPLASMIC:

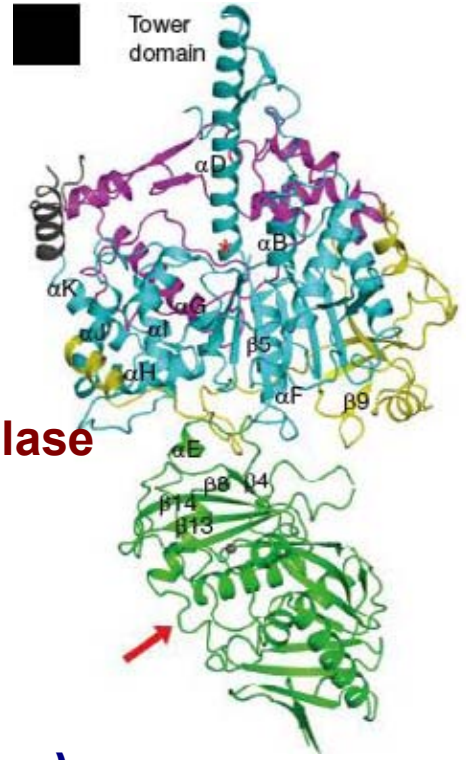
- generic mRNA decay
- specialised mRNA decay pathways: NMD, NSD, NO-GO decay, ARE-dependent decay



XRN family: 5'→3' processive exonucleases



Kastenmayer and Green, 2000, PNAS



Crystal structure of *S. pombe* Rat1/Rai1 complex

NUCLEAR

Rat1/XRN2 with Rai1 activator (5'-ppp pyrophosphohydrolase and phoshodiesterase-decapping nuclease)

- **5' end processing of 5.8S and 25S rRNAs, snoRNAs**
- **degradation of pre-mRNAs, tRNAs, sn/snoRNAs**
- **degradation of some ncRNAs: CUTs**
- **transcription termination of Pol I and II *(torpedo mechanism)***

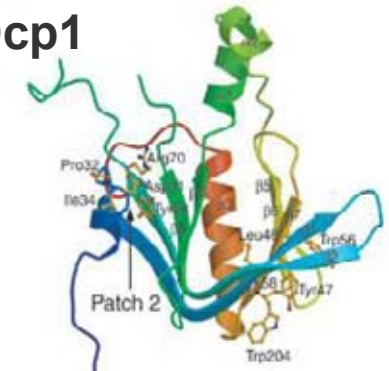
Xiang et al, 2009, Nature

CYTOPLASMIC XRN1

- **generic mRNA decay**
- **specialised mRNA decay pathways: NMD, NSD, NO-GO decay, ARE-dependent decay**
- **degradation of miRNA-dependent mRNA cleavage products *(in plants)***
- **degradation of some ncRNAs: CUTs, SUTs, XUTs**

DCP/NUDT- DECAPPING ENZYMES

Dcp1

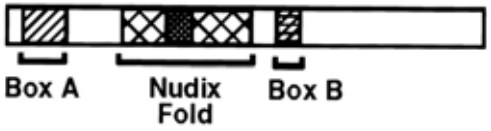


She et al. *Nat.Struct. Mol. Biol*, 2004

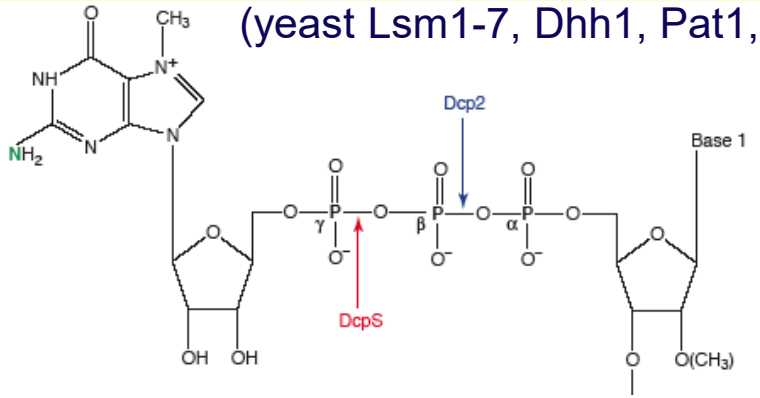
- **Dcp1/Dcp2 complex participates in mRNA 5' decay**
- **catalyses the reaction m^7GpppX-mRNA -> m^7GDP + 5'p-mRNA**
- **Dcp2 is the catalytic subunit (pyrophosphatase Nudix domain)**
- **Dcp1 is required for activity *in vivo*, interacts with other proteins**
- **Dcp1/Dcp2p is regulated by Pab1 and activating factors**

(yeast Lsm1-7, Dhh1, Pat1, Edc1-3, Upf1-3)

Dcp2

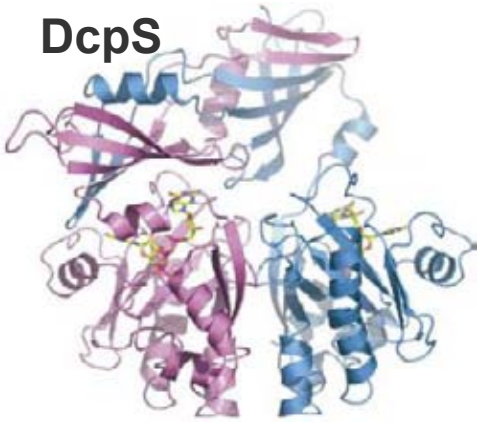


Wang et al. *PNAS*, 2002



Nudt proteins (22): Nudt16, Nudt3 *in vivo* decapping activity in mammals, plants…

DcpS

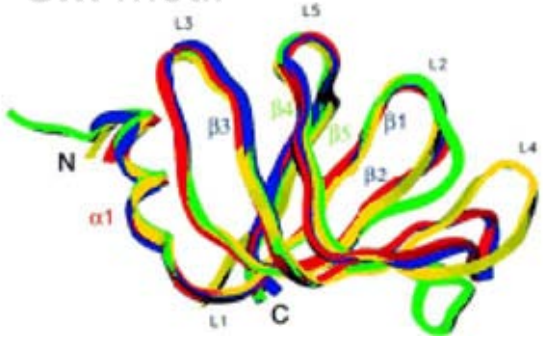


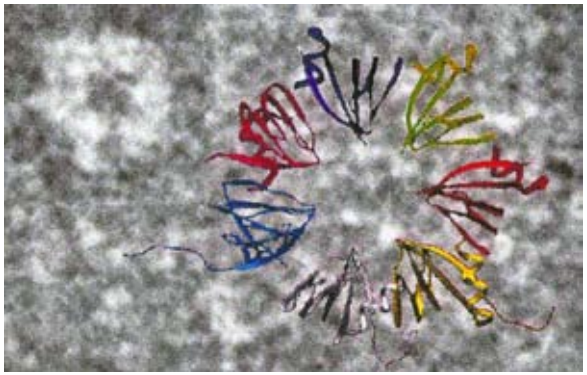
Gu et al., *M.Cell*, 2004

- **DcpS: HIT pyrophosphatase („histidine triad" on the C-terminus)**
- **catalyses the cleavage of m^7GDP -> m^7GMP + Pi remaining after decapping during mRNA 5' decay**
- **cooperates with the exosome during mRNA 3' decay (m^7GpppX-oligoRNA -> m^7GMP+ pp-oligoRNA)**
- **functions as an asymmetric dimer**

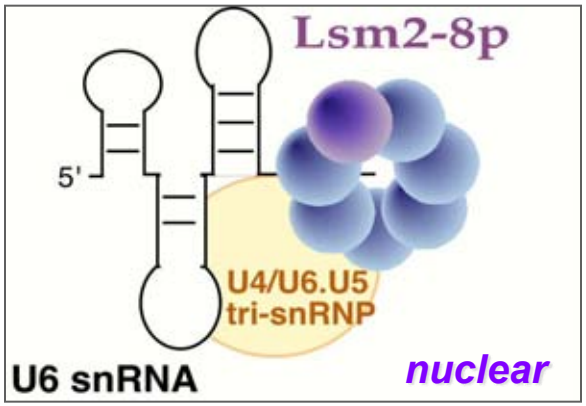
LSM PROTEINS

Sm motif



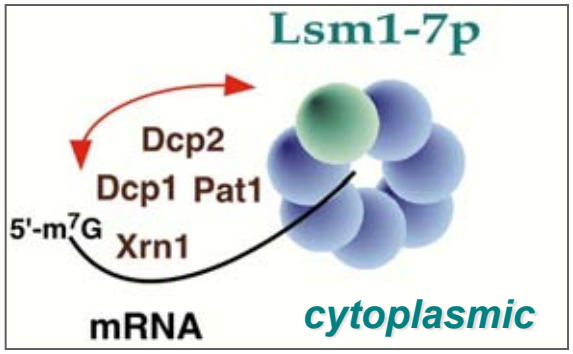


Achsel et al, *EMBO J,* 2001



Involved in pre-mRNA splicing

- associates with U6 snRNA
- required for U6 RNA accumulation and U6 snRNP biogenesis
- interacts with the U4/U6.U5 tri-snRNP

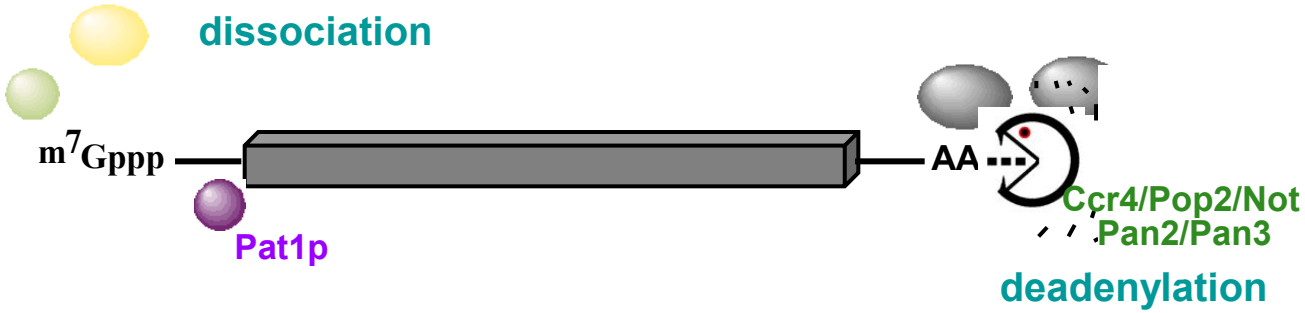


Functions in mRNA decapping and decay

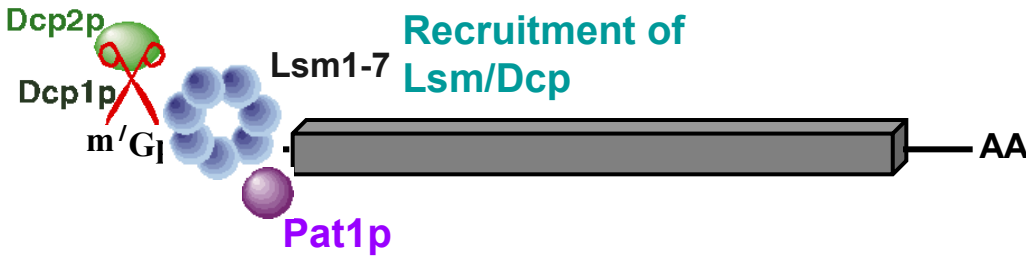
- activator of decapping
- interacts with components of the mRNA decapping and degradation machinery (XRN, DCP)

mRNA DECAY
IN THE CYTOPLASM

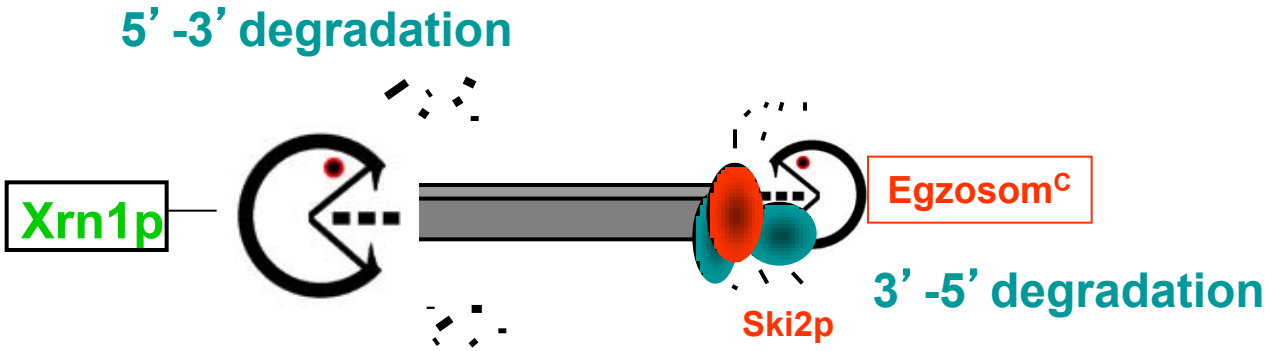
mRNA DECAY
IN THE CYTOPLASM



mRNA DECAY
IN THE CYTOPLASM

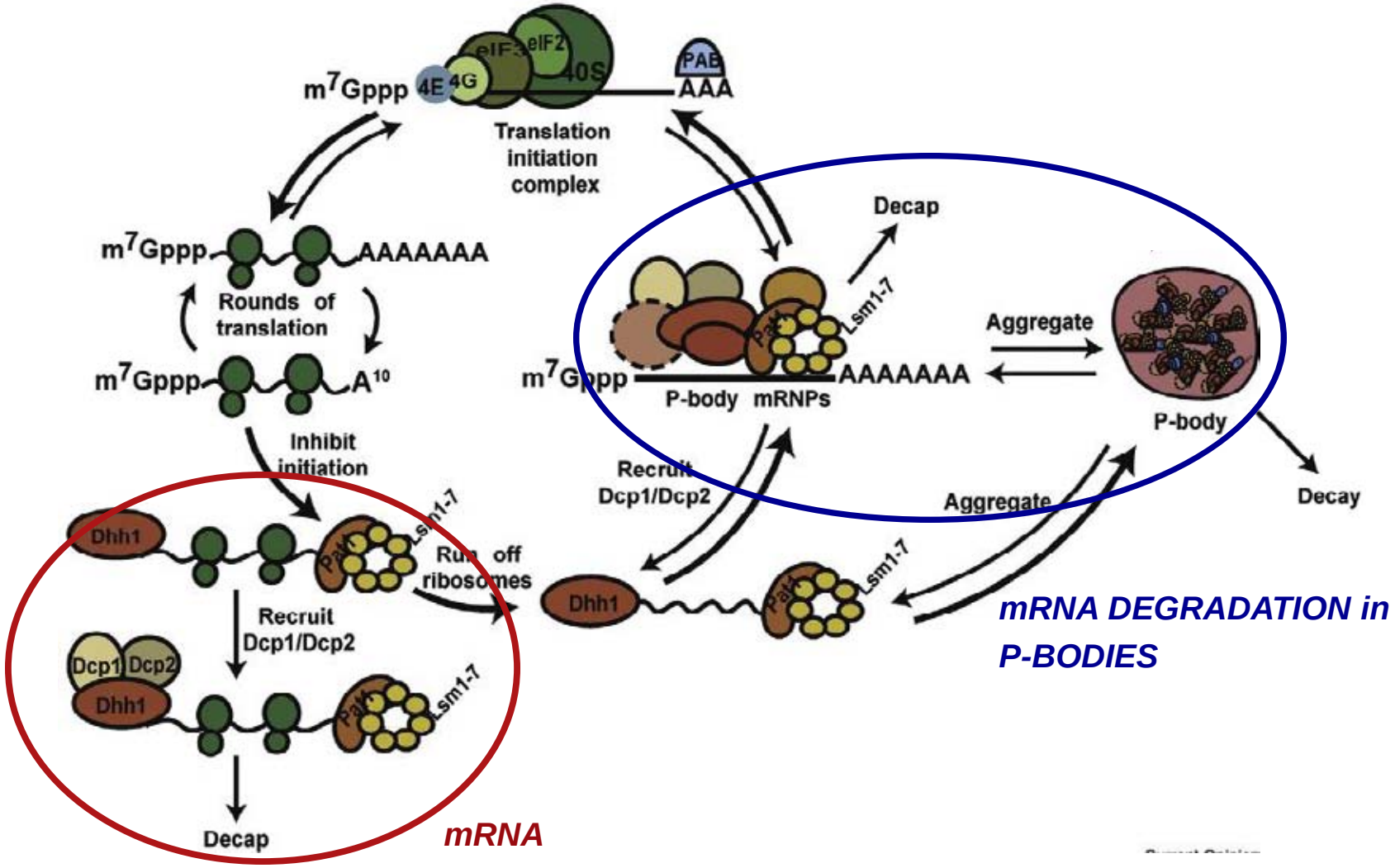


mRNA DECAY
IN THE CYTOPLASM



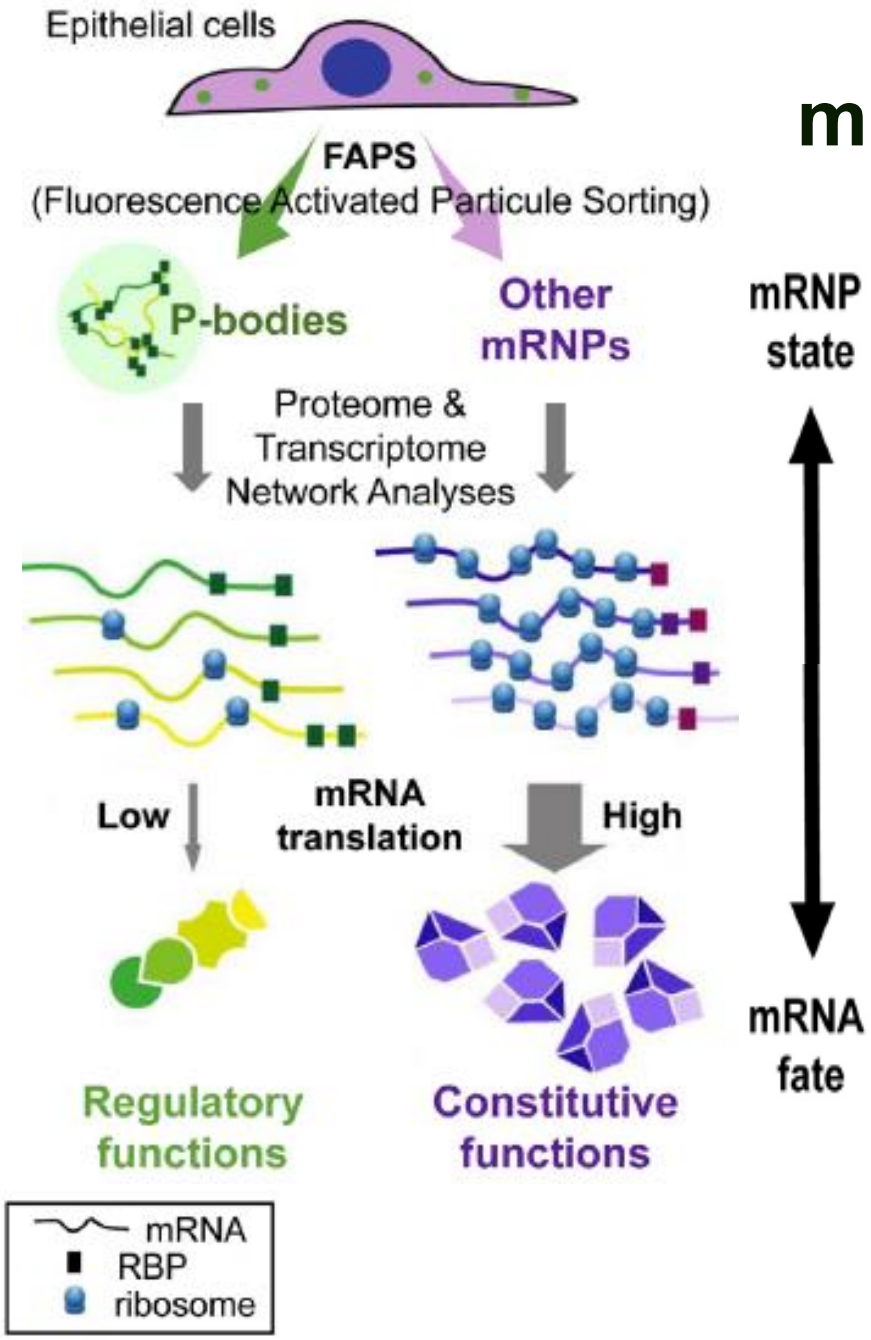
- normal mRNA decay involves deadenylation
- LSM/Pat1 binds and protects deadenylated mRNA 3’ ends against 3’-5’ degradation and recruite Dcp complex to activate 5’-3’ decay
- depending on organism different pathway (5’-3’ or 3’-5’) dominates

mRNA DEGRADATION in the CYTOPLASM



Balagopal and Parker, Cur.Op.Cel.Biol., 2009

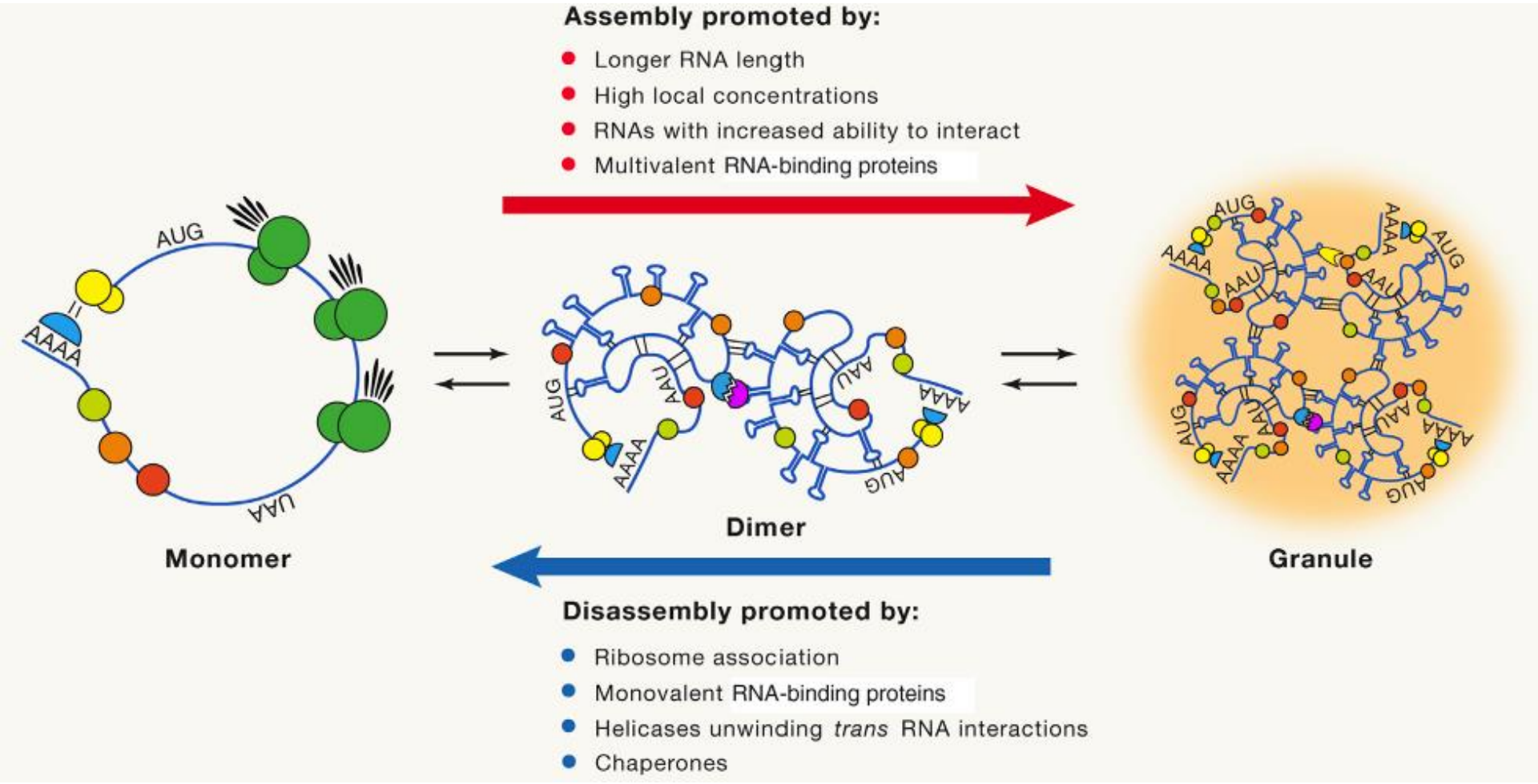
P-bodies: mRNA decay or storage?



- **Purified PB contain mRNA regulons: translationally repressed mRNAs with their regulatory proteins**
- **mRNAs with low protein yield are targeted to P-bodies**
- **mRNAs in PB are translationally repressed but not decayed**

Hubstenberger et al, Mol Cell, 2017

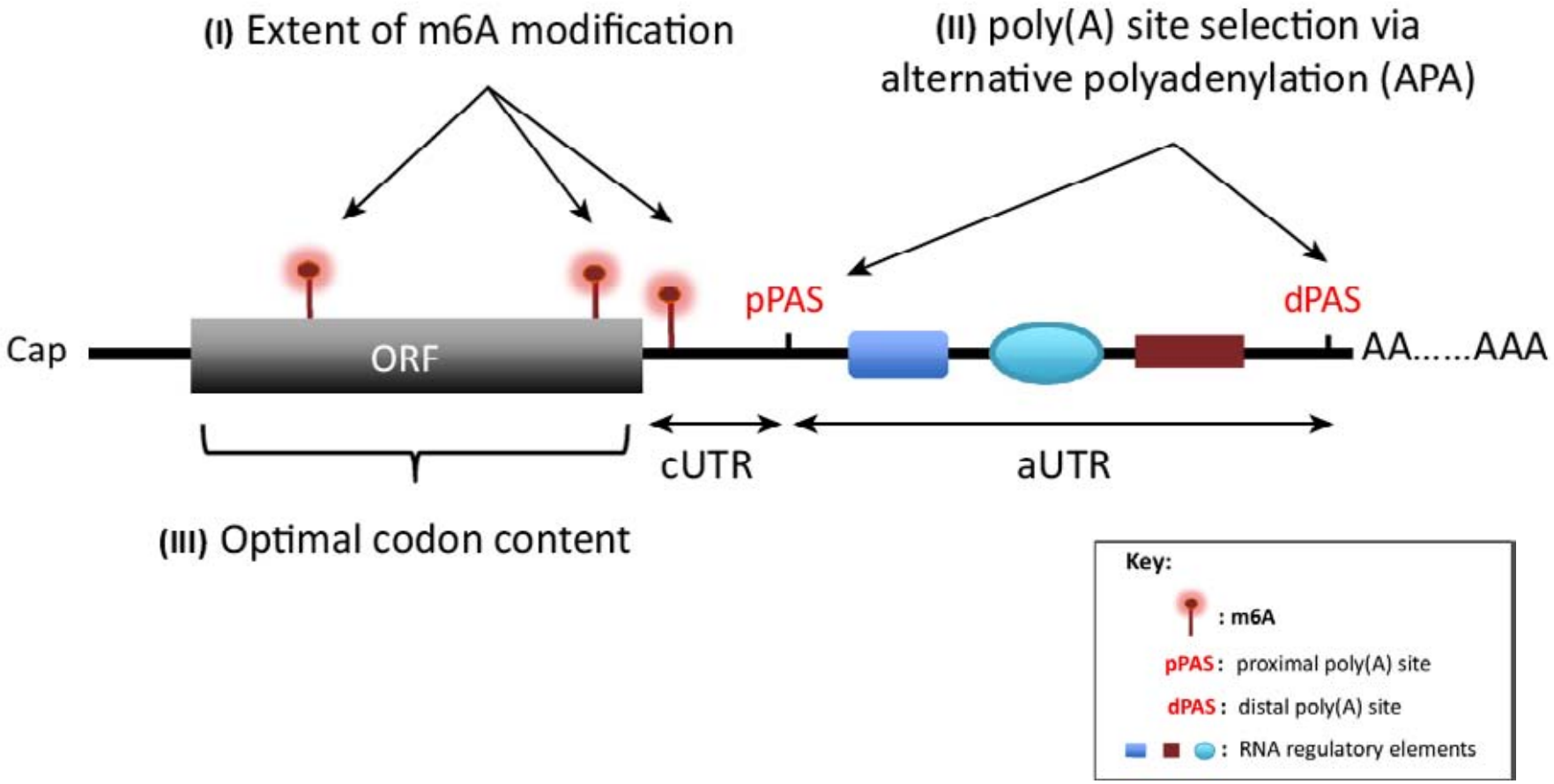
RNP granule assembly by protein-protein and RNA-RNA interactions



Treeck and Parker, Cell, 2018

mRNA STABILITY

Elements *in cis*:

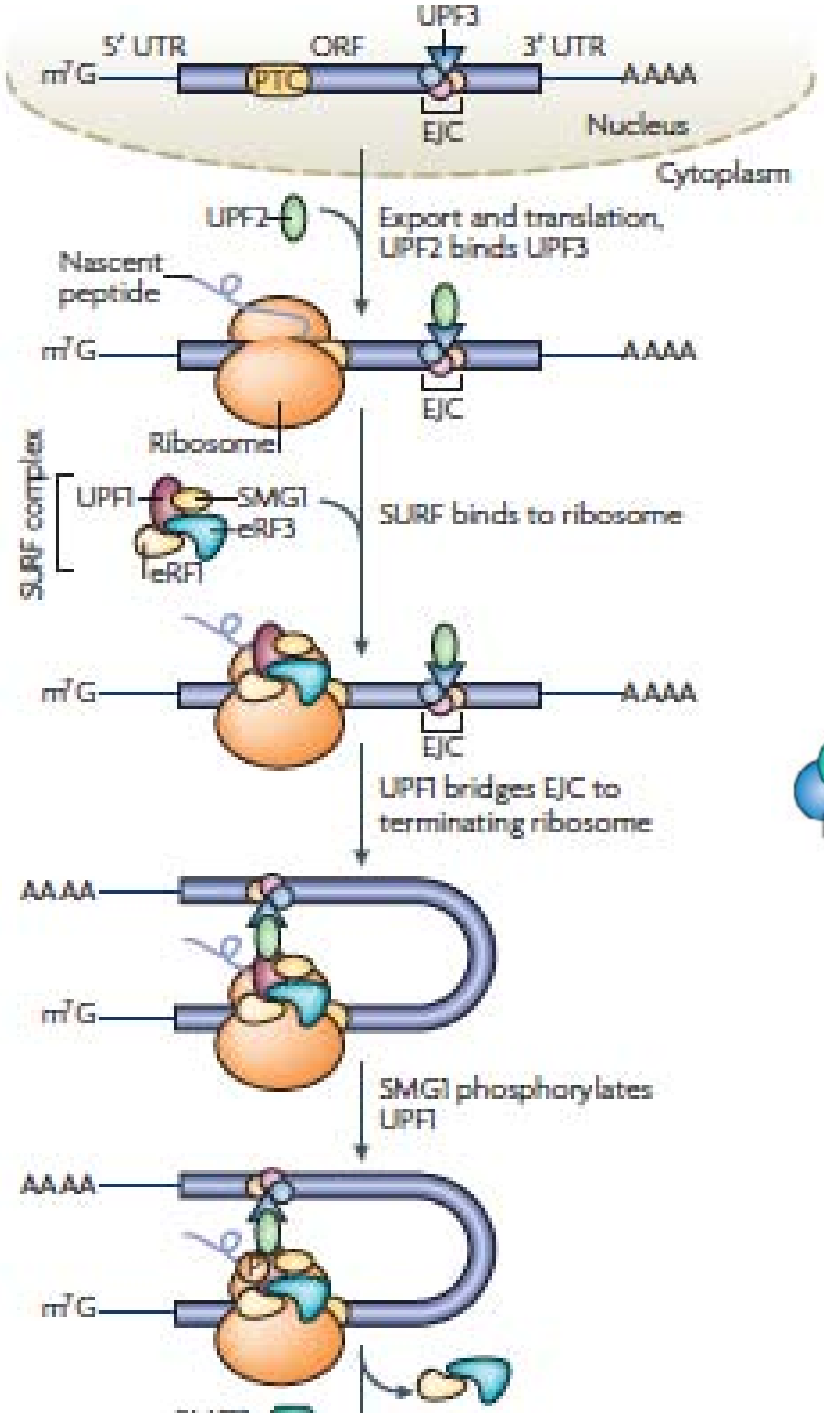


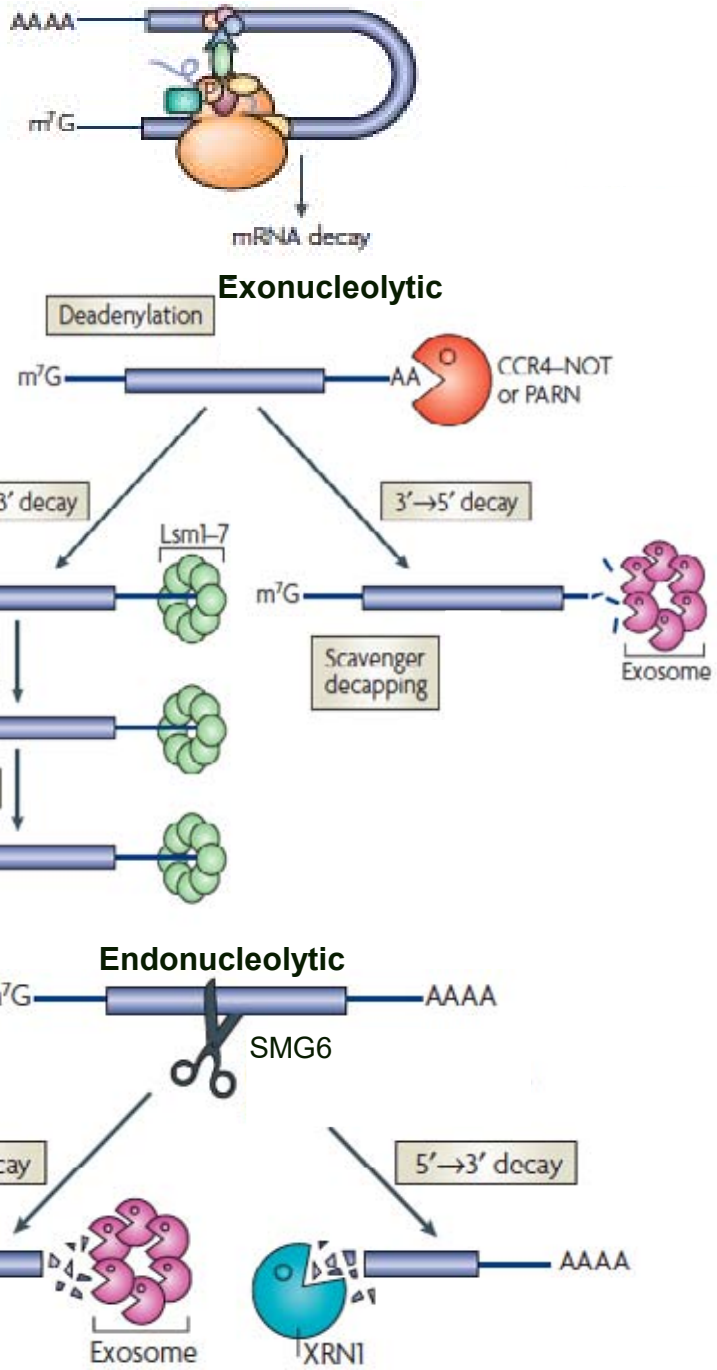
Chen and Shyu, TiBS 2016

RNA SURVEILLANCE = RNA QUALITY CONTROL MECHANISMS

- **NMD**- (nonsense mediated decay) - degradation of mRNAs with premature stop codons (PTC)
- **NSD**- (non-stop decay) - degradation of mRNAs with no stop codons
- **NO-GO** decay- degradation of mRNAs stalled in translation elongation
- **AMD**- **ARE** mediated decay- rapid degradation of mRNAs with specific instability elements (e.g. AU-rich)
- **nuclear RNA degradation** (mRNA, pre-mRNA, rRNA, tRNA) - degradation of RNA species that were not properly processed i.e. spliced, end-matured, modified....

NMD



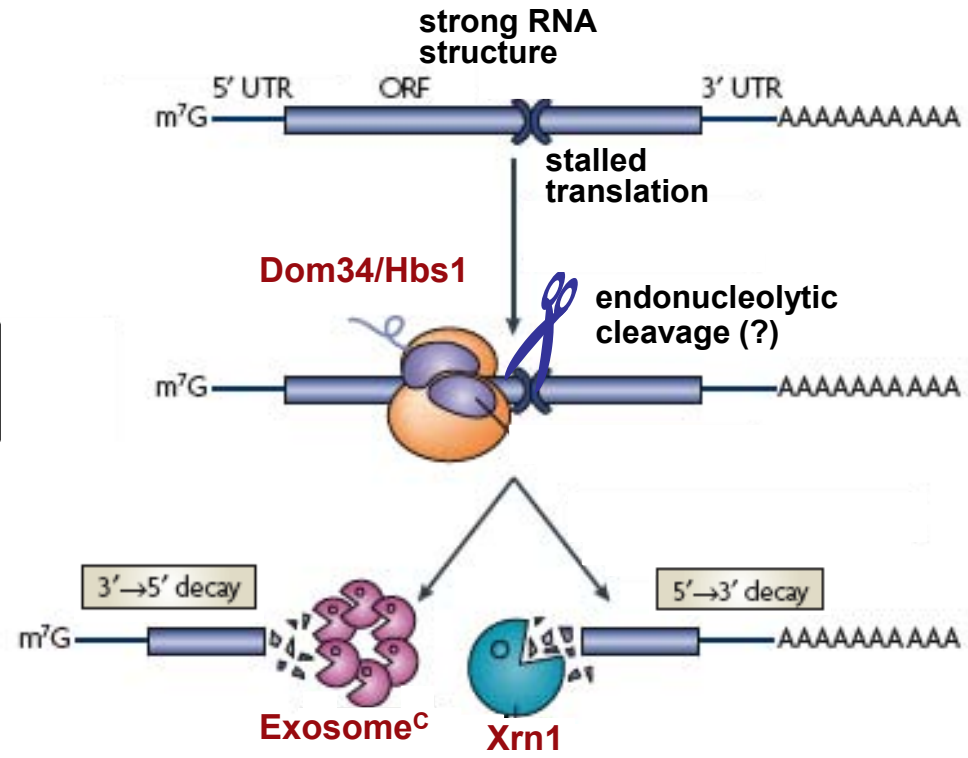


Garneau et al, Nat.Rev.Mol. el. iol. 2007

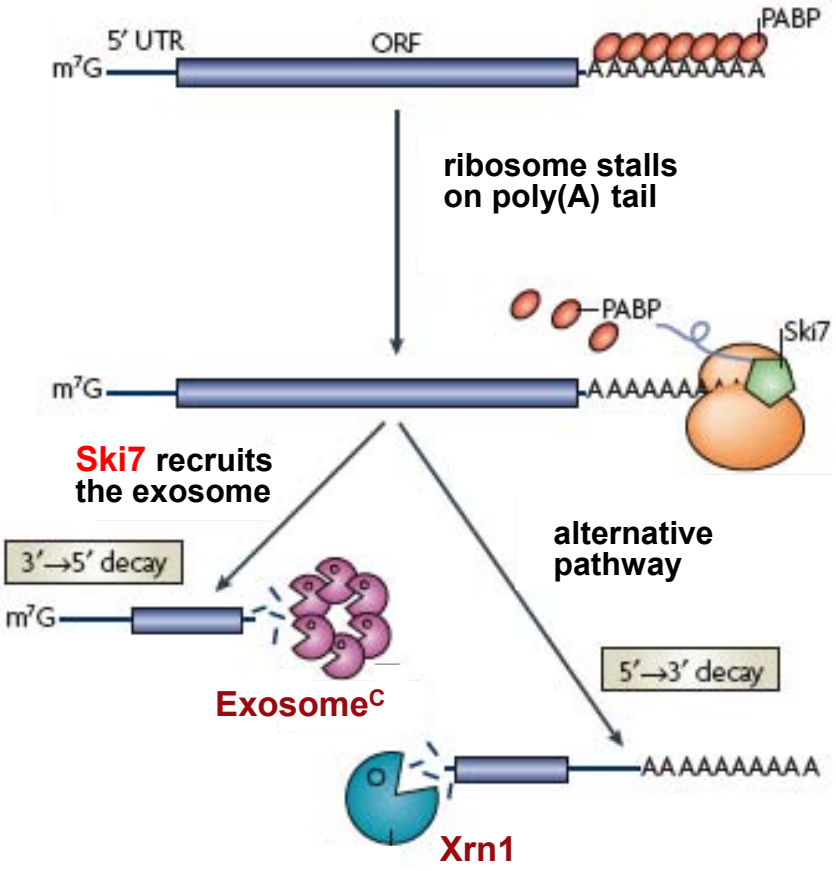
NGD and NSD

- **NGD** (non-go decay) - degradation of mRNAs stalled on ribosomes
- **NSD**- (non-stop decay) - degradation of mRNAs with no stop codons

NGD



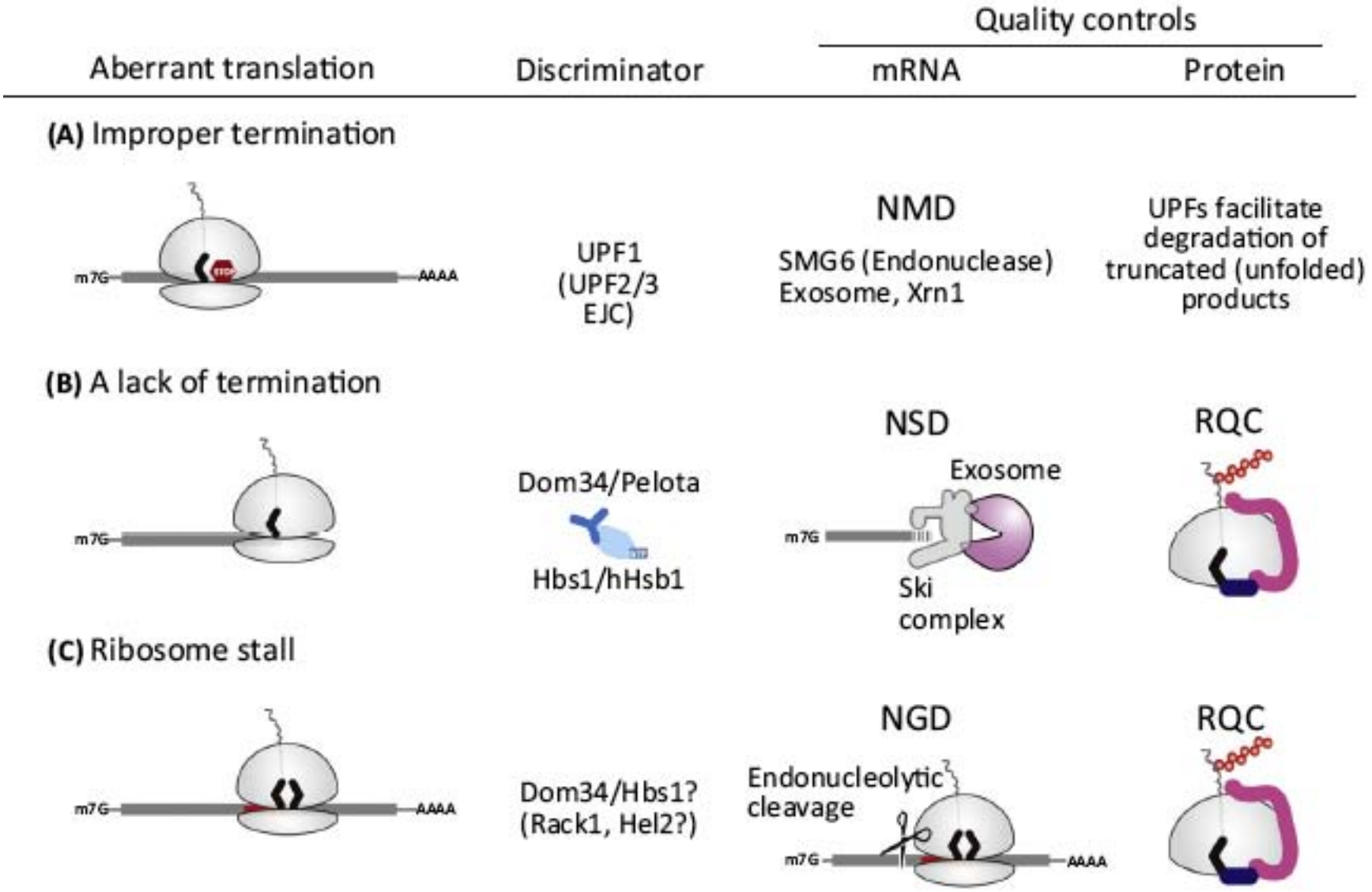
NSD



Dom34 – has RNA-binding Sm fold
Hbs1- GTPase binding activity

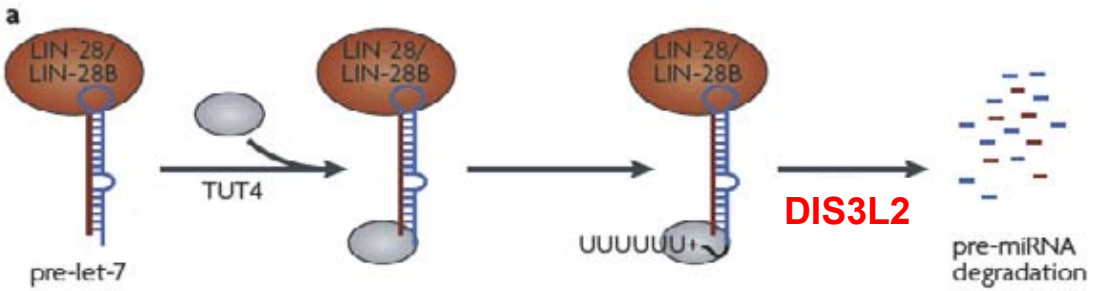
Garneau et al, Nat. Rev. Mol. Cel. Biol. 2007

NMD, NGD and NSD
problem with a stalling ribosome



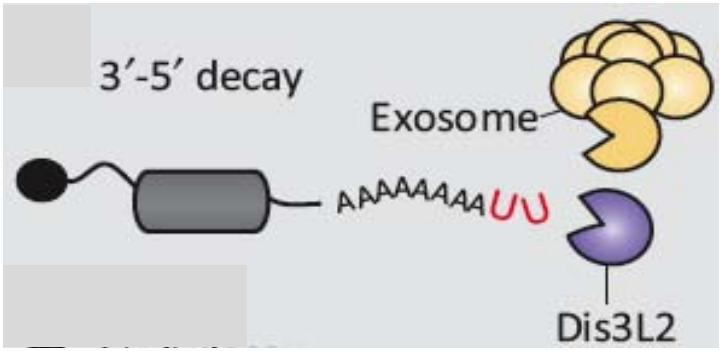
Inada, TiBS 2016

hDIS3L2 EXOSOME INDEPENDENT DECAY

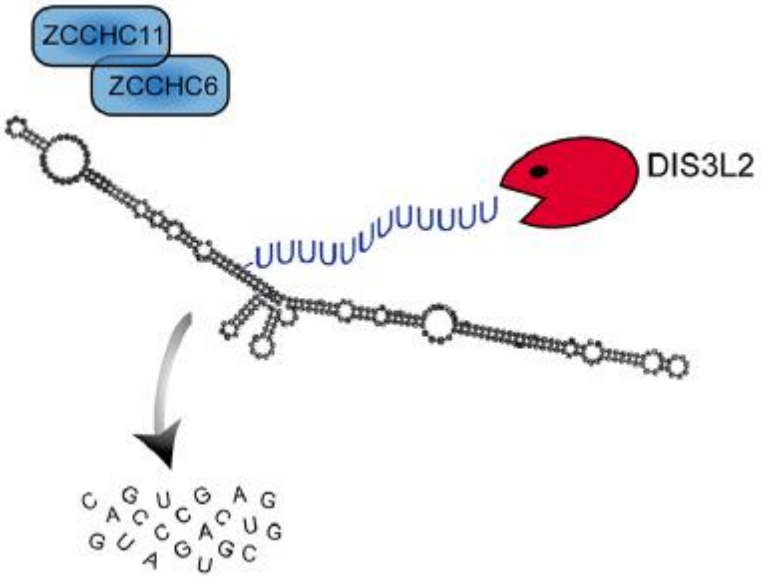


pre-miRNA degradation

Krol et al., Nat Rev Genet, 2010



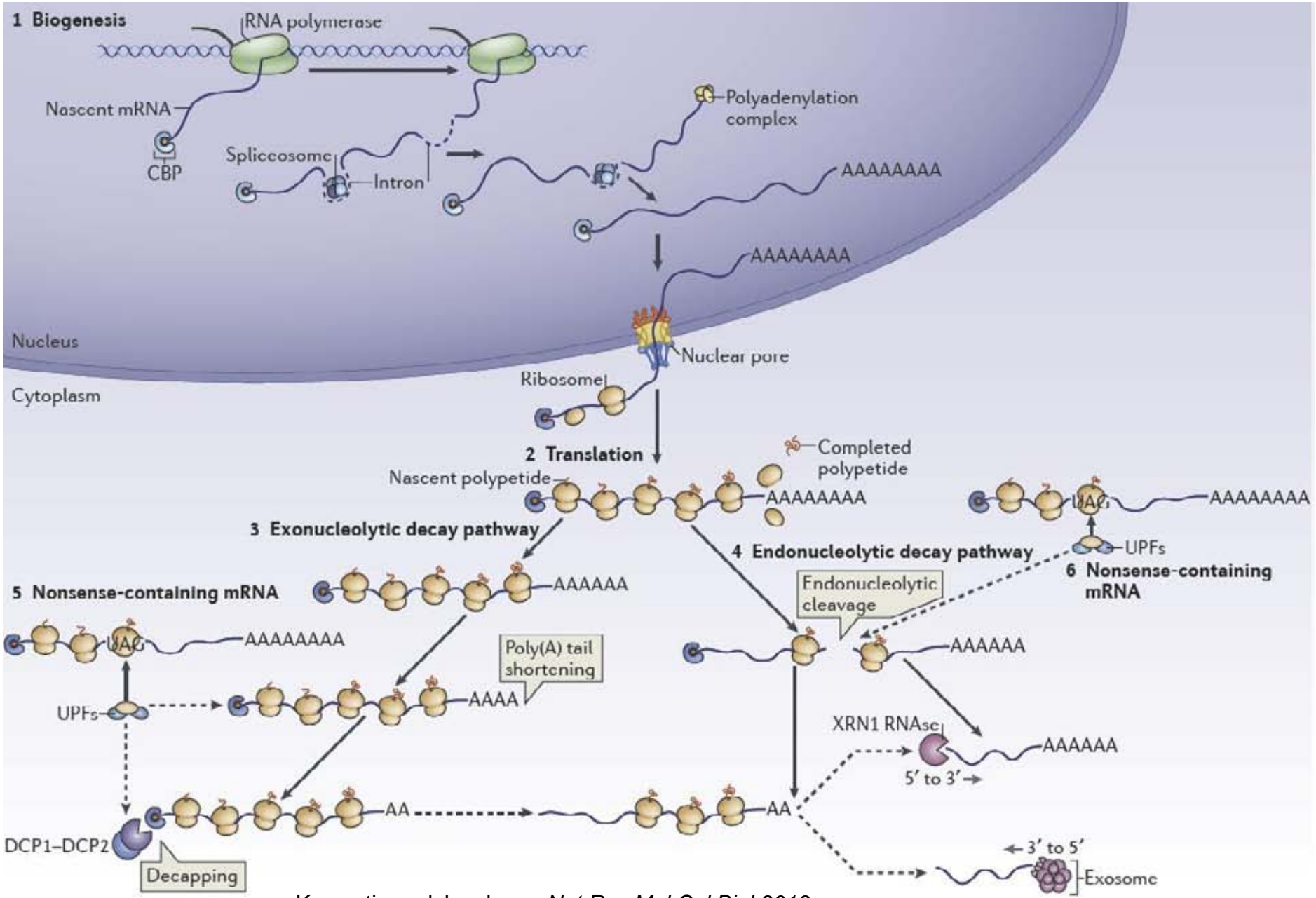
3’-5’ cytoplasmic mRNA decay



3’ degradation of aberrant, structured ncRNAs:
tRNA, sn/snoRNA, rRNA, lncRNA, Y RNA, vault RNA, surveillance of 3’ snRNA processing

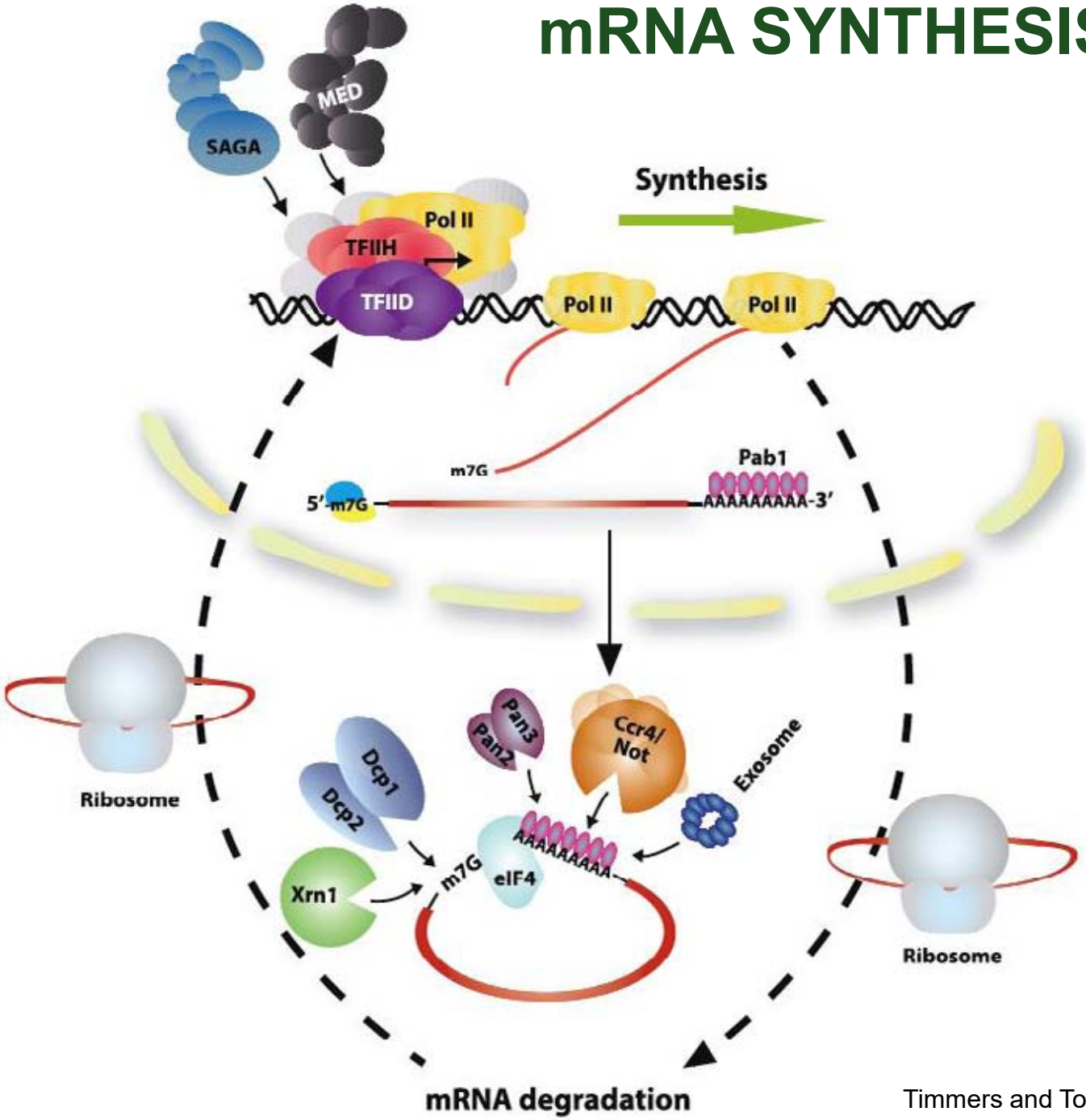
Pirouz et al., Cell Rep, 2016;
Ustianenko et al., EMBO, 2016

mRNA SYNTHESIS and DECAY



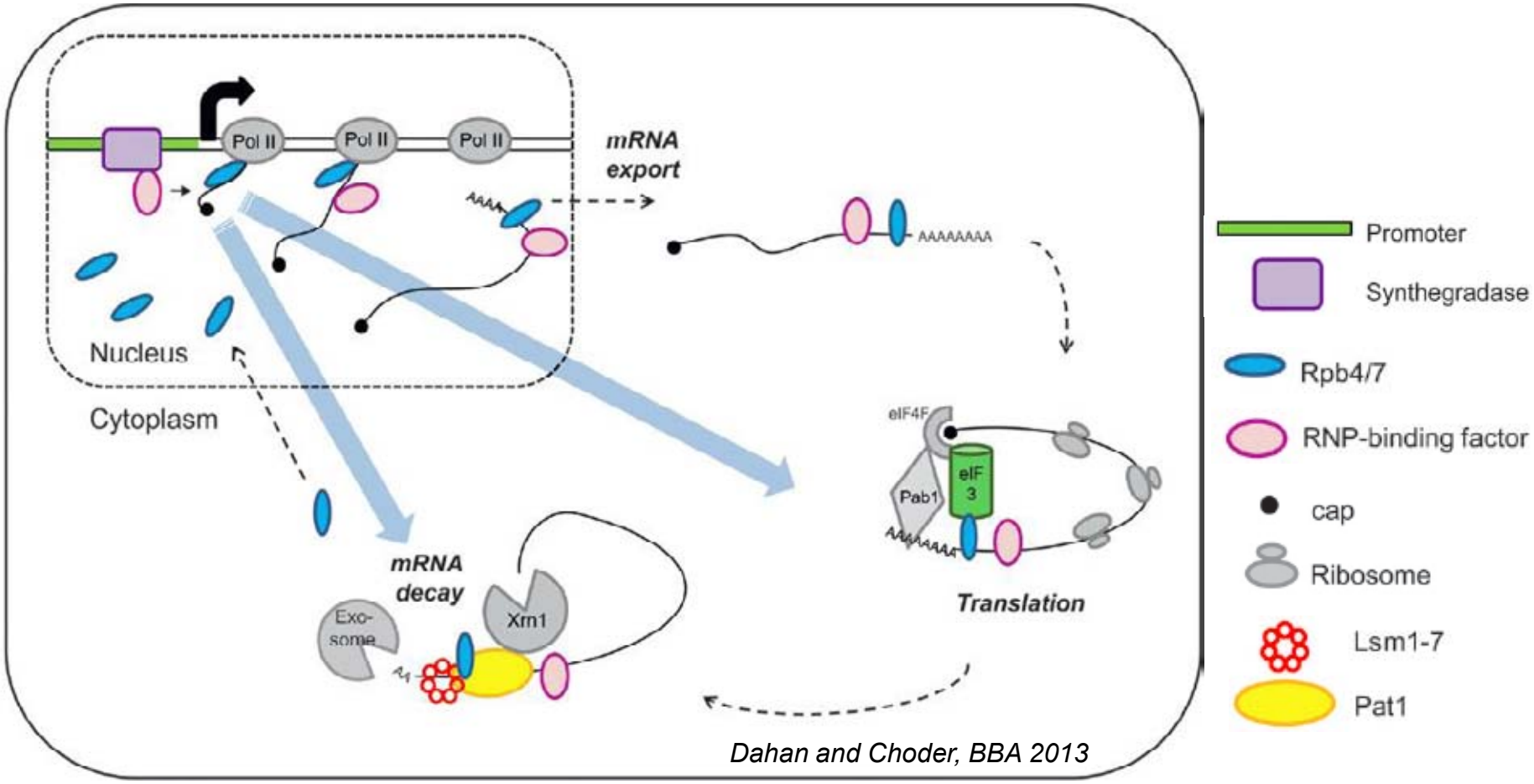
Kervestin and Jacobson, *Nat Rev Mol Cel Biol,2012*

mRNA SYNTHESIS and DECAY



Timmers and Tora, *Mol Cel,2018*

Coupling between transcription and mRNA decay



Dahan and Choder, BBA 2013

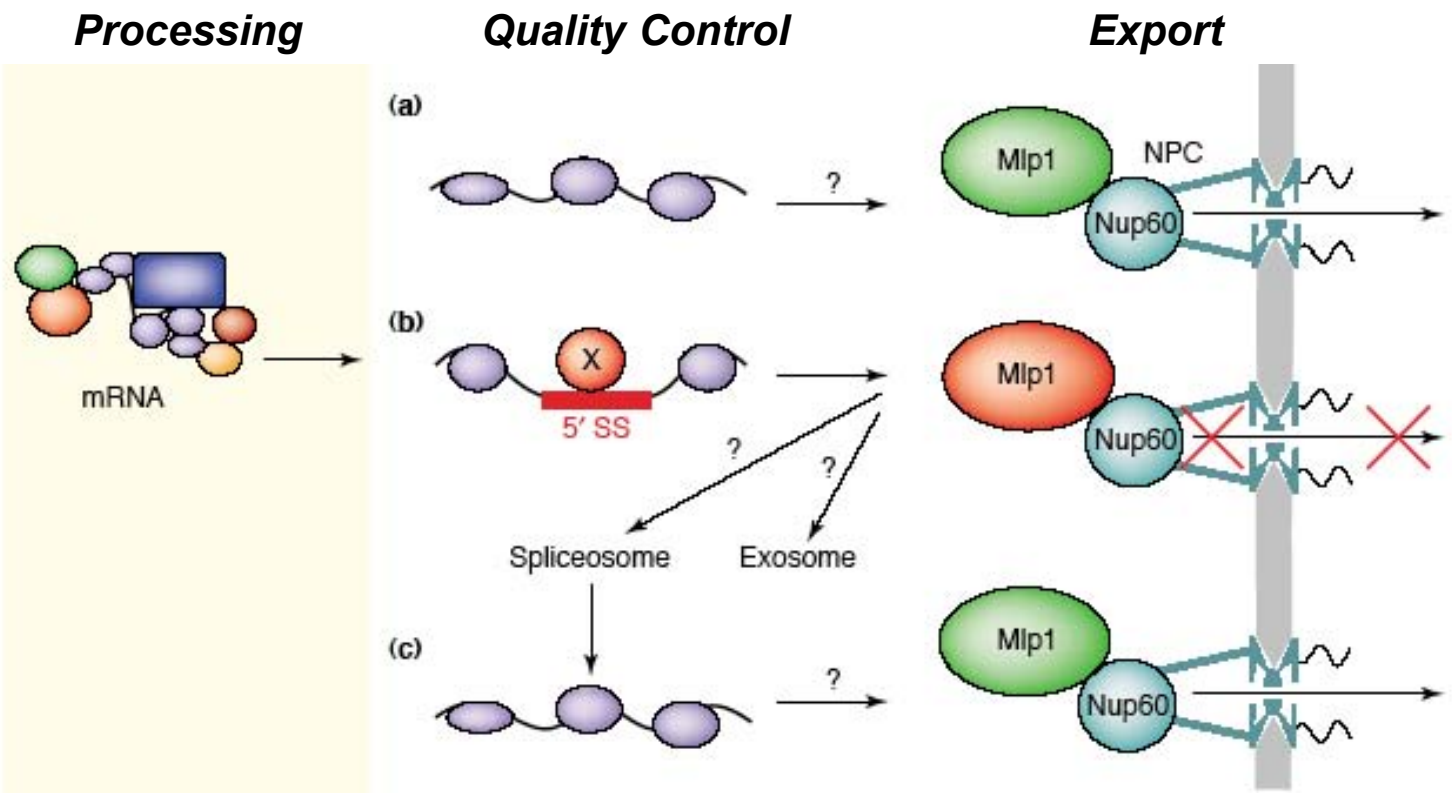
Transcriptional machinery regulates mRNA translation and decay in the cytoplasm

- PolII and promoters regulate cytoplasmic post-transcriptional stages

- Rpb4/7 subunits of PolII regulates trx initiation, elongation and polyadenylation by binding to the emerging transcript and remaining associated throughout its lifecycle: *(i)* mRNA export; *(ii)* translation initiation via interaction with eIF3; *(iii)* deadenylation and decay by Xrn1 and exosome via interaction with Pat1/Lsm1-7 complex

mRNA DECAY in the NUCLEUS

- **nuclear retention of intron-containing pre-mRNAs**

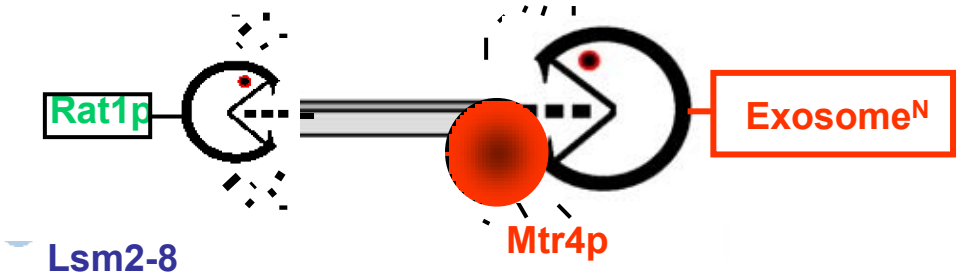


X **RES complex** (**RE**tention and **S**plicing): **Snu17/Bud13/Pml1**

Casolari and Silver, TiCB., 2004

mRNA DECAY in the NUCLEUS

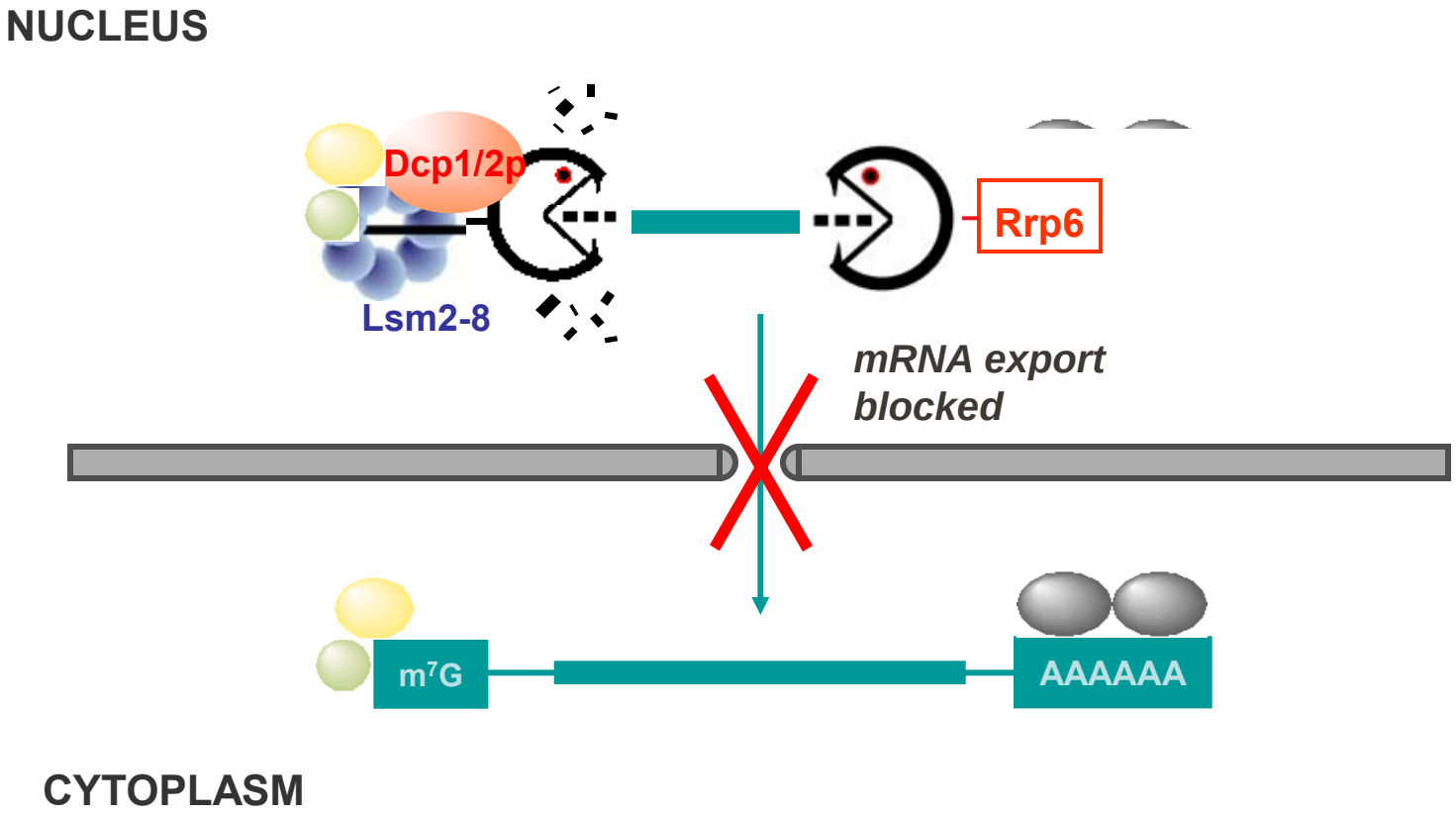
- **pre-mRNA with unspliced introns**



Bousquet-Antonelli et al., *Cell* 2000; Danin-Kreiselman et al., *Moll. Cell* 2003

mRNA DECAY in the NUCLEUS

- mRNA arrested in the nucleus

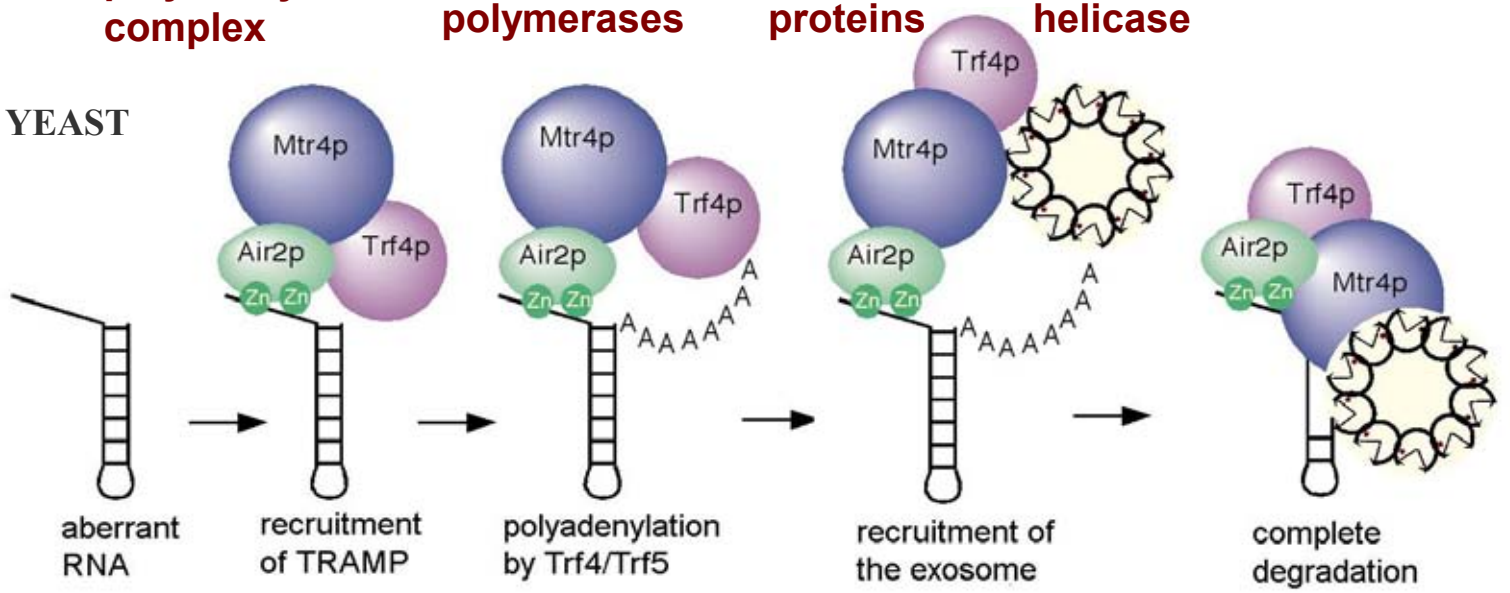


Hilleren et al., *Nature,* 2001; Das et al., *Mol.Cell. Biol.* 2003; Kufel et al., *Mol.Cell. Biol.* 2004, Milligan et al., *Mol.Cell. Biol.* 2008

TRAMP - EXOSOME COFACTORS (yeast)

TRAMP = Trf4/5 + Air1/2 + Mtr4

polyadenylation complex — **poly(A) polymerases** — **RNA binding proteins** — **RNA DEVH helicase**



some RNAs degraded by Rat1/Xrn1

Polyadenylation-mediated nuclear discard pathway for defective RNAs

- **hypomodified tRNAs**
- **CUTs (Cryptic Unstable Transcripts)**
- **ncRNAs: sn/snoRNAs, rRNAs, some mRNAs**

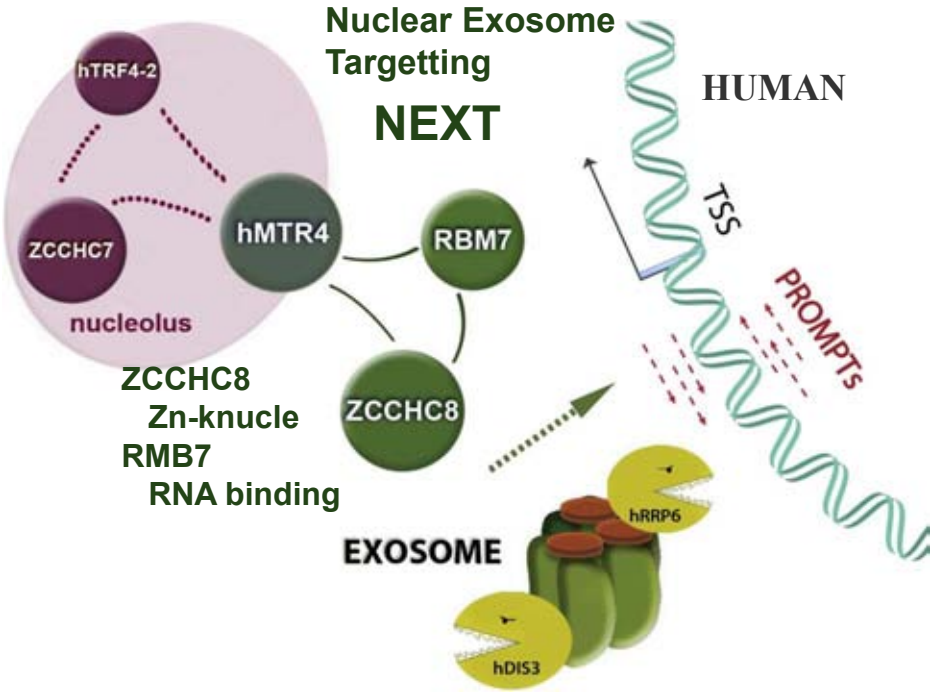
Interacts with

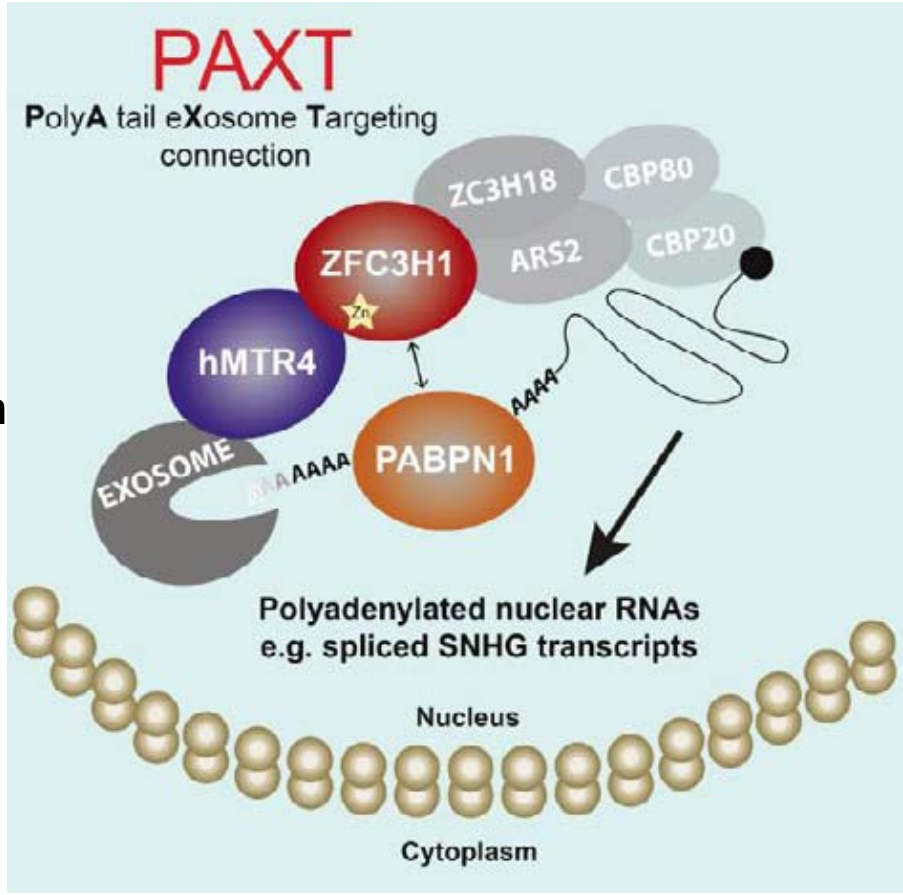
- exosome via Mtr4

- Nrd1/Nab3 complex

LaCava et al., *Cell,* 2005; Vanacova *et al., PLoS Biol.* 2005; Wyers *et al., Cell,* 2005; Lubas et al. *Mol. Cell*, 2011

NEXT and PAXT - EXOSOME COFACTORS (humans)

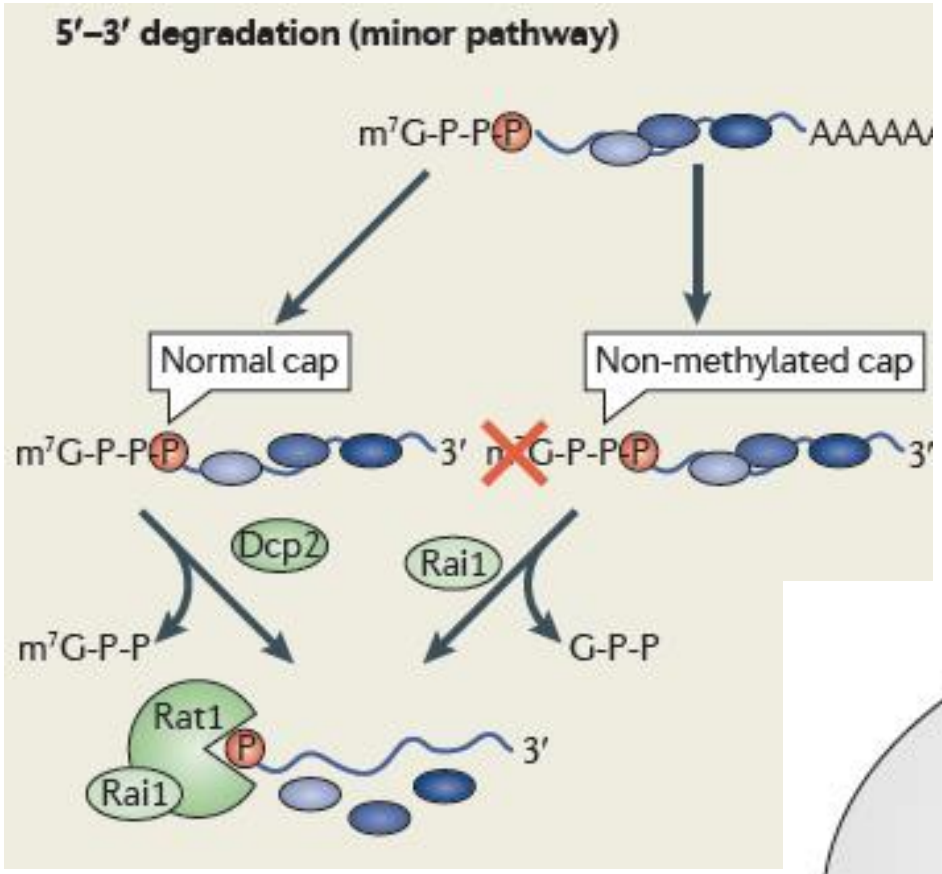




- **ZFC3H1 (Zn-knuckle protein) links MTR4 with PABPN1 in PAXT**
- **ZFC3H1/PABPN1 and RBM7/ZCCHC8 (NEXT) interact with MTR4 in a mutually exclusive manner**
- **PAXT and NEXT direct distinct RNA species for nuclear exosome degradation**
- **PAXT targets tend to be longer and more extensively polyadenylated than NEXT targets**

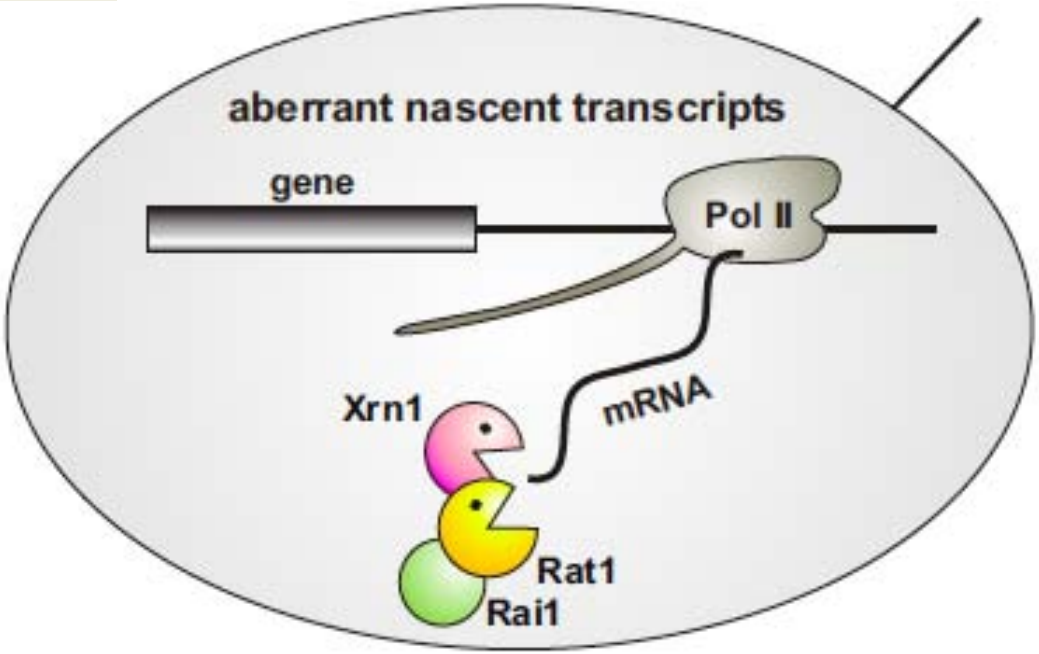
Lubas et al. *Mol. Cell*, 2011; Meola et al., . *Mol. Cell*, 2016

Rat1 - NUCLEAR RNA SURVEILLANCE 5'-3'



Rat1/Xrn2

- **decay of transcripts with aberrant cap structure**
- **degradation of prematurely terminated nascent transcripts**
- **degradation of readthrough transcripts**



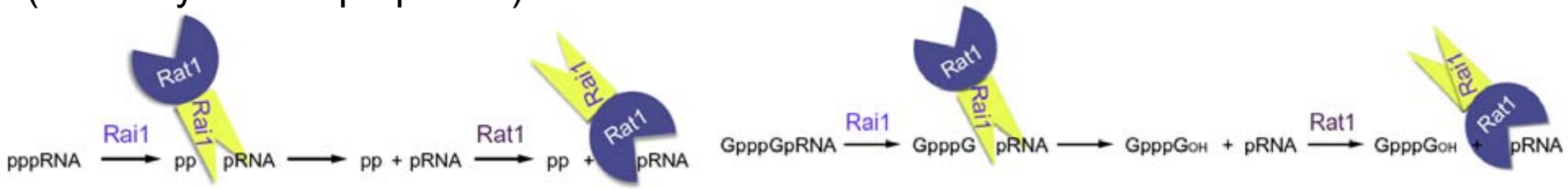
Tutucci and Stutz., Nat. Rev. Mol. Cel. Biol. , 2011

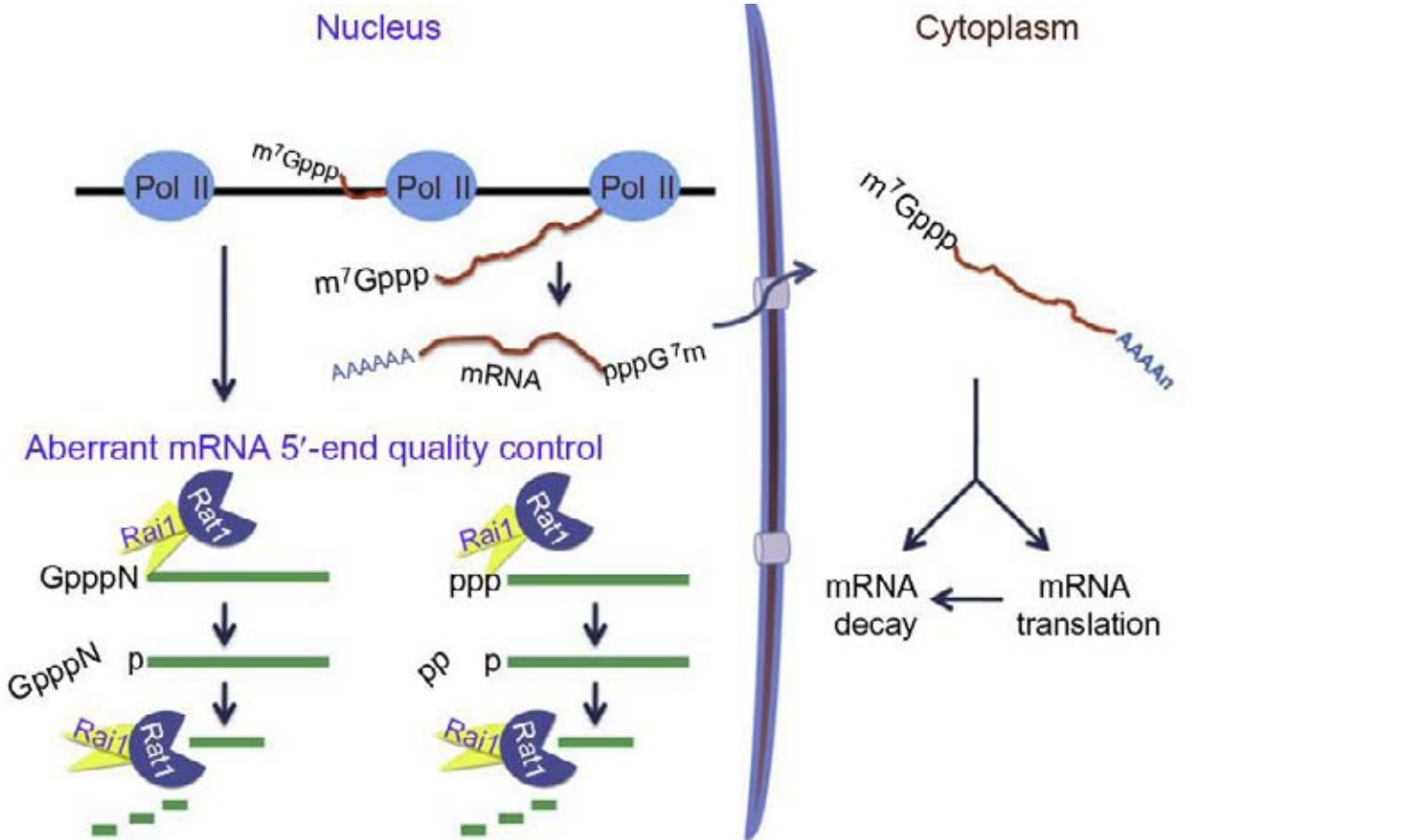
CAP NUCLEAR RNA SURVEILLANCE 5'-3'

S. cerevisiae Rai1 – Rat1 activator

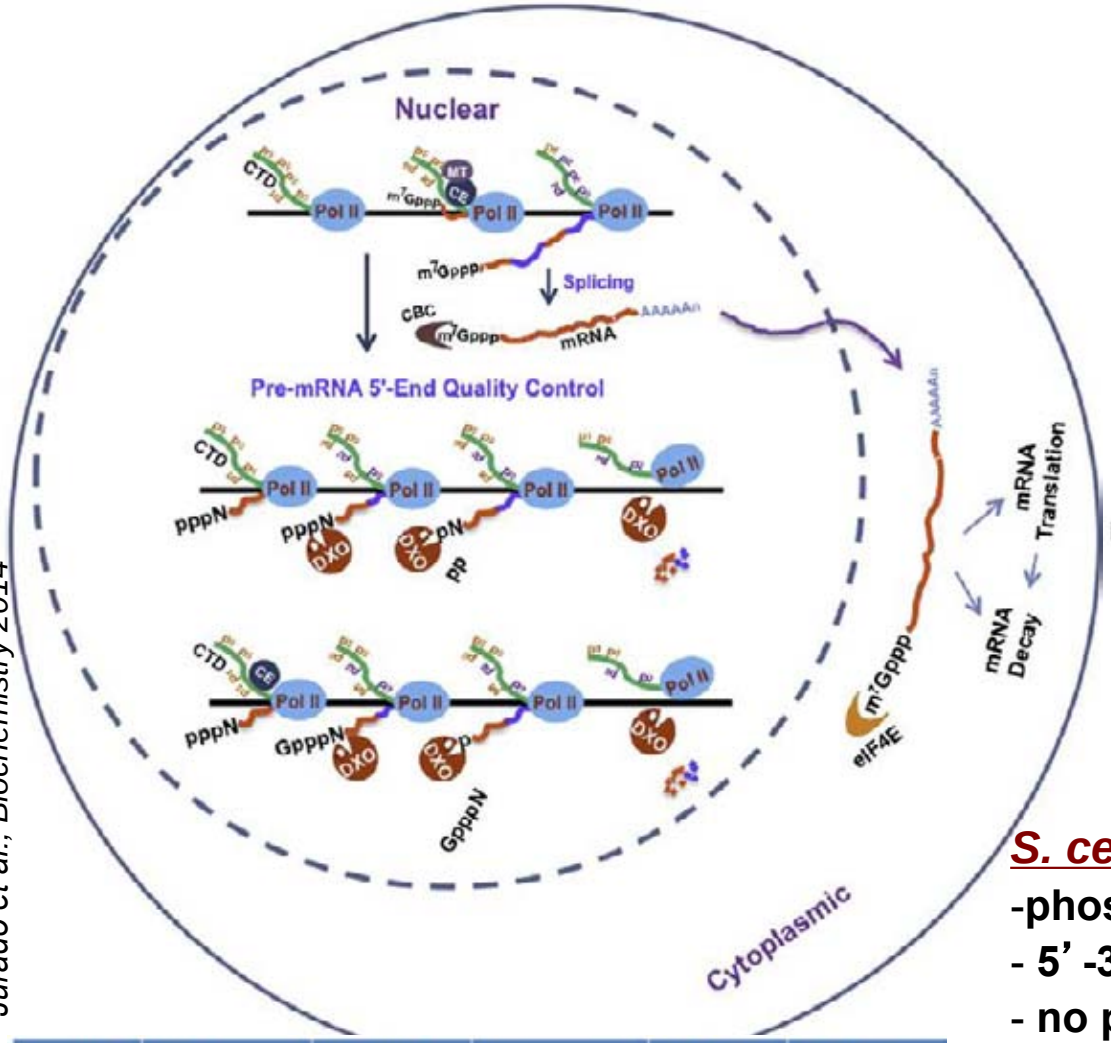
5' -ppp pyrophosphohydrolase and phoshodiesterase-decapping nuclease

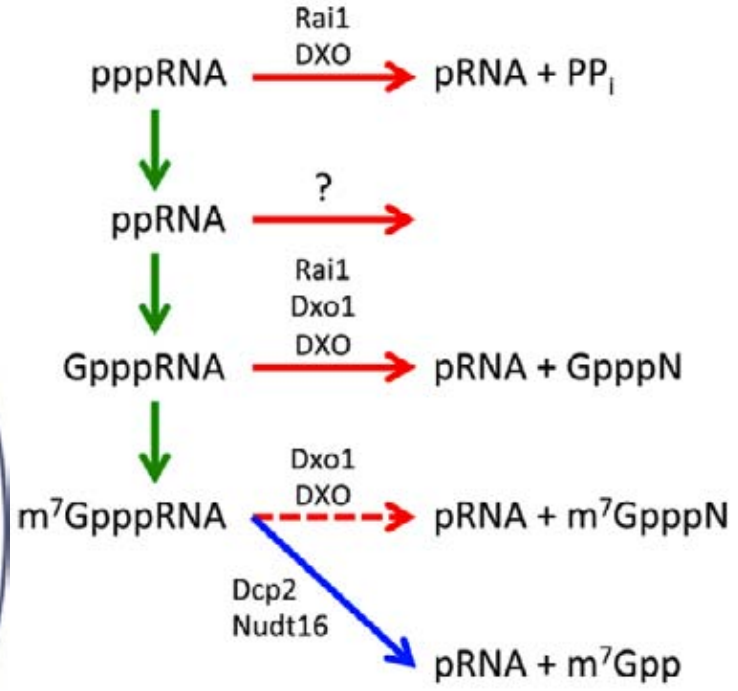
(unmethylated cap-specific)





CAP NUCLEAR RNA SURVEILLANCE 5'-3'





Jurado et al., Biochemistry 2014

Activity substrate product	PPH ppp-RNA PPi + pRNA	decapping Gppp-RNA GpppN+pRNA	decapping m7Gppp-RNA m7GpppN+pRNA	5'-3' Exo pRNA pN+pRNA	deNADing $N_n$ppAp-RNA $N_n$ppA+pRNA
ScRai1	-	+/-	-	-	nd.
ScDxo1	-	++	++	++	++
SpRai1	+	+	-	-	++
MmDXO	++	++	++	++	+++

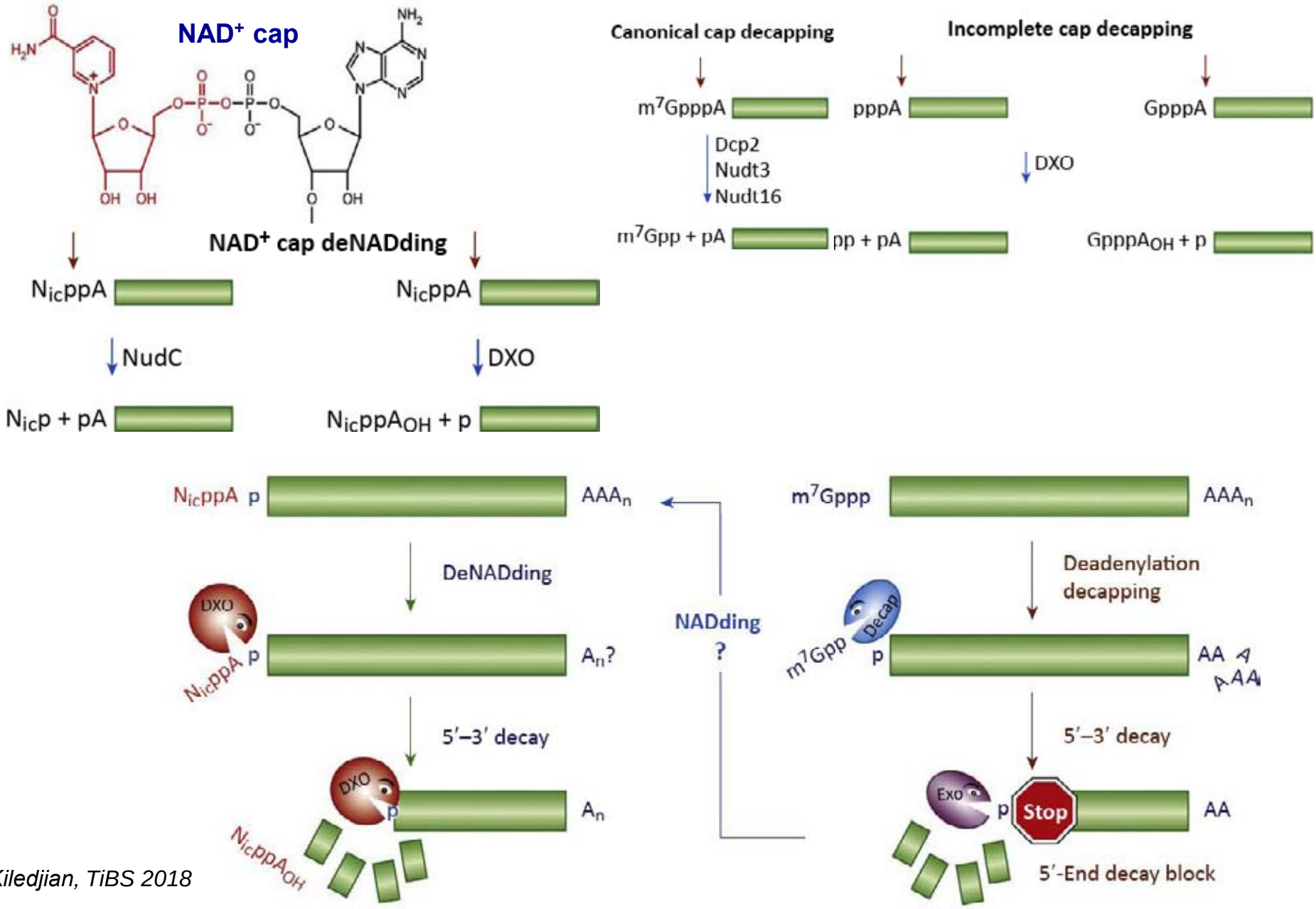
S. cerevisiae Dxo1

- phoshodiesterase -decapping nuclease
- 5' -3' exonuclease
- no pyrophosphohydrolase

Human DXO

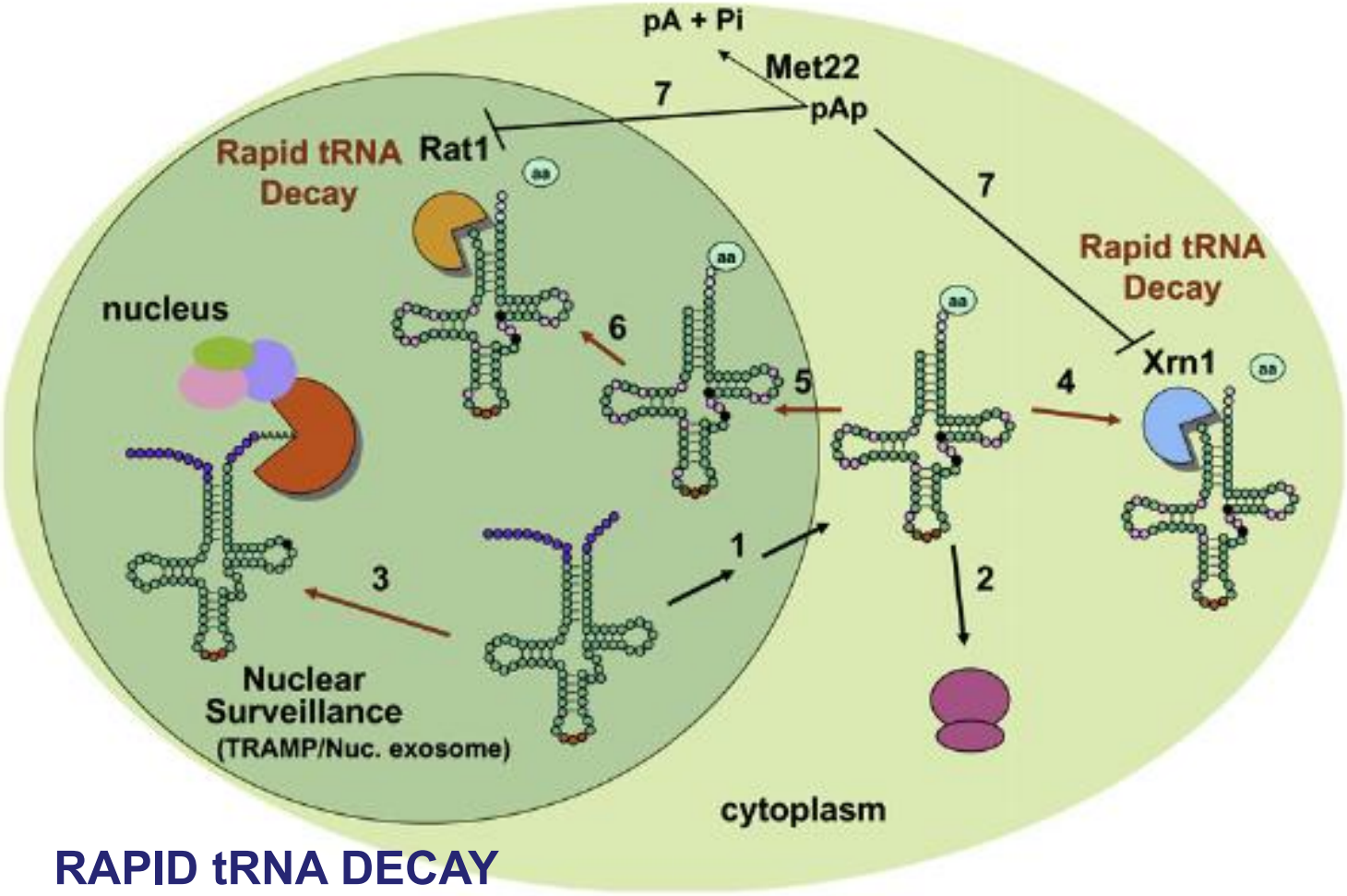
- 5' ppp pyrophosphohydrolase
- phoshodiesterase -decapping nuclease
- 5' -3' exonuclease

RNA 5'-end NAD+ capping and deNADding



Kiledjian, TiBS 2018

tRNA SURVEILLANCE



RAPID tRNA DECAY

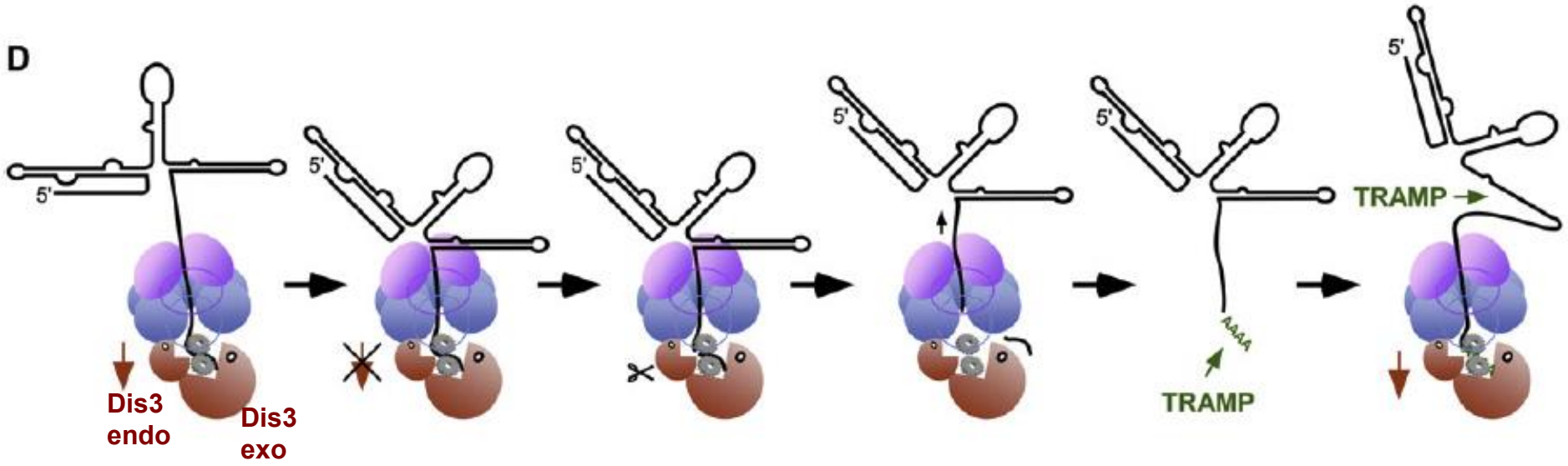
• occurs for precursors and mature tRNAs with mutations which destabilize tertiary structure (modifications)

- in the nucleus *(polyadenylation via TRAMP and degradation by the exosome or degradation by Rat1)*

- in the cytoplasm *(degradation by Xrn1)*

Phizicky and Hopper , GeneGev.,2010

pre-tRNAs are DEGRADED by the EXOSOME



Important contribution of the endo Dis3 activity to the degradation of structured substrates

Molecular Cell 2012

Transcriptome-wide Analysis of Exosome Targets

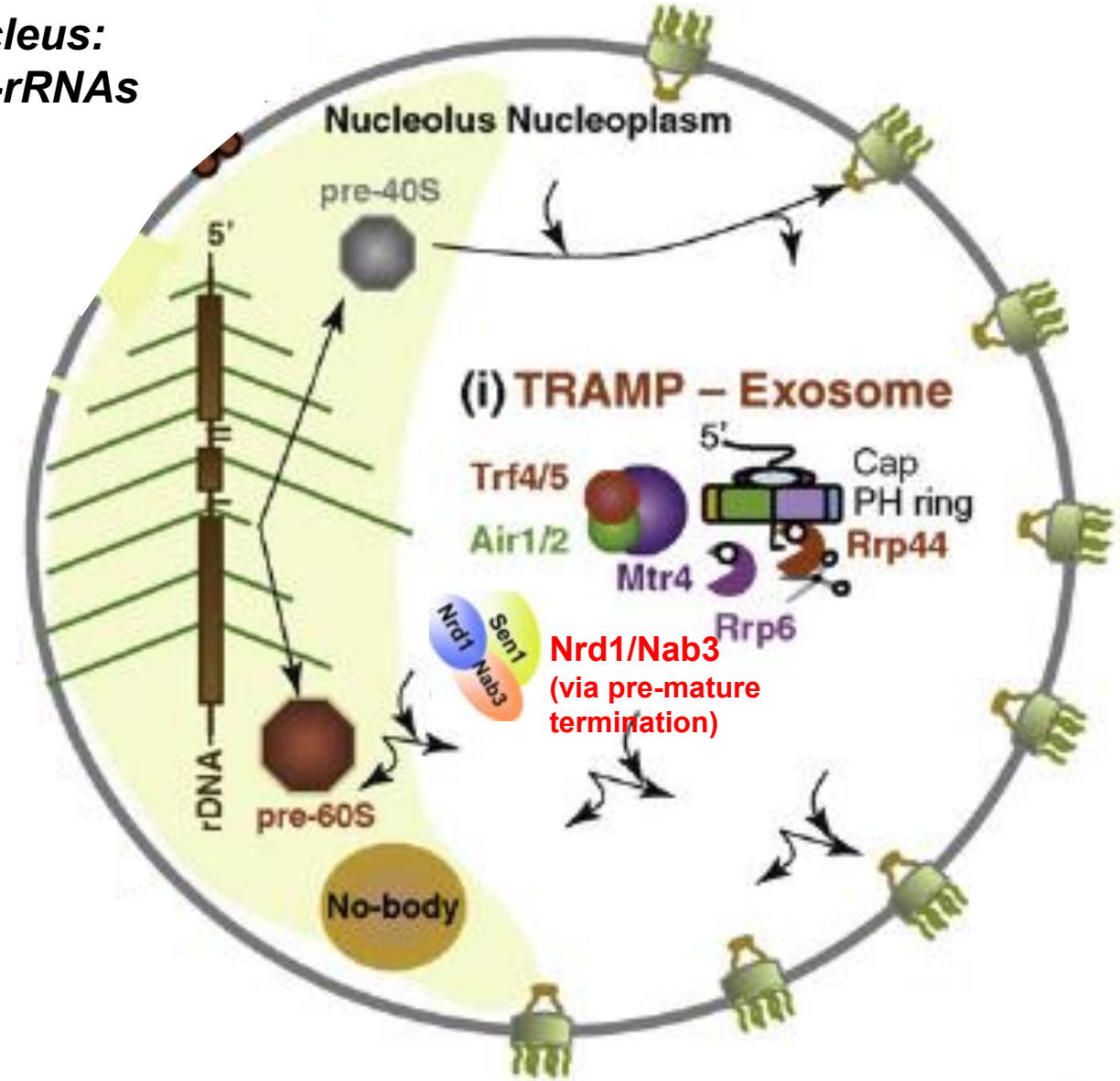
Claudia Schneider,[1,2,*] Grzegorz Kudla,[1,3] Wiebke Wlotzka,[1] Alex Tuck,[1] and David Tollervey[1,*]

Extensive Degradation of RNA Precursors by the Exosome in Wild-Type Cells

Rajani Kanth Gudipati,[1,3] Zhenyu Xu,[2] Alice Lebreton,[1,5,6] Bertrand Séraphin,[5] Lars M. Steinmetz,[2] Alain Jacquier, and Domenico Libri[1,*]

rRNA SURVEILLANCE

Nucleus:
pre-rRNAs

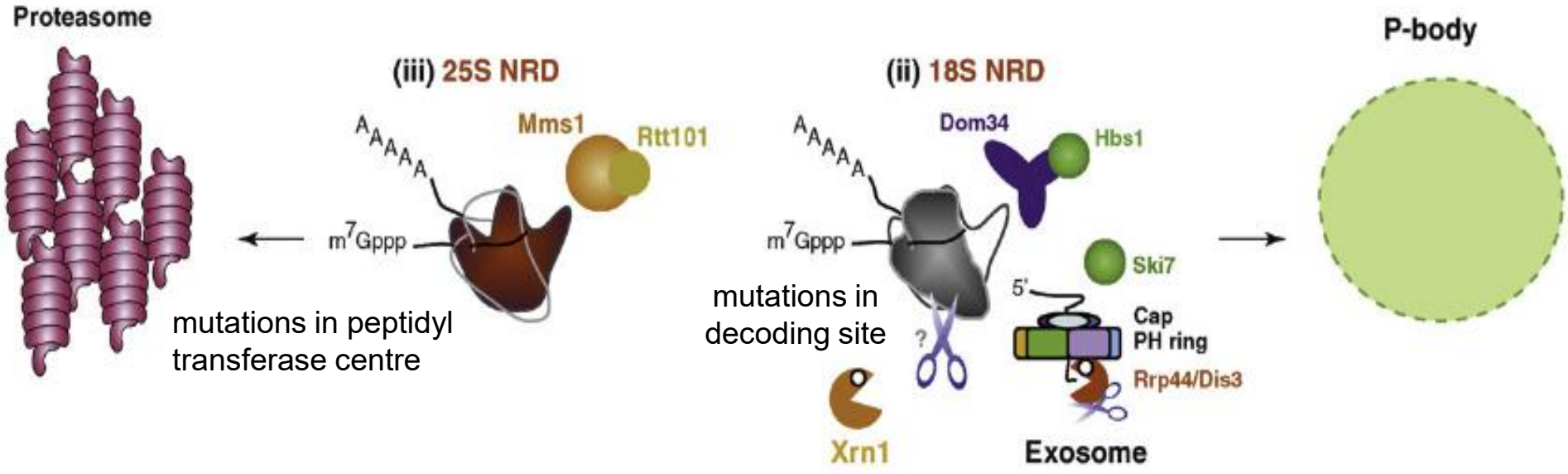


Lafontaine, TiBS.,2010

rRNA SURVEILLANCE

NRD- nonfunctional rRNA decay

Cytoplasm:
mature ribosomes

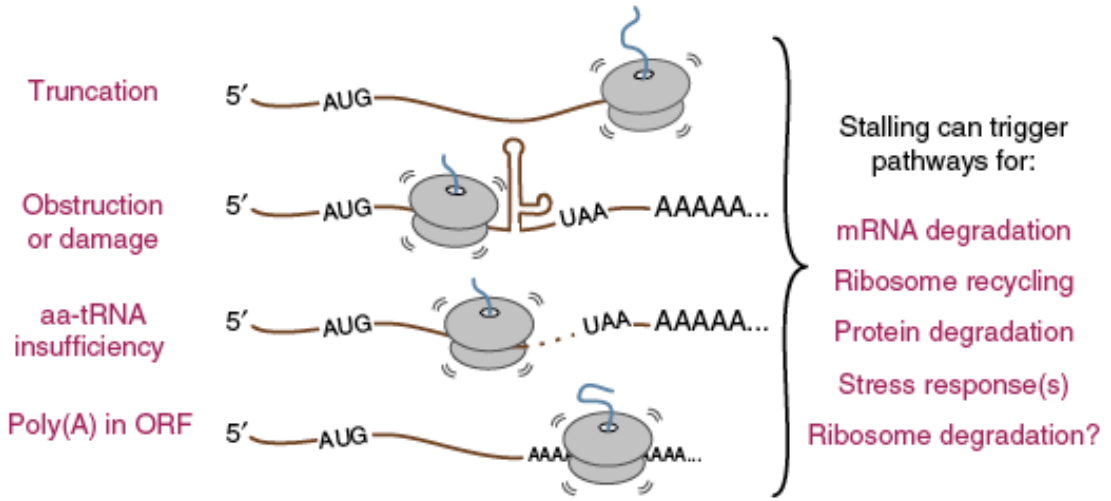


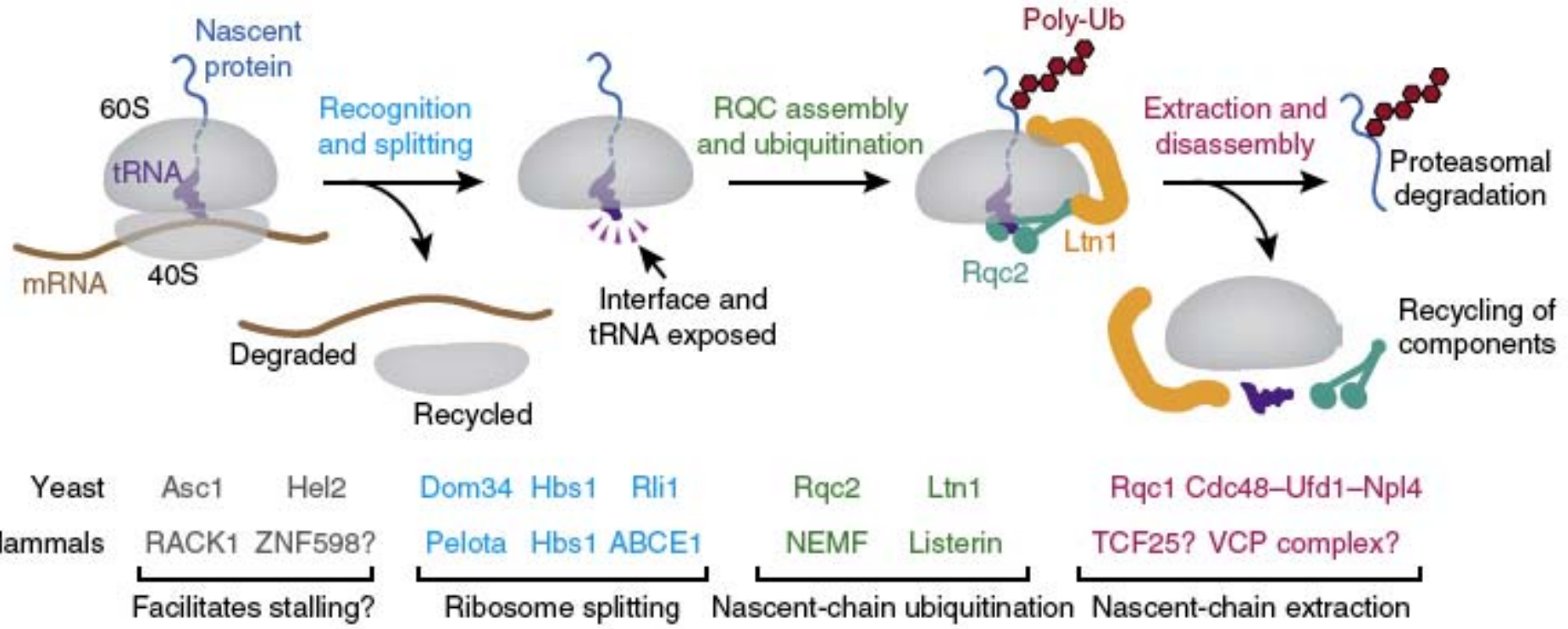
Mms1, Rtt101-
subunits of E3 ubiquitin ligase complex

Dom34::Hbs1
factors involved in NGD and NSD

Lafontaine, TiBS.,2010

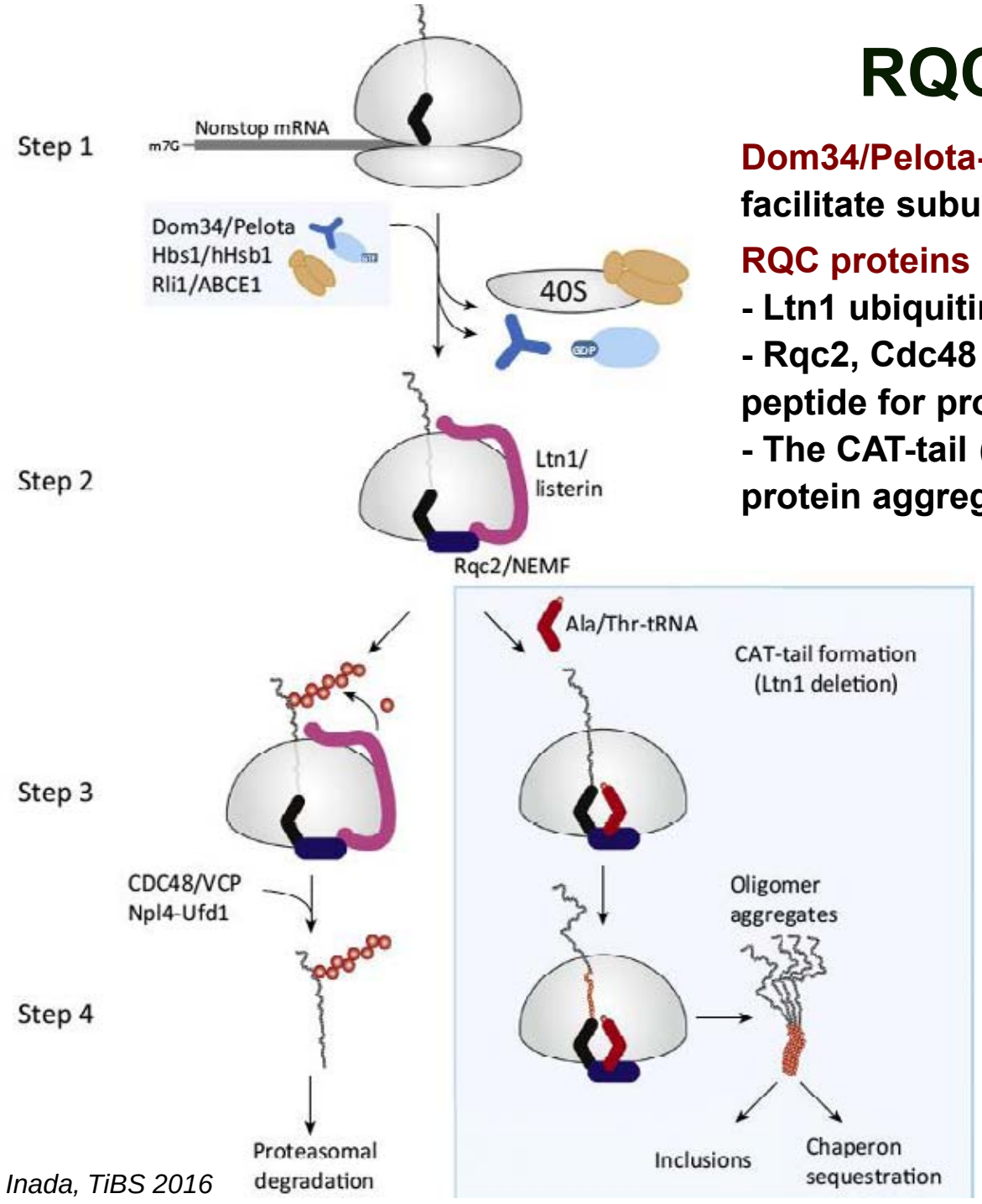
RIBOSOME QC (RQC): Rescuing stalled ribosomes





Brandman and Hegde, Nat. Struct. Mol. Biol. 2016

RQC mechanism

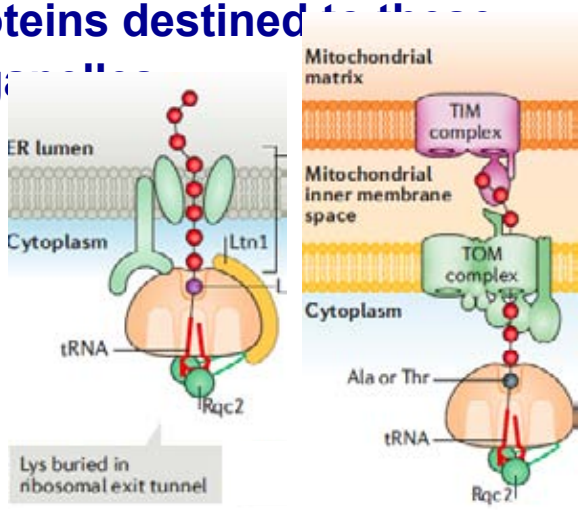


Inada, TiBS 2016

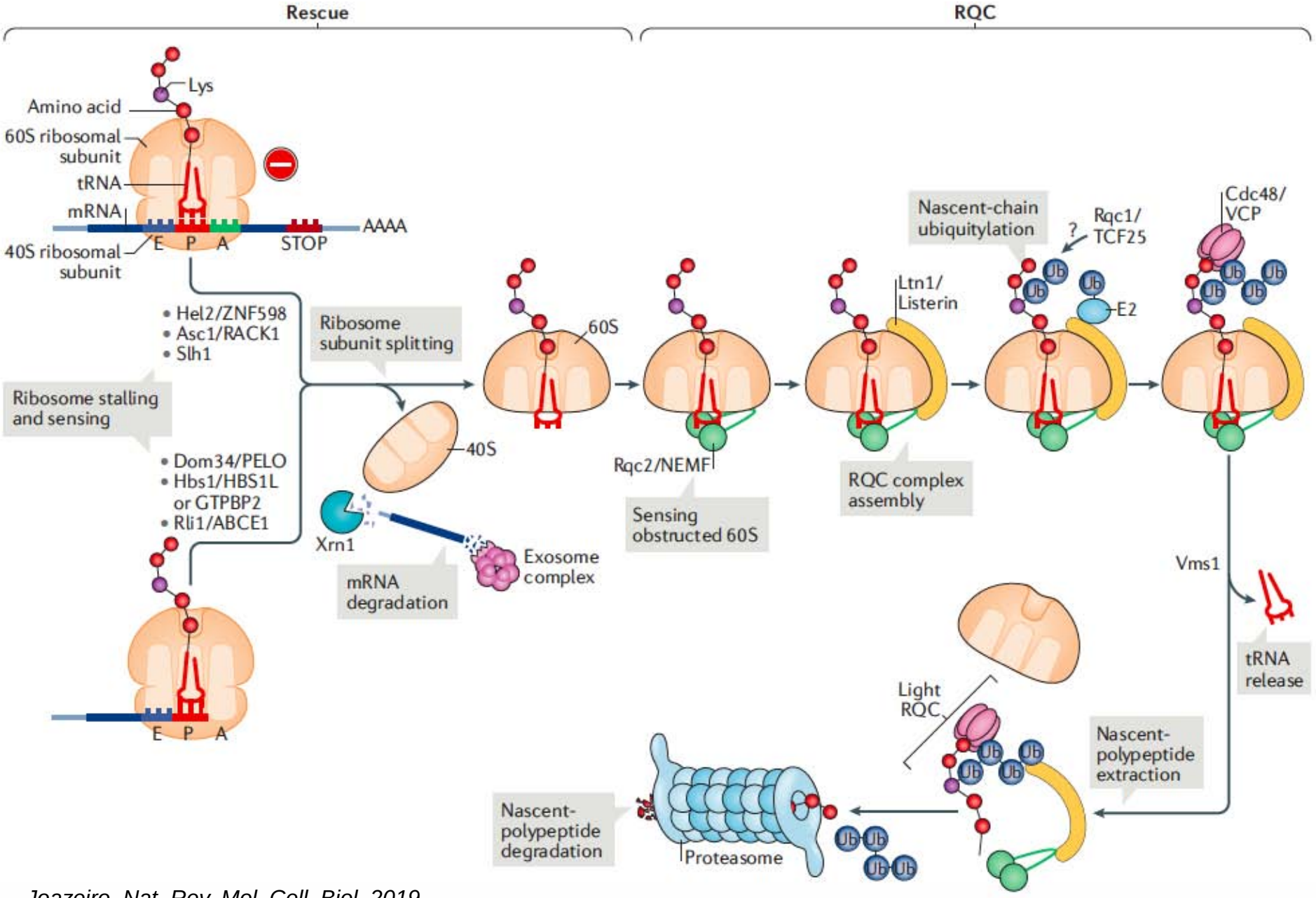
Dom34/Pelota-Hbs1
facilitate subunit dissociation of stalled ribosomes

RQC proteins assemble on 60S
- Ltn1 ubiquitinates the nascent peptide
- Rqc2, Cdc48 and cofactors remove nascent peptide for proteasomal degradation
- The CAT-tail (Ala and Thr extension) mediates protein aggregation and induces stress response

RQC occurs also on the ER and mitochondrial membranes for ribosomes stalled while translating proteins destined to these organelles



RQC mechanism

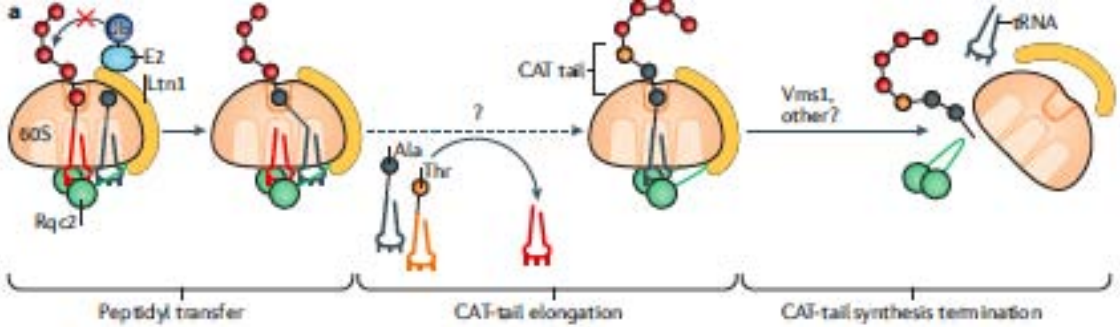


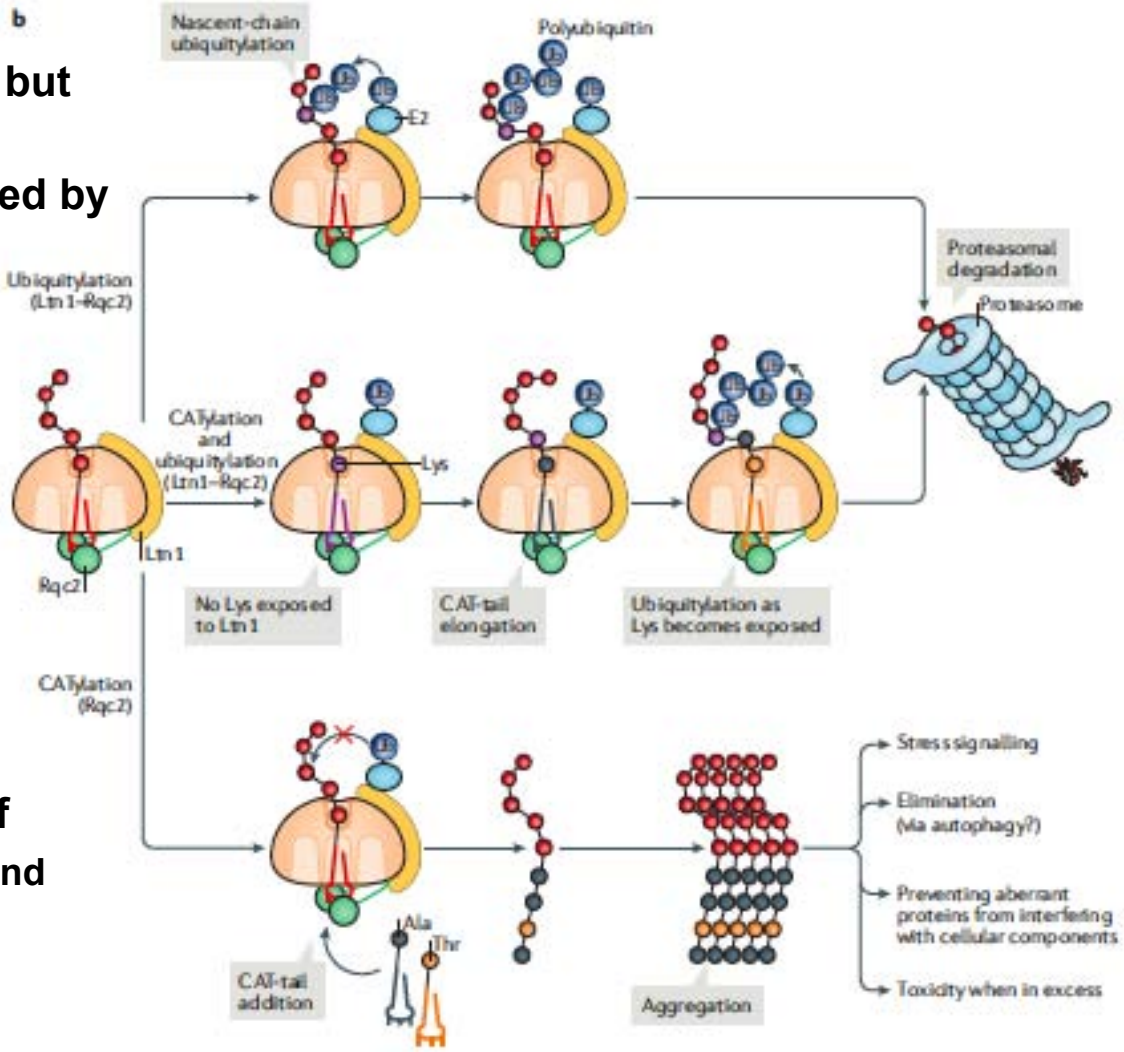
Joazeiro, Nat. Rev, Mol. Cell. Biol. 2019

RQC
CAT tailing

CAT tail: C - terminal untemplated Ala and Thr tail

The canonical RQC is preferred but ubiquitylation of the nascent polypeptide fails CAT tail is added by Rqc2 to rescue the trapped polypeptide when

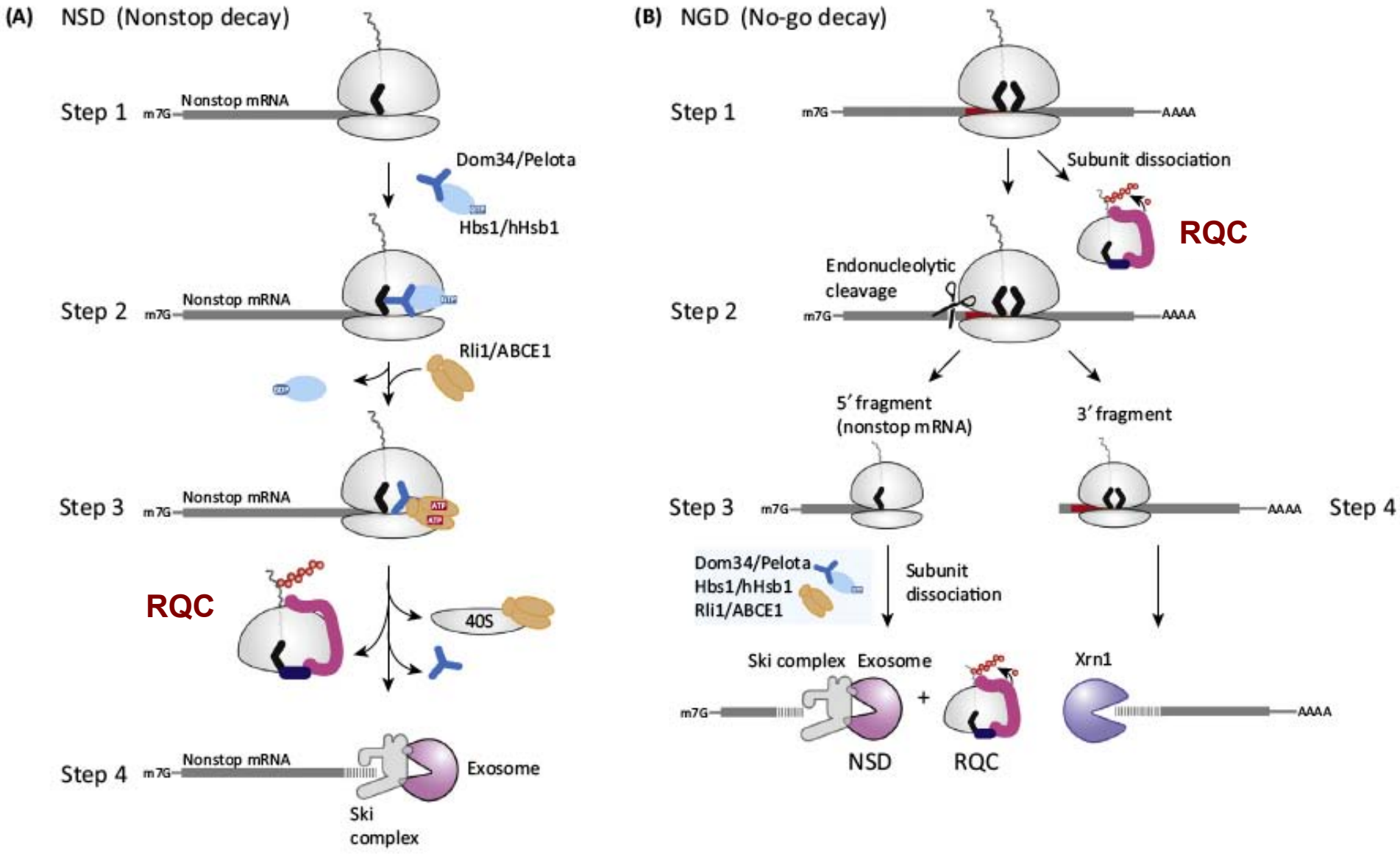




CATylation results in
- **Ltn1-dependent degradation of aberrant proteins (Lys exposure and ubiquitylation)**
- **nascent chain aggregation**
- **activation of stress signaling**

Joazeiro, Nat. Rev, Mol. Cell. Biol. 2019

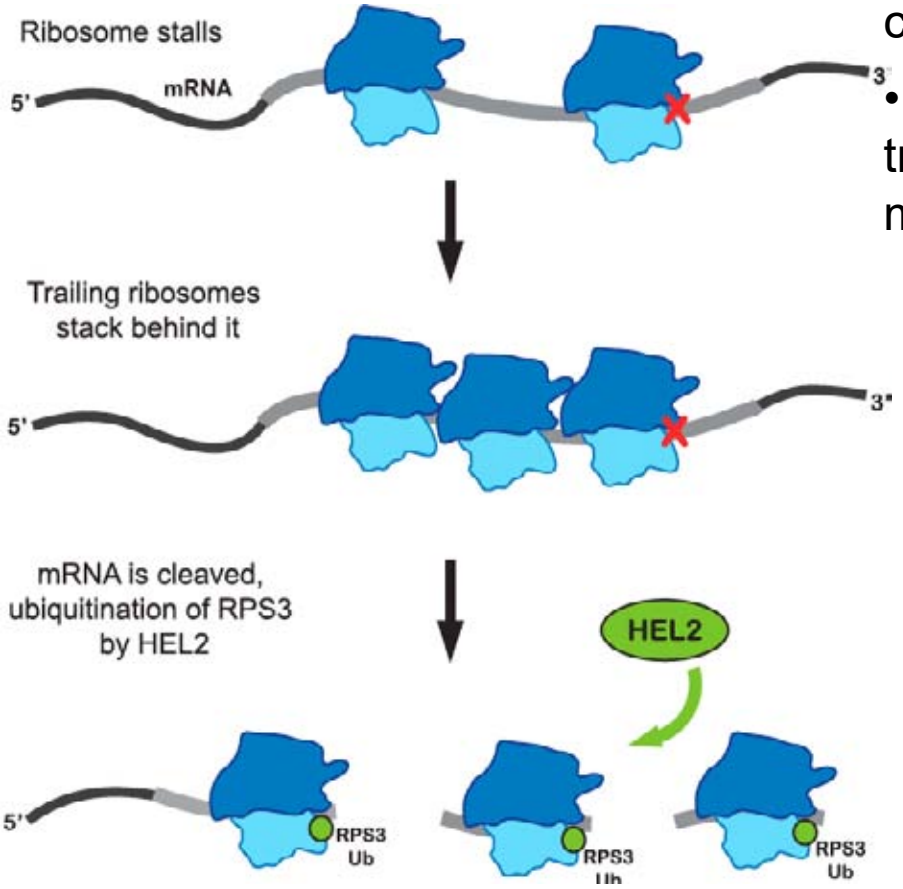
RQC in NSD and NGD



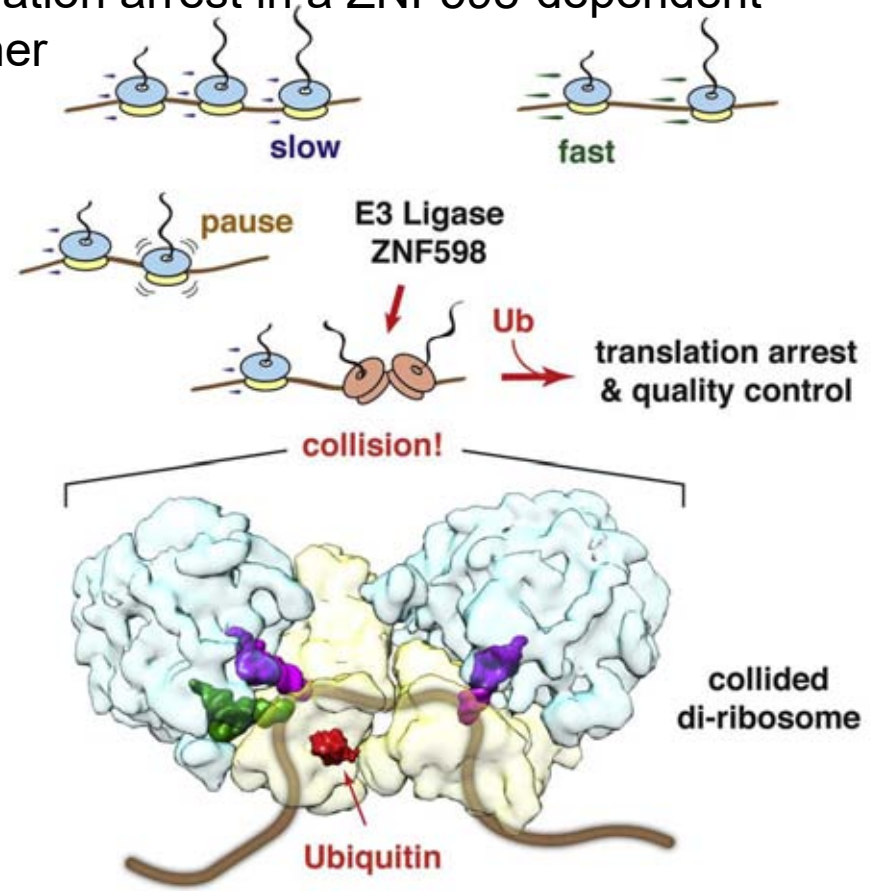
Inada, TiBS 2016

Ribosome collision in RQC during NGD

- Stacked or colliding ribosomes are required to elicit NGD
- Ubiquitination of RPS3 by HEL2 triggers RQC

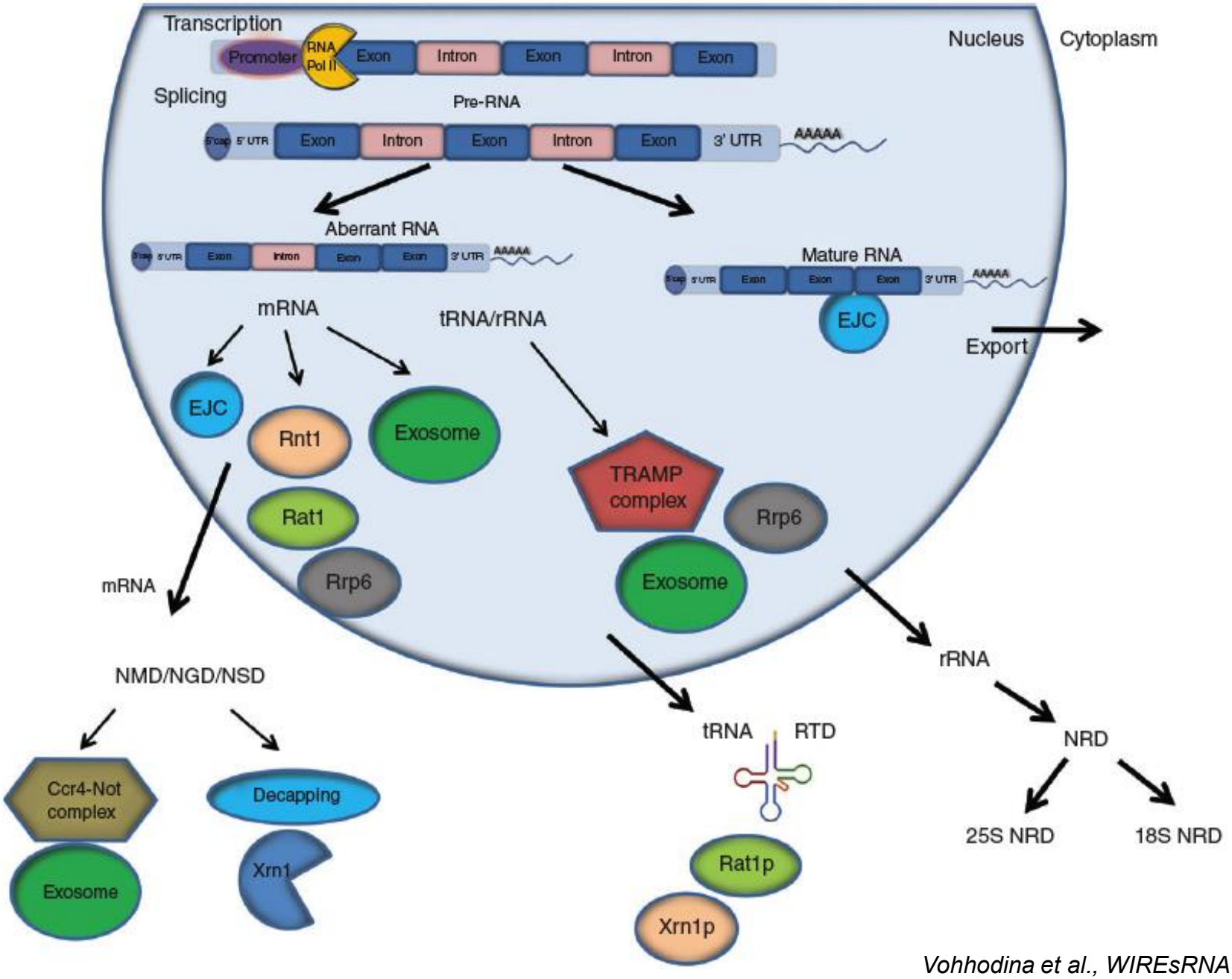


- RQC during aberrant translation/ribosome collisions is initiated by ubiquitin ligase ZNF598
- Collided di-ribosomes is a minimal target for translation arrest in a ZNF598-dependent manner



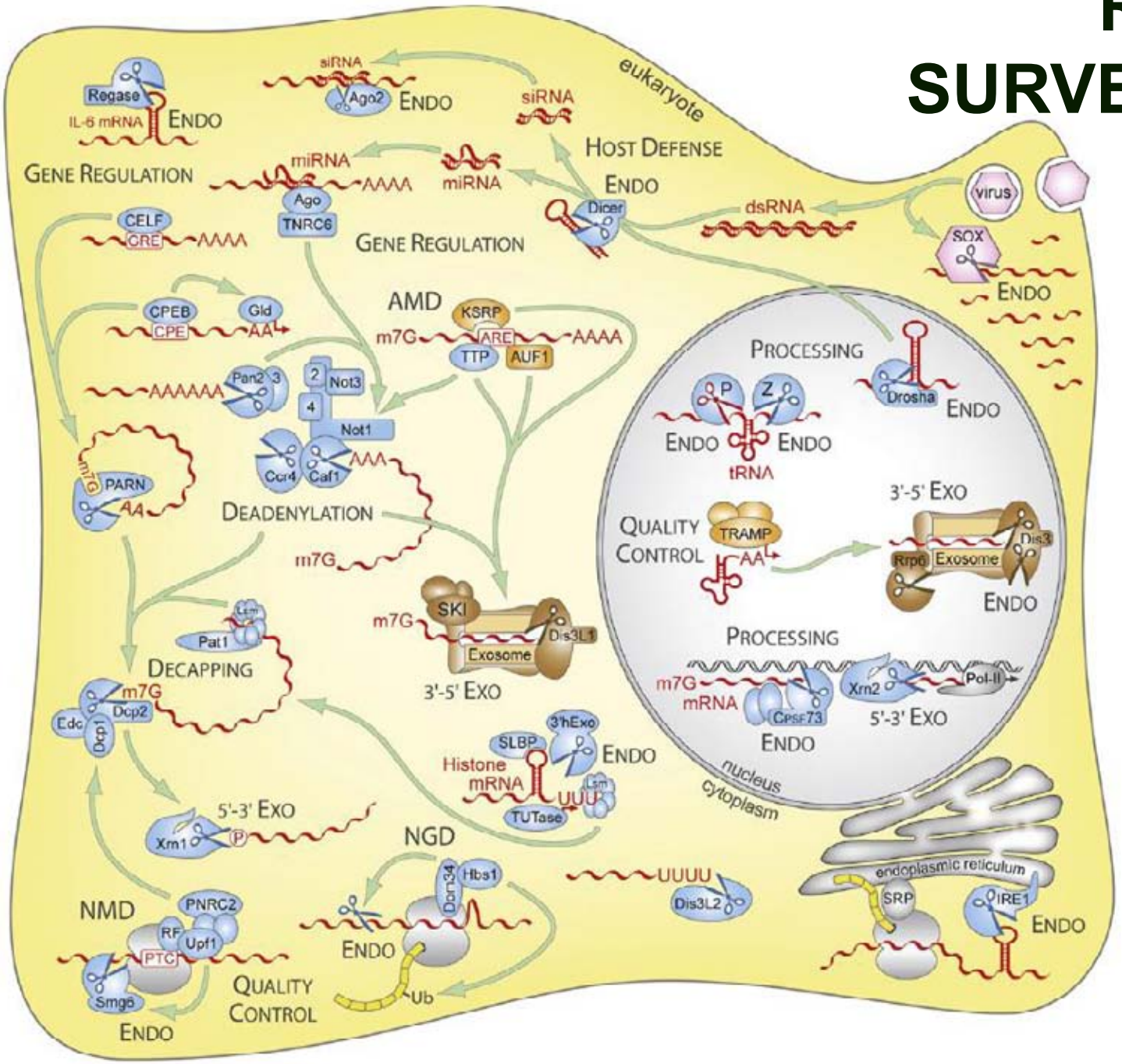
Simms et al, Mol Cell 2017; Juszkiewicz et al, Mol Cell 2018

RNA SURVEILLANCE

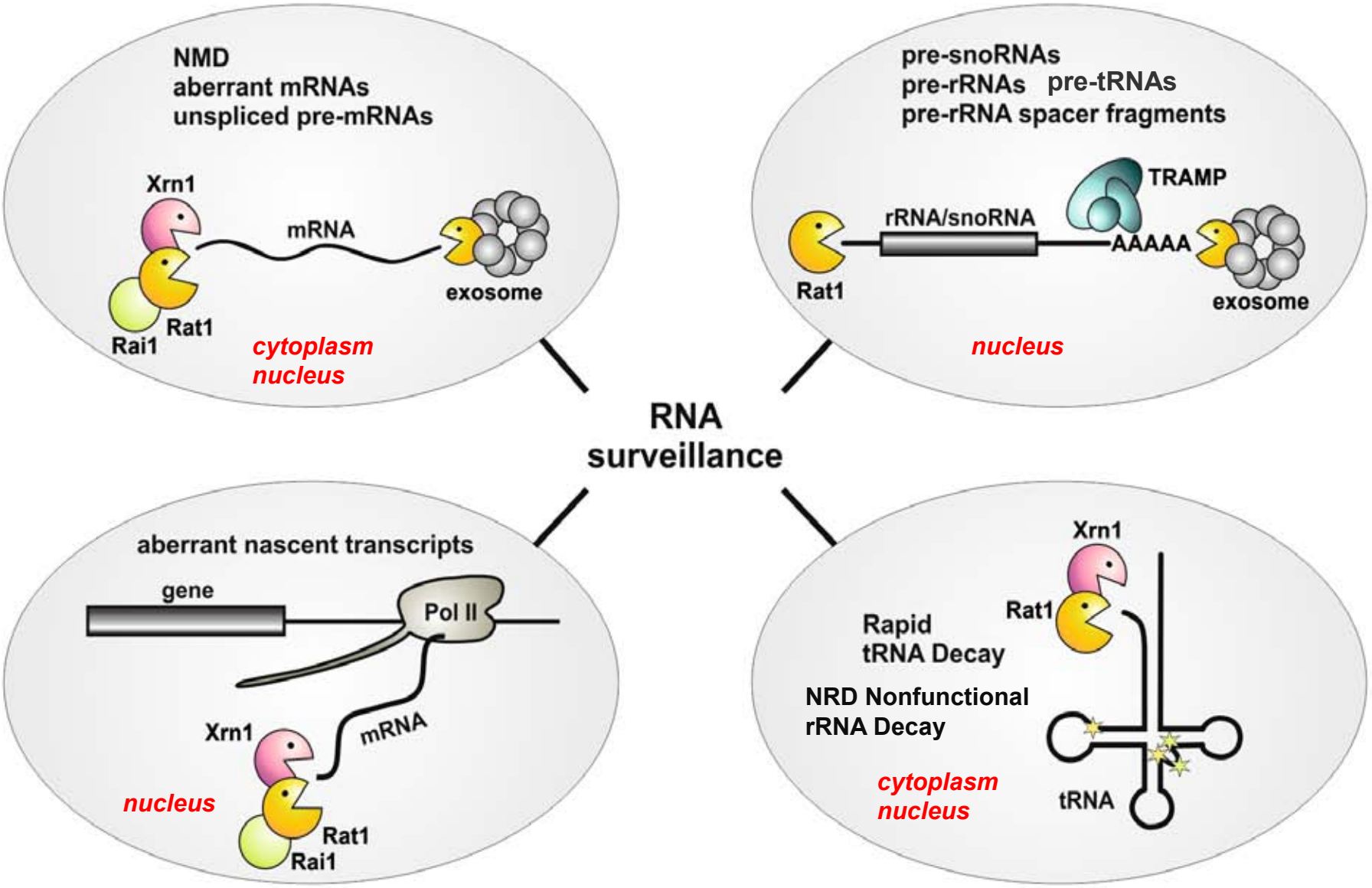


Vohhodina et al., WIREsRNA'16

RNA SURVEILLANCE



RNA SURVEILLANCE



Tuttuci and Stutz., *Nat. Rev. Mol. Cel. Biol.* , 2011

TAKEHOME MESSAGE

I. RNA WORLD

• **hypothesis – life started from prebiotic soup via self-sufficient RNA to DNA/RNA/protein world**

• **RIBOZYMES - catalytic RNAs, active without proteins**

- 2'-OH, Mg^{2+} , H_2O, nucleophilic attack

- self splicing introns, RNAse P RNA (bacterial, archaeal)

• **almost catalytic RNAs- SPLICEOSOME, RIBOSOME**

• **SELEX – procedure to select molecules with desired function**

• **RNA NOBELS: 1989 RIBOZYMES, 1993 SPLICING, 2006 RNAi, 2009 telomerase, ribosome structure**

TAKEHOME MESSAGE

II. MODERN RNA WORLD

- **replication (telomerase RNA, RNA primers)**
- **transcription regulation (ncRNAs, siRNA)**
- **RNA processing (snRNAs for pre-mRNA, snoRNA for pre-rRNA, gRNA for editing, RNAseP for pre-tRNA RNAseMRP for pre-rRNA)**
- **RNA stability (sRNAs, si/miRNAs)**
- **translation regulation (ncRNAs, miRNA)**
- **translation (rRNA, tRNA, mRNA)**
- **protein translocation (signal recognition particle)**

- **GENE EXPRESSION regulated at each step: transcription, processing (splicing, 3' end formation), RNP assembly, export, RNA decay/RNA surveillance, translation, protein stability**

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III. RNA METABOLISM

A.SYNTHESIS: 3 to 5 RNA polymerases, each makes specific RNAs

Pol I (rRNA); Pol II (mRNA, sn/snoRNA, CUT, miRNA); Pol III (5S rRNA, U6 snRNA, tRNA, other); Pol IV/V (siRNA pathway)

B. PROCESSING – all RNAs are processed from precursors and assembled into RNP structures

- **transcription termination**

- unified allosteric-torpedo model (Rat1 5'-3' exo)

- 3' cleavage and polyadenylation machinery (mRNA)

- Nrd1/Nab3/Sen1 mechanism (sn/snoRNA, CUT, short mRNA)

- Reb1, Rat1, Rnt1, Nrd1/Nab3/Sen1 (rRNA, and others)

- **pre-mRNA splicing (snRNA), polyadenylation, modification**
- **pre-rRNA processing – a very complex pathway (snoRNA)**
- **endo- (RNaseIII, RNase P/MRP) and exo- (exosome, Xrn1/Rat1) nucleolytic processing**

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IV. COTRANSCRIPTIONALITY

- **CTD of Pol II, Ser-P status** (S5-P initiation, S2-P elongation/termination)
- **m7G cap synthesis**
- **assembly of splicesome and processing factors** (cleavage and polyadenylation and Nrd1/Nab3 termination complexes, enzymes like Rat1, exosome)
- **assembly of export factors (e.g. Mex67, Yra1)**
- **splicing**
- **some processing (pre-rRNA cleavages) and modification**
- **connection between transcription, processing and export via THO/TREX and TREX-2 complexes (gene gating)**

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V. RNA DECAY

• **normal** (usually in the cytoplasm)

• **specialized, RNA surveillance: targeting aberrant, unstable transcripts for discard pathway (NMD, NSD, NGD, ARE, NRD etc)**

1. deadenylation ➔ decapping ➔ exonucleolytic degradation

5'-3' by Xrn1/Rat1 or 3'-5' by exosome

2. by endo- cleavage (miRNA-dependent, RNAse III/Rnt1, MRP, SMG6) followed by exo- digestion (Xrn1/Rat1, exosome)

• **nuclear RNA surveillance: polyadenylation by TRAMP (Trf4/5) followed by degradation by the exosome, Xrn1 or Rat1**

PROCESSING AND DEGRADATION ARE OFTEN CARRIED OUT BY THE SAME MACHINERIES