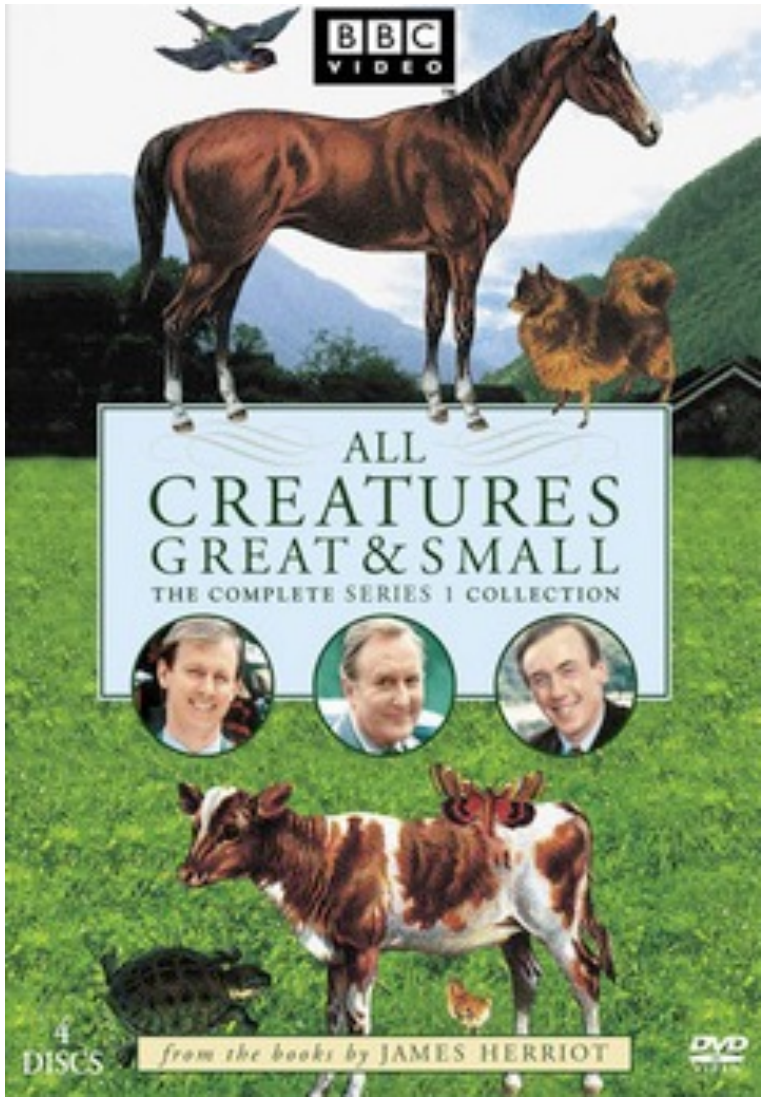
All RNAs great and small



lecture 3

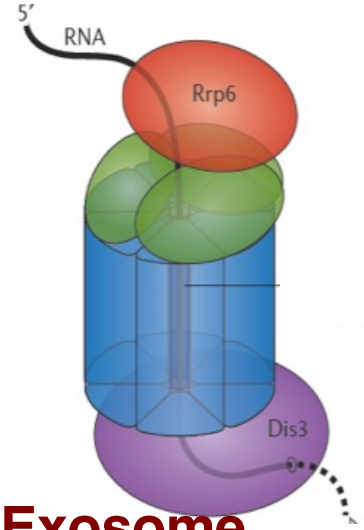
RNA enzymes and complexes

RNA granules

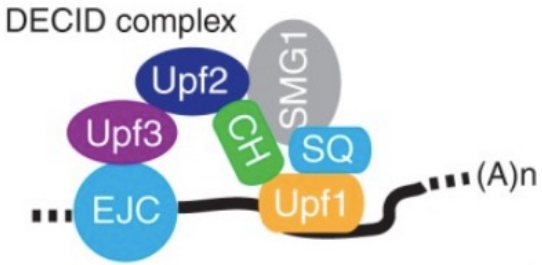
RNA decay

Institute of Genetics and Biotechnology
University of Warsaw

RNA enzymes and complexes

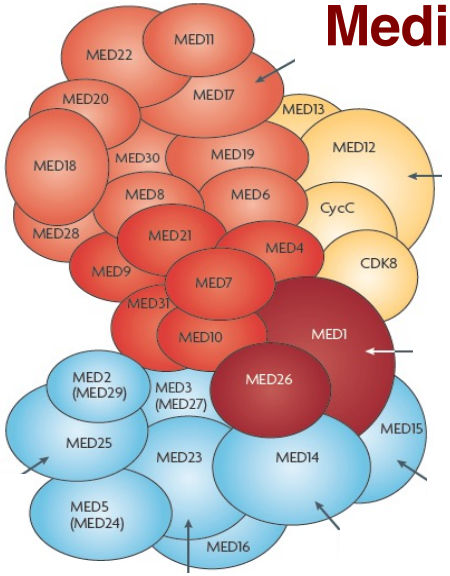


Exosome

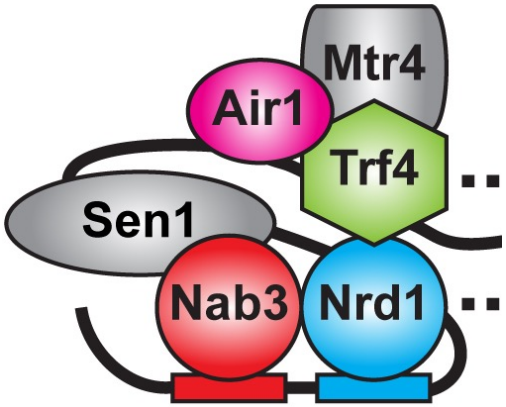


NMD

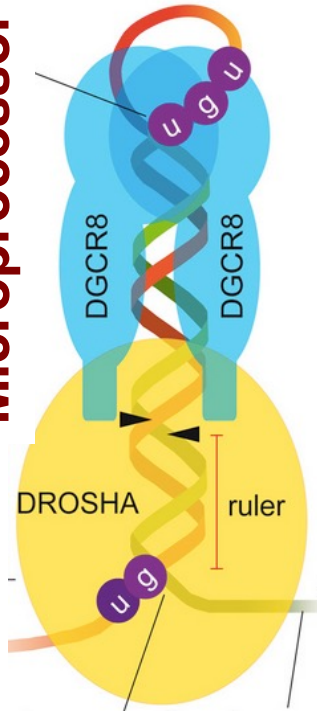
Mediator



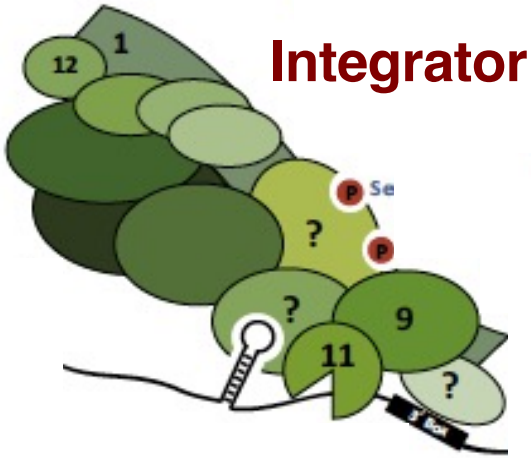
TRAMP/NNS



Microprocessor



Integrator



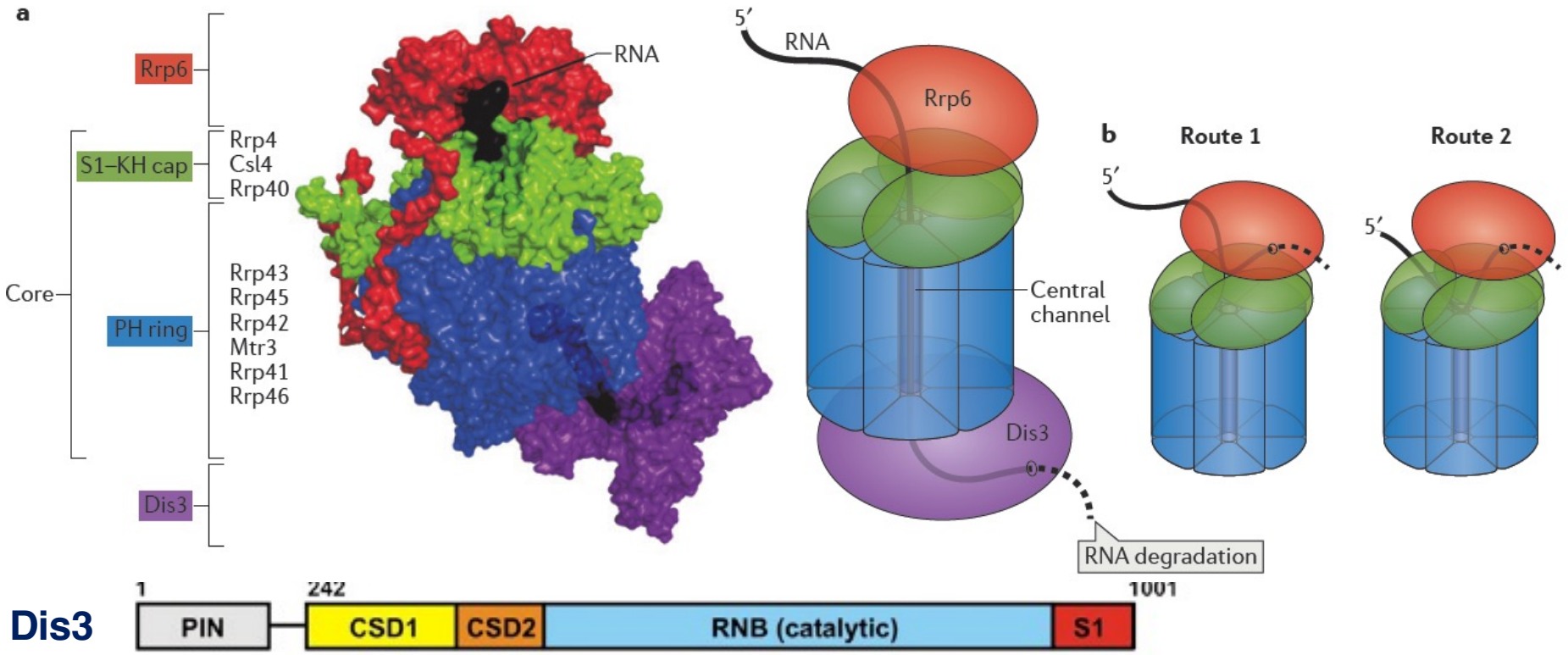
RNA processing and decay machinery: RNases

Protein	Function	Characteristics
Exonucleases 5'→3'		
Xrn1	cytoplasmic, mRNA degradation	processsive
Rat1/XRN2	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 PNPase
Rrp44/Dis3	catalytic subunit	Exo/PIN domains, processsive
Rrp4, Rrp40	pre-rRNA, sn/snoRNA processing, mRNA degradation	
Rrp41-43, 45-46	participates in NMD, ARE-dependent, non-stop decay	
Mtr3, Ski4		
Mtr4	nuclear helicase cofactor	DEAD box
Rrp6 (Rrp47)	nuclear exonuclease (Rrp6 BP, cofactor)	RNAse D homolog, processsive
Ski2,3,7,8	cytoplasmic exosome cofactors. SKI complex	helicase, GTPase
Other 3'→5' and 5'→3'		
Rex1-4	3'-5' exonucleases, rRNA, snoRNA, tRNA processing	RNase D homolog
DXO	5'-3' exonuclease in addition to decapping	
ERI1	3'-5' exonuclease, rRNA processing, histone mRNA decay	
mtEXO 3'→5'	mitochondrial degradosome RNA degradation in yeast	
Suv3/ Dss1	helicase/ 3'-5' exonuclease	DExH box/ RNase II homolog
Deadenylation		
Ccr4/NOT/Pop2	major deadenylase complex (Ccr, Caf, Pop, Not proteins)	Ccr4- Mg^{2+} dependent endonuclease
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
Utp24 Nob1 Las1	pre-rRNA processing at sites A0, D and C2	

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 repressio
DXO	pyrophoshohydrolase, 5' decapping endonuclease, deNADding, 5'OH hydrolase
TRAMP complex: exosome cofactors, nuclear RNA QC, polyadenylation-dependent degradation,	
Trf4/Trf5 (hTRF4-2)	nuclear alternative poly(A) polymerases
Mtr4 (hMTR4)	DEAD box helicase
Air1/Air2 (ZCCHC7)	RNA binding proteins
NEXT and PAXT complexes: exosome cofactors, nuclear RNA QC	
hMTR4	DEAD box helicase
RMB7/ZCCHC8	NEXT RNA binding proteins
ZFC3H1	PAXT RNA binding protein
PABPN1	PAXT nuclear polyA binding protein
Nrd1-Nab3-Sen1 complex: PolII termination of ncRNAs, TRAMP-depdendent degradation	
Nrd1	Pol II C-terminal domain (CTD) binding, RNA binding
Nab3	RNA binding
Sen1	RNA helicase
CBCA-NEXT, CBCA-PAXT and RESTRICTOR complexes: nuclear RNA QC	
CBC (CBCA)	nuclear cap binding complex
ARS2 (CBCA)	RNA binding, Pol II transcription, termination, RNA decay
ZC3H18	NEXT, zinc finger protein
ZFCH1	PAXT nuclear polyA binding protein
ZC3H4, WDR82	RESTRICTOR Pol II termination, RNA Decay by NEXT and exosome complexes

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

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

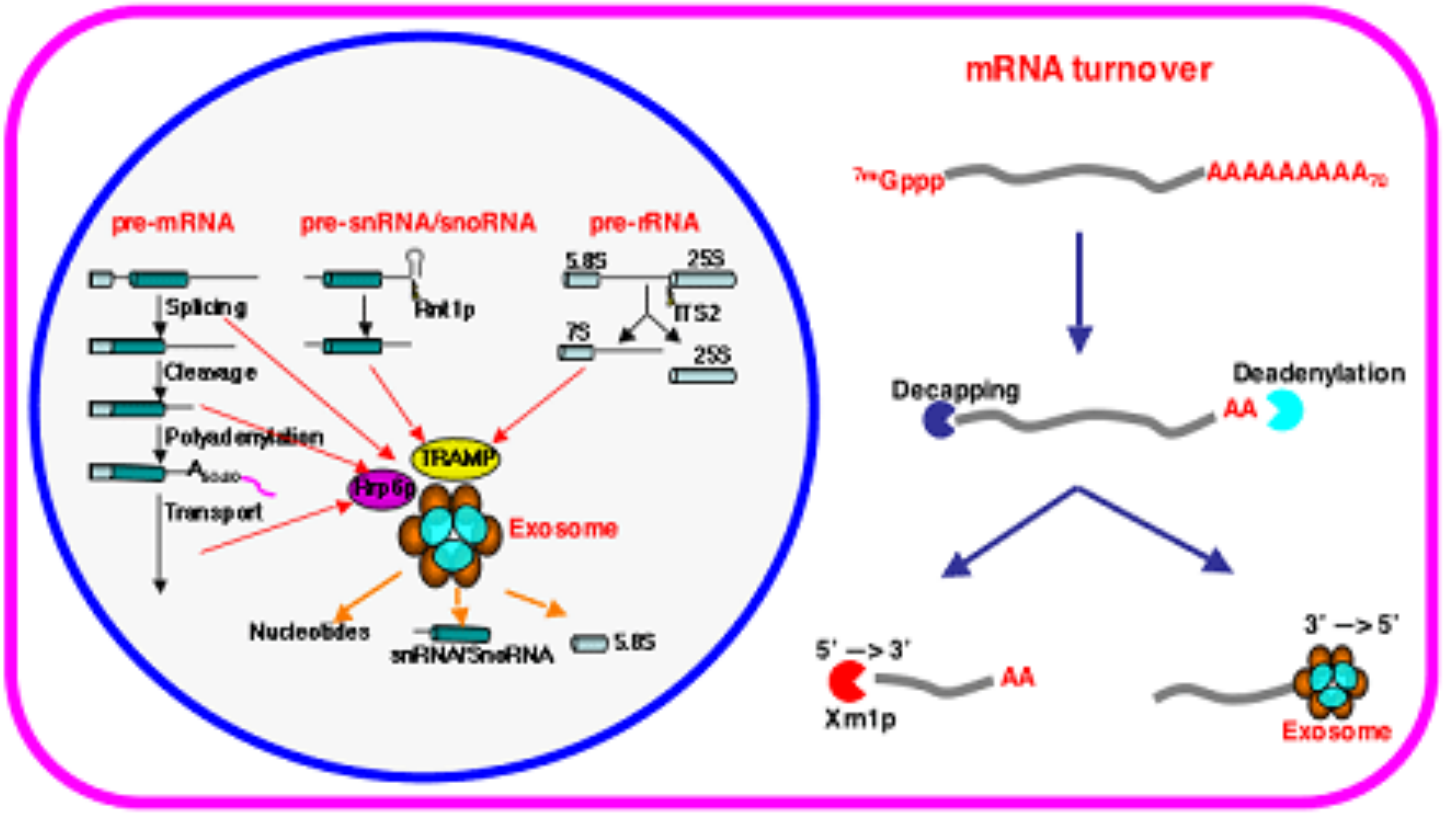
EXOSOME: 3’→5’ decay machinery: functions

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

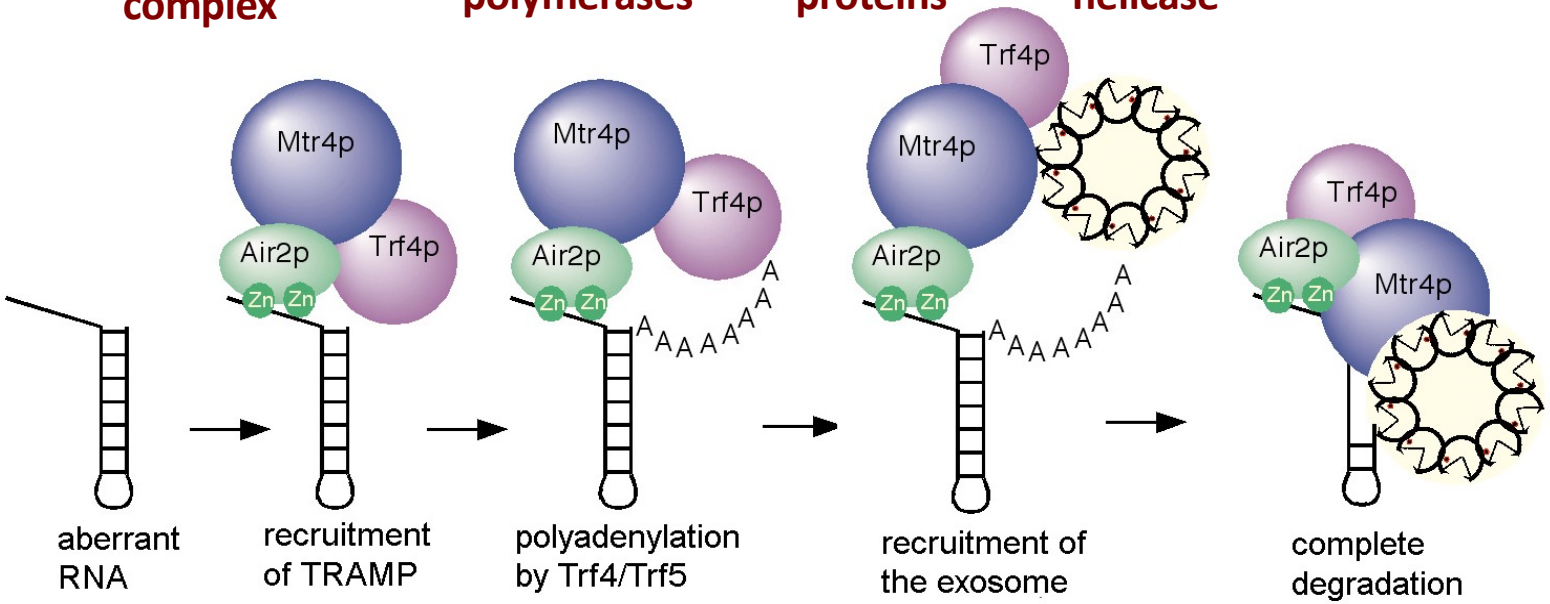


TRAMP – exosome cofactor

yeast

TRAMP = Trf4/5 + Air1/2 + Mtr4

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



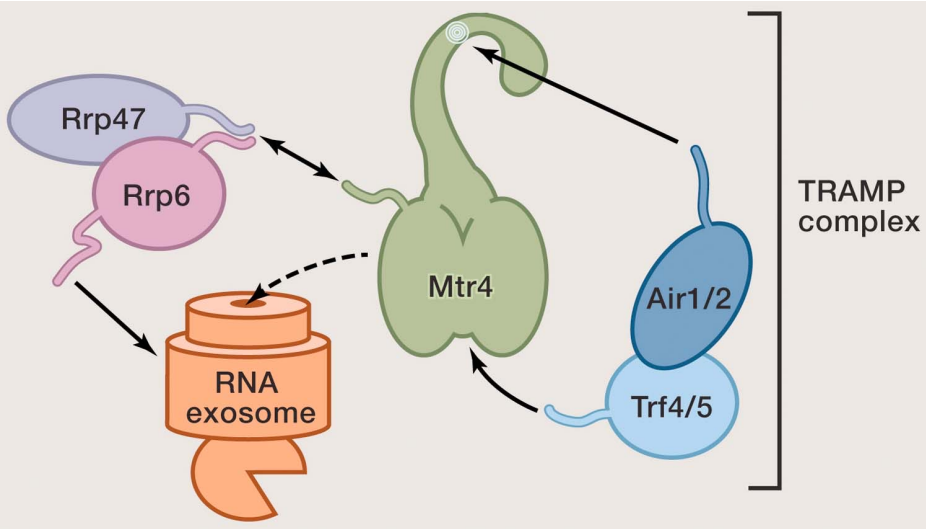
Polyadenylation-mediated nuclear discard pathway for defective and excessive RNAs

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

TRAMP interacts with
- exosome via Mtr4
- Nrd1/Nab3/Sen1 complex

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

TRAMP + Exosome = nuclear RNA surveillance



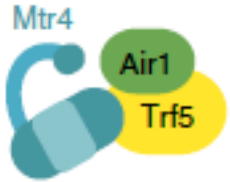
Mtr4 – DEAH box RNA helicase
Air1/2 – RNA binding proteins
Trf4/5 – poly(A) polymerases

Substrate specificity conferred by Trf4/5
Ai1/2 are highly redundant

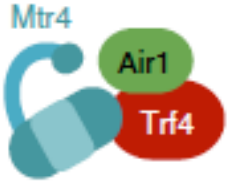
SUBSTRATES

TRAMP 4-2: mRNA, ncRNA

TRAMP 5-1: pre-rRNA



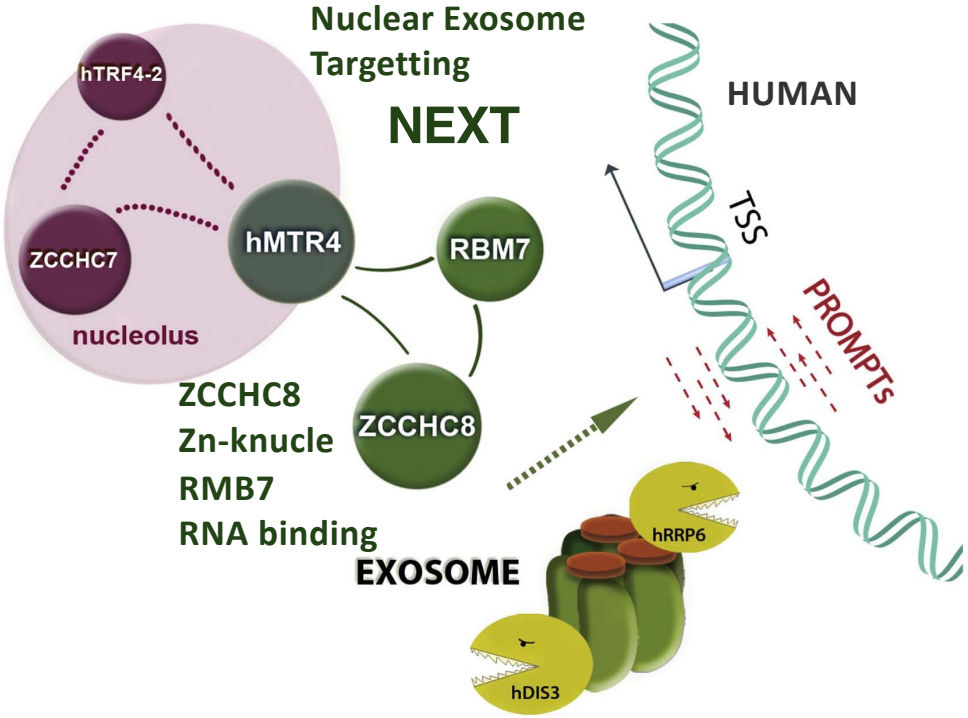
TRAMP 4-1: mRNA, introns



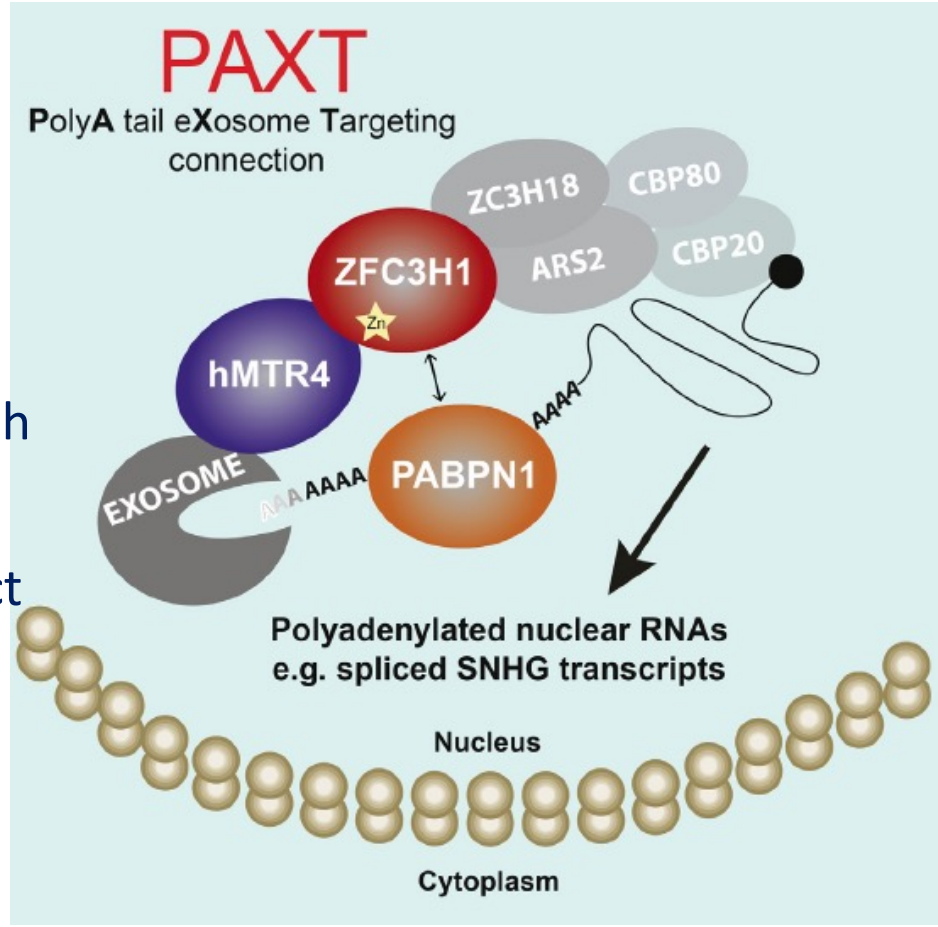
TRAMP
- interacts with the exosome via Mtr4 - role in degradation
- role in sn/snoRNA 3' end processing together with the exosome
- interacts with Nrd1/Nab3 complex - role in ncRNA Pol II termination
- role in transcription silencing in *S. cerevisiae* and *S. pombe* (Cid14)

NEXT and PAXT - exosome cofactors

mammals



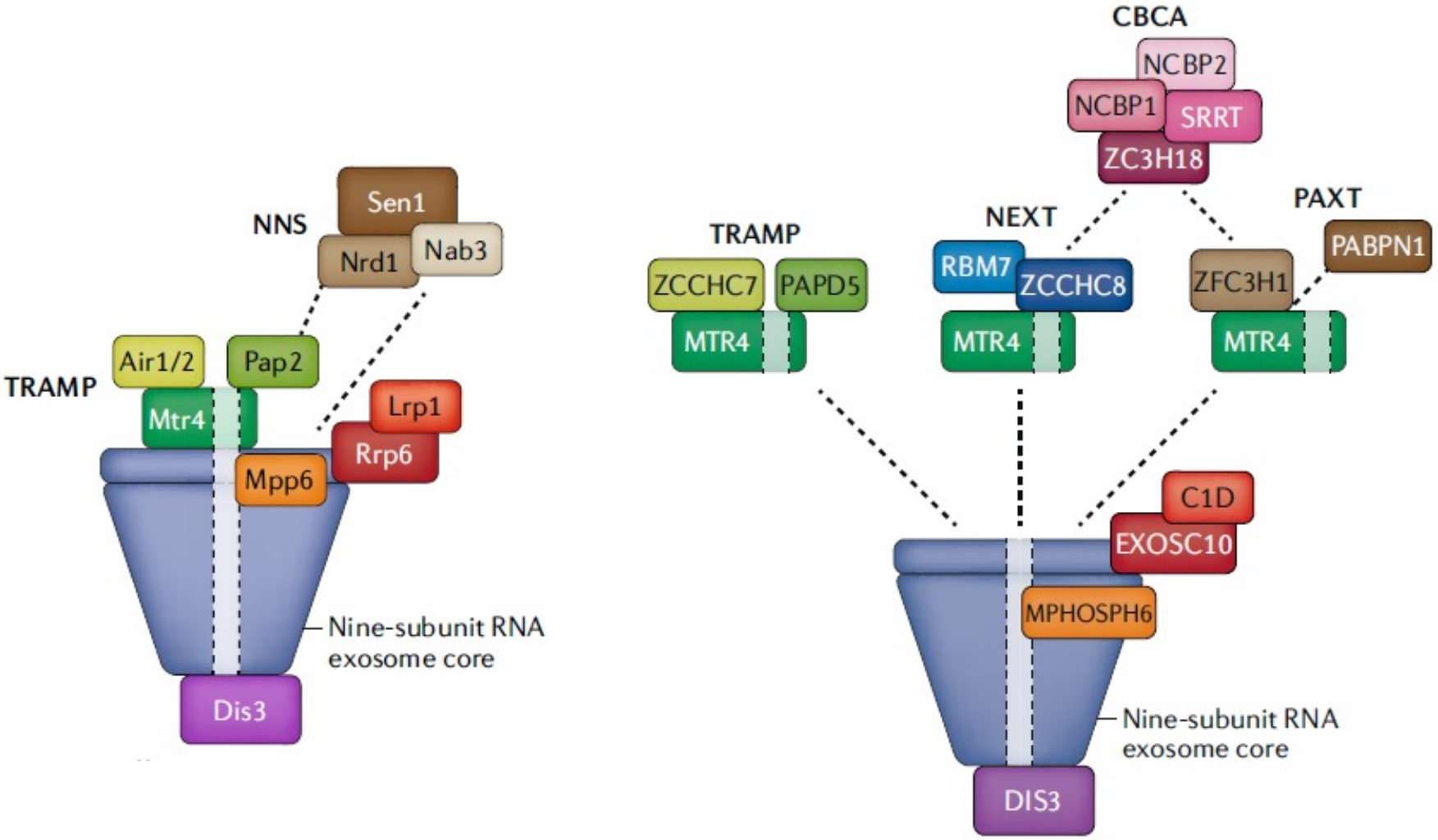
MTR4- associated complexes



- ZFC3H1 (Zn-knuckle protein) links MTR4 with PABPN1 in PAXT
- ZFC3H1/PABPN1 and RBM7/ZCCHC8 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

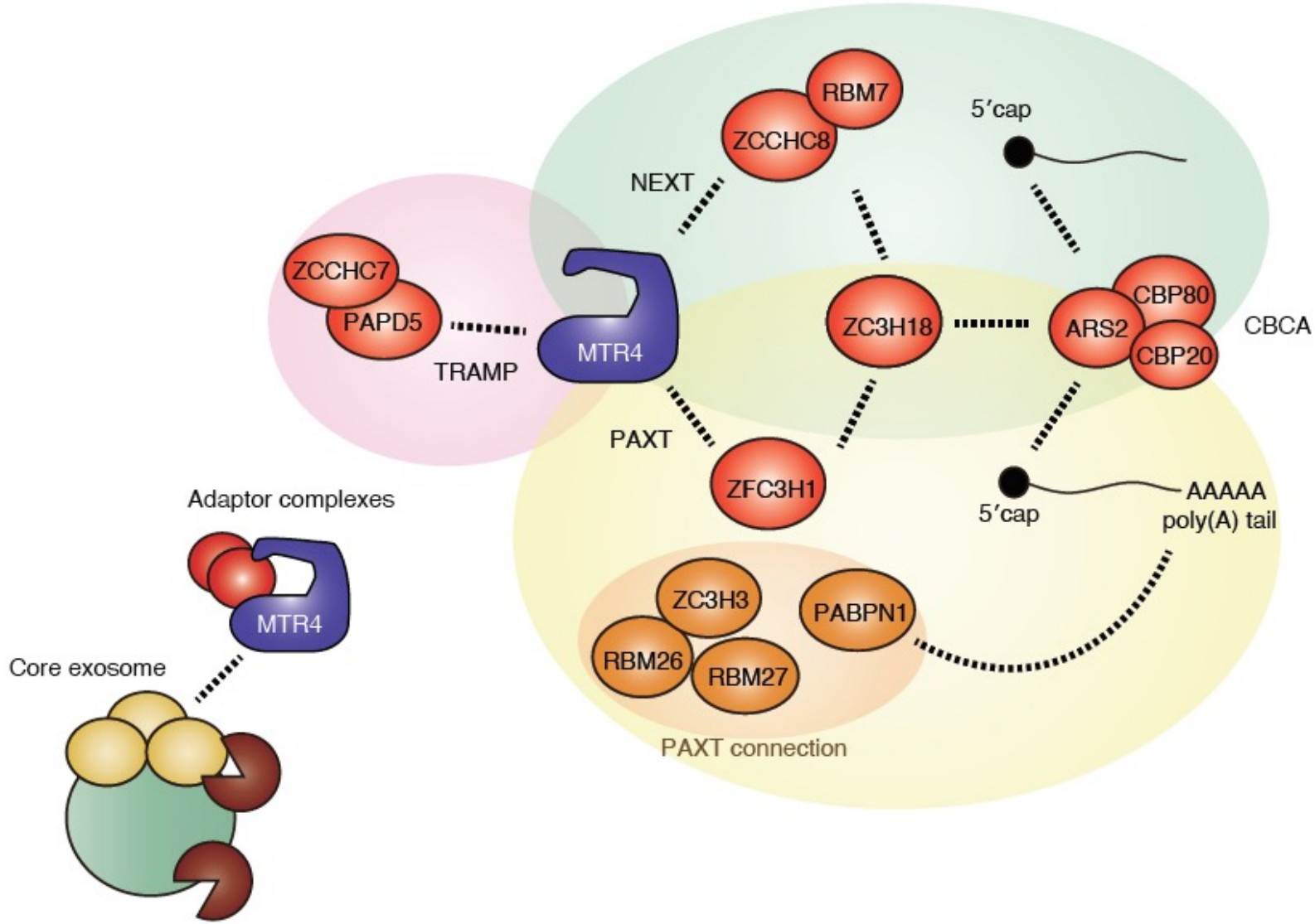
Lubas et al. *Mol. Cell*, 2011; Meola et al., . *Mol. Cell*, 2016

EXOSOME with TRAMP, NEXT and PAXT



Schmid and Jensen., Nat. Rev. Mol. Cel. Biol. , 2018

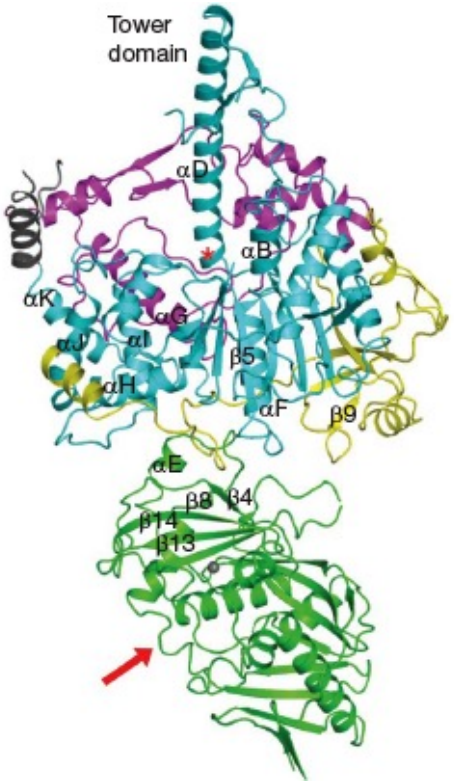
EXOSOME with TRAMP, NEXT and PAXT



Ogami and Suzuki., Int J Mol Sci, 2021

XRN family: 5’→3’ processive exonucleases

Kastenmayer and Green, 2000, PNAS



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

Xiang et al, 2009, Nature

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)*

CYTOPLASMIC XRN1

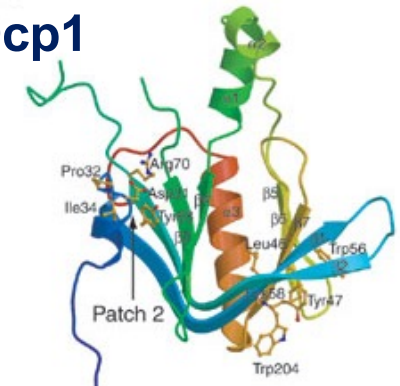
- generic mRNA decay
- specialised mRNA decay pathways: NMD, NSD, NO-GO decay, ARE-dependent decay
- degradation of miRNA-dependent mRNA cleavage products *(in plants)*
- degradation of some ncRNAs: CUTs, SUTs, XUTs

XRN1 and Rat1/XRN2 have deNADding and deFADding activity

Sharma et al, Nat Comm 2022; NAR 2922

DCP/NUDT- decapping enzymes

Dcp1

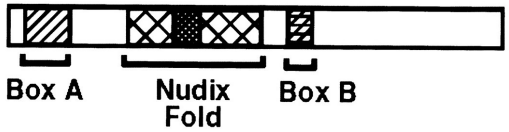


She et al. Nat.Struct. Mol. Biol, 2004

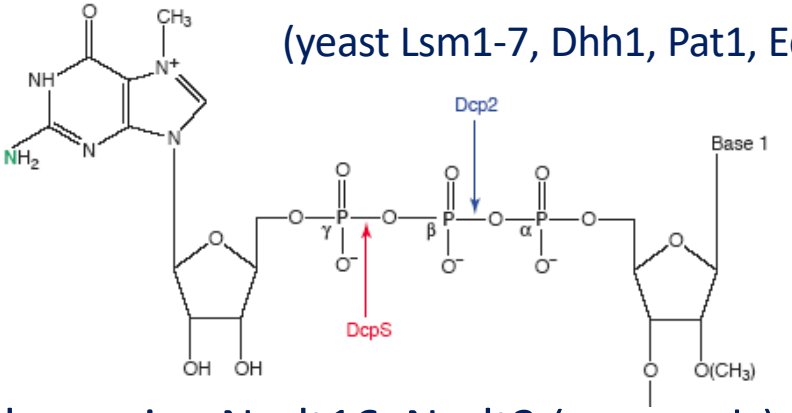
- **Dcp1/Dcp2** complex participates in mRNA 5' decay
- catalyses the reaction m^7GpppX-mRNA -> m^7GDP + 5'p-mRNA
- Dcp2 is the catalytic subunit (pyrophosphatase Nudix domain)
- Dcp1 is required for activity *in vivo*, interacts with other proteins
- Dcp1/Dcp2p is regulated by Pab1 and activating factors

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

Dcp2

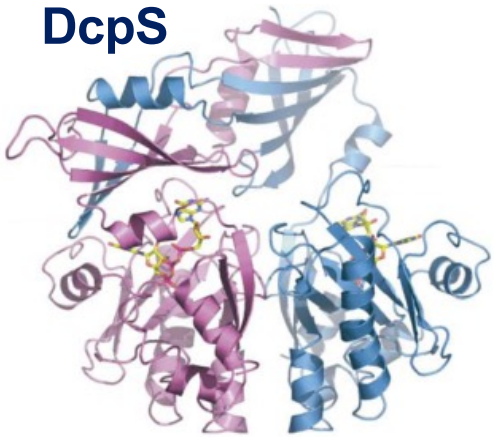


Wang et al. PNAS, 2002



NUDT proteins (22): *in vivo* decapping Nudt16, Nudt3 (mammals)
in vivo deNADding Nudt12 (mammals)

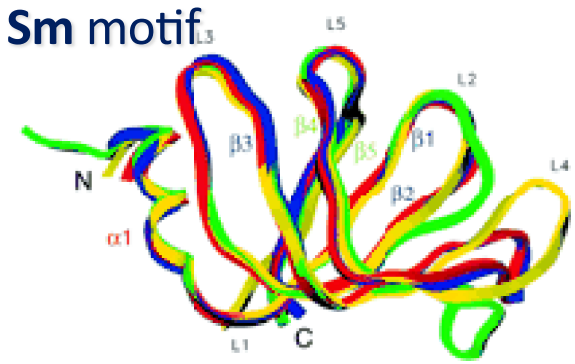
DcpS

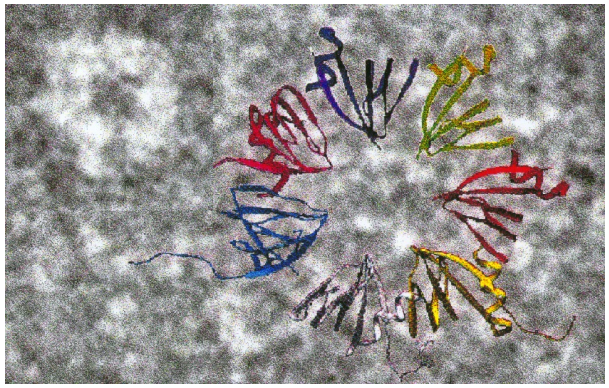


- **DcpS**: HIT pyrophosphatase („histidine triad" on the C-terminus)
- catalyses the cleavage of m^7GDP -> m^7GMP + Pi remaining after decapping during mRNA 5' decay
- cooperates with the exosome during mRNA 3' decay (m^7GpppX-oligoRNA -> m^7GMP+ pp-oligoRNA)
- functions as an asymmetric dimer

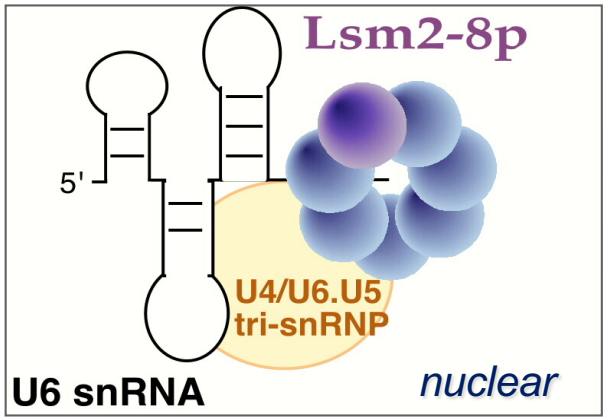
Gu et al., M.Cell, 2004

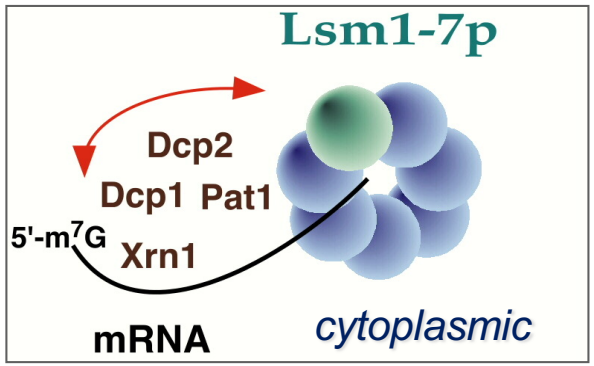
LSM proteins





Achsel et al, EMBO J, 2001





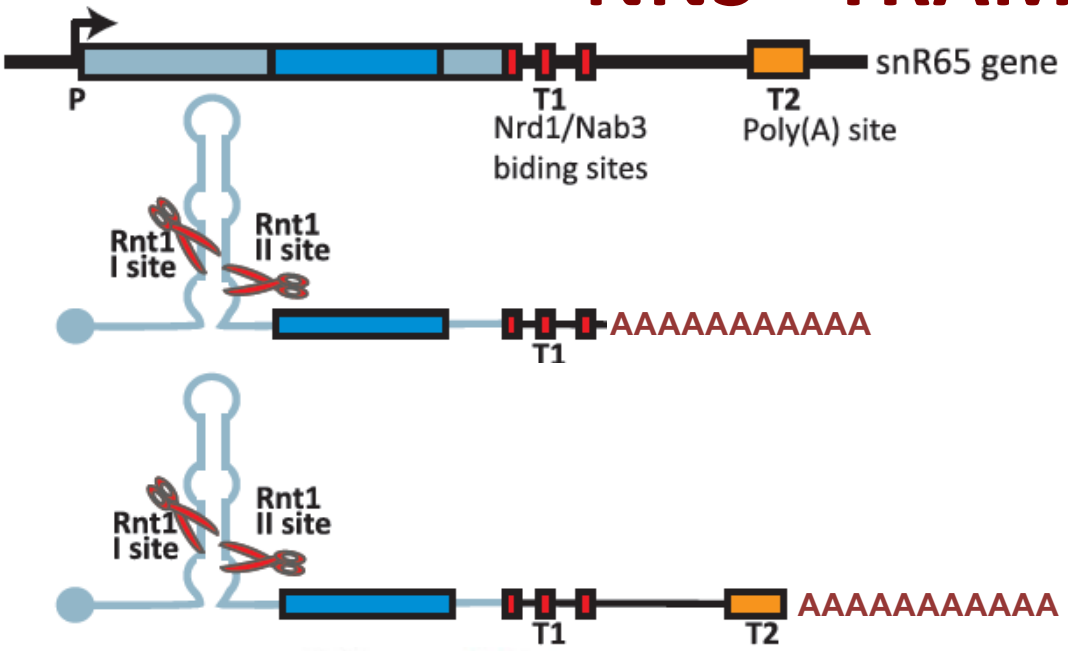
Involved in pre-mRNA splicing

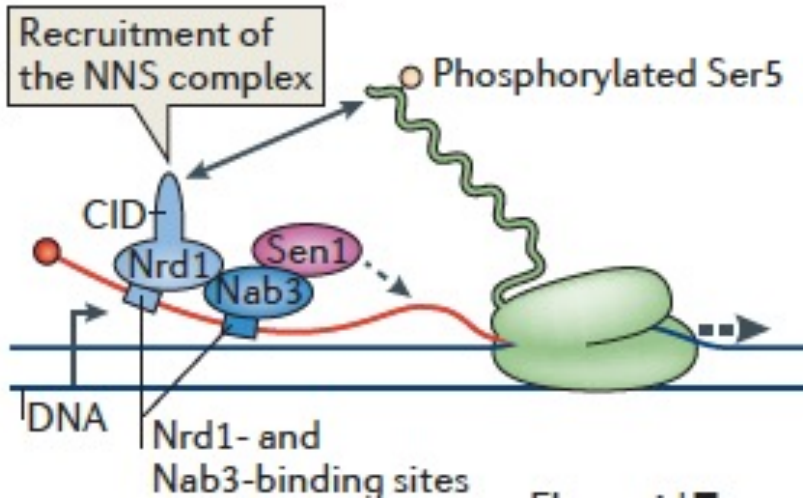
- associates with U6 snRNA
- required for U6 RNA accumulation and U6 snRNP biogenesis
- interacts with the U4/U6.U5 tri-snRNP

Functions in mRNA decapping and decay

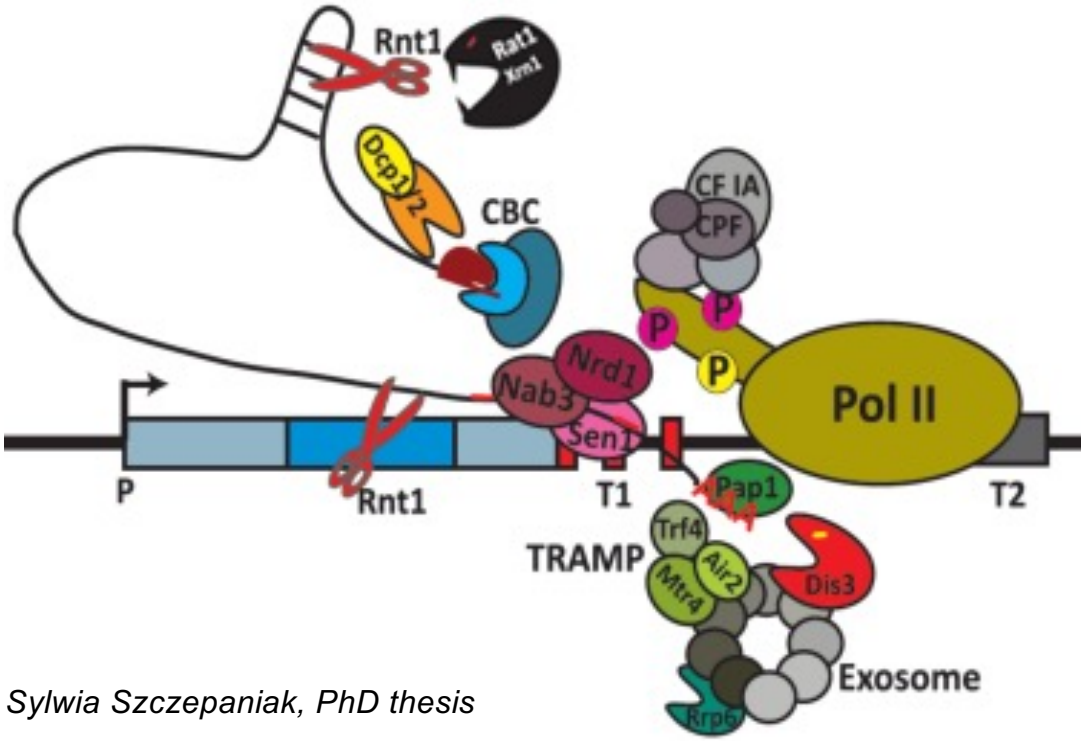
- activator of decapping
- interacts with components of the mRNA decapping and degradation machinery (XRN1, DCP1/2, PAT1)

NNS - TRAMP - exosome





Poruua, Libri, Nat Rev Mol Cell Biol, 2015

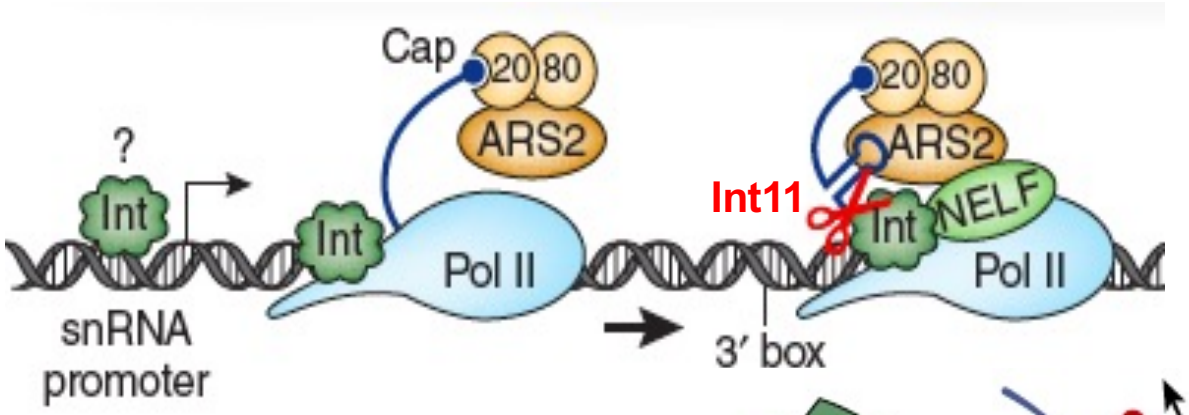


Sylwia Szczepaniak, PhD thesis

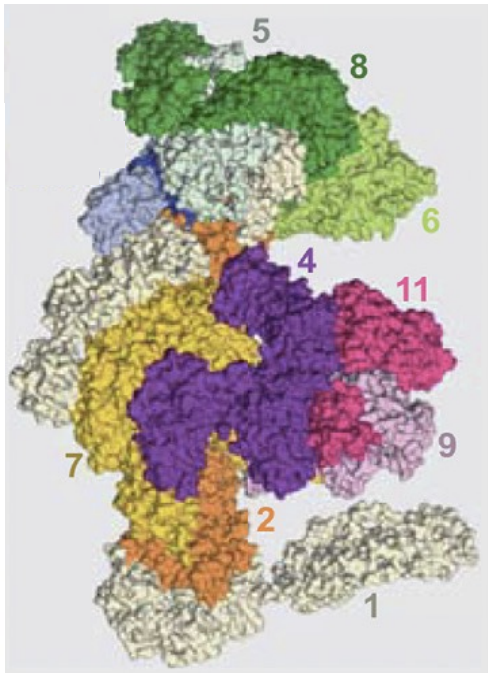
sn/snoRNA processing

yeast

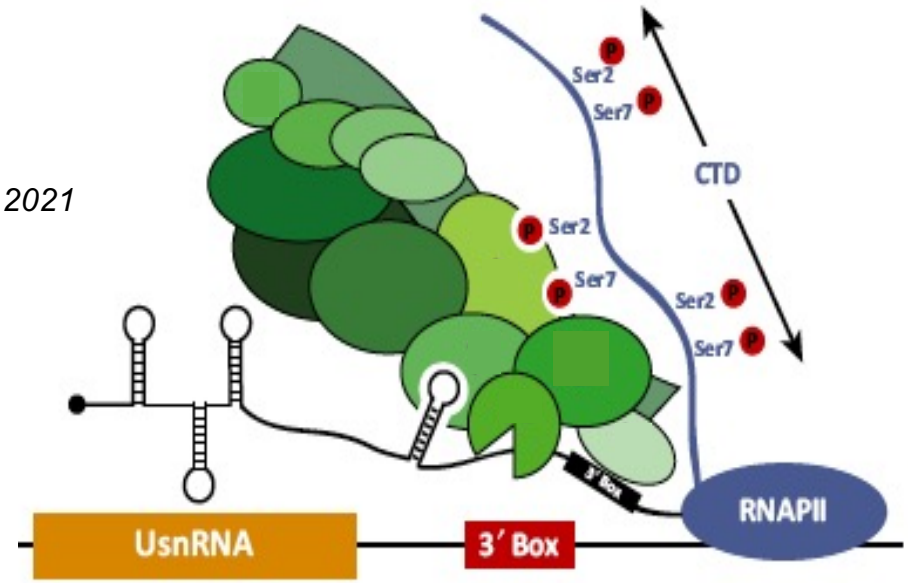
INTEGRATOR



snRNA processing
metazoa



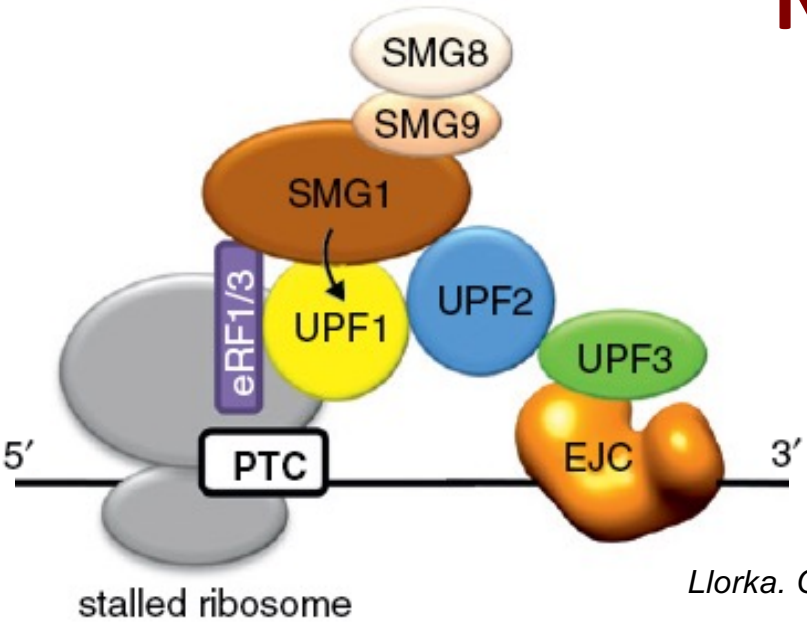
Baillat and Wagner, TiBS., 2015
Pfleiderer and Galej, Mol Cell, 2021
Sabath and Jonas, CurrOpStructBiol 2021



Integrator complex

- recruited contransctiptionaly to snRNA promoter
- interacts with Pol II CTD (Ser7-P/Ser2-P dyad)
- cleaves pre-snRNA at 3’box (endonuclease Int11)
- involved in transcription termination at snRNA genes
- contributes to transcription termination at mRNA genes (intronless in particular)
- promotes transcription elongation by nascent transcript cleavage (PolII release)

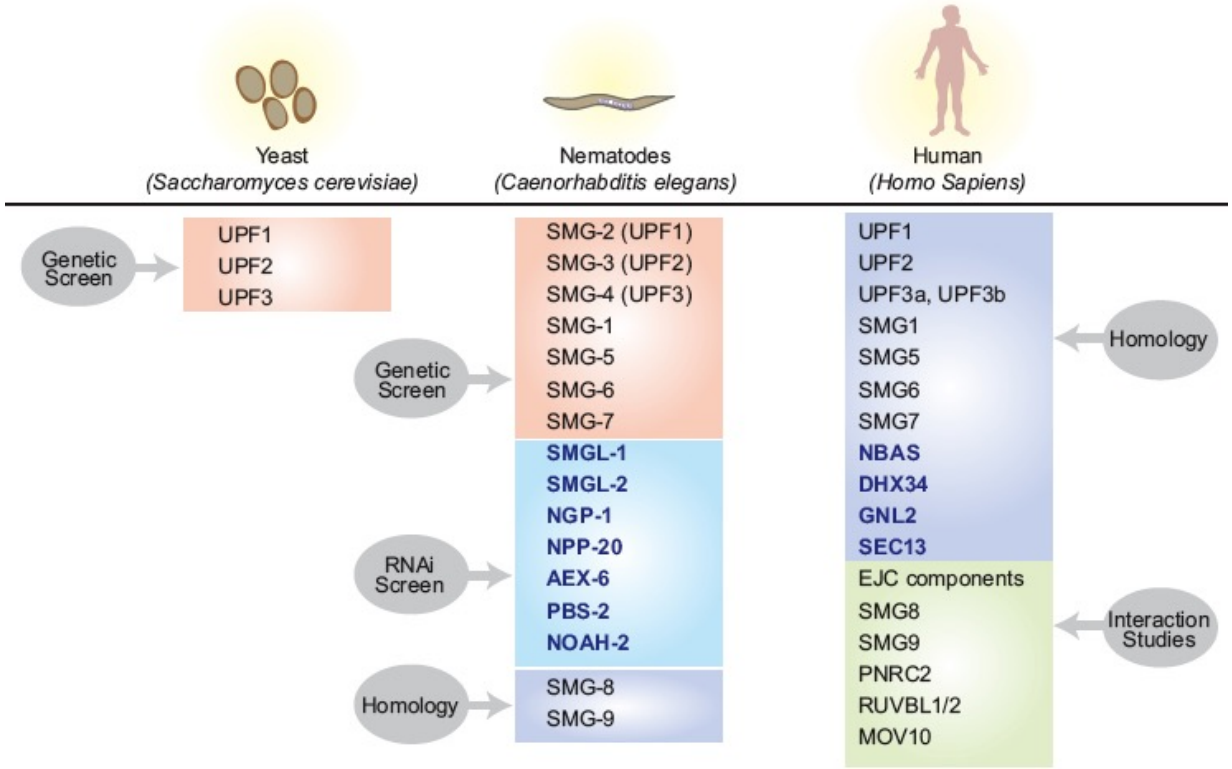
NMD factors



SURF complex
SMG1-UPFs-SMGs-Release Factors

DECID (decay inducing)
phoshoSMG1-UPFs-EJC

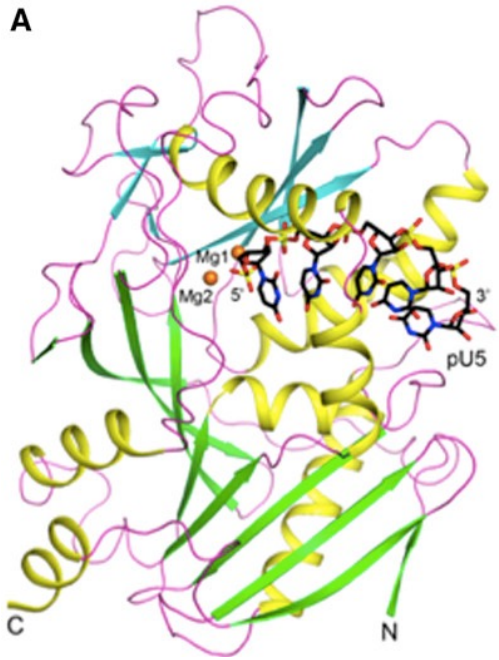
Llorka. Cur. Op. Chem. Biol. 2013



Hug et al., NAR, 2016

DXO/Rai1 family

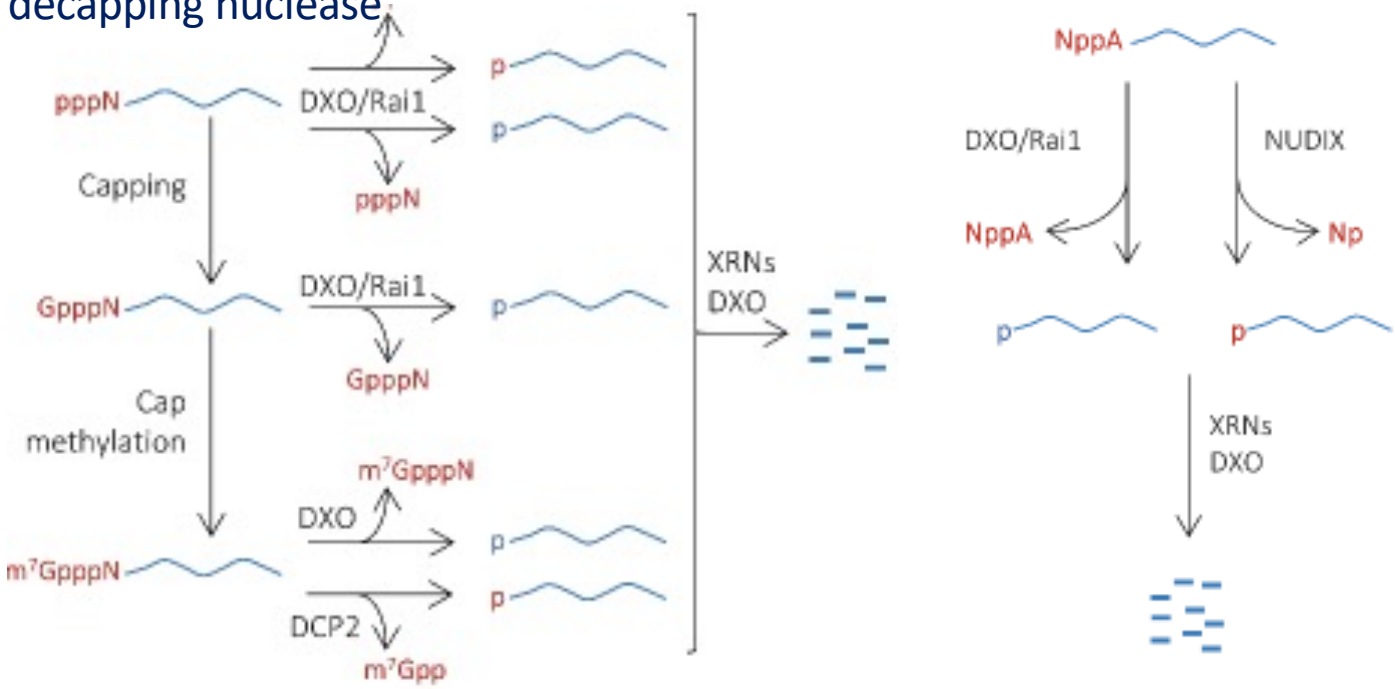
Cellular activities cap surveillance deNADding



pyrophosphohydrolase
decapping nuclease

5’ -3’ exonuclease

deNADding nuclease



ACTIVITY	SUBSTRATE	MmDXO	At DXO1
5'-3' exoribonuclease	p-RNA	+++	+
Pyrophosphohydrolase	ppp-RNA	+++	-
Decapping (unmethylated cap)	Gppp-RNA	+++	-
Decapping (mature cap)	m^{7}Gppp-RNA	+++	-
DeNADding	NppA-RNA	++++	+++

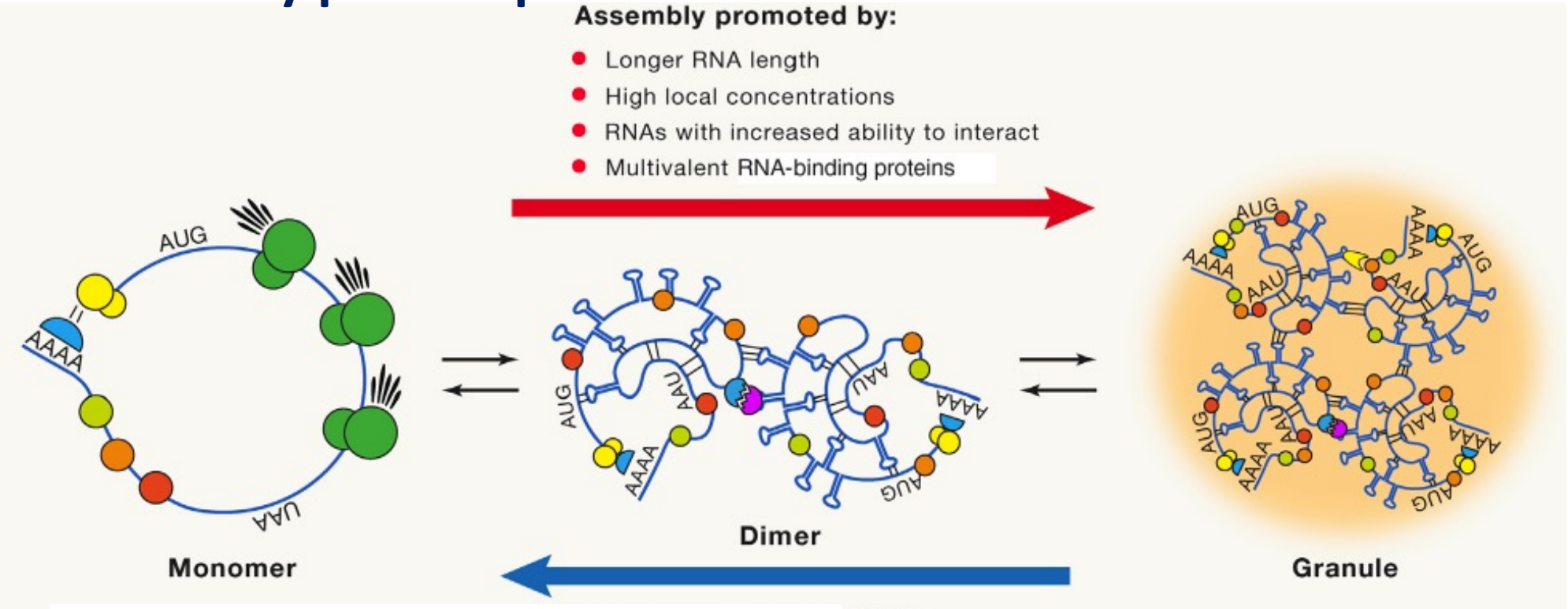
Additional activities:

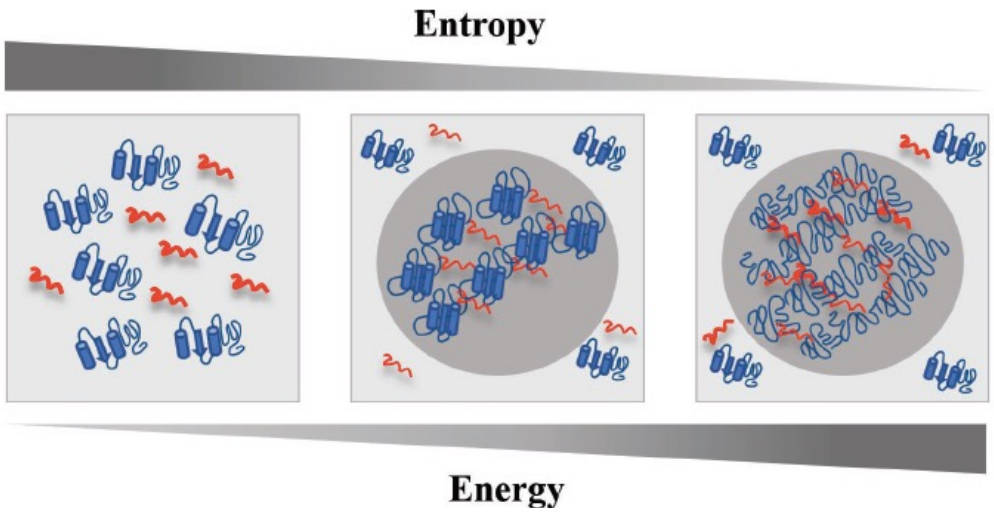
- 5’ OH RNA hydrolase
- FAD and CoA decapping nuclease

A. Kwaśnik, PhD thesis, 2019

RNP granule assembly

by protein-protein and RNA-RNA interactions





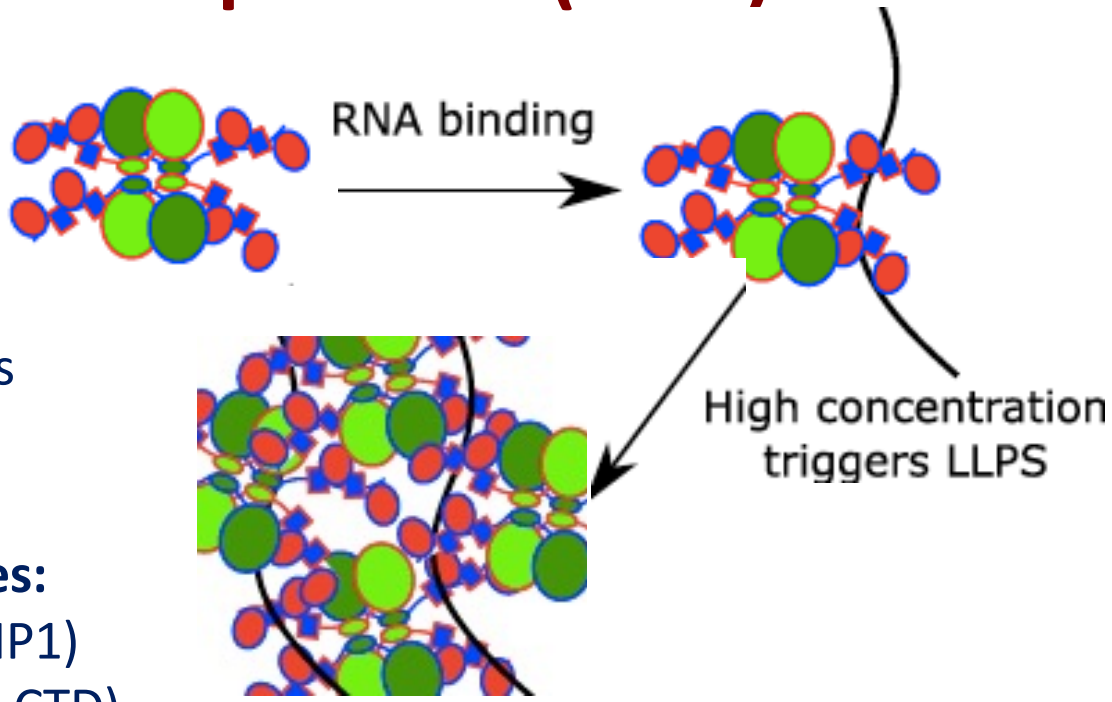
Treeck and Parker, Cell, 2018
Verdile et al, Front Genet, 2019

Phase transition

Droplets, MLOs (Membraneless Organelles)

Liquid-Liquid Phase Separation (LLPS)

Condensates



Formed by unstructured protein domains around RNAs

IDR - Intrinsic Disordered Domains

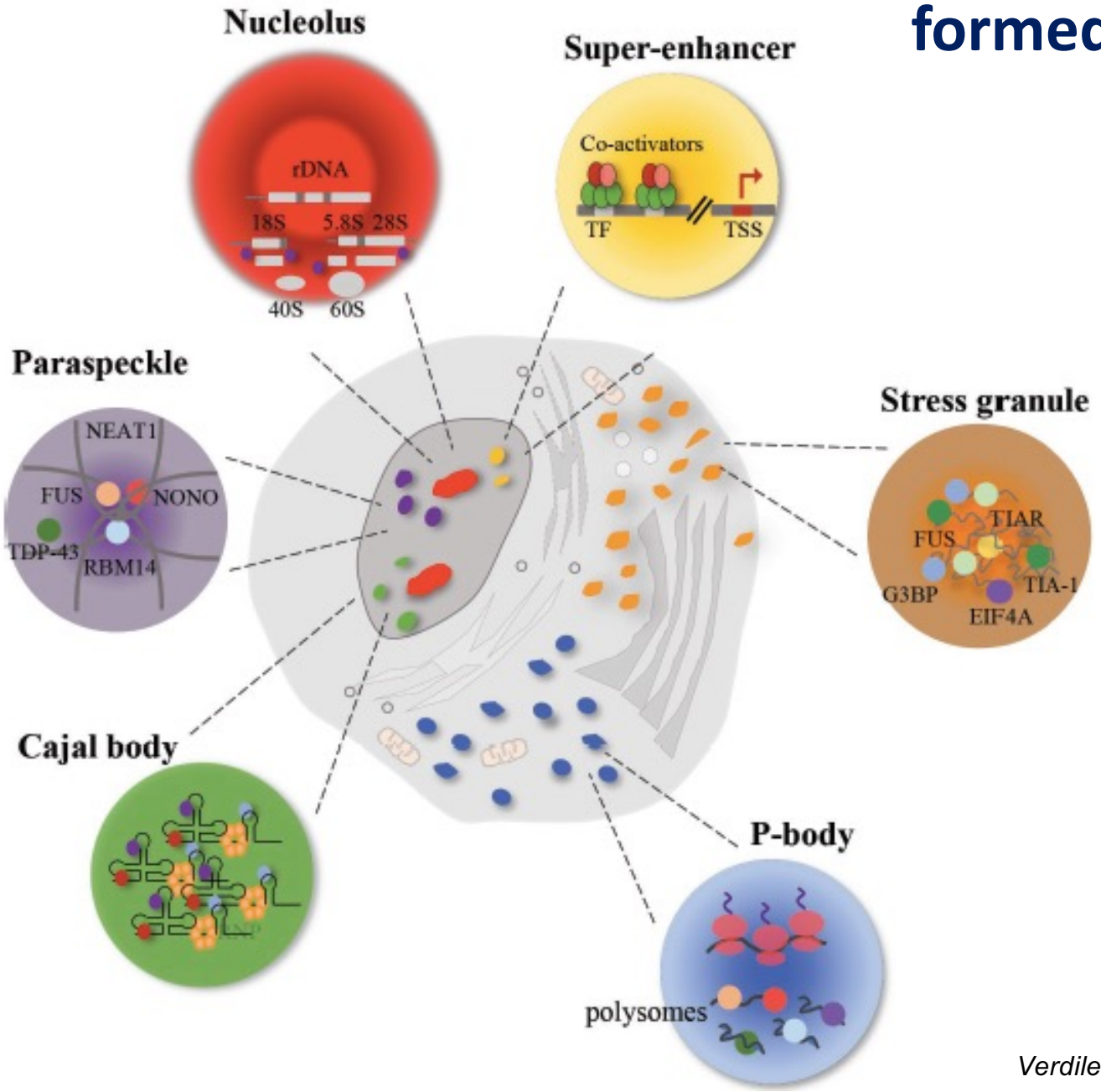
PLD - Prion-Like Domains

Organize several cellular processes:

- Heterochromatin structure (HP1)
- Transcription (Mediator, Pol II CTD)
- Processing (nucleolus, spliceosome, SR proteins, Cajal bodies)
- RNA retention and storage
  (Nuclear speckles, Paraspeckles, P-bodies, Stress Granules)
- RNA decay (degradosome)
- Protein modificarion and degradation (autophagosome, proteasome)

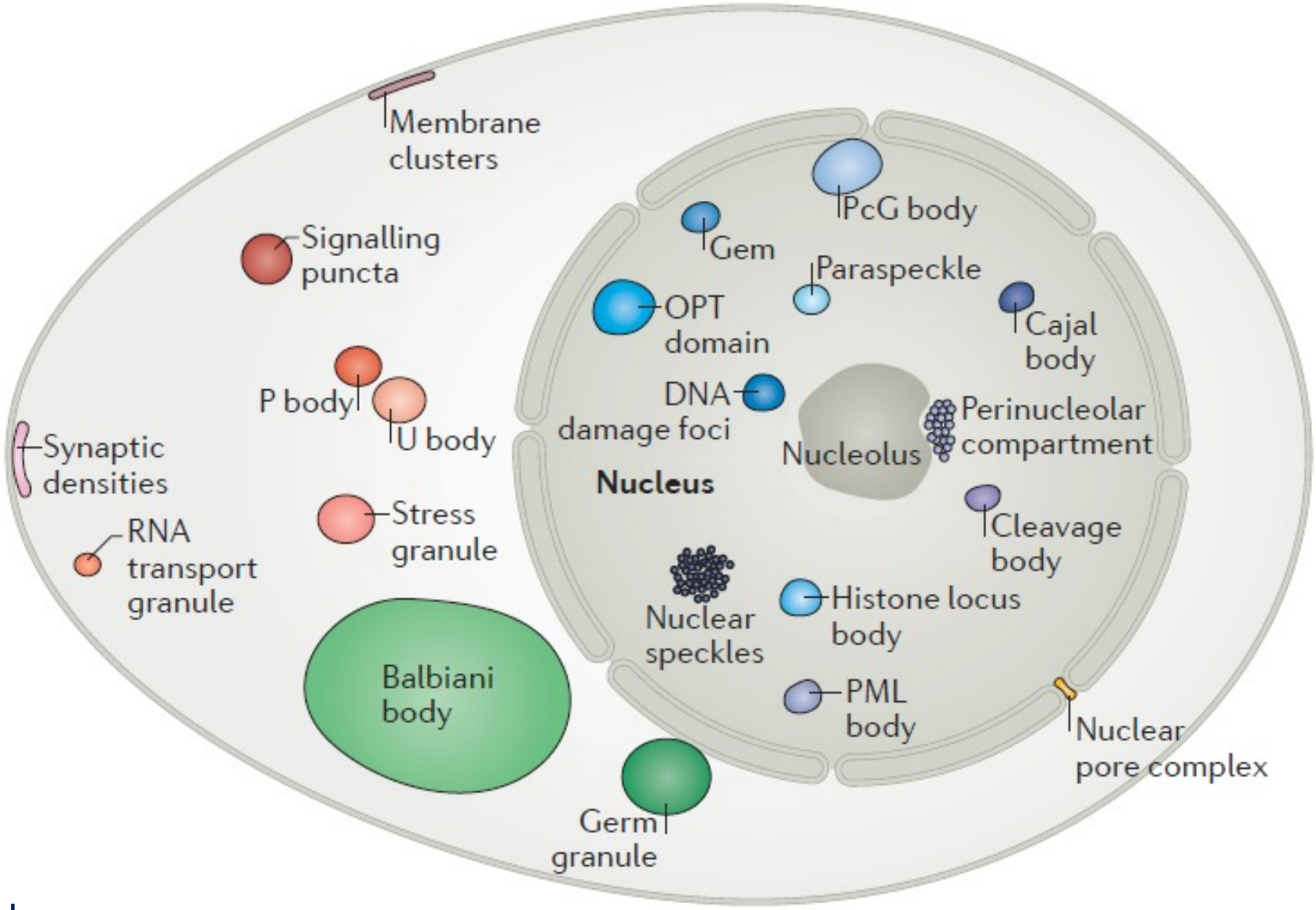
Membraneless Organelles

formed by LLPS



Verdile et al, Front Genet, 2019

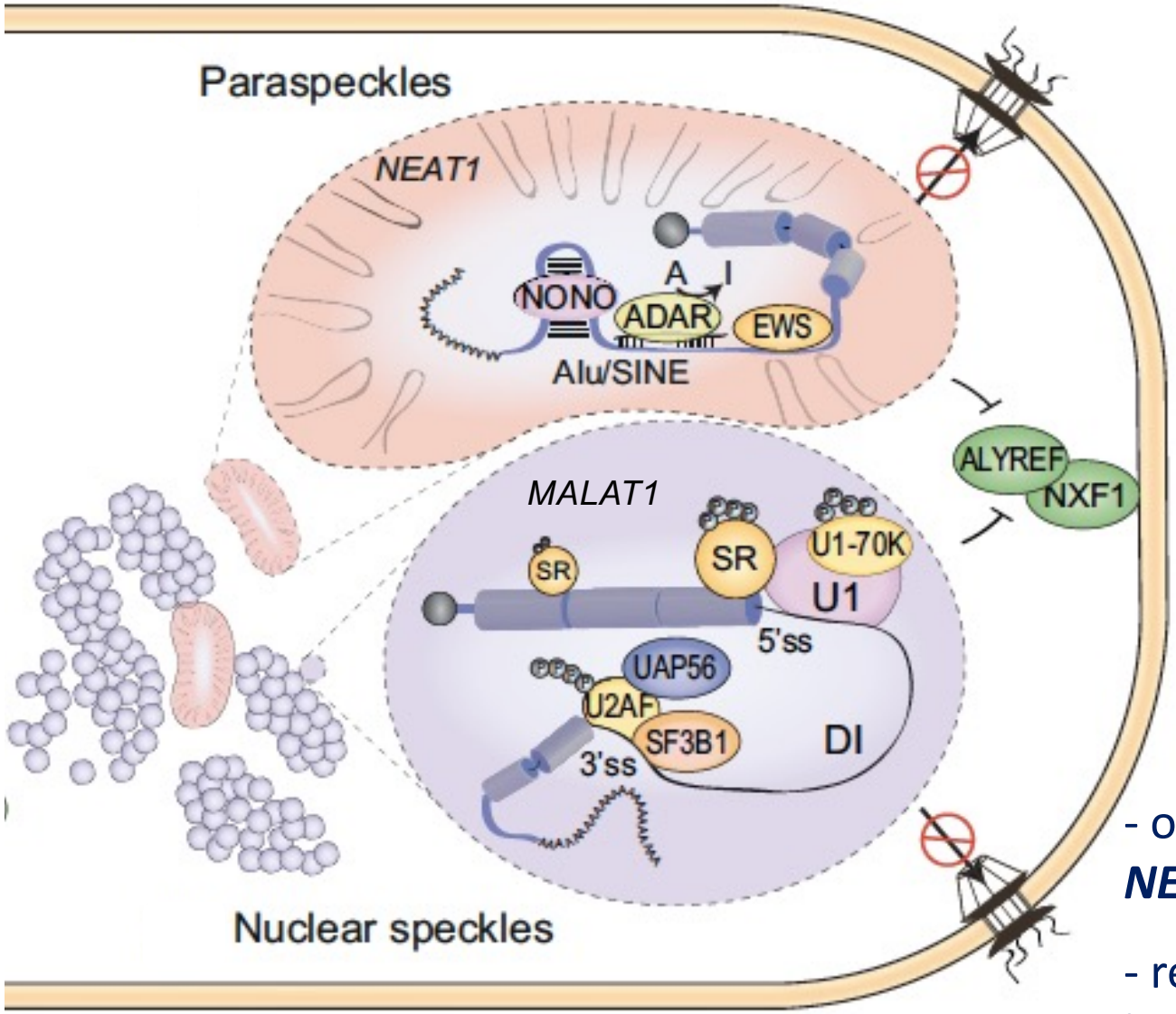
Cellular Condensates

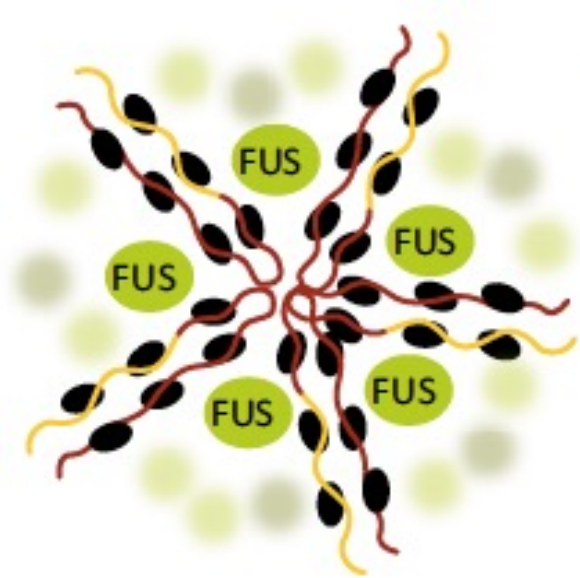


and many more...

Banani et al, Nat Rev Cell Mol Biol, 2017

Paraspeckles
Nuclear speckles



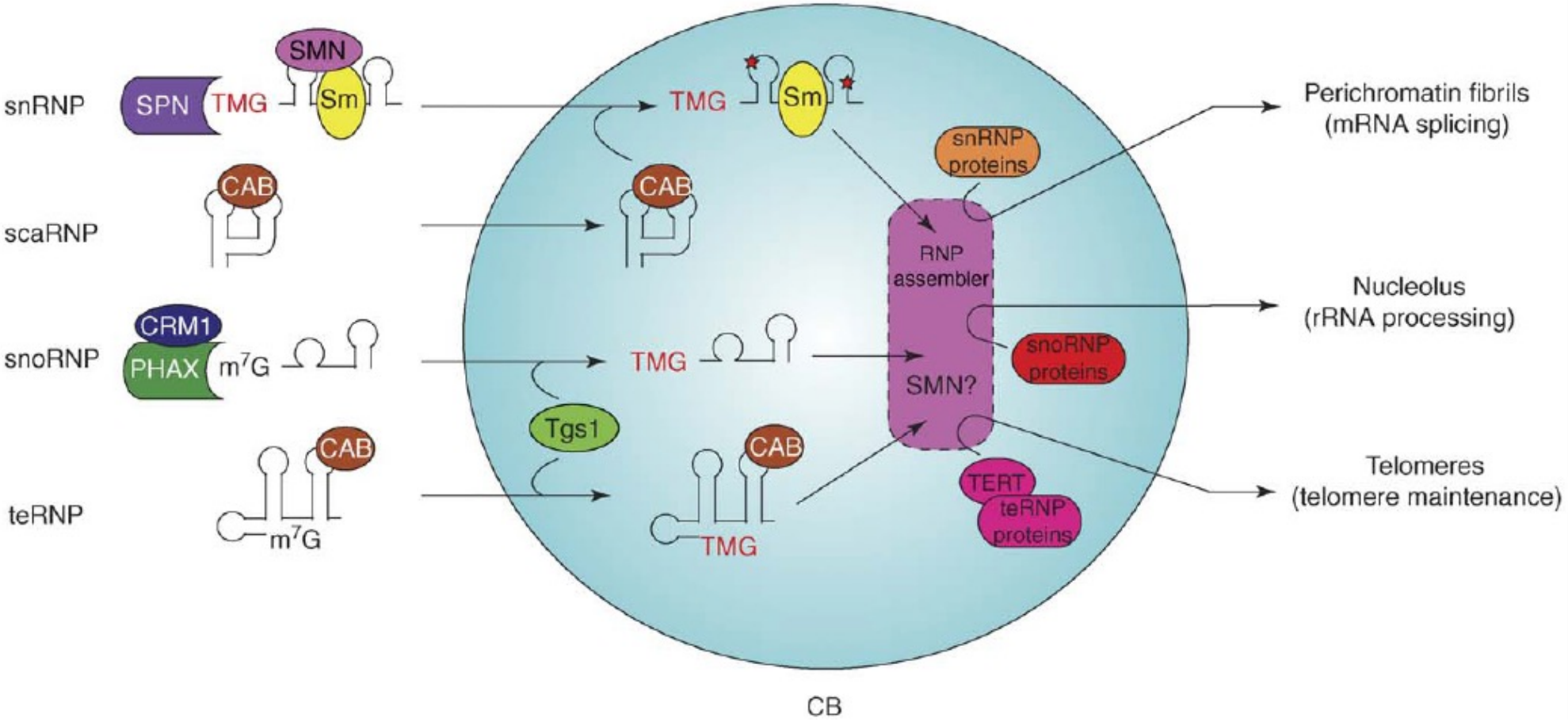


- organized around lncRNAs: ***NEAT1*** (PS) or ***MALAT1*** (NS)

- regulate gene expression by mRNA nuclear retention

Wegener and Müller-McNicoll, Sem Cell Dev Biol 2018

Cajal bodies

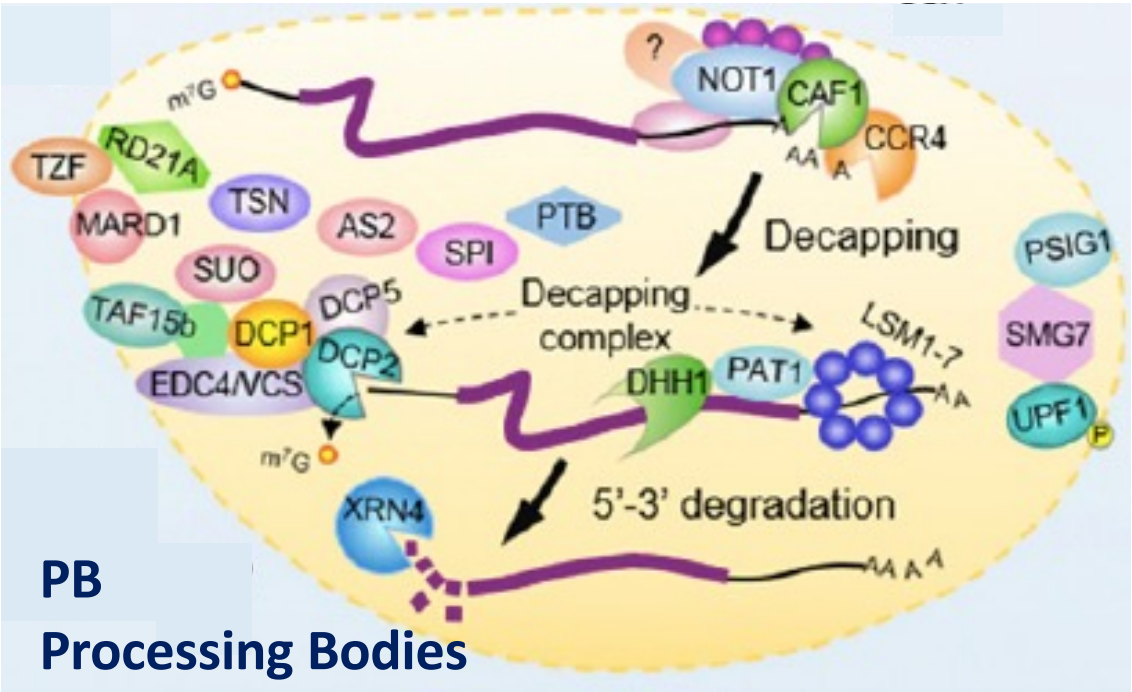


- contain CB-specific scaRNA

- sites of snRNA modification (capping, 2'O-Me, pseudoU) and RNA processing

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

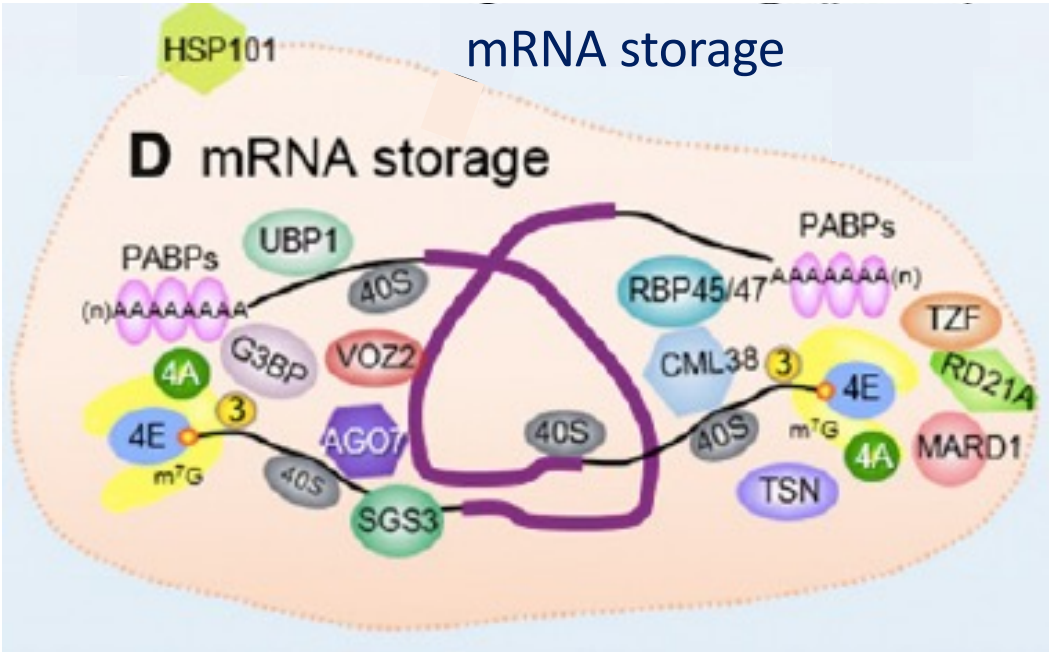
Cytoplasmic P-bodies and Stress Granules



PB
Processing Bodies
mRNA storage
mRNA decay

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)

SG Stress Granules
mRNA storage

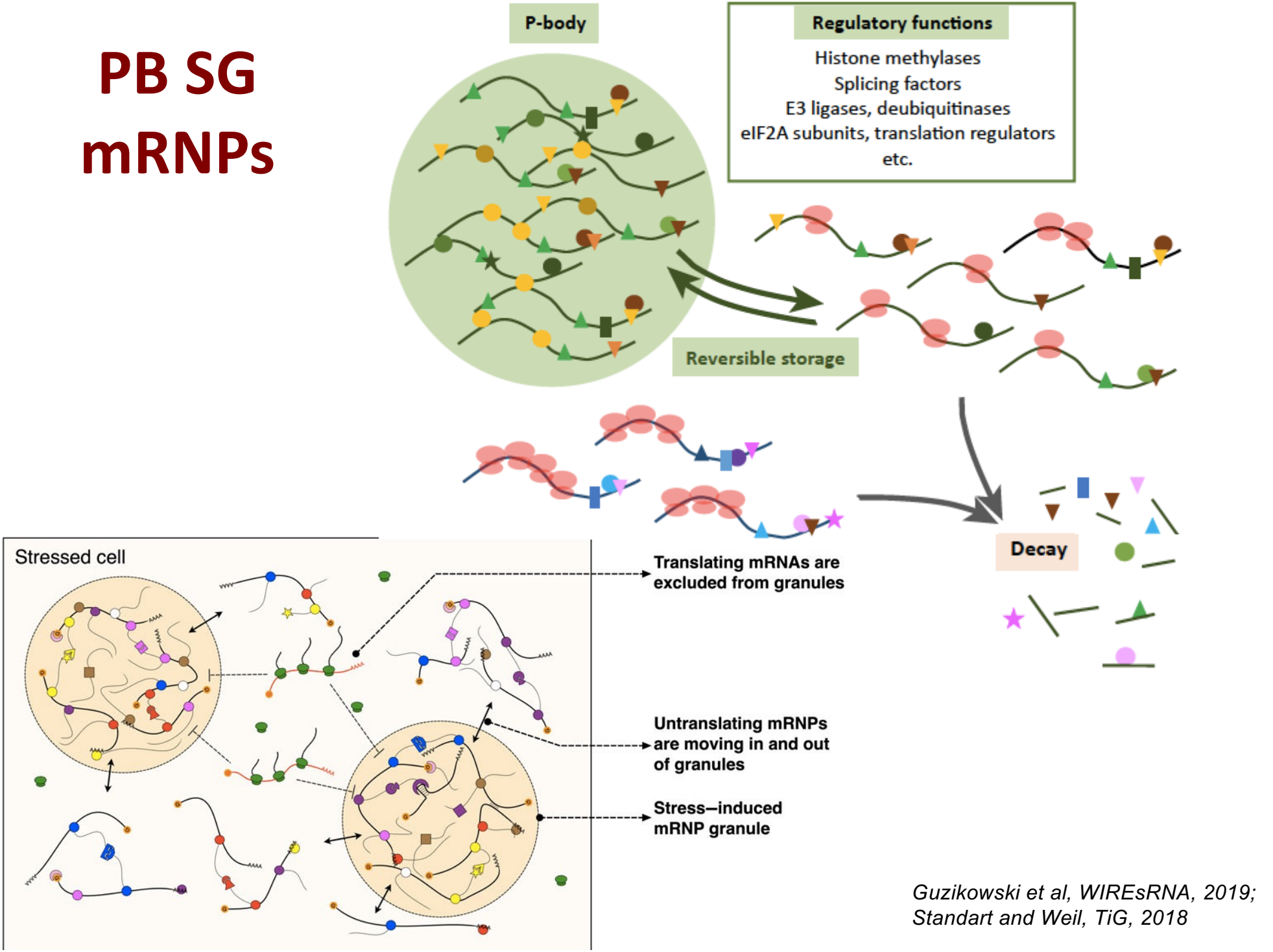


SG: global translation halts upon stress, mRNAs bound to the translational machinery and other proteins form SGs.

PB: translationally stalled mRNAs devoid of initiation factors shuttle to PBs.

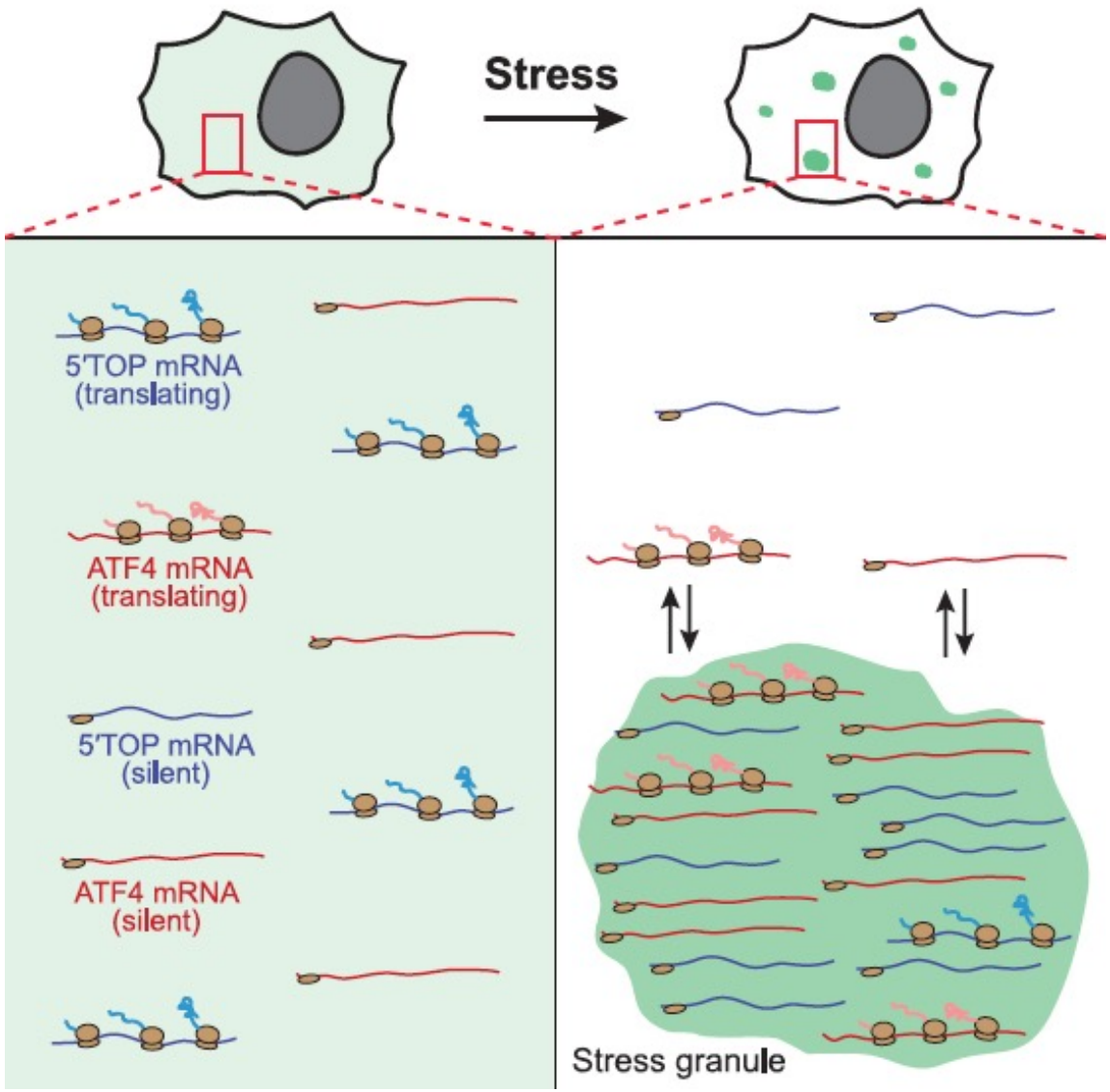
Chantarachot and Bailey-Serres, Plant Phys, 2018

PB SG mRNPs



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

Translation in SGs



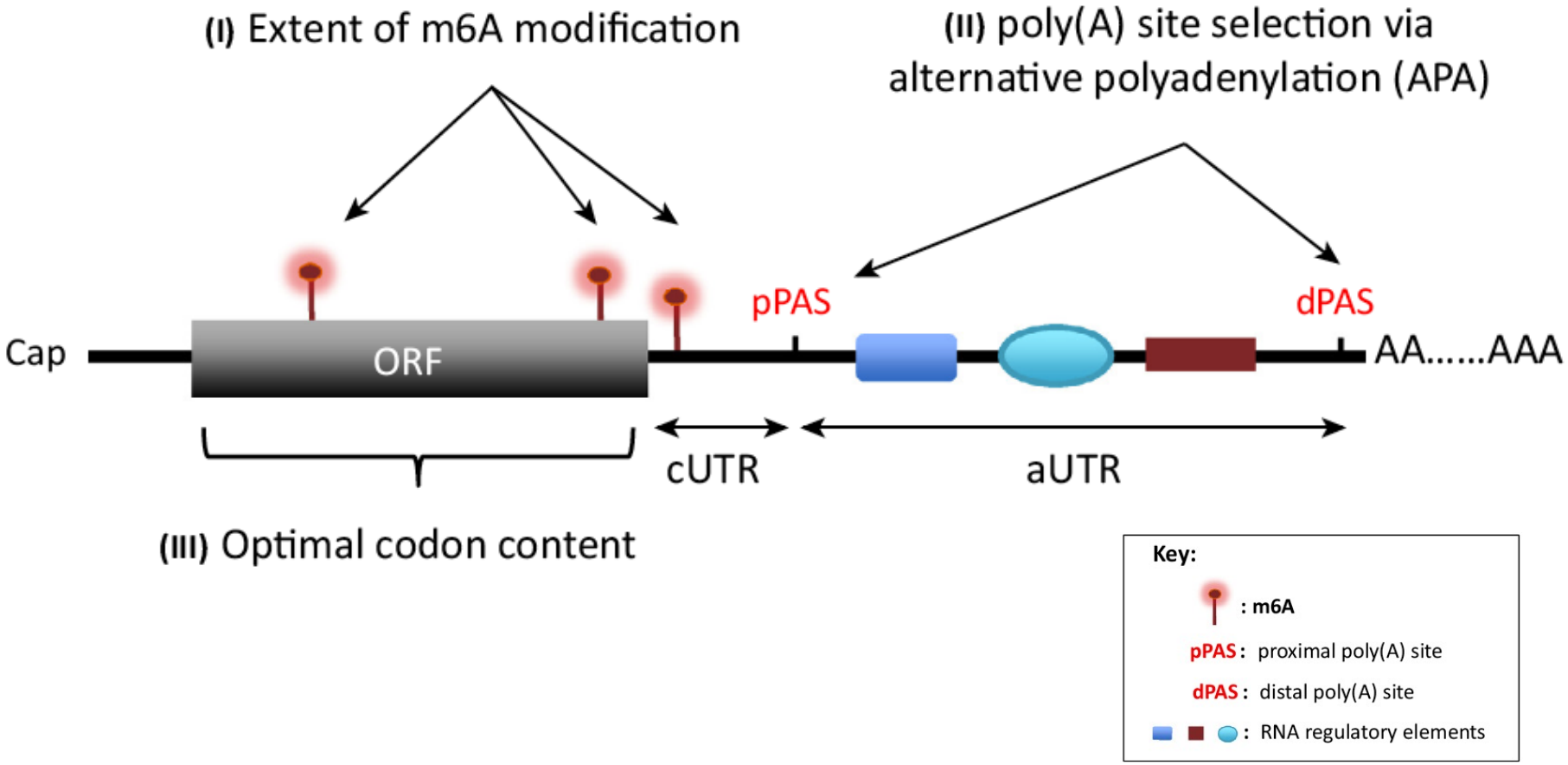
- nontranslating mRNAs are preferentially recruited to SGs
- mRNAs in SGs can undergo translation (complete cycle)
- translating mRNAs can enter, leave, or stably localize to SGs
- translation in SGs mainly, but not only, occurs on mRNAs enhanced under stress

(shown using single-molecule mRNA imaging, SunTag)

Mateju et al., Cell, 2020

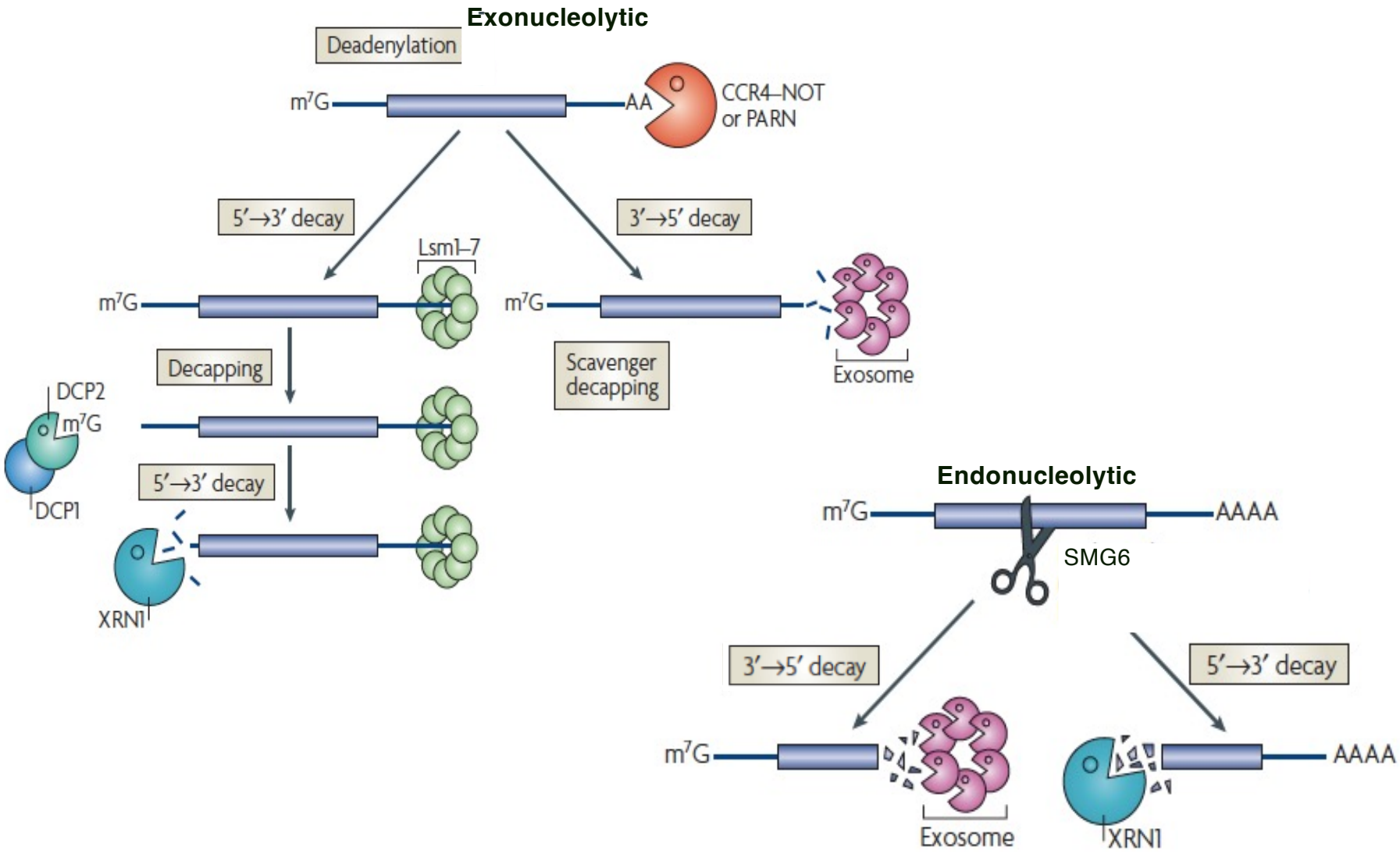
mRNA STABILITY

Elements *in cis*:



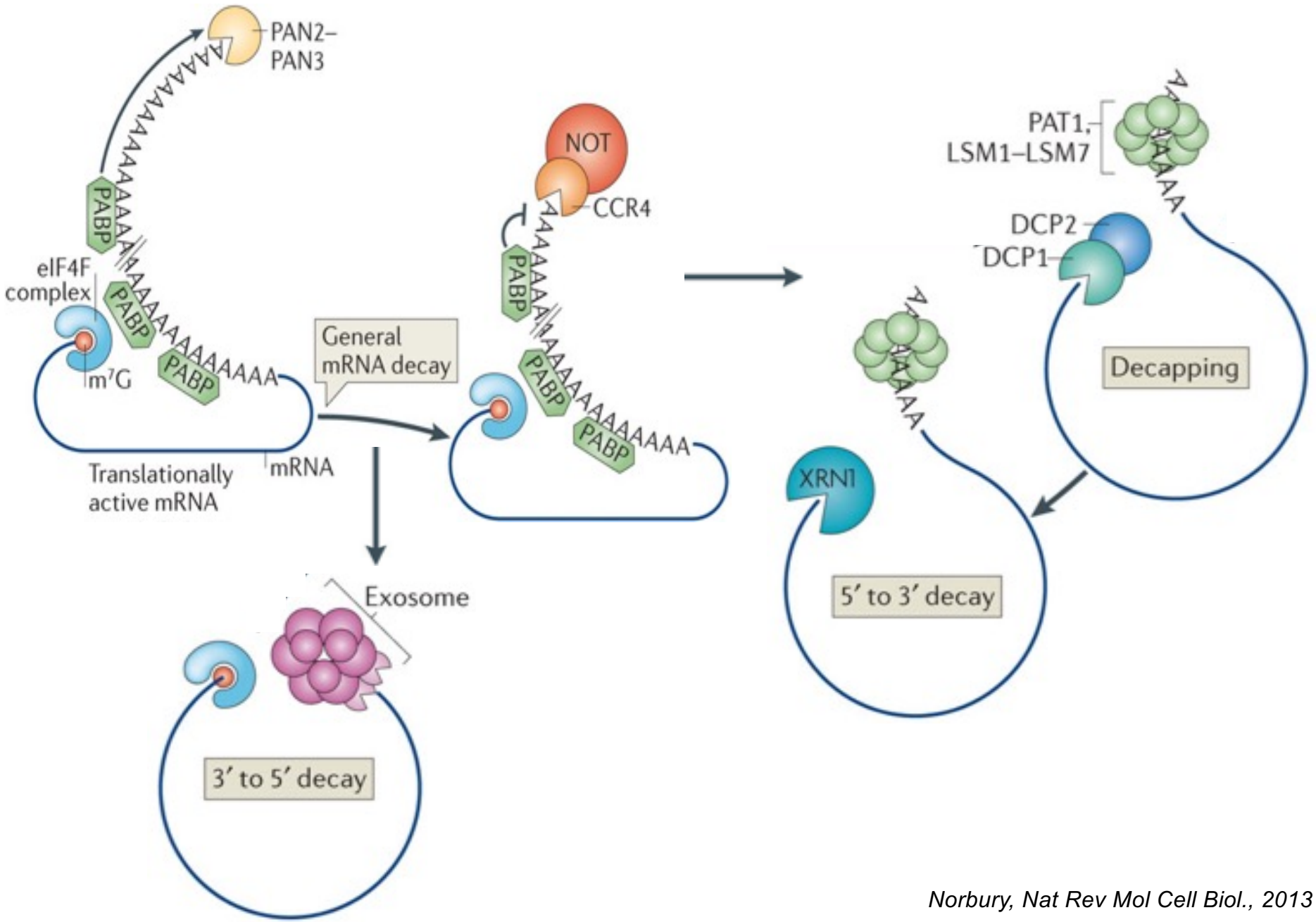
Chen and Shyu, TiBS 2016

mRNA general decay in the cytoplasm



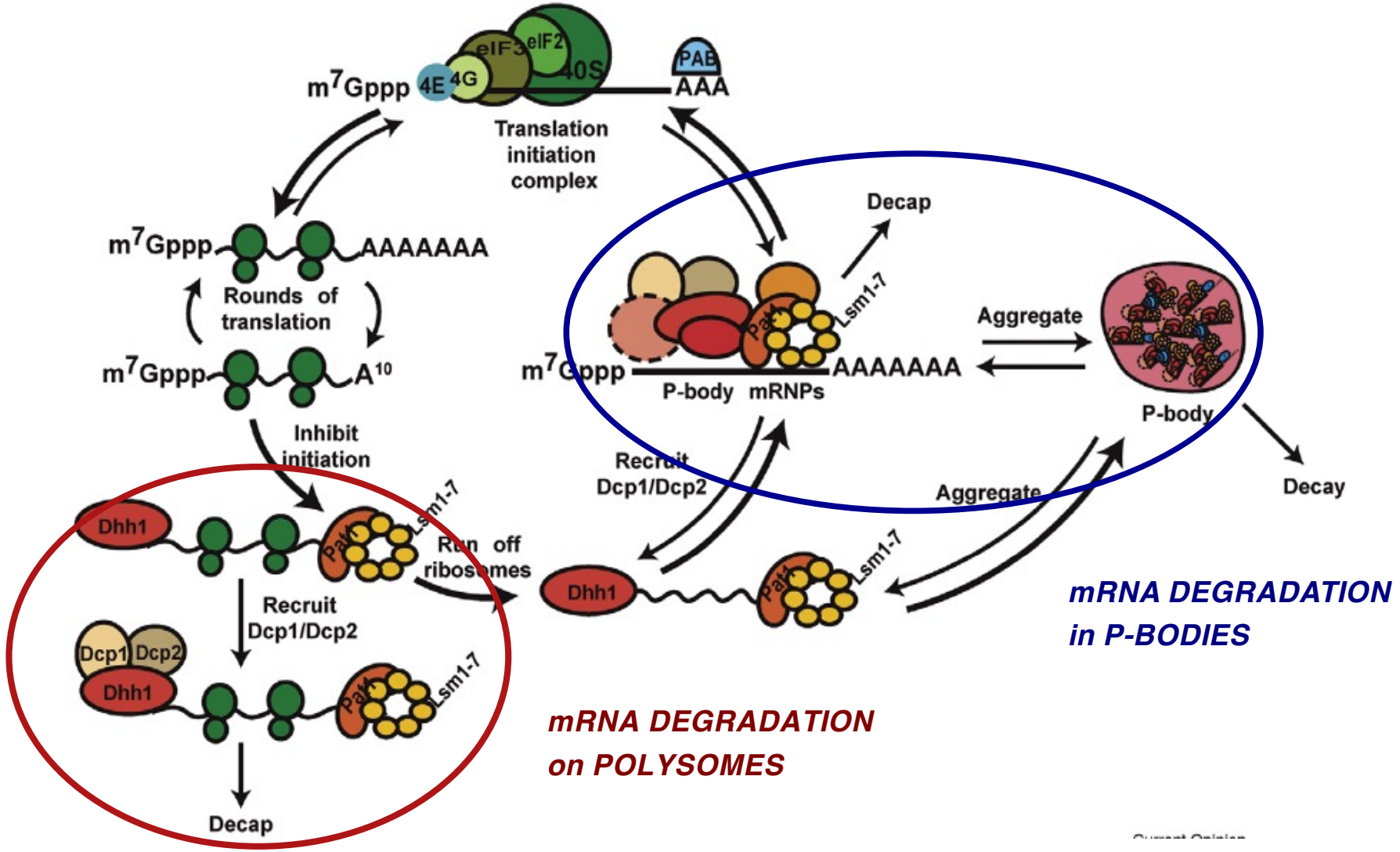
Garneau et al, Nat.Rev.Mol. Cell. Biol. 2007

mRNA degradation in the cytoplasm



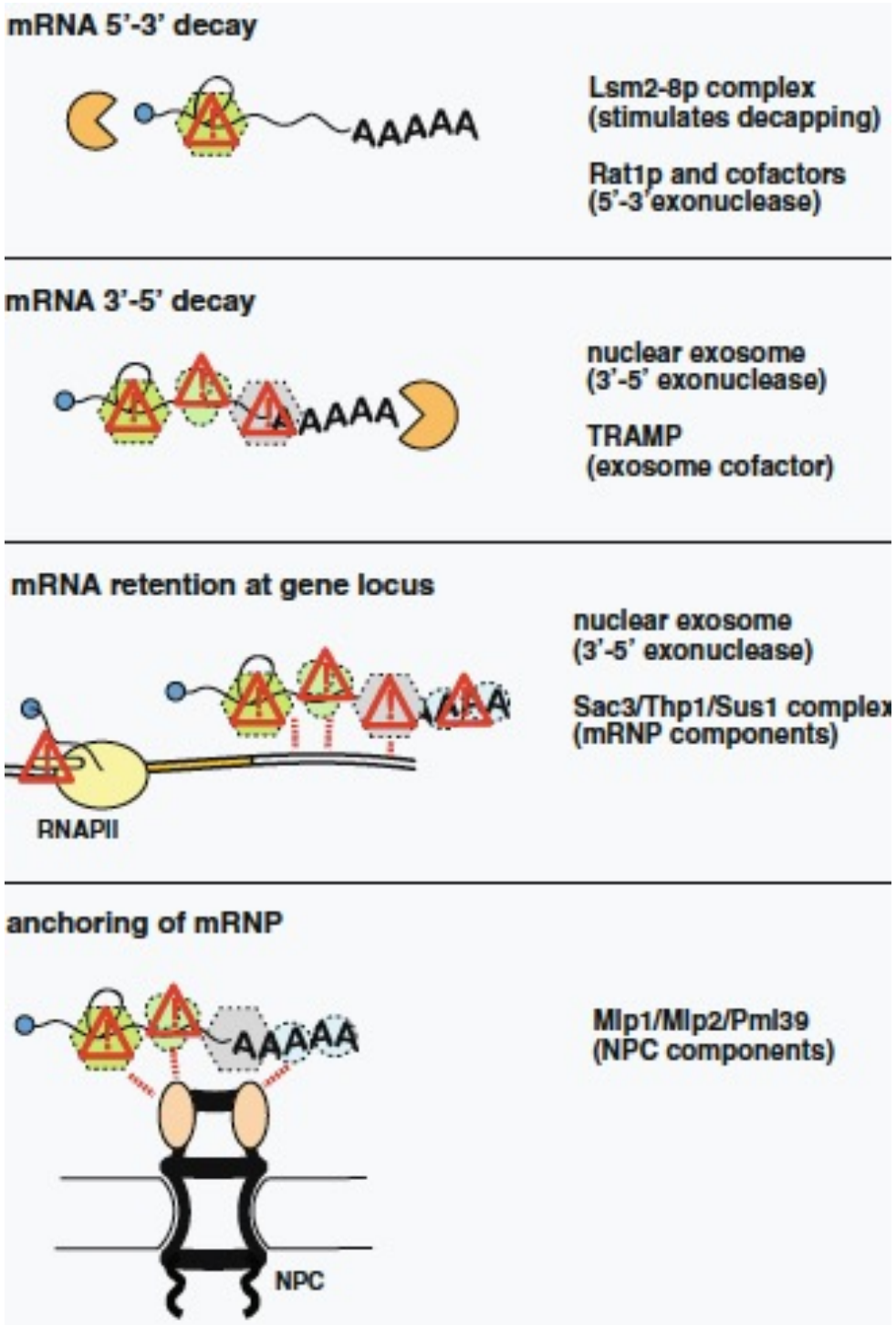
Norbury, Nat Rev Mol Cell Biol., 2013

mRNA degradation in the cytoplasm



Balagopal and Parker, Curr. Op.Cell Biol., 2009

DEADENYLATION → RELEASE OF RIBOSOMES → RELEASE OF TRANSLATION FACTORS → RECRUITMENT OF DECAY FACTORS → RNA DECAY



RNA decay in the nucleus

- Unspliced pre-mRNAs
- 3'-end unprocessed pre-mRNAs
- Unpackaged mRNAs
  (wrong mRNPs)
- mRNAs retained in the nucleus
  (export defect)
- Transcripts retained at chromatin
- Aberrant ncRNAs
- Unmodified tRNAs
- Excessive rRNAs and tRNAs

Schmid and Jensen, Chromosoma., 2008

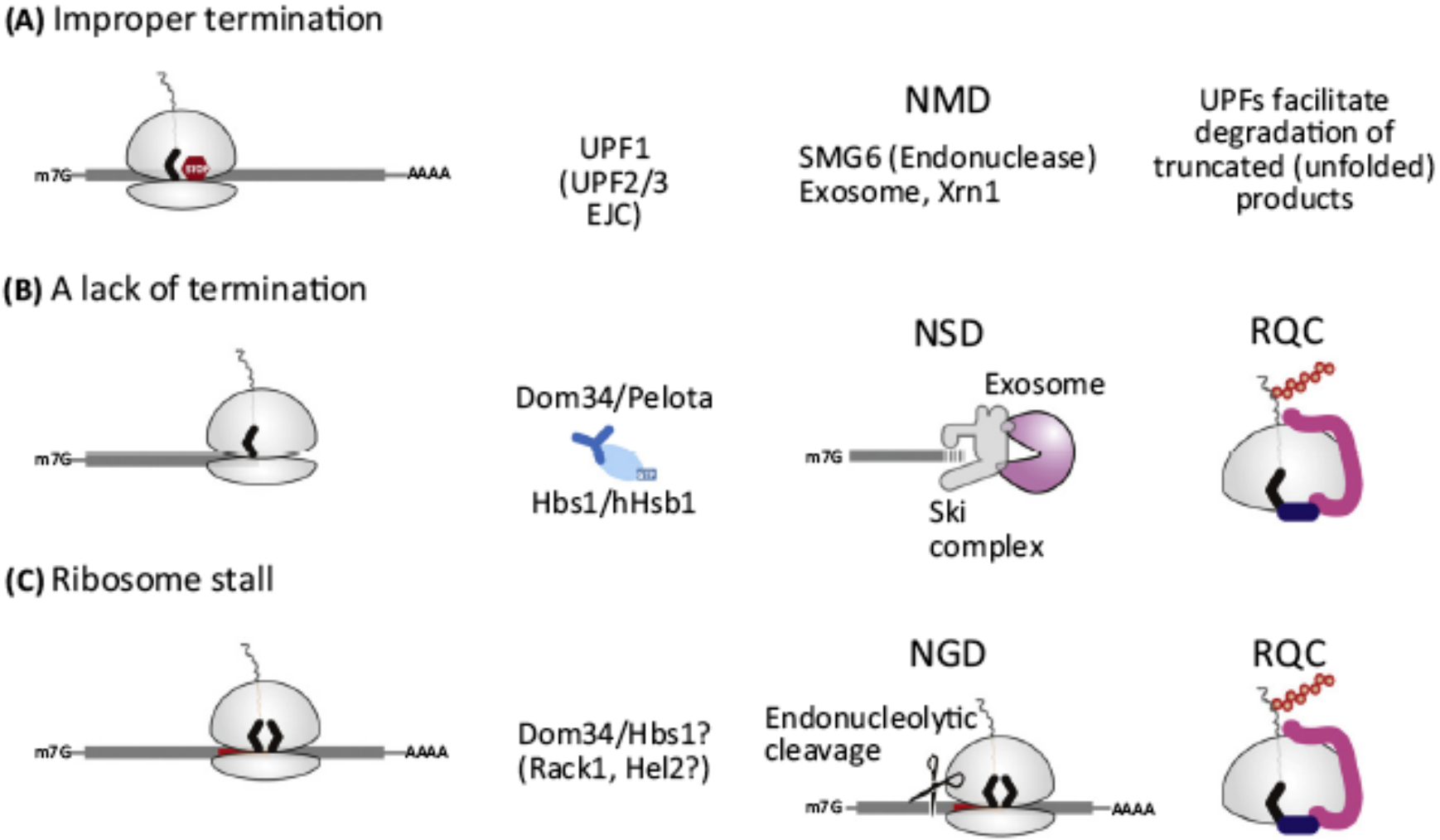
mRNA quality control decay in the cytoplasm

NMD – Nonsense Mediated Decay (mRNAs with premature STOP codon)

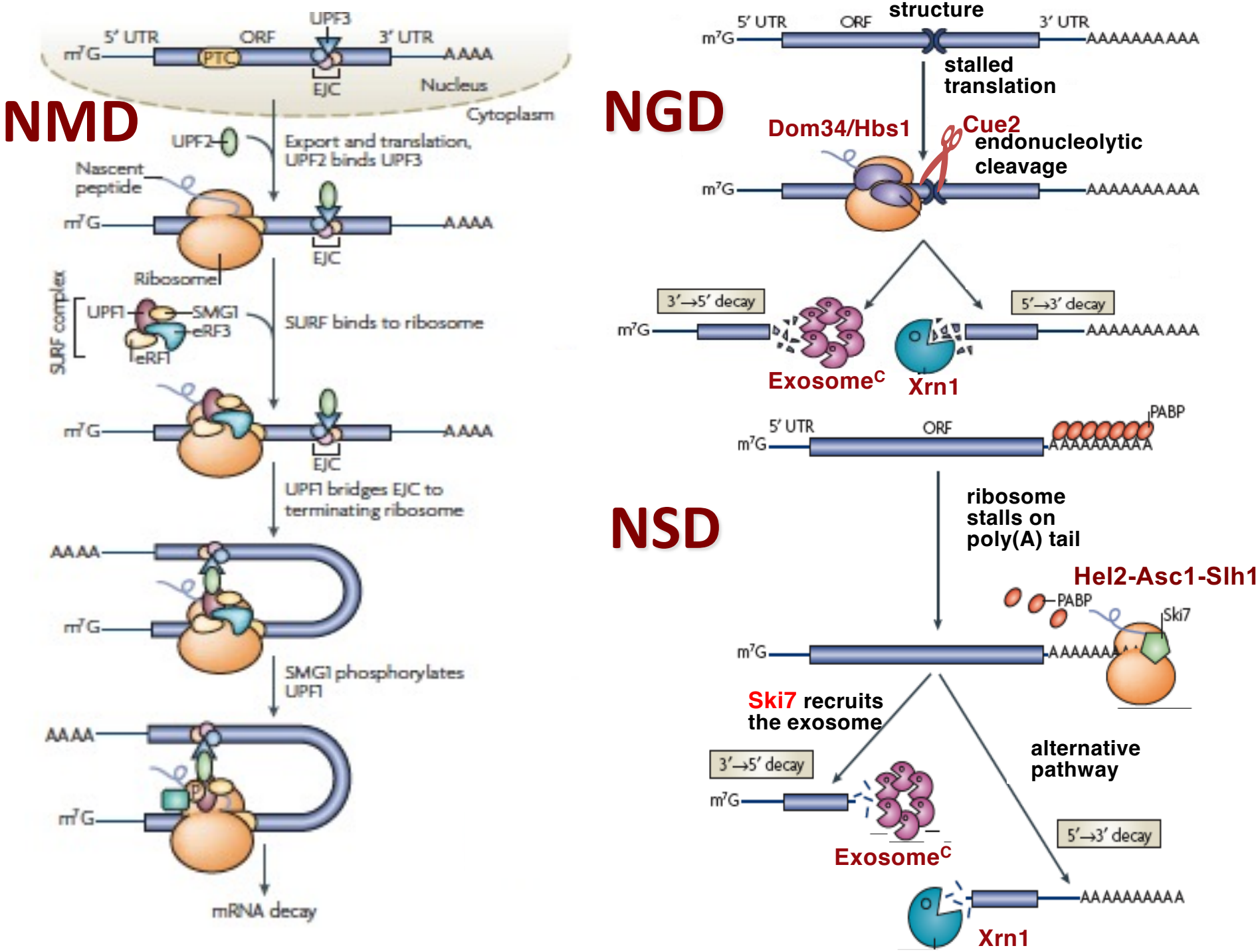
NGD – No-Go Decay (ribosome stuck on an obstacle)

NSD – Non-Stop Decay (mRNAs with no STOP codon)

Problems with a stalling ribosome during translation



Inada, TiBS 2016

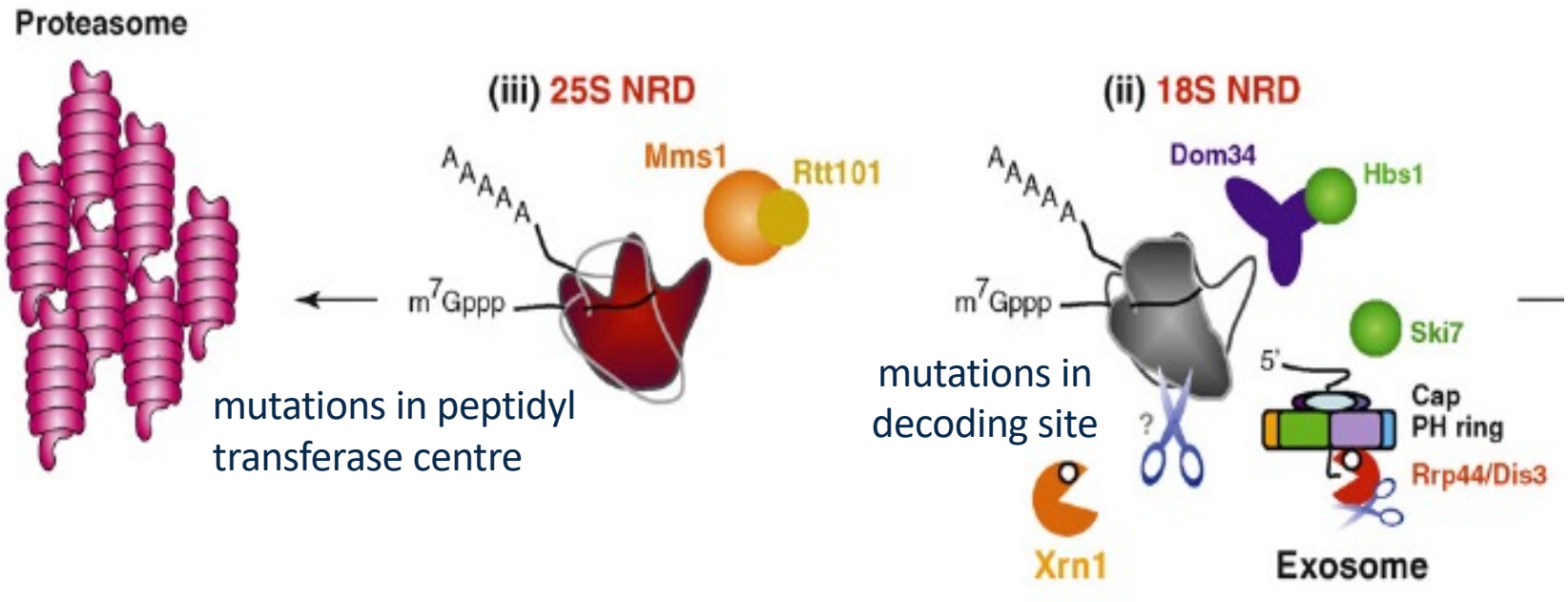


Garneau et al, Nat.Rev.Mol. el. iol. 2007

rRNA surveillance

NRD- Nonfunctional rRNA Decay

Mature aberrant ribosomes are eliminated in the cytoplasm

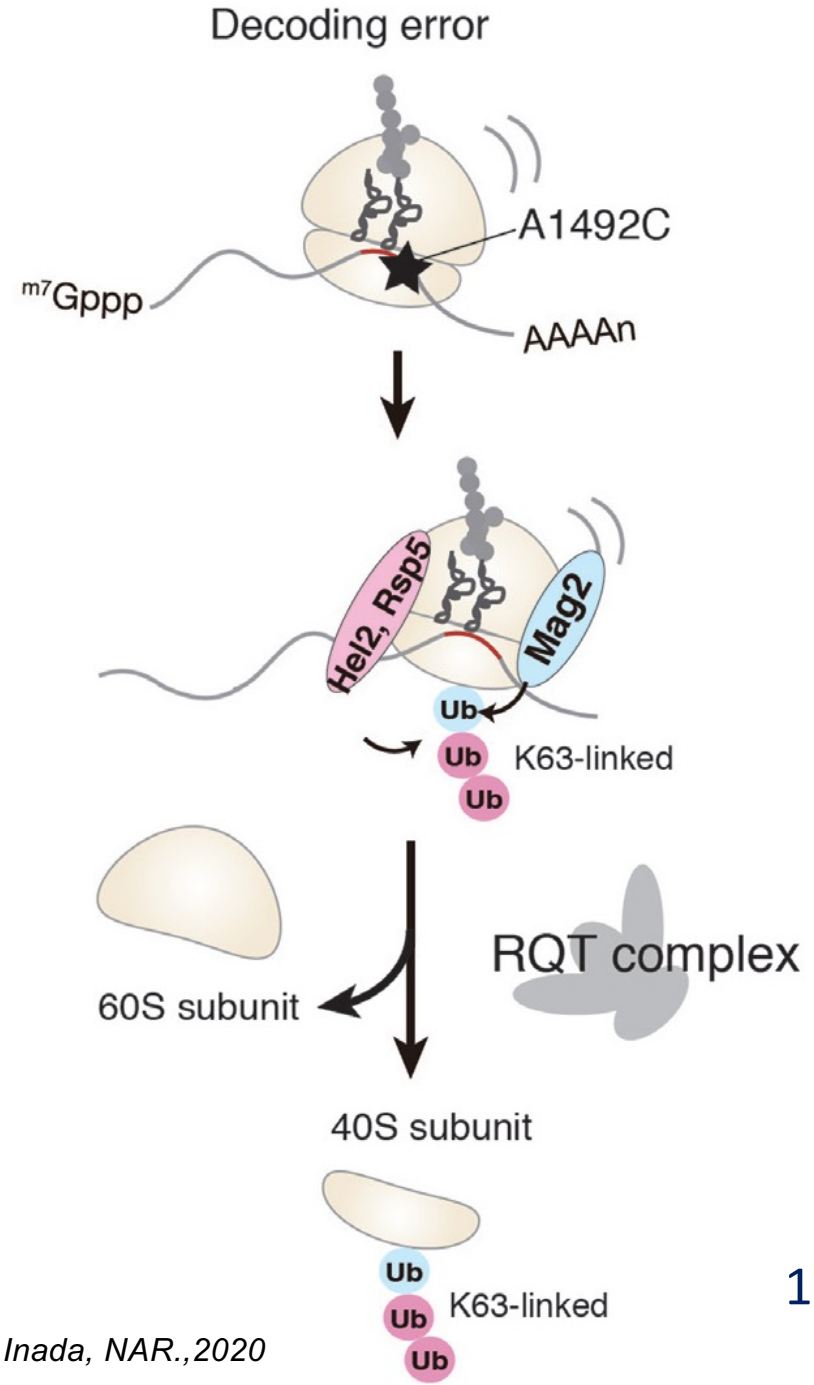


Mms1 & **Rtt101**
subunits of E3 ubiquitin ligase complex

Dom34 & **Hbs1**
factors involved in NGD and NSD

Lafontaine, TiBS.,2010

18S NRD



Inada, NAR.,2020

- **Ribosome stalling due to decoding error**
- **Recognition of the stalled ribosome**

- **Ribosome ubiquitination**

K212 of RPS3 is monoubiquitinated by **Mag2** followed by polyubiquitination by **Hel2** or **Rsp5**

- **Ribosome dissociation**

Subunit dissociation by the Ski2-like RNA helicase **Slh1** in the **RQT** complex

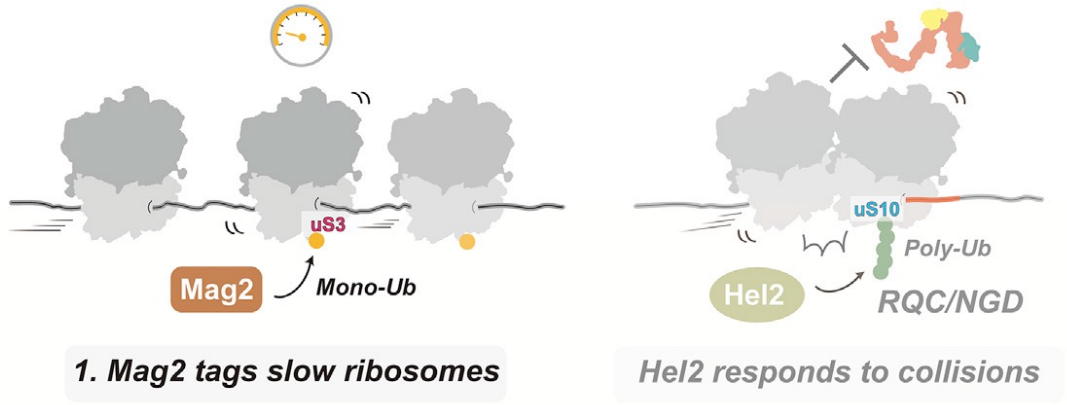
- 18s rRNA degradation

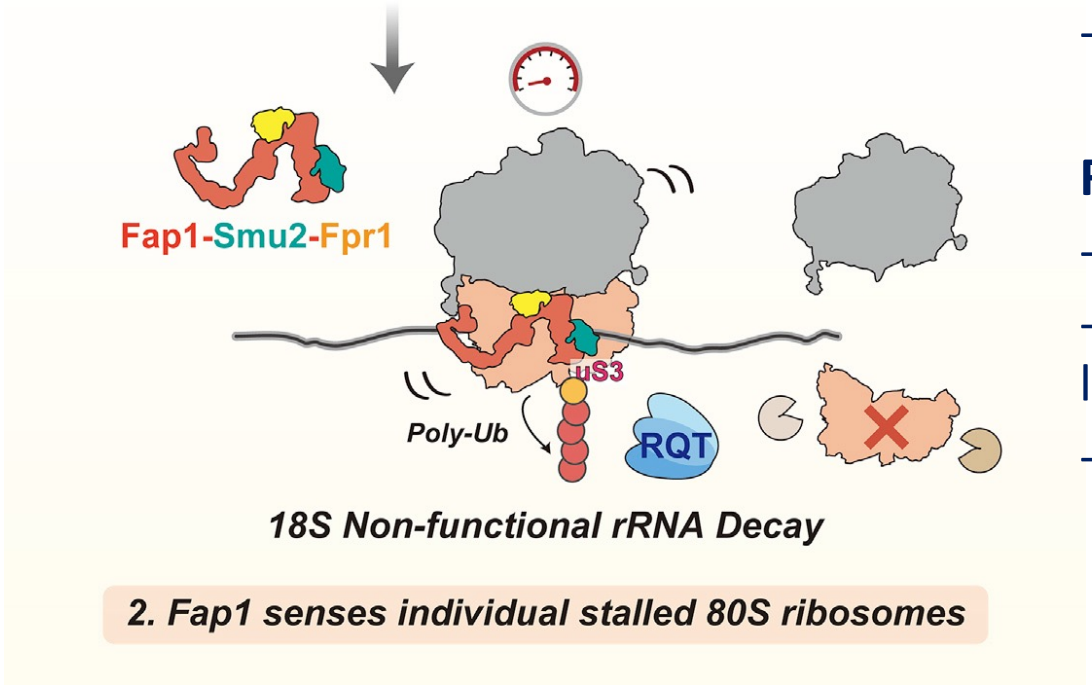
by **Xrn1** or **exosome**

18S NRD factors are also involved in RQC

rRNA surveillance

18S NRD versus RQC





18S NRD

- detects non-functional ribosomes (mutation in the decoding center 18S rRNA)
- or stalled monosomes
- stalled ribosomes recognized by Mag2 E3 ligase, RPS3 monoUb Fap1 E3 ligase, RPS3 polyUb
- non-functional ribosomes are degraded

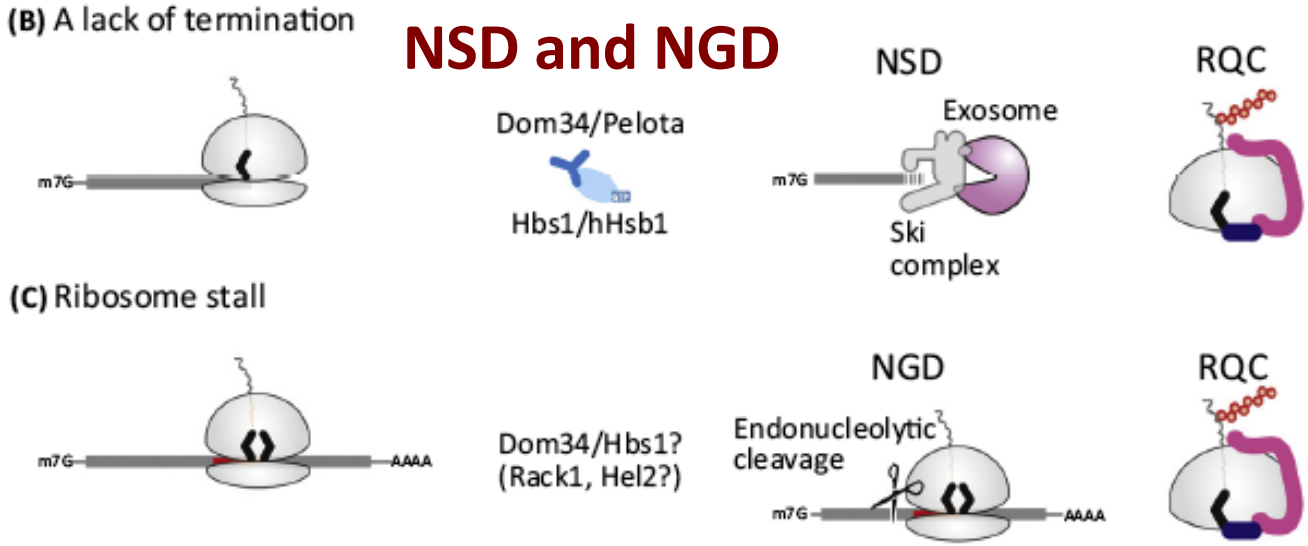
Ribosome Quality Control - RQC

- detects collided ribosomes
- stalled disome recognized by Hel2 E3 ligase, RPS10-polyUb
- ribosomes are released and recycled

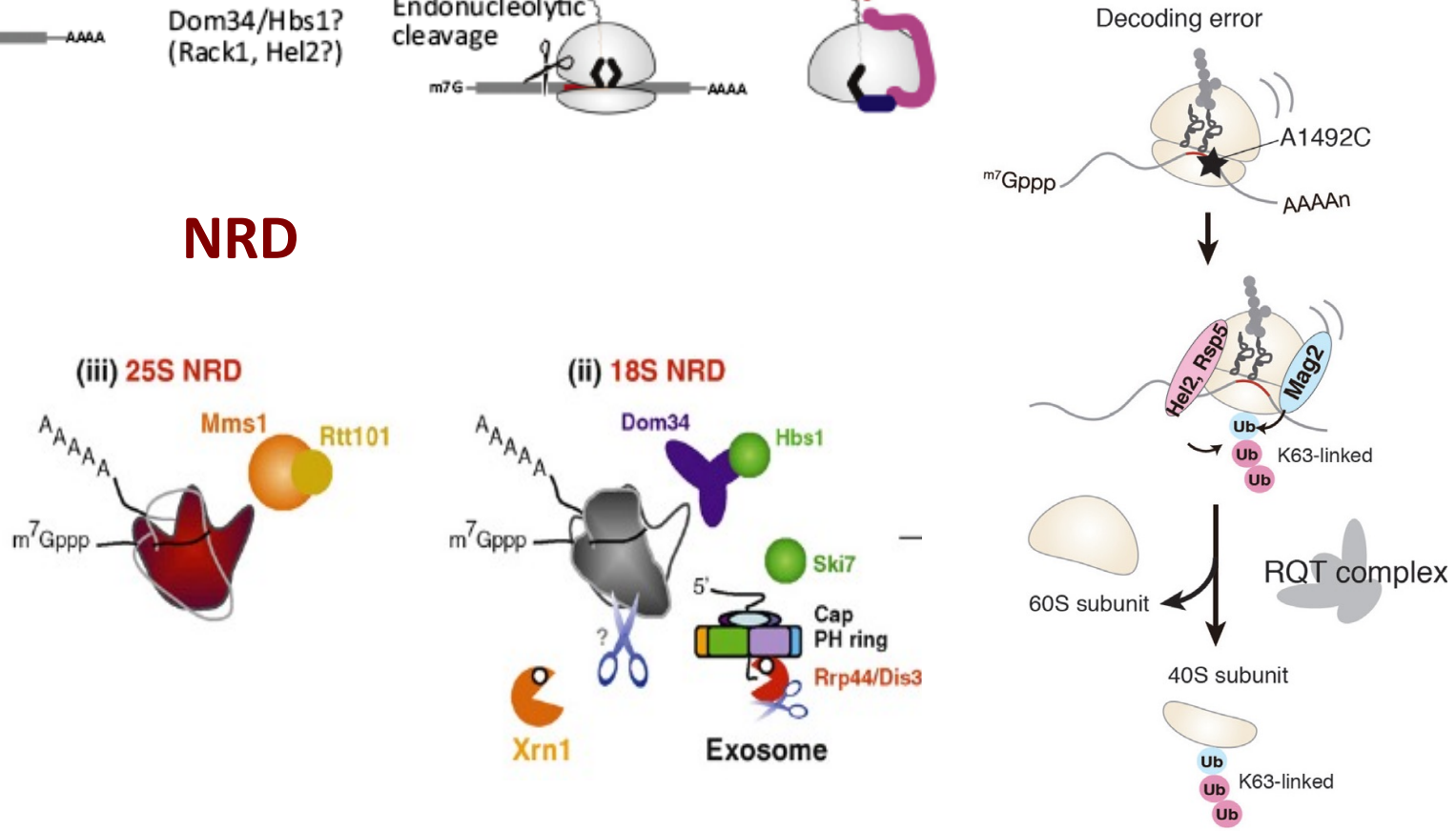
Li et al, Mol Cell.,2022

Ribosome-associated Quality Control - RQC

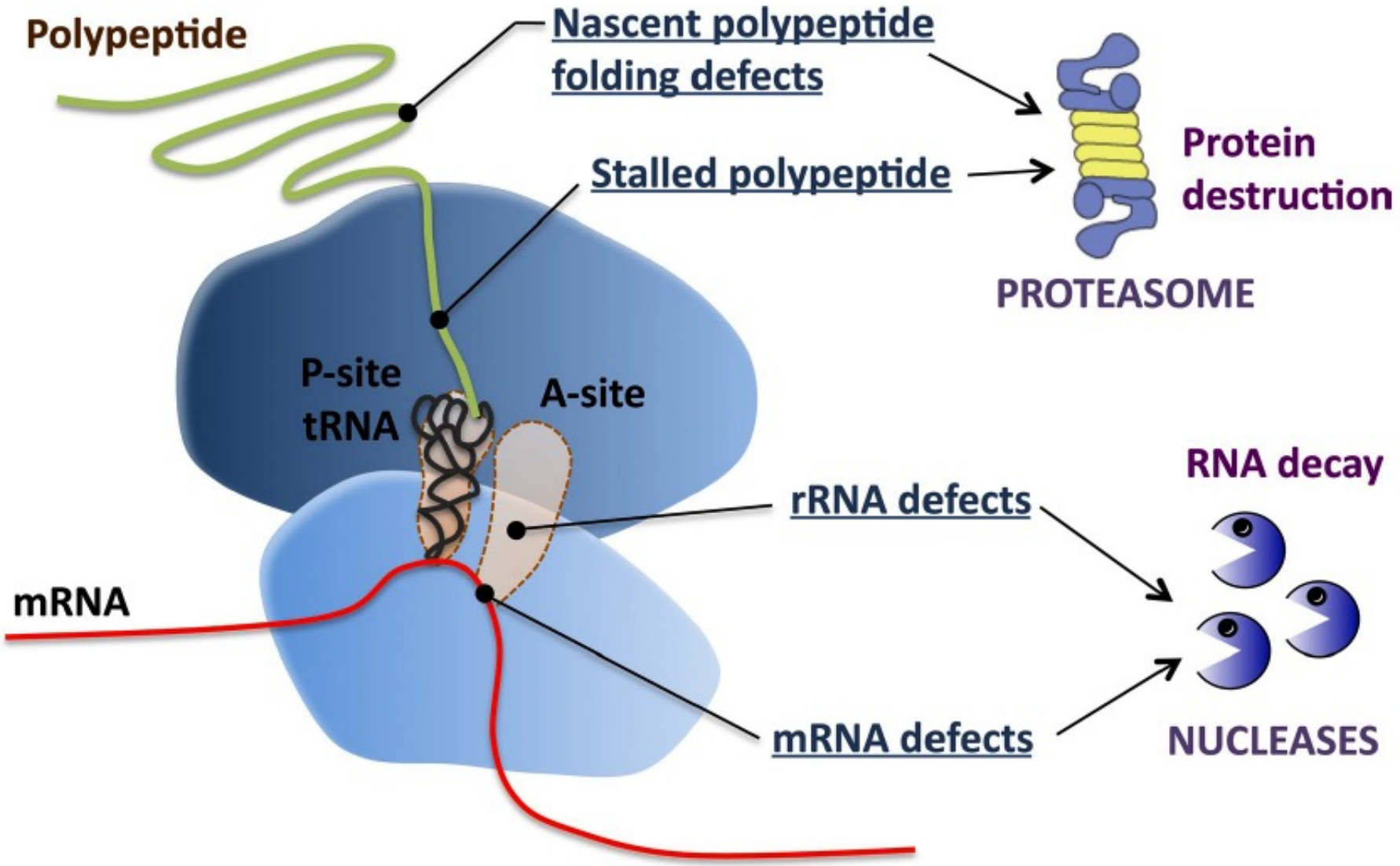
NSD and NGD



NRD

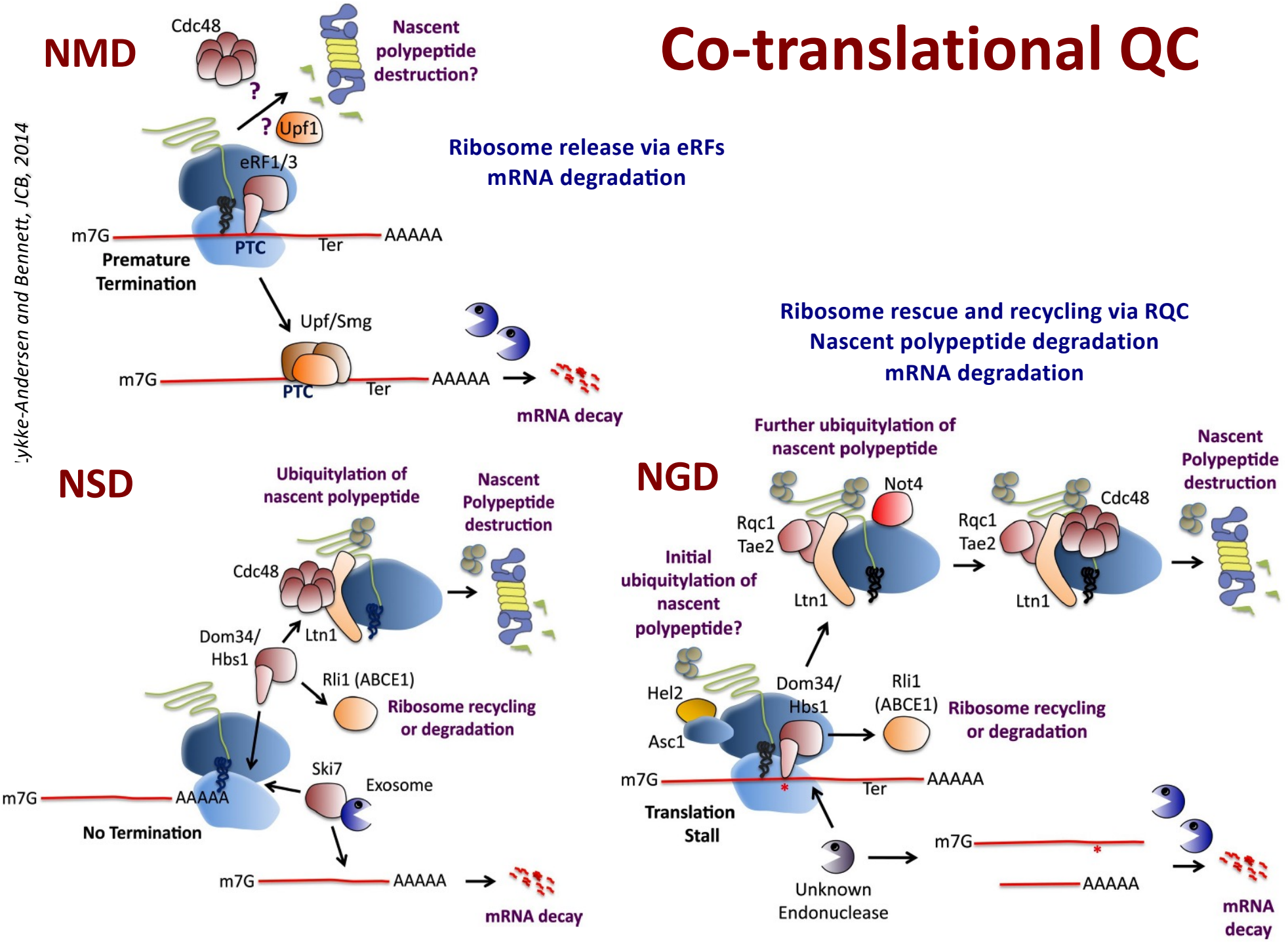


Co-translational mRNA, peptide and ribosome QC

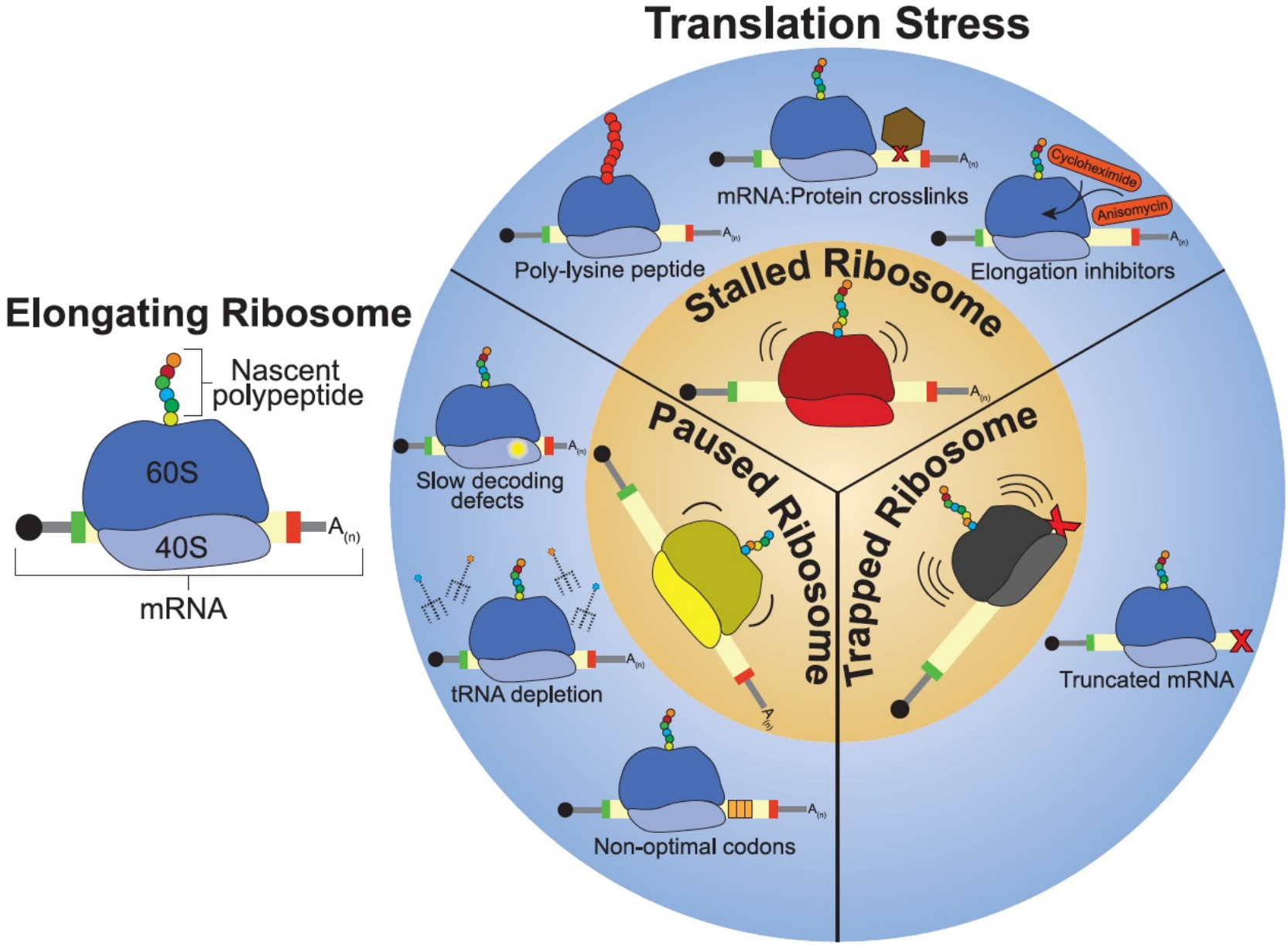


Lykke-Andersen and Bennett, JCB, 2014

Co-translational QC

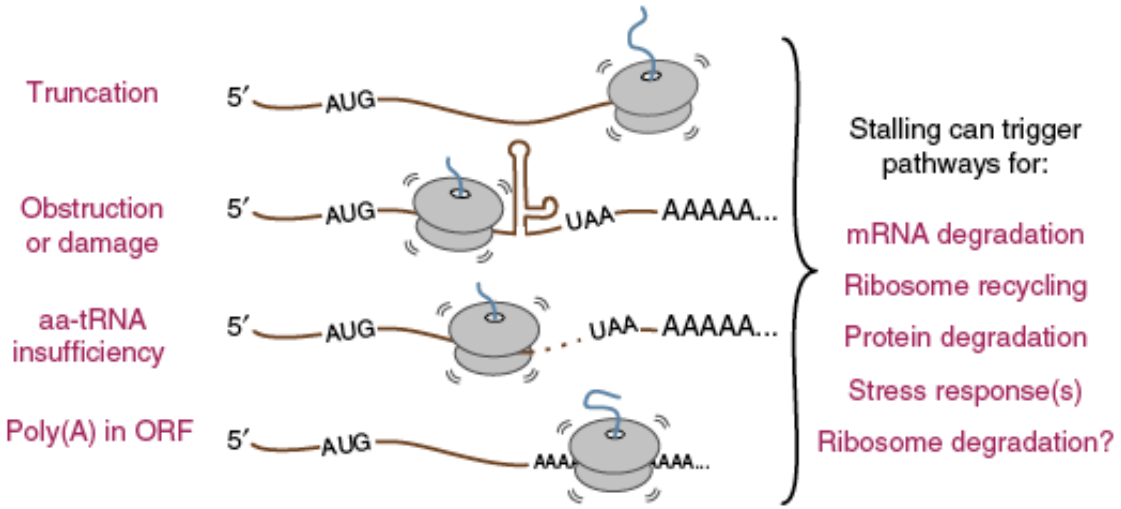


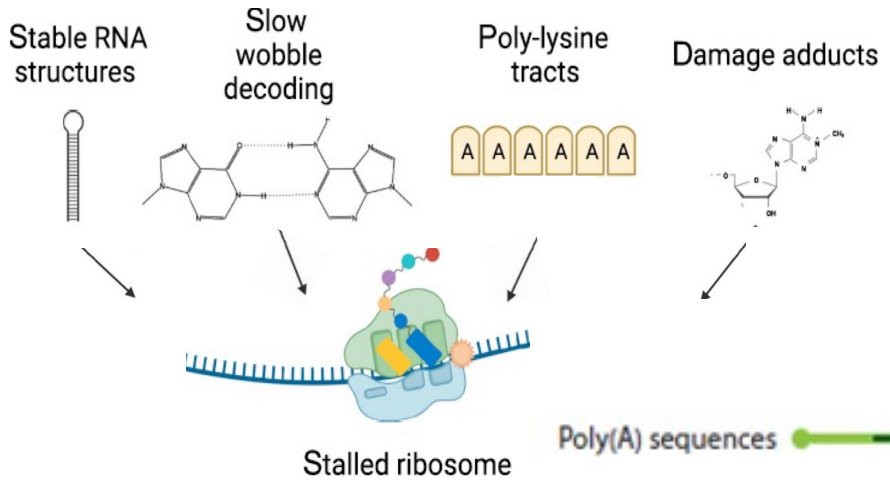
Ribosome-associated Quality Control - RQC

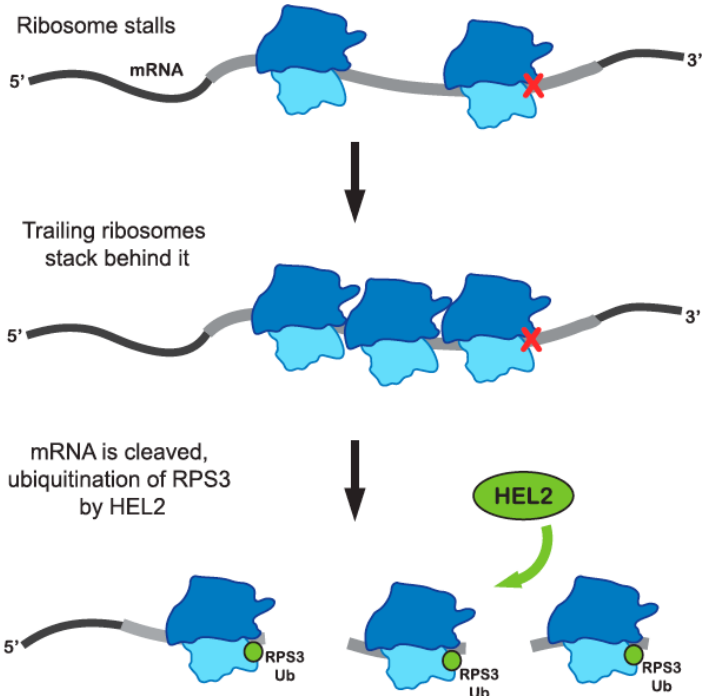


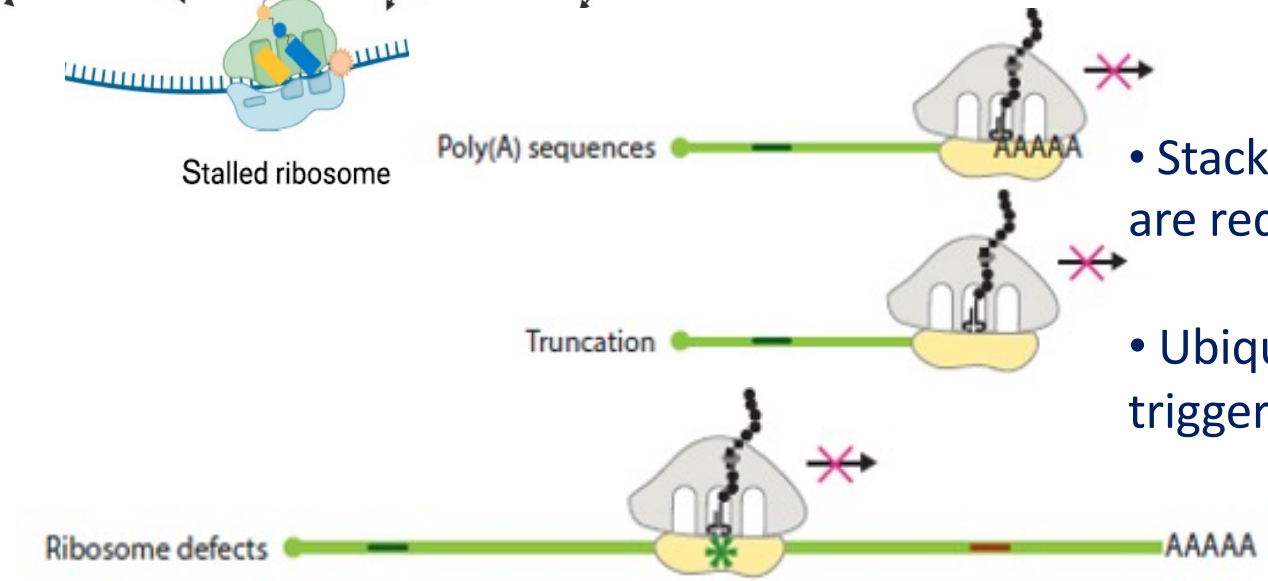
Ford et al, Cell Rep, 2024

Ribosome stalling





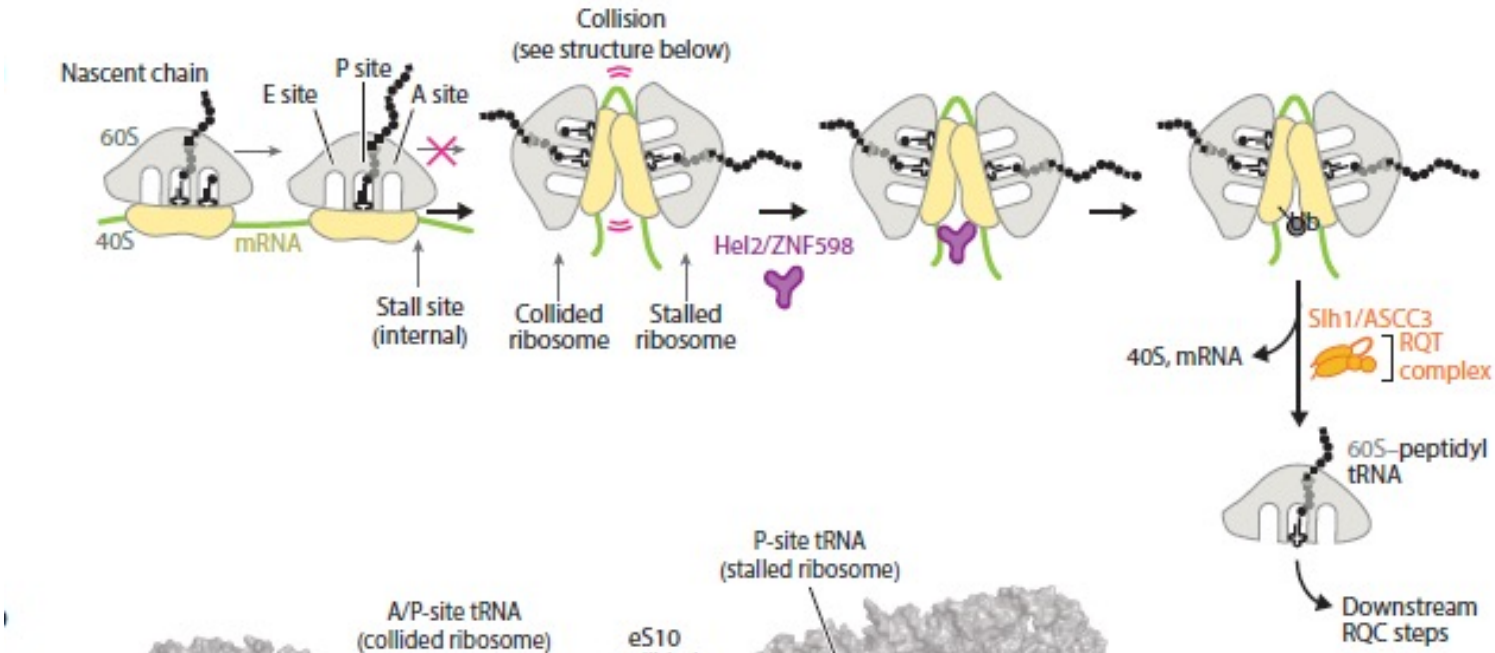


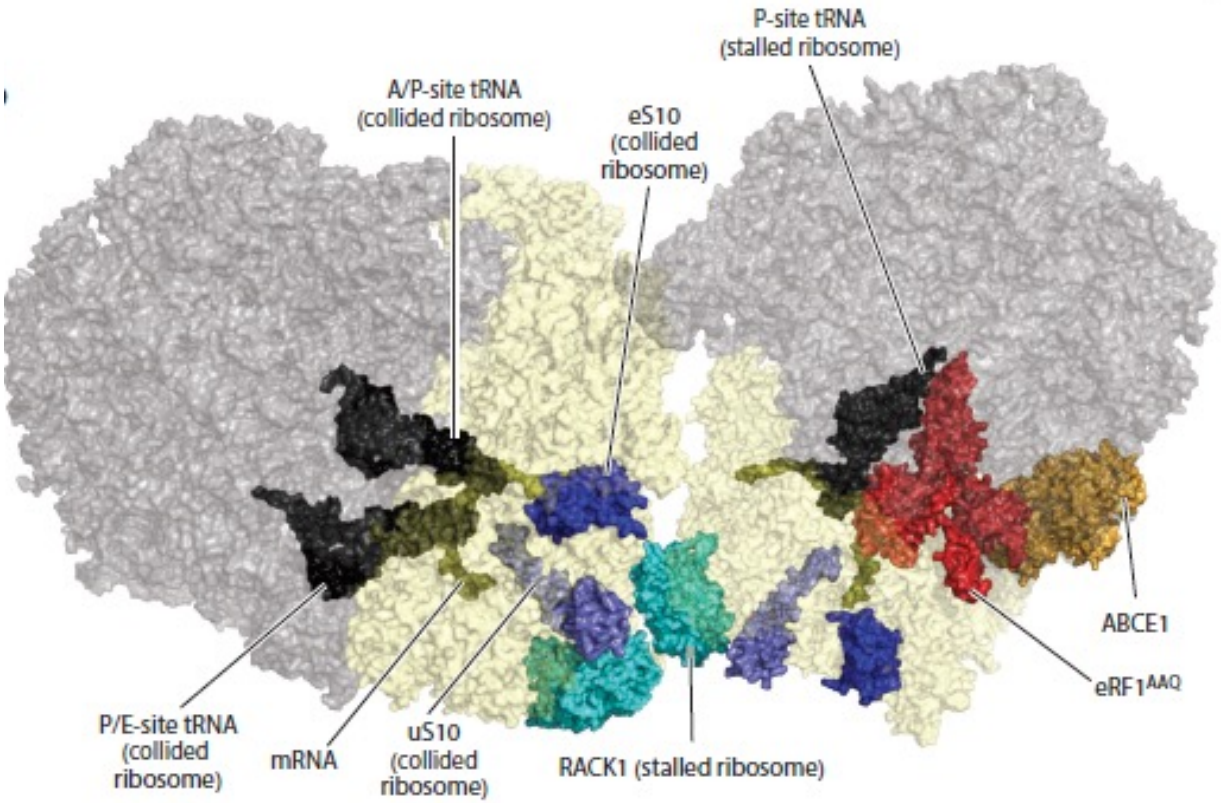


- Stacked or colliding ribosomes are required to elicit NGD
- Ubiquitination of RPS3 by Hel2 triggers RQC

Brandman and Hegde, NatStrMolBiol 2016; Sitron and Brandman, AnnRevBiochem, 2020; Kim and Zaher TiBS, 2021

Ribosome stalling and collision



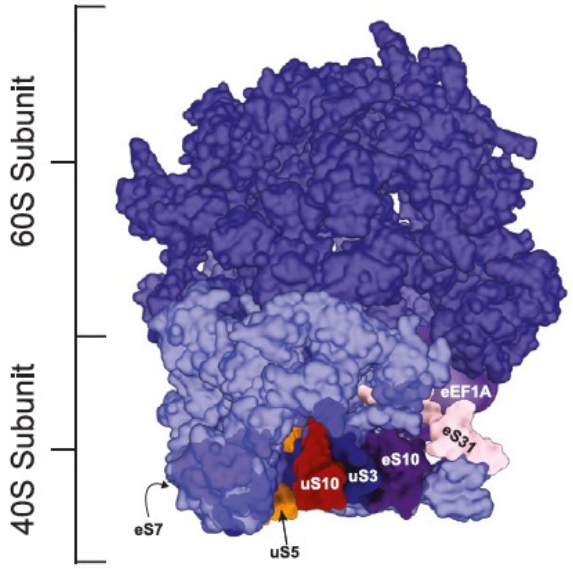


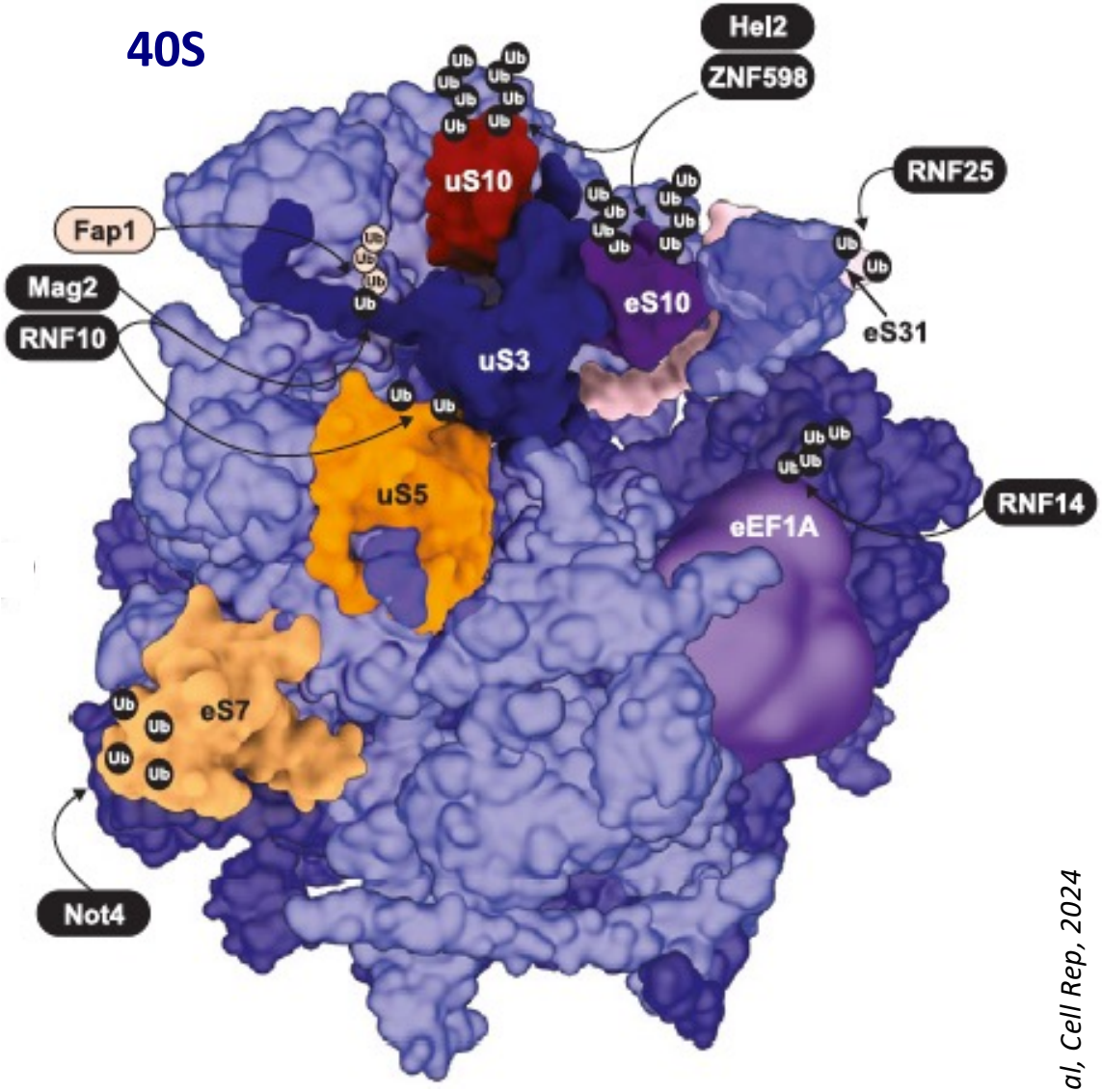
Ribosome stalling →
Ribosome collision →
Recognition by the E3 Ub ligase Hel2/ZNF589 →
Ubiquitination of ribosomal proteins (RPS3, RPS20, RPS19)

But ribosome stalling does not always lead to collision

Sitron and Brandman, AnnRevBiochem, 2020

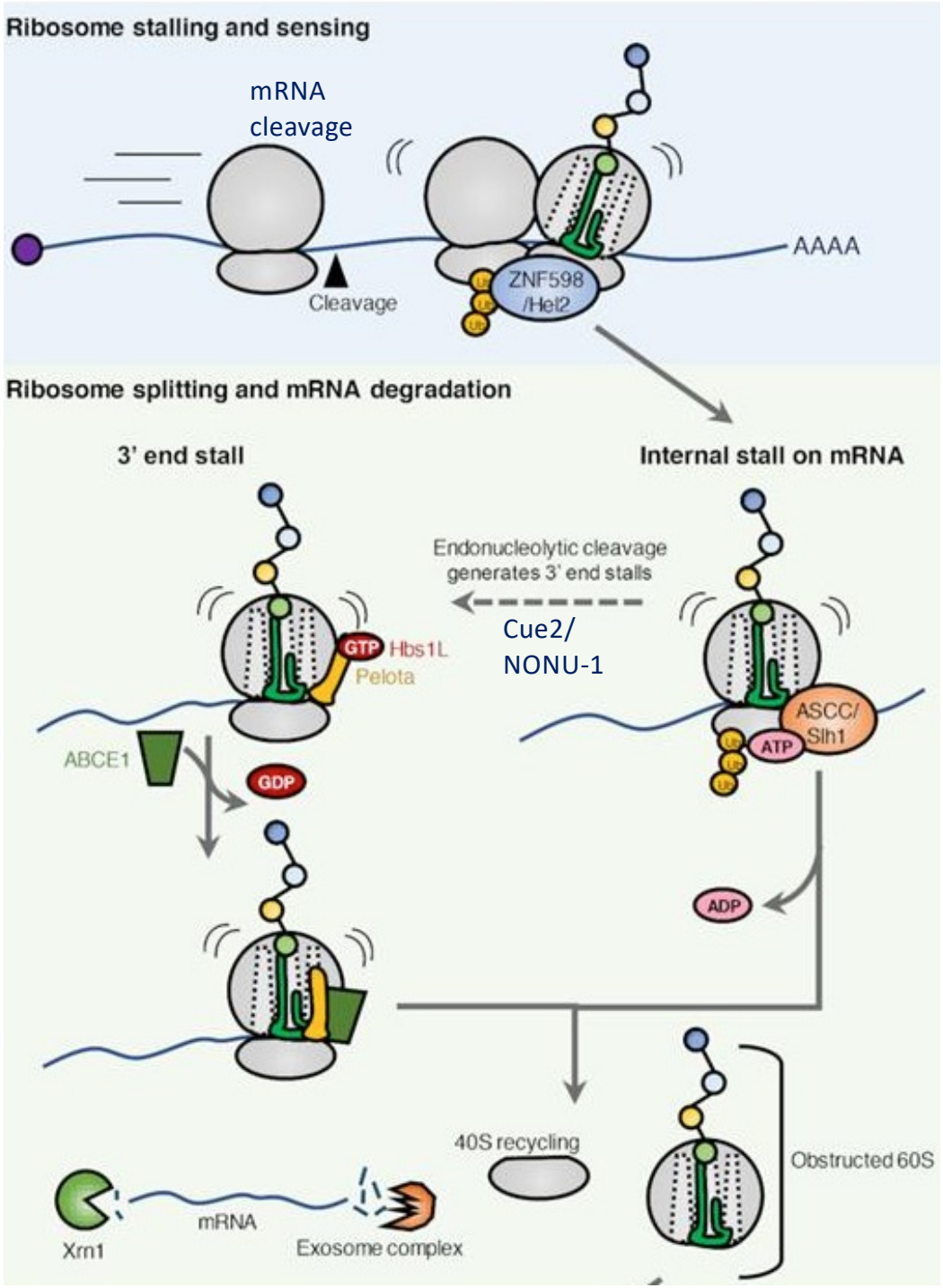
RP ubiquitination in the 40S





Ubiquitination of specific ribosomal proteins (RPs) by ubiquitin ligases serves to resolve stalled ribosomes

Ford et al, Cell Rep, 2024



Ribosome rescue

Ribosome collision

Recognition by Hel2/ZNF589

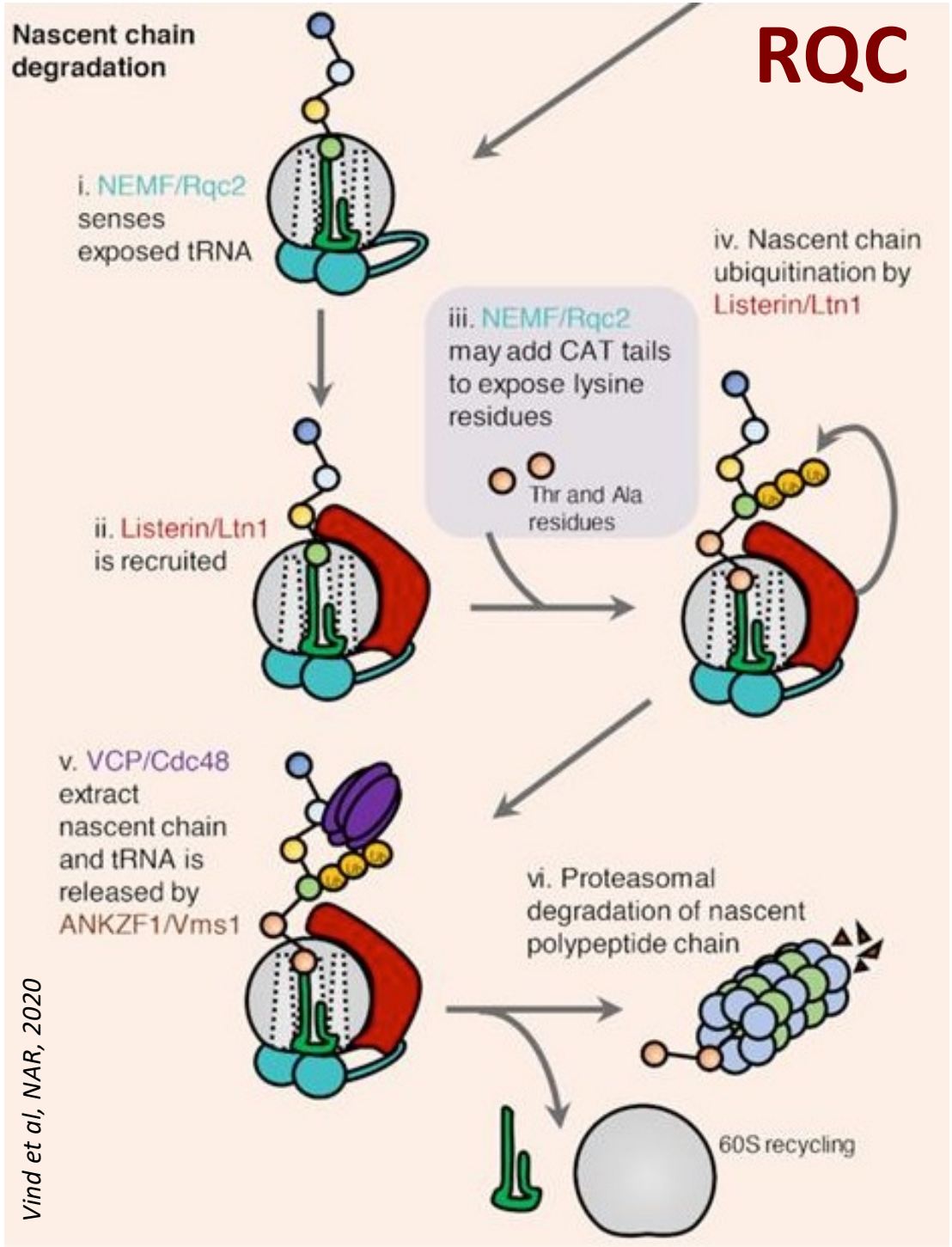
Ubiquitination of RPs (RPS3, RPS20, RPS19)

Endonucleolytic mRNA cleavage by Cue2/NONU-1

Ribosome splitting/disassembly
- by Dom34-Hbs1-Rli1
(Pelota/HBS1L or GTPBP2 /ABCE1)
or
- RQT (ribosome quality control trigger) complex Slh1, Cue3/Rqt3, and Rqt4

mRNA degradation (optional)

Vind et al, NAR, 2020



Recognition of tRNA-obstructed 60S by Rqc2/NEMF component of RQC. Recruitment of Ltn1/Listerin

Ubiquitination of the nascent chain Lys residues by E3 Ub ligase Ltn1/Listerin

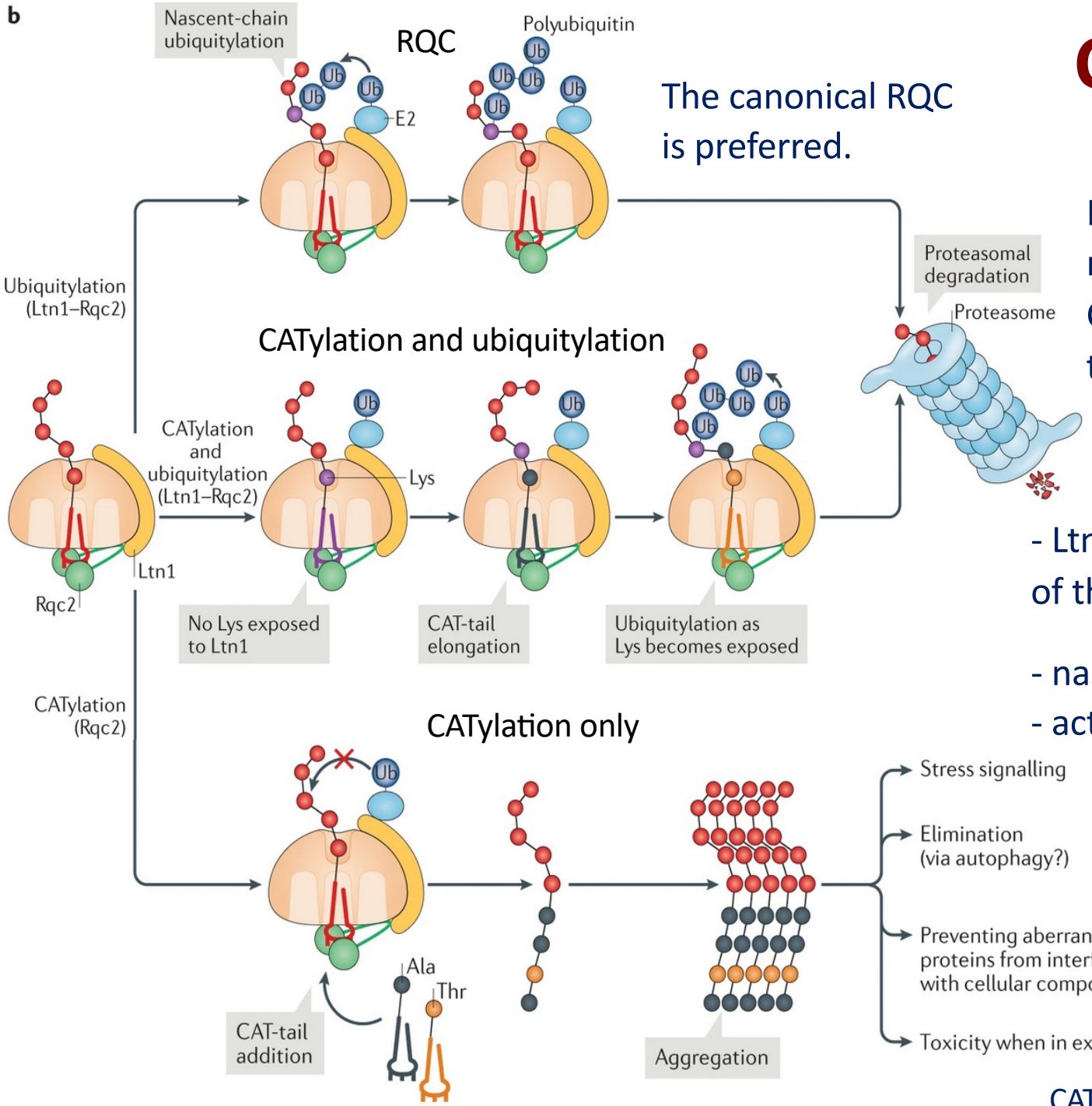
Alernative: CAT-tailing by Rqc2/ NEMF to expose Lys residues buried in the ribosome

Recruitment of ATPase Cdc48/VCP by ubiquitination

Extraction of the nascent chain by Cdc48/VCP and tRNA by Vms1/ ANKZF1

Delivery of the polypeptide to the proteasome by Cdc48/VCP. Polypeptide degradation.
60S recycling

CATylation



The canonical RQC is preferred.

If ubiquitylation of the nascent polypeptide fails, CAT tail is added by Rqc2 to extract the polypeptide

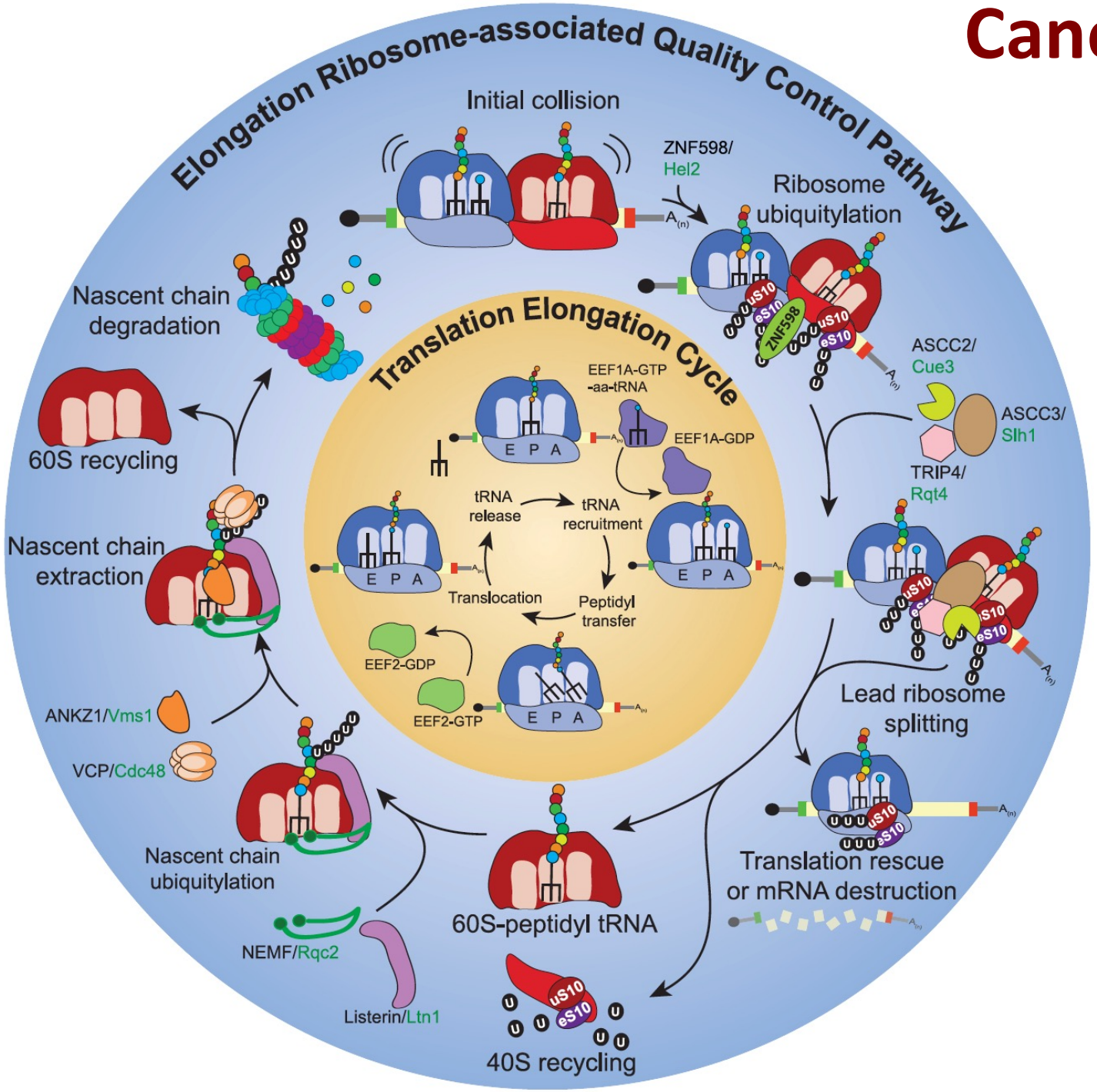
CATylation results in
- Ltn1-dependent degradation of the nascent peptide

- nascent chain aggregation
- activation of stress signaling

CAT tail - Ala and Thr extension

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

Canonical RQC



Ford et al, Cell Rep, 2024

NEXT LECTURE:

Global analyses of RNAs and RNPs

RQC factors

Recognition of stalled ribosomes differs for internally and terminally stalled ribosomes

Hel2/ZNF598: detection of internally stalled ribosomes

Hel2/ZNF598 probably (i) recognizes the rotated conformation of stalled ribosomes or (ii) senses collisions between adjacent ribosomes and binds to disomes

Hel2/ZNF598 marks 80S ribosomes by ubiquitylating their 40S subunits

Hel2 mono- and diubiquitylates uS10 and uS3, forming K48- and K63-linked ubiquitin chains on uS10

ZNF598 monoubiquitylates the 40S ribosomal proteins eS10 and uS10

Asc1/RACK1: detection of internally stalled ribosomes. Probably stabilizes the collided disome species that initiates RQC (also have RQC-independent function in translation)

Sitron and Brandman, Annu Rev Biochem, 2020

RQC factors

Ribosome splitting

Dom34/Pelota-Hbs1: ribosome splitting. Acts similarly to eRF1-eRF3 and also uses Rli1/ABCE1 to dissociate 40S, but does not cleave the peptidyl tRNA bond, leaving the nascent chain attached to the 60S subunit. Dom34/Pelota does not require the presence of an A-site stop codon and preferentially targets ribosomes that lack mRNA in the A site, which occurs when ribosomes reach the 3' end of truncated mRNAs and can no longer elongate. Dom34/Pelota may act as a stall sensor like Hel2/ZNF598 but does not identify collided ribosomes and rather may identify ribosomes with empty A sites and target them for RQC.

There is additional ribosome splitting pathway, because CGA-mediated RQC does not require Dom34 in yeast and ribosomes stalled by truncated mRNAs in the absence of Dom34 still undergo RQC. Dom34/Pelota- andHbs1-independent splitting in yeast could involve the RNA helicase Slh1, which functions in RQC after Asc1/RACK1 and Hel2/ZNF598

Slh1/ASCC3: ribosome splitting of internally stalled ribosomes.

RQT (RQC-trigger) complex: yeast Slh1, Cue3/Rqt3, Ykr023w/Rqt4

Functions in RQC downstream of Asc1/RACK1 and Hel2/ZNF598; may be involved in splitting internally stalled ribosomes. Slh1 is strictly required, the other RQT proteins promote RQC but are not required.

RQC factors

Marking nascent chain for degradation

Ltn1: ribosome splitting.Sspecifically associates with the 60S subunit

Rqc2/NEMF:

Rqc1/TCF25:

CAT Tails:

RQC factors

RQC termination

Vms1/ANKZF1: extraction phase of RQC Contains a domain that folds similarly to eRF1 release factor. Releases nascent chains from 60S RQC complexes. Inside the 60S subunit, Vms1/ANKZF1 positions its catalytic loop toward the tRNA's 3'-CCA, where the tRNA conjugates to the nascent chain, then it cleaves the 3'-CCA from the tRNA to produce a 3'-CCA-linked nascent chain and a cleaved tRNA

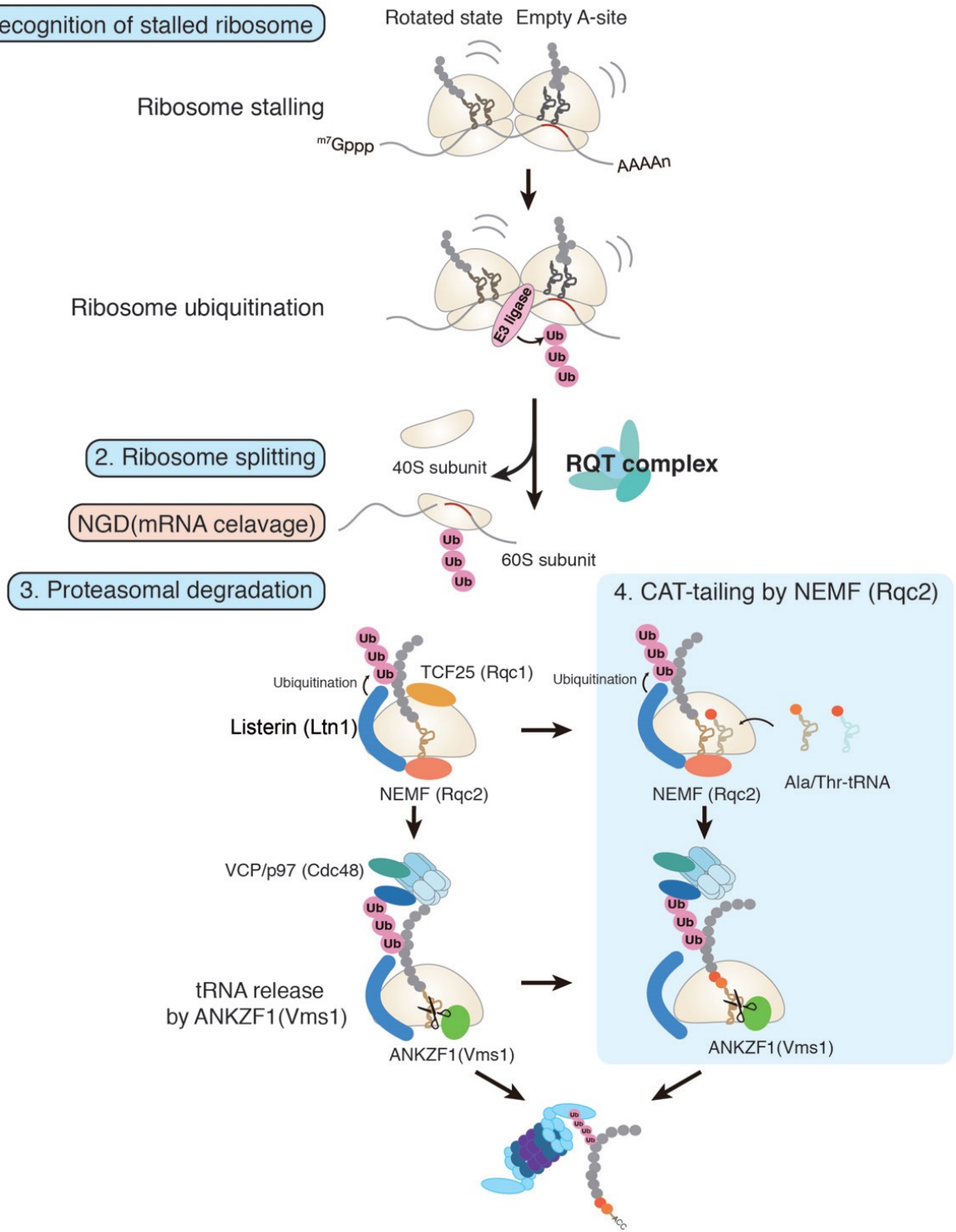
Arb1: Vms1/ANKZF1 cofactor, adenosine triphosphatase. Stimulates tRNA cleavage by Vms1/ANKZF1

Cleavage of tRNA releases the nascent chain from the ribosome to permit proteasomal degradation. tRNA lacking the CCA is checked for errors by 3'-CCA-adding enzyme TRNT1, and then returned to the translational tRNA pool after addition of CCA.

Cdc48/p97: nascent chain delivery to the proteasome. Proteasomal degradation of ubiquitylated nascent chain occurs after it is removed from 60S. Cdc48/p97, together with Ufd1/UFDL1 and Npl4/NPLOC4 (UN complex) bind to K48-linked ubiquitin. Cdc48/p97 hydrolyses ATP, unfolds polyubiquitylated protein by pulling it through the central pore.

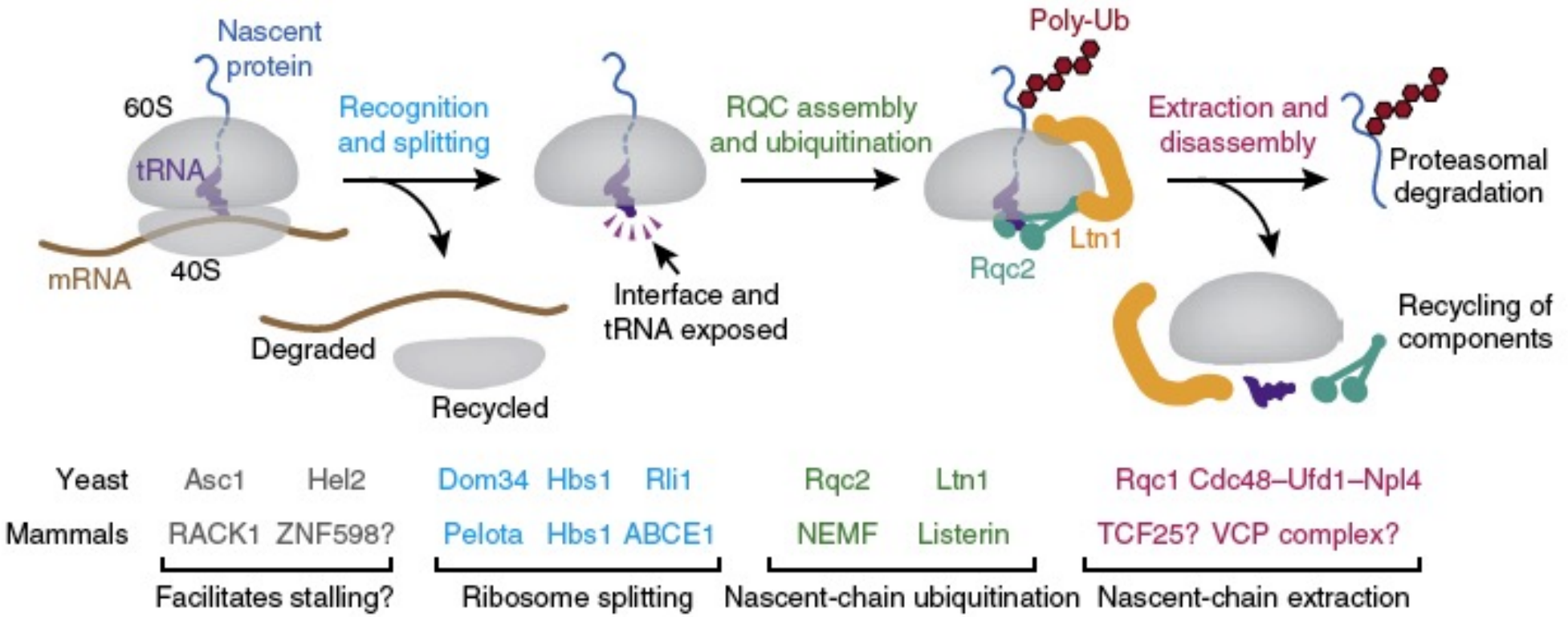
Cdc48, other components of the yeast RQC complex (Ltn1, Rqc1, and Rqc2) and E3 ligase Tom1 bind to nascent chains in nonribosomal fractions. This complex also interacts with the proteasome .

RIBOSOME QC (RQC)



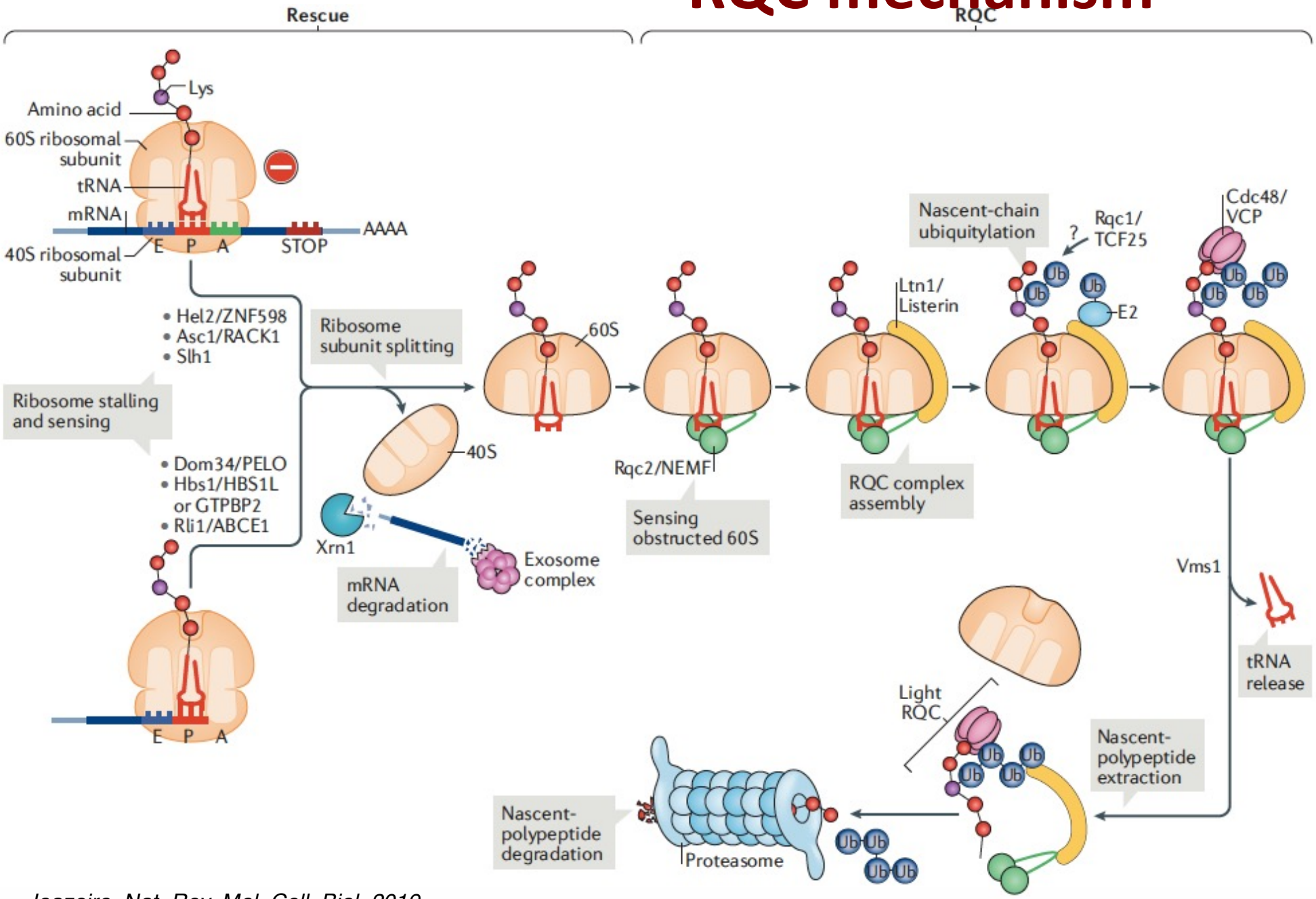
Inada, NAR.,2020

RQC pathway



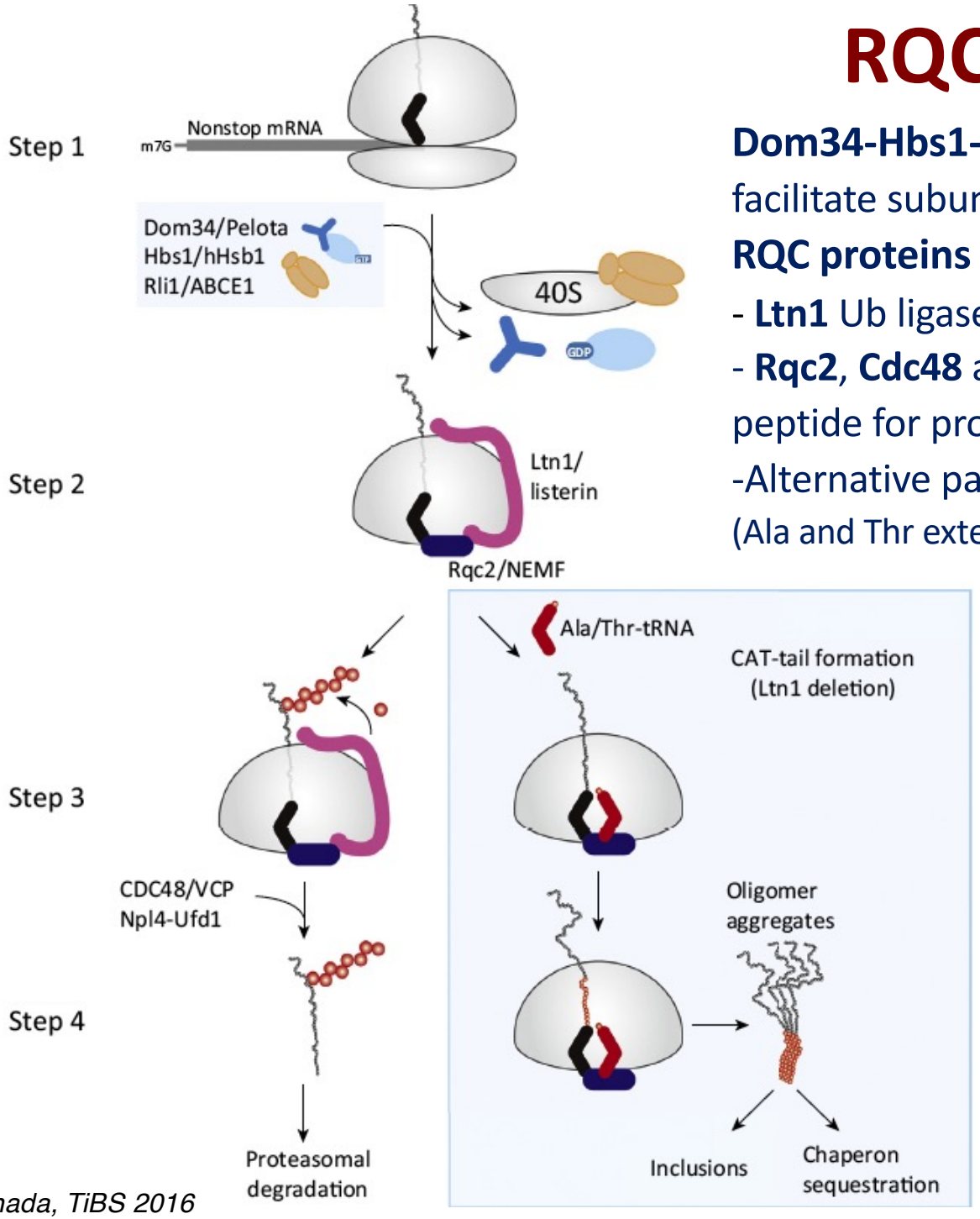
Brandman and Hegde, NatStrMolBiol 2016

RQC mechanism



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

RQC mechanism



Inada, TiBS 2016

Dom34-Hbs1-Rli1 or Hel2-Asc1-Slh1
facilitate subunit dissociation of stalled ribosomes

RQC proteins assemble on 60S

- **Ltn1** Ub ligase ubiquitinates the nascent peptide
- **Rqc2**, **Cdc48** and cofactors remove nascent peptide for proteasomal degradation
-Alternative pathways: via addition of CAT-tail (Ala and Thr extension)

CATylation

The canonical RQC is preferred but if ubiquitylation of the nascent polypeptide fails, CAT tail is added by Rqc2 to extract the trapped polypeptide
CATylation results in
- Ltn1-dependent degradation of aberrant proteins
- nascent chain aggregation
- activation of stress signaling
- nascent chain proteolysis