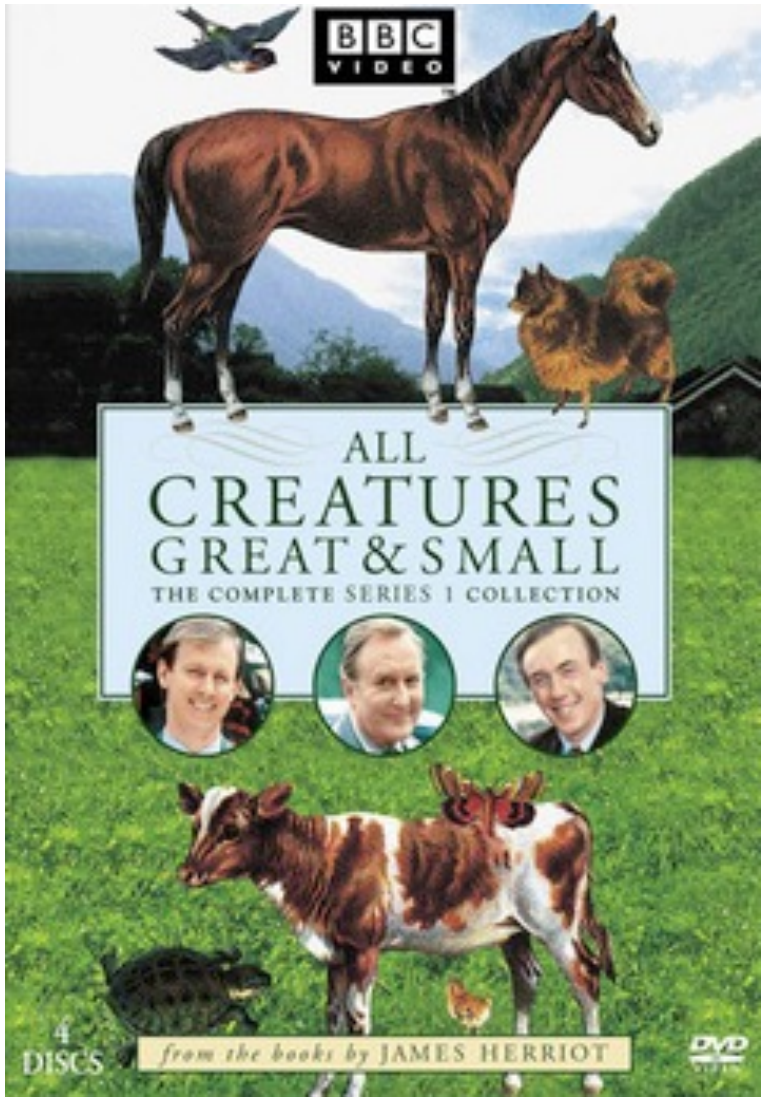
All RNAs great and small



lecture 3

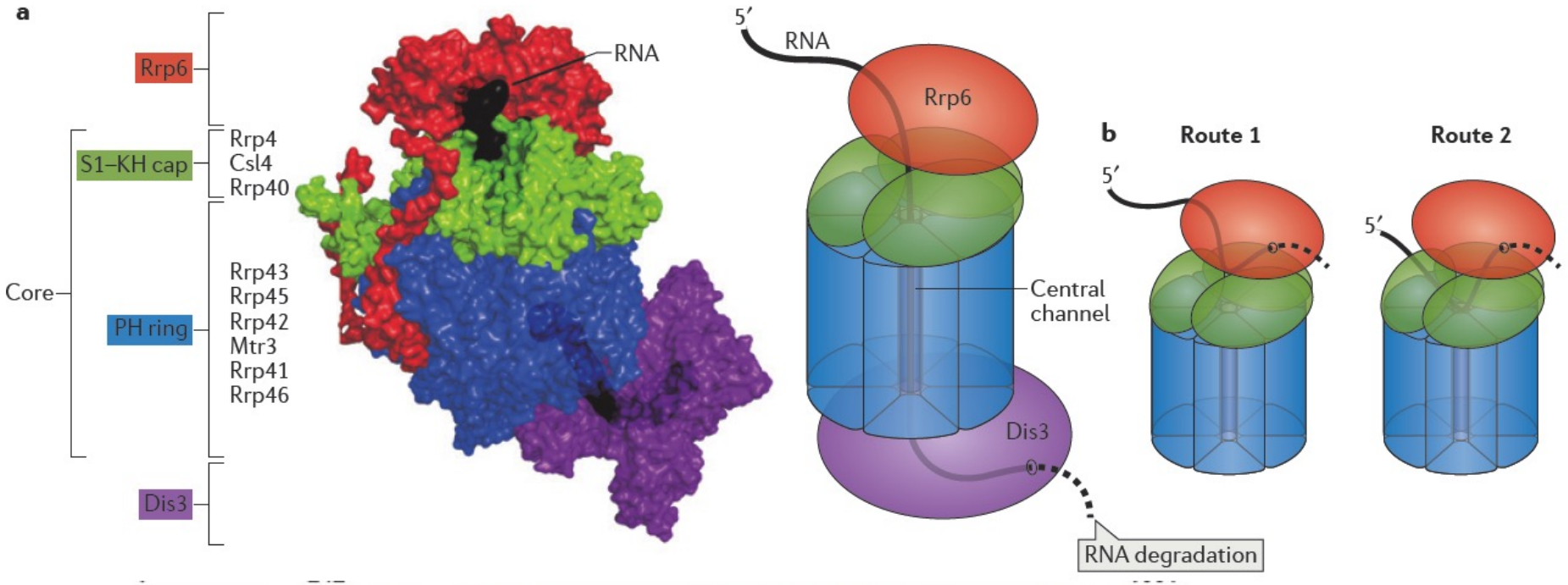
RNA enzymes and complexes

RNA granules

RNA decay

Institute of Genetics and Biotechnology
University of Warsaw

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 Tomecki

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

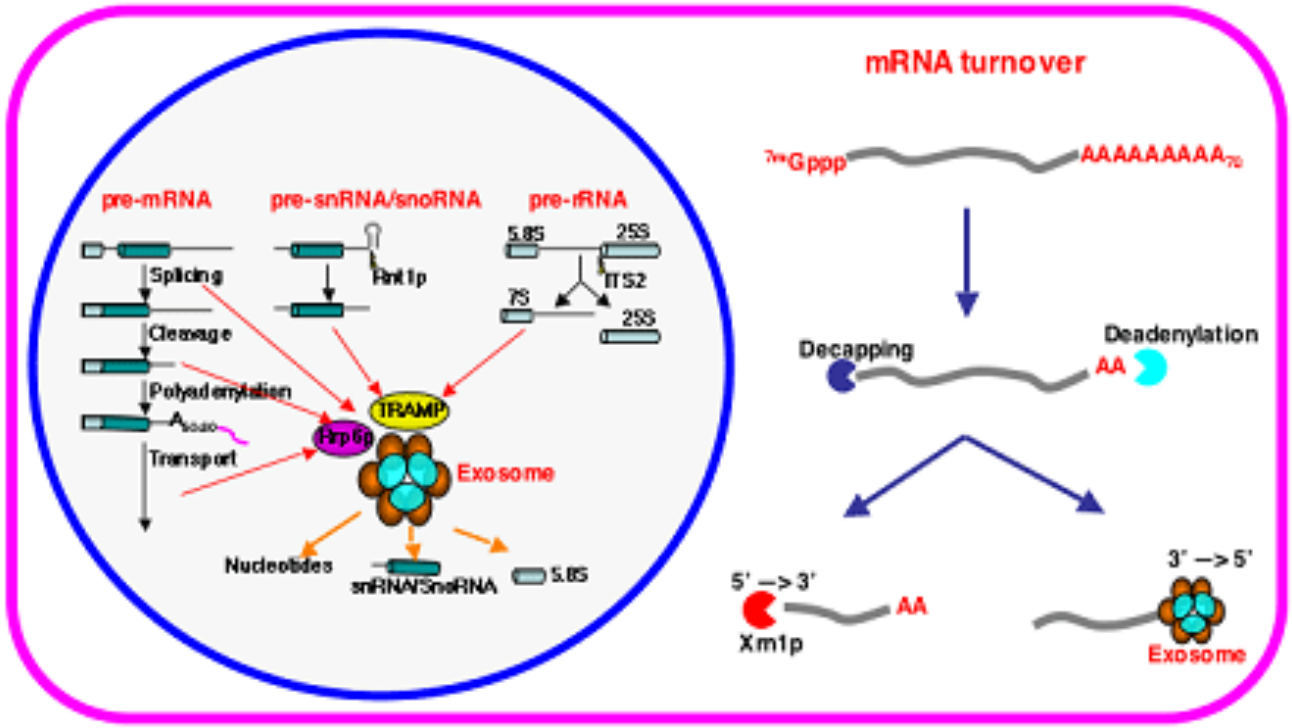
EXOSOME: 3’→5’ decay: FUNCTION

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:

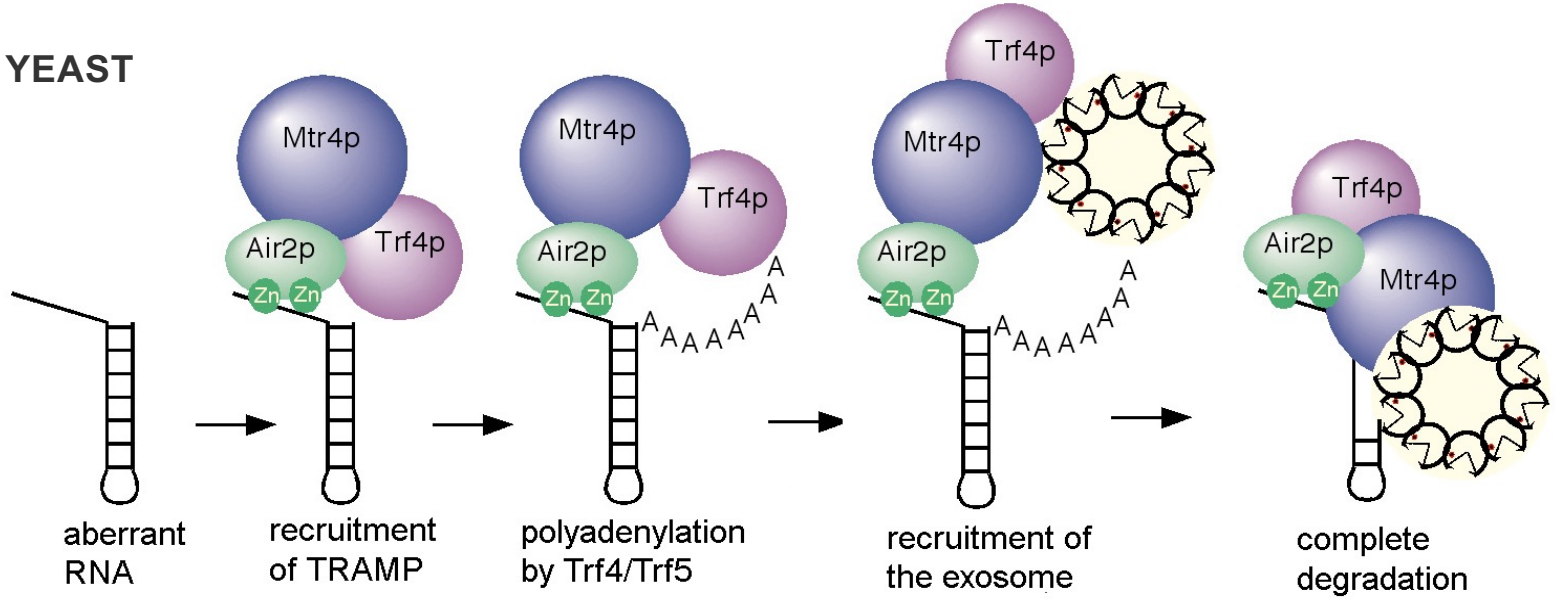
- generic mRNA decay
- specialised mRNA decay pathways: NMD, NSD, NO-GO decay, ARE-dependent decay



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**

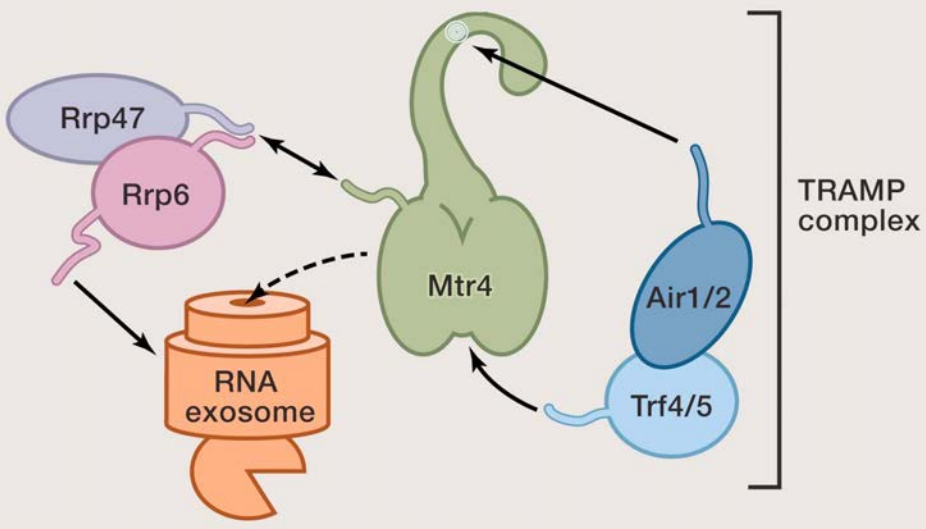
Interacts with

- 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

TRAMP + Exosome = nuclear RNA surveillance

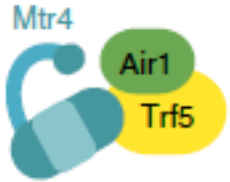


Mtr4 – DEAH box RNA helicase
Air1/2 – RNA binding proteins
Trf4/5 – poly(A) polymerases

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

TRAMP 4-2: mRNA, ncRNA

TRAMP 5-1: pre-rRNA

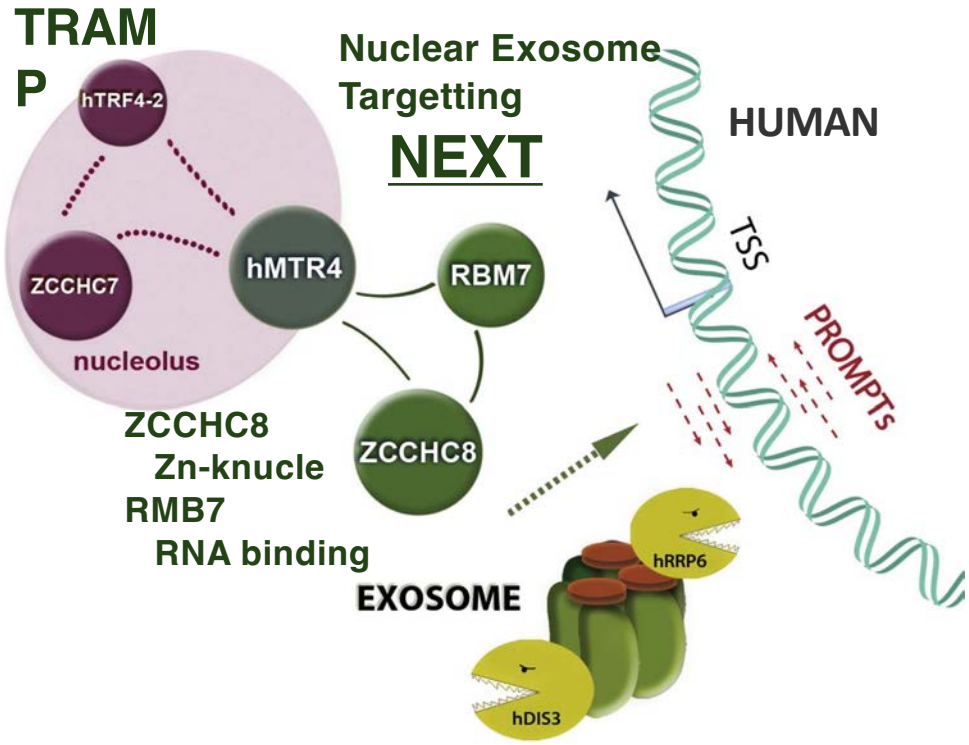


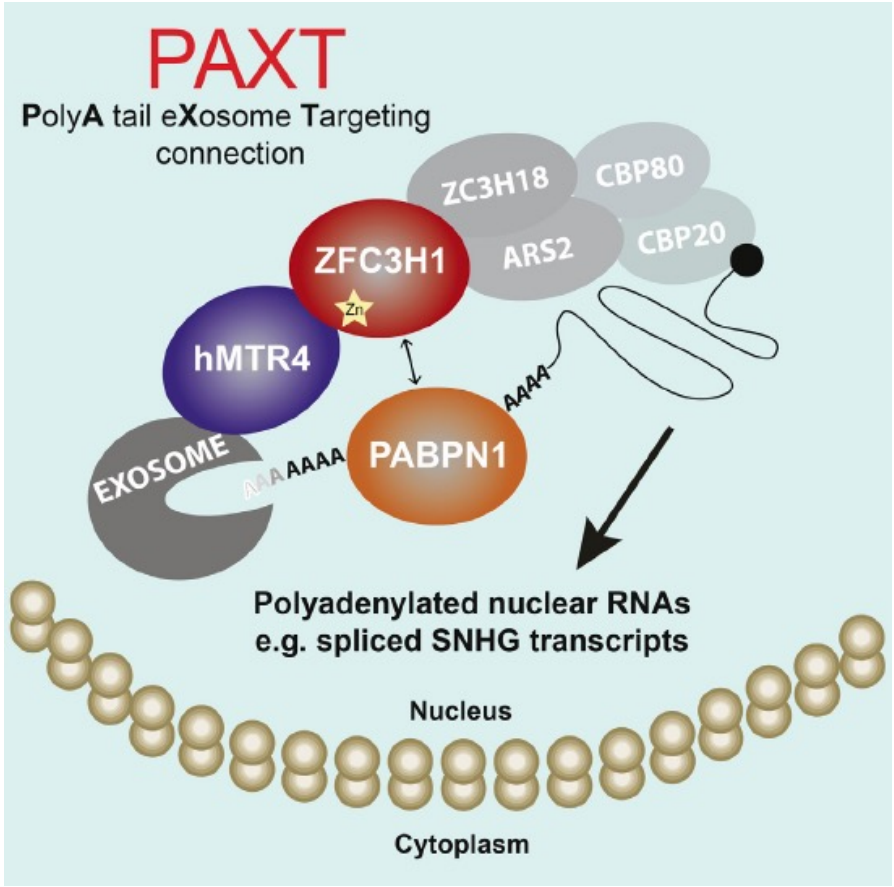
TRAMP 4-1: mRNA, introns

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 (humans)

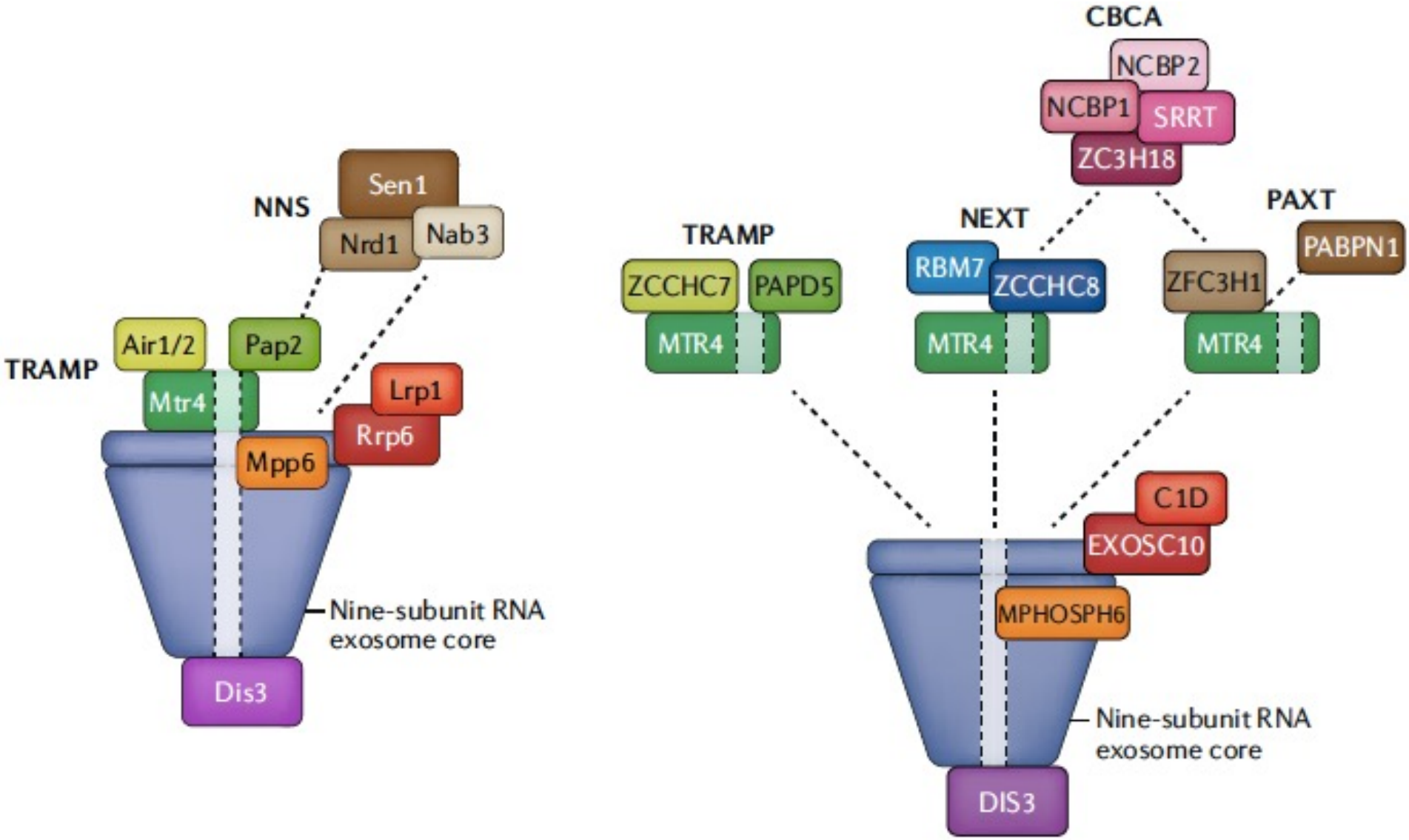




- **ZFC3H1 (Zn-knuckle protein) links MTR4 with PABPN1 in PAXT**
- **ZFC3H1/PABPN1 and RBM7/ZCCHC8 (NEXT) interact with MTR4 in a mutually exclusive manner**
- **PAXT and NEXT direct distinct RNA species for nuclear exosome degradation**
- **PAXT targets tend to be longer and more extensively polyadenylated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

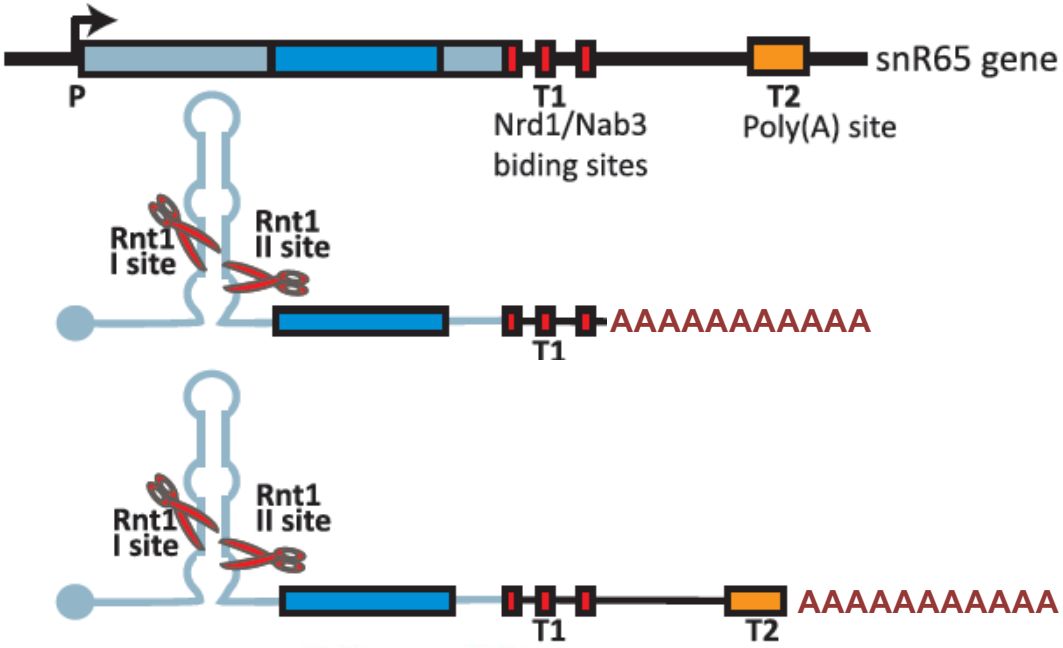
Lubas et al. *Mol. Cell*, 2011; Meola et al., . *Mol. Cell*, 2016

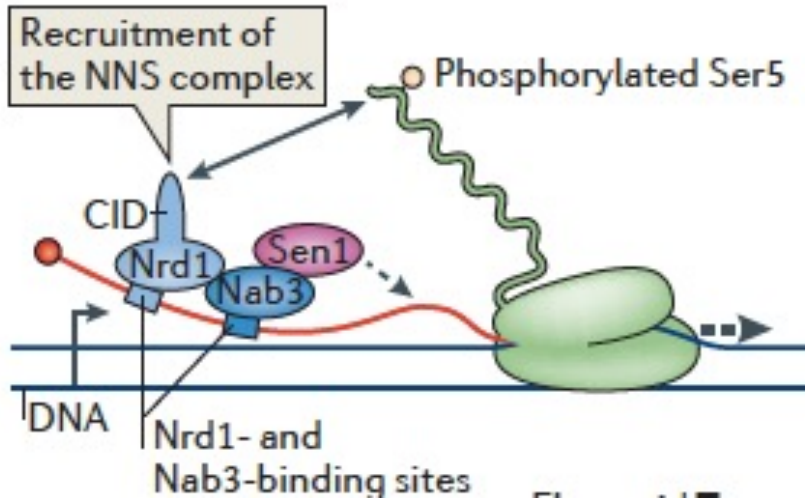
EXOSOME with TRAMP, NEXT, PAXT



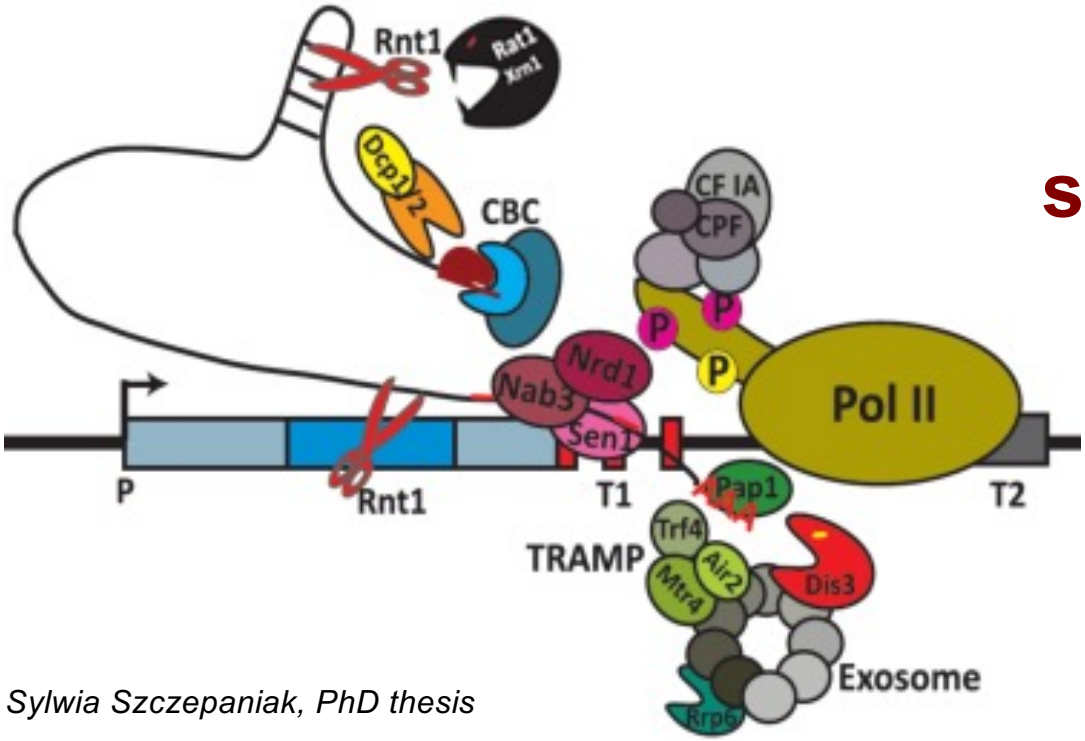
Schmid and Jensen, *Nat Rev Genet*, 2018

NNS-TRAMP-exosome





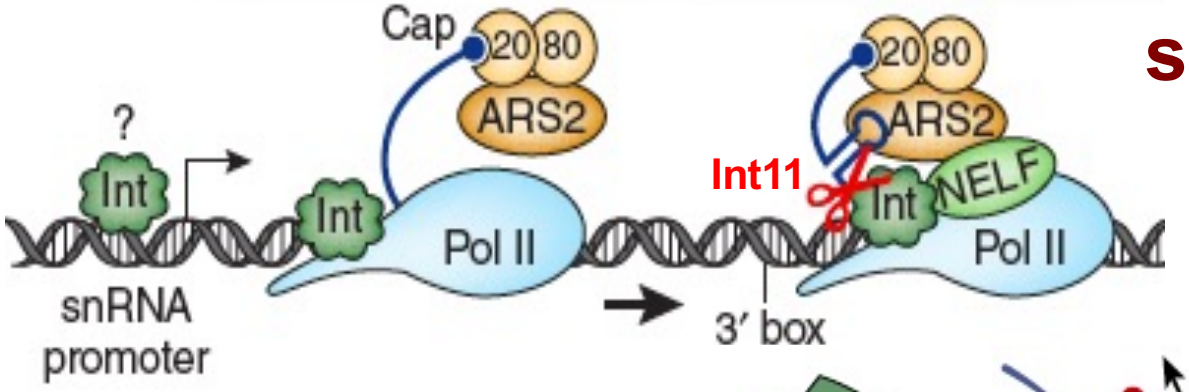
Poruua, Libri, Nat Rev Mol Cell Biol, 2015



sn/snoRNA processing (yeast)

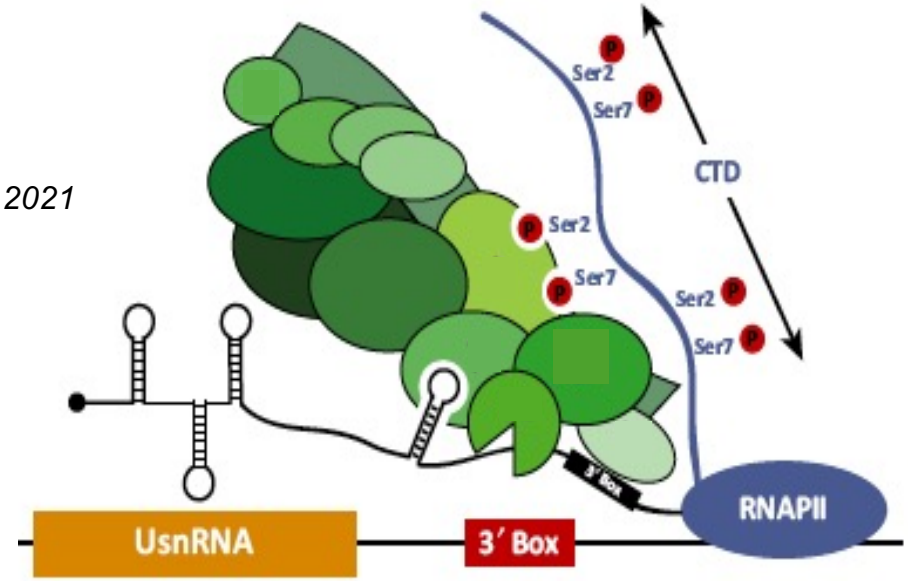
Sylwia Szczepaniak, PhD thesis

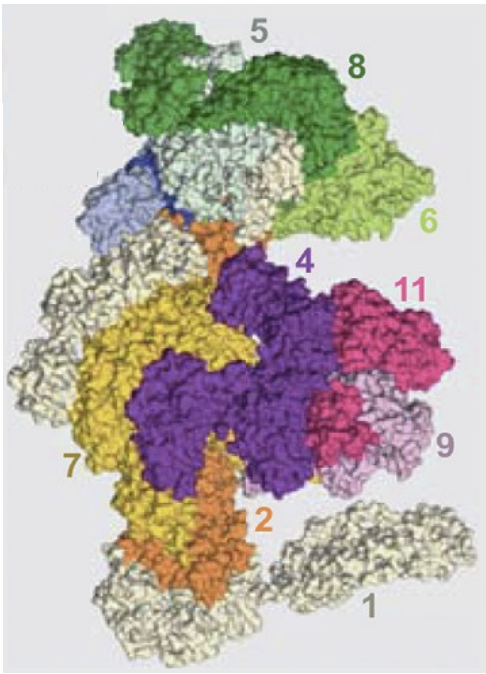
INTEGRATOR



snRNA processing (metazoa)

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

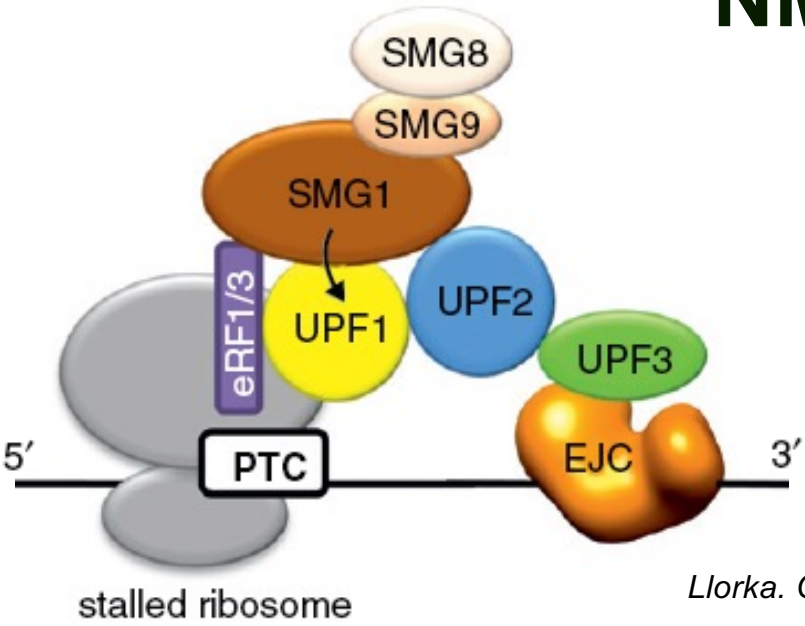




Integrator complex

- **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)

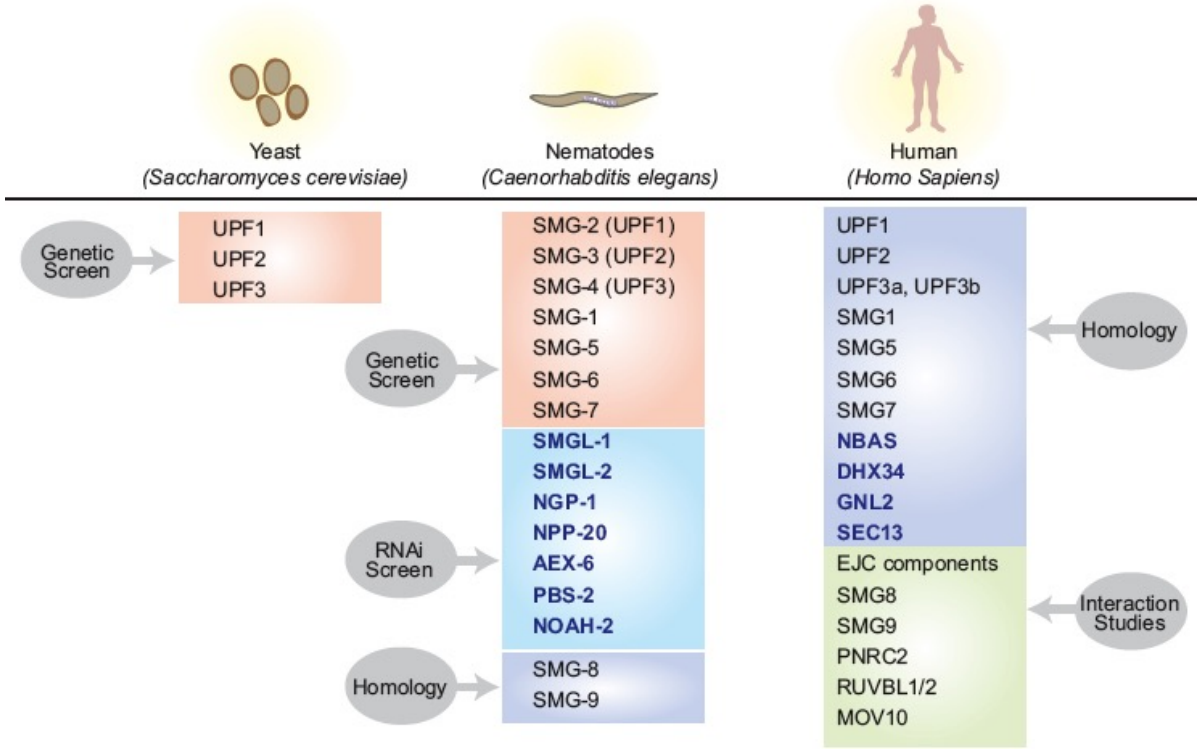
NMD FACTORS



SURF complex
SMG1-UPFs-SMGs-Release Factors

DECID (decay inducing)
phoshoSMG1-UPFs-EJC

Llorka. Cur. Op. Chem. Biol. 2013

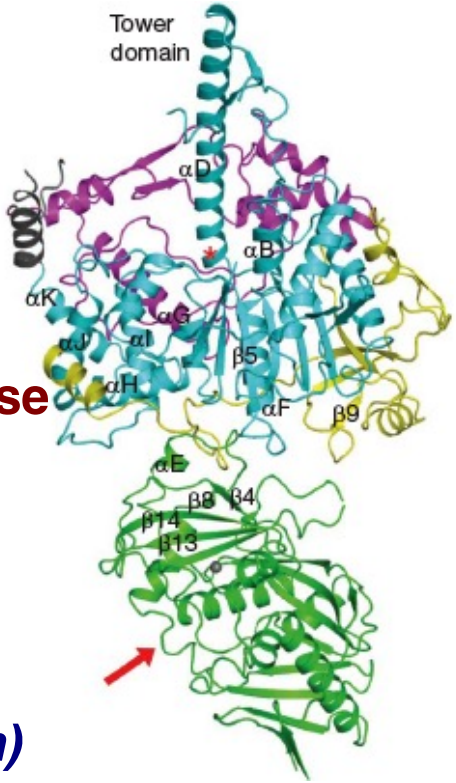


Hug et al., NAR, 2016

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)***

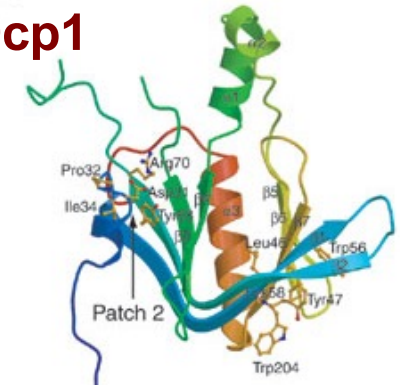
CYTOPLASMIC XRN1

- **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**

Yeast Rat1 and Xrn1 have also deNADding activity

DCP/NUDT- DECAPPING ENZYMES

Dcp1

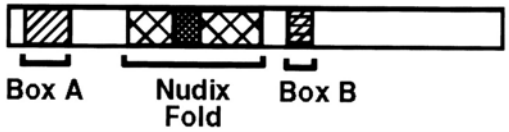


She et al. *Nat.Struct. Mol. Biol*, 2004

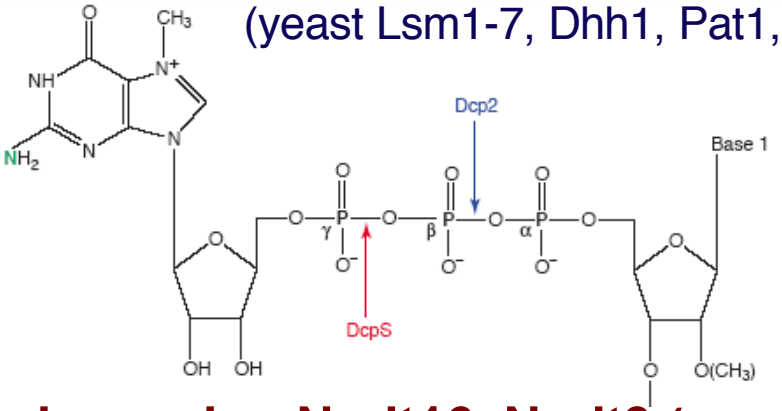
- **Dcp1/Dcp2 complex participates in mRNA 5' decay**
- **catalyses the reaction m^7GpppX-mRNA -> m^7GDP + 5'p-mRNA**
- **Dcp2 is the catalytic subunit (pyrophosphatase Nudix domain)**
- **Dcp1 is required for activity *in vivo*, interacts with other proteins**
- **Dcp1/Dcp2p is regulated by Pab1 and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

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

Dcp2

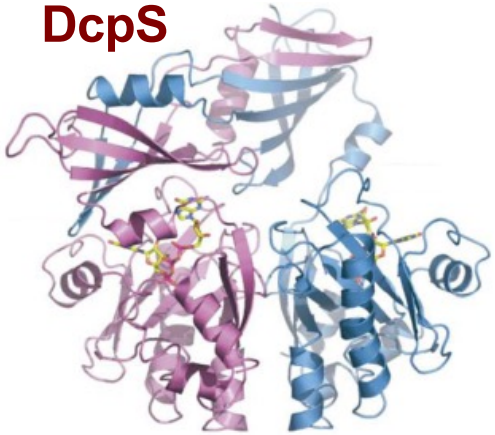


Wang et al. *PNAS*, 2002



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**

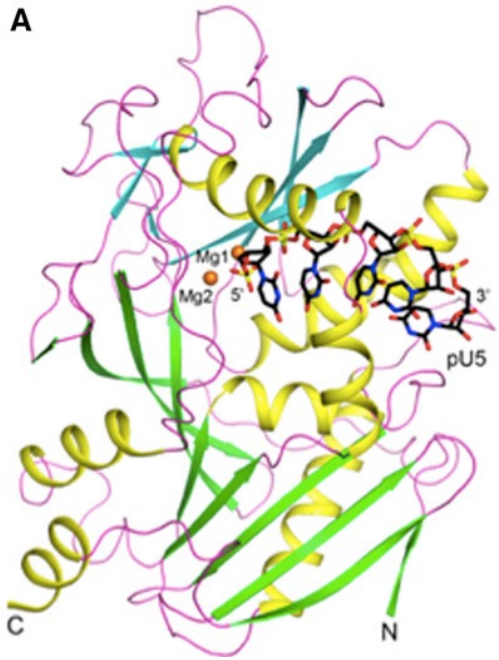
Gu et al., *M.Cell*, 2004

DXO/Rai1 family

Cellular activities

cap surveillance

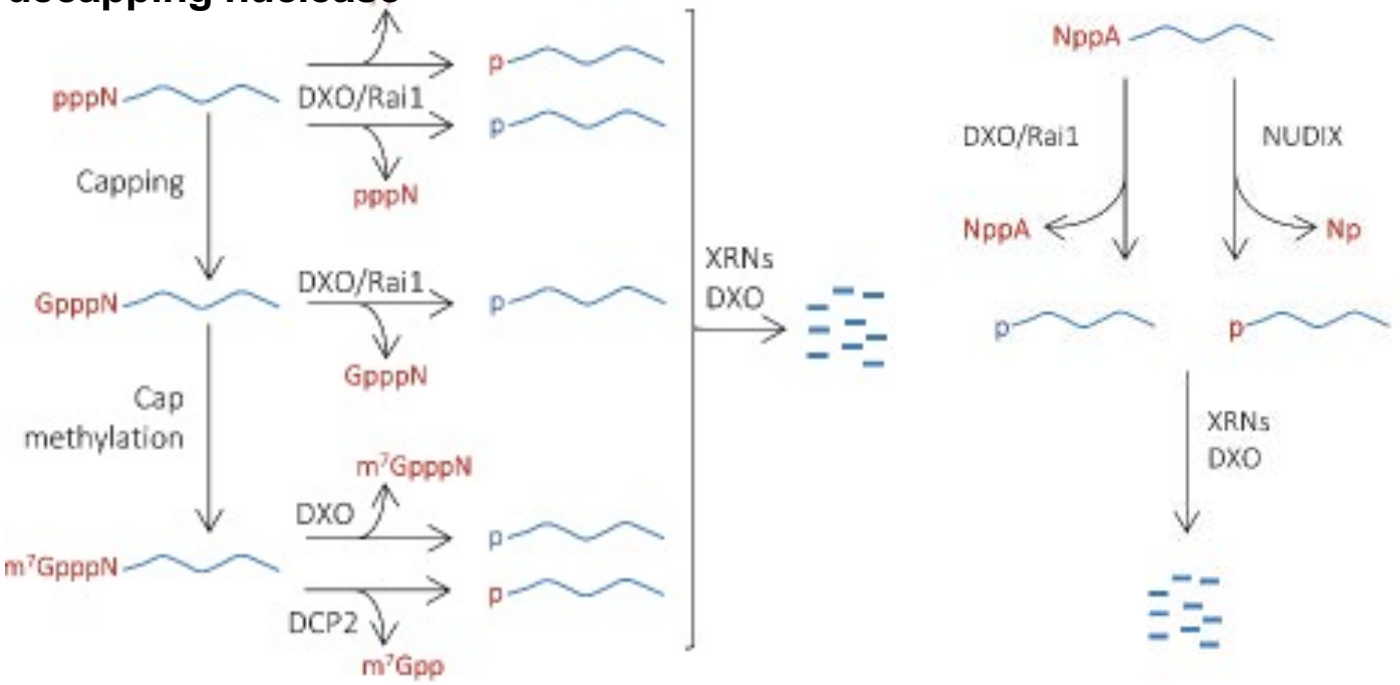
deNADding



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^7Gppp-RNA	+++	-
DeNADding	NppA-RNA	++++	+++

Additional activities:

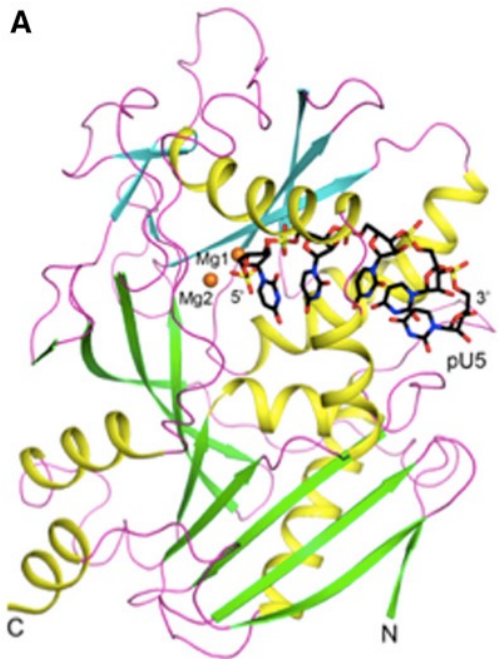
- 5' OH RNA hydrolase

- FAD and CoA decapping nuclease

A. Kwaśnik, PhD thesis, 2019

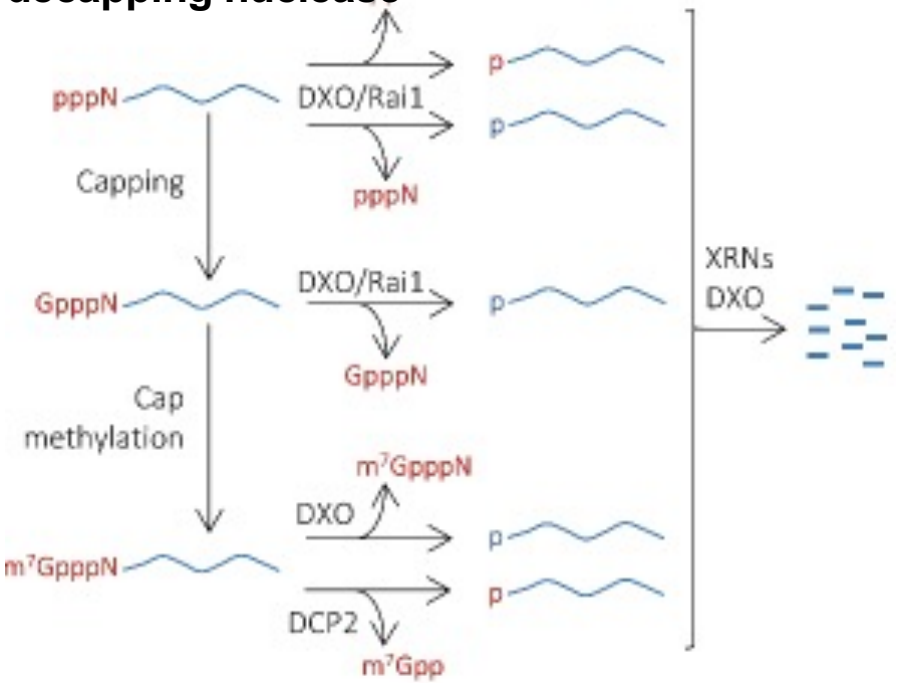
DXO/Rai1 family

Cellular activities cap surveillance deNADding

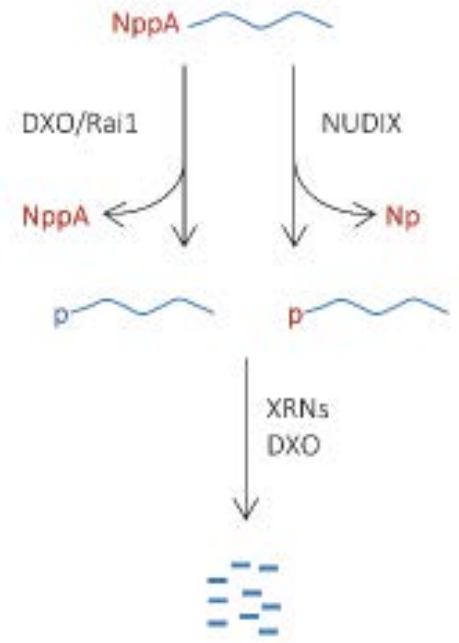


pyrophosphohydrolase decapping nuclease

5' -3 exonuclease



deNADding nuclease



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Decapping (unmethylated cap)	Gppp-RNA	+++	-
Decapping (mature cap)	m^{7}Gppp-RNA	+++	-
DeNADding	NppA-RNA	++++	+++

Additional activities:

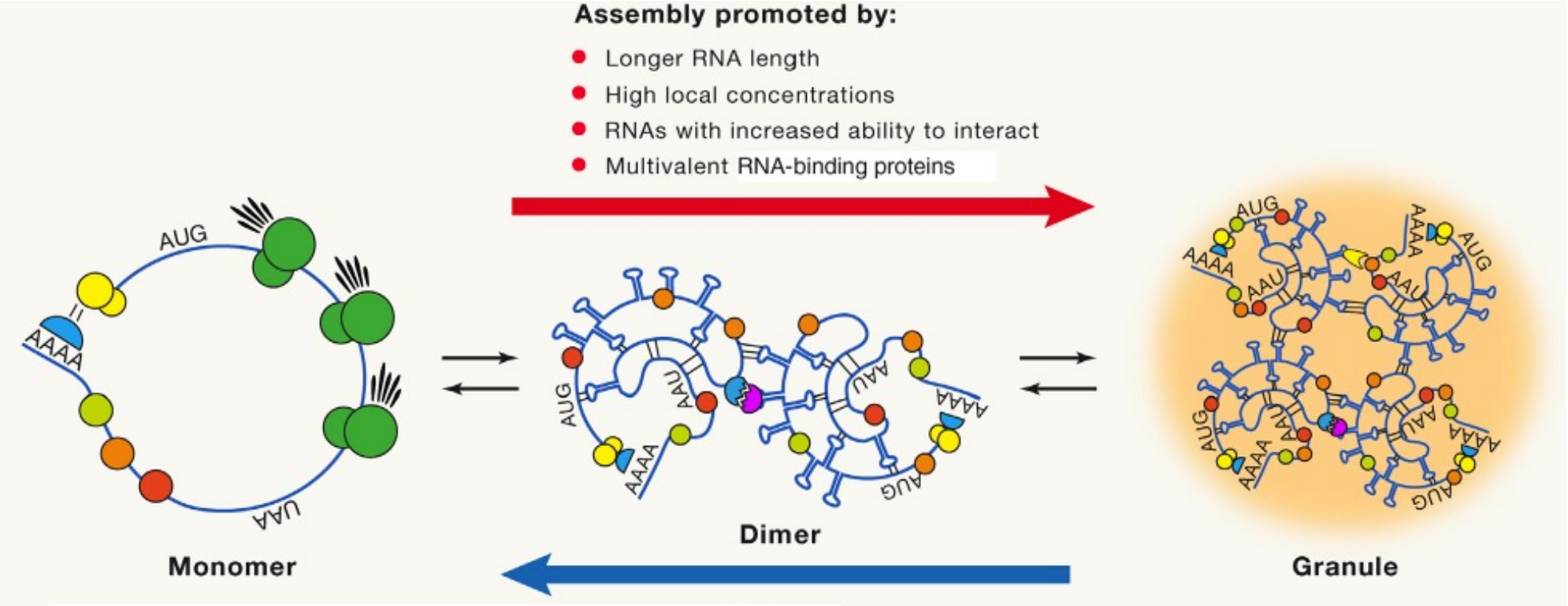
- 5' OH RNA hydrolase

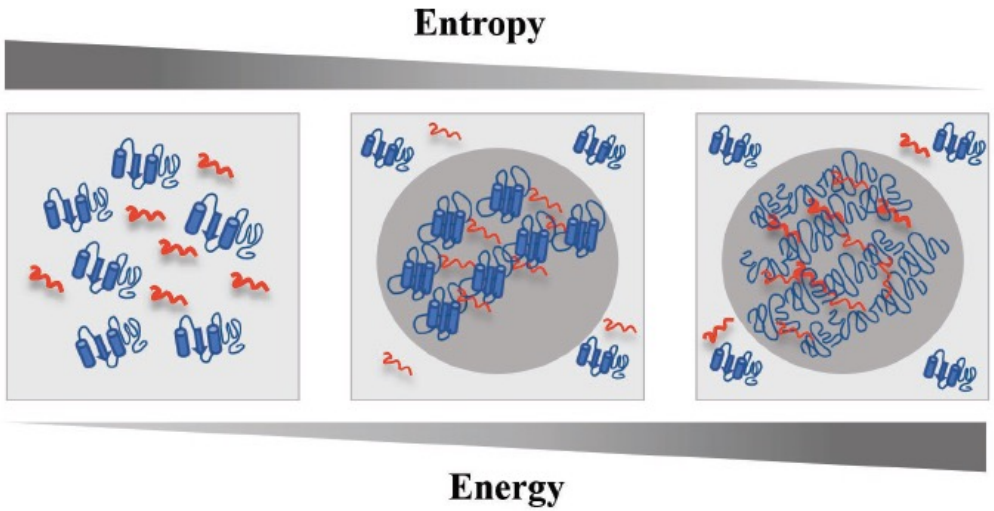
- FAD and CoA decapping nuclease

A. Kwaśnik, PhD thesis, 2019

RNP granule assembly

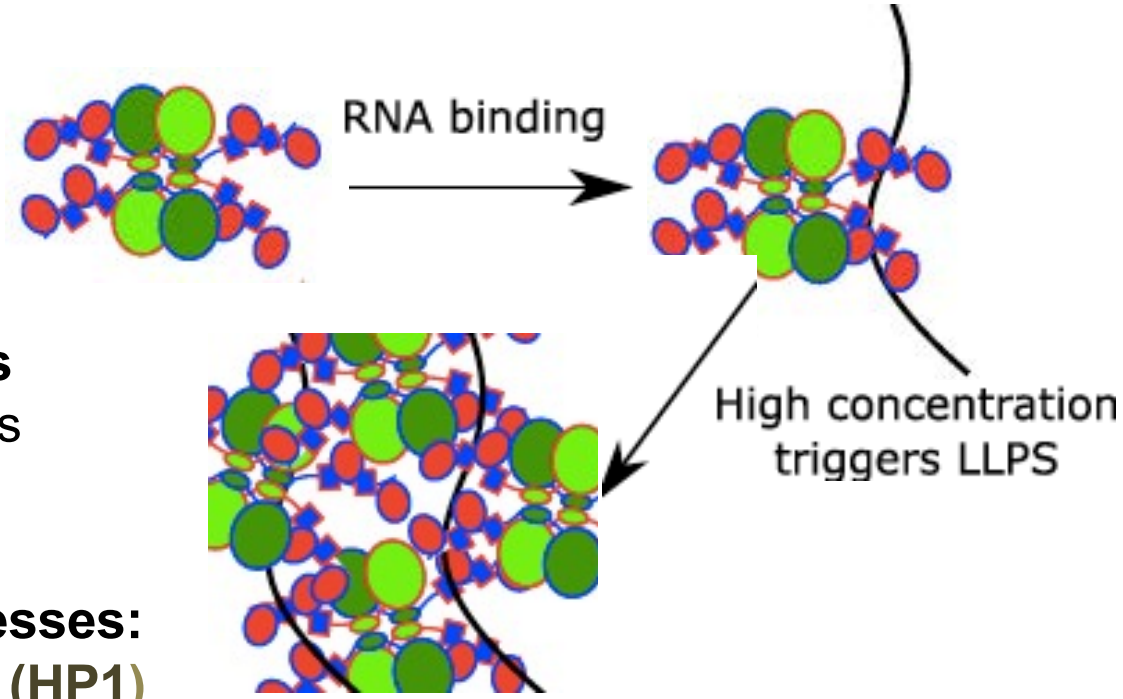
by protein-protein and RNA-RNA interactions





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

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

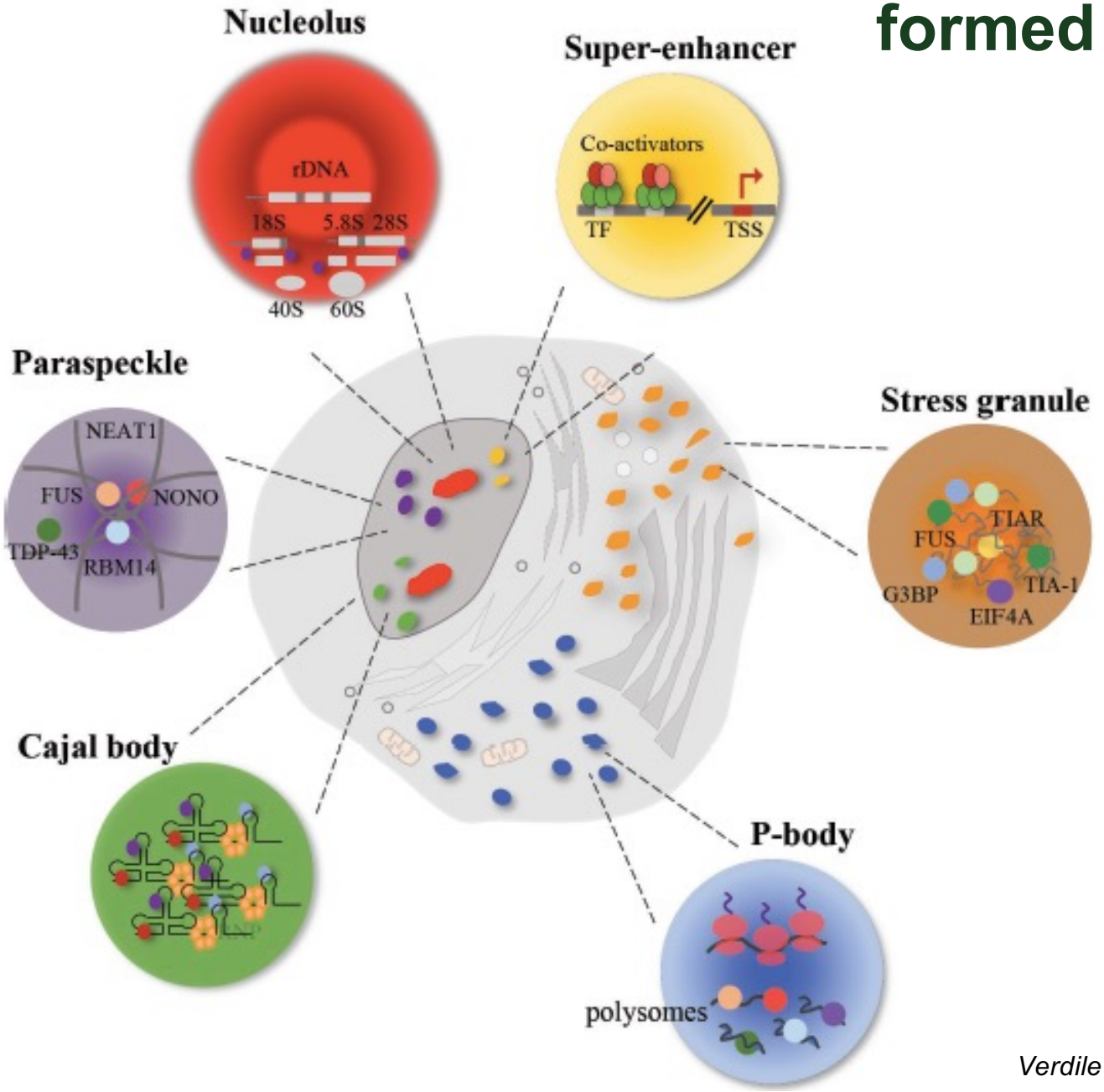


Formed by unstructured protein domains around RNAs
IDR - Intrinsic Disordered Domains
PLD - Prion-Like Domains

Organize several cellular processes:

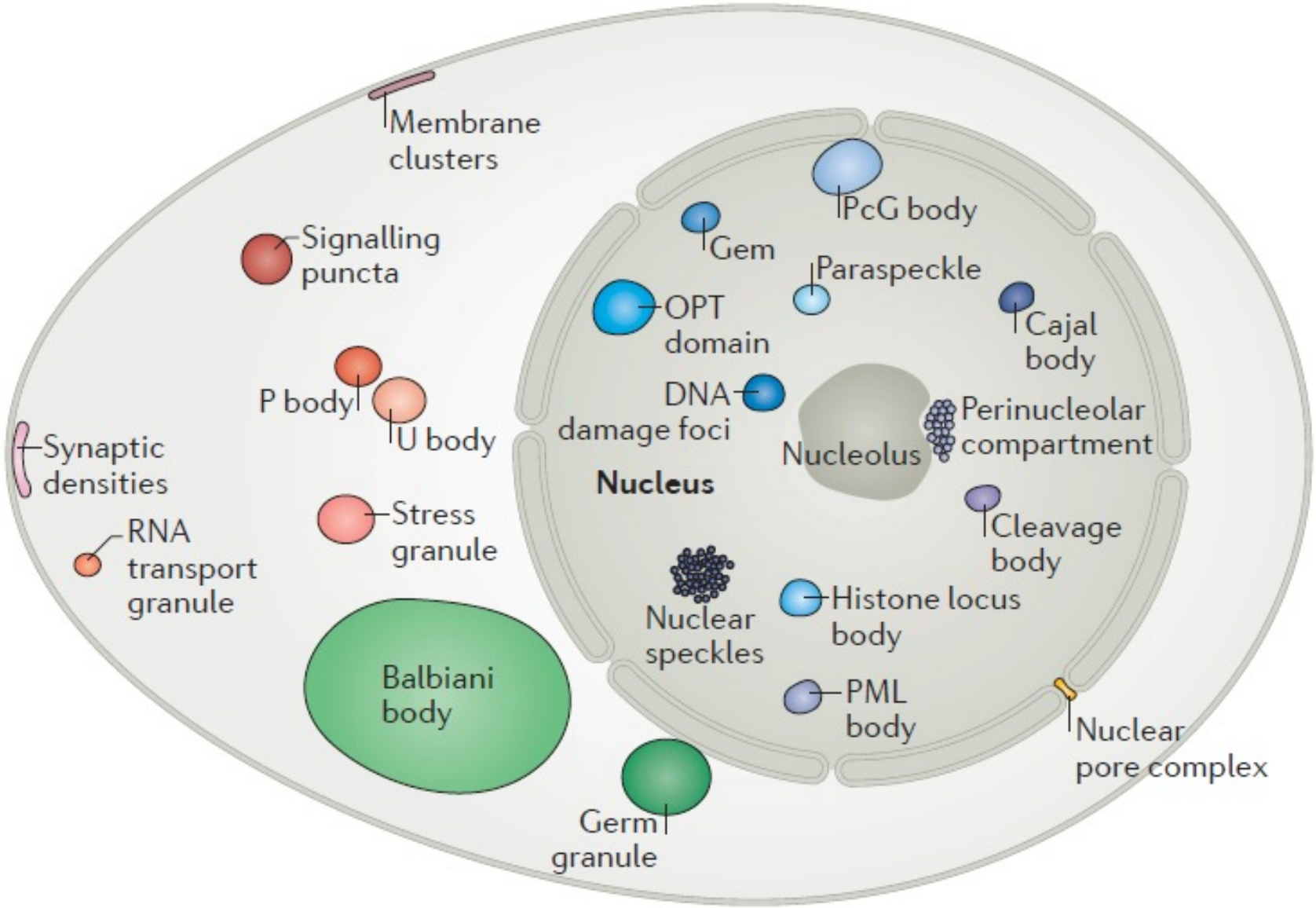
- **Heterochromatin structure** (HP1)
- **Transcription** (Mediator, Pol II CTD)
- **Processing** (nucleolus, spliceosome, SR proteins, Cajal bodies)
- **RNA retention and storage**
  (Nuclear speckles, Paraspeckles, P-bodies, Stress Granules)
- **RNA decay** (degradosome)
- **Protein modificarion and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

Membraneless Organelles formed by LLPS



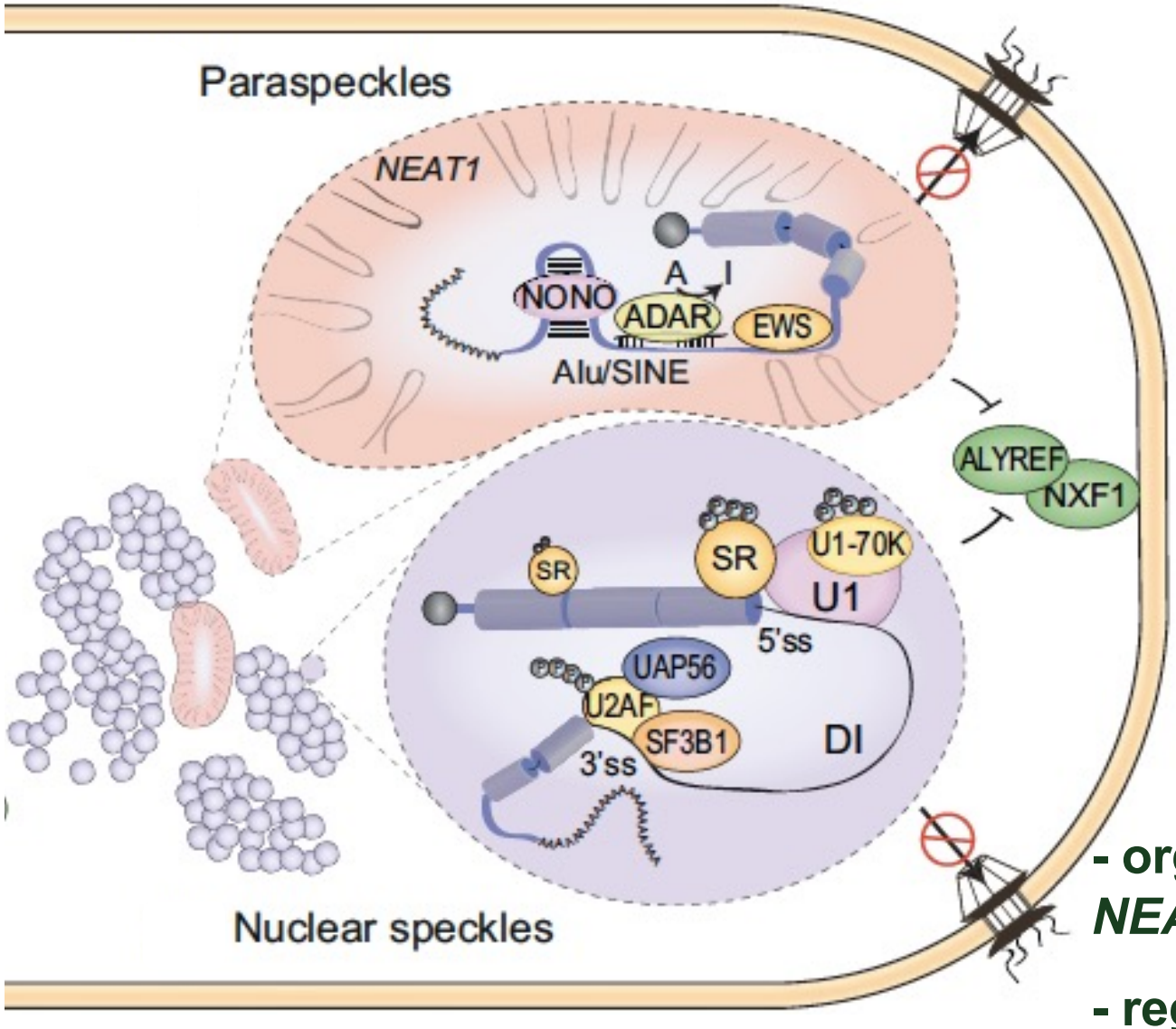
Verdile et al, Front Genet, 2019

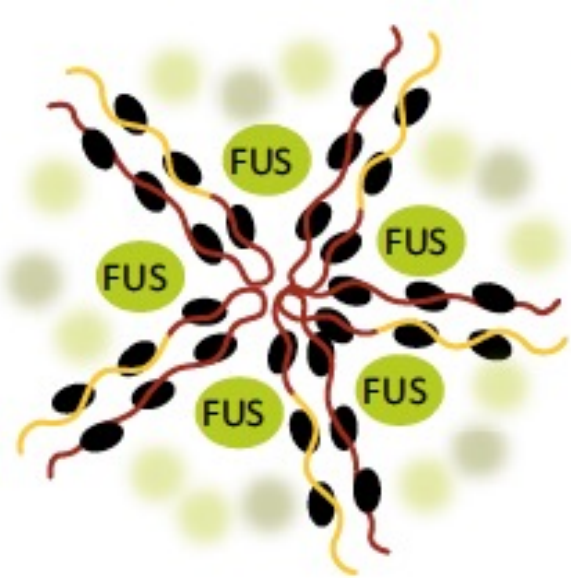
Cellular Condensates



Banani et al, Nat Rev Cell Mol Biol, 2017

Paraspeckles
Nuclear speckles



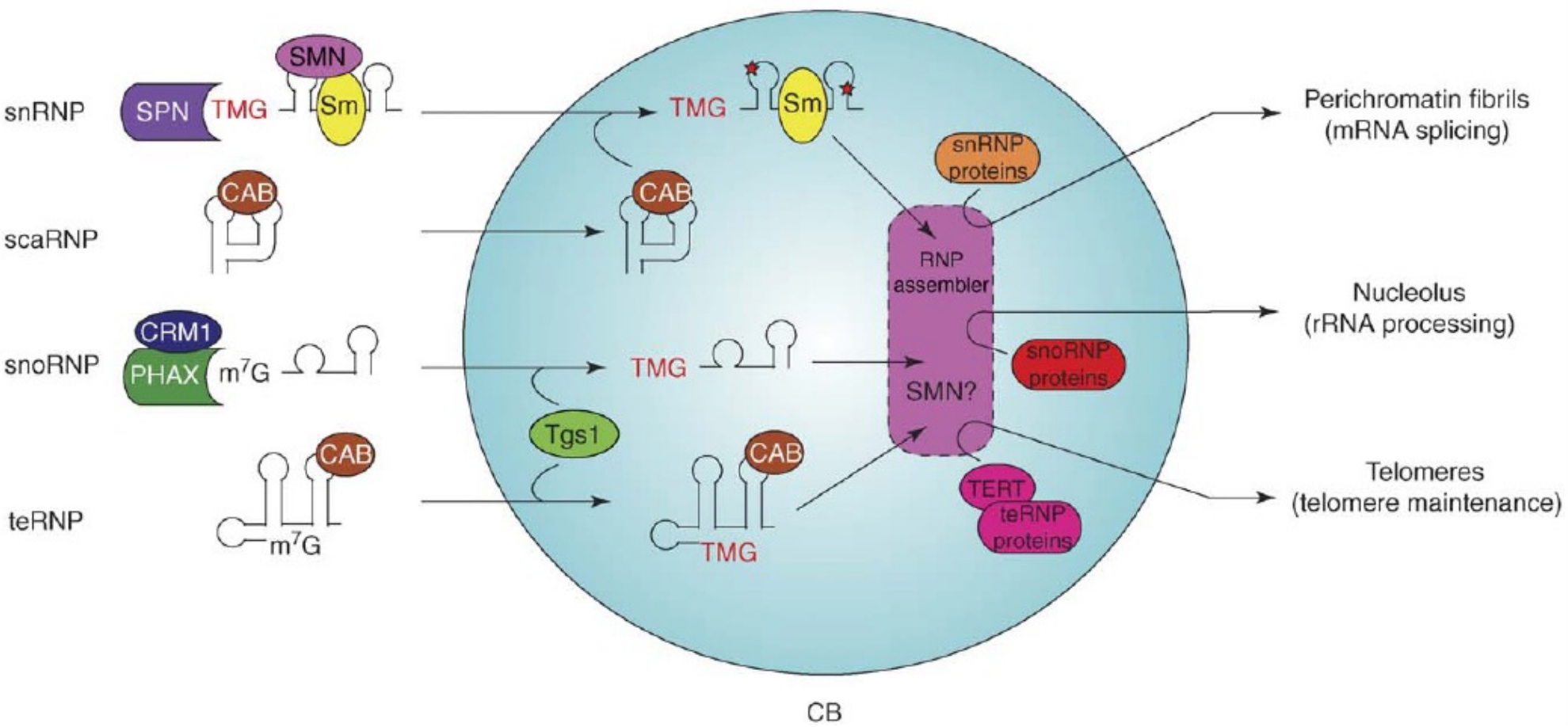


- organized around lncRNAs: *NEAT1* (PS) or *MALAT1* (NS)

- regulate gene expression by mRNA nuclear retention

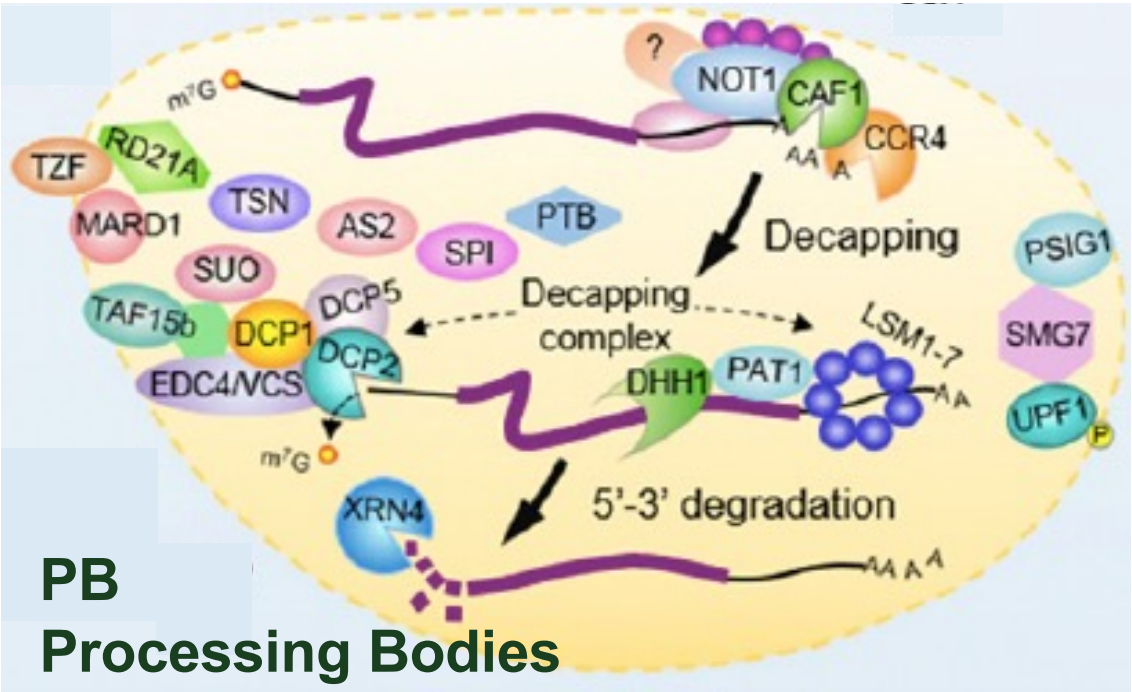
Wegener and Müller-McNicoll, Sem Cell Dev Biol 2018

Cajal bodies



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

Cytoplasmic P-bodies and Stress Granules



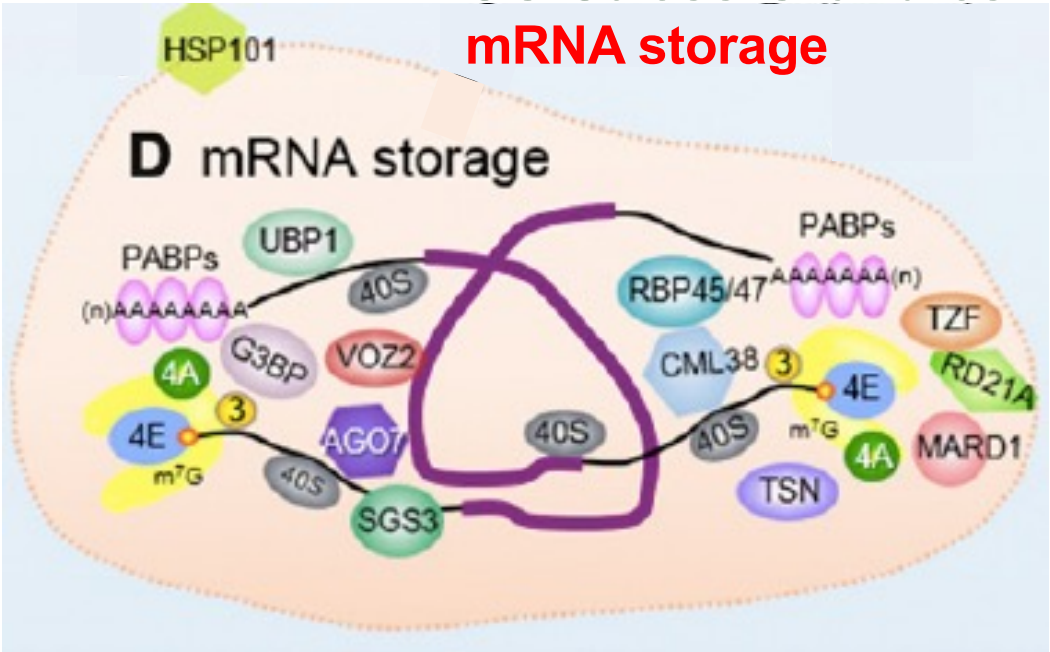
PB
Processing Bodies

mRNA storage
mRNA decay

Dynamic biomolecular condensates
Form 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

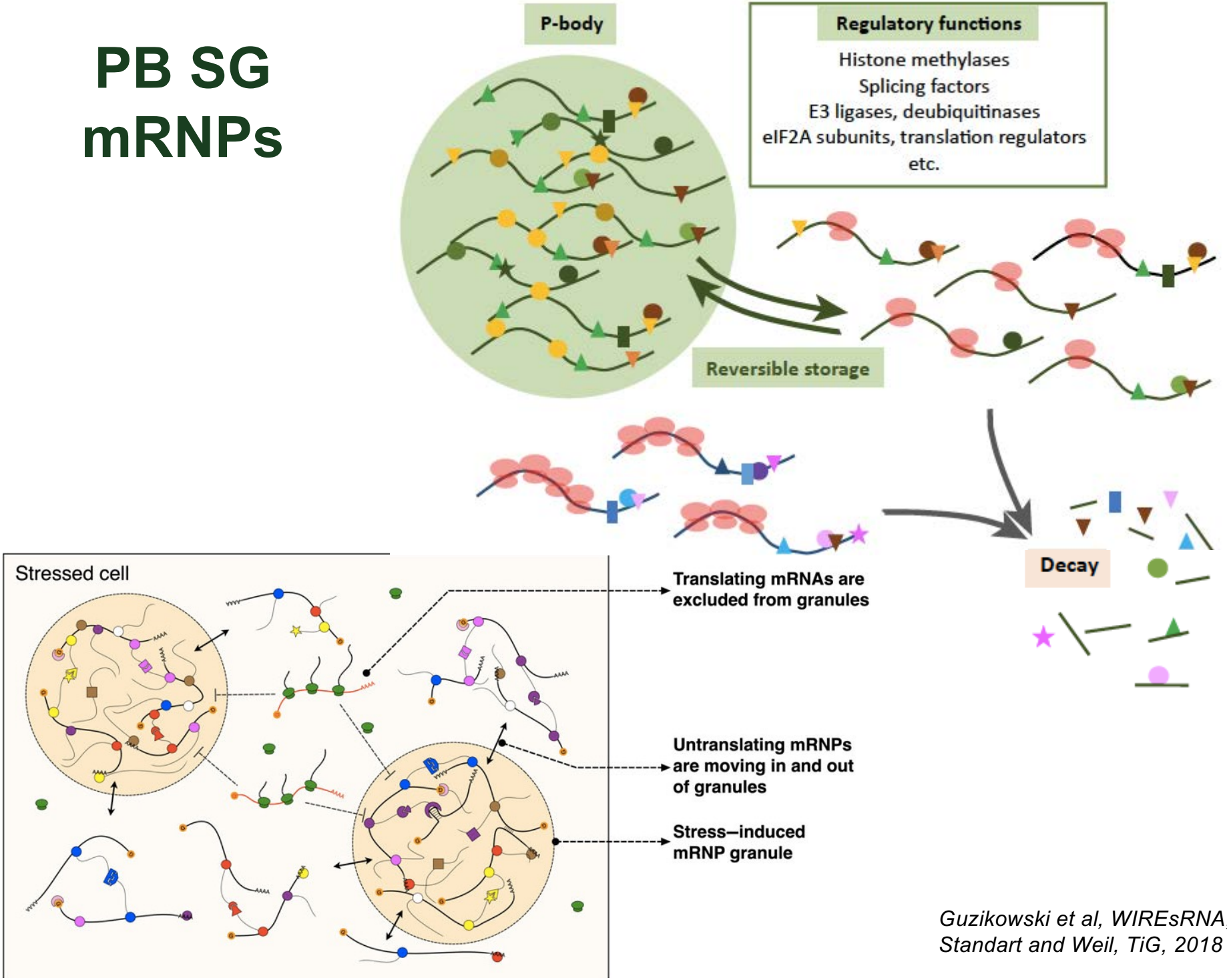


SG: global translation halts upon stress, mRNAs bound to the translational machinery and other proteins form SGs.

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

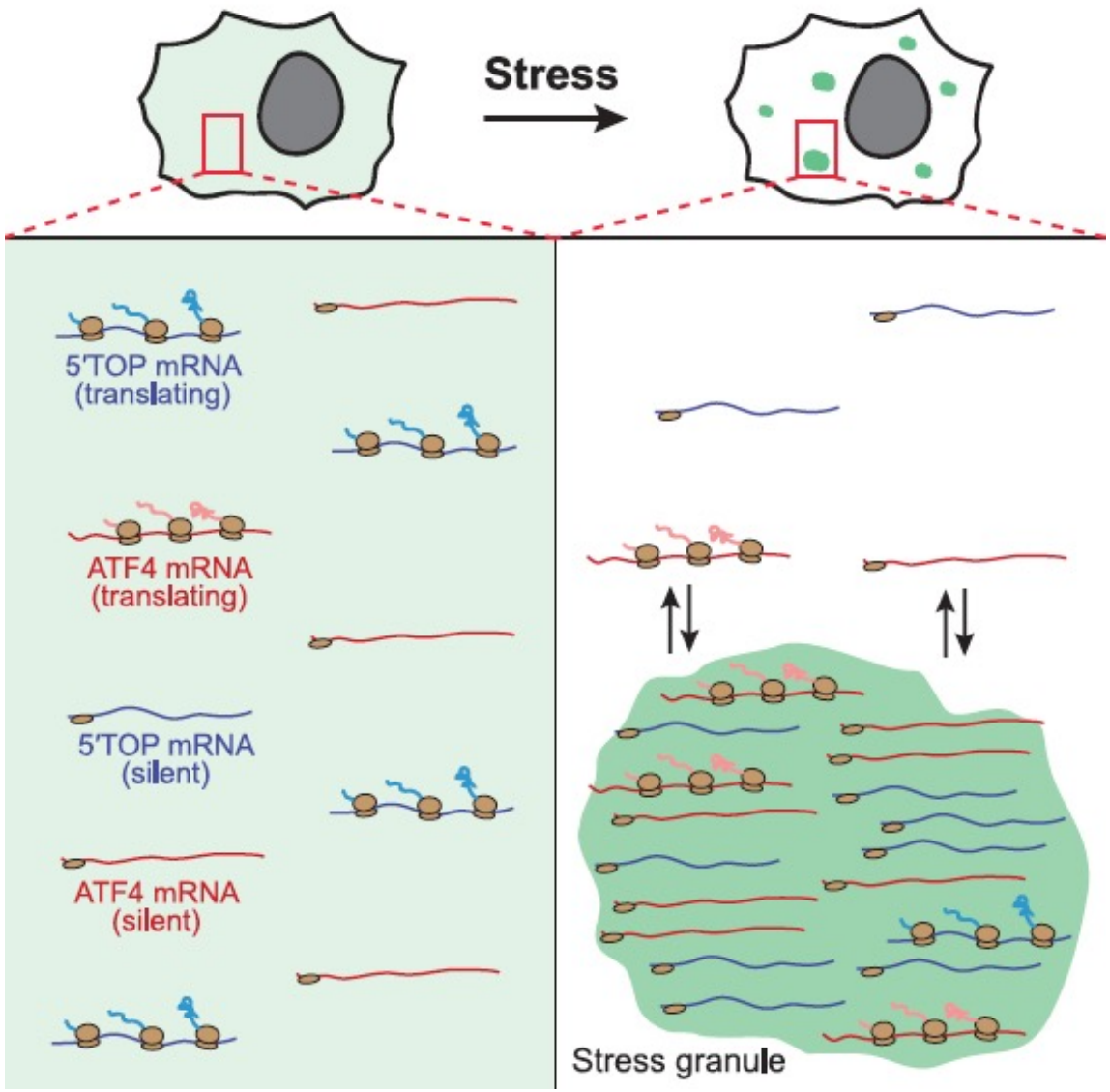
Chantarachot and Bailey-Serres, Plant Phys, 2018

PB SG mRNPs



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

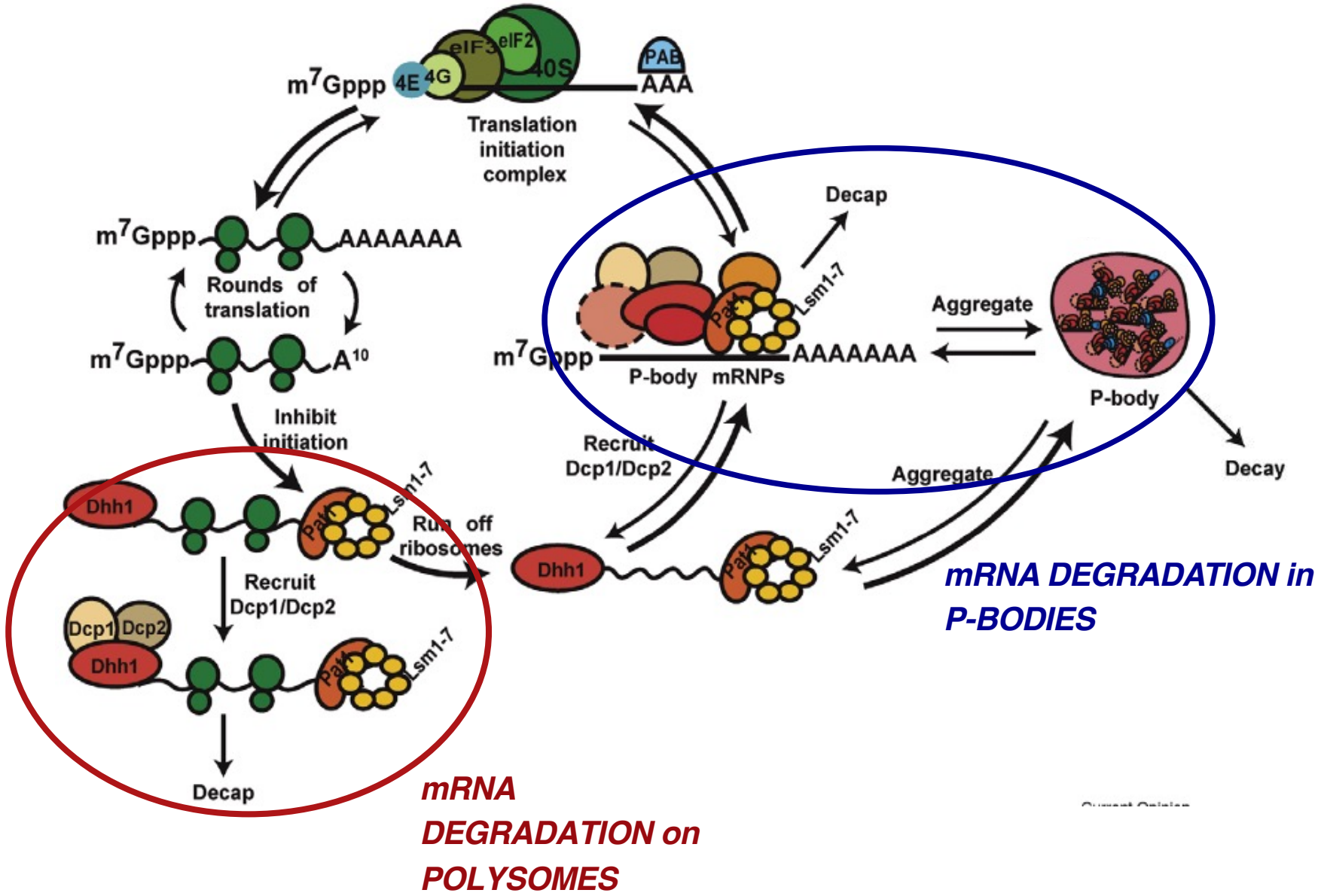
Translation in SGs



- **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)*

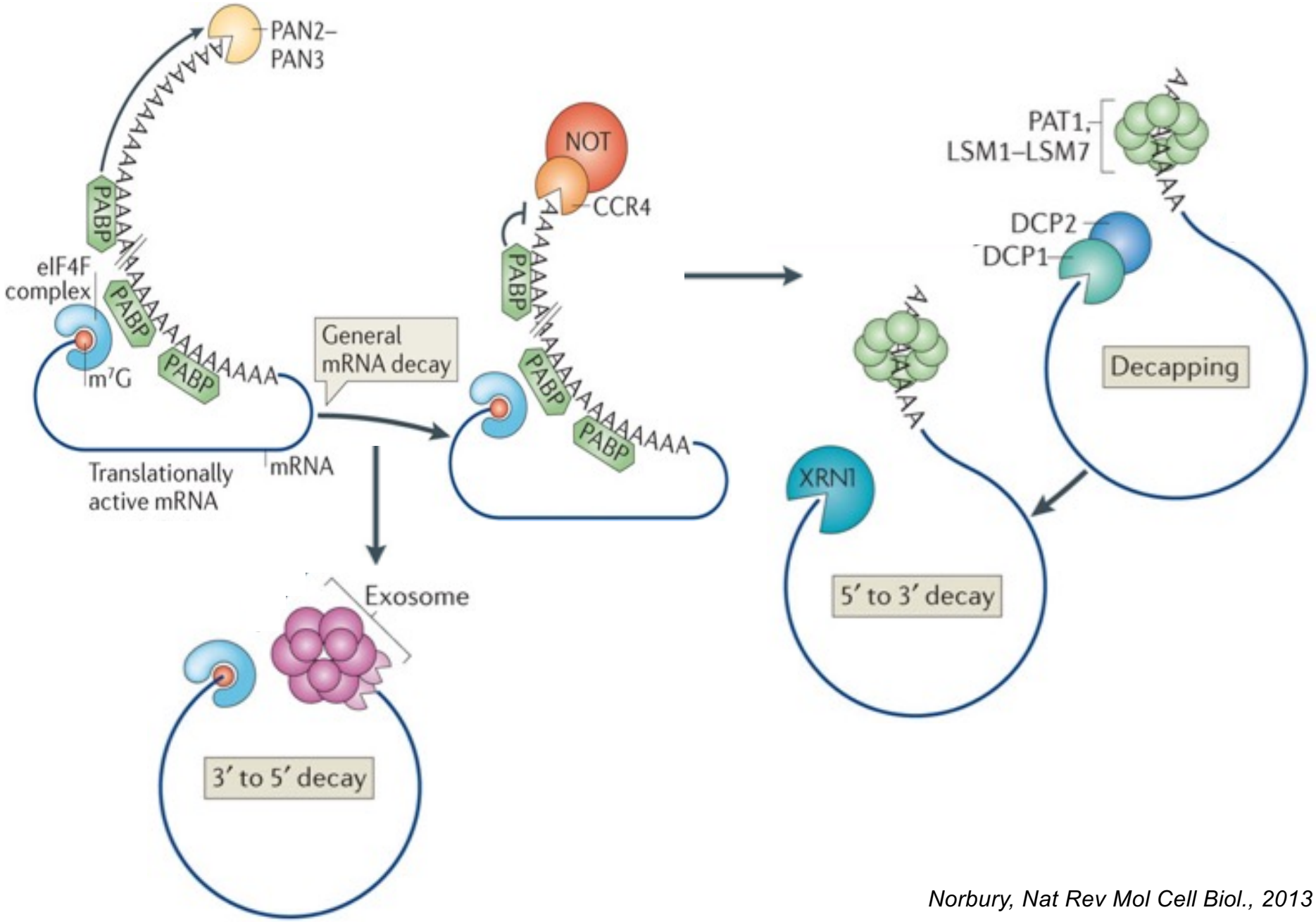
Mateju et al., Cell, 2020

mRNA DEGRADATION in the CYTOPLASM



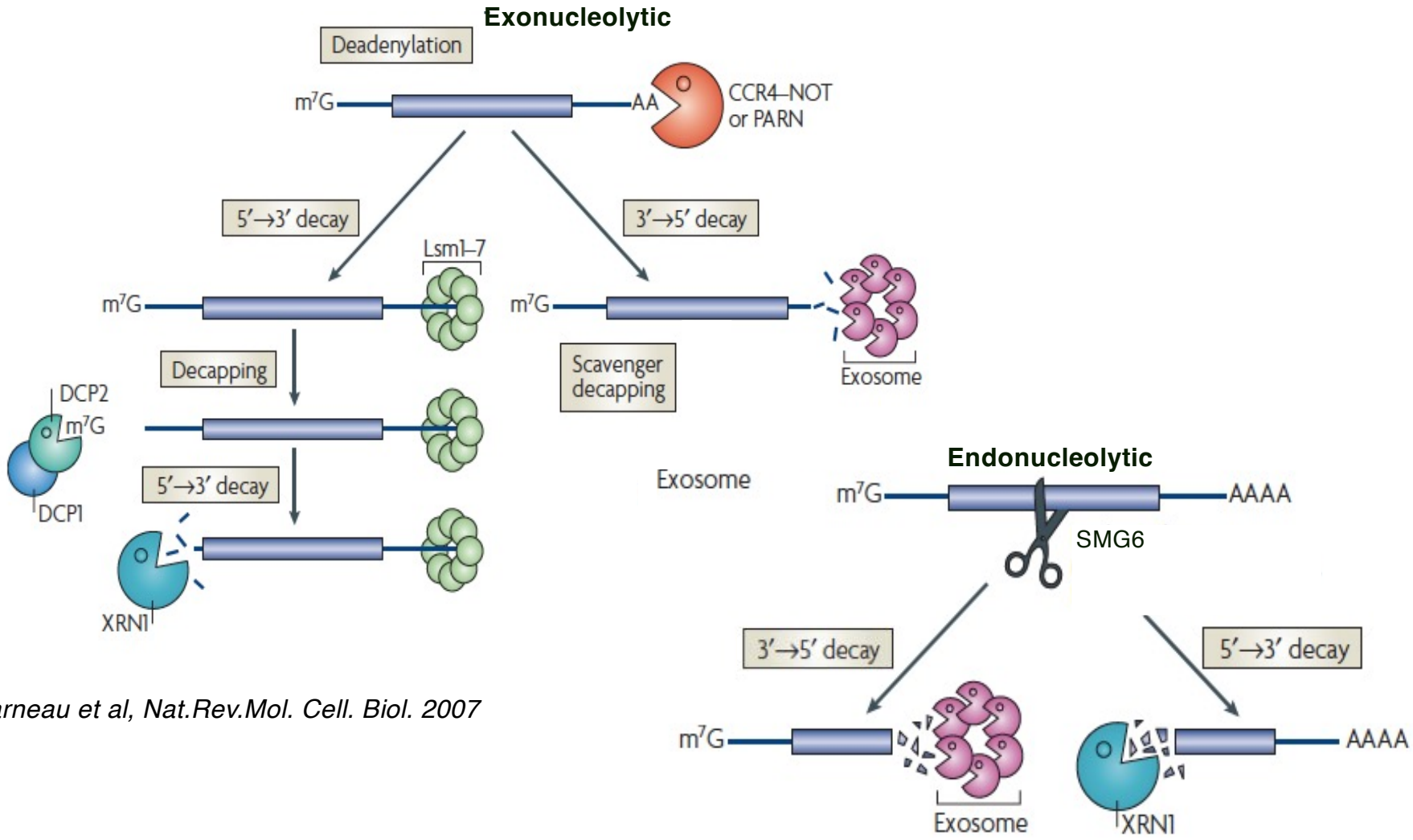
Balagopal and Parker, Cur.Op.Cel.Biol., 2009

mRNA general decay in the cytoplasm



Norbury, Nat Rev Mol Cell Biol., 2013

mRNA general decay in the cytoplasm



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

RNA is also degraded in the nucleus:

- unspliced, unporcessed or unexported mRNAs
- aberrant ncRNAs, unmodified tRNAs, excessive rRNAs and tRNAs

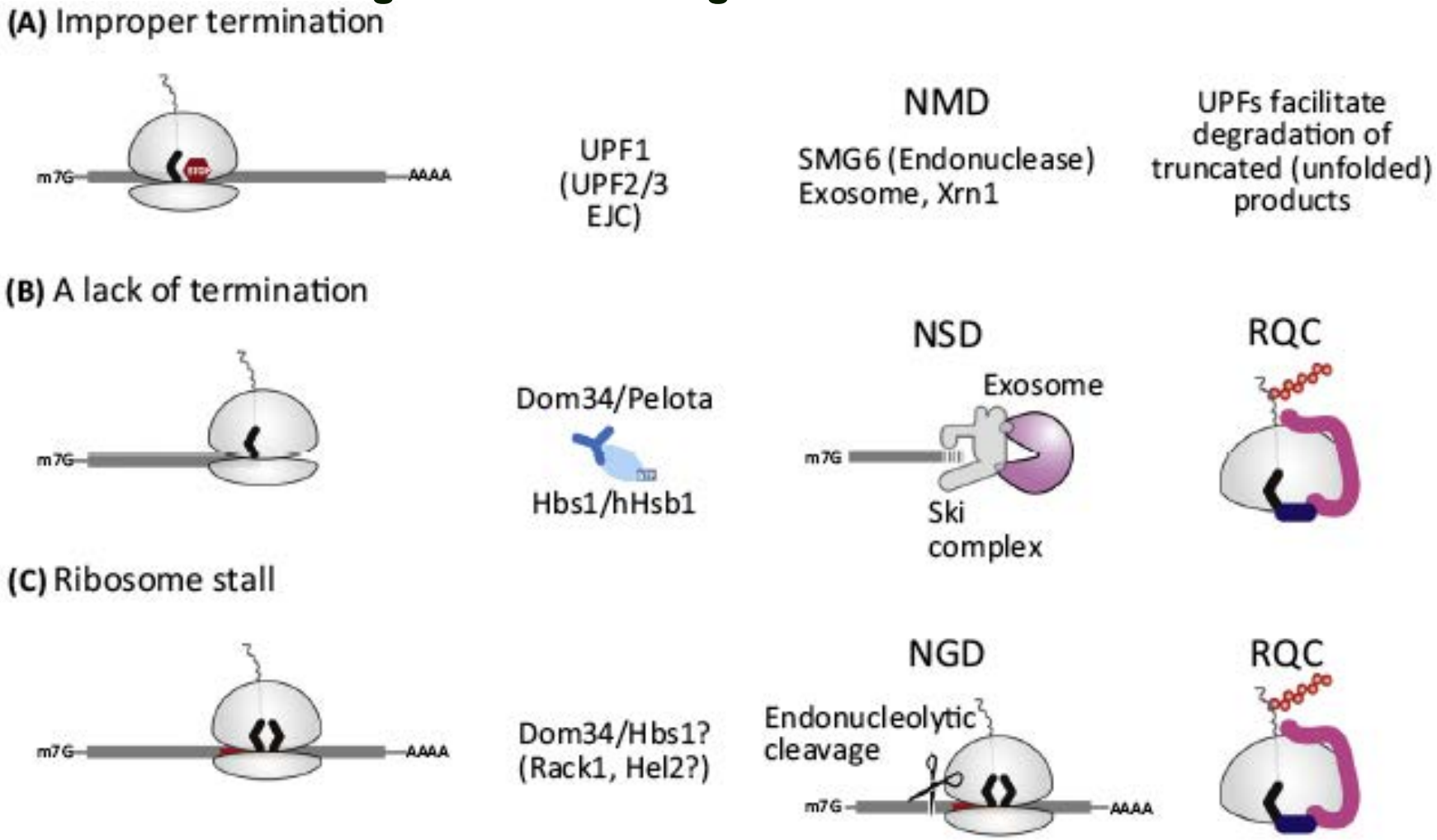
mRNA quality control decay in the cytoplasm

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

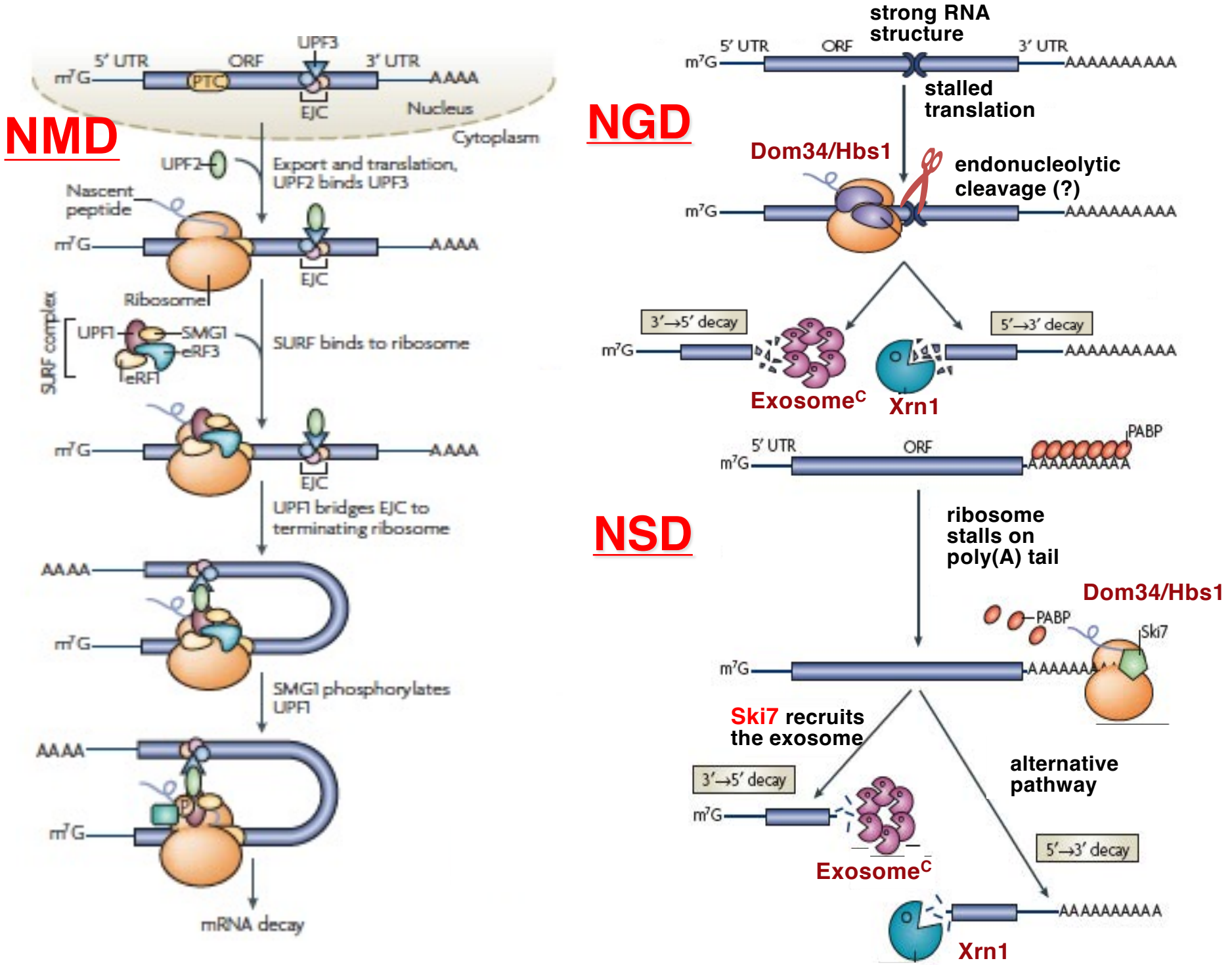
NGD – No-Go Decay (ribosome stuck on an obstacle)

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

Problems with a stalling ribosome during translation



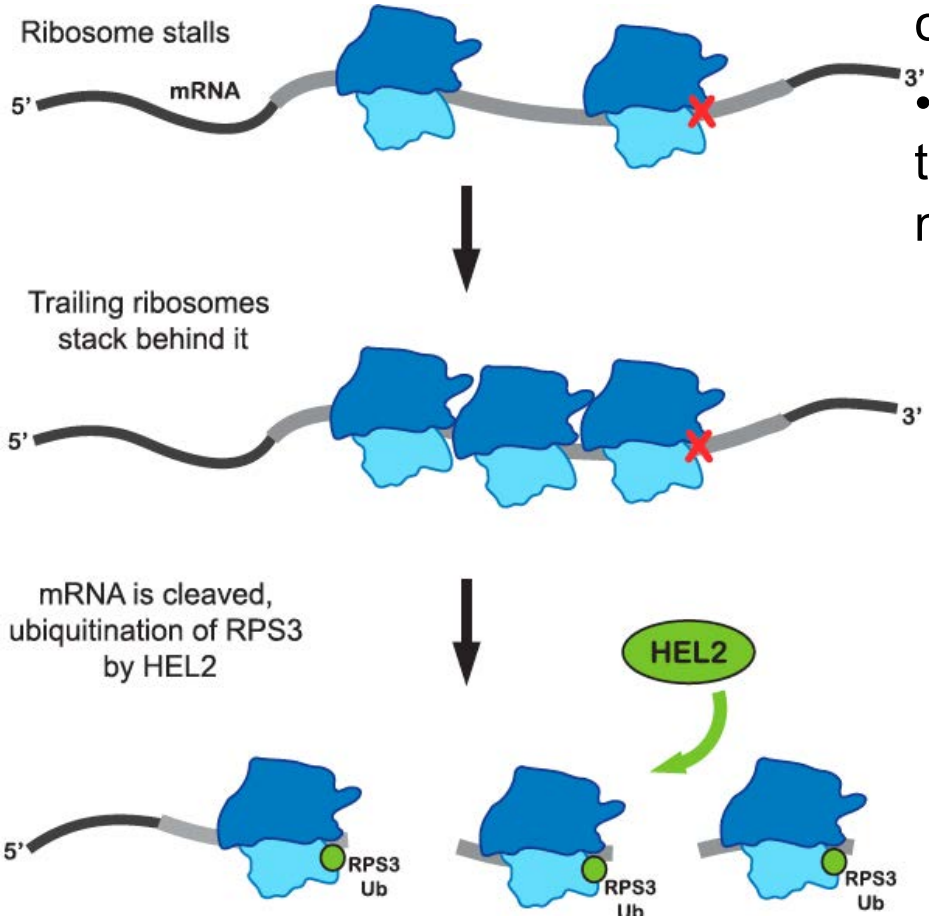
Inada, TiBS 2016



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

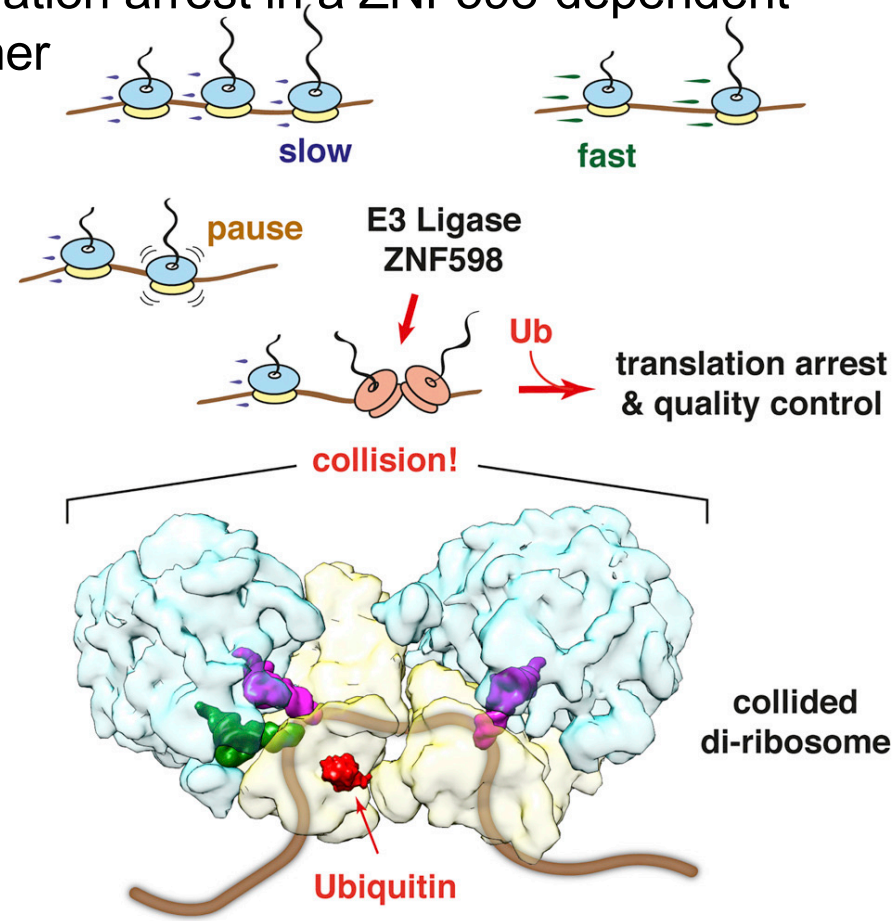
Ribosome collision in RQC during NGD

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

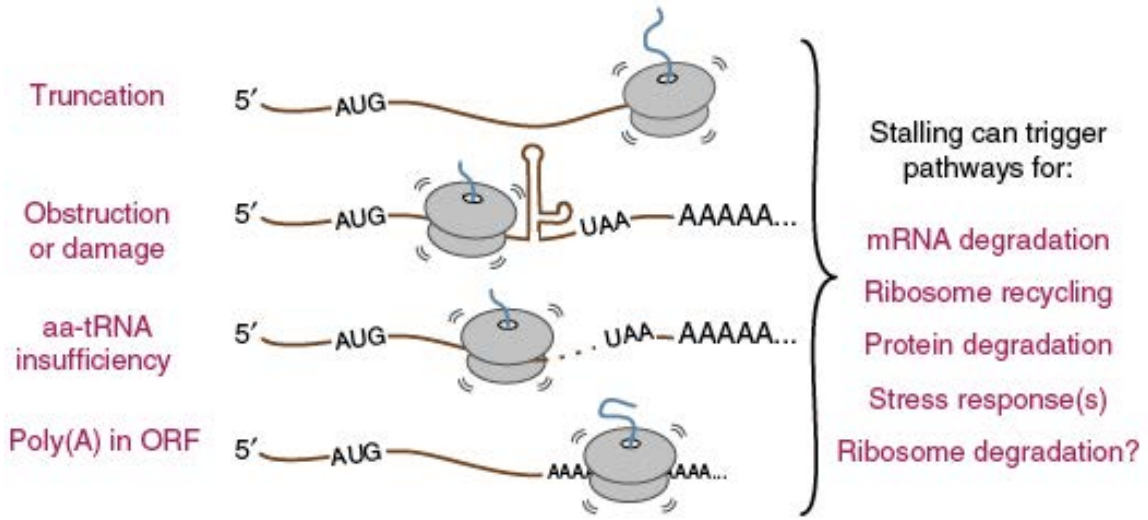


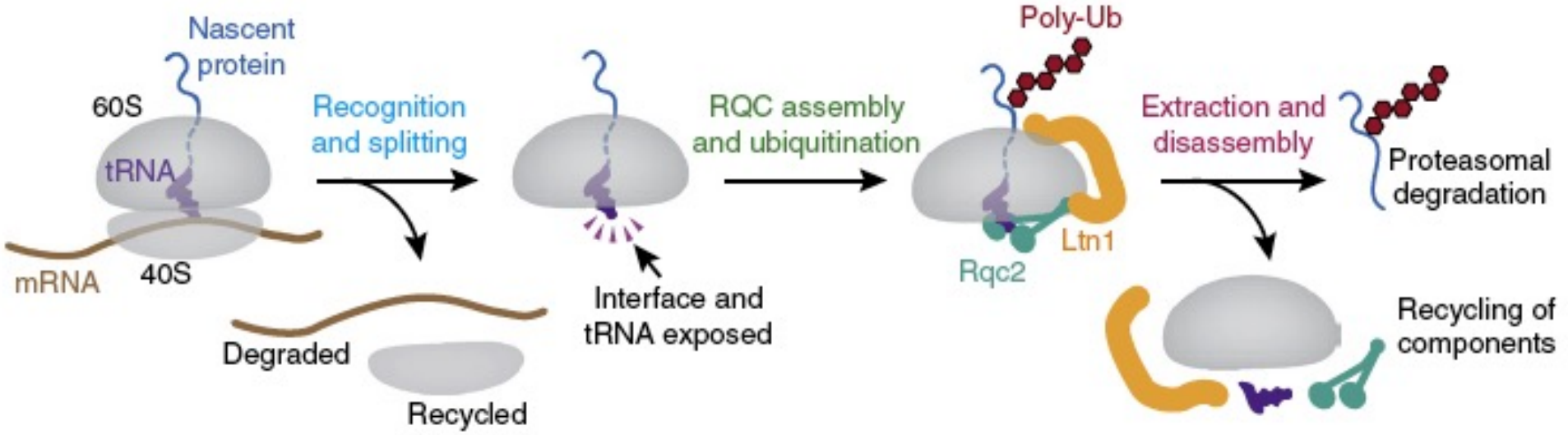
Simms et al, Mol Cell 2017; Juszkiewicz et al, Mol Cell 2018

- 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



RIBOSOME QC (RQC)

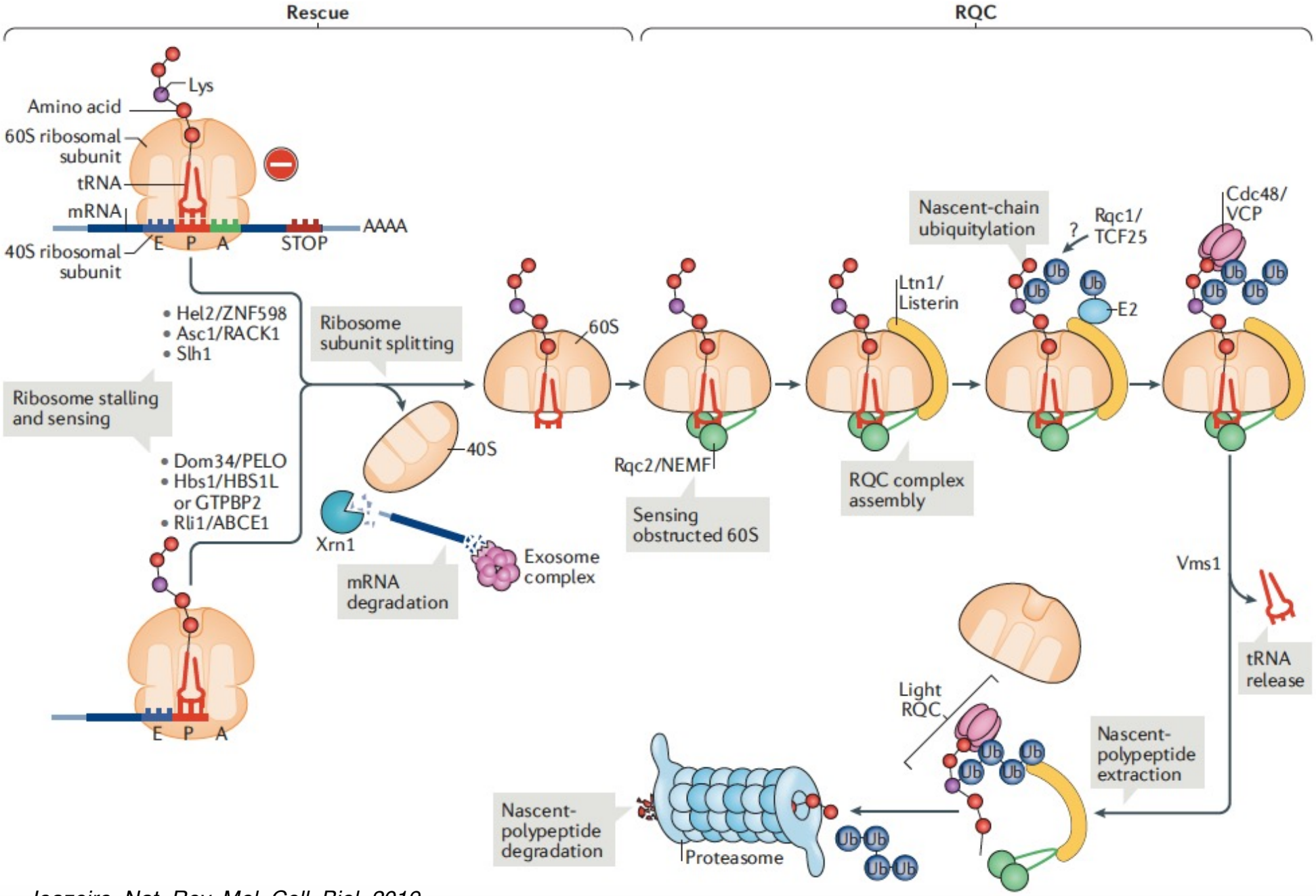




Yeast	Asc1	Hel2	Dom34	Hbs1	Rli1	Rqc2	Ltn1	Rqc1	Cdc48–Ufd1–Npl4		
Mammals	RACK1	ZNF598?	Pelota	Hbs1	ABCE1	NEMF	Listerin	TCF25?	VCP complex?		
	Facilitates stalling?		Ribosome splitting			Nascent-chain ubiquitination		Nascent-chain extraction			

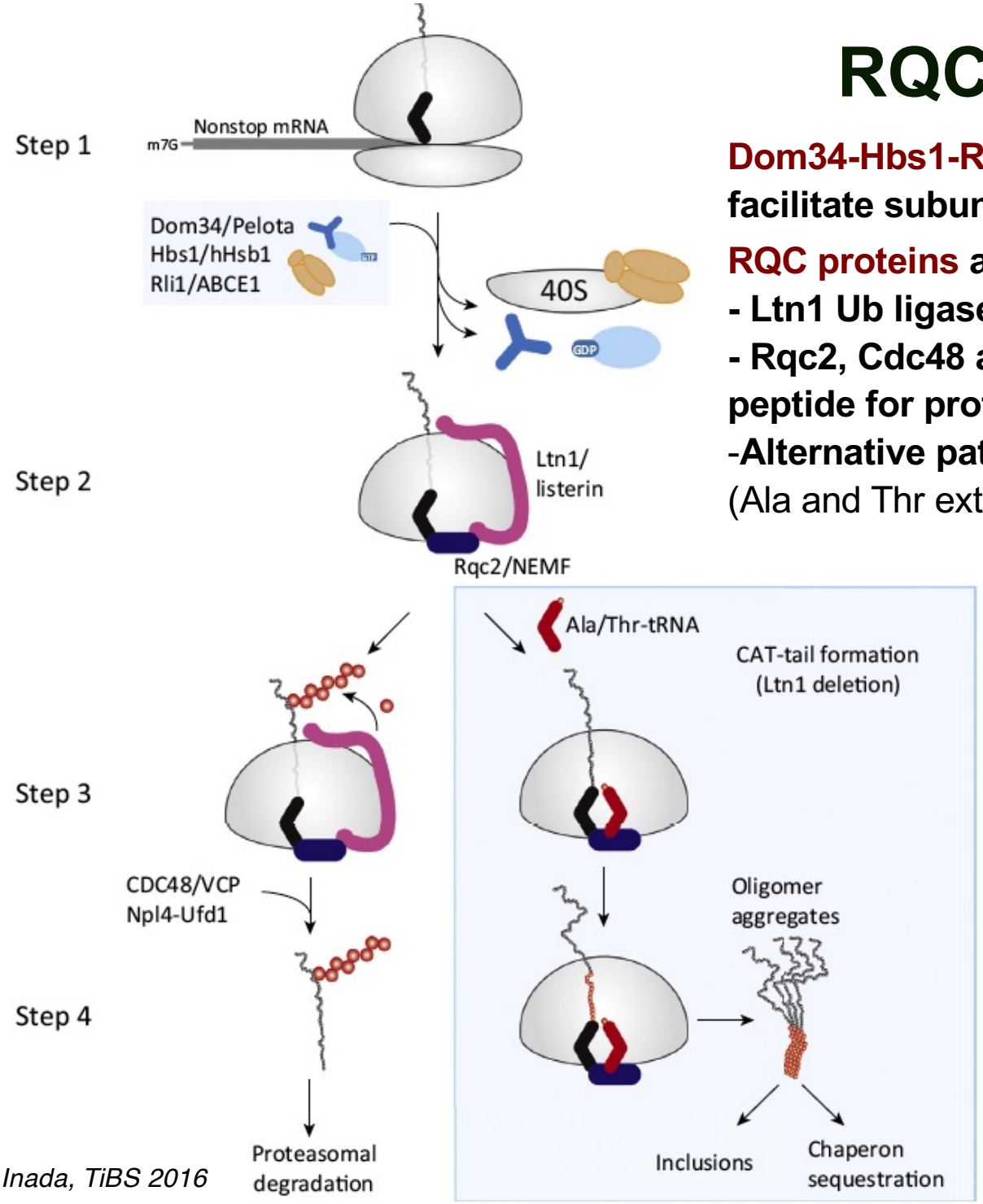
Brandman and Hegde, NatStrMolBiol 2016

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

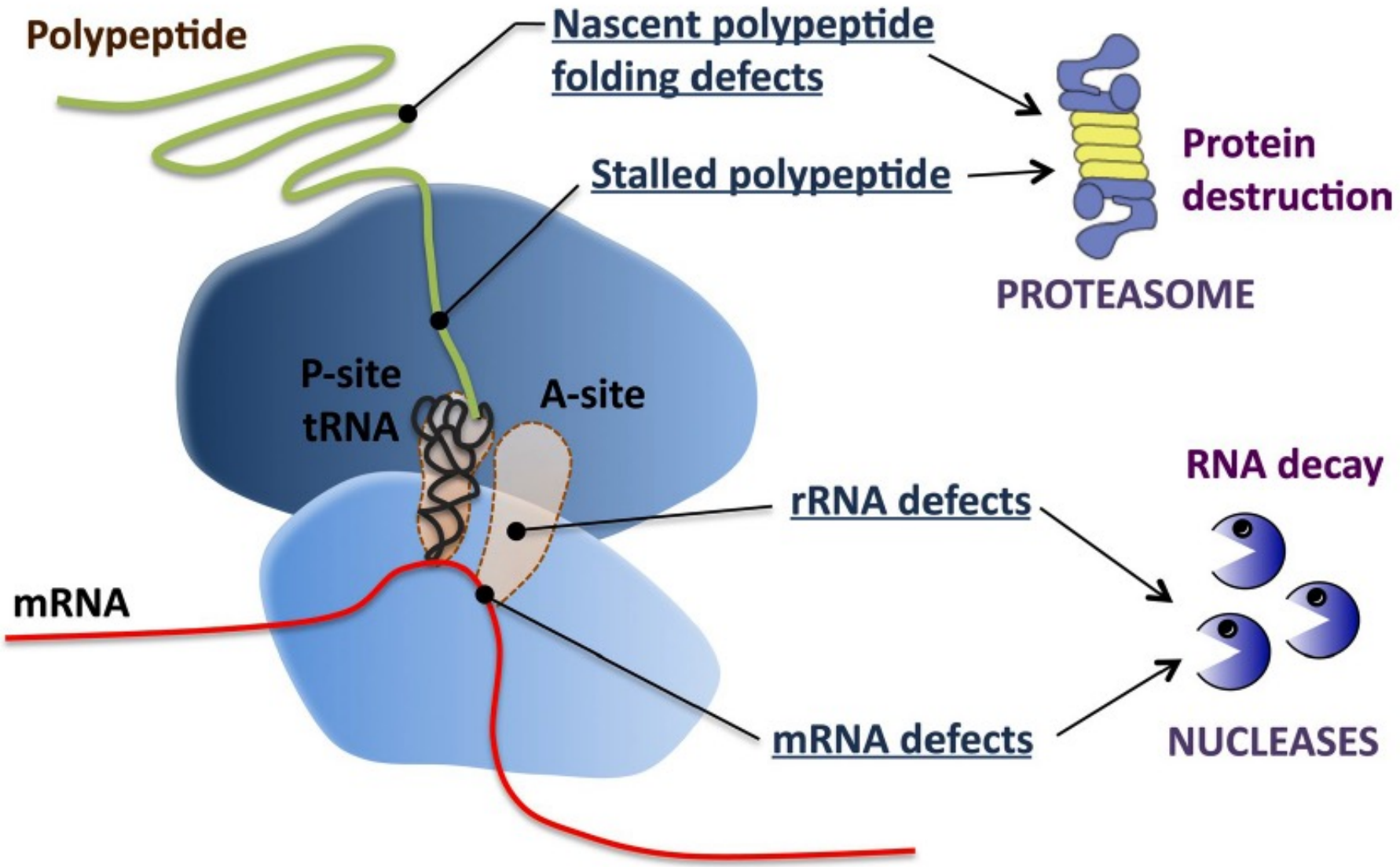
- degradation of aberrant proteins

- nascent chain aggregation

- activation of stress signaling

- nascent chain proteolysis

Co-translational protein and mRNA QC



Lykke-Andersen and Bennett, JCB, 2014

NEXT LECTURE:

Global analyses of RNAs and RNPs