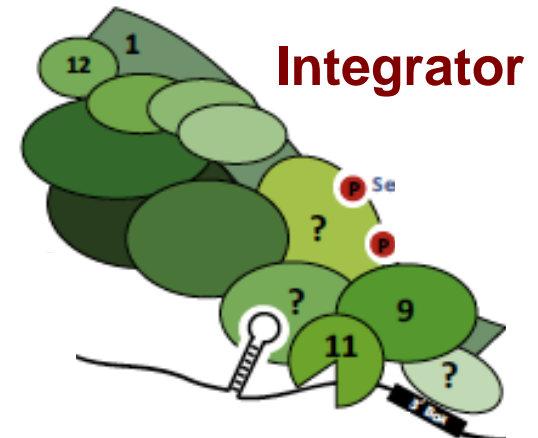
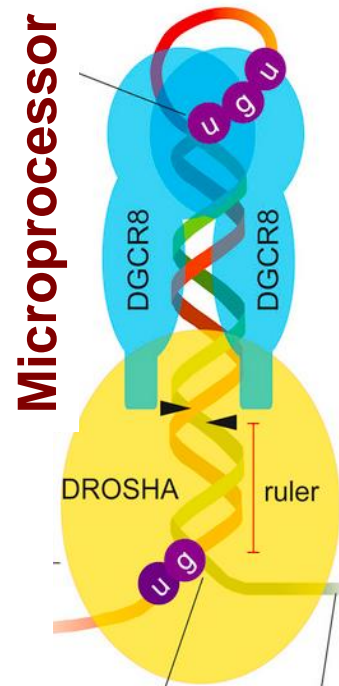
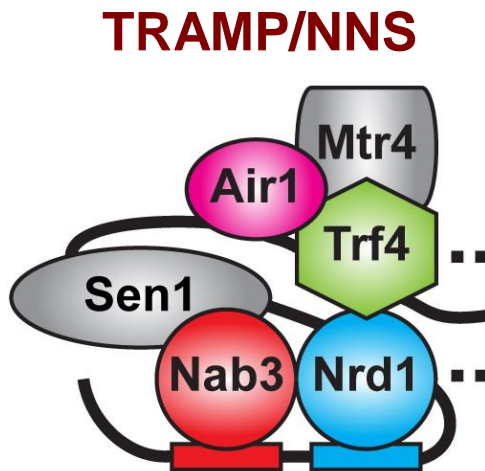
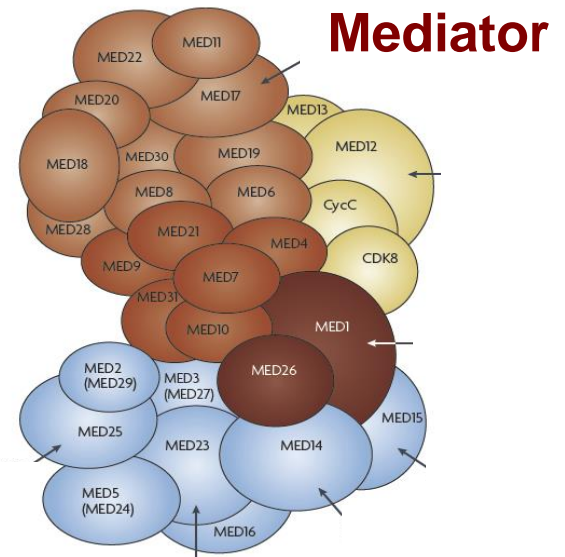
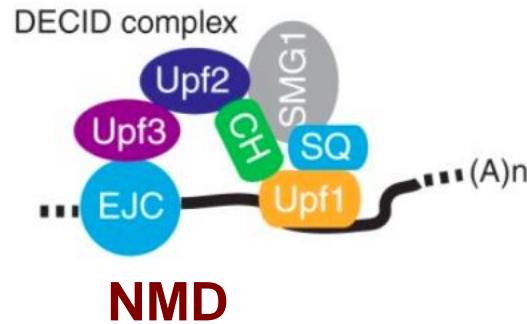
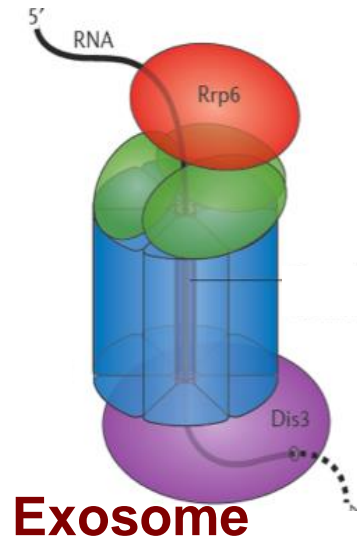


ENZYMES AND RNA COMPLEXES



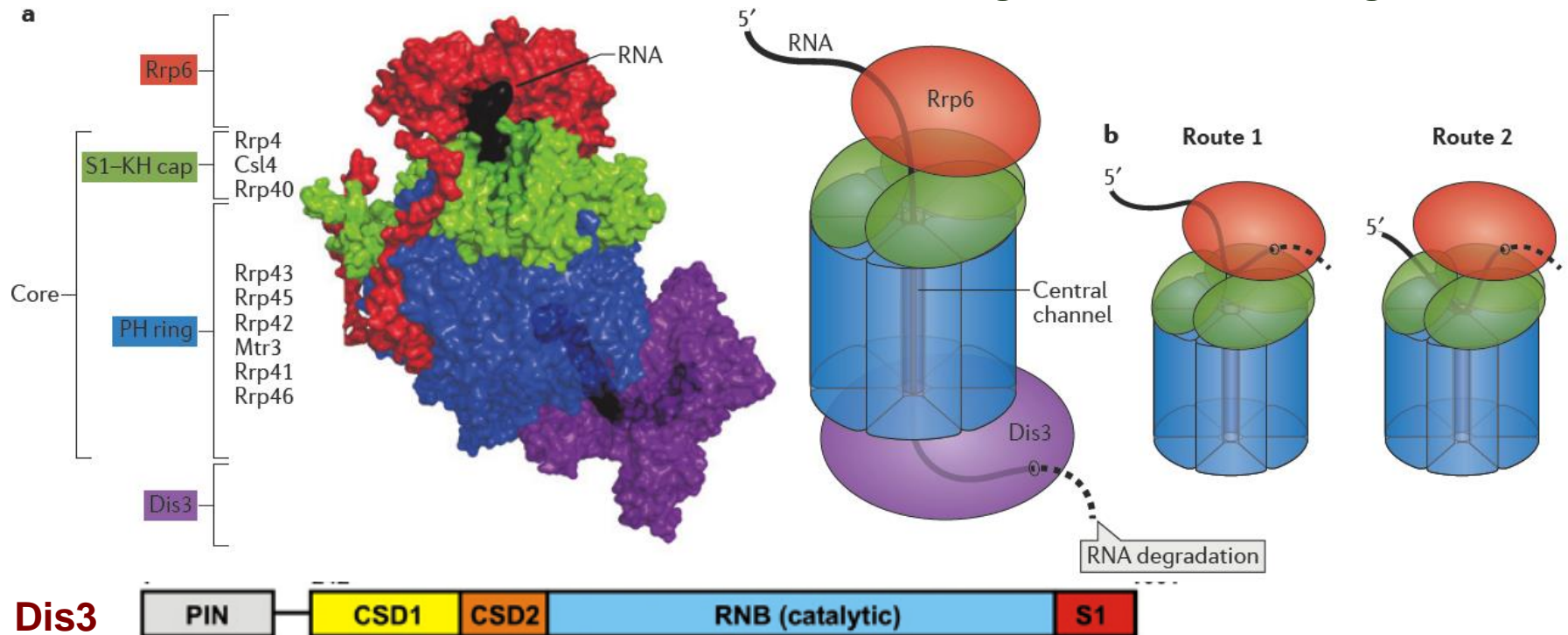
RNA PROCESSING and DECAY machinery: RNases

Protein	Function	Characteristics
<u>Exonucleases 5' → 3'</u>		
Xrn1	cytoplasmic, mRNA degradation	processive
Rat1	nuclear, pre-rRNA, sn/snoRNA, pre-mRNA processing and degradation	
Rrp17/hNol12	nuclear, pre-rRNA processing	
<u>Exosome 3' → 5'</u> multisubunit exo/endo complex		subunits organized as in bacterial PNPase
Rrp44/Dis3	catalytic subunit	Exo/PIN domains, processive
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, processive
Ski2,3,7,8	cytoplasmic exosome cofactors. SKI complex	helicase, GTPase
<u>Other 3' → 5'</u>		
Rex1-4	3'-5' exonucleases, rRNA, snoRNA, tRNA processing	RNase D homolog
DXO	3'-5' exonuclease in addition to decapping	
<u>mtEXO 3' → 5'</u> mitochondrial degradosome RNA degradation in yeast		
Suv3/ Dss1	helicase/ 3'-5' exonuclease	DExH box/ RNase II homolog
<u>Deadenylation</u>		
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
<u>Endonucleases</u>		
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) _n A)
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 factors

Protein	Function / Characteristics
<u>5'→3' decay: decapping</u>	
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 eukaryotes)
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
DXO	pyrophosphohydrolase, 5' decapping endonuclease, deNADding, 5'OH hydrolase
<u>TRAMP complex: nuclear RNA surveillance, polyadenylation-dependent degradation</u>	
Trf4/Trf5	nuclear alternative poly(A) polymerases
Mtr4	DEAD box helicase
Air1/Air2	RNA binding proteins, also nuclear exosome cofactor
<u>Nrd1-Nab3-Sen1 complex: PolII termination of small RNAs, TRAMP-dependent degradation</u>	
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
- substrates- processing and/or degradation of almost all RNAs

Lecture on the exosome by Rafał Tomecki

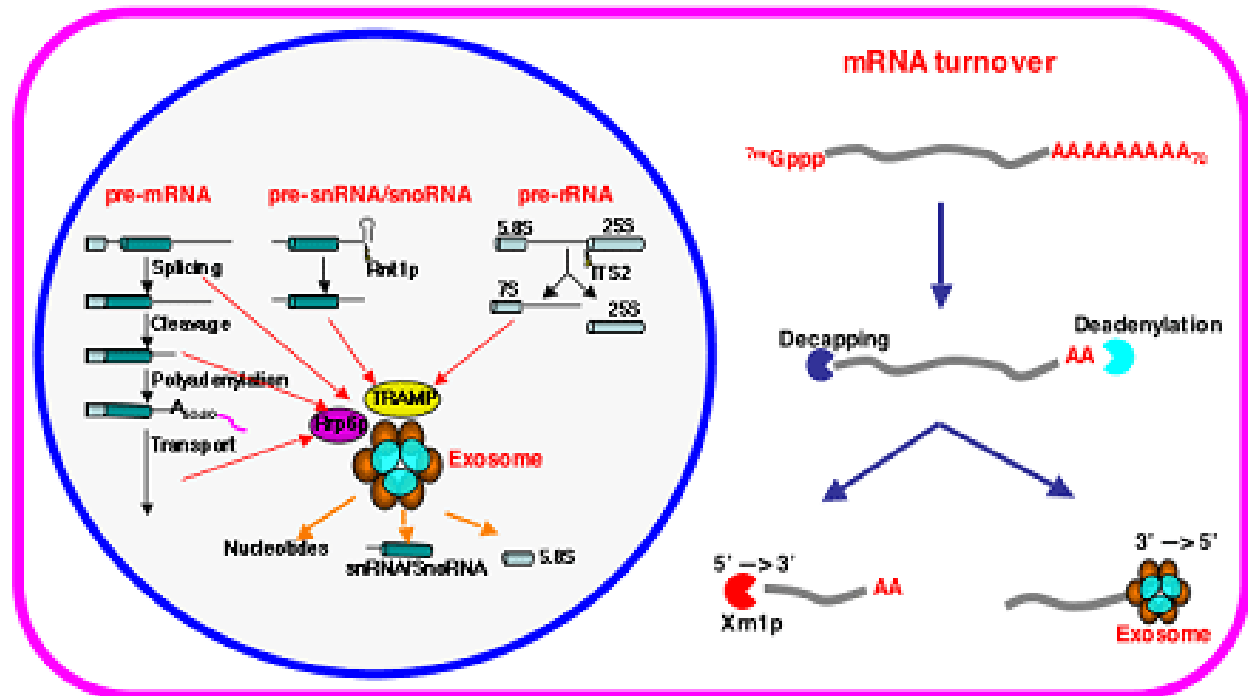
EXOSOME: 3' → 5' decay: FUNCTION

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:

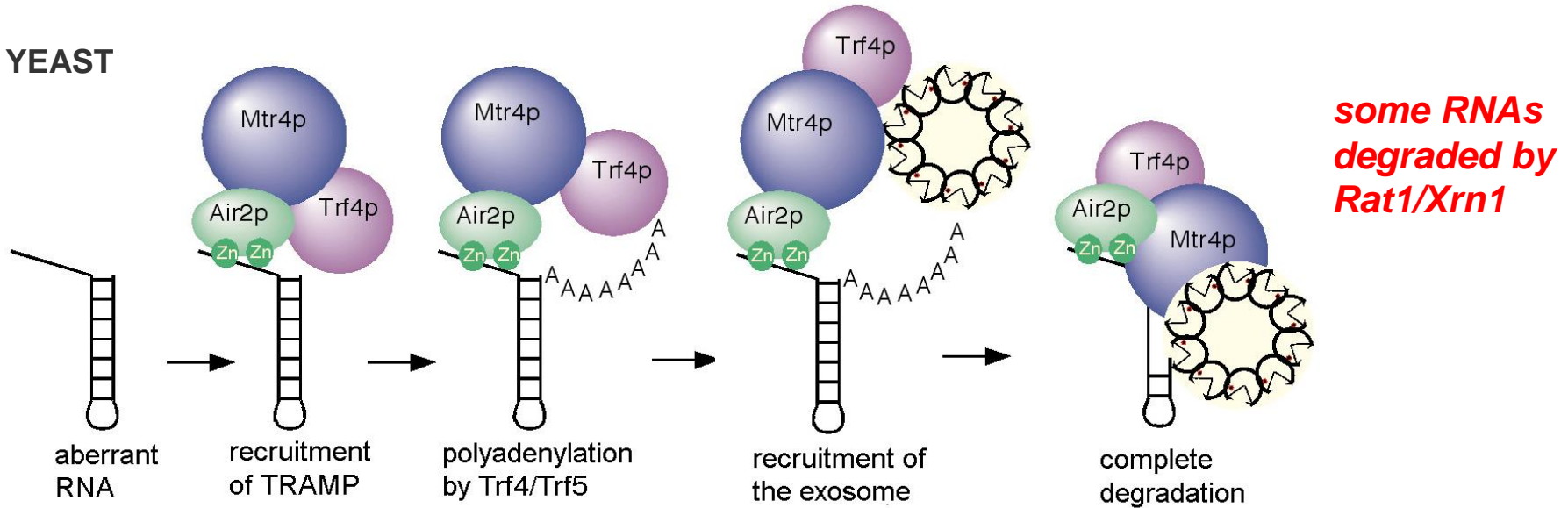
- generic mRNA decay
- specialised mRNA decay pathways: NMD, NSD, NO-GO decay, ARE-dependent decay



TRAMP - EXOSOME COFACTORS (yeast)

TRAMP = Trf4/5 + Air1/2 + Mtr4
polyadenylation complex poly(A) polymerases RNA binding proteins RNA DEVH helicase

YEAST



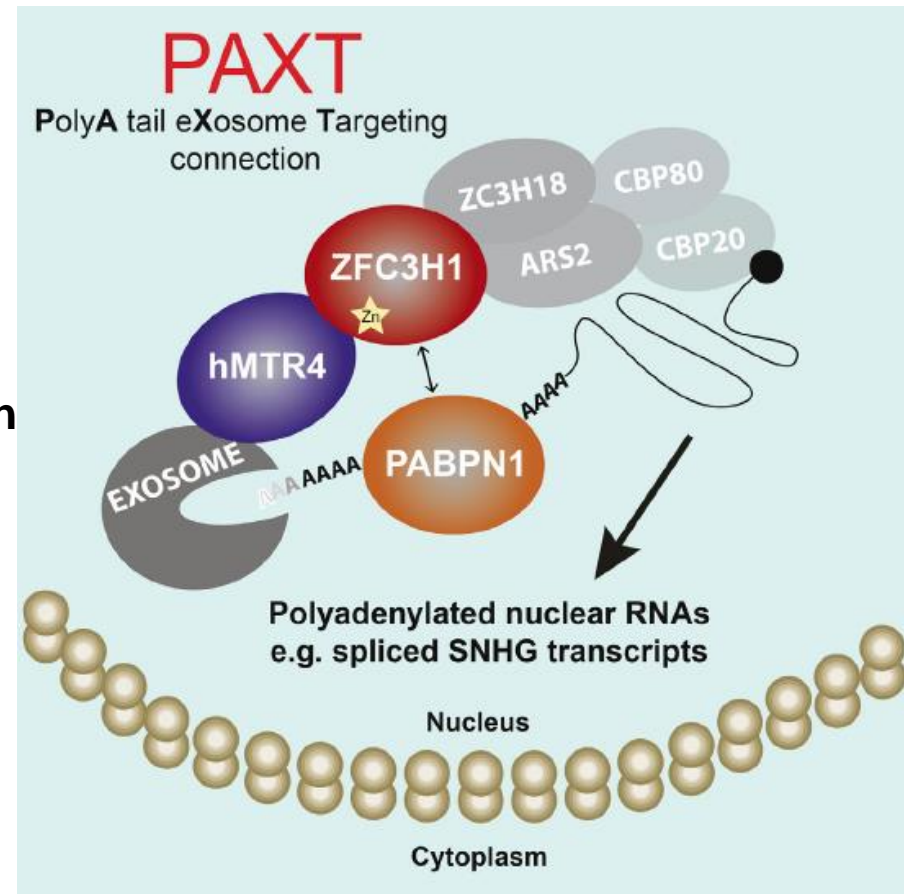
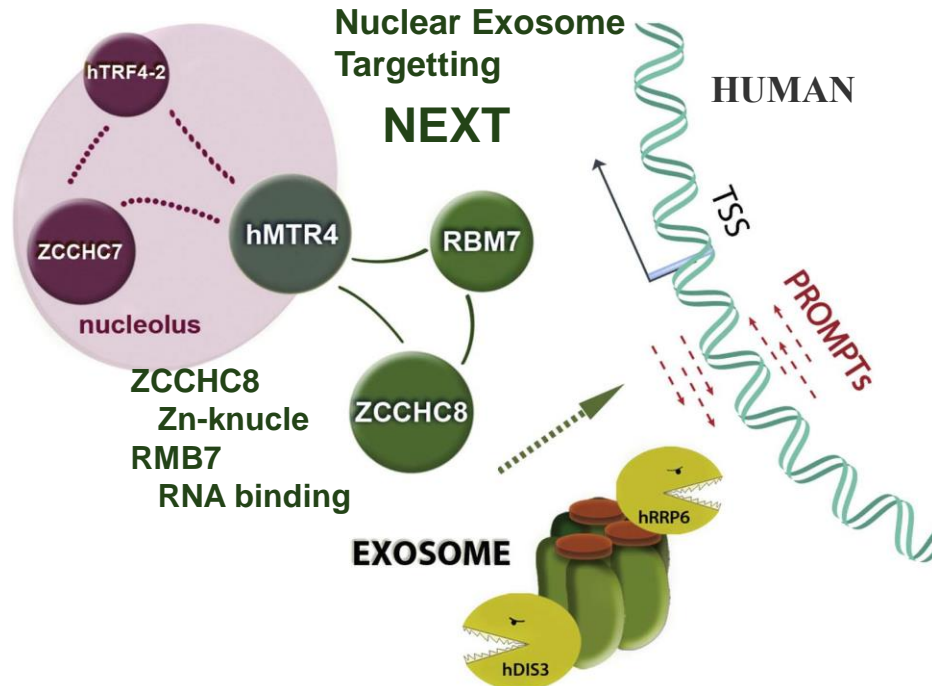
Polyadenylation-mediated nuclear discard pathway for defective RNAs

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

Interacts with

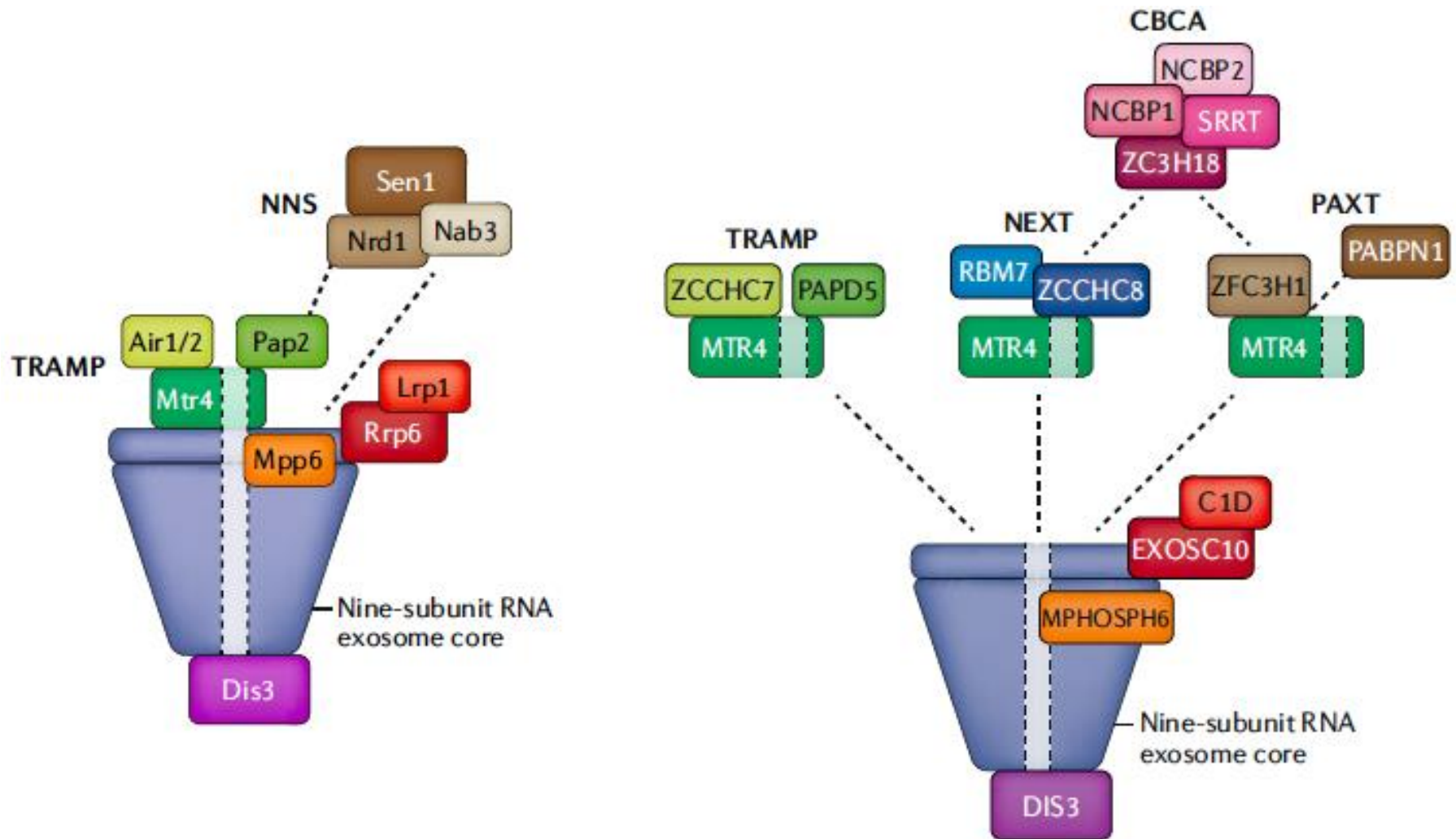
- exosome via Mtr4
- Nrd1/Nab3 complex

NEXT and PAXT - EXOSOME COFACTORS (humans)

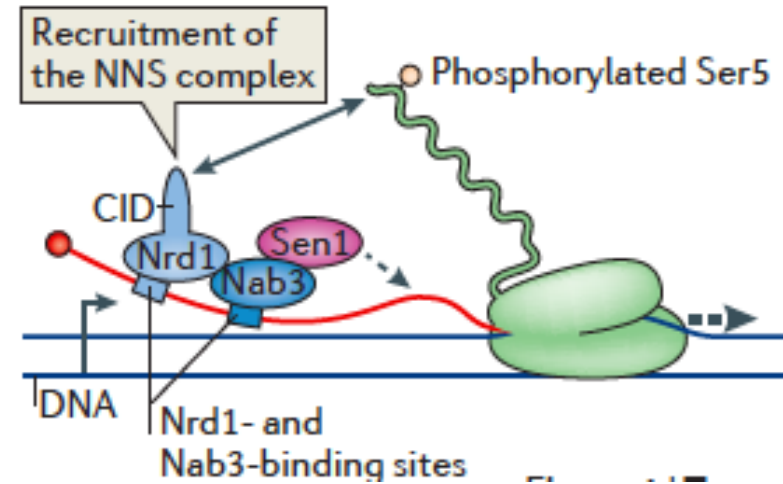
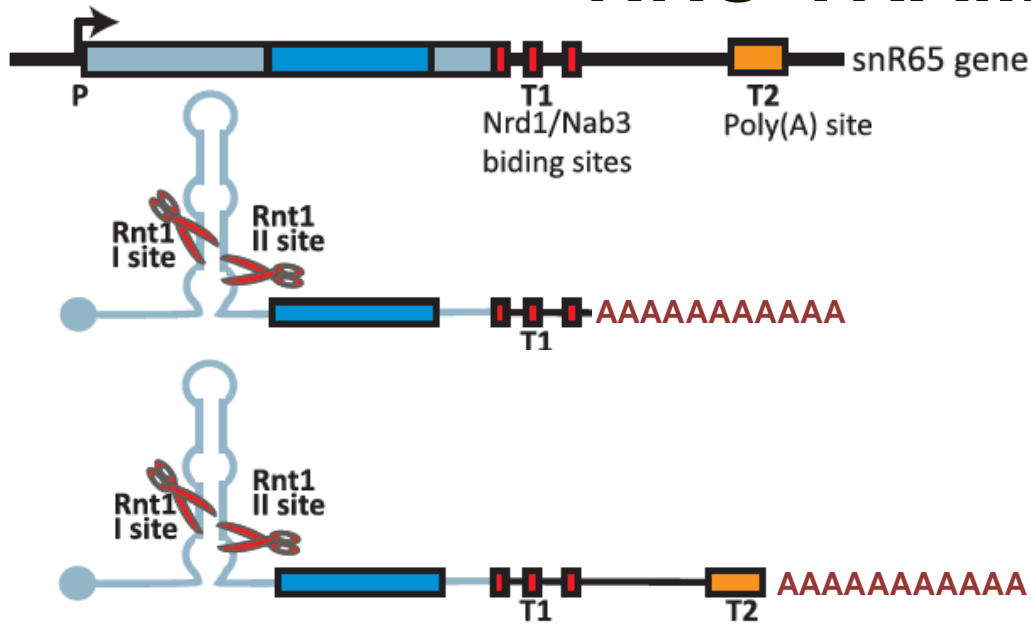


- ZFC3H1 (Zn-knuckle protein) links MTR4 with PABPN1 in PAXT
- ZFC3H1/PABPN1 and RBM7/ZCCHC8 (NEXT) interact with MTR4 in a mutually exclusive manner
- PAXT and NEXT direct distinct RNA species for nuclear exosome degradation
- PAXT targets tend to be longer and more extensively polyadenylated than NEXT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

EXOSOME with TRAMP, NEXT, PAXT

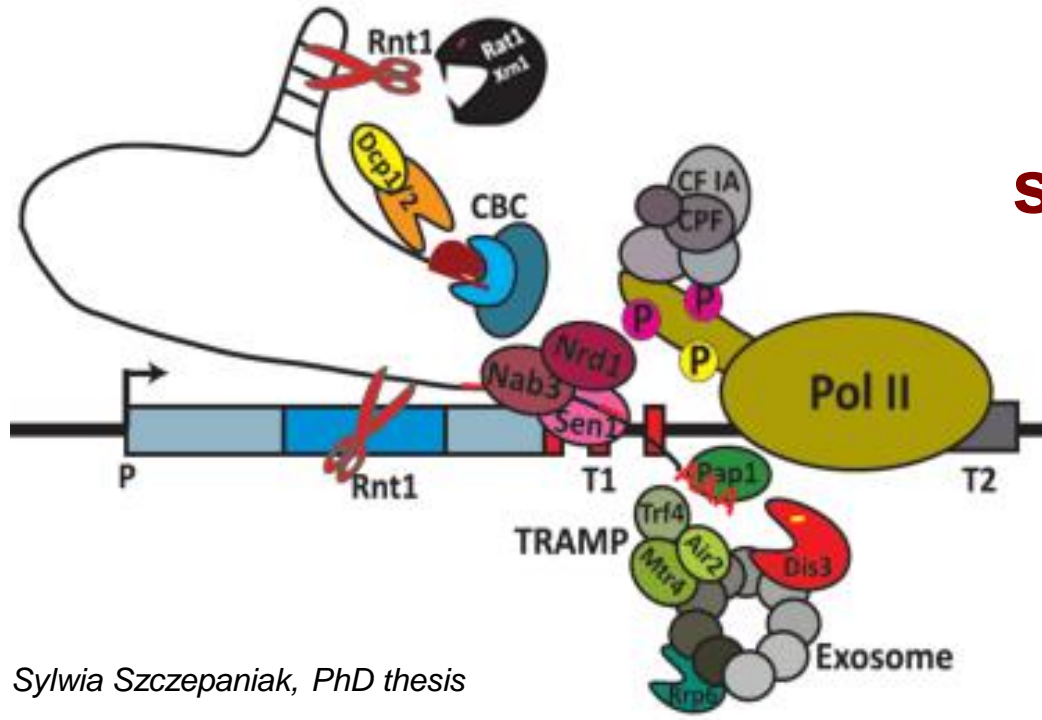


NNS-TRAMP-exosome



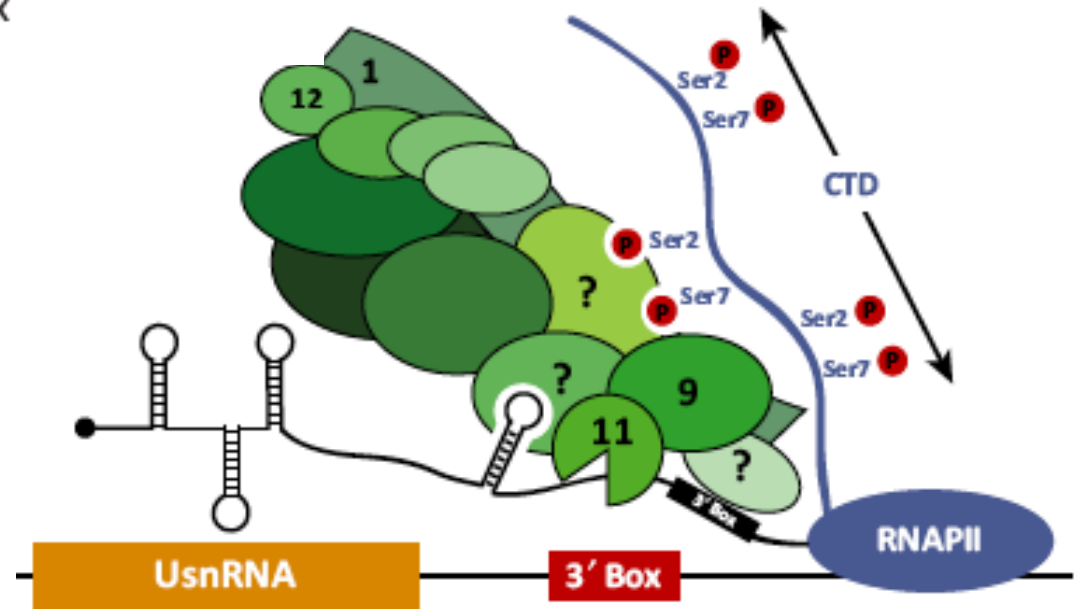
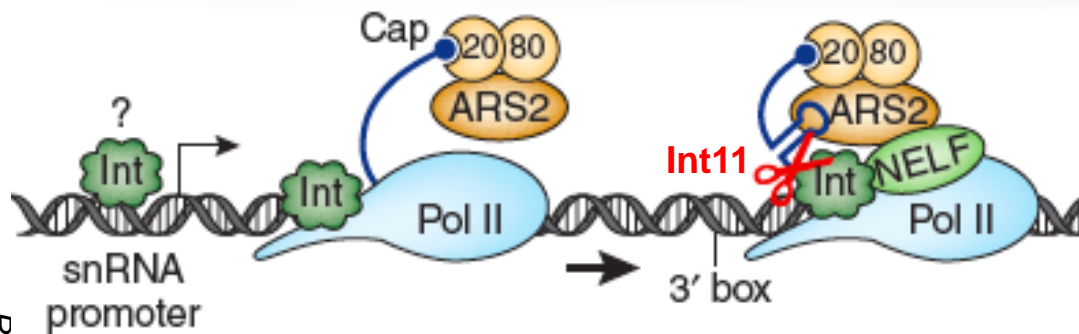
Poruua, Libri, Nat Rev Mol Cell Biol, 2015

sn/snoRNA processing (yeast)



INTEGRATOR

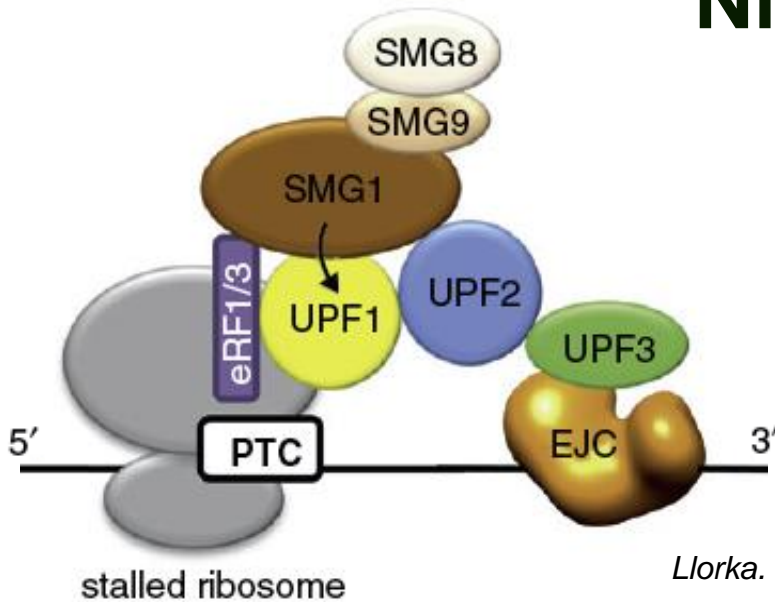
snRNA processing (metazoa)



INT - Integrator complex

- recruited contrancriptionally 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)

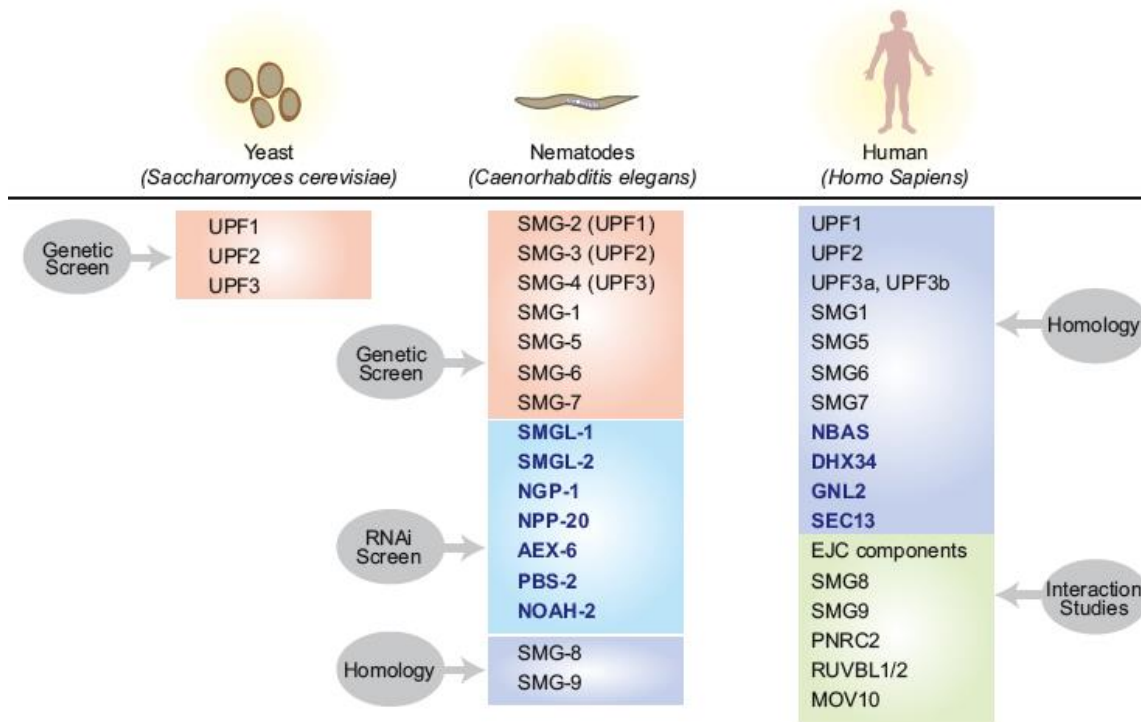
NMD FACTORS



SURF complex
SMG1-UPFs-SMGs-
Release Factors

DECID (decay inducing)
phosphoSMG1-UPFs-EJC

Llorka. Cur. Op. Chem. Biol. 2013



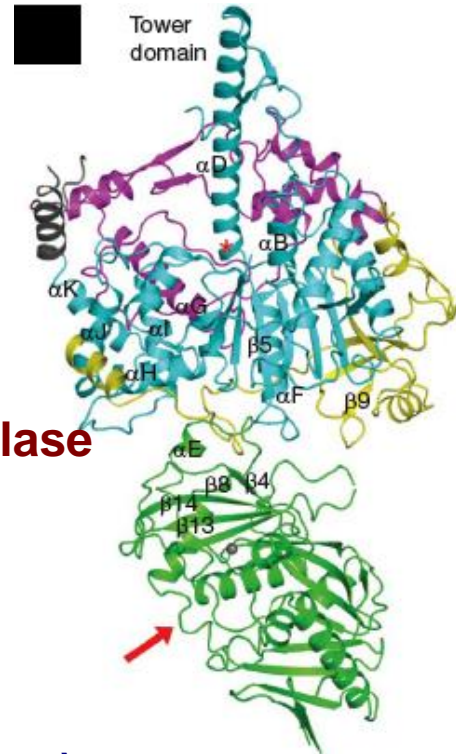
Hug et al., NAR, 2016

XRN family: 5'→3' processive exonucleases



Kastenmayer and Green, 2000, PNAS

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



NUCLEAR

Rat1/XRN2 with Rai1 activator (5' -ppp pyrophosphohydrolase and phosphodiesterase-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*)

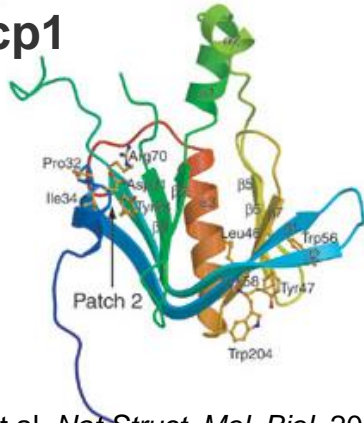
Xiang et al, 2009, Nature

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

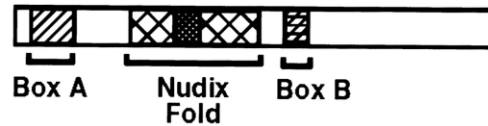
DCP/NUDT- DECAPPING ENZYMES

Dcp1



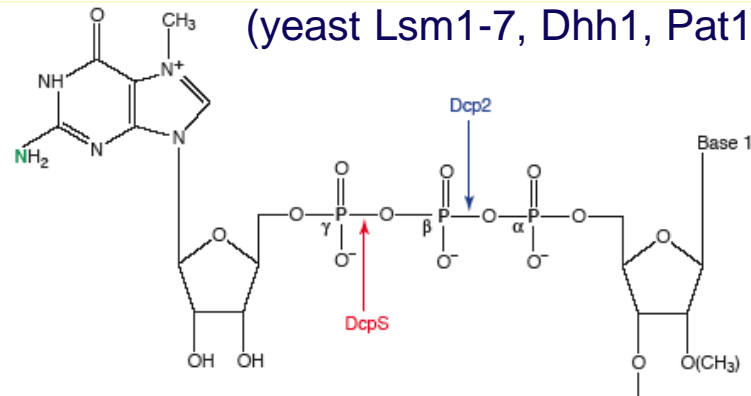
She et al. *Nat. Struct. Mol. Biol.*, 2004

Dcp2



Wang et al. *PNAS*, 2002

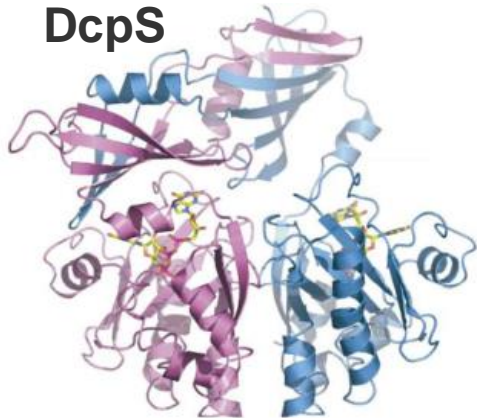
- Dcp1/Dcp2 complex participates in mRNA 5' decay
- catalyses the reaction $m^7GpppX\text{-mRNA} \rightarrow m^7GDP + 5'p\text{-mRNA}$
- Dcp2 is the catalytic subunit (pyrophosphatase Nudix domain)
- Dcp1 is required for activity *in vivo*, interacts with other proteins
- Dcp1/Dcp2p is regulated by Pab1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



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

NUDT proteins (22): *in vivo* decapping Nudt16, Nudt3 (mammals)
in vivo deNADding Nudt12 (mammals), Nudt7 (plants)

DcpS



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

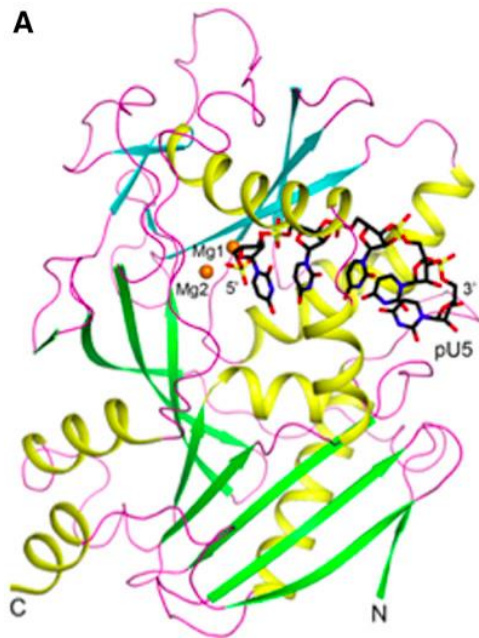
Gu et al., *M. Cell*, 2004

DXO/Rai1 family

Cellular activities

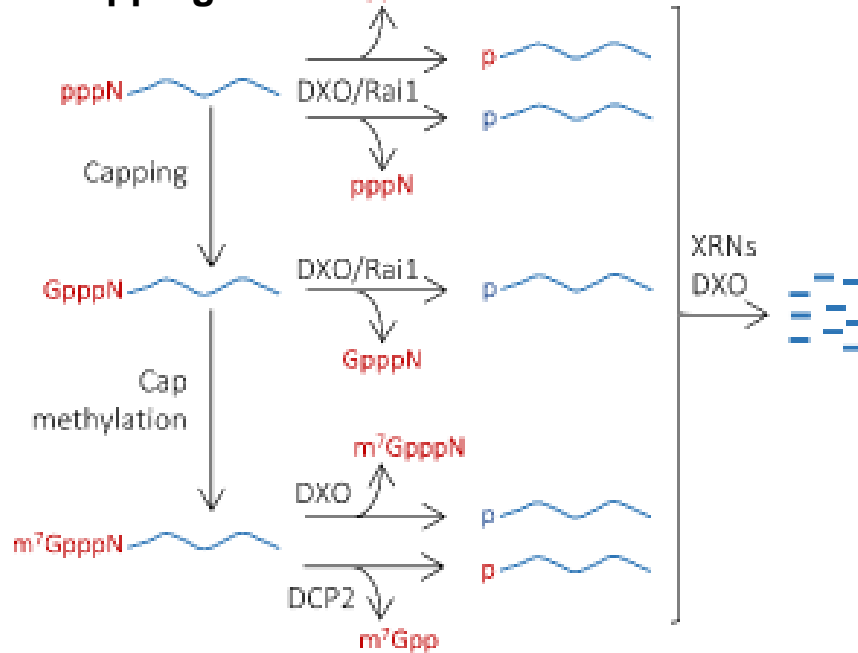
cap surveillance

deNADding

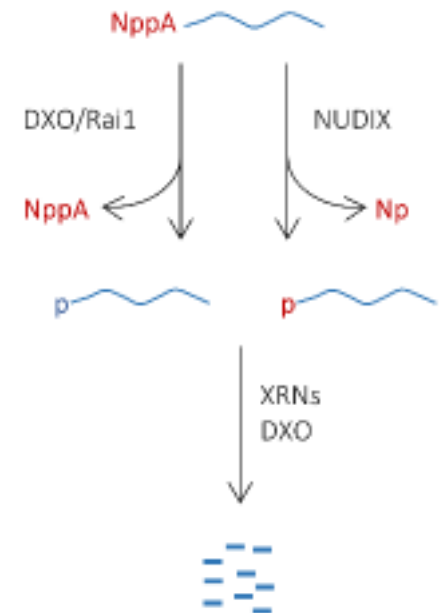


pyrophosphohydrolase decapping nuclease

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

Additional activities:

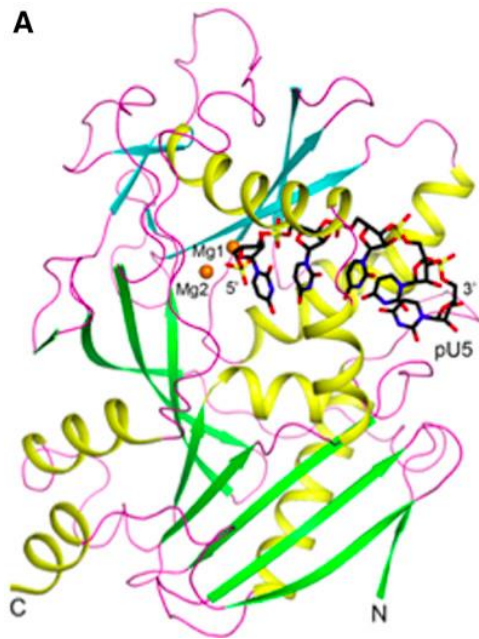
- 5' OH RNA hydrolase
- FAD and CoA decapping nuclease

DXO/Rai1 family

Cellular activities

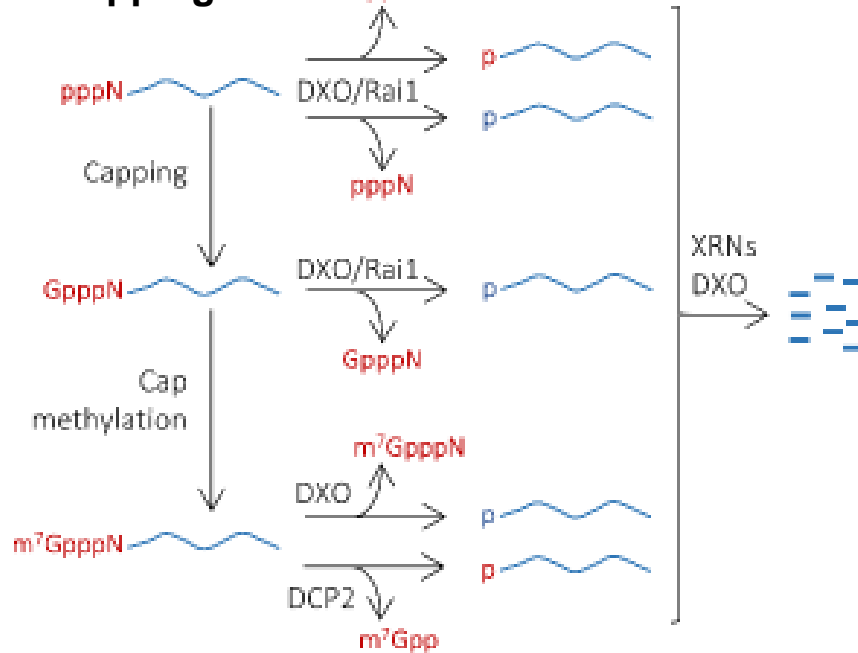
cap surveillance

deNADding

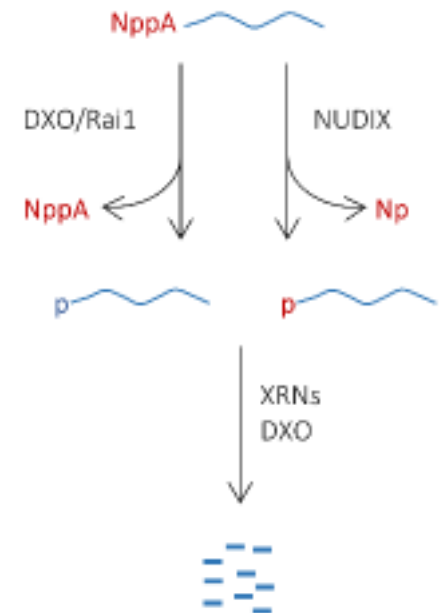


pyrophosphohydrolase decapping nuclease

5' -3' exonuclease



deNADding nuclease

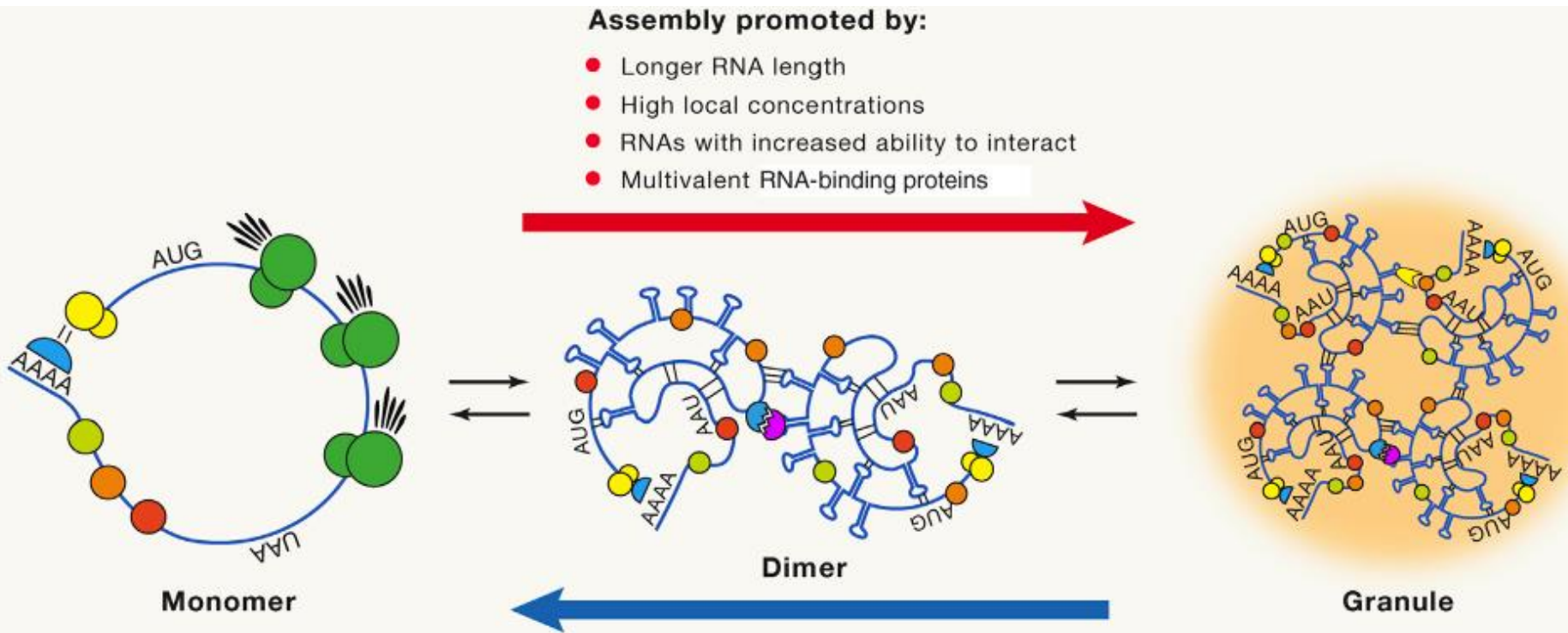


ACTIVITY	SUBSTRATE	MmDXO	At DXO1
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Pyrophosphohydrolase	ppp-RNA	+++	-
Decapping (unmethylated cap)	Gppp-RNA	+++	-
Decapping (mature cap)	m ⁷ Gppp-RNA	+++	-
DeNADding	NppA-RNA	++++	+++

Additional activities:

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

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

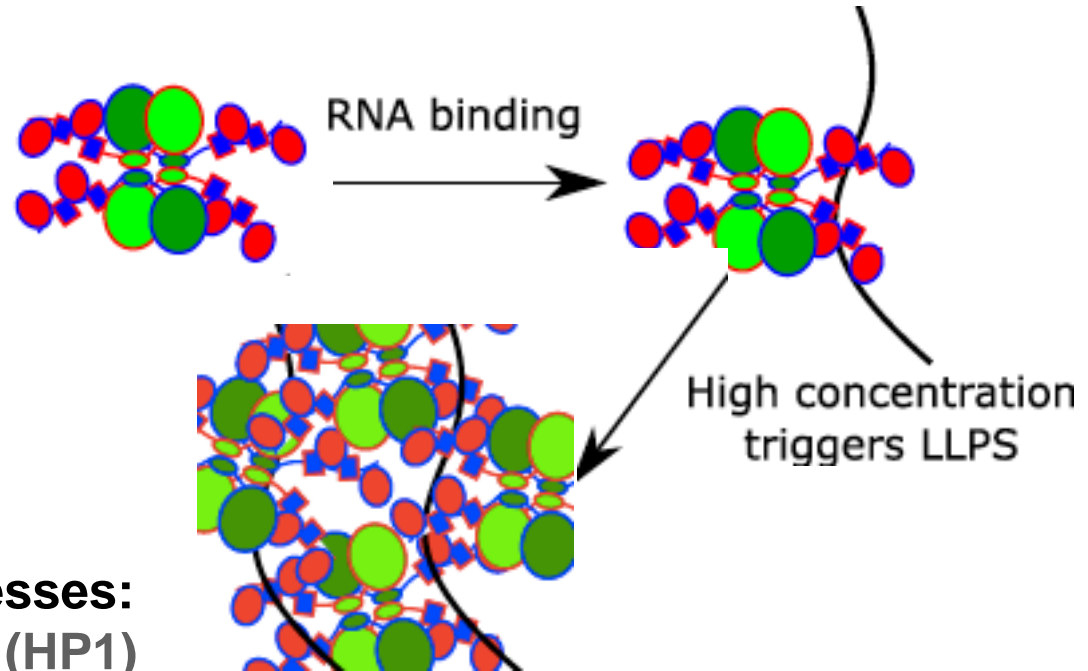


Phase transition

Droplets, MLOs (Membraneless Organelles)

Liquid-Liquid Phase Separation (LLPS)

Formed by unstructured disordered protein domains around RNAs

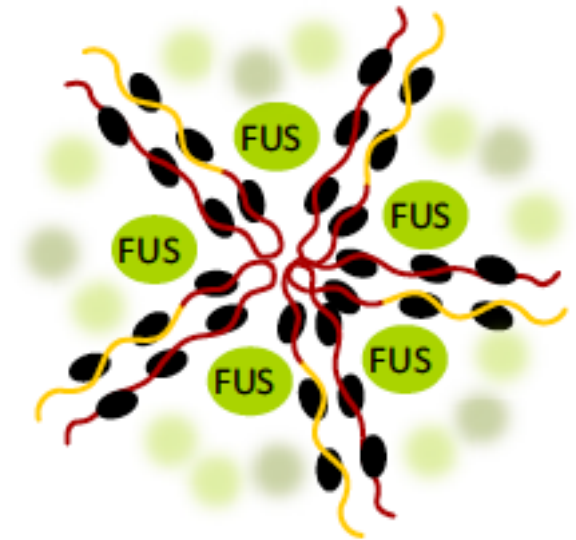
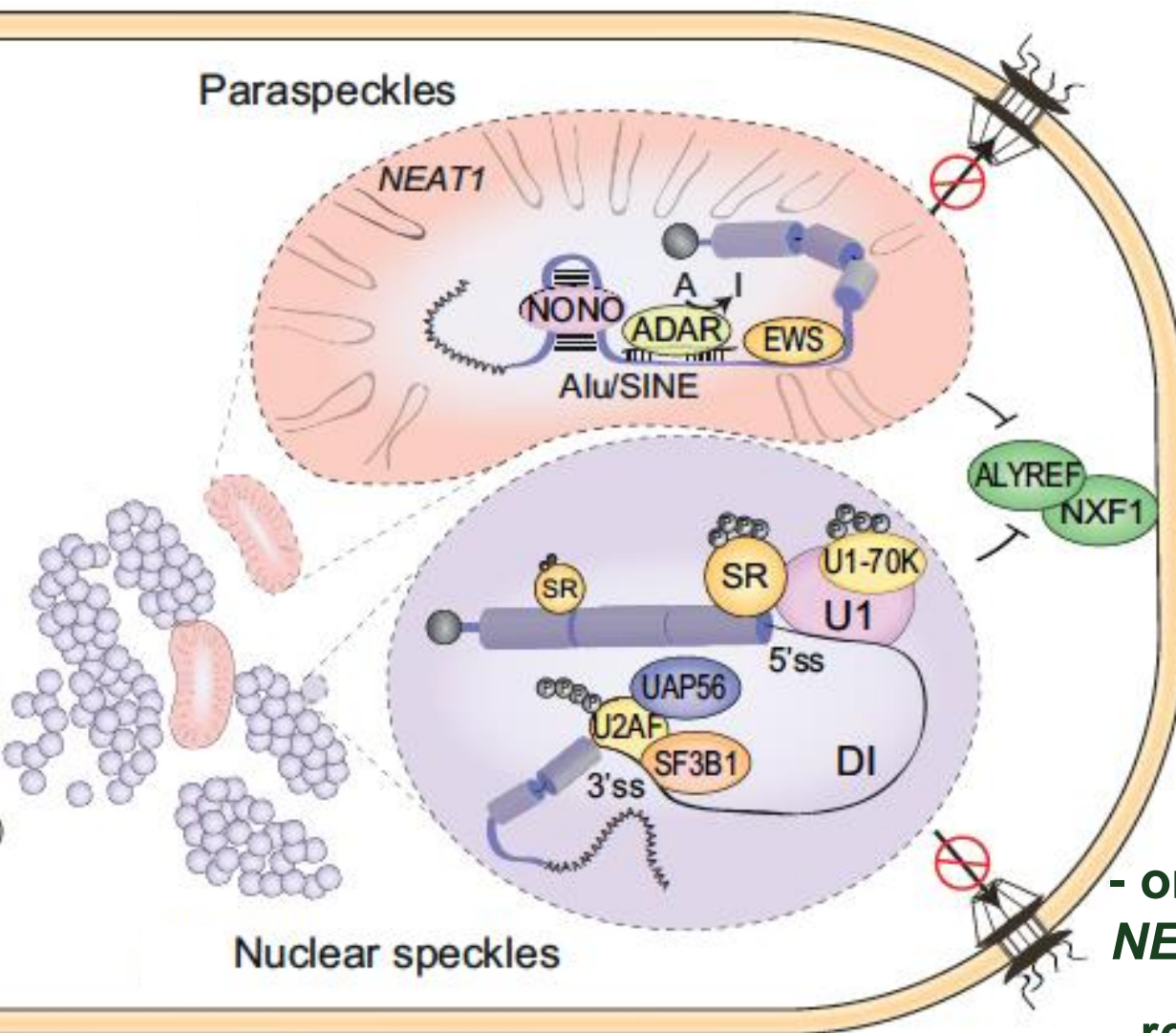


Organize several cellular processes:

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

Paraspeckles

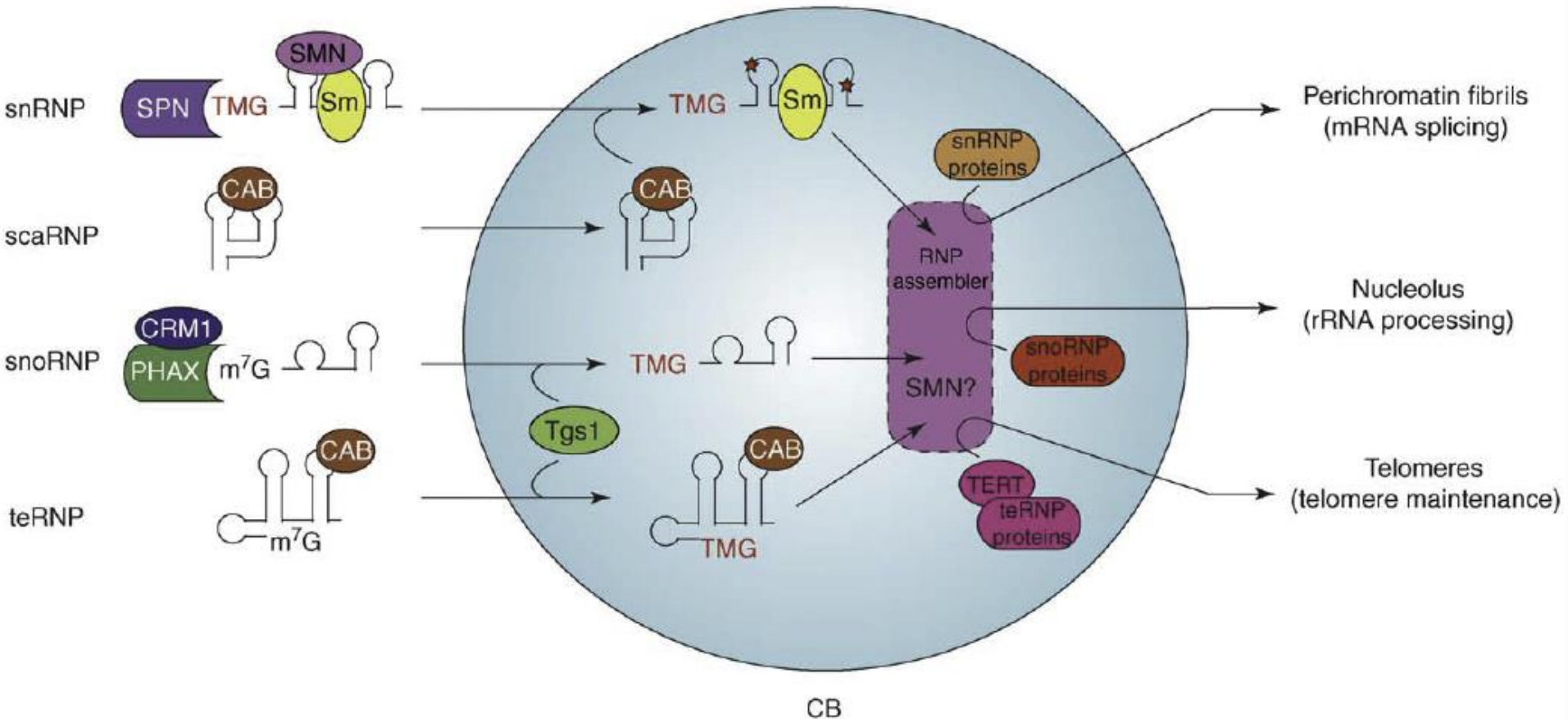
Nuclear speckles



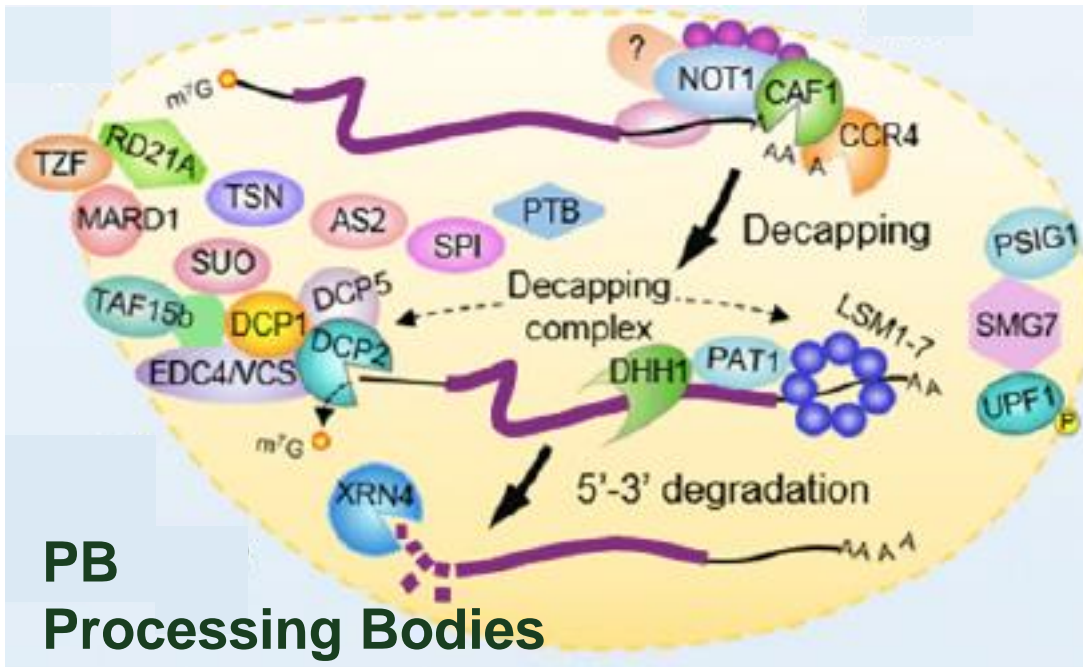
- organized around lncRNAs:
NEAT1 (PS) or *MALAT1* (NS)

- regulate gene expression
by mRNA nuclear retention

Cajal bodies



Cytoplasmic P-bodies and Stress Granules



PB Processing Bodies

mRNA storage
mRNA decay

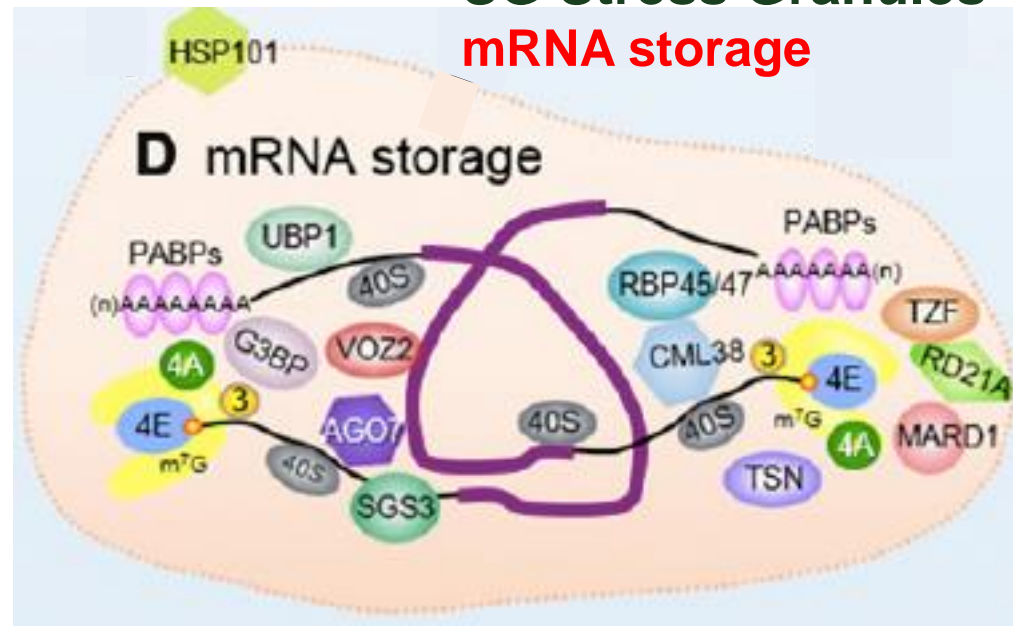
SG: global translation halts upon stress, mRNAs bound to the translational machinery and other proteins form SGs.

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

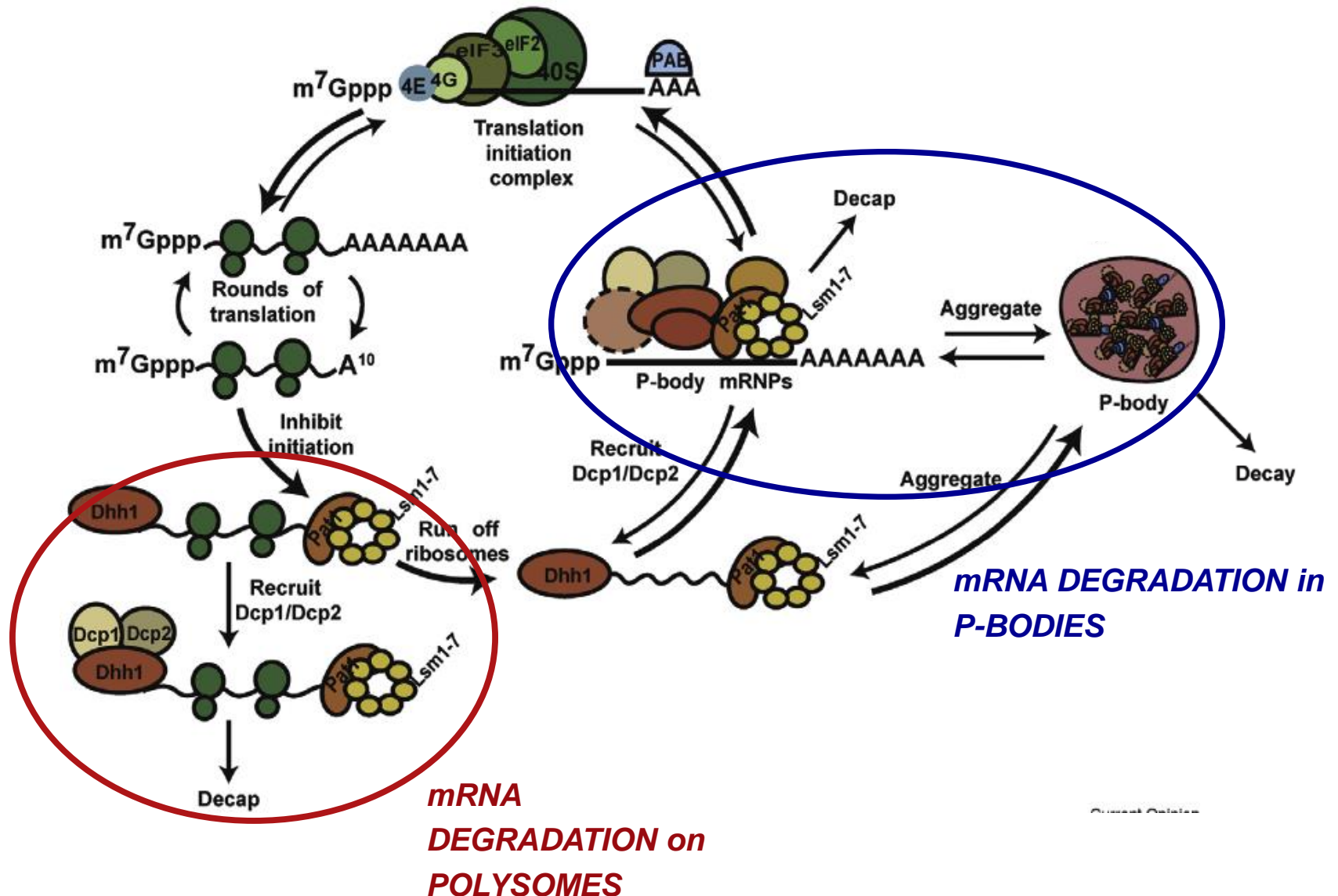
Dynamic biomolecular condensates
Form by phase separation of RNAs and proteins
Role in translational control and proteome buffering upon translational arrest (PB) and stress (SG)

SG Stress Granules

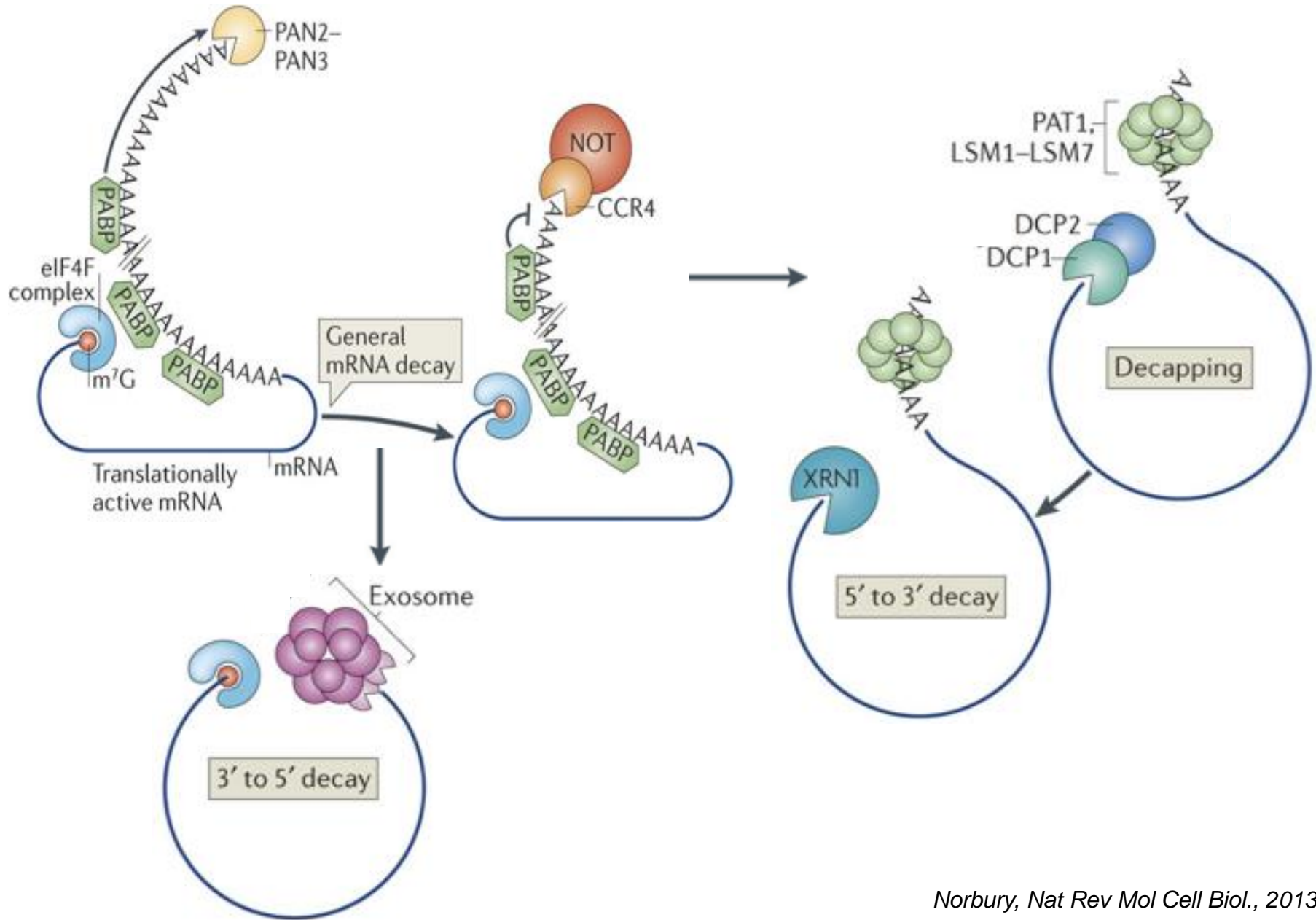
mRNA storage



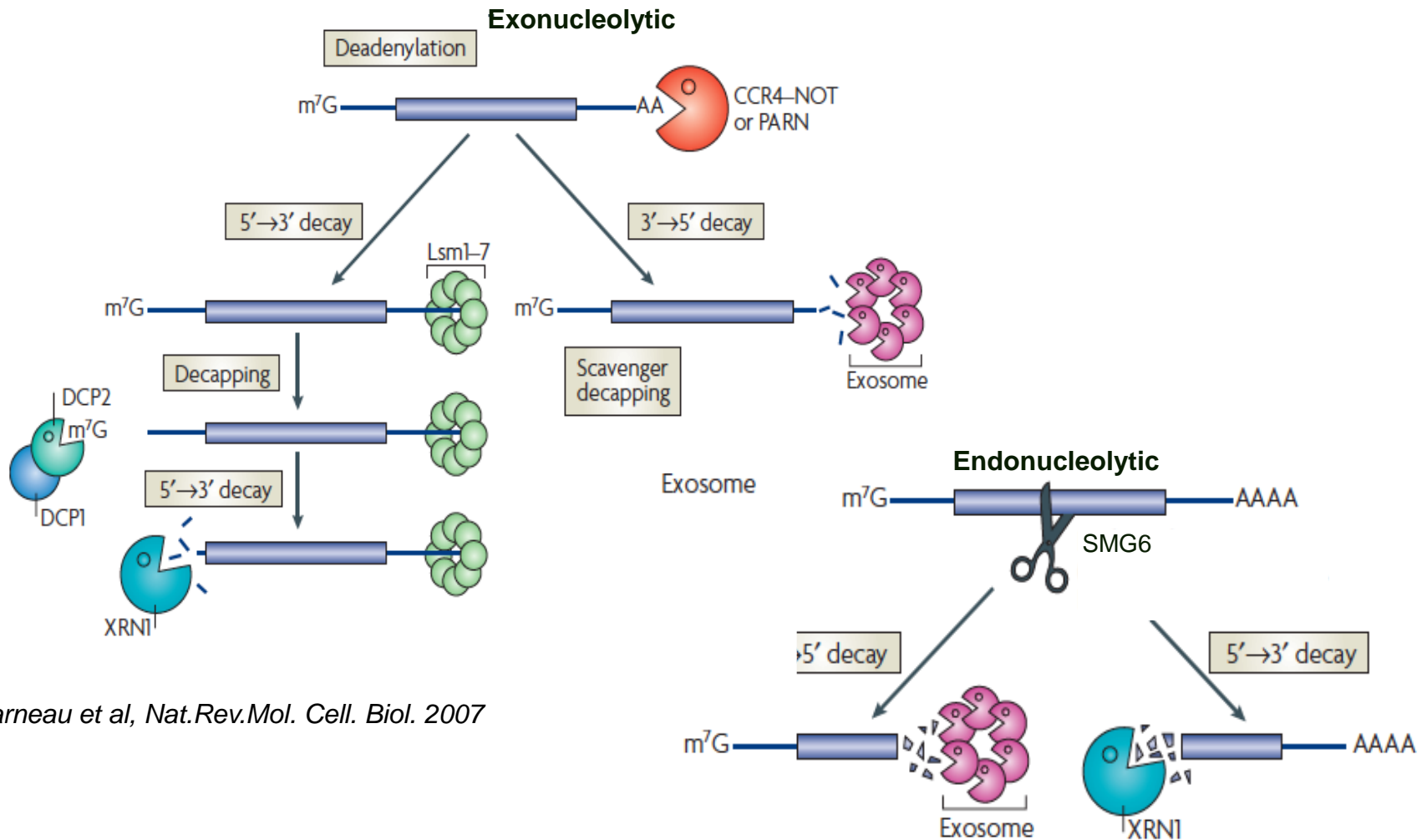
mRNA DEGRADATION in the CYTOPLASM



mRNA general decay in the cytoplasm



mRNA general decay in the cytoplasm

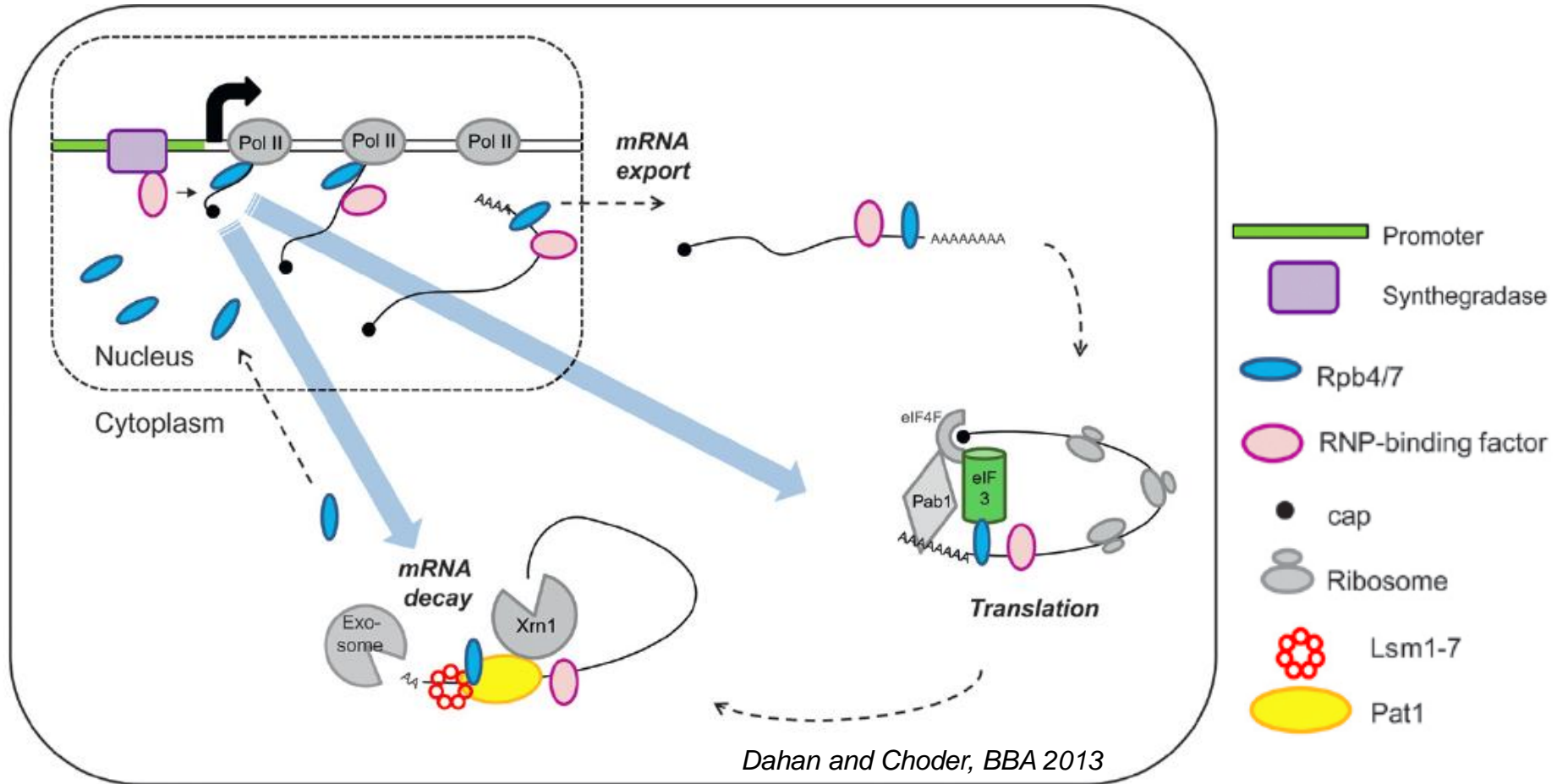


Garneau et al, Nat.Rev.Mol. Cell. Biol. 2007

RNA is also degraded in the nucleus:

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

Coupling between transcription and mRNA decay



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 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

(A) Improper termination



UPF1
(UPF2/3
EJC)

NMD

SMG6 (Endonuclease)
Exosome, Xrn1

UPFs facilitate
degradation of
truncated (unfolded)
products

(B) A lack of termination



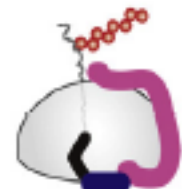
Dom34/Pelota
Hbs1/hHsb1

NSD

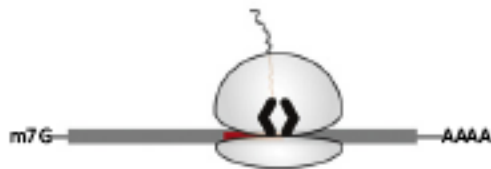
Exosome



RQC



(C) Ribosome stall



Dom34/Hbs1?
(Rack1, Hel2?)

NGD

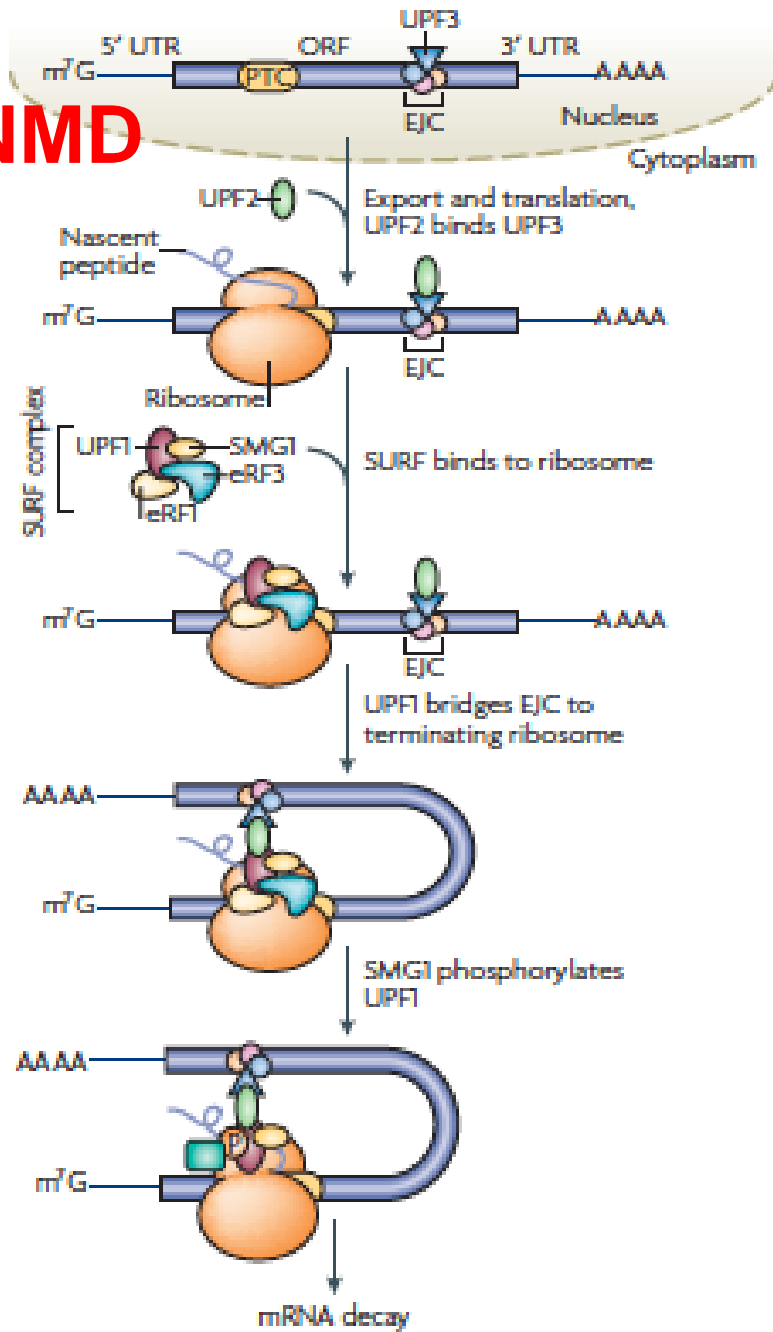
Endonucleolytic
cleavage



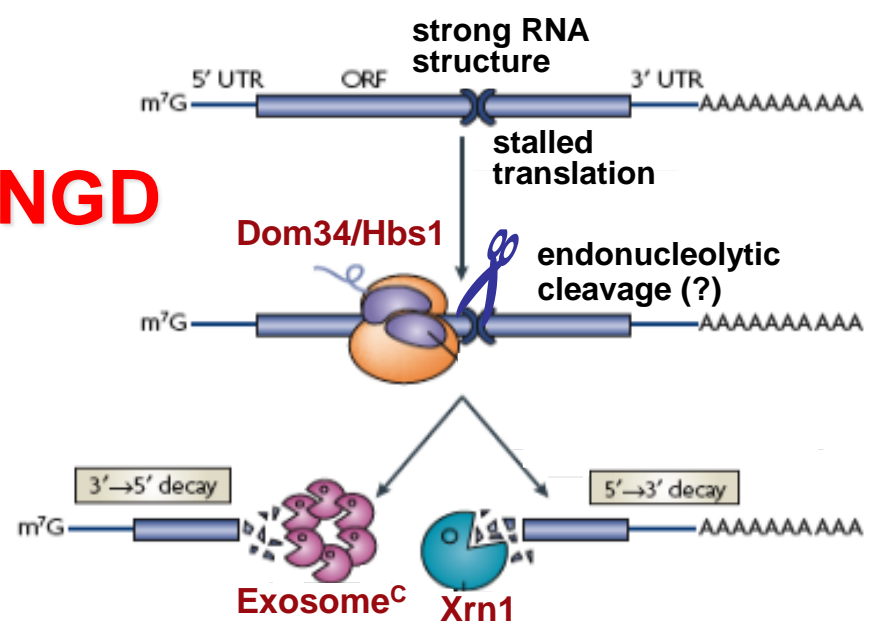
RQC



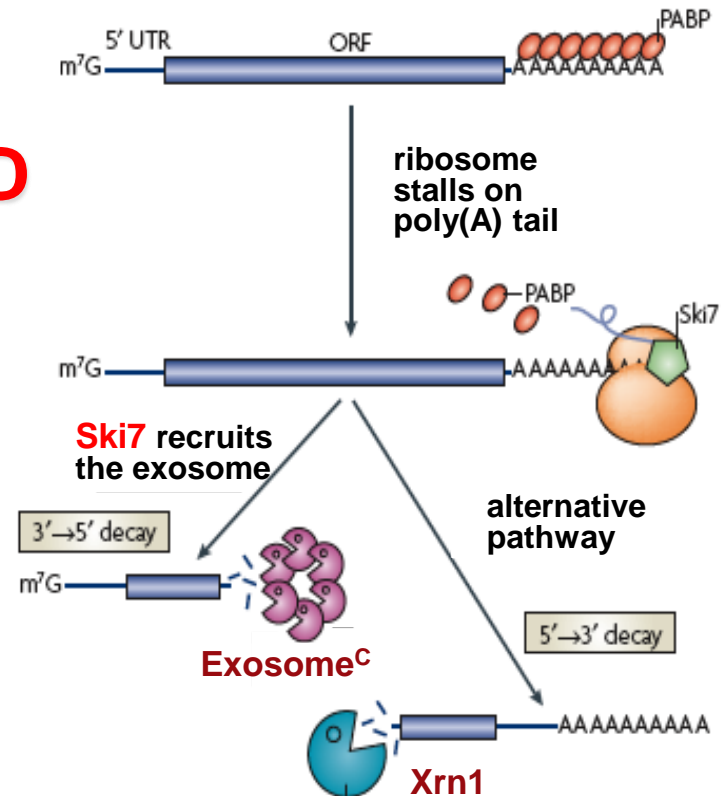
NMD



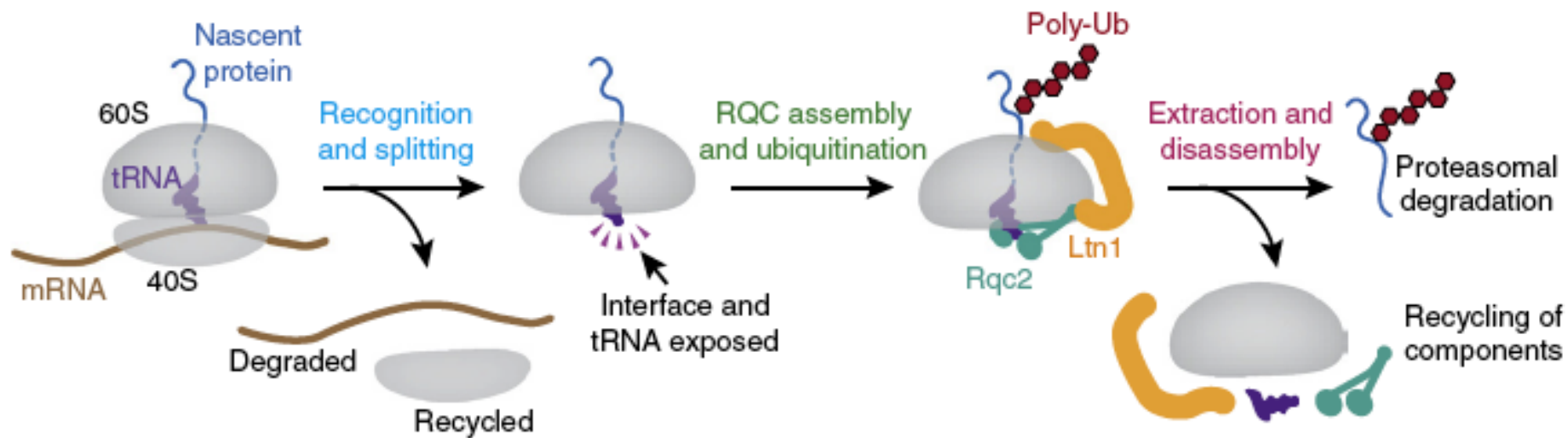
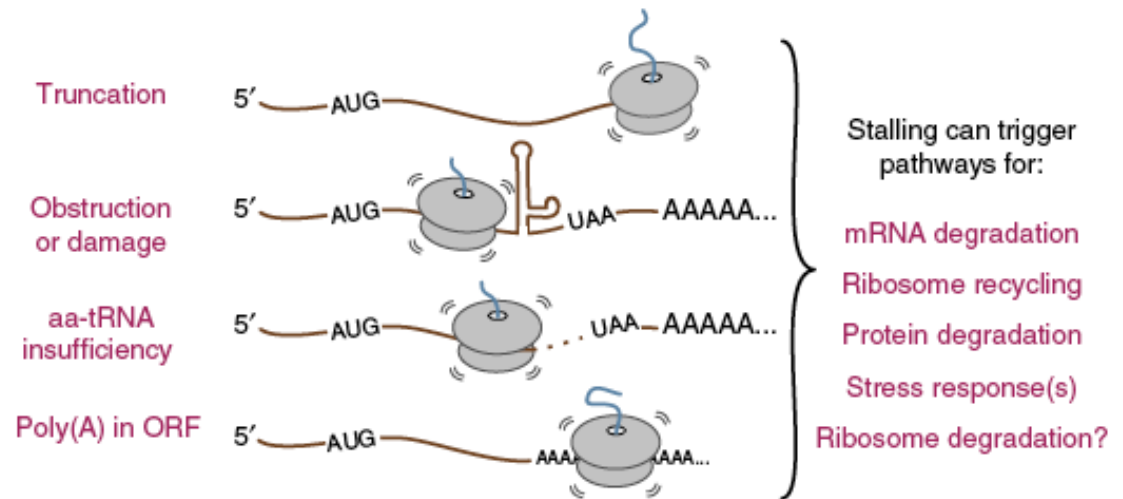
NGD



NSD



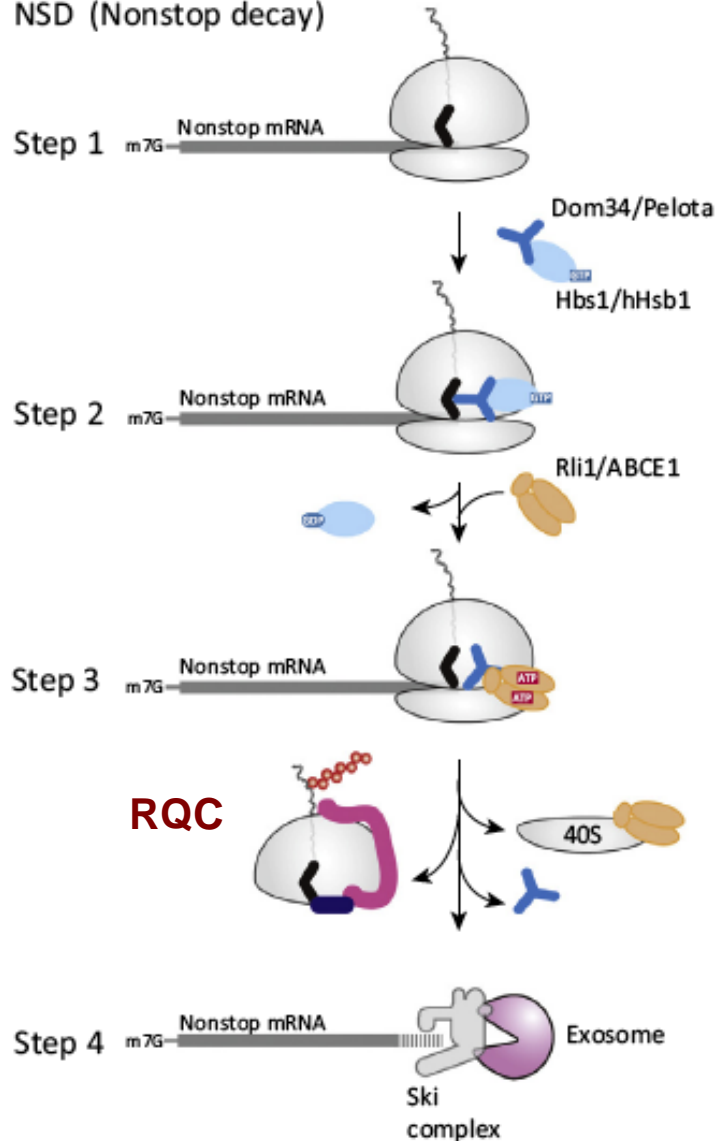
RIBOSOME QC (RQC)



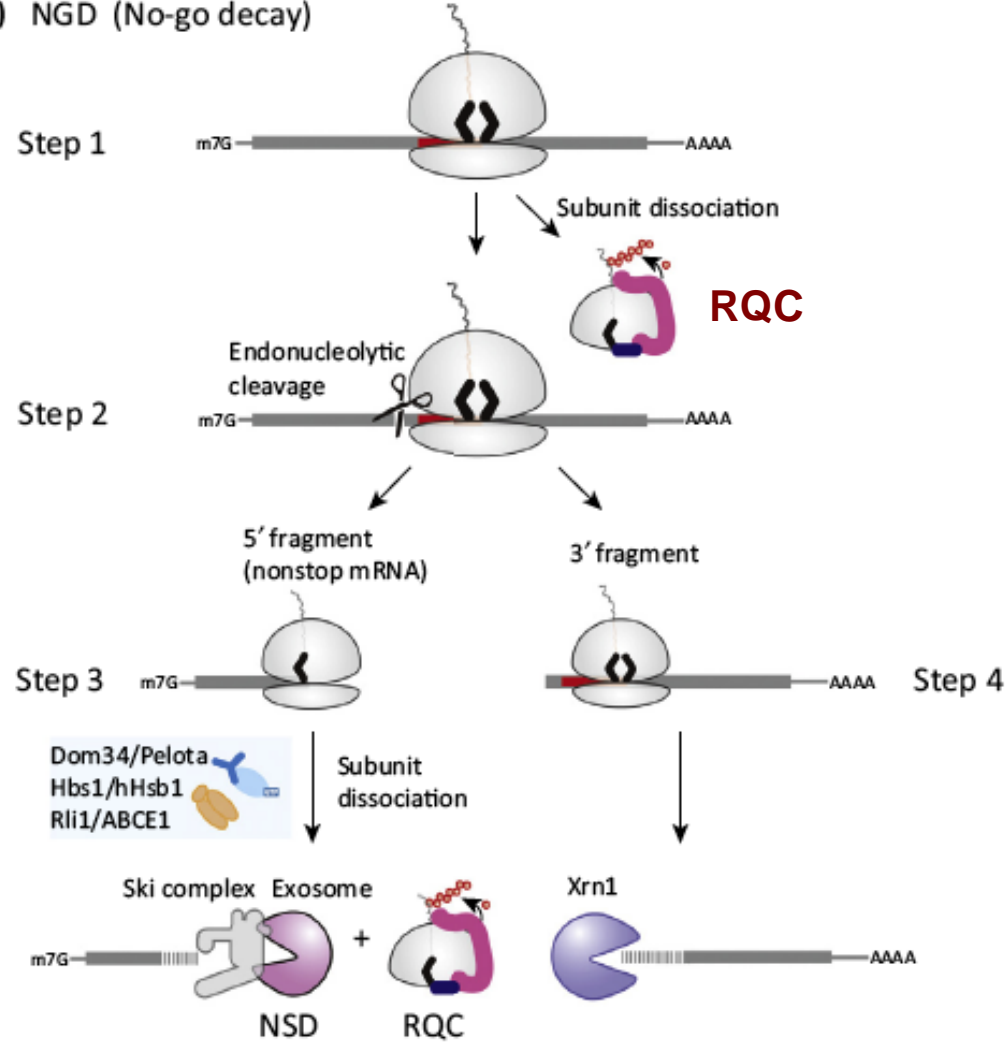
Yeast	Asc1	Hel2	Dom34	Hbs1	Rli1	Rqc2	Ltn1	Rqc1	Cdc48-Ufd1-Npl4
Mammals	RACK1	ZNF598?	Pelota	Hbs1	ABCE1	NEMF	Listerin	TCF25?	VCP complex?
Facilitates stalling?			Ribosome splitting			Nascent-chain ubiquitination		Nascent-chain extraction	

RQC in NSD and NGD

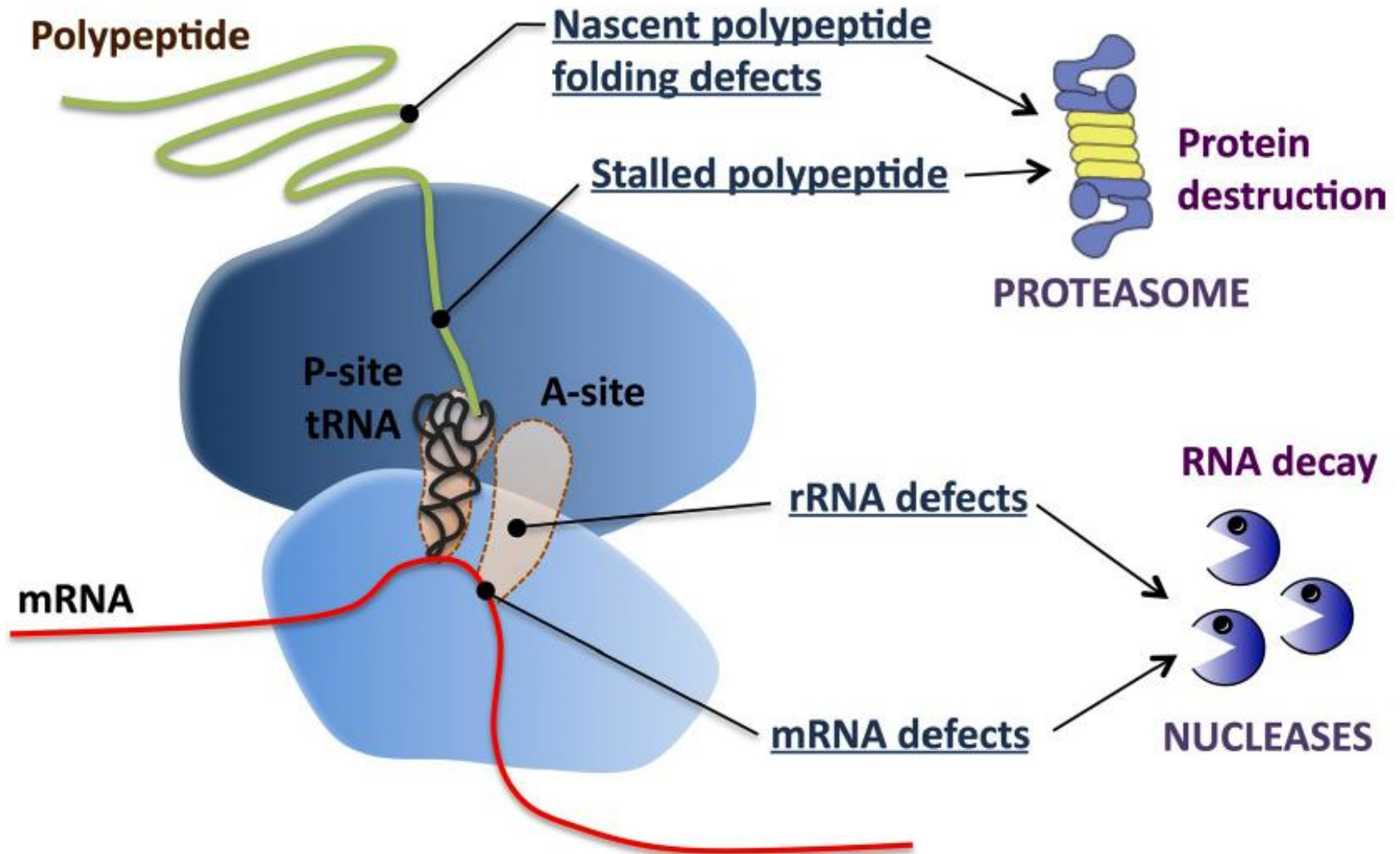
(A) NSD (Nonstop decay)



(B) NGD (No-go decay)

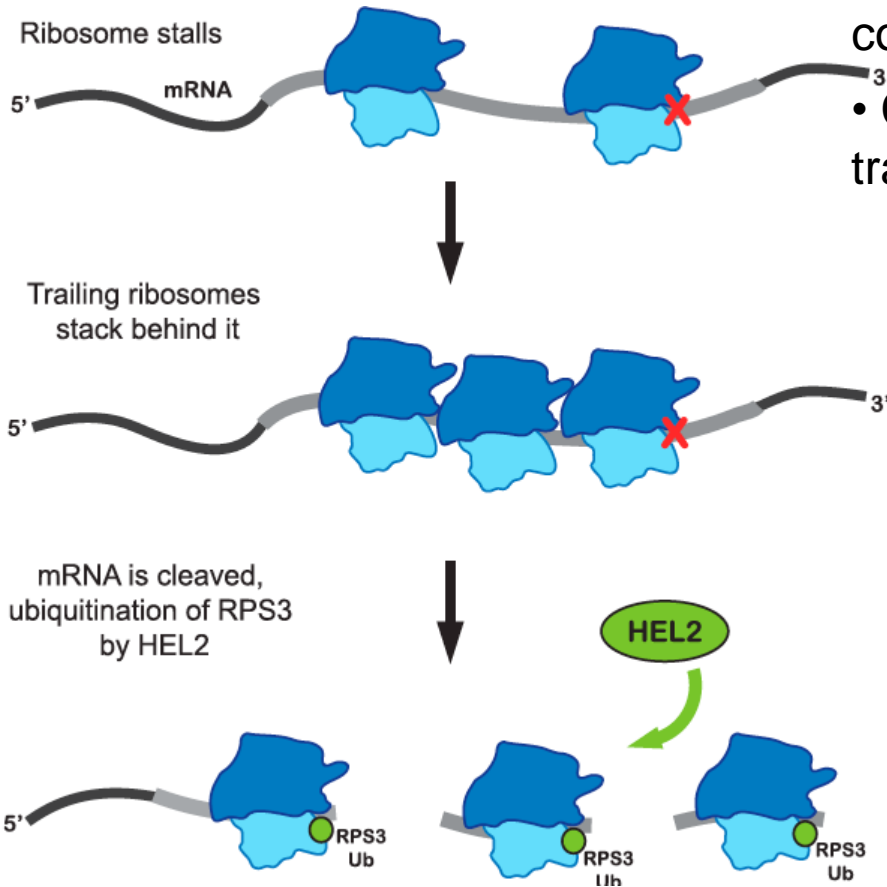


Co-translational protein and mRNA QC



Ribosome collision in RQC during NGD

- Stacked or colliding ribosomes are required to elicit NGD
- Ubiquitination of RPS3 by HEL2/ZNF598 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

