RNA Quality Control Pathways

PART I - GENERAL MECHANISMS

PART II - SPECIFIC PATHWAYS

RNases

Endonucleases

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

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

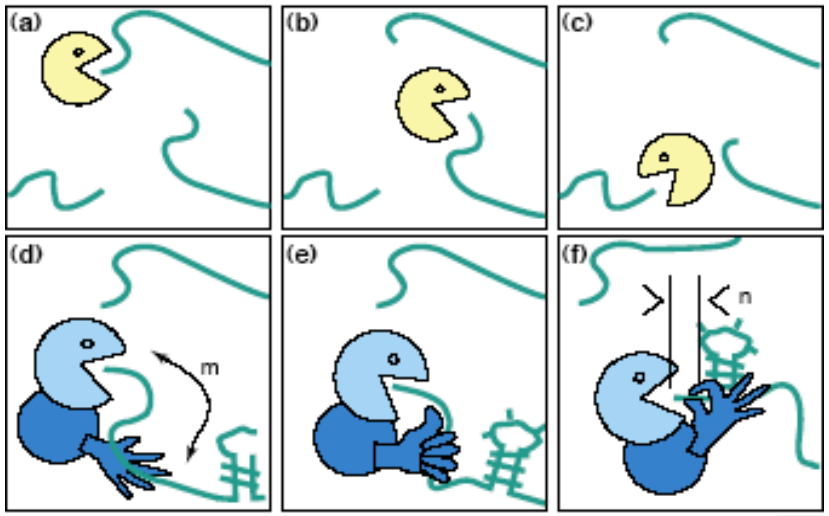
degrading (RNase I, RNAse A):

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

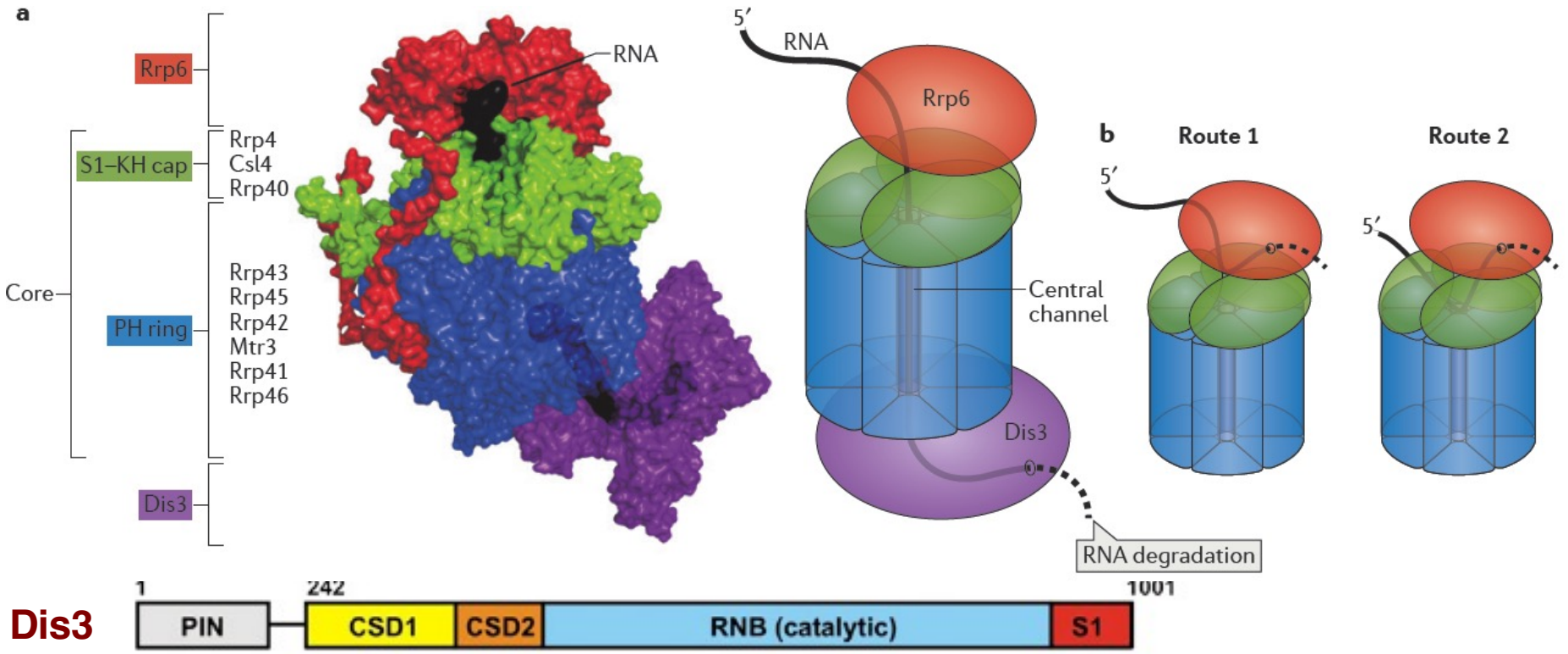
RNA processing and decay machinery: RNases

Protein	Function	Characteristics
Exonucleases 5'→3'		
Xrn1	cytoplasmic, mRNA degradation	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, also necessary for interaction of Dis3 with the core
- 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

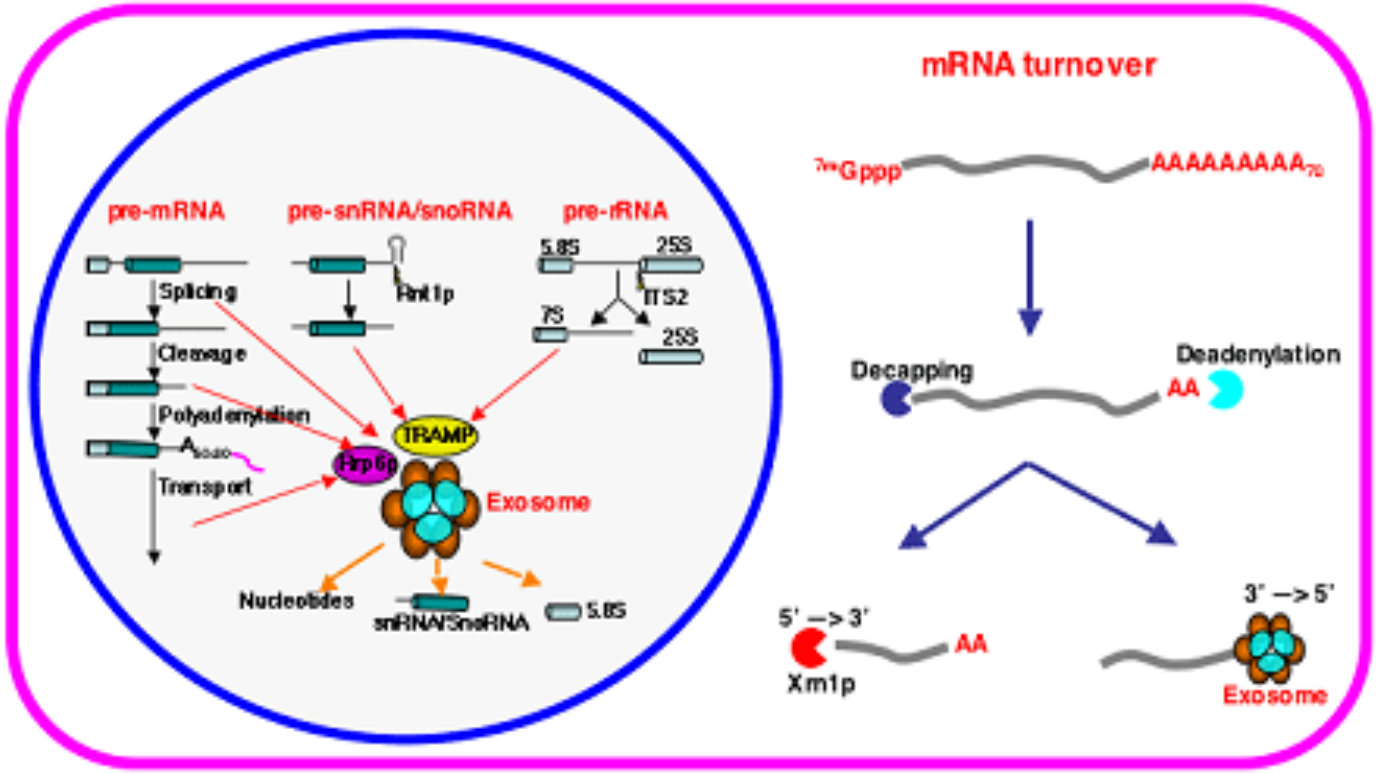
EXOSOME: 3’→5’ decay machinery: functions

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

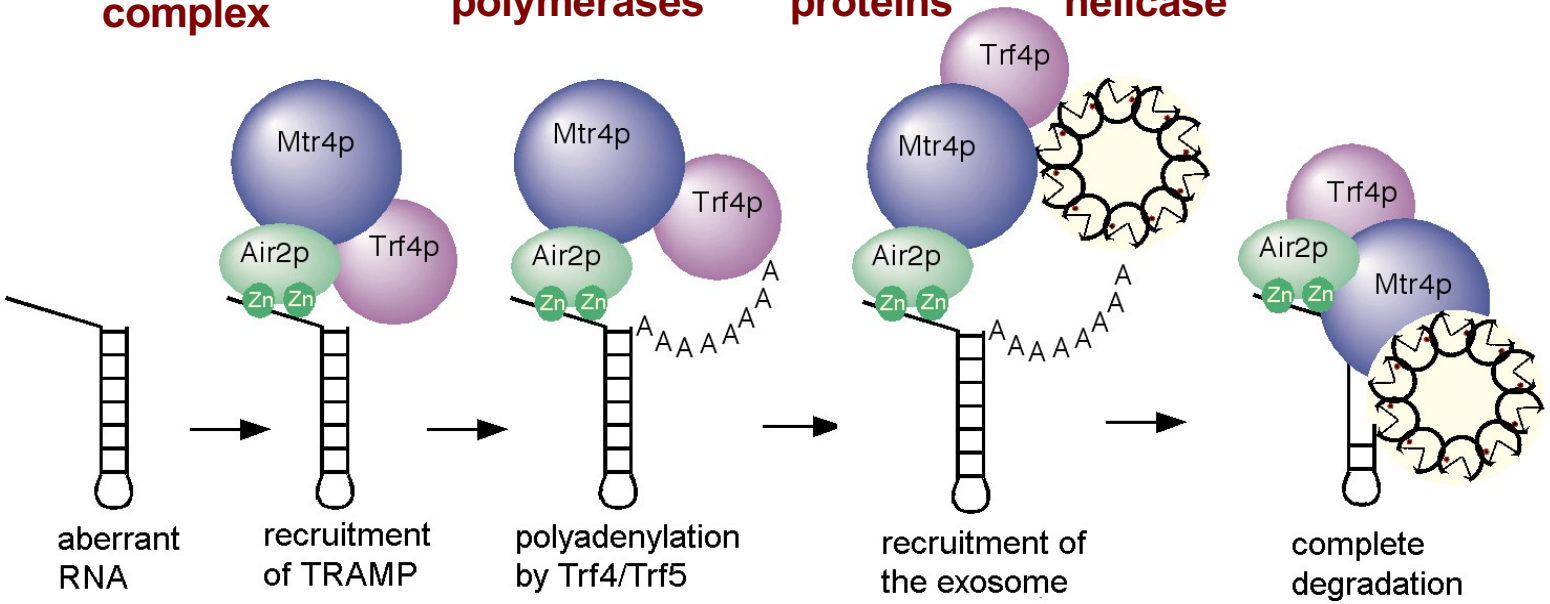


TRAMP – exosome cofactor

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 RNAs

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

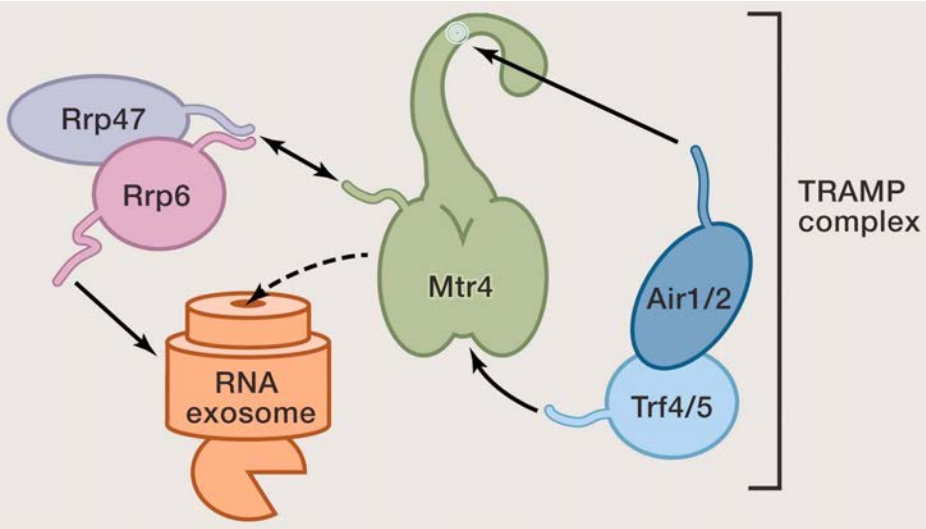
TRAMP interacts with

- 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



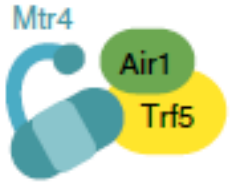
Mtr4 – DEAH box RNA helicase
Air1/2 – RNA binding proteins
Trf4/5 – poly(A) polymerases

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

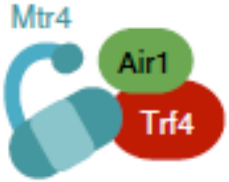
SUBSTRATES

TRAMP 4-2: mRNA, ncRNA

TRAMP 5-1: pre-rRNA



TRAMP 4-1: mRNA, introns

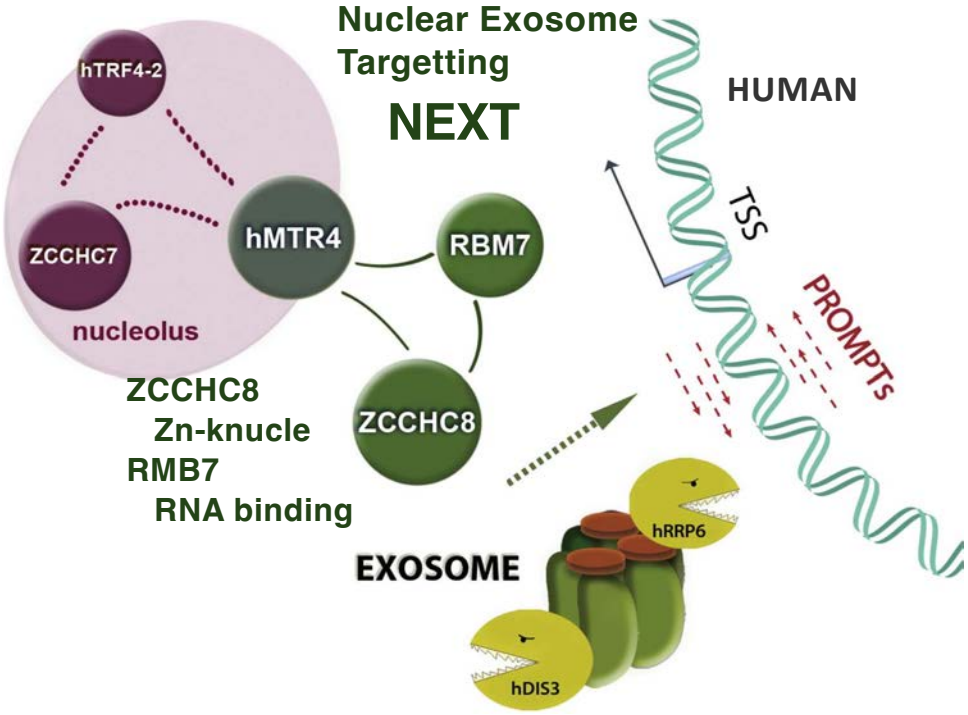


TRAMP

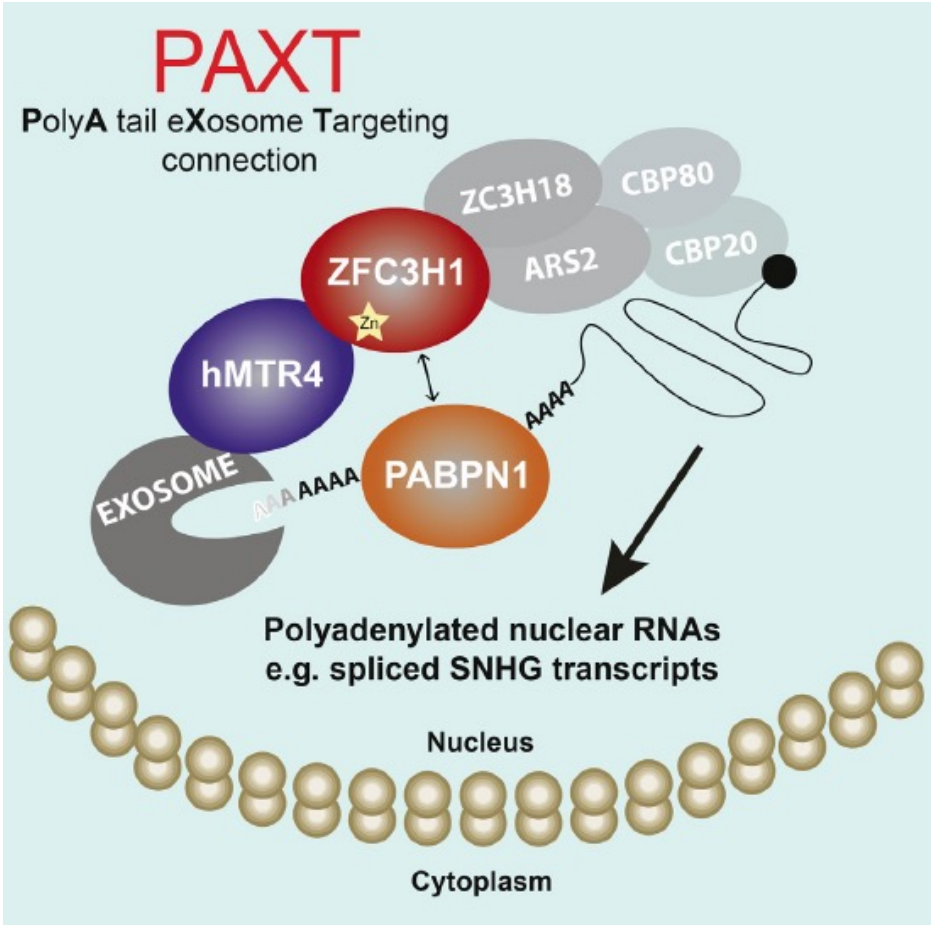
- **interacts with the exosome via Mtr4 - role in degradation**
- **role in sn/snoRNA 3' end processing together with the exosome**
- **interacts with Nrd1/Nab3 complex - role in ncRNA Pol II termination**
- **role in transcription silencing in *S. cerevisiae* and *S. pombe* (Cid14)**

NEXT and PAXT - exosome cofactors

mammals



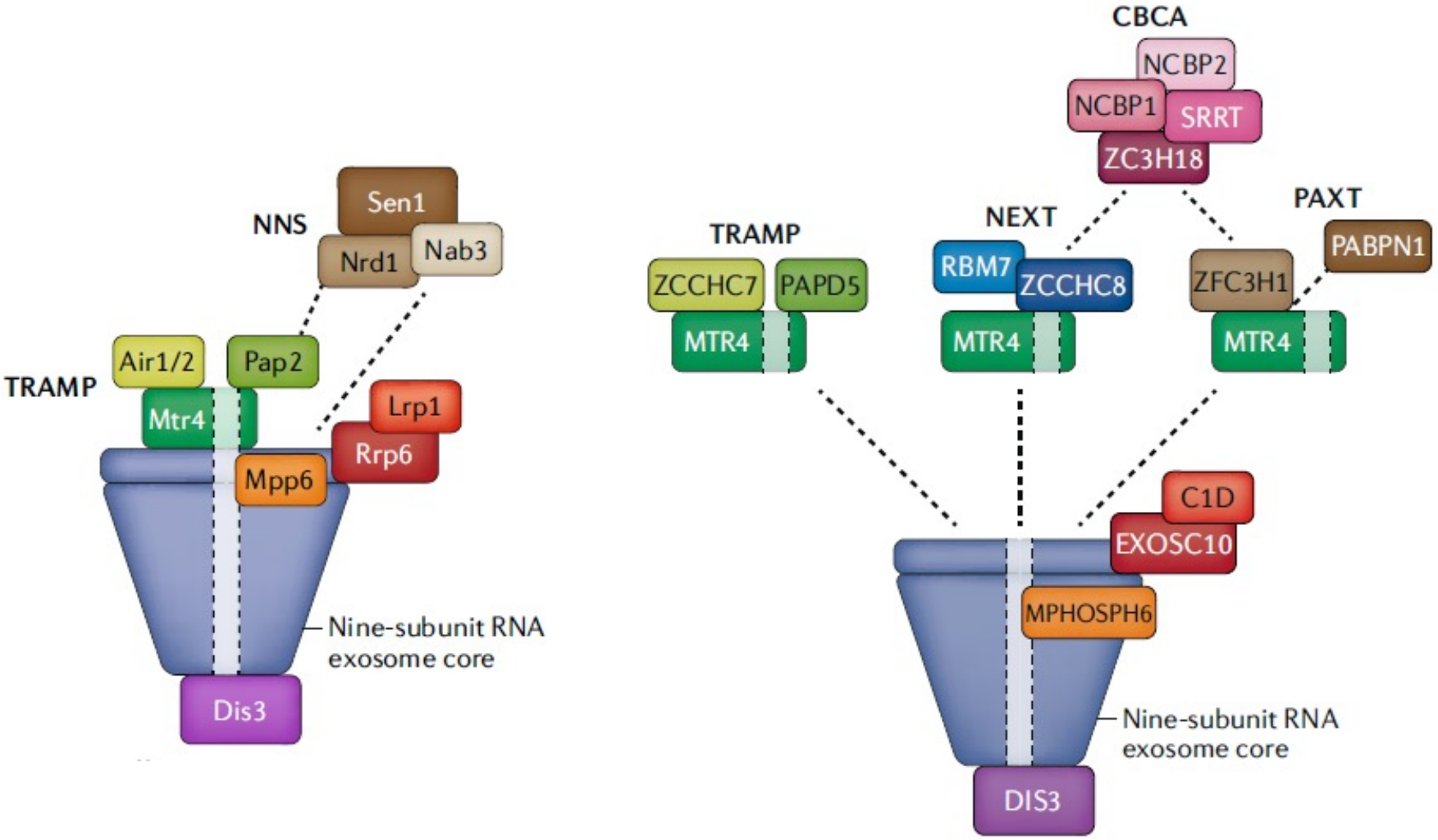
MTR4- associated complexes



- **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**

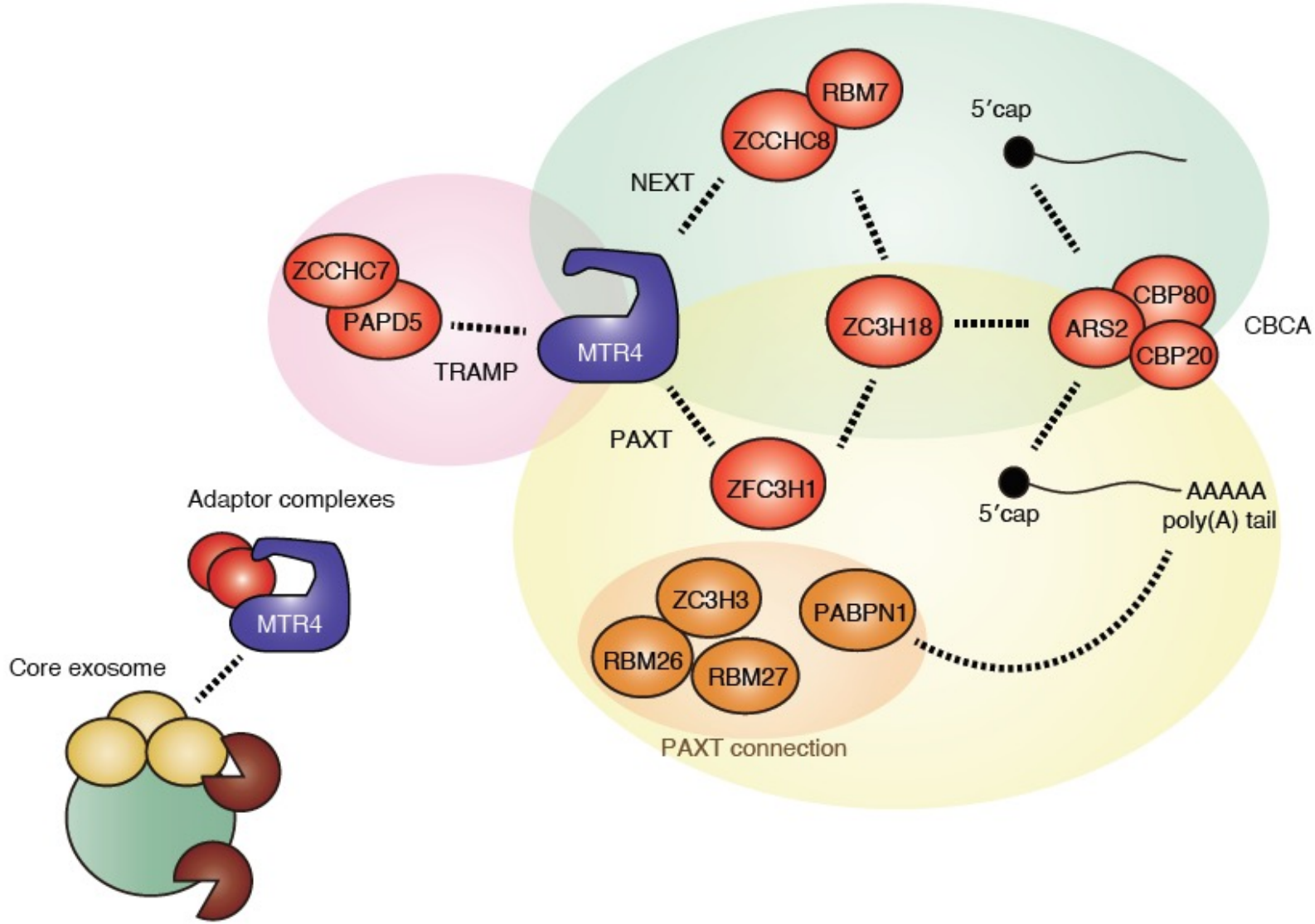
Lubas et al. *Mol. Cell*, 2011; Meola et al., . *Mol. Cell*, 2016

EXOSOME with TRAMP, NEXT and PAXT



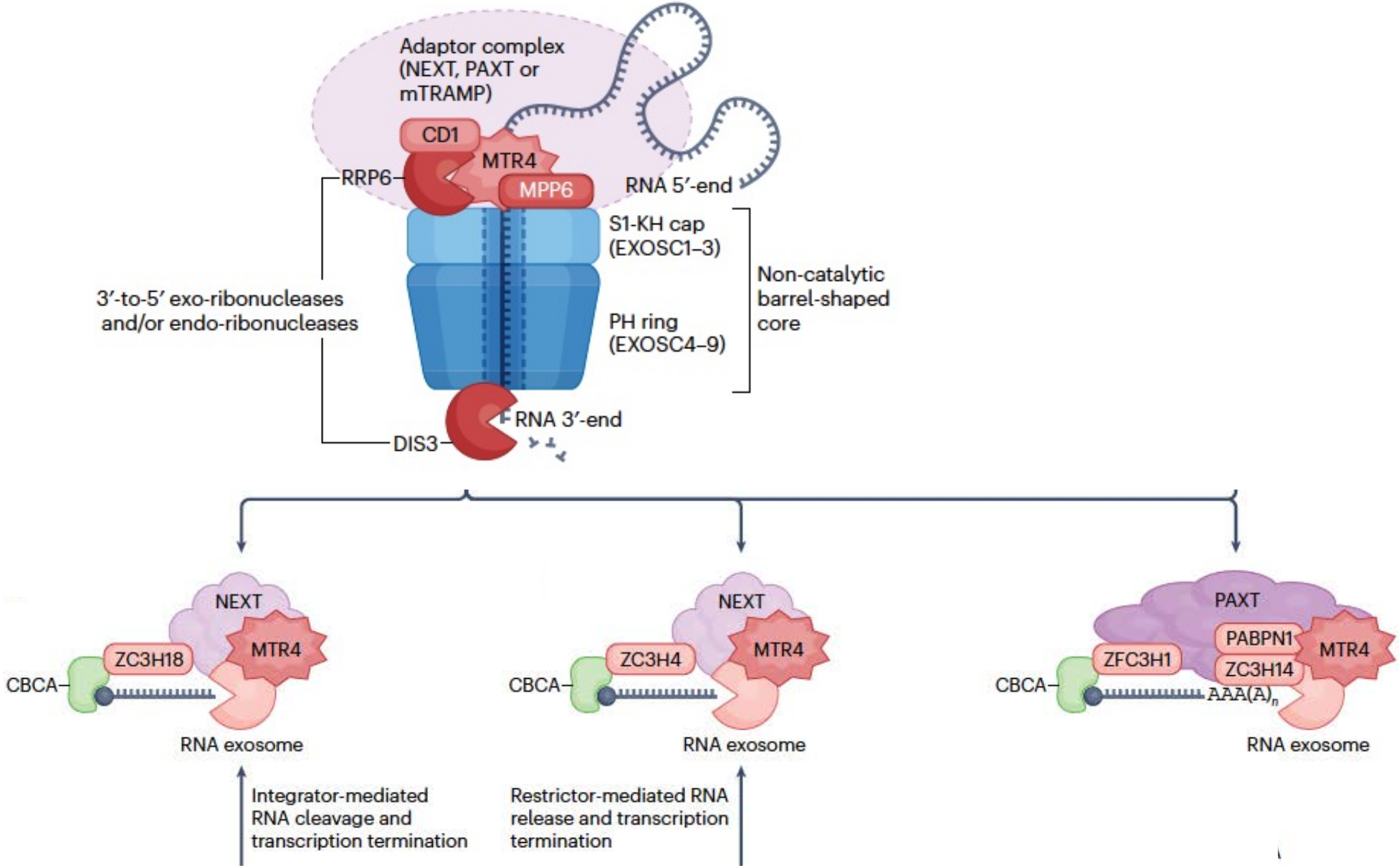
Schmid and Jensen., Nat. Rev. Mol. Cel. Biol. , 2018

EXOSOME with TRAMP, NEXT and PAXT



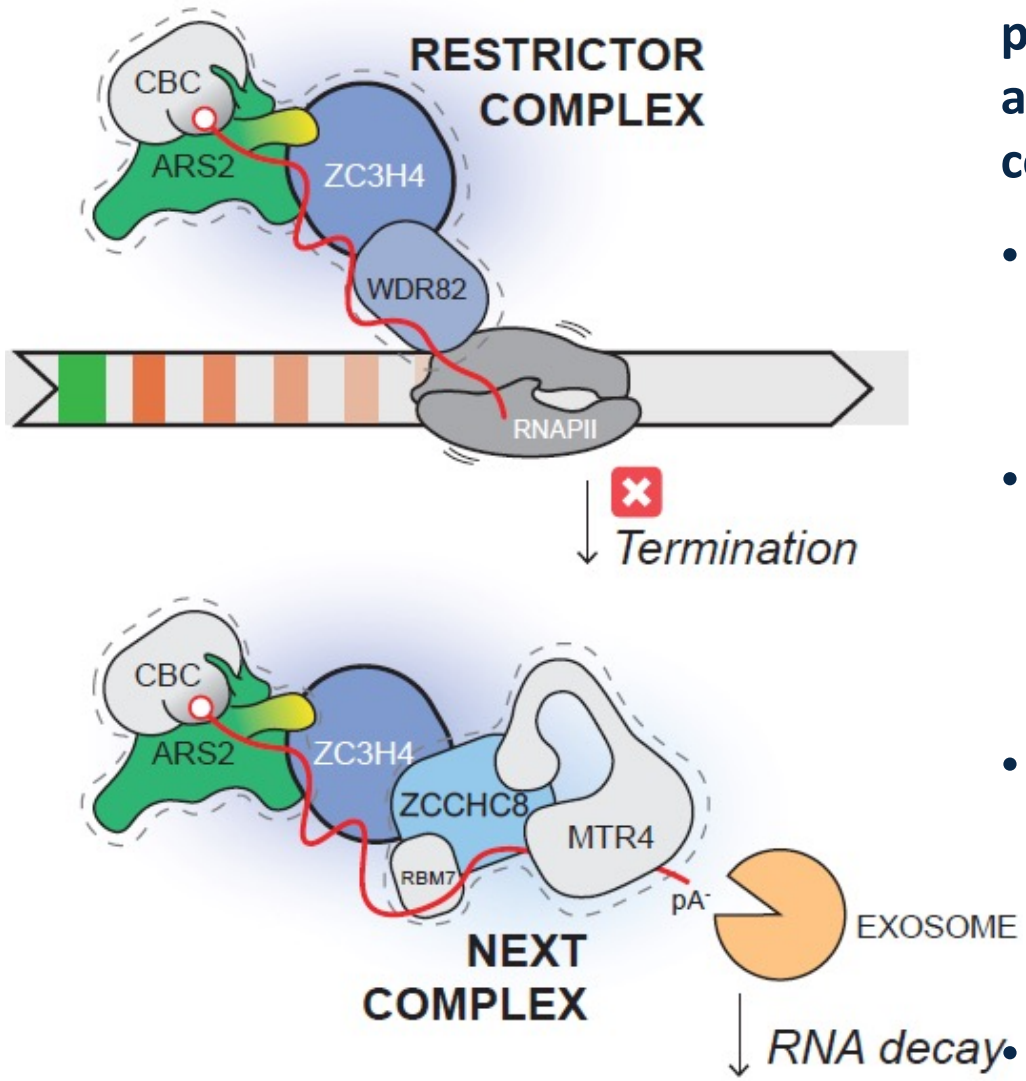
Ogami and Suzuki., Int J Mol Sci, 2021

EXOSOME with TRAMP, NEXT and PAXT



Rambout et al, Nat Rev Genet, 2024

EXOSOME with Restrictor



Rouviere et al, Mol Cell, 2023; Estell et al, Mol Cell, 2023

Restrictor:

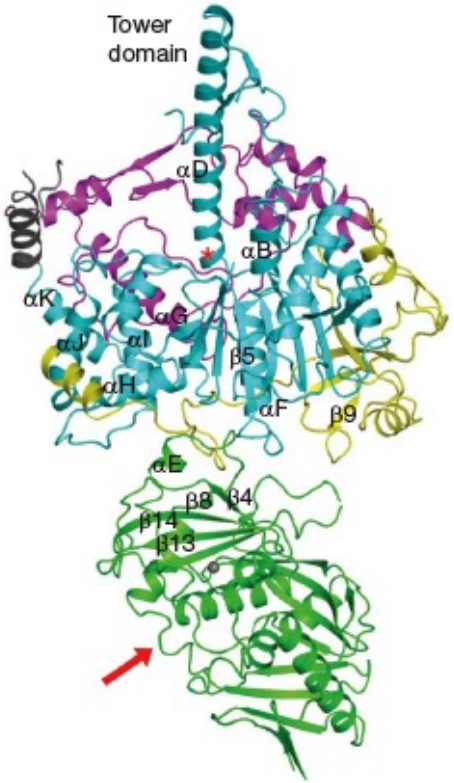
ZC3H4 Zn-finger protein

WDR82 component of PNUTS (protein phosphatase 1 nuclear-targeting subunit) and SET1 histone methyltransferase complexes

- **Transcription termination activity of ARS2 is independent of CPA and Integrator pathways**
- **ARS2 recruits ZC3H4/Restrictor to chromatin at a number of loci and, together with PNUTS, terminate common ncRNAs**
- **ARS2-ZC3H4 commits terminated non-coding transcripts for degradation by the nuclear exosome via a direct recruitment of the NEXT complex**
- **At protein coding genes the action of Restrictor/PNUTS is counteracted by U1 snRNP**

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

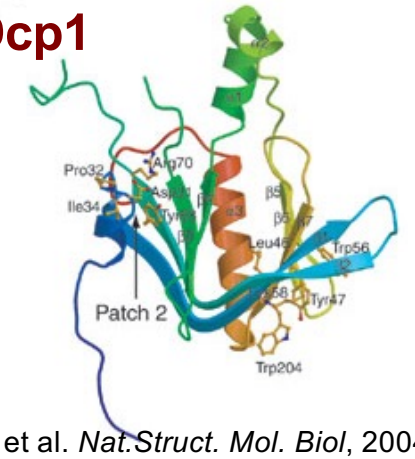
- **generic mRNA decay**
- **specialised mRNA decay pathways: NMD, NSD, NO-GO decay, ARE-dependent decay**
- **degradation of miRNA-dependent mRNA cleavage products *(in plants)***
- **degradation of some ncRNAs: CUTs, SUTs, XUTs**

XRN1 and Rat1/XRN2 have deNADding and deFADding activity

Sharma et al, Nat Comm 2022; NAR 2922

DCP/NUDT- decapping enzymes

Dcp1

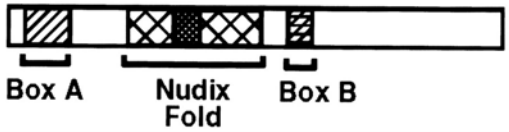


She et al. *Nat.Struct. Mol. Biol*, 2004

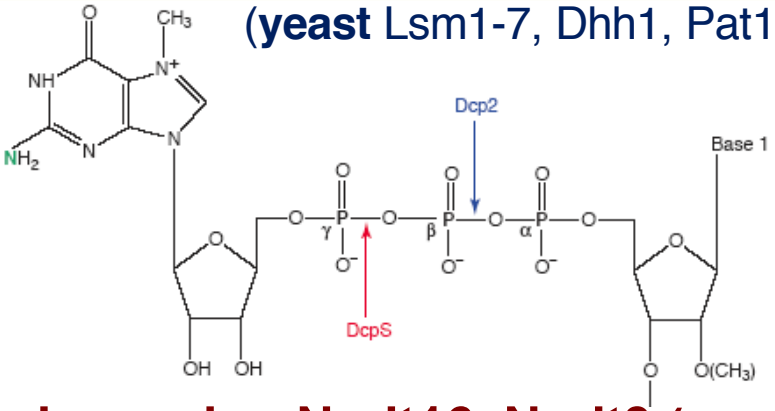
- **Dcp1/Dcp2 complex participates in mRNA 5' decay**
- **catalyses the reaction m^7GpppX-mRNA -> m^7GDP + 5'p-mRNA**
- **Dcp2 is the catalytic subunit (pyrophosphatase Nudix domain)**
- **Dcp1 is required for activity *in vivo*, interacts with other proteins**
- **Dcp1/Dcp2p is regulated by Pab1 and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

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

Dcp2

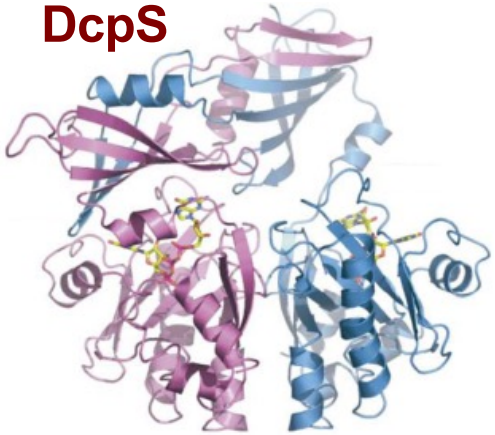


Wang et al. *PNAS*, 2002



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

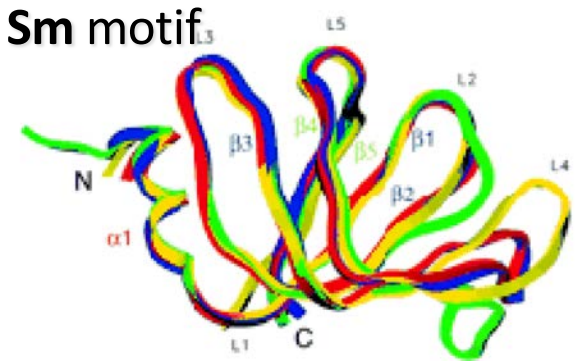
DcpS

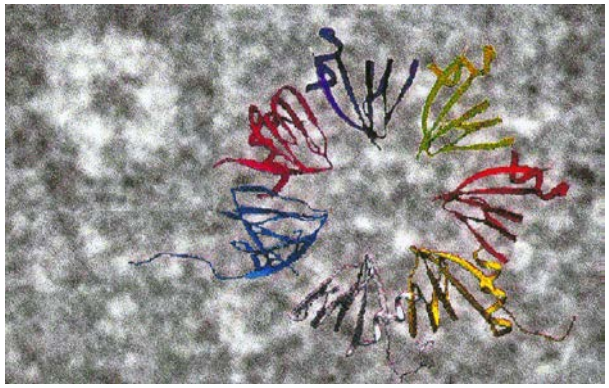


- **DcpS: HIT pyrophosphatase („histidine triad" on the C-terminus)**
- **catalyses the cleavage of m^7GDP -> m^7GMP + Pi remaining after decapping during mRNA 5' decay**
- **cooperates with the exosome during mRNA 3' decay (m^7GpppX-oligoRNA -> m^7GMP+ pp-oligoRNA)**
- **functions as an asymmetric dimer**

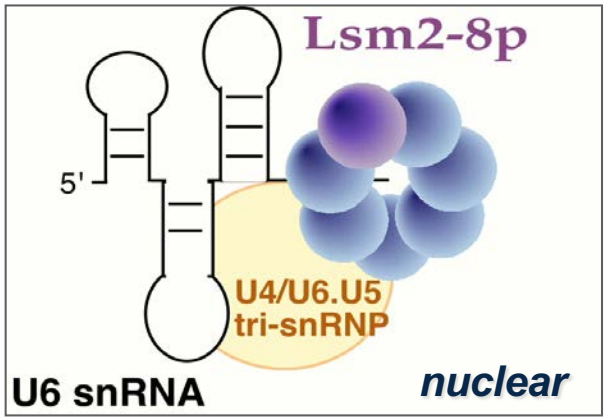
Gu et al., *M.Cell*, 2004

LSM proteins



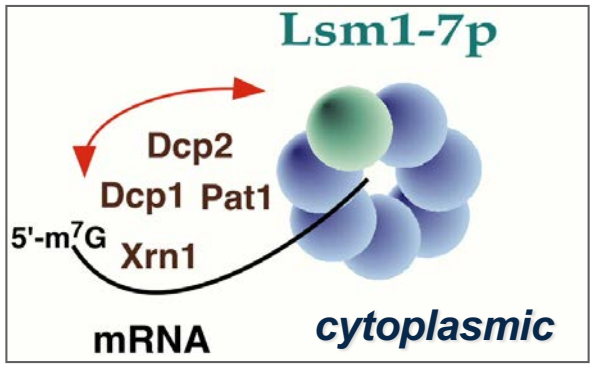


Achsel et al, EMBO J, 2001



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

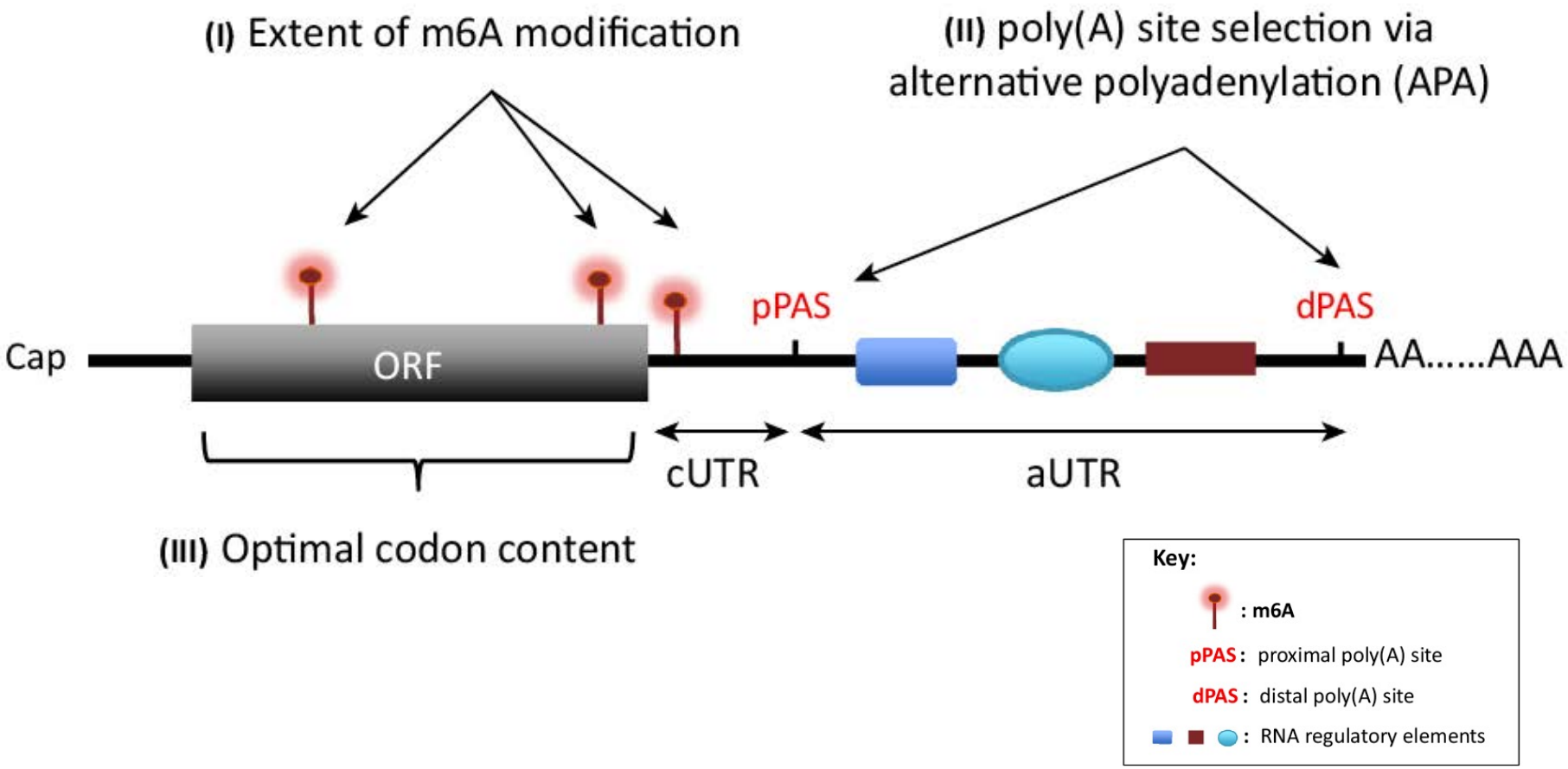


Functions in mRNA decapping and decay

- activator of decapping
- interacts with components of the mRNA decapping and degradation machinery (XRN, DCP, Pat1)

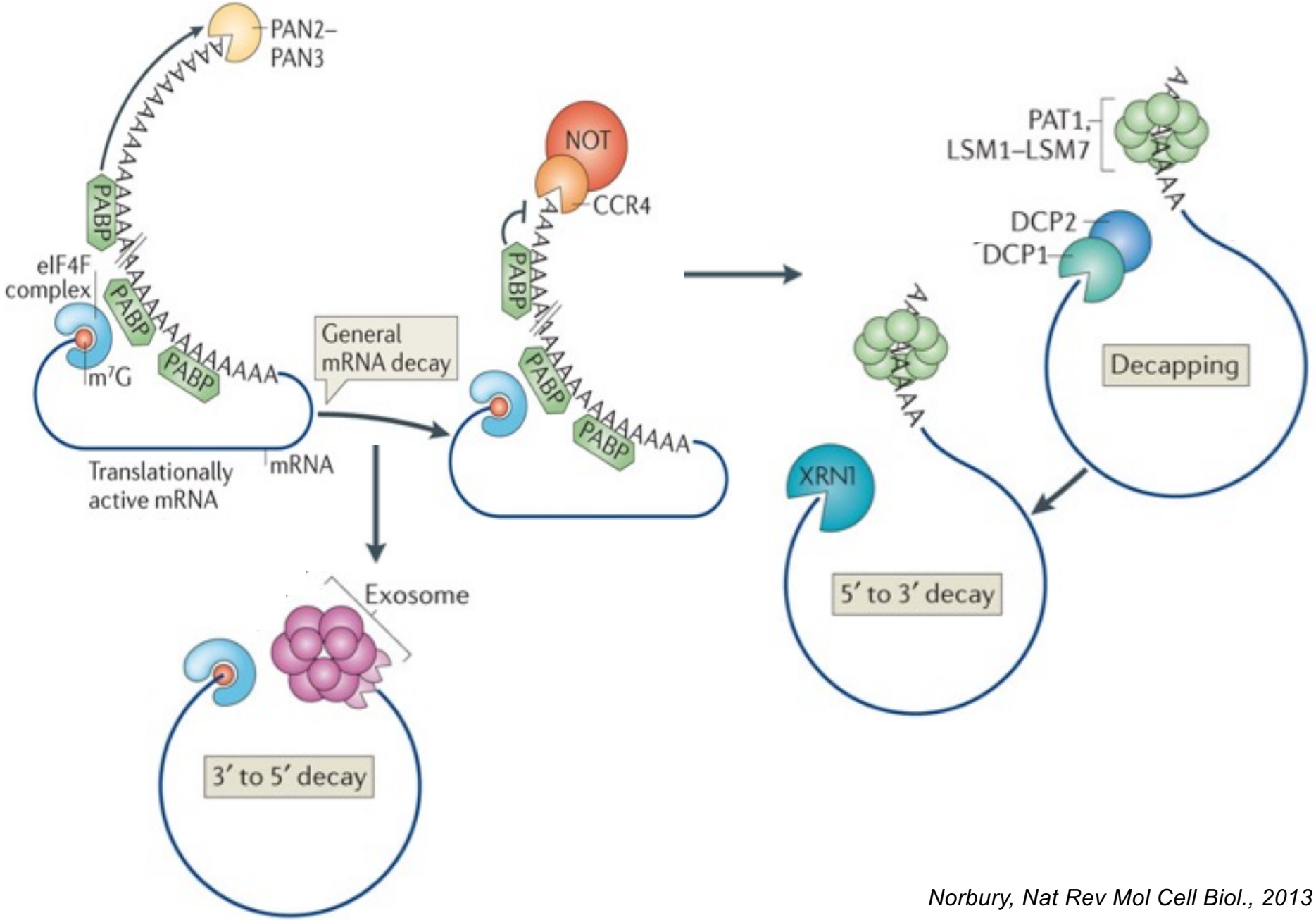
mRNA STABILITY

Elements *in cis*:



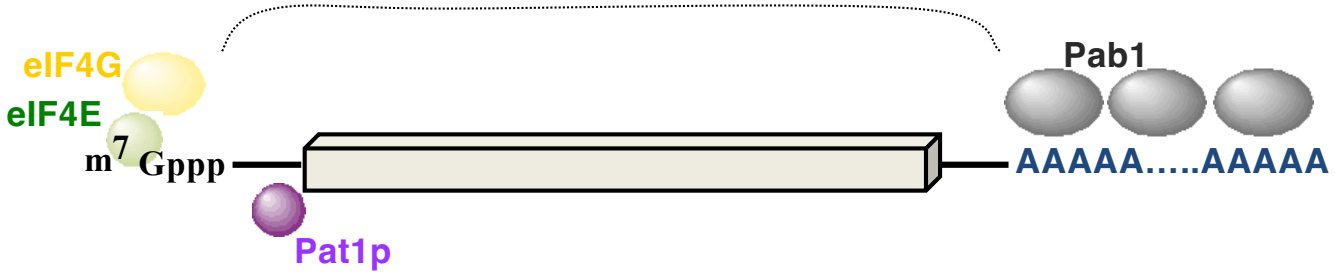
Chen and Shyu, TiBS 2016

mRNA degradation in the cytoplasm

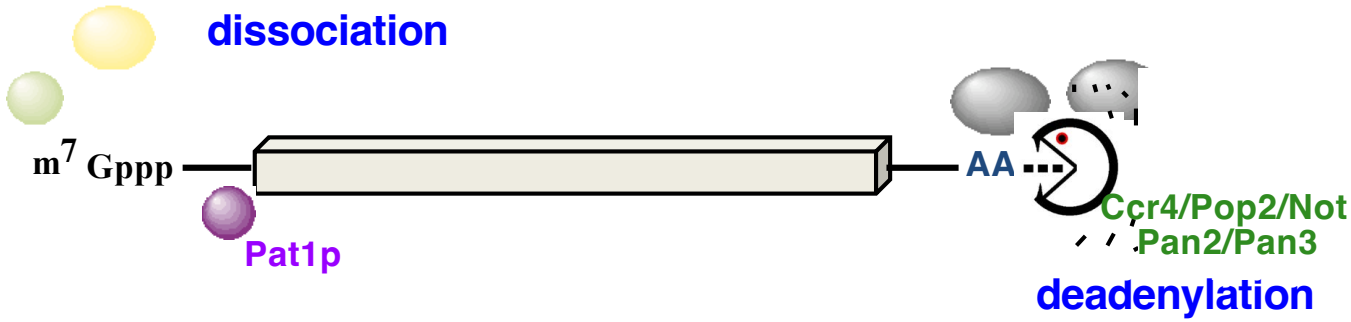


Norbury, Nat Rev Mol Cell Biol., 2013

mRNA degradation in the cytoplasm

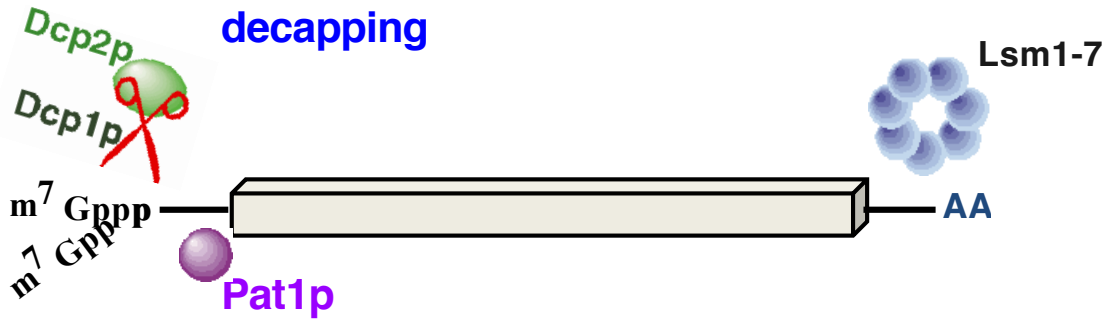


mRNA degradation in the cytoplasm

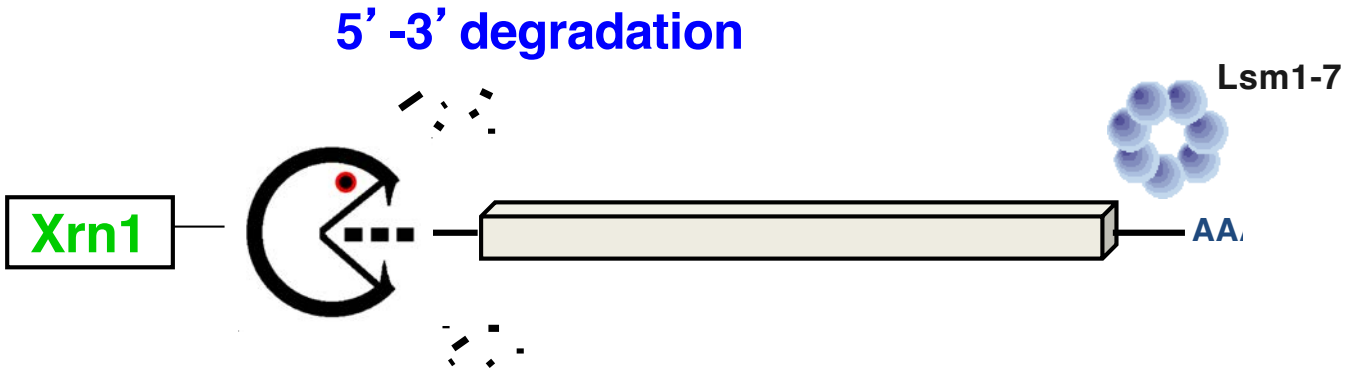


mRNA degradation in the cytoplasm

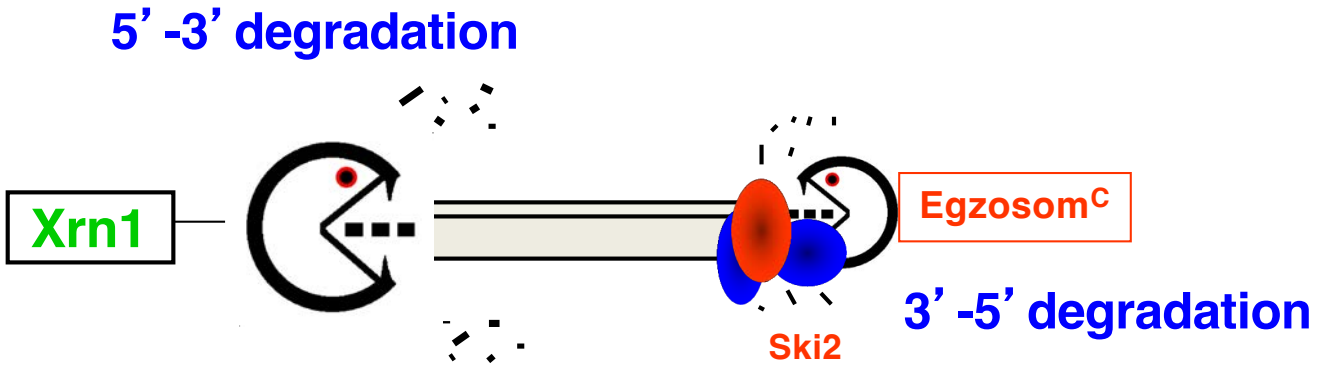
mRNA degradation in the cytoplasm



mRNA degradation in the cytoplasm

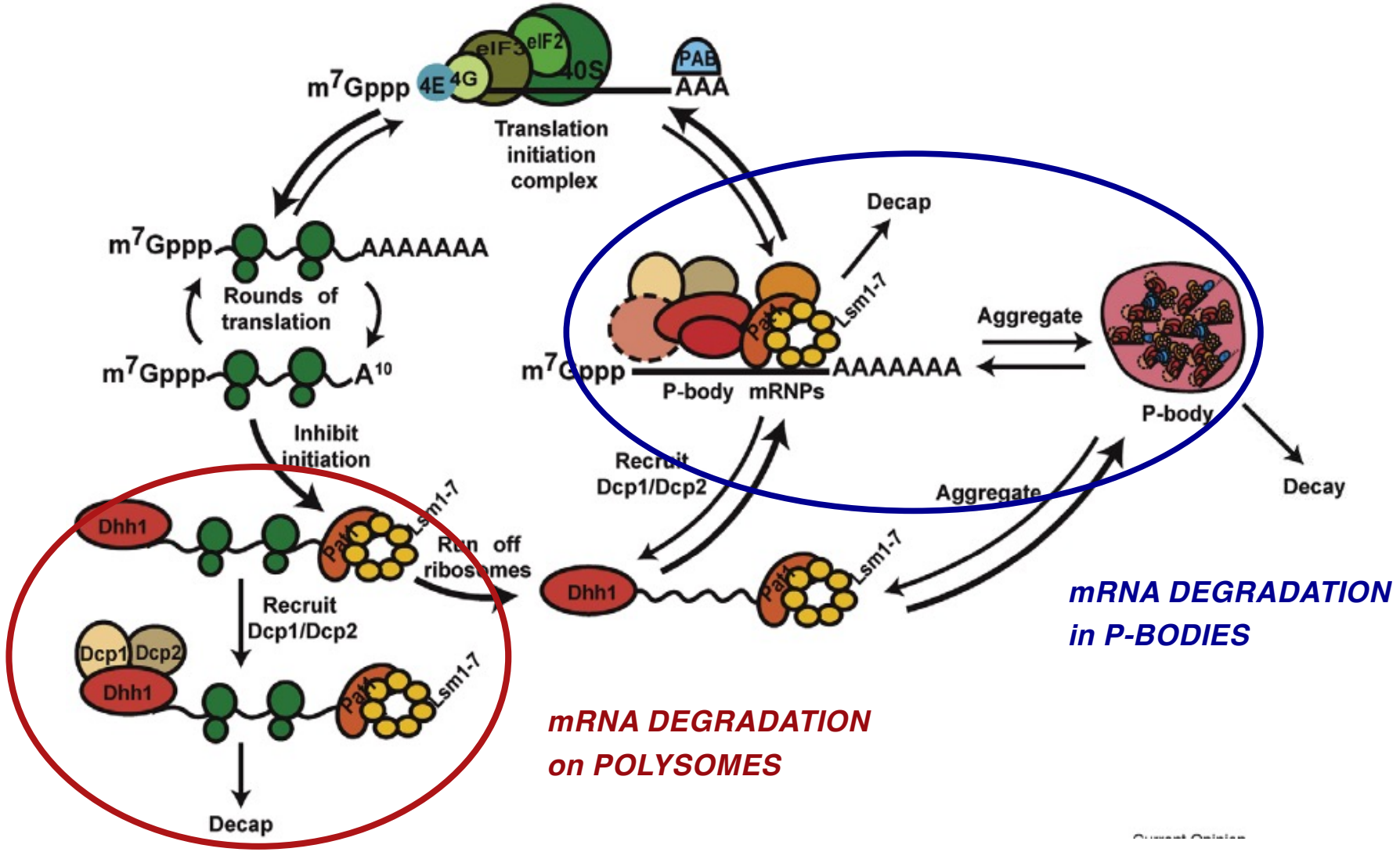


mRNA degradation in the cytoplasm



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

mRNA degradation in the cytoplasm



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

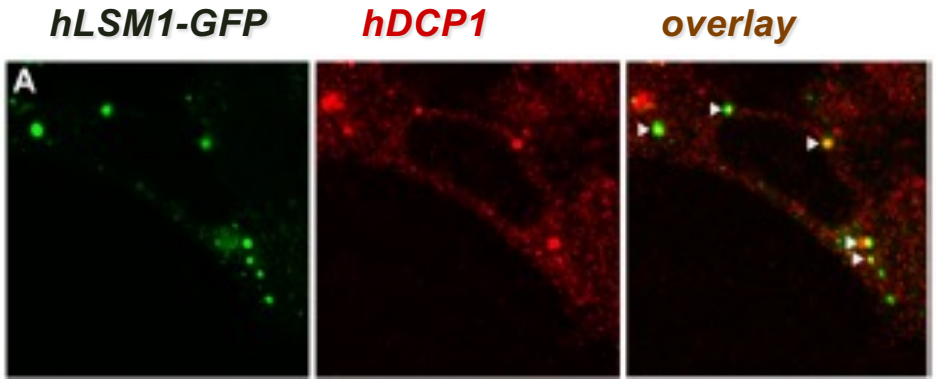
DEADENYLATION → RELEASE OF RIBOSOMES → RELEASE OF TRANSLATION FACTORS → RECRUITMENT OF DECAY FACTORS → RNA DECAY

P bodies- processing bodies

(decay bodies, DCP bodies, GW bodies)

P bodies (PBs)

- *cytoplasmic dynamic structures of mRNA and decay factors storage (LSM, DCP, XRN, GW182)*
- *sites of mRNA storage and/or degradation (?)*

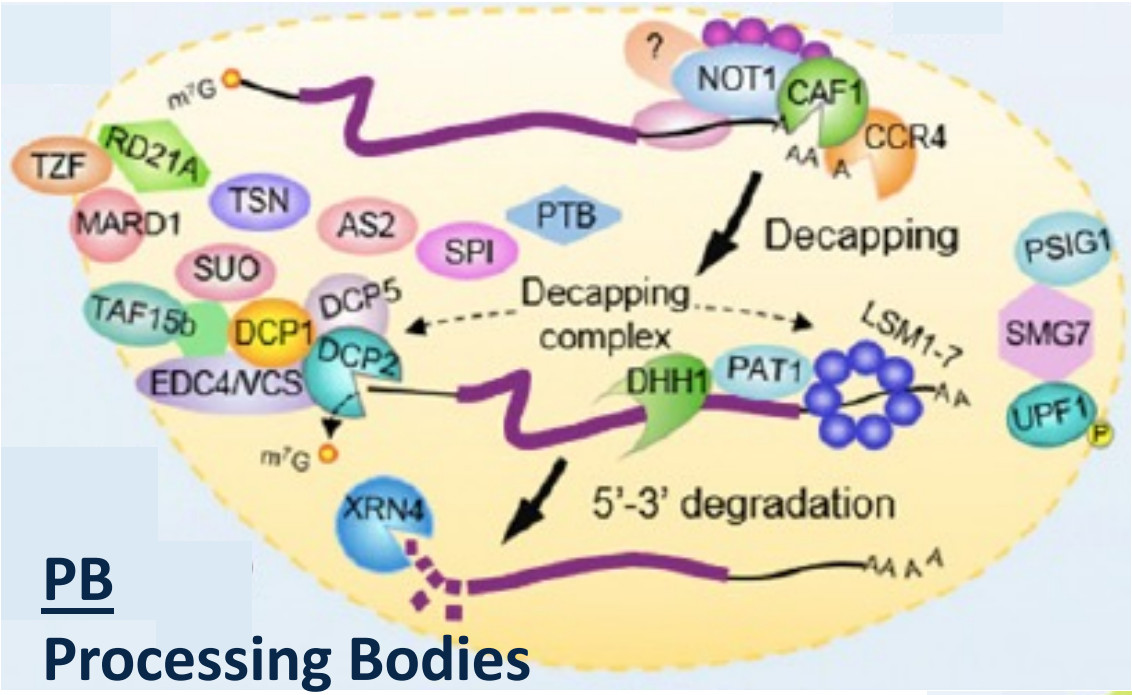


Cougot et al, JCB, 2004

SPECIES:	CONTENT:	
Yeast	Dcp1/2	general
Human cells	Lsm	general
Drosophila	Edc1/2/3	general
C. elegans	Dhh1, Pat1	general
	SMG5-7	human
	GW182	human
	AIN-1, ALG-1	C. elegans miRISC

- **mRNA decay factors co-localize and polyA$^+$ RNA accumulates in P bodies**
- **PBs differ from stress granules (SG)**
- **PB are activated by translation inhibition, stress and mutations in mRNA decay factors**

Cytoplasmic P-bodies and Stress Granules



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

PB
Processing Bodies

mRNA storage
mRNA decay?

PB

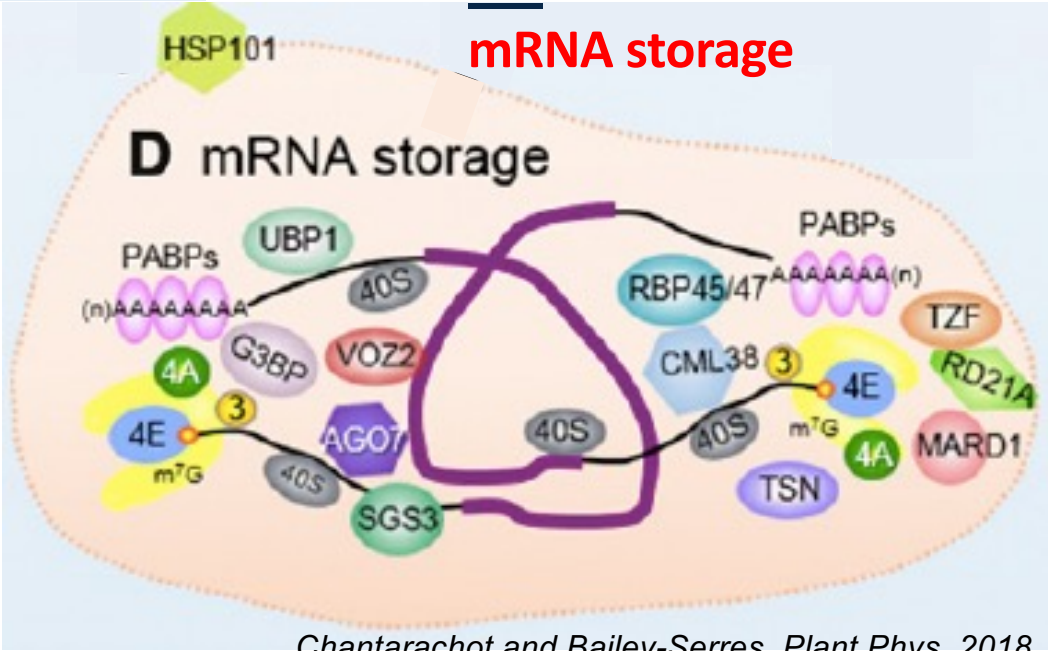
Translationally stalled mRNAs devoid of initiation factors shuttle to PBs

SG

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

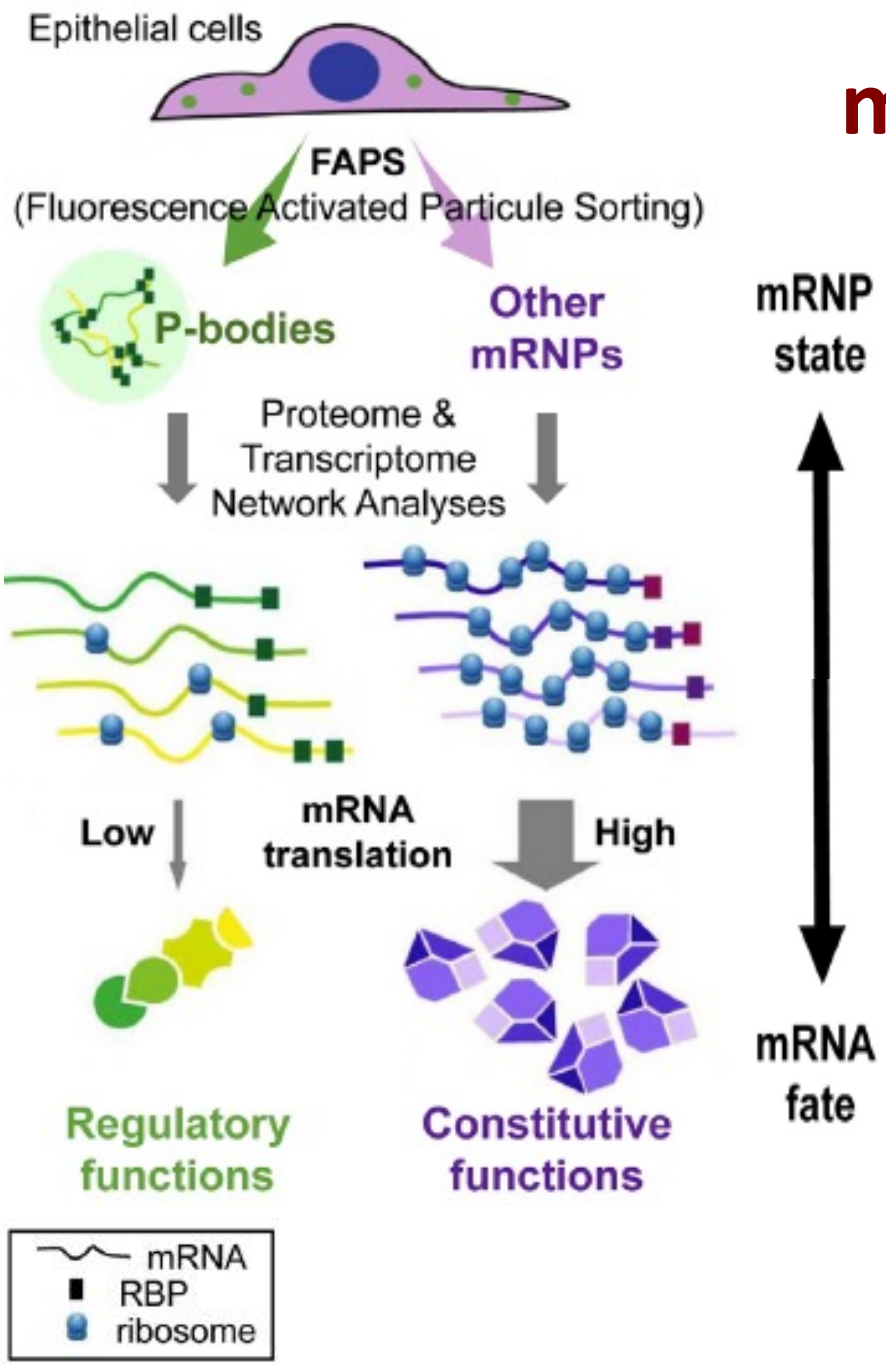
SG Stress Granules

mRNA storage



Chantarachot and Bailey-Serres, Plant Phys, 2018

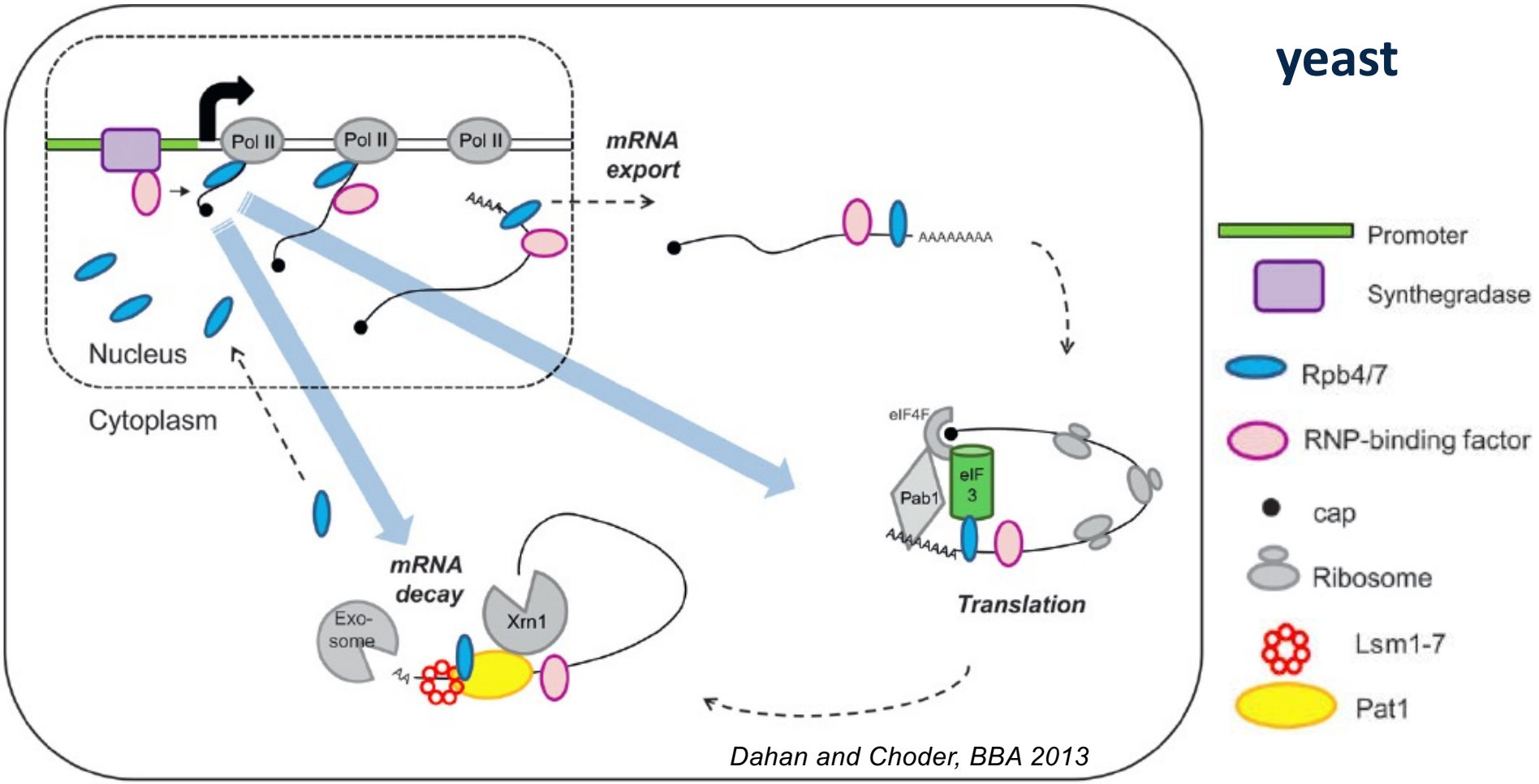
PB mRNPs:
mRNA storage and decay



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

Hubstenberger et al, Mol Cell, 2017

Transcription and mRNA decay are coupled



- Promoters regulate cytoplasmic mRNA decay via Rap1 transcription factor
- Pol II subunits, Rbp4/7, and Xrn1 shuttle between the nucleus and cytoplasm
- Rpb4/7 regulate transcription, processing and decay by binding to the emerging transcript and remaining associated throughout its lifecycle
- Xrn1 also act as a transcription factor

RNA Quality Control Pathways

PART I - GENERAL MECHANISMS

PART II - SPECIFIC PATHWAYS

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)
- **NRD -** Non-functional rRNA decay
- **nuclear RNA degradation** (mRNA, pre-mRNA, rRNA, tRNA, ncRNAs) - degradation of RNA species that were not properly processed i.e. spliced, end-matured, modified or of unstable species (CUTs)

The importance of RNA QC

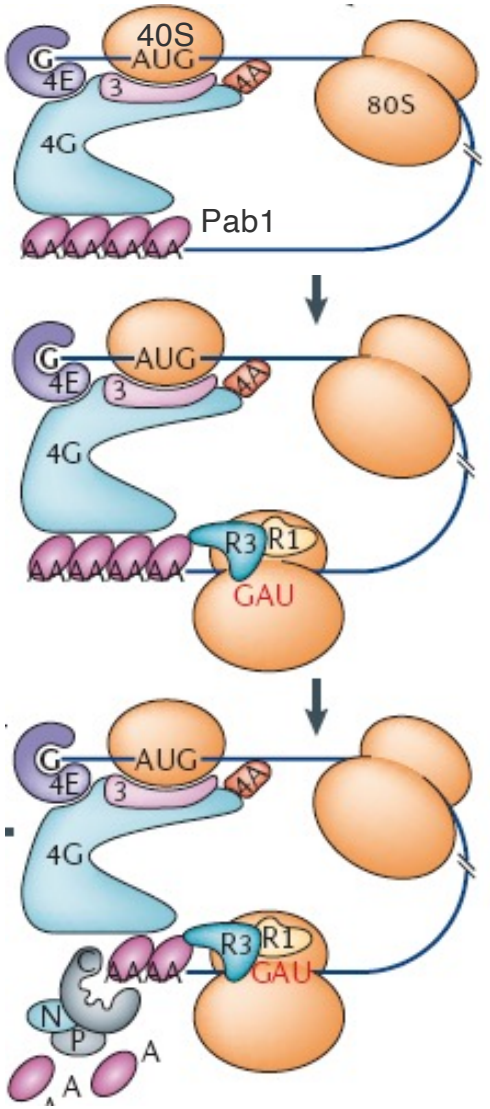
- **These mechanisms control the synthesis, integrity and lifespan of all cellular RNA molecules to assure optimal functioning of the cell**
- **Deficient QC and mutations in QC components lead to severe defects and diseases**

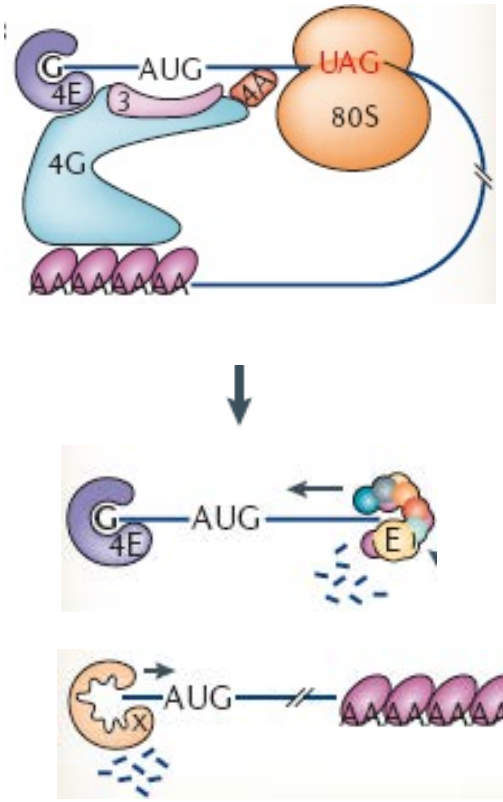
- several genetic disorders (30% - e.g. β-thalasemia, ostegenesis imperfecta, Marfan syndrome, Stickler's syndrome, neurologic syndromes) result from inefficient NMD and other QC mechanisms due to frameshift mutations and premature translation termination

- mutations in RNA enzymes confer autoimmune diseases in humans and developmental defects in plants, worms and flies

NMD

- degradation of mRNAs containing premature STOP codons (PTC)
- prevents expression of truncated, possibly harmful, proteins
- 33% of yeast intron-containing mRNAs undergo NMD
- 30% of alternatively spliced human mRNAs generate NMD substrates





Translation termination problem:

- premature termination

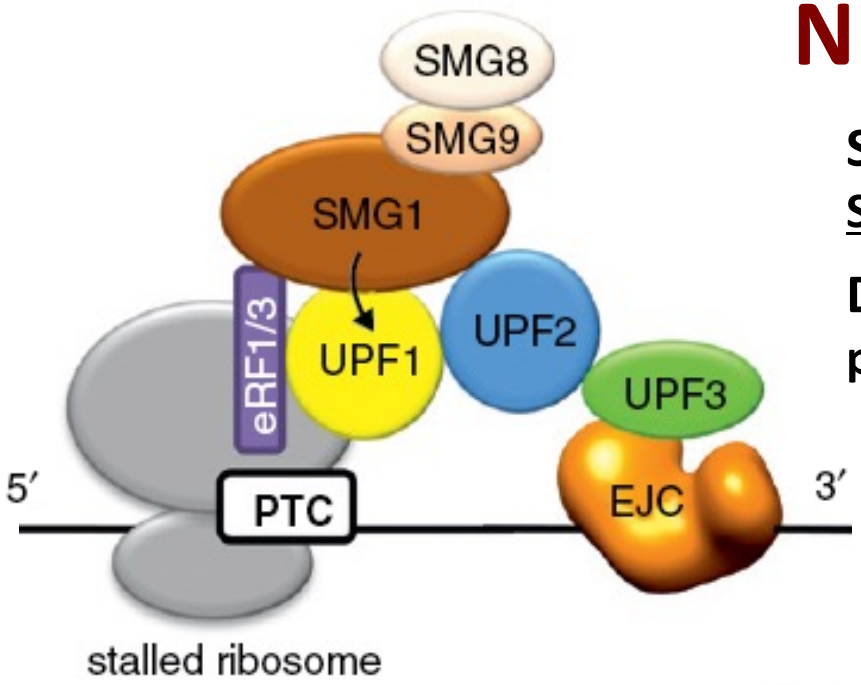
or

- ribosome stalling on PTC

mRNA degradation

Amrani et al. Nat.Rev.Cel.Biol., 2006

NMD factors



Llorka. Cur. Op. Chem. Biol. 2013

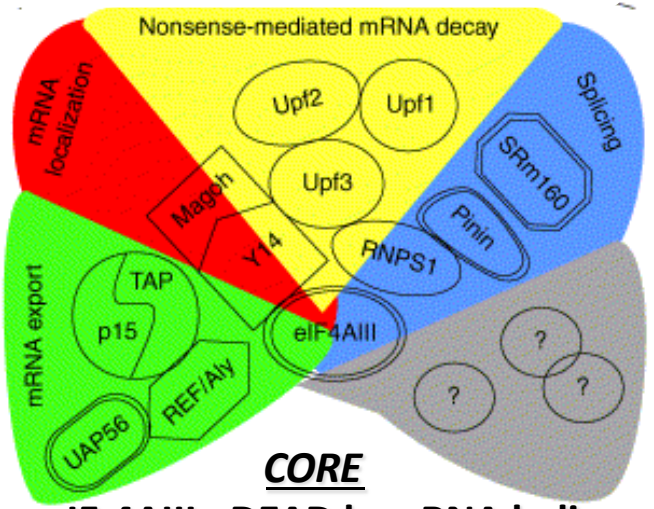
SURF complex

SMG1-UPFs-SMGs-Release Factors

DECID (decay inducing)

phoshoSMG1-UPFs-EJC

EJC: splicing, export, NMD



CORE

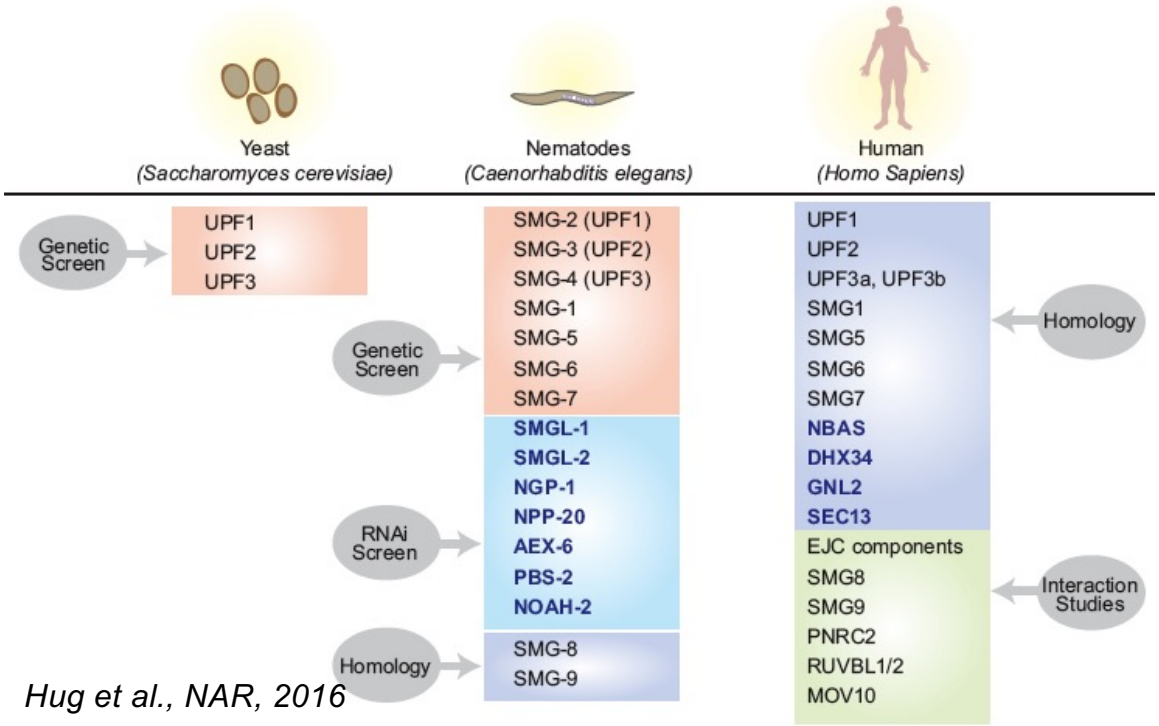
eIF-4AIII - DEAD box RNA helicase

MLN51 - stimulates eIF-4AIII

Magoh/Y14 - binds to eIF-4AIII

ASSOCIATED

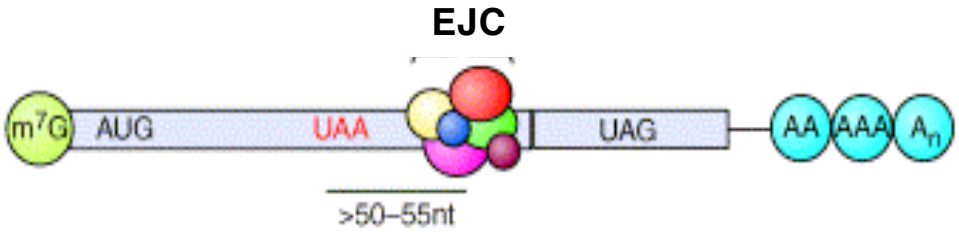
UAP56, SRm160, Acinus, SAP18,
Pinin, RNPS1 (splicing)
REF, TAP, p15 (export)
Upf1, Upf2, Upf3 (NMD)



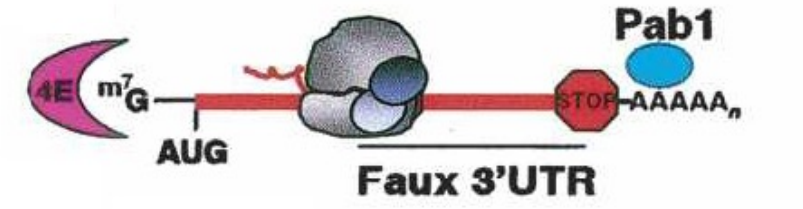
Hug et al., NAR, 2016

NMD mechanism

1. Recognition of premature stop codon during translation

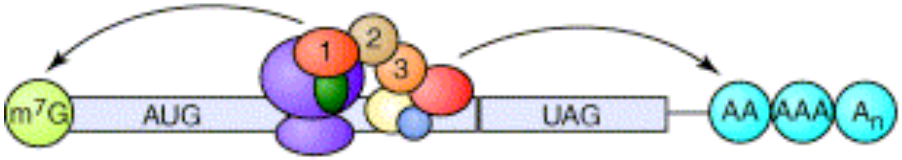


splicing-related mechanism
- EJC deposited as a mark of splicing
- Upf3 is bound to mRNA via EJC
- mRNA is exported and Upf2 joins Upf3



translation termination and unified 3'UTR mechanism:
ribosome not interacting with 3'UTR factors is arrested on the PTC

2. Assembly of the active NMD complex and repression of translation



active NMD complex
Upf1-3 + SMG proteins

EJC downstream of PTC is not removed by the advancing ribosome

SURF complex, Upf1.SMG1 and eRF1-2, is recruited by the stalled ribosome

Upf1 is phosphorylated by SMG1
eRF1-eRF2 are released

mRNA is directed for degradation

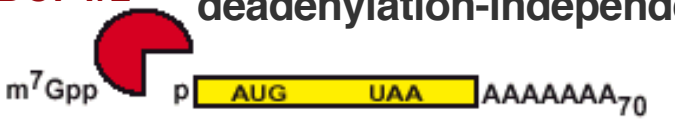
Singh and Lykke-Andersen, TiBS 2003; Isken and Maquat, Gene Dev. 2007

NMD mechanism

3. mRNA degradation

NMD 5'→3'

DCP1/2 deadenylation-independent decapping

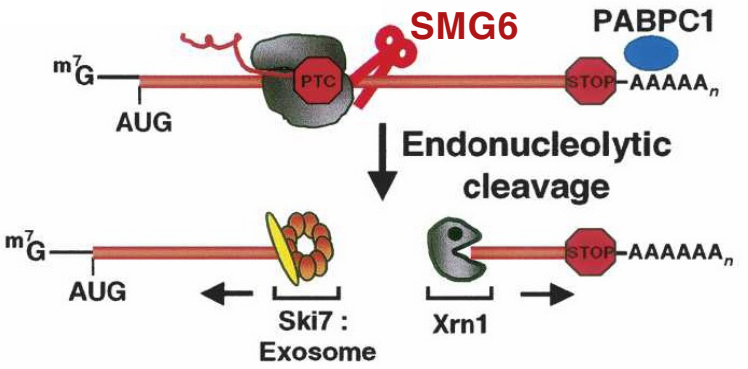


XRN1 exonucleolytic 5'→3' degradation (XRN1)

decapping is triggered by SMG7 recruitment or dephosphorylation of Upf1

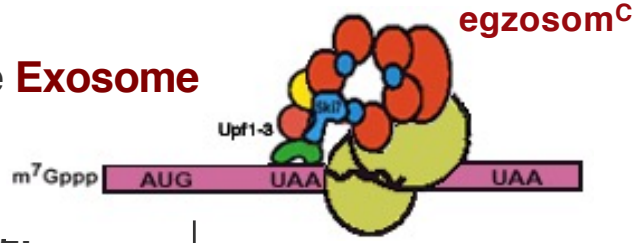
NMD endonucleolytic cleavage

(Drosophila melanogaster, humans)



NMD 3'→5'

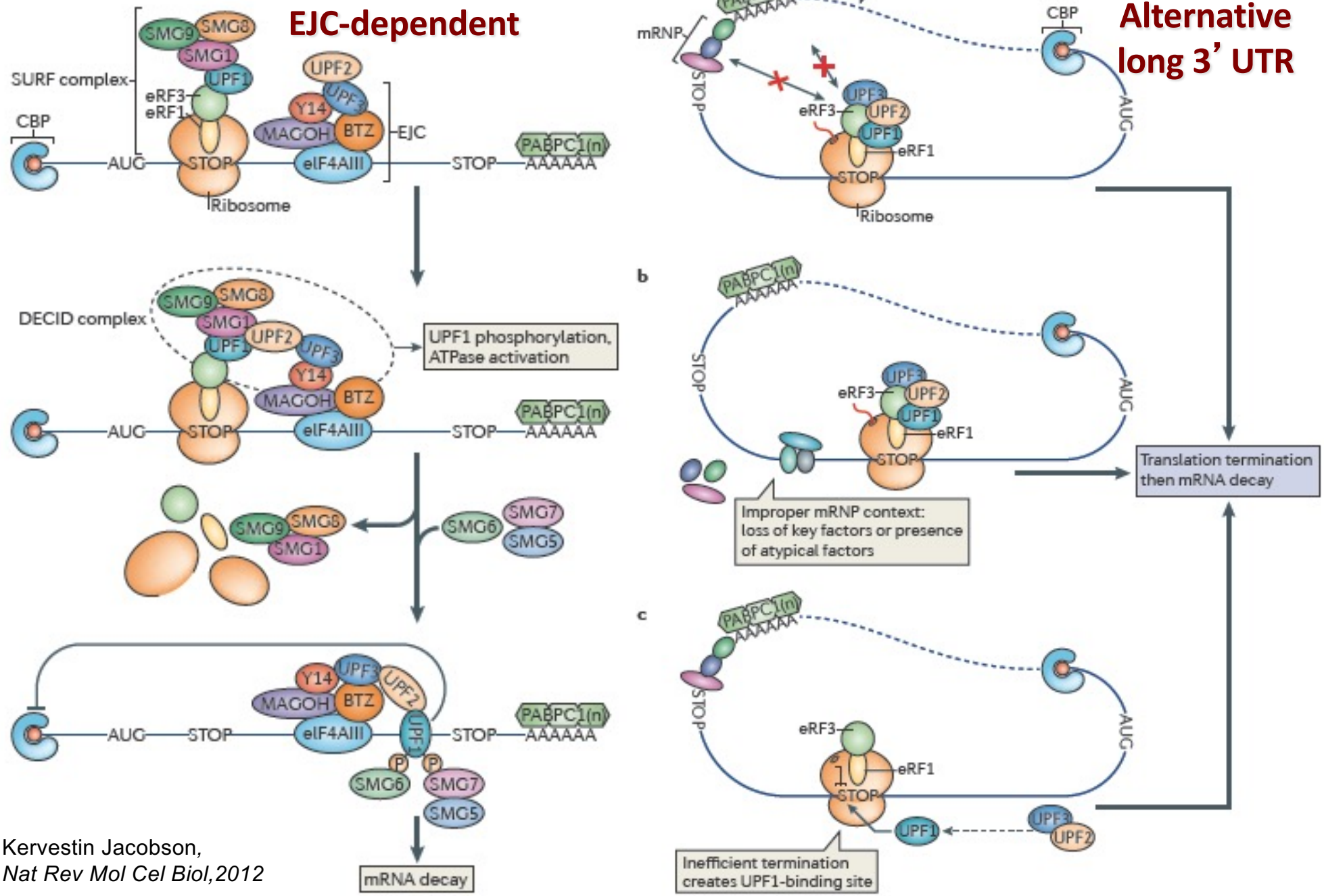
recruitment of the Exosome by Upf1



exonucleolytic 3'→5' degradation

Parker and Song, Nat. Struct. Mol. Biol. 2004; Isken and Maquat, Gene Dev. 2007

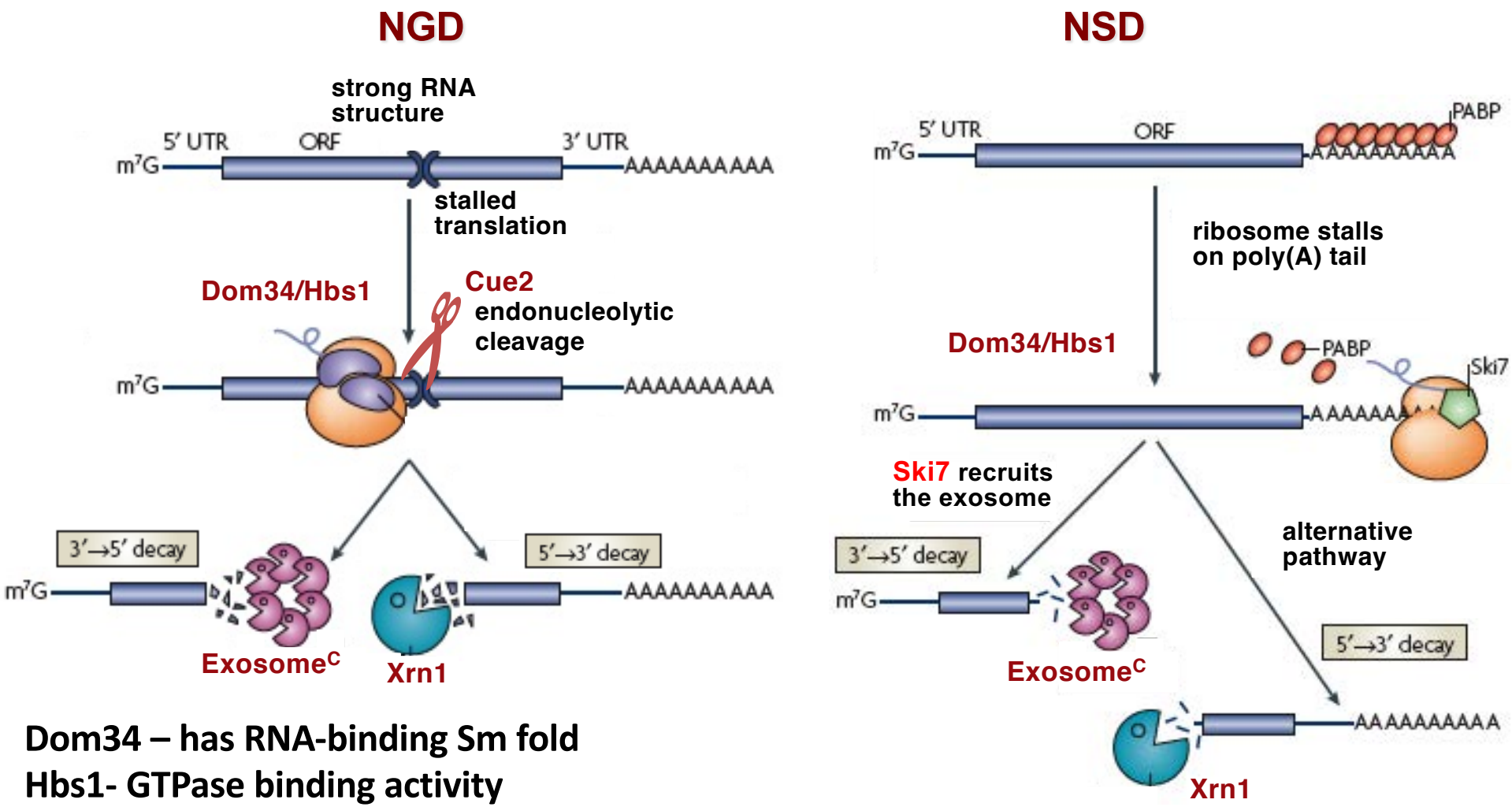
NMD mechanism



Kervestin Jacobson,
Nat Rev Mol Cel Biol,2012

NGD and NSD

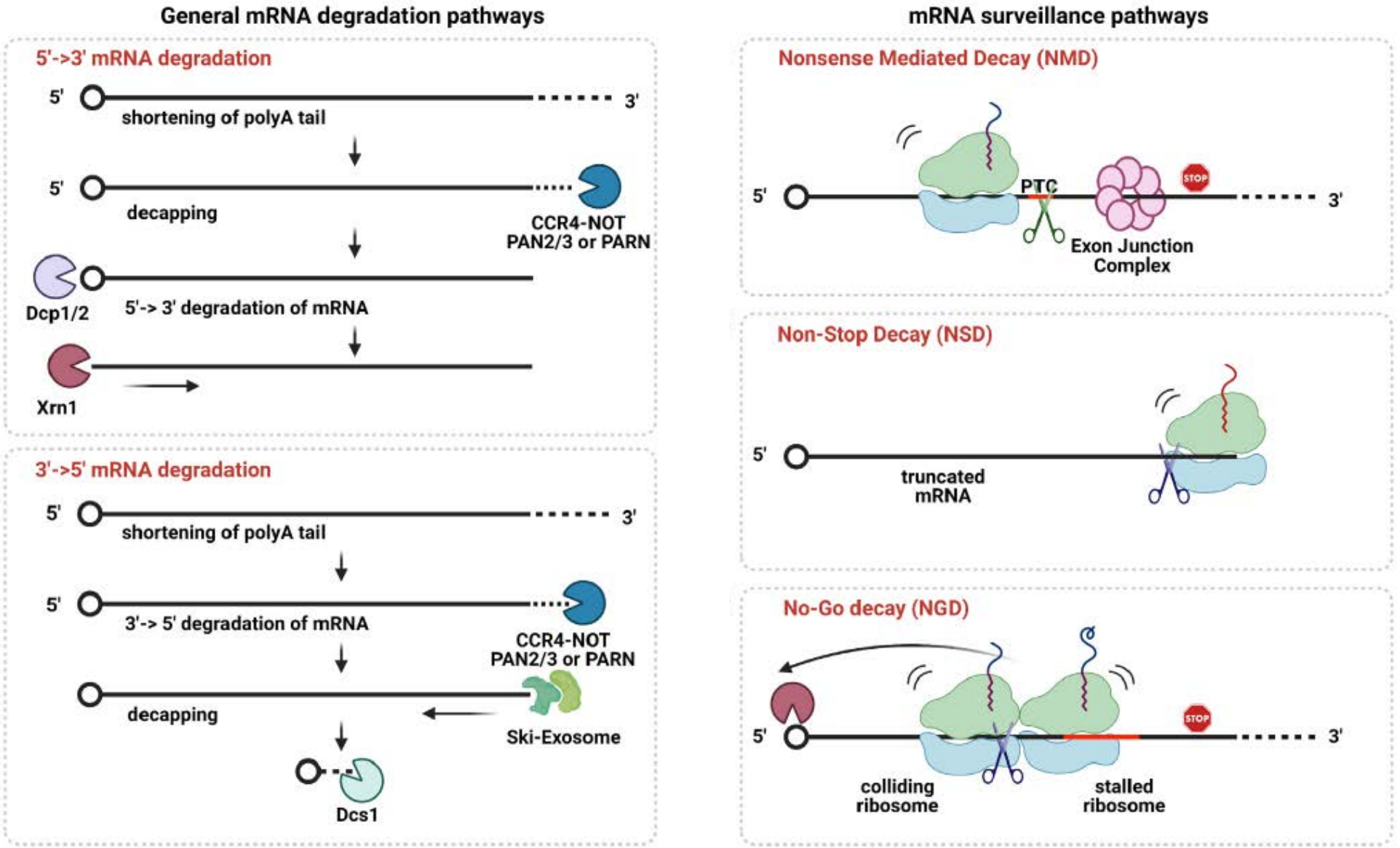
- **NGD** (non-go decay) - degradation of mRNAs stalled on ribosomes
- **NSD**- (non-stop decay) - degradation of mRNAs with no stop codons



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

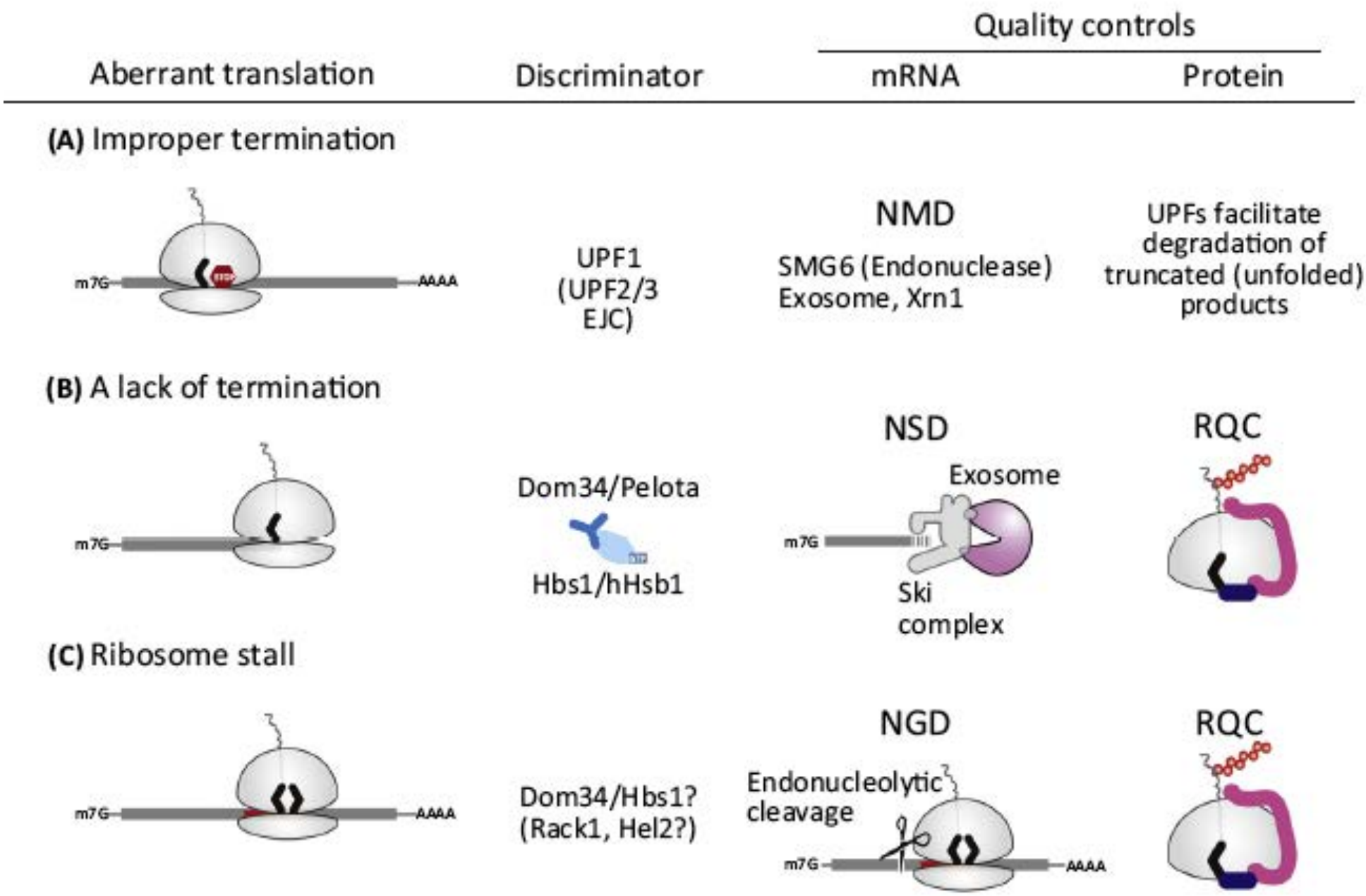
Garneau et al, Nat. Rev. Mol. Cel. Biol. 2007

NMD, NGD and NSD



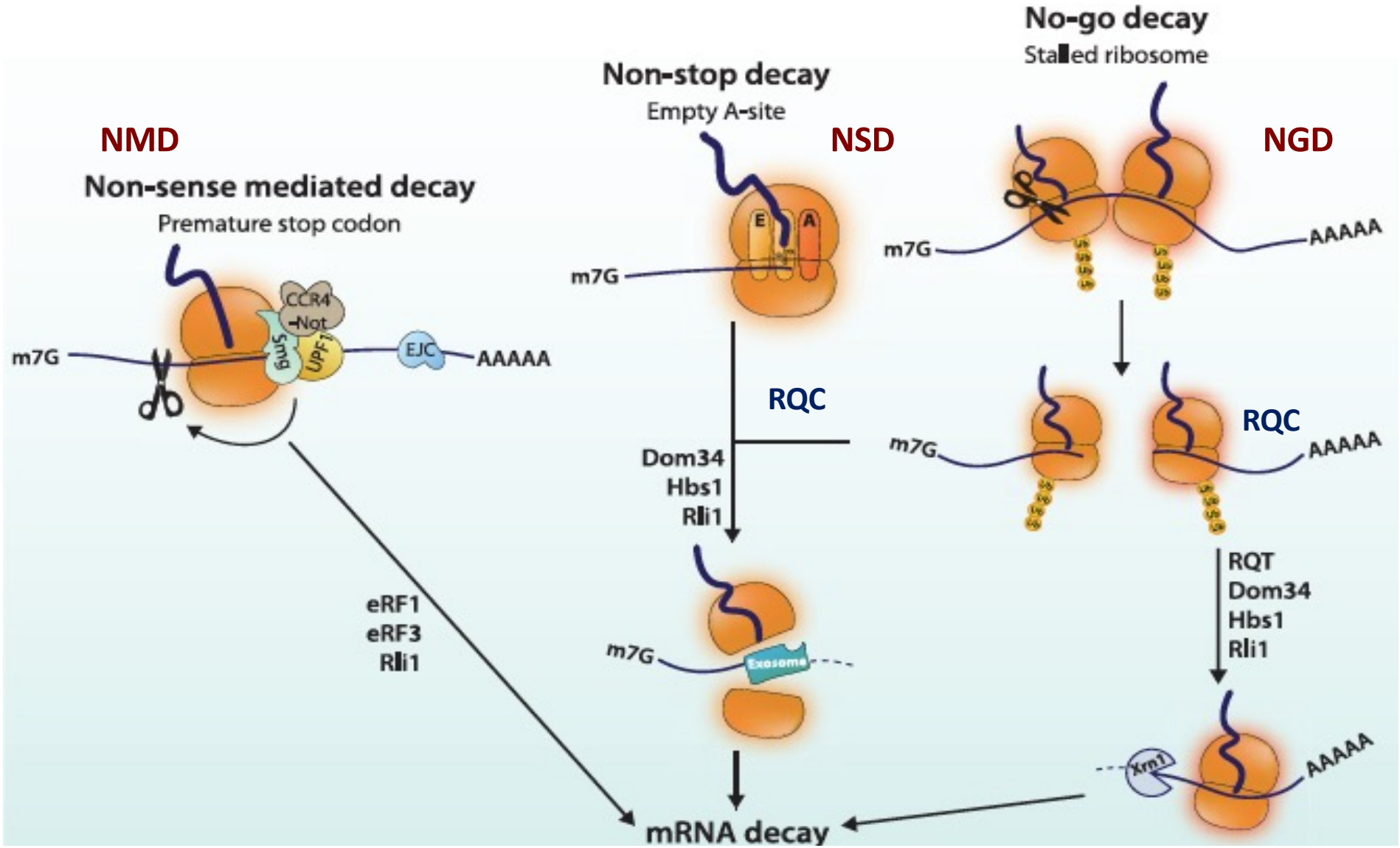
Boopathy et al, WIREsRNA, 2023

NMD, NGD and NSD



Inada, TiBS 2016

Co-translational mRNA QC



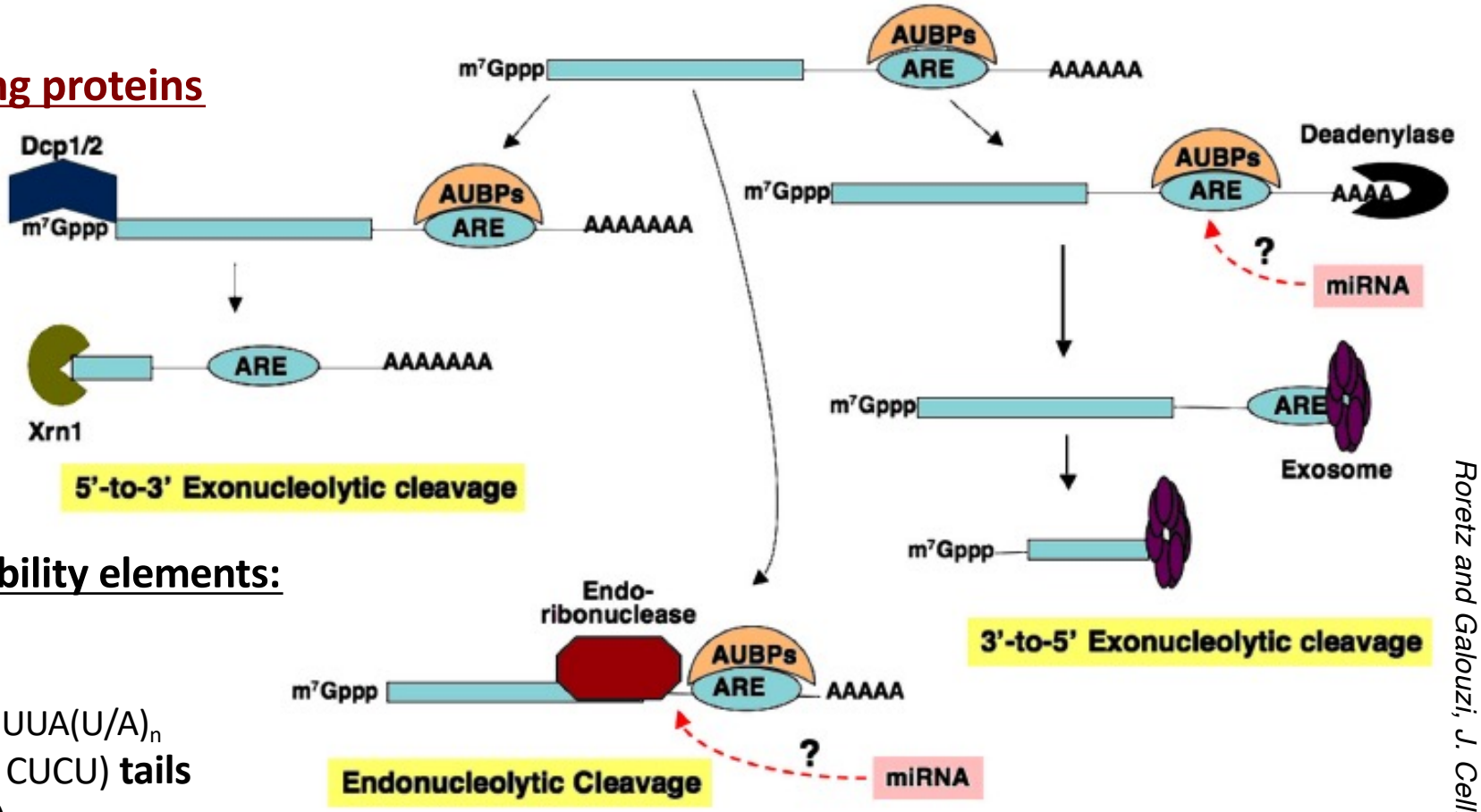
Collart and Weiss, NAR.,2019

AMD ARE - mediated decay

- **AMD** degradation of mRNAs containing AU-rich elements present in mRNA 3'UTR

ARE-binding proteins (AUBP)

- TTP
- BRF1
- HuR
- AUF1
- KSRP



mRNA instability elements:

- **U rich**
- **AU rich**

AUUUA, UUAUUUA(U/A)$_n$

- **oligo(U)** (or CUCU) **tails**
- **DST** (plants)

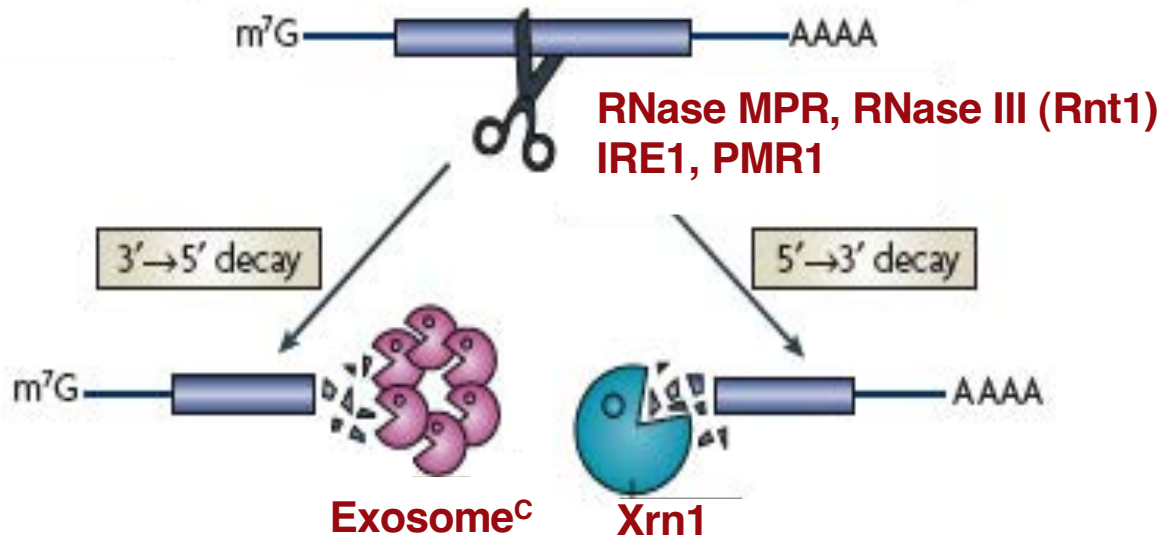
GGAnnAUAGAUUnnnCAUUnnGUAU

Roretz and Galouzi, J. Cell. Biol. 2008

- **Exosome (RRP45, RRP41, RRP43) is recruited by ARE-binding proteins AUBP (AUF1)**
- **Exosomal subunits interact directly with ARE sequences**
- **PARN and CCR4, deadenylases and XRN1, DCP1/2 interact with AUBP (TTP, KSRP, BRF1)**

Other mechanisms

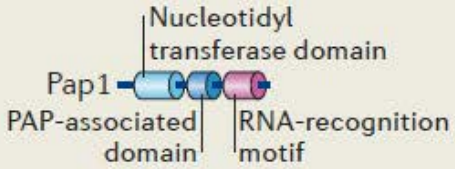
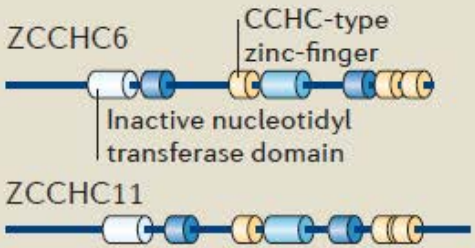
Endonuclease mediated decay



- PMR1 - degradation of translationally active mRNAs on polysomes
- IRE1 - degradation of mRNAs in ER during Unfoded Protein Response stress
- MRP - cleaves *CLB2* mRNA within its 5' UTR in yeast
- Rnt1 - cleaves stem-loops structures in some ribosomal protein mRNAs

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

3’ end decay: Nucleotidyl Transferases

RNA nucleotidyl transferase subclass	Budding yeast	Fission yeast	Mammals	Reported activity
PAP				Polyadenylation
Trf4 and Trf5-like				Oligodenylation
Mitochondrial PAP	NA	Unknown		Oligoadenylation
GLD2-like	NA			Polyadenylation, monoadenylation
U6TUT	NA	NA		Oligouridylation, polyadenylation
Cid1-like	NA			Monouridylation, oligouridylation
Other	NA		NA	Unknown

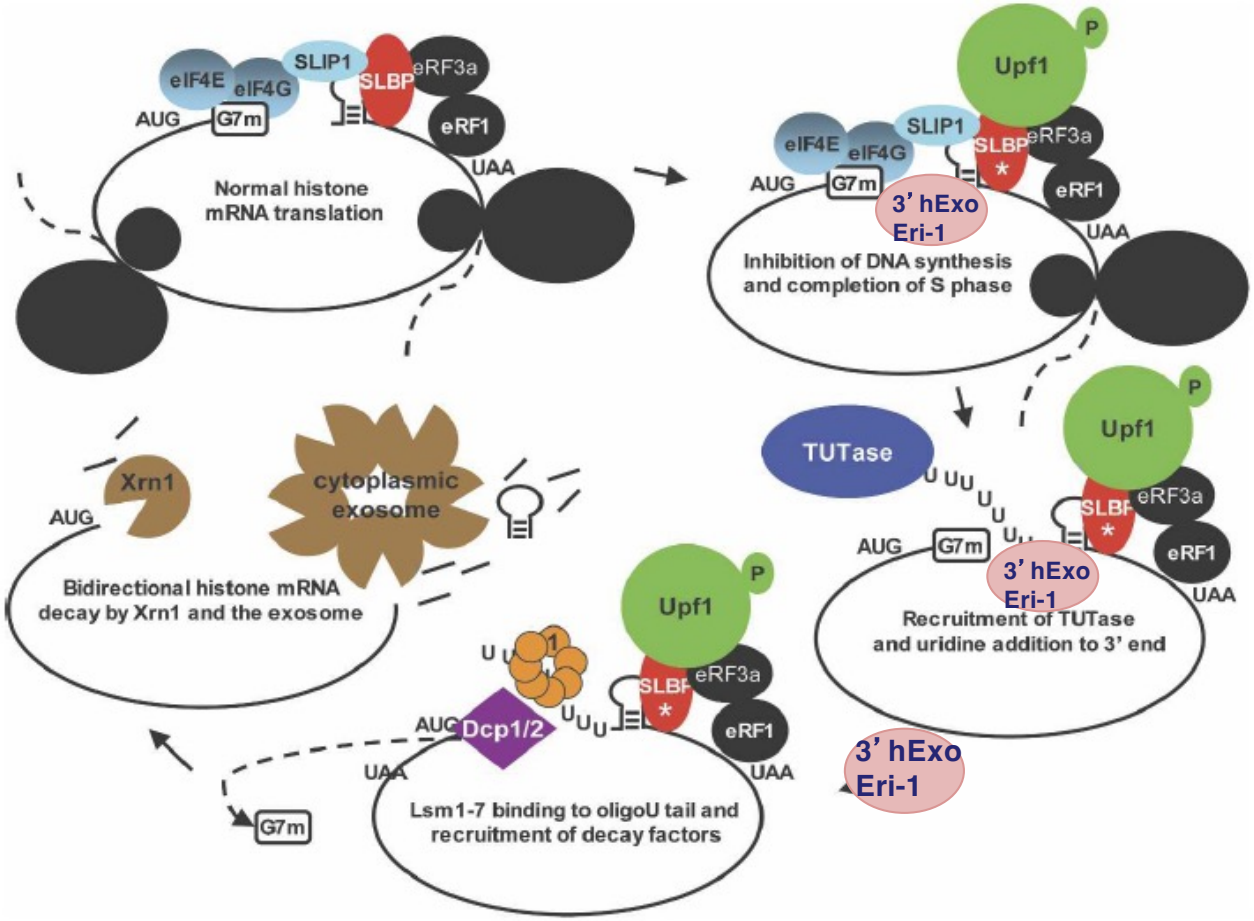
Norbury, Nat. Rev. Mol. Cel. Biol. 2013

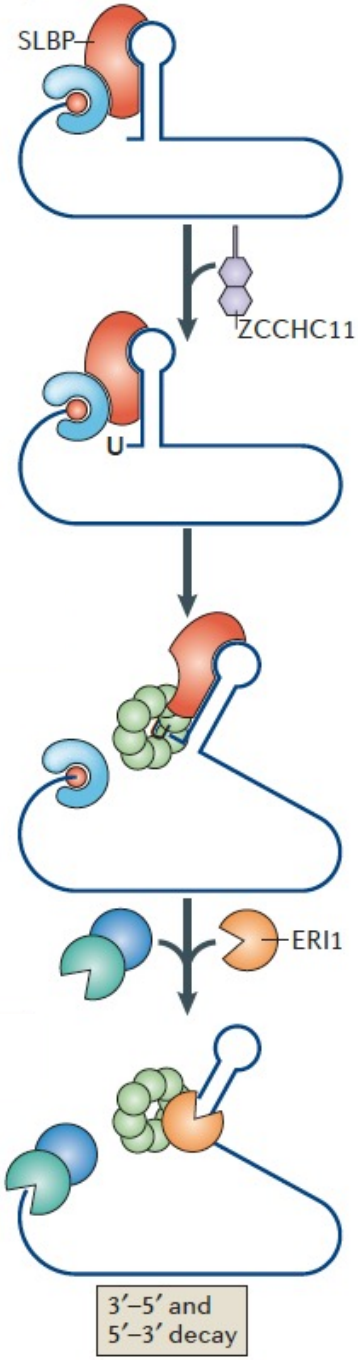
3’-Uridylation

TUTases Terminal Uridylyl Transferases

PUPases Poly(U) Polymerases

Histone mRNA degradation (metazoa)

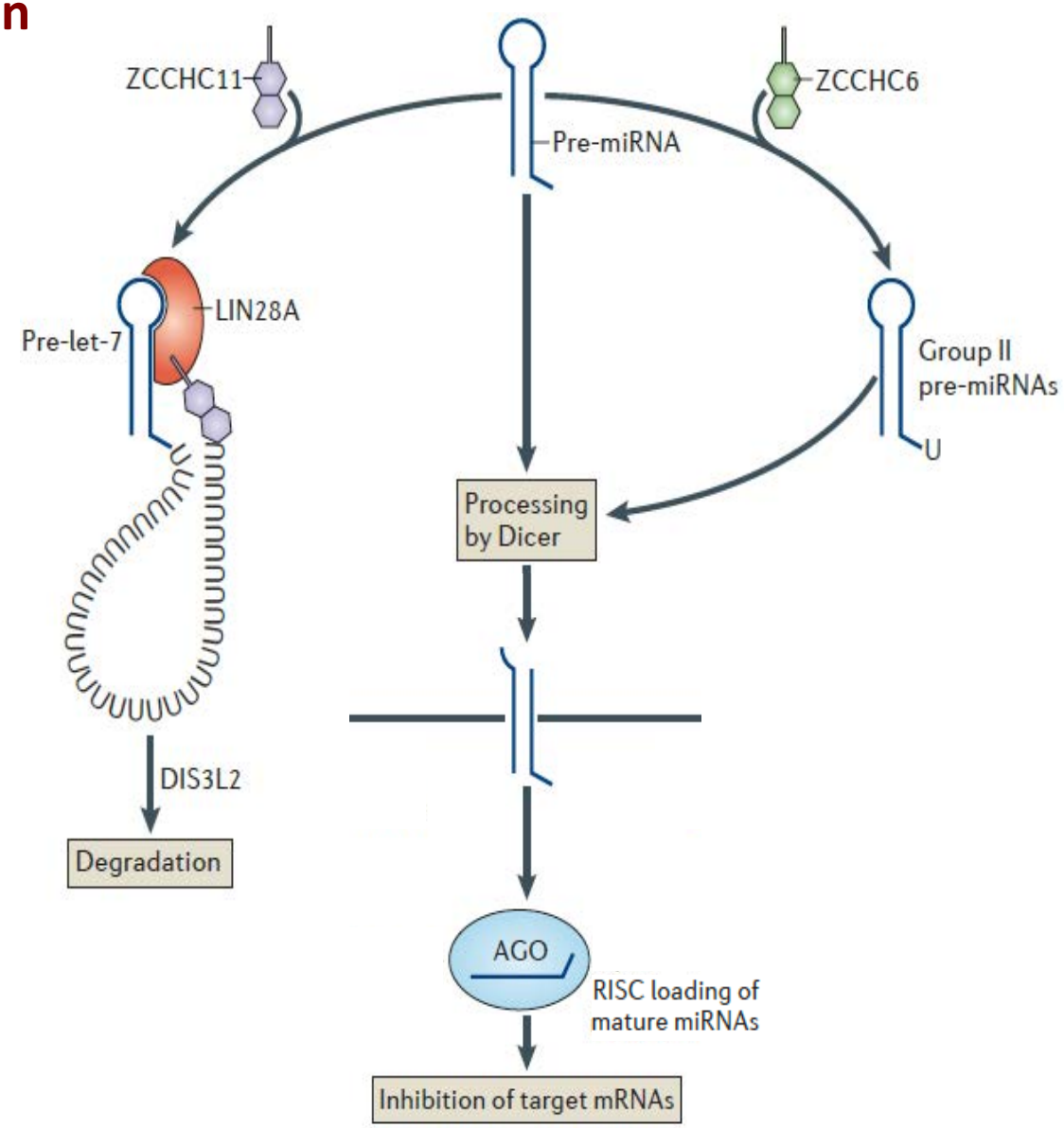




Mullen and Marzluff, Genes Dev., 2008; Norbury, Nat. Rev. Mol. Cel. Biol. 2013

3’-Uridylation

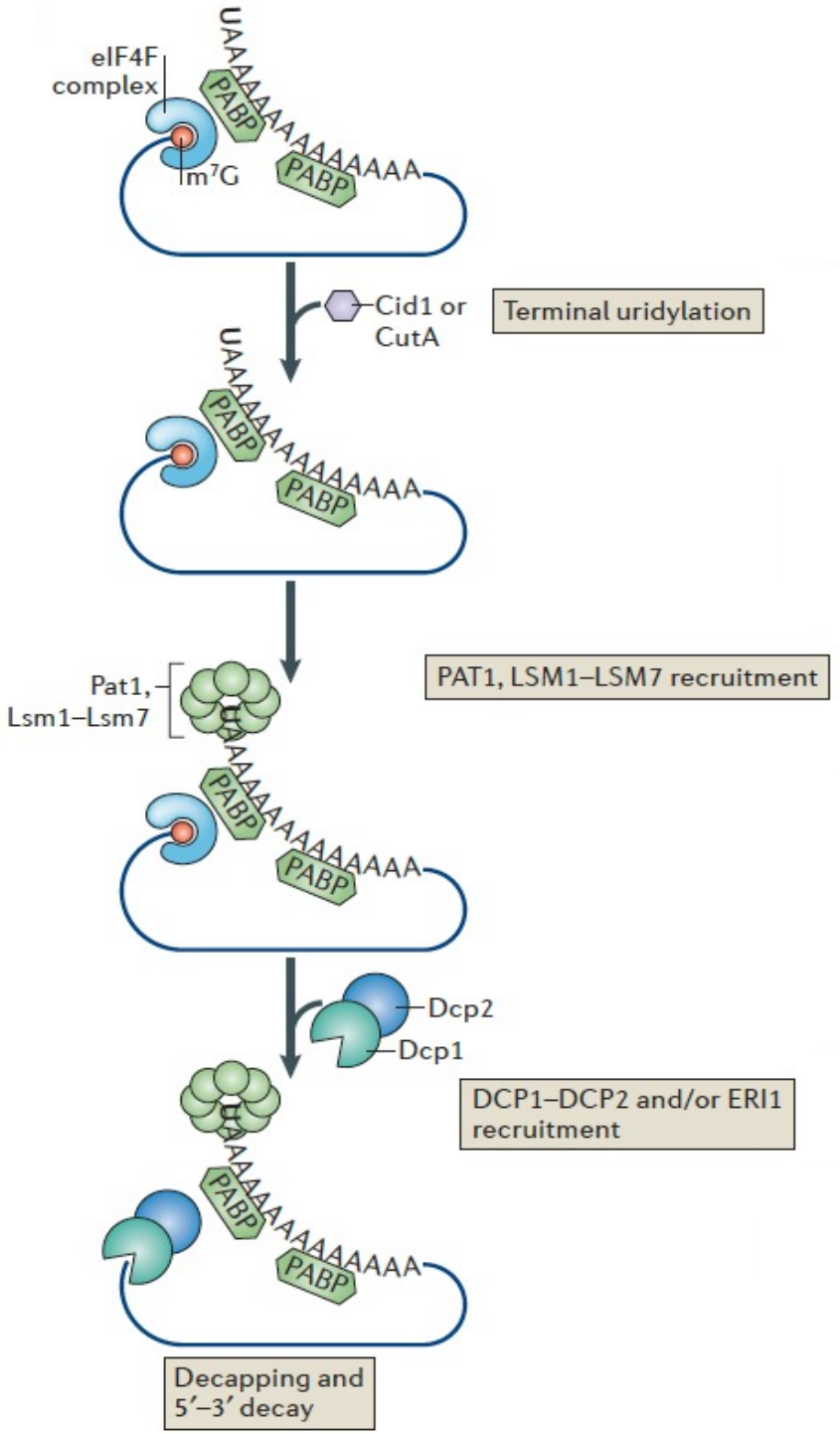
pre-miRNA degradation



Norbury, Nat. Rev. Mol. Cel. Biol. 2013

3’-Uridylation

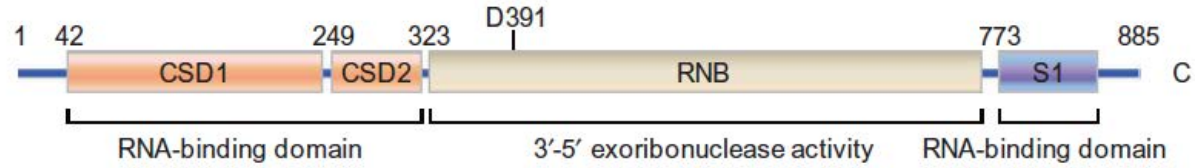
Cytoplasmic mRNA degradation

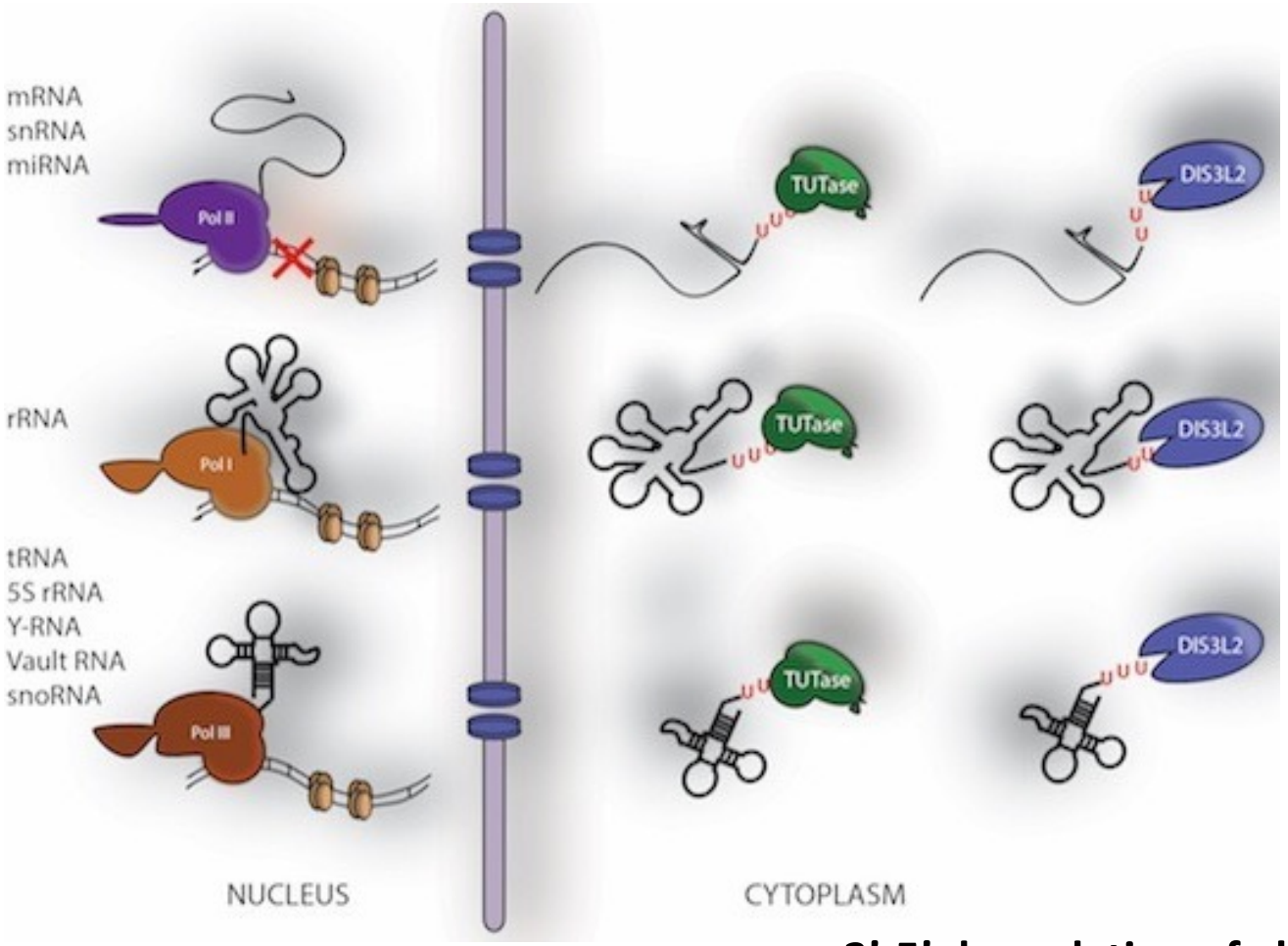


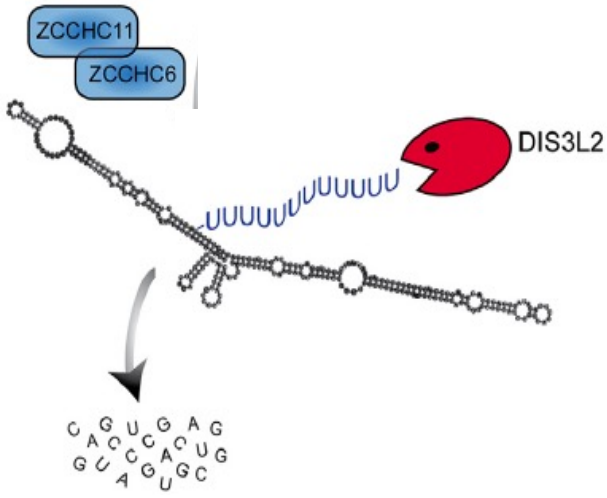
Norbury, Nat. Rev. Mol. Cel. Biol. 2013

hDIS3L2 – exosome independent decay

TUT-DIS3L2 (TDS) - mediated
3’-5’ cytoplasmic RNA decay

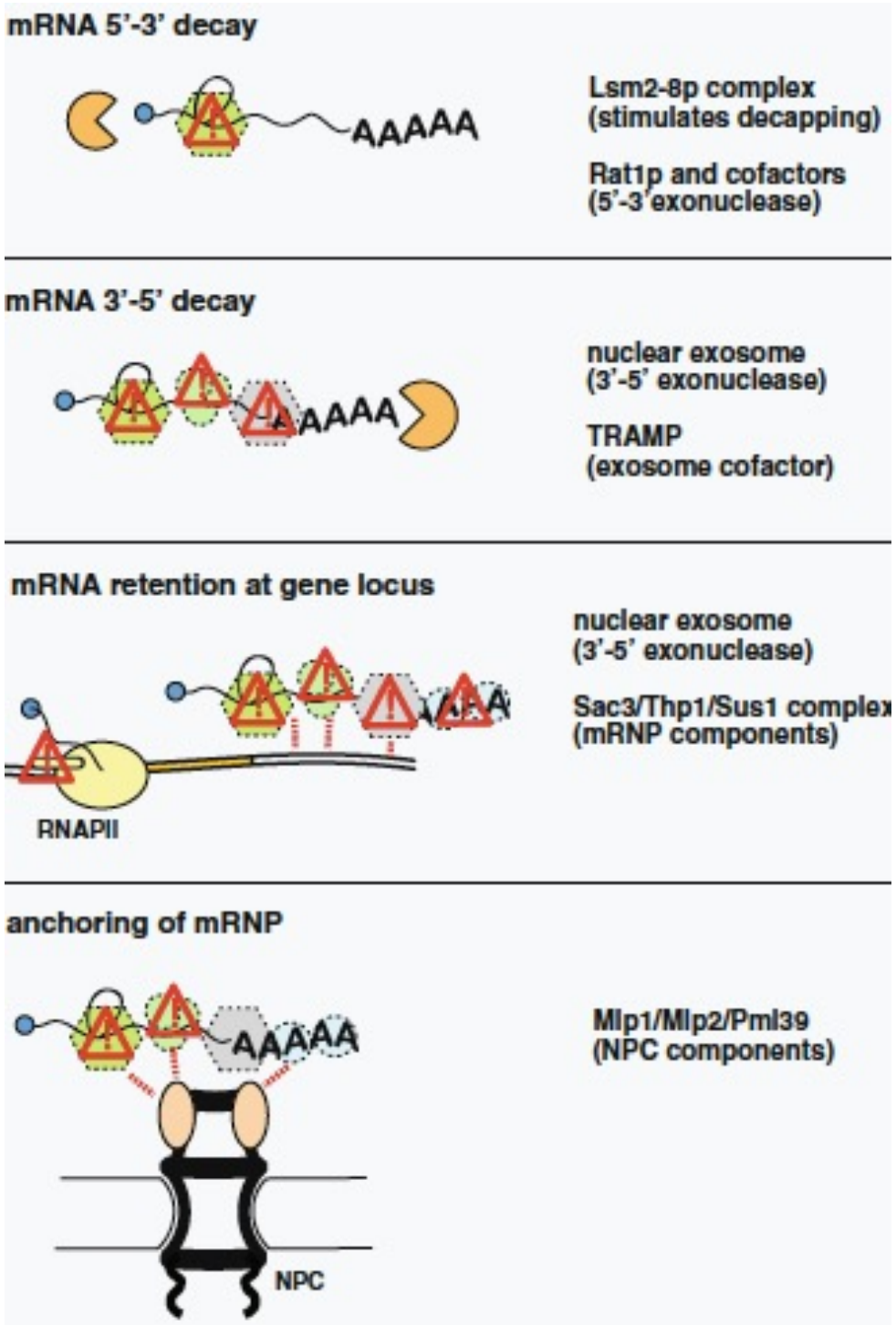






3’-5’ degradation of aberrant, structured ncRNAs
tRNA, sn/snoRNA, rRNA, lncRNA, Y RNA, vault RNA, surveillance of 3’ snRNA processing

Ustianenko et al., EMBO, 2016



mRNA decay in the nucleus

Unspliced pre-mRNAs

3'-end unprocessed pre-mRNAs

Unpackaged mRNAs

(wrong mRNPs)

mRNAs retained in the nucleus

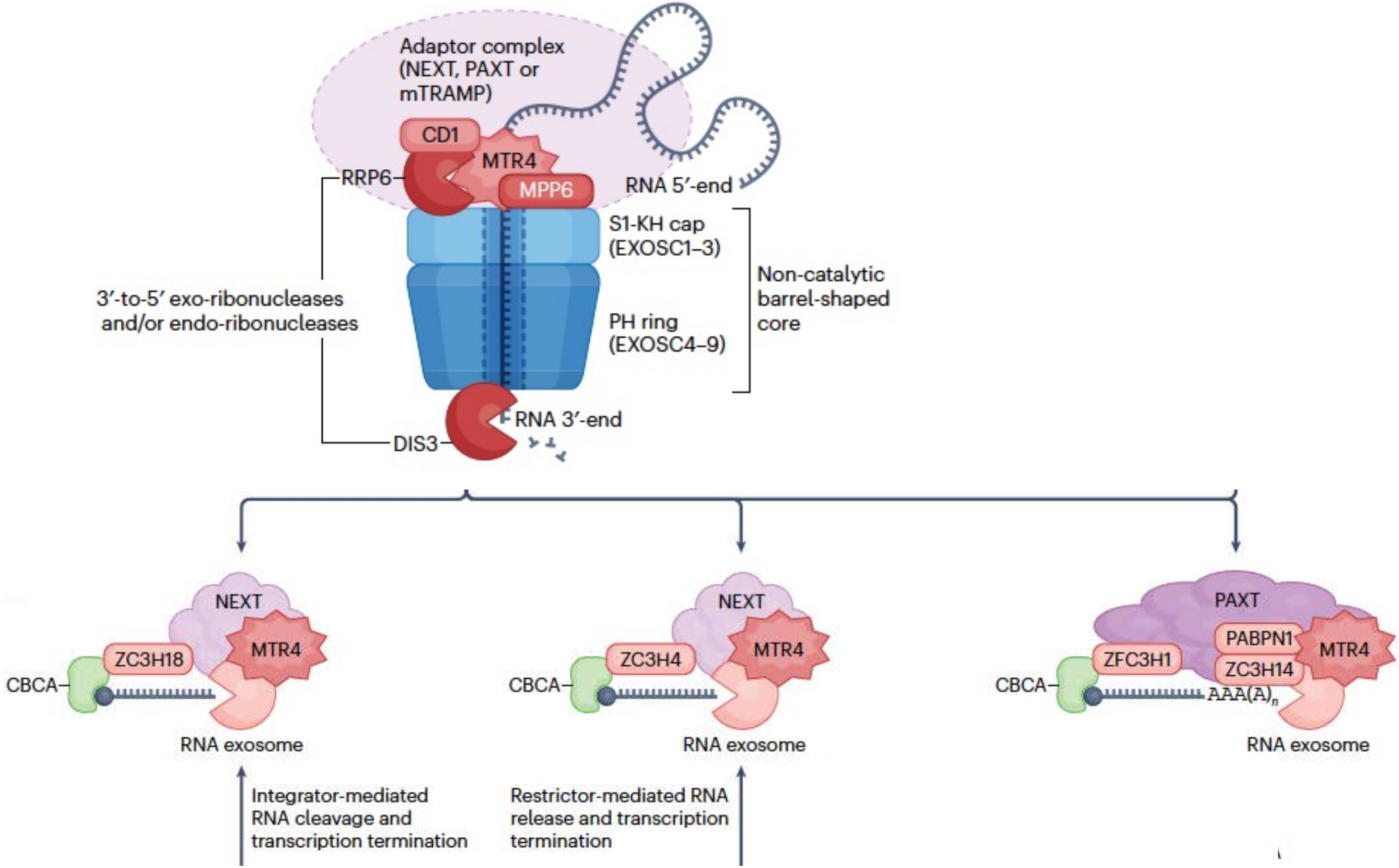
(export defect)

Transcripts retained at chromatin

Aberrant ncRNAs

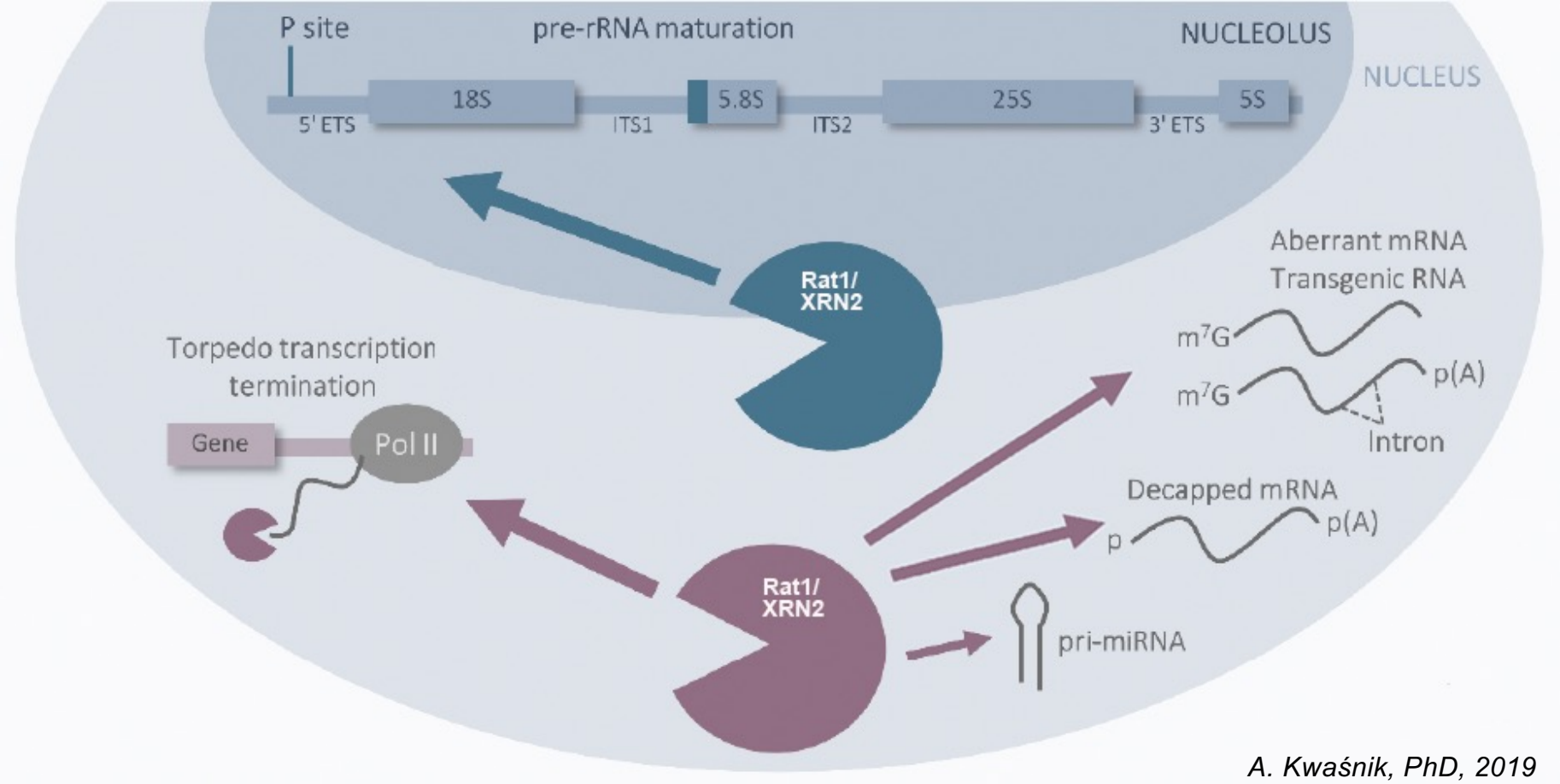
Schmid and Jensen, Chromosoma., 2008

EXOSOME with TRAMP, NEXT and PAXT



Rambout et al, Nat Rev Genet, 2024

Rat1/Xrn2 - nuclear RNA surveillance 5'→3'



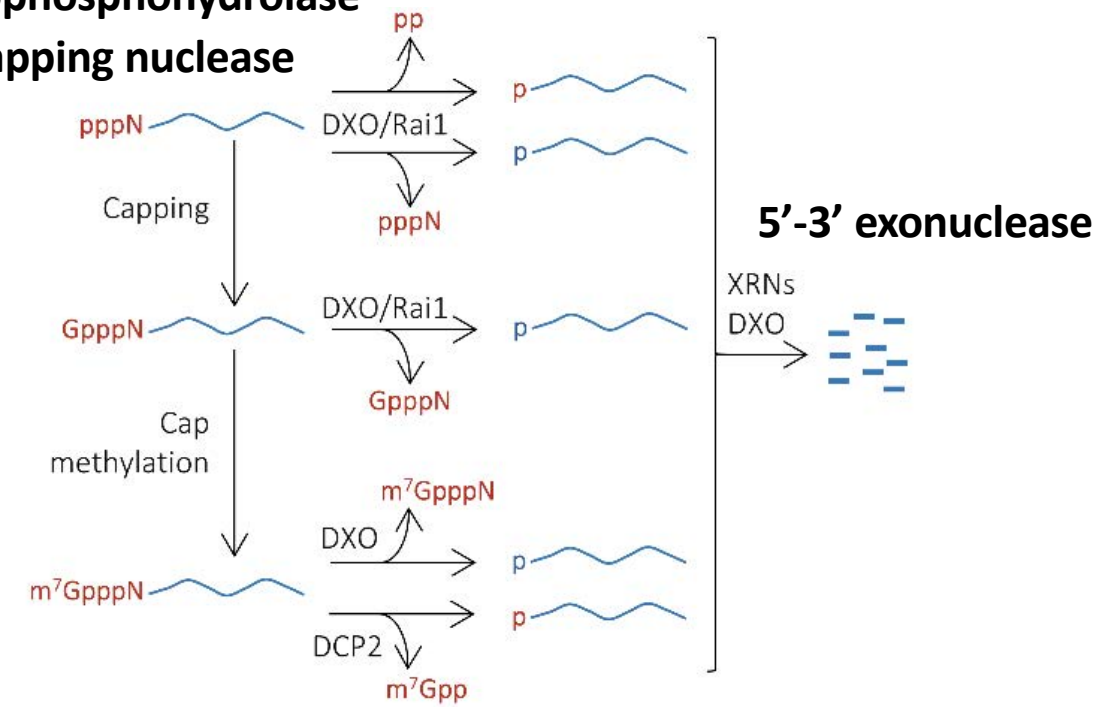
Rat1/XRN2

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

Cap nuclear RNA surveillance 5’→ 3’

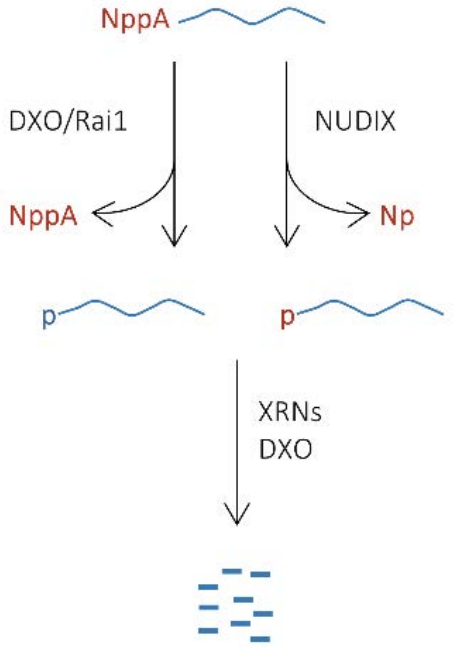
Activities of the DXO/Rai1 family

pyrophosphohydrolase decapping nuclease



5’-3’ exonuclease

deNADding nuclease



A. Kwaśnik, PhD thesis, 2019

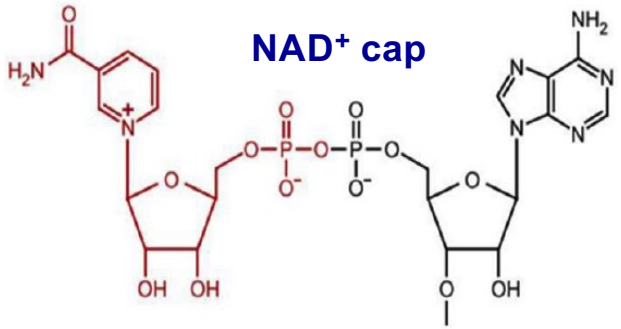
Activity	Substrate	yRai1	yDXO1	mDXO	AtDXO1	Rat1/ Xrn1
Pyrophospohydrolase	ppp-RNA	+++	-	+++	-	-
Decapping (unmethyl)	Gppp-RNA	+++	+++	+++	-	-
Decapping (mature)	m7Gppp-RNA	++	++	+++	-	-
5’-3’ exoribonuclease	p-RNA	-	+++	+++	+	++++
DeNADding	NppA-RNA	++	++	++++	+++	++

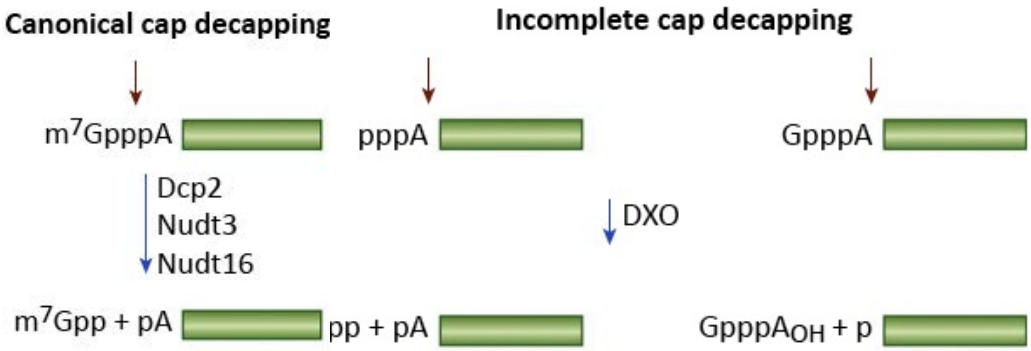
Decapping by Nudix family: m^7G, NAD, NADH, CoA, alarmone caps

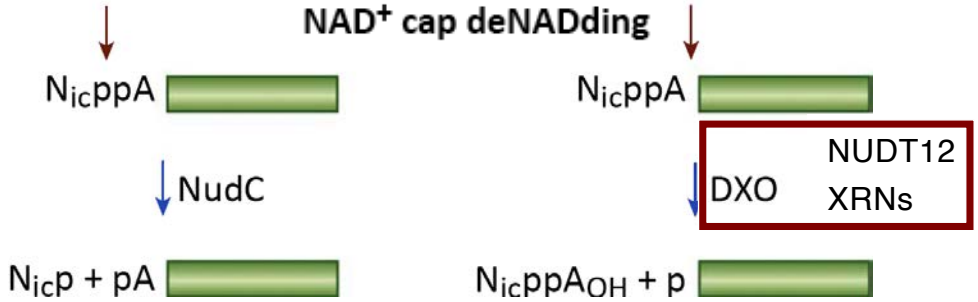
Additional DXO activities: 5’ OH RNA hydrolase; decapping FAD, CoA, NADH

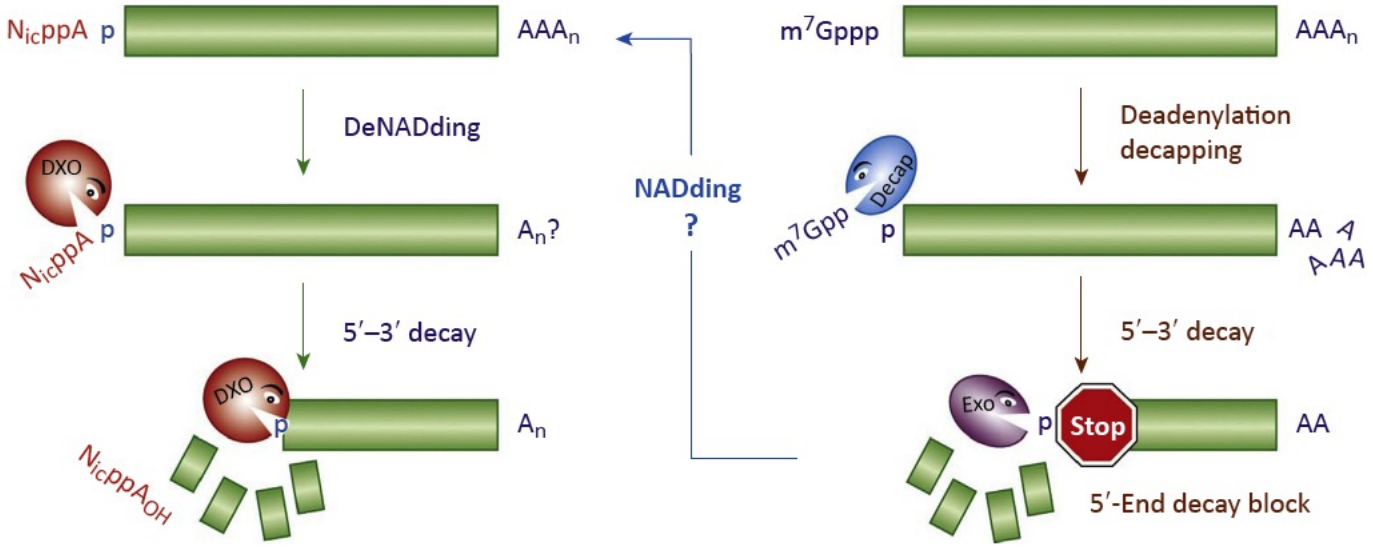
XRN – deNADding, deFADding

RNA 5’-end deNADding: DXO, NUDTs and XRNs



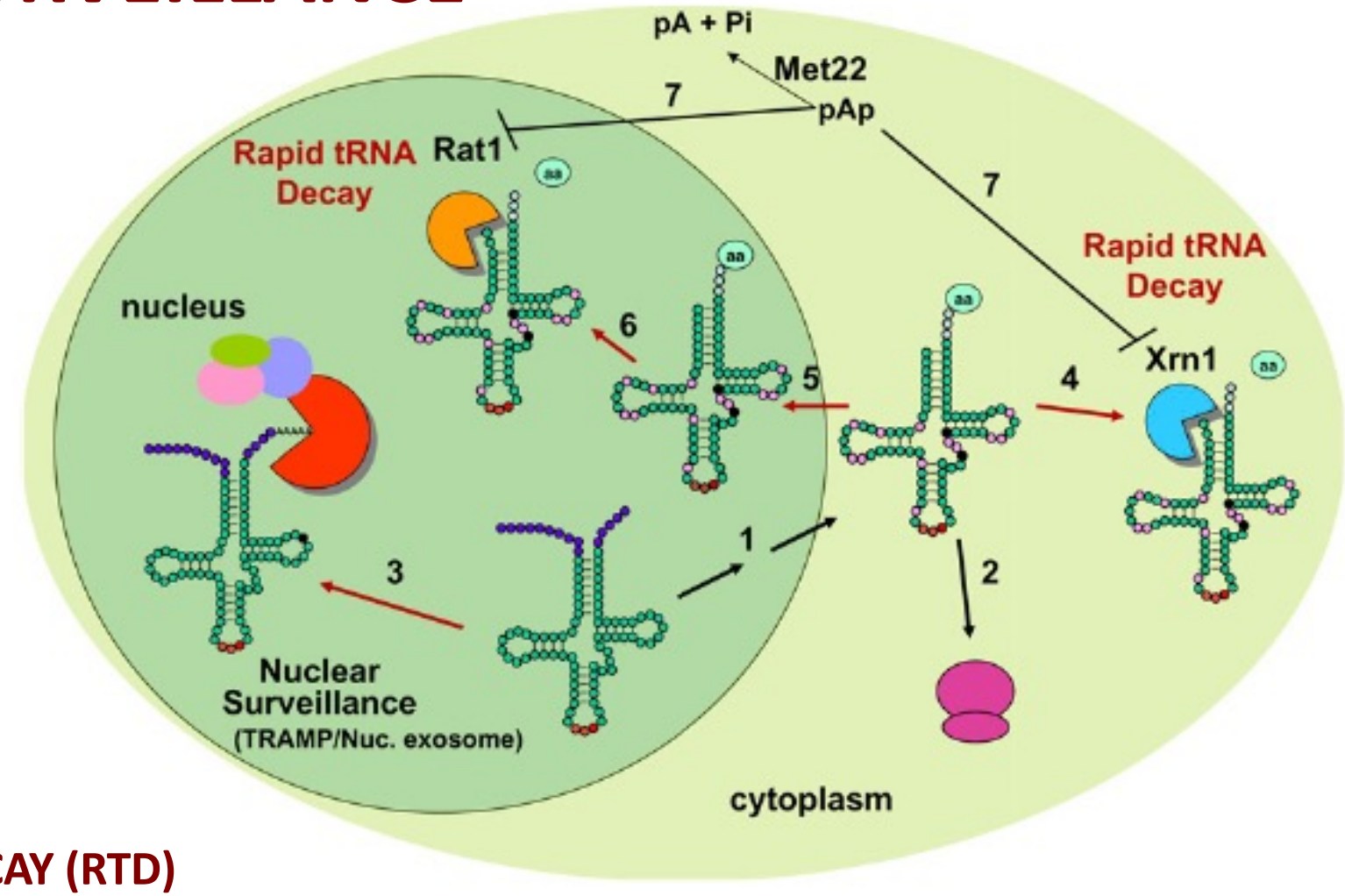






Kiledjian, TiBS 2018

tRNA SURVEILLANCE



RAPID tRNA DECAY (RTD)

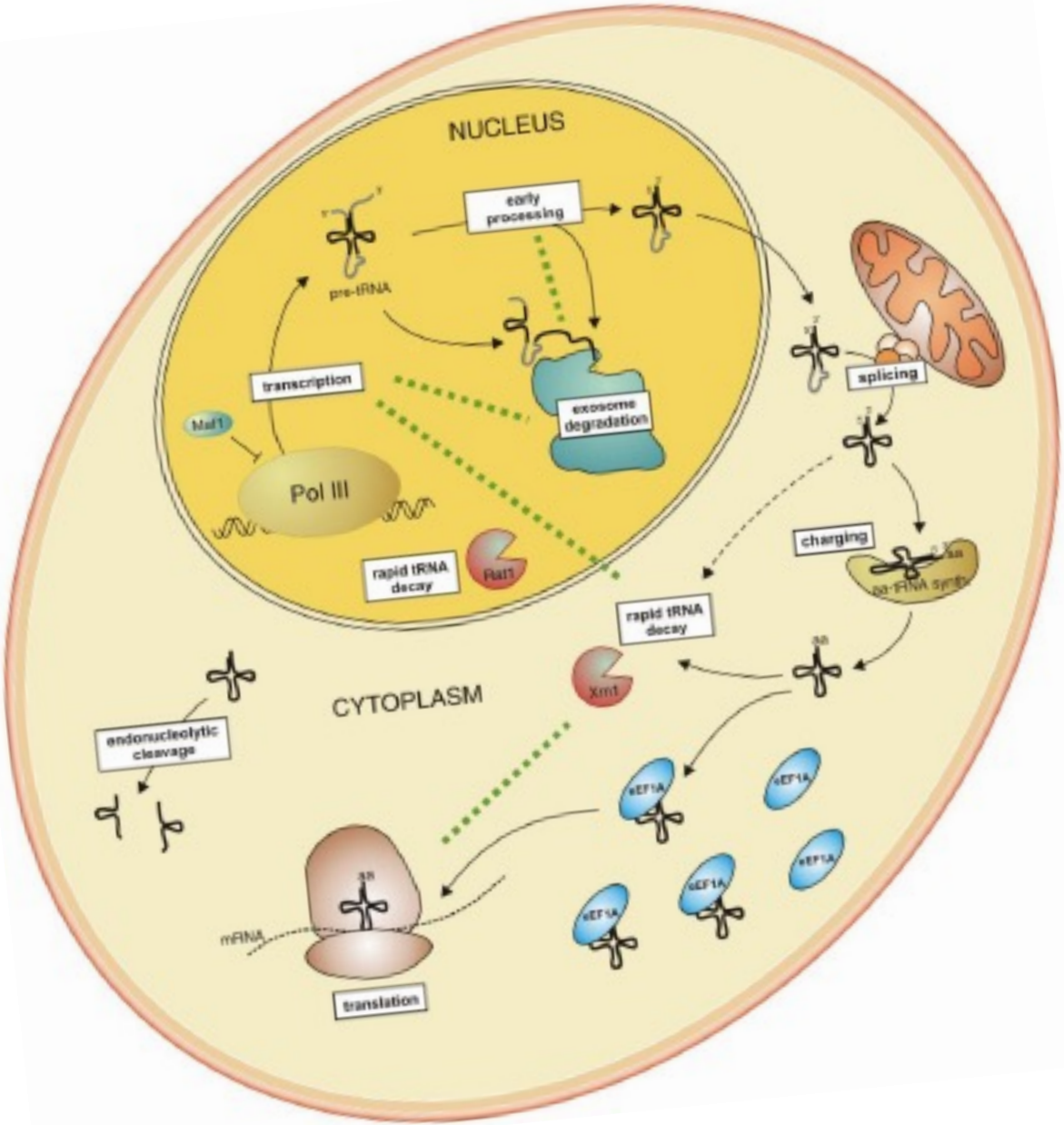
Precursors and mature tRNAs with mutations which destabilize tertiary structure (e.g. lack of modifications)

- in the nucleus: polyadenylation by **TRAMP** and degradation by the **exosome** or **Rat1**
- in the cytoplasm: degradation by **Xrn1** (Xrn1-mediated RTD)

Mature tRNA can be cleaved into tRNA halves under stress

Phizicky and Hopper , GeneGev.,2010

tRNA synthesis and degradation



Wichtowska, Turowski, Boguta, WIREsRNA, 2013

- **tRNA primary transcript** synthesized by RNA Pol III, regulated by Maf1
- **Initial processing in the nucleus**:

5’ leader and 3’ trailer removed

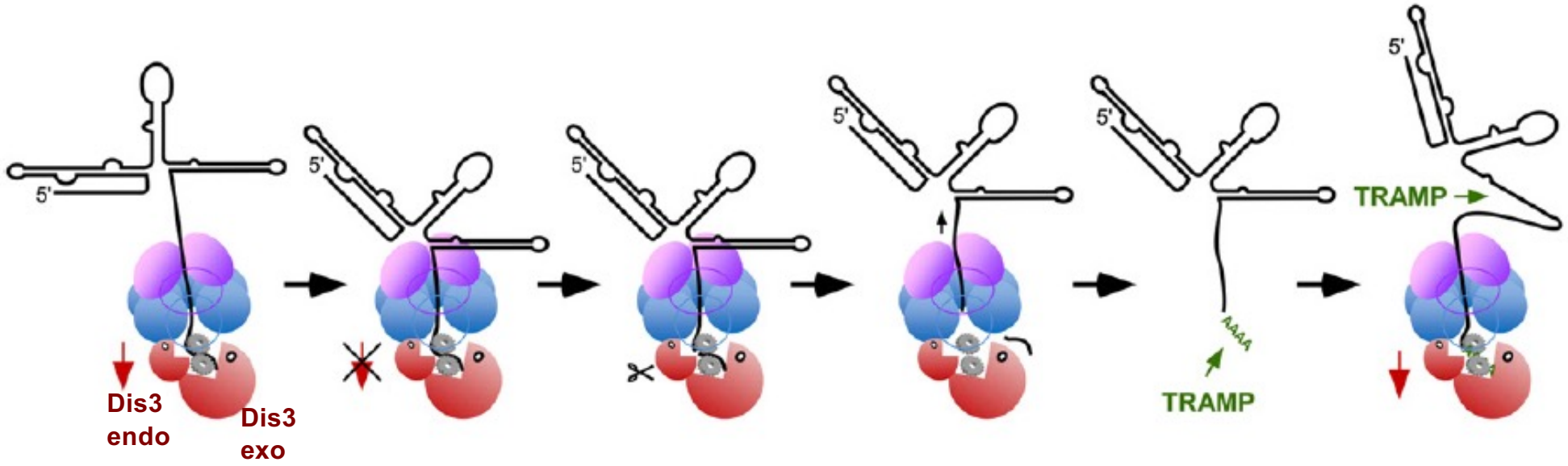
- **pre-tRNA exported to the cytoplasm**
- **CCA** on the 3’ terminus **modifications** added **to** pre-tRNA
- **Intron spliced out** on the outer surface of the mitochondrial membrane
- **tRNA charged** by tRNA synthetase, bound by elongation factor eEF1A and delivered to ribosomes for translation
- **tRNA turnover**:

In the nucleus pre-tRNAs degraded by the exosome or by Rat1 in RTD

In the cytoplasm mature tRNAs degraded by Xrn1-mediated RTD.

Mature tRNA cleaved into tRNA halves under stress

pre-tRNAs are degraded by the exosome



The endo activity of Dis3 contributes to the degradation of structured RNAs

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,*]

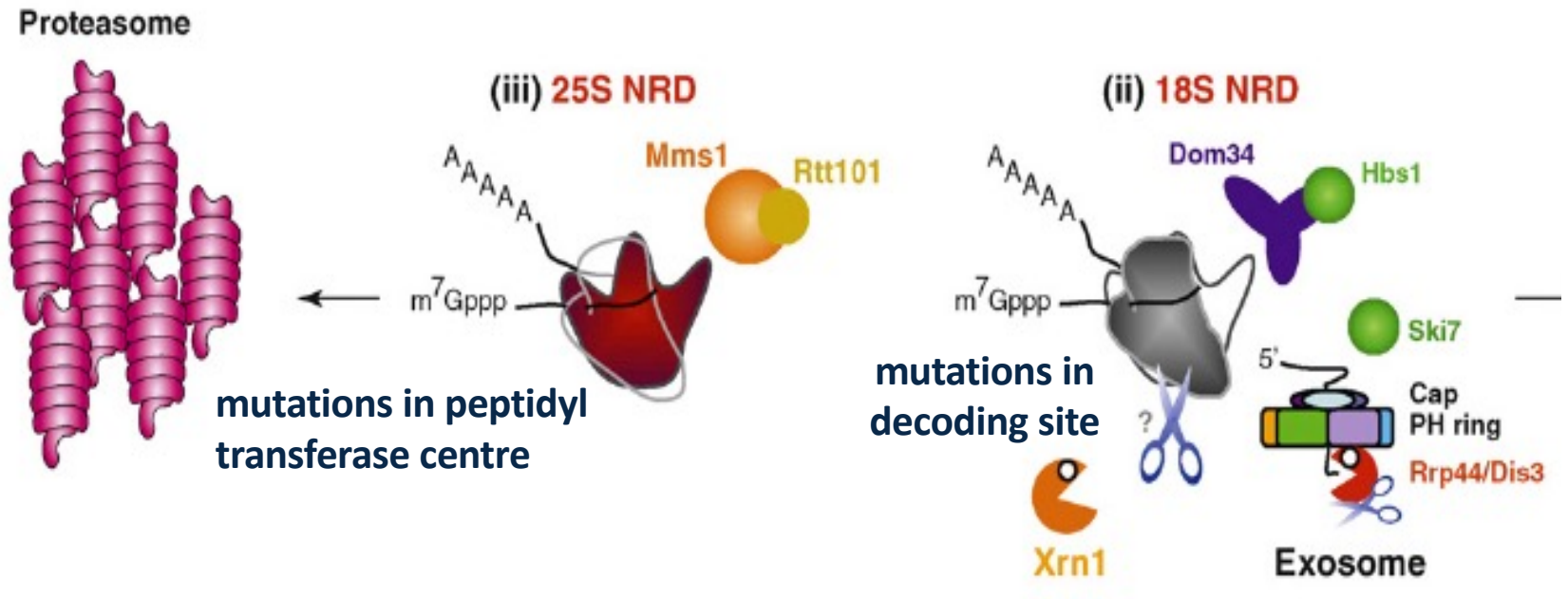
Extensive Degradation of RNA Precursors by the Exosome in Wild-Type Cells

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

NRD- Nonfunctional rRNA Decay

Mature aberrant ribosomes are eliminated in the cytoplasm

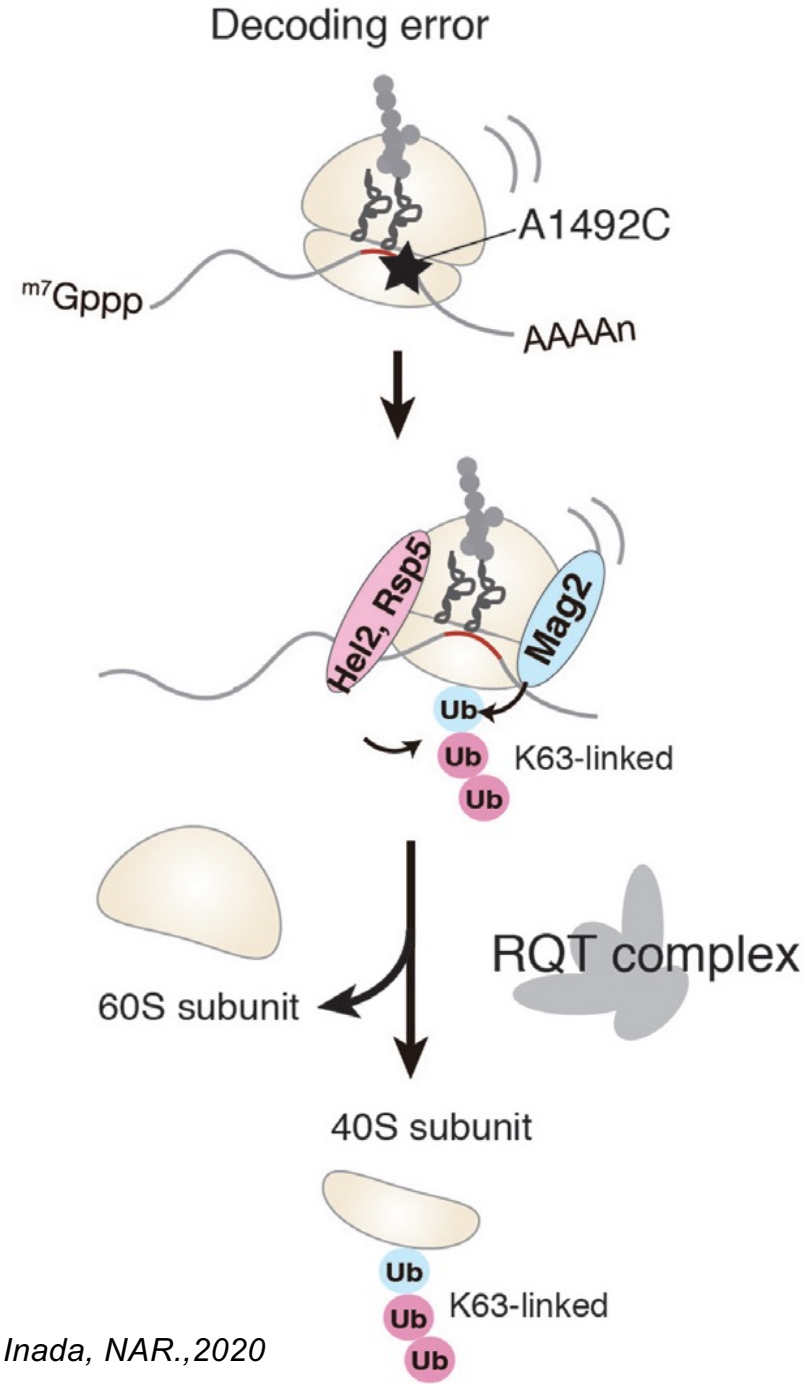


Mms1, Rtt101-
subunits of E3 ubiquitin ligase complex

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

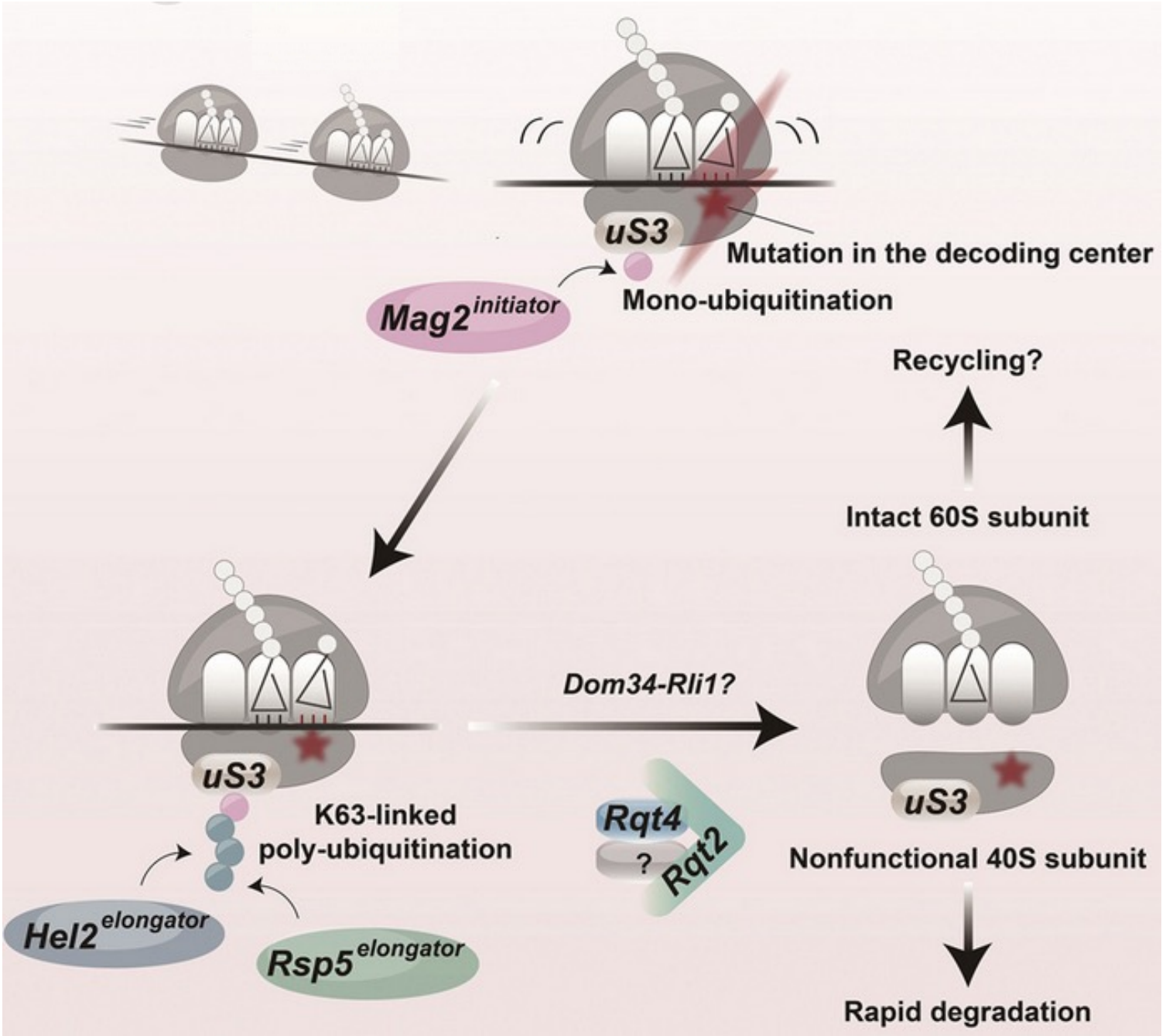
Ribosome ubiquitination
K212 of RPS3 is monoubiquitinated by Mag2 followed by polyubiquitination by Hel2 or Rsp5

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

18s rRNA degradation
by Xrn1 or exosome

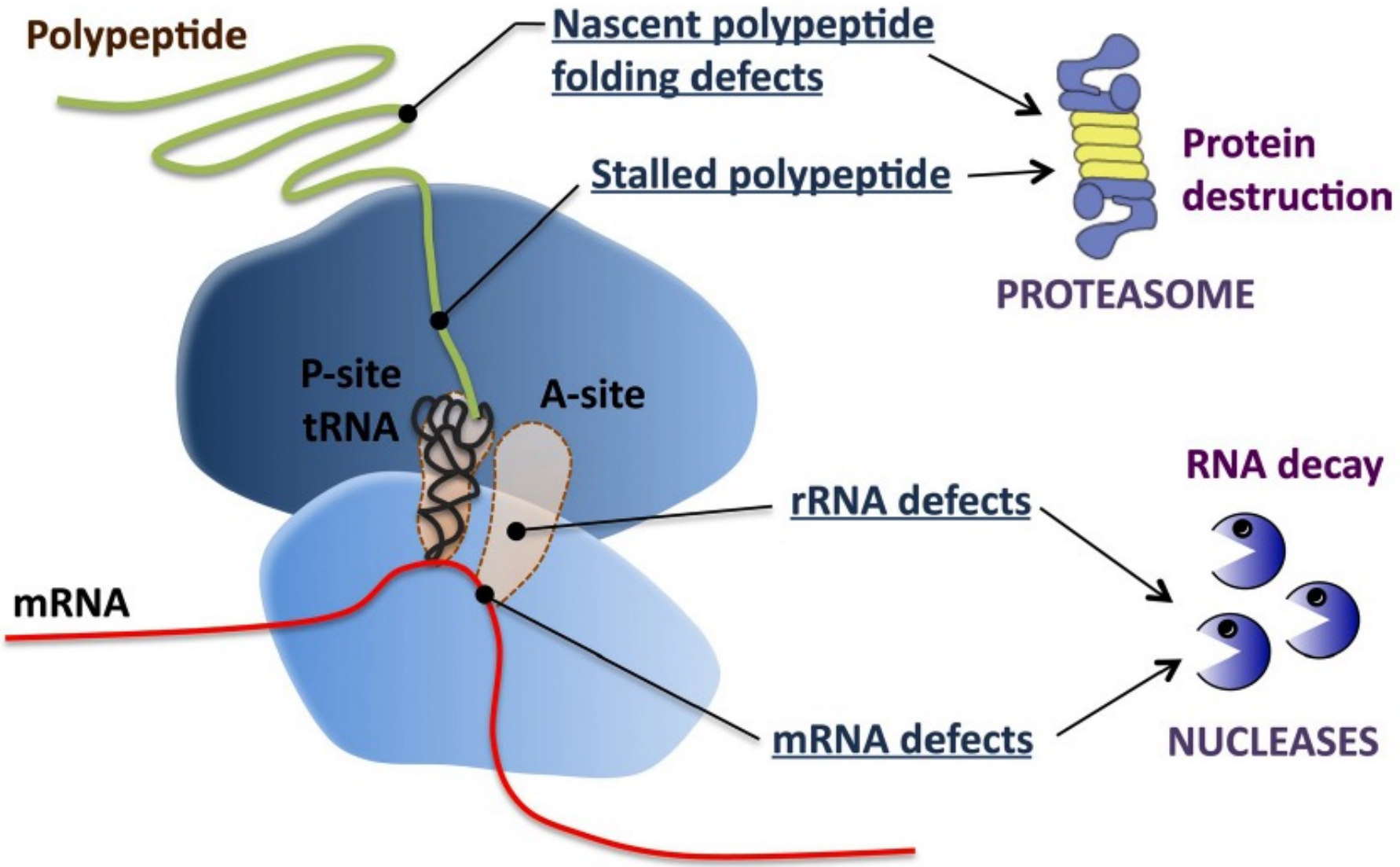
Factors involved in 18S NRD are also involved in RQC

18S NRD



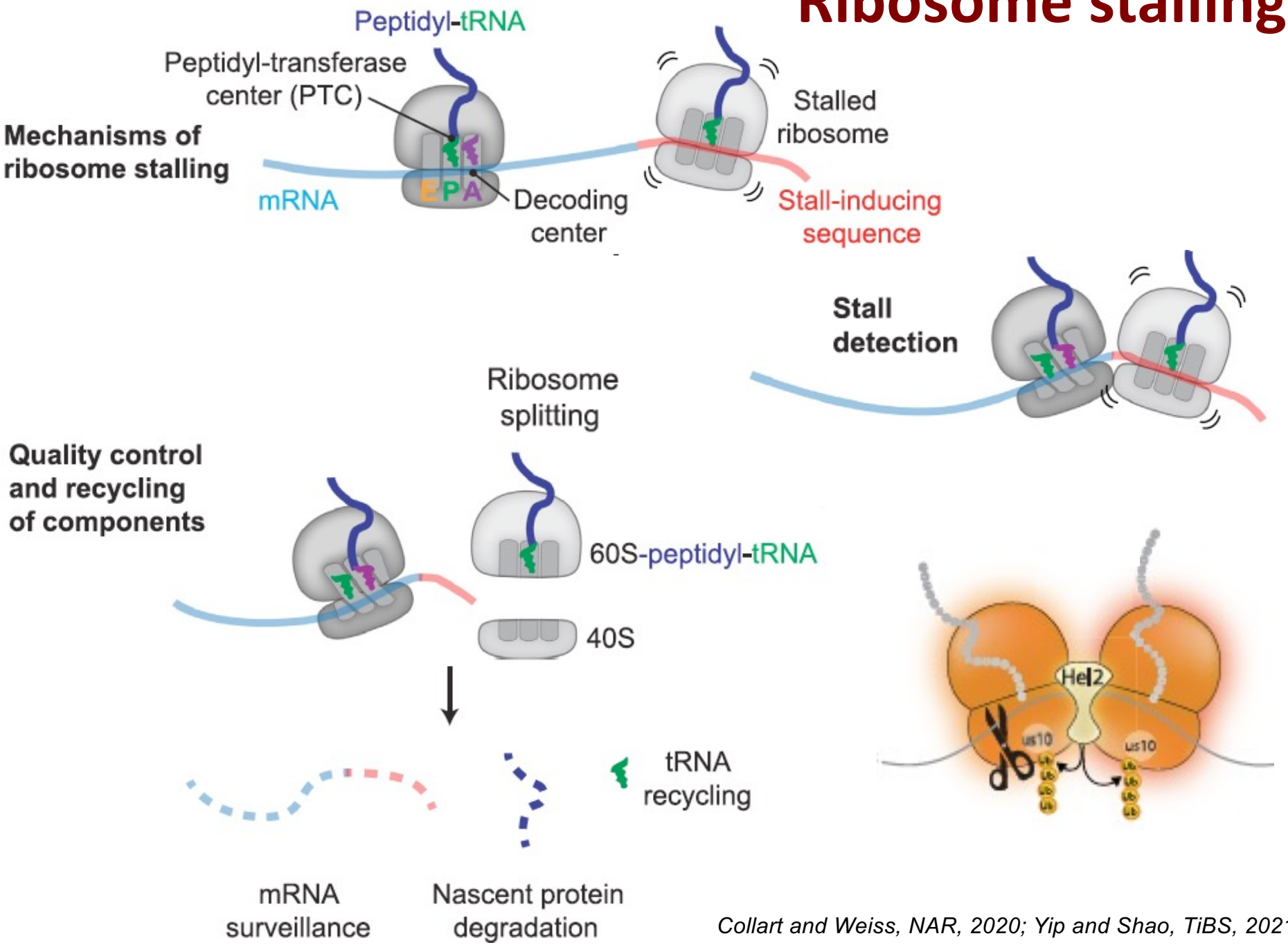
Sugiyama, Cell Rep.,2019

Co-translational ribosome, peptide and mRNA QC



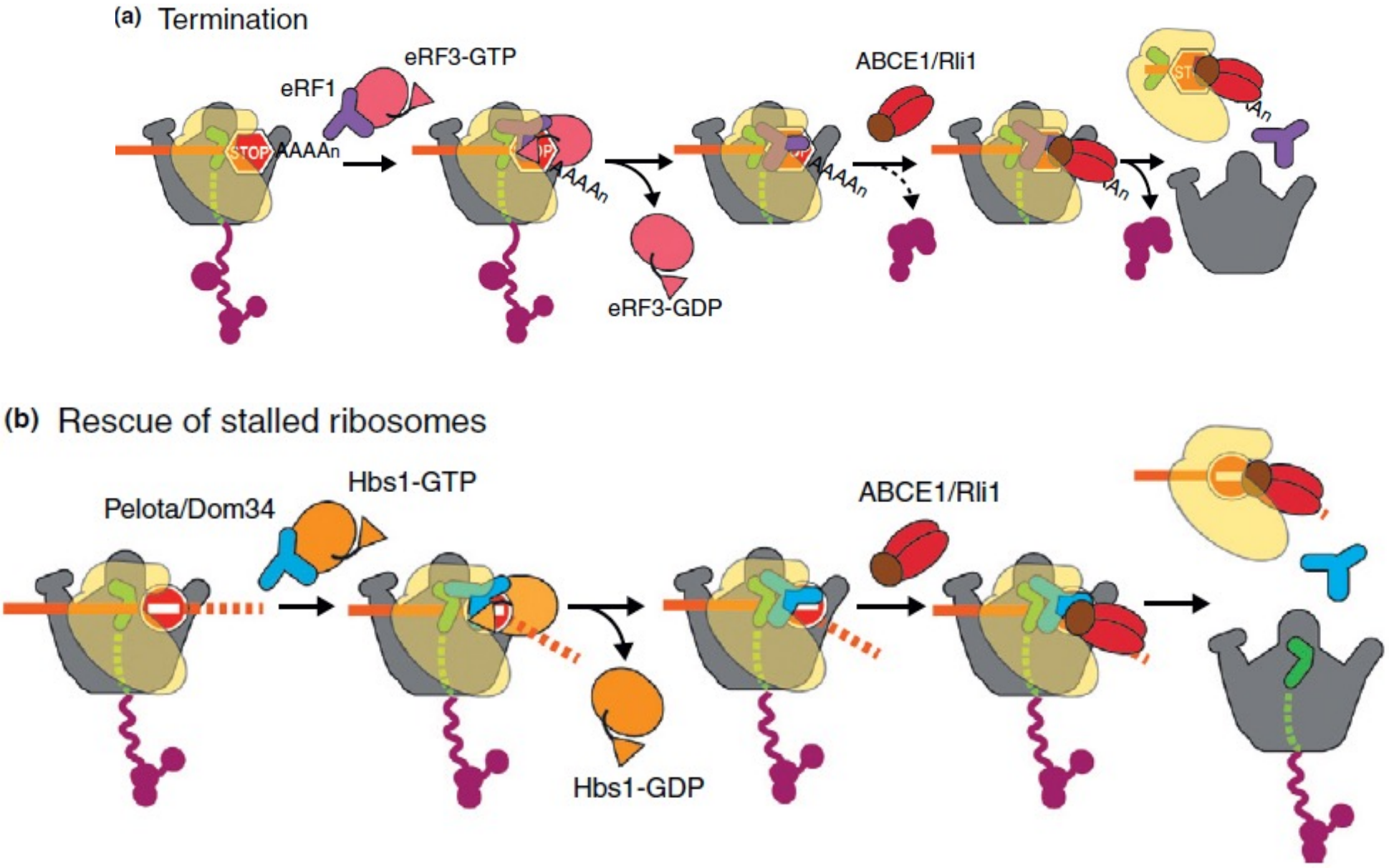
Lykke-Andersen and Bennett, JCB, 2014

Ribosome stalling



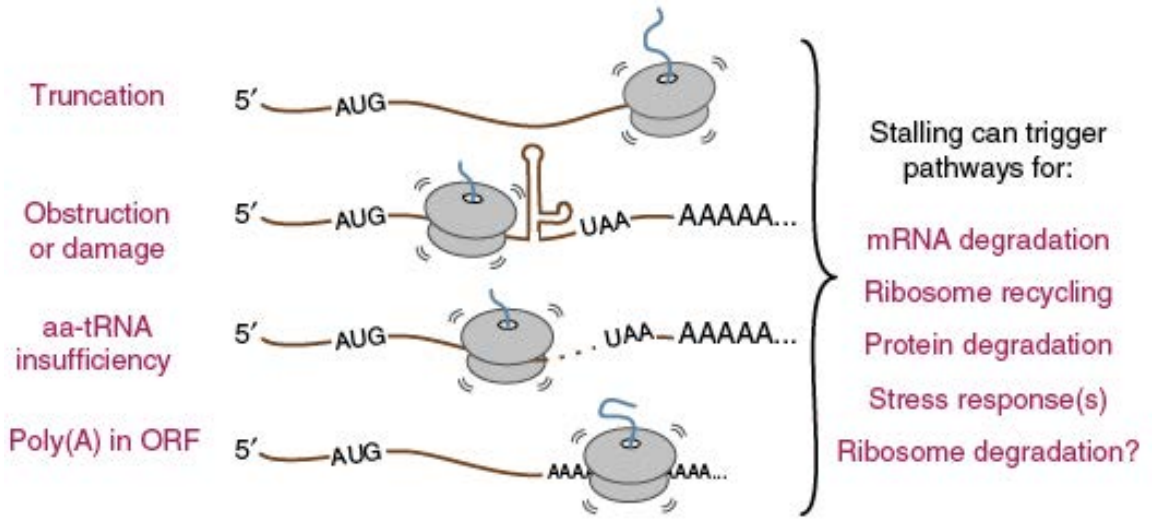
Collart and Weiss, NAR, 2020; Yip and Shao, TiBS, 2021

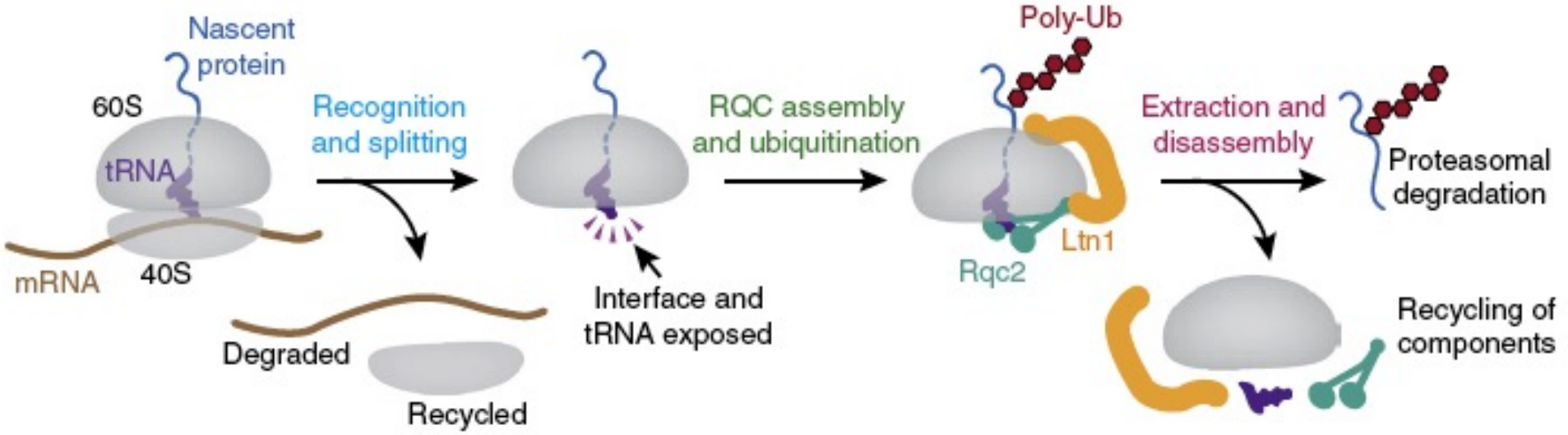
Rescue of stalled ribosomes



Franckenberg et al., Curr Op Struct Biol 2012

RIBOSOME QC (RQC)

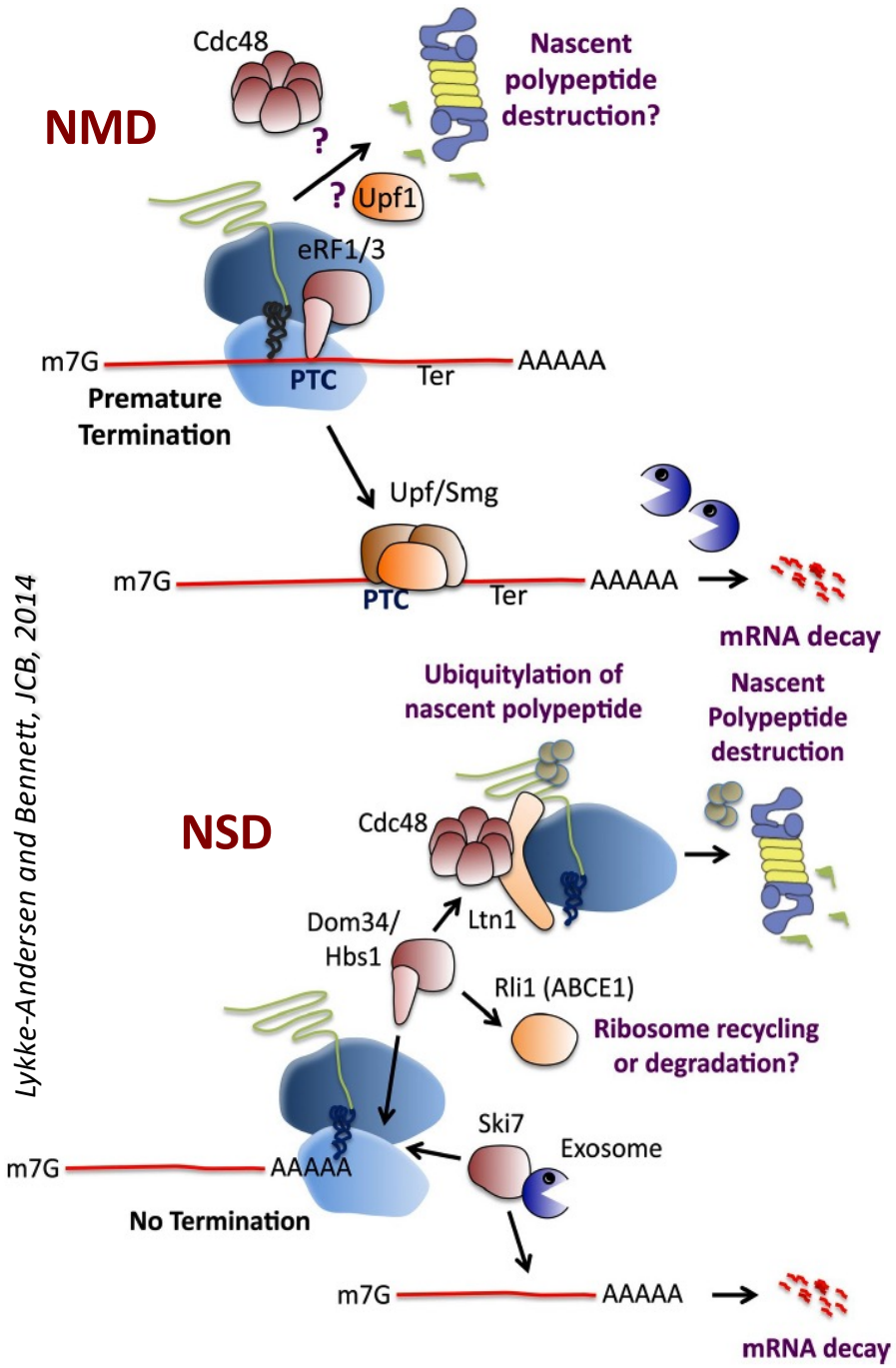


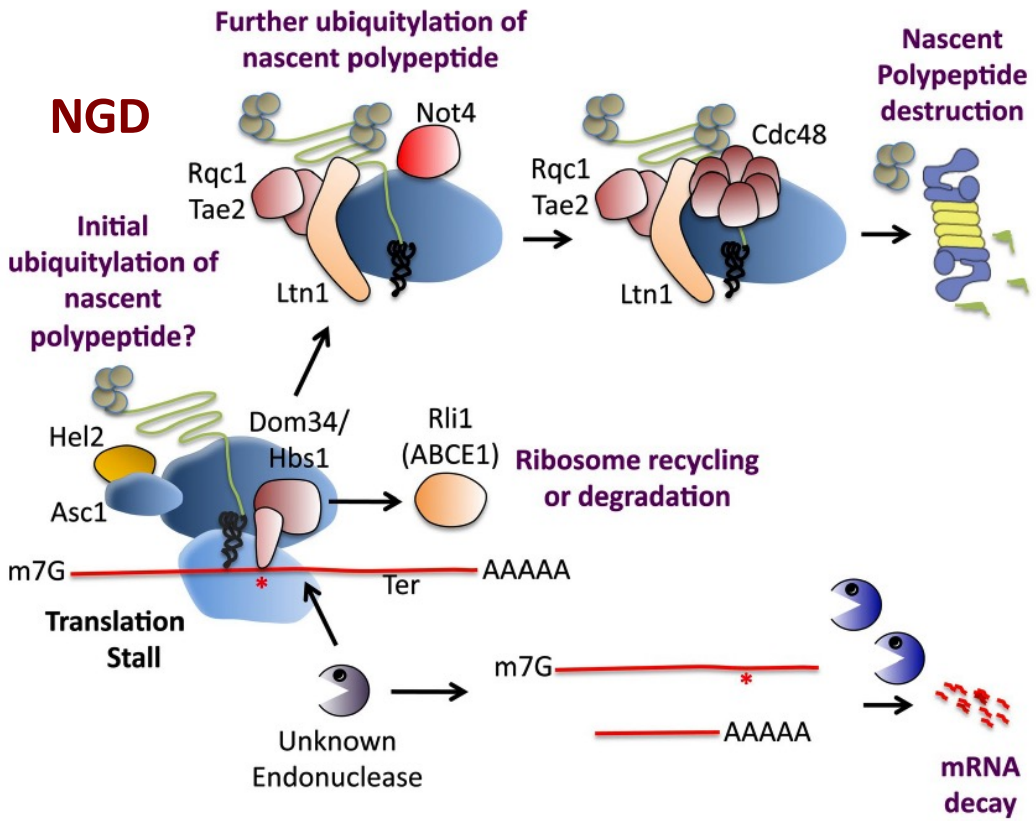


Yeast	Asc1	Hel2	Dom34	Hbs1	Rli1	Rqc2	Ltn1	Rqc1	Cdc48–Ufd1–Npl4		
Mammals	RACK1	ZNF598?	Pelota	Hbs1	ABCE1	NEMF	Listerin	TCF25?	VCP complex?		
	Facilitates stalling?		Ribosome splitting			Nascent-chain ubiquitination		Nascent-chain extraction			

Brandman and Hegde, NatStrMolBiol 2016

Co-translational QC





RQC- ribosome QC complex

Ltn1, Cdc48, Tae2, Rqc1

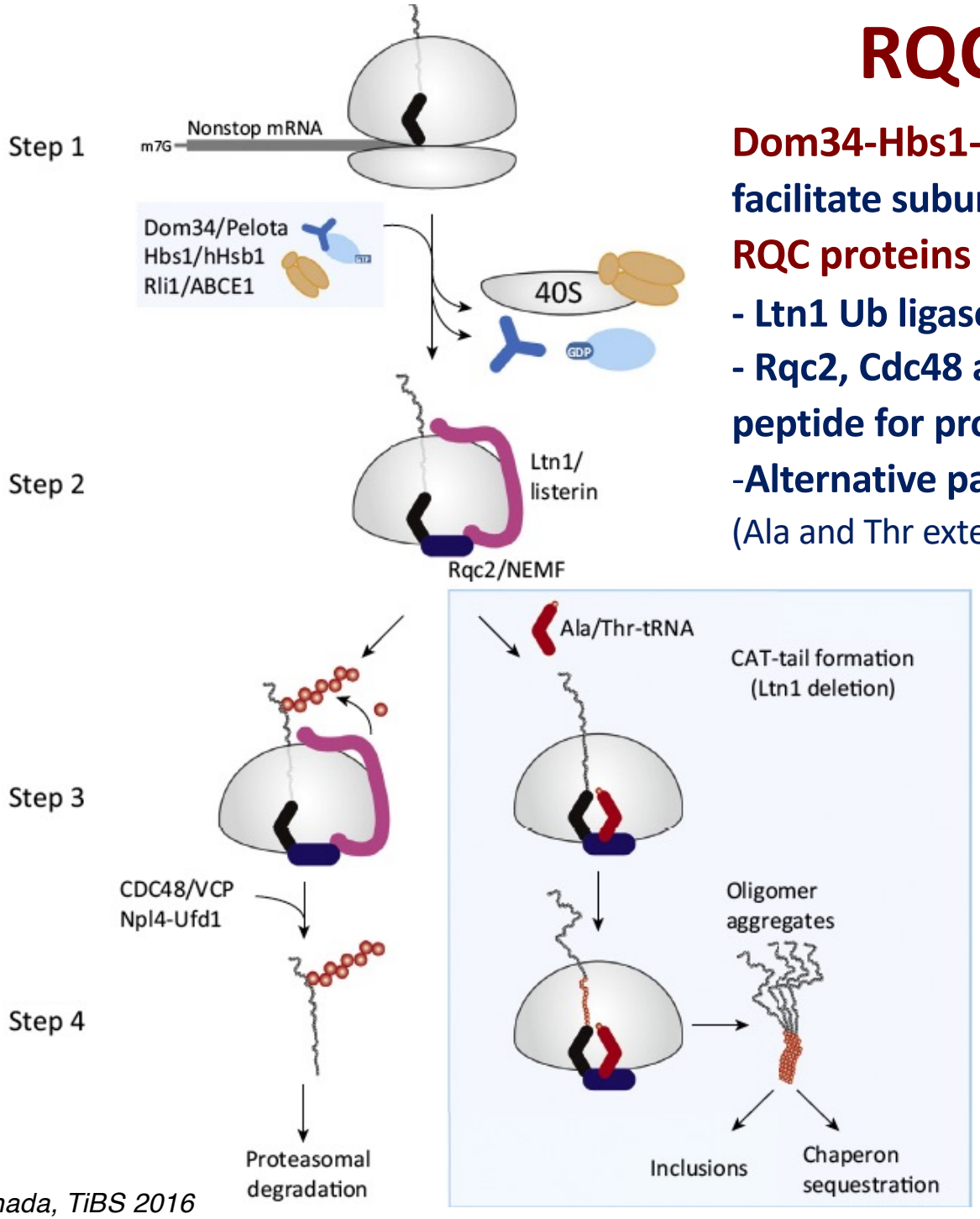
Ribosome release - Dom34/Hbs1/Rli1

Ubiquitin pathway components-

Ltn1, Cdc48, Not4

Hel2, Asc1 (target nascent protein chain)

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

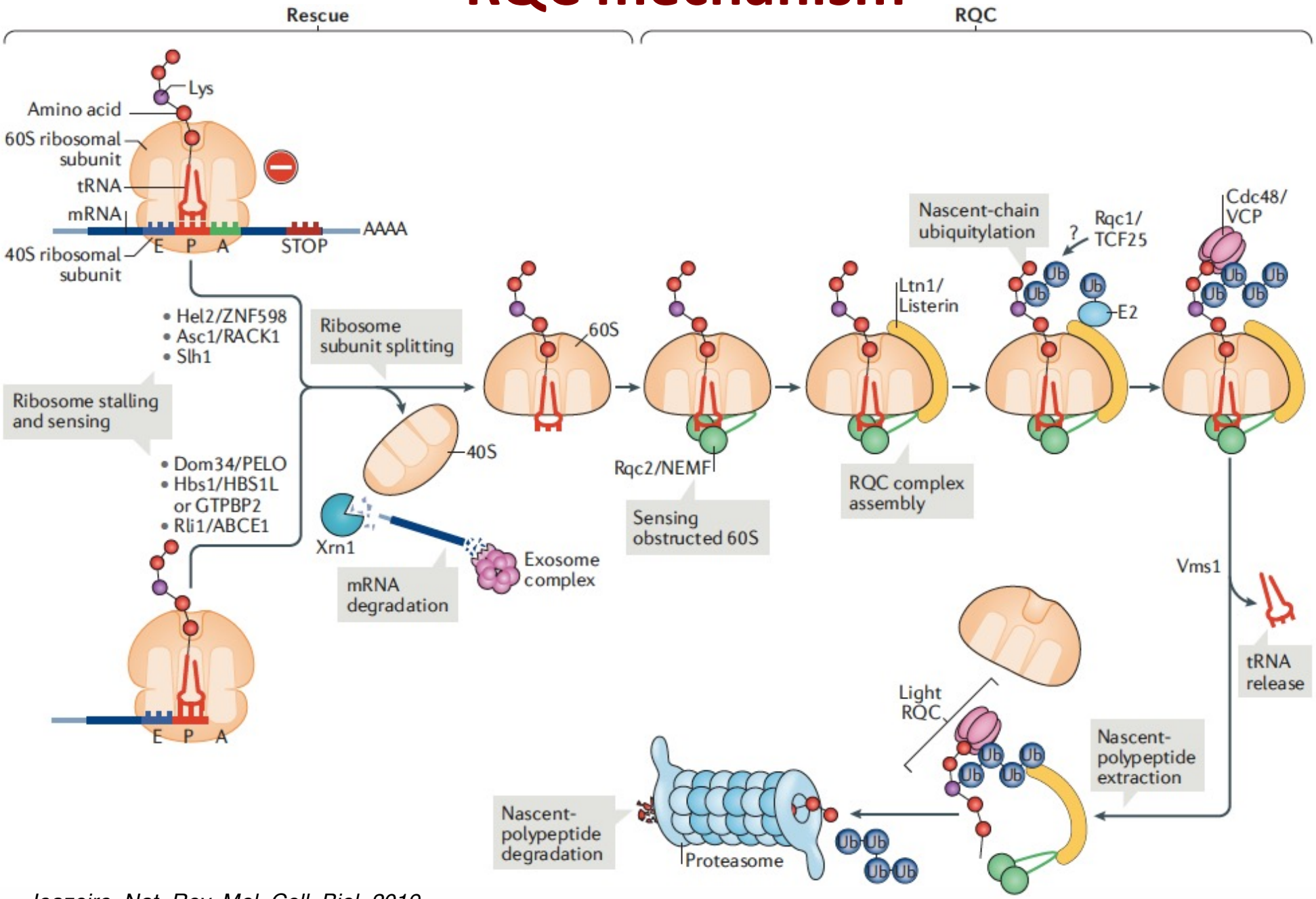
- Ltn1-dependent degradation of aberrant proteins

- nascent chain aggregation

- activation of stress signaling

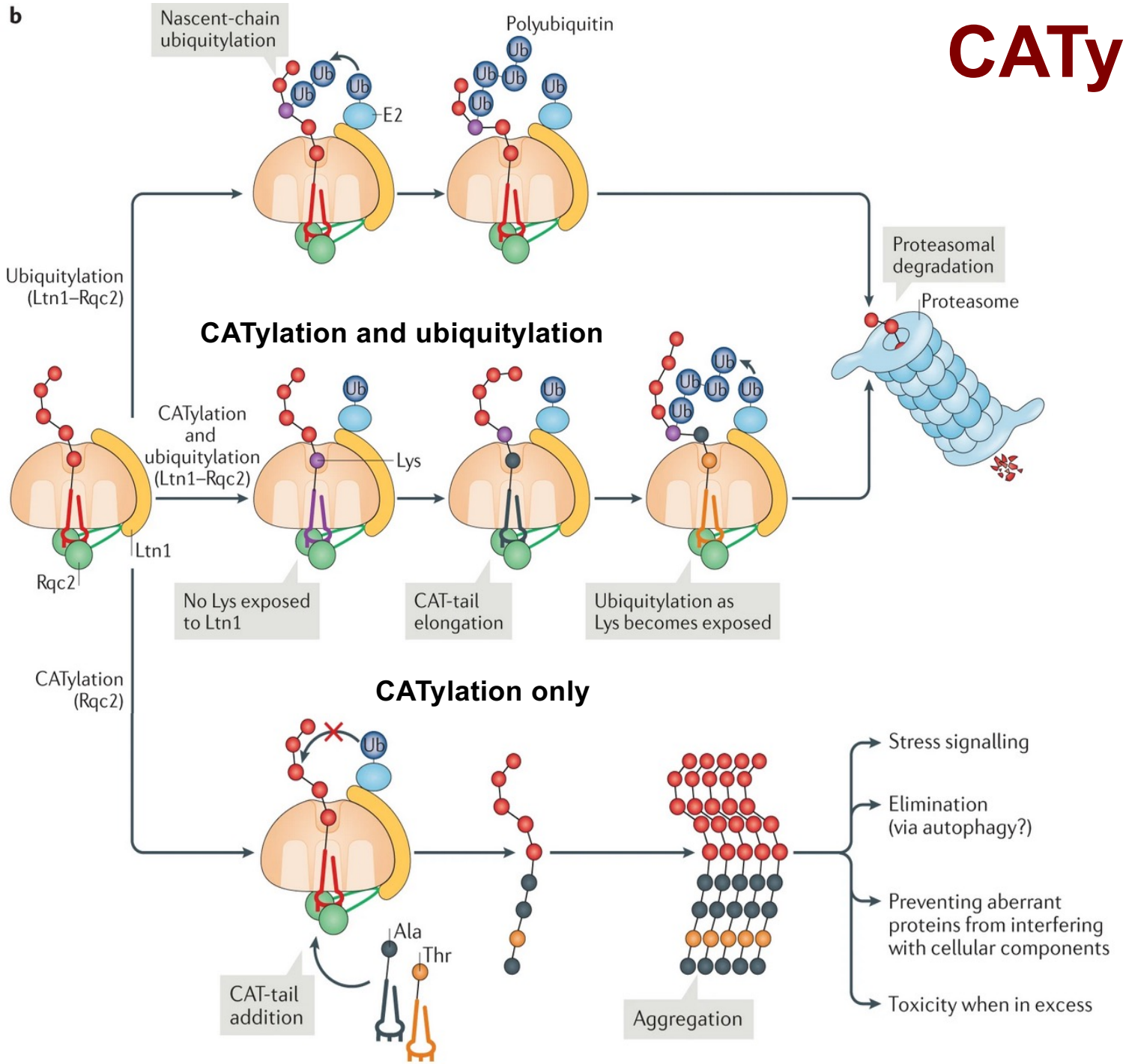
- nascent chain proteolysis

RQC mechanism



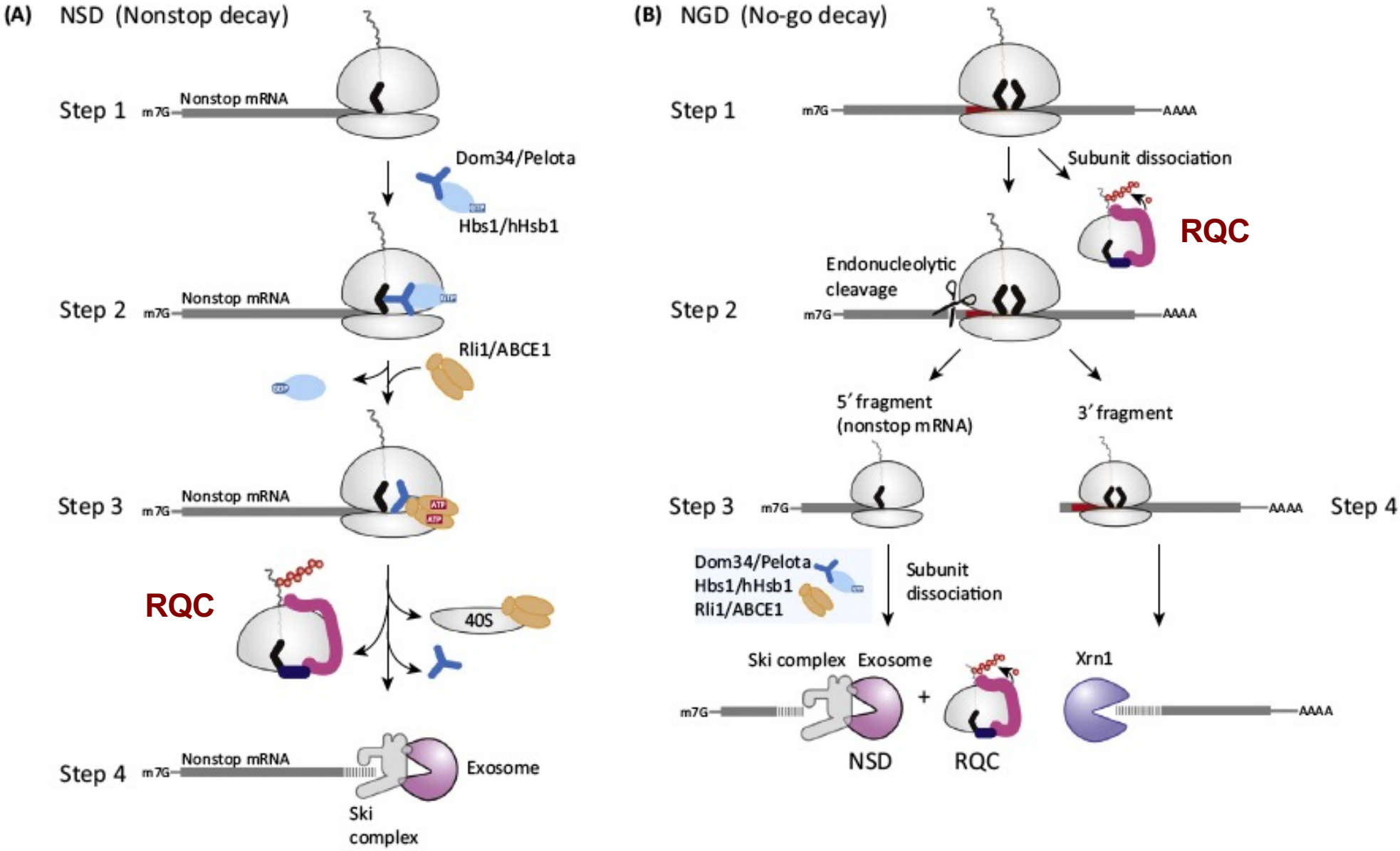
Joazeiro, Nat. Rev, Mol. Cell. Biol. 2019

CATylation



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

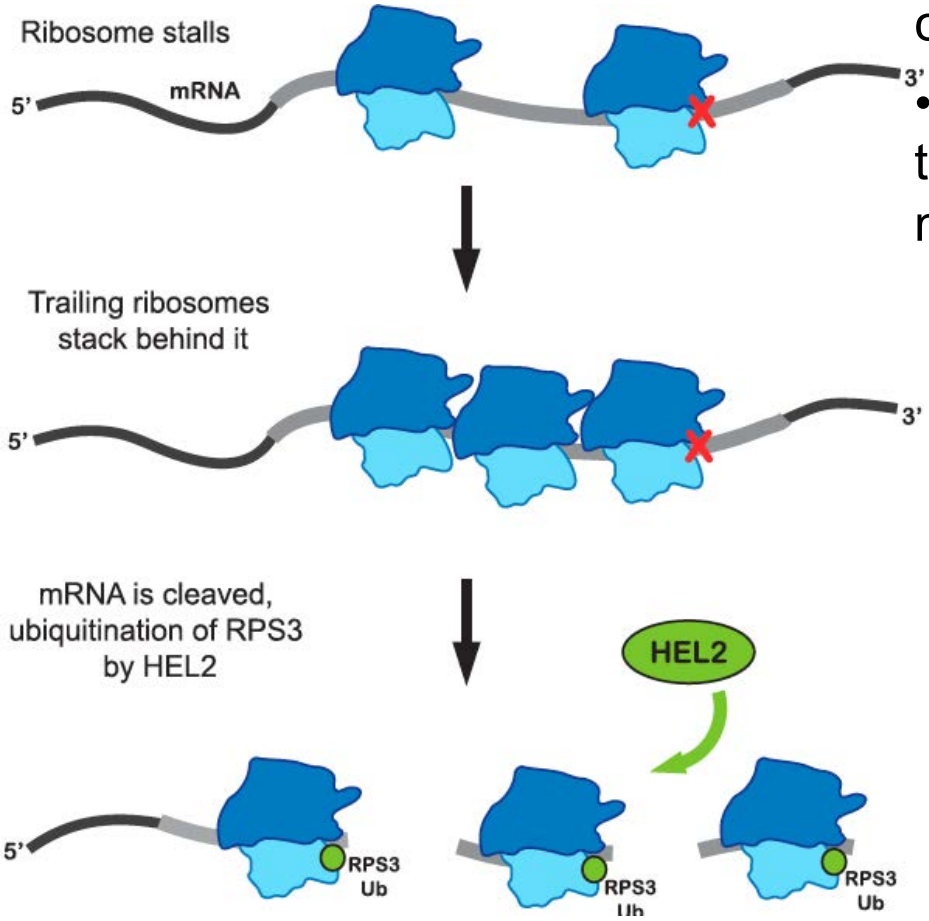
RQC in NSD and NGD



Inada, TiBS 2016

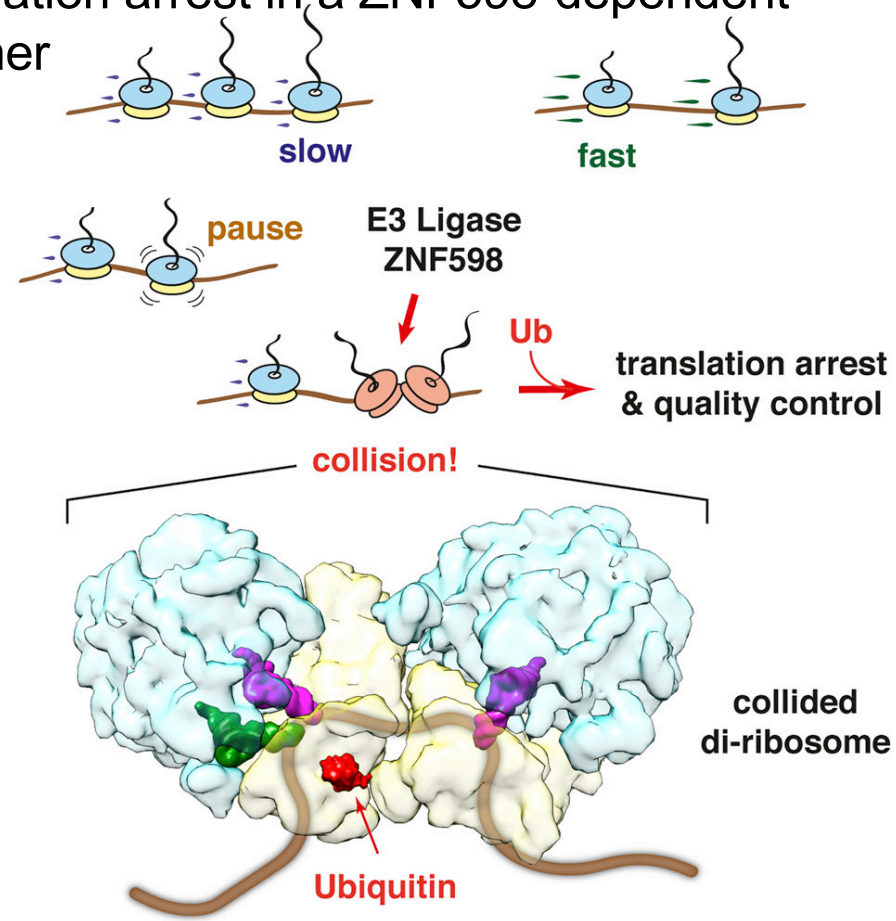
Ribosome collision in RQC during NGD

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

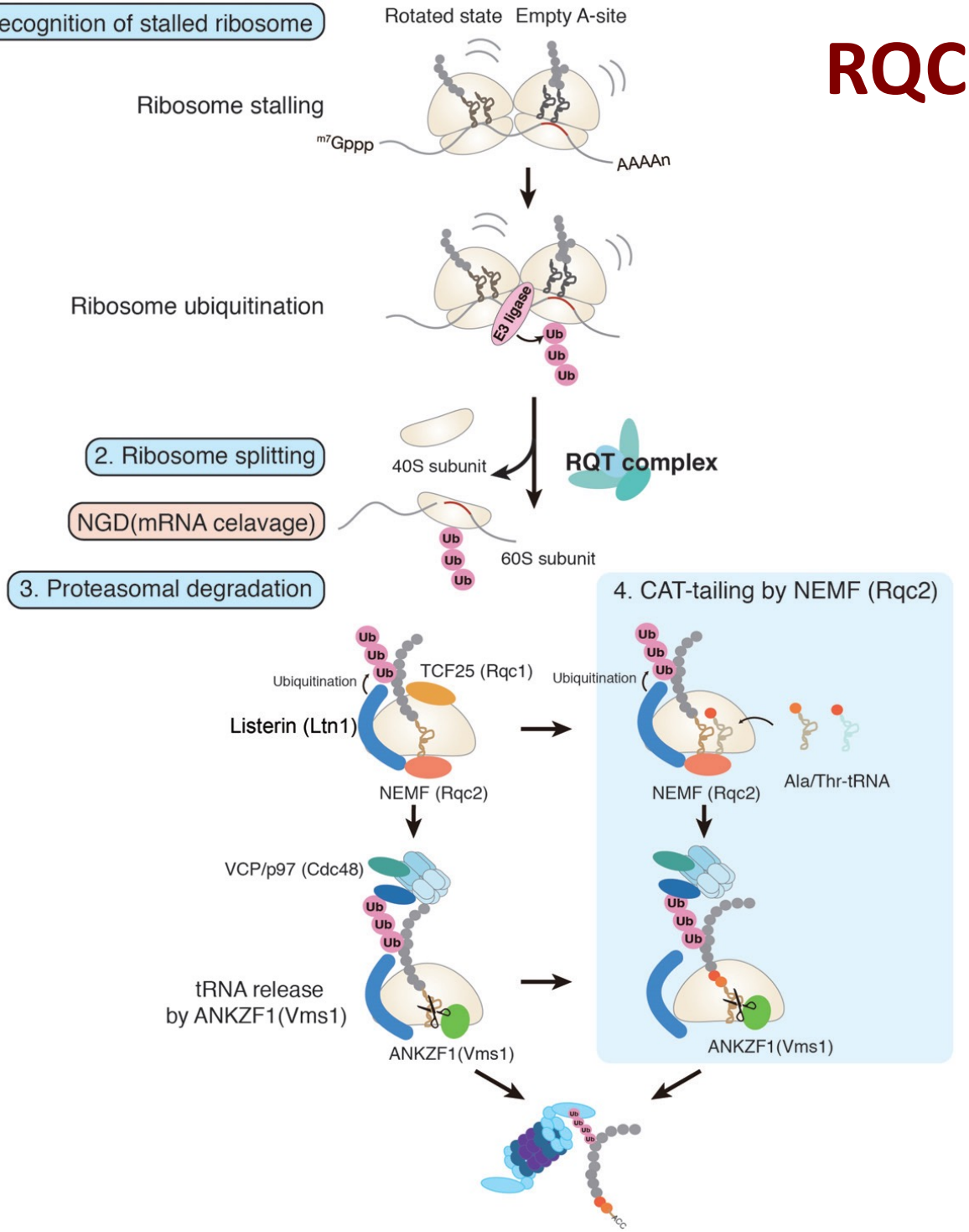


Simms et al, Mol Cell 2017; Juszkiewicz et al, Mol Cell 2018

- 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



RQC mechanism

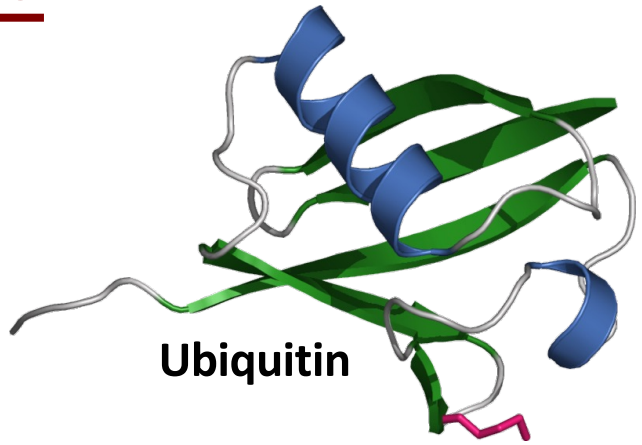


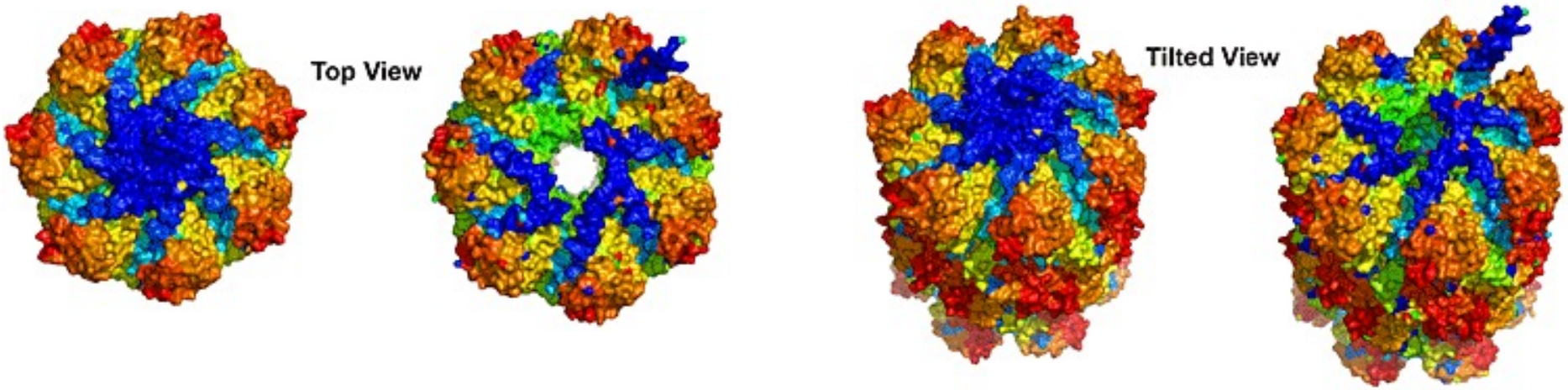
Inada, NAR.,2020

PROTEIN DEGRADATION:

UBIQUITINATION

PROTEASOME

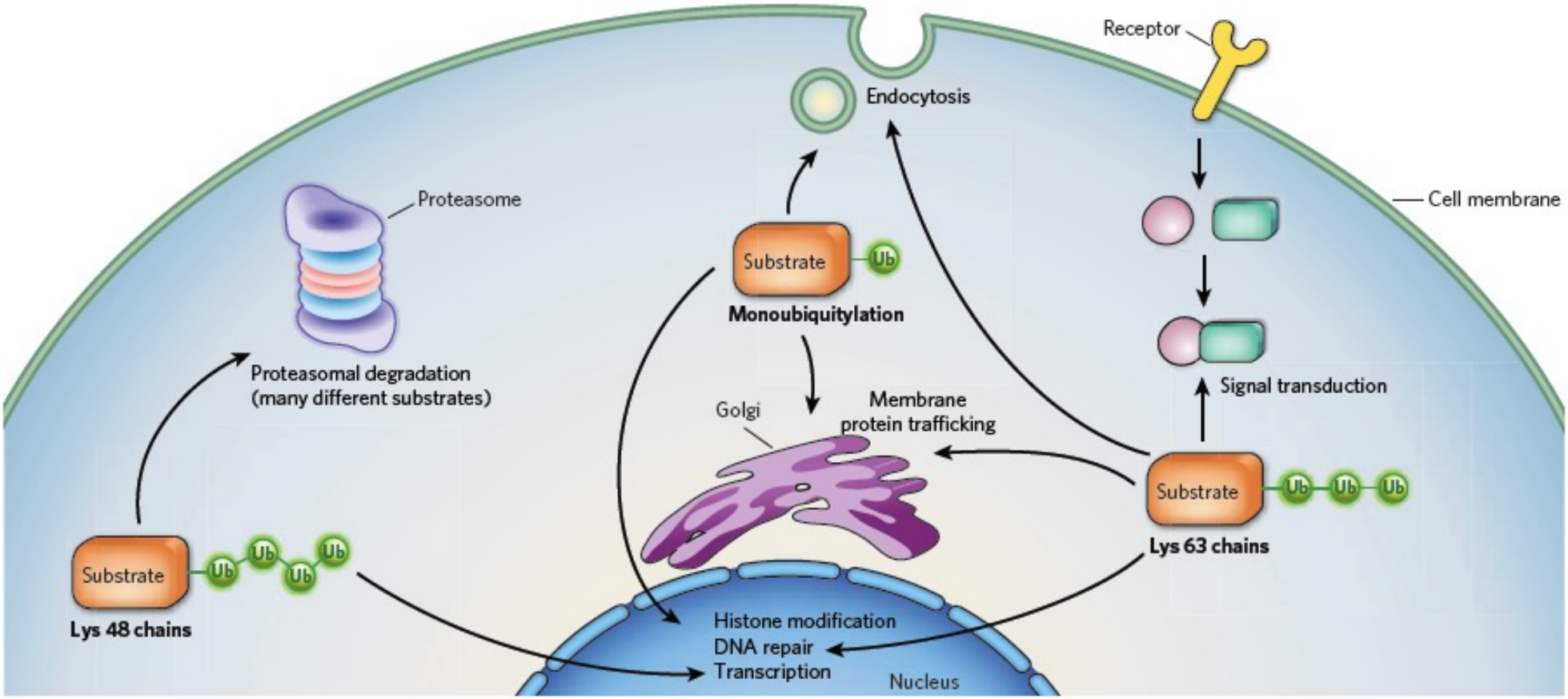




Regulation of specific proteins by proteolytic destruction

Occurs in the cytoplasm and the nucleus

Processes regulated by ubiquitination



Hochstrasser., Nature, 2011

UBIQUITIN (Ub)

- **highly conserved 76 aa polypeptide**

  (3 aa differences between yeast and human homologues)

- **C-Terminal Gly residue is activated via an ATP to form a thiol ester**

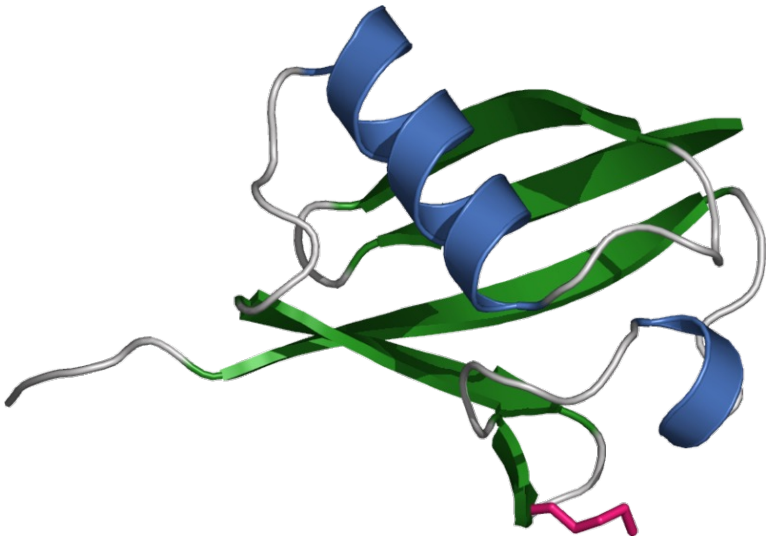
```
1-MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIF-45
1-MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIF-45
1-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIF-45
1-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIF-45

46-AGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-76
46-AGKQLEDGRTLADYNIQKESTLHLVLRLRGG-76
46-AGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-76
46-AGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-76
```

Fission yeast

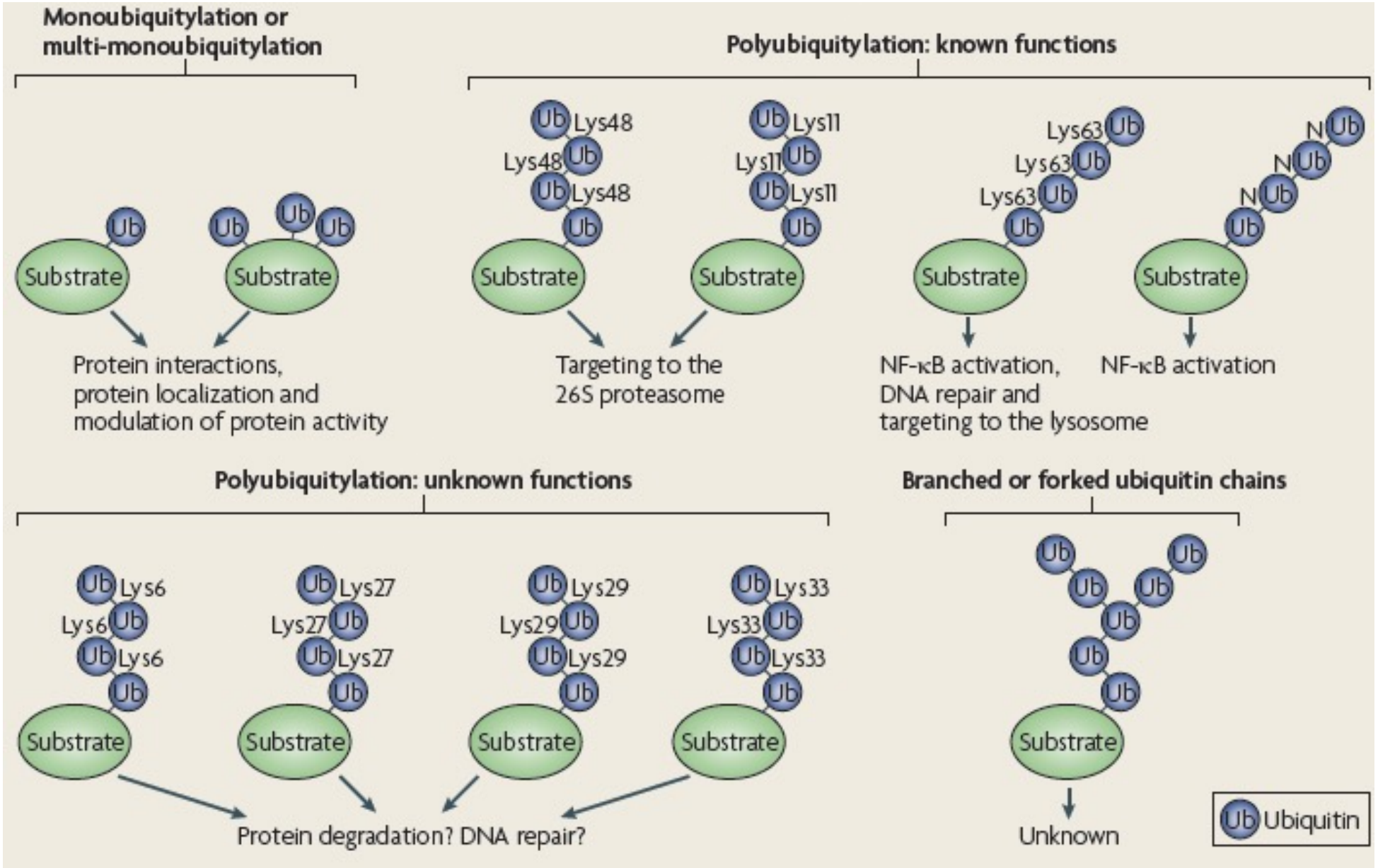
Green pea

fruitfly

human

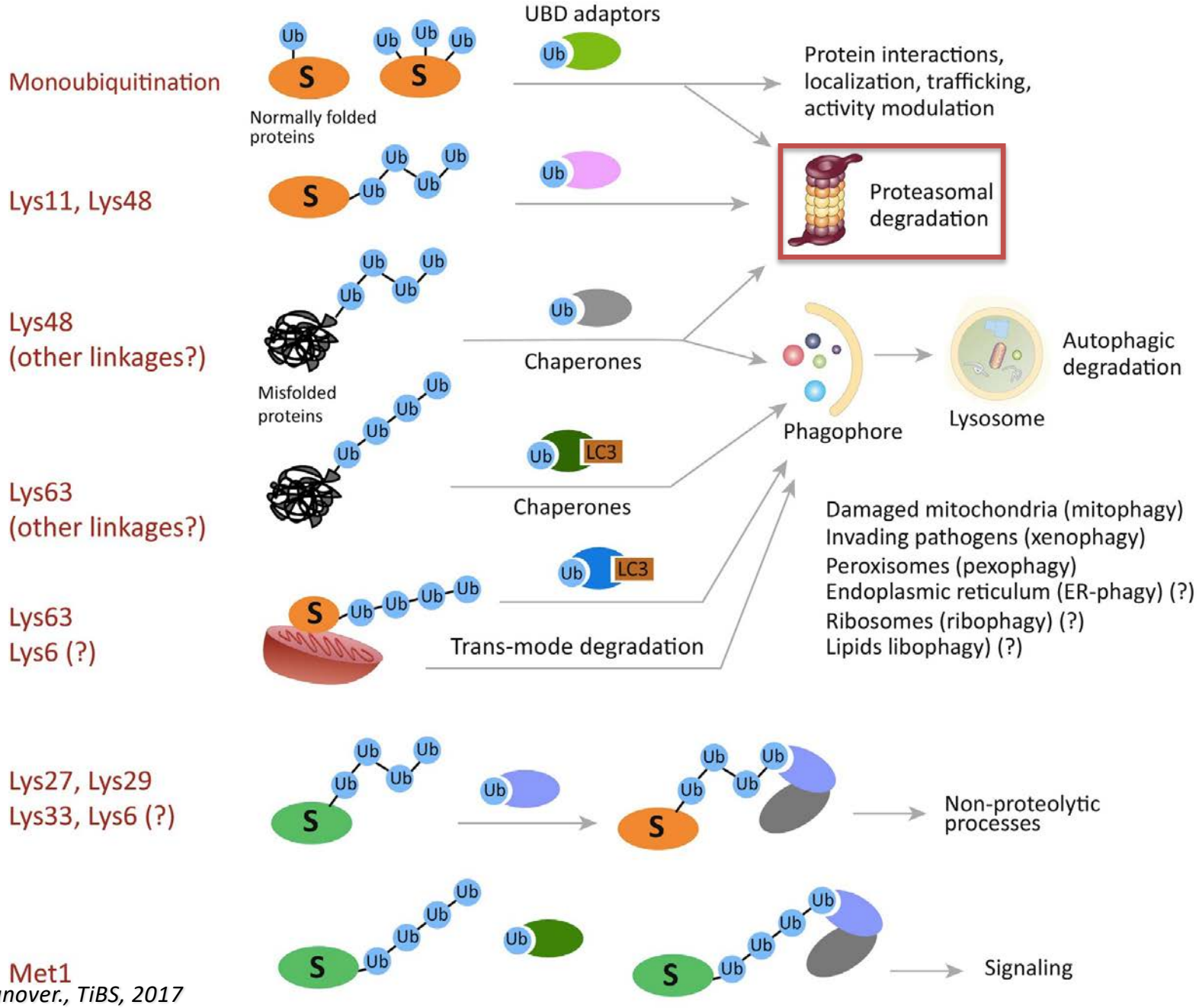


Ub chains



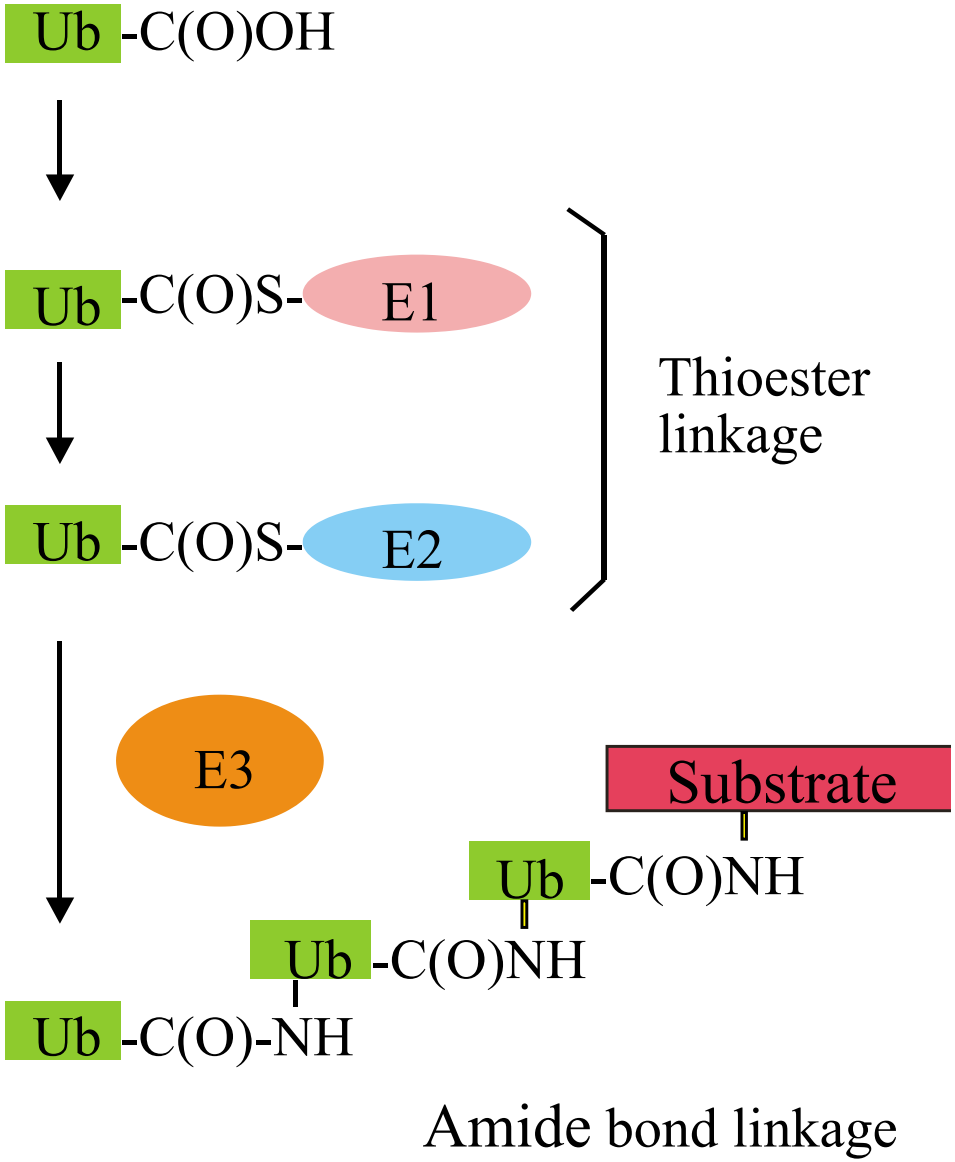
Ye and Rape., Nature Rev Cel Mol Biol, 2009

Function of Ub chains



Kwon and Ciechanover., TiBS, 2017

Ub Chain assembly



3-step Ub conjugation

Ub activating enzyme E_1	High energy thiol ester is formed between C-terminal Gly of ubiqutin and a Cys in the E_1 active site (ATP/AMP)
Ub conjugating enzymes E_2	Ub is transferred to a Cys of E_2 forming a new thiol ester
Ub ligase E_3	Ub forms isopeptide bond between C-terminal Gly of Ub and ε-amino group of Lys on a target protein

Increasing level of regulatory specificity:

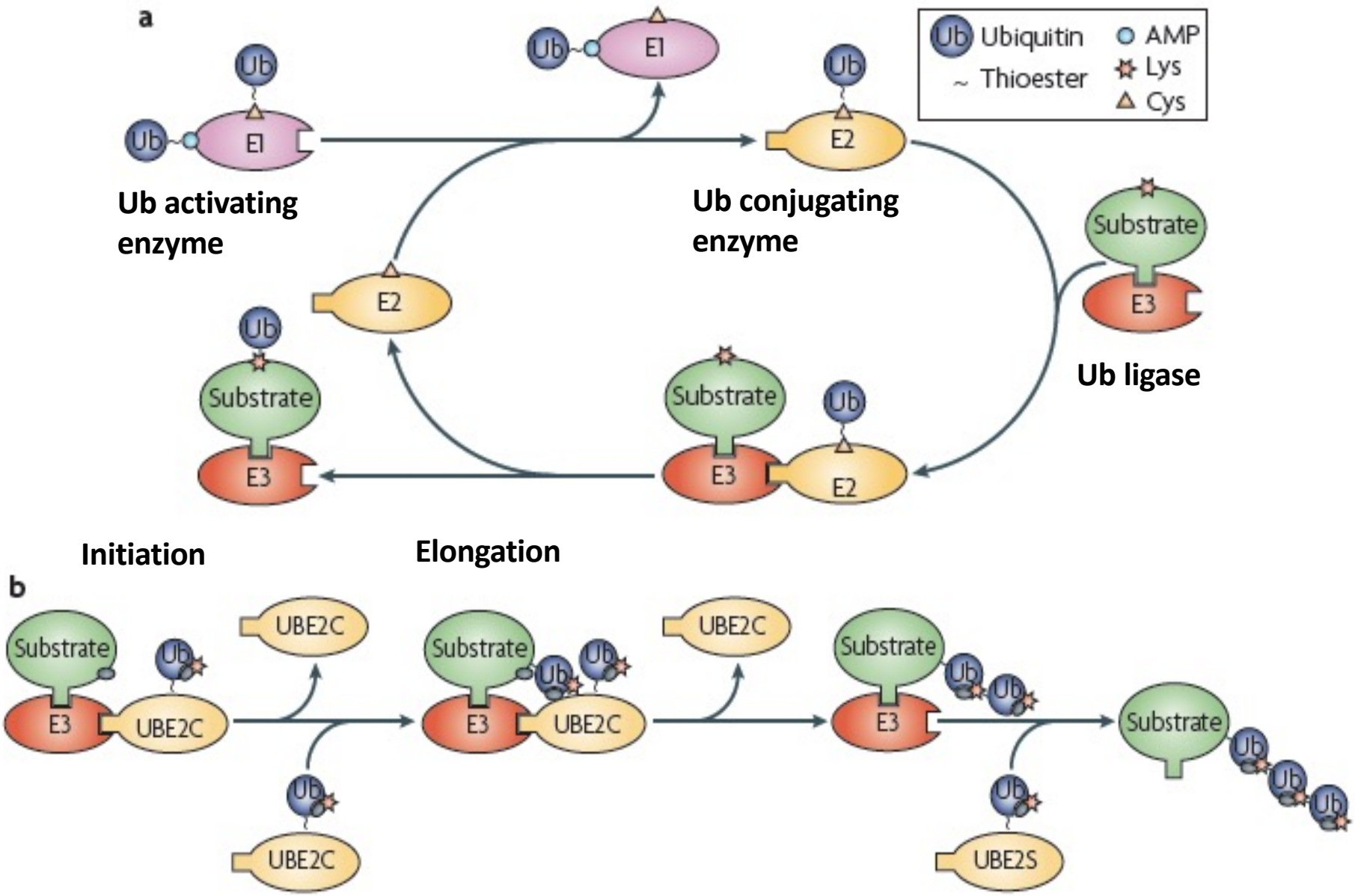
E1: 1

E2: 10-12 (homologous family)

E3: many and structurally unrelated: RING, F, HECT classes

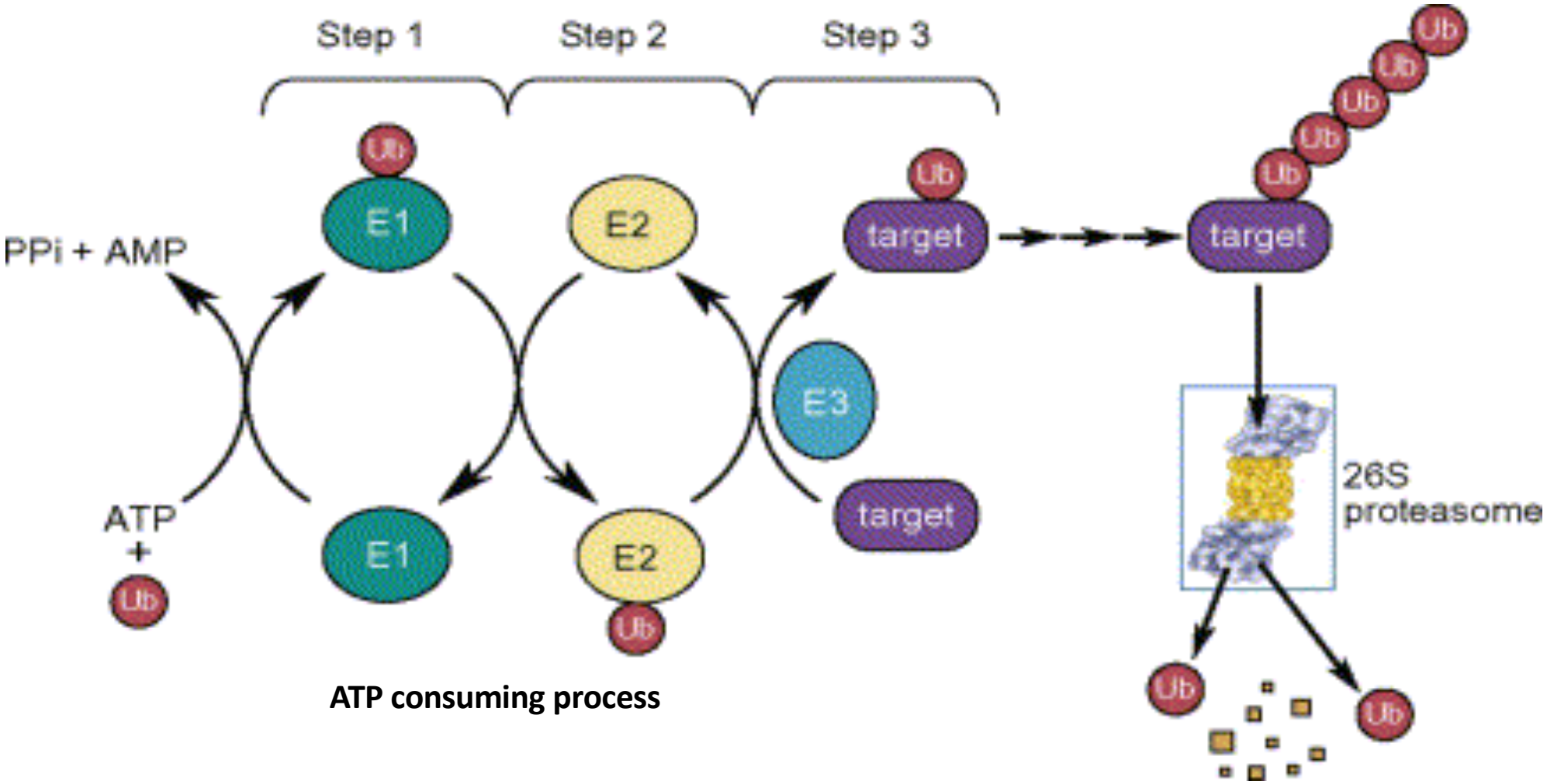
UBIQUITINATION

Covalent attachment of multiple ubiquitins (Ub) to a substrate via Lys48 in Ub



Ye and Rape., Nature Rev Cel Mol Biol, 2009

Protein degradation via ubiquitination

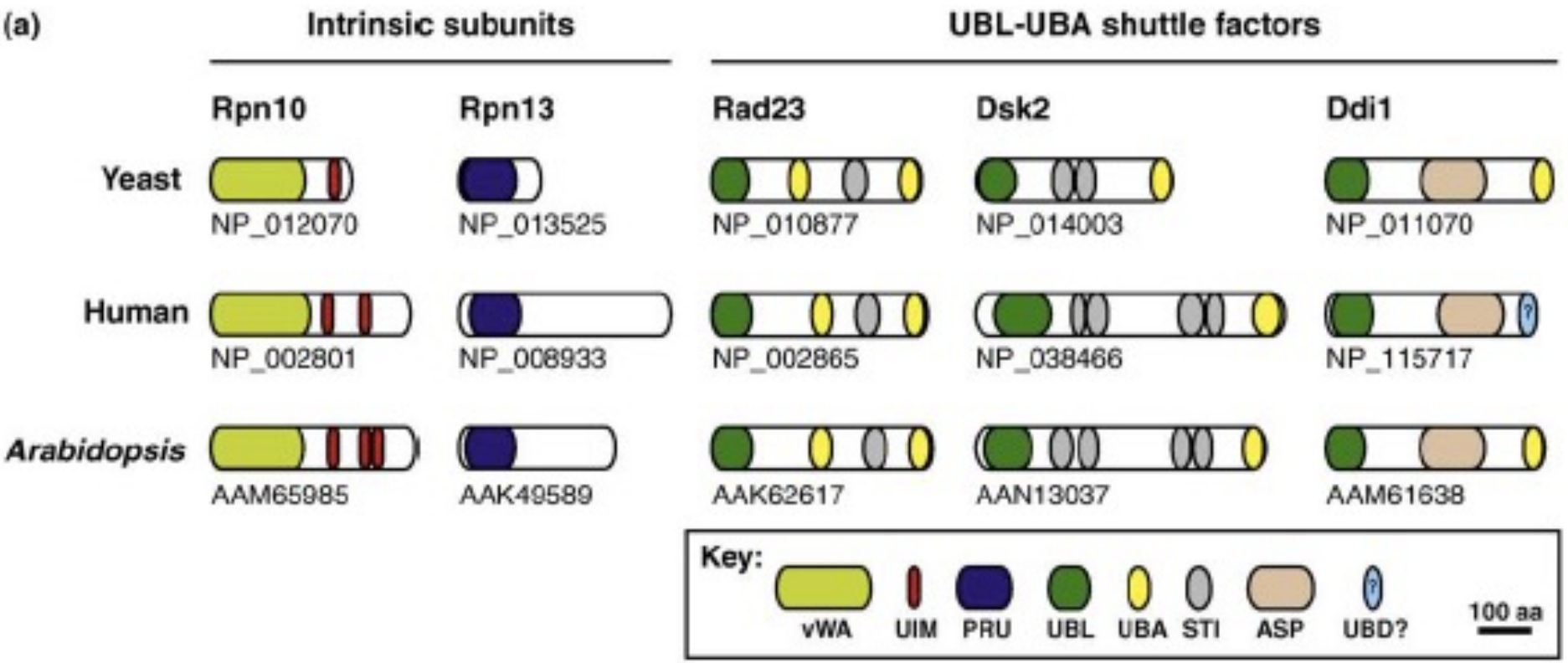


ATP consuming process

Tagged proteins are degraded by the 26S proteasome

Ubiquitin is recycled

26S PROTEASOME



Composed of 43 subunits with a molecular mass of about 2500 kD

Tunnel-like 20S catalytic core particle

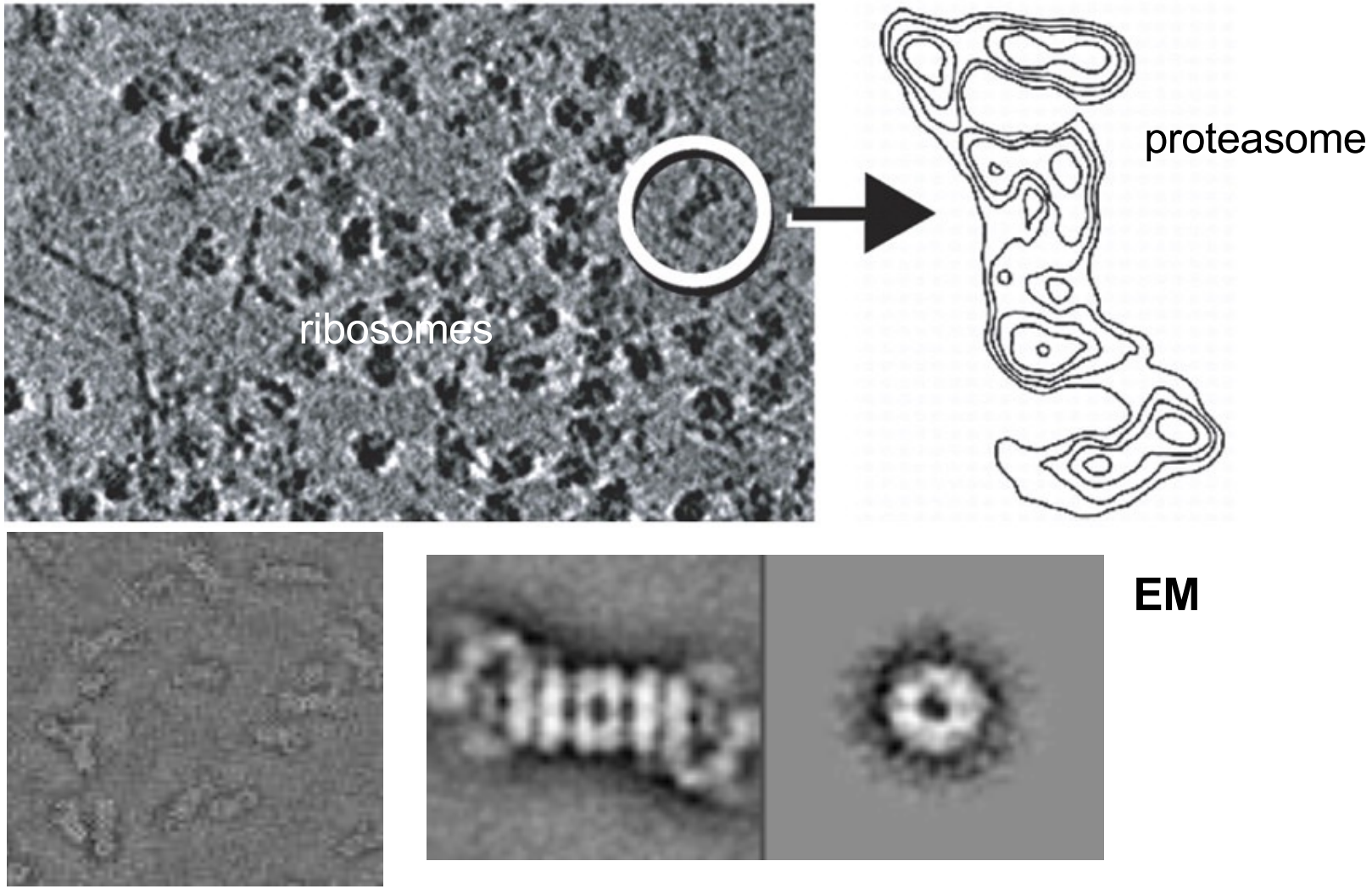
Two 19S regulatory cap particles

Major substrates: polyubiquitinated proteins

Cleaves proteins in an ATP dependent manner

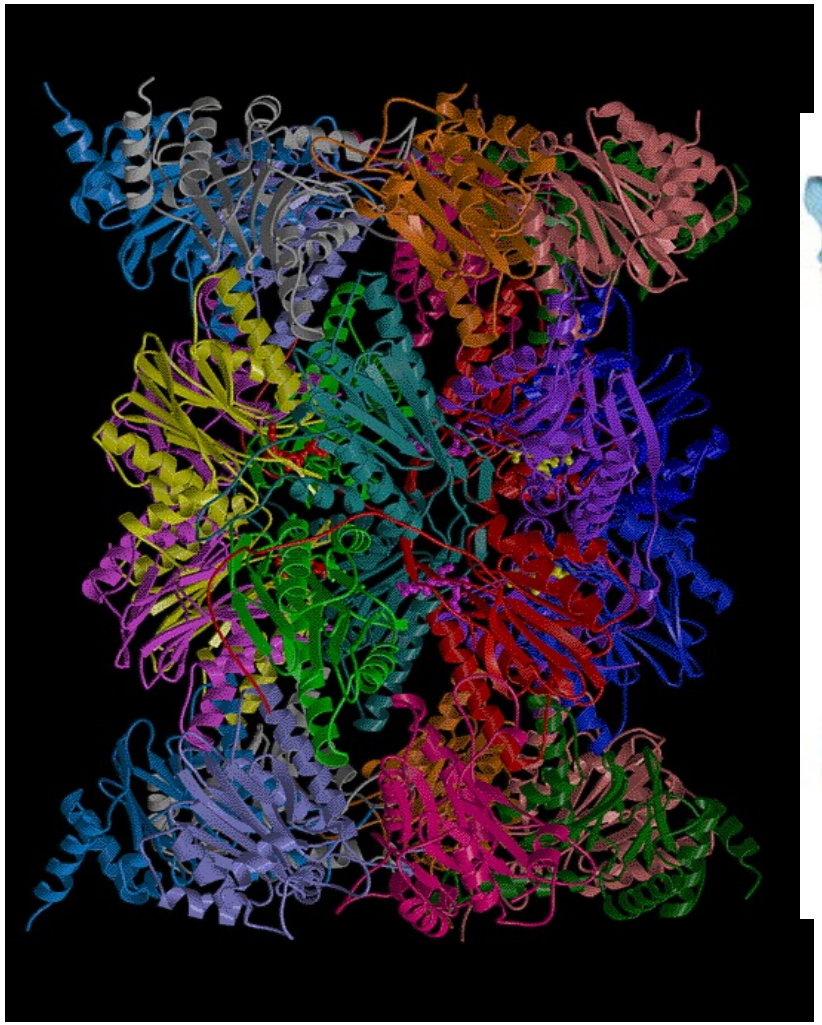
Fu et al, TiPlSci, 2010

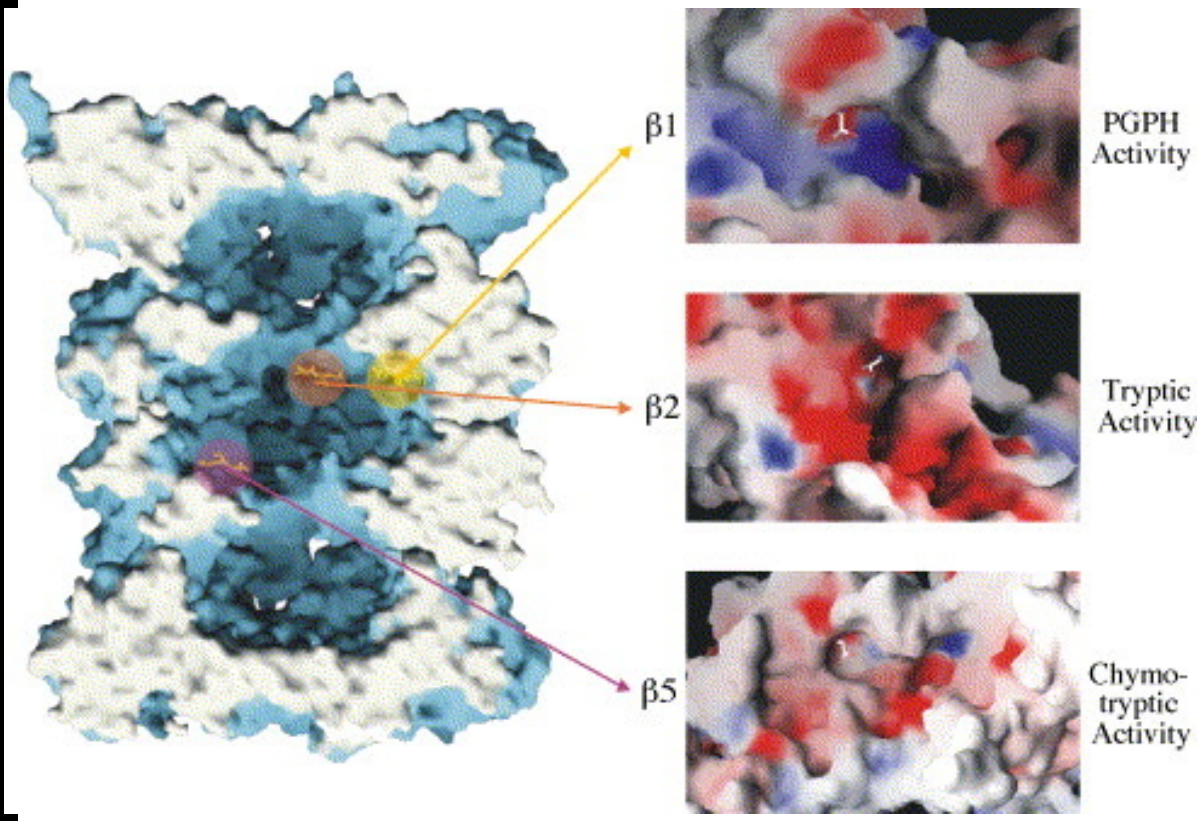
26S PROTEASOME



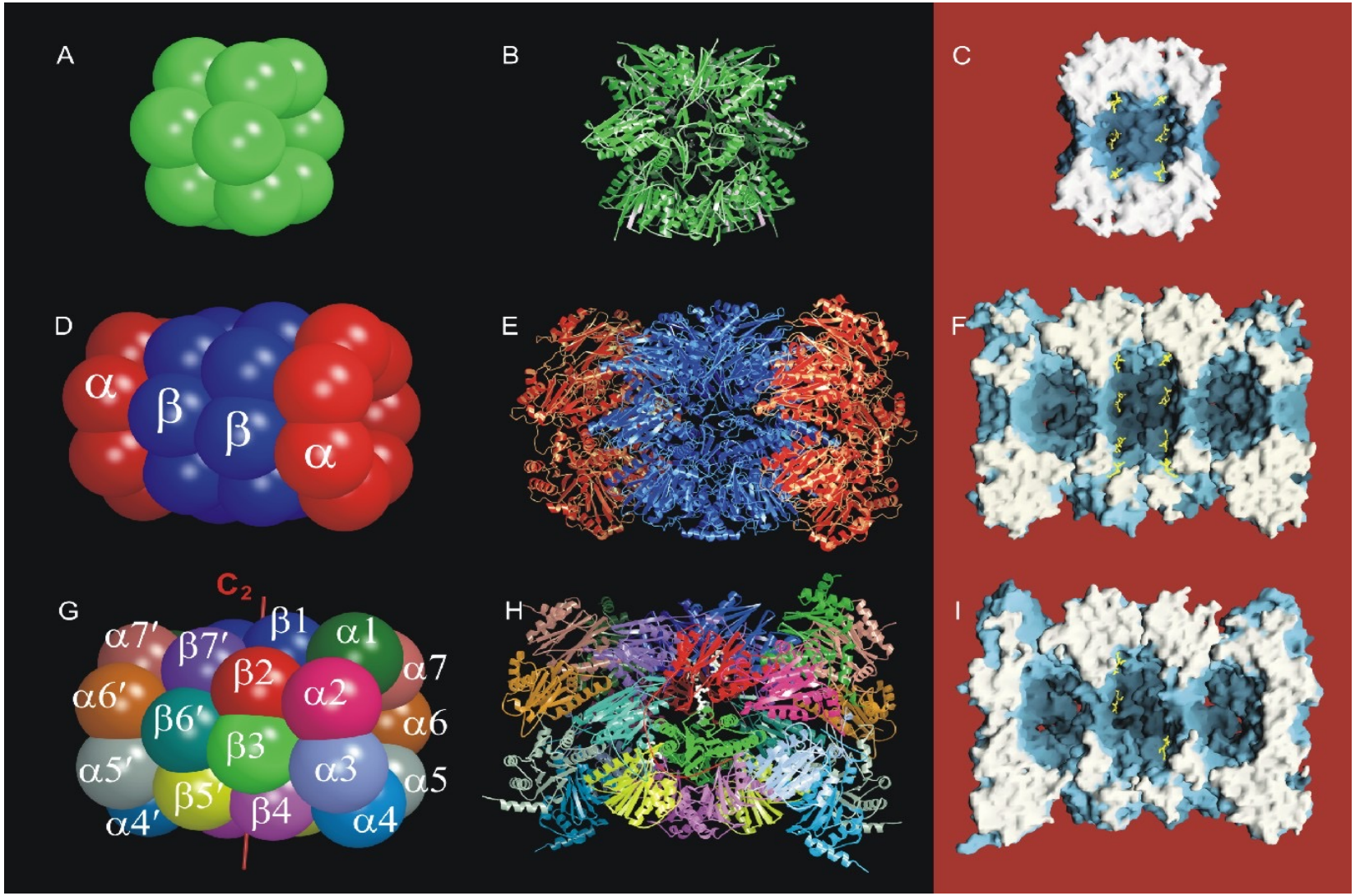
Baumeister, W. (2005) Protein Science, 14 (1), 257-269

26S PROTEASOME



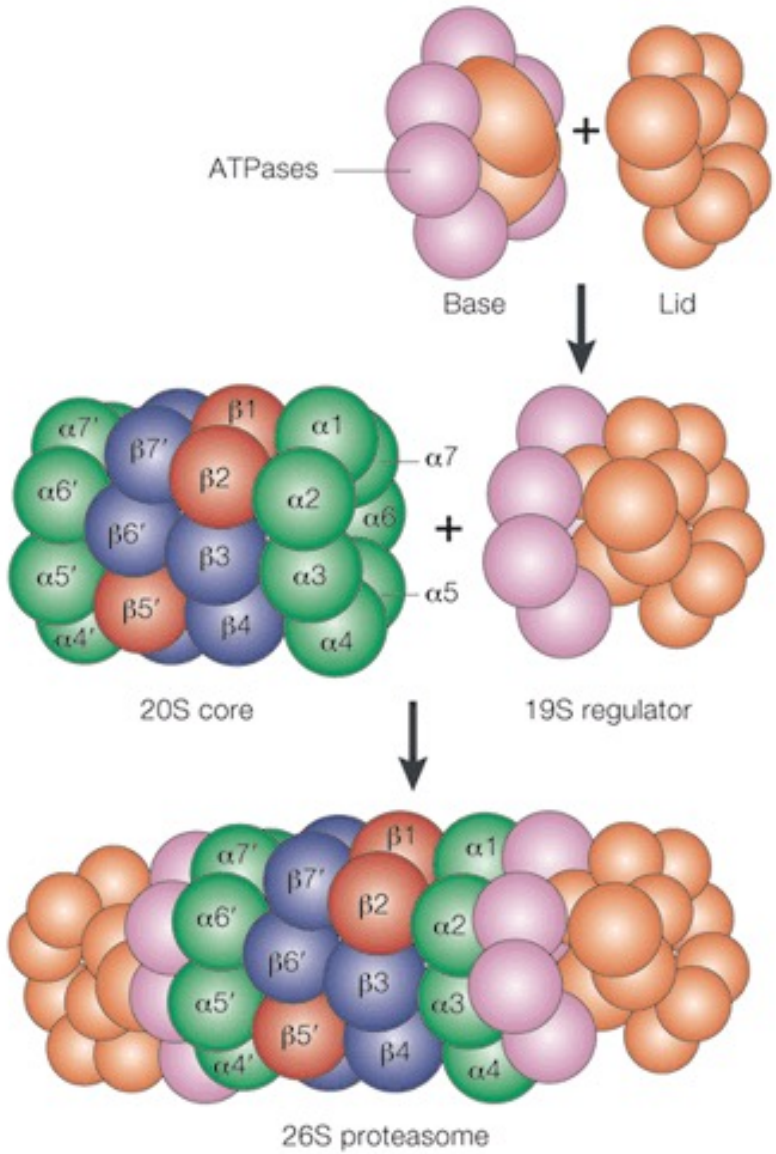


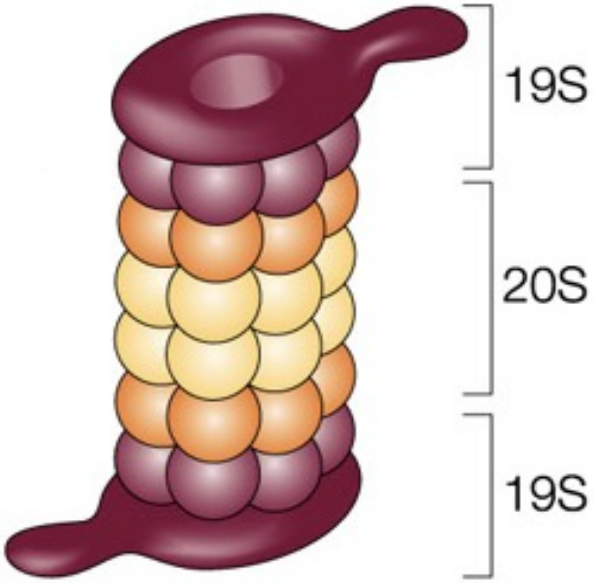
26S PROTEASOME

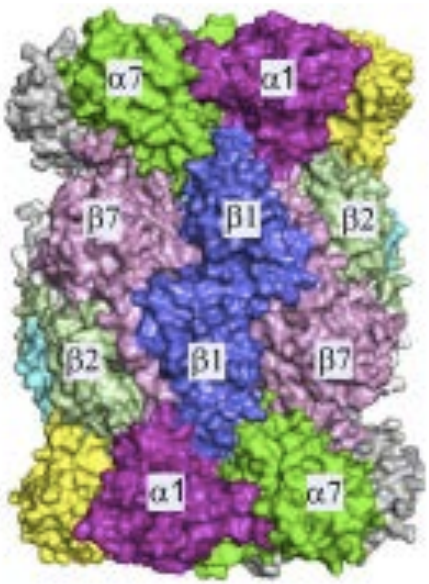


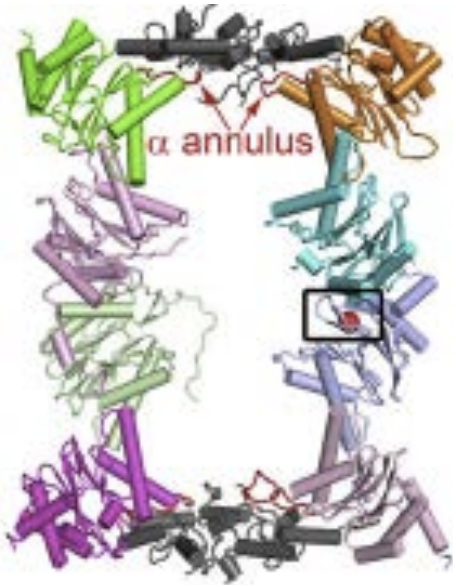
Bochtler et al., Annual Reviews of Biophysics and Biomolecular Structure 1999

26S PROTEASOME



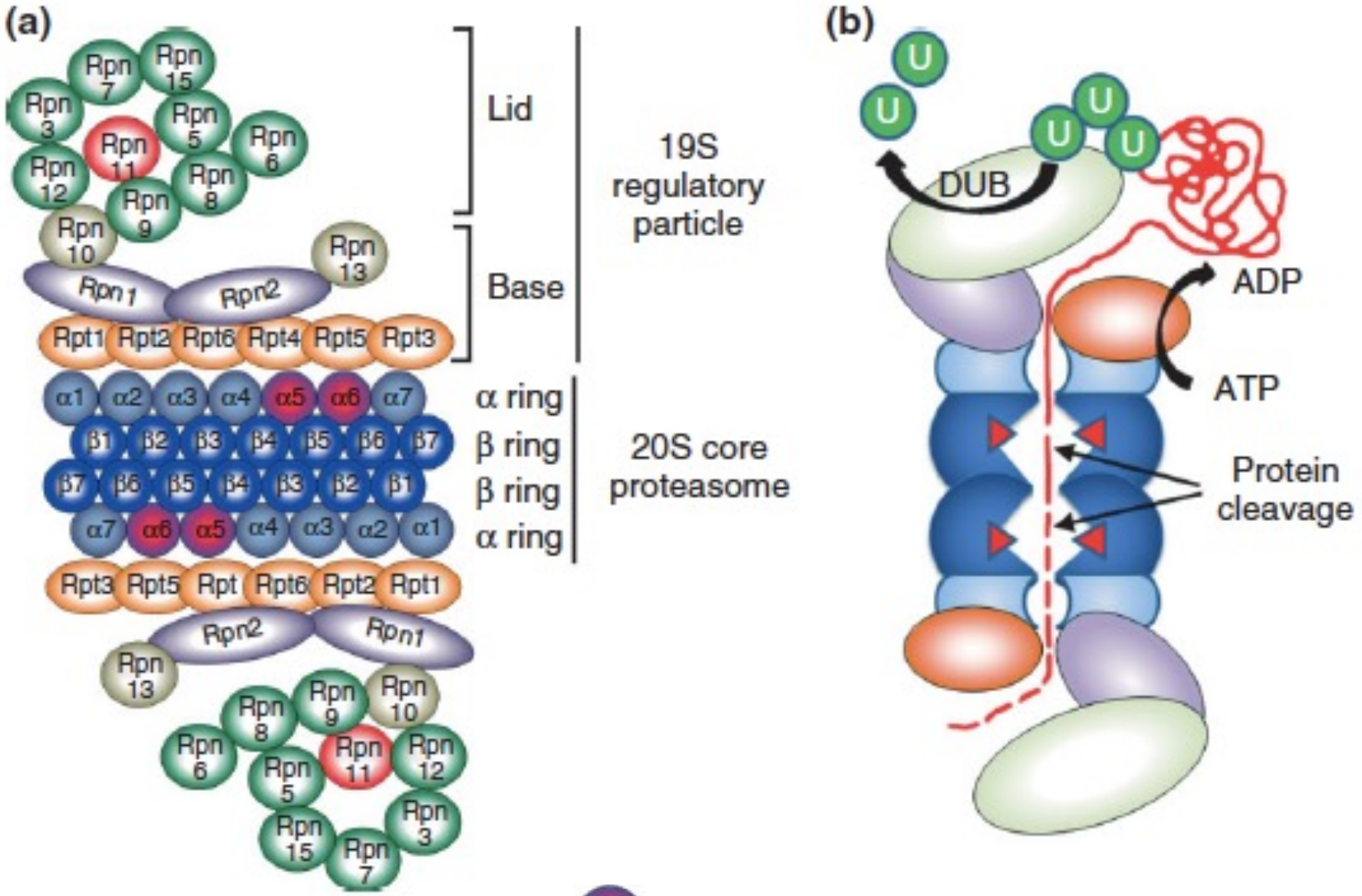






Nature Reviews | Molecular Cell Biology

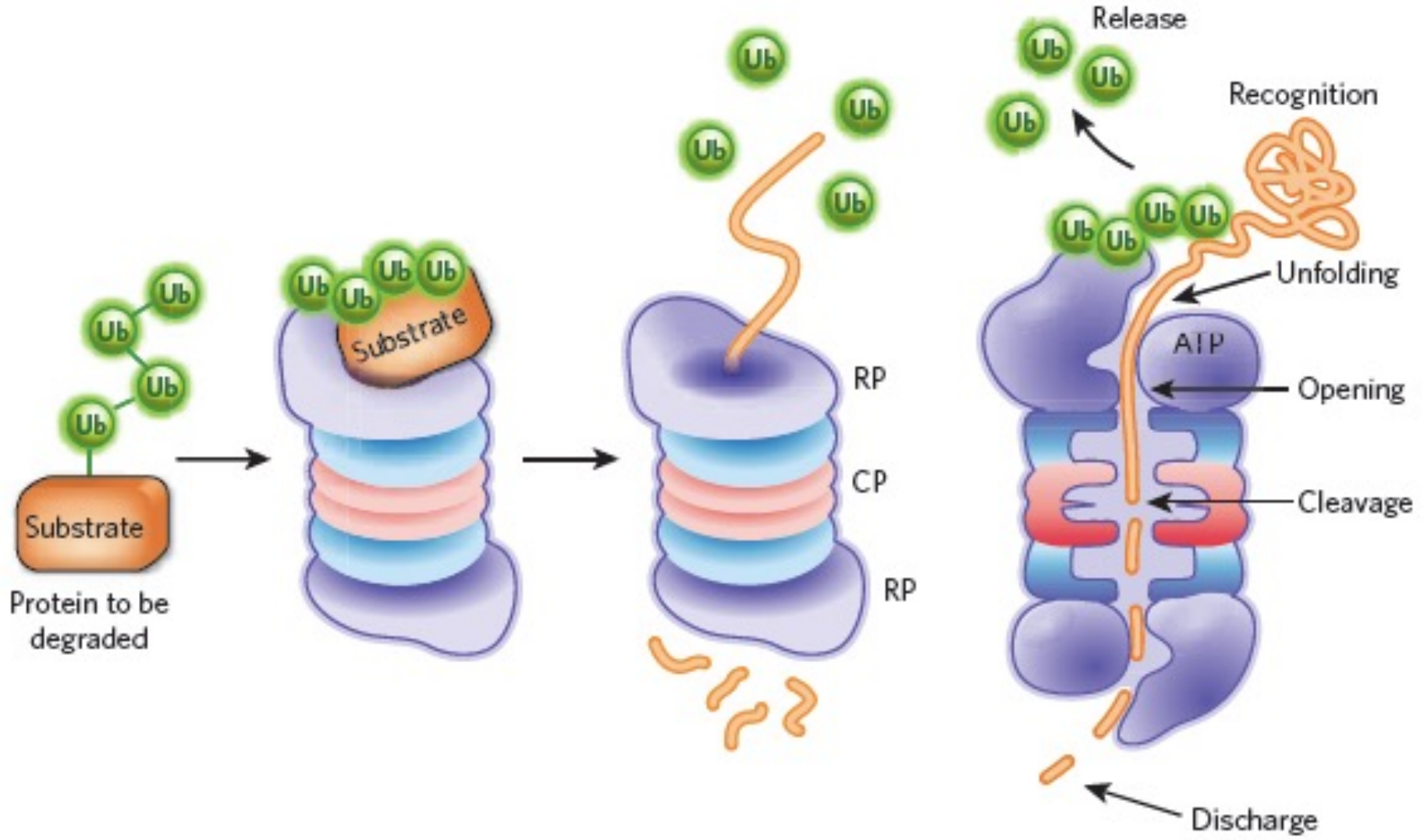
26S PROTEASOME



α5 **RNAse activity**

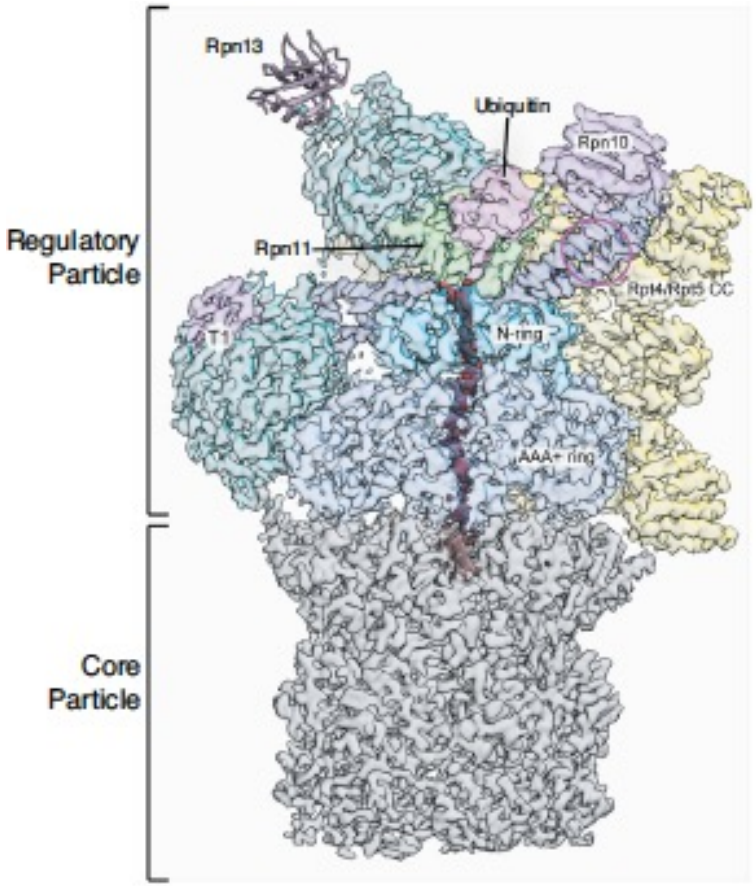
Brooks, WIREs RNA, 2010

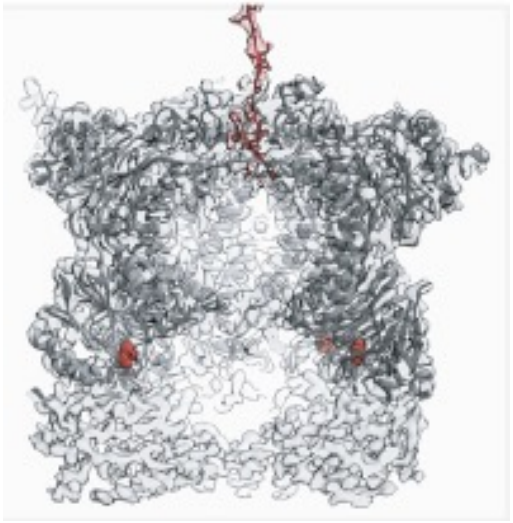
Protein degradation inside the proteasome

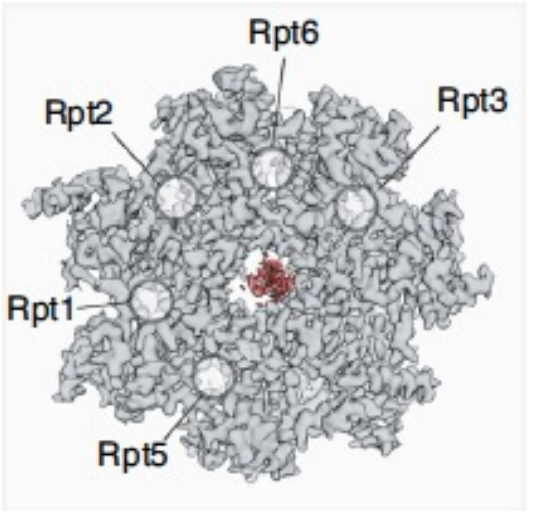


Hochstrasser., Nature, 2011

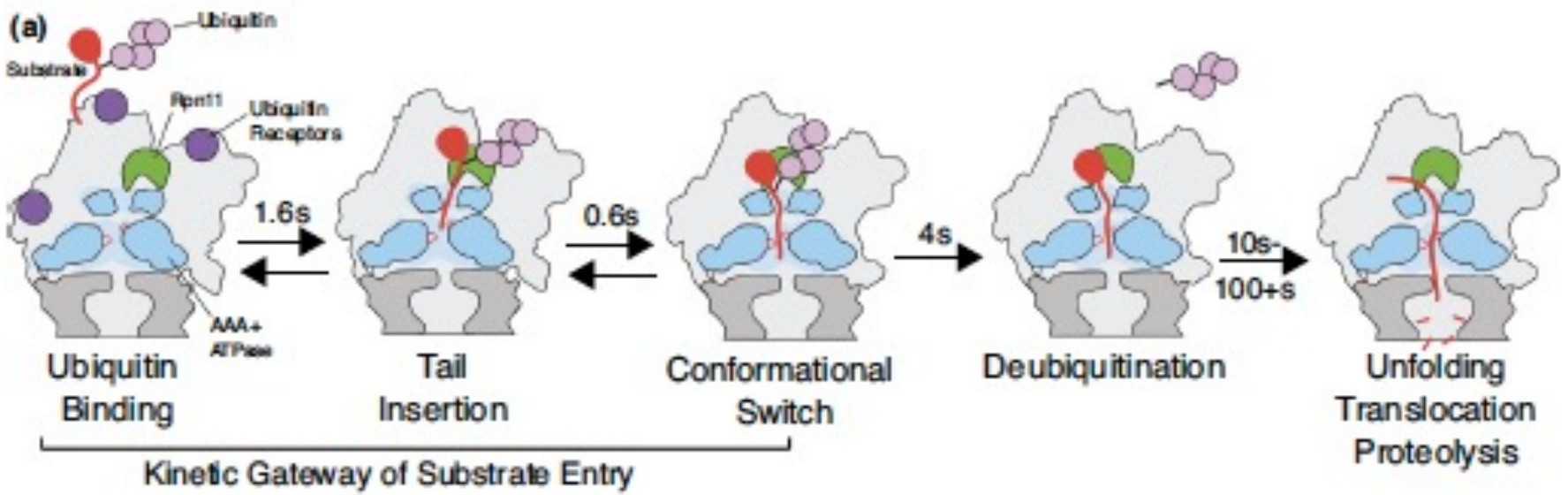
Protein degradation inside the proteasome





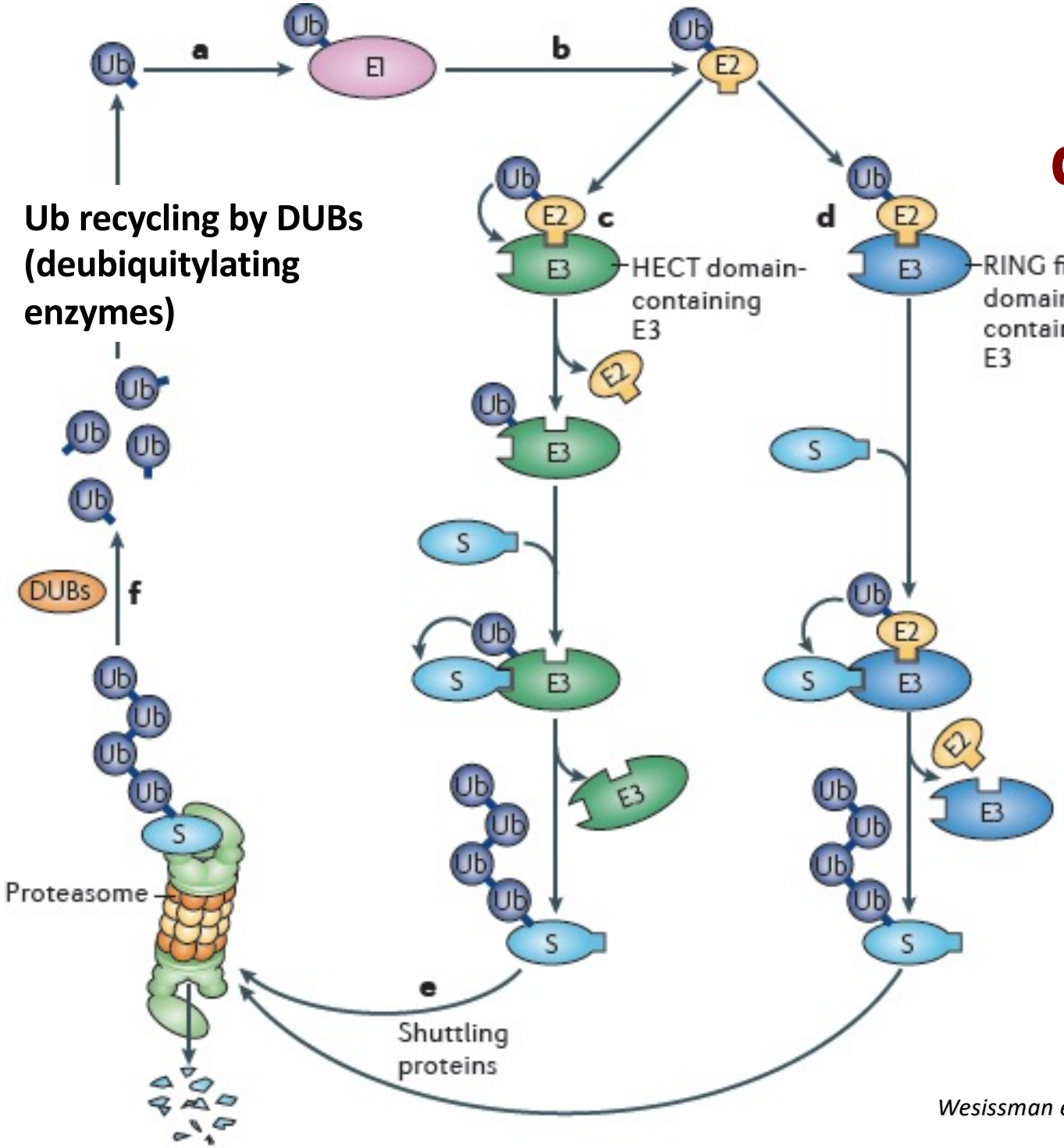


CryoEM



Greene et al., Curr Op Struct Biol , 2020

Protein degradation

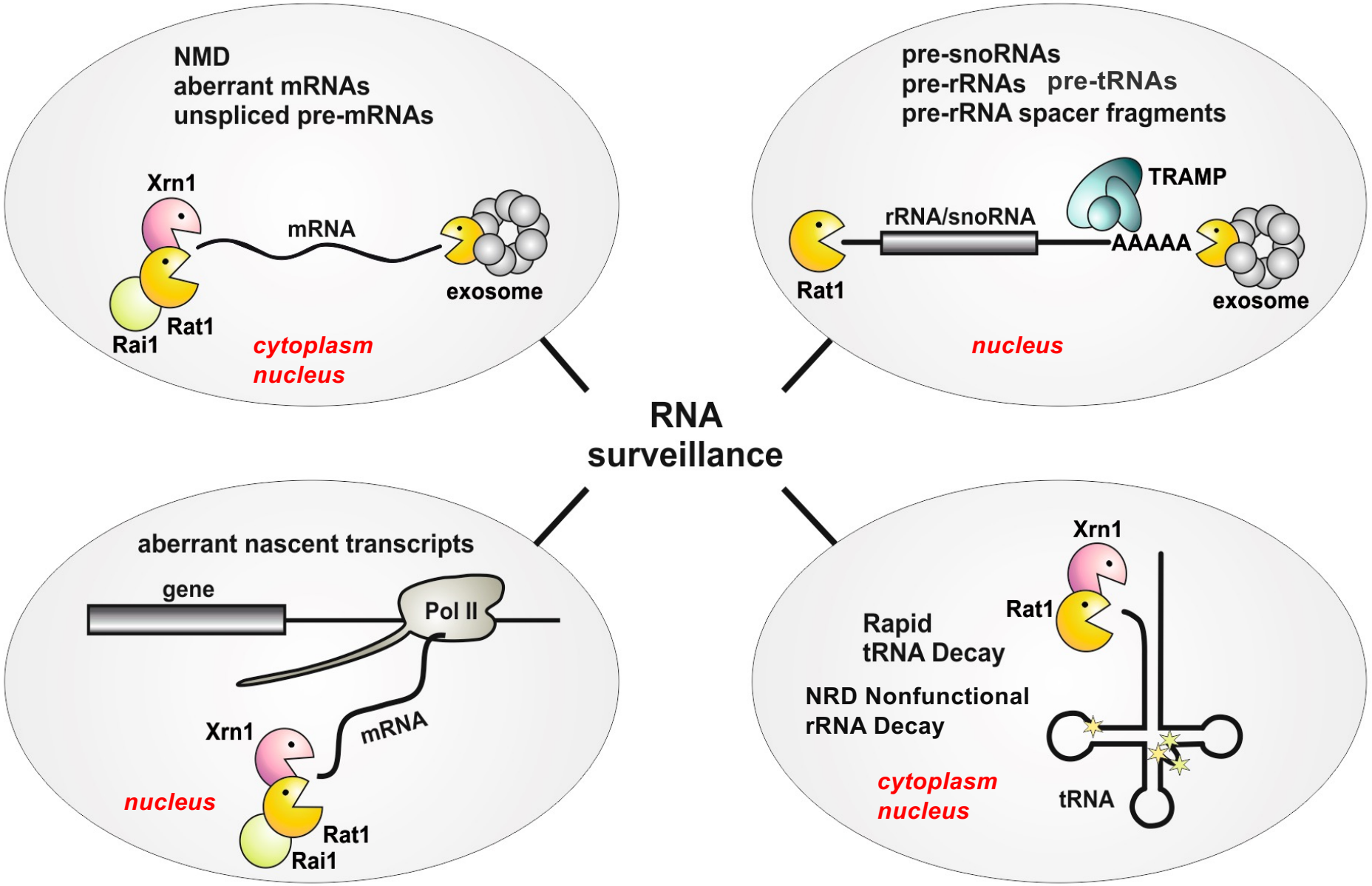


Protein degradation is regulated by protein degradation
- degradation of Ub
- degradation of E1-3
- degradation of proteasomal subunits

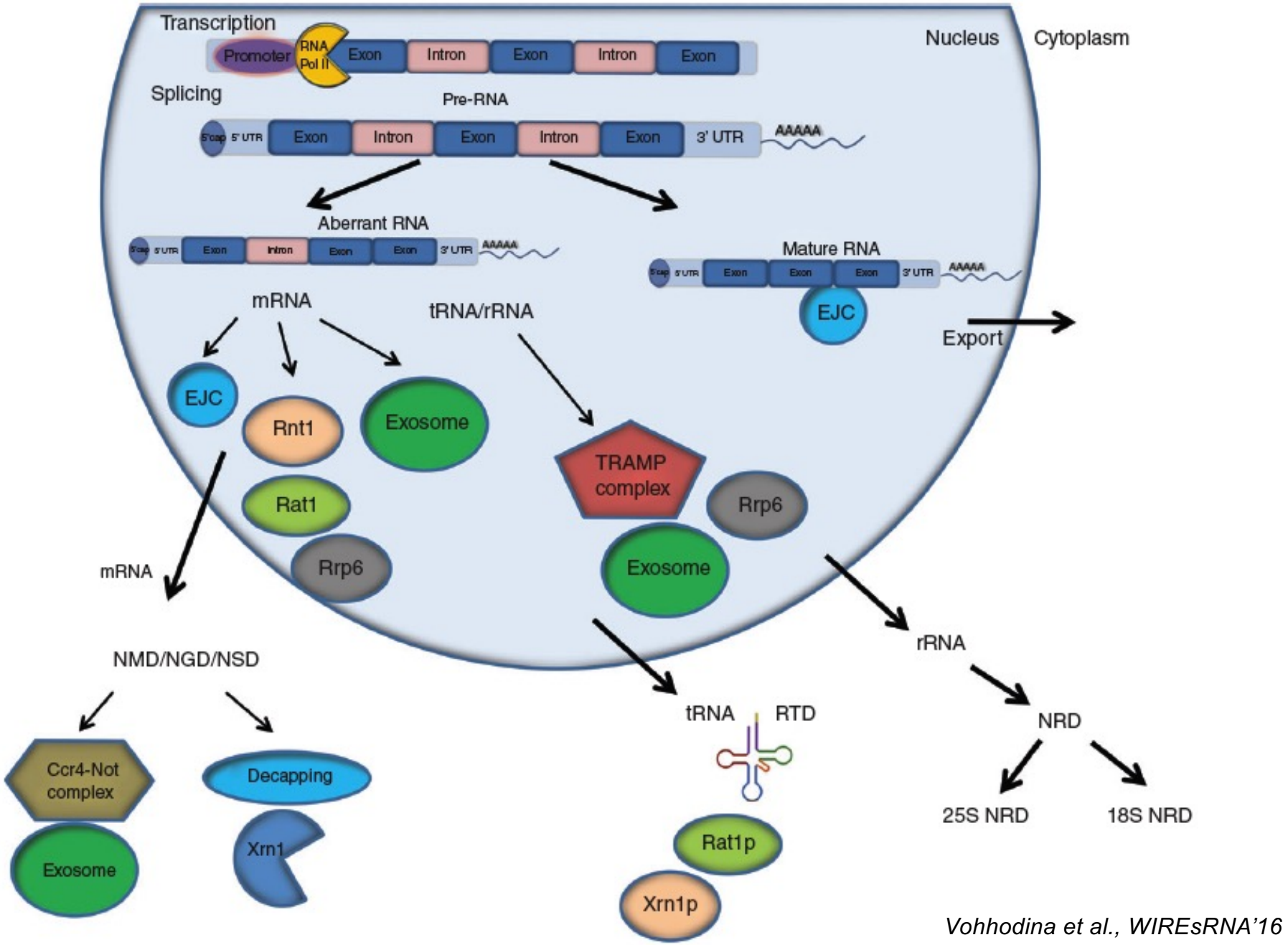
(e.g. during stress)

Wesissman et al, Nat Rev Mol Cell Biol, i, 2011

RNA surveillance

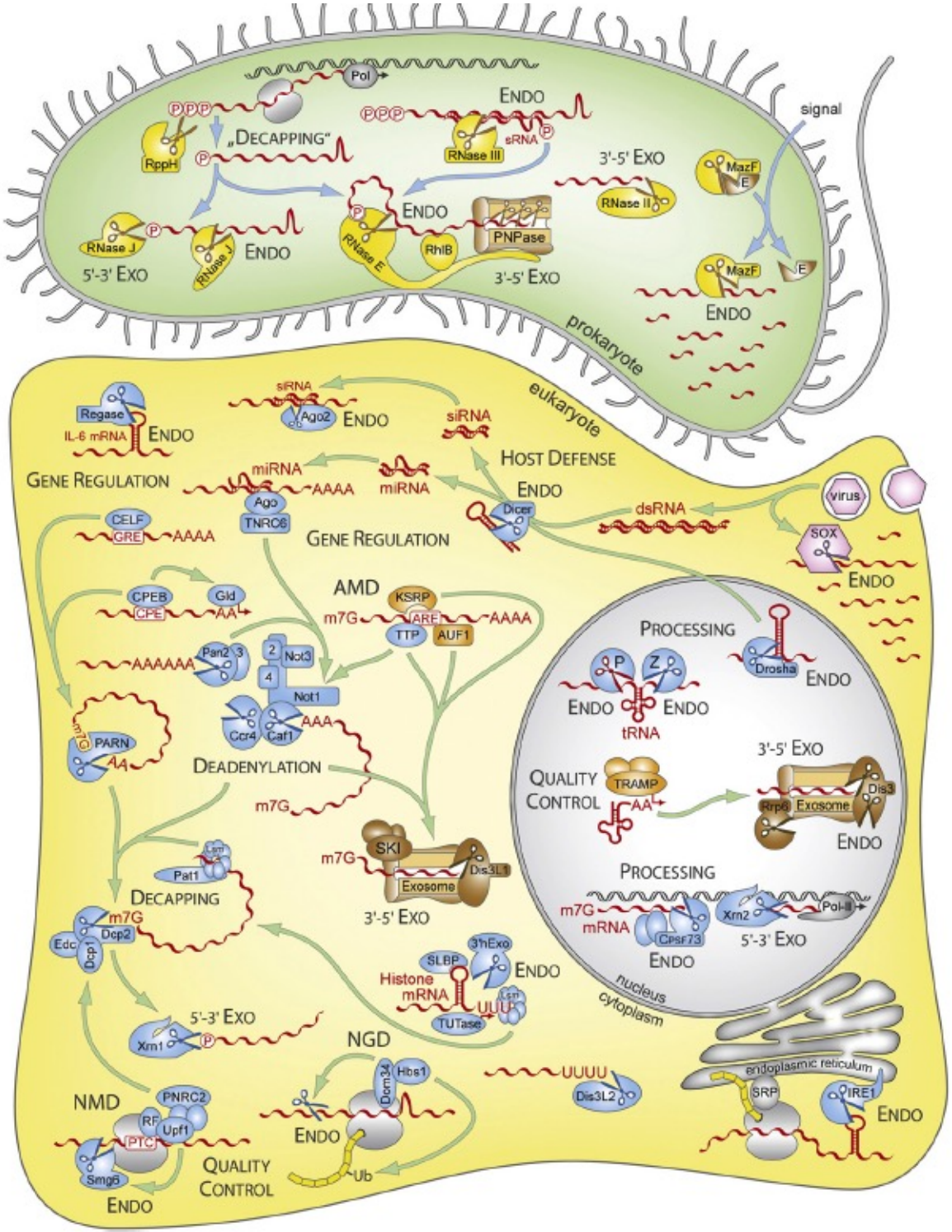


RNA surveillance



Vohhodina et al., WIREsRNA'16

RNA surveillance



Stoecklin and Mühlemann, BBA, 2013