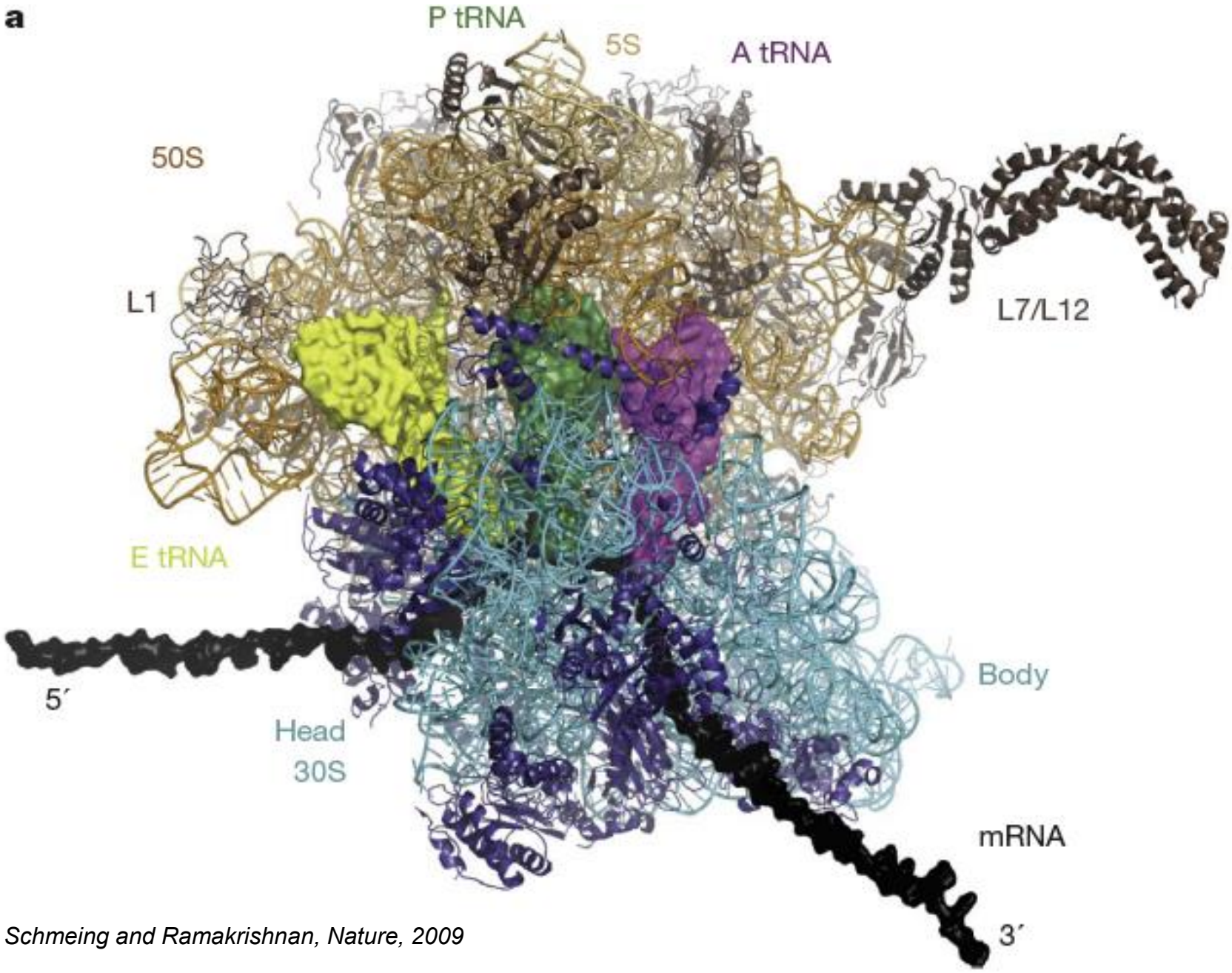
THE RIBOSOME



Schmeing and Ramakrishnan, Nature, 2009

RNA DECAY

RNases

Endonucleases

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

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

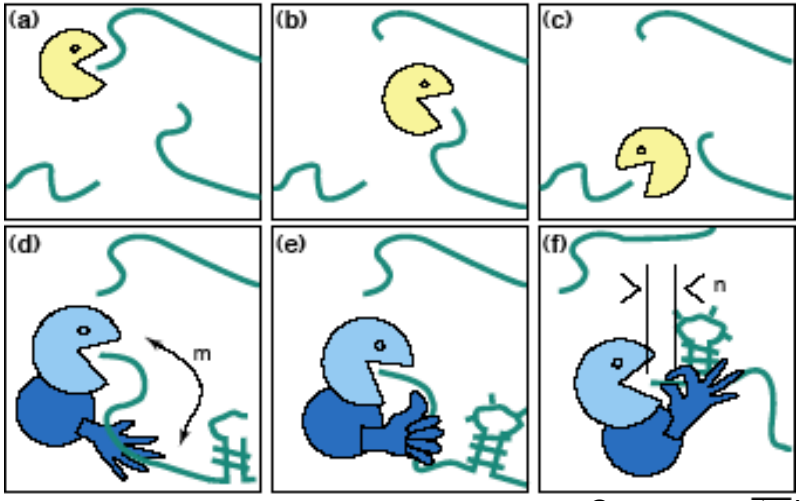
degrading (RNase I, RNAse A):

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

Exonucleases

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

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



distributive

processive

Symmons et al, TiBS, 2002

RNA PROCESSING and DECAY machinery: RNases

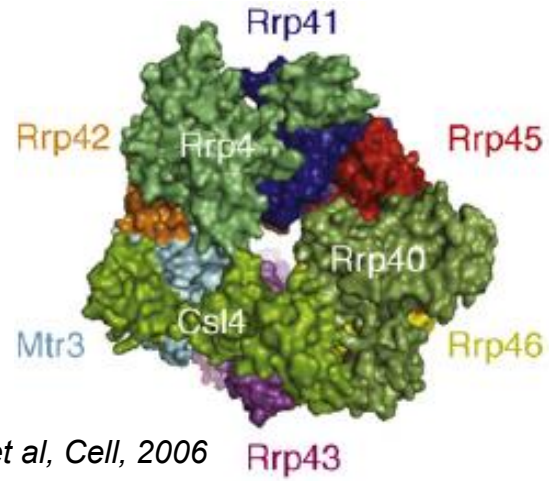
Protein	Function	Characteristics
Exonucleases 5'→3'		
Xrn1	cytoplasmic, mRNA degradation	
Rat1	nuclear, pre-rRNA, sn/snoRNA, pre-mRNA processing and degradation	
Rrp17/hNol12	nuclear, pre-rRNA processing	
Exosome 3'→5'	multisubunit exo/endo complex	subunits organized as in bacterial PNPazy
Rrp44/Dis3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

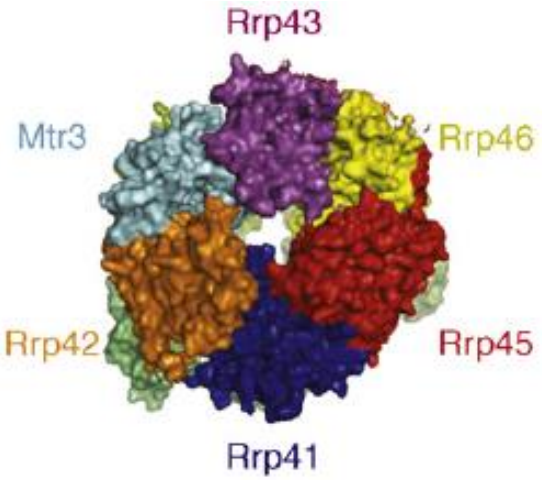
Eukaryotic auxiliary decay factors

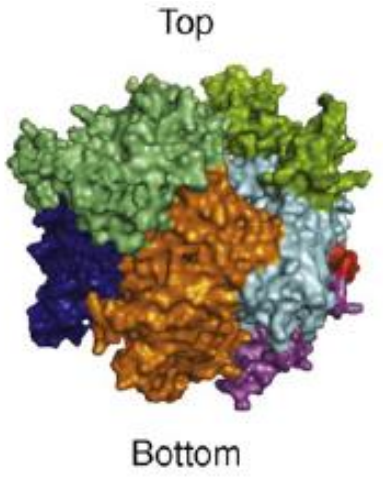
Protein	Function / Characteristics
5'→3' decay: decapping	
Dcp1/Dcp2	Dcp2- pyrophosphatase catalytic activity, Nudix domain, Dcp1- protein binding
Hedls/Ge-1/Edc4	decapping cofactor, WD40 domain
Edc1,2,3	decapping enhancers, stimulate cap binding/catalysis, Edc1-2 (yeast), Edc3 (all eykaryotes)
Dhh1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

EXOSOME: 3'→5' decay machinery

Crystal structure of human core exosome

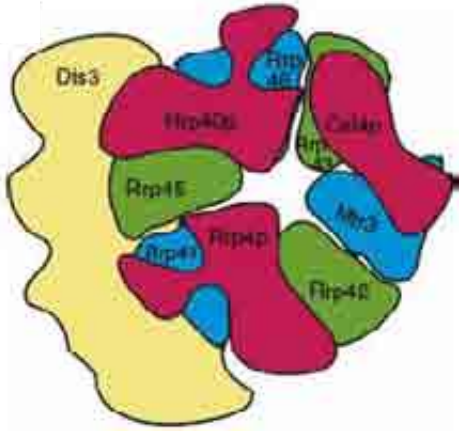






Liu et al, Cell, 2006

Dziembowski et al, Mol.Cell, 2008; Nature, 2008



- **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**

EXOSOME: 3’→5’ decay machinery
FUNCTIONS

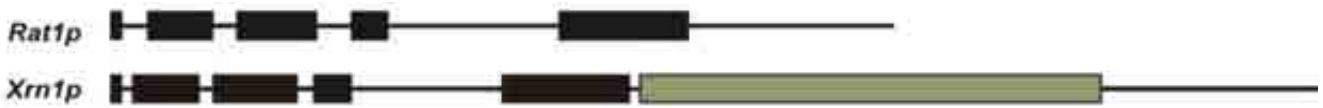
NUCLEAR: Rrp6 and core components have partly separate functions

- 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

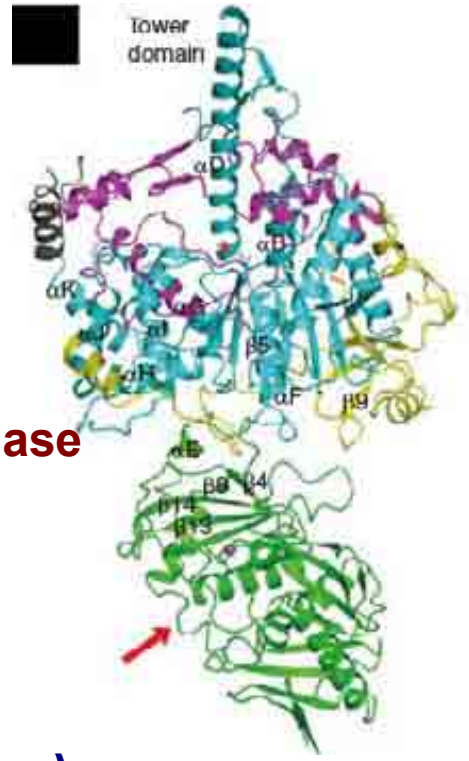
CYTOPLASMIC:

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

XRN family: 5’→3’ processive exonucleases



Kastenmayer and Green, 2000, PNAS



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

NUCLEAR

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

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

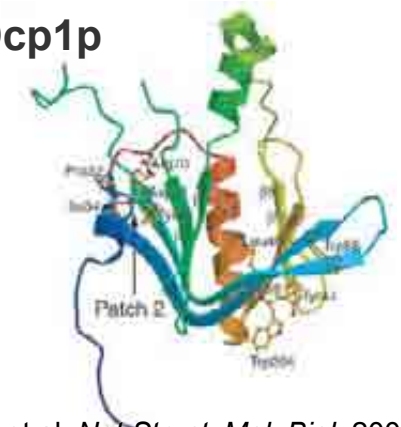
Xiang et al, 2009, Nature

CYTOPLASMIC XRN1

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

DCP- decapping enzymes

Dcp1p

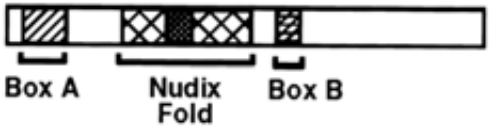


She et al. *Nat.Struct. Mol. Biol*, 2004

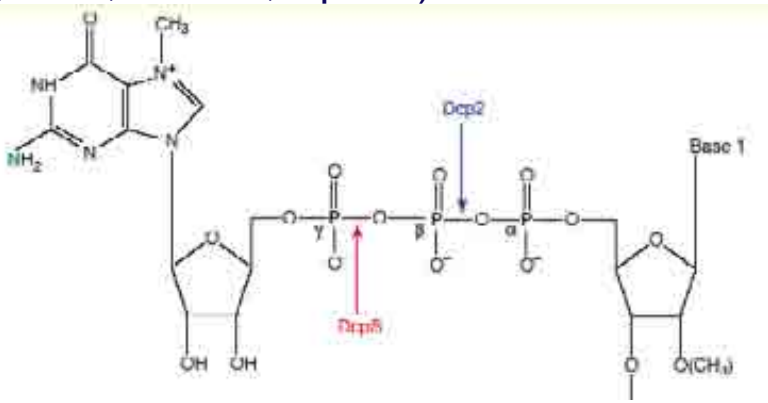
- **Dcp1/Dcp2 complex participates in mRNA 5' decay**
- **catalyses the reaction m^7GpppX-mRNA → m^7GDP + 5'p-mRNA**
- **Dcp2 is the catalytic subunit (pyrophosphatase Nudix domain)**
- **Dcp1 is required for activity *in vivo*, interacts with other proteins**
- **Dcp1/Dcp2p is regulated by Pab1 and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

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

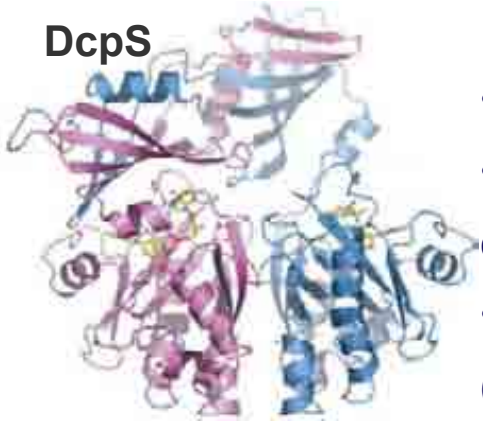
Dcp2p



Wang et al. *PNAS*, 2002



DcpS

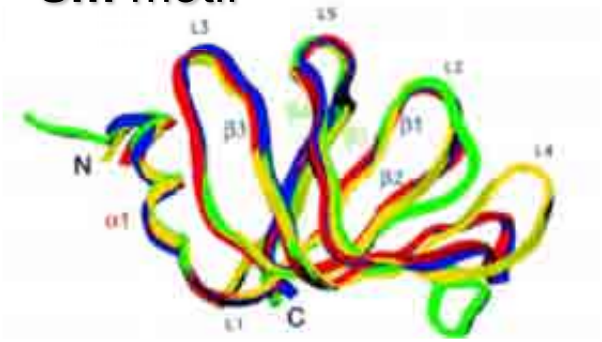


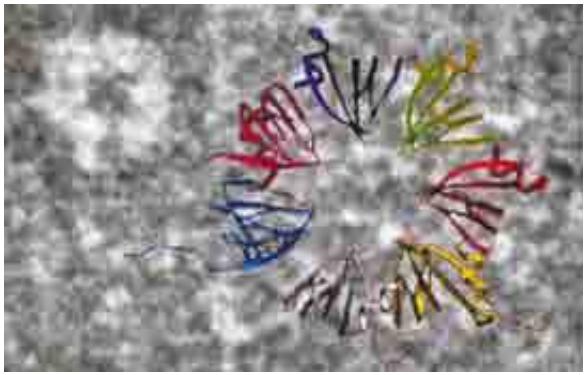
Gu et al., *M.Cell*, 2004

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

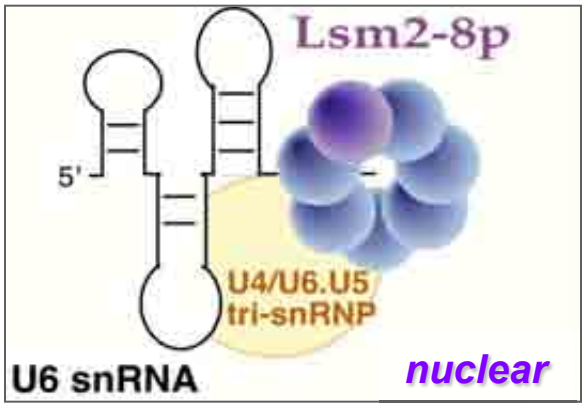
LSM PROTEINS

Sm motif



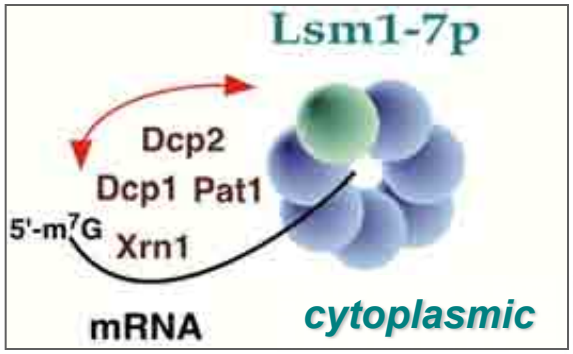


Achsel et al, *EMBO J,* 2001



Involved in pre-mRNA splicing

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

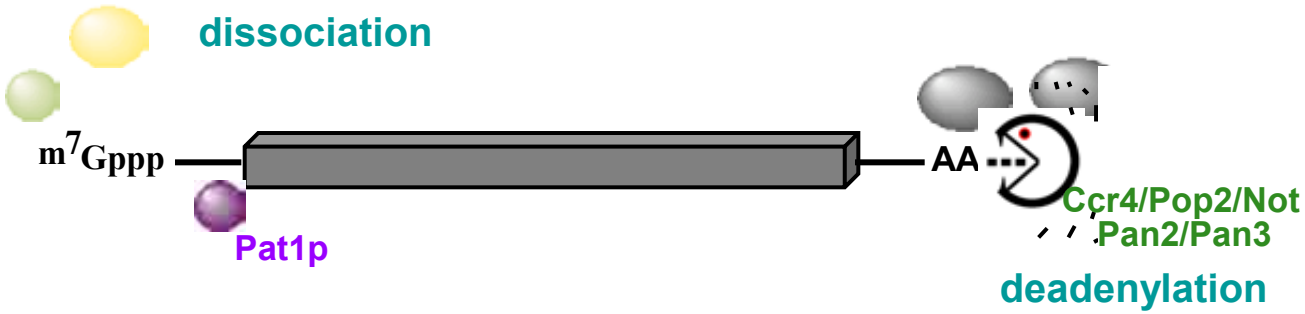


Functions in mRNA decapping and decay

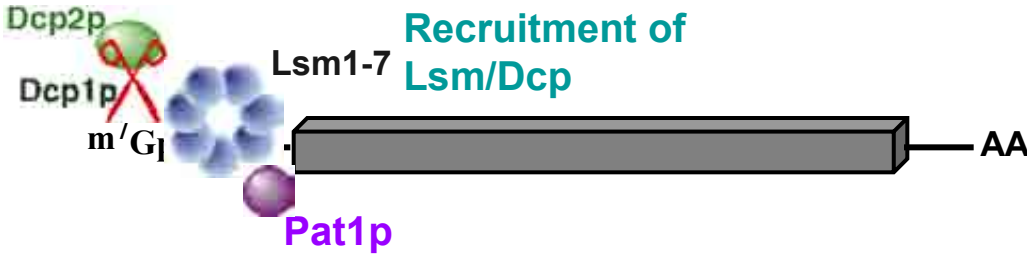
- activator of decapping
- interacts with components of the mRNA decapping and degradation machinery (XRN, DCP)

mRNA DECAY
IN THE CYTOPLASM

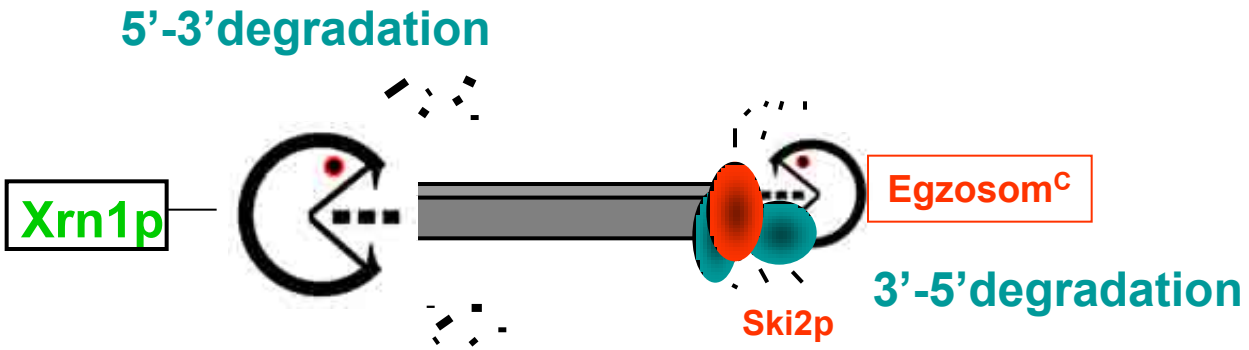
mRNA DECAY
IN THE CYTOPLASM



mRNA DECAY
IN THE CYTOPLASM

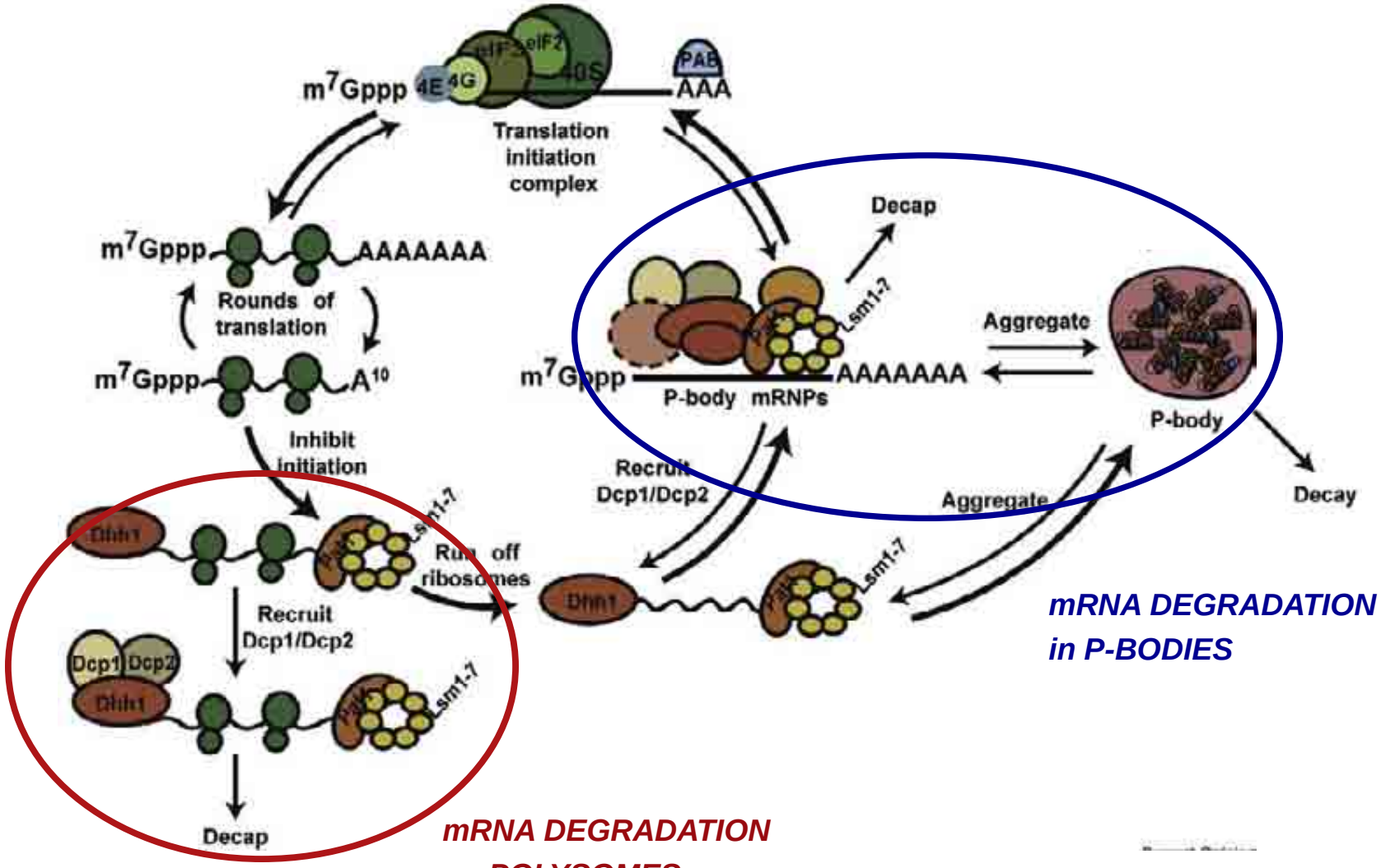


mRNA DECAY
IN THE CYTOPLASM



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

mRNA DEGRADATION in the CYTOPLASM



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

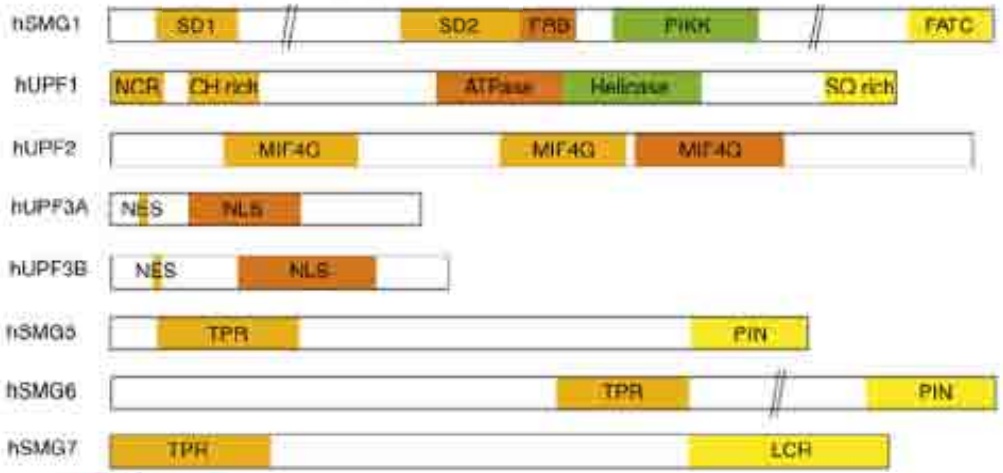
RNA SURVEILLANCE = RNA QUALITY CONTROL MECHANISMS

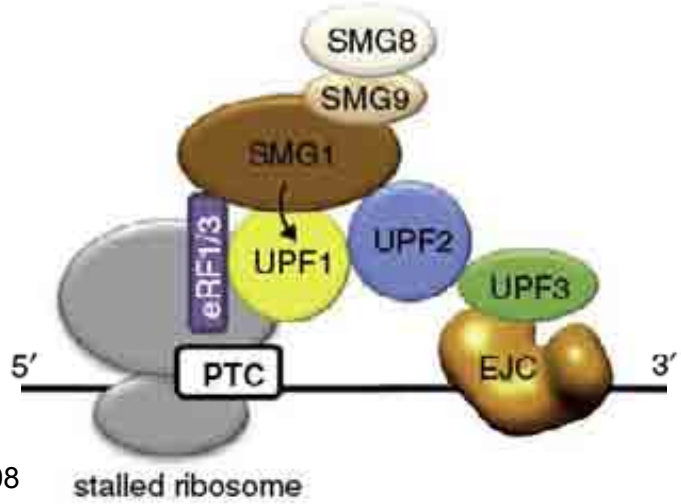
- **NMD**- (nonsense mediated decay) - degradation of mRNAs with premature stop codons (PTC)
- **NSD**- (non-stop decay) - degradation of mRNAs with no stop codons
- **NO-GO** decay- degradation of mRNAs stalled in translation elongation
- **AMD**- **ARE** mediated decay- rapid degradation of mRNAs with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

NMD

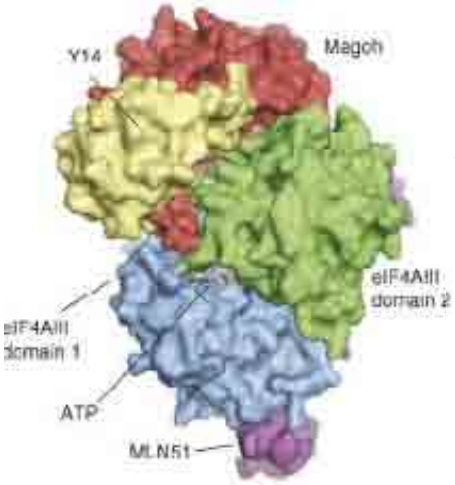
- degradation of mRNAs containing premature STOP codons (PTC)
- prevents expression of truncated, possibly harmful, proteins
- 33% of yeast intron-containing mRNAs undergo NMD
- 30% of alternatively spliced human mRNAs generate NMD substrates

NMD factors





Stalder and Muhlemann, *TiCB*, 2008



Bono et al., *Cell*, 2006

Exon-Junction Complex EJC

core

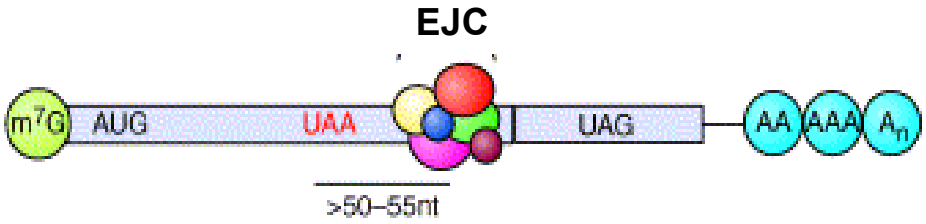
- eIF-4AIII - DEAD box RNA helicase
- MLN51 - stimulates eIF-4AIII
- Magoh/Y14 - heterodimer binds to eIF-4AIII

Associated factors

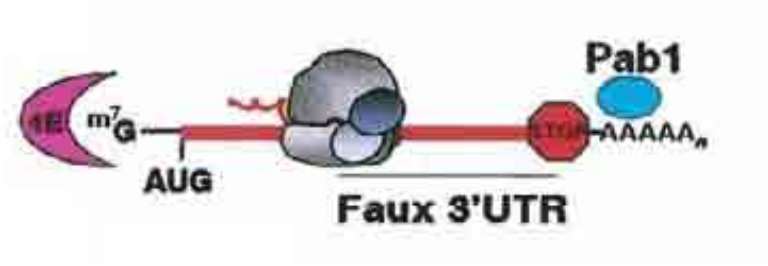
- UAP56, SRm160, Acinus, SAP18, Pinin, RNPS1 (splicing)
- REF, TAP, p15 (export)
- Upf2, Upf3 (NMD)

MECHANISM OF NMD

1. Recognition of premature stop codon during translation

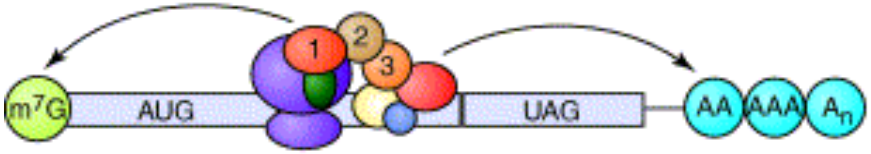


splicing-related mechanism
- EJC deposited as a mark of splicing
- Upf3 is bound to mRNA via EJC
- mRNA is exported and Upf2 joins Upf3



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

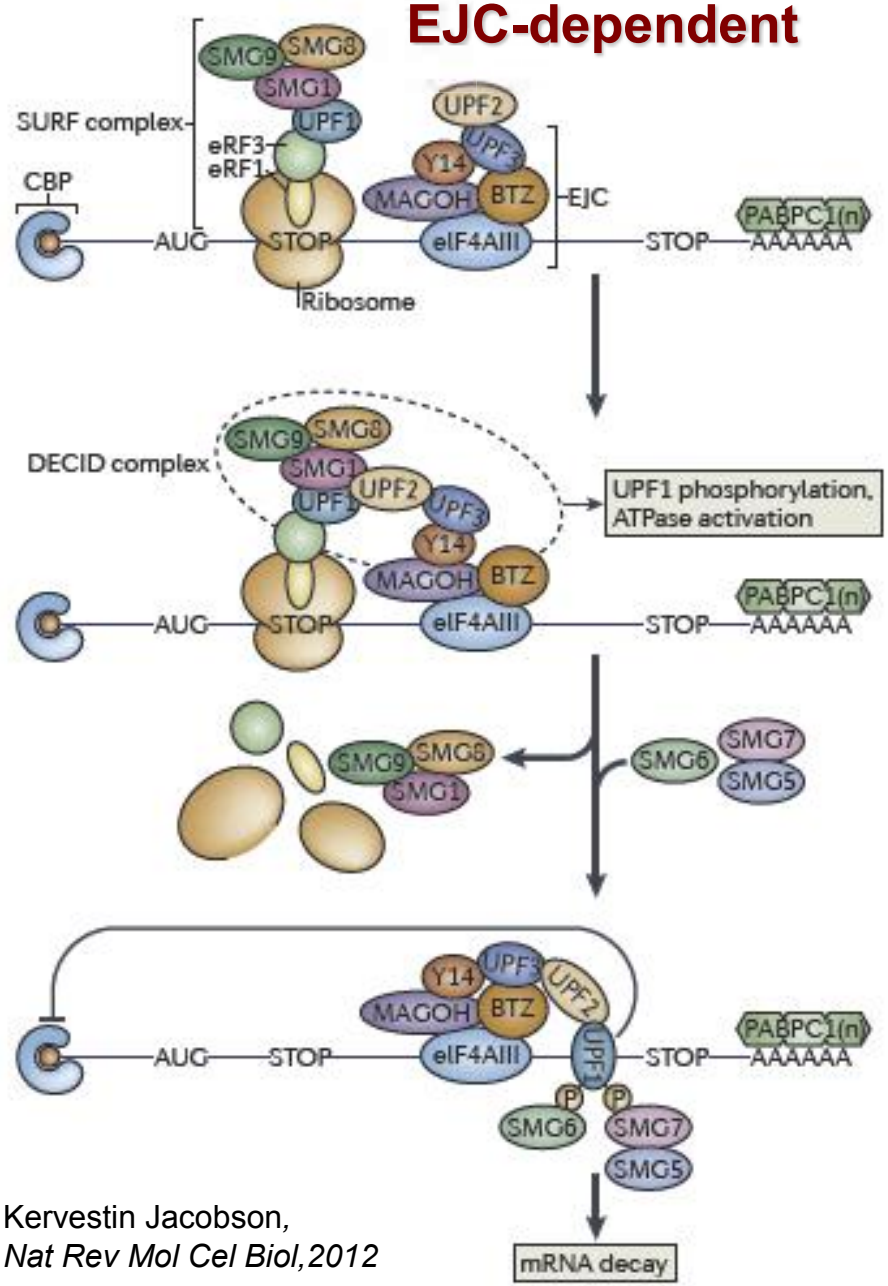
SURF complex, Upf1.SMG1 and eRF1-2, is recruited by the stalled ribosome

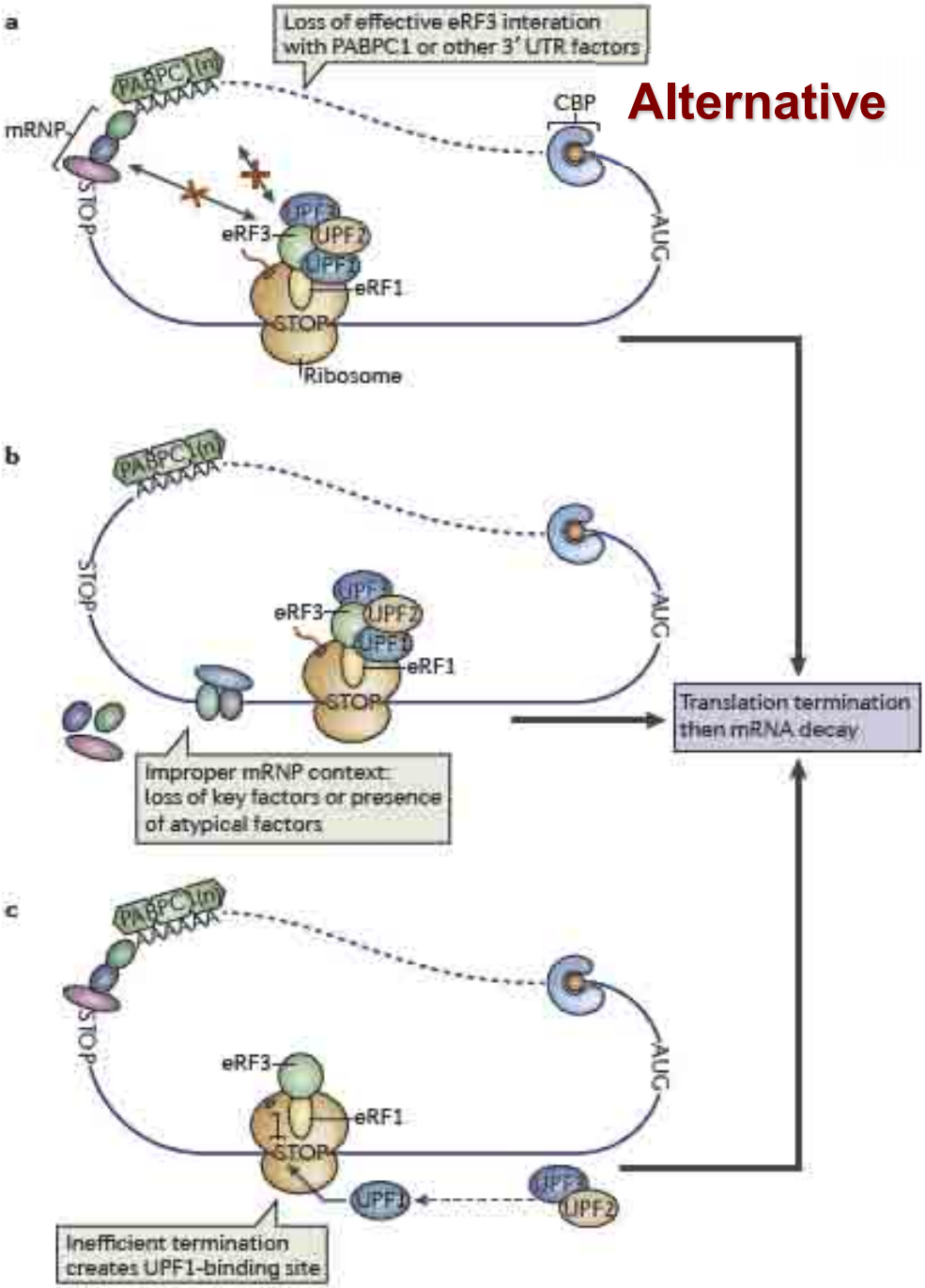
Upf1 is phosphorylated by SMG1
eRF1-eRF2 are released

mRNA is directed for degradation

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

MECHANISMS OF NMD





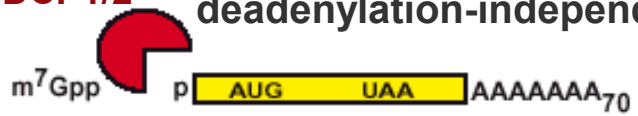
Kervestin Jacobson,
Nat Rev Mol Cel Biol,2012

MECHANISM OF NMD

3. mRNA degradation

NMD 5'→3'

DCP1/2 deadenylation-independent decapping

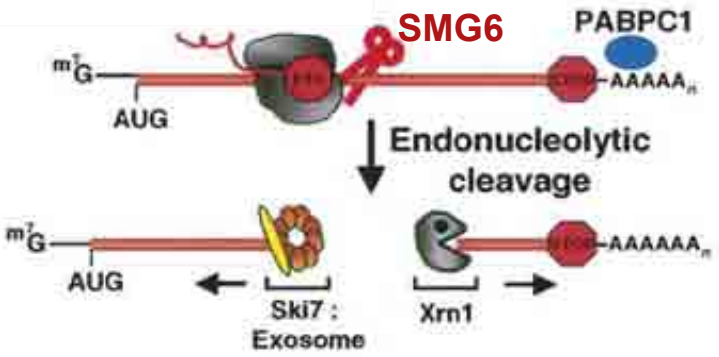


XRN1 exonucleolytic 5'→3' degradation (XRN1)

decapping is triggered by SMG7 recruitment or dephosphorylation of Upf1

NMD endonucleolytic cleavage

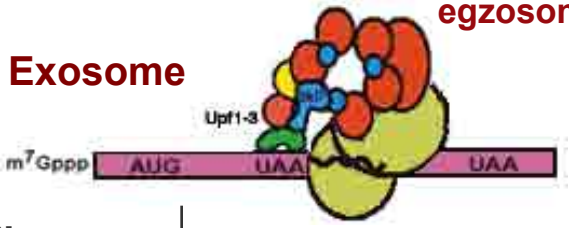
(Drosophila melanogaster)



NMD 3'→5'

recruitment of the Exosome by Upf1

egzosomc



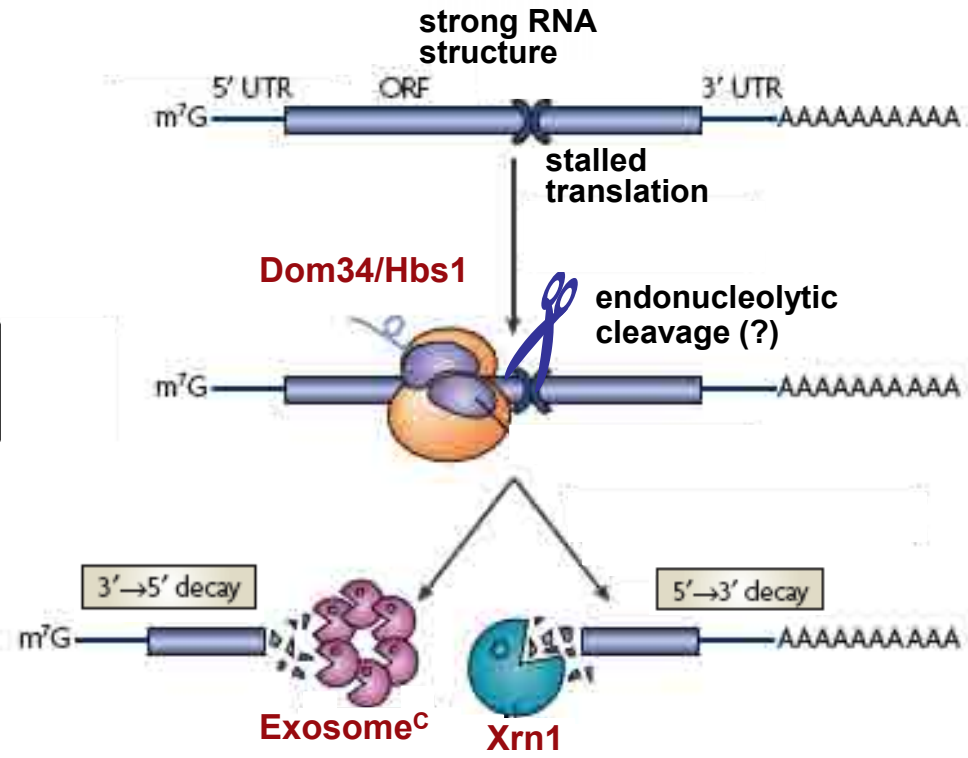
exonucleolytic 3'→5' degradation

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

NGD and NSD

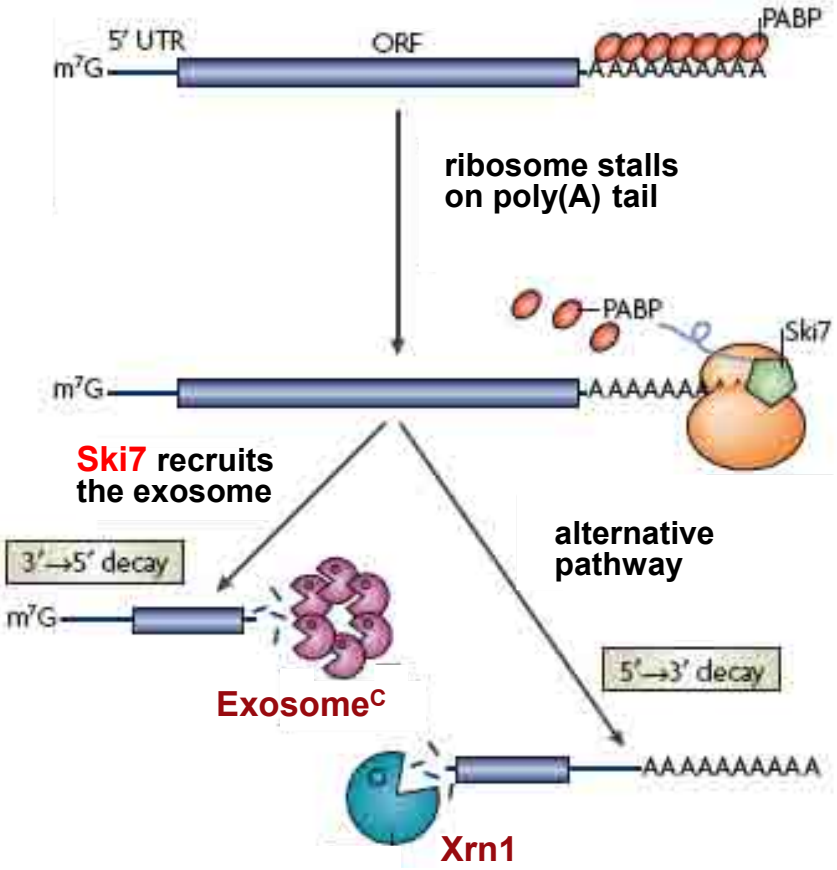
- **NGD** (non-go decay) - degradation of mRNAs stalled on ribosomes
- **NSD**- (non-stop decay) - degradation of mRNAs with no stop codons

NGD



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

NSD



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

NGD and NSD

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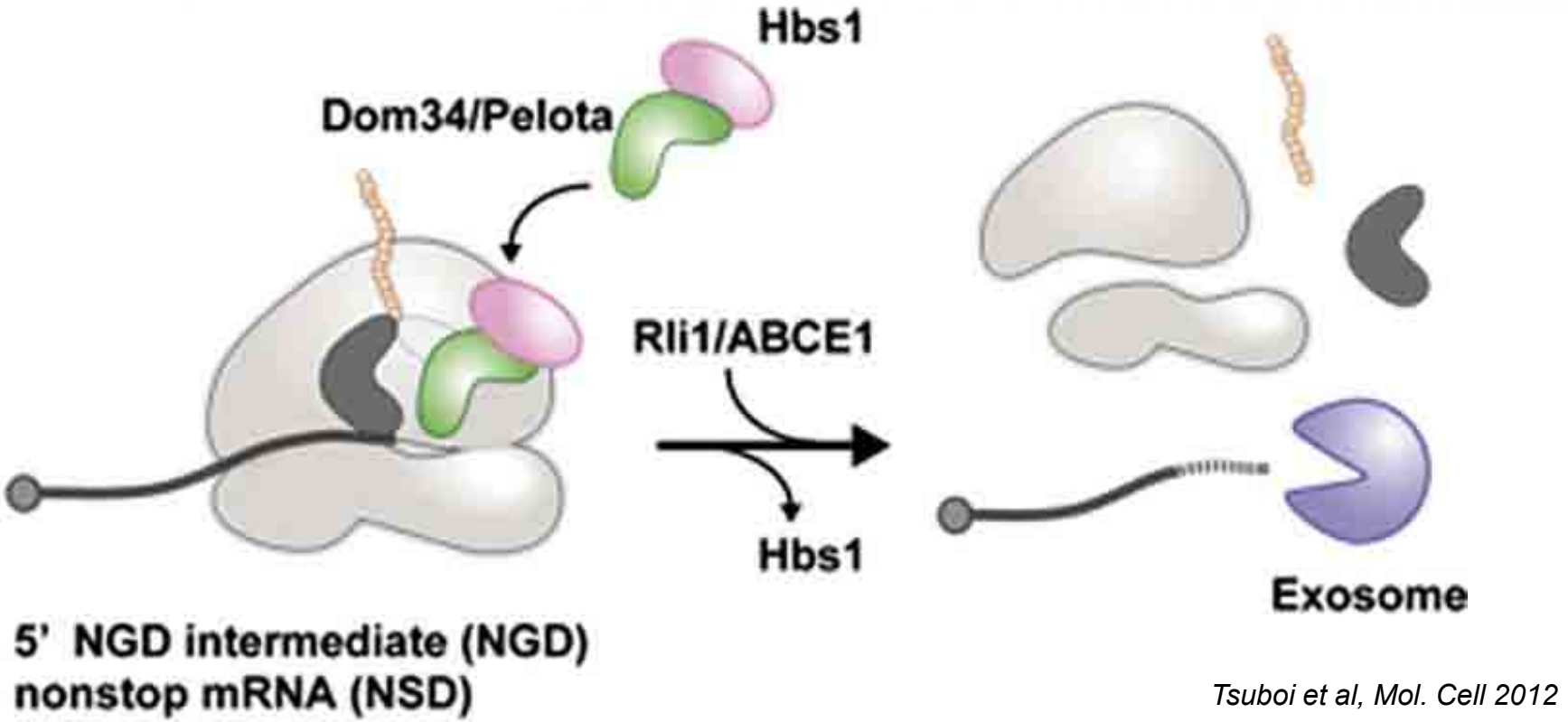
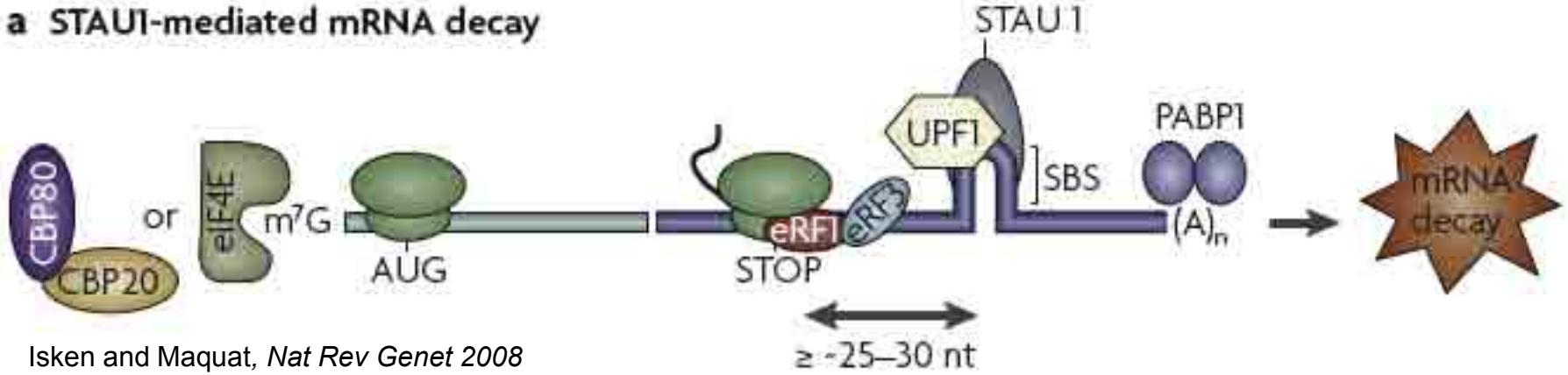
- **NSD**- (non-stop decay) - degradation of mRNAs with no stop codons



Tsuboi et al, Mol. Cell 2012

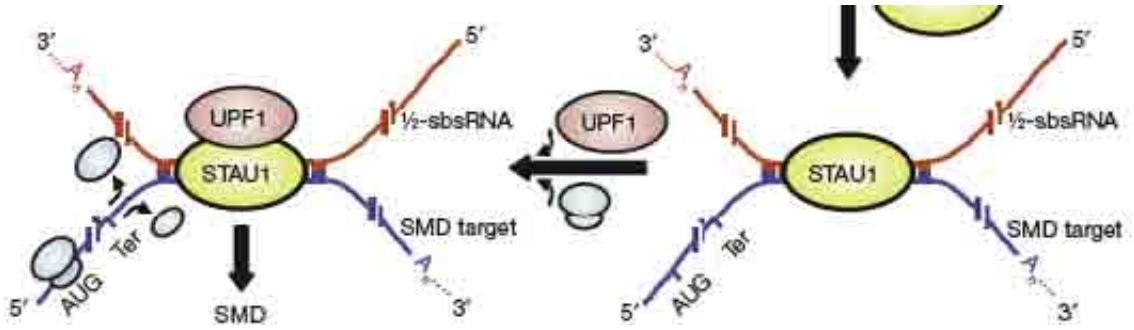
Dom34:Hbs1 stimulates degradation of the 5'-NGD intermediate and nonstop mRNA by dissociating the ribosome that is stalled at the 3' end of the mRNA

STAUFEN-mediated DECAY (SMD)



Isken and Maquat, *Nat Rev Genet 2008*

STAU1, a dsRNA binding protein, recruits UPF1 to target mRNA 3'UTRs to elicit SMD in a translation-dependent fashion.
SMD targets contain a STAU1 binding site (SBS) within their 3' UTR. They include newly synthesized CBC-bound mRNAs and steady-state eIF4E-bound mRNAs.

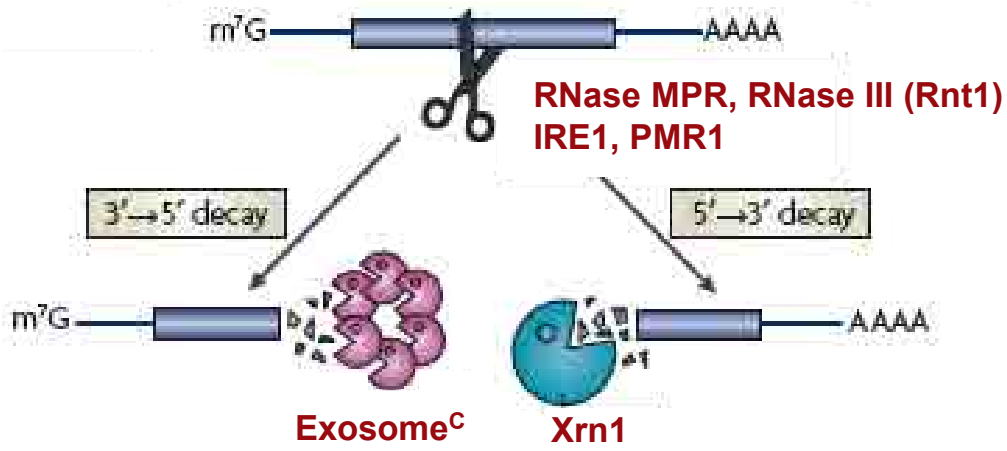


Some mRNAs are targeted to SMD by lncRNAs *(Alu elements)* which form SBS with SMD substrates.

Gong and Maquat, *Nature 2011*

OTHER MECHANISMS

Endonuclease mediated decay

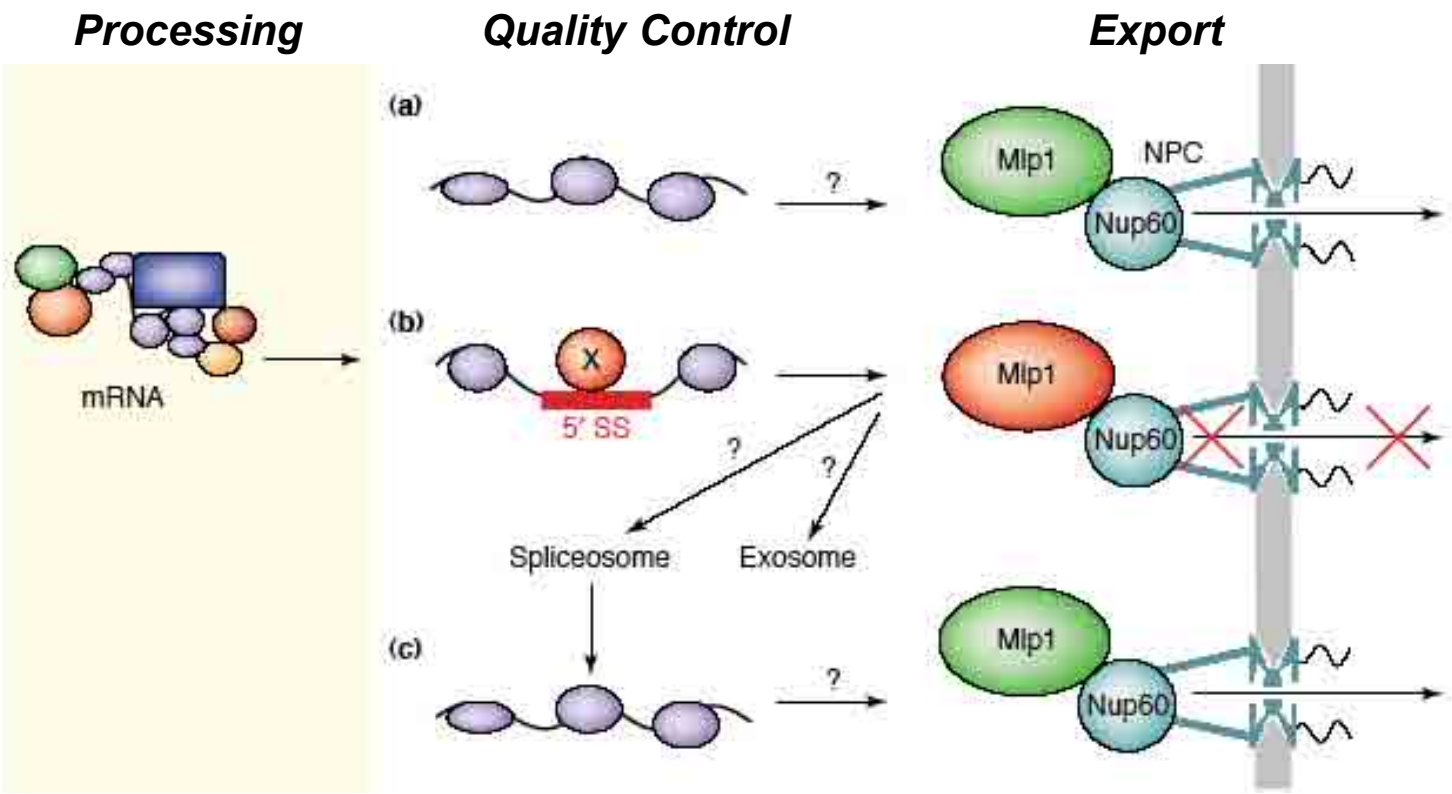


- **PMR1 - degradation of translationally active mRNAs on polysomes**
- **IRE1 - degradation of mRNAs in endoplasmic reticulum during Unfoded Protein Response stress**
- **MRP** ***(RNP responsible for pre-rRNA processing in the nucleolus)*** **- cleaves** ***CLB2*** **mRNA within its 5' UTR in yeast**
- **Rnt1** ***(RNAse III endonuclease, involved in pre-rRNA and pre-sn/snoRNA processing)*** **- cleaves stem-loops structures in some ribosomal protein mRNAs**

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

mRNA DECAY in the NUCLEUS

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

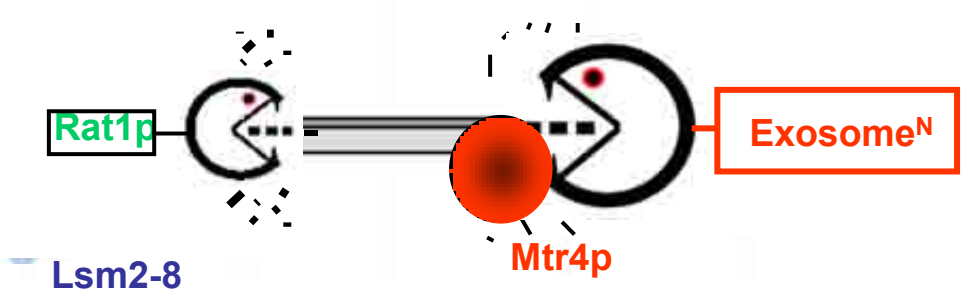


X **RES complex** (**RE**tention and **S**plicing): **Snu17/Bud13/Pml1**

Casolari and Silver, TiCB., 2004

mRNA DECAY
IN THE NUCLEUS

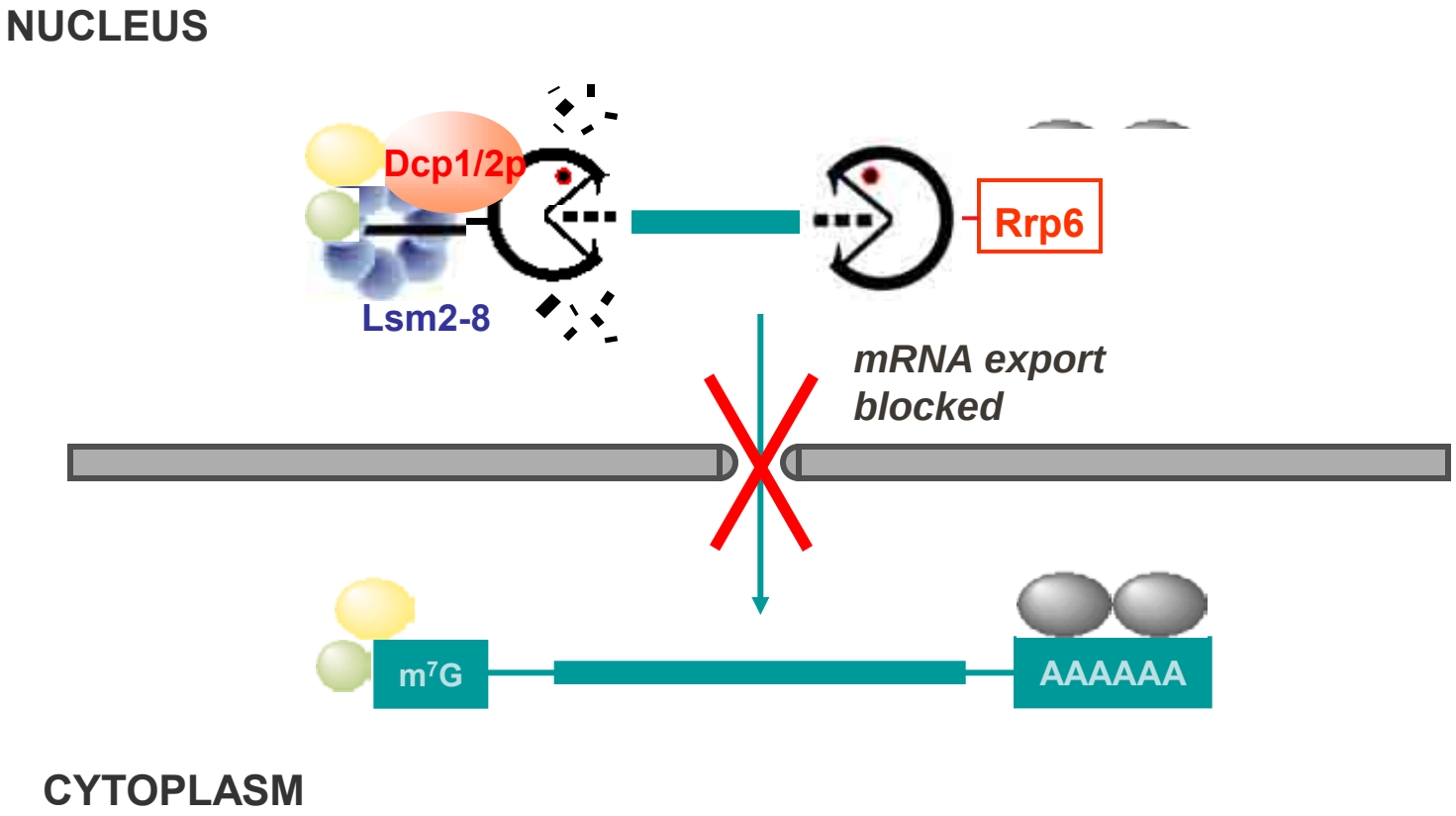
- **pre-mRNA with unspliced introns**



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

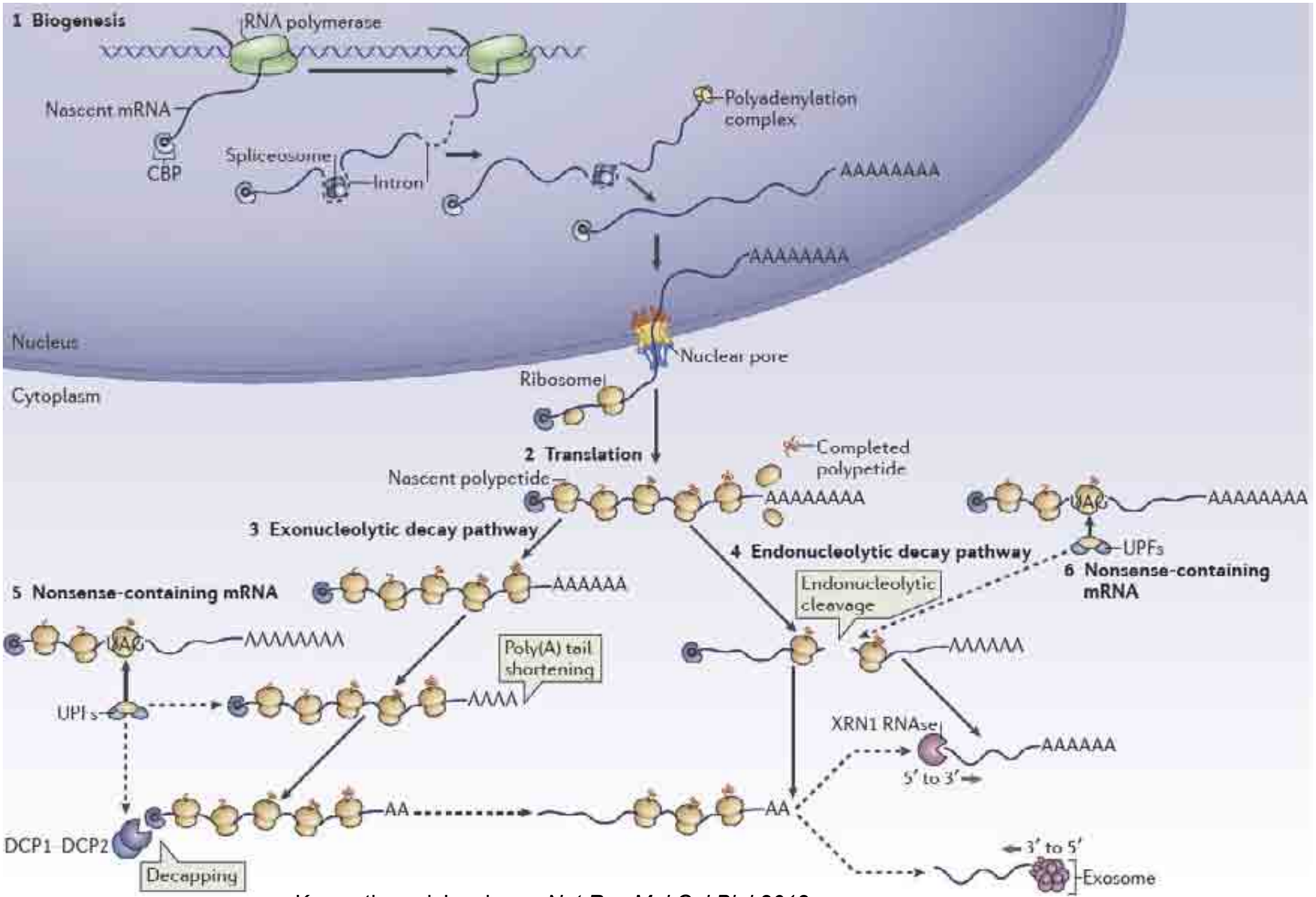
mRNA DECAY
IN THE NUCLEUS

- **mRNA arrested in the nucleus**



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

mRNA SYNTHESIS and DECAY



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

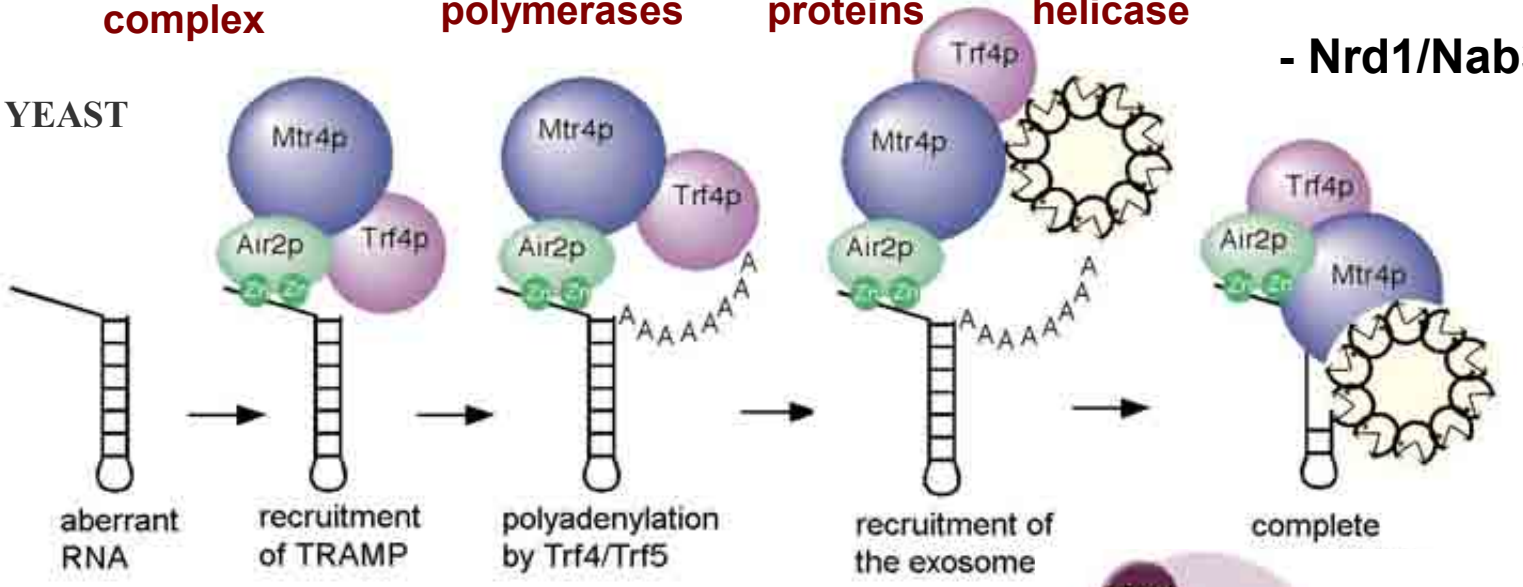
TRAMP/NEXT EXOSOME COFACTORS

TRAMP = **Trf4/5** + **Air1/2** + **Mtr4**

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

Interacts with

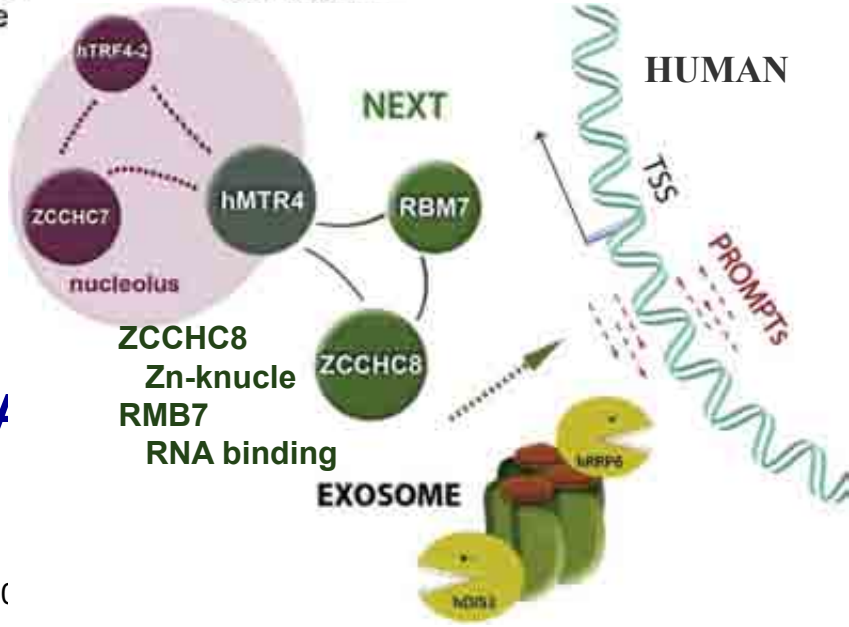
- **exosome via Mtr4**
- **Nrd1/Nab3 complex**



some RNAs degraded by Rat1/Xrn1

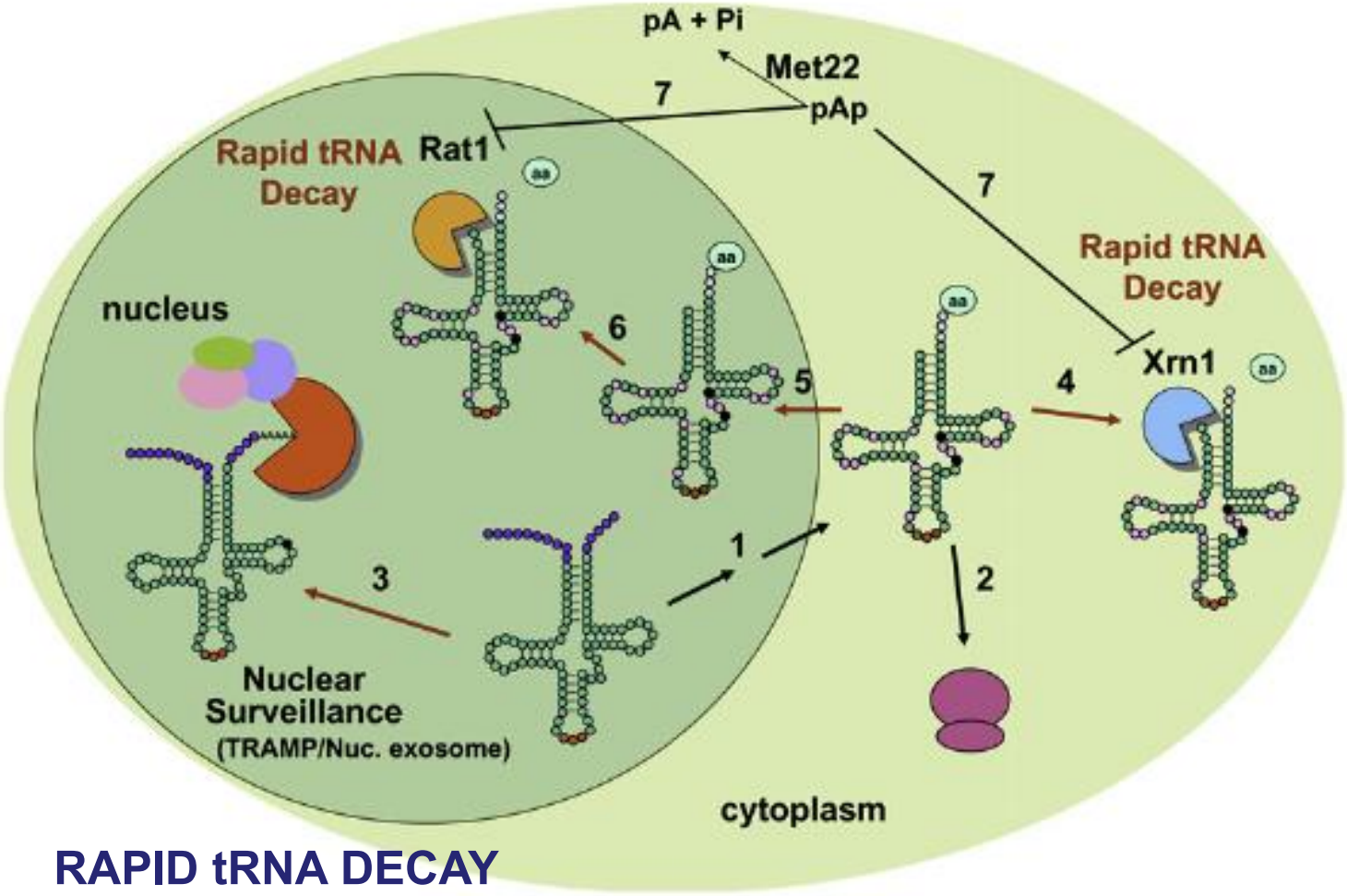
Polyadenylation-mediated nuclear discard pathway for defective RNAs

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



LaCava et al., *Cell,* 2005; Vanacova *et al., PLoS Biol.* 2005; Wyers *et al., Cell,* 20(

tRNA SURVEILLANCE



RAPID tRNA DECAY

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

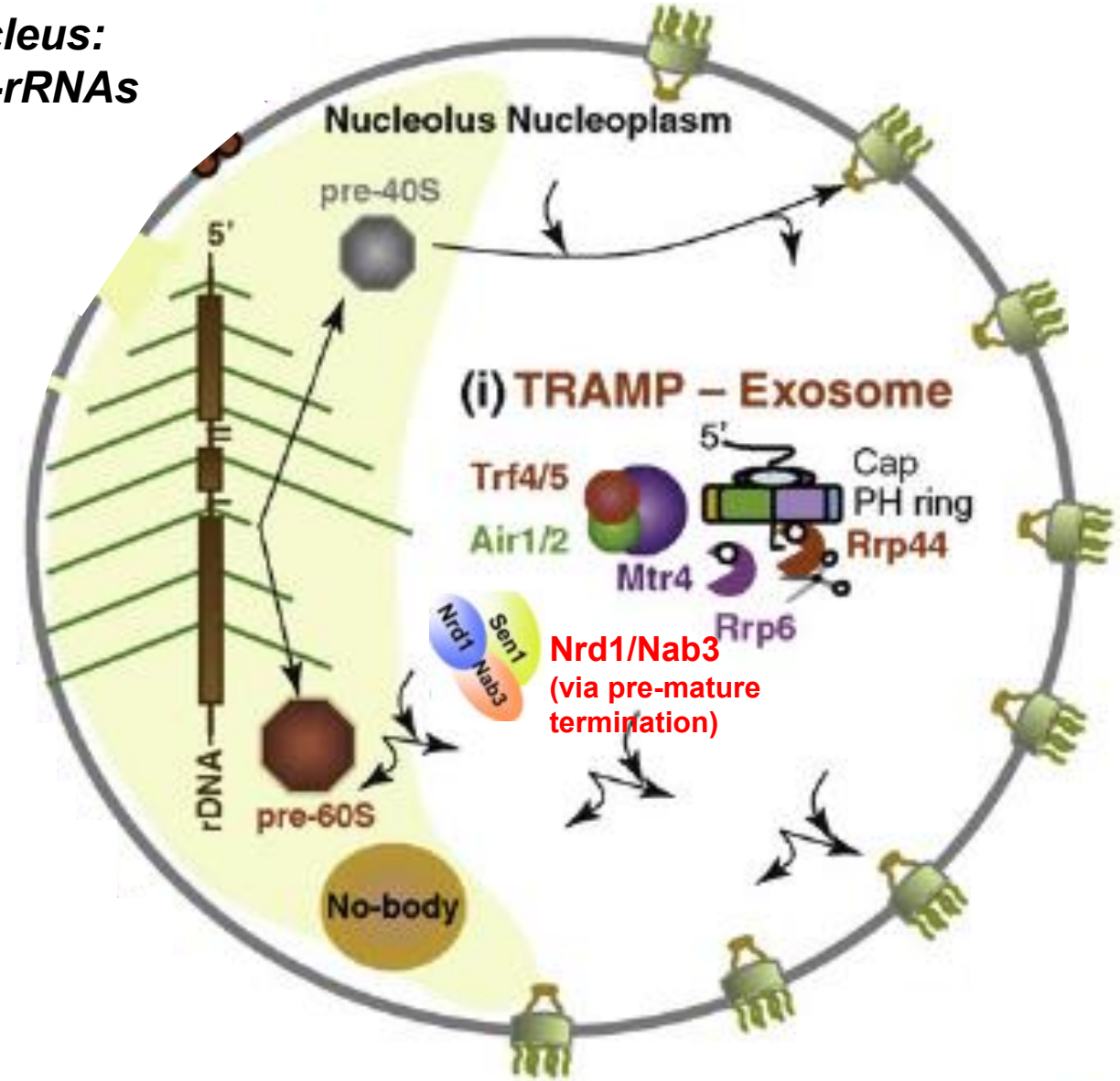
- in the nucleus *(polyadenylation via TRAMP and degradation by the exosome or degradation by Rat1)*

- in the cytoplasm *(degradation by Xrn1)*

Phizicky and Hopper , GeneGev.,2010

rRNA SURVEILLANCE

Nucleus:
pre-rRNAs

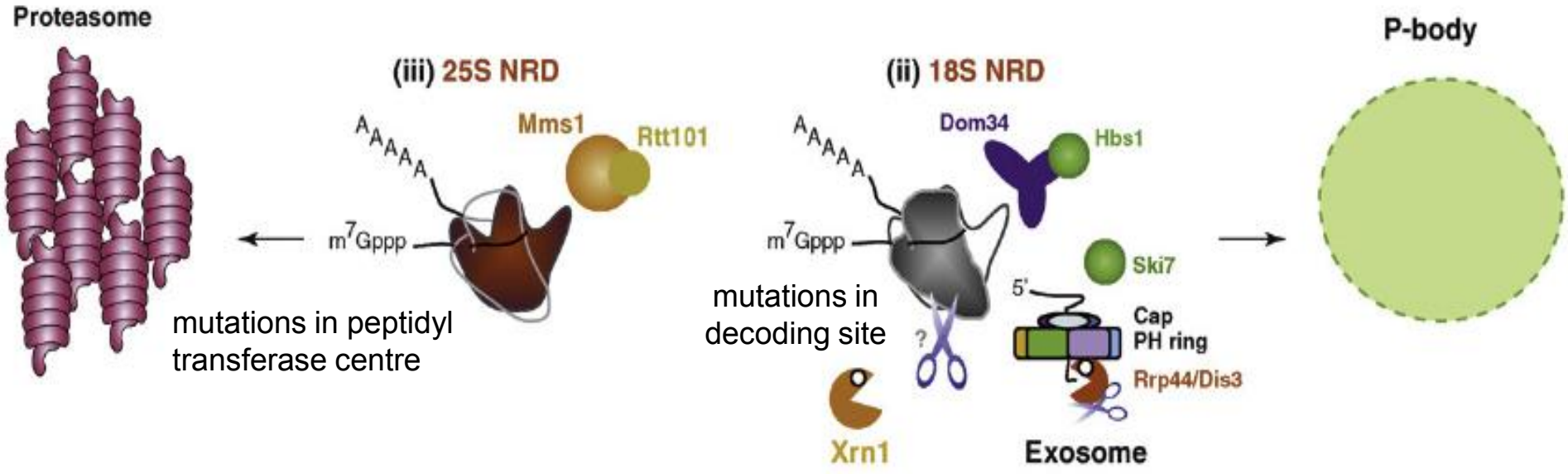


Lafontaine, TiBS.,2010

rRNA SURVEILLANCE

NRD- nonfunctional rRNA decay

Cytoplasm:
mature ribosomes

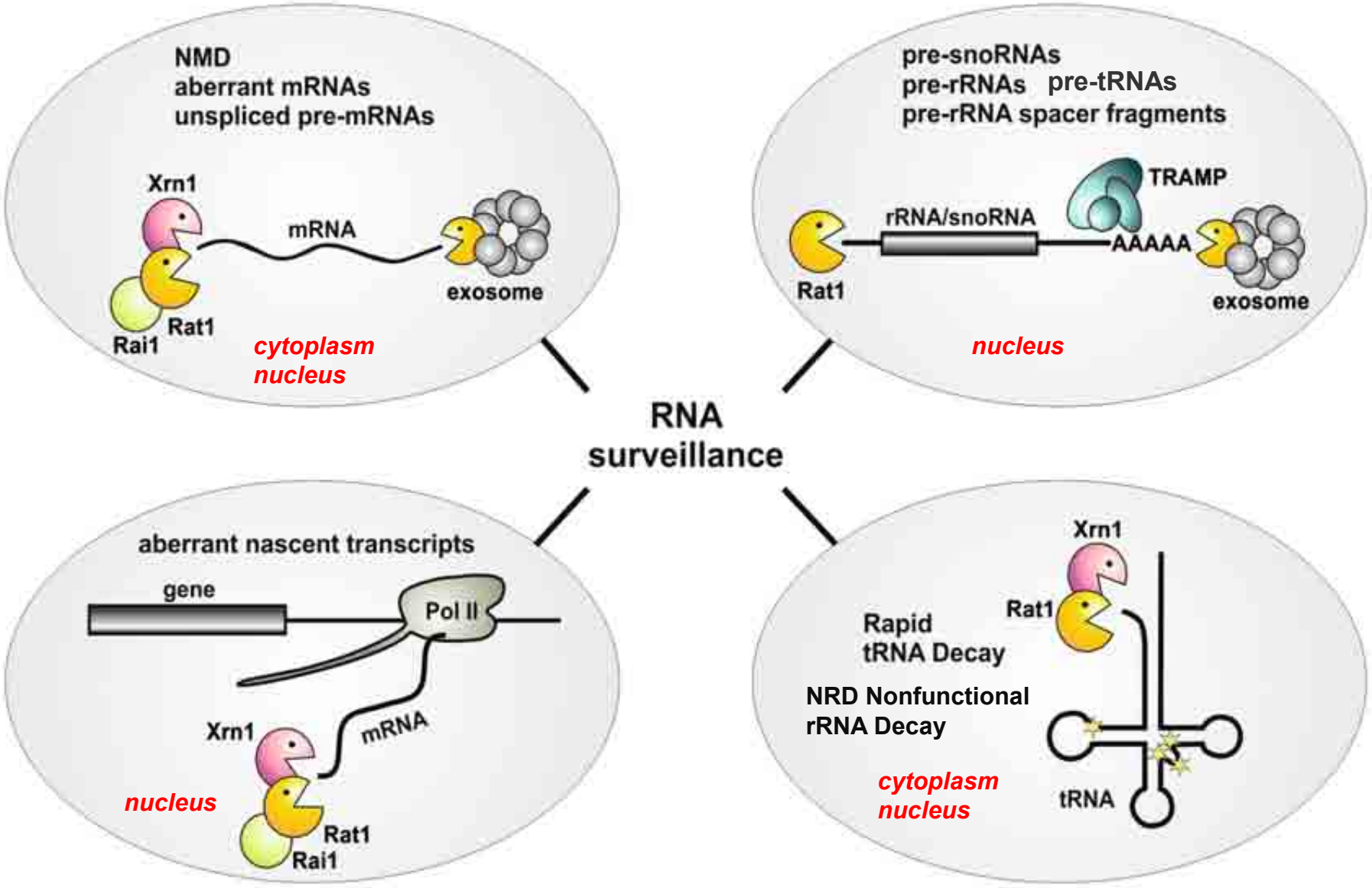


Mms1, Rtt101-
subunits of E3 ubiquitin ligase complex

Dom34::Hbs1
factors involved in NGD and NSD

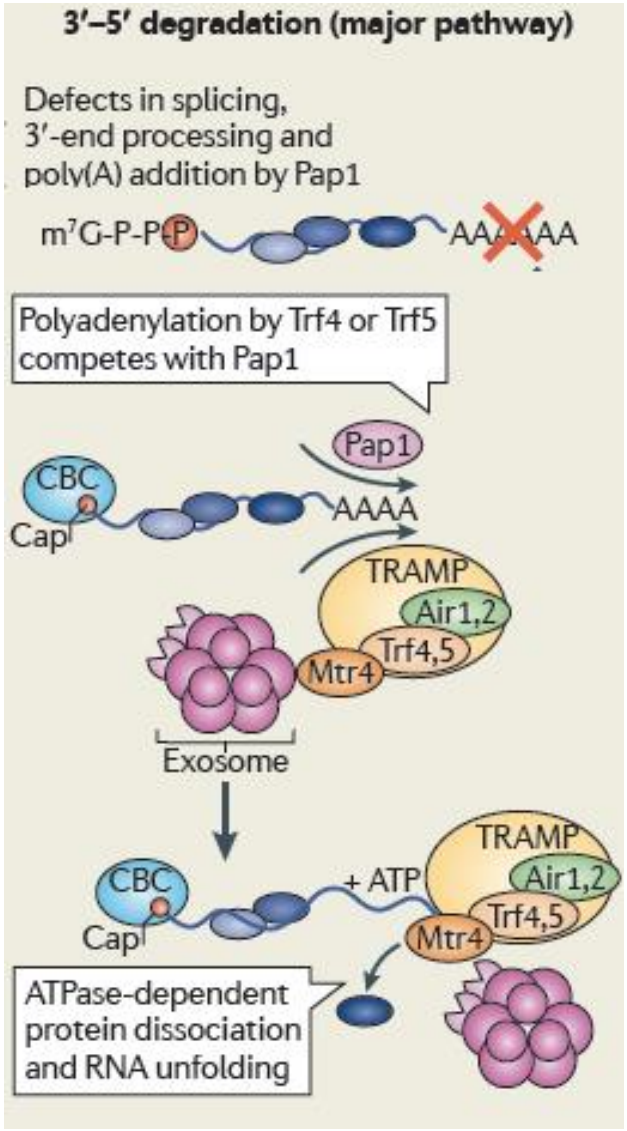
Lafontaine, TiBS.,2010

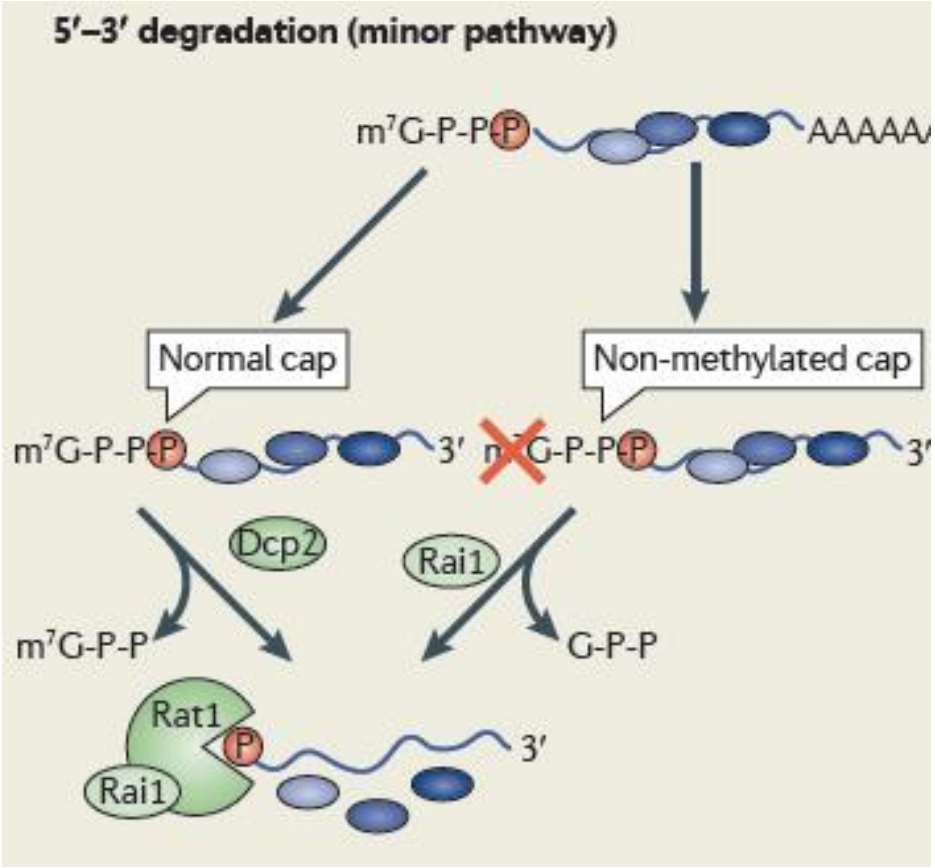
RNA SURVEILLANCE



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

NUCLEAR RNA SURVEILLANCE





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

TAKEHOME MESSAGE

I. RNA WORLD

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

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

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

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

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

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

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

TAKEHOME MESSAGE

II. MODERN RNA WORLD

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

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

TAKEHOME MESSAGE

III. RNA METABOLISM

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

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

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

- **transcription termination**

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

- 3' cleavage and polyadenylation machinery (mRNA)

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

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

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

TAKEHOME MESSAGE

IV. COTRANSCRIPTIONALITY

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

TAKEHOME MESSAGE

V. RNA DECAY

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

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

1. deadenylation ➔ decapping ➔ exonucleolytic degradation

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

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

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

PROCESSING AND DEGRADATION IS OFTEN CARRIED OUT BY THE SAME MACHINERIES