RNA DEGRADATION AND QUALITY CONTROL

RNA metabolism 21st November 2023

LECTURE CONTENTS

RNA degradation

-general principles

-RNA degradation machines

RNA surveillance

-noncoding RNAs

-coding RNAs (mRNAs)

(m)RNA Turnover: Why Should We Care?

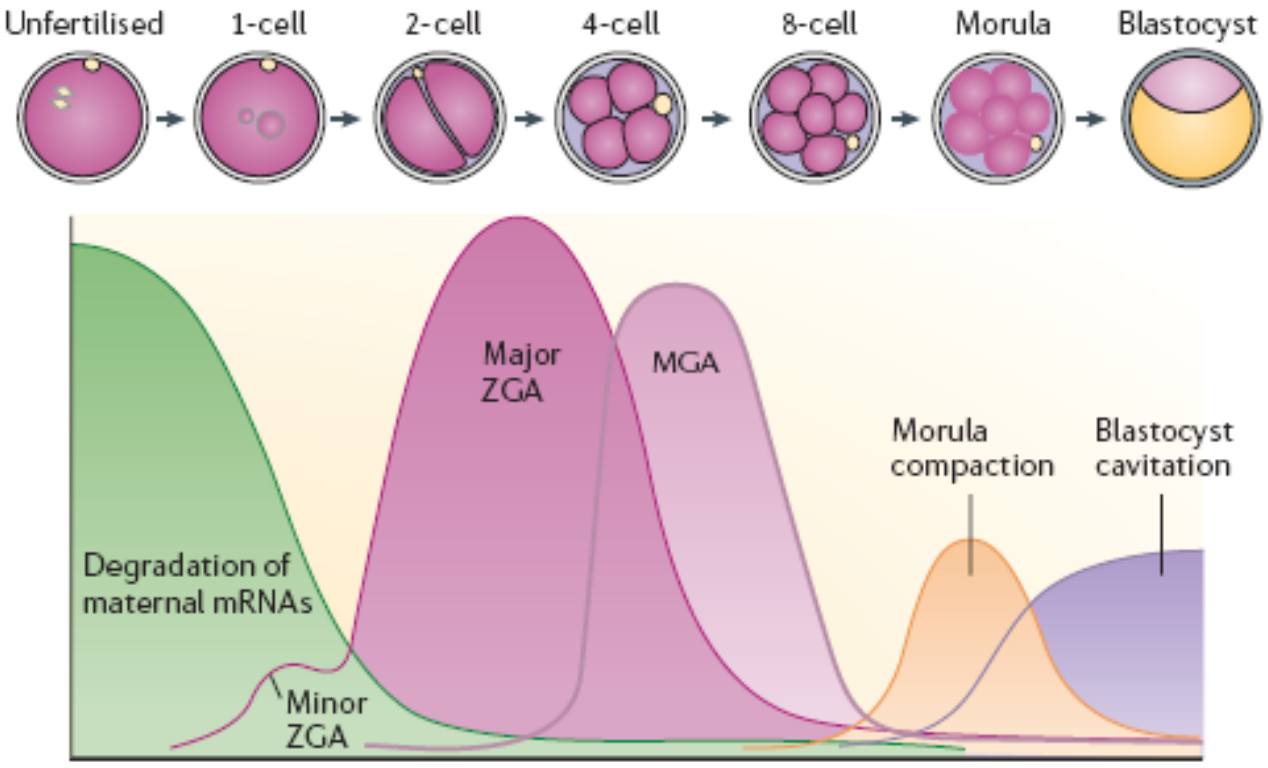
1. Control of Gene Expression

2. Quality Control of RNA Biogenesis

RNA degradation in development

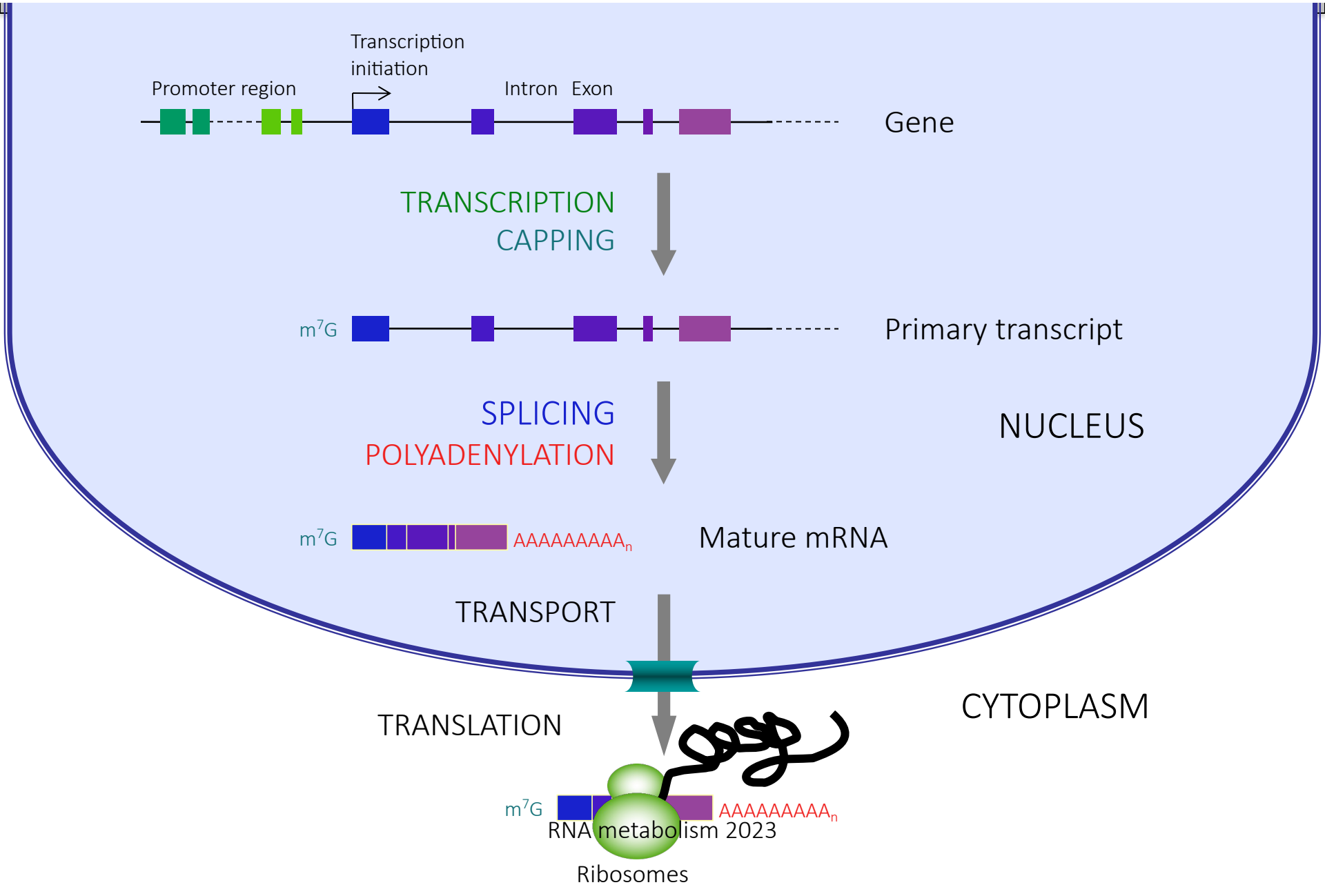
Some examples

Gene expression during preimplantation embryo development

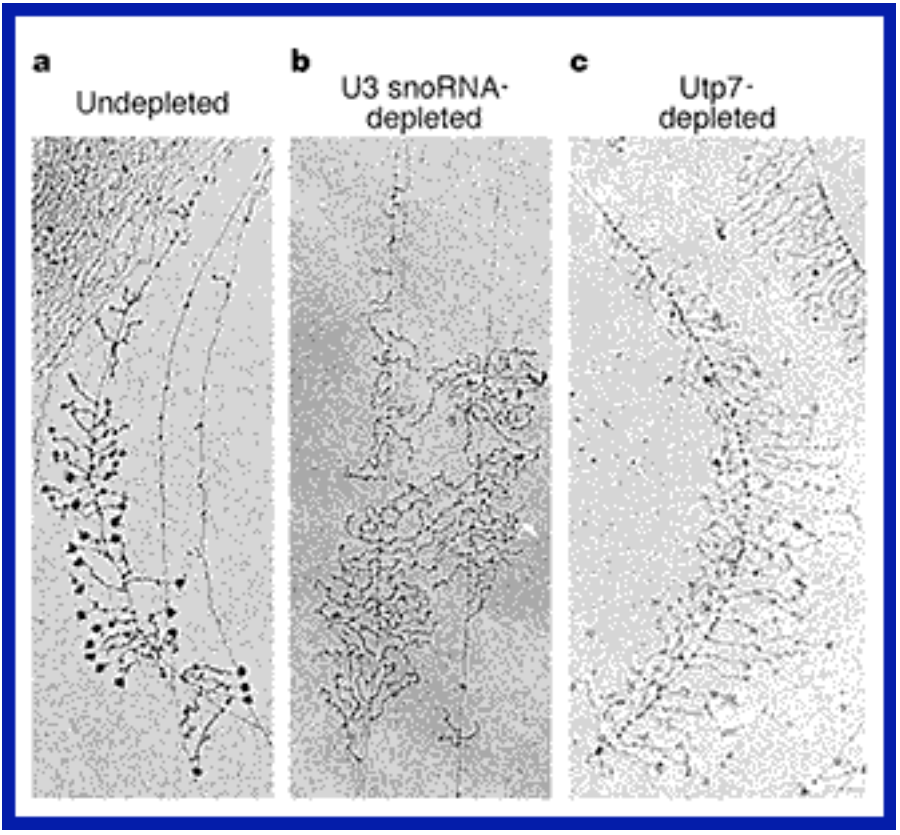


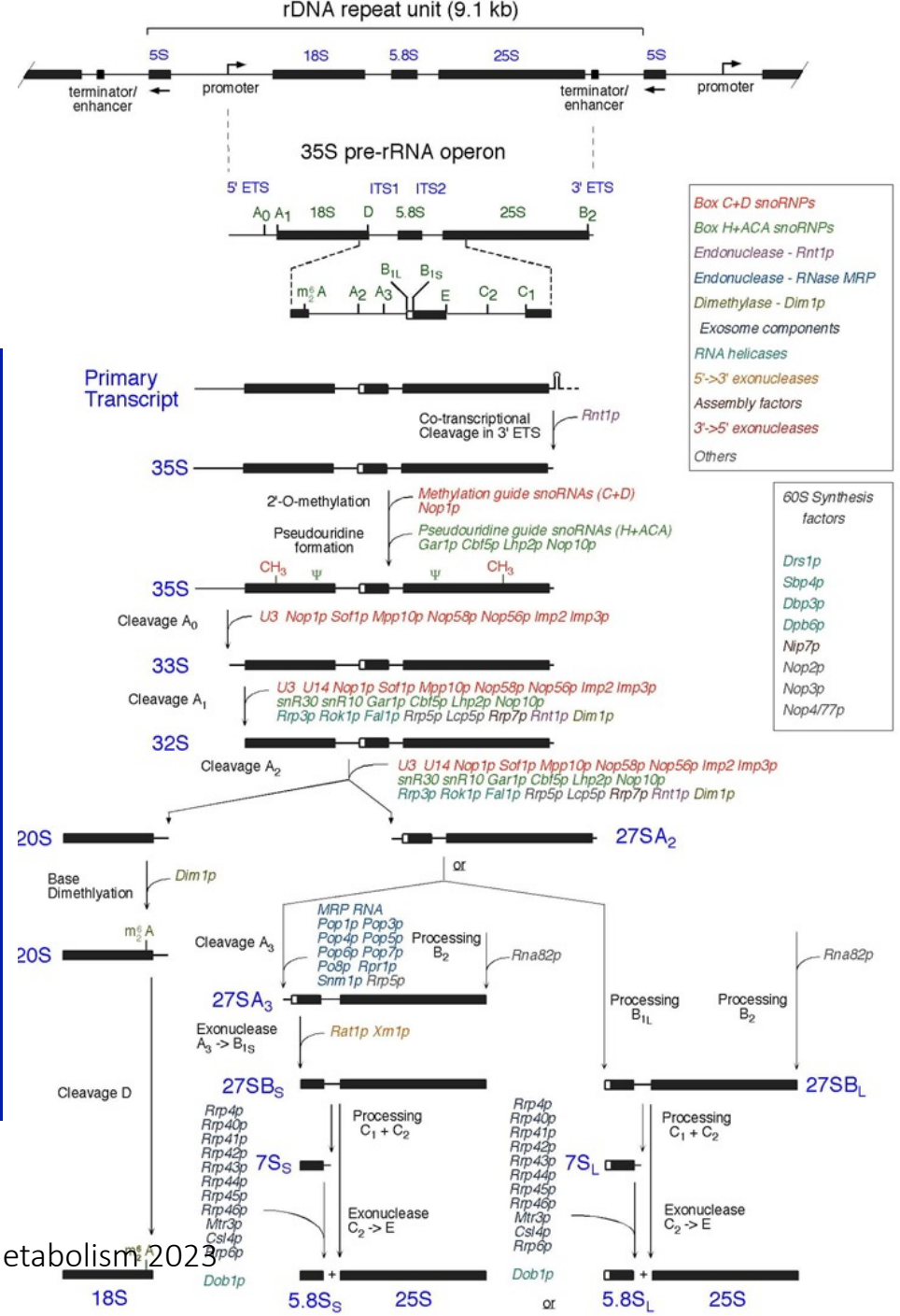
Wang et al. Nature Reviews Genetics 7, 185–199 (March 2006) | doi:10.1038/nrg1808

All steps of mRNA production are controlled

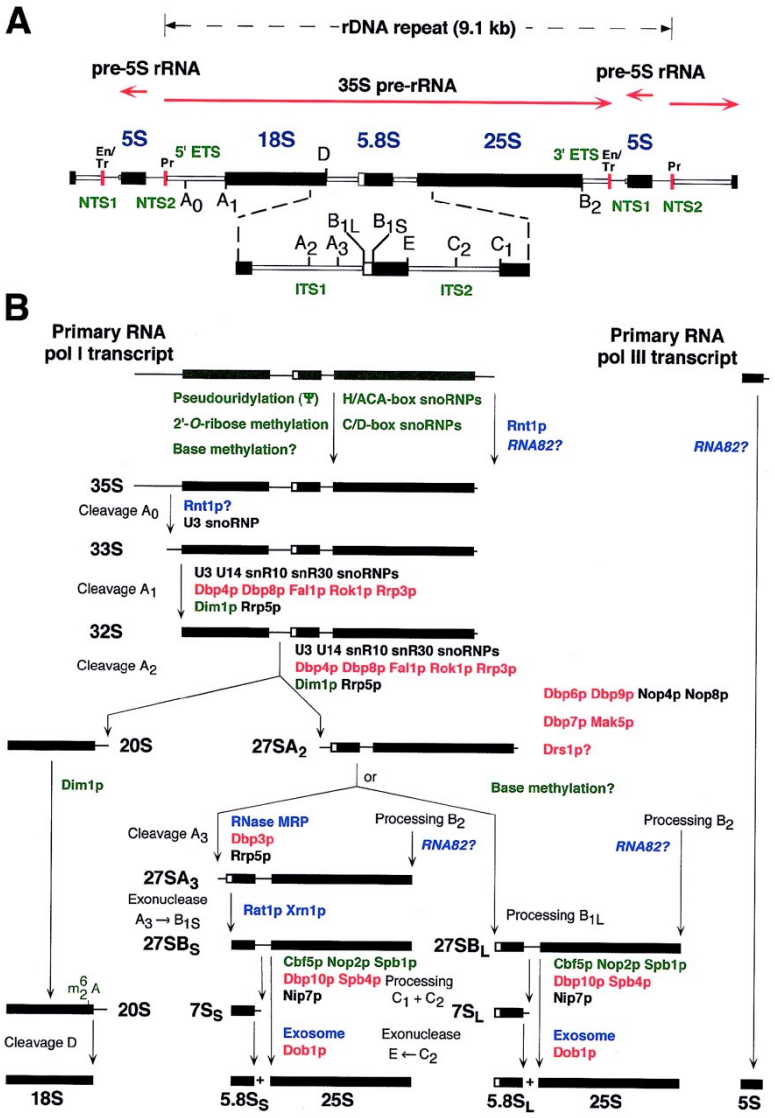


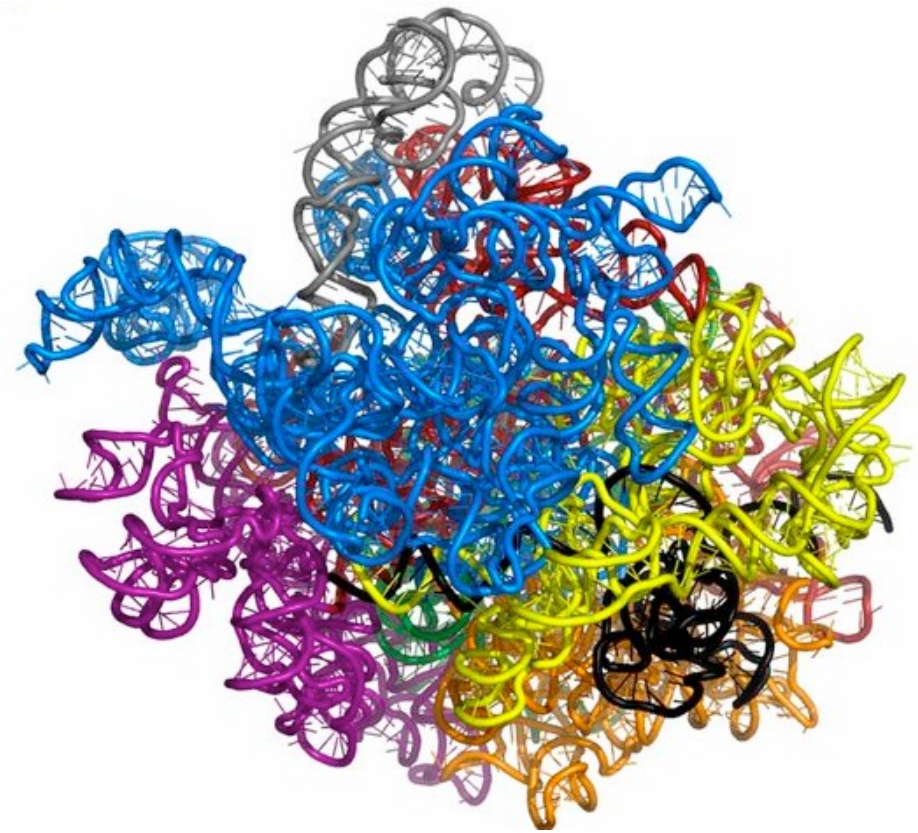
Ribosomal RNA maturation is one of the most complex RNA linked processes in the cell and must be tightly controlled.





rRNA production is highly complex and energetically expensive





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When the removal of decay products goes wrong

AVERAGE mRNA HALF LIFE

AVERAGE mRNA HALF LIFE

E. coli: 4 min (2-10 min)

Yeast: 22 min (4-40 min)

Humans: 10 hours (0.5-24 hours)

RNA degradation: → typical mRNAs in a somatic cell last from minutes to hours and this is a function of the balance between synthesis and degradation

mRNA stability and turnover

• The concentration of mRNA is a function of both the rate of mRNA synthesis and the rate of mRNA degradation

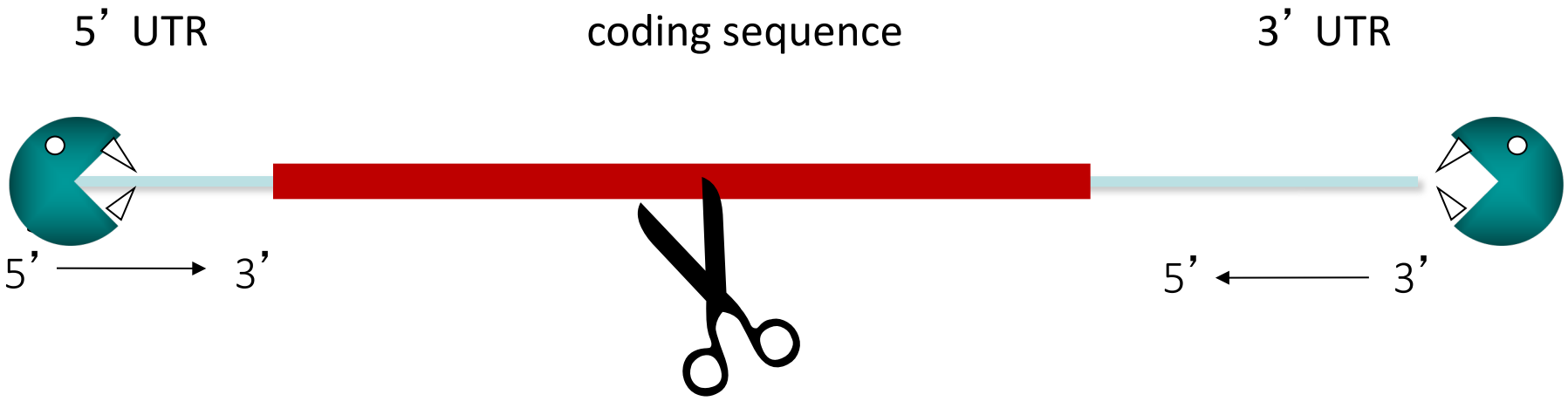
• The stability of mRNA also determines how rapidly synthesis of the encoded protein can be shut down → e.g. for a stable mRNA, protein synthesis can persist long after transcription of the gene is repressed → mRNA half life of most multicellular eukaryotic cells is many hours (compared to just a few minutes for bacteria)

• Some proteins in eukaryotic cells are required for very short periods of time and are expressed in bursts (e.g. many signaling molecules like cytokines or cell cycle regulated transcription factors, such as c-fos)

• Regulating the stability of mRNA is one way of ensuring that proteins are present for only short bursts or for longer periods of time, as is needed

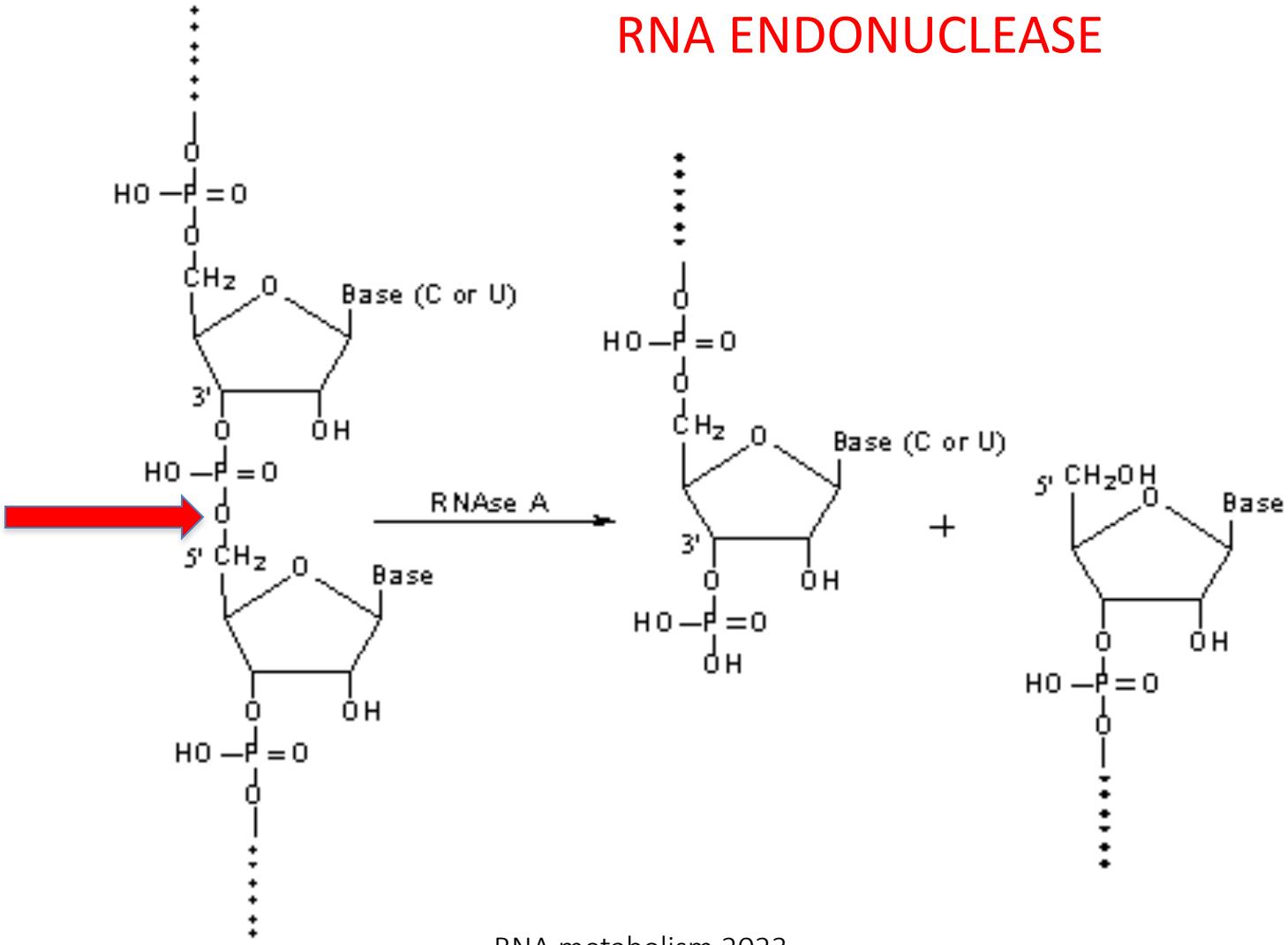
RNA degradation mechanisms

RNA is prone to nucleolysis



Endonuclease

RNA degradation by nucleases



RNA degradation by nucleases

RNaseA

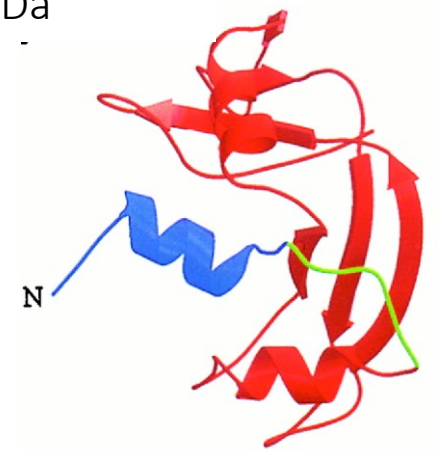
pankreatic endoribonuclease

Highly stable,

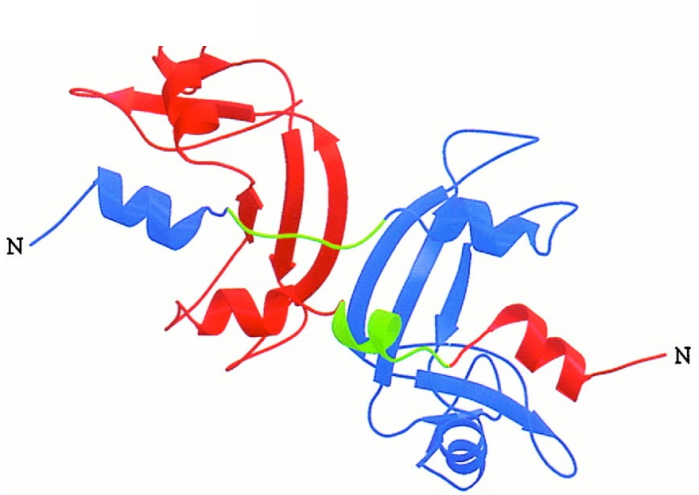
Heat resistant

Small – hard to remove

Monomer, 14 kDa



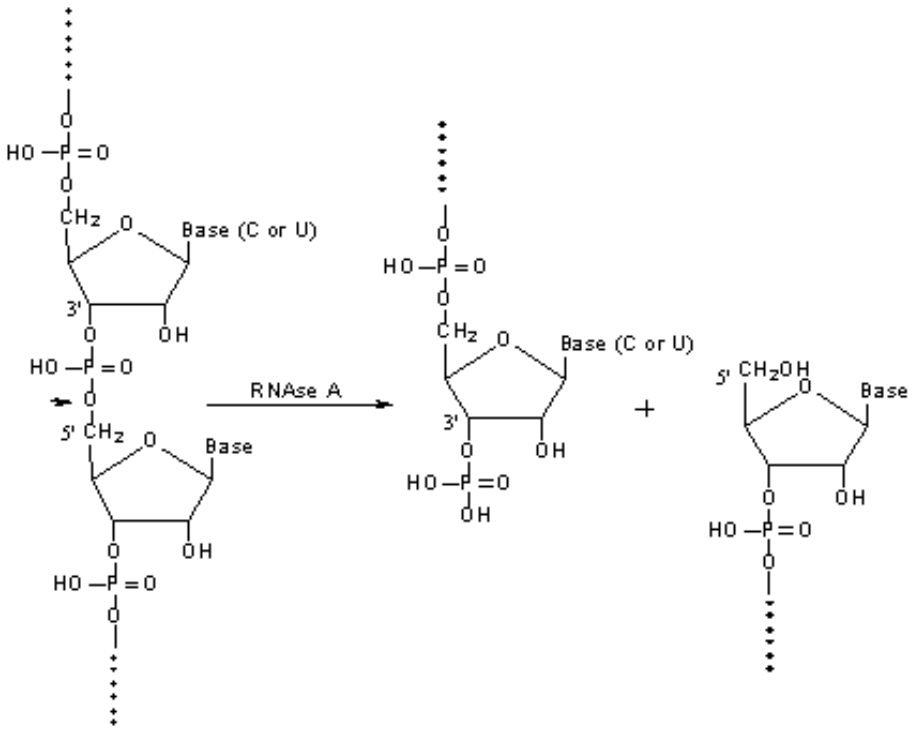
Dimer



Liu et al., PNAS 1998

RNA degradation by nucleases

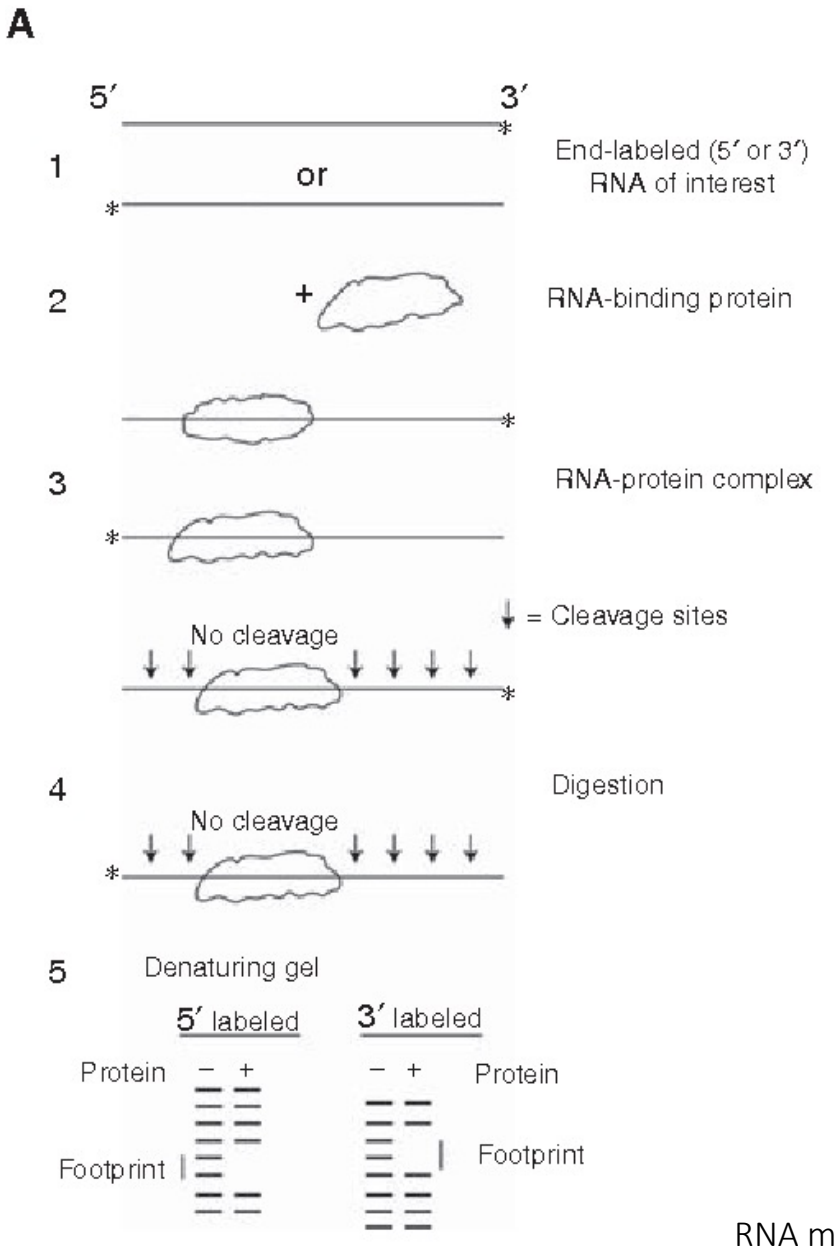
RNA ENDONUCLEASE

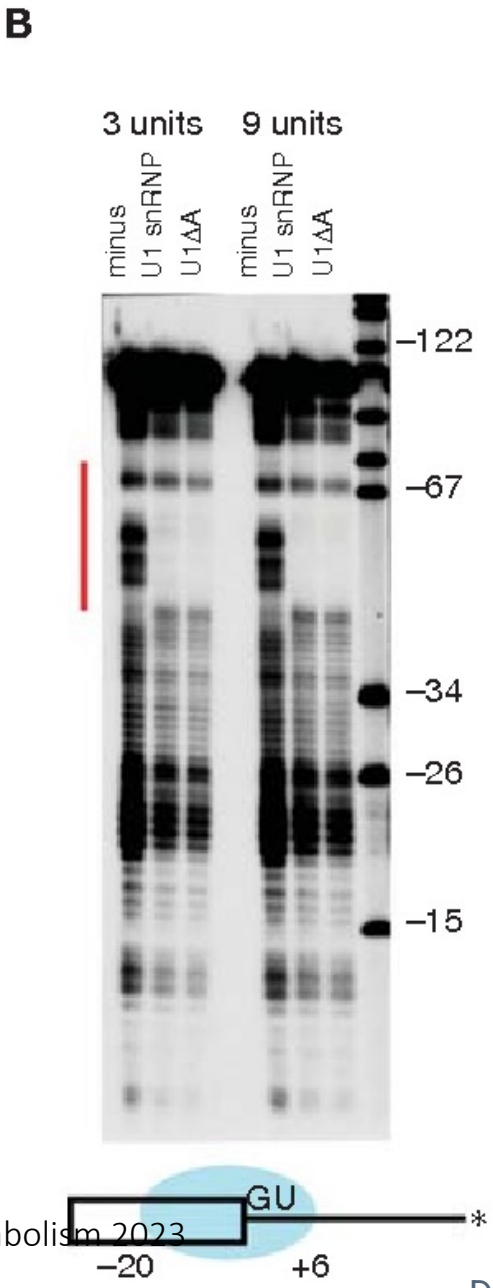


	cleaves 3'of
RNase A	C,U
RNase T1	G
RNase T2	GACU
RNase U2	A in ssRNA
RNase I	GACU in ssRNA
RNase III	GACU in dsRNA
RNase H	RNA:DNA hybrid

(often used to remove specific parts of RNA)

RNAse protection assay





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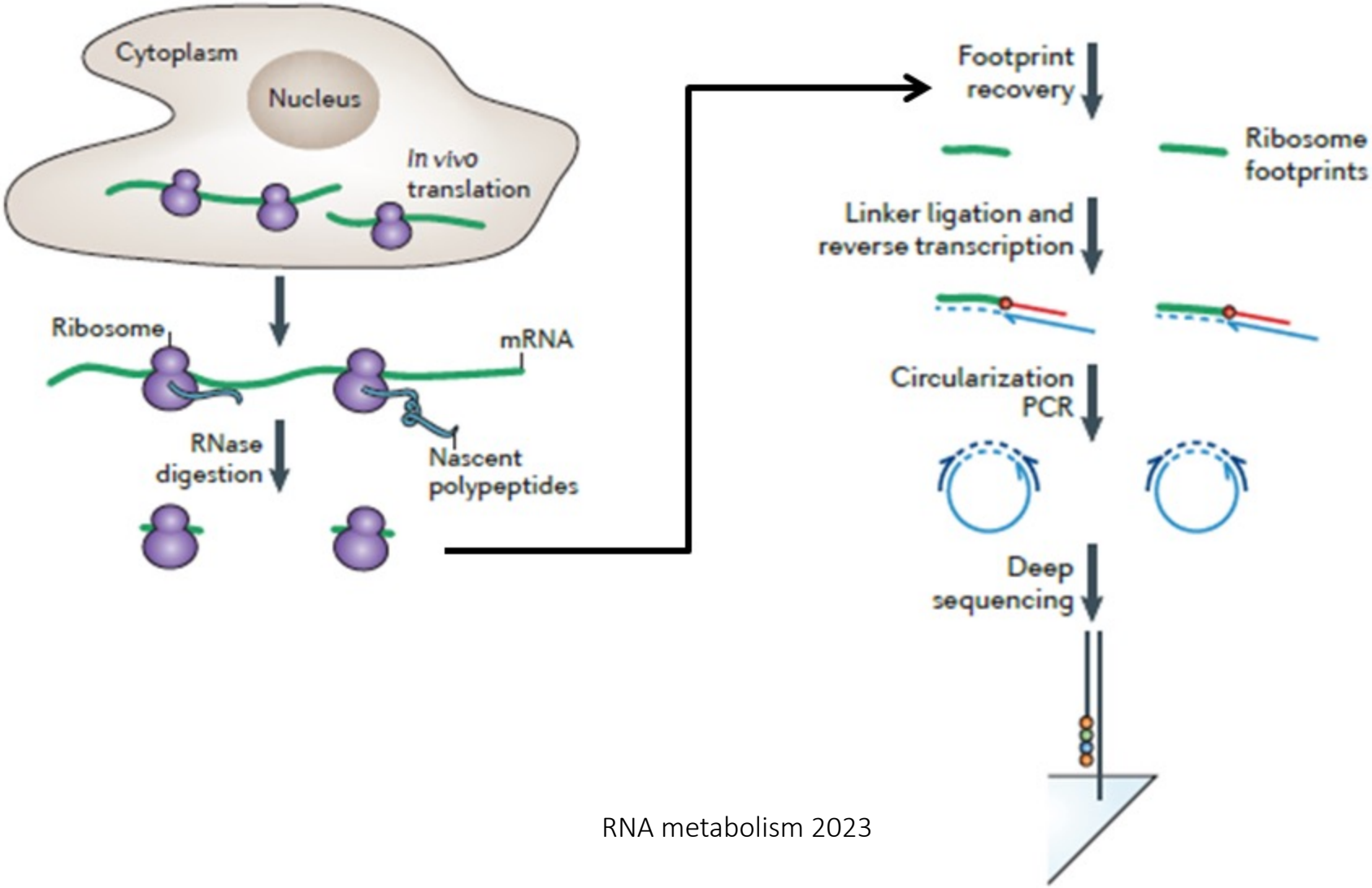
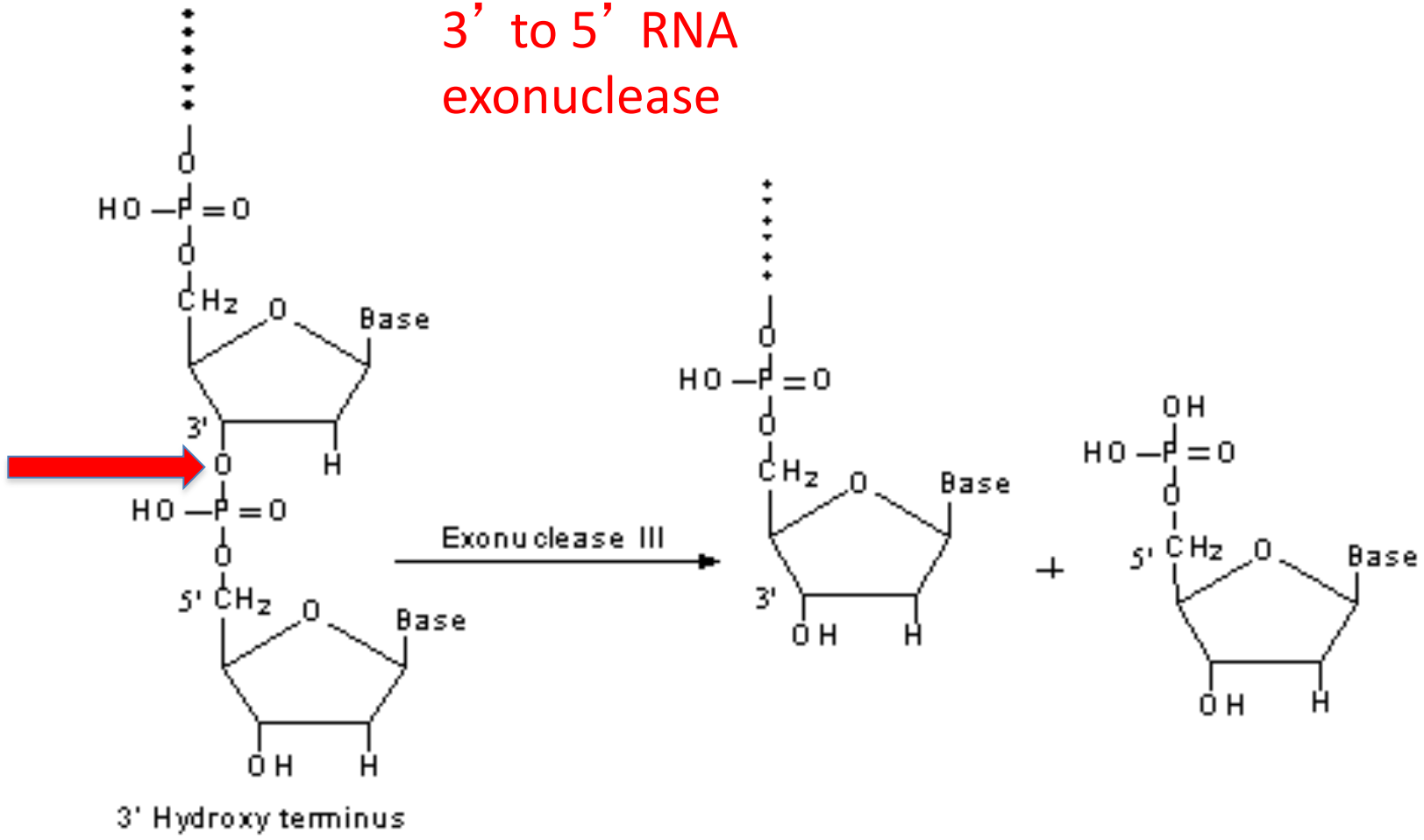
•DOI:10.1101/pdb.prot080788

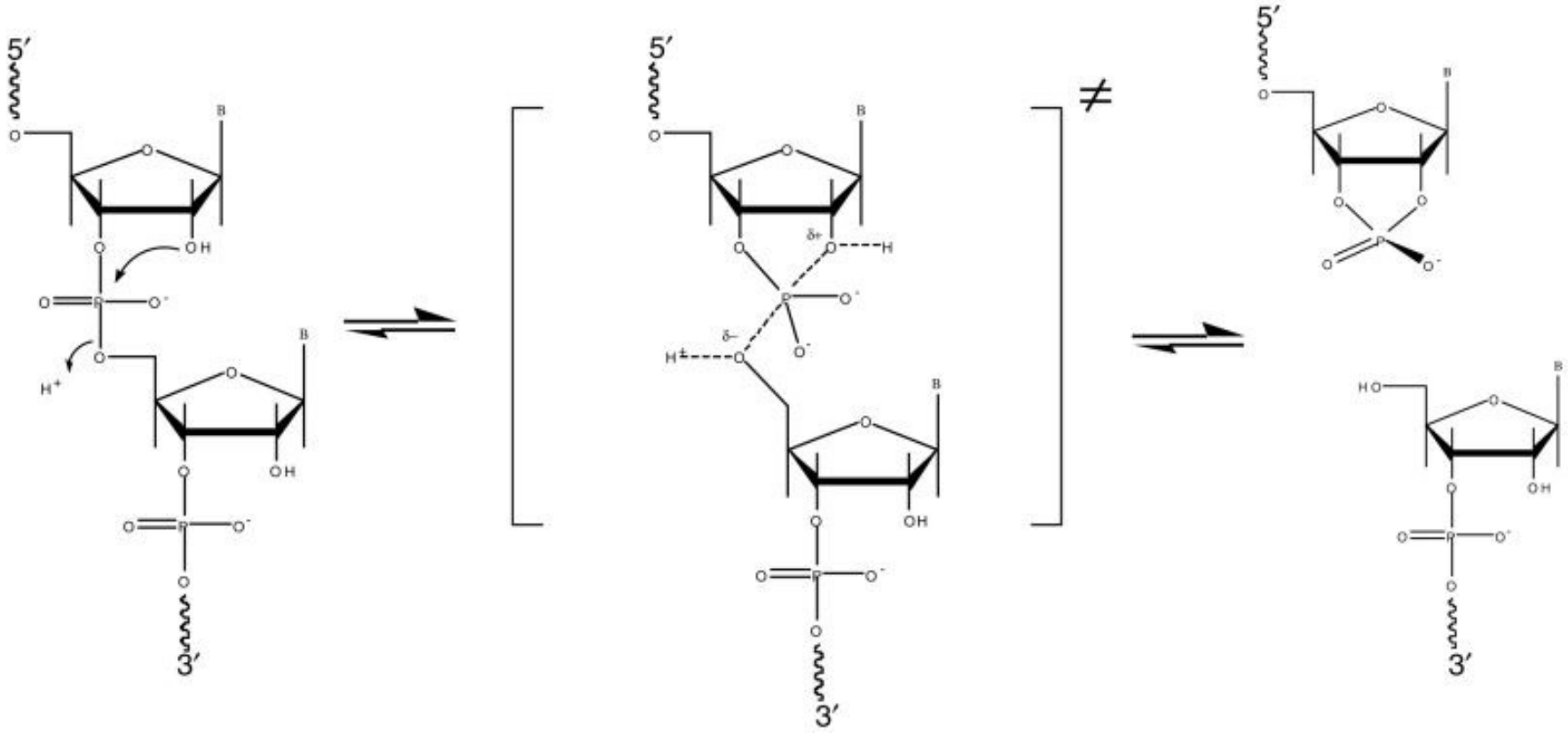
Ribosome footprinting analysis



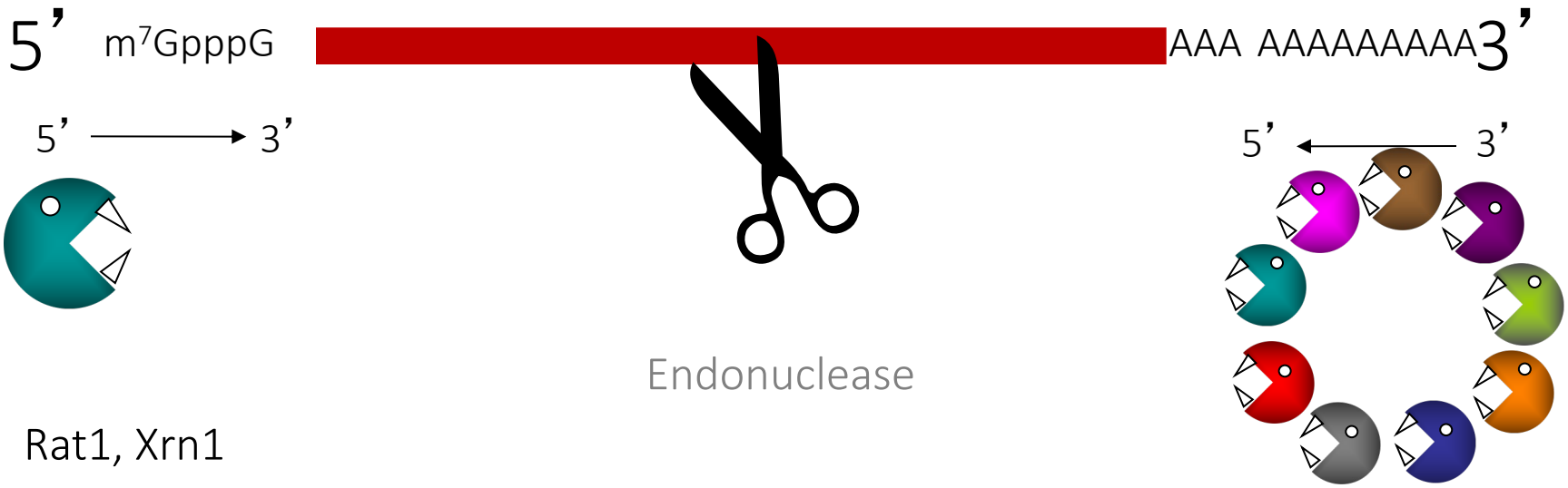
3’ to 5’ RNA exonuclease



RNA degradation by hydrolysis!!



mRNA DEGRADATION



The degradation must be closely regulated in order to prevent wholesale elimination of all transcripts.

RNA DECAY

- Is mainly exonucleolytic – RNAs can escape the decay by simply protecting their ends with proteins and/or by structural elements.

Examples:

<u>mRNA</u>:

5’ 7mGpppG cap plus cap-binding proteins

3’ poly(A) tail with poly(A) binding proteins bound

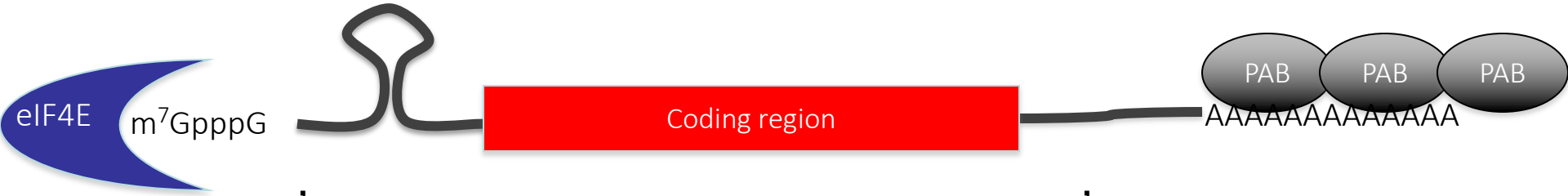
<u>tRNA, rRNA, snRNA, snoRNA</u>:

complex secondary and tertiary structures

Base modifications

mRNA stabilizing and destabilizing features

- Protein binding elements
- RNA binding sequence elements
- Structural elements



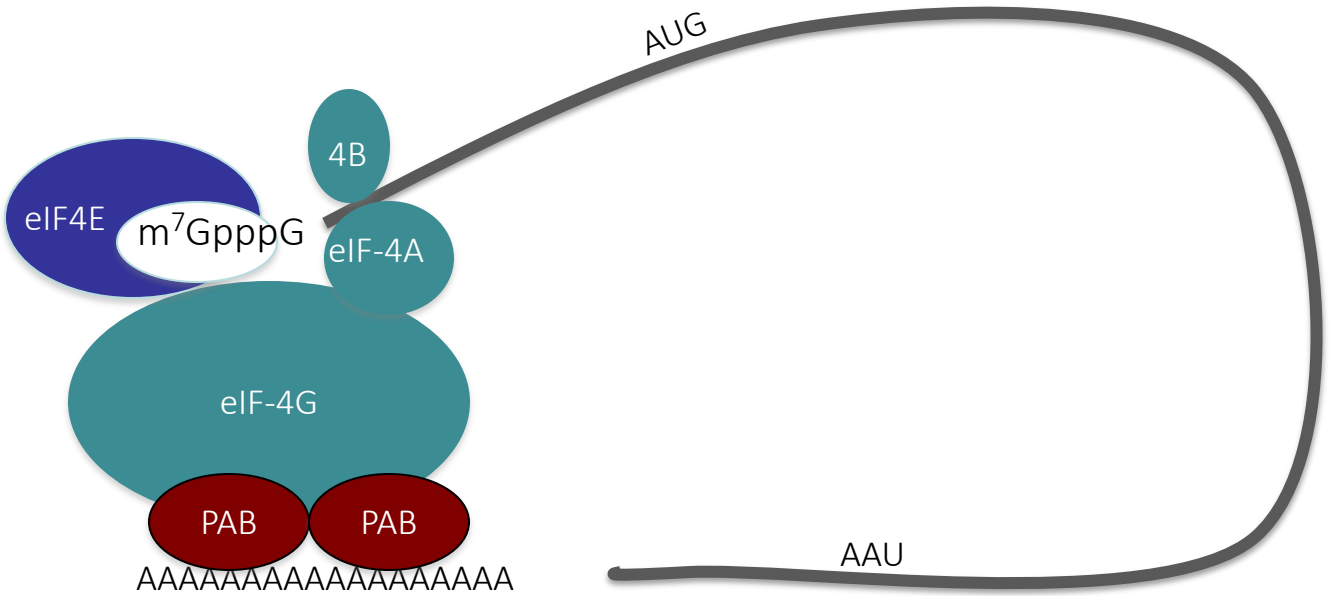
5’ UTR

Protein binding and secondary structures

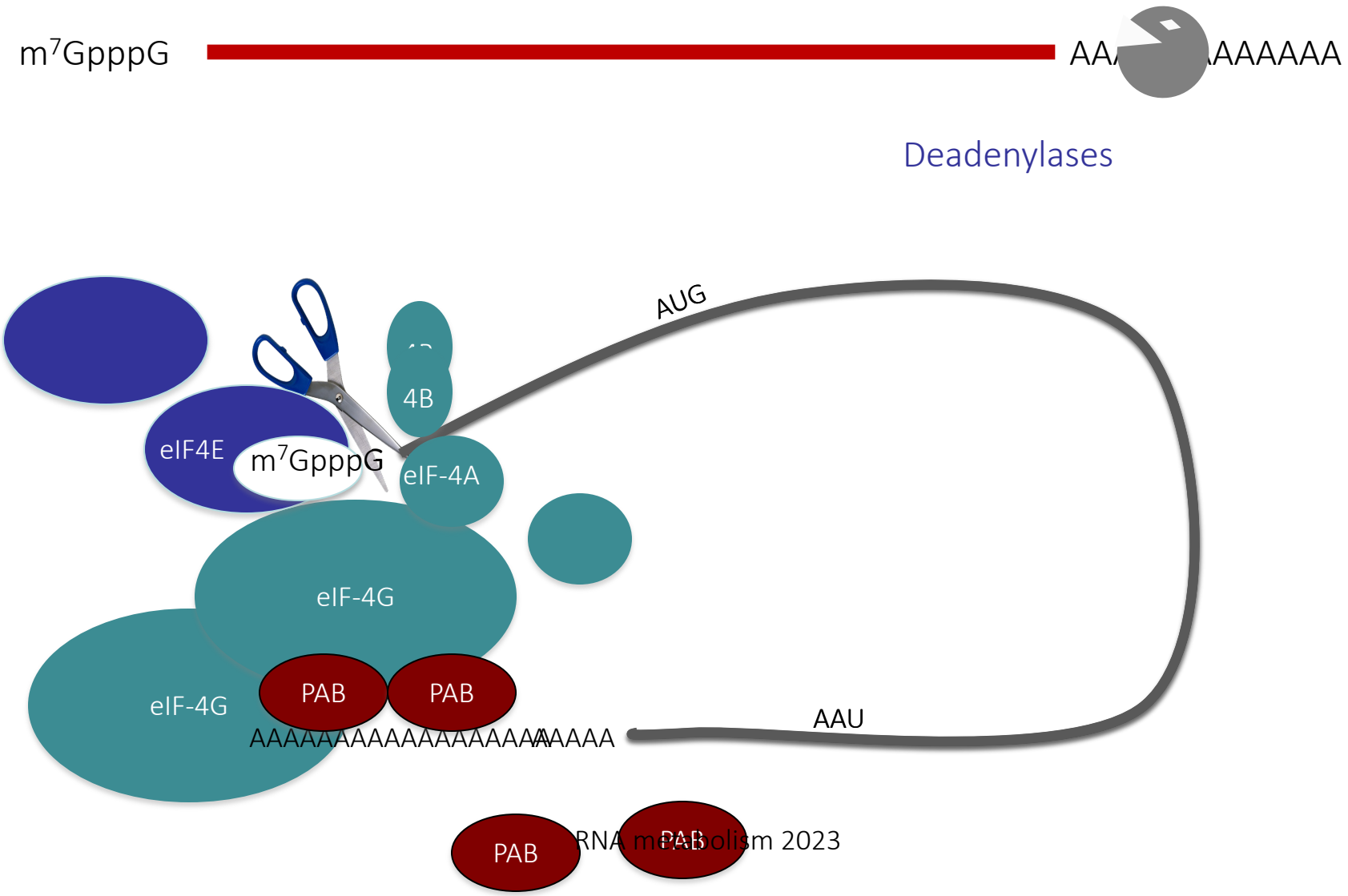
3’ UTR

Protein and RNA binding

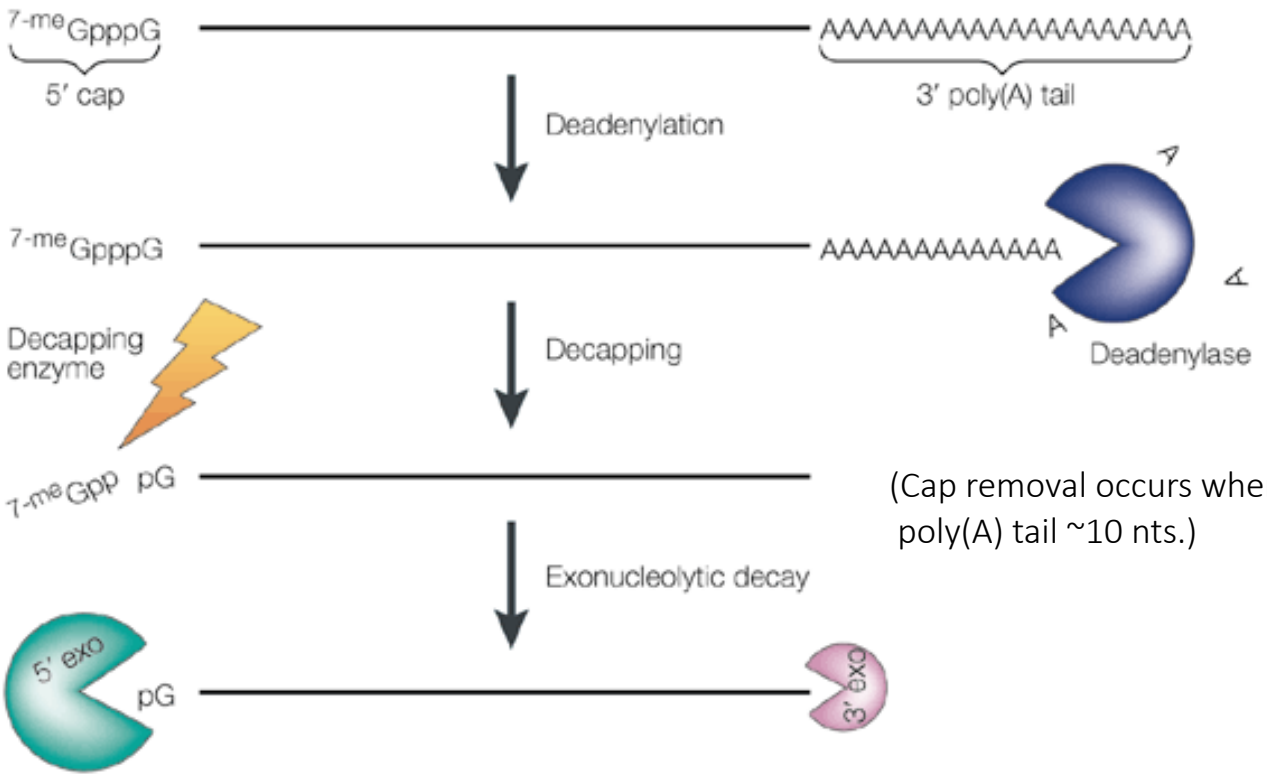
Translation initiation complex



‘Normal’ mRNA degradation is initiated by deadenylation



Deadenylation-dependent mRNA decay (in yeast)



(Dcp1/Dcp2)

(Cap removal occurs when poly(A) tail ~10 nts.)

(Xrn1)

RNA metabolism 2023

Wilusz et al. Nat. Rev. Molec Cell Biol. 2:237; 2001

Molecular machines in mRNA degradation

Exonuclease

Rat1, Xrn1

Monomer, very potent, highly processive

Exosome

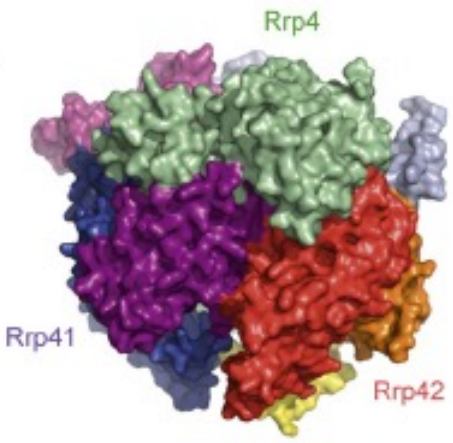
needs cofactors and activation

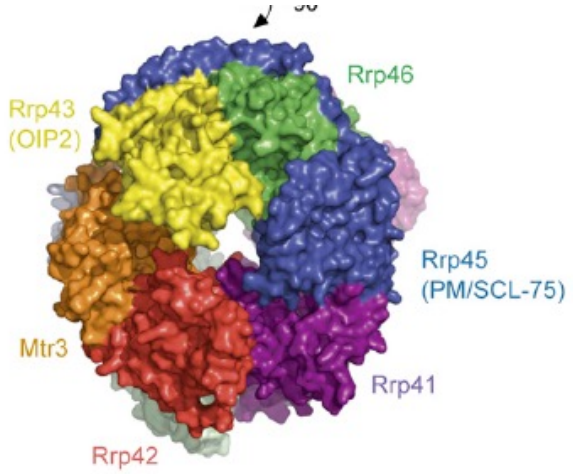
The exosome

Associates with specific co-factors depending on localization

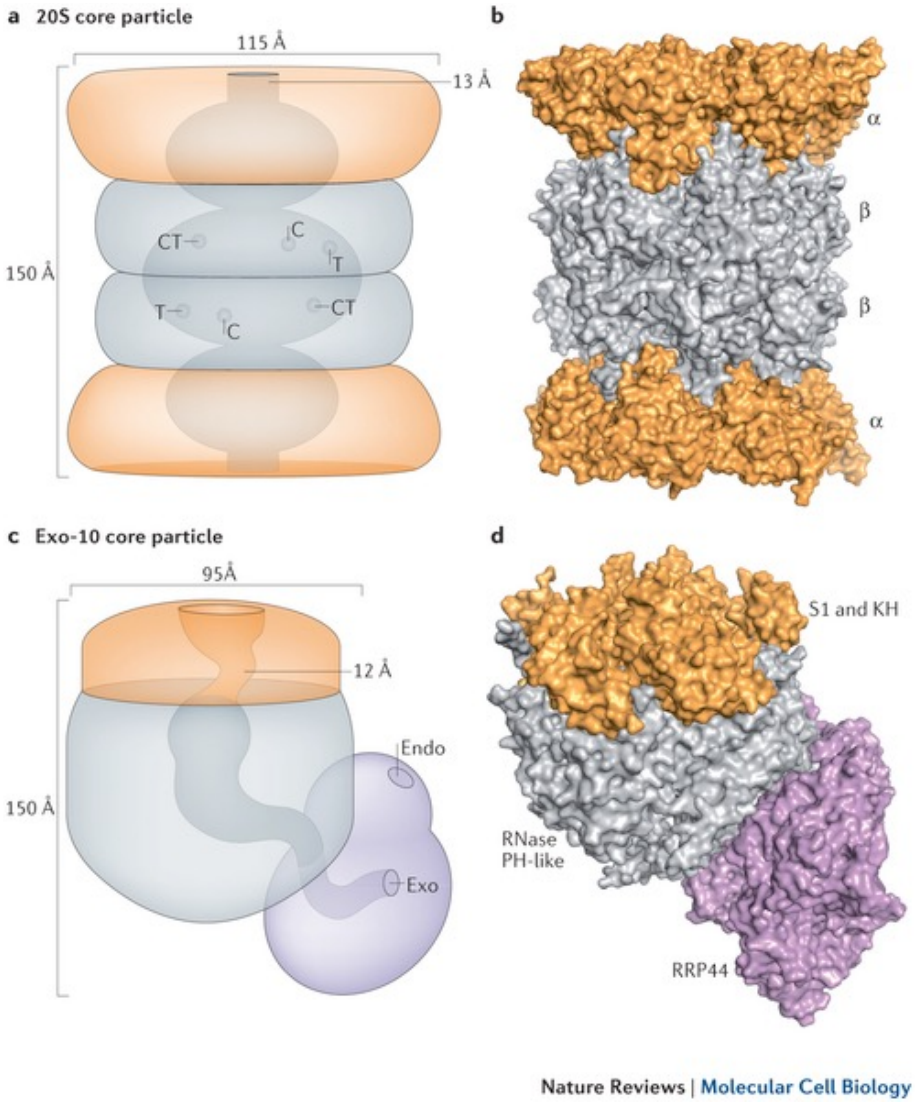
2 forms: nuclear and cytoplasmic

Exosome is poorly active in vitro and needs cofactors for activation

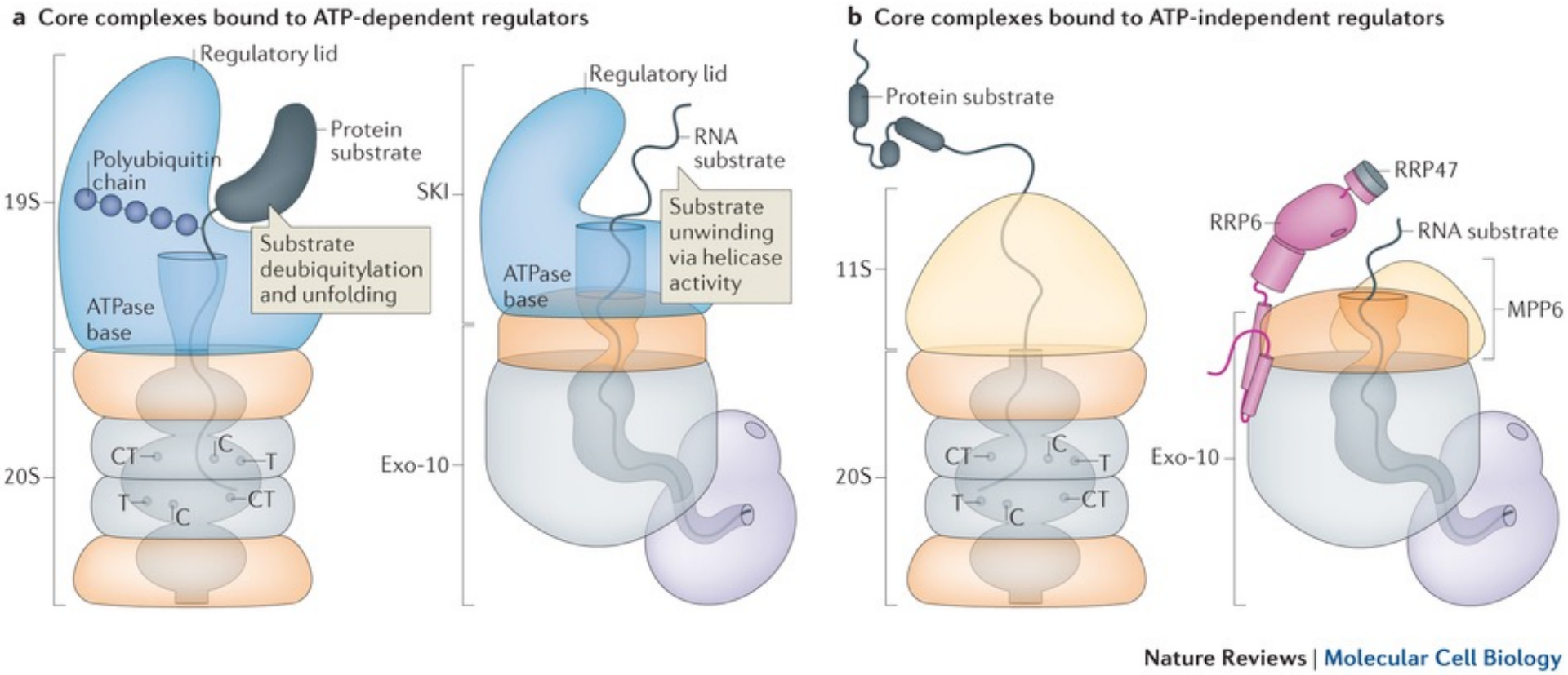




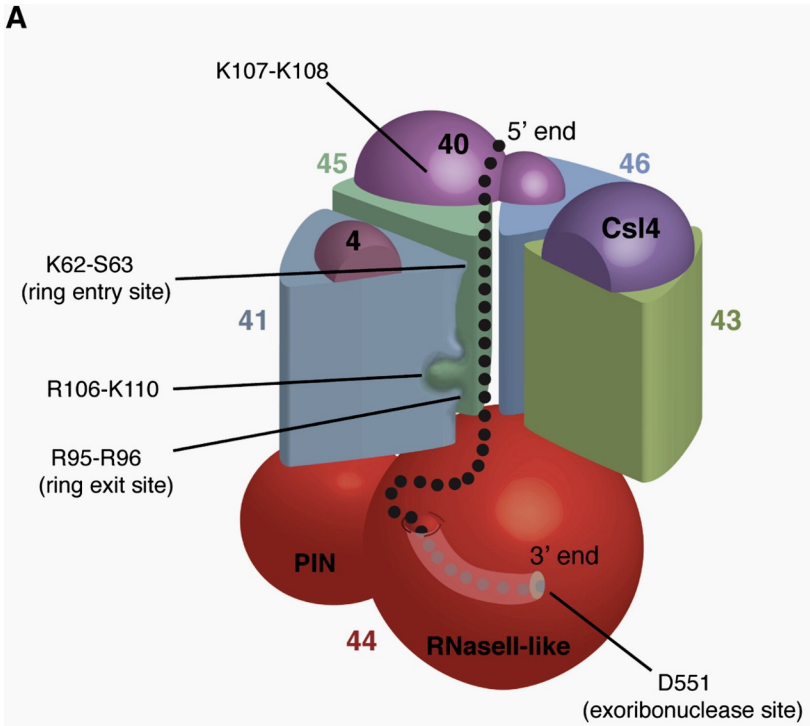
The RNA exosome and proteasome: common principles of degradation control

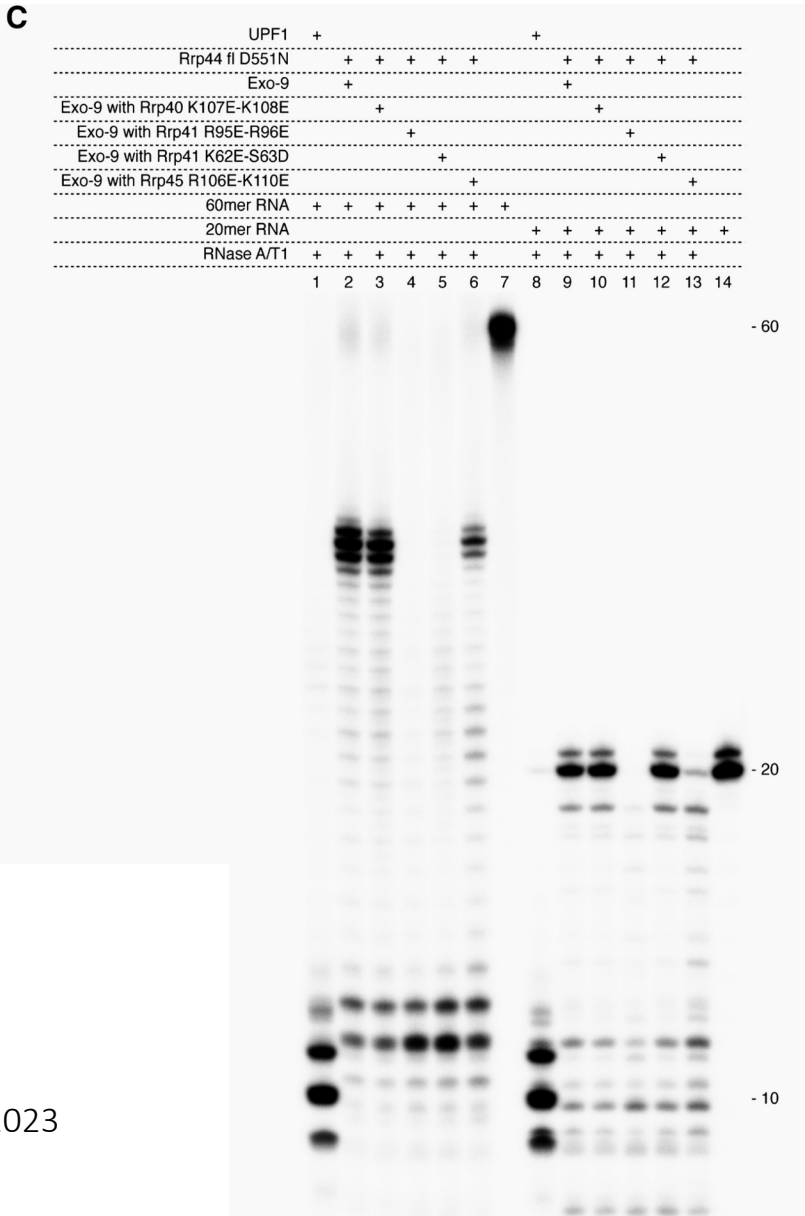


The RNA exosome and proteasome: common principles of degradation control

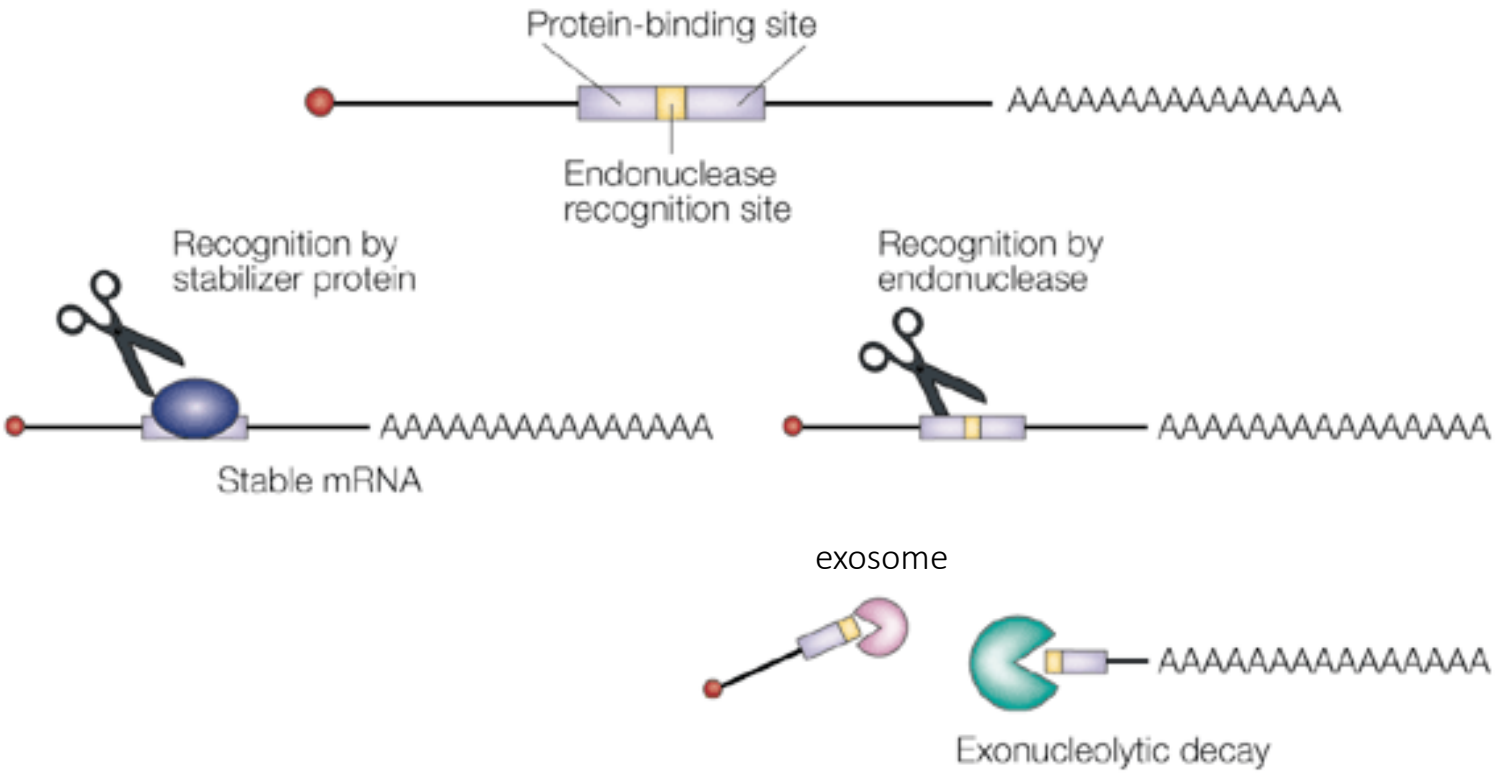


RNA is fed through the barrel





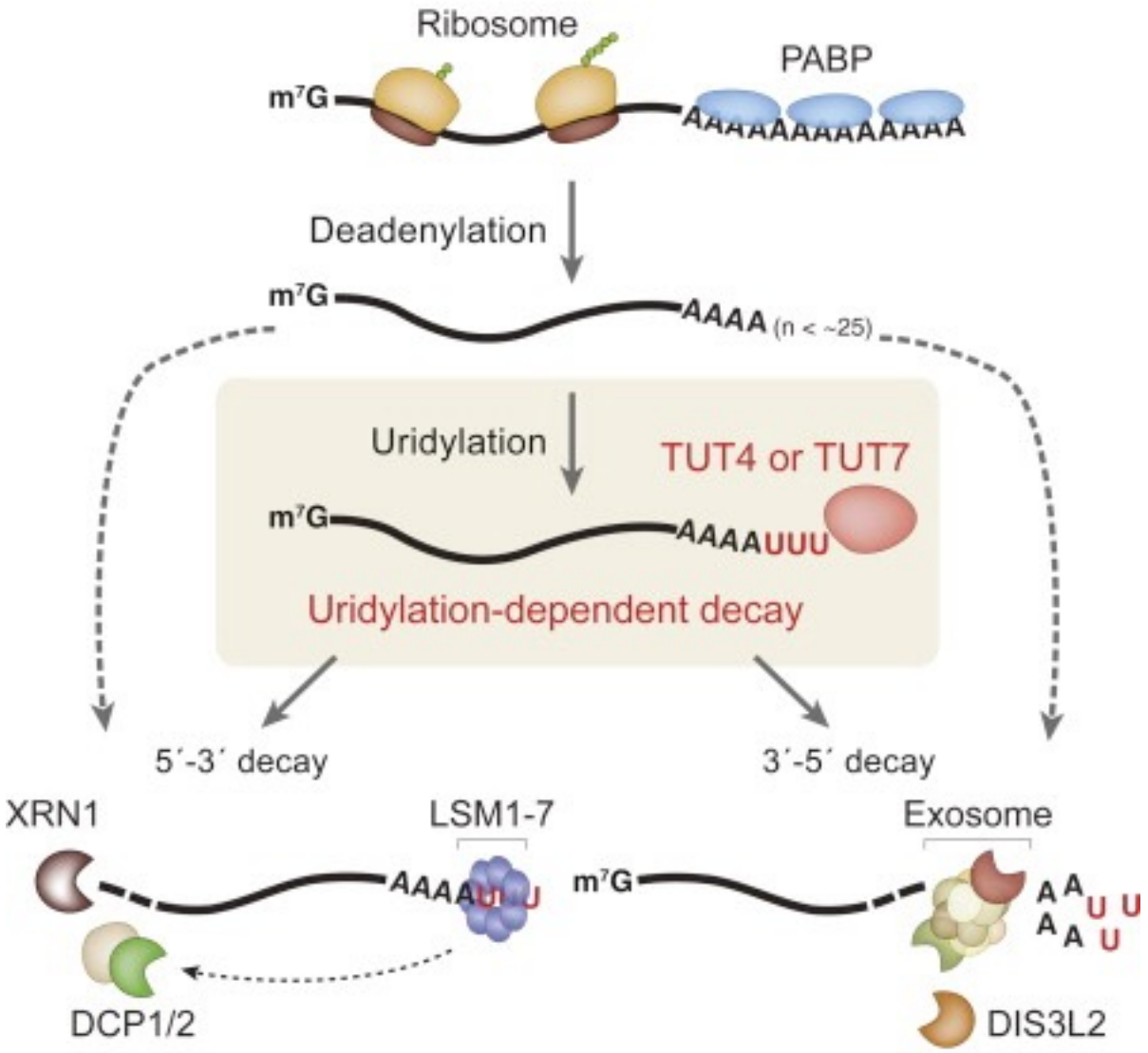
Endoribonucleolytic decay



(Xrn1)

Wilusz et al. Nat. Rev. Molec Cell Biol. 2:237; 2001

Uridylation marks mRNA for decay



Lim et al. Cell 2014

Deadenylation dependent mRNA degradation

• The poly(A) tail is progressively shortened by a deadenylase enzyme until it reaches ~20 A residues or less

• The PABPI becomes destabilized and weakening its interaction with the 5’ cap and translation initiation factors and also leads to an exposed 5’ cap

•Some mRNAs are cleaved internally by endonucleases (e.g. the miRISC) before they are further degraded by 3’-5’ exonucleases

• 5’ caps can then be removed by decapping enzymes and unprotected 5’ end is degraded by 5’-3’ exonucleases

• The shortened poly(A) tail is also susceptible to 3’-5’ exonucleases

•Oligouridylation of short poly(A) tail recruits Lsm complex, which in turn recruits decaping aparatus and induces degradation at the 5’end

• 5’ decapping and subsequent degradation (from the 5’ end) can occur independently of deadenylation

Regulatory sequence elements in mRNAs

Encoded:

- AU rich elements (ARE) in 3’ UTRs – binding of specific proteins that recruit the exosome
- Iron-responsive element (IRE) and iron regulatory protein (IRP)
- Cell cycle-regulated histone mRNA stem-loop determinant (SL/SLBP)
- Cytoplasmic polyadenylation element (CPE)……

Specialized mRNA turnover pathways

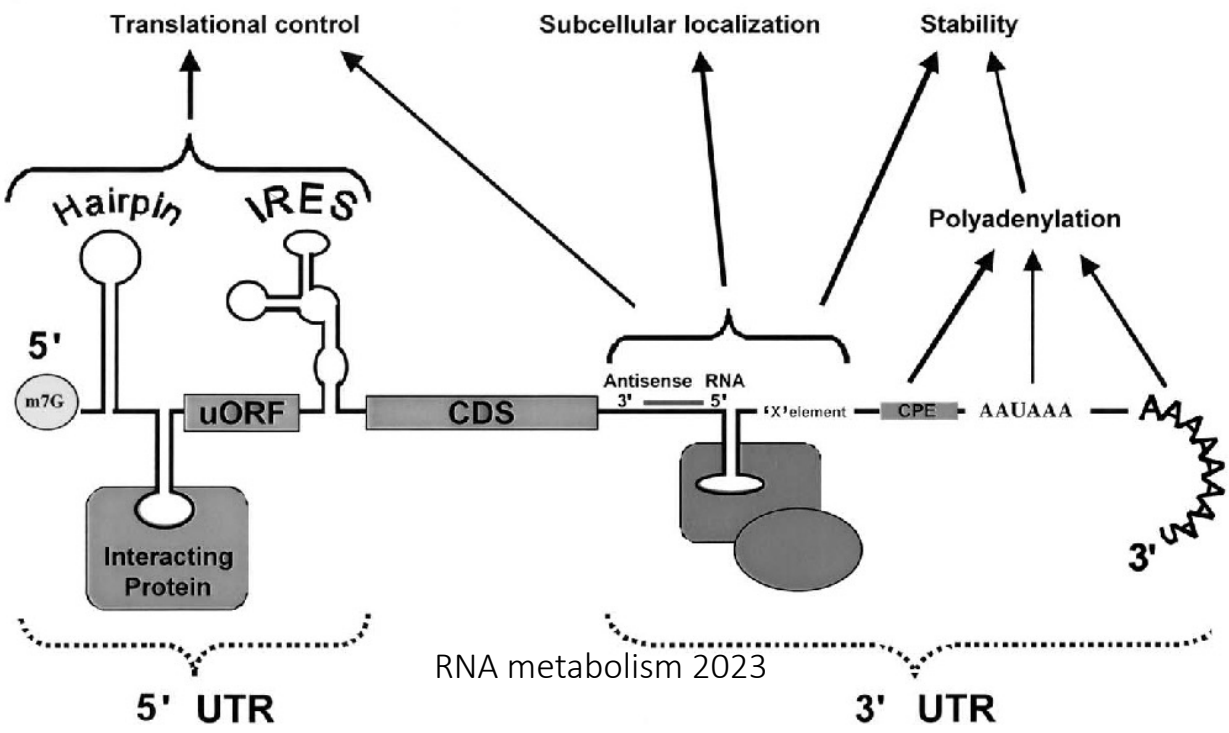
- ARE-mediated mRNA decay

Regulatory sequences in mammalian mRNAs

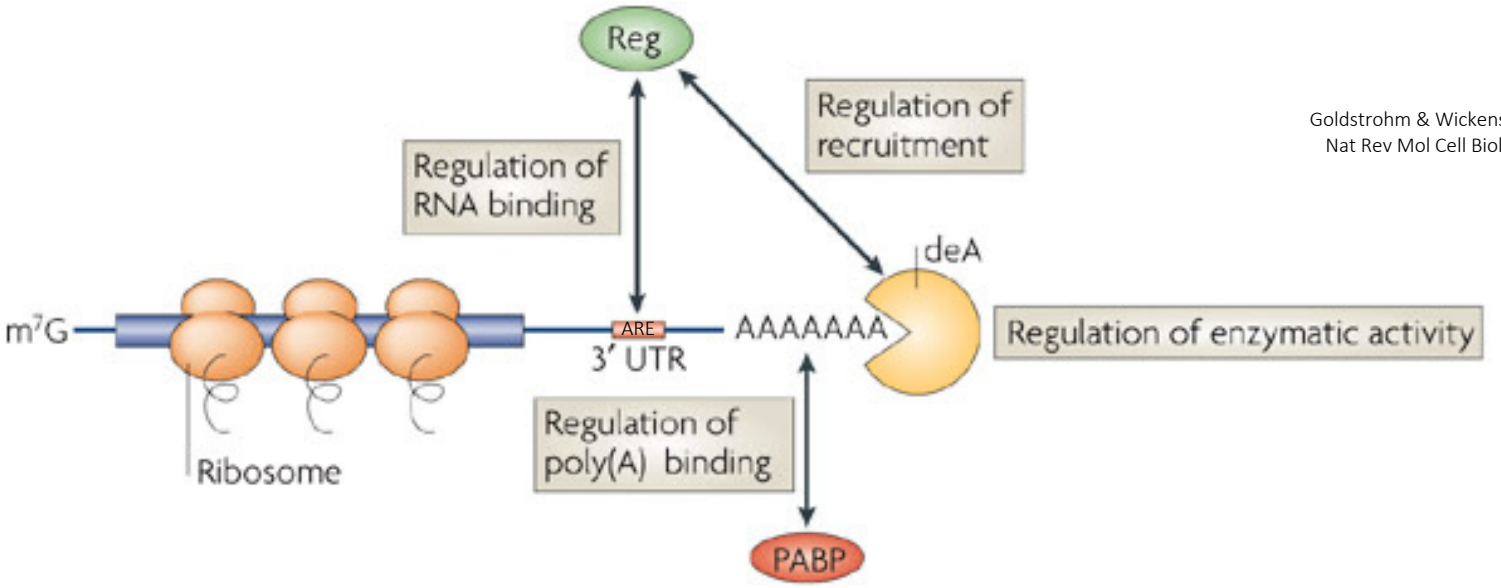
ARE-binding proteins affect mRNA stability, translation and subcellular localization.

- ARE's (adenylate- uridylate-rich instability elements) in mRNA's enhance deadenylation and decay rates (but some ARE's can stabilize mRNA's);
- ARE's are bound by factors (e.g., HuR/HuA, AUF1/hnRNP D) that modulate stability of ARE-containing mRNA's; mechanism of action not clear

Other elements found in the 5' UTR and coding regions also modulate transcript stability.



ARE-Mediated mRNA Turnover

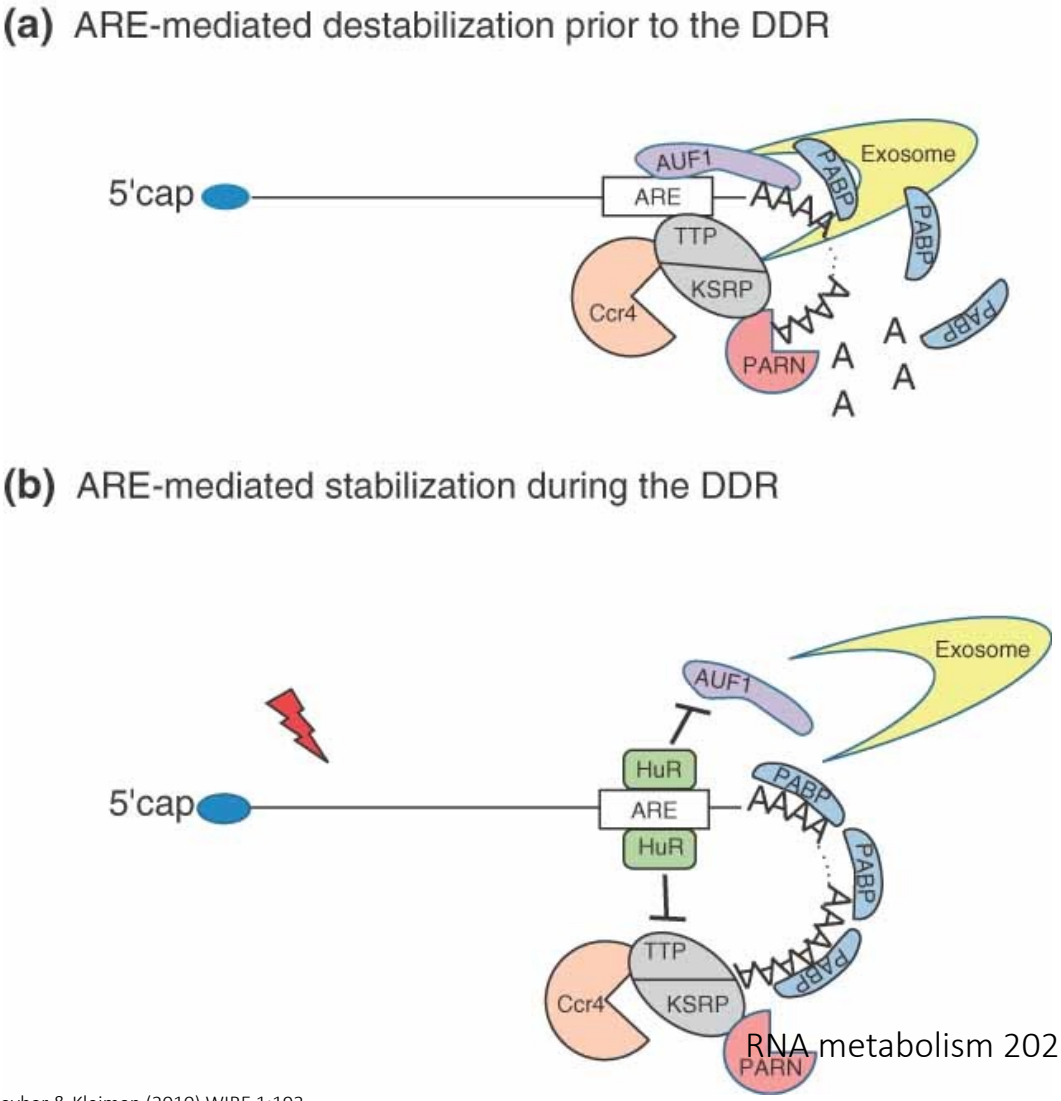


Goldstrohm & Wickens (2008)
Nat Rev Mol Cell Biol 9:337

- AU rich elements (50-150nts) AUUUA; UUAUUUA(U/A)(U/A); or U-rich
- cis-acting element located in 3' UTRs of mRNAs
- transcripts that encode proteins that require rapid changes in response to stimuli such changes in the cell cycle, growth factors, response to microorganisms, inflammatory stimuli, and environmental factors
- 10% of mammalian mRNAs contain AREs

- A diverse set of trans-acting proteins bind to AREs. These proteins can mediate other protein interactions that modulate mRNA stability. Various ARE-associated proteins can promote rapid mRNA turnover by promoting enhanced decapping, deadenylation, exosome recruitment, endonucleolytic cleavage or combinations of these. Alternatively, some proteins that bind to AREs can stabilize the mRNA.

Example of ARE-mediated change in mRNA Levels before and after the DNA damage response



Under non-damage conditions:

- AUF1 competes with the PABP for poly(A) tail binding, exposing it to PARN; TTP (tristetraproline) and KSRP (KH-type splicing regulatory protein) recruit PARN and CCR4 to deadenylate prior to degradation by the exosome.

Under DNA damage conditions:

- Genes involved in the DNA damage response pathway are up-regulated. HuR is up-regulated and competes with AUF1 for binding to the same ARE region. Loss of AUF1 binding stabilizes PABP association with the poly(A) tail. HuR also competes with TTP and KRSP to prevent recruitment of the deadenylases and exosome.

Cevhar & Kleiman (2010) WIRE 1:193

Extremely long lived mRNAs in humans?

MINIREVIEW

Regulation of α-Globin mRNA Stability

SHELLY A. WAGGONER AND STEPHEN A. LIEBHABER[1]
Department of Genetics and Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

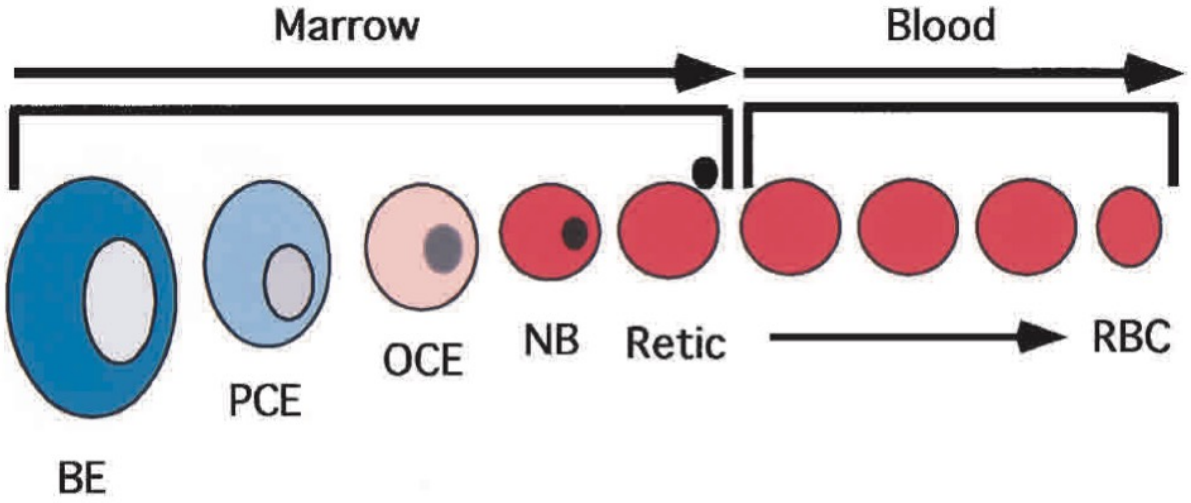


Figure 2. Stages of erythropoiesis. The name of each cell type is indicated by its abbreviation (see text for full names). The nucleus is shown as progressively darkened and condensed and is eventually extruded from the cell during the transition to the reticulocyte (retic) stage. The colors of the cells are shown as visualized by Wright/Giemsa staining.

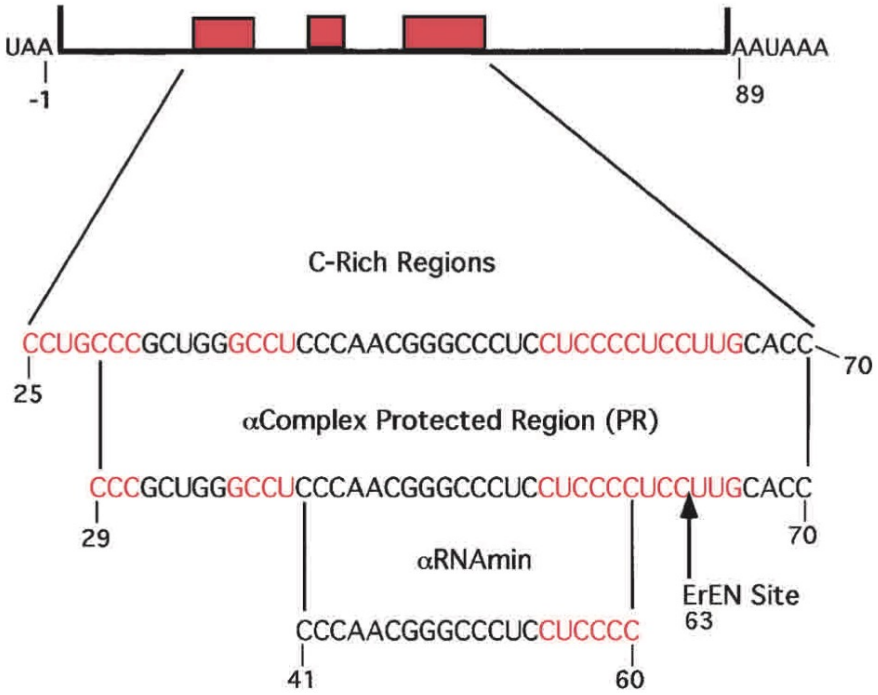
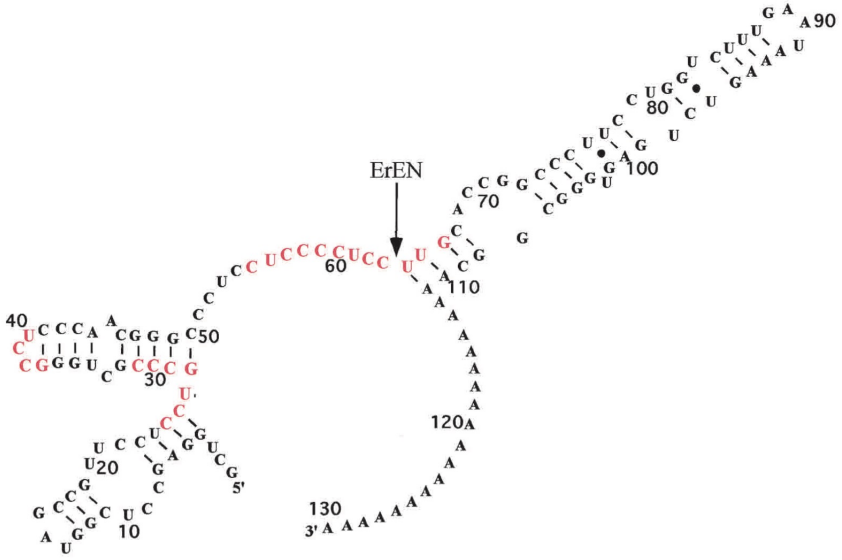
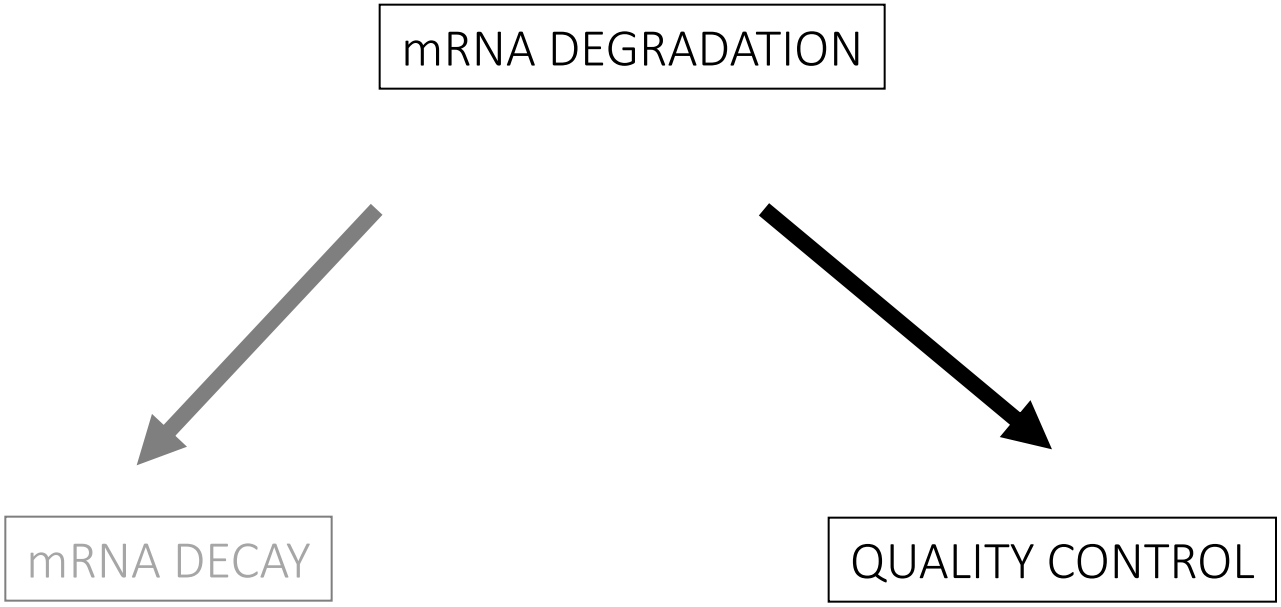


Figure 3. Important sequence determinants in the α-globin 3′-UTR. A segment of the α-globin 3′–UTR is illustrated, with the translational stop codon (UAA) and cleavage and polyadenylation signal (AAUAAA) indicated. The nucleotides are numbered in this and the following figures such that the first nucleotide 3′ to the stop codon is nucleotide 1. The red boxes and red sequences represent the C-rich regions that comprise the stability determinant. Also shown are the sequences of the α-complex protected region (PR) that contains the ErEN Site and the minimal α-complex binding site (αRNAmin).




mRNA DEGRADATION
mRNA DECAY
QUALITY CONTROL

mRNA quality control

RNA quality control and degradation in the cytoplasm

Aim: To prevent translation of mRNAs that would generate aberrant proteins

-targets mainly mRNAs

- Almost 20% of mRNAs in humans have a premature stop codon. All of these are degraded by NMD. Where do you think all these mistakes are coming from? That is, which process in the biogenesis of an mRNA molecule is the most prone to errors?

Alternative pre-mRNA splicing can create enormous diversity

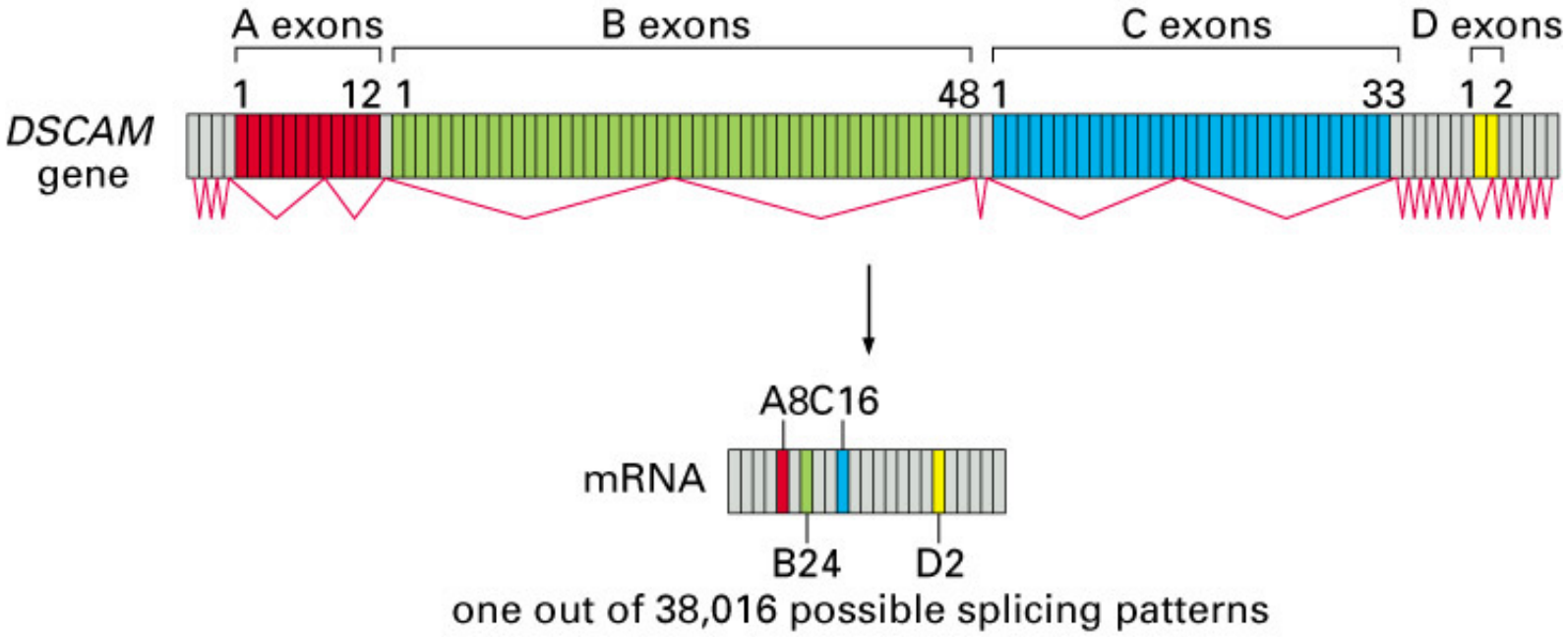
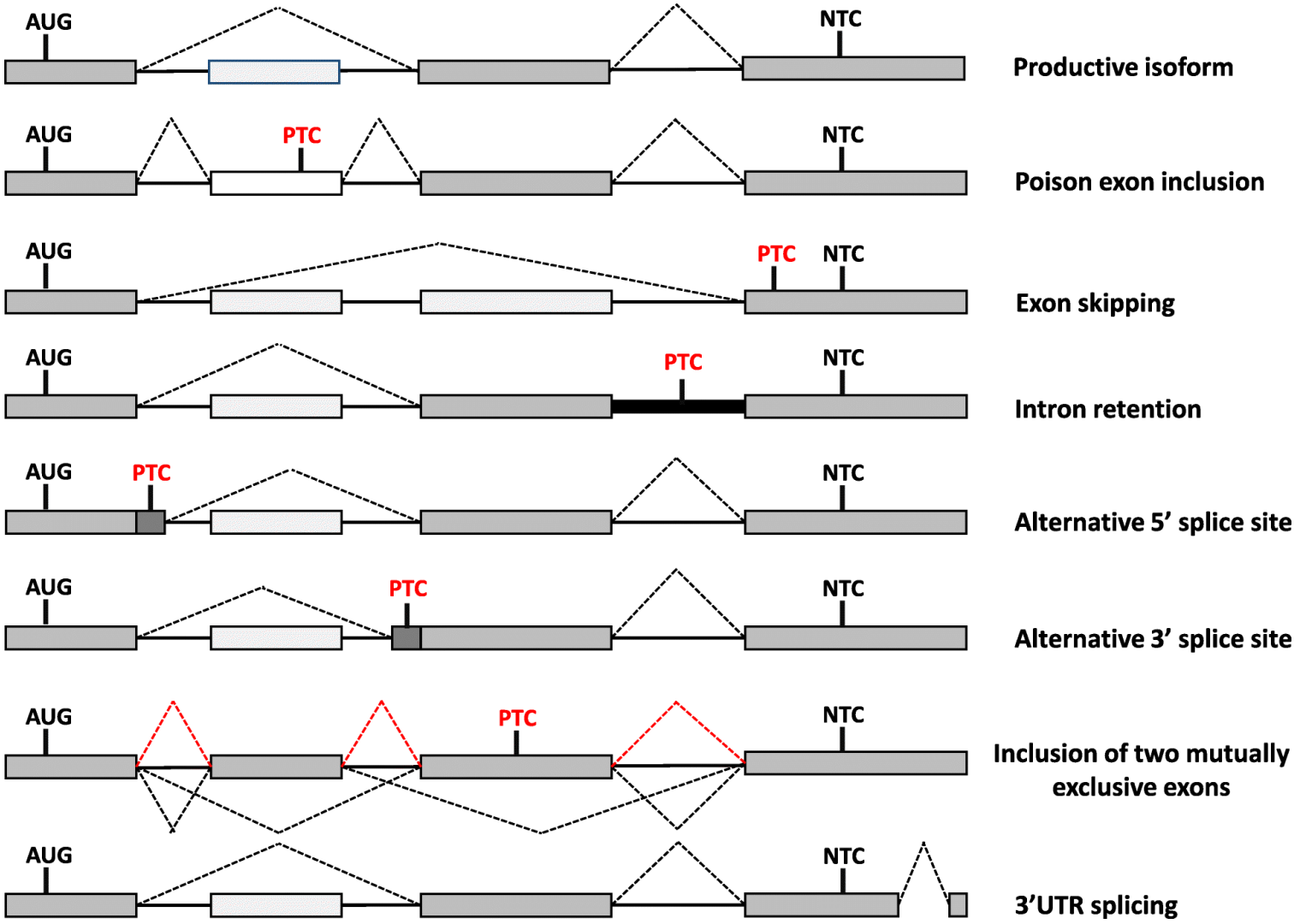


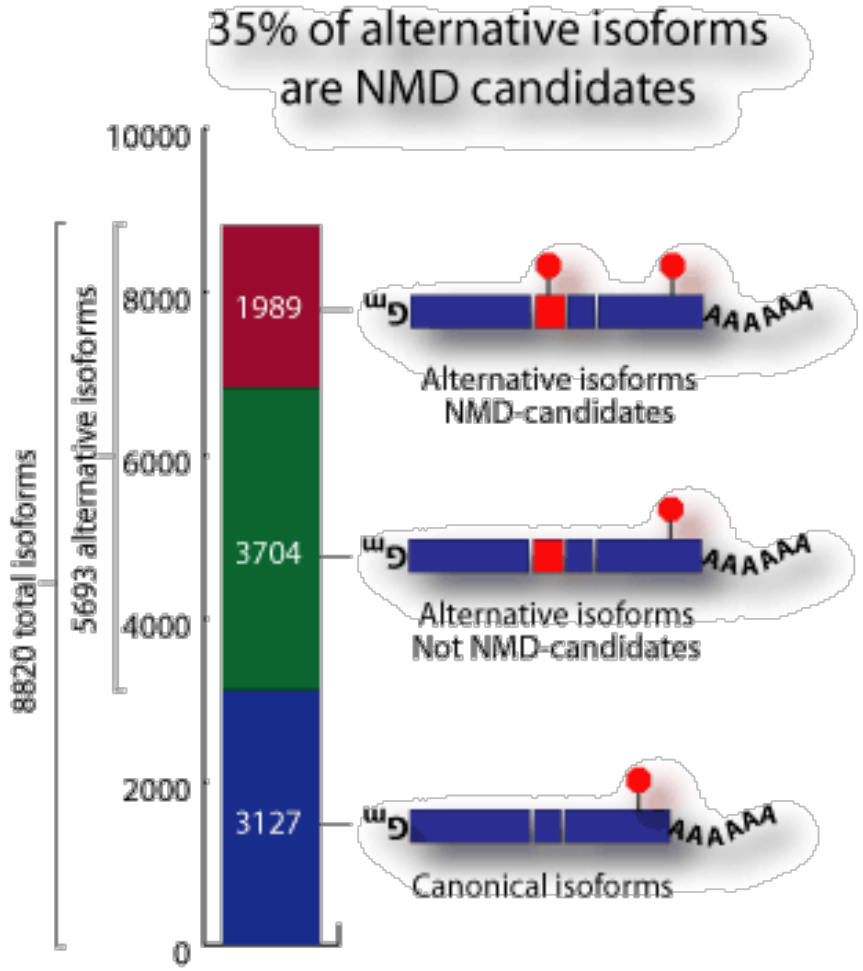
Figure 7–89. Molecular Biology of the Cell, 4th Edition.

Schematic representation of alternative splicing (AS) patterns inducing nonsense-mediated mRNA decay (NMD)-sensitive mRNA isoforms



Nogueira et al., 2021

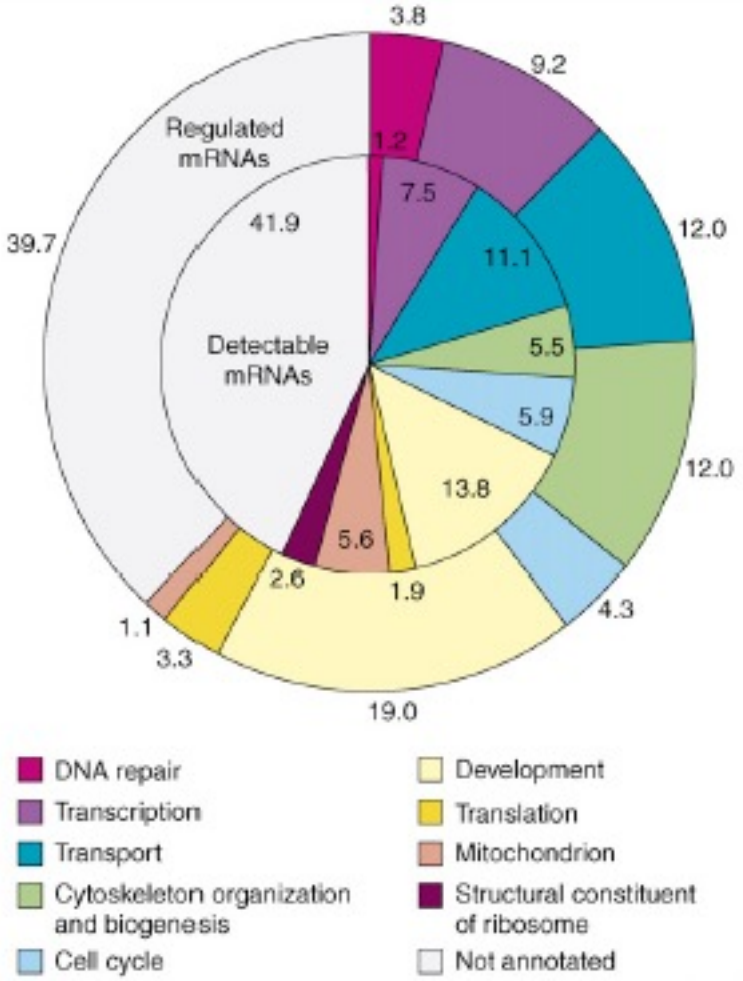
RNA quality control in the cytoplasm: NMD



RNA metabolism 2023

Rehwinkel, Raes, Izaurralde, 2006

NMD regulates the expression of transcripts associated with diverse cellular processes.



RNA metabolism 2023

Rehwinkel, Raes, Izaurralde, 2006

Aberrant mRNA Decay Pathways

A. Nonsense-mediated mRNA decay (NMD)
- Degrades mRNAs with premature stop codons

B. Nonstop mRNA decay (NSD)
- Degrades mRNAs without a stop codon

C. No-go mRNA decay (NGD)
- Degrades mRNAs that have a stalled ribosome

D. Ribosome extension-mediated decay (REMD)
- Degrades mRNAs where ribosome translates past the stop codon and into the 3’ UTR

RNA quality control in the cytoplasm

Nonsence mutations in the coding regions of mRNAs can lead to:

1. Generation of a premature termination codon (PTC) → shorter protein product

NONSENCE MEDIATED DECAY = NMD, SMD

2. Loss of the termination codon- nonstop message → longer protein product

NONSTOP DECAY = NSD

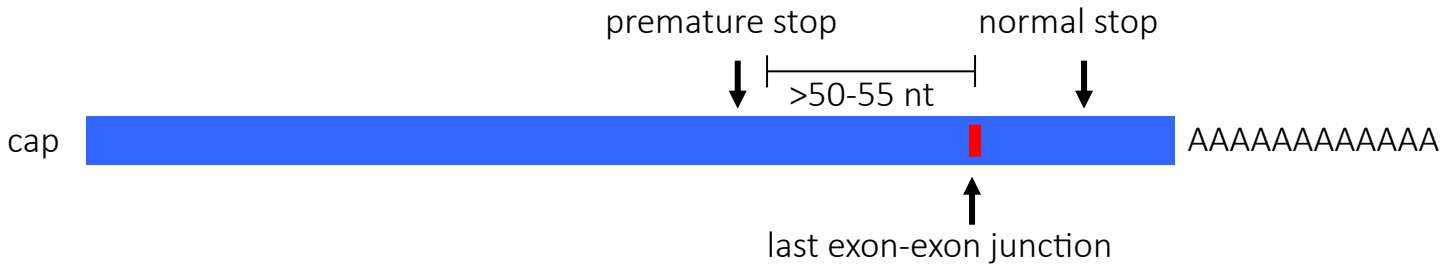
Both these surveillance pathways depend on a 'test' round of translation that can detect the presence of stop codons.

RNA quality control in the cytoplasm: NMD

NMD = Nonsense-Mediated Decay

Is initiated when mRNA contains:

- a premature stop codon
- an in-frame stop codon within a retained intron
- an extended 3' UTR due to improper polyadenylation site use
- an ORF in their 5' UTR



It has been estimated that 30% of inherited genetic disorders in humans result from nonsense mutations or frameshift mutations, which generate PTCs

Yet, most of these diseases are recessive (i.e. the truncated protein is not made and thus cannot interfere with the function of the wild type protein)

Nonsense-Mediated mRNA Decay

- Specialized pathway that degrades mRNAs that contain premature translation termination signals

Czapllinski et al. (1999)
Bioeassay 21:685

- Protects the cell from translating mRNAs that might produce truncated peptides that could lead to harmful dominant negative effects
- Occurs in all eukaryotes.
- 30% of disease-generating mutations result in premature stop codons
- Up to 10-20% of the transcriptome is regulated by NMD
- PTC-containing transcripts caused by point mutations, frameshift mutations, mRNAs with faulty alternative splicing, pre-mRNAs that escape nuclear retention, mRNAs that contain upstream open reading frames, mRNAs that carry introns in 3´ untranslated regions, or mRNAs with long 3´ untranslated regions

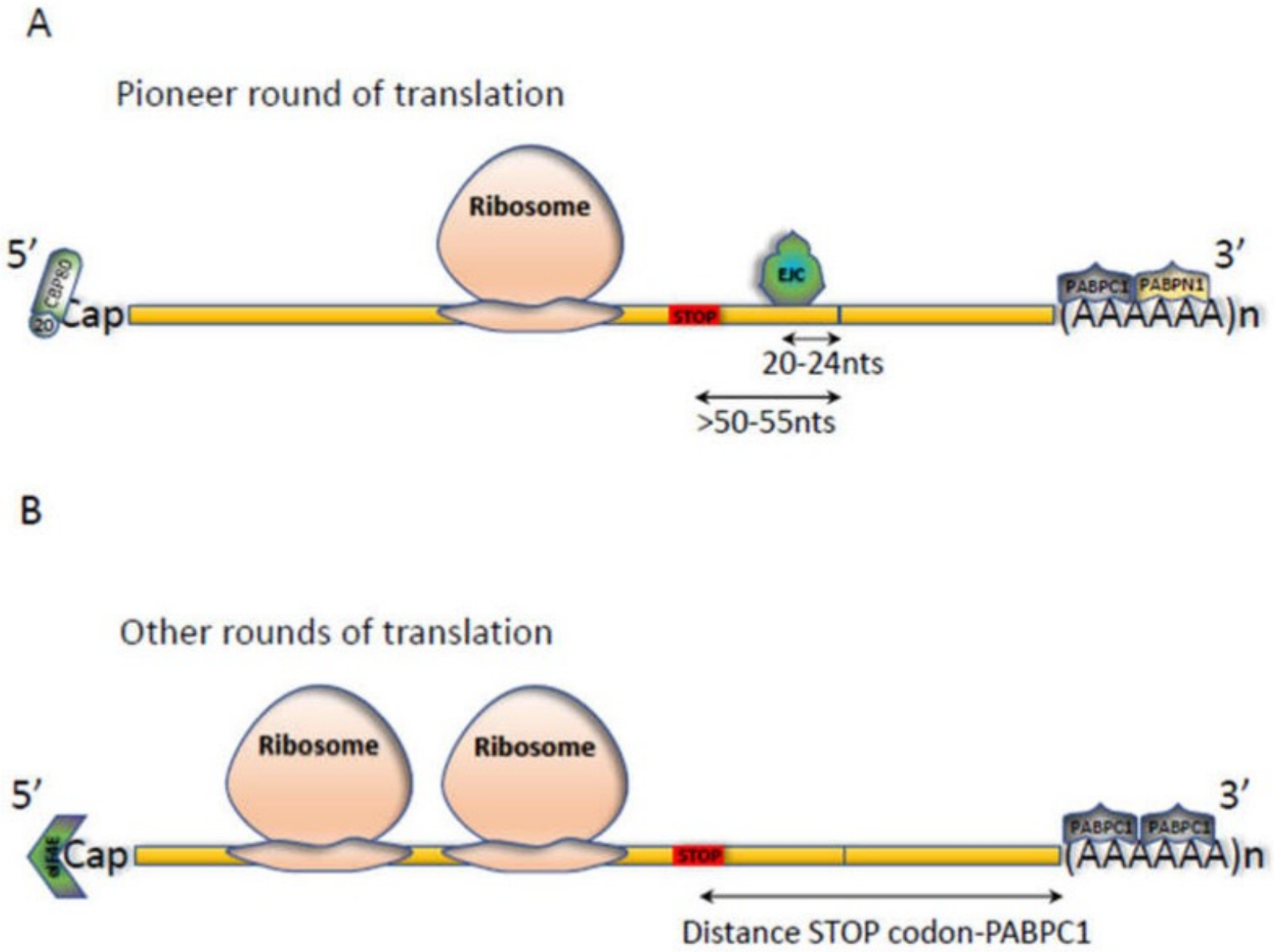
NMD = Nonsense-Mediated Decay

Two main steps:

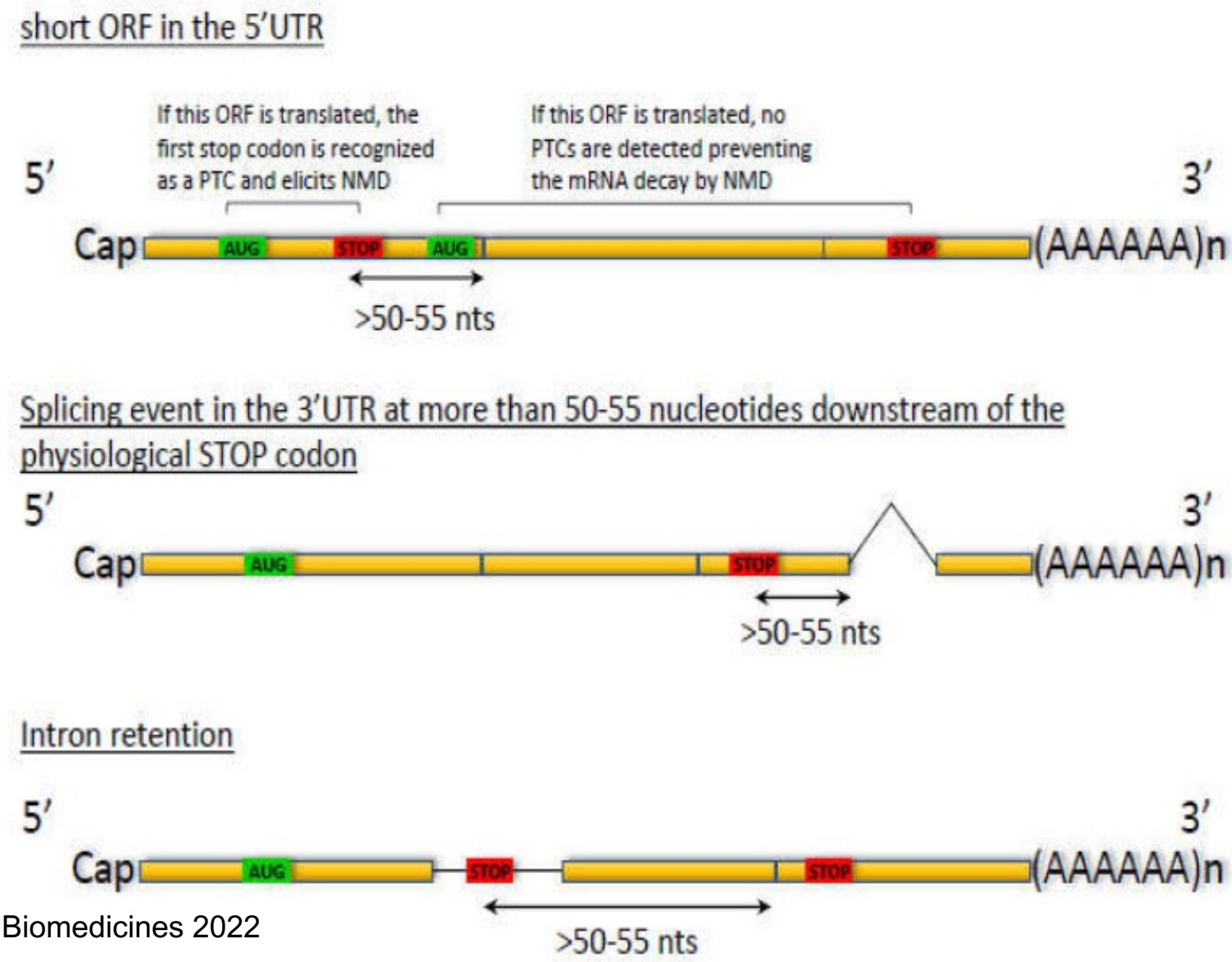
1. PTC recognition

2. Initiation of mRNA degradation

Rules for eliciting NMD according to the two models of NMD activation in human cells.

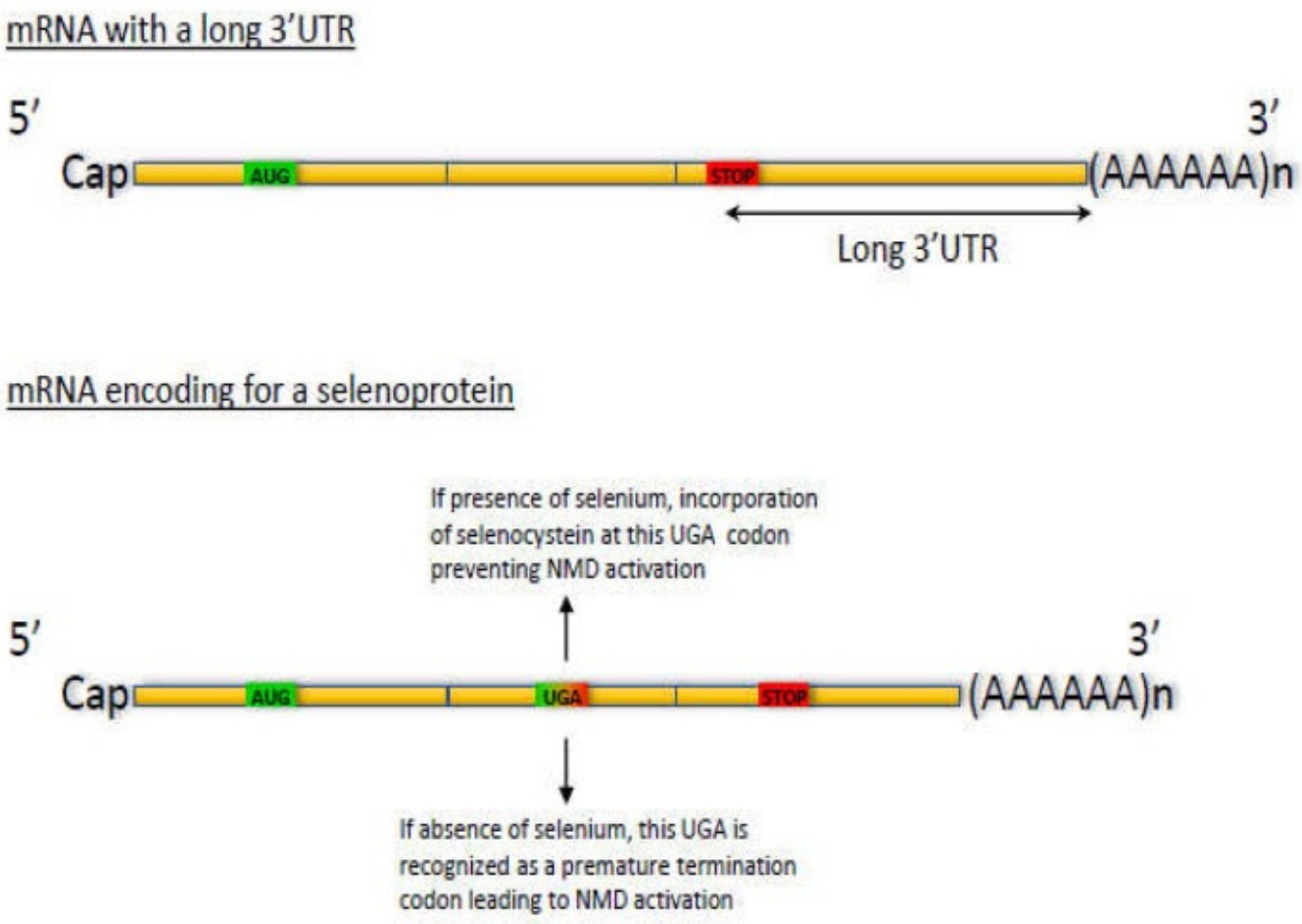


Examples of natural NMD substrates



Lejeune, Biomedicines 2022

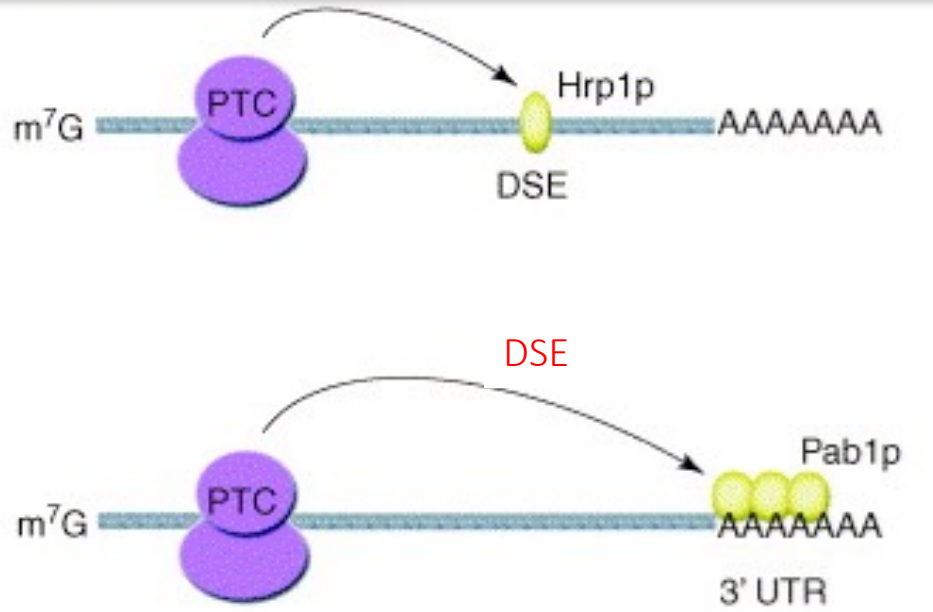
Examples of natural NMD substrates



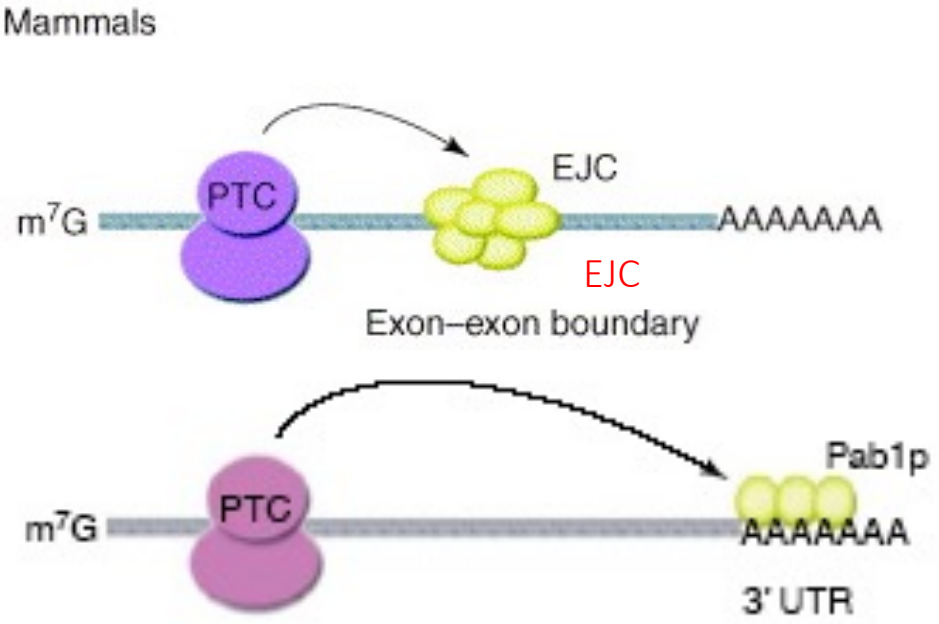
Lejeune, Biomedicines 2022

PTC recognition

Yeast



Mammals



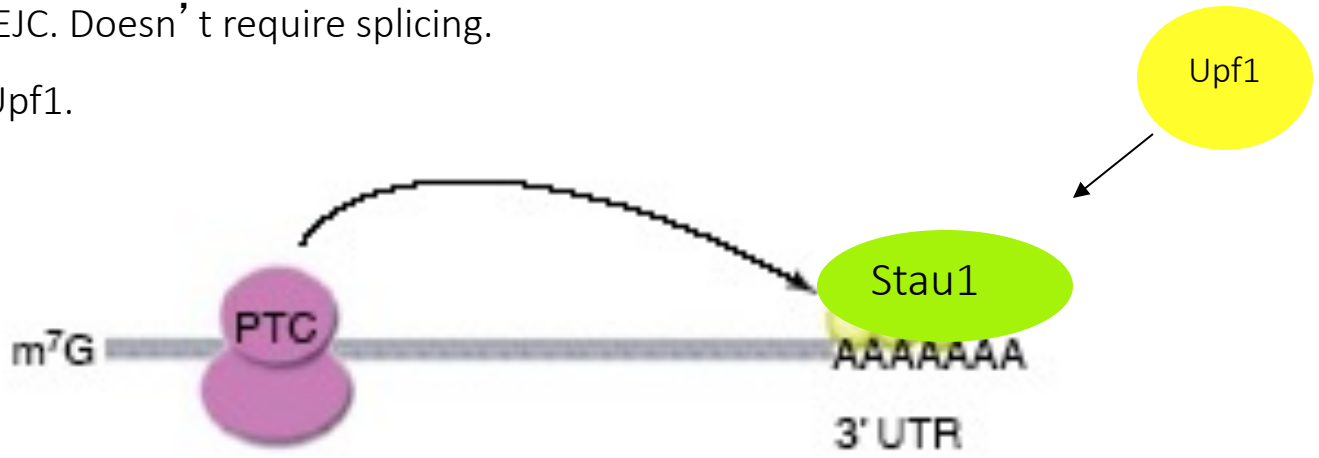
Conti and Izaurralde, 2005)

SMD in mammals

SMD = Staufen1 (Stau1) mediated decay.

Independent of EJC. Doesn't require splicing.

Involves Stau1, Upf1.



Stau1 binds to the 3' UTR of a subset of particular mRNAs

NMD versus Staufen1 decay pathways

PTC recognition

Mechanism by which a stop codon is defined premature and the targeted mRNA is degraded differ across species: but in all depends on a test round of translation

1. Mammals

-Cross-talk between terminating ribosome and downstream EJC (exon-junction complex)

-Premature Stop Codons (PTC) defined as premature if they are located >50 nt upstream of an exon-junction complex

- Mammals: PTC recognition relies on splicing or on Staufen protein (SMD)

- depends on a 'test' round of translation that can detect the presence of stop codons.

2. Yeast and invertebrates

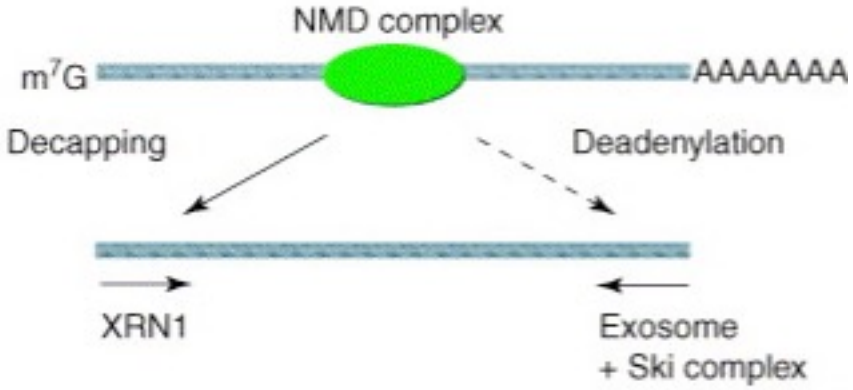
- PTC recognition doesn't depend on splicing, intronless mRNAs subjected to NMD

- depends on loosely defined sequence elements, and/or on the interaction with other proteins bound downstream on the mRNA

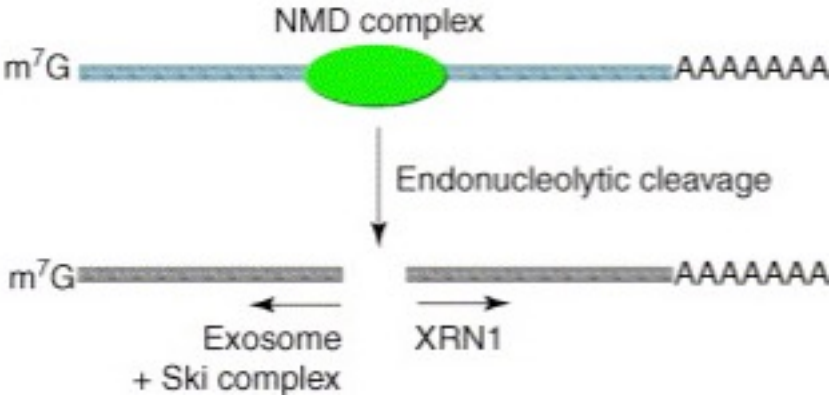
- stop codon has to be in the "appropriate context"

Decay of NMD targets

Yeast and mammals

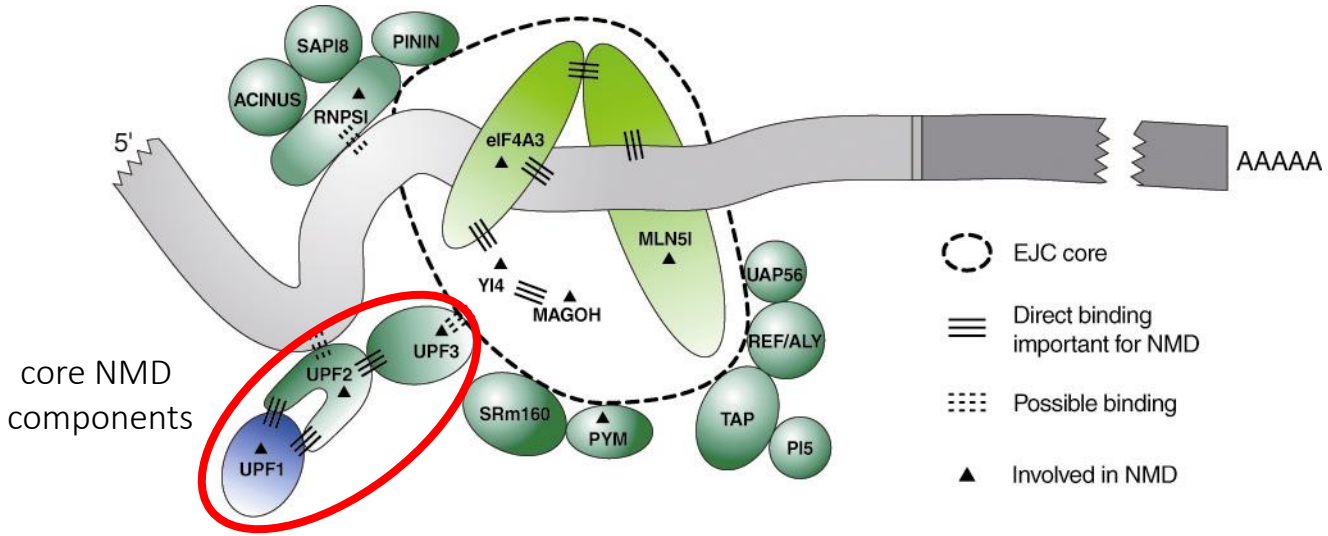


Drosophila



NMD substrates are targeted for degradation via interaction with Upf proteins

NMD Factors Associate With the EJC



Chang Y-F, et al. 2007.
Annu. Rev. Biochem. 76:51–74

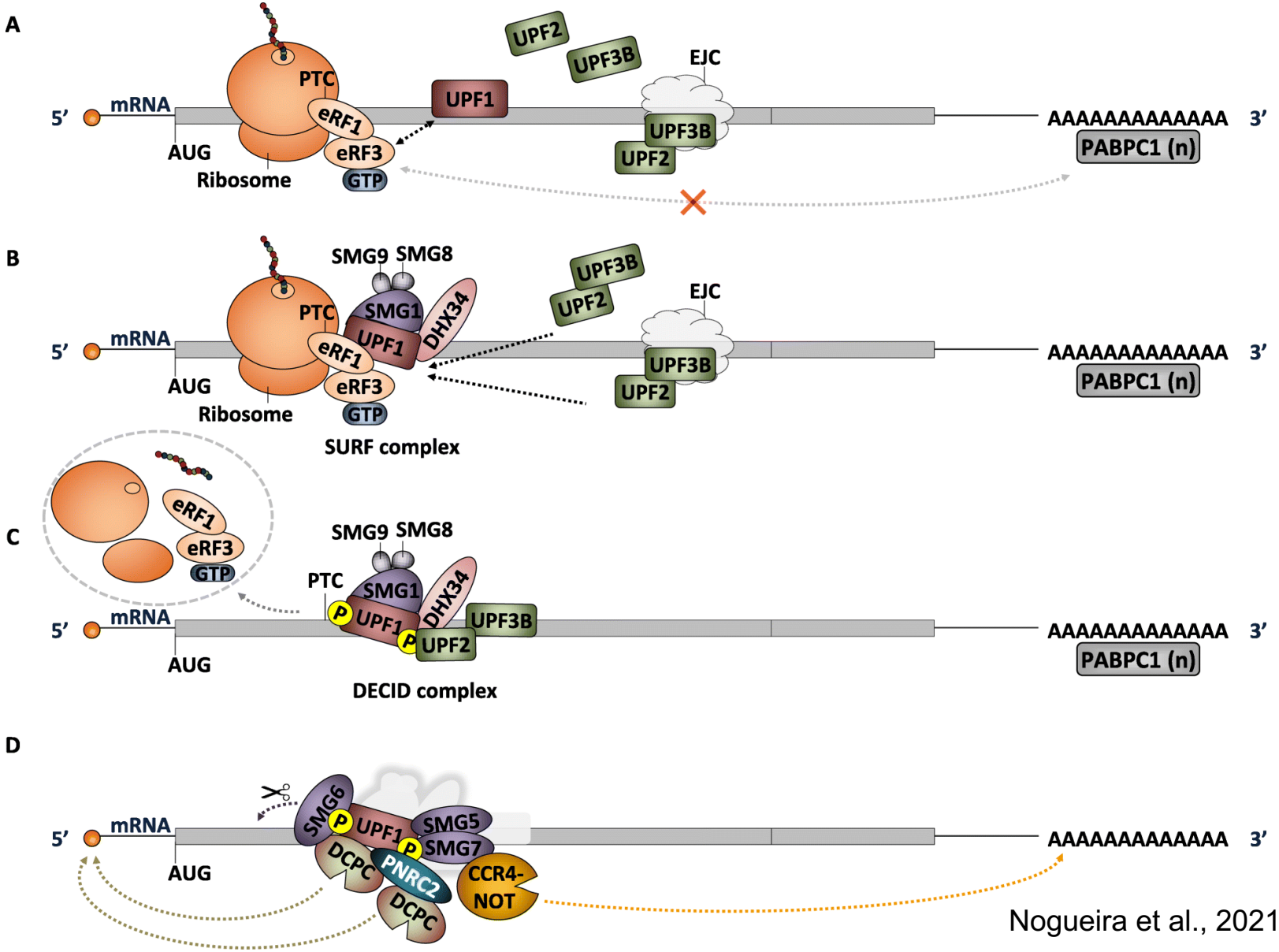
Core NMD Components:

UPF3: associates with the EJC in the nucleus

UPF2: perinuclear and binds to Upf3 as the mRNA is exported

UPF1: associates at the stop codons in mRNAs during translation

Simplified representation of the nonsense-mediated mRNA decay (NMD) model in mammalian cells



Nogueira et al., 2021

Initiation of mRNA degradation

Major role of 3 conserved proteins UPF1,2,3 (Up-Frameshift):

UPF3 - nuclear protein, associates with EJC of spliced mRNAs (positioned 20-24 nt upstream of exon-exon boundary)

has an RBD that contacts UPF2

UPF2 - perinuclear, associates with Upf3, UPF2/3 dimer binds mRNA via UPF2 surface, it can also interact with UPF1

UPF1 - cytoplasmic, RNA helicase, key component of NMD

associates with eRF1 and eRF3

binds to Upf2/3 dimer

regulated by phosphorylation in multicellular organisms - role for SMG1-7 proteins

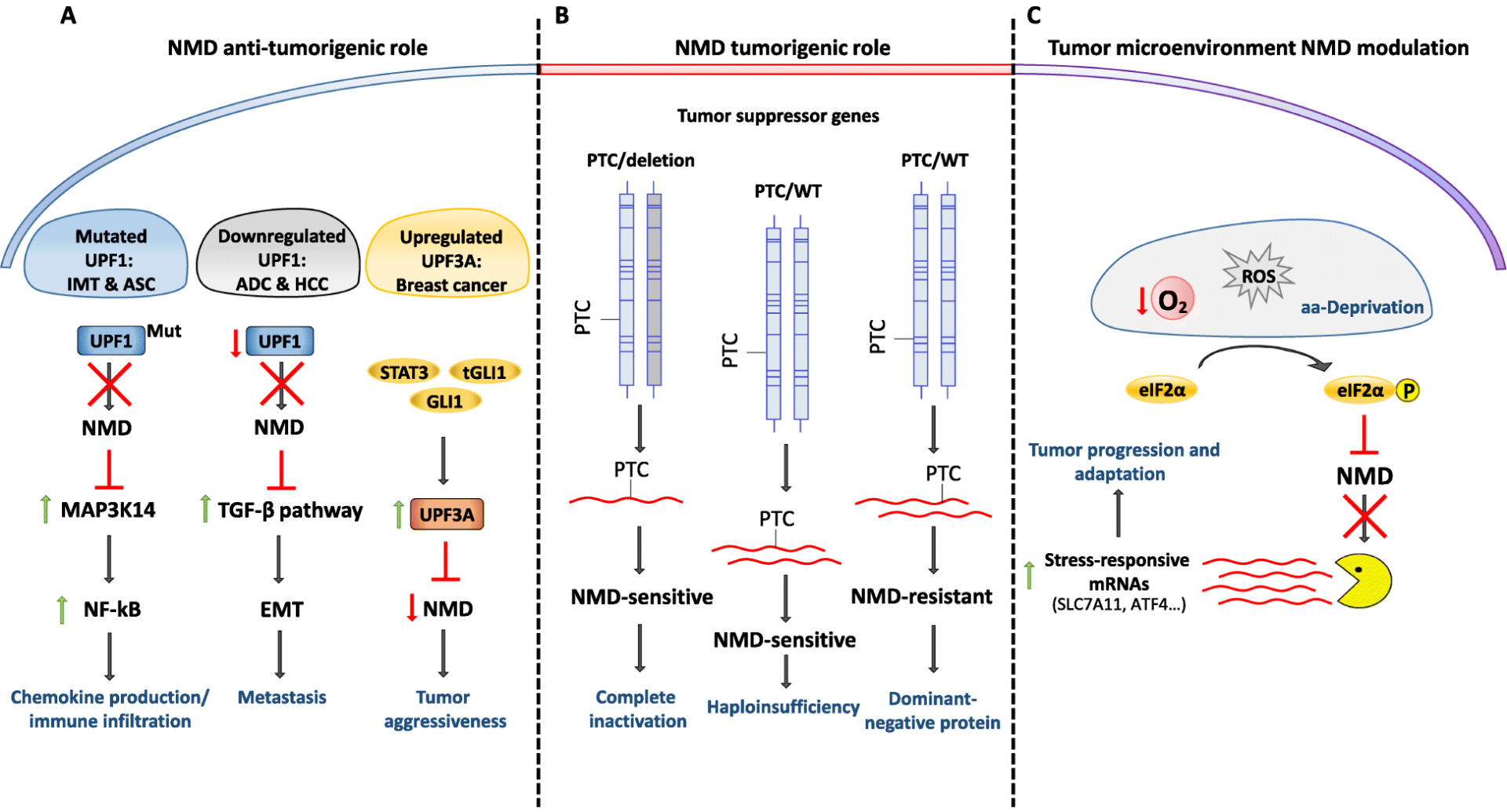
Recognition of PTC recruits UPF1 binding that can then interact with downstream UPF2/3 forming surveillance complex

NMD effectors and phenotypes across species

Organism	Effectors	Phenotype
Yeast: S. cerevisiae	Upf1 Upf2 (Nmd2) Upf3	Not essential
Worm: C. elegans	SMG-2 (UPF1) SMG-3 (UPF2) SMG-4 (UPF3) SMG-1 SMG-5 SMG-6 SMG-7	Viable worms with morphological effects on genitalia
Fruitfly: D. melanogaster	UPF1 UPF2 UPF3 SMG1 SMG5 SMG6	Required for cell-cycle progression and proliferation
Mammals: Mus musculus	Upf1 (Rent1) Upf2 Upf3 Smg1 Smg5 Smg6 Smg7	Upf1 KO: embryonic lethal; required for cell cycle progression Upf2 KD: no effect on cell viability

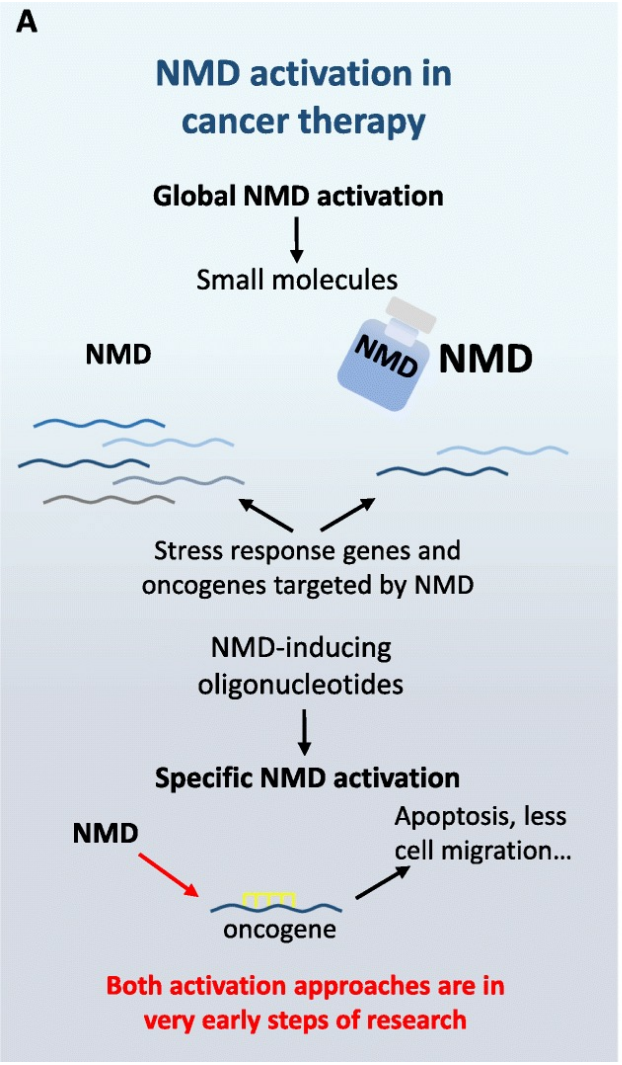
Rehwinkel, Raes, Izaurralde, 2006

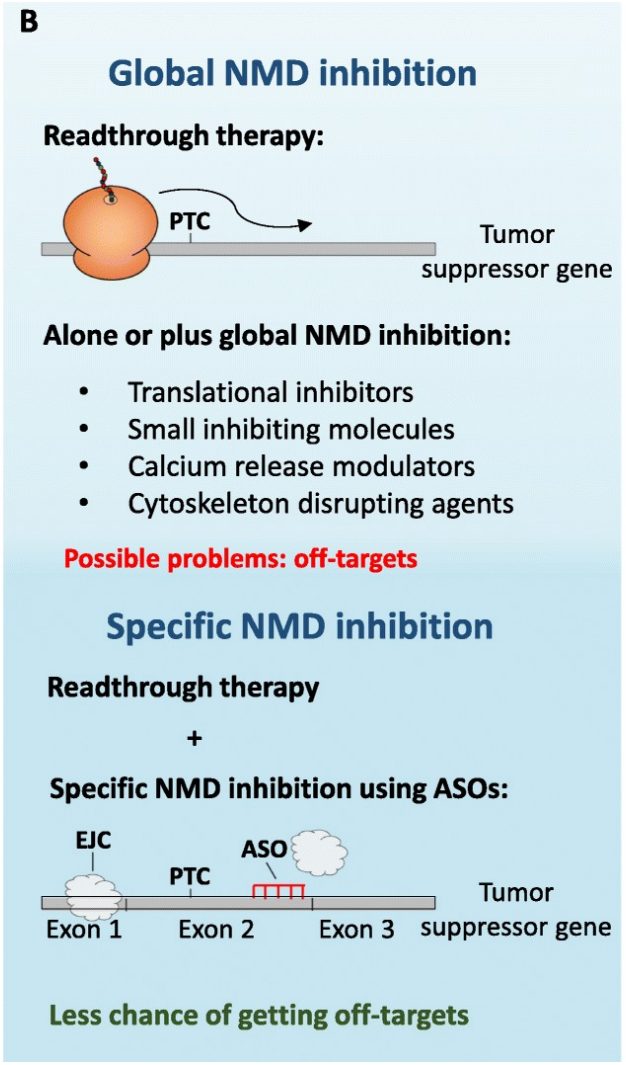
Nonsense-mediated RNA decay and its bipolar function in cancer

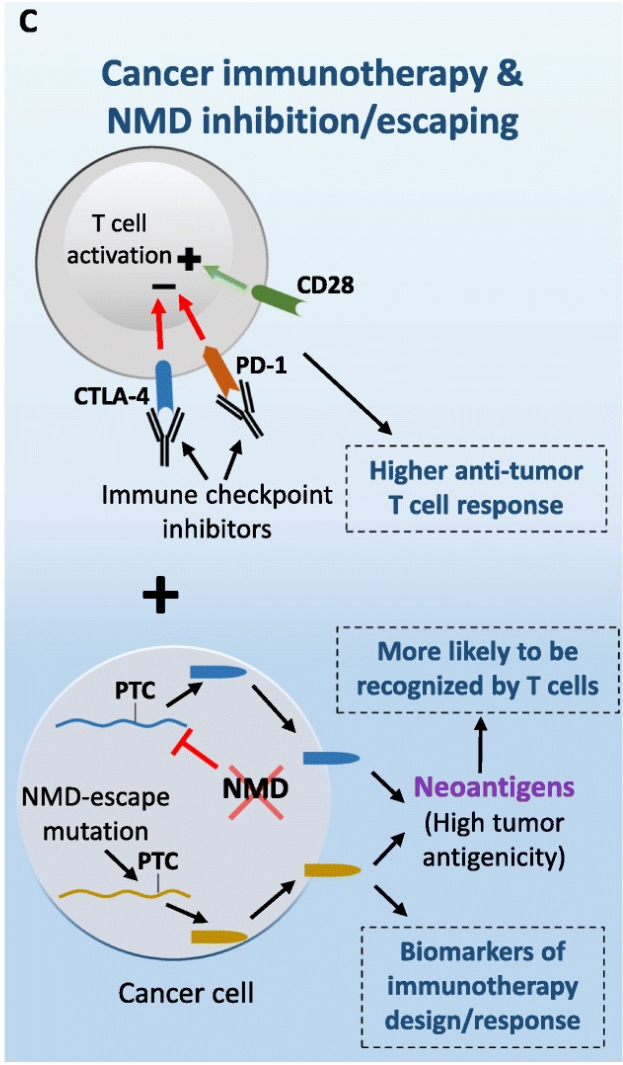


Nogueira et al., 2021

Summary of nonsense-mediated mRNA decay (NMD) inhibition/escaping and activation strategies for cancer treatment







Nogueira et al., 2021

RNA quality control in the cytoplasm: ND

Nonstop decay

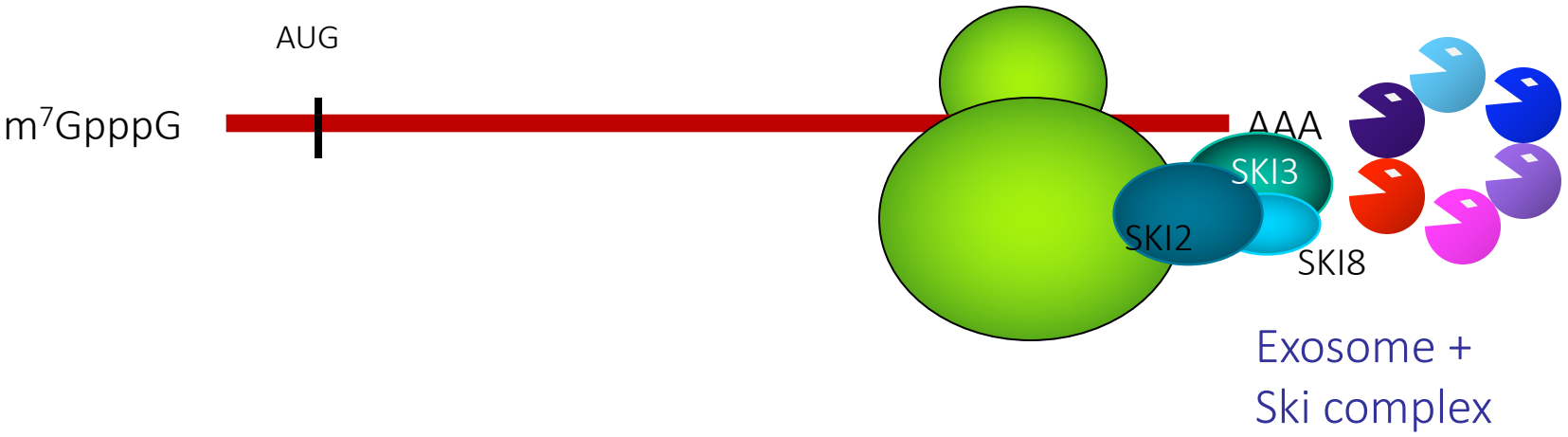
Nonstop decay (ND) targets mRNAs lacking an in-frame termination codon

Occurs by different mechanism than NMD, but is also translation dependent

ND requires the cytoplasmic exosome and the associated Ski complex

Independent of the decapping and deadenylation factors

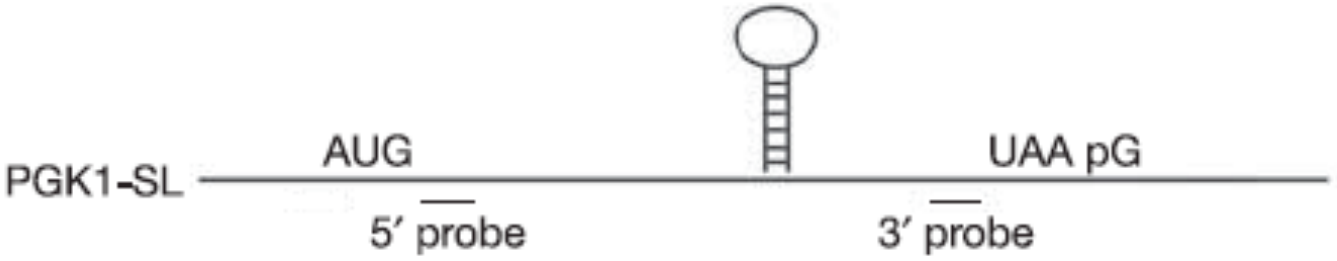
Ski7 C-terminal domain binds to free A-site of ribosome, and recruites the exosome and the Ski complex



RNA quality control in the cytoplasm: NoGo

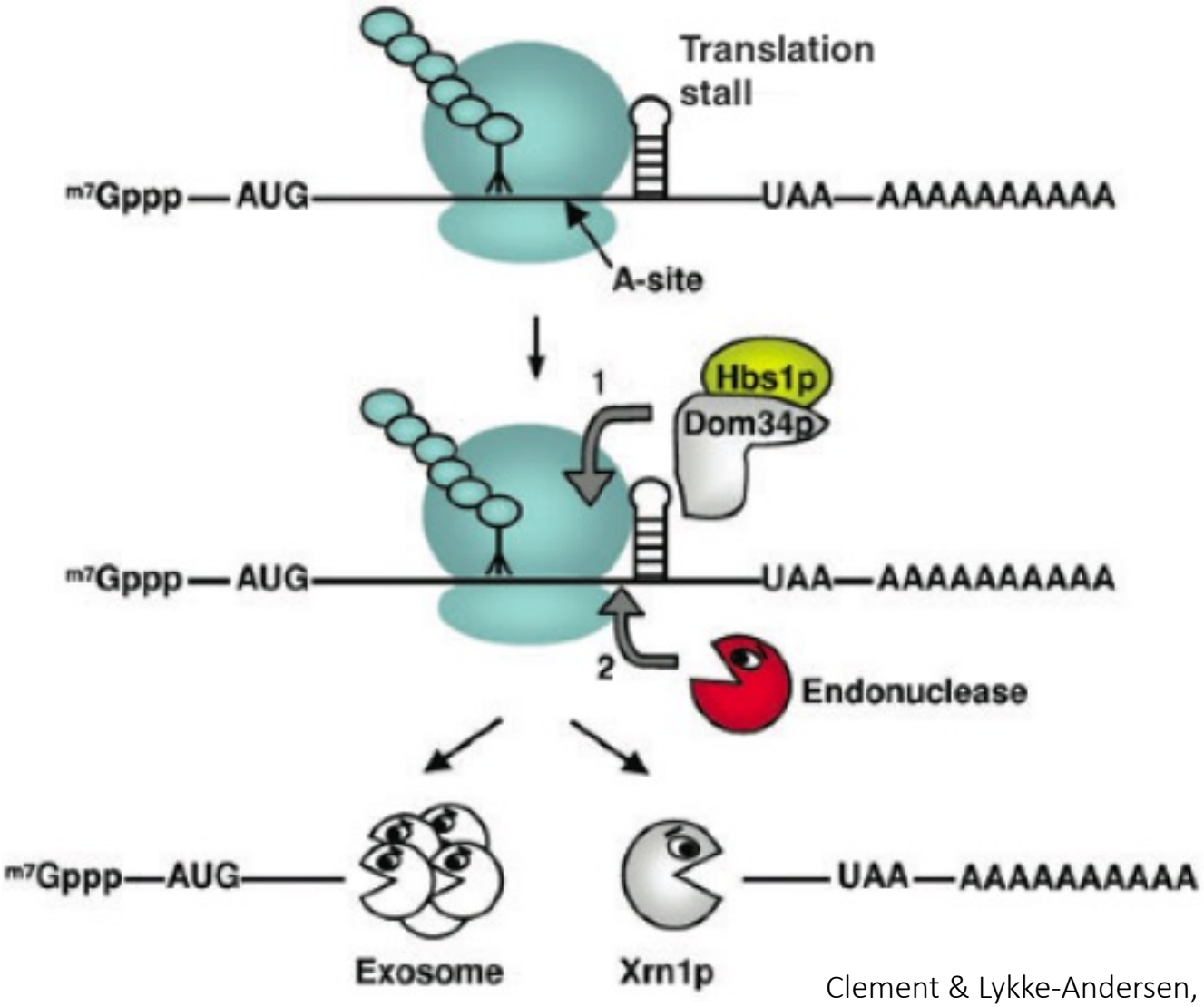
NoGo decay

What happens when ribosomes are stalled on mRNAs due to mRNA defects, or defects in ribosomes?



Doma & Parker, 2006

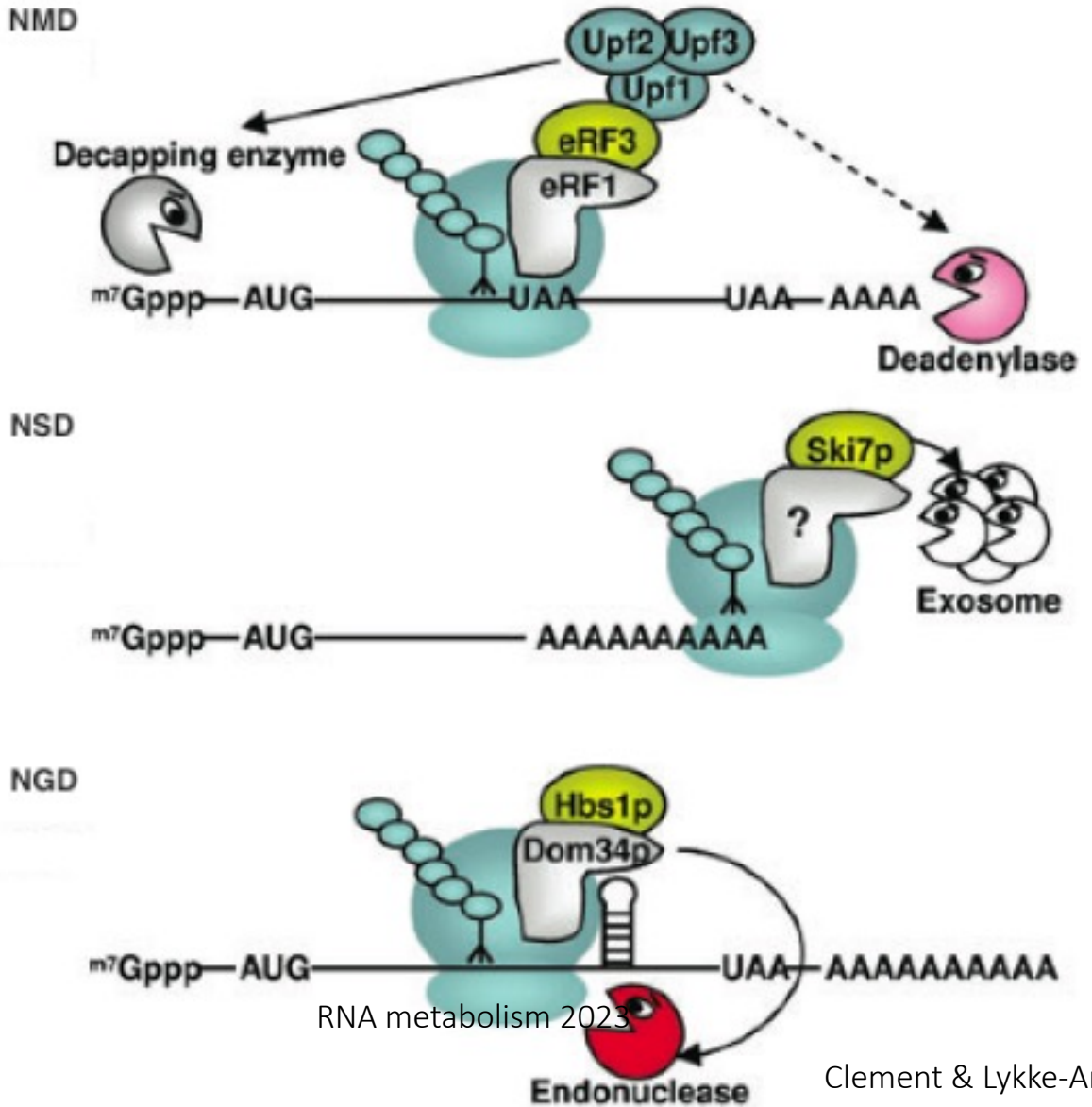
NoGo decay



Clement & Lykke-Andersen, 2006

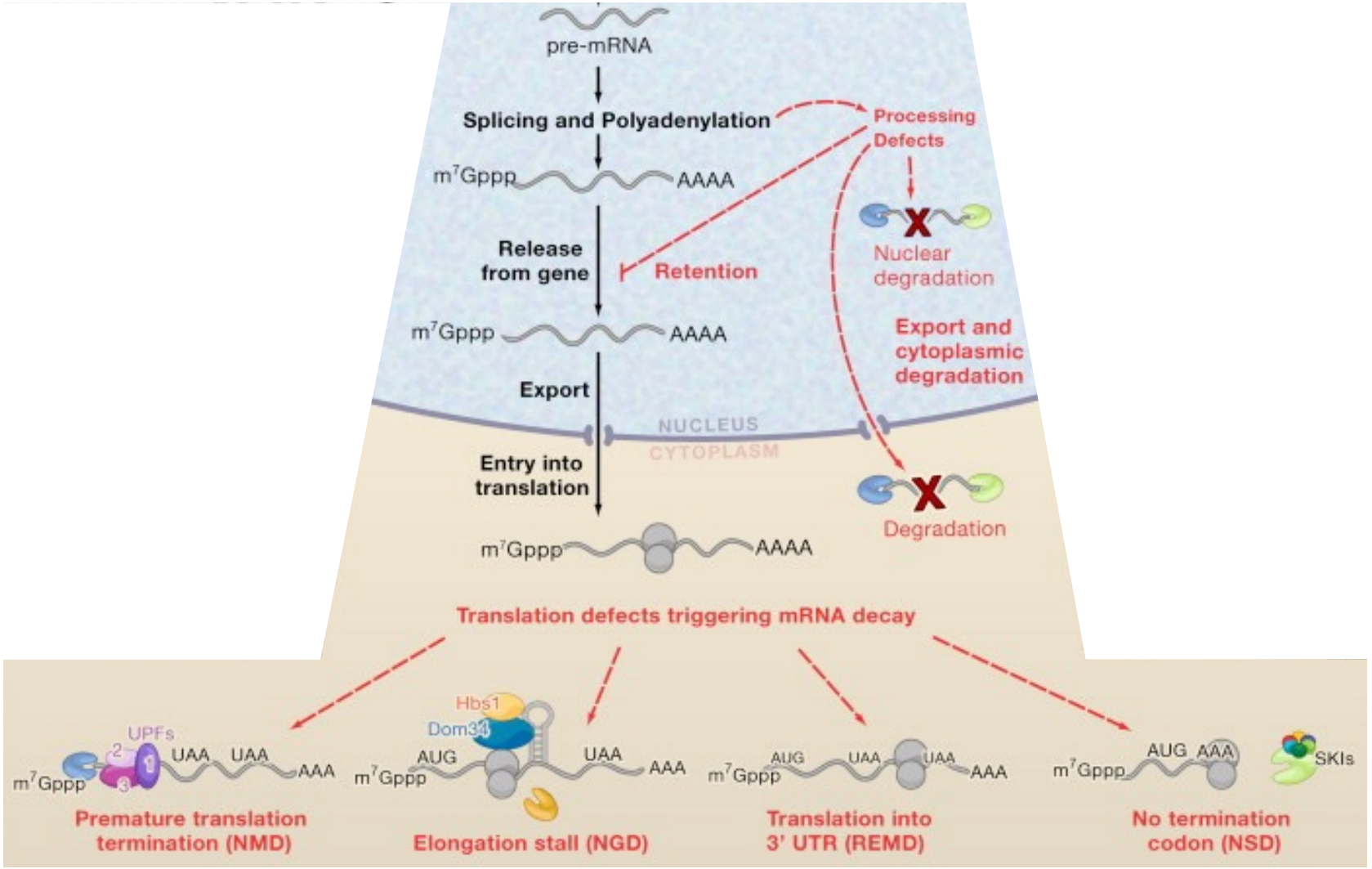
Doma & Parker, 2006

Summary of mRNA surveillance pathways in the cytoplasm



Clement & Lykke-Andersen, 2006

Summary of mRNA Surveillance Pathways

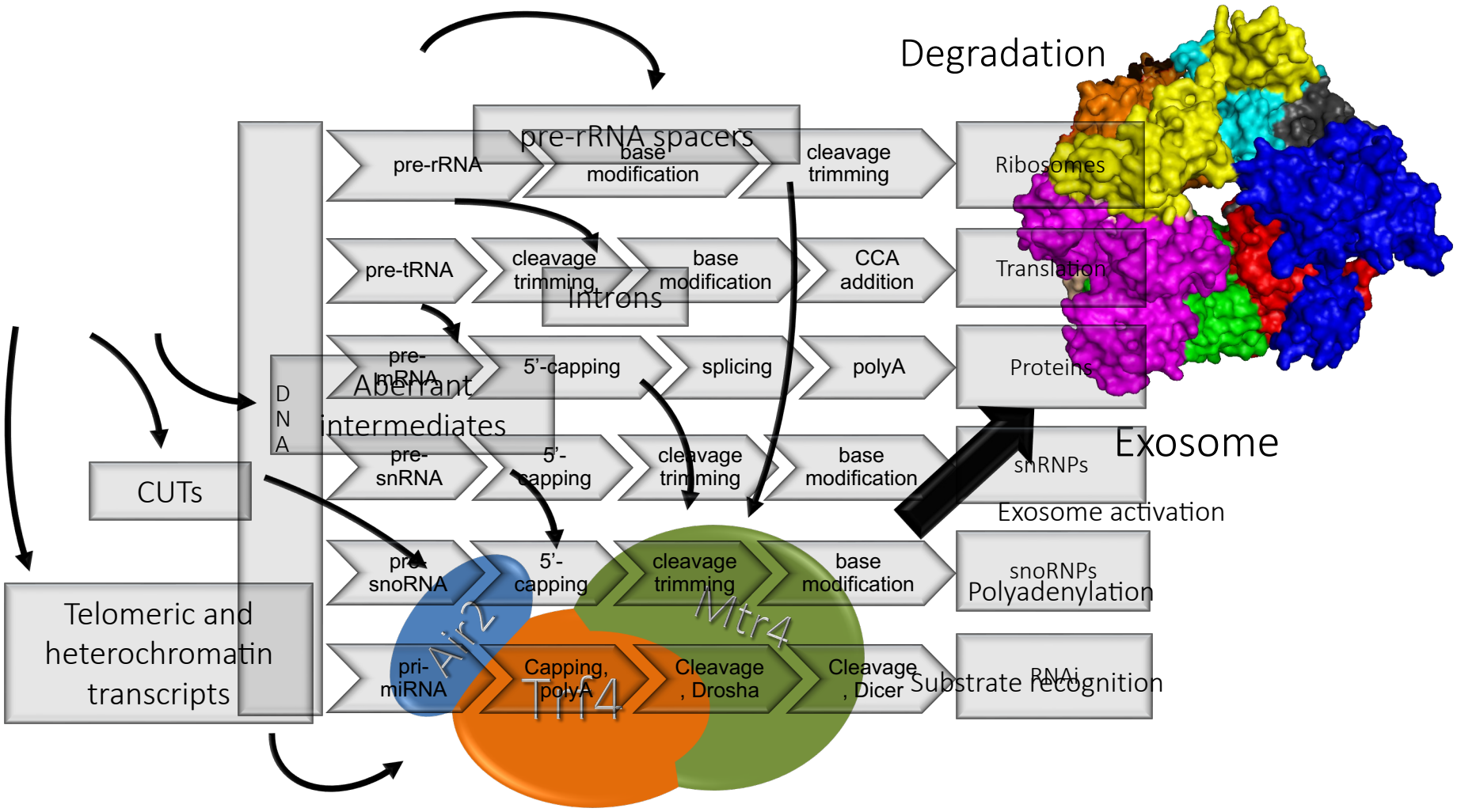


RNA metabolism 2023

Doma & Parker (2007) Cell 131:660

RNA quality control of noncoding RNAs

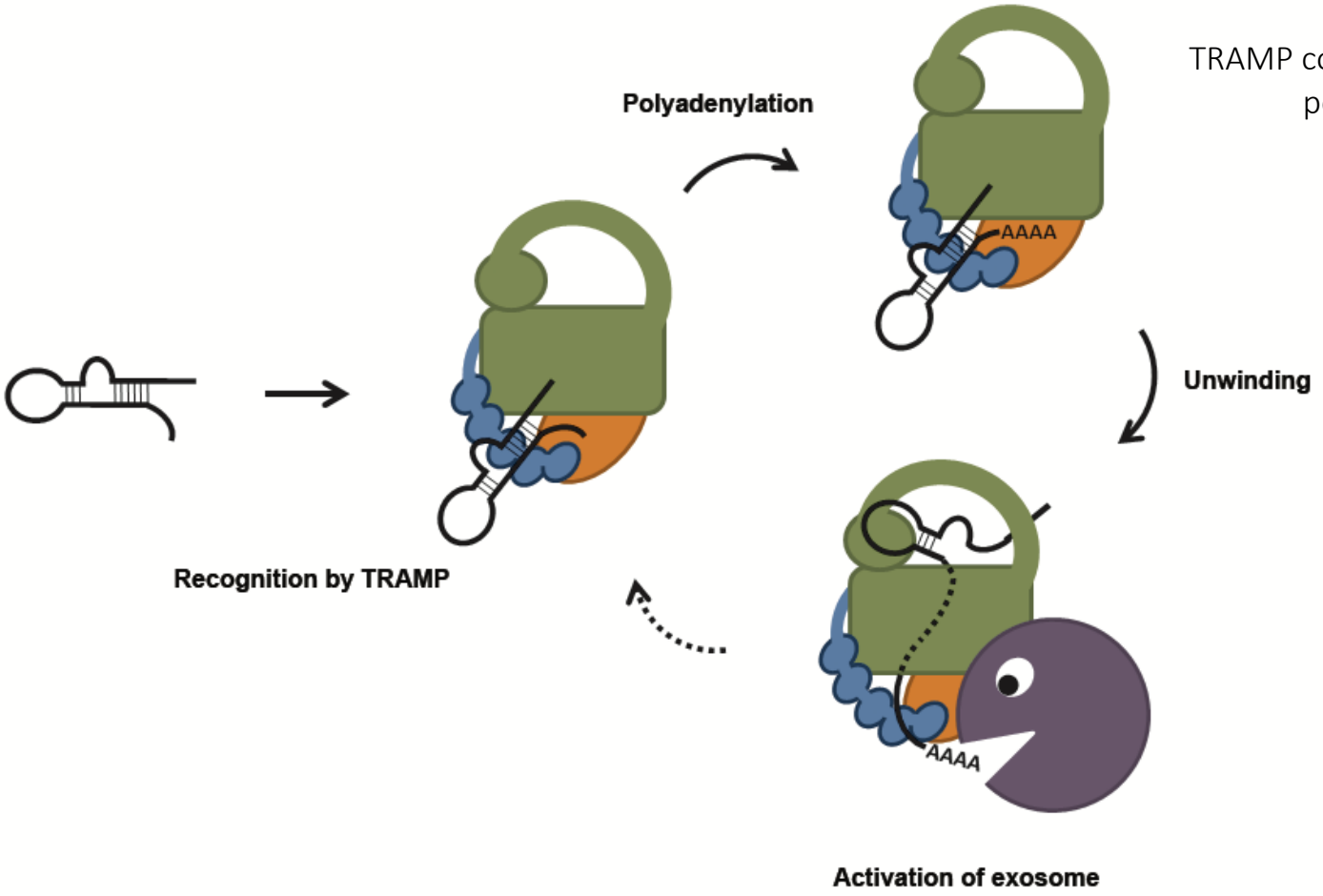
Nuclear RNA surveillance of noncoding RNAs



RNA metabolism 2023

TRAMP complex

Polyadenylation mediates surveillance of ncRNAs in the nucleus



TRAMP complex adds polyA

RNA metabolism 2023

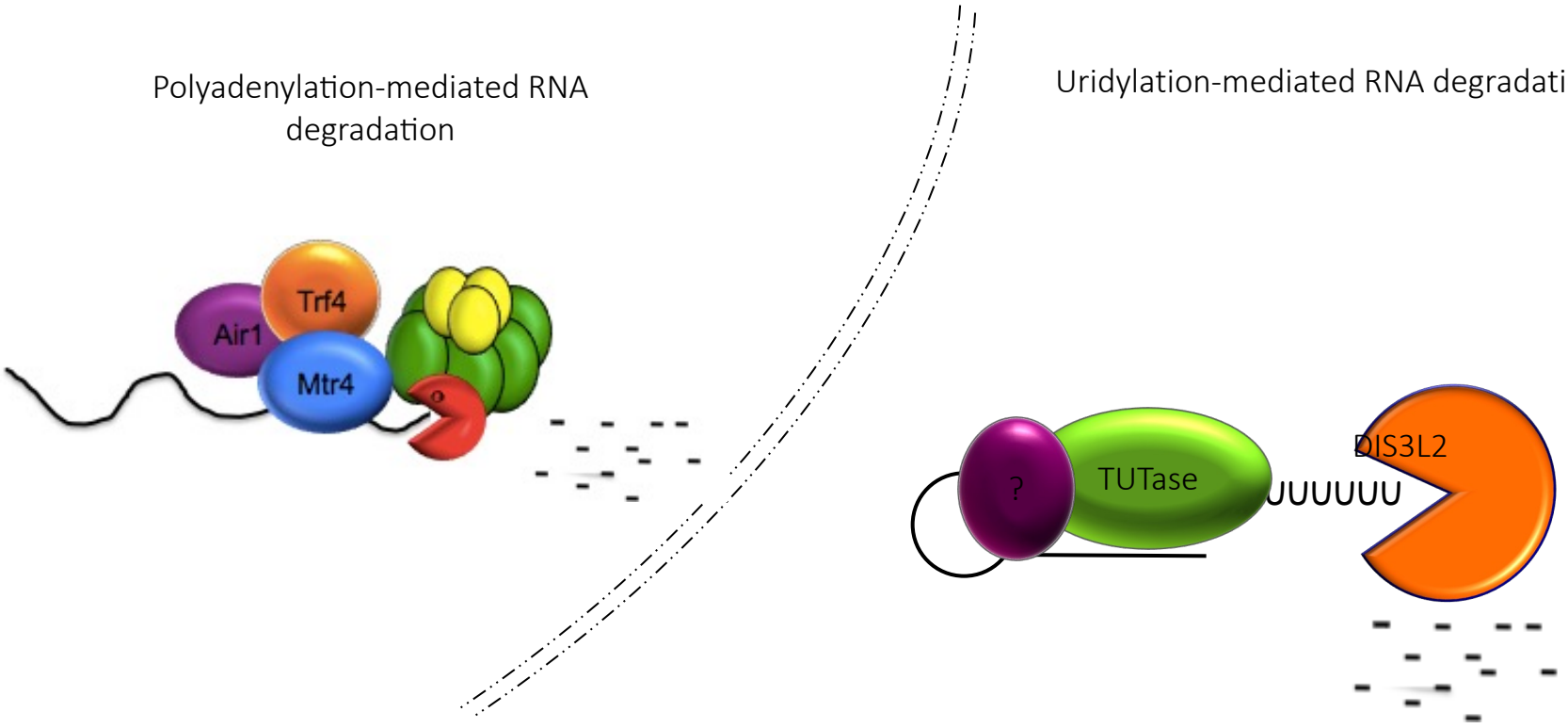
RNA tagging for degradation

NUCLEUS

CYTOPLASM

Polyadenylation-mediated RNA degradation

Uridylation-mediated RNA degradation

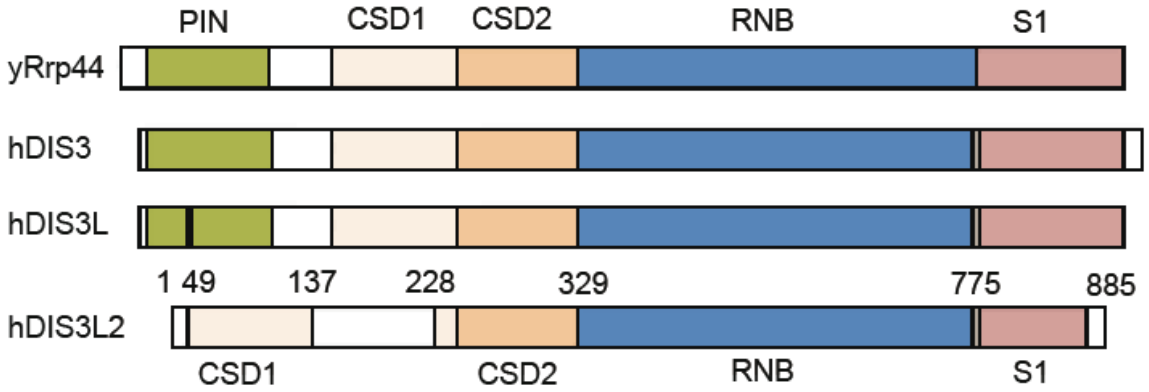


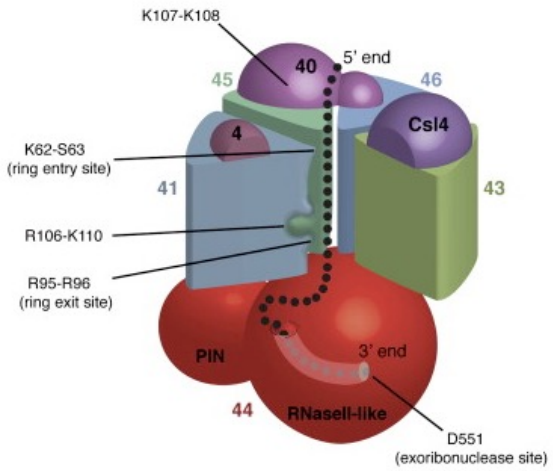
TRAMP + DIS3-EXOSOME

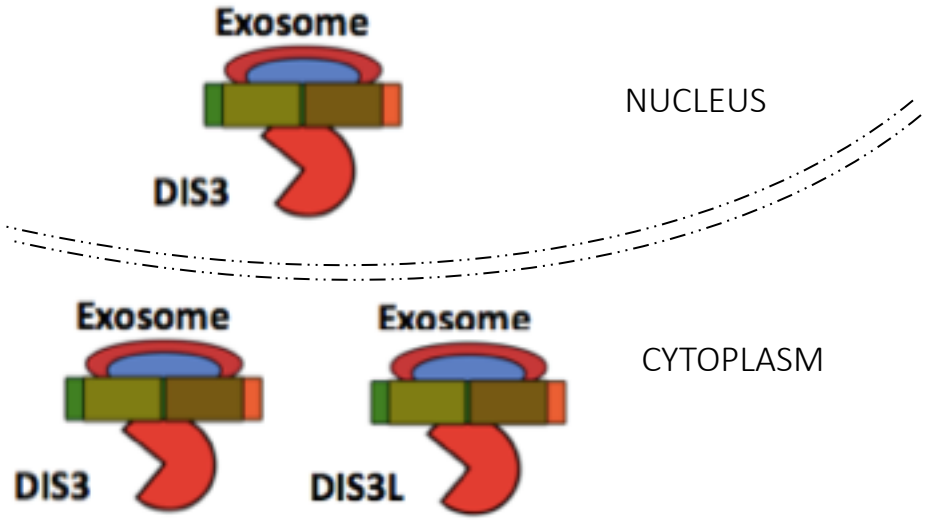
TUTase + DIS3L2

RNA surveillance of noncoding RNAs in the cytoplasm

The domain organization of DIS3 proteins





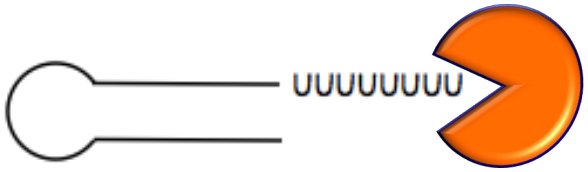


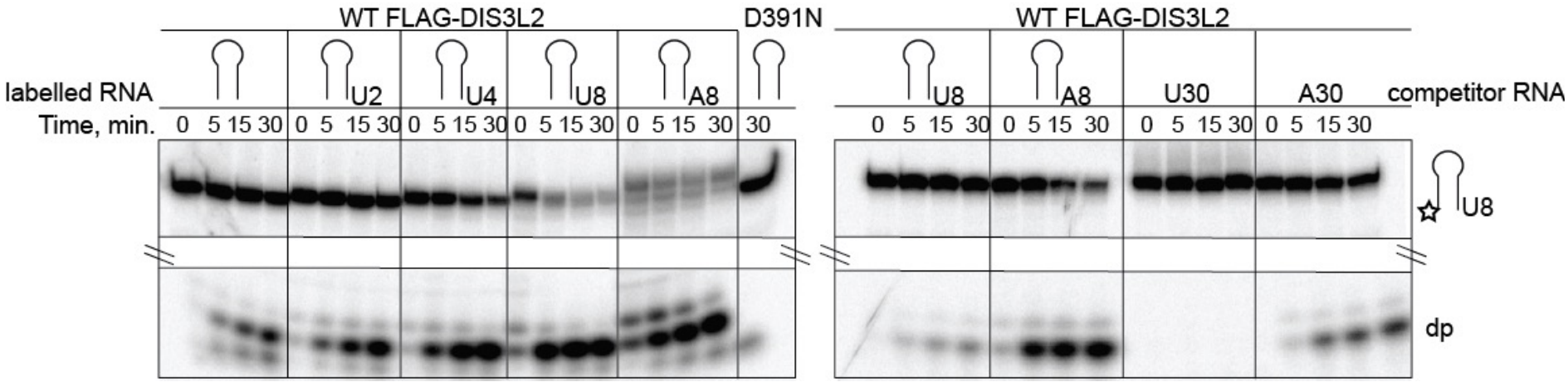
RNA metabolism 2023

Bonneau, 2009, Lebreton 2008, Schaeffer 2009

Tomecki et al. EMBOJ 2010, Staals et al., EMBOJ 2010

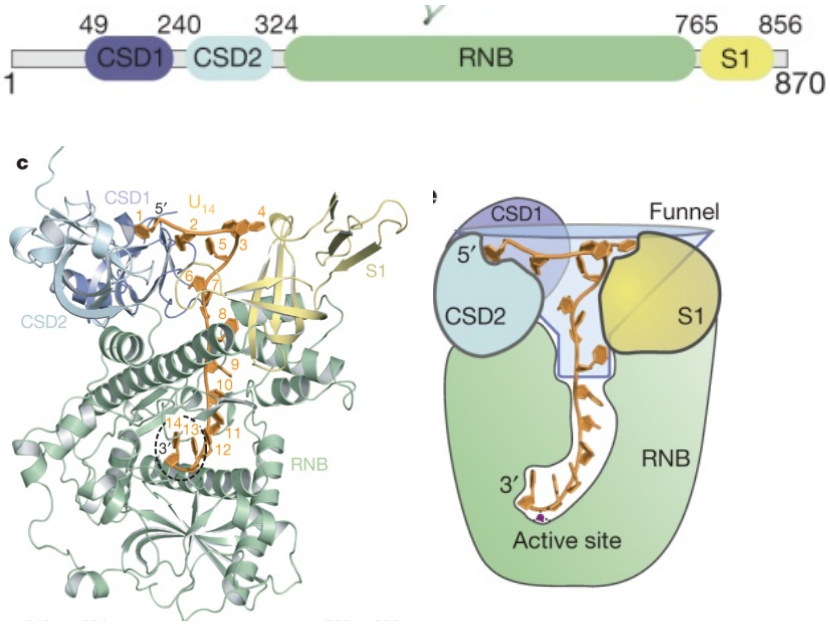
DIS3L2 degrades uridylated RNAs





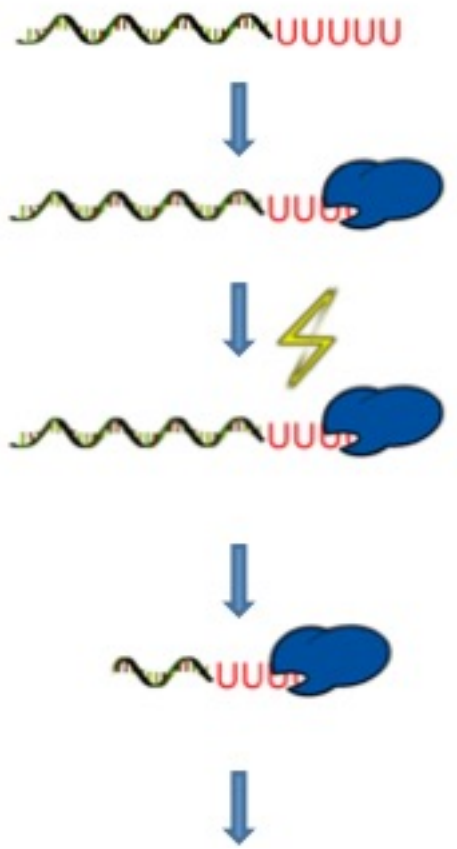
Ustianenko et al., 2013

CLIP-seq approach to identify DIS3L2 targets



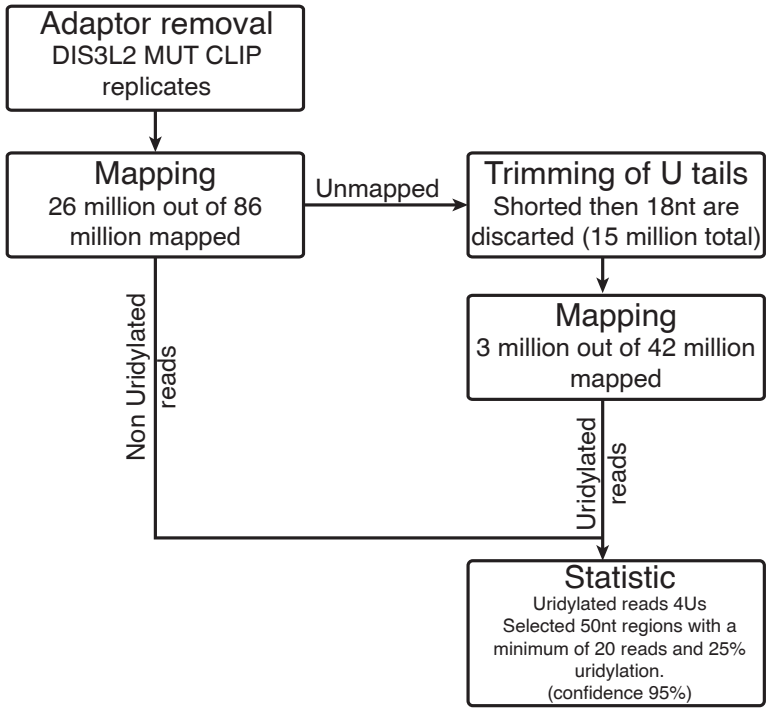
Faehnle et al.,Nature 2014

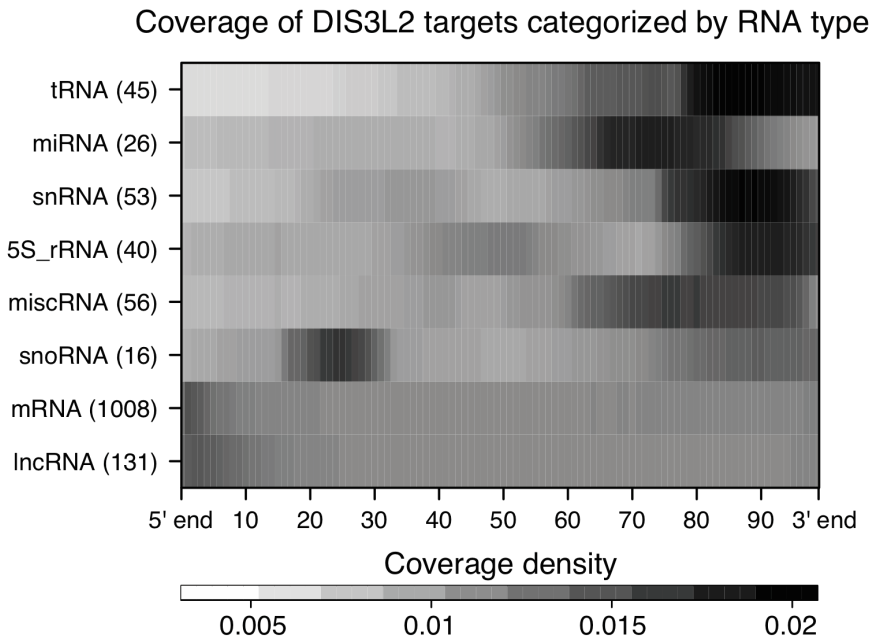
In vivo crosslinking



RNA metabolism 2023

The U-tail distribution on DIS3L2 bound RNAs



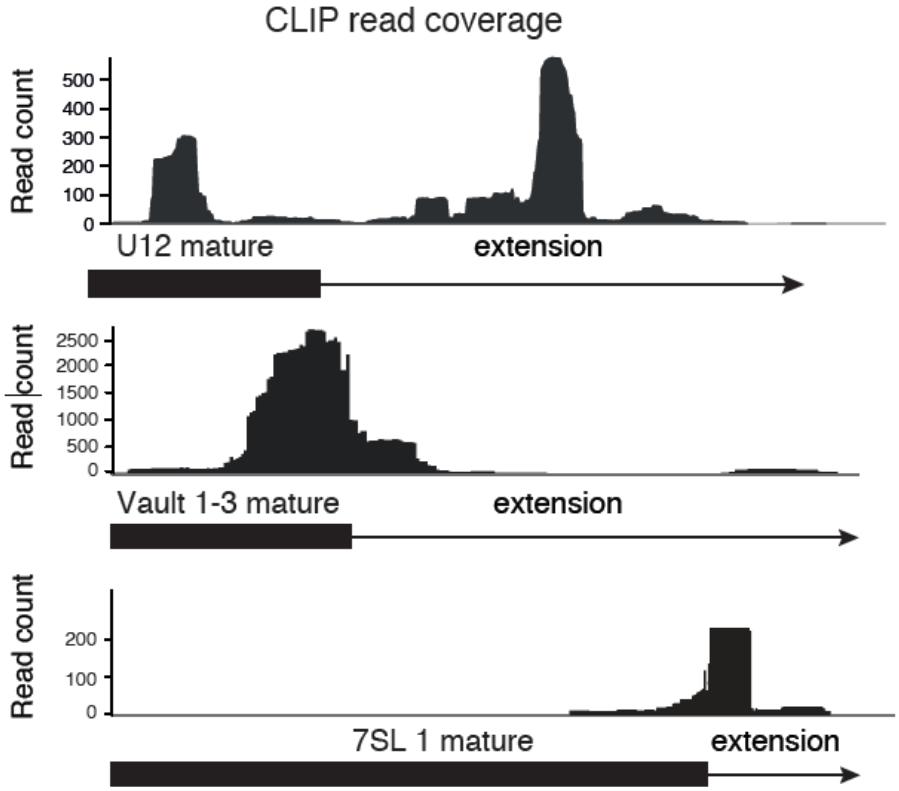


RNA metabolism 2023

Ustianenko et al., EMBO J 2016

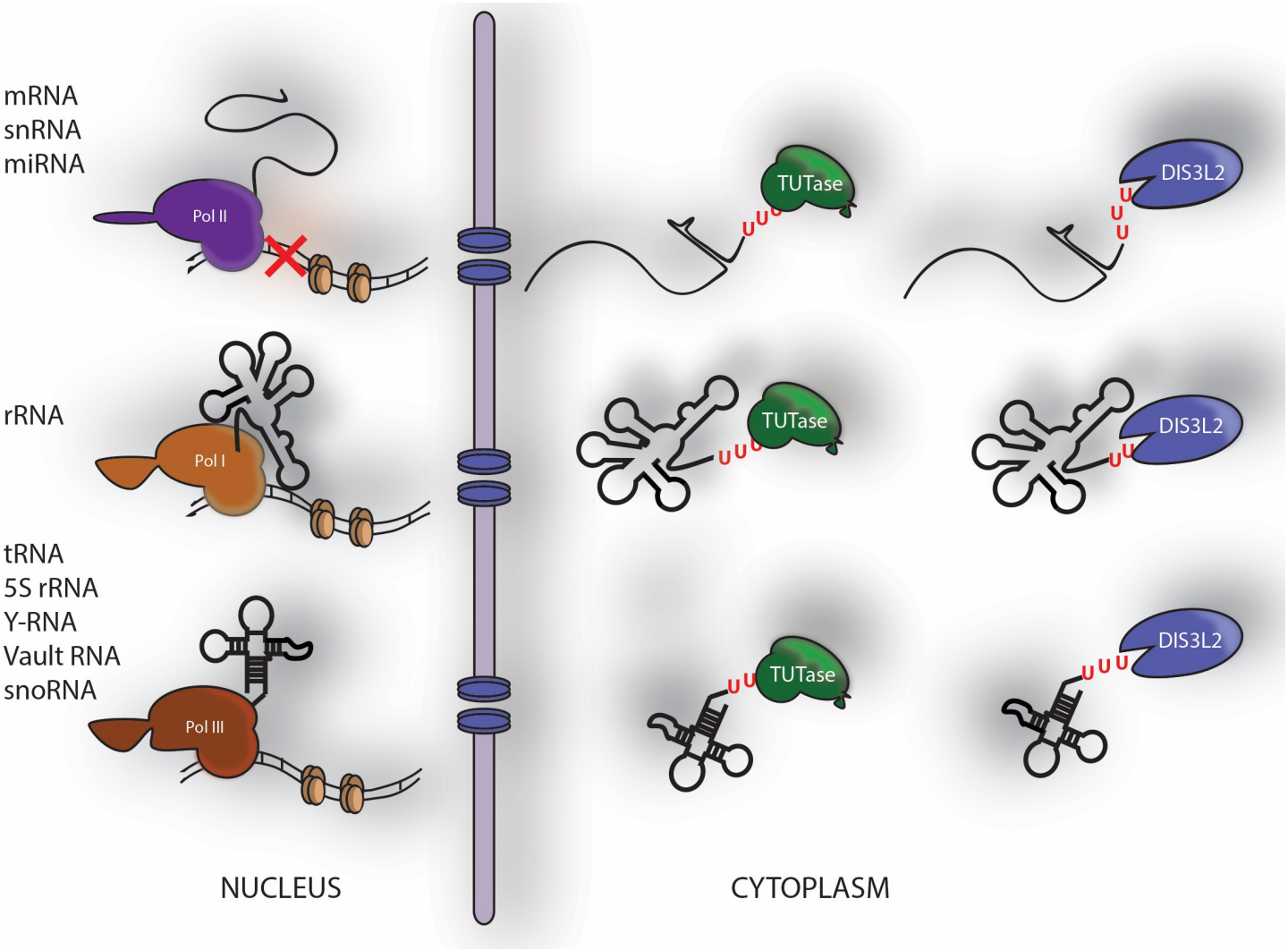
Missprocessed ncRNAs and transcripts from pseudogenes are urydilated

RNA type	Genes	Pseudogenes
snRNA	55	48
snoRNA	17	1
5S rRNA	43	43
Vault RNA	3	0
Y RNA	47	13
7SL RNA	6	5
RNAseP	1	0
mtRNAseP	1	0



Ustianenko et al., EMBO J 2016

TDS = TUT-DIS3L2 sureillance of ncRNAs in the cytoplasm



RNA metabolism 2023

Ustianenko et al., EMBO J 2016

Lecture overview

1. General mRNA turnover pathways
   - 5’→3 & 3’→5’
   - Deadenylases
   - Decapping complex
   - Xrn1, exosome, DcpS

2. Aberrant RNA turnover pathways
   - Premature stop codons: nonsense-mediated mRNA decay (NMD)
   - No stop codons: non-stop mRNA decay (NSD)
   - Elongation stall: no-go mRNA decay (NGD)
   - Translation into the 3’ UTR: ribosome extension-mediated mRNA decay (REMD

3. Specialized mRNA turnover pathways
   - ARE-mediated mRNA turnover: AU-rich elements in the 3’ UTR are bound by proteins that modulate the stability of mRNAs in response to regulatory signals

4. RNA surveillance of noncoding RNAs
   - oligo(A) mediated RNA quality control in the nucleus
   - oligo(U) mediated RNA surveillance in the cytoplasm