RNA DEGRADATION AND QUALITY CONTROL

RNA structure and function 23.11. 2020

LECTURE CONTENTS

RNA degradation

- -general principles
- -RNA degradation machines

RNA surveillance

- -coding RNAs (mRNAs)
- -noncoding RNAs

(m)RNA Turnover: Why Should We Care?

1. Control of Gene Expression

2. Quality Control of RNA Biogenesis

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

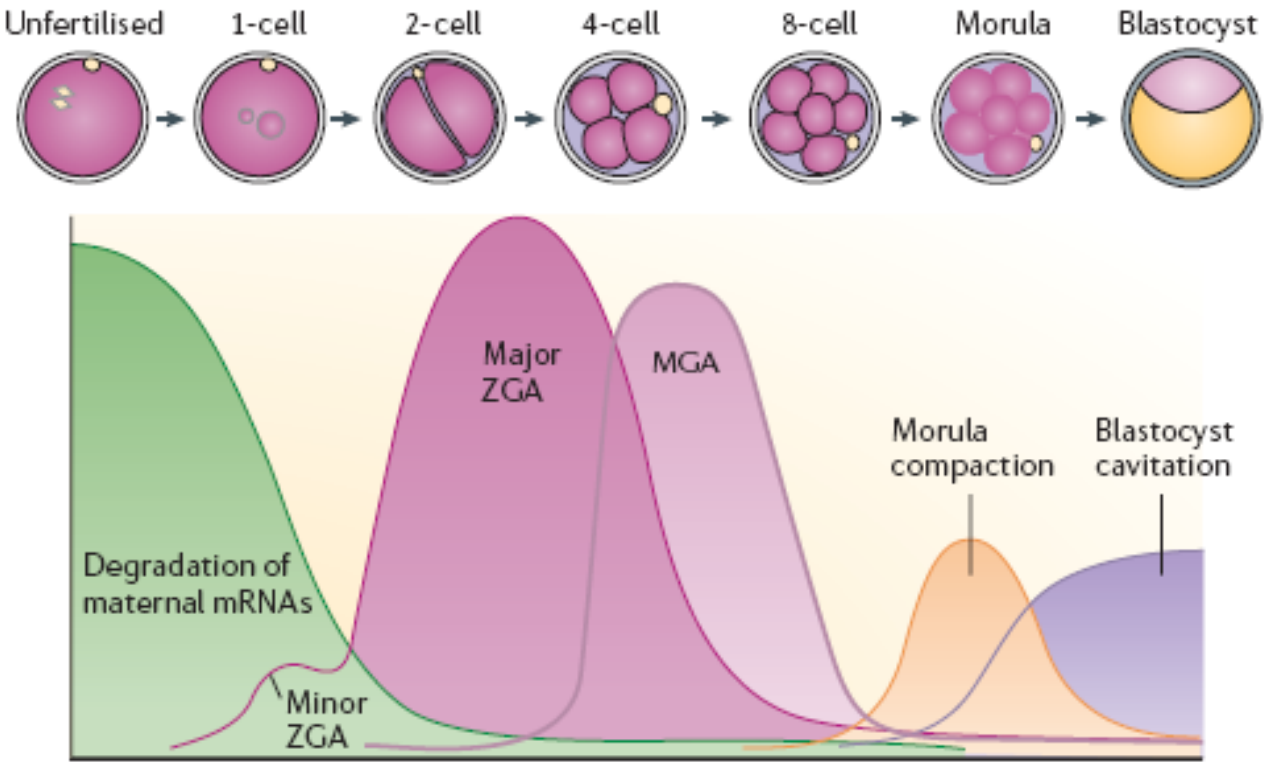
• The concentration of mRNA is a function of both the rate of mRNA synthesis and the rate of mRNA degradation

• 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

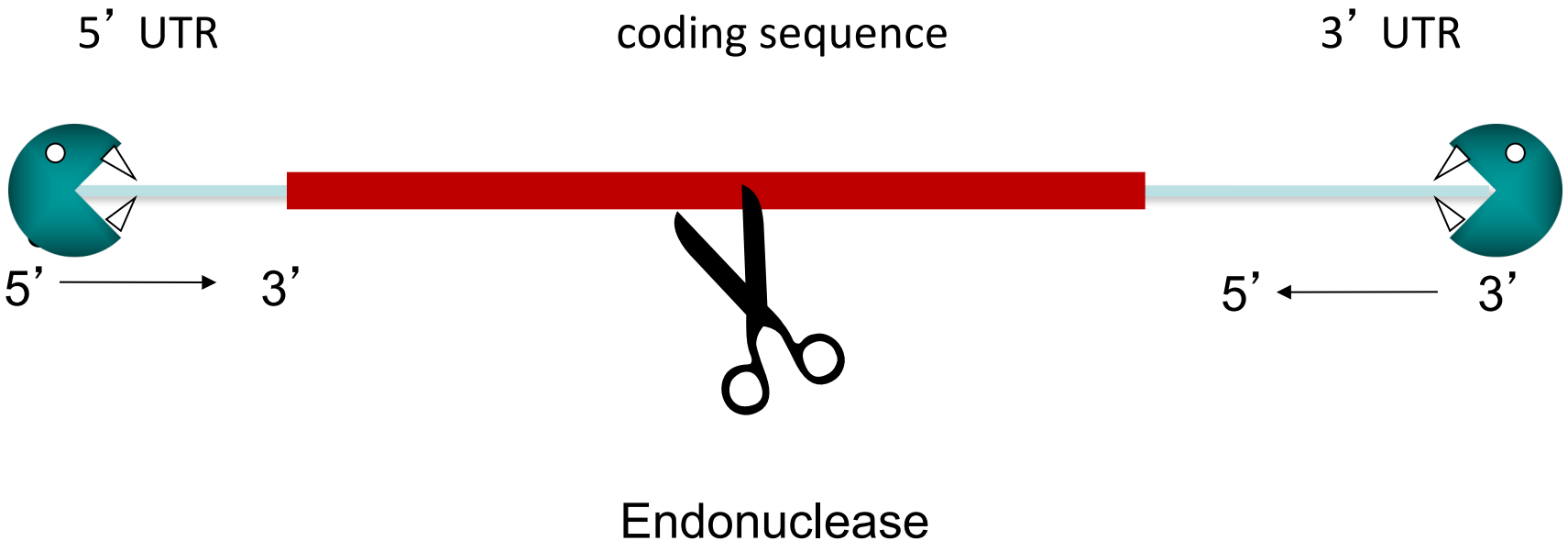
Gene expression during preimplantation embryo development



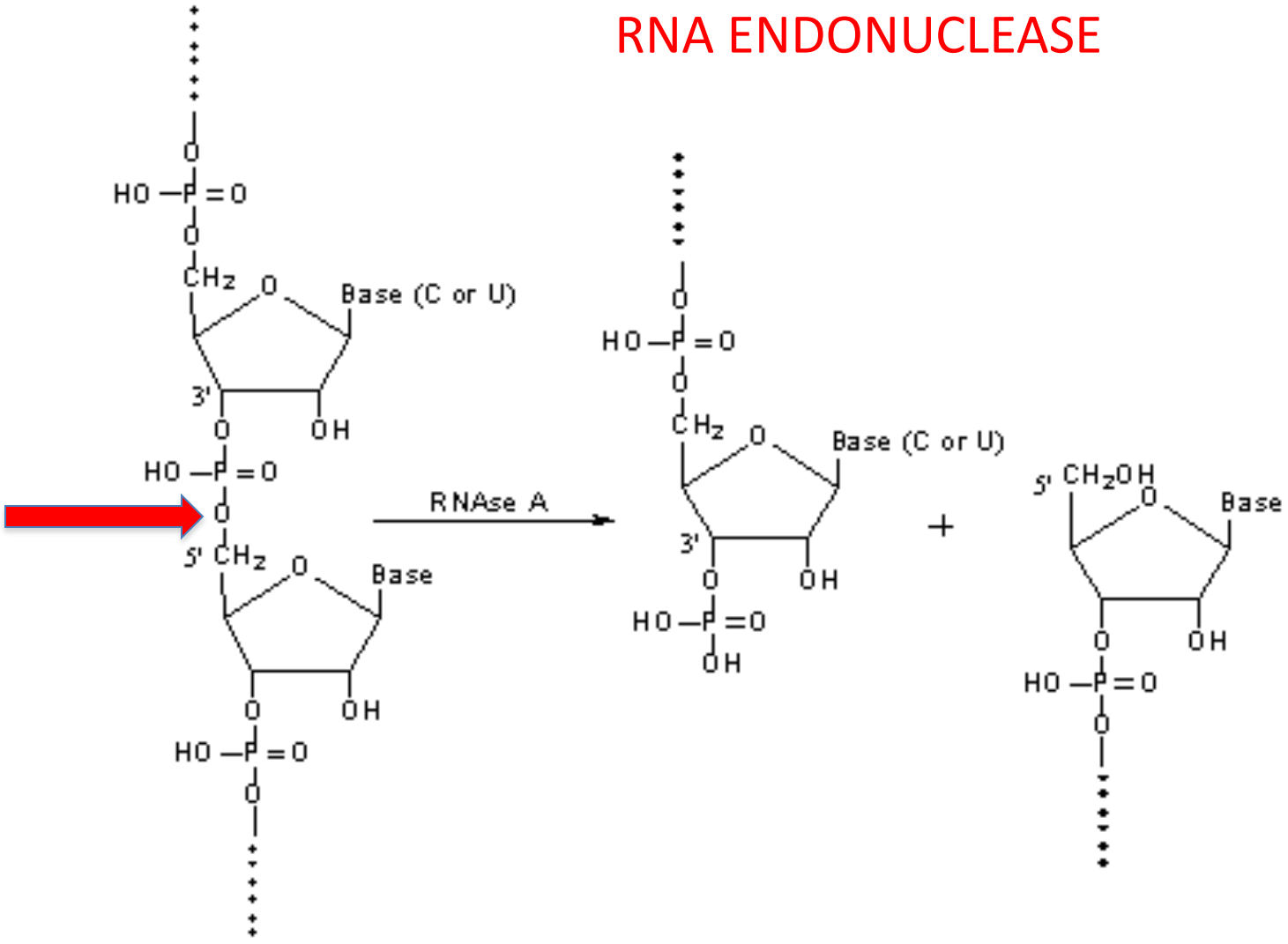
Wang *et al. Nature Reviews Genetics* **7**, 185–199 (March 2006) | doi:10.1038/nrg1808

RNA degradation mechanisms

RNA is prone to nucleolysis



RNA degradation by nucleases



RNA degradation by nucleases

RNaseA

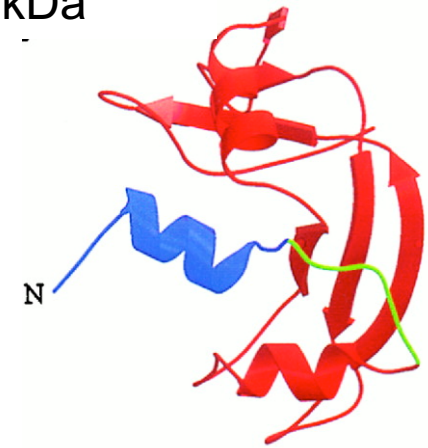
pankreatic endoribonuclease

Highly stable,

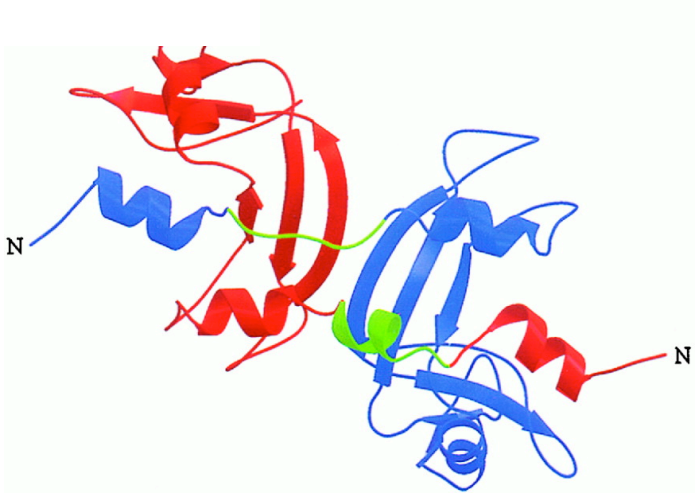
Heat resistant

Small – hard to remove

Monomer, 14 kDa



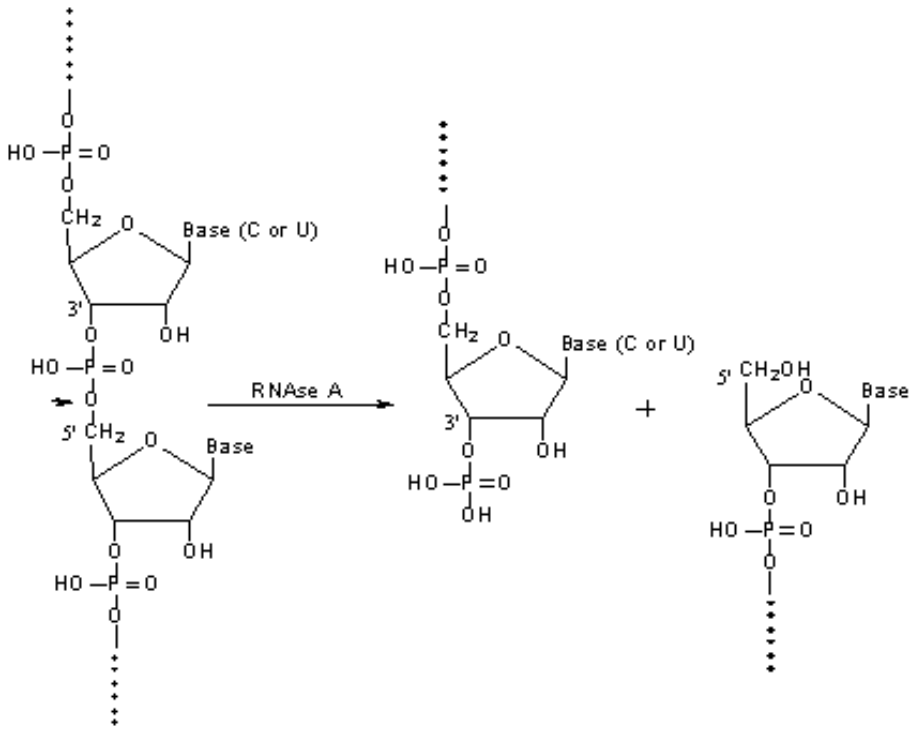
Dimer



Liu et al., PNAS 1998

RNA degradation by nucleases

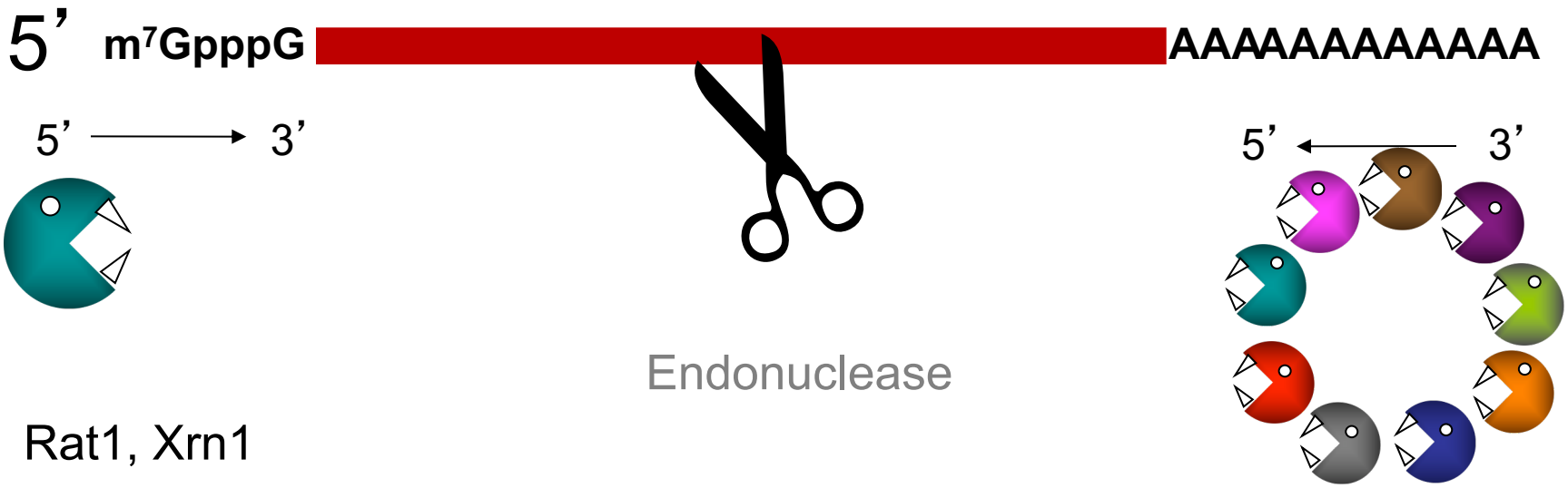
RNA ENDONUCLEASE



	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)

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

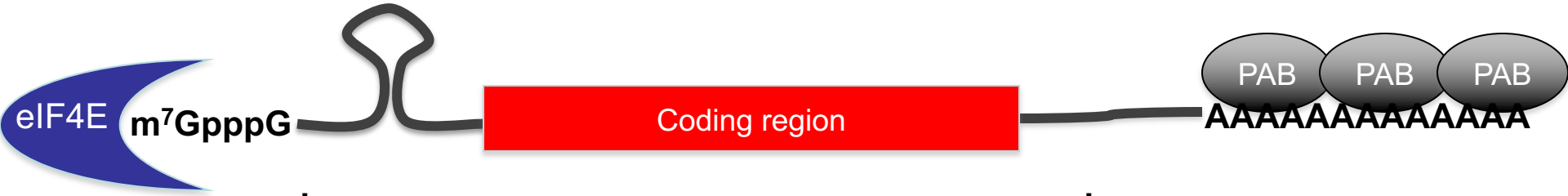
<u>tRNA, rRNA, snRNA, snoRNA</u>:

complex secondary and tertiary structures

Base modifications

mRNA stabilizing and destabilizing features

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



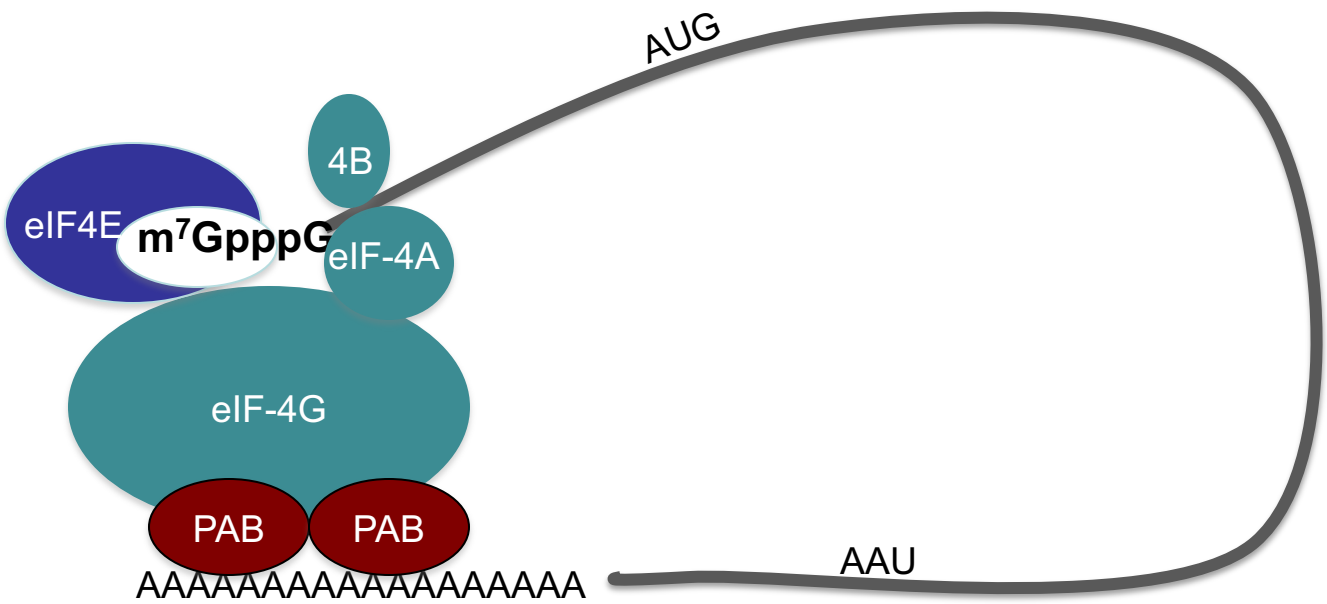
5’ UTR

Protein binding and secondary structures

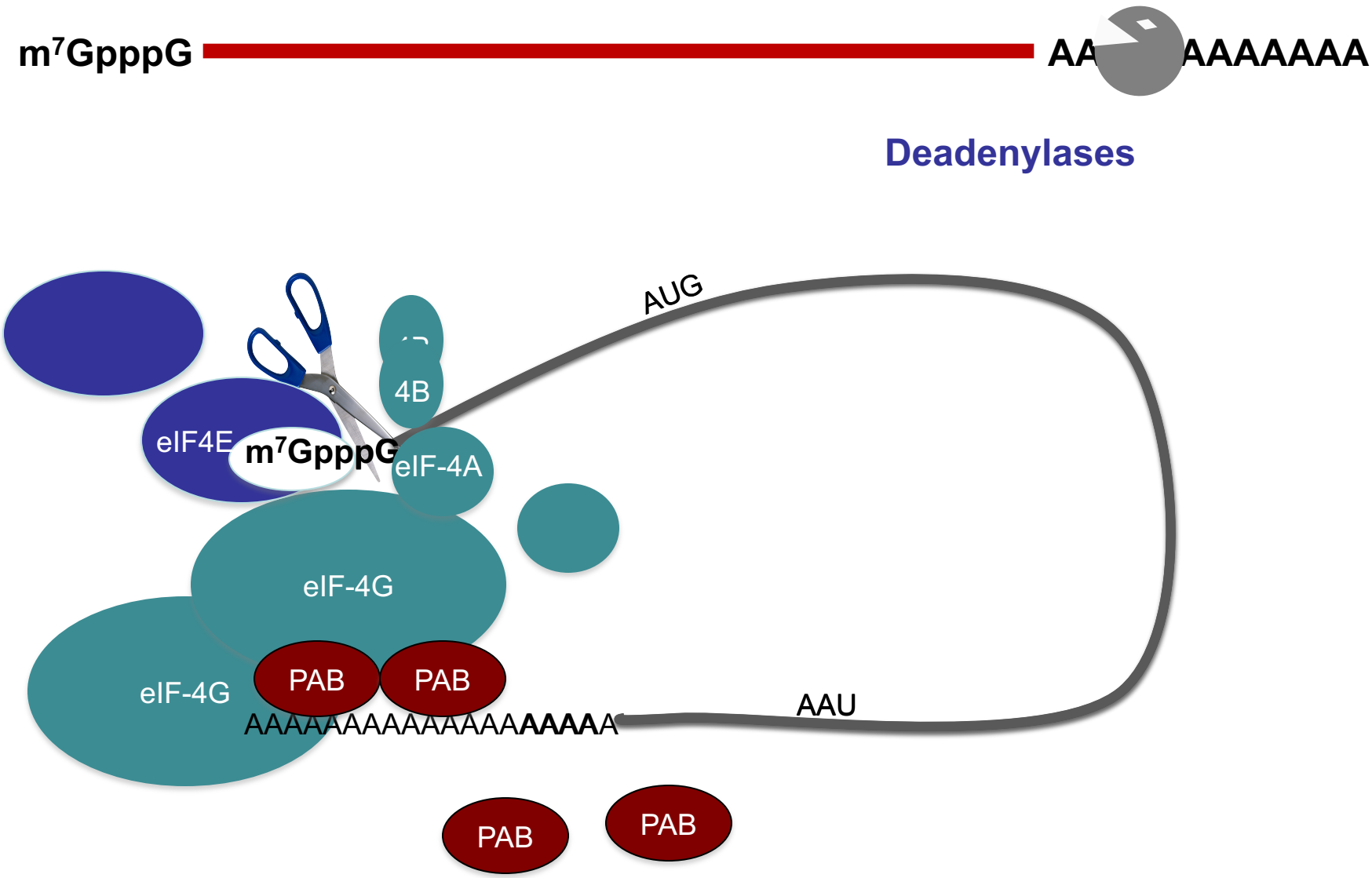
3’ UTR

Protein and RNA binding

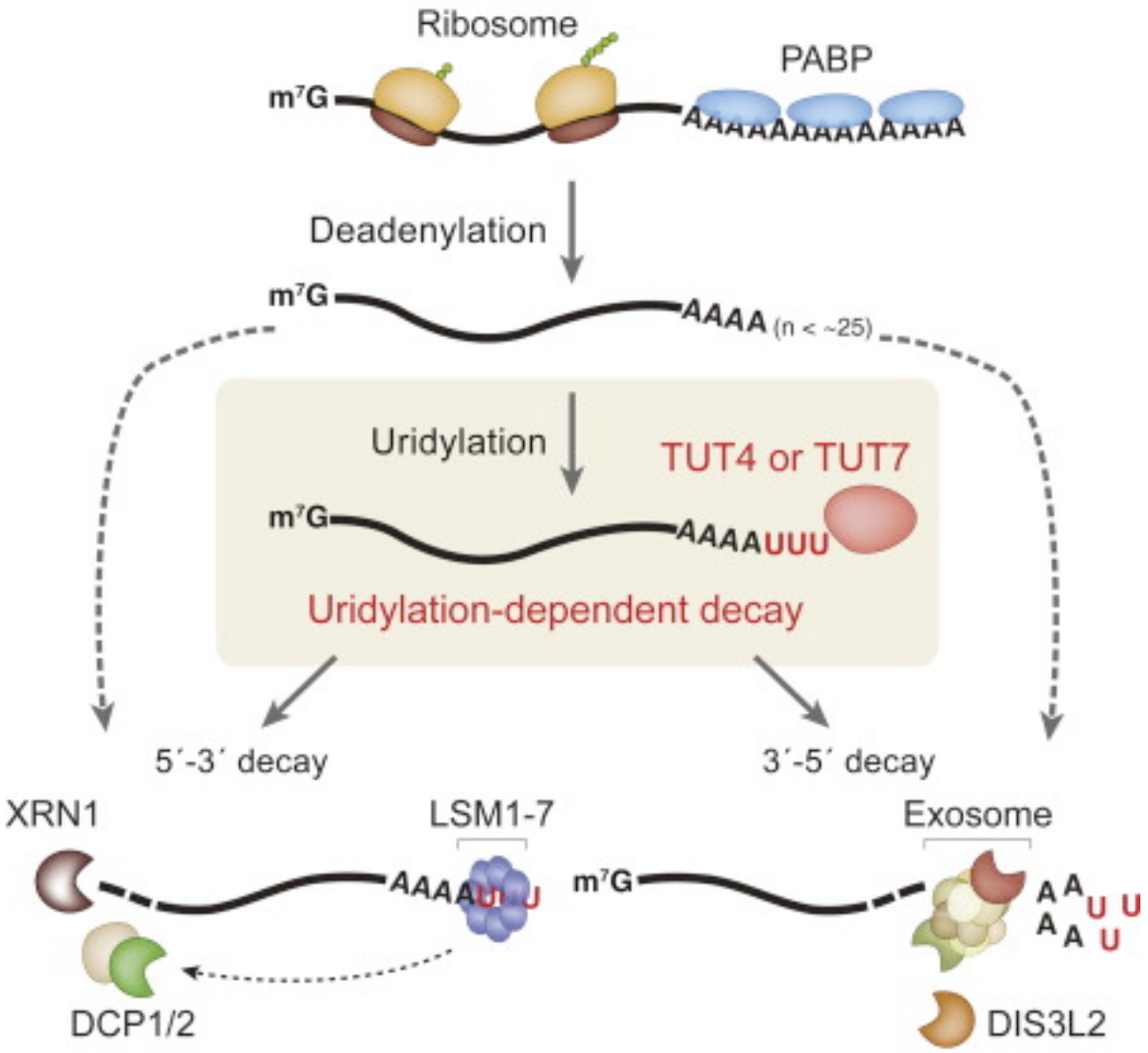
Translation initiation complex



‘Normal’ mRNA degradation is initiated by deadenylation



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

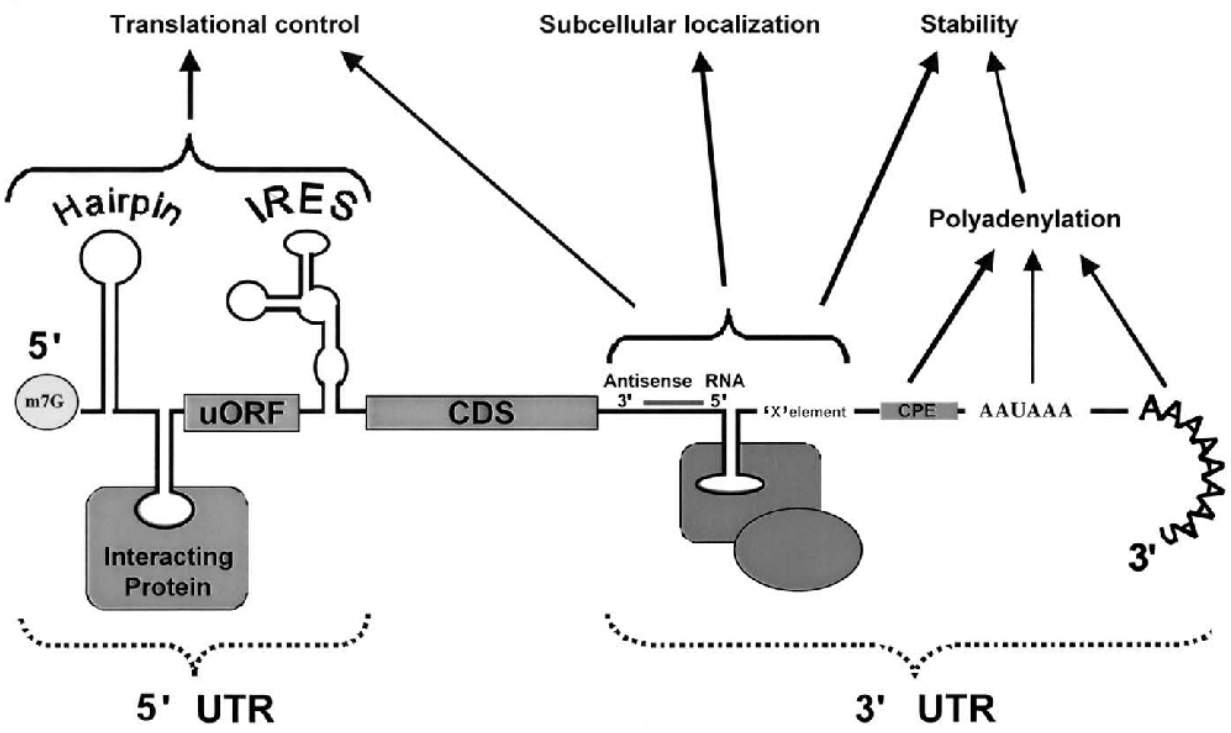
•Oligouridylation of short poly(A) tail recruits Lsm complex, which in turn recruits decaping aparatus and induces degradation at the 5’end

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

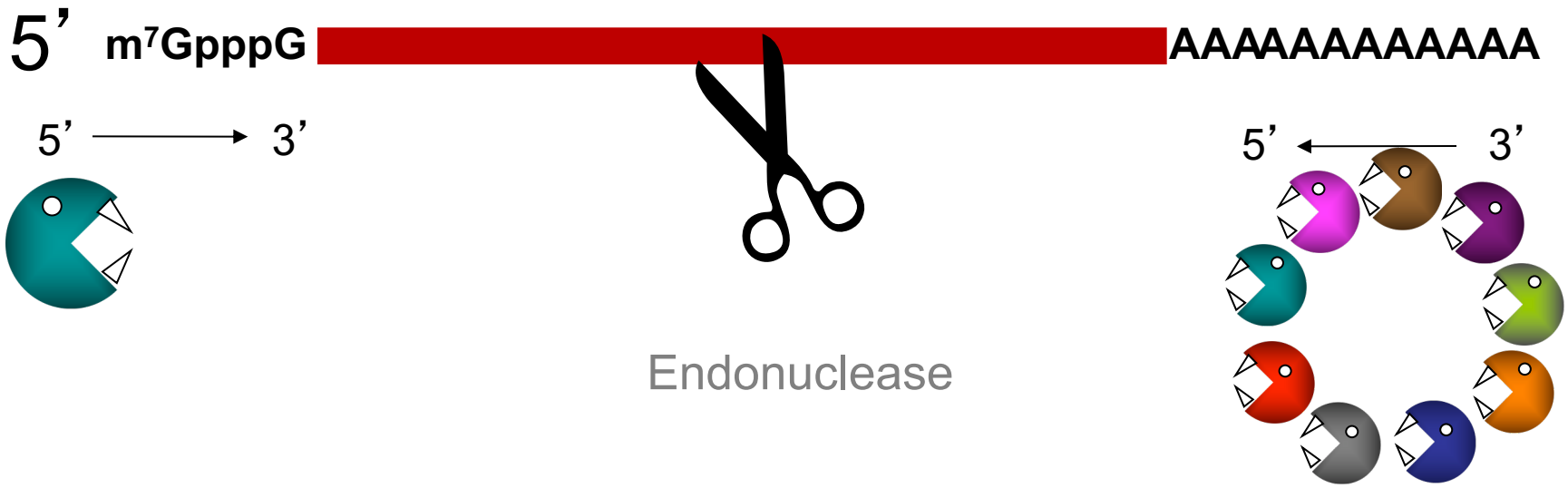
Regulatory sequence elements in mRNAs

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)……



Molecular machines in mRNA degradation



Exonuclease

Rat1, Xrn1

Monomer, very potent, highly processive

Exosome

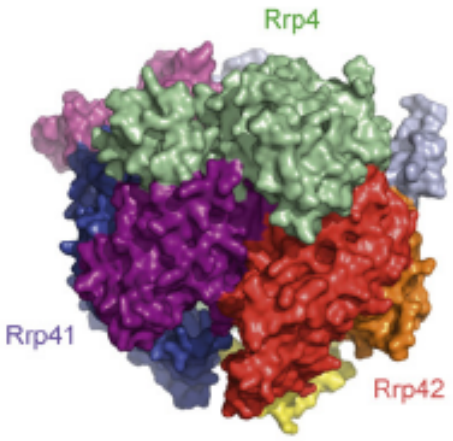
needs cofactors and activation

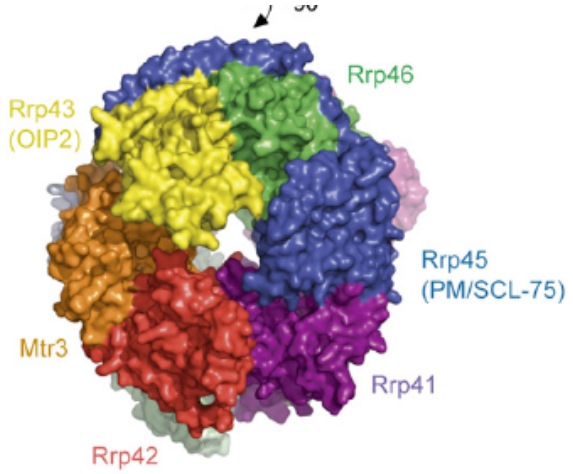
The exosome

Associates with specific co-factors depending on localization

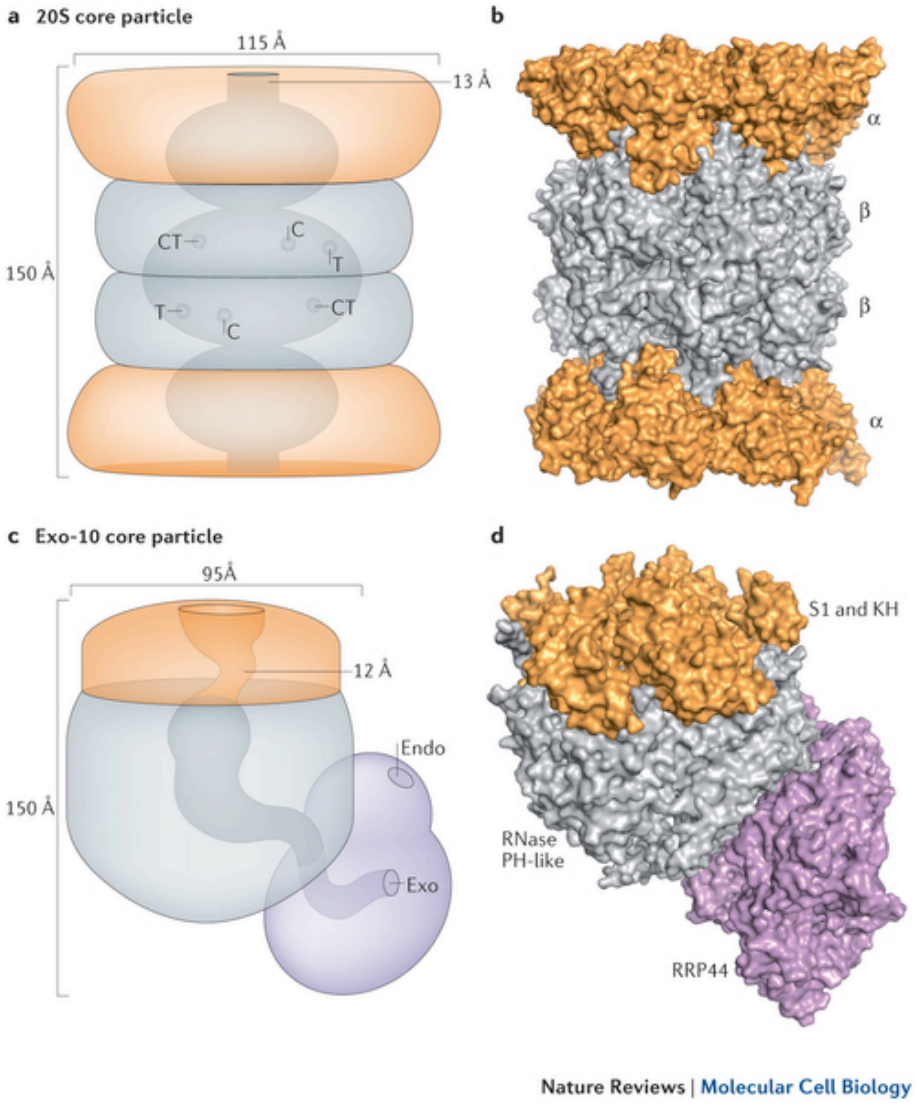
2 forms: nuclear and cytoplasmic

Exosome is poorly active *in vitro* and needs cofactors for activation





The RNA exosome and proteasome: common principles of degradation control



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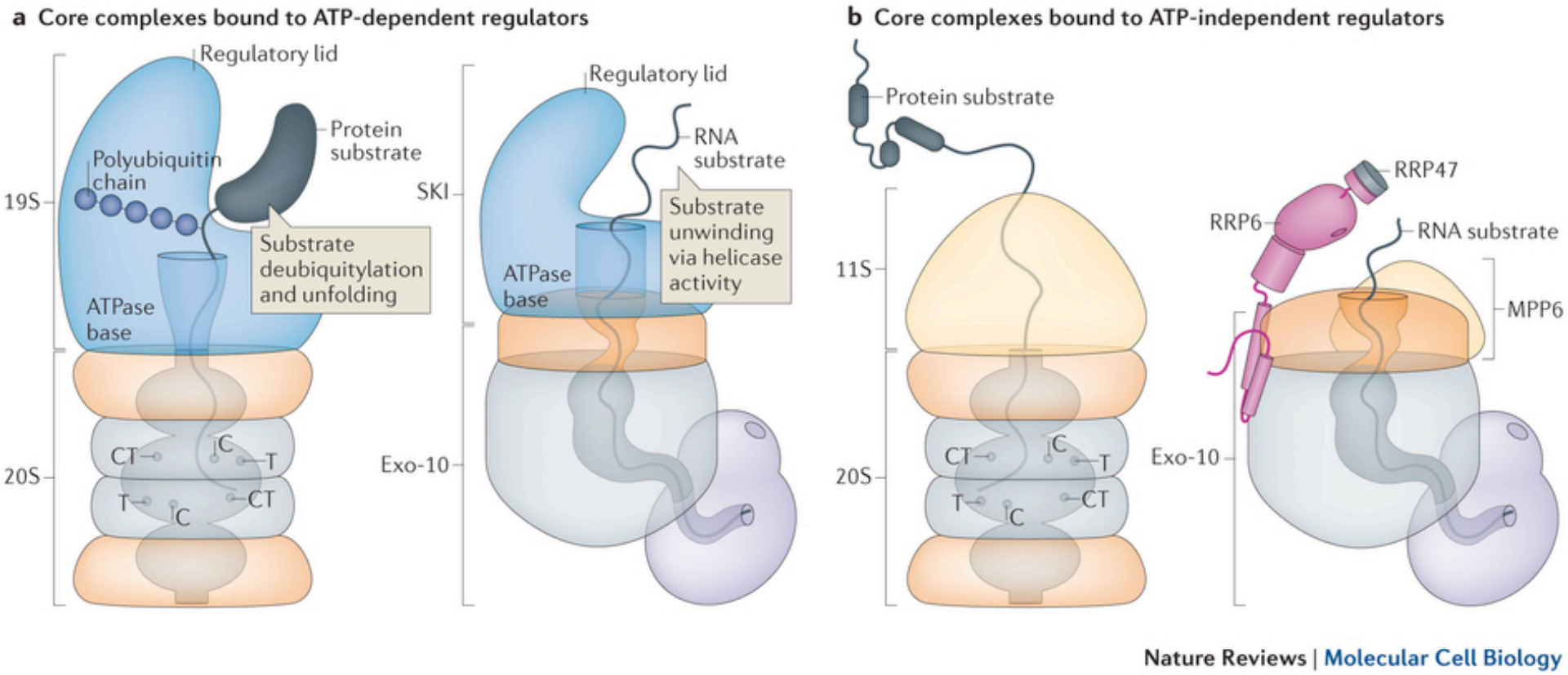
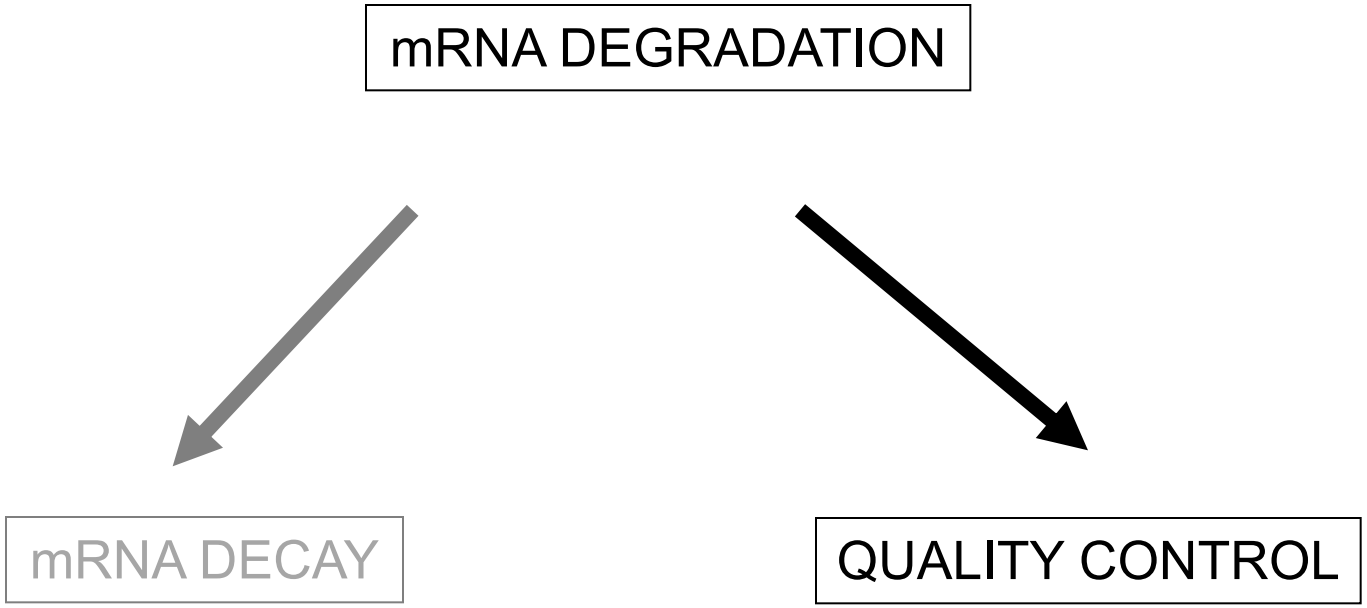
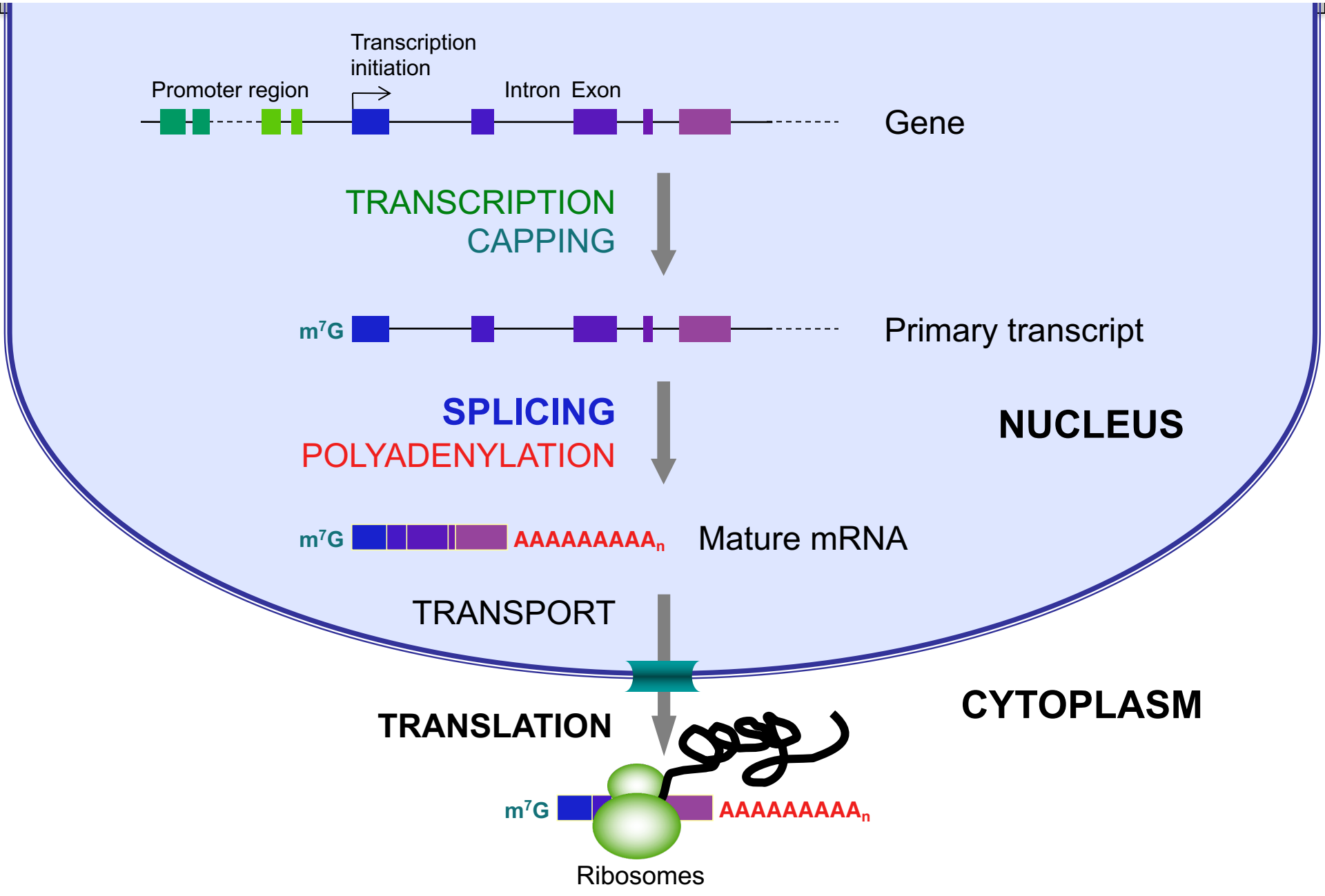


Nature Reviews | Molecular Cell Biology

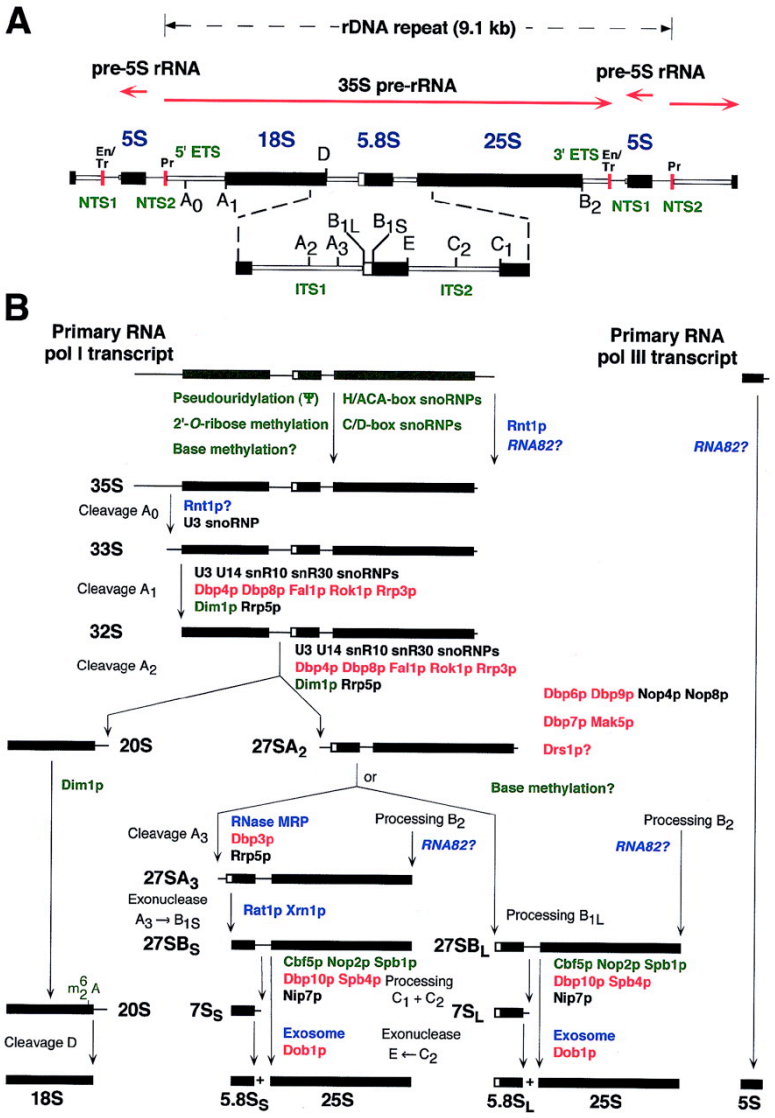

mRNA DEGRADATION
mRNA DECAY
QUALITY CONTROL

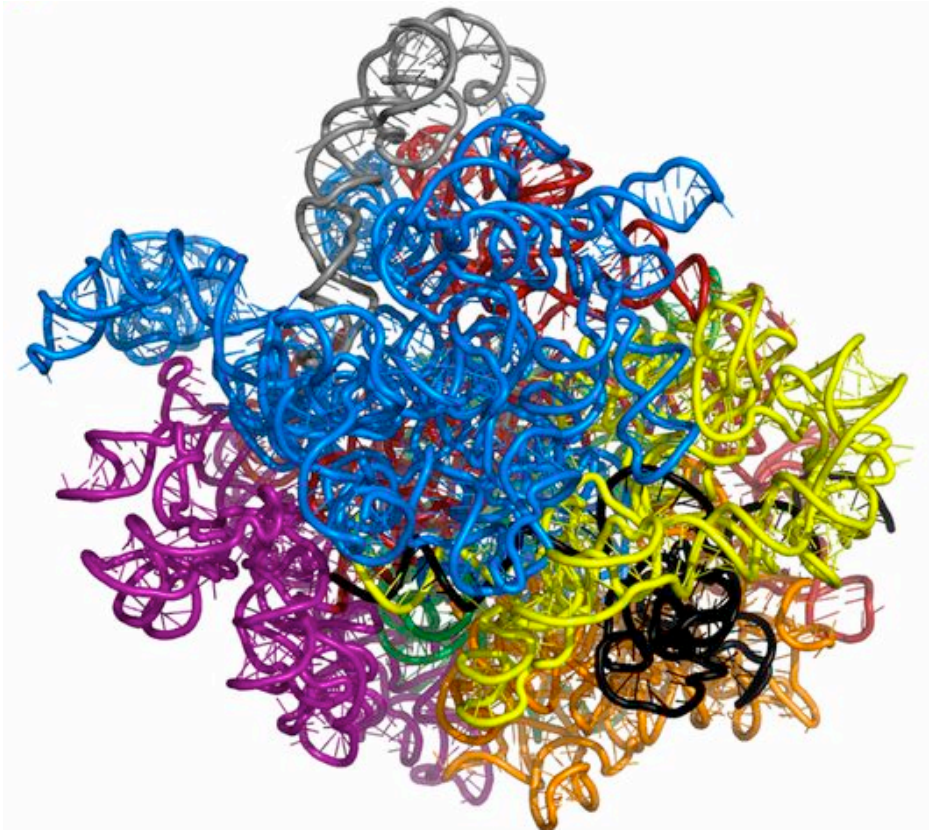
mRNA quality control

All steps of mRNA production are controlled

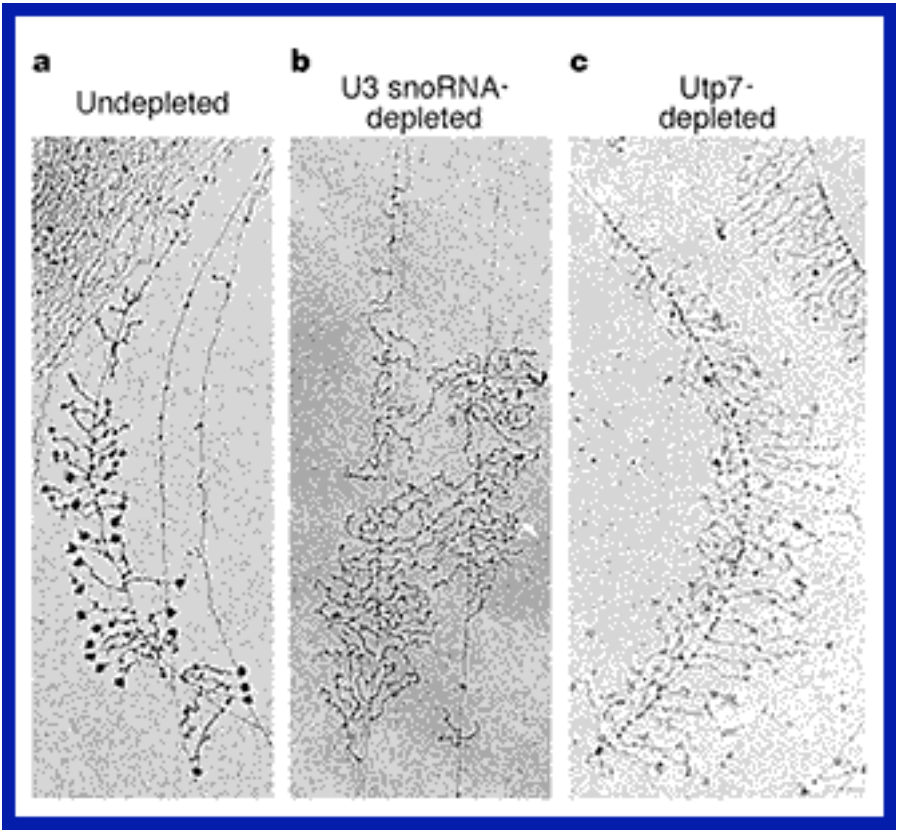


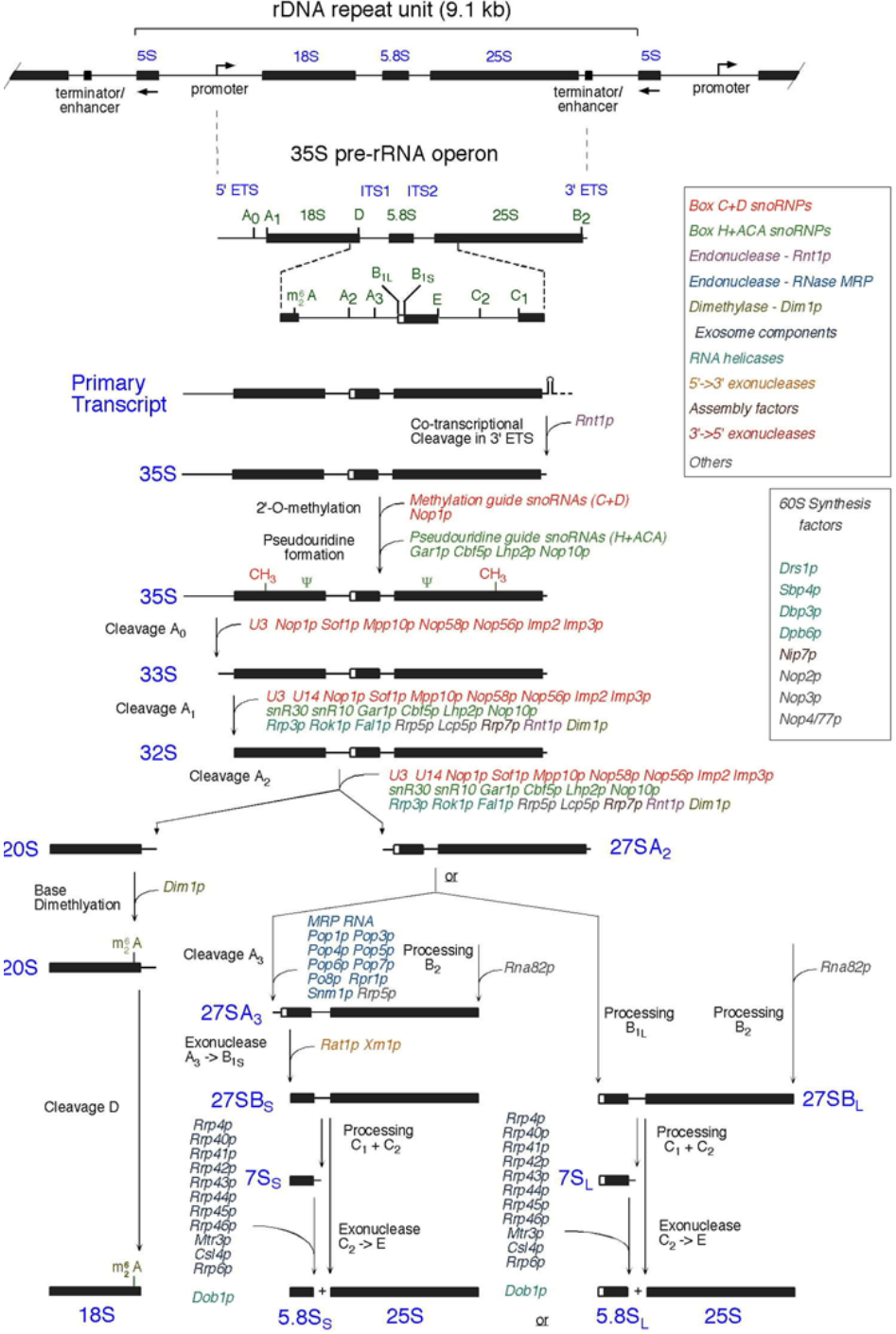
rRNA production is highly complex and energetically expensive





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





When the removal of decay products goes wrong

mRNA 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

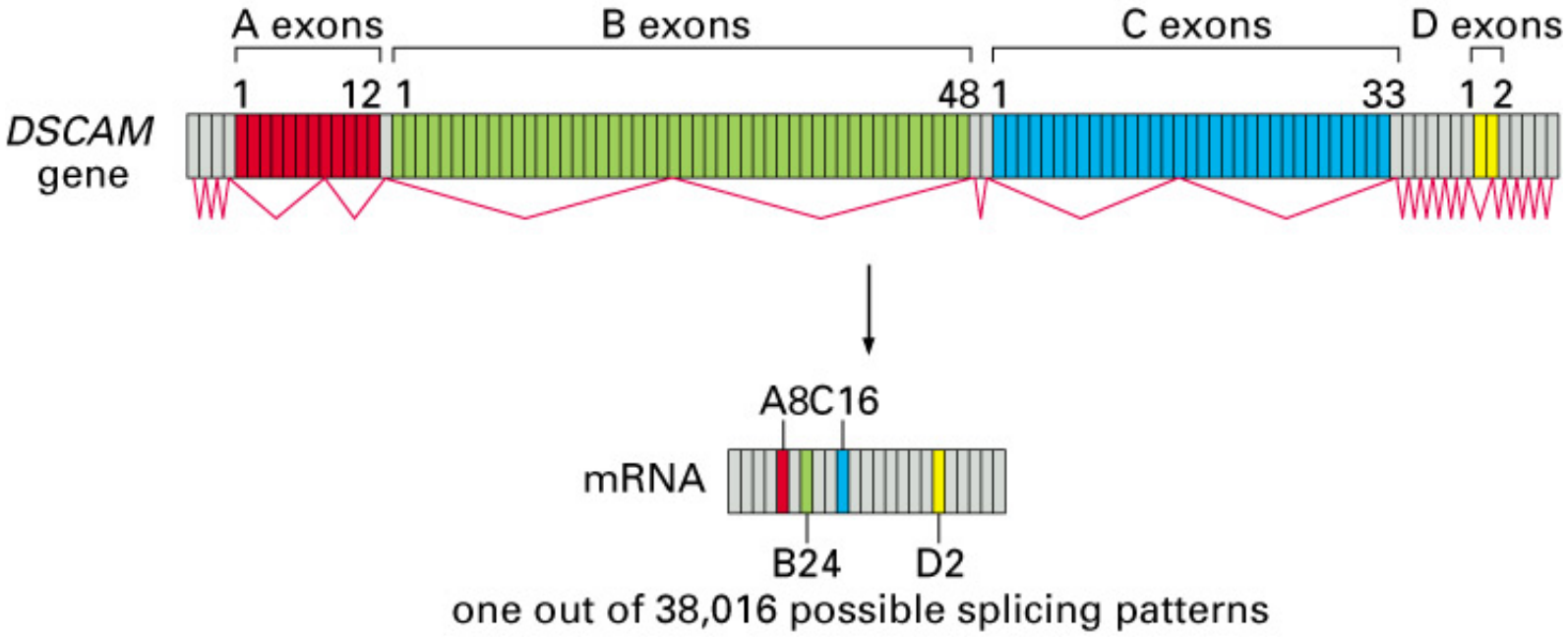
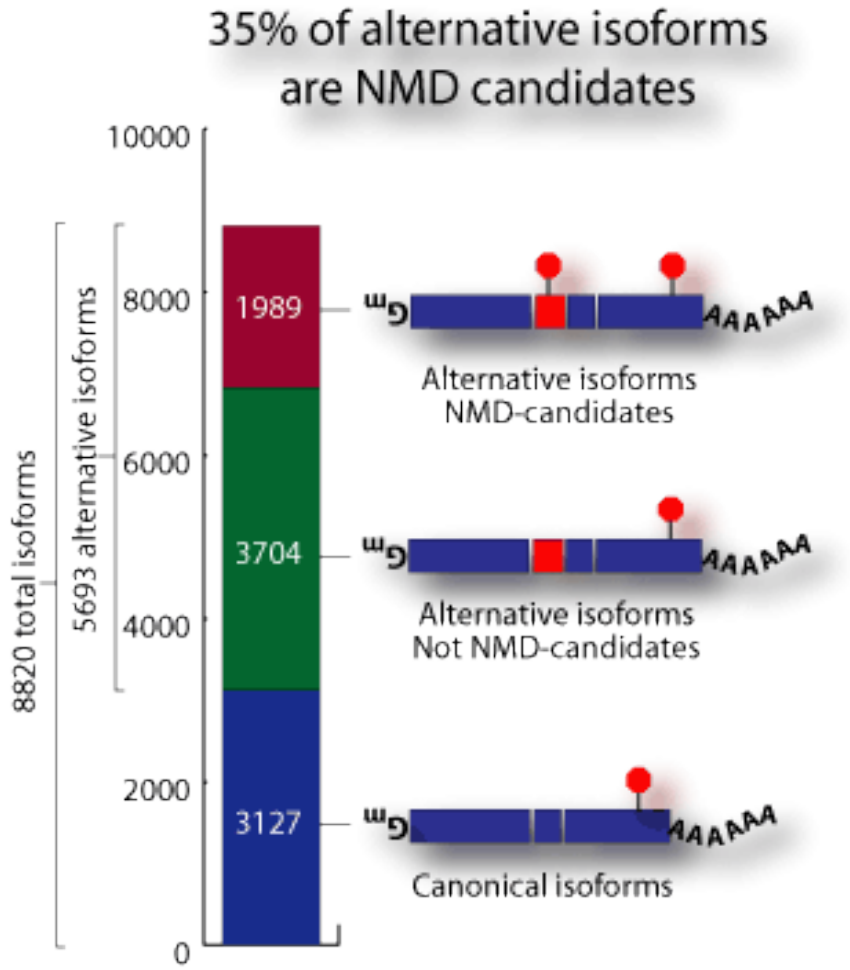


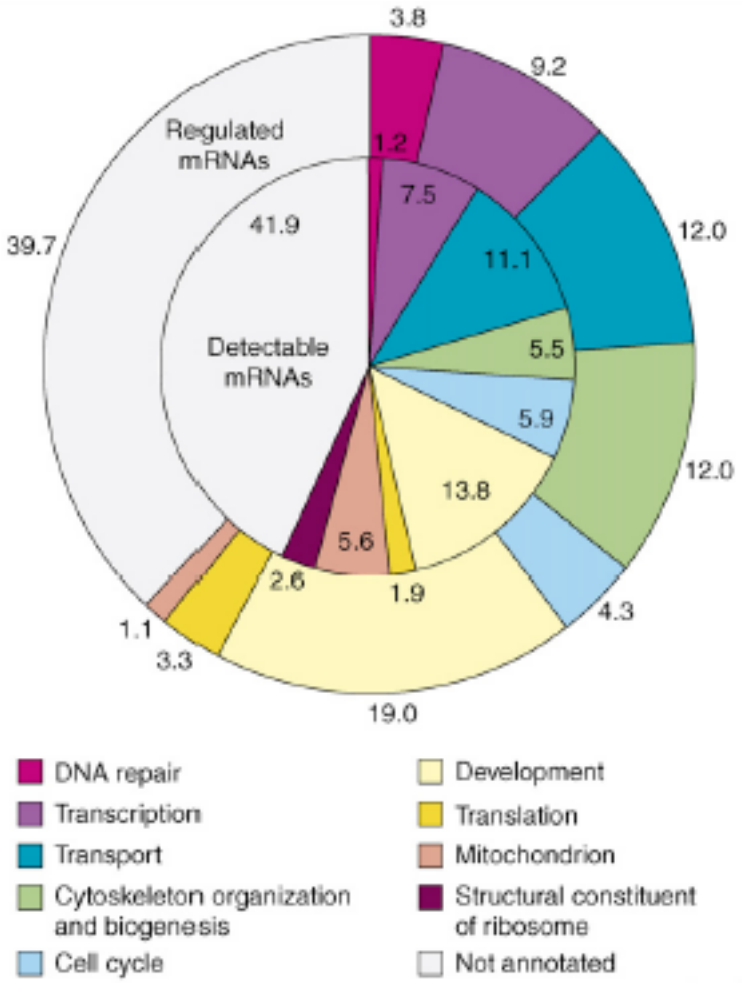
Figure 7–89. Molecular Biology of the Cell, 4th Edition.

RNA quality control in the cytoplasm: NMD



Rehwinkel, Raes, Izaurralde, 2006

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



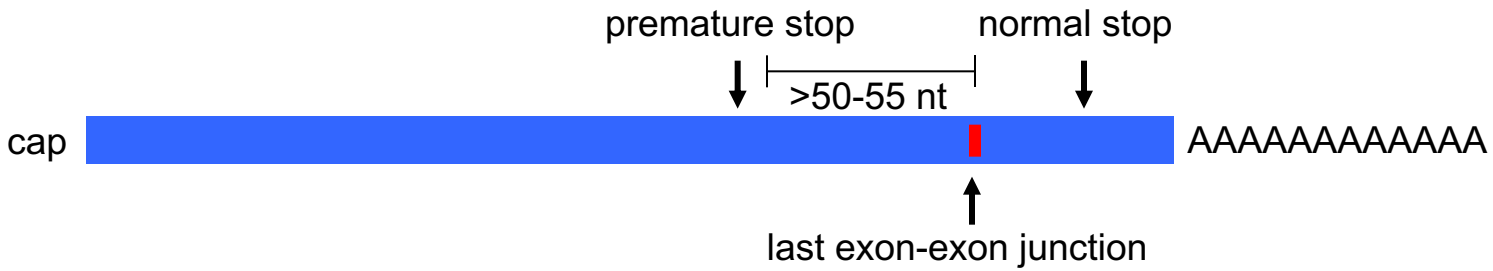
Rehwinkel, Raes, Izaurralde, 2006

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)
*Bioeas*say 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

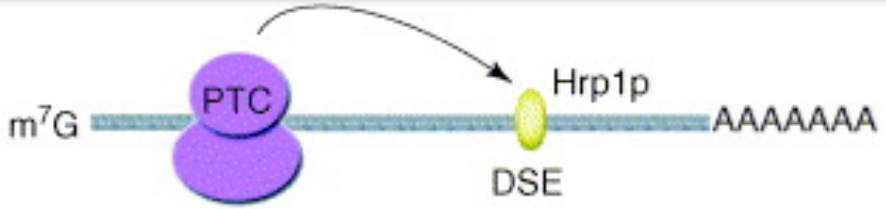
NMD = Nonsense-Mediated Decay

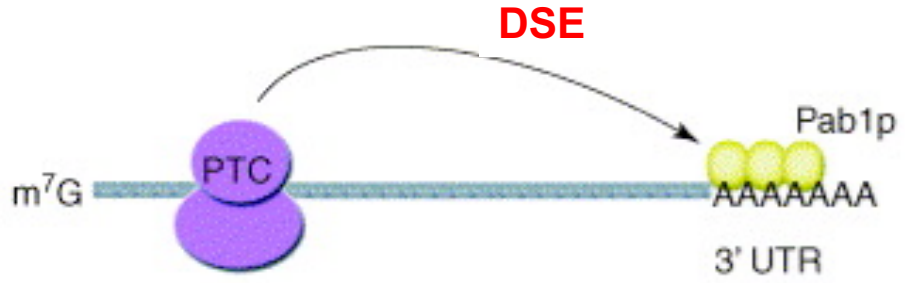
Two main steps:

1. PTC recognition
2. Initiation of mRNA degradation

PTC recognition

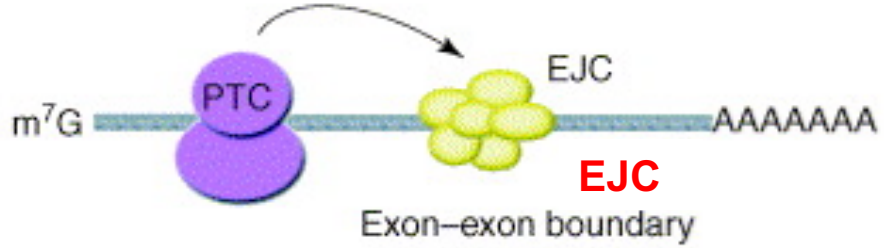
Yeast

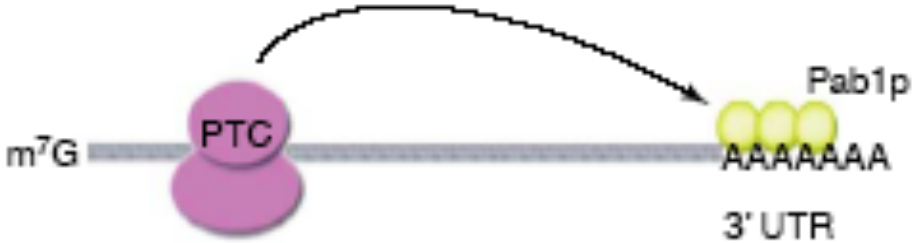




Mammals

Mammals





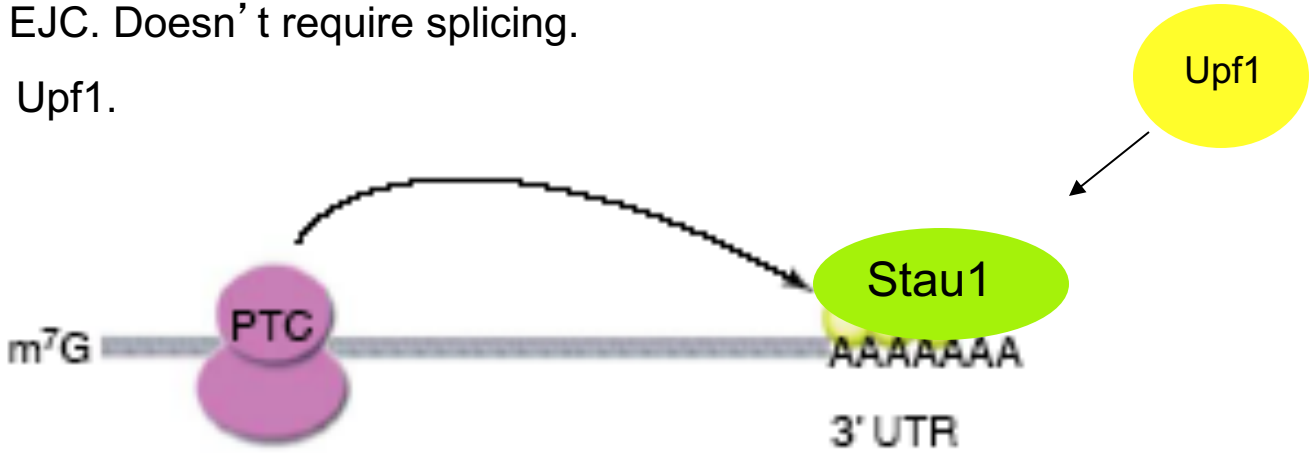
(Conti and Izaurralde, 2005)

SMD in mammals

SMD = Staufen1 (Stau1) mediated decay.

Independent of EJC. Doesn't require splicing.

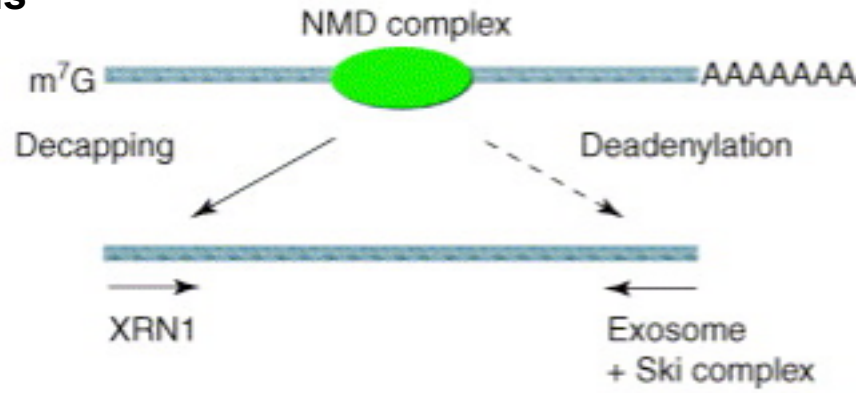
Involves Stau1, Upf1.



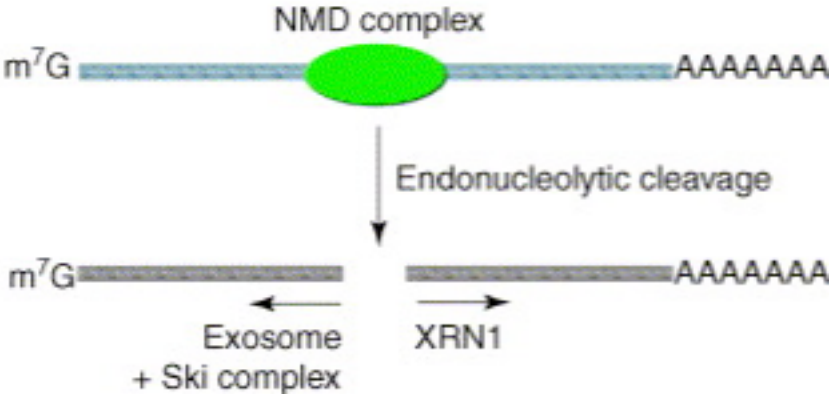
Stau1 binds to the 3' UTR of a subset of particular mRNAs

Decay of NMD targets

Yeast and mammals

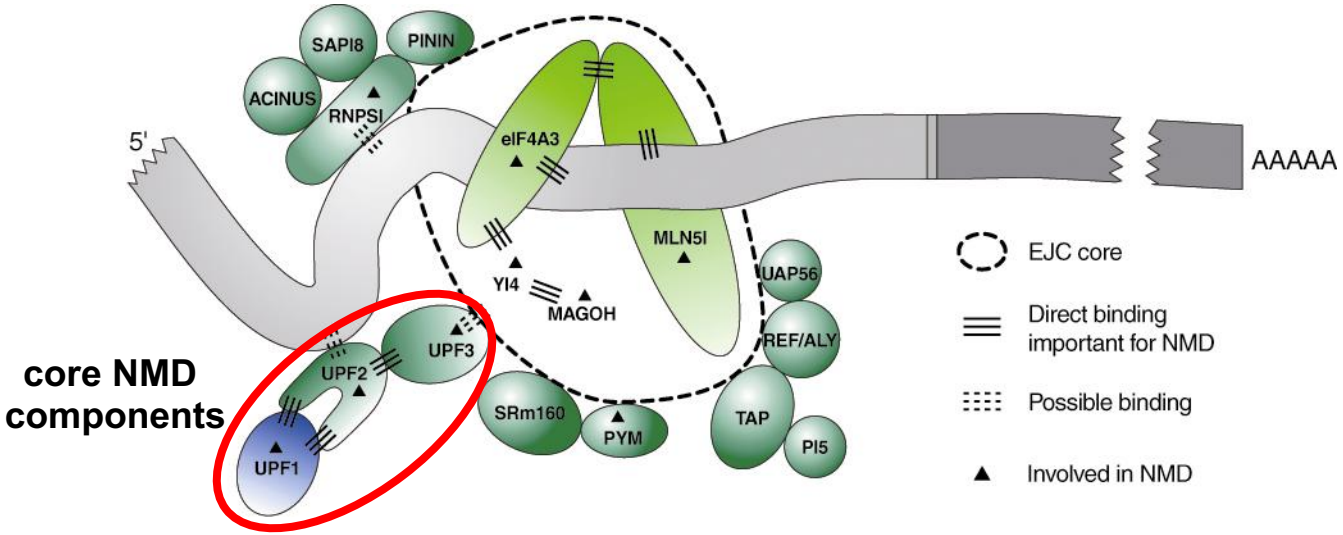


Drosophila



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

Aberrant mRNA Decay Pathways

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

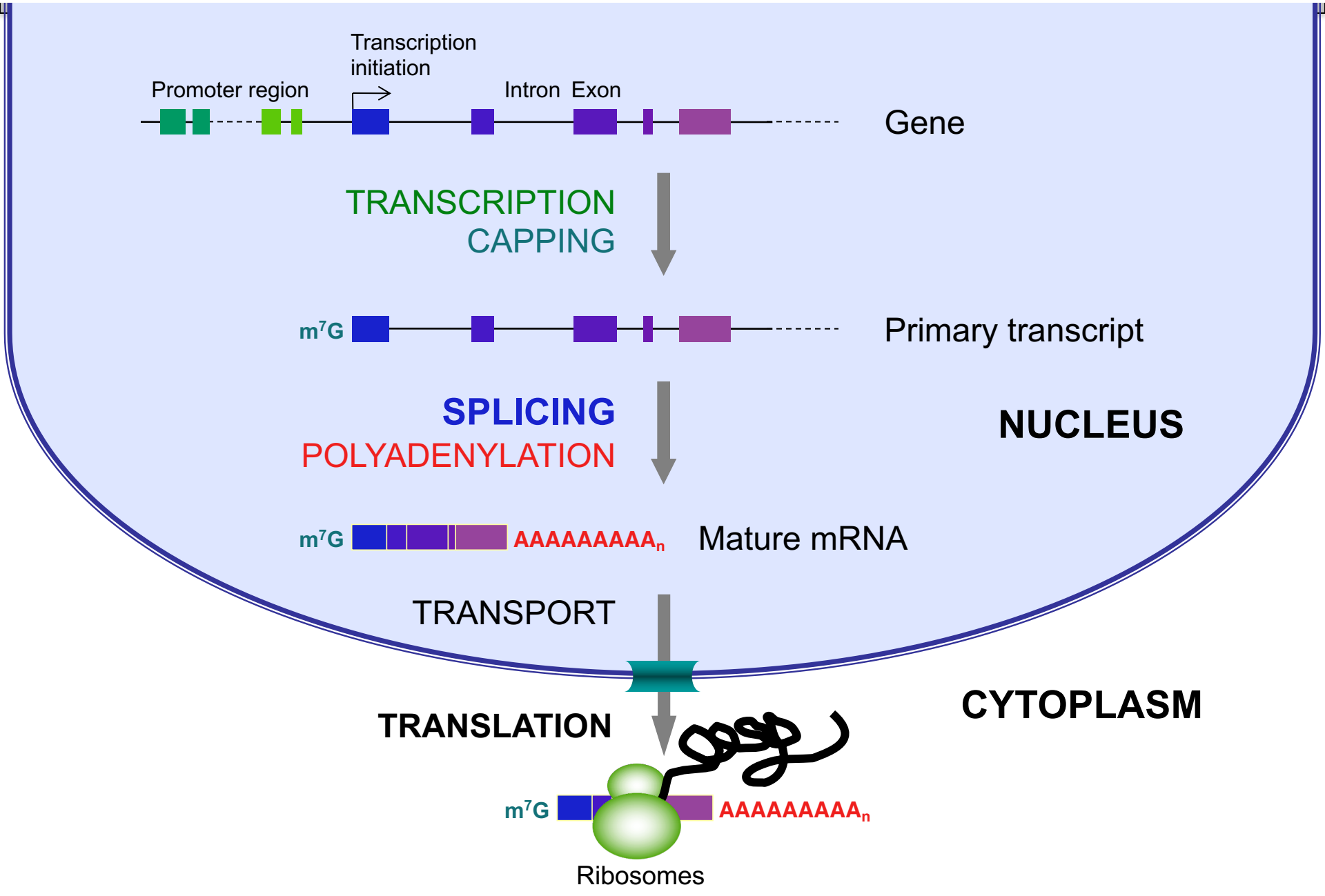
B. Nonstop mRNA decay (NSD)
- Degrades mRNAs without a stop codon

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 of noncoding RNAs

All steps of mRNA production are controlled



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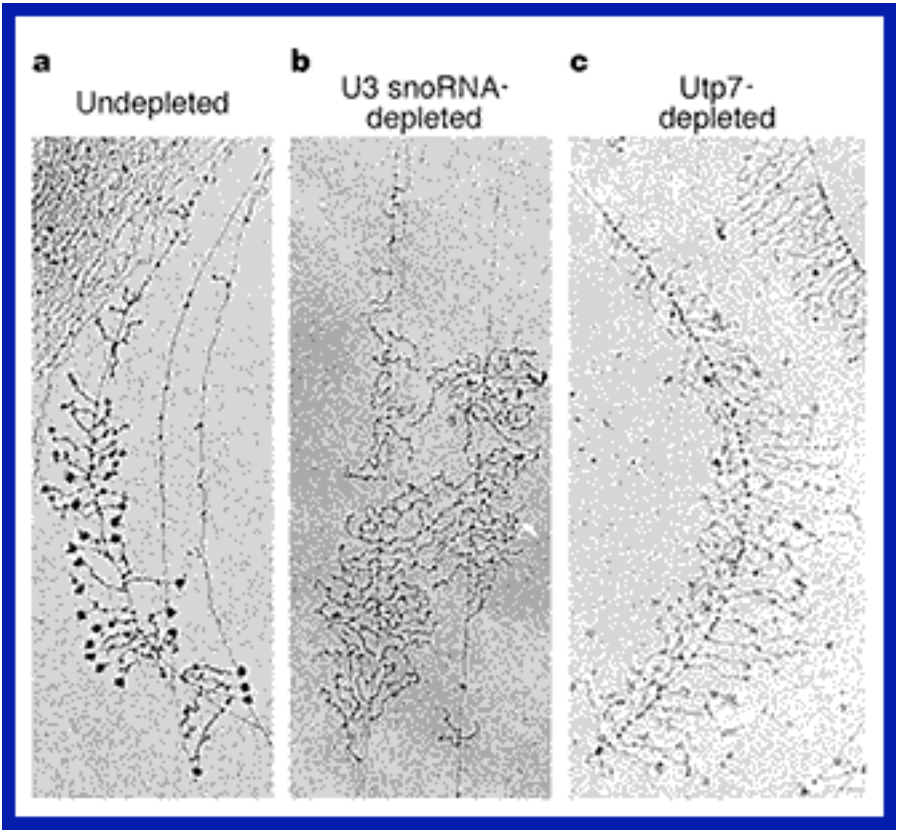
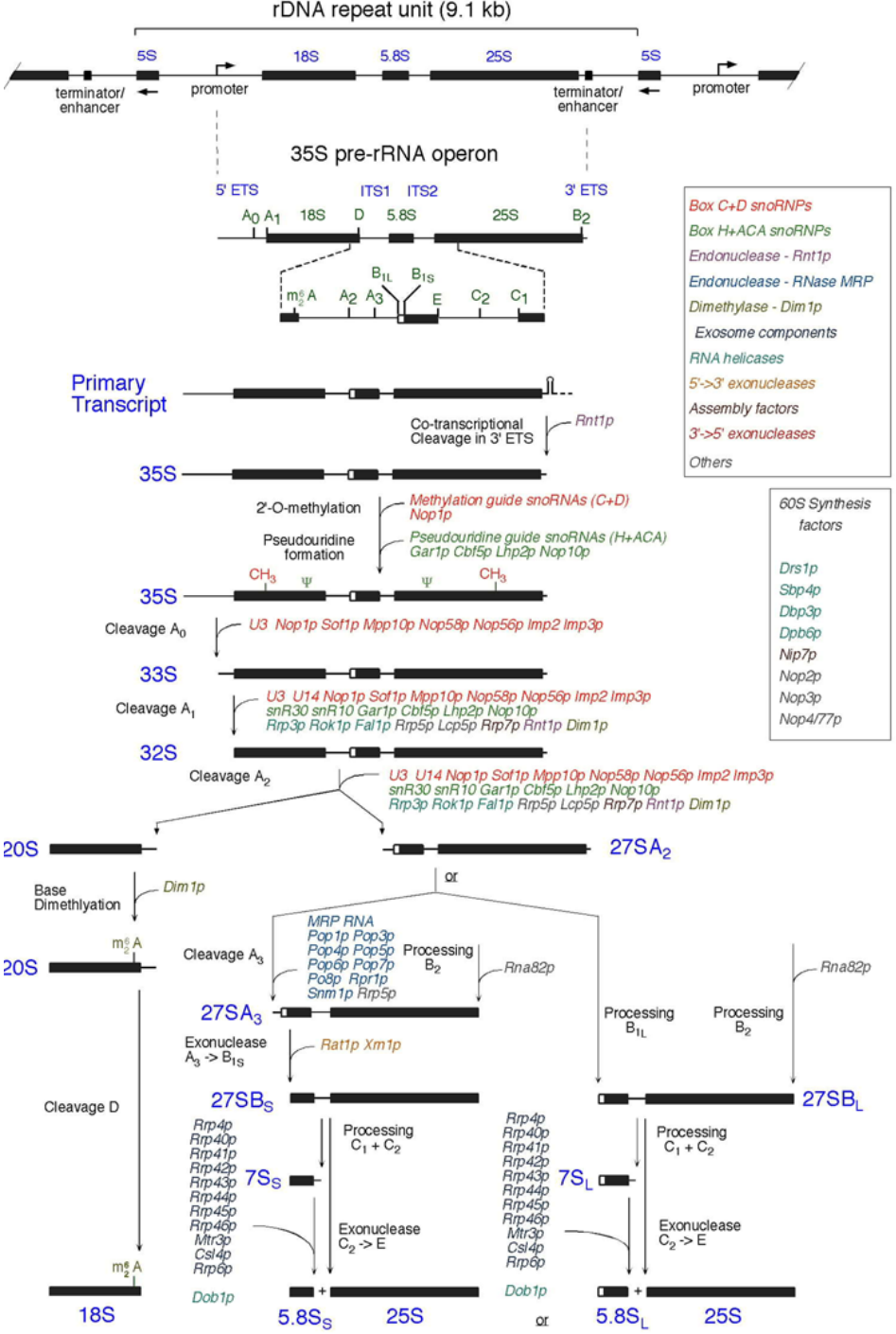
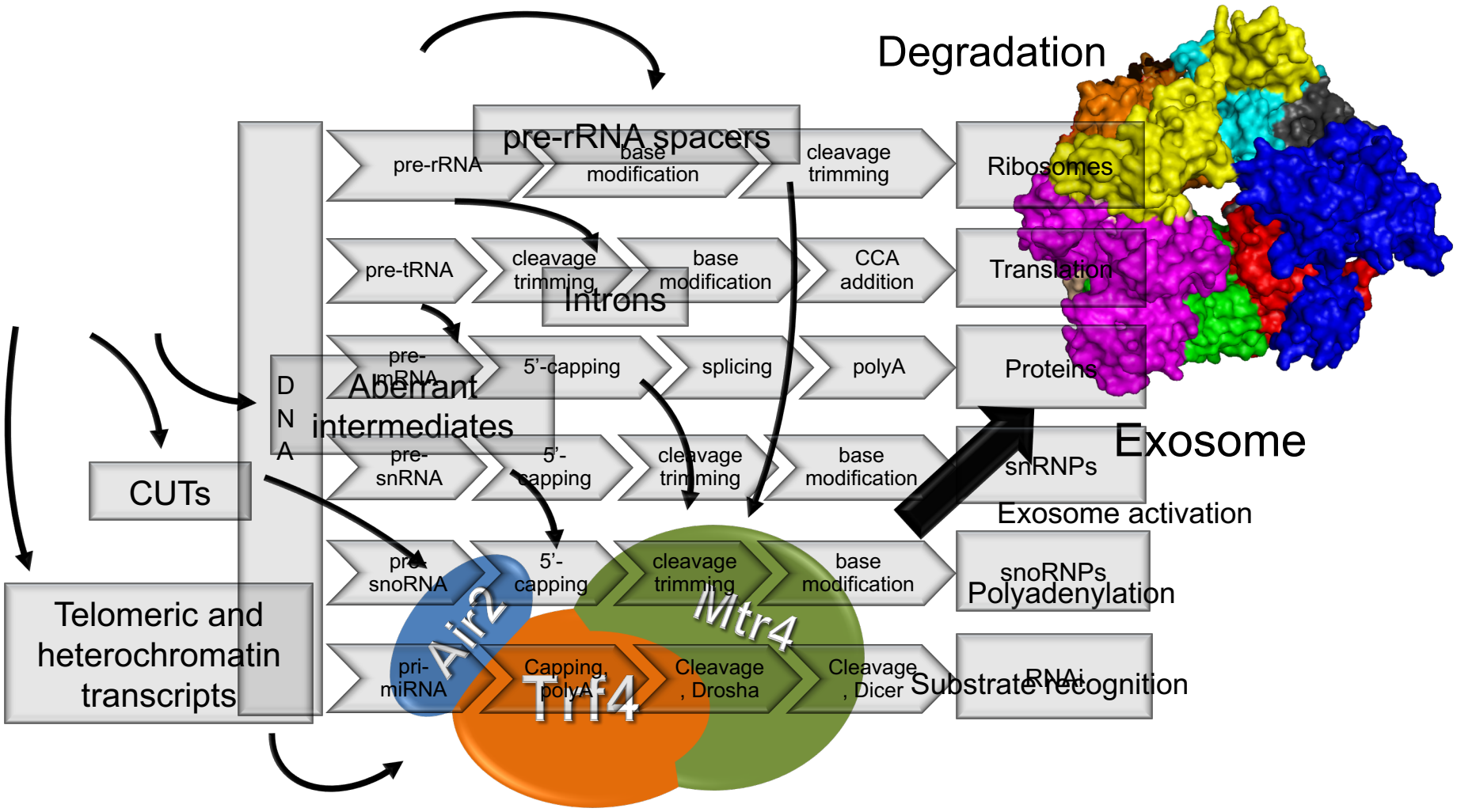
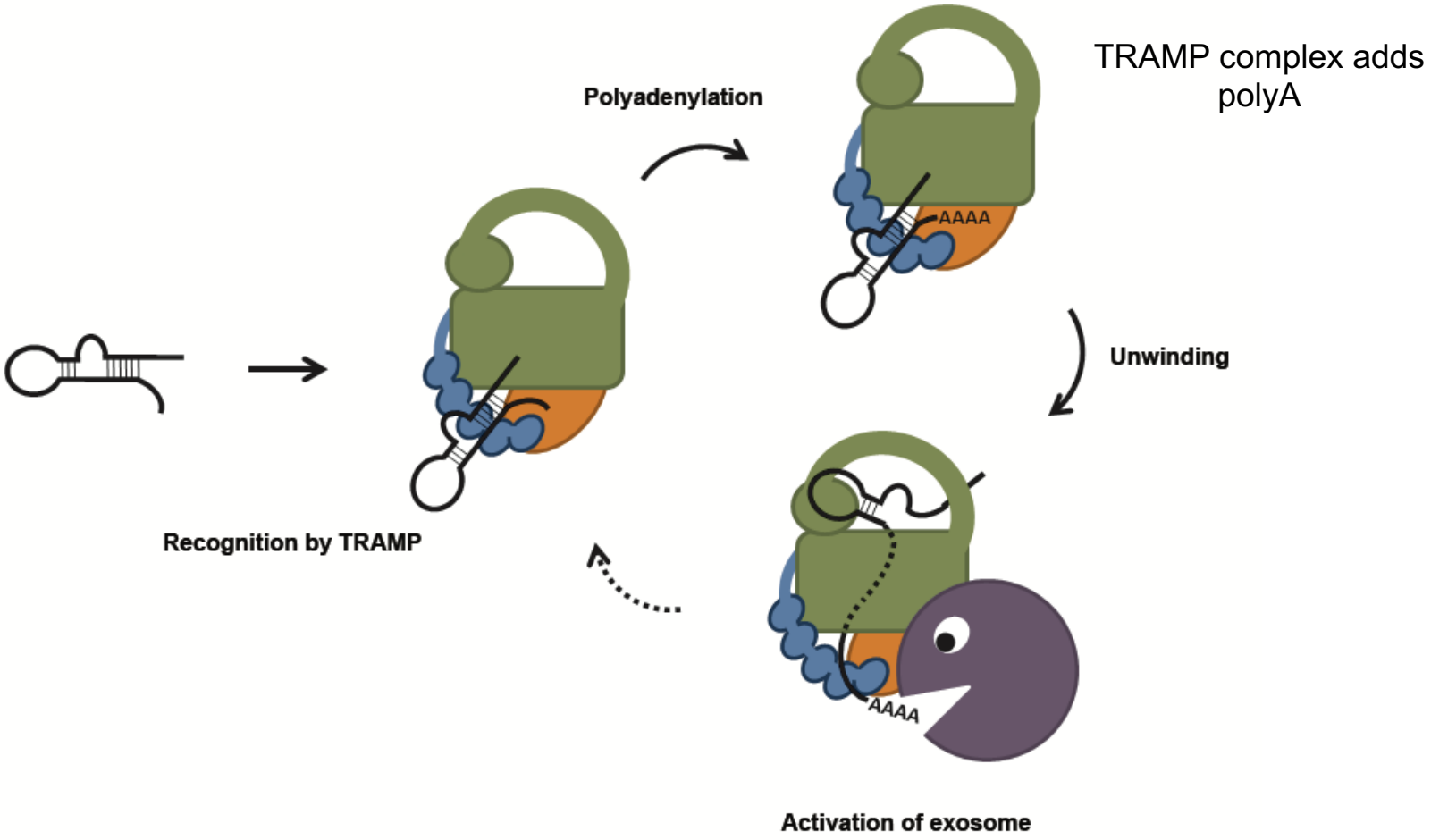




Nuclear RNA surveillance of noncoding RNAs



Polyadenylation mediates surveillance of ncRNAs in the nucleus

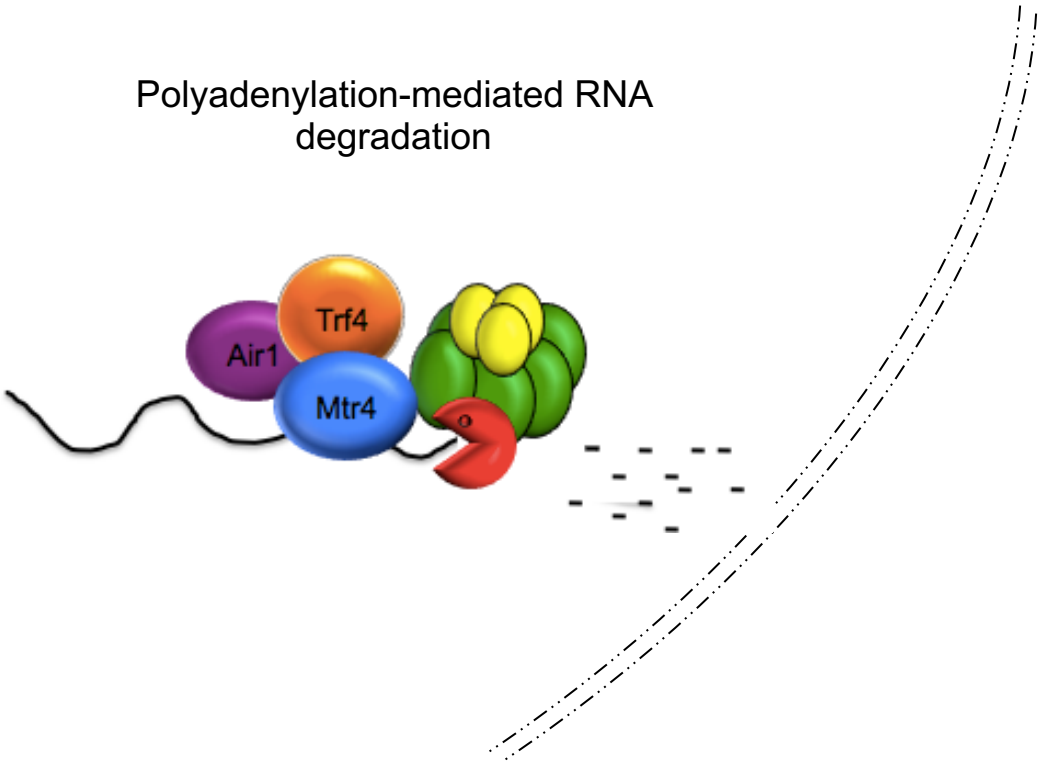


Nuclear and cytoplasmic RNA surveillance

NUCLEUS

CYTOPLASM

Polyadenylation-mediated RNA degradation



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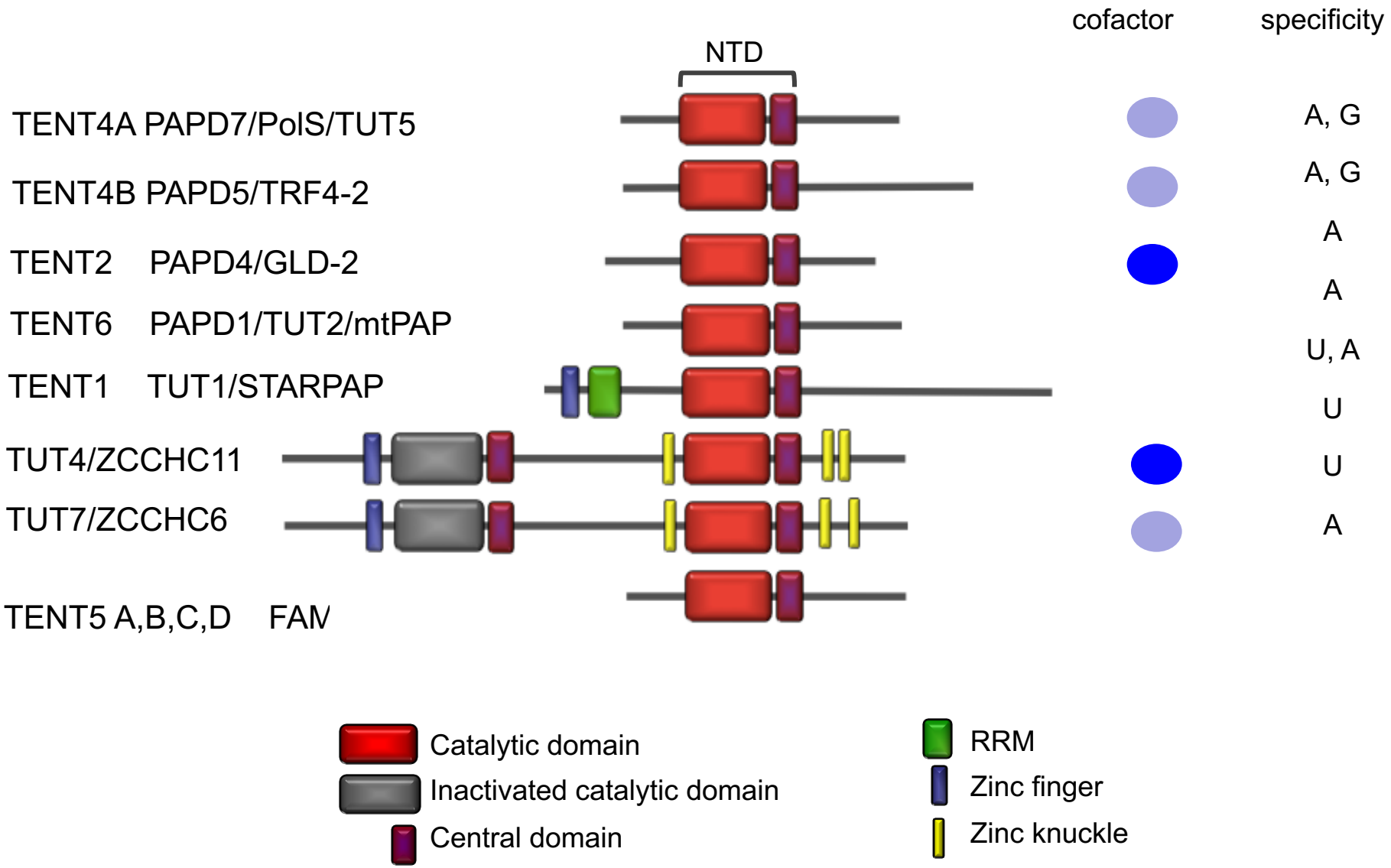
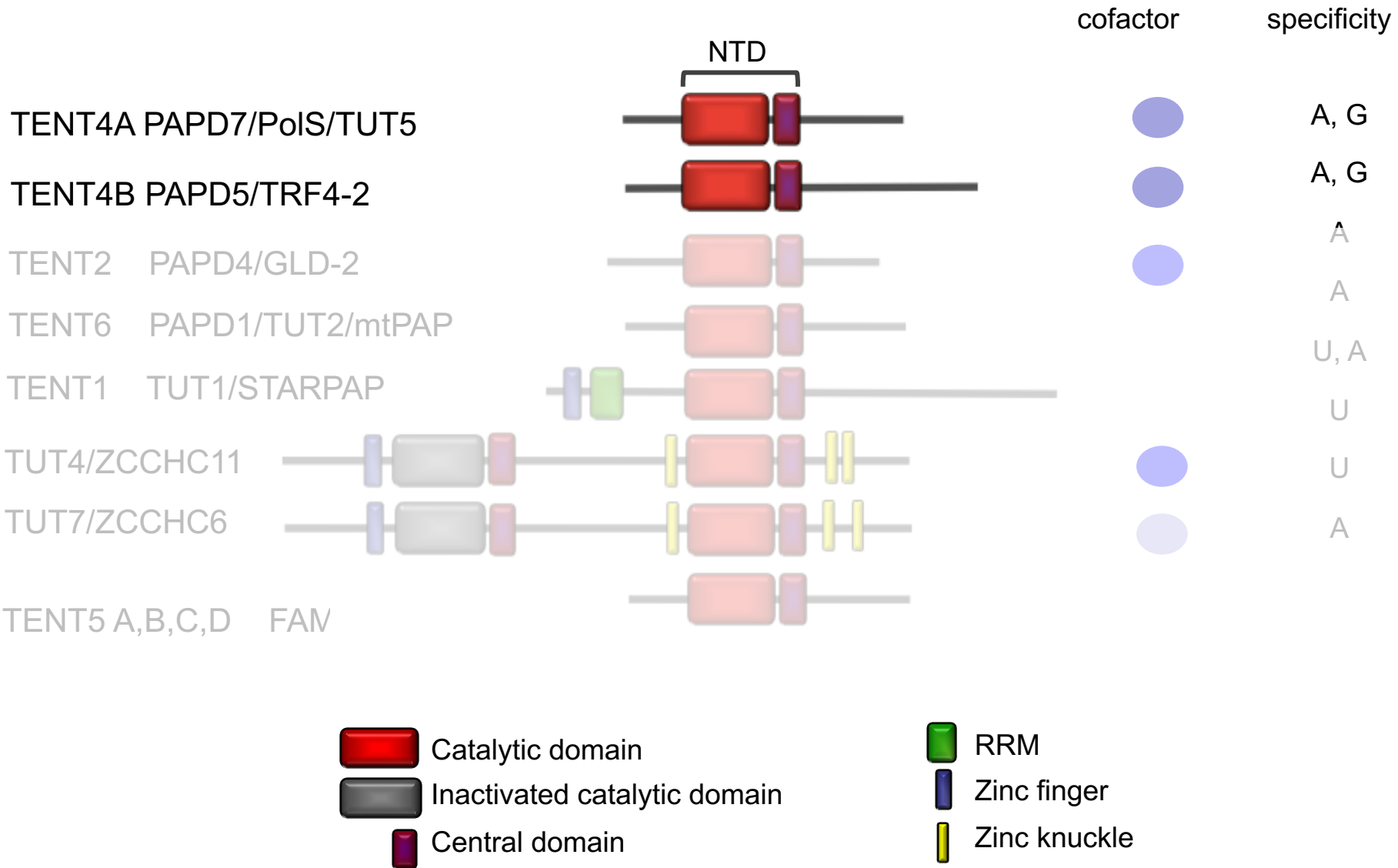
TRAMP + DIS3-EXOSOME

Vanacova et al., 2005, La Cava et al., 2005, Wyers et al., 2005

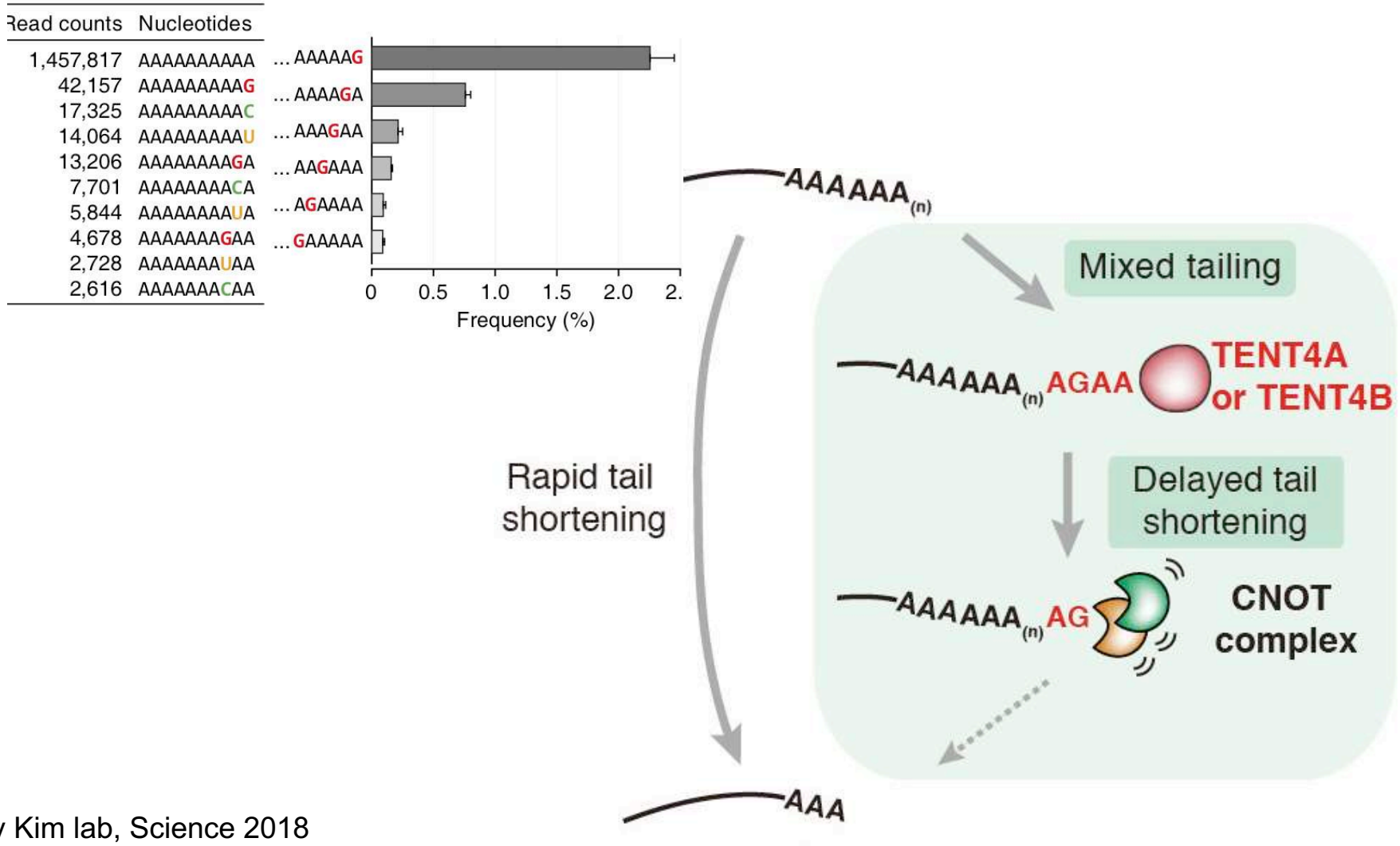
Mammalian TErminal NucleoTidyltransferases



Mammalian TErminal NucleoTidyltransferases

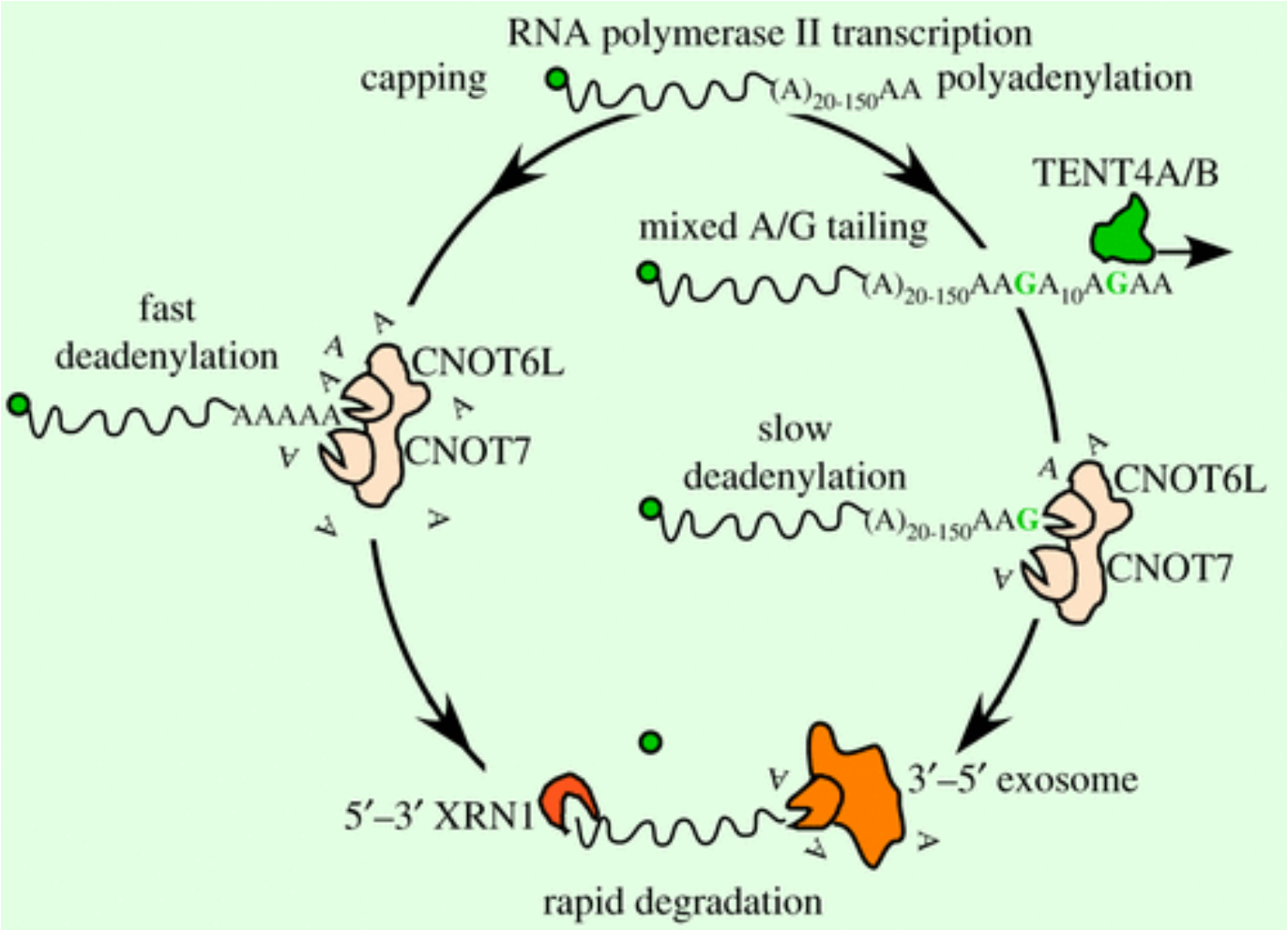


Mixed A/G tailing by TENT4A/B stabilizes mRNAs



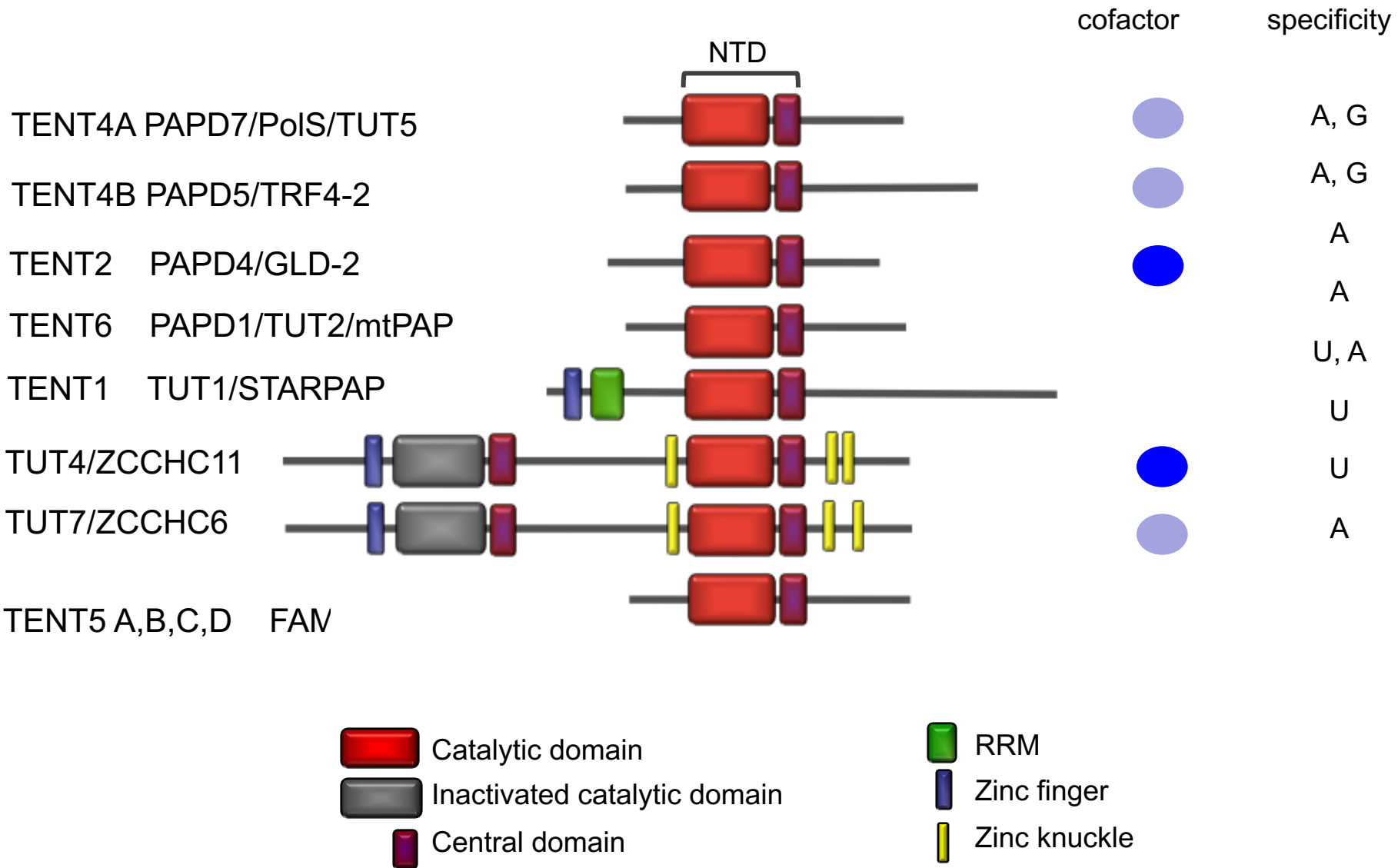
Narry Kim lab, Science 2018

Mixed A/G tailing by TENT4A/B stabilizes mRNAs

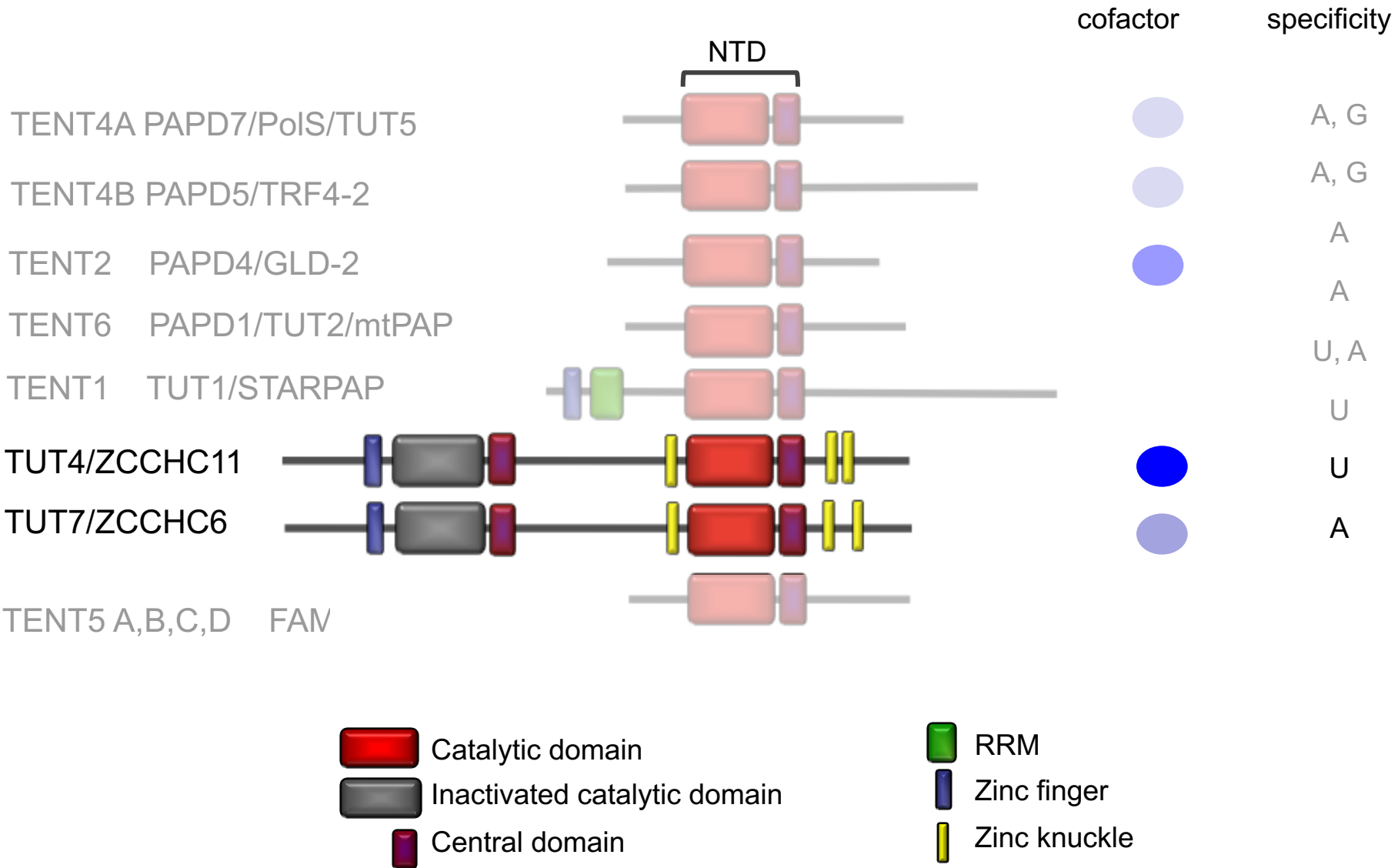


Terminal nucleotidyl transferases (TENTs) in mammalian RNA metabolism, Volume: 373, Issue: 1762, DOI: (10.1098/rstb.2018.0162)

Mammalian TErminal NucleoTidyltransferases



Mammalian TENTs



TUT4 & TUT7

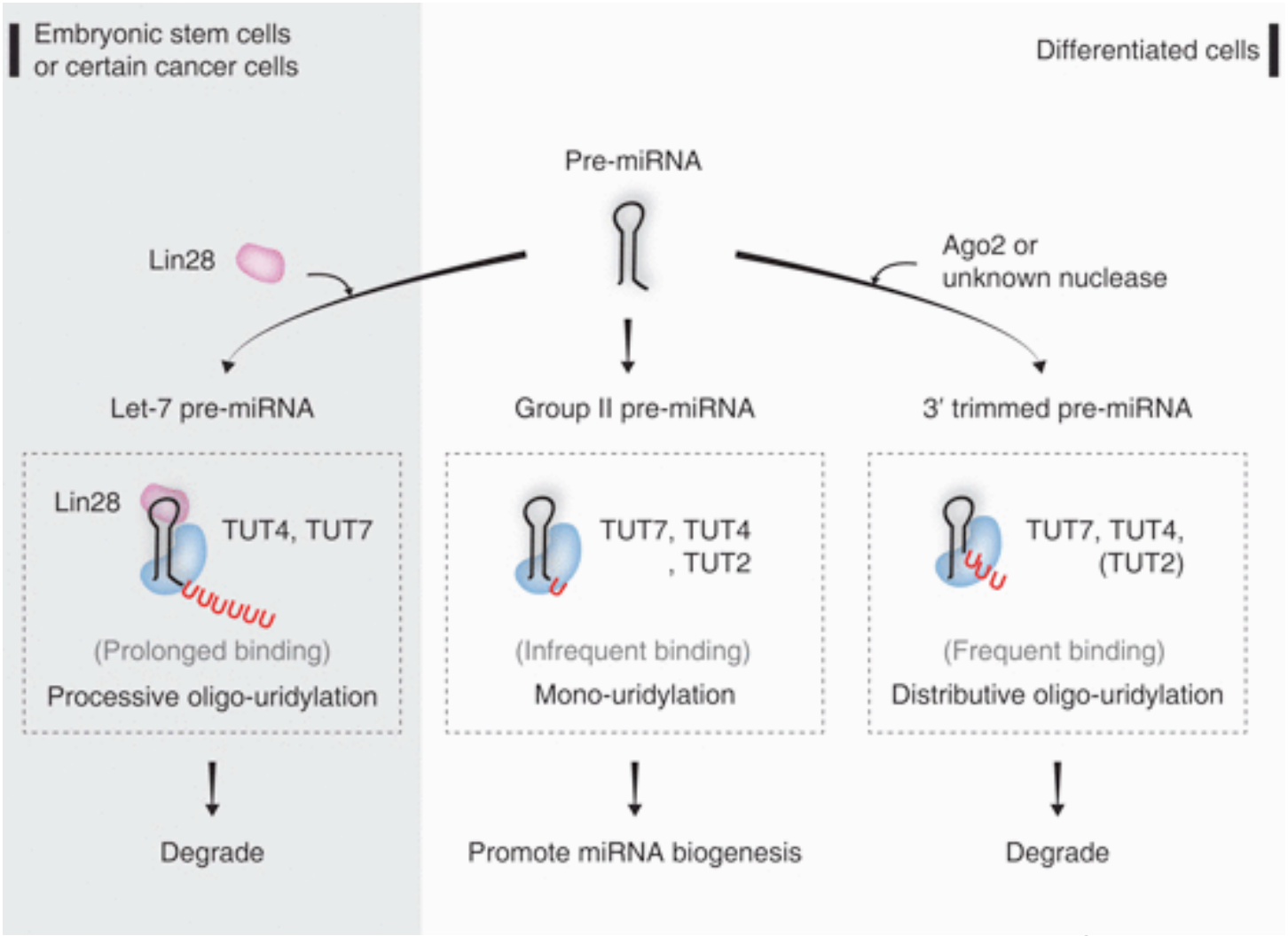
The most extensively studied protein from the whole family
Multiple diverse roles
Multiple targets: mRNA, ncRNAs
Act in both: RNA processing and degradation

Essential for:

- Germline development
- Differentiation
- Viral infection
- Stress response
- Apoptosis
- Cancer progression
- Inhibition of retrotransposition
- ….

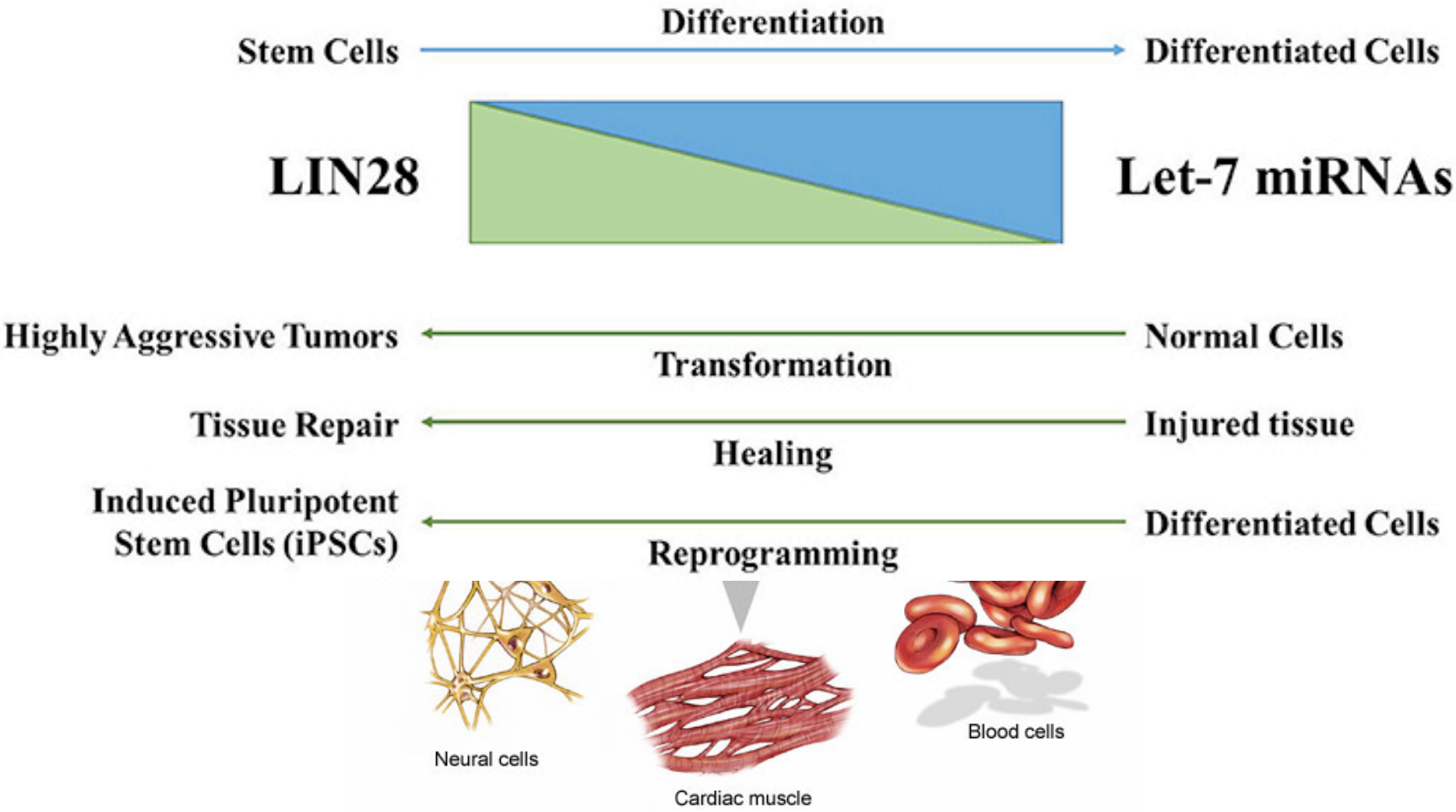
TUT4/7 in noncoding RNA metabolism

Uridylation of miRNA precursors can either stimulate processing or trigger RNA degradation.

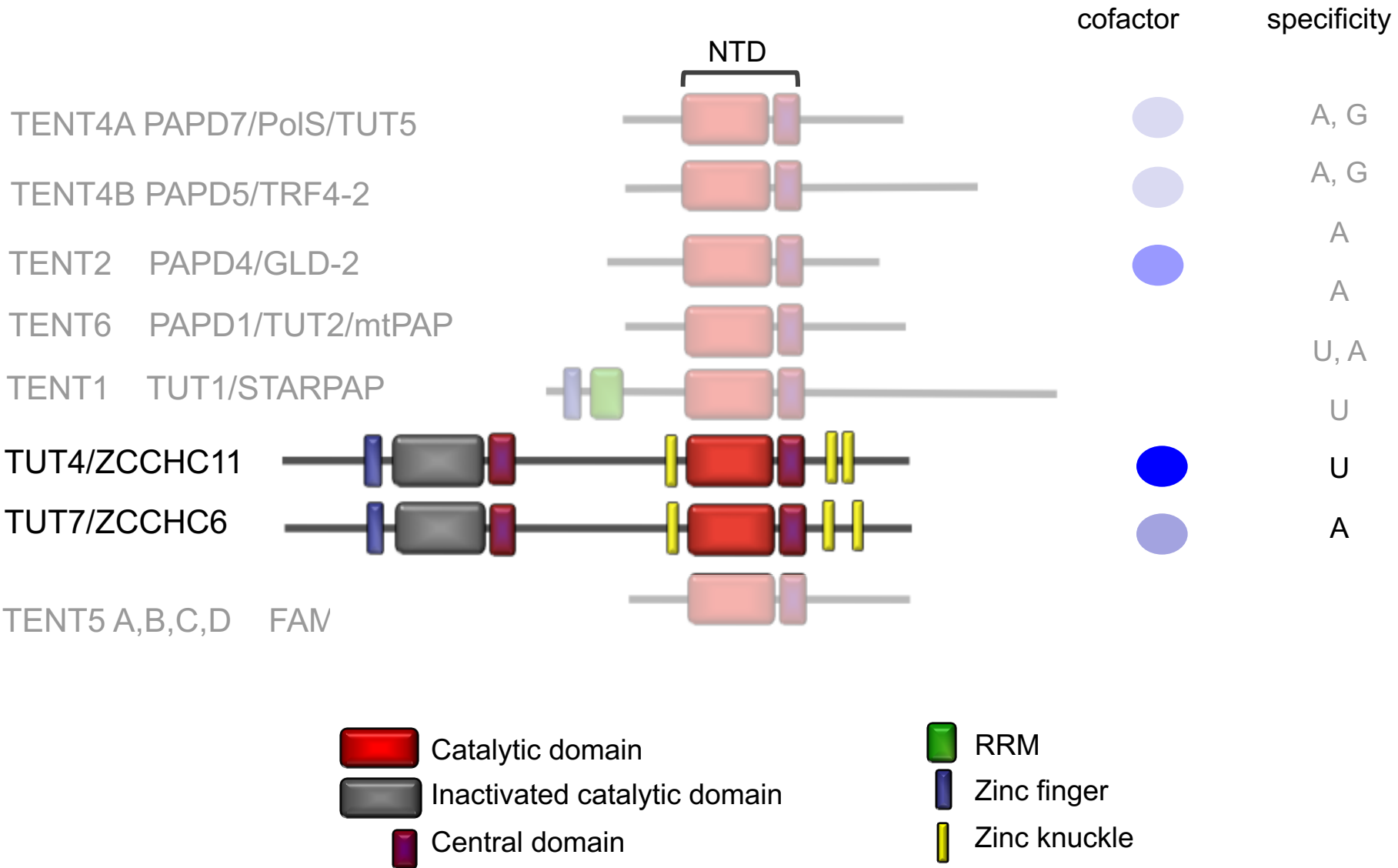


Narry Kim, EMBOJ 2014

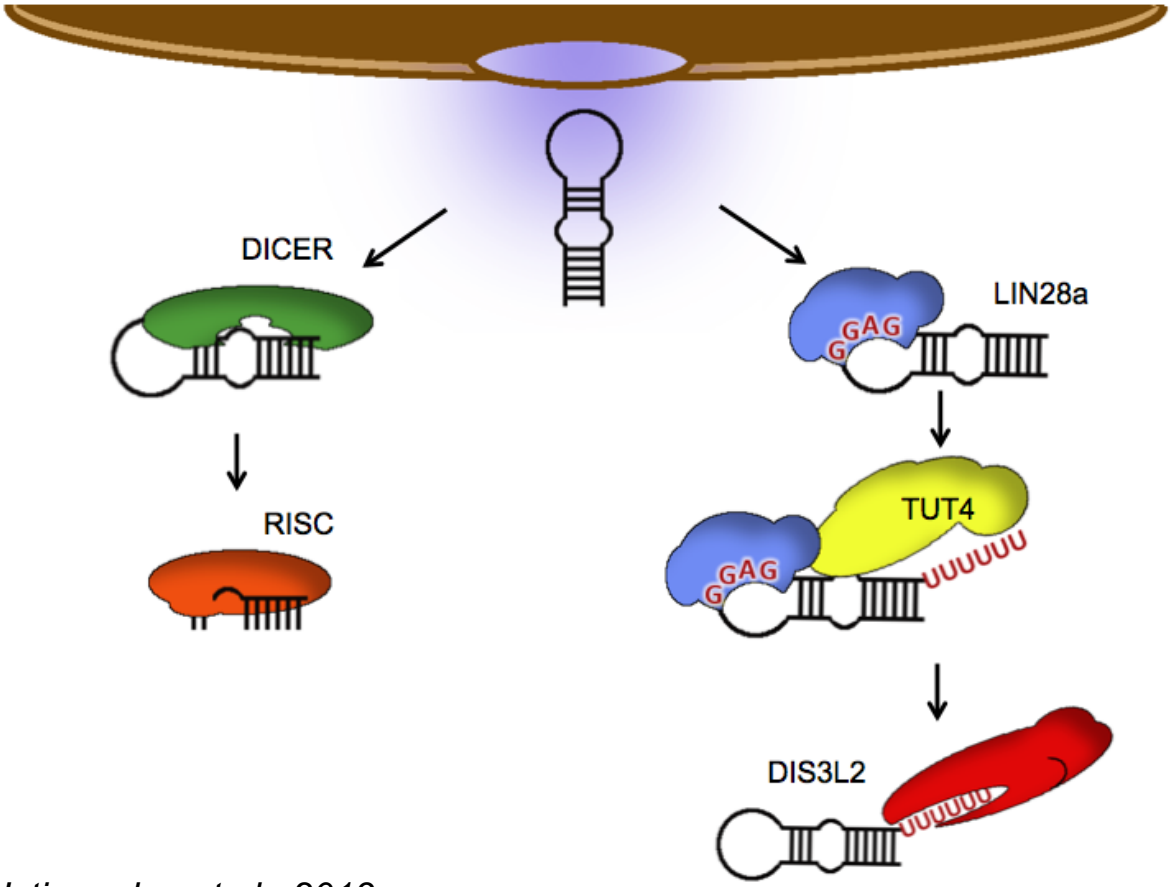
The role of let-7 in differentiation



Mammalian TENTs



DIS3L2 targets uridylated precursors of let-7 miRNA



Chang et al., 2013, Ustianenko et al., 2013

DIS3L2 in cancer and dissease

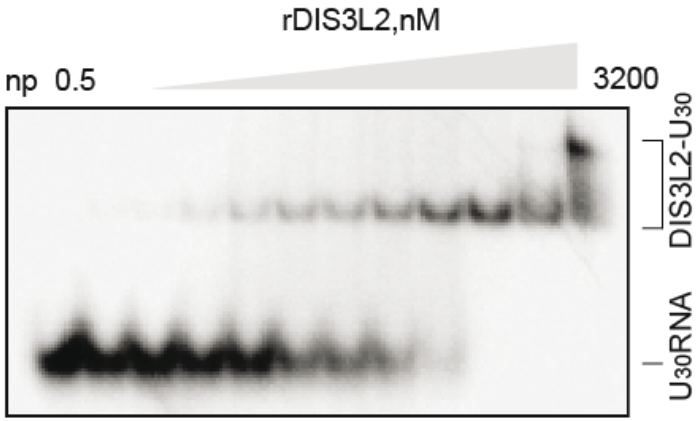
nature genetics 2012

Germline mutations in *DIS3L2* cause the Perlman syndrome of overgrowth and Wilms tumor susceptibility

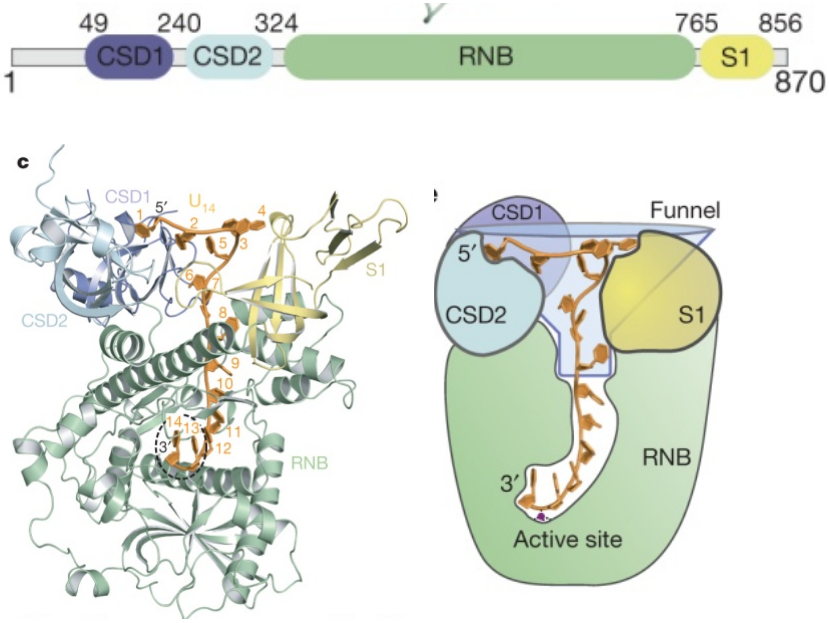
Dewi Astuti[1,9], Mark R Morris[1,2,9], Wendy N Cooper[1], Raymond H J Staals[3], Naomi C Wake[1], Graham A Fews[4], Harmeet Gill[1], Dean Gentle[1], Salwati Shuib[1], Christopher J Ricketts[1], Trevor Cole[4], Anthonie J van Essen[5], Richard A van Lingen[6], Giovanni Neri[7], John M Opitz[8], Patrick Rump[5], Irene Stolte-Dijkstra[5], Ferenc Müller[1], Ger J M Pruijn[3], Farida Latif[1] & Eamonn R Maher[1,4]

DIS3L2 oligo(U) specificity

EMSA assay

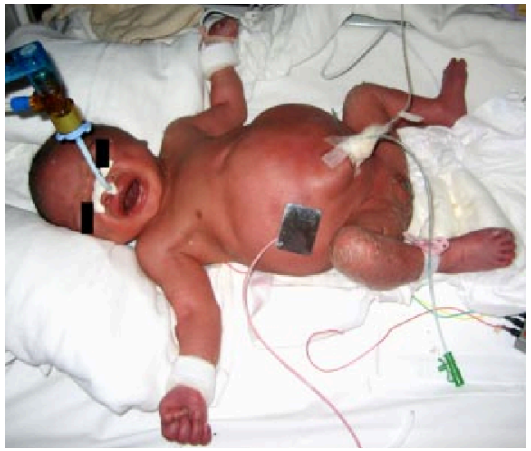


Ustianenko et al., 2013



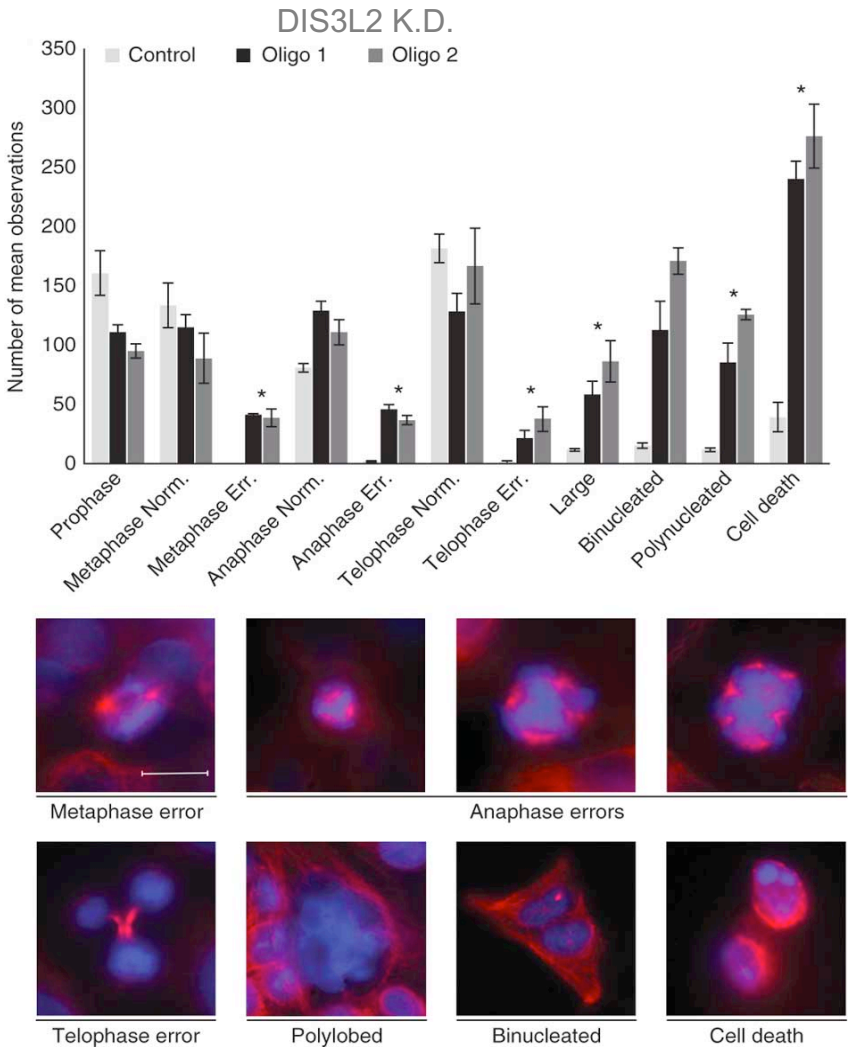
Faehnle *et al.*, 2014

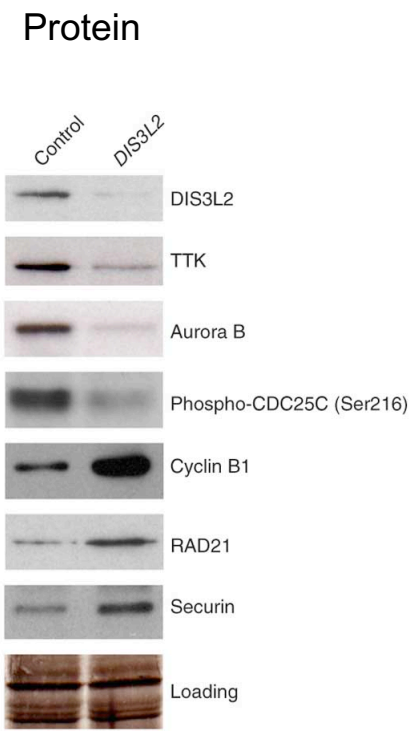
Perlman syndrome

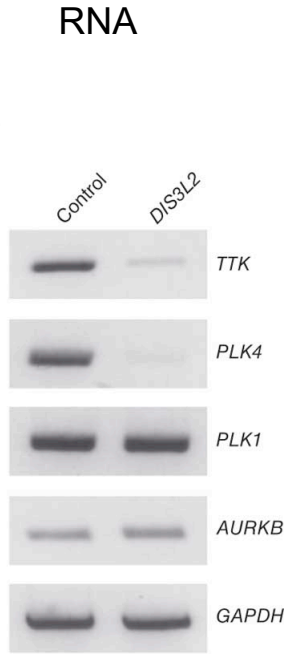


- association with DIS3L2 mutation
- *(Astuti et al., Nature Genetics, 2012)*
- rare genetic disorder
- fetal overgrowth
- developmental delay
- kidney abnormalities
- high risk of bilateral tumors and Wilms' tumor – a rare kidney cancer
- poor prognosis, high neonatal mortality
- link between DIS3L2 dysfunction and a disease phenotype remains unknown

DIS3L2 knock down in somatic cells results in cell cycle defects



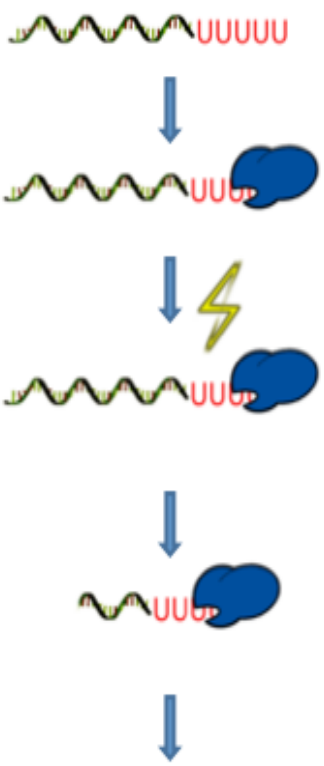




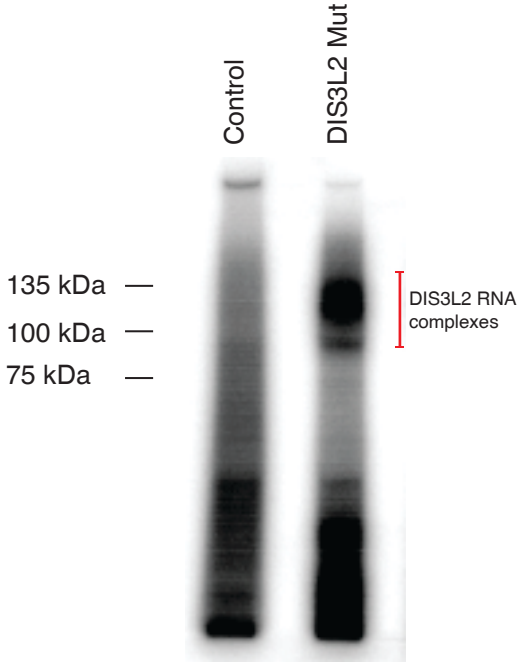
Astuti et al. Nature 2012

DIS3L2 bound RNAs contain 3’ uridylyl residues

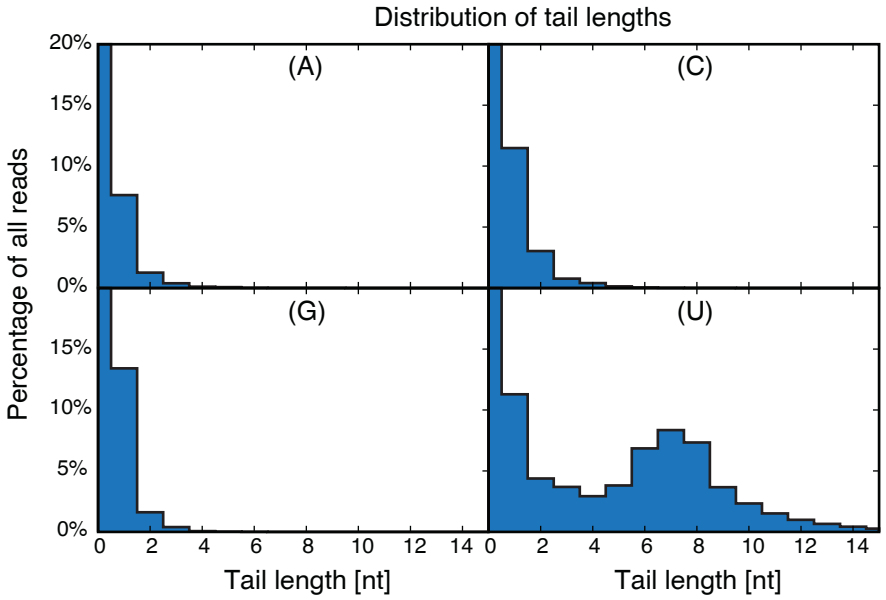
In vivo crosslinking



Protein-RNA complexes



Reads containing homogeneous oligo(N) tails

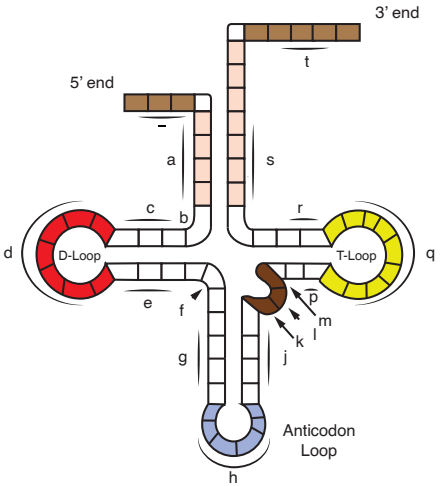


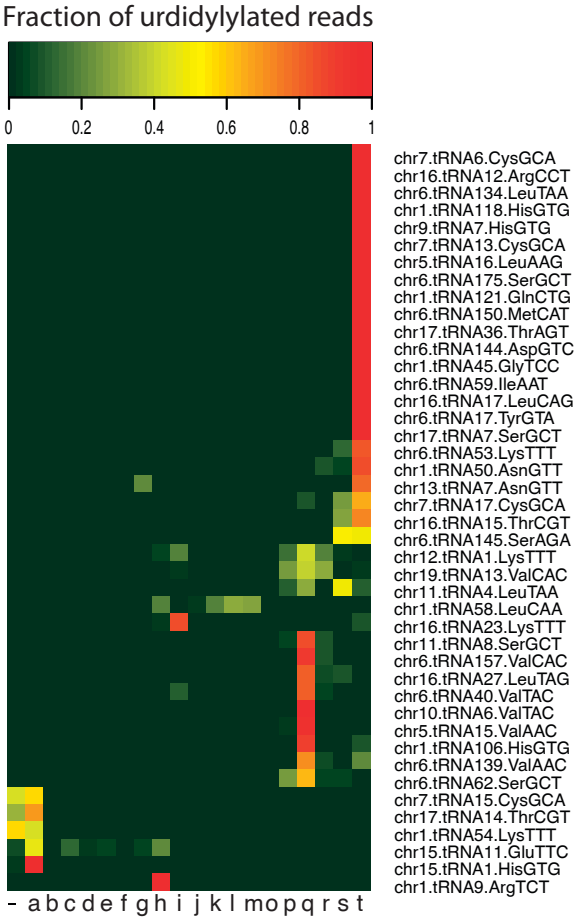
Ustianenko et al., EMBO J 2016

DIS3L2 targets miRNAs and tRNAs

miRNA	Reads
hsa-let-7f-1	23331
hsa-let-7g	9646
hsa-mir-98	9012
hsa-let-7b	6379
hsa-let-7i	6060
hsa-mir-484	1939
hsa-mir-320a	1846
hsa-let-7d	1078
hsa-let-7f-2	573
hsa-mir-6821	395
hsa-mir-4521	310
hsa-let-7e	261
hsa-mir-4741	119
hsa-mir-18b	99

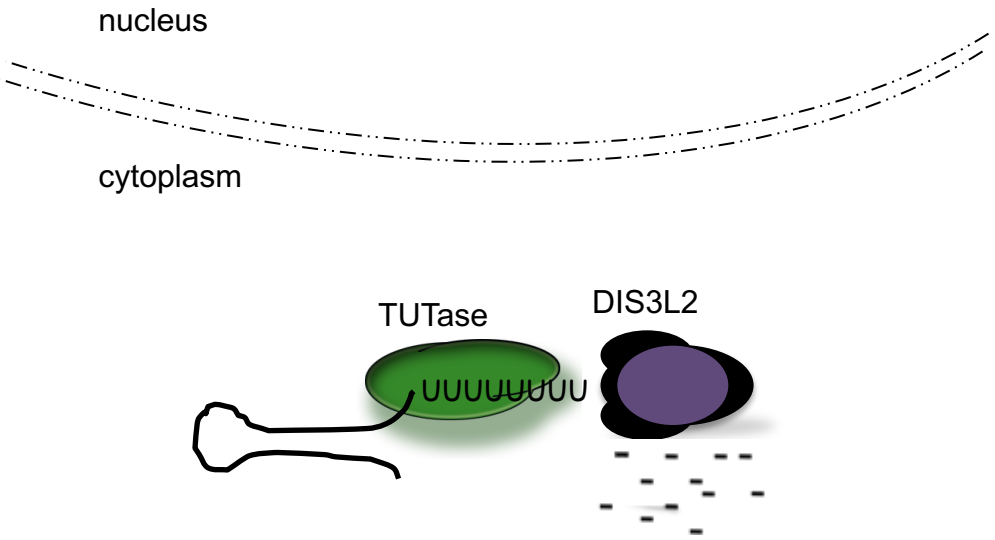
tRNA scheme





Ustianenko et al., EMBO J 2016

TDS= TUT-DIS3L2 SURVEILLANCE

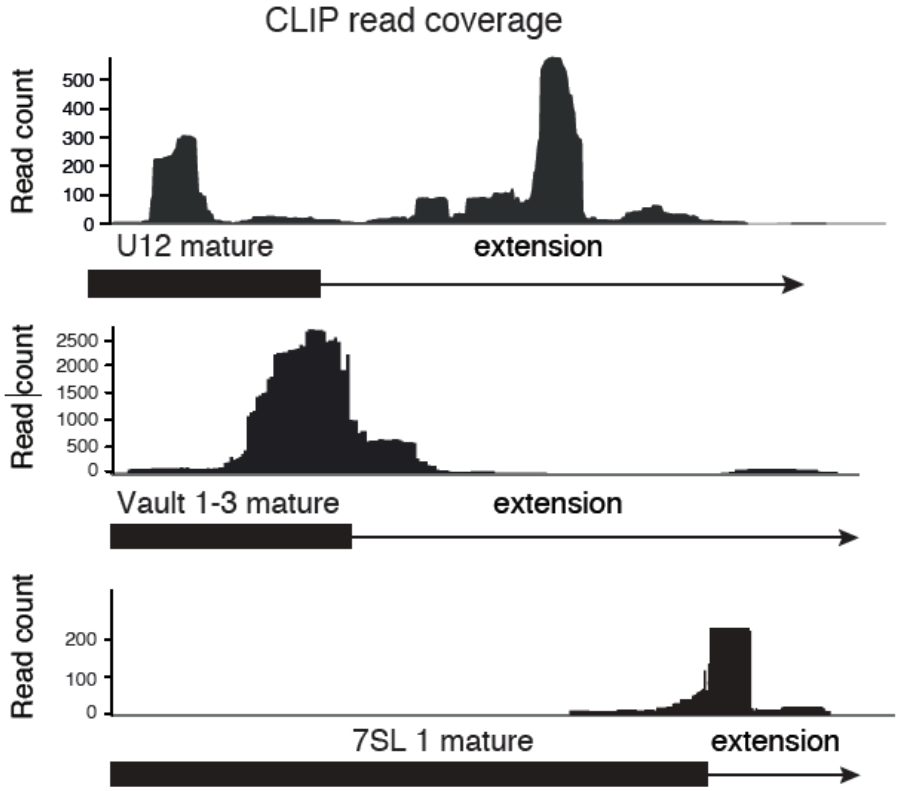


TDS targets:

- Highly structured aberrant RNAs

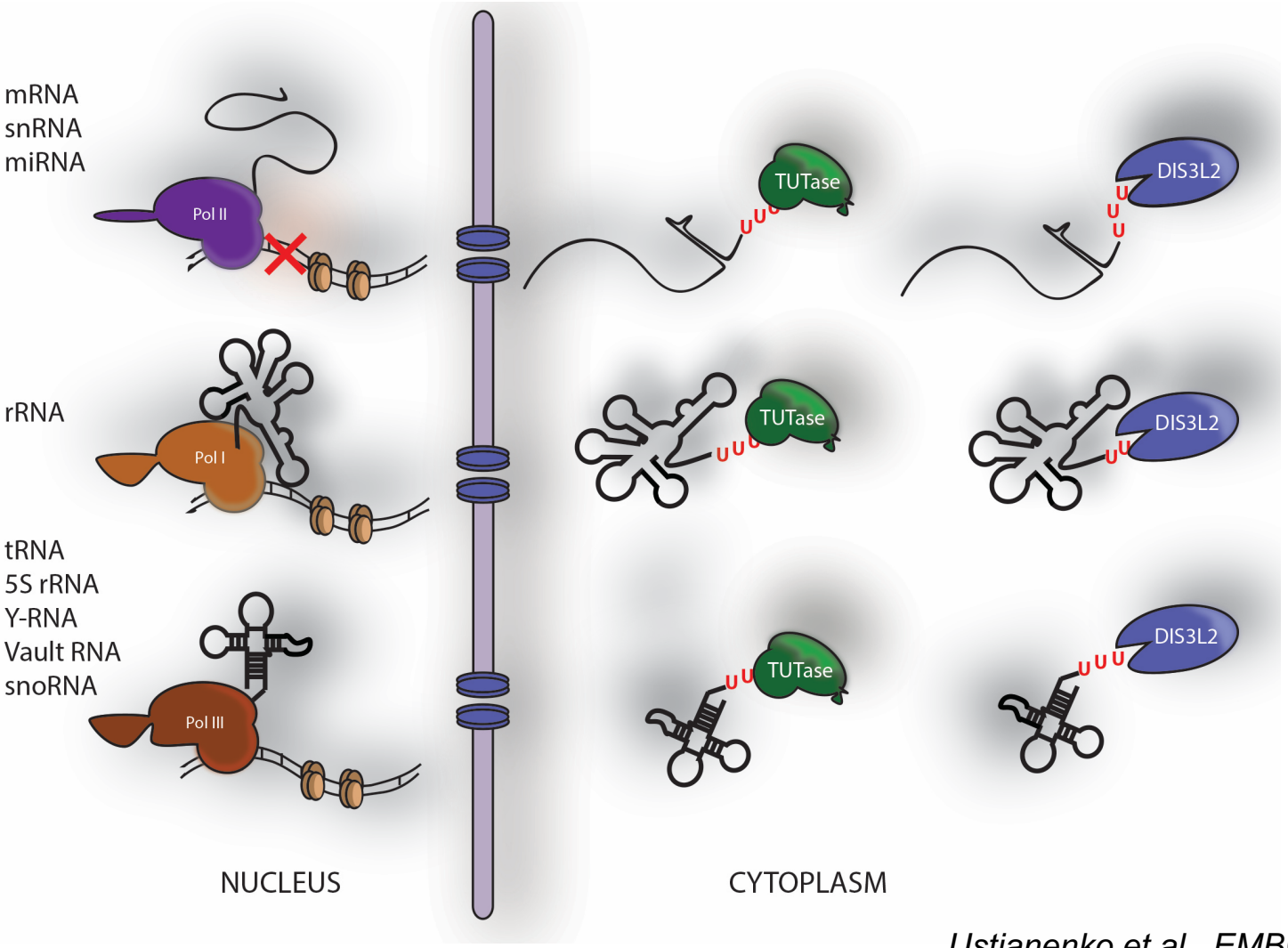
DIS3L2 targets aberrant forms of numerous highly structured ncRNAs and transcripts from pseudogenes

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 summary



Ustianenko et al., EMBO J 2016

TUT-DIS3L2 cytoplasmic RNA surveillance is a conserved mechanism

Dis3l2-Mediated Decay Is a Quality Control Pathway for Noncoding RNAs.

Pirouz M, Du P, Munafò M, Gregory RI.

Cell Rep. 2016 Aug 16;16(7):1861-73. doi: 10.1016/j.celrep.2016.07.025. Epub 2016 Aug 4.

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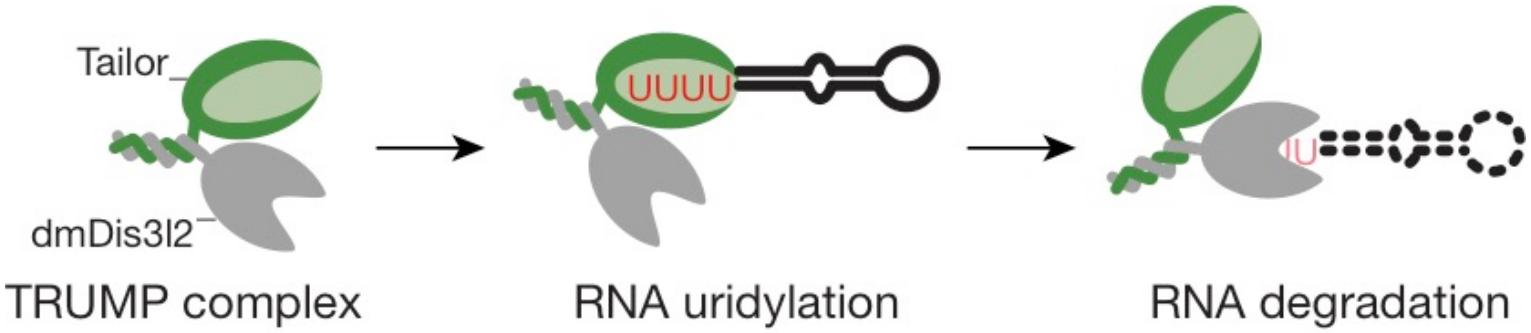


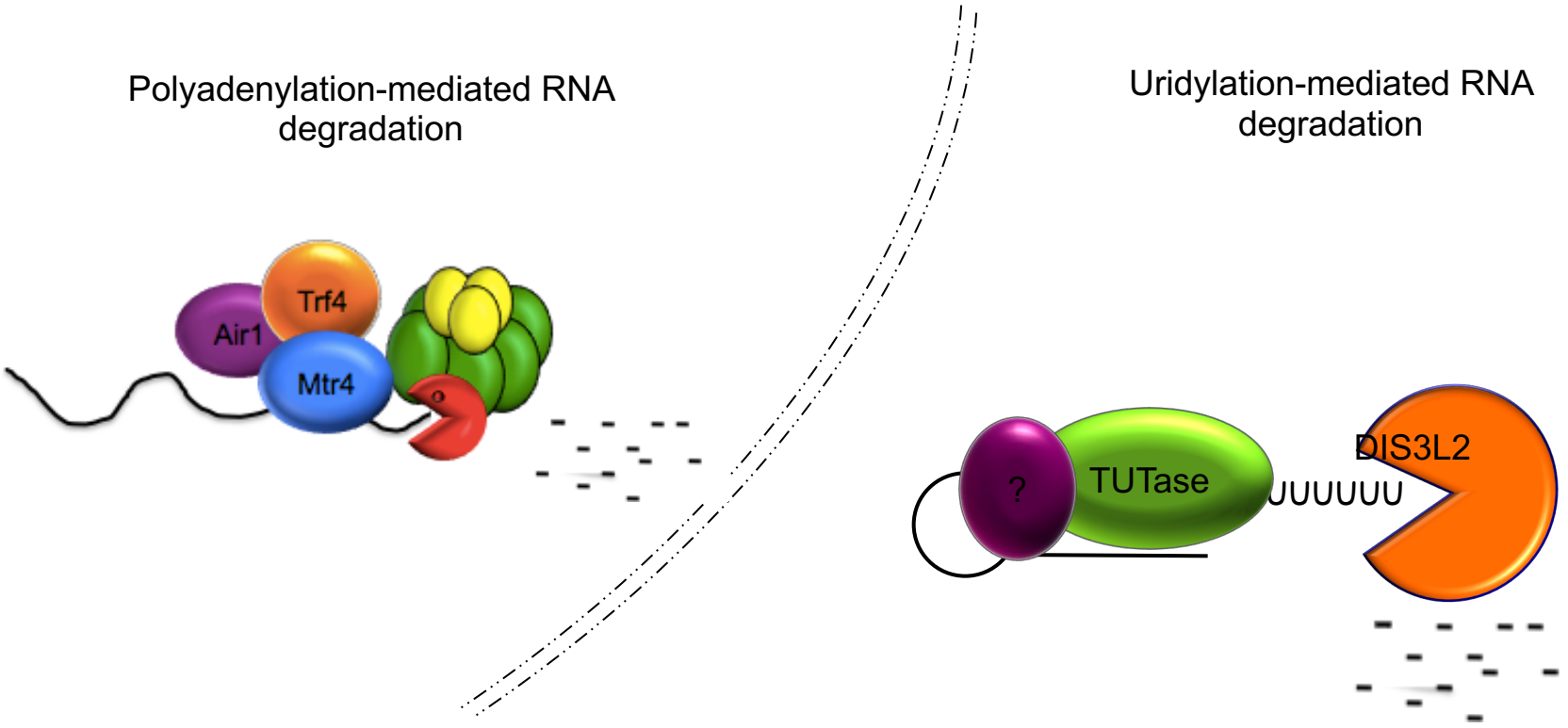
Nuclear and cytoplasmic RNA surveillance

NUCLEUS

CYTOPLASM

Polyadenylation-mediated RNA degradation

Uridylation-mediated RNA degradation



TRAMP + DIS3-EXOSOME

TRUMP + DIS3L2

Lecture summary

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. Surveillance and decay of noncoding RNAs
   mediated by nontemplated 3’ end tailing
   -oligoadenylation in the nucleus
   -oligouridylation in the cytoplasm