mRNA Decay

mRNA Decay

Considerations:

1. mRNA steady-state levels do not directly correlate with the rate of transcription

2. the coordinated mRNA decay of groups of transcripts involved in specific metabolic pathways introduced the concept of mRNA **decay regulons**.

3. mRNAs encoding housekeeping proteins tend to have considerably longer half-lives than those encoding regulatory proteins

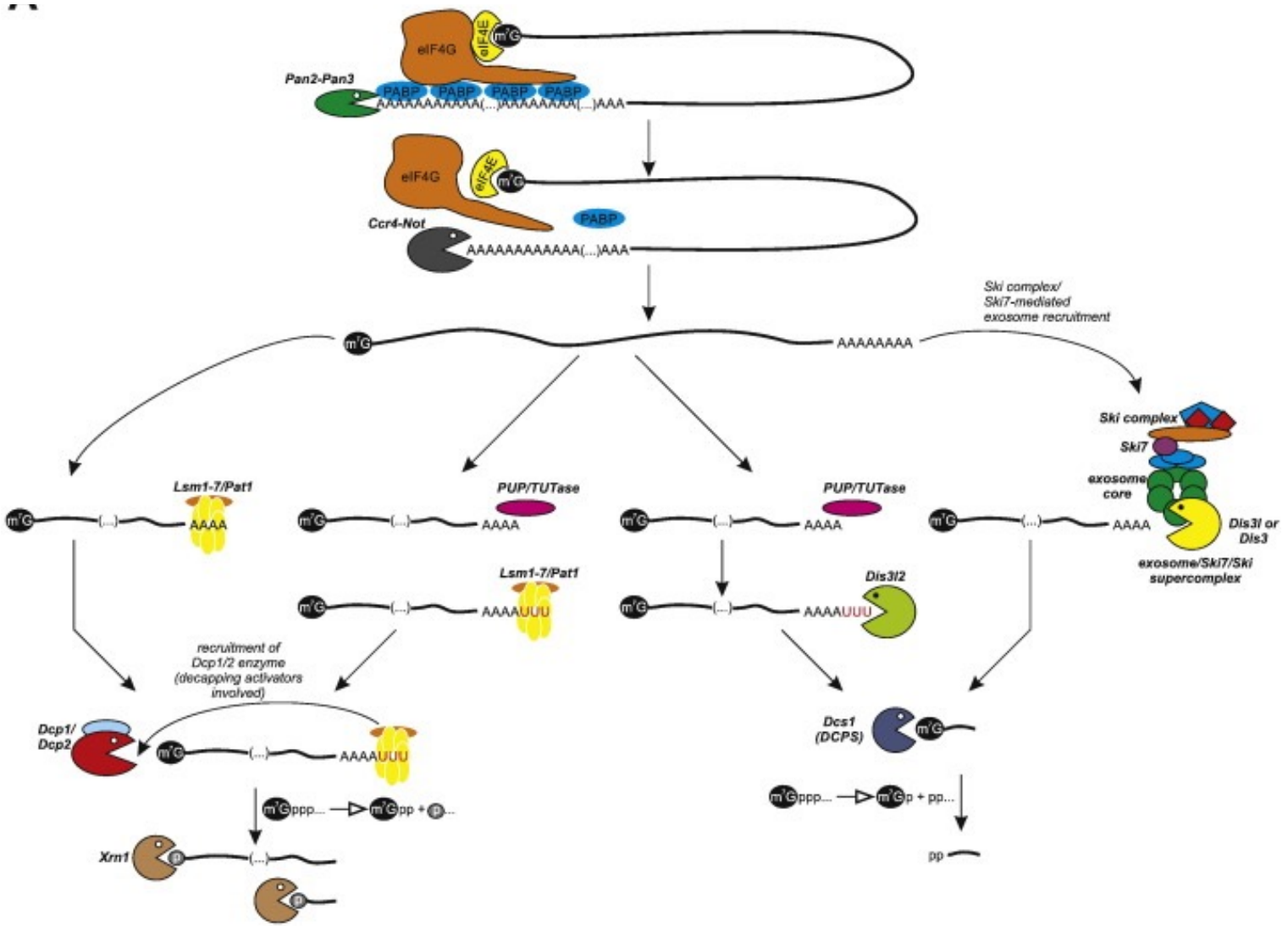
4. multiple parallel, partially redundant, mRNA decay pathways operate in the cytoplasm.

5. dysregulation of mRNA stability may underlie aspects of diseases such as obesity [1], Alzheimer's disease [2], and autism spectrum disorders [3].

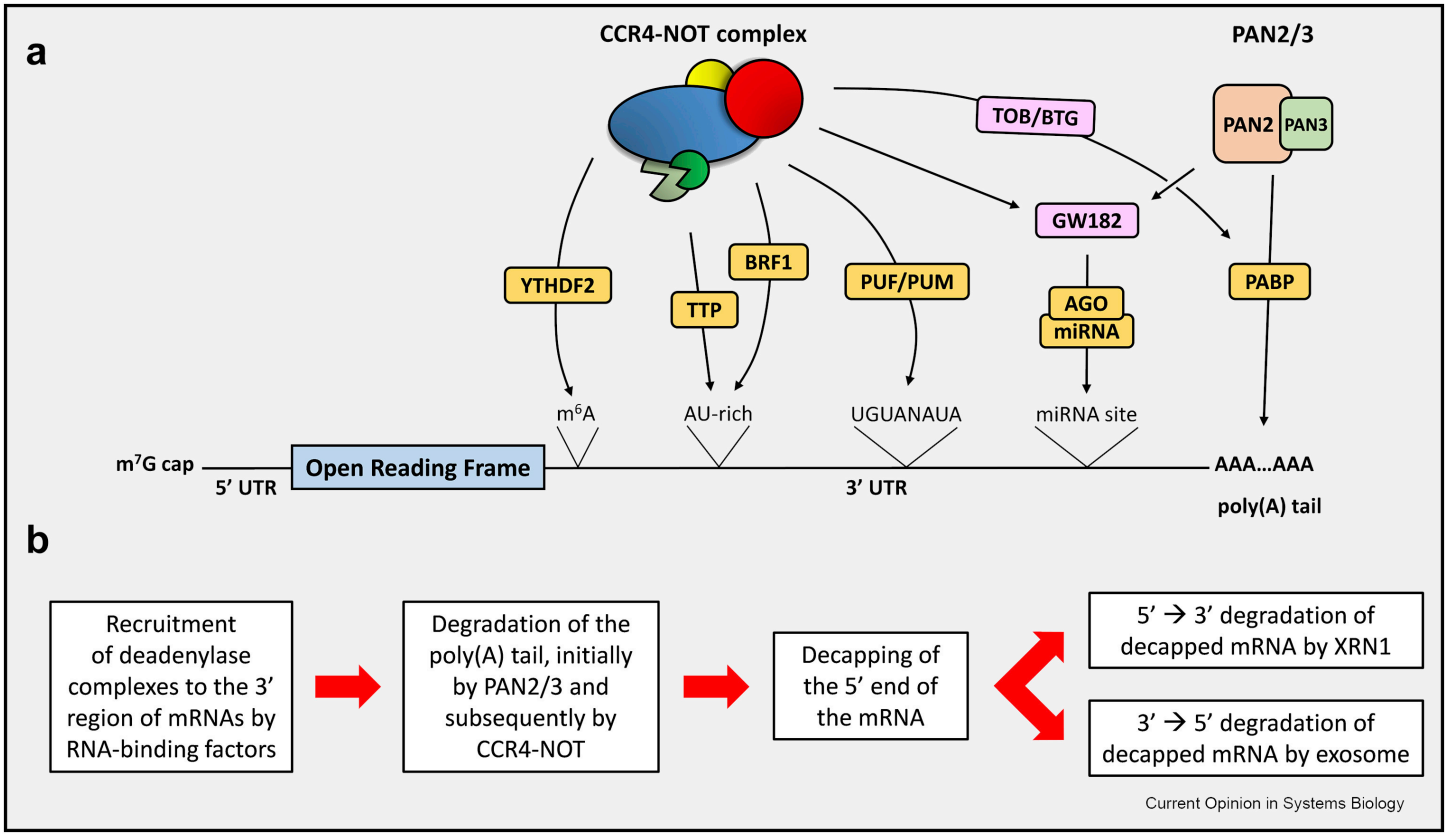
6. activating mechanisms dependent on both *cis*-acting and *trans*-acting protein/ncRNA regulatory factors exist

7. mRNA degradation is coupled to translation.

PolyA mRNA


eIF4G
eIF4E
m7G
Pan2-Pan3
PABP
AAAAAAAAAAA(...)AAAAAAAAA(...)AAA
Ccr4-Not
AAAAAAAAAAAA(...)AAA
Ski complex/
Ski7-mediated
exosome recruitment
AAAAAAAA
Lsm1-7/Pat1
PUP/TUTase
AAAA
AAAAUUU
Dis3l2
Ski complex
Ski7
exosome
core
Dis3l or
Dis3
exosome/Ski7/Ski
supercomplex
recruitment of
Dcp1/2 enzyme
(decapping activators
involved)
Dcp1/
Dcp2
Dcs1
(DCPS)
m7Gppp...
m7Gpp + p...
m7Gp + pp...
Xrn1
pp

The mechanism of mRNA decay



Deadenylation: Ccr4-Not

In Yeast **Ccr4** and **Caf1/Pop2** are the **catalytically active subunits**, both acting as 3'-5' poly(A)-specific exoribonucleases (major and minor, respectively), with the prevailing hypothesis that these deadenylases act exclusively as parts the Ccr4-Not complex and not on their own.

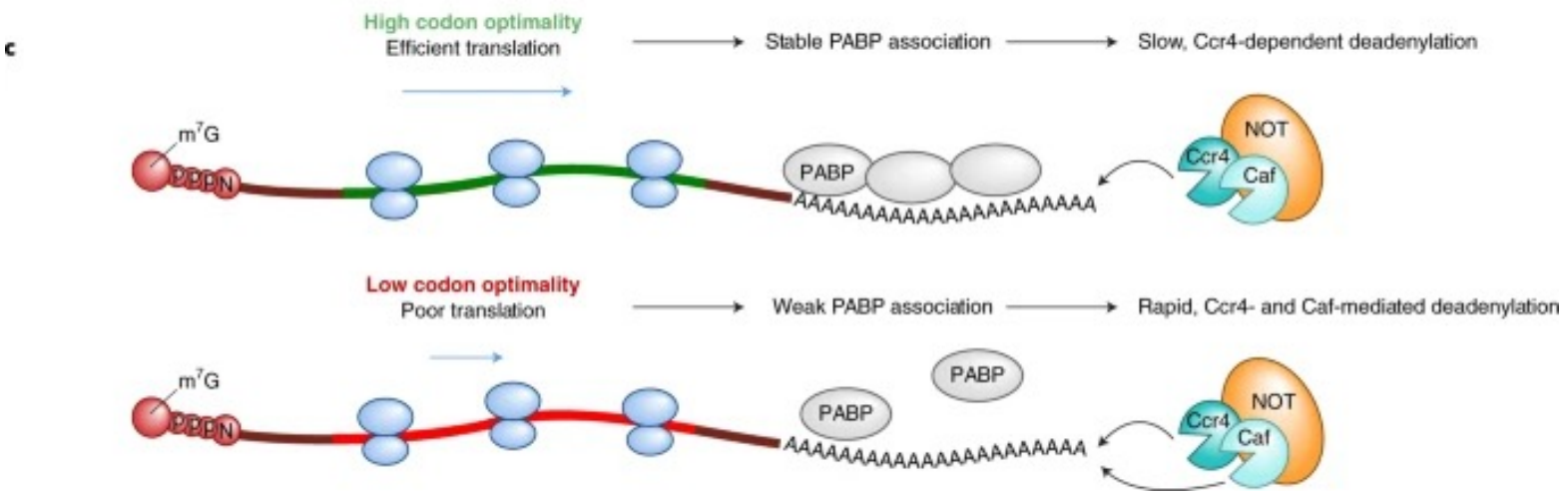
Mammalian genomes encode several **homologues** of Ccr4 (Ccr4a, Ccr4b, NOC/Ccr4c, ANGEL2/Ccr4d, ANGEL1/Ccr4e).

Three Caf1 homologues have been identified in human: CNOT7 (hCaf1/hCaf1a), CNOT8 (hPOP2/hCaf1b) and CAF1Z.

Human CAF1Z, in addition to its deadenylation activity, catalyzes 3'-5' decay, shuttles between cytoplasm and nucleus and localizes to nuclear foci (Cajal bodies).

Pop2 protein may be responsible for deadenylation of mRNAs enhance the function of the Ccr4 deadenylase, either by stabilizing the deadenylase complex or by perhaps providing additional interactions between the deadenylase complex and mRNA

Deadenylation: Ccr4-Not

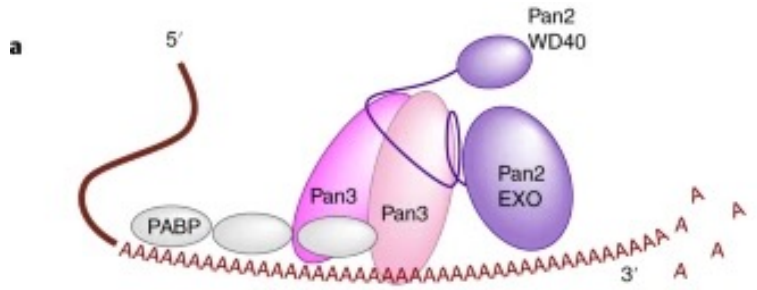


Adaptor proteins such as Mmi1, TTP, Tob/BTG, DDX6, GW182, and others can bind to different surfaces of the CCR4–NOT complex to activate and recruit deadenylase activity to specific mRNAs and decay pathways.

Ccr4 interacts with and is stimulated by PABP, but **Caf1 activity is inhibited by PABP**: consistent with this, Caf1 was found to preferentially deadenylate inefficiently translated mRNAs with poor codon optimality and reduced PABP occupancy in the poly(A) tail, whereas Ccr4 is necessary for deadenylation of efficiently translated mRNAs with high codon optimality and more-stably bound PABP.

Thus translation elongation and codon optimality appear to be tightly linked to deadenylation activity and decay via CCR4–NOT, with PABP acting as a critical modulator of this coupling.

Deadenylation: Pan2-Pan3 complex



Pan2/Pan3 does not degrade poly(A) tails completely *in vitro* or *in vivo* so it is thought to be responsible for initial poly(A) tail trimming.

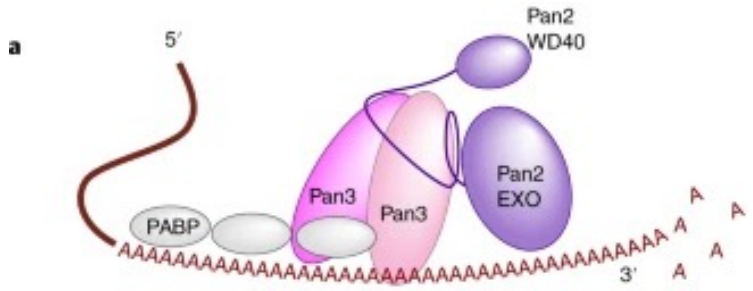
Pan2 acts as a **catalytic** subunit.

Pan2 stimulated by PABPs both in yeast and mammals .

Additionally, yeast and human Pan2 interacts with Pan3, and human **PAN3 mediates PAN2 recruitment to mRNA through PABPs**

PAN3 is important not only for initial PAN2 recruitment, but also for eliciting further steps, resulting in complete mRNA degradation.

Deadenylation: Pan2-Pan3 complex

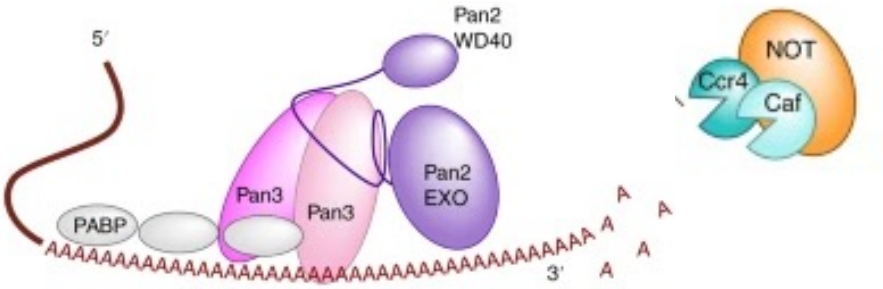


PAN3 recognizes both **PABP and poly(A)** RNA to recruit the complex to RNA 3’ tails and simultaneously stimulates PAN2 deadenylase activity.

PAN3 dimer also directly binds **GW182** proteins.

It is possible that **PAN3 might also recruit additional decay factors during bulk mRNA decay**, as suggested by the observation that PAN2 deadenylation activity was insufficient to stimulate decay of mRNAs in the absence of PAN3.

Deadenylation model



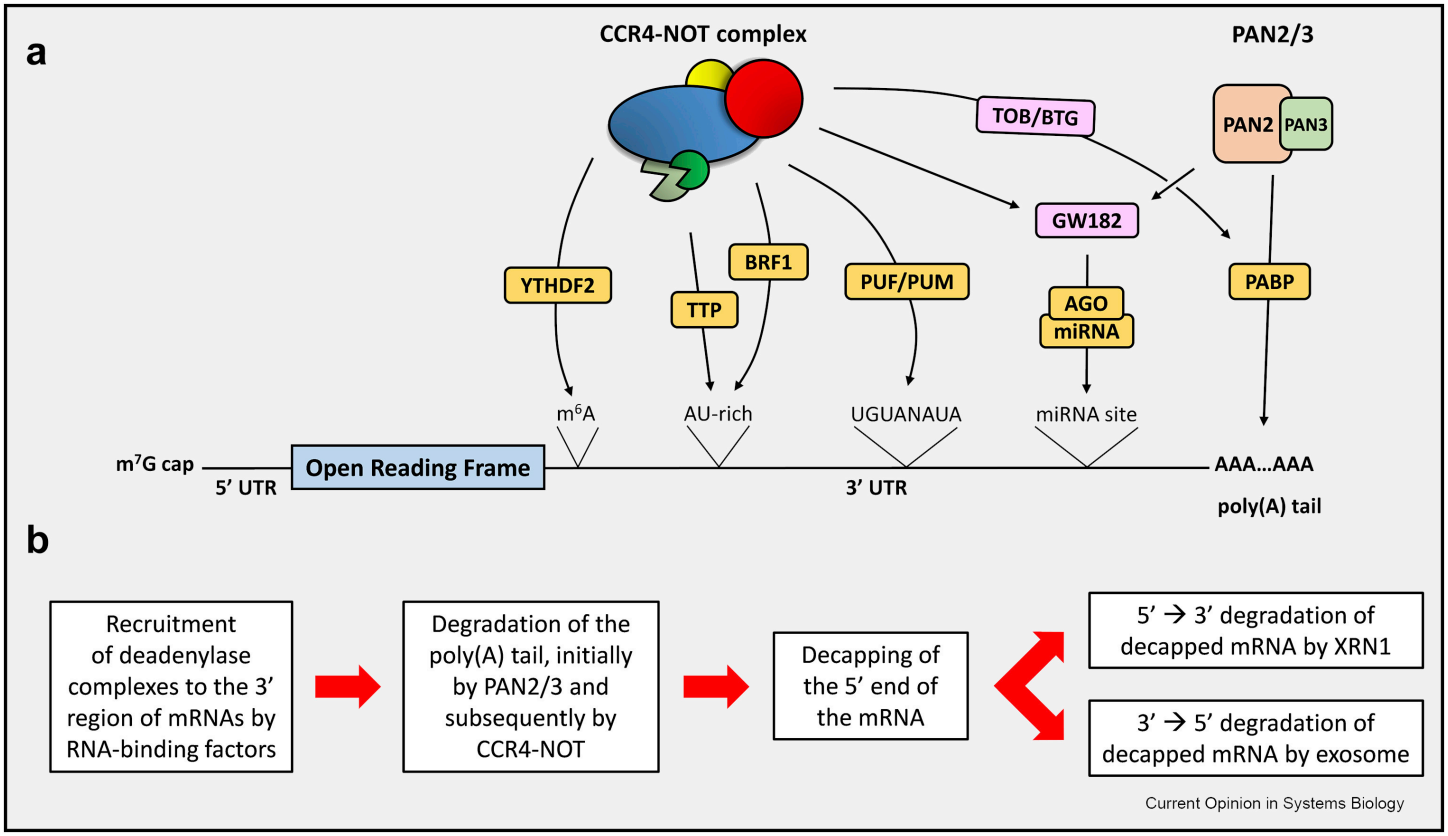
The sequential model for mRNA deadenylation, in which PAN2–PAN3 carries out initial 3′ poly(A) tail shortening followed by more complete deadenylation by CCR4–NOT to trigger 5′ decay, is an oversemplification.

PAN2–PAN3 and CCR4–NOT have different substrate preferences

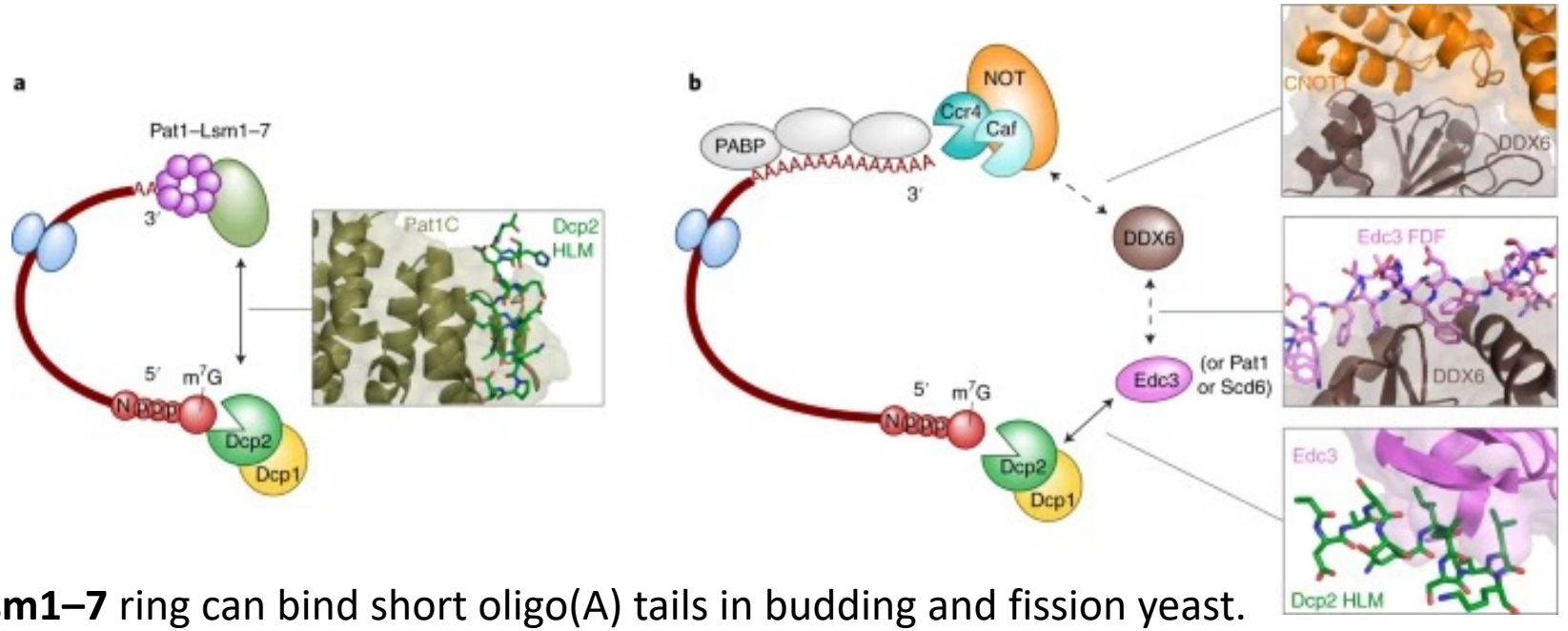
PAN2–PAN3 and CCR4–NOT complexes may target different pools of RNA for **deadenylation to tune their poly(A) tail length, PABP occupancy, translational properties, and stability.**

PARN, but also Nocturnin and Angel1/2 further expands the repertoire of poly(A) tail length regulators in eukaryotes

Deadenylation…what's next?



Linking 3′ deadenylation to 5′ end decay in yeast

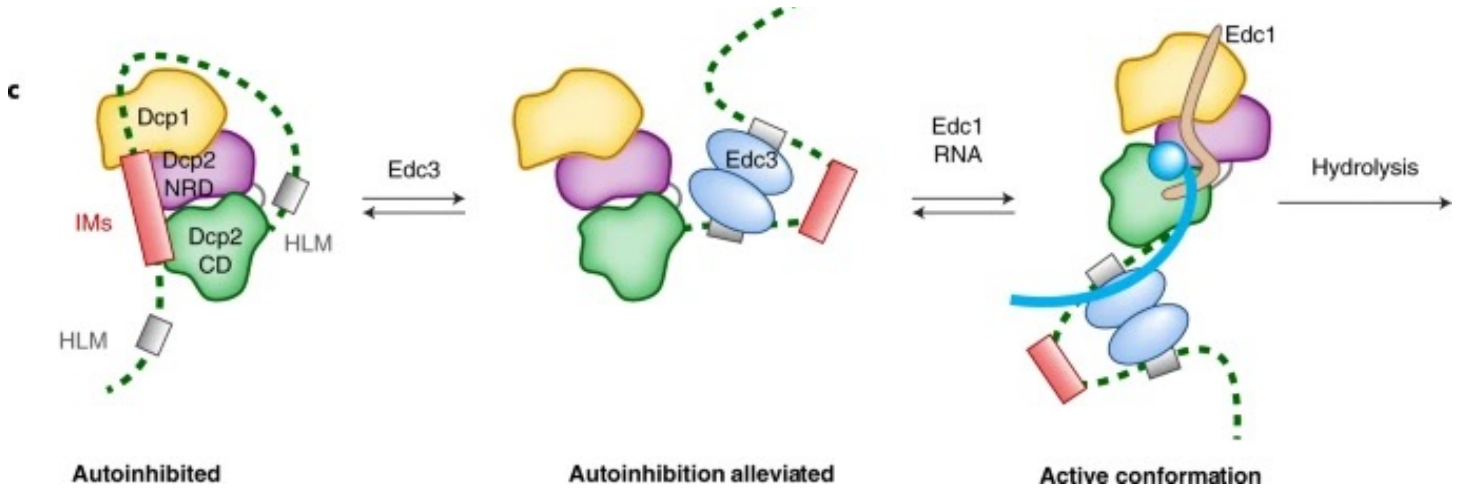


a) The **Lsm1–7** ring can bind short oligo(A) tails in budding and fission yeast. **Pat1** interacts with HLMs of Dcp2 in budding and fission yeast.
These interactions may recruit the decapping complex directly to deadenylated/urydilated 3′ tails.

b) **DDX6** (Dhh1) binds CNOT1 and a variety of decapping coactivators including Edc3, Pat1, and Scd6. Genome-wide studies indicate that these decapping coactivators control different subsets of mRNAs.

The precise mechanisms controlling formation of the decapping mRNP remain unclear

mRNA decapping



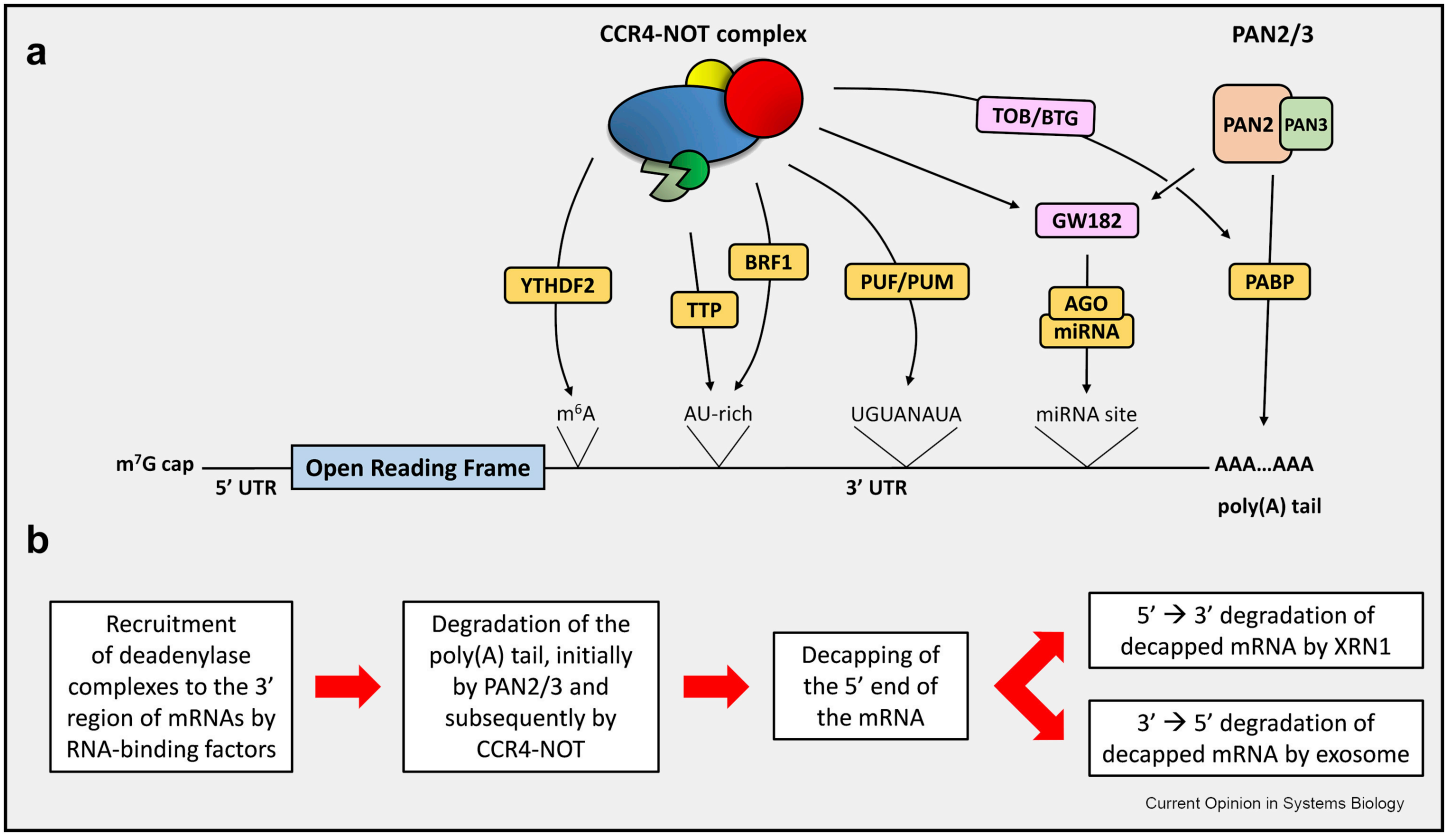
Core decapping complex: Dcp2 is the decapping enzyme, the essential activator **Dcp1**, and **scaffolding proteins**. In mammals there are many different decapping enzymes.

Dcp1–Dcp2 catalytic core exists initially in an inactive conformation.

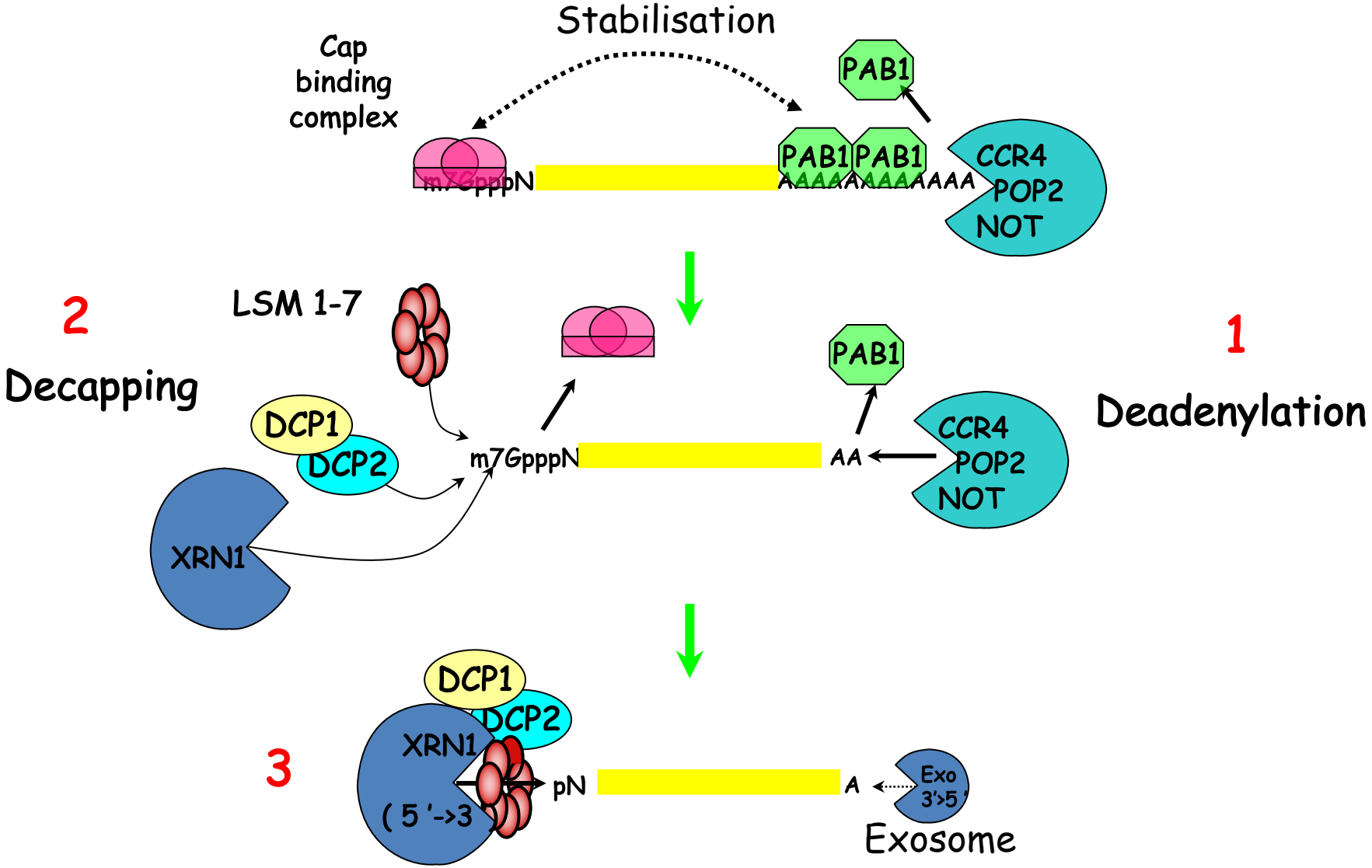
Binding of the coactivator **Edc3** to HLMs in the Dcp2 C terminus activates decapping by multiple mechanisms: **it alleviates autoinhibition, promotes RNA binding**, and allows efficient binding and activation of the decapping complex by **Edc1-like coactivators**.

Binding of substrate and Edc1-like coactivators promotes the **catalytically active conformation of Dcp2 and hydrolysis of the 5′ cap.**

Deadenylation…what's next?

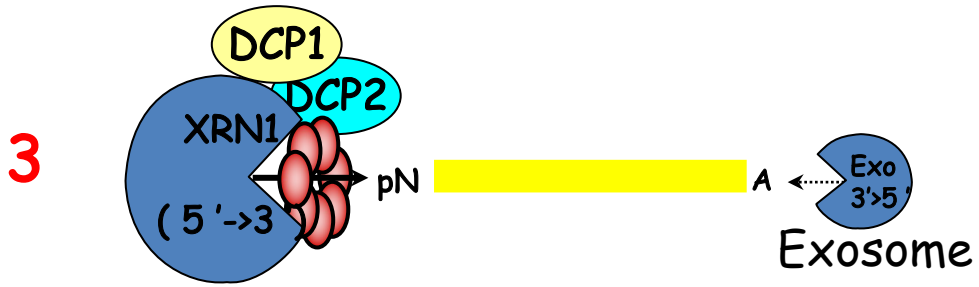


Decapping…what's next?



the conserved cytoplasmic exonuclease **Xrn1** recognizes the newly exposed 5' monophosphate of the RNA and rapidly, processively degrades the transcript body

Decapping…what's next?

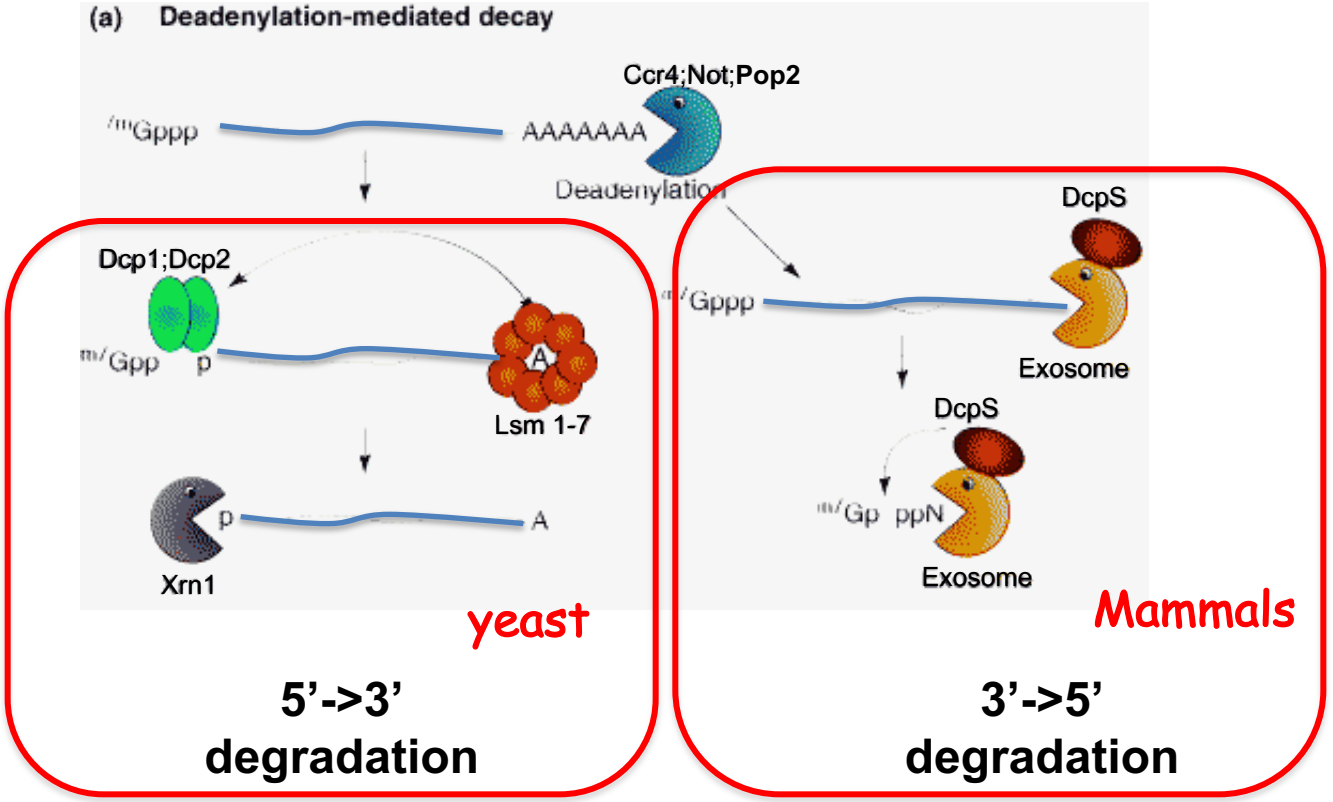


Xrn1 and the decapping complex itself (Dcp1 or Edc4 in metazoans) or decapping coactivators (Pat1 in yeast) can interact.

Following removal of the 5′ cap by the decapping machinery, the conserved cytoplasmic exonuclease Xrn1 recognizes the newly exposed 5′ monophosphate of the RNA and rapidly, processively degrades the transcript body.

Exosome can also partecipate in degradation.

Different mRNA decays operate in yeast and mammals

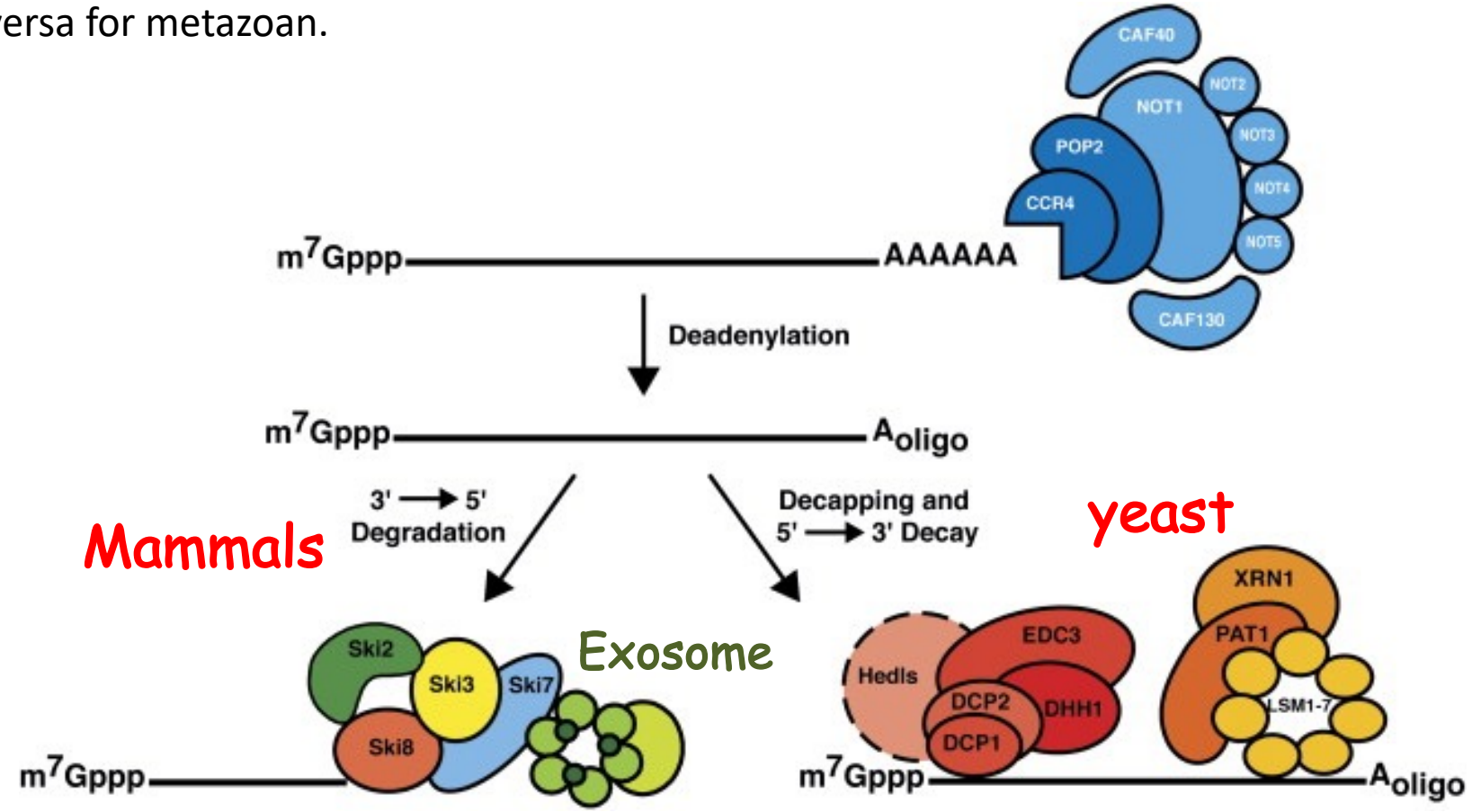


The Exosome

Deadenylation leads to the release of PABPs from the mRNA 3' –end which allows for a direct attack by 3' -5' exoribonucleases.

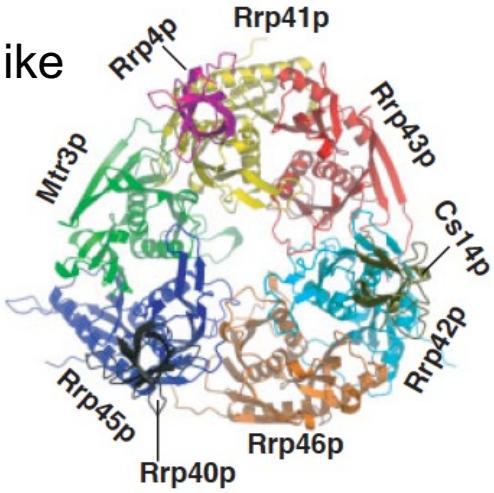
A major eukaryotic exoribonuclease degrading transcripts from 3' -end is the **RNA exosome complex**

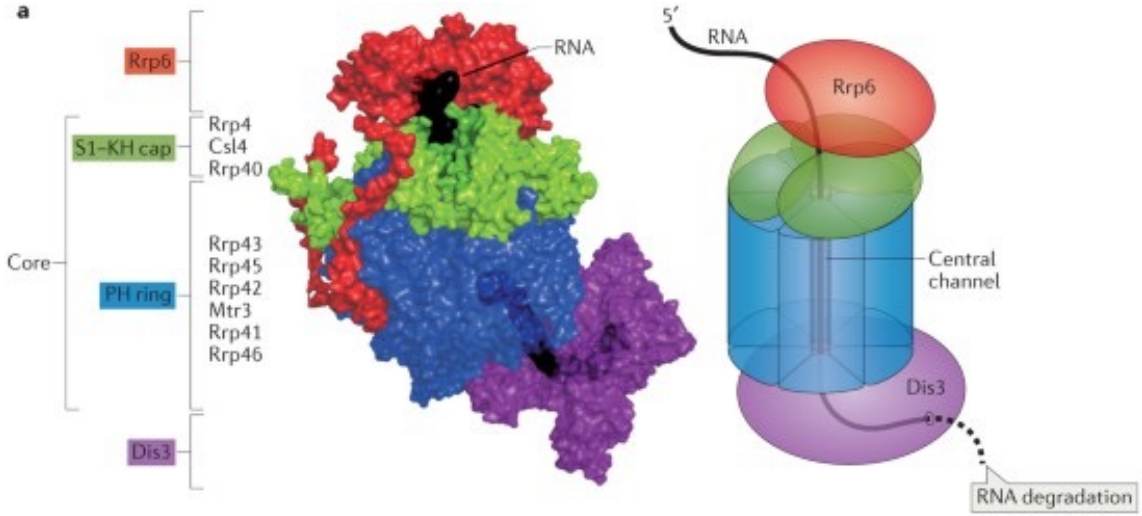
In yeast, exosome-mediated 3' -5' decay pathway, although functional, does not play a major role in the control of mRNA stability, and decapping-dependent 5' -3' pathway prevails. Viceversa for metazoan.



The Exosome: 3’-5’ decay

Barrel like

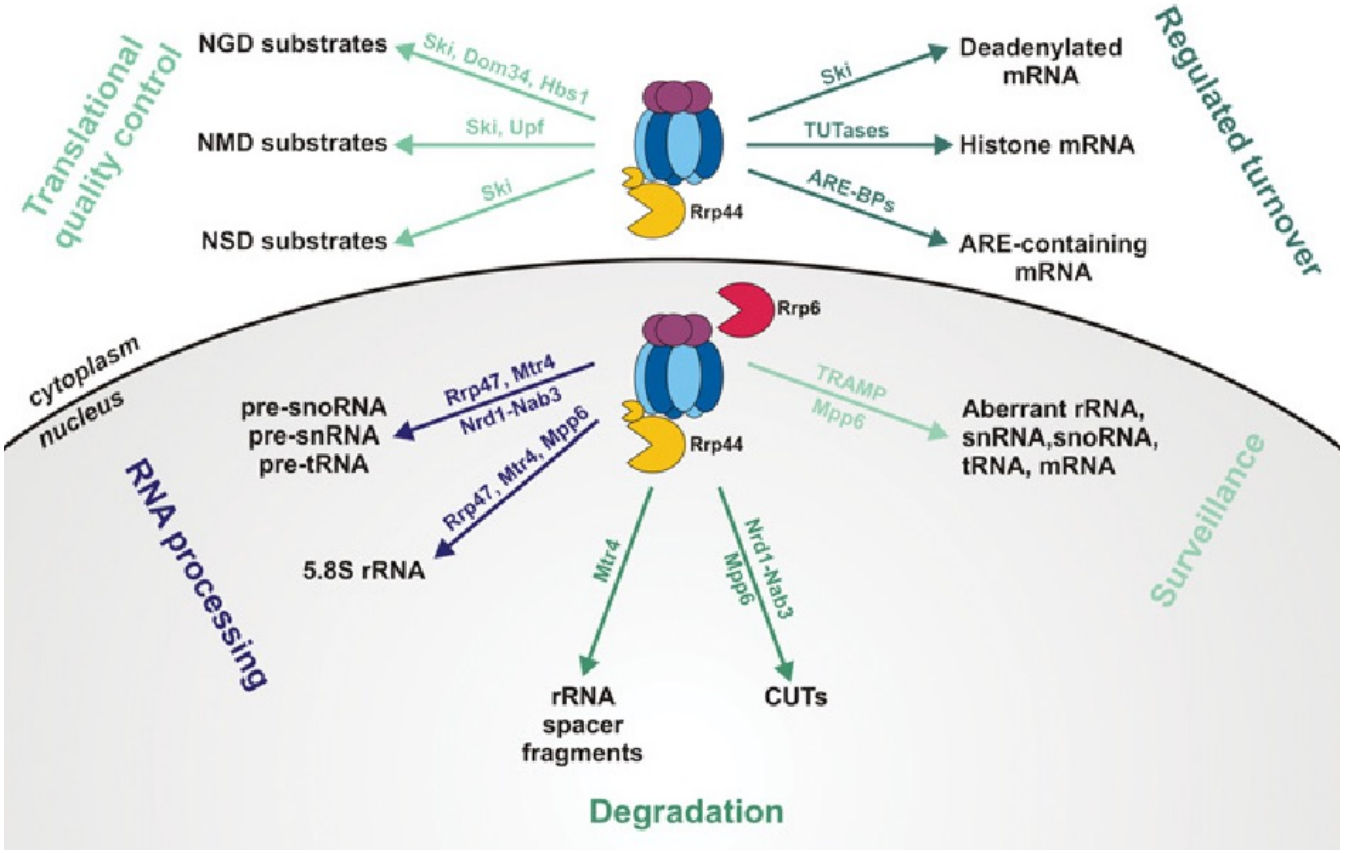




The eukaryotic exosome is a **major ribonuclease for RNA decay and processing.**
The repertoire of catalytic subunits varies among Eukaryota.
Some catalytic subunits are compartment-specific and give rise to exosome isoforms.

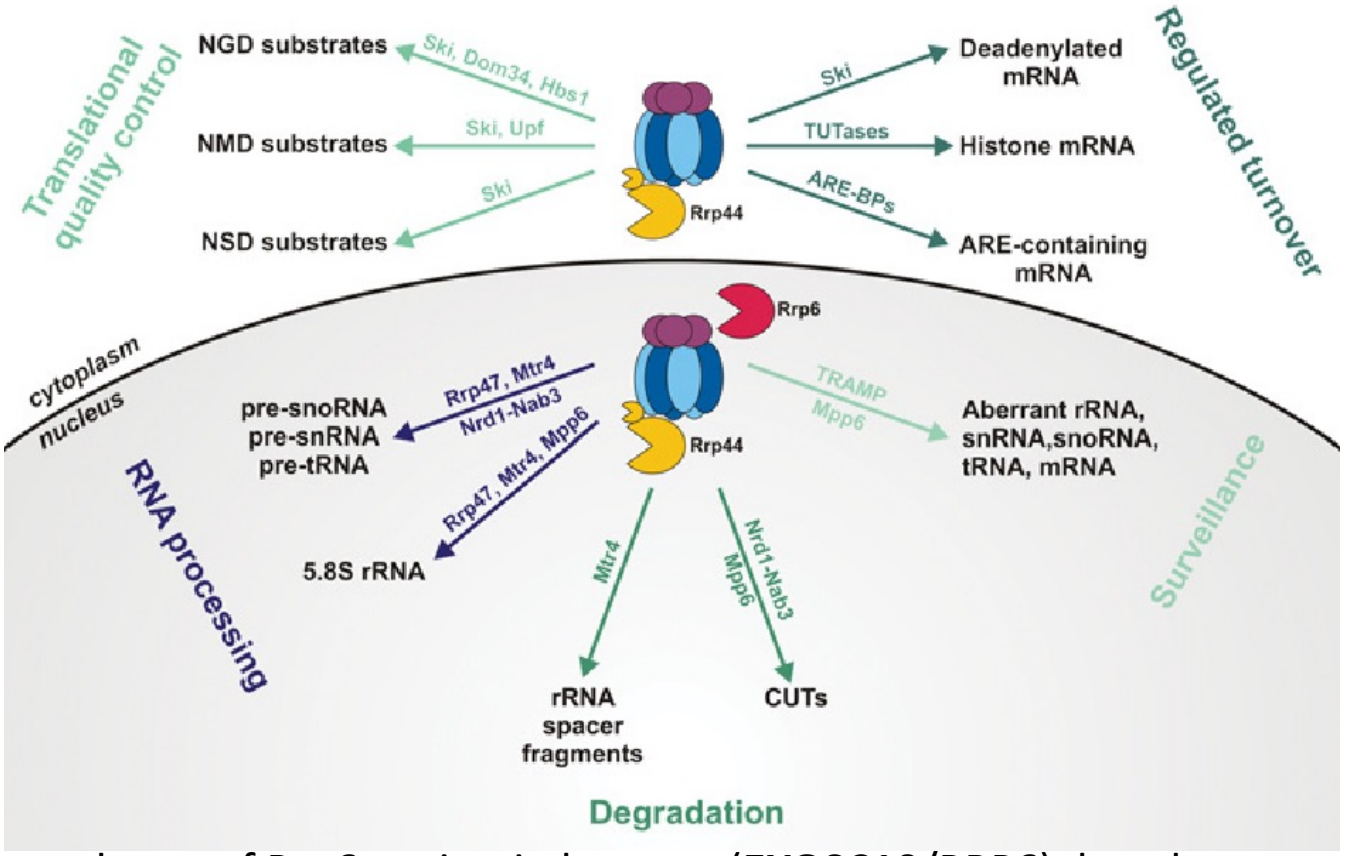
In *S. cerevisiae*, the exosome is the only essential 3’-5’ exoribonuclease , which is highly conserved in eukaryotic kingdom, with exosome core forming **a barrel-like assembly** (6 subunits – Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, Mtr3) and cap (3 subunits – Rrp4, Rrp40, Csl4).

The Exosome: 3’-5’ decay



The core complex in *S. cerevisiae*, which is the best studied model in exosome research, lacks catalytic activity which is instead provided in the cytoplasm by a stable interaction with **Dis3p** (Rrp44), **a nuclease possessing both endo- and exonucleolytic activity**.
Dis3p is also present in the **nucleus**, where the exosome core associates with an additional catalytic subunit, Rrp6p.

The Exosome: 3’-5’ decay



Only one homologue of Rrp6p exists in humans (**EXOSC10**/RRP6), but there are three proteins of the DIS3 (Rrp44) family: **DIS3, DIS3L and DIS3L2.**

DIS3L and DIS3L2 are cytoplasmic, **DIS3** is primarily localized to the **nucleus**, and **EXOSC10** to the **nucleolus**.

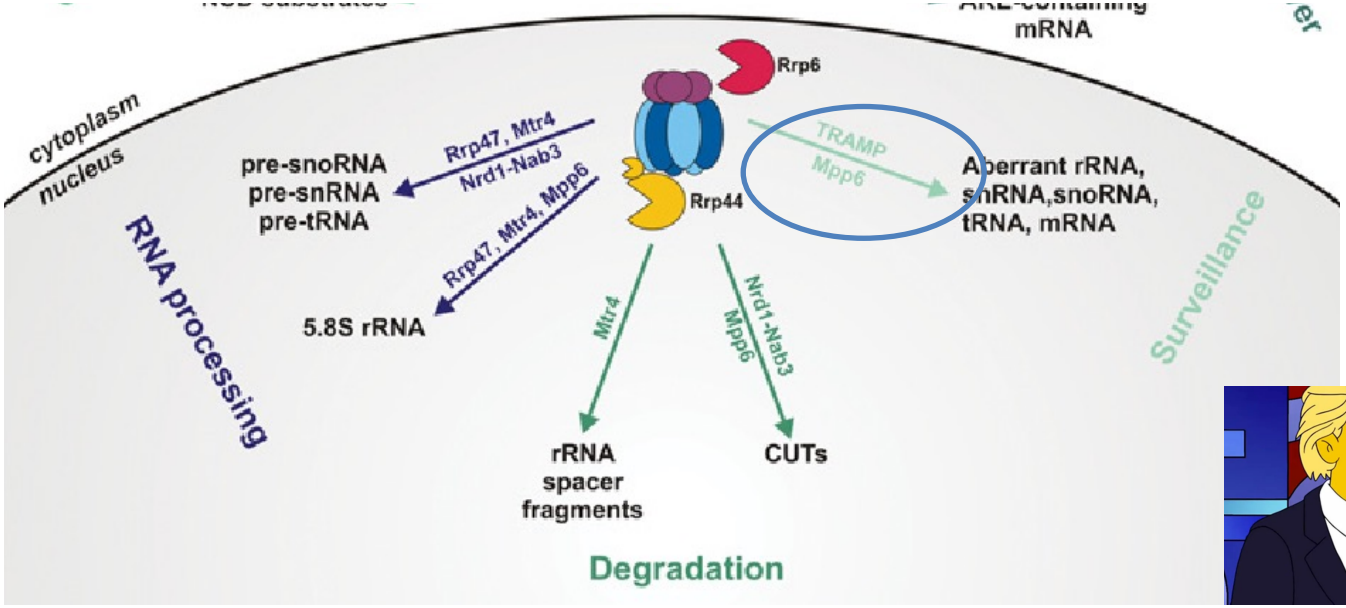
DIS3L2 does not interact with exosome core functions in a **exosome-independent** cytoplasmic RNA degradation pathway.

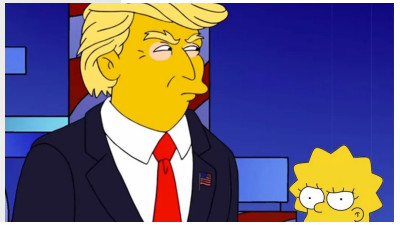
mRNA EXPORT: YEAST
mRNA quality control and export check-point

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/

The nuclear removal of **faulty RNAs** relies on the **TRAMP** (Trf4/5, Air1/2, Mtr4) **complex** that marks these RNAs with a **short oligo(A) tail** for subsequent **degradation by the nuclear exosome**

The Exosome in the nucleus





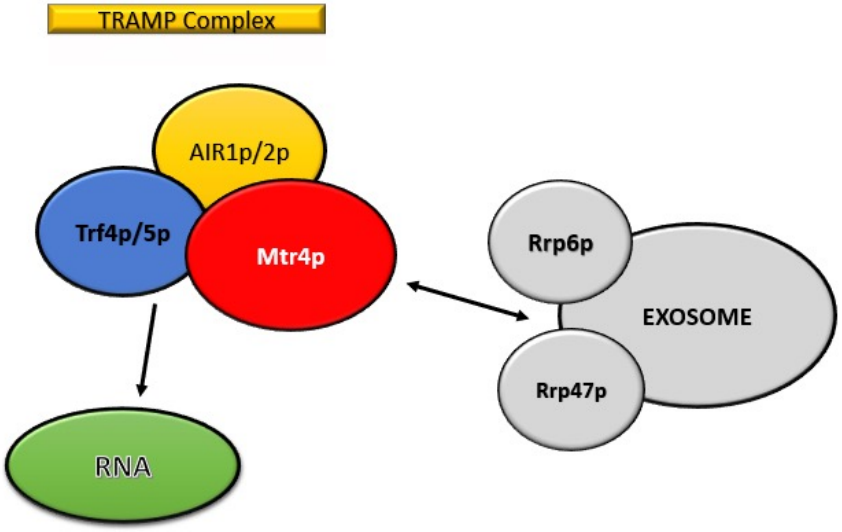
Exosome cofactors:

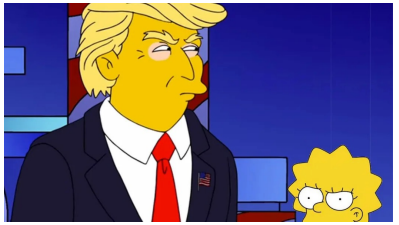
TRAMP facilitates and/or enhances exosome activity due to the helicase activity of Mtr4p as well as the addition of unstructured oligo(A) stretches to RNA 3' ends by Trf4/5p.

S. cerevisiae TRAMP acts on most nuclear exosome substrates for **degradation or processing** (often in competition)

The **human** version of **Mtr4** forms at least two distinct complexes: the Nuclear Exosome Targeting (**NEXT**, with RBM7 and ZCCHC8) complex in the **nucleoplasm** and the '**human TRAMP**' in the **nucleolus** (with TRF4-2 and ZCCHC7)

The Exosome in the nucleus





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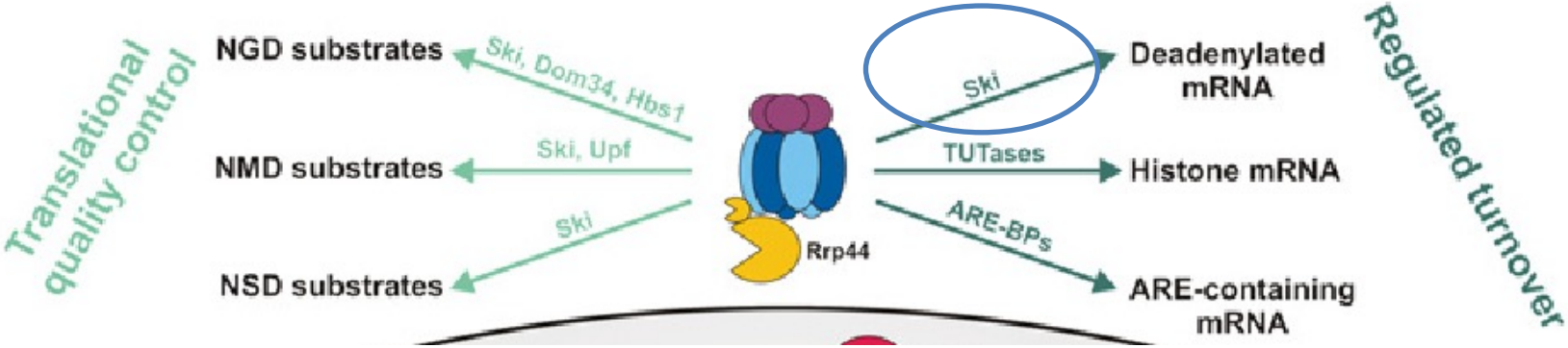
The Exosome in the cytoplasm

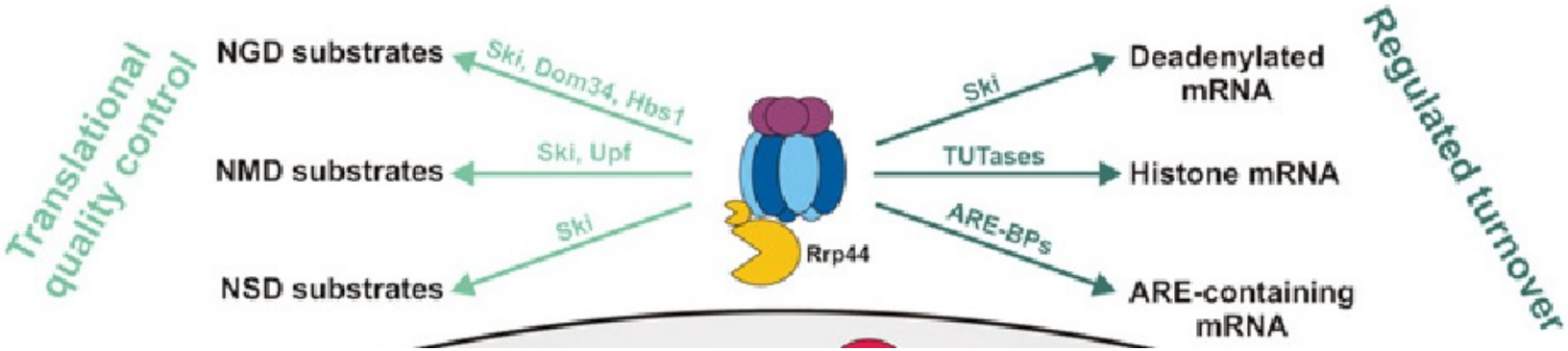


The cytoplasmic exosome is usually assisted by the **superkiller (SKI) complex**.

In yeast the interaction between the exosome and SKI is mediated by the **Ski7p** protein. The human version of Ski7p does not appear to exist. However, Ski7p has a paralogue – Hbs1p – that is involved in the NSD and NGD pathways, and Hbs1p has a human counterpart – HBS1L – that co-purifies with the exosome and perhaps has taken over the functions of Ski7p.

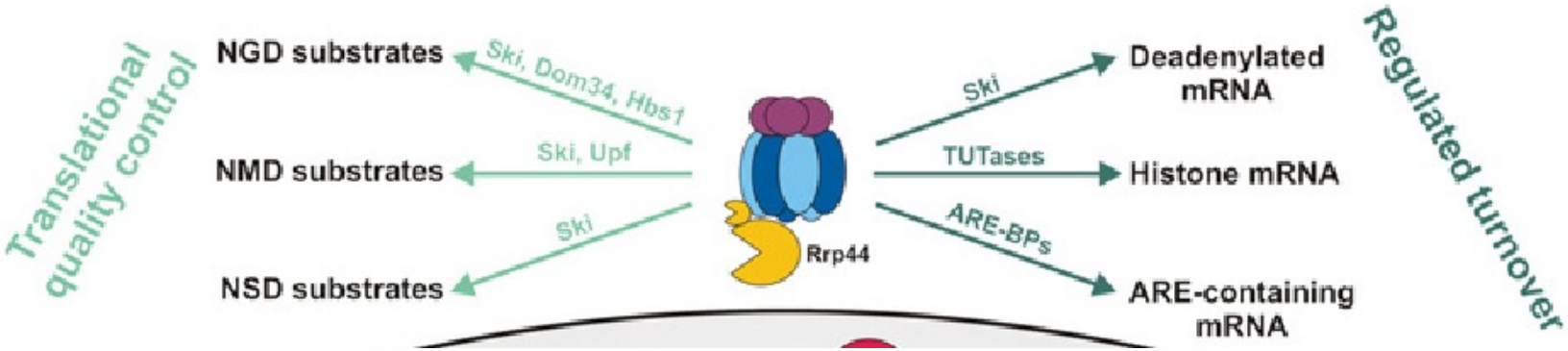
The **SKI complex is necessary for RNAi** and for decay of several kinds of faulty mRNAs: ones with premature termination codons (PTCs; the nonsense mediated decay(**NMD**) pathway), ones lacking termination codons altogether (the non-stop decay (**NSD**) pathway) and ones where ribosomes stall (the no-go decay (**NGD**) pathway).

NGD, NMD, and NSD



NGD, NMD, AND NSD systems all detect defects that interfere with translation efficiency.

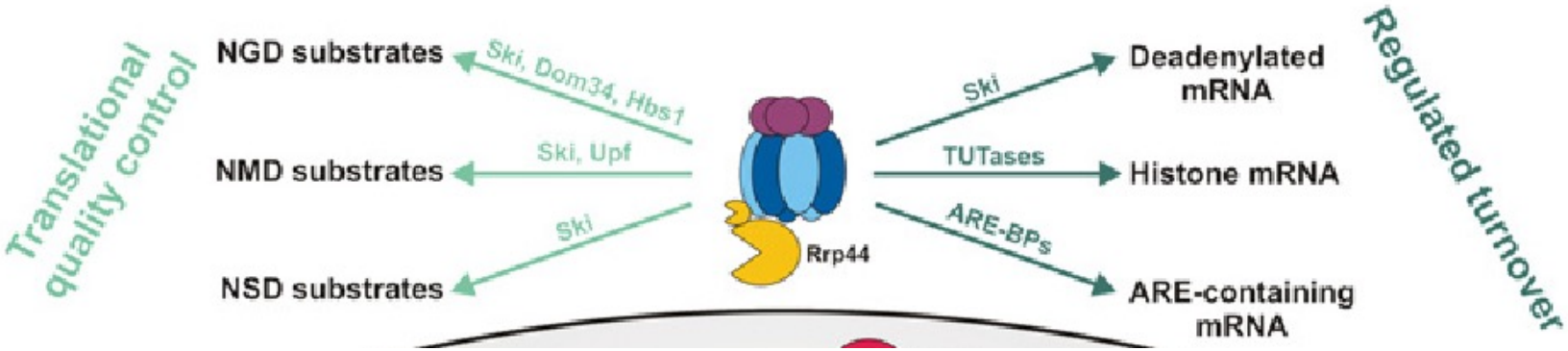
the no-go decay (NGD)



NGD, NMD, AND NSD systems all detect defects that interfere with translation efficiency.

In **NGD**, ribosomes stop translation because of **rare codons or stable secondary structures**. Here, recognition occurs by the proteins **Dom34p and Hbs1p** (the Ski7p paralogue), after which the obstructing **secondary structure is cleaved by an unknown endonuclease**, enabling mRNA degradation by **SKI–exosome**.

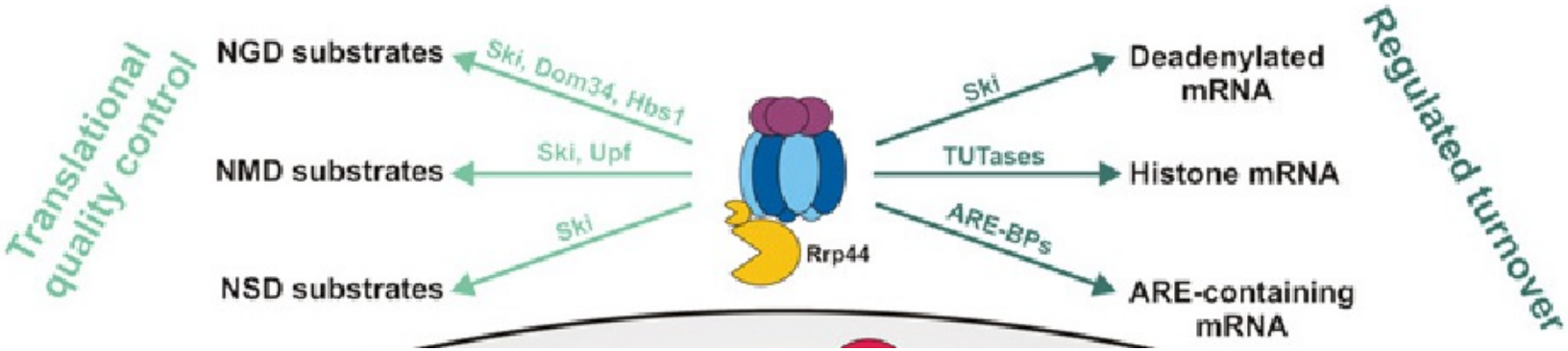
the non-stop decay (NSD)



NGD, NMD, AND NSD systems all detect defects that interfere with translation efficiency.

In NSD, **ribosomes that stall at 3' ends of mRNA are identified by a domain of Ski7p that resembles ribosome release factors**, followed by SKI and the exosome.

the nonsense mediated decay (NMD)



NGD, NMD, AND NSD systems all detect defects that interfere with translation efficiency.

In NMD, **mRNA bearing a PTC is recognized by Upf proteins**, deadenylated or cleaved and then SKI and the exosome are recruited to destroy it.

How is the PTC recognized?

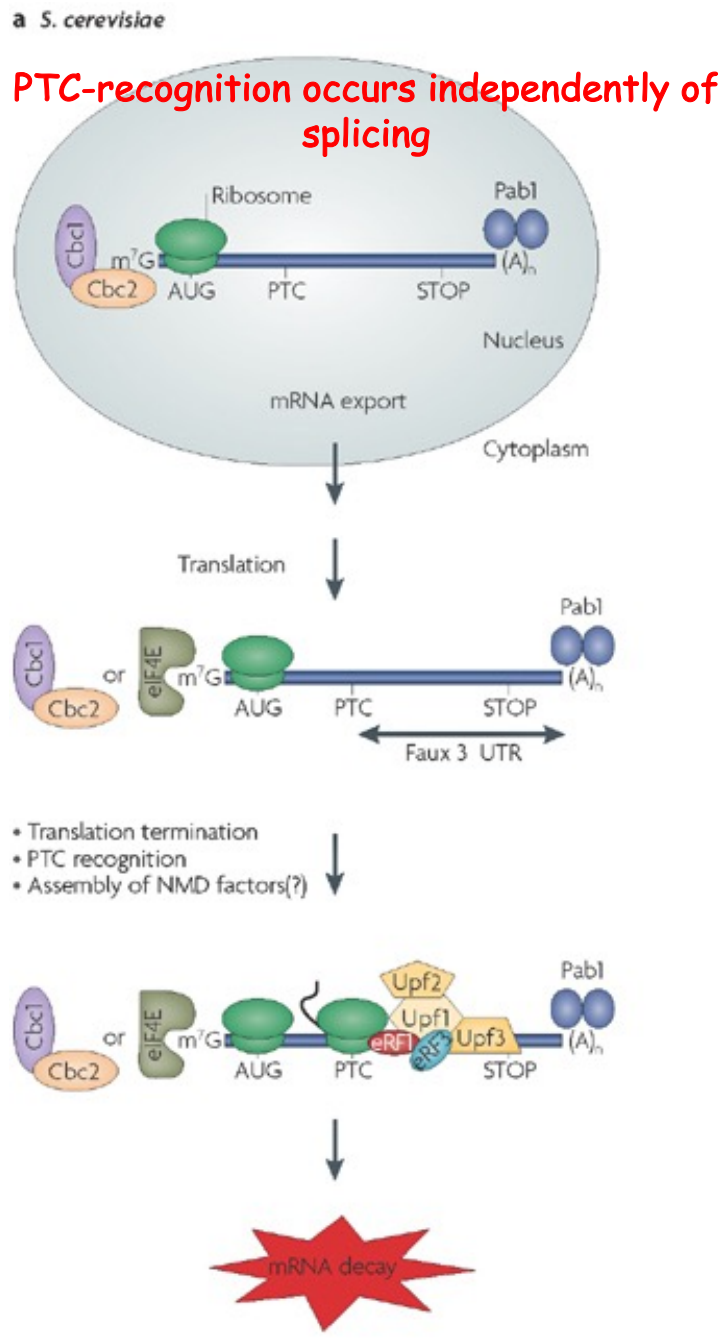
NMD (Yeast)

•In this model a central role is played by **'marker' proteins** that are deposited on the mRNA downstream of the PTC and upstream of a normal termination codon.

•**Abnormally long 3'-UTR** (**"faux"**) or a **downstream sequence element** (**DSE**) recruits proteins required for the identification of a nonsense codon as a PTC.

•**In a normal mRNA, the translating ribosomes displace these marker proteins so that they cannot trigger NMD.** However, in a PTC-containing mRNA, the marker proteins would still be bound when the translational apparatus recognizes the PTC.

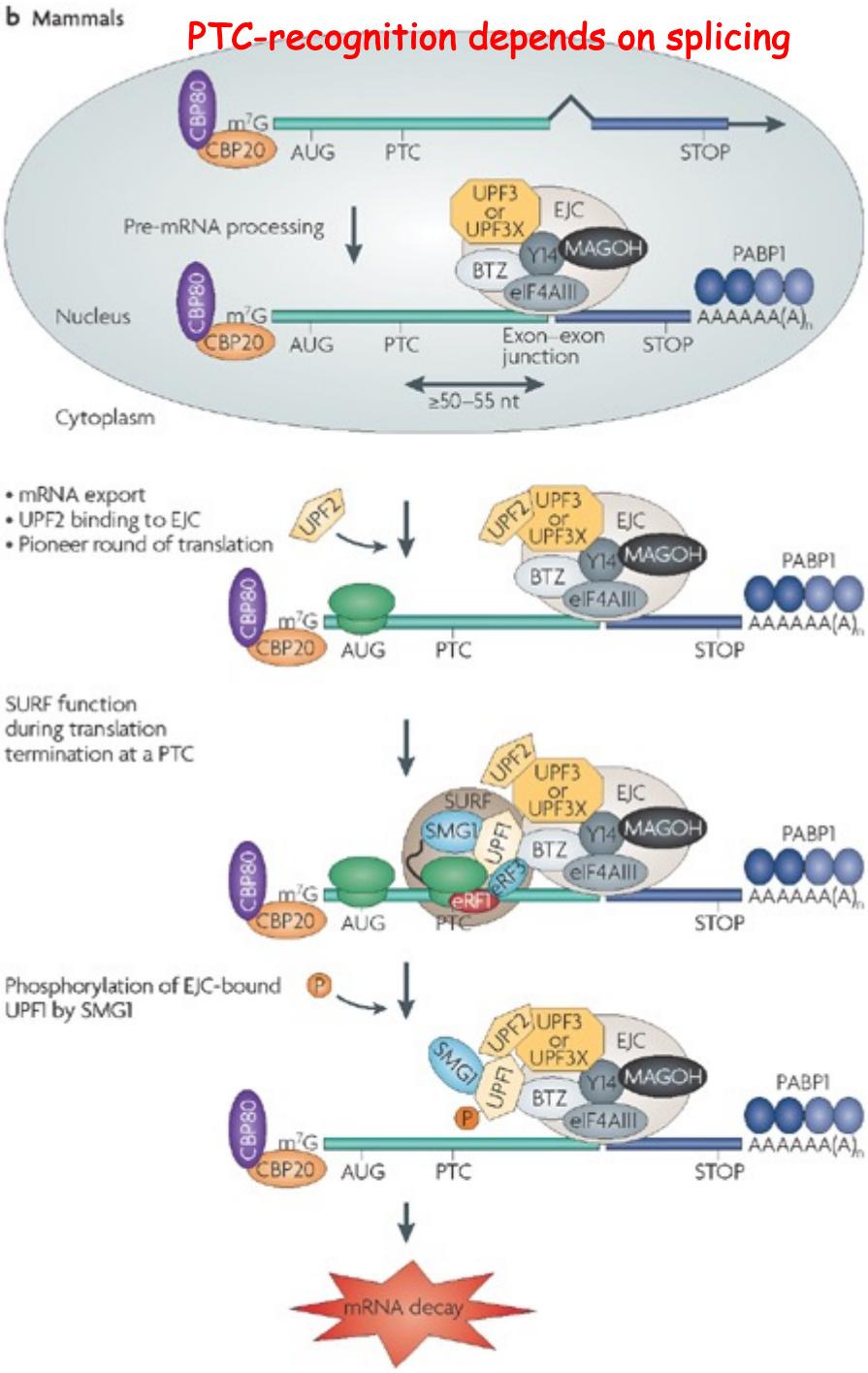
•Interaction of these marker proteins with translation termination factors recruited to the PTC leads to rapid mRNA degradation



NMD (mammals)

•In mammalian cells, a **large exon junction protein complex (EJC)** deposited about 20–24 nucleotide (nt) upstream of exon–exon junctions during RNA splicing, is widely considered to **be <u>a mark that triggers NMD</u>**

•**Nonsense codons more than 55-nt upstream of the last intron generally trigger NMD**

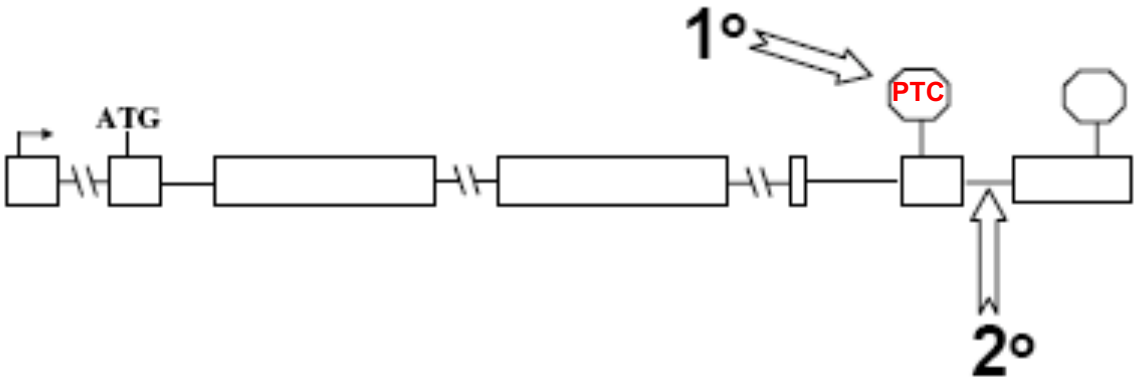


Detection of mRNAs with PTCs in mammals

Two signals are required for NMD:

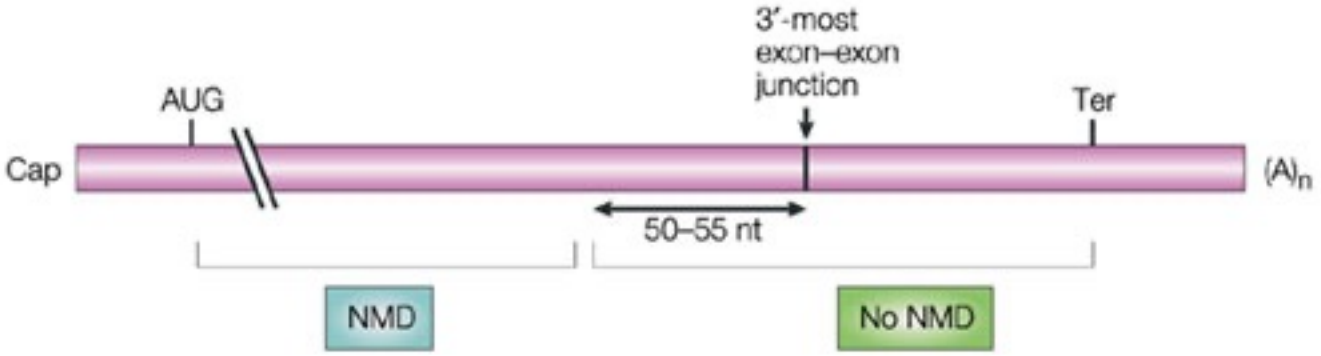
1. Premature Stop Codon (PTC)
   (more than 55-nt upstream of the last intron)

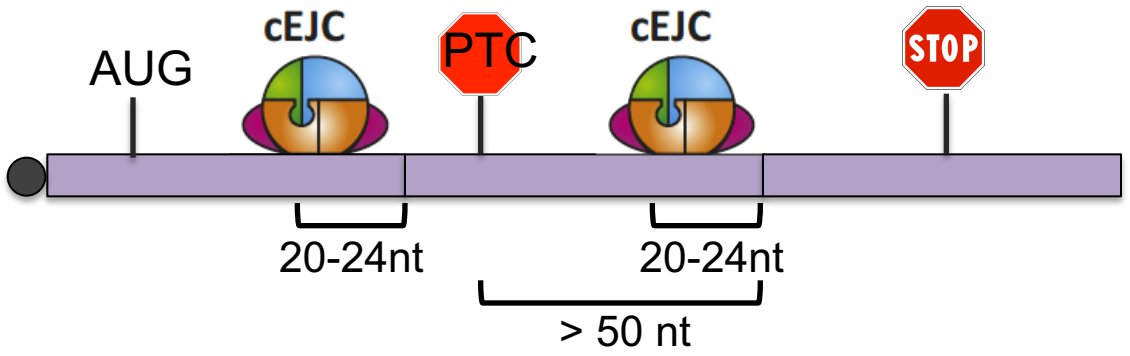
2. Intron



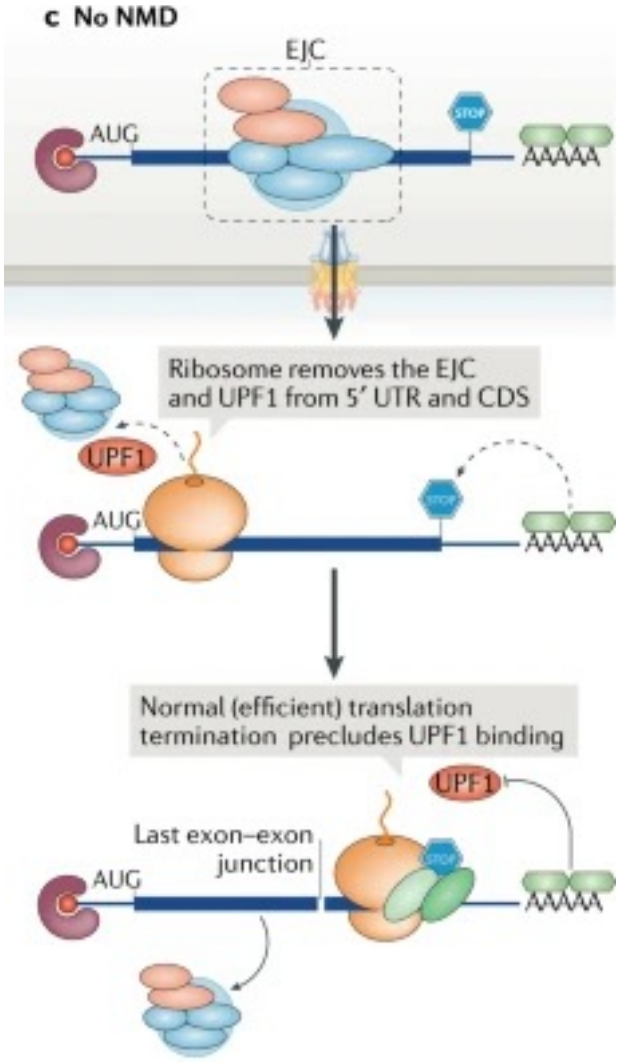
The 'position-of-an-exon–exon-junction' rule

A **premature termination codon (PTC)** that is located in the region indicated in blue, which is followed by an exon–exon junction more than 50–55 nucleotides (nt) downstream, elicits **NMD**, whereas a PTC that is located in the region indicated in green fails to elicit NMD.





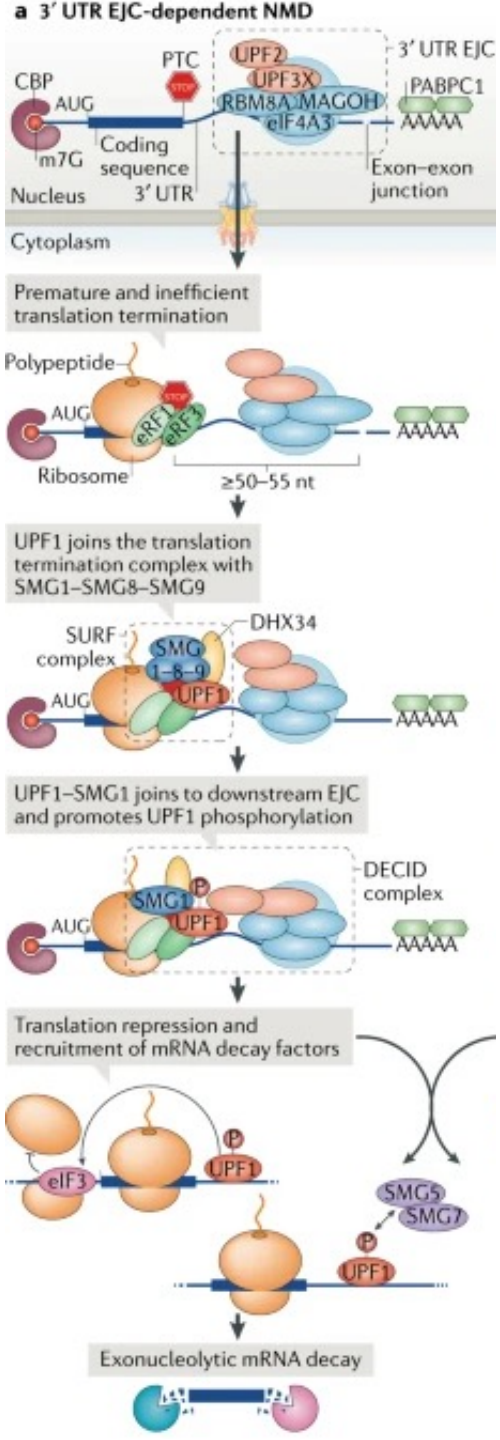
NMD



Normally, **translating ribosomes remove EJCs and any promiscuously bound UPF1** from the 5′ UTR and from the coding sequence (CDS) but do not travel beyond the stop codon into the 3′ UTR.

PABPC1, through its interactions with eRF1–eRF3 or translation initiation factor eIF4G (not shown), **is thought to preclude UPF1 from joining the termination complex**. CBP, cap-binding protein; eIF4A3, eukaryotic initiation factor 4A3; RBM8A, RNA-binding protein 8A

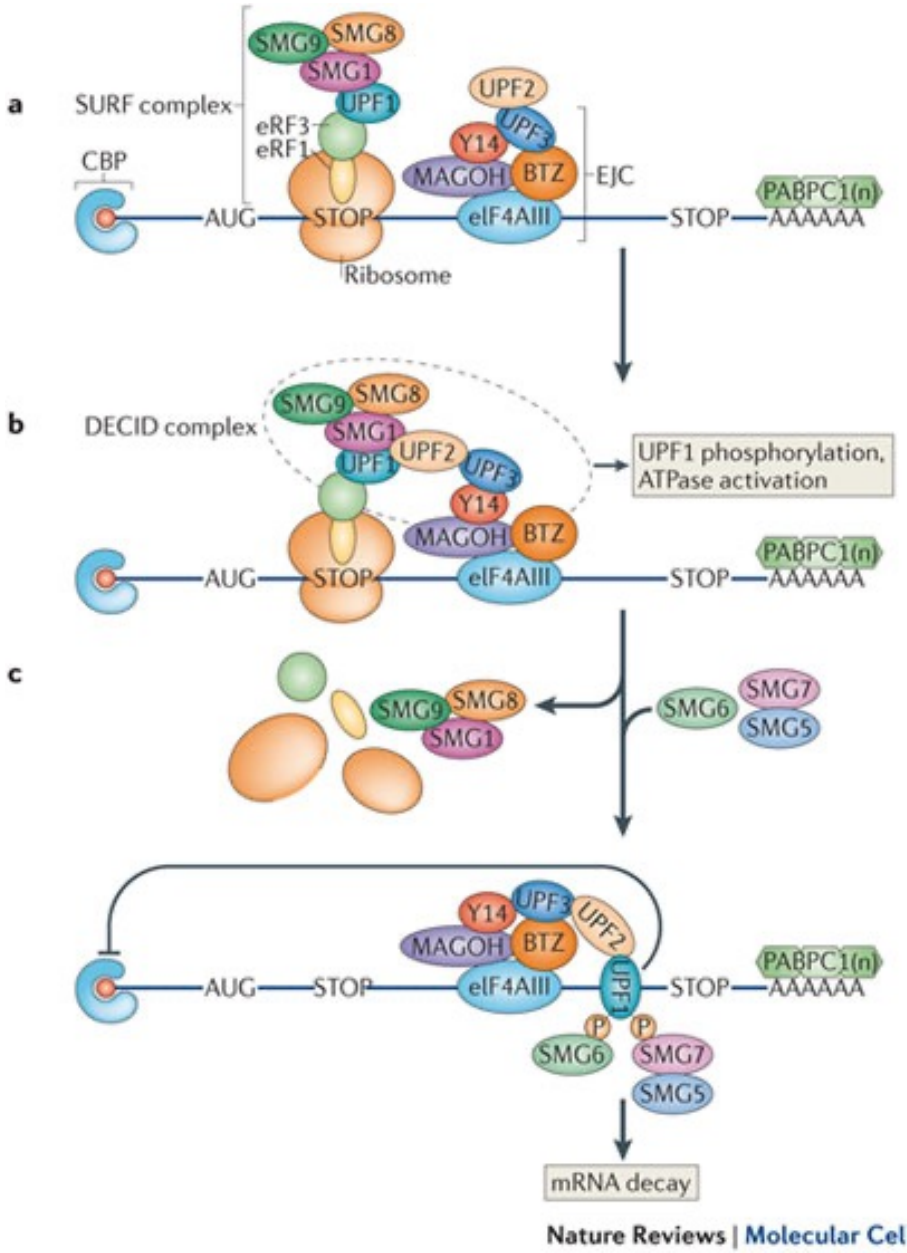
EJC-dependent NMD



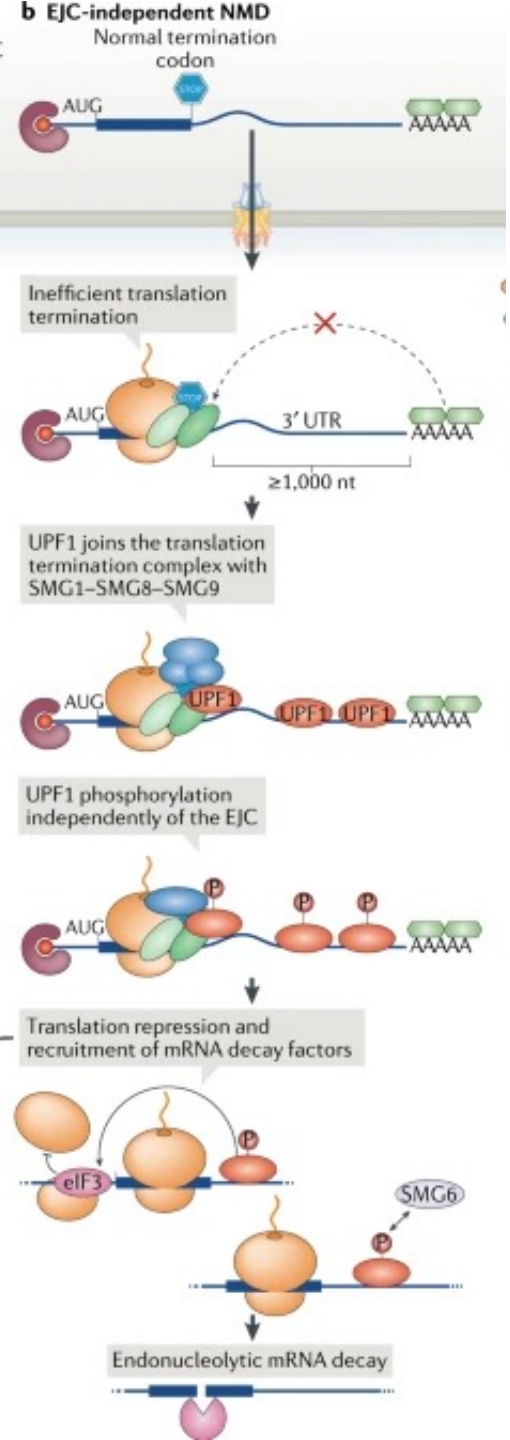
- the **ribosome will not dislocate the EJC**, which is effectively in the 3′ UTR. Because most termination codons are located in the final exon and thus downstream of exon–exon junctions, this situation is abnormal, since an EJC is located downstream of the termination codon.
- Such translation **termination is inefficient**, presumably because the EJC interferes with the interaction between polyadenylate-binding protein 1 (PABPC1) and eukaryotic release factor 3 (eRF3).
- **UPF1 and the complex of serine/threonine kinases SMG1–SMG8–SMG9** then join the eRF1–eRF3 **translation termination complex and form the SMG1–UPF1–eRFs (SURF)** complex.
- Next, **UPF1–SMG1 join the downstream EJC and form the decay-inducing (DECID) complex, where UPF1 is activated by SMG1-mediated phosphorylation (P)**. UPF1 phosphorylation represses further translation initiation and triggers mRNA decay, which is accomplished by recruiting nucleases, either directly, as in the case of endonucleolytic decay by SMG6 (not shown), or indirectly, as in the case of exonucleolytic decay through the SMG5–SMG7 heterodimer.

UPF1-P is essential for NMD

SMG-1 forms a complex (**SURF**) with **Upf1**, **eRF1**, and **eRF3**, most likely just after the recognition of the translation termination codon on post-spliced mRNAs. If the **SURF** can recognize downstream **Upf2-EJC**, the SURF associates with Upf2-EJC to form the **"decay inducing complex" (DECID)** to induce Upf1 phosphorylation and NMD.
Phosphorylated UPF1 induces <u>various mRNA decay activities</u> by recruiting decay factors or adaptor proteins for decay complexes through its N- and C-terminal phospho-sites.



EJC-independent NMD



At unusually long, unstructured 3′ UTRs, **PABPC1 is too distant from the PTC to efficiently recruit eRF1–eRF3 to initiate translation termination.**

The presence of UPF1 on the 3′ UTR increases the probability of UPF1 activation by phosphorylation and thus the probability of NMD.

UPF1-P is essential for NMD

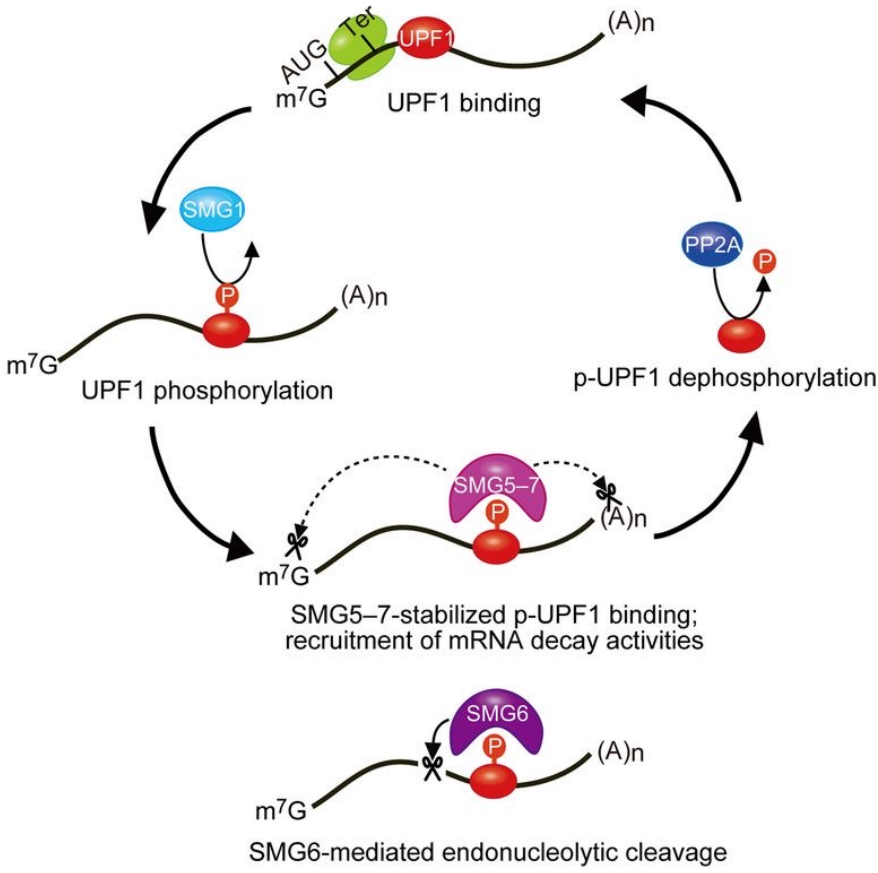
NMD is an evolutionarily conserved process that is mediated in all tested eukaryotes by the RNA-dependent helicase and ATPase **UPF1.**

UPF1 induces various mRNA decay activities.

NMD is regulated by **SMG factors**, which are proteins that are involved in a cycle of UPF1 phosphorylation and dephosphorylation.

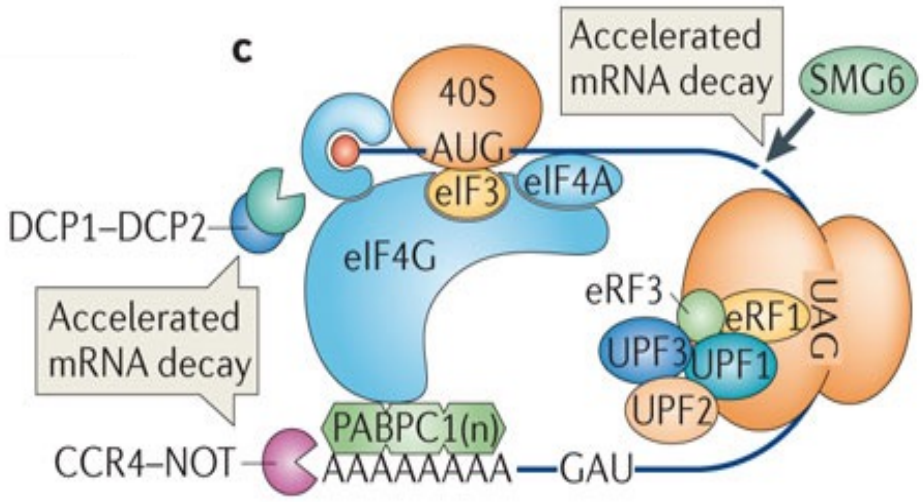
Regulated UPF1 phosphorylation by **SMG1** requires UPF1 recognition of a termination codon as one that triggers NMD. p-UPF1 recruits **SMG6** and/or **SMG5–SMG7 or DCP**, which directly or indirectly trigger(s) mRNA decay, respectively.

PP2A returns p-UPF1 to a dephosphorylated state after mRNA decay is initiated.



Degradation of NMD-targeted mRNAs

In mammals, rapid degradation of NMD targets can be achieved through at least **three different pathways**

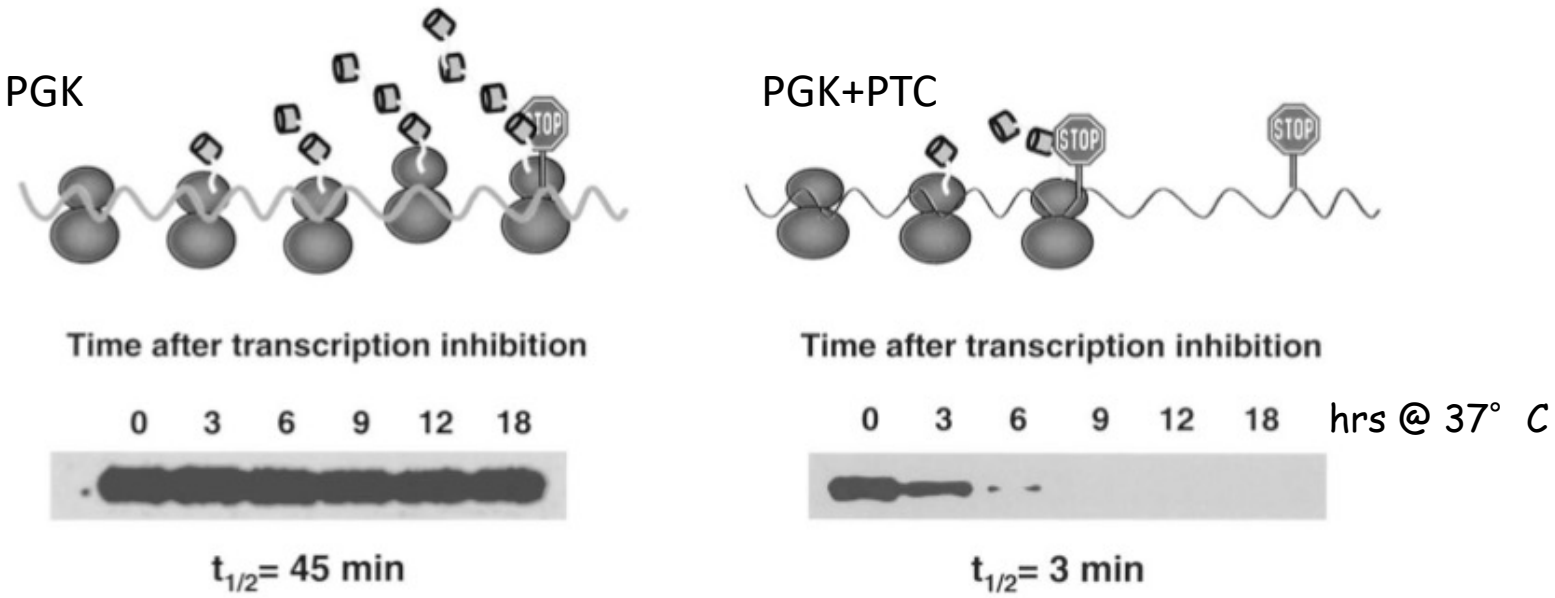


1. **SMG6 cleaves mRNA endonucleolytically** close to PTCs and in human cells the majority of NMD targets are apparently degraded through this pathway. After SMG6-mediated cleavage, for which **SMG6 needs to interact with UPF1**, the 3' RNA fragment is rapidly degraded by XRN1 and the 5' fragment seems to be **digested by the exosome**.

2. **The heterodimer SMG5–SMG7, which interacts with phosphorylated UPF1, Interacts the deadenylase CNOT8 (POP2**) interacts with this C-terminal of SMG7, indicating that SMG5-SMG7 recruits the CCR4-NOT complex to NMD targets to induce their deadenylation-dependent decapping and subsequent XRN1-mediated degradation. Consistently, SMG7-mediated mRNA decay requires the presence of DCP2 and XRN1 but not of SMG6.

3. **UPF1 also associates with decapping complex subunits DCP1A, DCP2, and PNRC2, indicating that direct deadenylation-independent decapping** might constitute a third route to degrade aberrantly terminating mRNAs

Nonsense mutations in a gene can reduce the steady-state levels of the mRNA

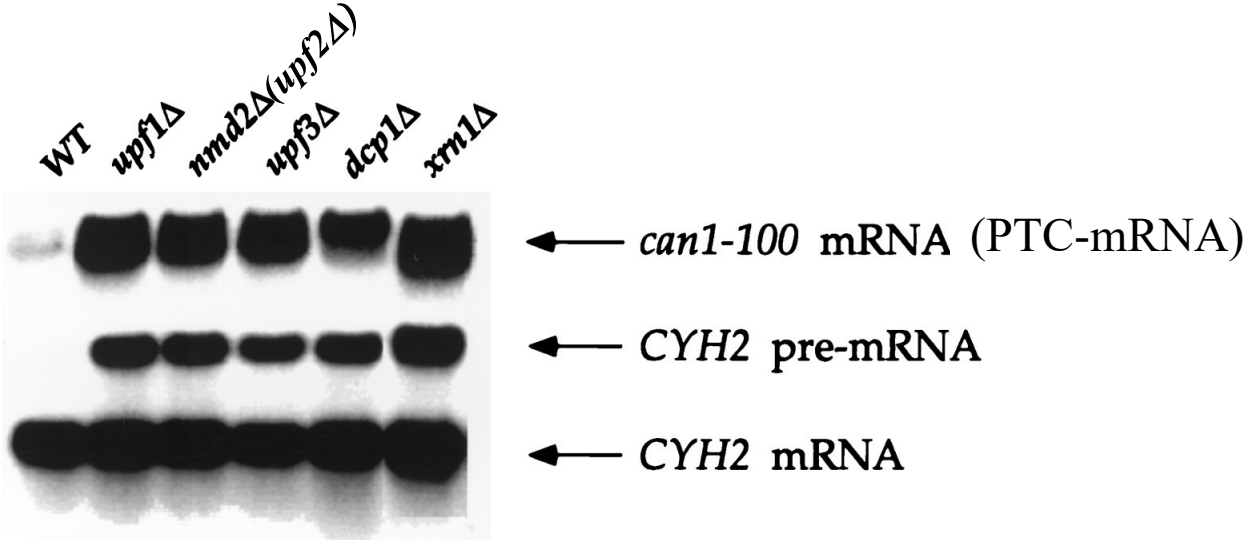


yeast strain harboring a temperature-sensitive RNA polymerase II

Upf1, Upf2 and Upf3

•They were identified by genetic screening as **suppressors of nonsense mutations**

•Deletion of either Upf1, Upf2 or Upf3 leads to a nonsense suppression phenotype



NMD is also crucial to regulate gene expression and for maintaining genome stability

There is a growing appreciation that NMD and proteins that are key players in the NMD pathway have important functions other than mRNA quality control.

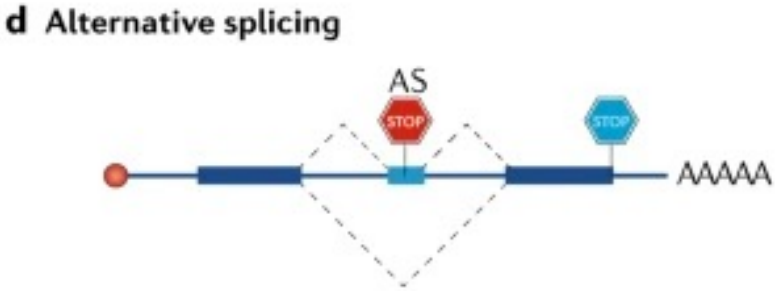
These functions include **regulation of the expression of certain classes of genes**, roles in specialized pathways of mRNA decay, functions in DNA synthesis and cell-cycle progression, and contributions to the maintenance of telomere.

Gene expression profiles of *S. cerevisiae*, *D. melanogaster* and human cells that completely or partially lack an NMD factor have indicated that **a significant fraction of cellular transcripts (1–10%) are upregulated and thus affected by NMD.**

NMD is also crucial to regulate gene expression and for maintaining genome stability

NMD can be associated to alternative splicing to regulate gene expression

Alternative splicing (AS) that generates a termination codon that can function as a PTC with any of the above-described features. Alternative splicing may also convert a normal termination codon into one that triggers NMD

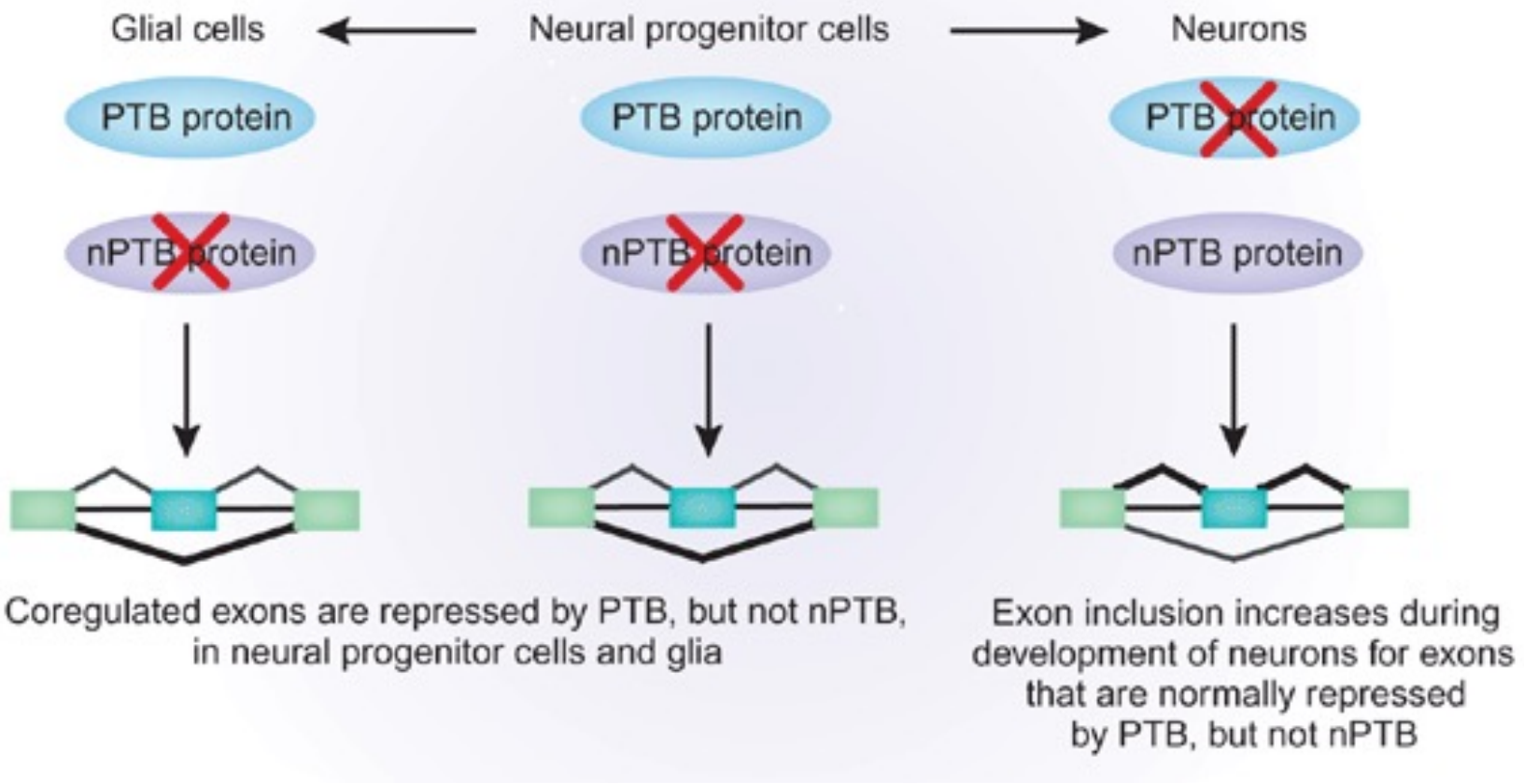


Role of NMD in the production of PTB proteins

Polypyrimdine tract binding proteins (PTB)

PTB is expressed in many tissues but not in neurons and muscles

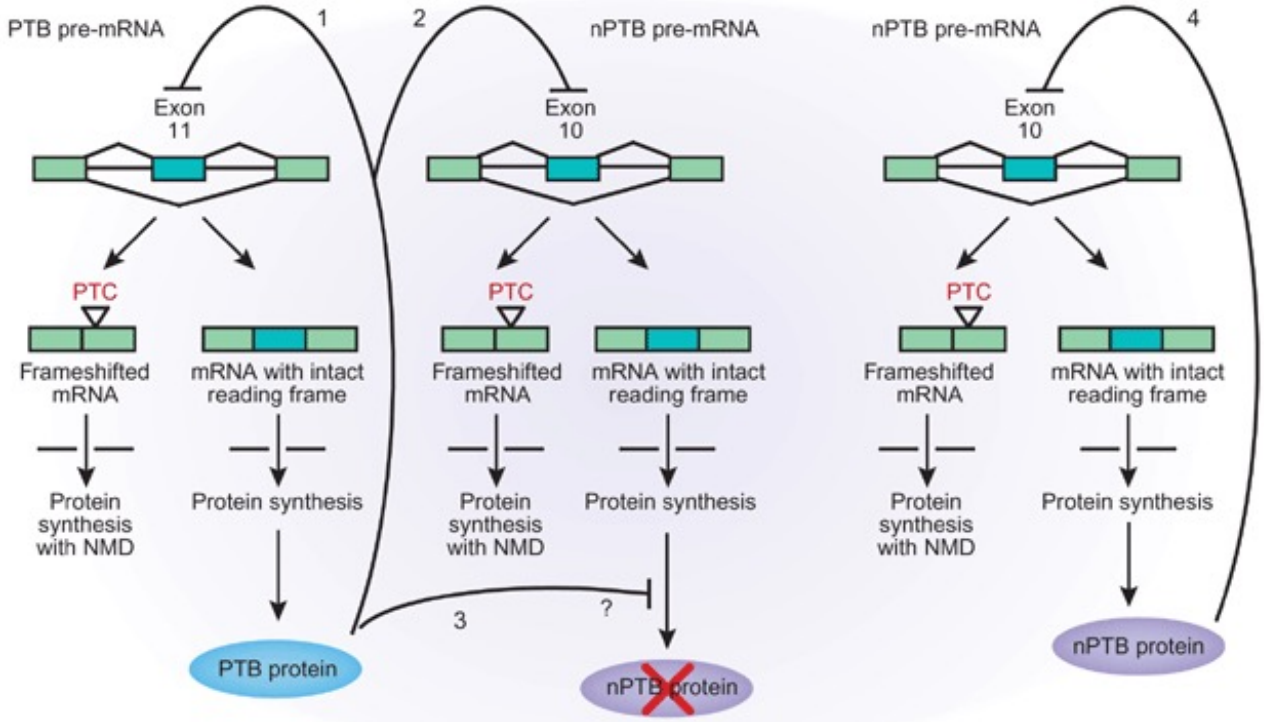
nPTB is expressed in neurons



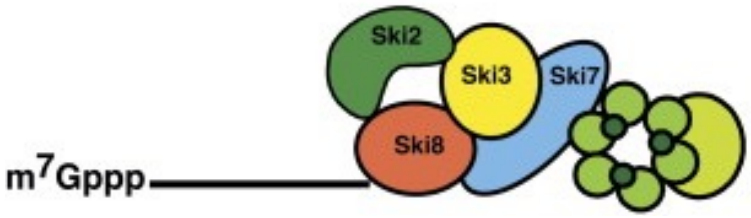
PTB/nPTB regulation

PTB regulates its own expression by inducing the skipping of exon 11 leading to the formation of a **PTC**, which promotes NMD. **PTB also represses nPTB** expression at the level of splicing by inducing exon 10 skipping. As with PTB autoregulation, the frameshifted mRNA contains a PTC, which targets it for destruction by NMD.

In neuronal cells, **nPTB accumulates because PTB is no longer expressed**. nPTB autoregulates its own synthesis by NMD at the level of exon 10 skipping when PTB is absent.



3’-5’ decay…what’s next?



the remaining mRNA fragment with its 5’-cap (m7GpppG) is degraded by **scavenger decapping enzymes**: **Dcs1p** in *S. cerevisiae*, DCPS in mammals and possibly also in other organisms, as it is evolutionarily conserved.

DCPS is capable of efficiently hydrolyzing capped RNA substrate when its length **does not exceed 10 nt.**

Human DCPS localizes to both cytoplasm and nucleus, in contrast to its mostly cytoplasmic homologues from lower eukaryotes, suggesting that mammals may have adopted DCPS to act in both compartments.

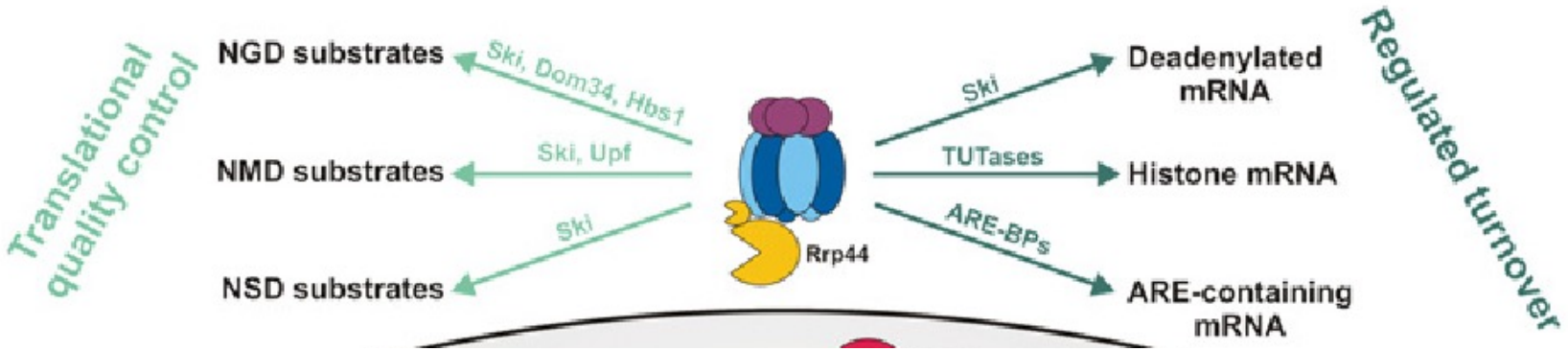
ARE-mediated decay (AMD)

•Several sequence elements within transcripts have been linked to the control of mRNA turnover. **AU-rich elements (ARE)** in 3'-UTR characterize most short-lived transcripts

•Destabilizing **RNA-binding proteins** have been shown to direct ARE-containing mRNA to the exosome

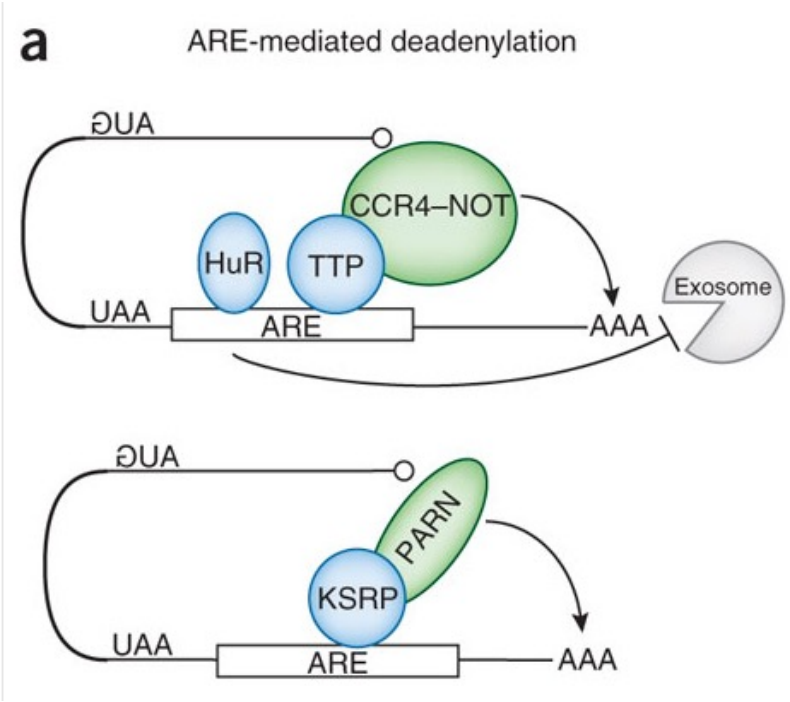
•RNA can be stabilized by proteins that compete for ARE binding and direct transcripts to the polysome for translation

•**AREs** are found in mRNAs involved in cellular responses to environmental and/or metabolic changes: cyclins, cytokines (GM-CSF, IL-3 etc.), oncogenes (c-myc, c-fos etc.)……



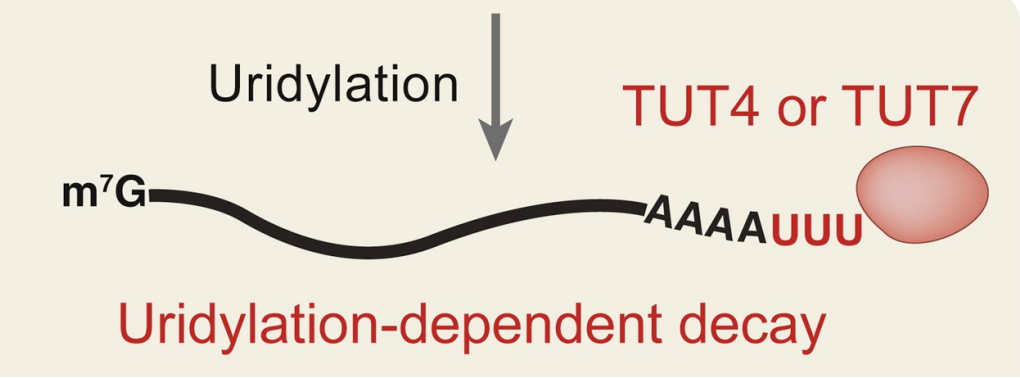
AREs drive mRNA deadenylation

The binding of the ARE-BPs **TTP** and **KSRP** on AREs induces rapid **mRNA deadenylation** by the recruitment of the deadenylases CCR4–NOT complex and PARN, respectively. The binding of **HuR** stabilizes the transcript by inhibiting **exosome recruitment**.



Uridylation by TUT4 and TUT7 Marks mRNA for Degradation

Over the past few years it has become evident that not only deadenylation, but also extension of the 3' -ends of protein-coding transcripts with stretches of uridine residues, i.e. uridylation, may serve as an initial signal triggering mRNA decay in the cytoplasm.

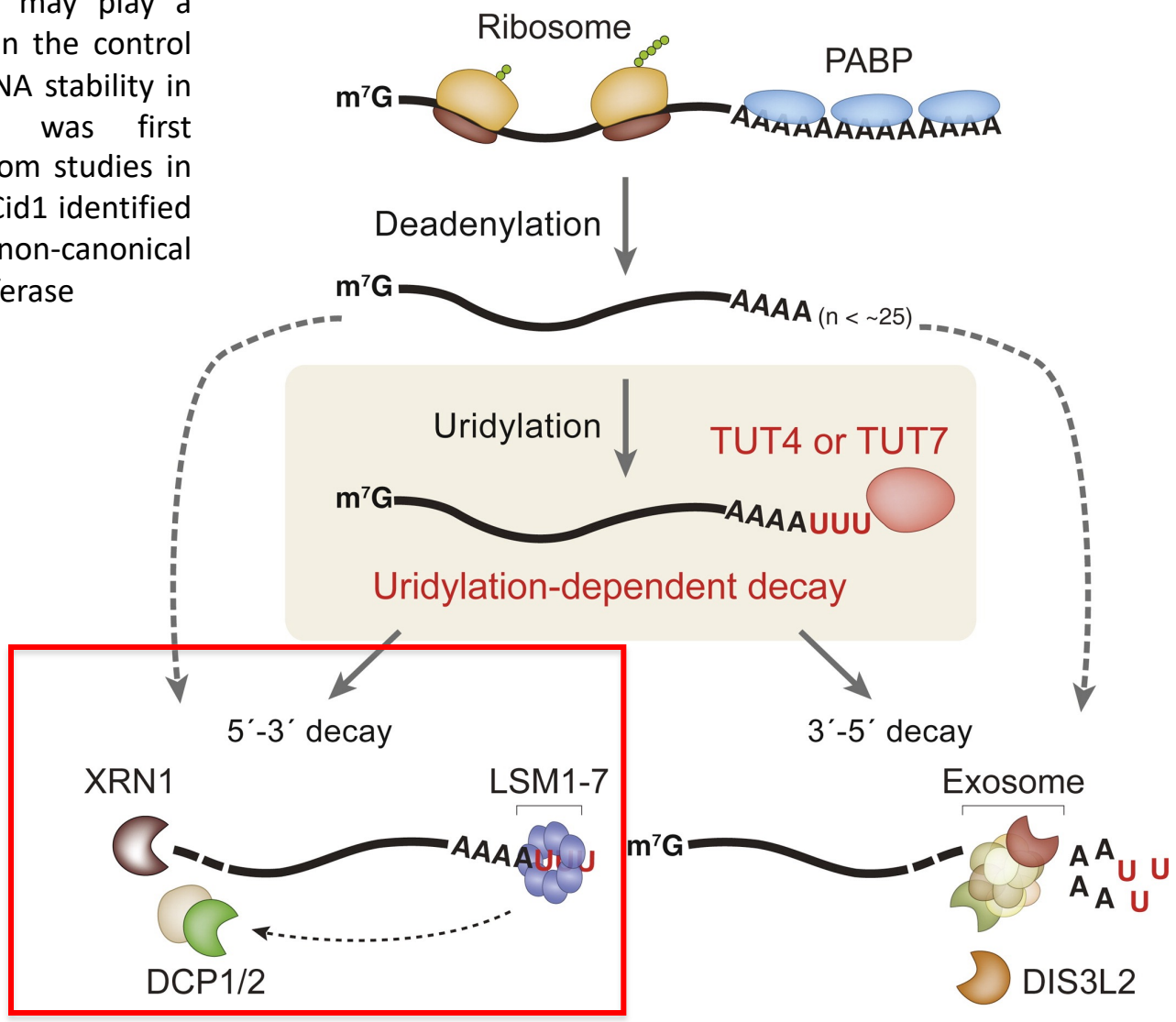


The human genome encodes 7 non-canonical RNA nucleotidyltransferases, with some of them preferentially adding uridine instead of adenine, functioning more as terminal uridyltransferases (TUTases) or poly(U) polymerases (PUPs)

Uridylating enzymes have been found in all eukaryotes, with the exception of S. cerevisiae

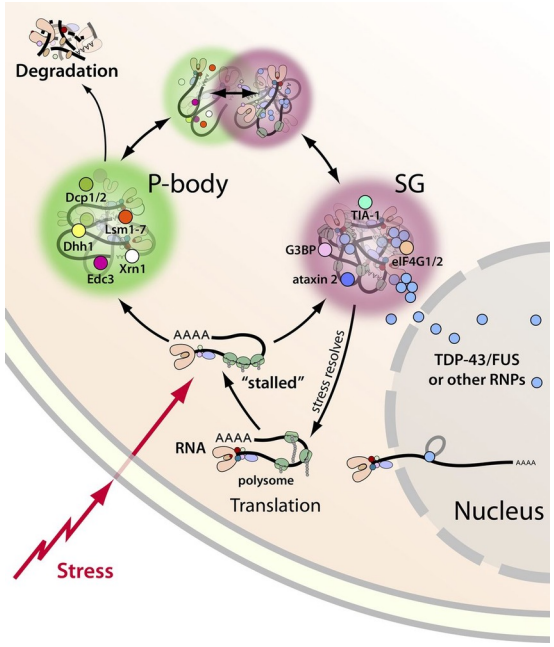
Uridylation-dependent mRNA decapping

That uridylation may play a prominent role in the control of poly(A)+ mRNA stability in the cytoplasm was first demonstrated from studies in S. pombe with, Cid1 identified as the first non-canonical nucleotidyltransferase



Evolutionary conserved mechanism

P-bodies and Stress granules



SGs and PBs are transient cytoplasmic membraneless compartments of RNA and protein, and represent new frontiers for understanding translational control.

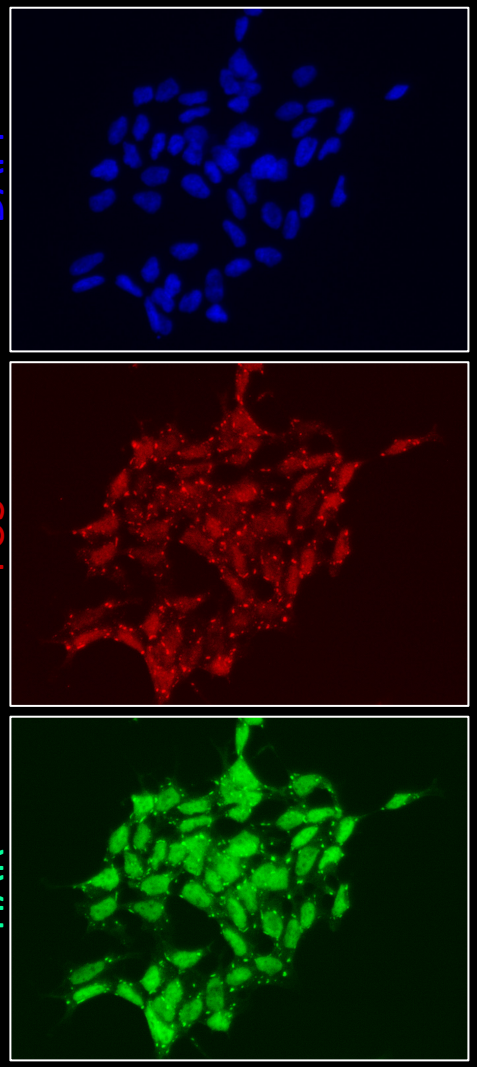
Stress granules (SGs) and processing bodies (PBs) are microscopically visible cytoplasmic membraneless organelles, consisting of RNA and proteins.

SG formation is stress induced, whereas PBs may exist constitutively but increase in size and number with stress

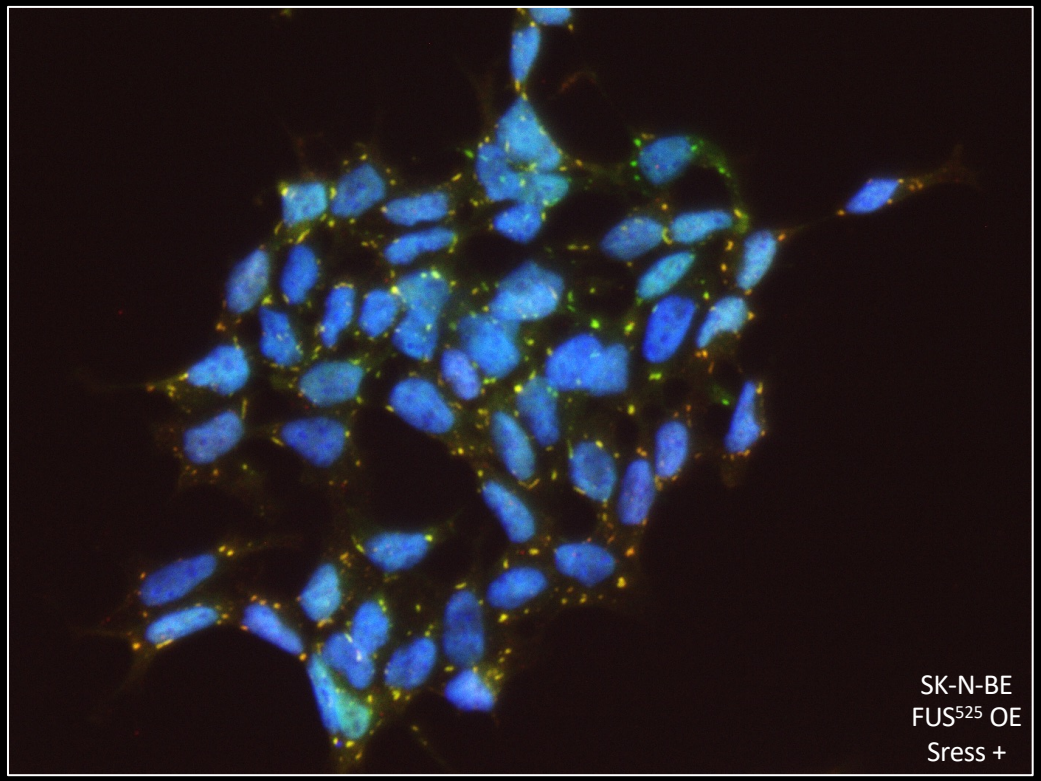
SGs and PBs condense into being through liquid–liquid phase separation (LLPS), a fundamental process that creates liquid-like compartments of RNA and proteins, and is crucial to the integrity of SGs, PBs

They contain mRNA and components of the nonsense-mediated decay pathway, and the 5'–3' mRNA decay machinery, including decapping enzymes and endonucleases. They also contain factors involved in small RNA-guided gene silencing.

P-bodies and Stress granules



MERGE



Suggested Readings:
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