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

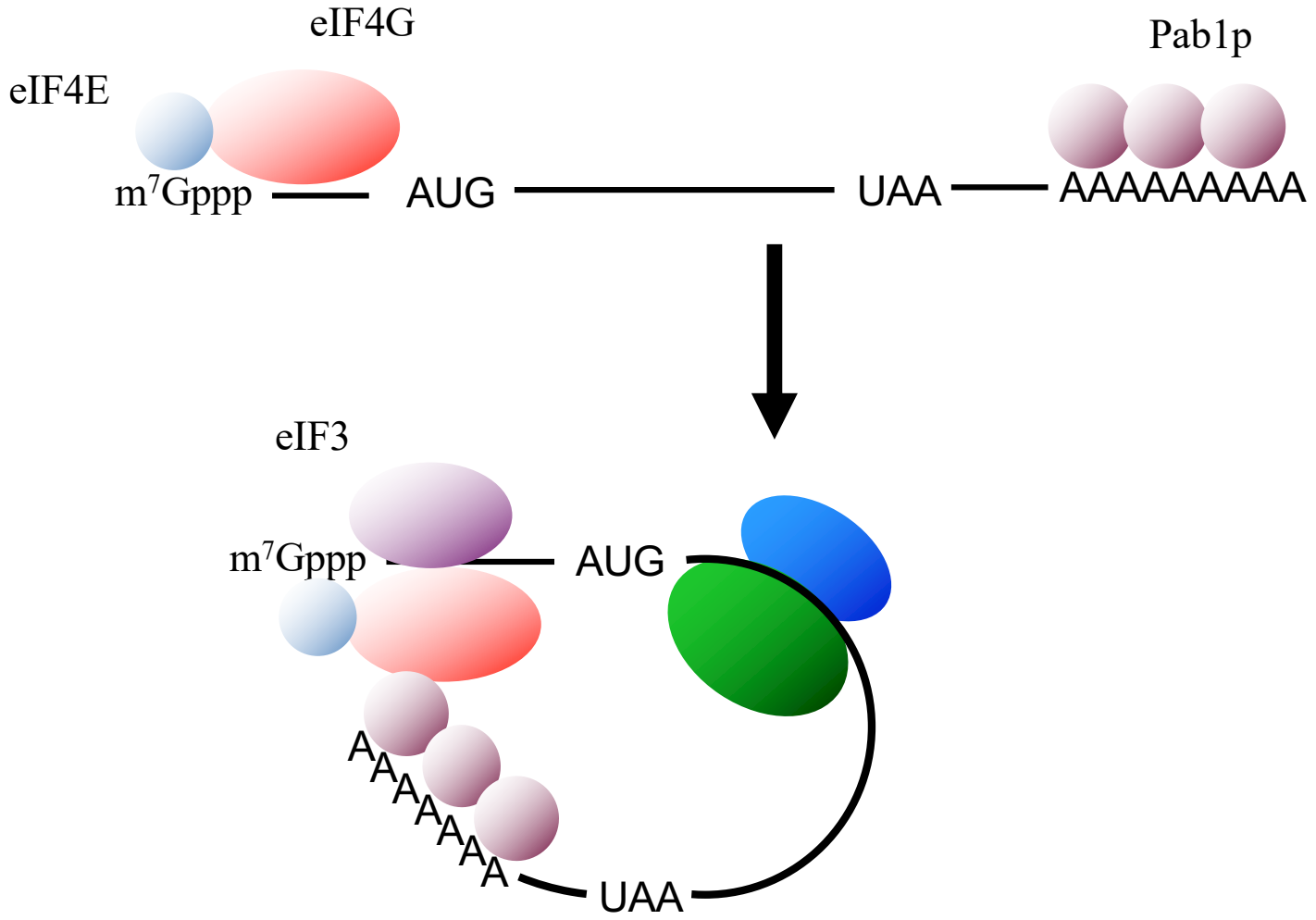
4. multiple parallel, partially redundant, mRNA decay pathways operate in the cytoplasm.

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

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

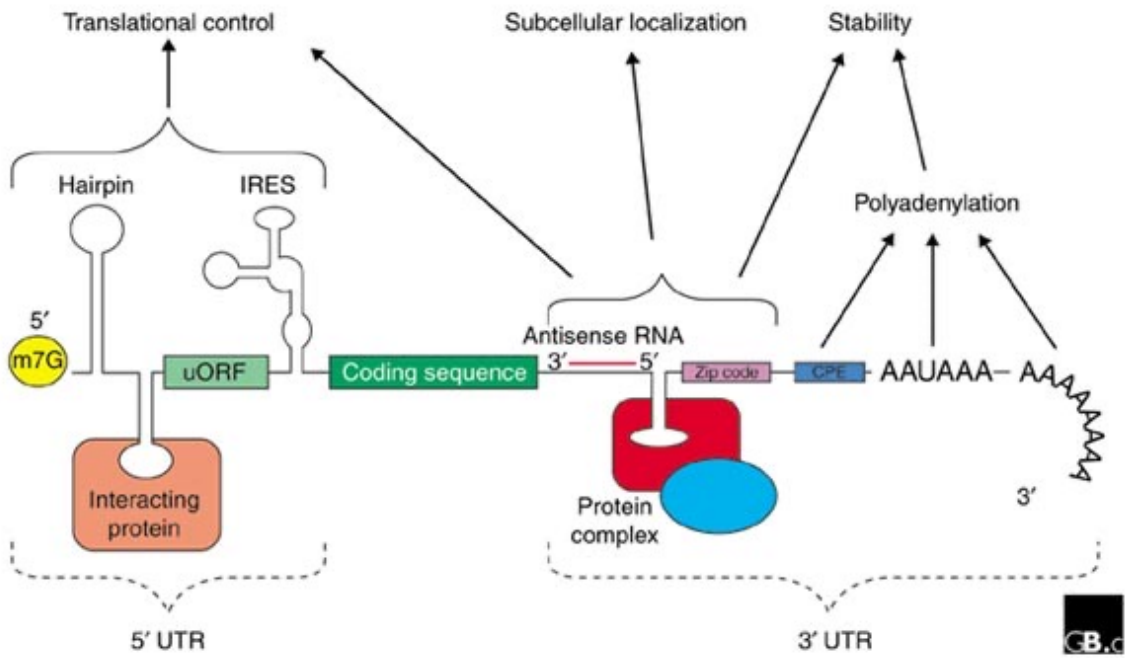
7. mRNA degradation is coupled to translation.

mRNA

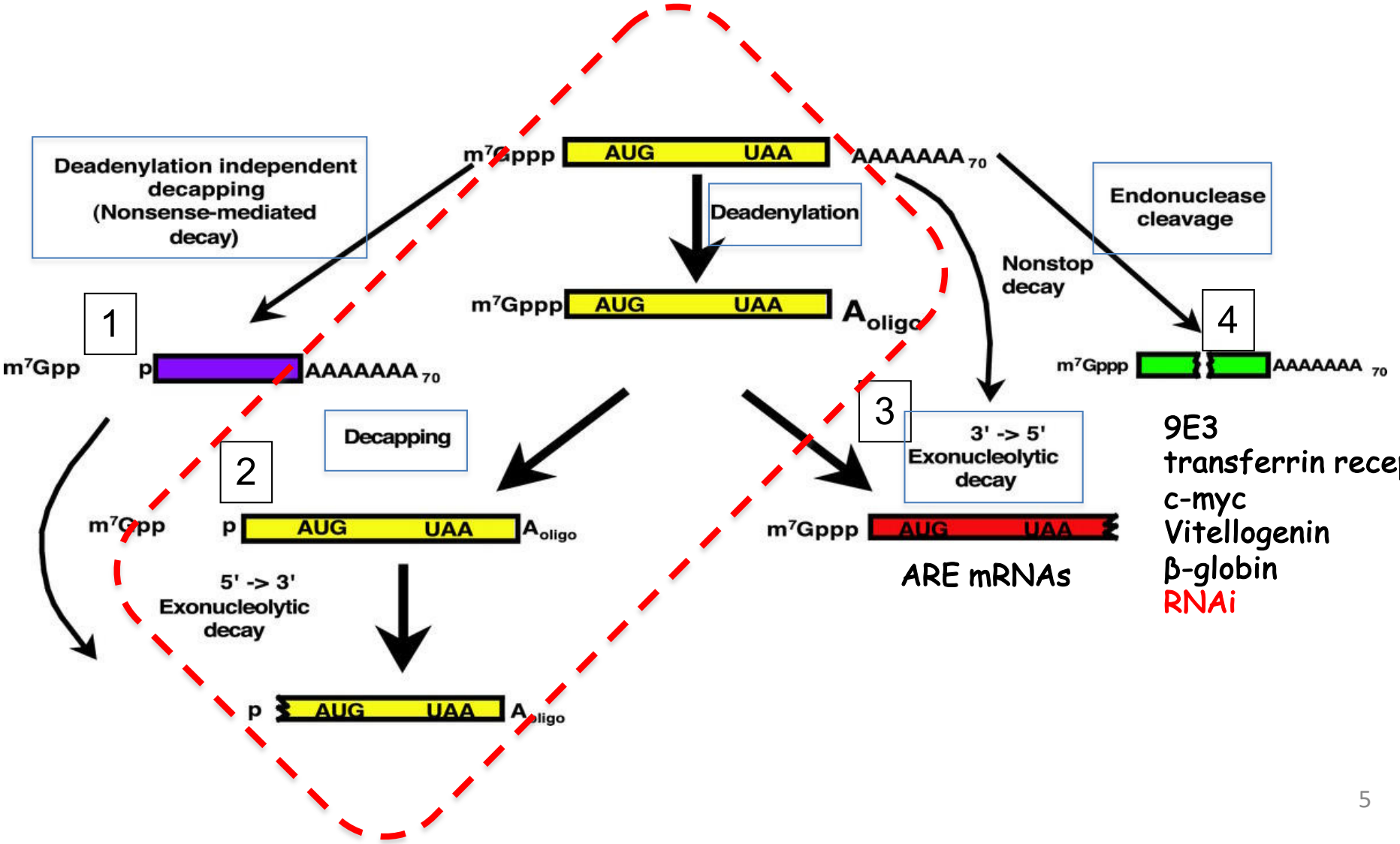

eIF4G
eIF4E
Pab1p
m7Gppp
AUG
UAA
AAAAAAAAA
eIF3
m7Gppp
AUG
AAAAAAA
UAA

Regulatory elements of mRNA

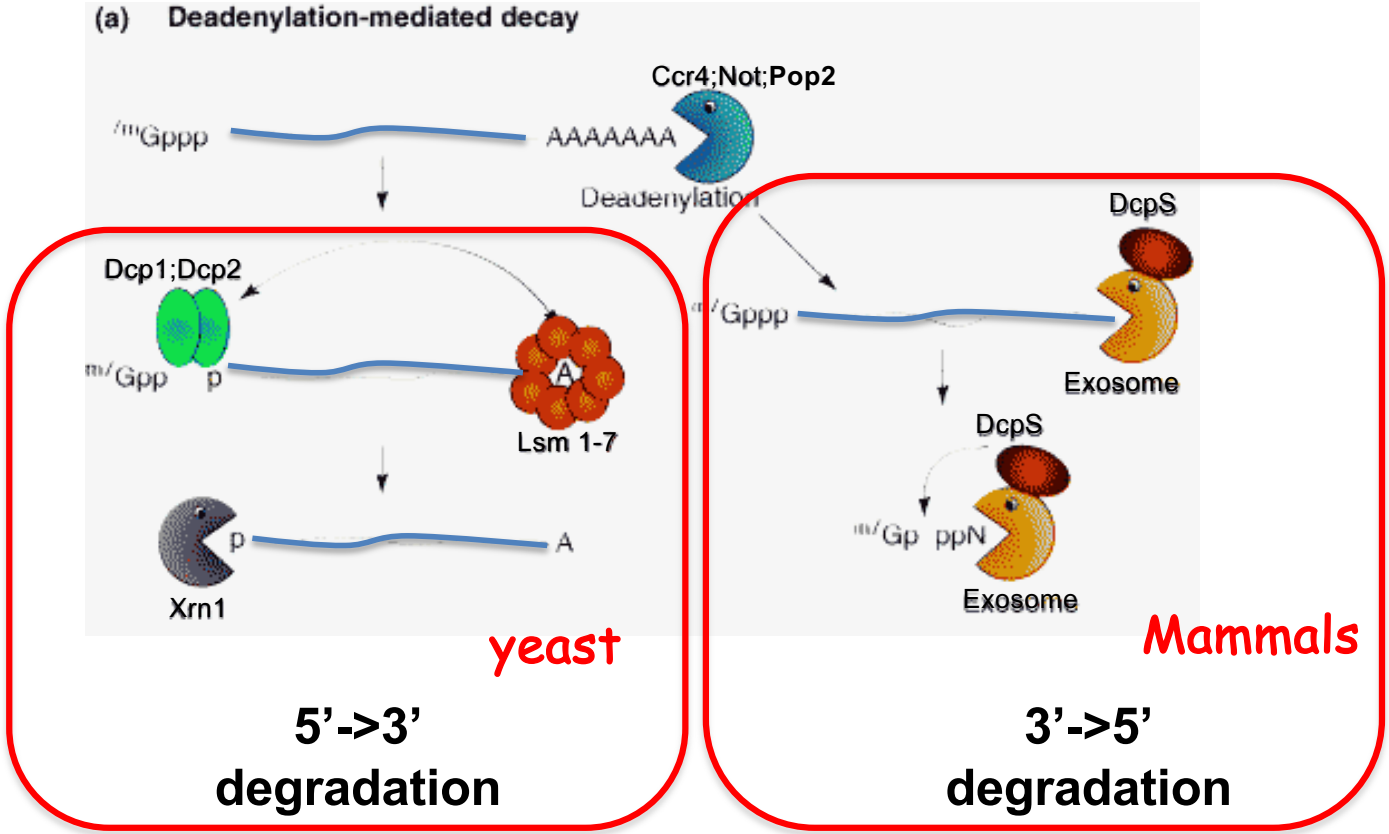
mRNAs contain several regulatory elements recognized by specific regulatory factors: **proteins** and **RNAs**, which allow fine-tuned regulation of gene expression by controlling its translation, degradation and localization.



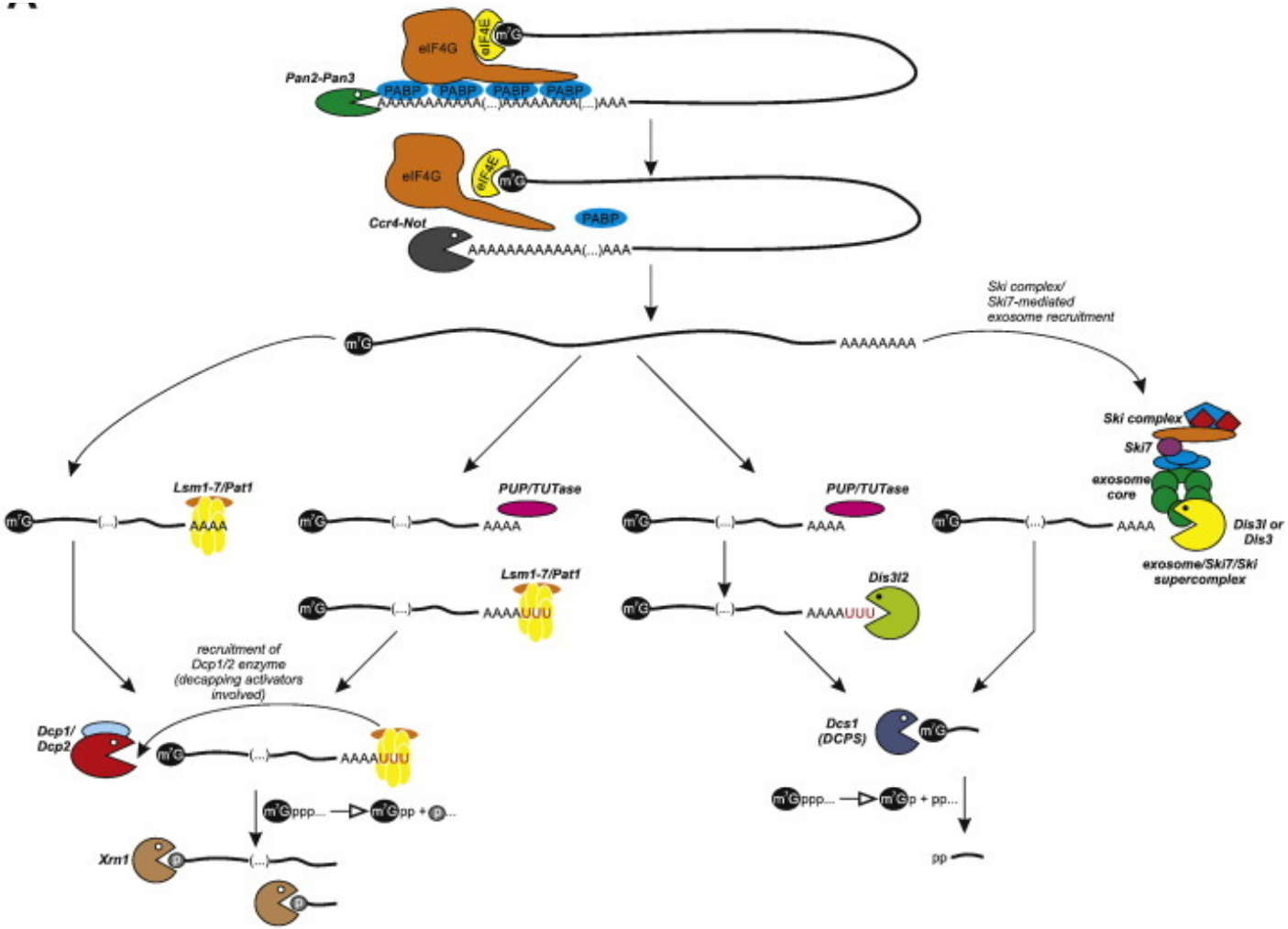
Pathways by which eukaryotic mRNAs are degraded



Different mRNA decays operate in yeast and mammals



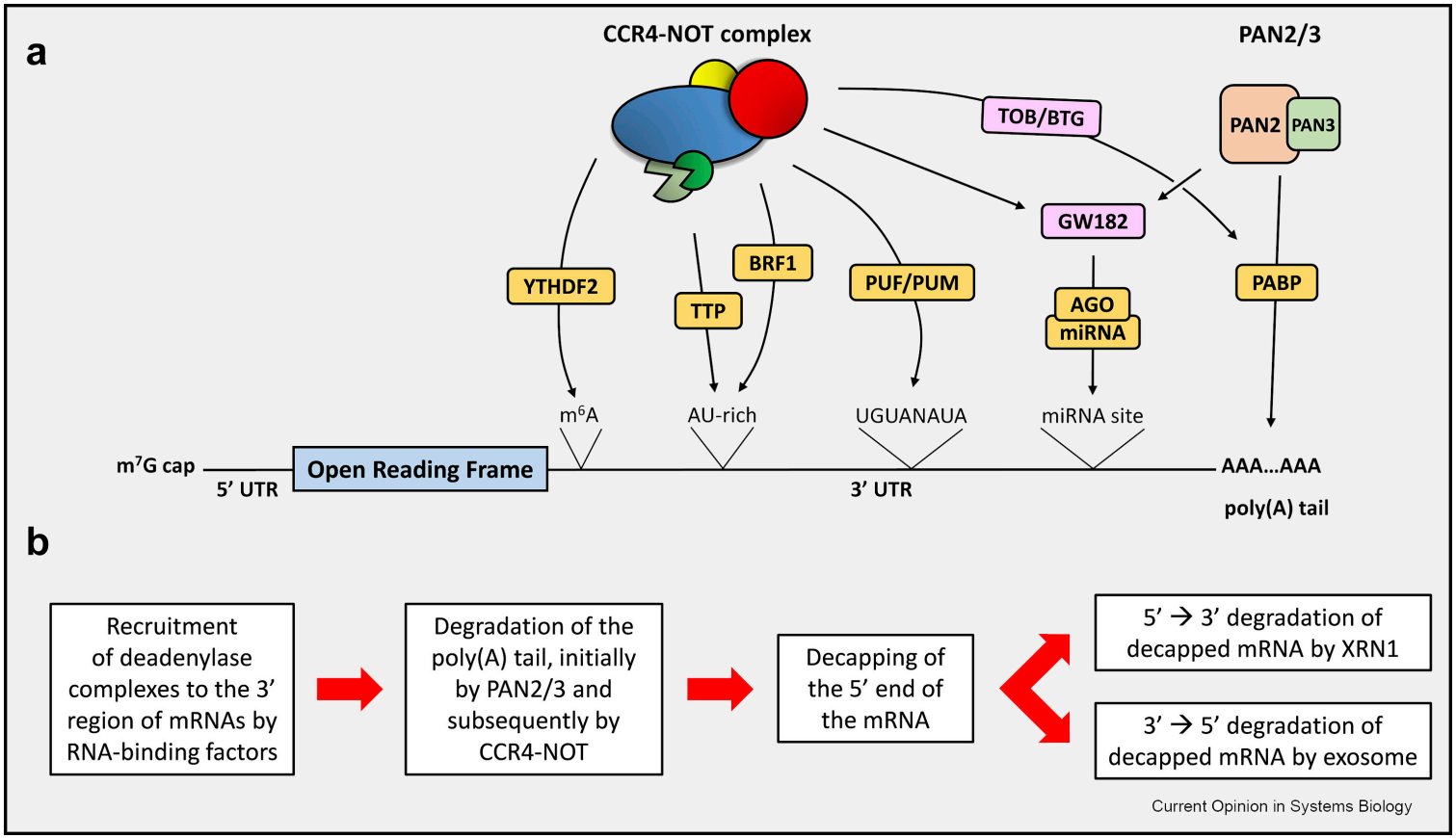
PolyA mRNA



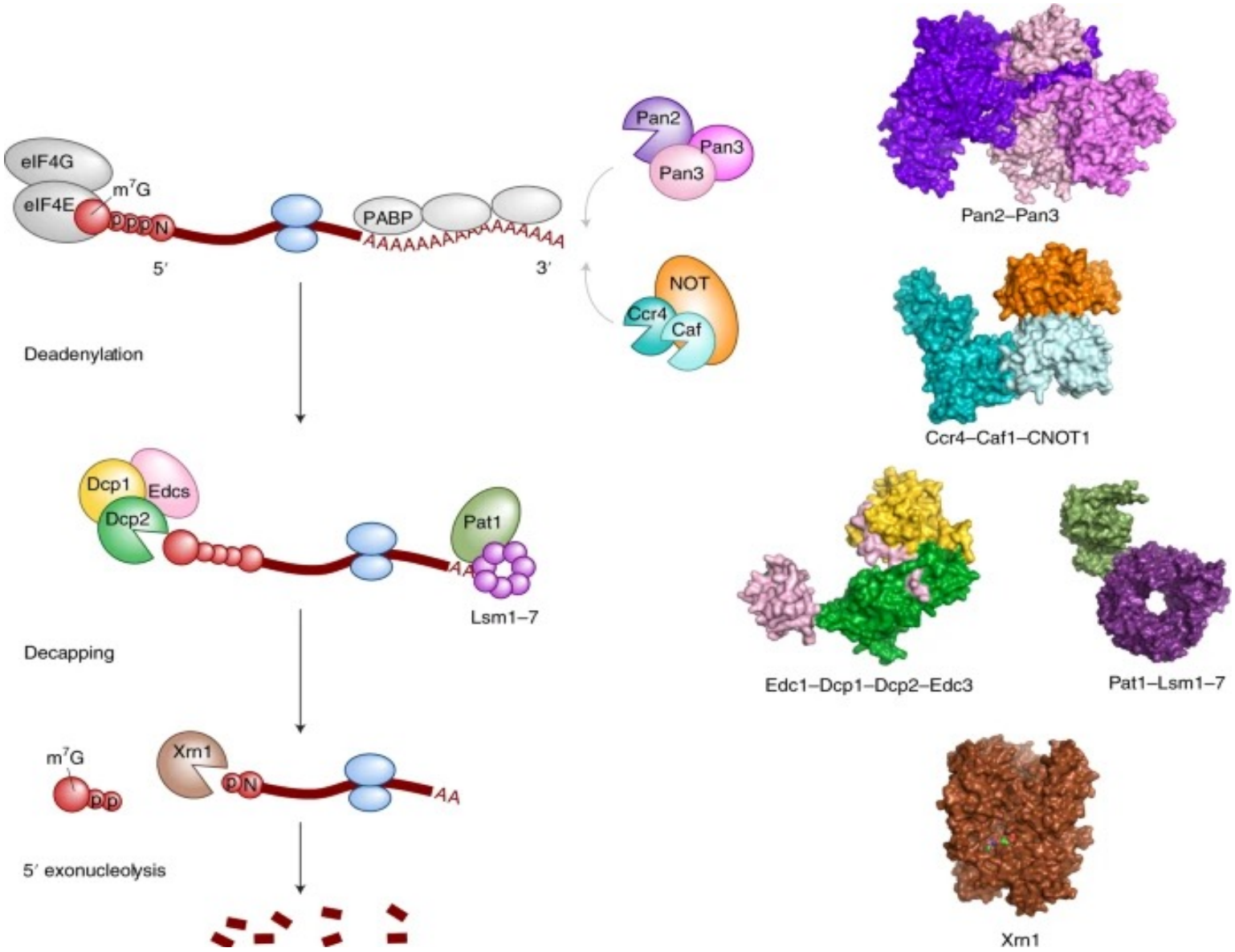
While deadenylation and decapping were long considered as consecutive events, it is now known that cap removal can be uncoupled from poly(A) tail shortening.

Therefore, rates of deadenylation, decapping or exoribonucleolytic digestion can each be limiting to the overall transcript degradation speed.

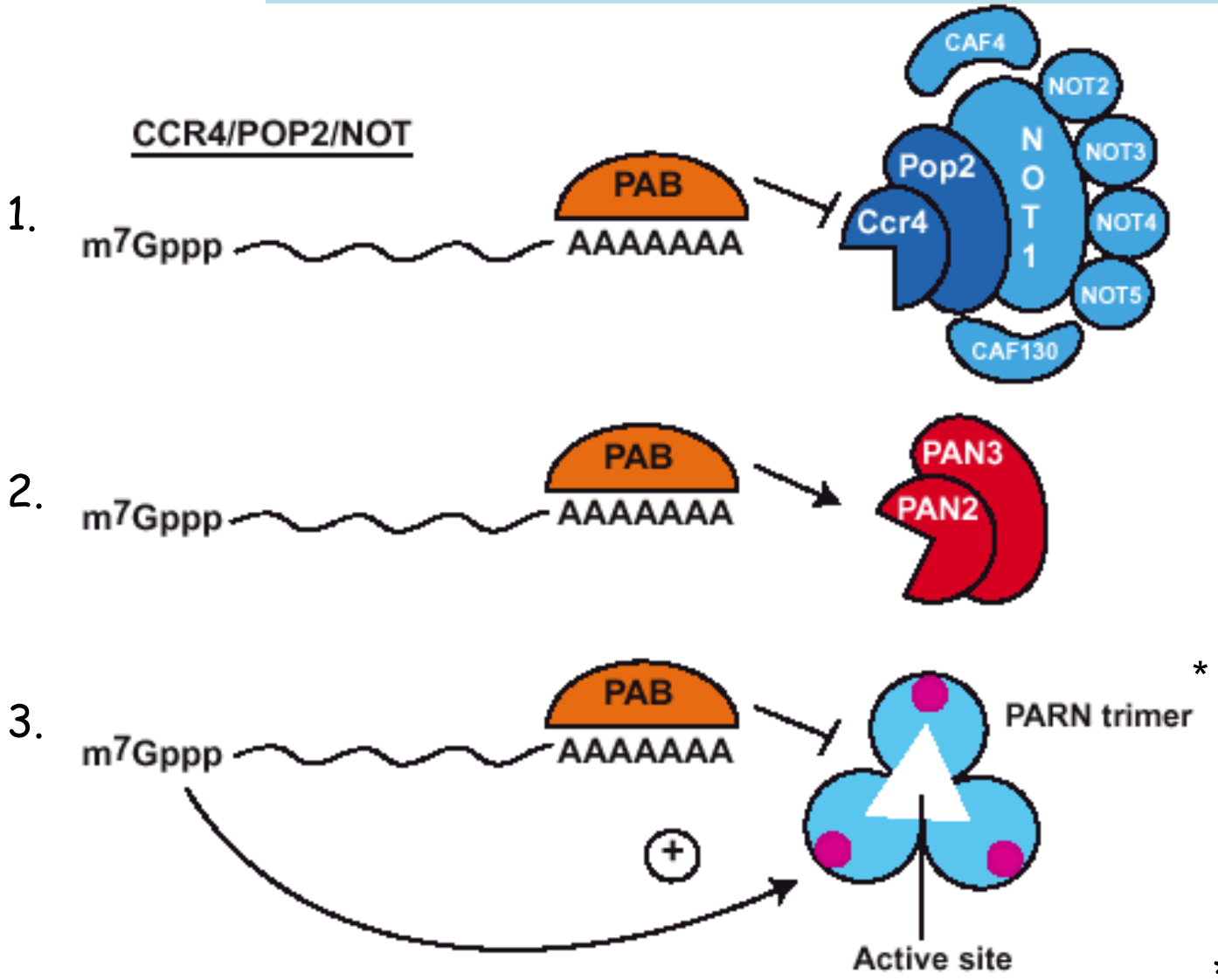
The mechanism of mRNA decay



The mechanism of mRNA decay



Eukaryotic mRNA deadenylases



*Only in metazoa

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

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.

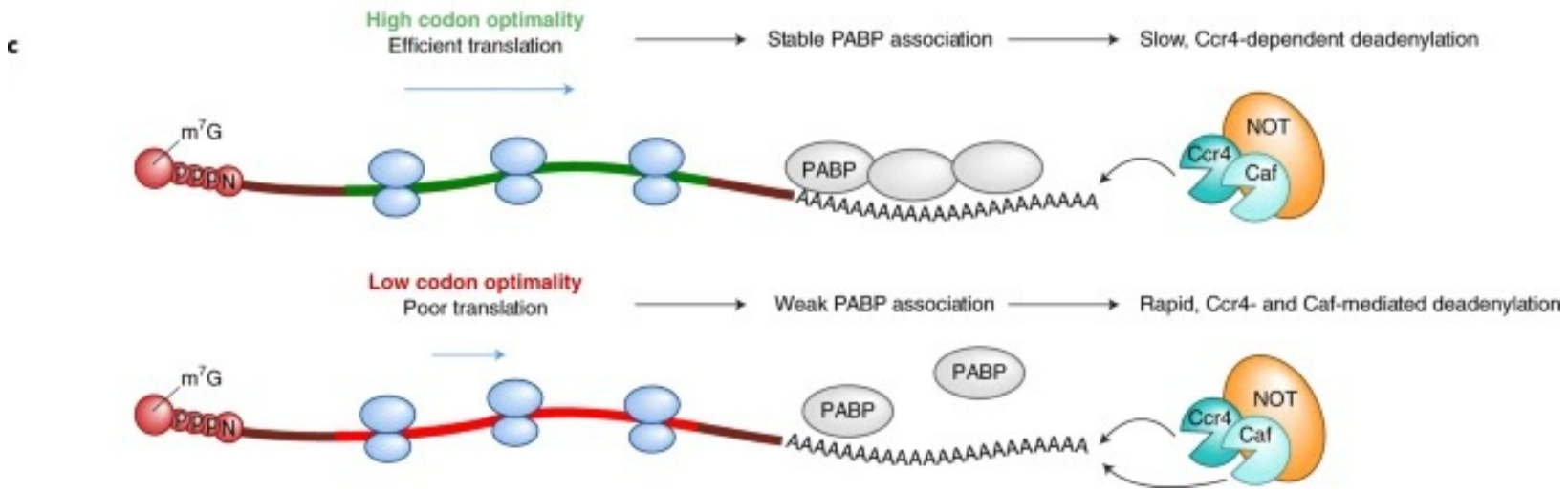
Mammalian genomes encode several **homologues** of Ccr4 (Ccr4a, Ccr4b, NOC/Ccr4c, ANGEL2/Ccr4d, ANGEL1/Ccr4e).

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

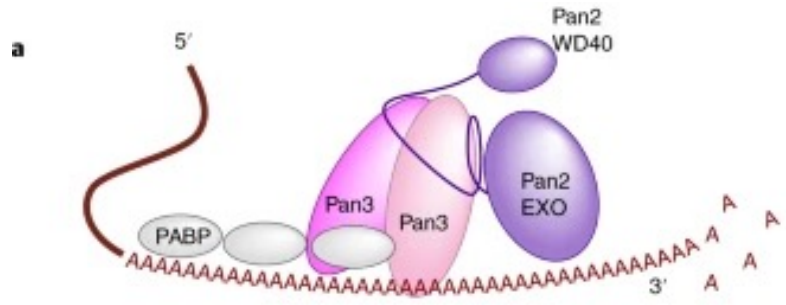


Adaptor proteins such as Mmi1, TTP, Tob/BTG, DDX6, GW182, and others can bind to different surfaces of the CCR4–NOT complex to activate and recruit deadenylase activity to specific mRNAs and decay pathways.

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.

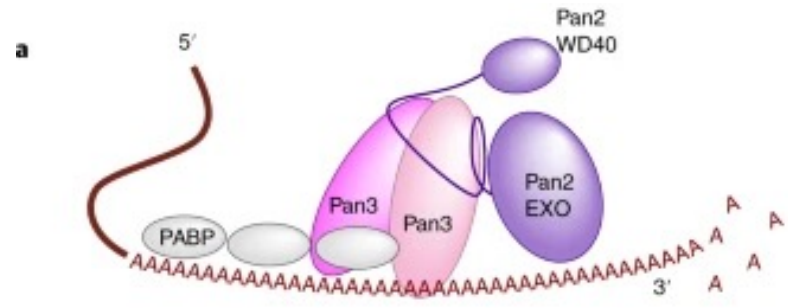
Pan2 acts as a **catalytic** subunit.

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

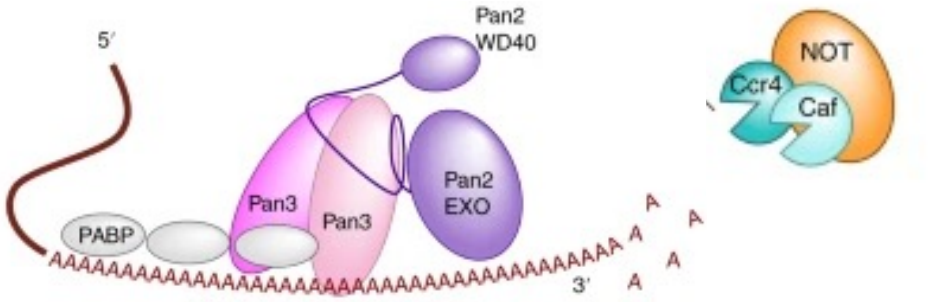


PAN3 recognizes both **PABP and poly(A)** RNA to recruit the complex to RNA 3’ tails and simultaneously stimulates PAN2 deadenylase activity.

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



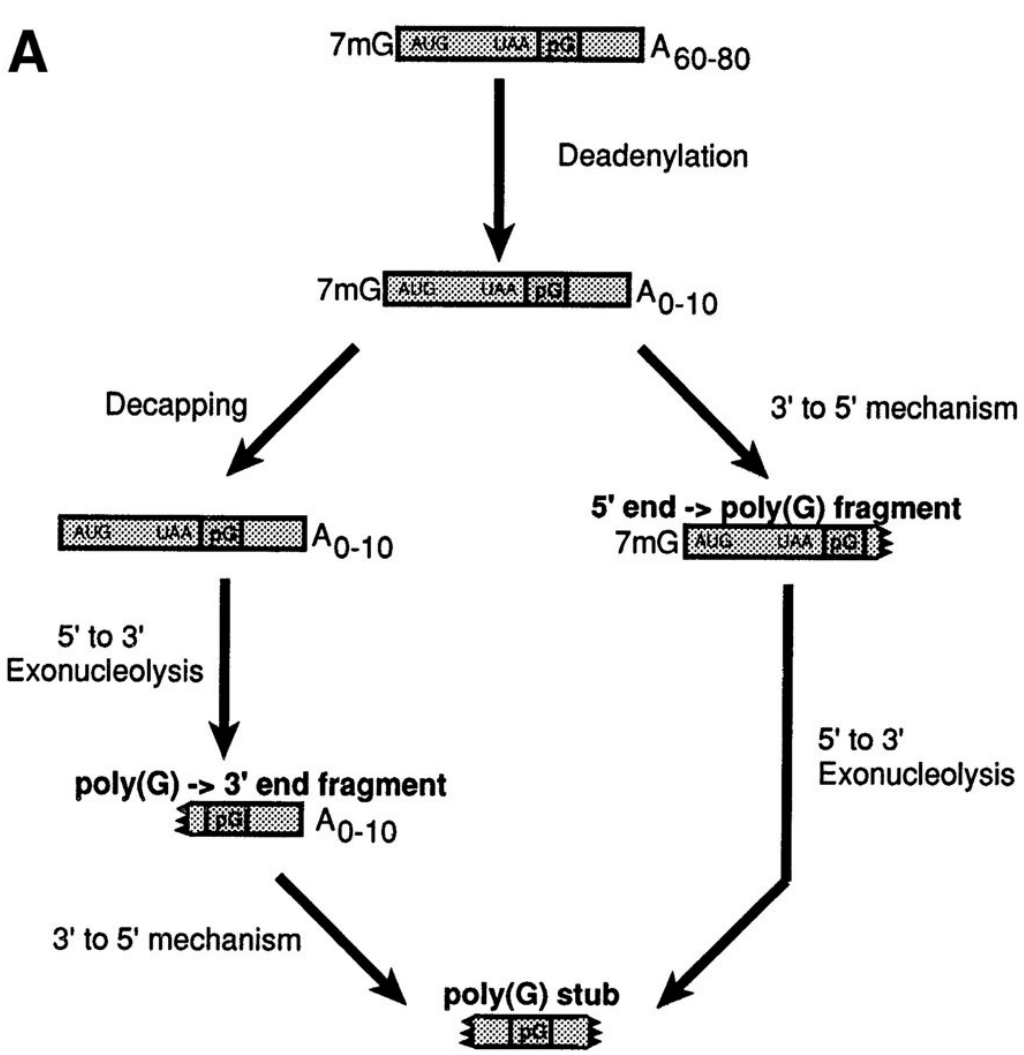
The sequential model for mRNA deadenylation, in which PAN2–PAN3 carries out initial 3′ poly(A) tail shortening followed by more complete deadenylation by CCR4–NOT to trigger 5′ decay, is an oversemplification.

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

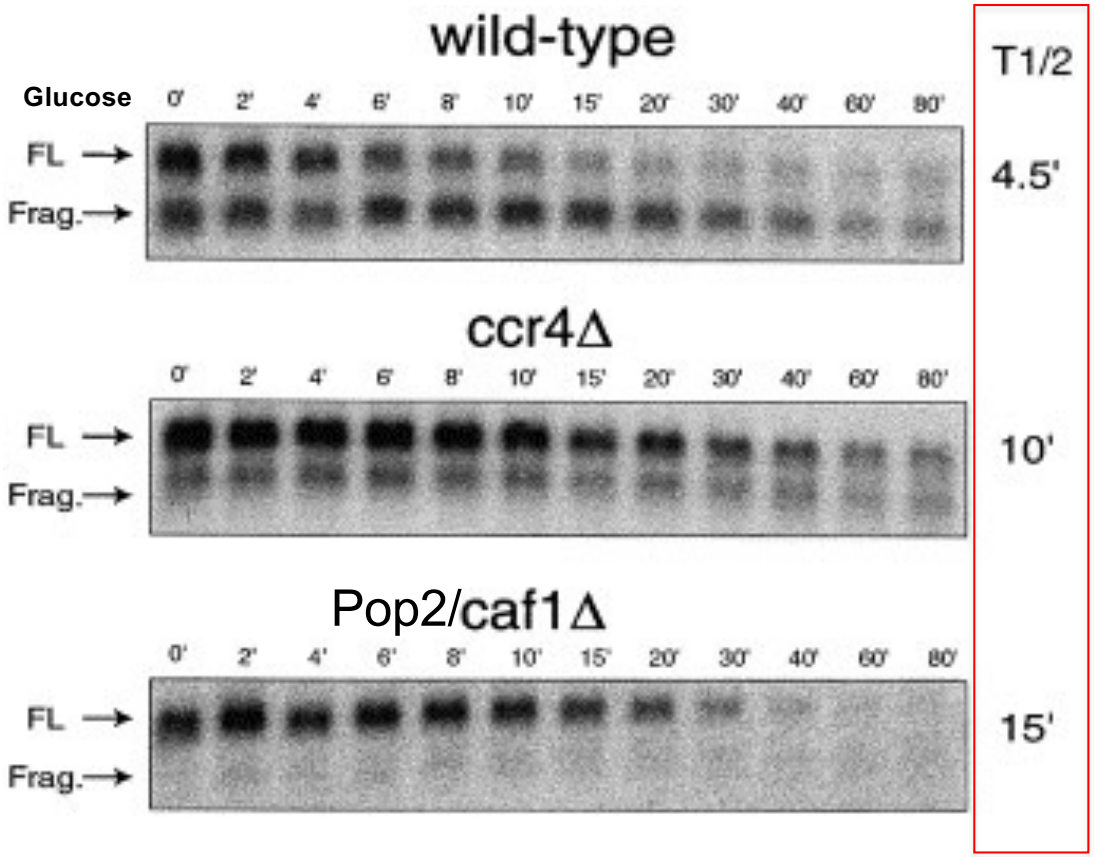
How to study mRNA decay in yeast



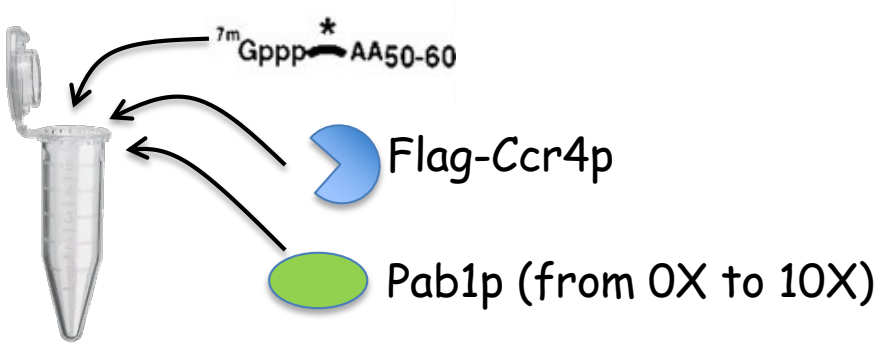
- Transcription must be off (GAL or Tet-OFF promoter)
- The PolyG cassette (pG) blocks exonuclease activity

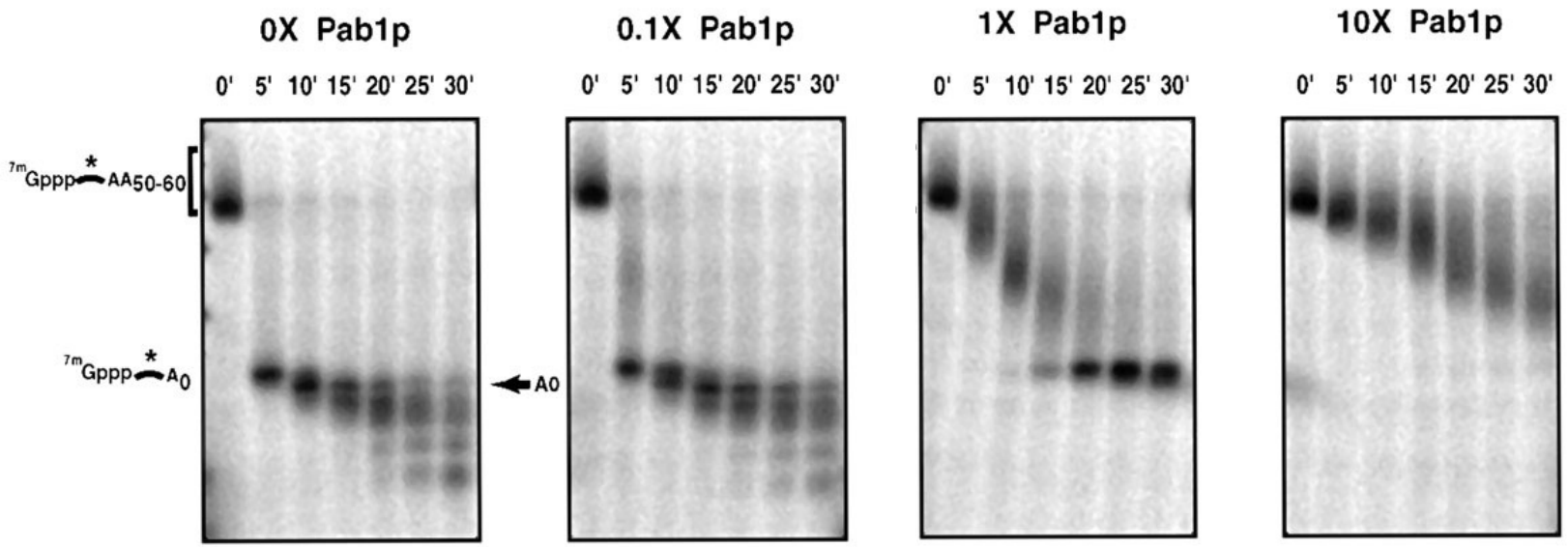
Ccr4p and Pop2/Caf1p Are Required for Normal Rates of mRNA Turnover

Measurement of the Decay Rate of the MFA2pG mRNA:



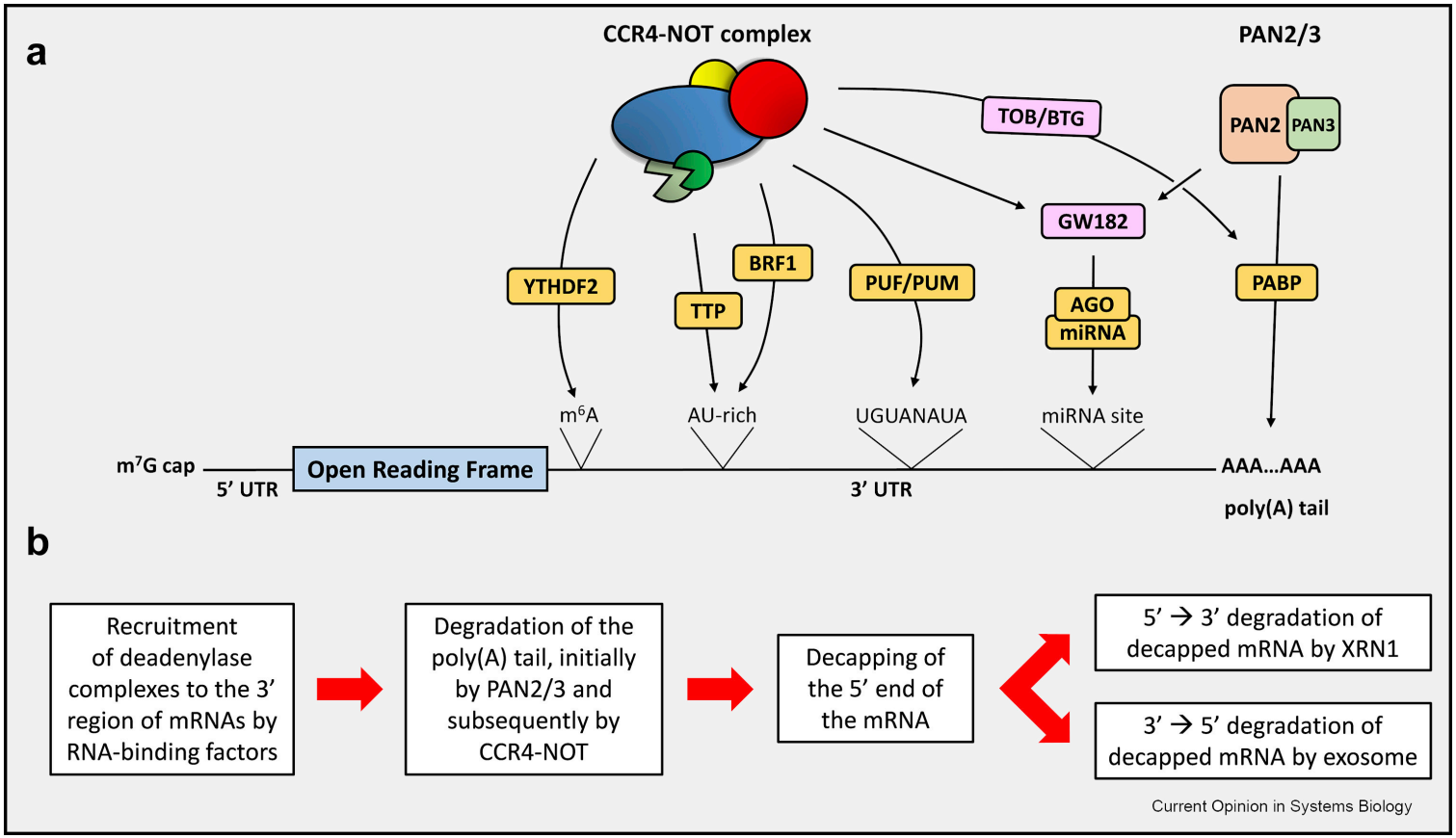
Pab1p inhibits Ccr4p deadenylation activity



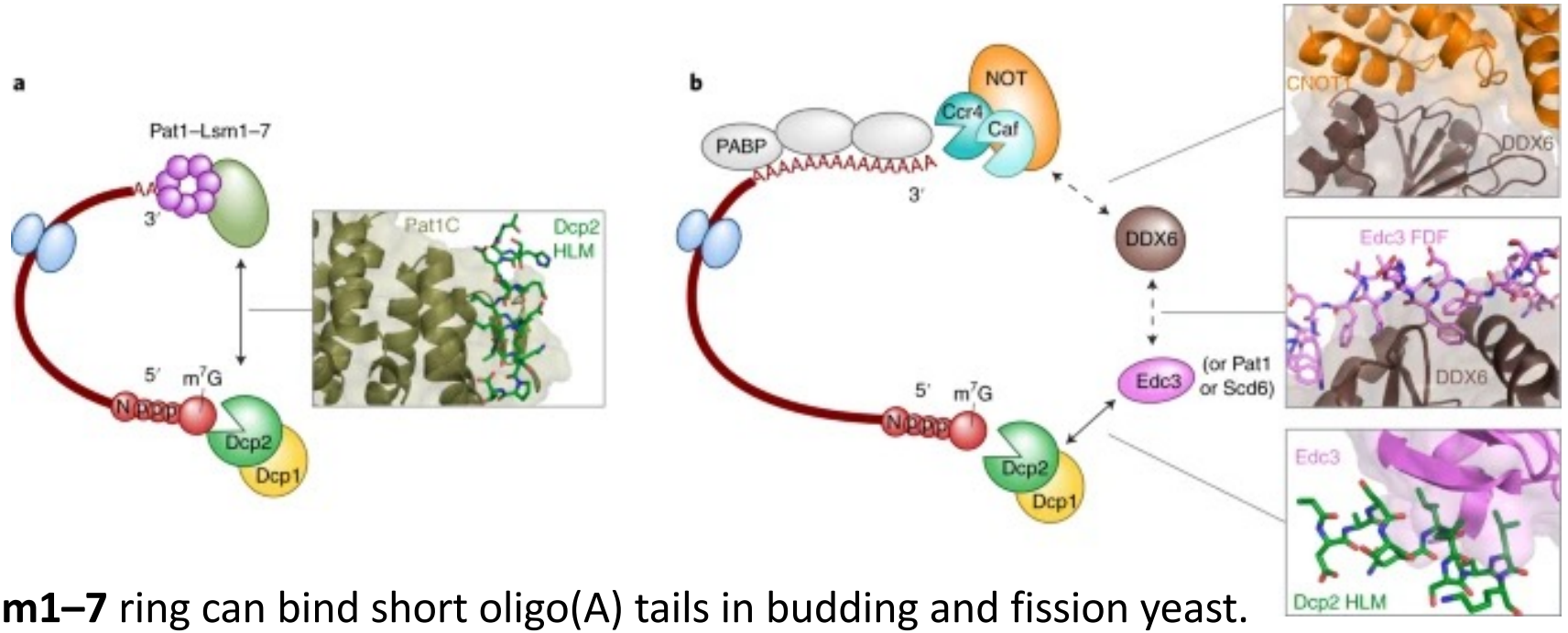


Analysis of deadenylation activity in Flag-Ccr4p purified fractions with addition of increasing amounts of purified Pab1p

Deadenylation…what's next?



Linking 3’ deadenylation to 5’ end decay in yeast

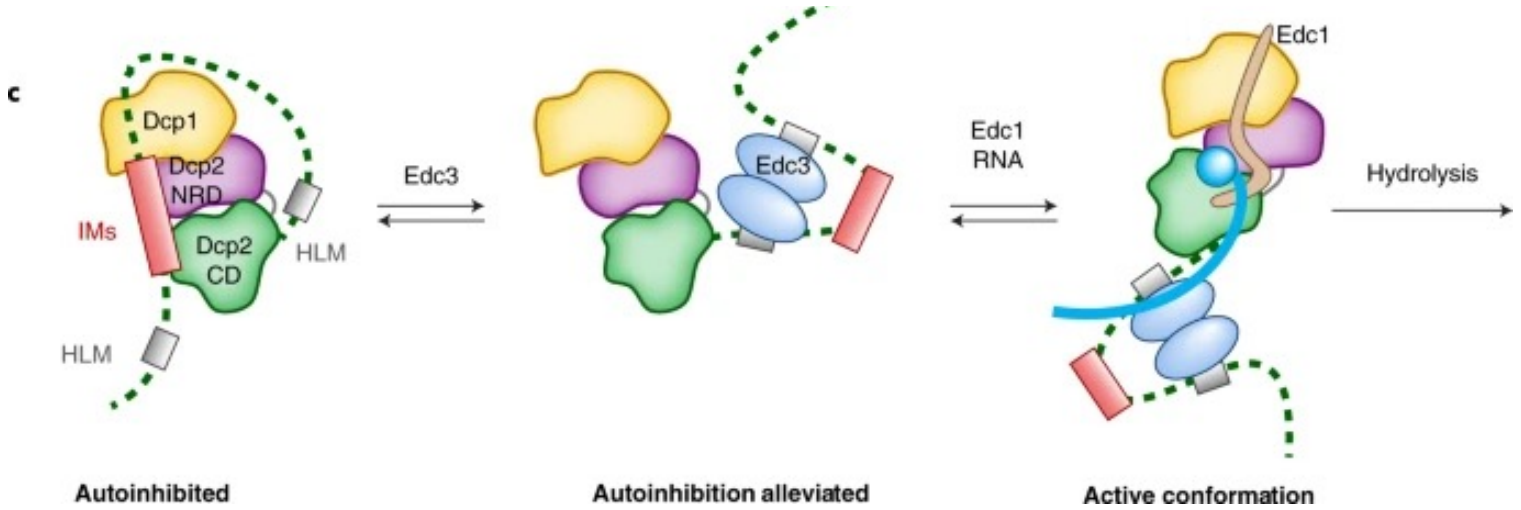


a) The **Lsm1–7** ring can bind short oligo(A) tails in budding and fission yeast.
Pat1 interacts with HLMs of Dcp2 in budding and fission yeast.
These interactions may recruit the decapping complex directly to deadenylated/urydilated 3’ tails.

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 still unclear

mRNA decapping



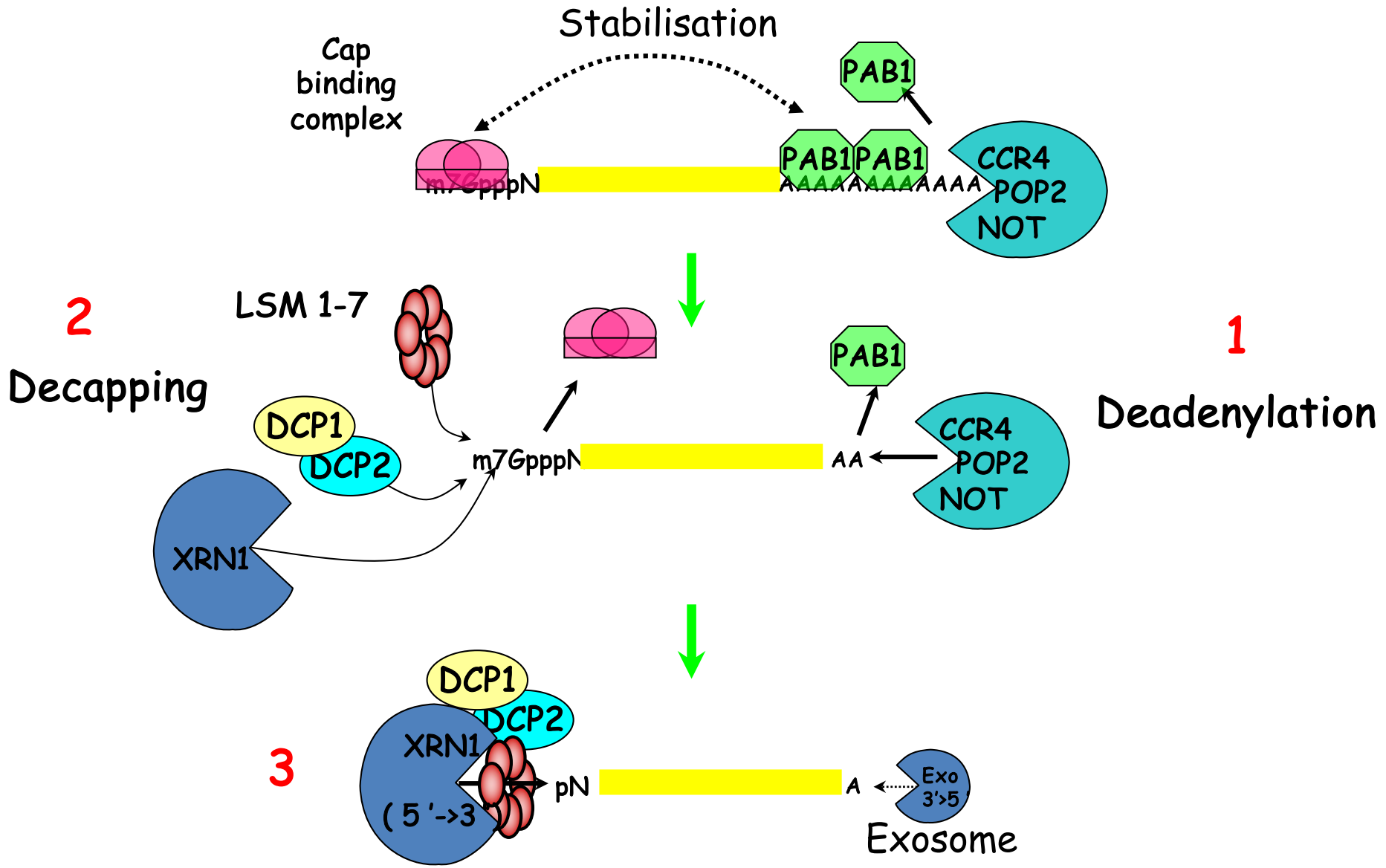
Core decapping complex: Dcp2 is the decapping enzyme, the essential activator **Dcp1**, and **scaffolding proteins**. In mammals there are many different decapping enzymes.

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**.

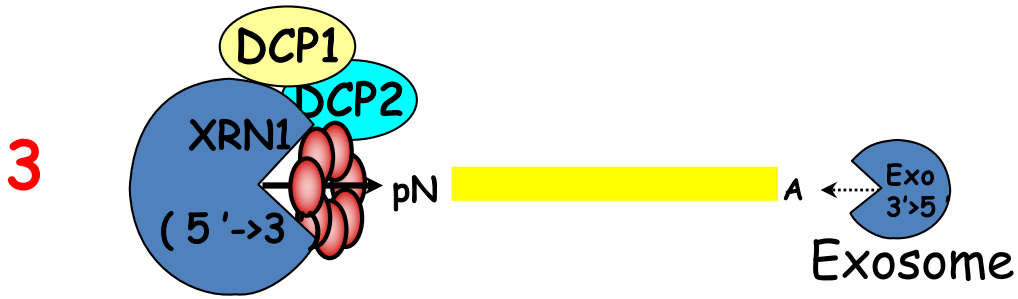
Binding of substrate and Edc1-like coactivators promotes the **catalytically active conformation of Dcp2 and hydrolysis of the 5' cap.**

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?

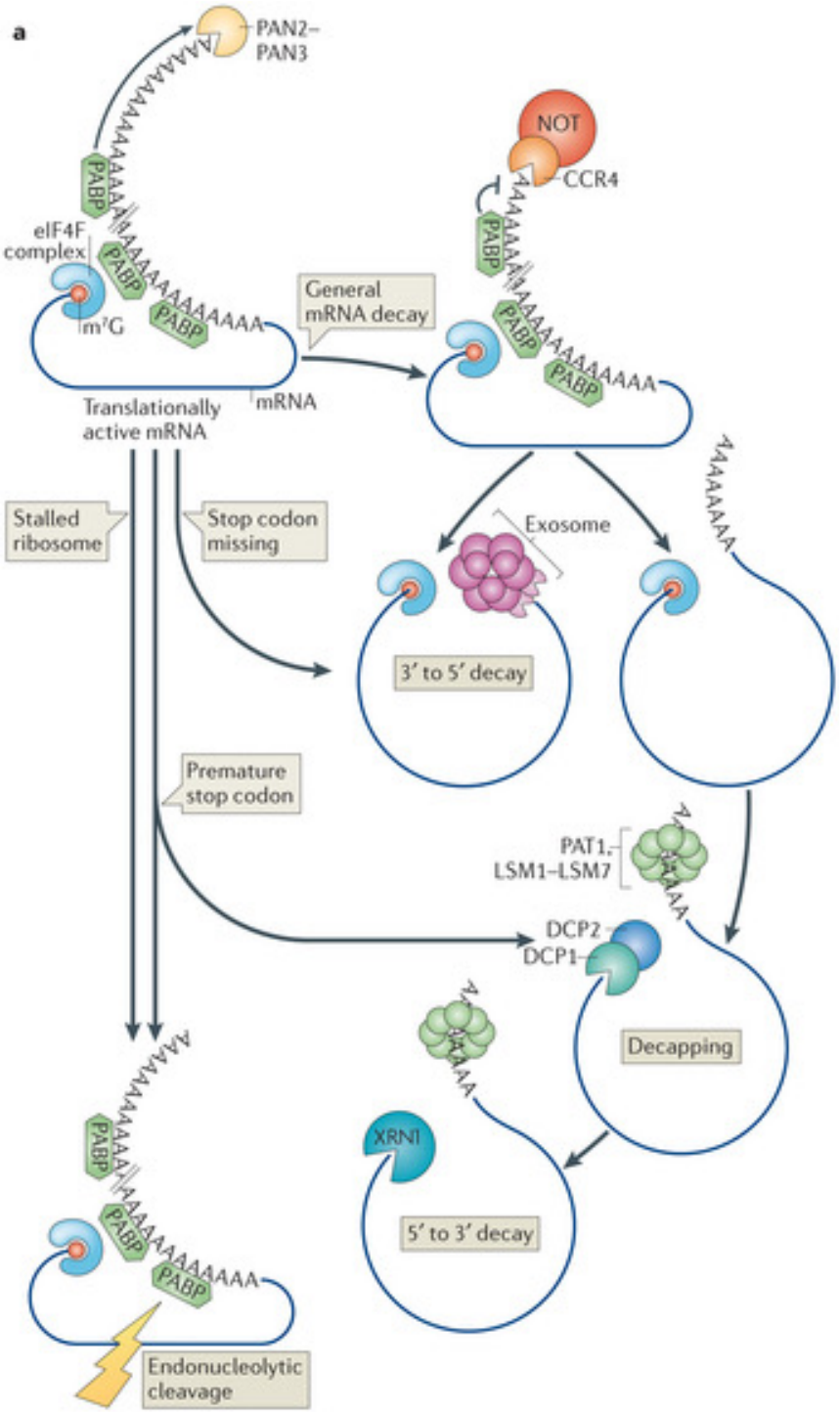


Xrn1 and the decapping complex itself (Dcp1 or Edc4 in metazoans) or decapping coactivators (Pat1 in yeast) can interact.

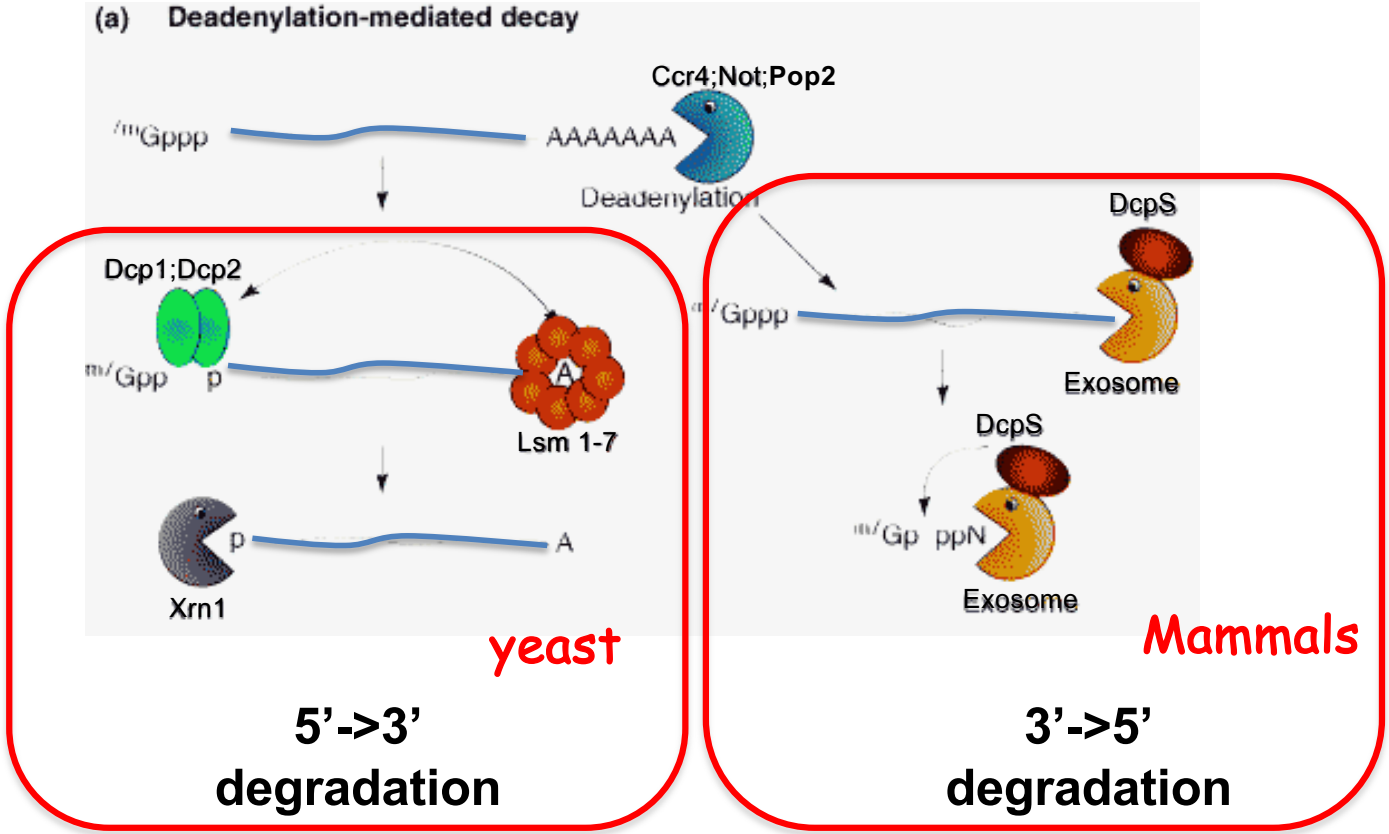
Following removal of the 5' cap by the decapping machinery, the conserved cytoplasmic exonuclease Xrn1 recognizes the newly exposed 5' monophosphate of the RNA and rapidly, processively degrades the transcript body.

Exosome can also partecipate in degradation.

The initial phase of poly(A) trimming is carried out by **PAN2**, which interacts directly with and is stimulated by PABP and is additionally regulated by its binding partner **PAN3**. Subsequently, bulk mRNA deadenylation is the responsibility of the conserved **CCR4–NOT complex**, which contains the **Ccr4** and **Pop2** (Caf1) 3′–5′ exonucleases and the scaffold protein **Not1**. **CCR4**, the major deadenylase of this complex, is inhibited rather than activated by PABP. As it is also required for translation initiation, it seems that PABP plays a central part in coordinating poly(A) tail length, translation and deadenylation.

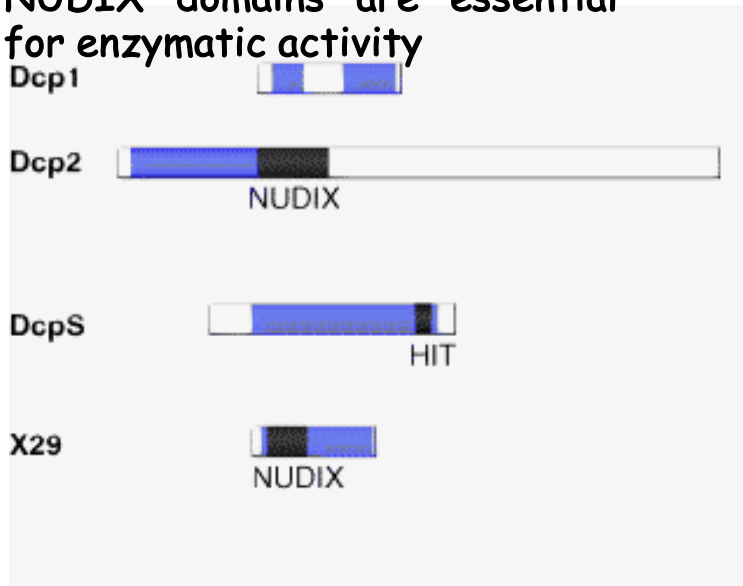


Different mRNA decays operate in yeast and mammals



Eukaryotic decapping proteins

NUDIX domains are essential for enzymatic activity



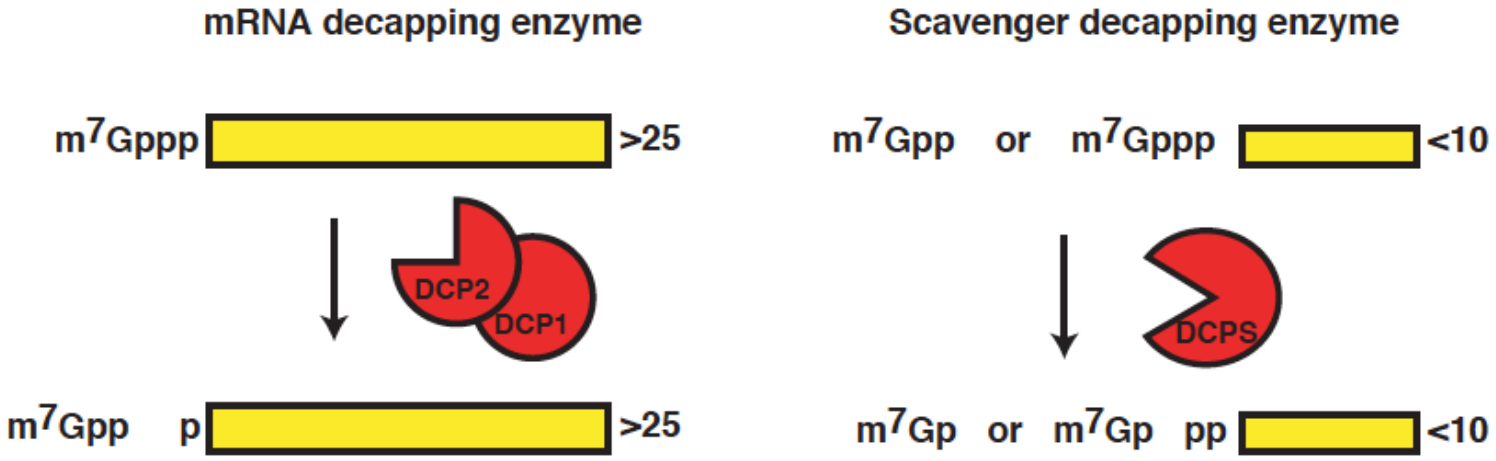
- The Dcp1:Dcp2 complex catalyzes the removal of ^{m7}GDP (7-methyl-GDP) from a capped RNA of more than 25 nucleotides

- DcpS hydrolyzes the m^{7}GpppN cap releasing m^{7}GMP, from capped oligonucleotides produced by exosome and results from complete mRNA degradation. It is unable to decap long substrates.

DcpS exists in complex with the exosome and is believed to play an important role in ensuring that no excess unhydrolyzed cap accumulates, which could titrate the cap-binding translation initiation factor, eIF4E, away from translated mRNAs

- X29 (Nudt16) was identified in Xenopus as specific decapping enzyme for U8 snoRNA. It is the main decapping activity in mammalian cells.

Eukaryotic decapping proteins



In yeast, major cytoplasmic mRNA decay mechanism is:

1) deadenylation
2) decappinng
3) 5′ → 3′ exo digestion

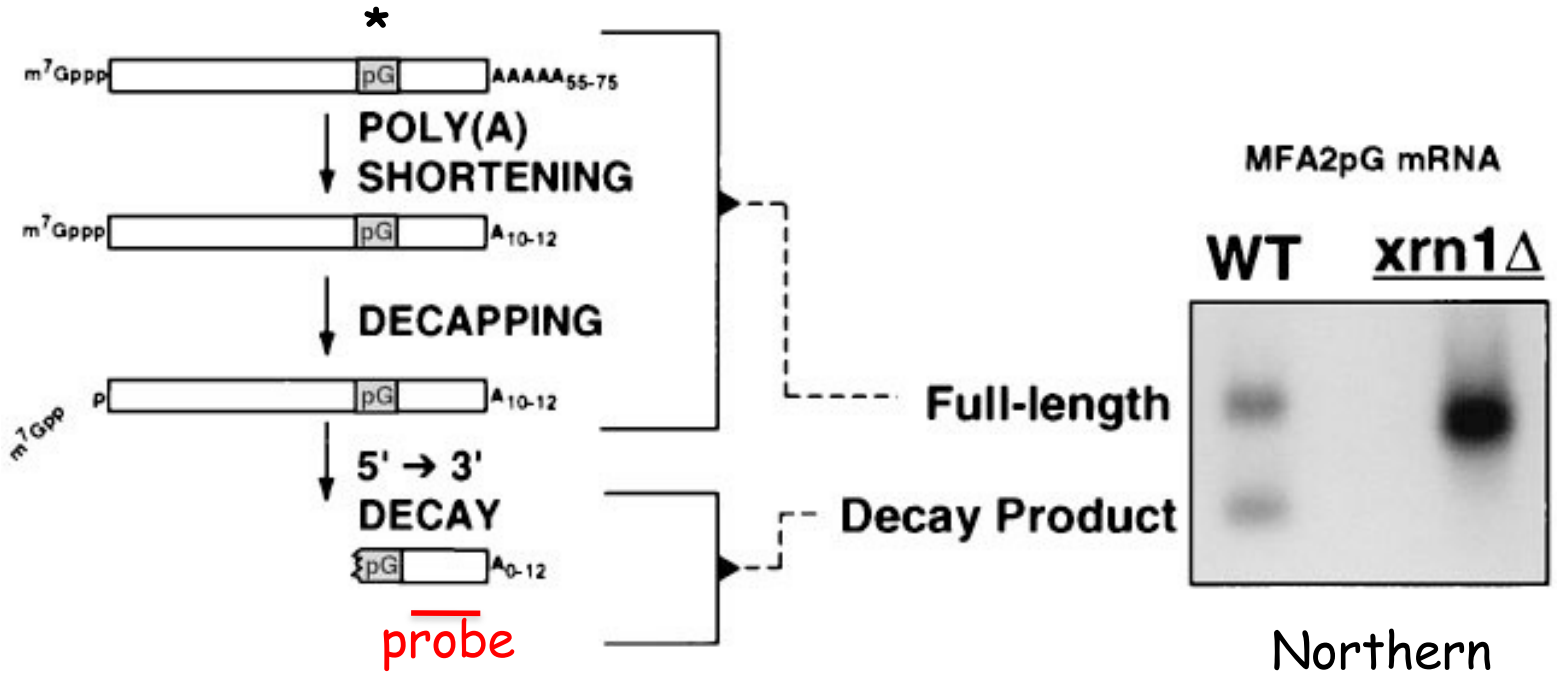
(can also detect minor 3′ → 5′ degradation pathway)

In mammals, major cytoplasmic mRNA decay mechanism is:

1) deadenylation
2) 3′ → 5′ digestion by exosome
3) decapping by scavenger decapping enzyme

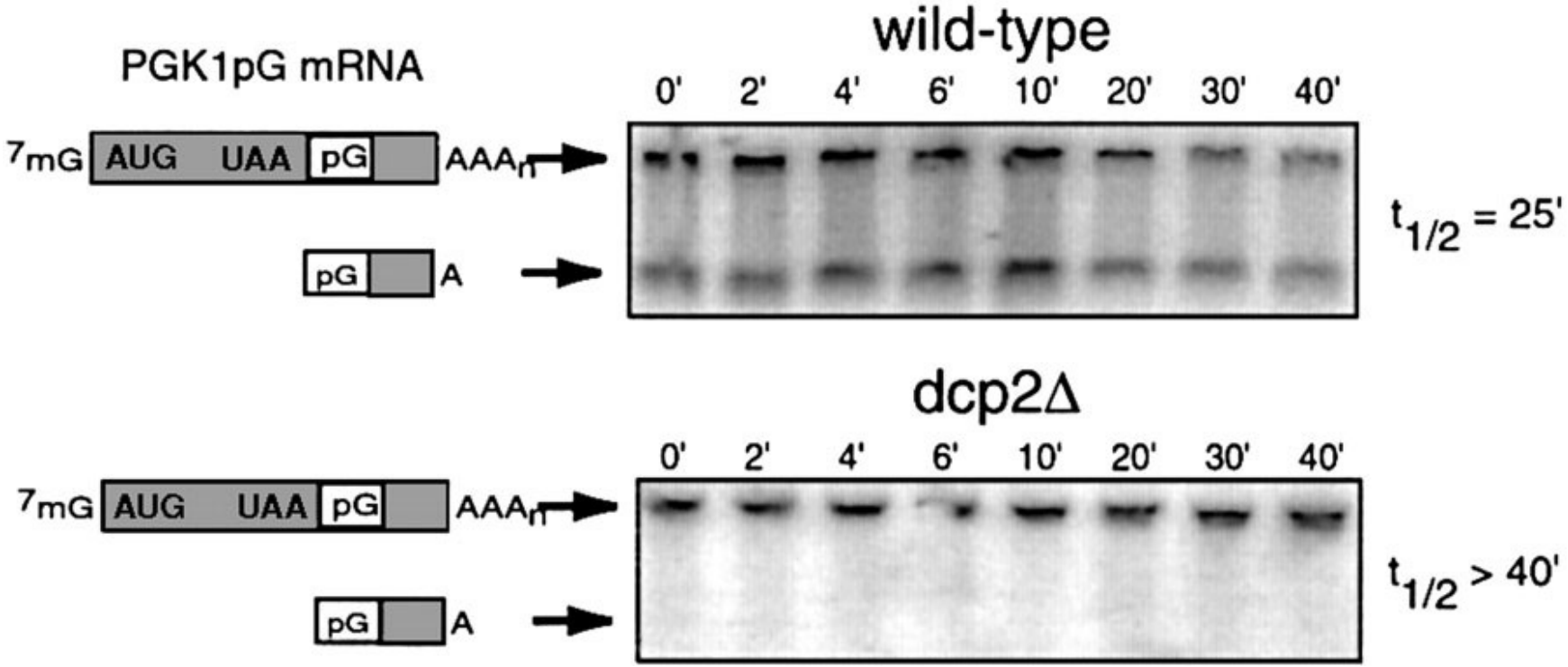
(can also detect minor 5′ → 3′ degradation pathway)

Strategy to isolate mutations affecting mRNA decay



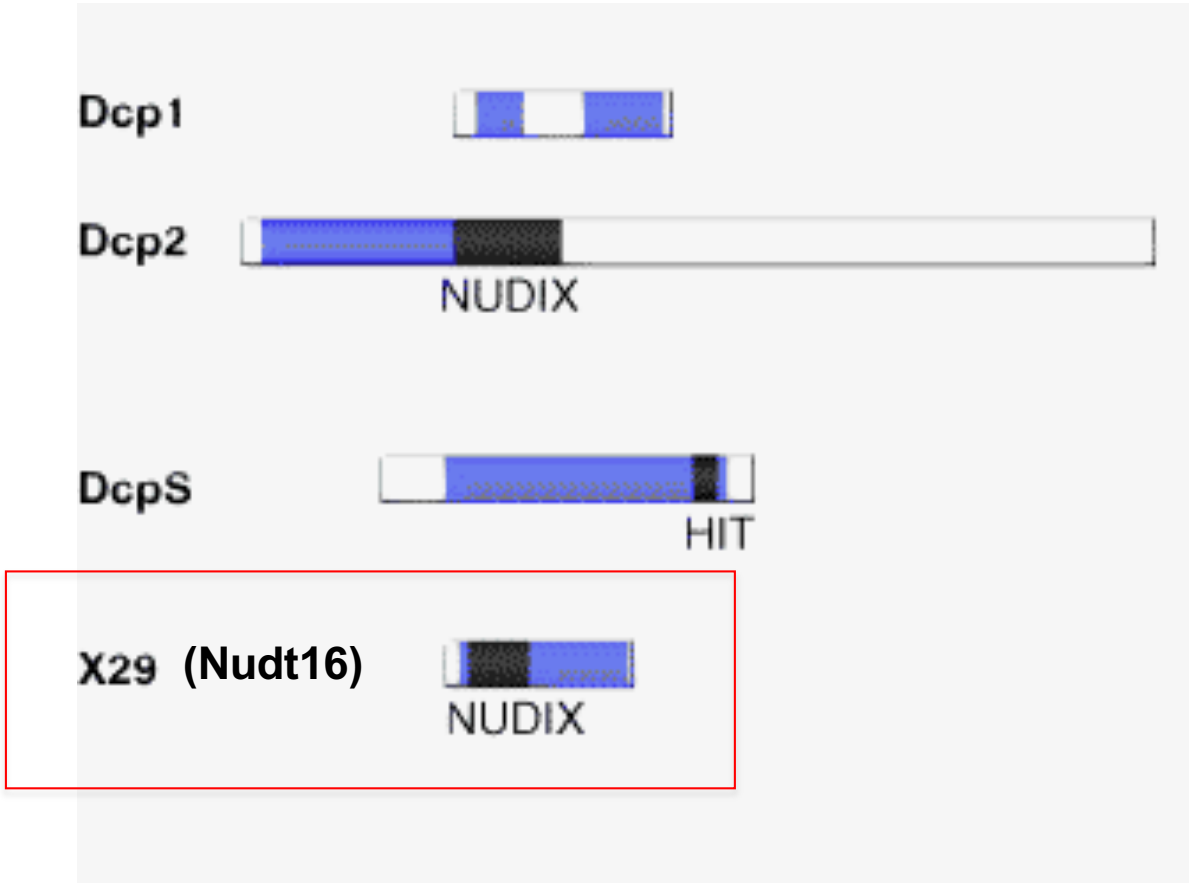
*pG cassette blocks exonucleases

DCP2 is required for 5' to 3' mRNA decay



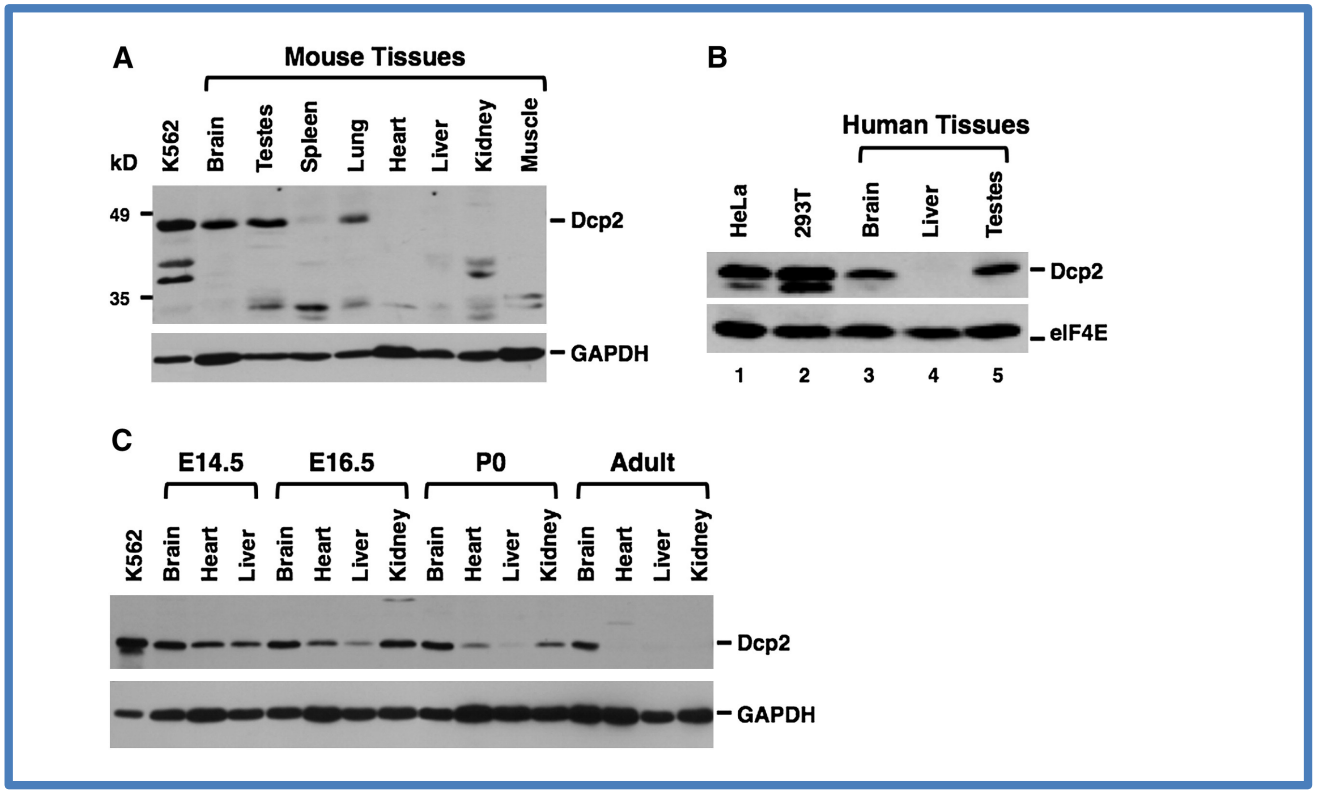
mRNA Decapping Enzymes in Mammalian Cells

Eukaryotic decapping proteins

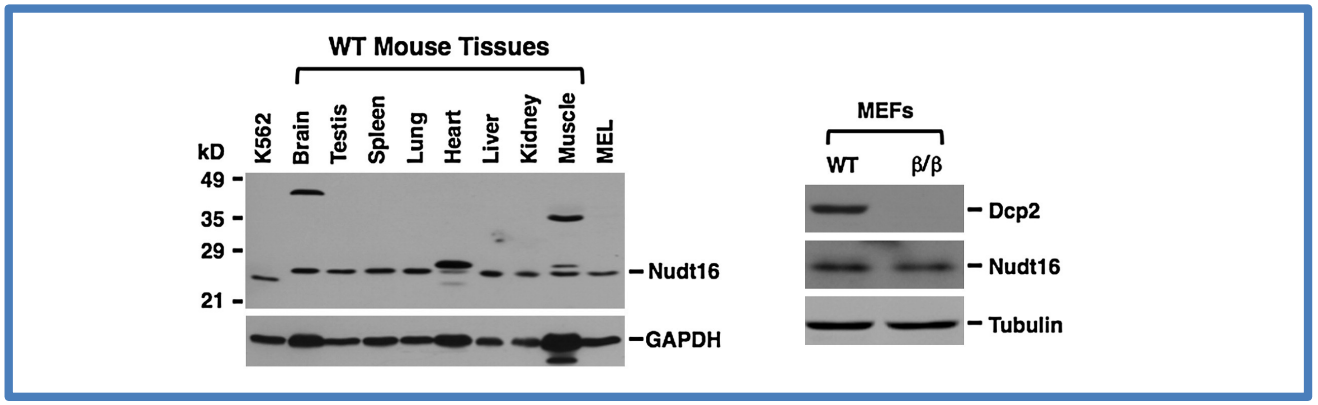


NUDIX domains are essential
for enzymatic activity

Dcp2 and *Nutd16* expression



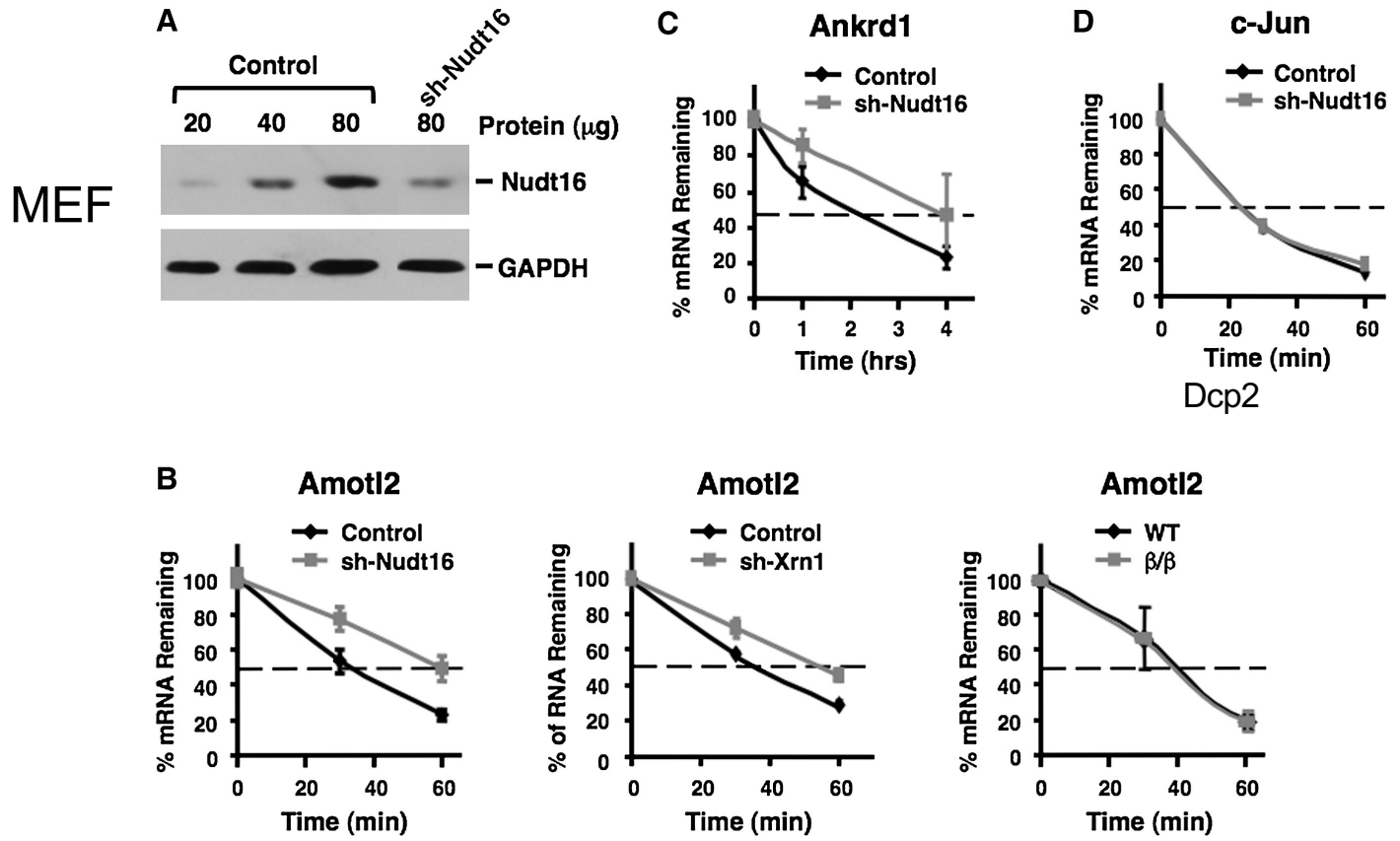
Dcp2



Nudt16

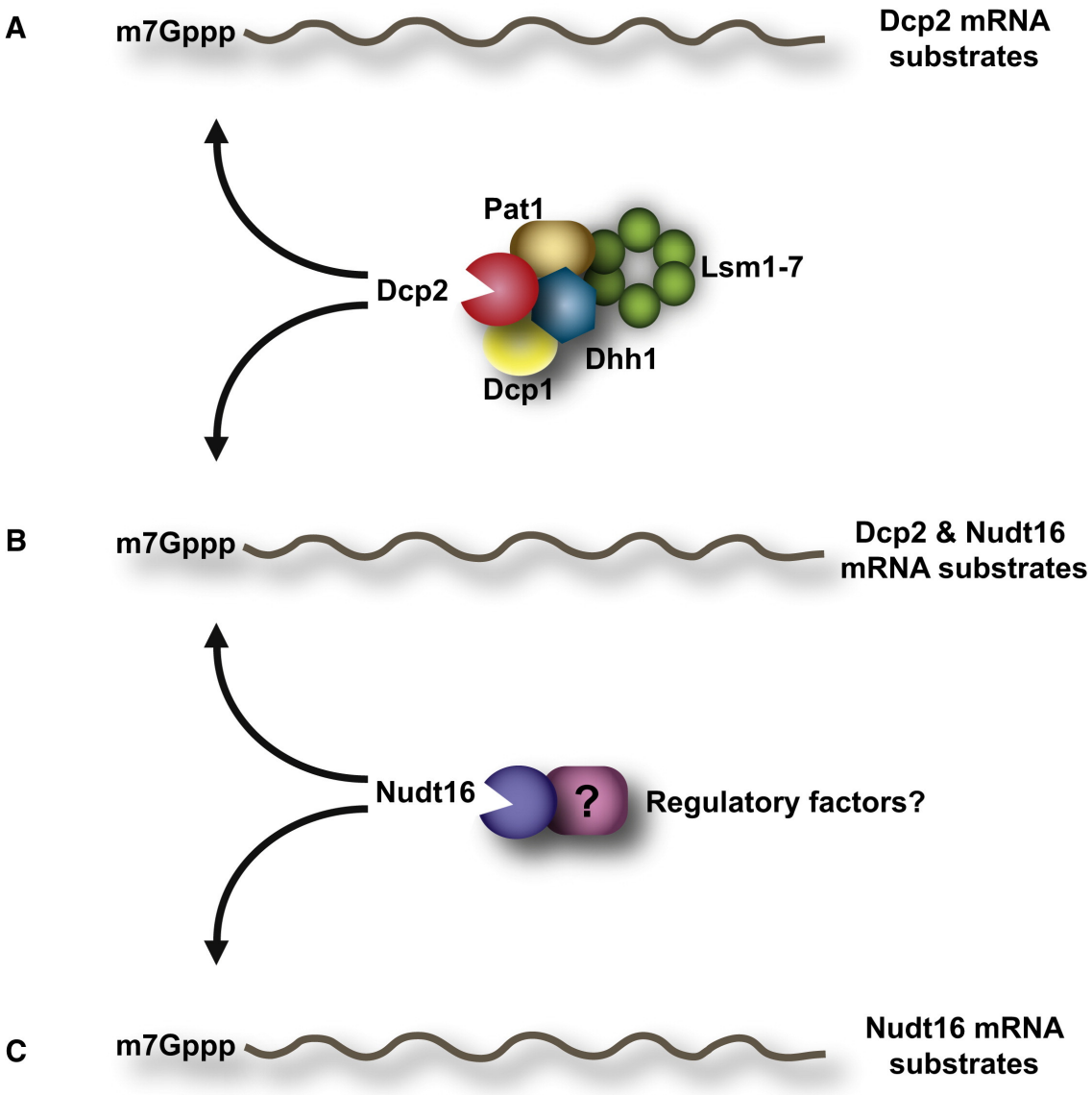
Nudt16 Is Involved in the Decay of a Subset of mRNAs

MEF cells were infected with lentivirus carrying shRNA against Nutd16 or Xrn1



Transcription was arrested by the addition of Actinomycin D 48 hrs post lentiviral infection and mRNA levels tested at the times indicated.

mRNA Decapping Enzymes in Mammalian Cells



Regulators of yeast mRNA decapping

Proteins that associate with the Dcp1:Dcp2 complex can facilitate its recruitment and stimulate mRNA decapping:

Protein	Properties	Function	Significant interactions
Pab1p	Contains four N- terminal RRM domains and a proline-rich C terminus	Major protein associated with poly(A) tail. Blocks mRNA decapping and stimulates translation. Primary coupler of deadenylation and decapping.	eIF-4G, eRF3, Pan2/3p
eIF-4E	Cap-binding protein	Component of the eukaryotic translational initiation complex, eIF-4F. Blocks mRNA decapping by competing with Dcp1/2p for access to the cap.	Lsm7p, eIF-4G, eIF- 4A, eIF-4B, Pab1p
Lsm1–7p	Sm-like proteins	Required for the efficiency of decapping in vivo. Forms a heteroheptameric ring complex and interacts with the mRNA after deadenylation. May facilitate the assembly of the decapping complex.	Dcp1p, Dcp2p, Dhh1p, Pat1p, Xrn1p, Upf1p
Pat1p	8kDa protein with no recognizable sequence motifs	Interacts with both polyadenylated and deadenylated transcripts. Required for efficiency of both decapping and formation of P bodies in vivo. May "seed" the decapping complex on the mRNA.	Dcp1p, Dcp2p, Lsm1–7p, Dhh1p, Xrn1p, Crm1p
Dhh1p	Member of the ATP-dependent DExD/H box helicase family	Required for the efficiency of decapping in vivo. Homologs across species are required for translational repression during mRNA storage events.	Dcp1p, Dcp2p, Lsm1–7p, Ccr4p, Pop2p, Caf17p, Pbp1p, Edc3p
Edc1p, Edc2p	Small, basic proteins with weak homology to each other	Required for efficient decapping in vitro. Directly binds to the mRNA substrate.	Dcp1p, Dcp2p
Edc3p	Contains five conserved domains	A general and mRNA-specific regulator of decapping. Regulates the decapping of the RPS28a mRNA.	Dcp1p, Dcp2p, Dhh1p, Crm1p, Rps28ap, Nup157p, Lsm8p
Puf3p	Pumillo-like protein, contains eight PUF repeats	Messages specific activator of mRNA deadenylation and decapping. Homologs facilitate translational repression. Regulates the decapping of the COX17 mRNA.	
Upf1p, Upf2p, Upf3p	Upf1p is an ATP-dependent RNA helicase	Required for non-sense- mediated decapping.	eRF1, eRF3, Dcp2p, Upf2p, Lsm1p
eIF-5a, Vps16p, Mrt4p, Sla2p, Gcr5p, Ths1p		Additional proteins suggested to be involved in mRNA turnover but functions remain unclear.	

Decapping regulators bind to Dcp2p but differ in mechanisms of decapping enhancement

Dhh1p and **Pat1p** repress translation directly, which enhances decapping because translation and decapping are in competition.
Edc1p-3p and Pat1p are able to stimulate Dcp2p catalytic activity directly,

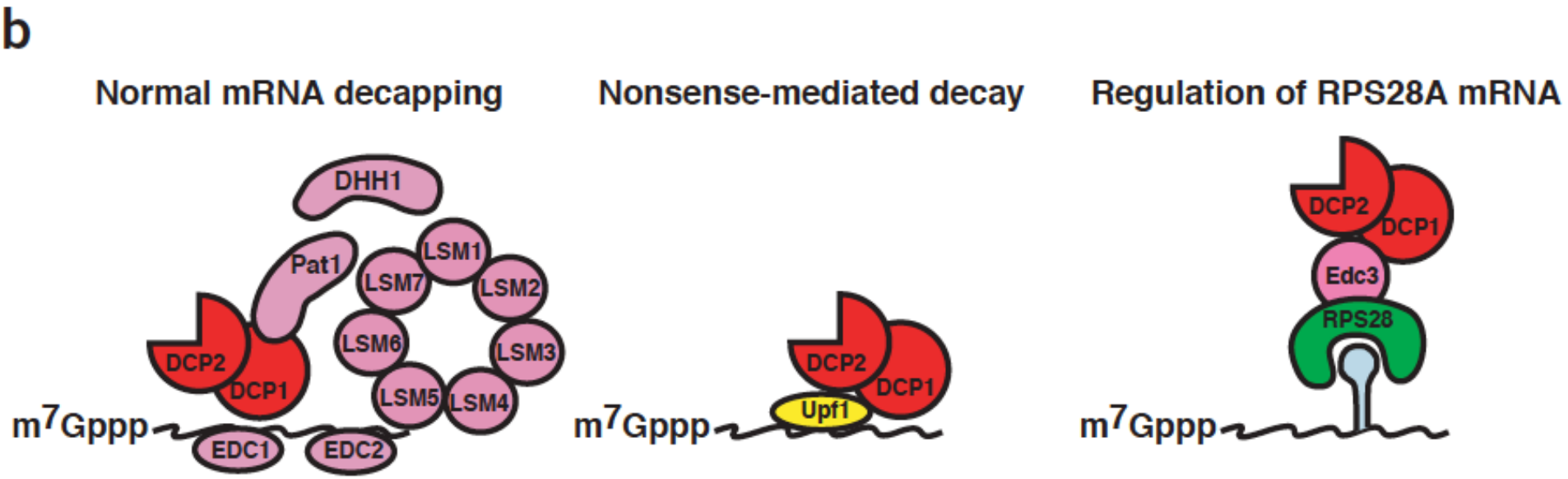
Pat1p serves as a scaffold for recruitment of other proteins, including Lsm1p-7p heptamer. In agreement with its multiple roles in the regulation of decapping, Pat1p deletion induces the most severe decapping defect among known yeast EDCs.

Lsm1p-7p complex forms a ring, binds to shortened poly(A) tail after deadenylation and enhances interaction of Dcp2p with mRNA. Lsm1p-7p together with Pat1p also bind to uridine stretches near the 3′ end of transcript, explaining why Lsm1-7/Pat1 complex stimulates decapping following both mRNA deadenylation and uridylation.

Xrn1 ribonuclease interacts with EDCs and this provides direct connection between decapping and 5′ -3′ degradation.

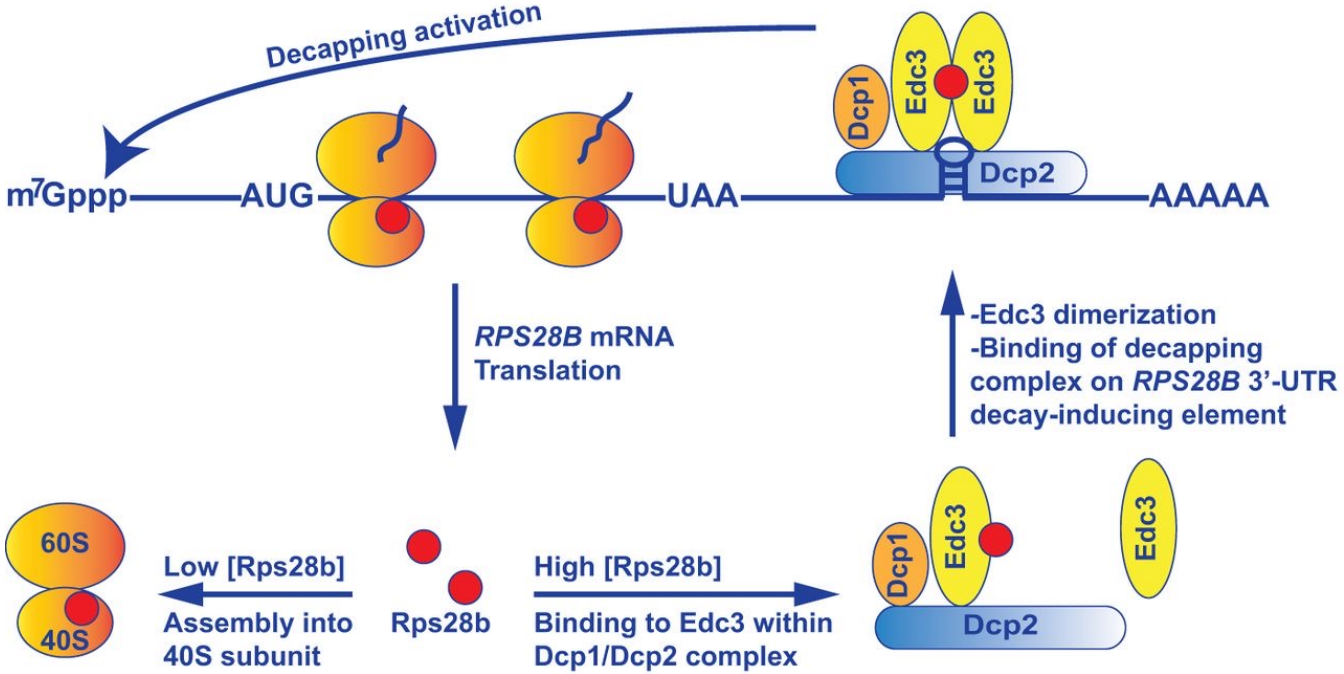
Generally, Dcp1/Dcp2 are deposited on mRNAs as parts of a ribonucleoprotein complex, and the composition of this complex varies in different organisms.

decapping complexes recruit Dcp1-Dcp2p to different mRNA substrates



A Model for the Edc3-Mediated Autoregulation of the *RPS28B mRNA*

RPS28B mRNA is translated to produce Rps28b protein that are usually assembled into ribosomal subunits. However, once the Rps28b protein concentration reaches a certain threshold, the excess Rps28b molecules that are produced bind to Edc3 within the Dcp1/Dcp2 complex. Rps28b binding to Edc3 promotes Edc3 dimerization and leads to the formation of an active decapping complex. This active decapping complex possesses unique substrate specificity for *RPS28B* mRNA, binds to the *RPS28B* 3' UTR decay-inducing element, and catalyzes the removal of the cap structure from the *RPS28B* mRNA.

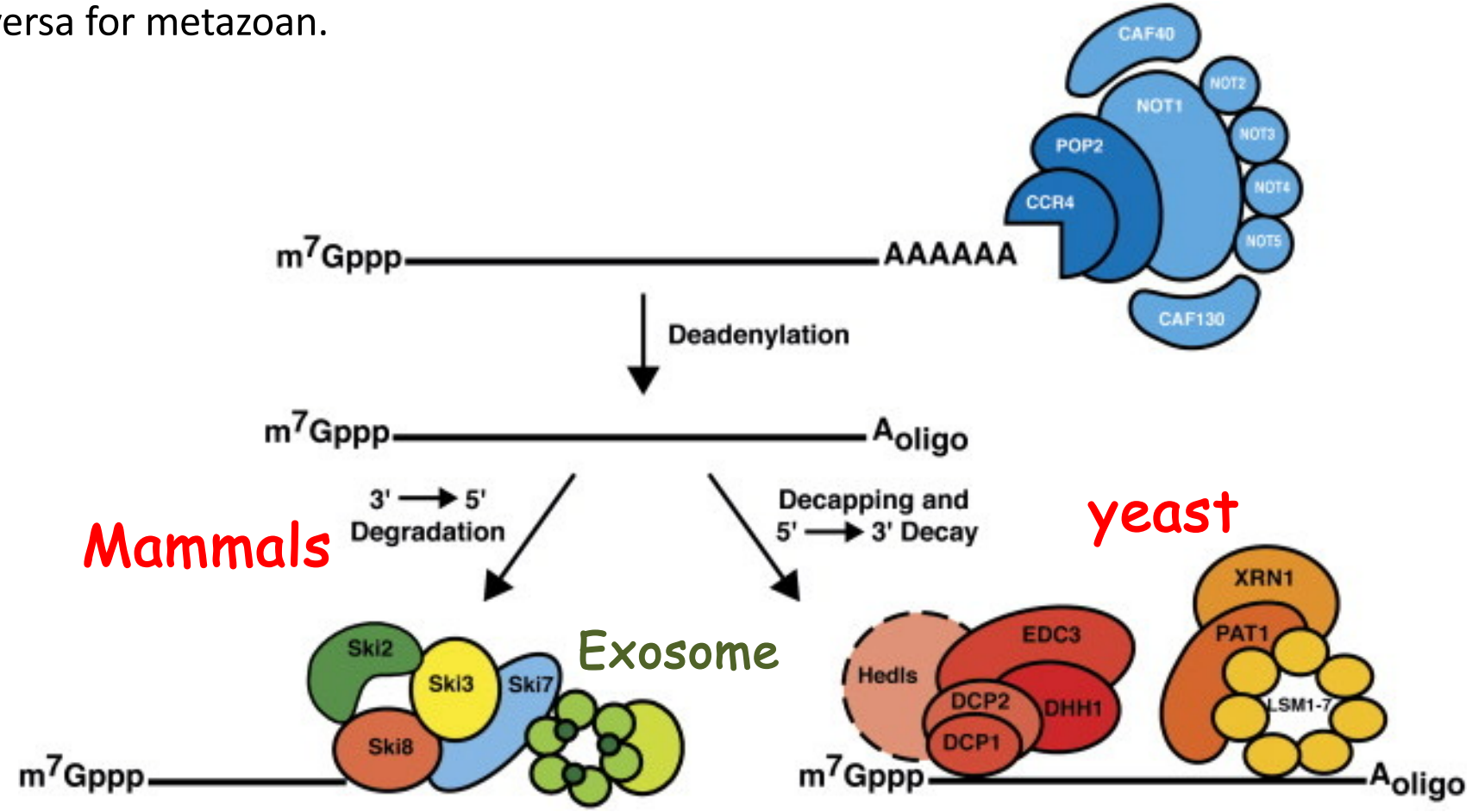


The Exosome

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

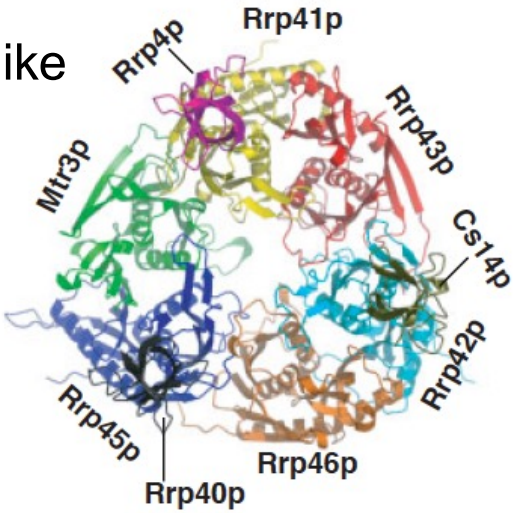
A major eukaryotic exoribonuclease degrading transcripts from 3' -end is the **RNA exosome complex**

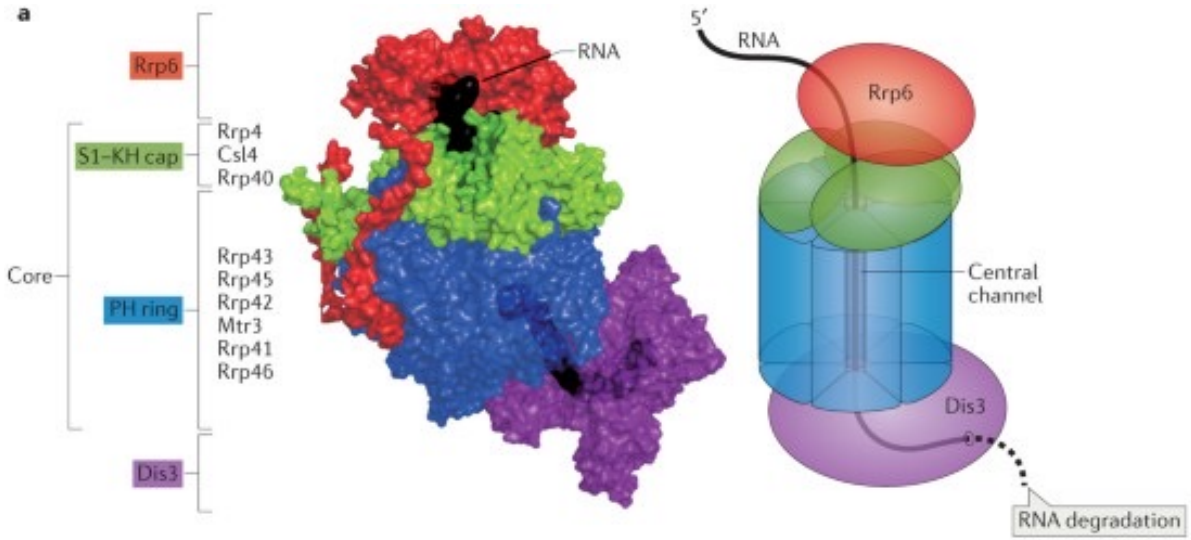
In yeast, exosome-mediated 3' -5' decay pathway, although functional, does not play a major role in the control of mRNA stability, and decapping-dependent 5' -3' pathway prevails. Viceversa for metazoan.



The Exosome: 3’-5’ decay

Barrel like



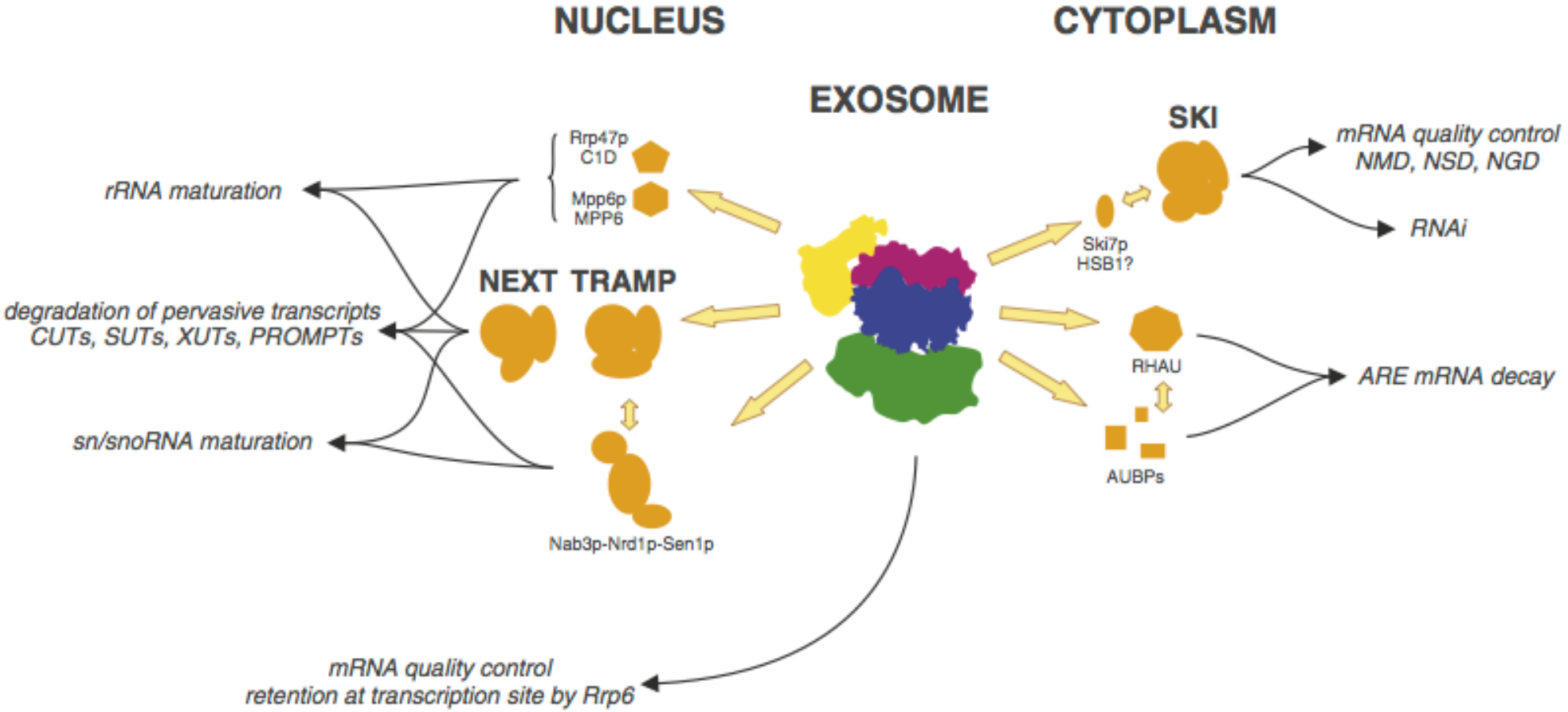


The eukaryotic exosome is a **major ribonuclease for RNA decay and processing.**
The repertoire of catalytic subunits varies among Eukaryota.
Some catalytic subunits are compartment-specific and give rise to exosome isoforms.

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).

Exosome's cellular functions and cofactors

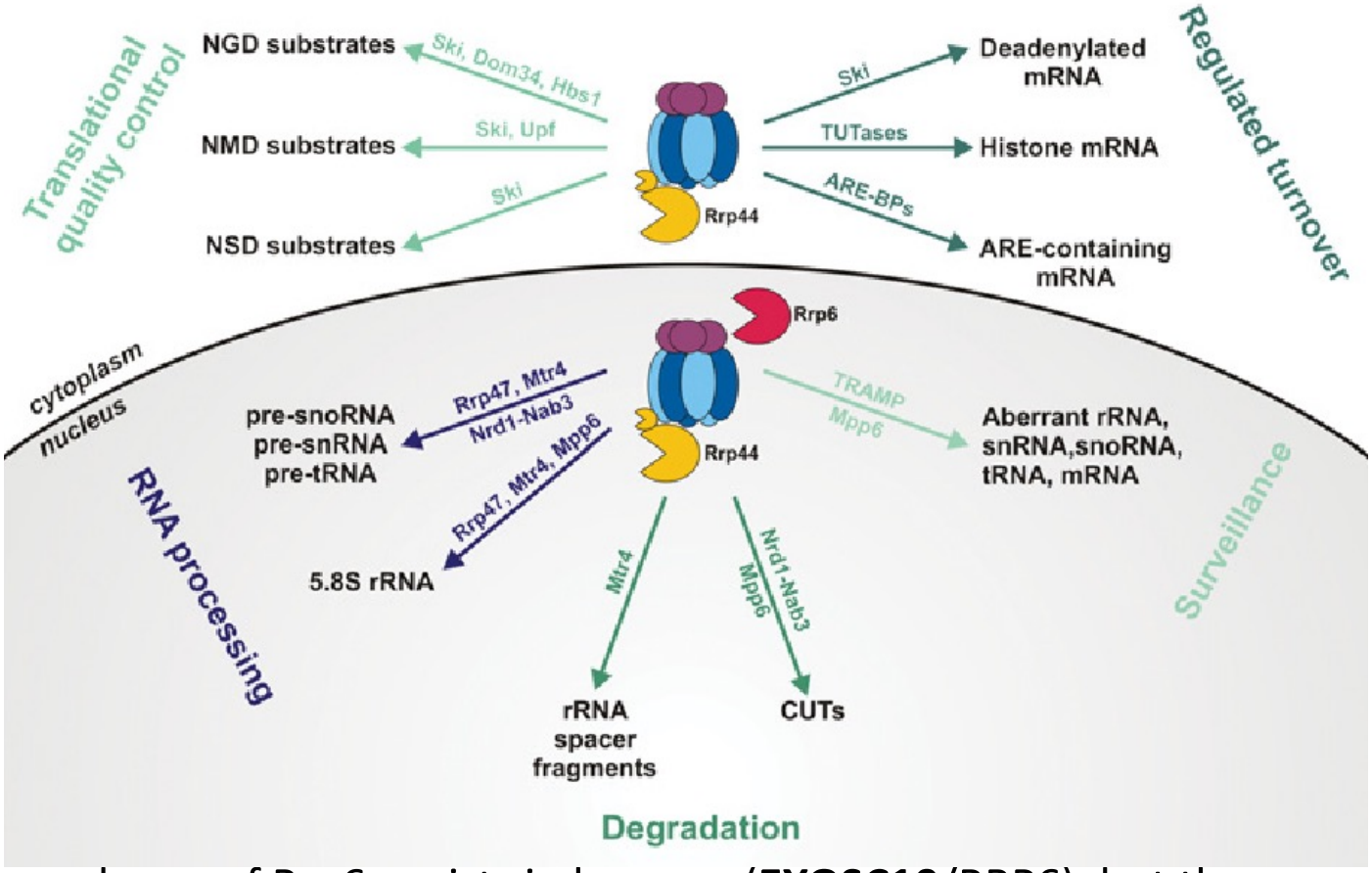
The exosome is a large protein complex containing multiple 3′→5′ exonucleases that also functions in a variety of nuclear RNA processing reactions



The core complex in *S. cerevisiae*, which is the best studied model in exosome research, lacks catalytic activity which is instead provided 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



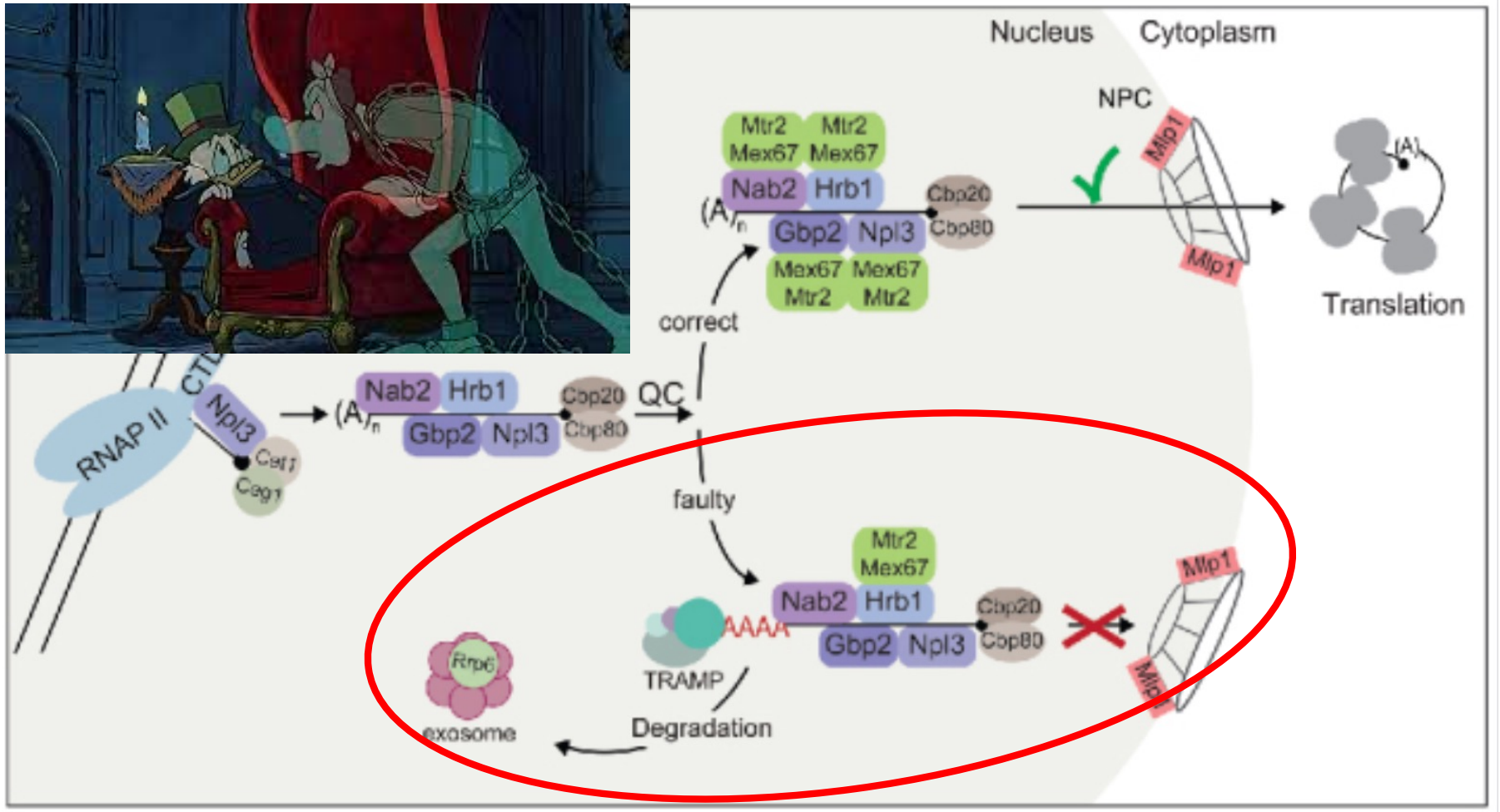
Only one homologue of Rrp6p exists in humans (**EXOSC10**/RRP6), but there are three proteins of the DIS3 (Rrp44) family: **DIS3, DIS3L and DIS3L2.**

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

DIS3L2 does not possess a domain responsible for interaction with exosome core and is responsible for functioning of an exosome-independent, conserved cytoplasmic RNA degradation pathway.

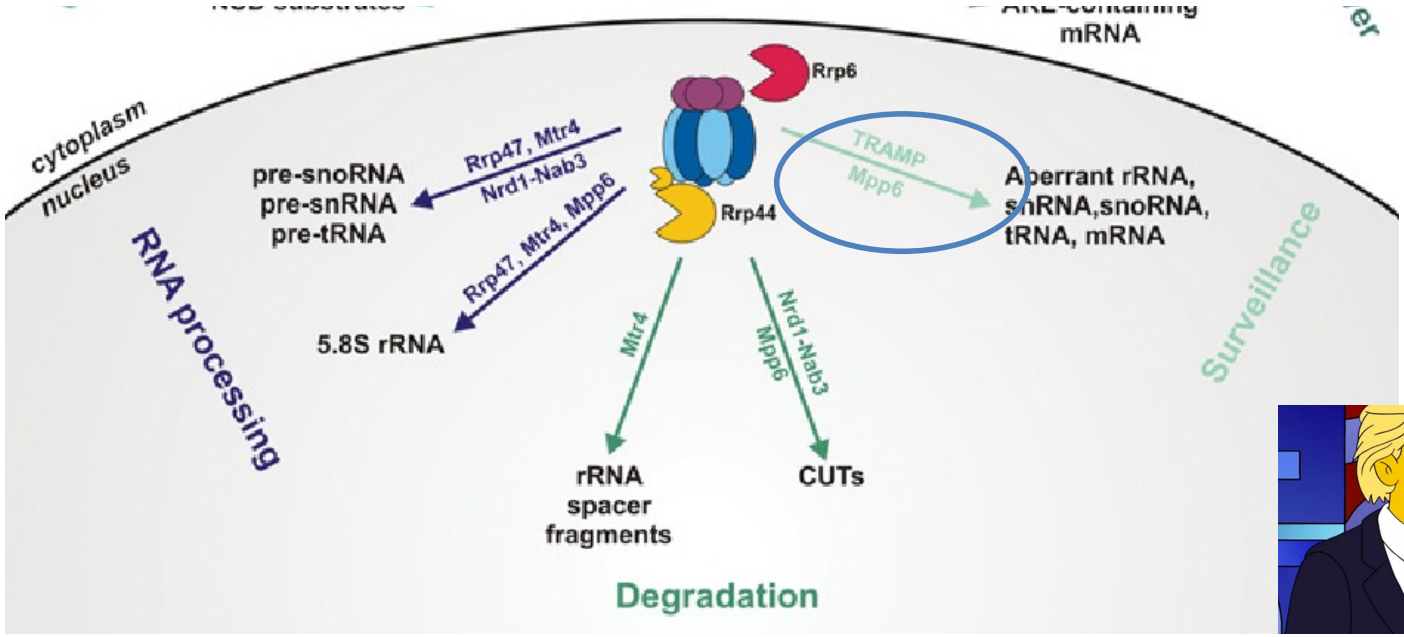
mRNA EXPORT: YEAST
mRNA quality control and export check-point

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



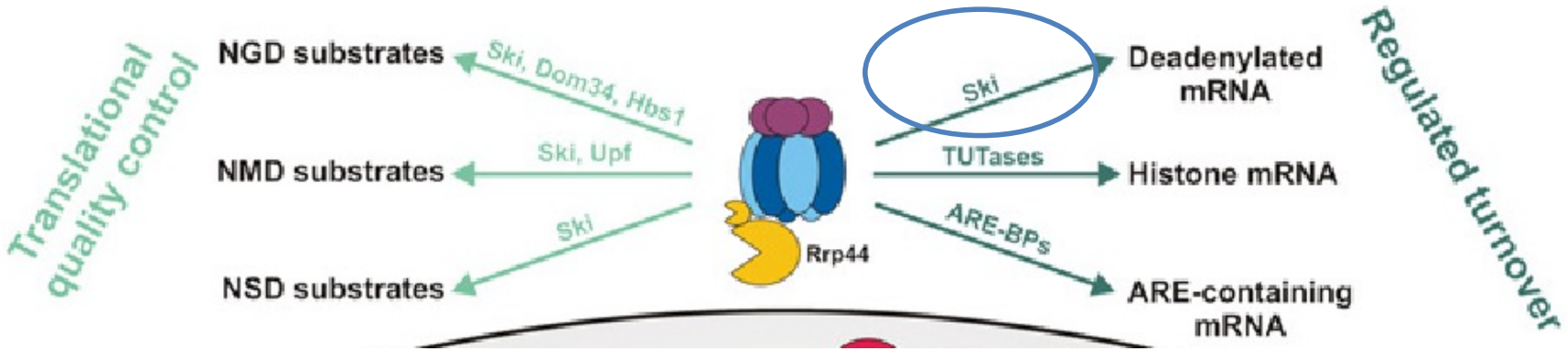
Exosome cofactors:

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 cytoplasm

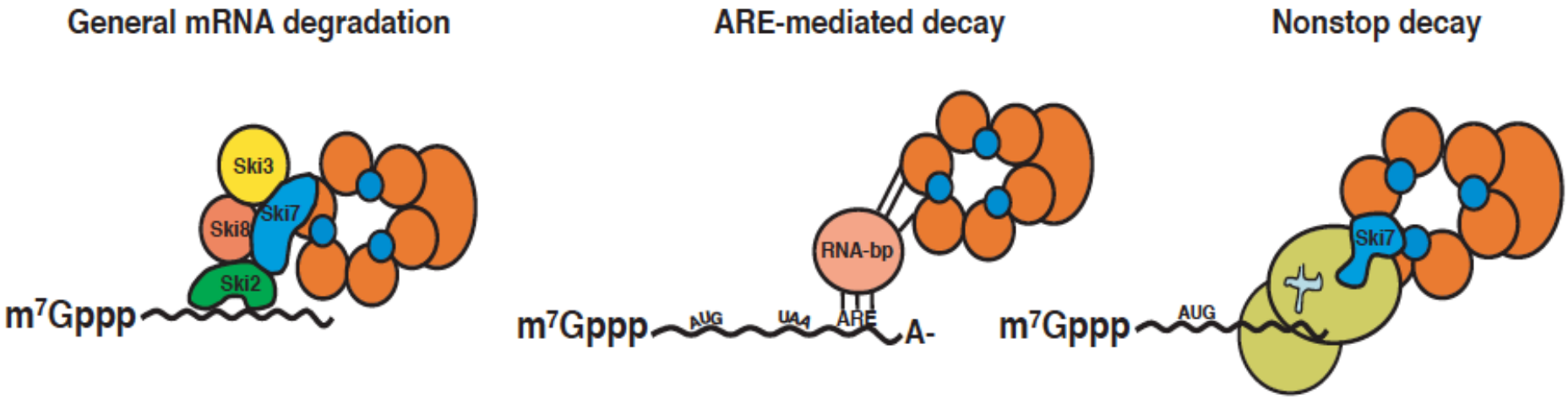


The cytoplasmic exosome is usually assisted by the **superkiller (SKI) complex**.

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).

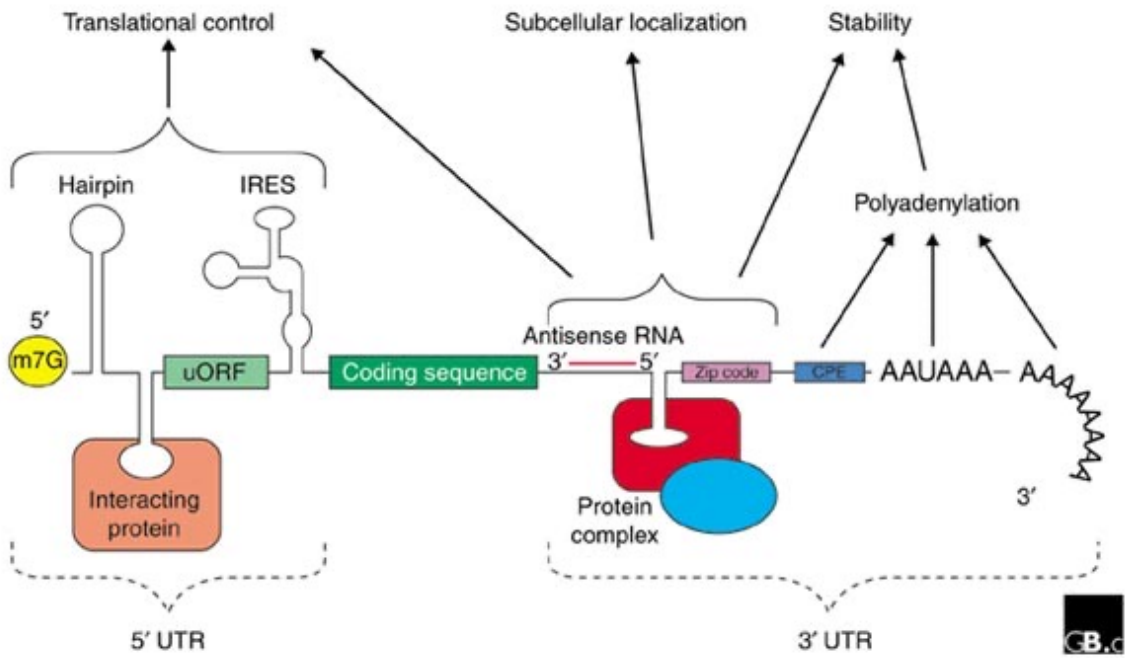
In yeast the interaction between the exosome and SKI is mediated by the **Ski7p** protein.
The human version of Ski7p does not appear to exist. However, Ski7p has a paralogue – Hbs1p – that is involved in the NSD and NGD pathways, and Hbs1p has a human counterpart – HBS1L – that co-purifies with the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

mechanisms of exosome recruitment to different mRNA substrates



Regulatory elements of mRNA

mRNAs contain several regulatory elements recognized by specific regulatory factors: **proteins** and **RNAs**, which allow fine-tuned regulation of gene expression.



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.).....

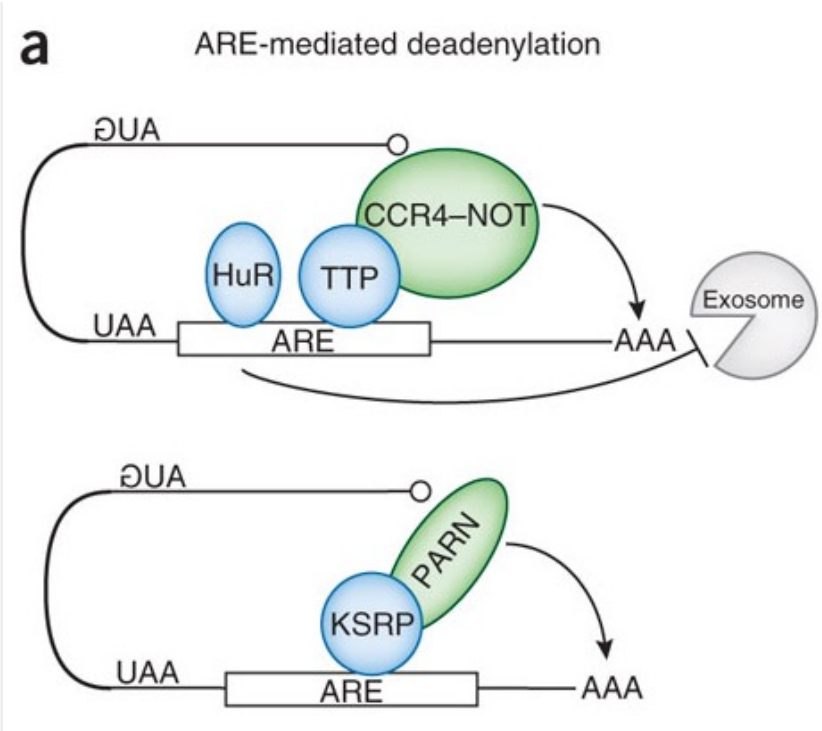
ARE binding proteins

Protein	Nucleus/Cytoplasm	Role in mRNA decay
AUF-1/hnRNP D 4 isoforms	p42, p45 nuclear, p37, p40 cytoplasmic	Destabilizing, stabilizing
HuR/HuA	Nuclear	Stabilizing
HuD, Hel-N1 /HuB, HuC	Nuclear	Stabilizing
TTP (tristetrapolin)	Nuclear, cytoplasmic	Destabilizing
KSRP	Nuclear, cytoplasmic	Destabilizing
Hsp70	Cytoplasmic	Destabilizing
TIA-1,TIAR	Nuclear, cytoplasmic	Destabilizing, translation
AUH	Cytoplasmic	Enzymatic activity ?
hnRNPA1	Nuclear	Destabilizing
hnRNPC	Nuclear	Destabilizing
GAPDH	Nuclear, cytoplasmic	Enzymatic activity ?
AUBF	Cytoplasmic	?
AU-A, AU-B, AU-C	AU-A nuclear, AU-B, AU-C cytoplasmic	Destabilizing

These proteins have been identified in cell extracts by ultraviolet (UV)-crosslinking and gel-shift assays

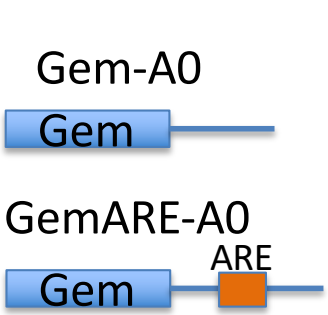
AREs drive mRNA deadenylation

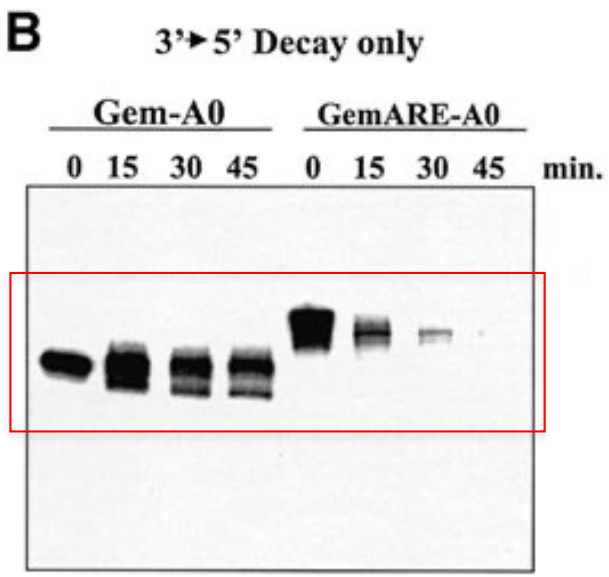
The binding of the ARE-BPs **TTP** and **KSRP** on AREs induces rapid mRNA deadenylation by the recruitment of the deadenylases CCR4–NOT complex and PARN, respectively. The binding of **HuR** stabilizes the transcript by inhibiting exosome recruitment.

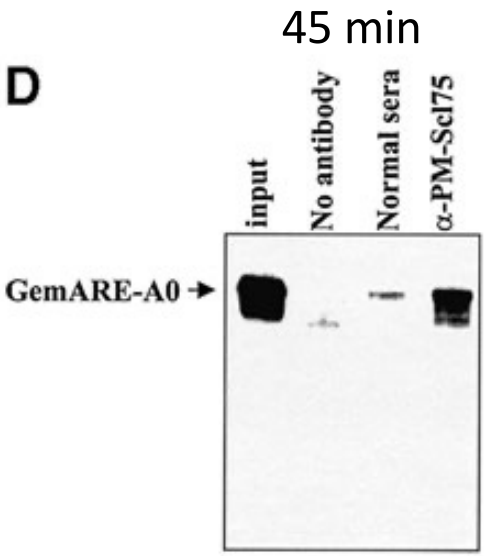


The mammalian exosome mediates the degradation of mRNAs that contain ARE

•Capped but non-polyadenylated RNAs were prepared that lacked (Gem-A0) or contained the ARE (GemARE-A0) and incubated in HeLa S100 extracts (Figures B and C)



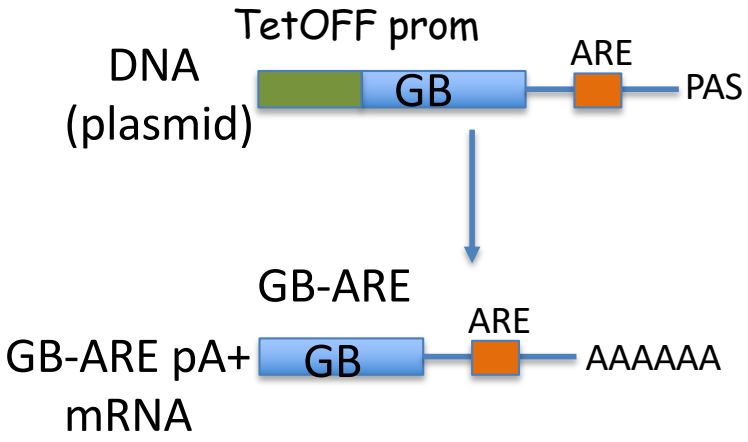


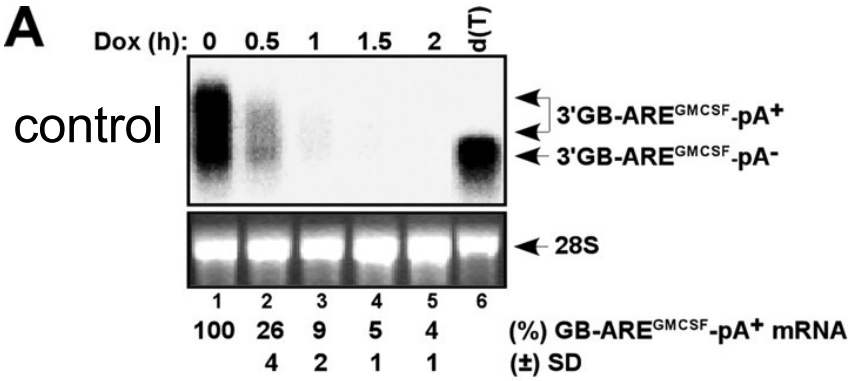


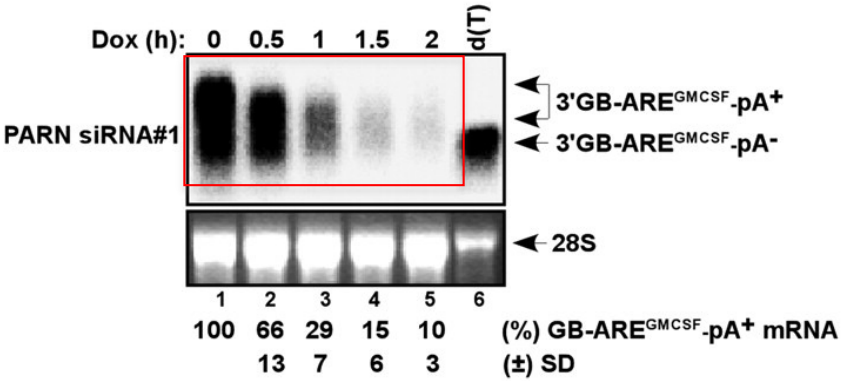
•immunodepletion of HeLa extracts with antibodies against the exosomal protein PM-Scl75 reduced the efficiency of 3'–5' exonucleolytic decay in these assays

Down-regulation of PARN inhibits deadenylation step of ARE-mediated Decay (AMD)

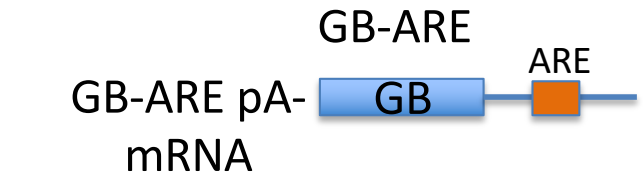
HeLa cells were transfected with a construct expressing beta globin (GB)-ARE^{GMCSF} under the control of a TetOFF promoter





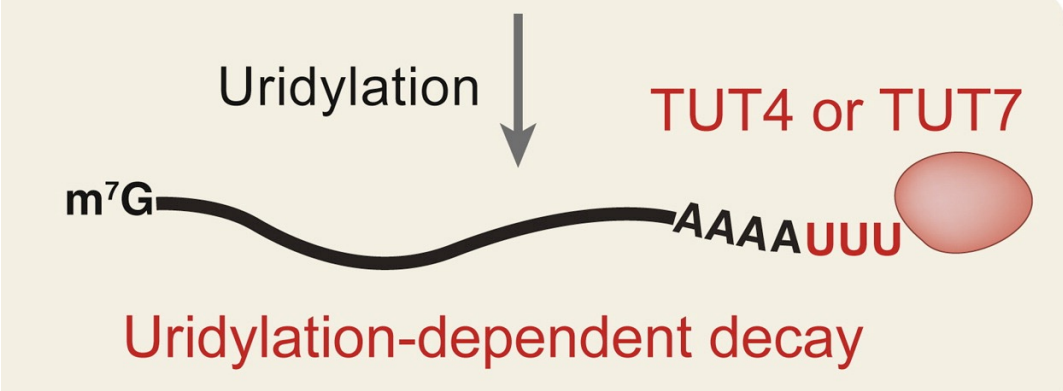


In vitro transcribed



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.

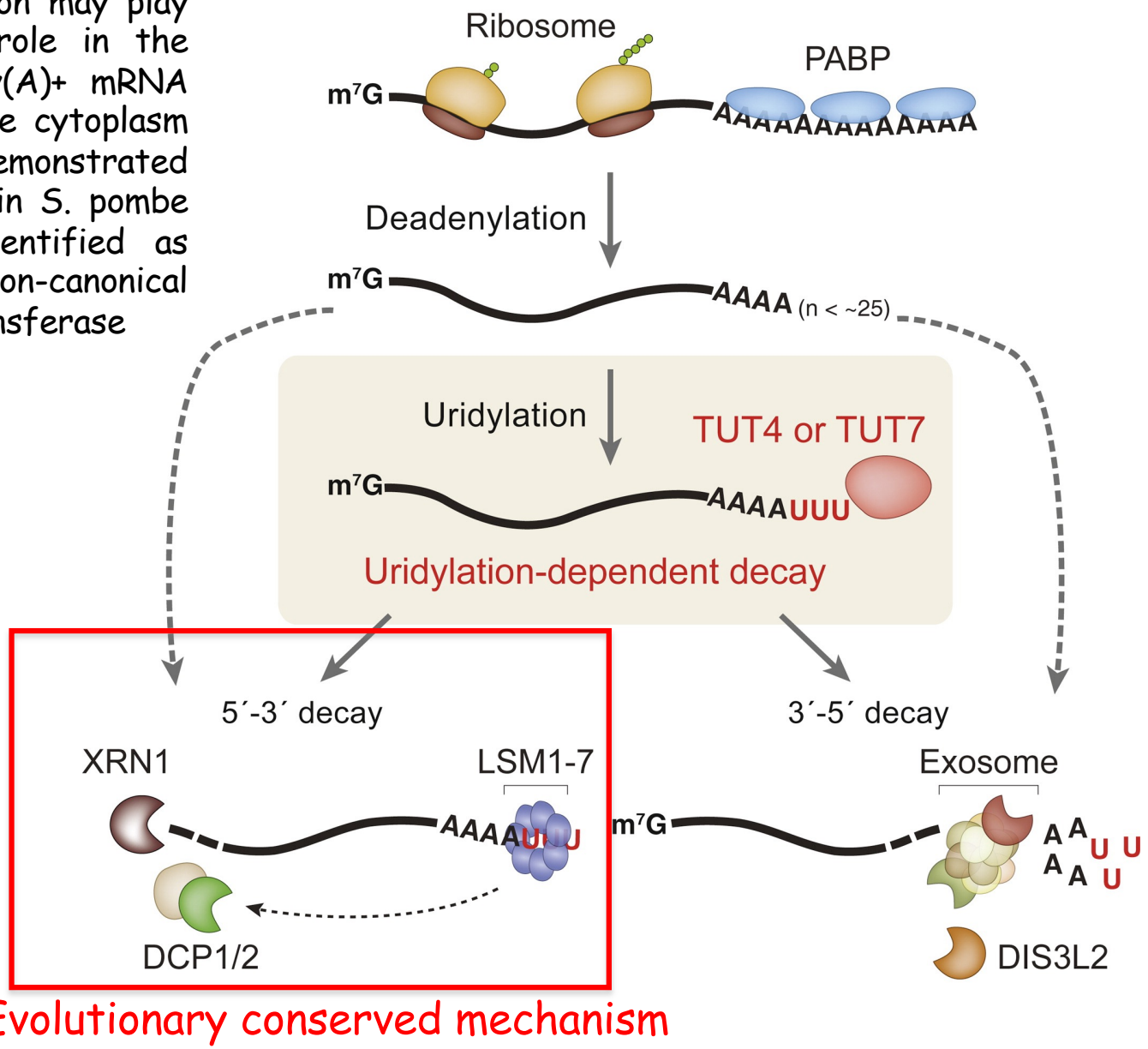


The human genome encodes 7 non-canonical RNA nucleotidyltransferases, with some of them preferentially adding uridine instead of adenine, functioning more as terminal uridyltransferases (TUTases) or poly(U) polymerases (PUPs)

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

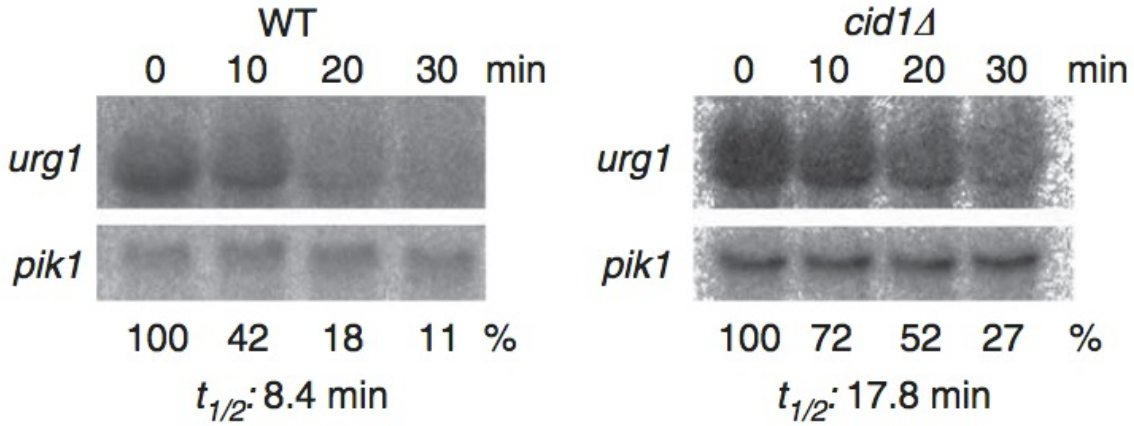
Uridylation-dependent mRNA decapping

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

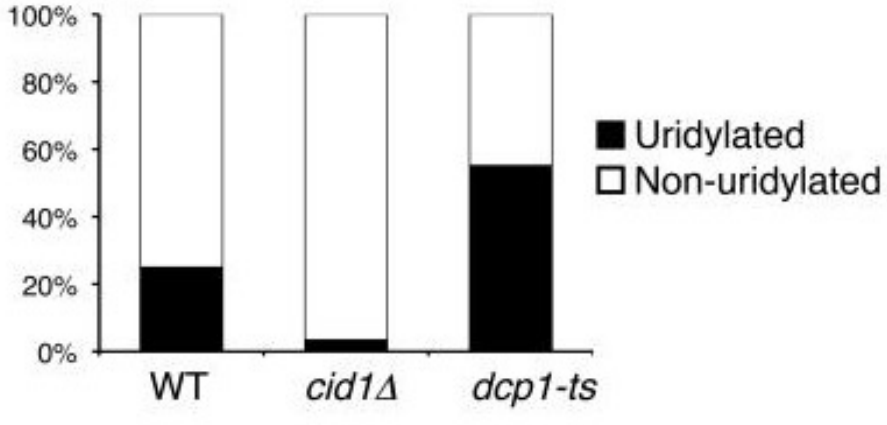


Evolutionary conserved mechanism

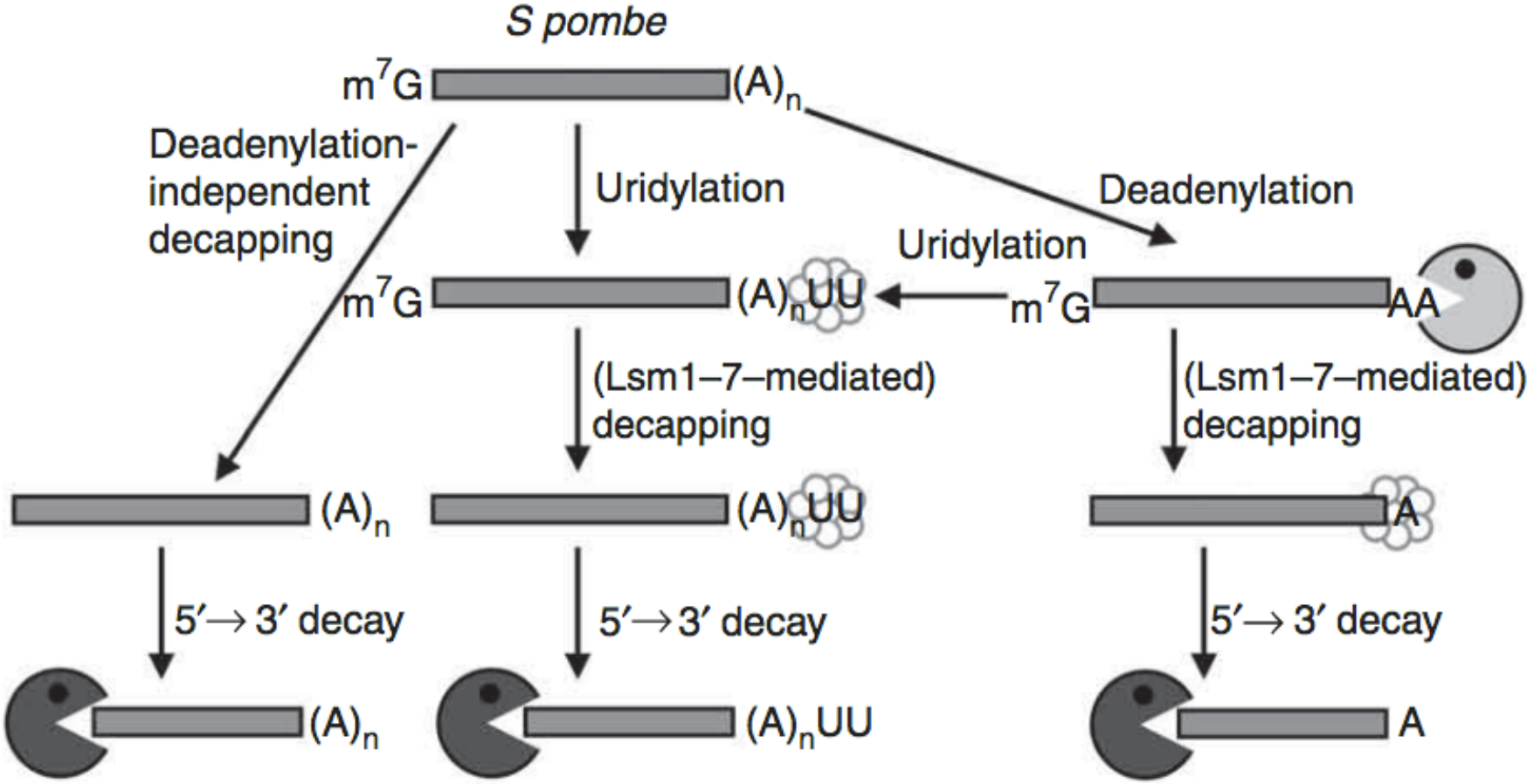
mRNA stability increases substantially in a cid1 deletion strain



oligouridylation occurs prior to decapping



Uridylation is largely Cid1 dependent and precedes decapping

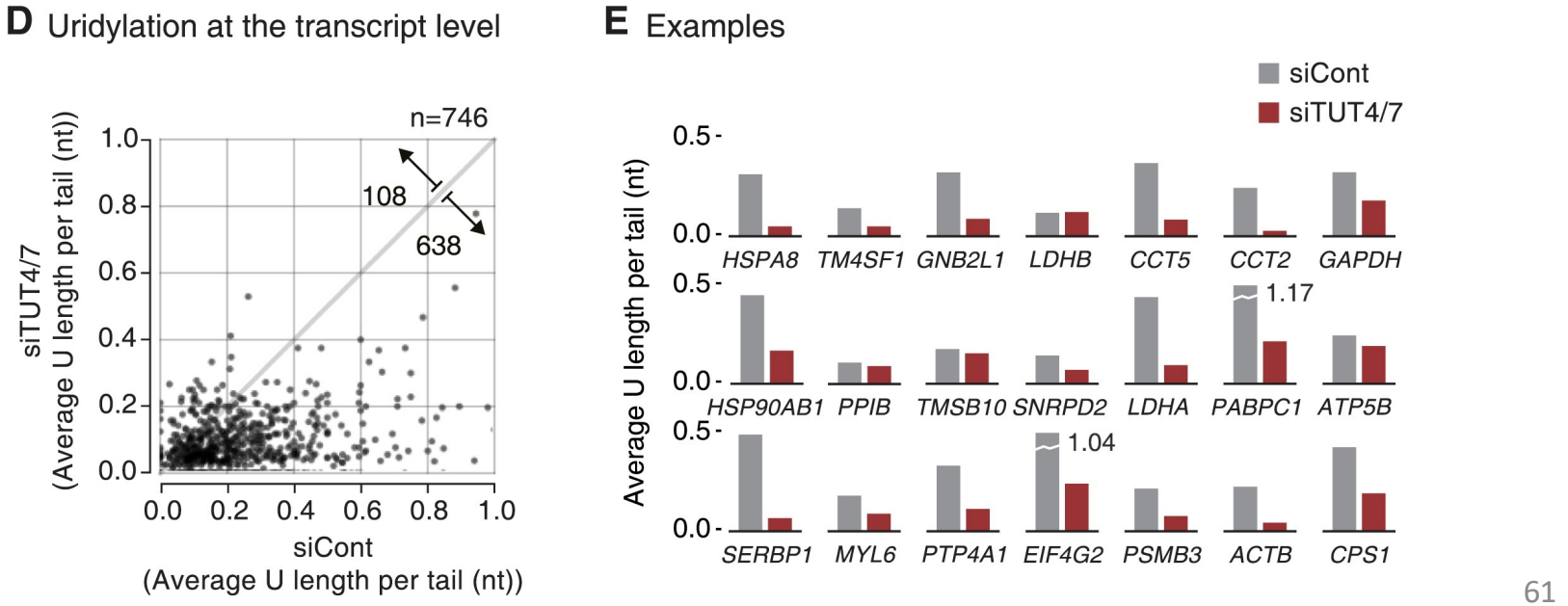


It was postulated that uridylation-dependent decay may be of particular importance in S. pombe , since poly(A) tails present on its mRNAs are considerably shorter than in other eukaryotes .

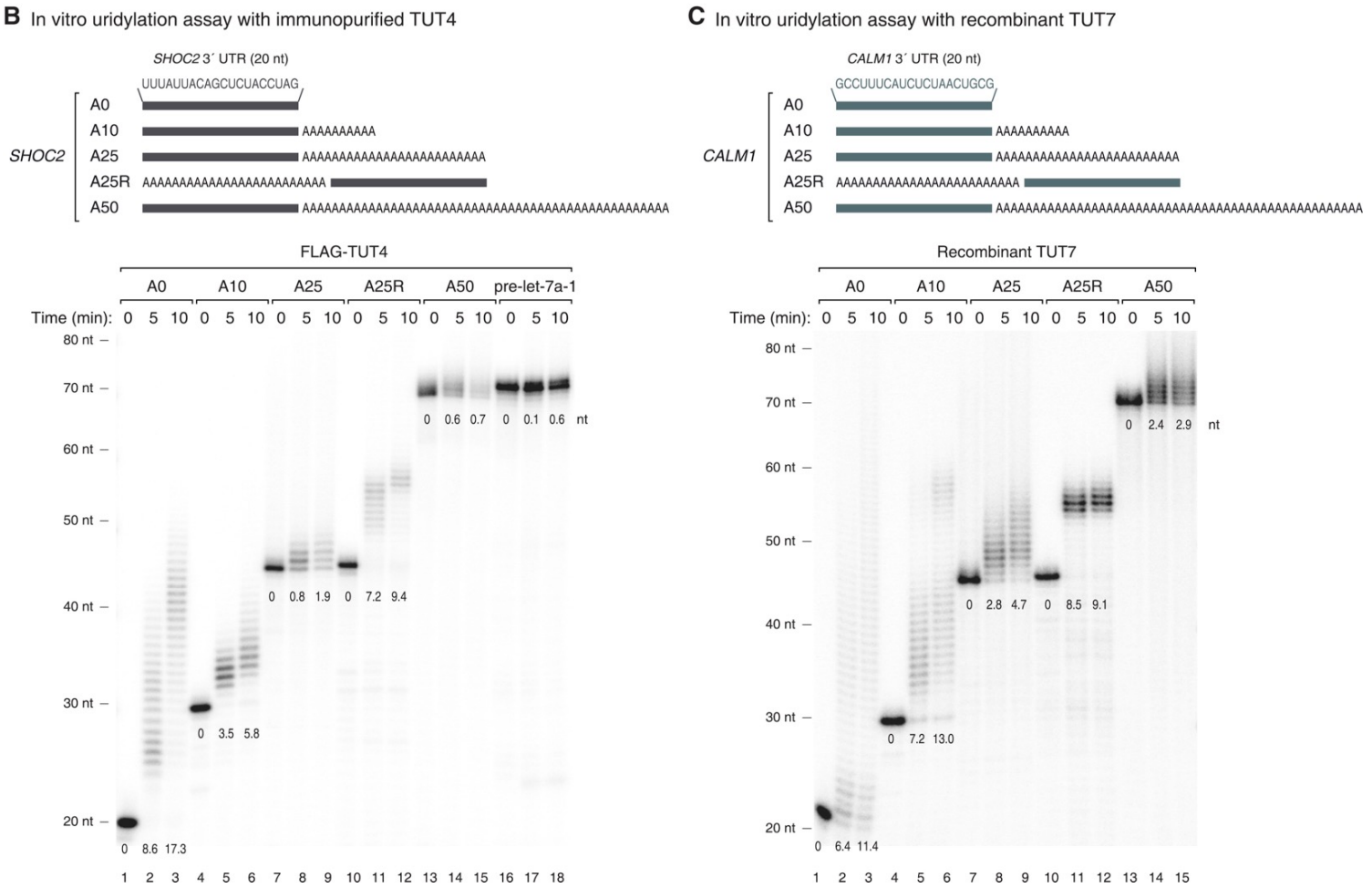
TUTase in human cells

TUTase-4 (ZCCHC11) and TUTase-7 (ZCCHC6) proteins, non-canonical nucleotidyltransferases homologous to fission yeast Cid1, were identified as enzymes responsible for the uridylation of mRNA 3' -ends in human cells. In concordance with previous findings, siRNA-mediated

Gene-level analyses revealed that the majority of mRNA species (638 out of 746 genes, 85.5%) are decreased in uridylation following TUT4/7 knockdown (p = 7.69 × 10–100, one-tailed Mann-Whitney U test) (Figure 1D; Table S1). This result strongly indicates that TUT4/7 uridylate most, if not all, mRNAs



U-rich extensions were added more efficiently to shortened poly(A) tails by TUTase-4/7 both in vivo and in vitro

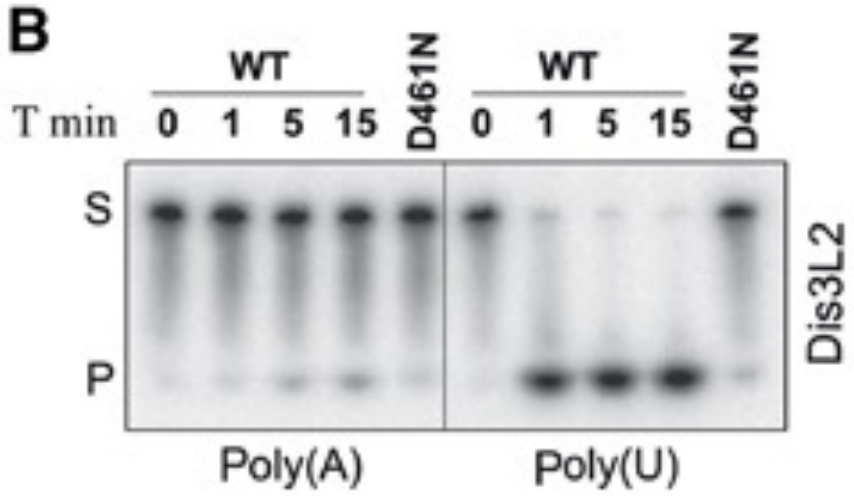


in human cells, in contrast to S. pombe (possessing intrinsically shorter poly(A) tails), mRNA deadenylation precedes uridylation

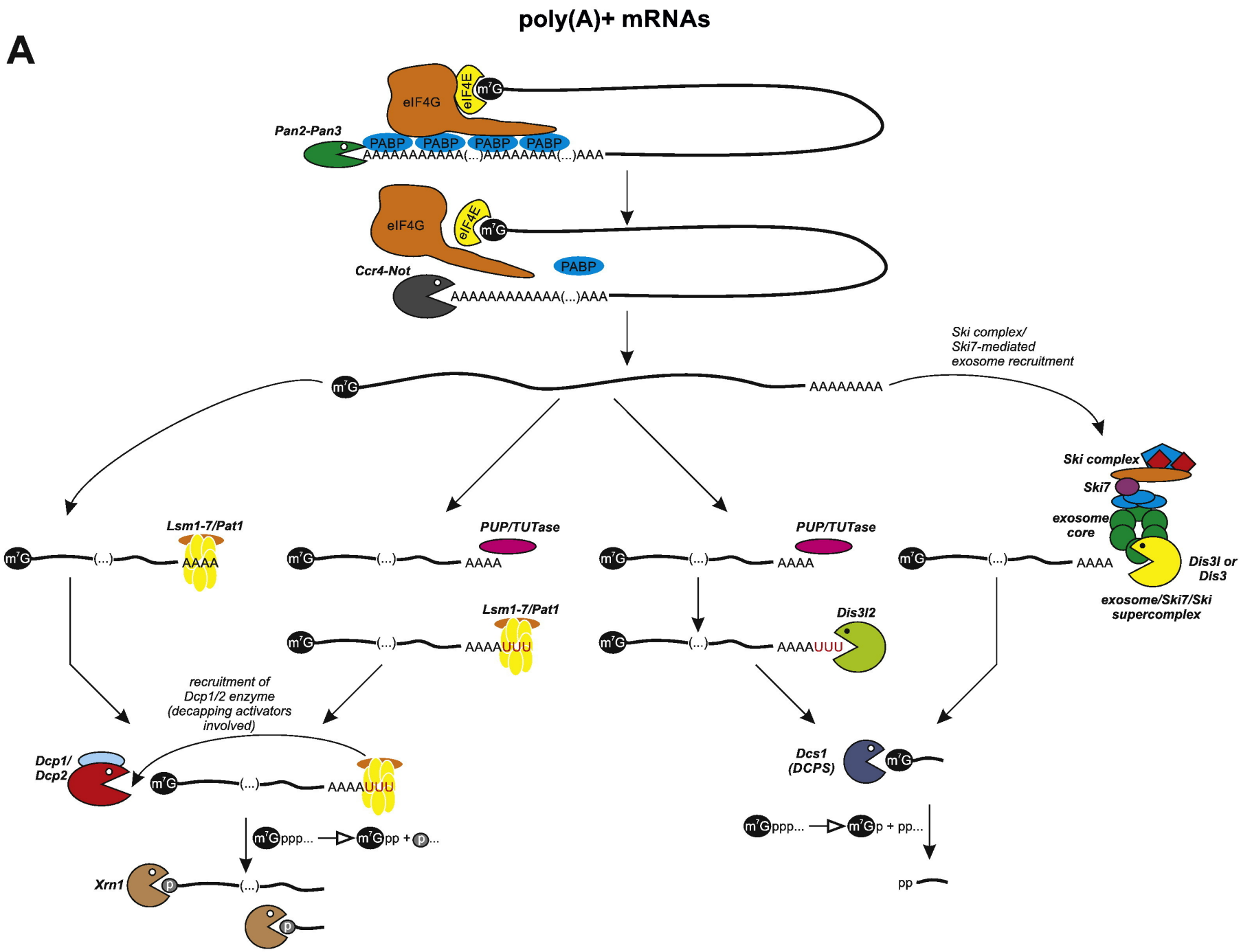
Dis3l2 is the central player of the novel, exosome independent mRNA decay pathway in the cytoplasm.

Dis3l2 is absent from S. cerevisiae , but was shown to participate in mRNA degradation in S. pombe plants and human cells,,

Recent studies carried out in both S. pombe and mammalian cells revealed that Dis3l2 nuclease, a paralogue of the exosome complex Dis3/Dis3l catalytic subunits, works on its own preferentially degrading 3' -uridylated mRNAs in the 3' -5' direction



In addition to activation of decapping and 5' -3' degradation, uridylation apparently also stimulates mRNA decay in the other direction by enhancing Dis3l2 exoribonuclease activity


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

TUTase-4 is the most likely enzyme involved in histone mRNA decay