Nonsense-mediated decay
NMD

"Nonsense-mediated decay (NMD)"

•NMD was initially described as a mechanism for recognizing and degrading faulty transcripts harbouring a **premature translation-termination codon (PTC)**

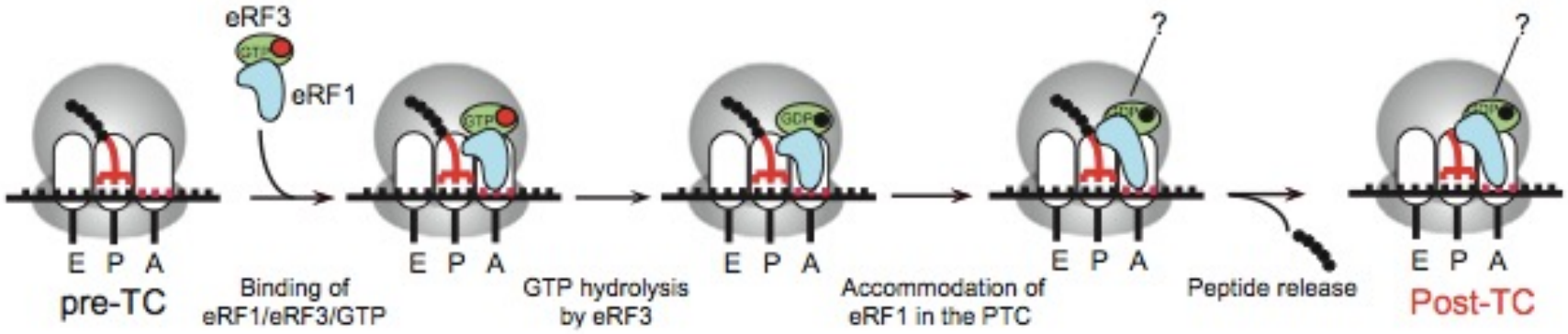
•PTCs can arise either from mutations at the DNA level (e.g. nonsense mutations, frame-shifting deletions and insertions) or from altered splicing signals that induce production of alternatively spliced mRNA isoforms with truncated reading frames

•it has been estimated that 30% of the known disease-associated mutations in humans generate a PTC-containing mRNA

•Among pre-mRNAs that undergo alternative splicing, 45% generate at least one splice form predicted to be an NMD substrate

•NMD not only degrades faulty transcripts but also regulates the steady-state level of many physiological mRNAs involved in a variety of different cellular processes

Normal Translation termination



The **pre-termination complexes** (pre-TCs) contain peptidyl-tRNA in the ribosomal P-site. **eRF1** and **eRF3** bind to pre-TCs as an **eRF1/eRF3/GTP ternary complex**. The stop codon is recognized by eRF1. After GTP hydrolysis by eRF3, eRF1 induces peptide release. At least one release factor, eRF1, remains associated with post-termination complexes (post-TCs). Dissociation and recycling of the termination complex is facilitated by the **ABCE1 ATPase** and the eukaryotic translation initiation factors **eIF3**, **eIF1**, **eIF1A** and **eIF3.**

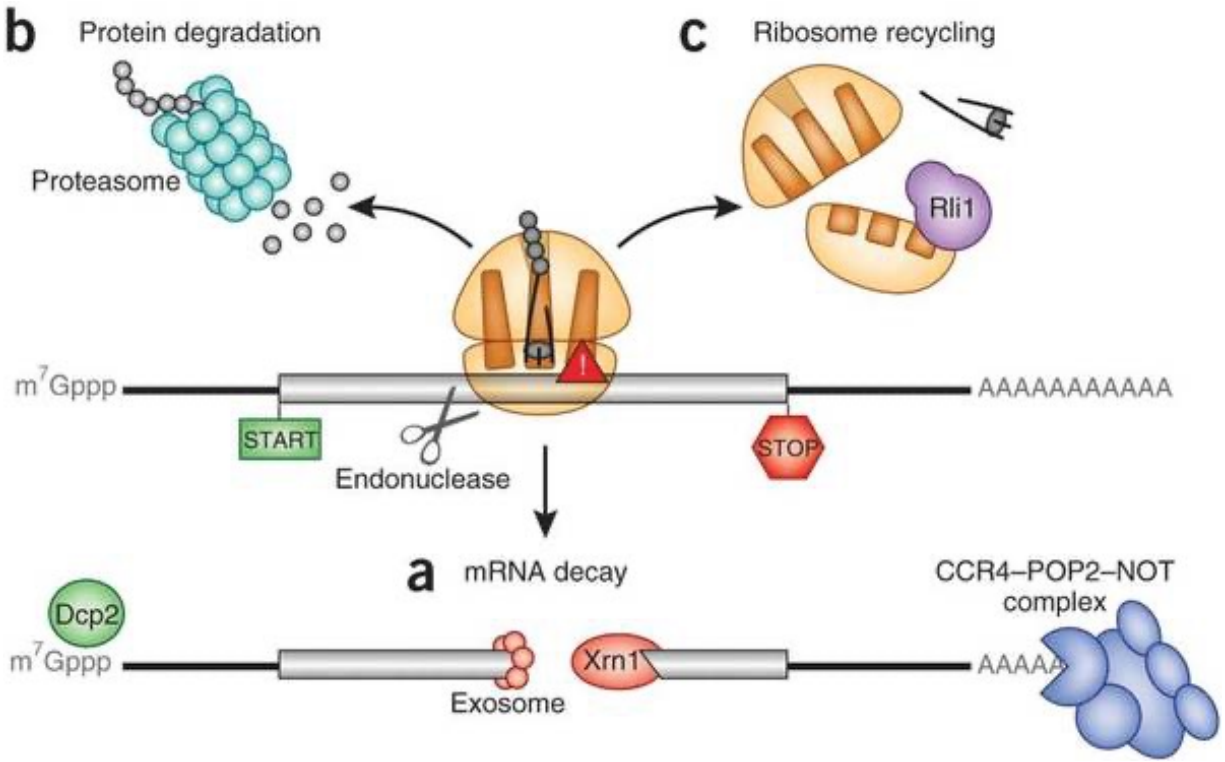
The aberrant nature of premature termination

In both yeast and human cells, translation termination at a PTC is less efficient than normal termination and leads to a pause of the ribosome at the nonsense codon.

There is no evidence that peptide hydrolysis is slower at PTCs than at NTCs, thus implicating reductions in the rate of later steps in the termination process.

Most evidence points to decreased efficiency of ribosome and mRNP dissociation subsequent to peptide hydrolysis.

NMD Outcomes



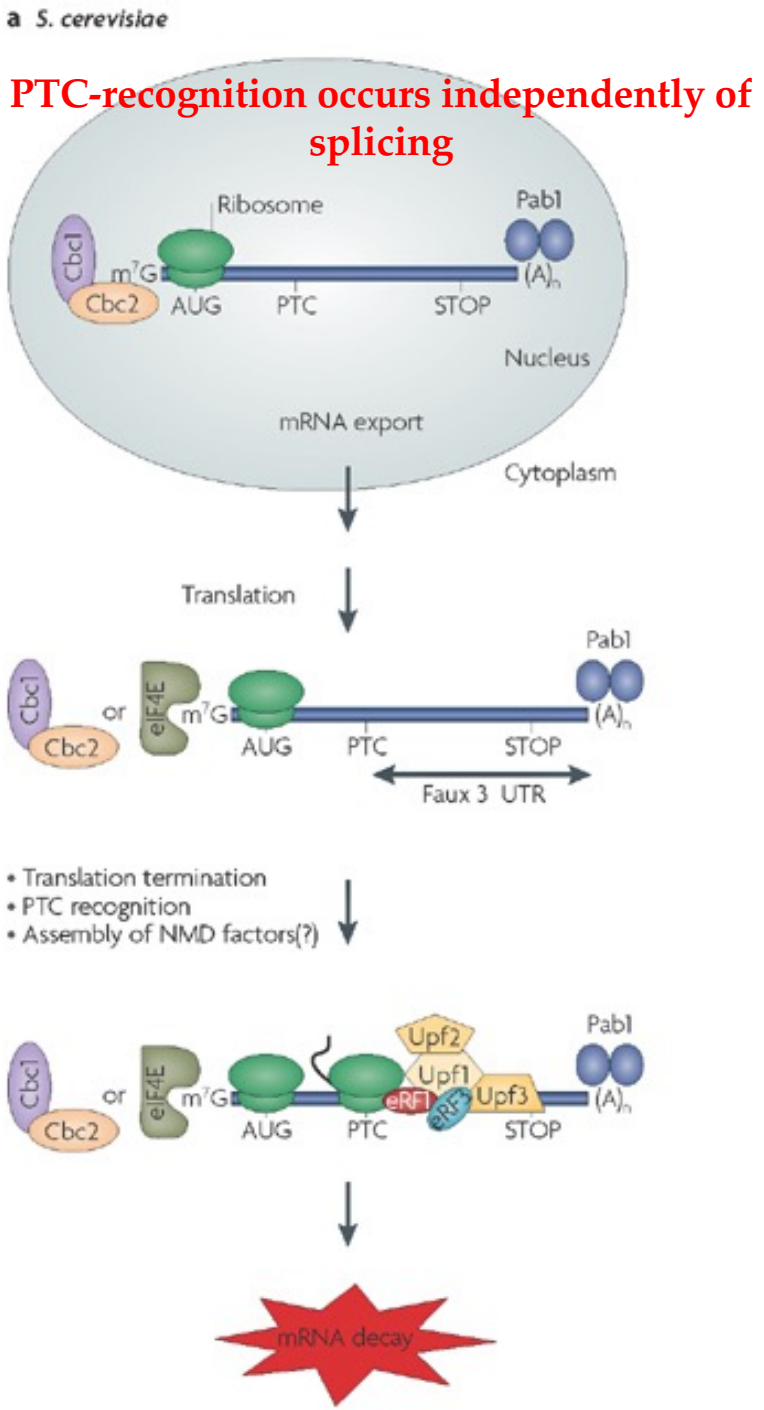
NMD (Yeast)

•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.

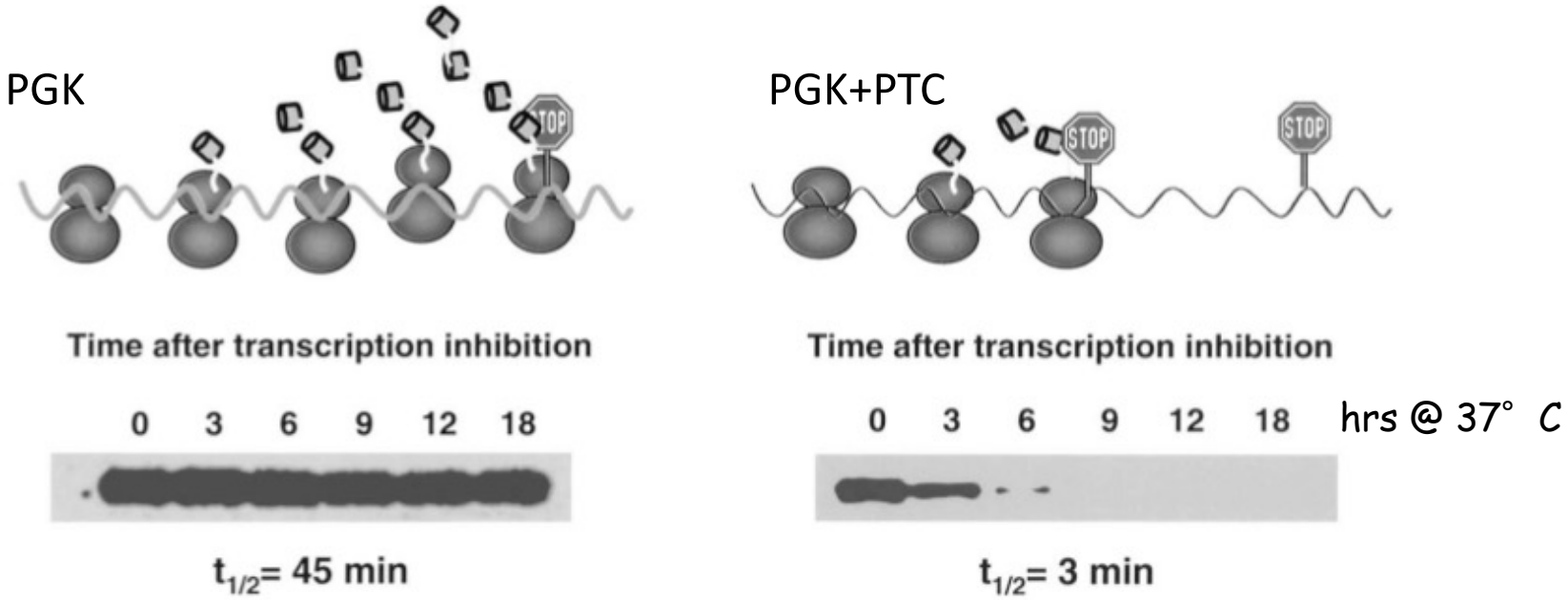
•**Abnormally long 3'-UTR ("faux") or a downstream sequence element (DSE)** recruits proteins required for the identification of a nonsense codon as a PTC.

•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



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

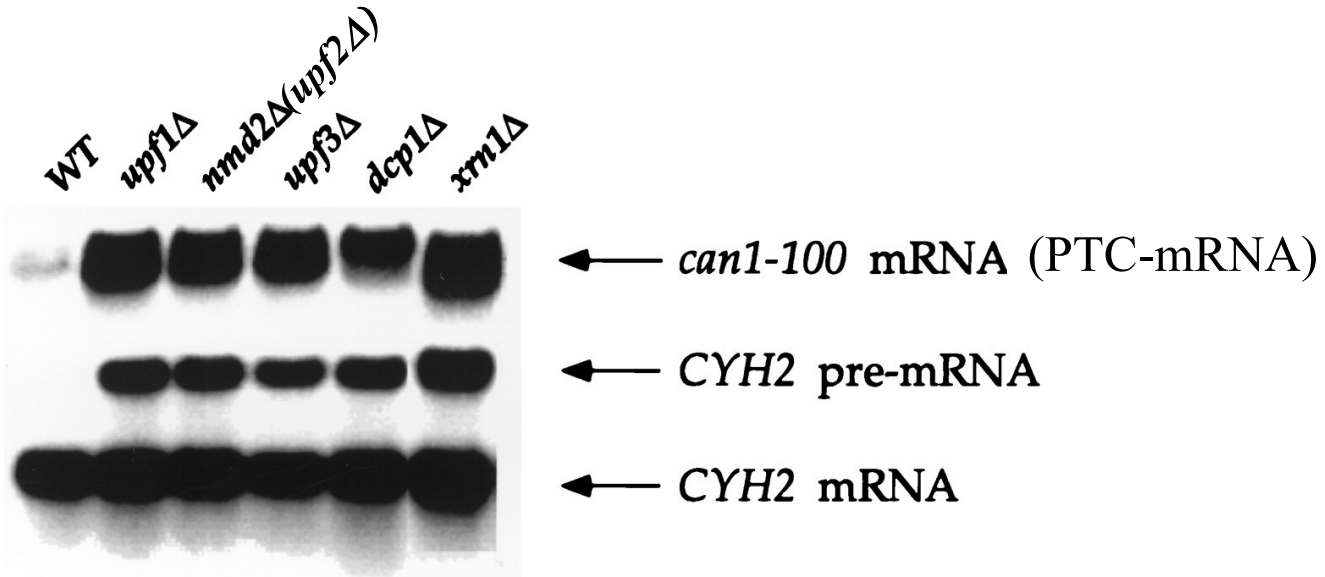


yeast strain harboring a temperature-sensitive RNA polymerase II

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



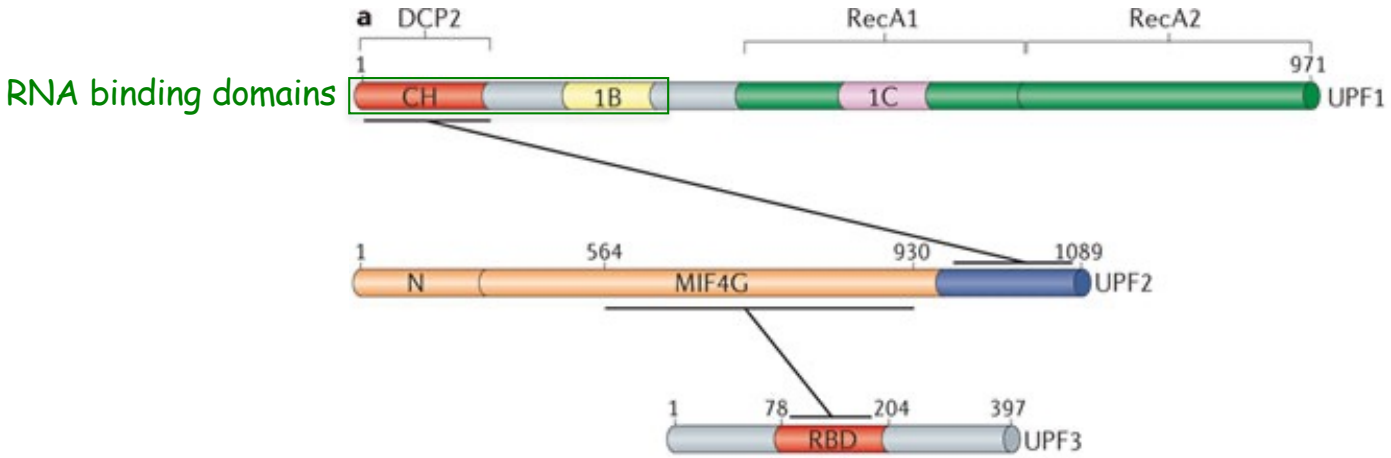
Upf1, Upf2 and Upf3

The proteins encoded by ***UPF1*** (*smg-2* in *C.elegans*), ***UPF2*** (*smg-3* in *C. elegans*) and ***UPF3*** (*smg-4* in *C. elegans*) are the principal NMD regulators in eukaryotes

The three UPF proteins interact, with UPF2 acting as a bridge between UPF1 and UPF3

UPF1 can bind RNA and has helicase activity. **UPF2** interact with **UPF3**, a protein with an RNP-type RNA-binding domain

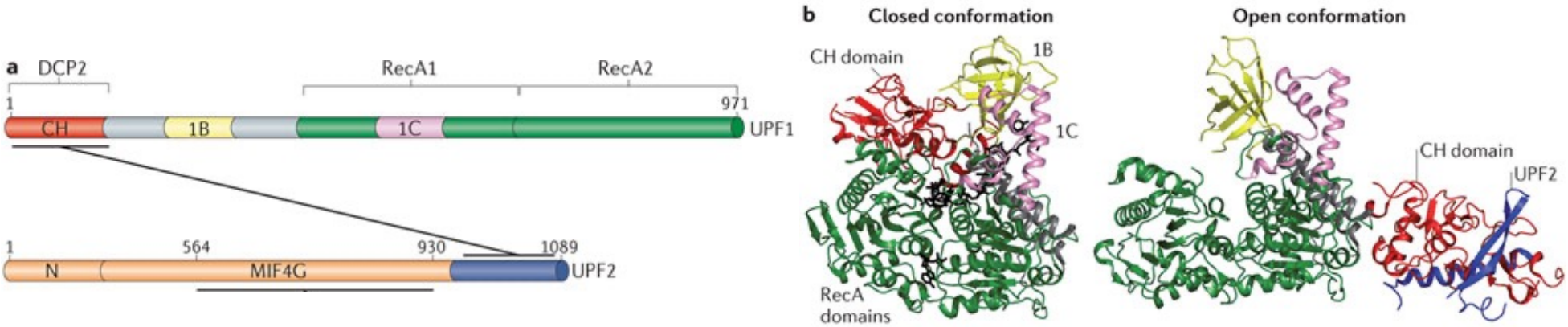
UPF1 is the key effector of NMD, with UPF2 and UPF3 regulating UPF1 function. In yeast, overexpression of *UPF1* can compensate for mutations in *UPF2* and *UPF3* but not *vice versa*.



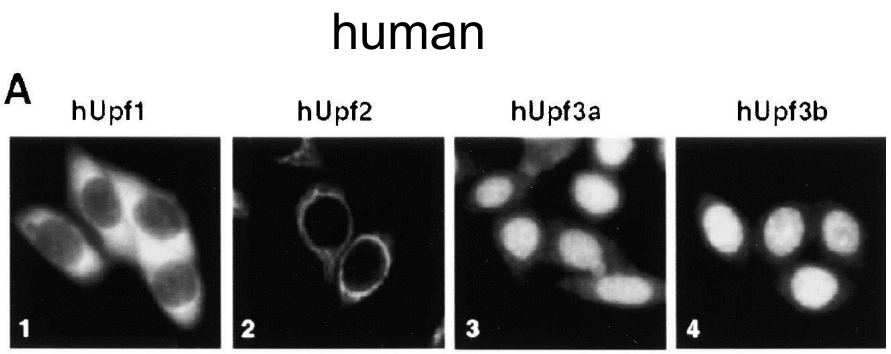
Upf1, Upf2 and Upf3

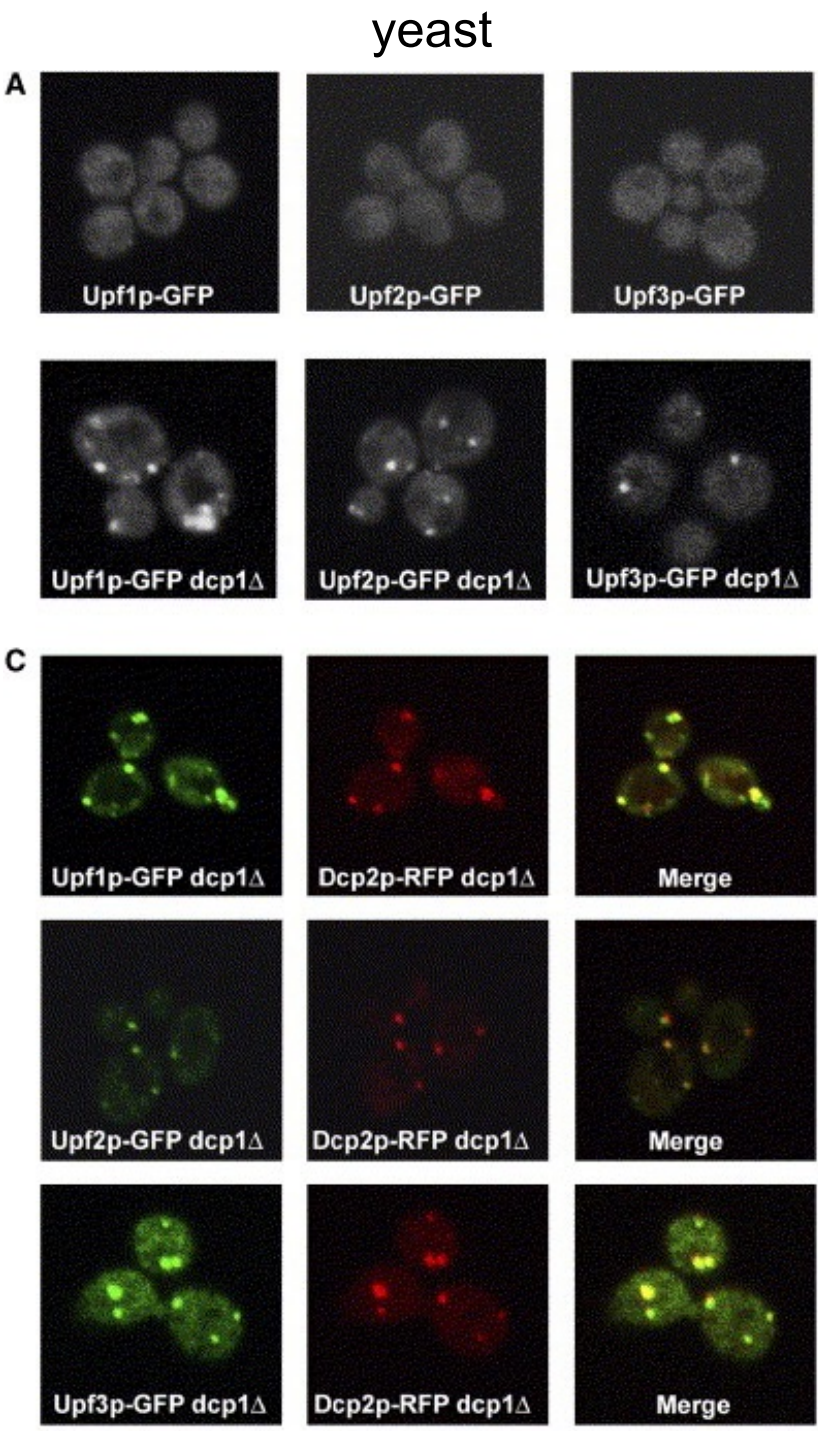
In the absence of **UPF2**, **UPF1** exists in a closed conformation in which the CH domain (RNA binding domain) interacts with the RecA-like domains (helicase domain). In this conformation, UPF1 binds to RNA, thus increasing the overall extent of RNA binding by UPF1 and decreasing its ATPase and helicase activities.

Upon binding to **UPF2**, UPF1 undergoes substantial conformational change, with the CH domain switching from a position near the RNA-binding site to a more distal position. This open form binds RNA less extensively and has increased levels of ATPase and helicase activities; that is, **it switches from a state in which it is tightly bound to RNA to one in which it can unwind RNA.**



Yeast **Upf** proteins have cytoplasmic localisation and accumulate in **P-bodies** if 5′->3′degradation of mRNA was blocked. In human only **Upf1** has a cytoplasmic localization, while **Upf3** is nuclear and **Upf2** perinuclear.

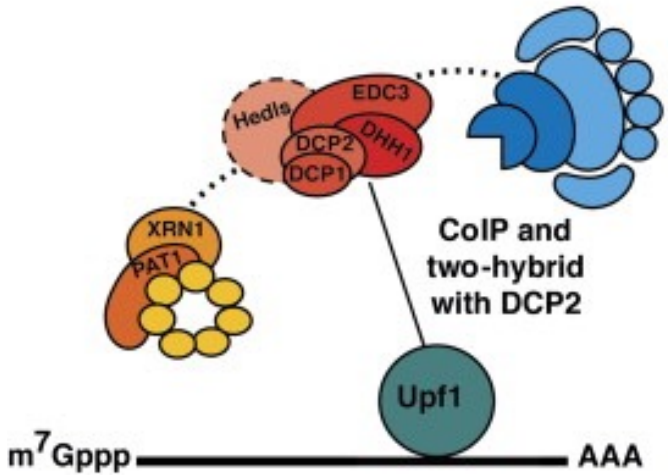




Nonsense mutations in a gene can reduce the steady- state levels of the mRNA

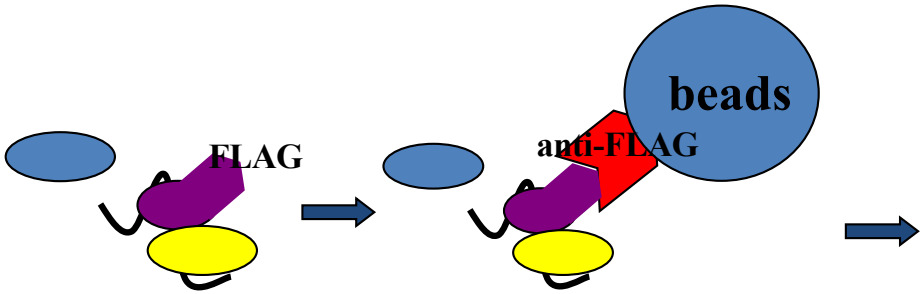
•The turn-over of nonsense-containing mRNAs is deadenylation-independent, entering the predominant 5 -> 3 decay pathway with an intact poly(A) tail

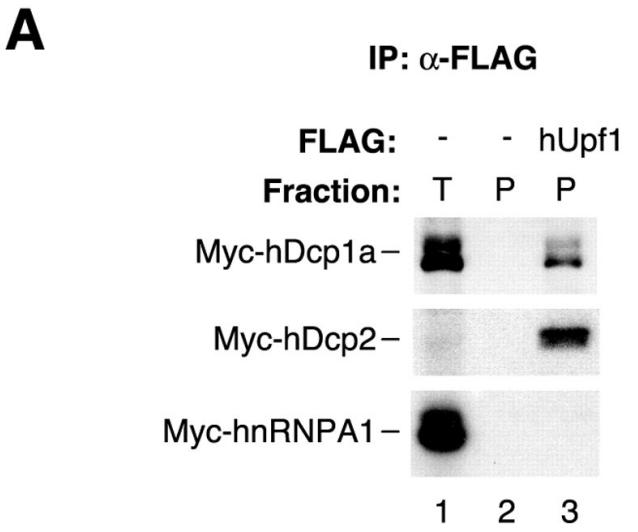
•These nonsense-containing mRNAs are decapped by **Dcp2p**, followed by degradation of the body of the transcript by **Xrn1p.**

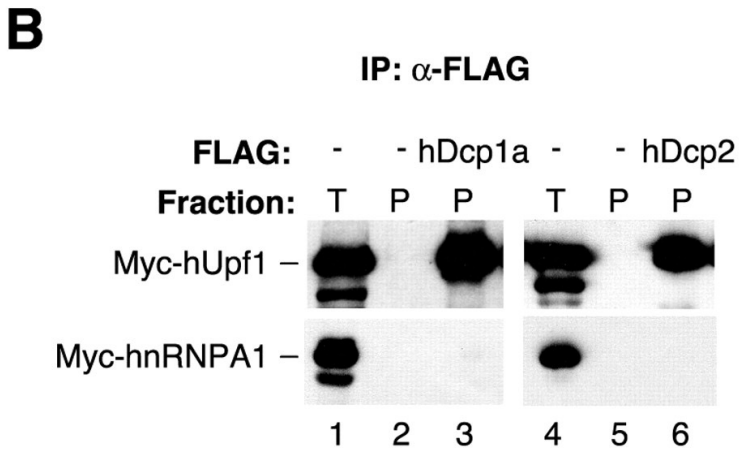


The Upf1 protein interacts specifically with Dcp1p and Dcp2p

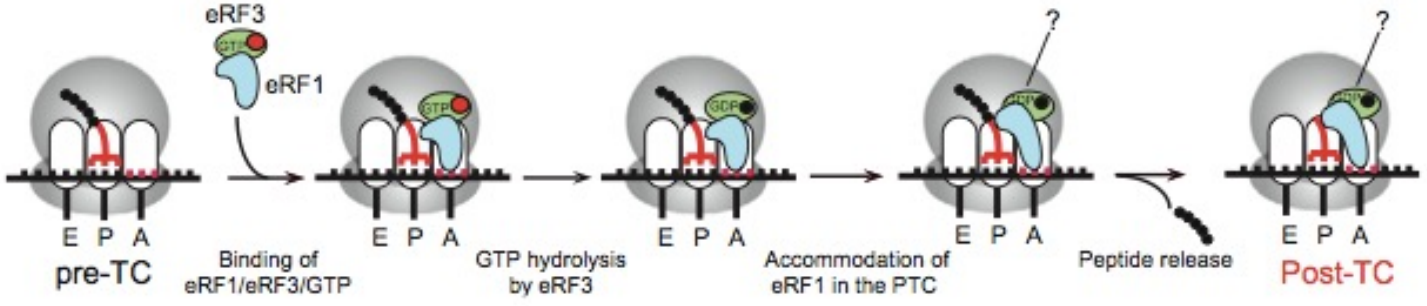
Upf1 interacts with **Dcp1** and **Dcp1** by 2YS and co-IP (human and yeast)



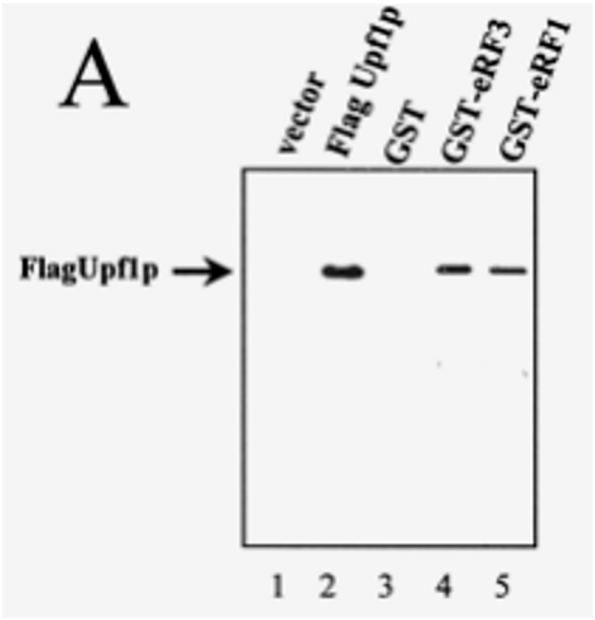




The Upf1 protein interacts specifically with the peptidyl release factors eRF1 and eRF3

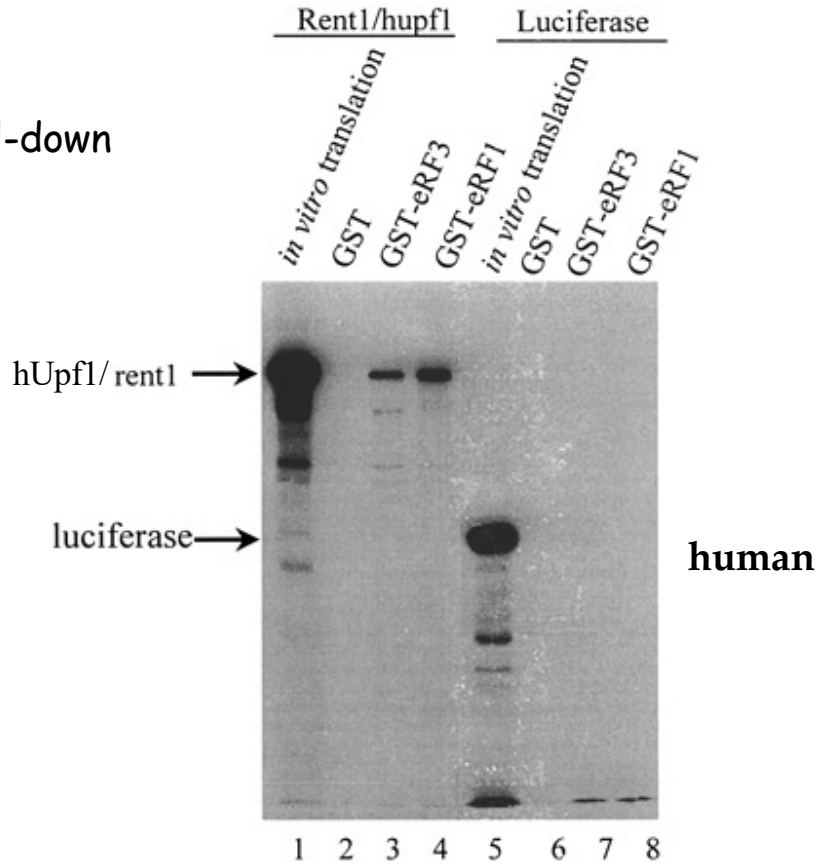


GST pull-down with GST-eRF1 or GST-eRF3:



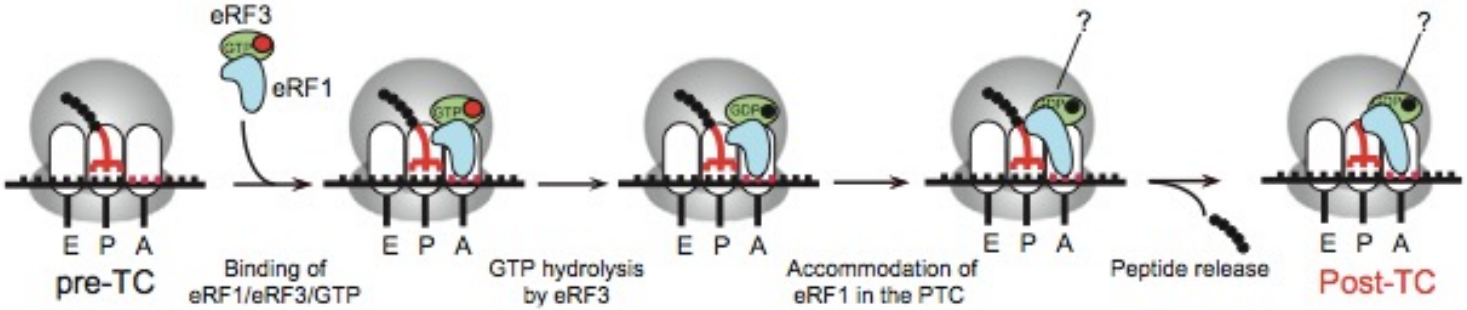
Yeast

GST Pull-down

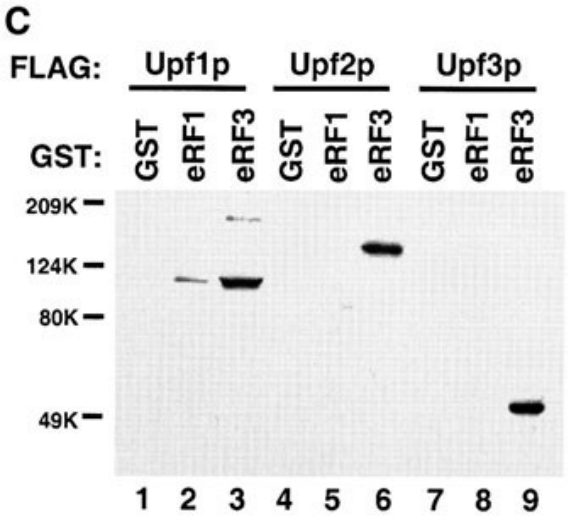


human

The Upf2 and Upf3 proteins interact with the release factor eRF3



GST Pull-down with GST-eRF1 or GST-eRF3 and extracts containing FLAG-Upf2 or 3:



NMD pathway in yeast is a translational-dependent event

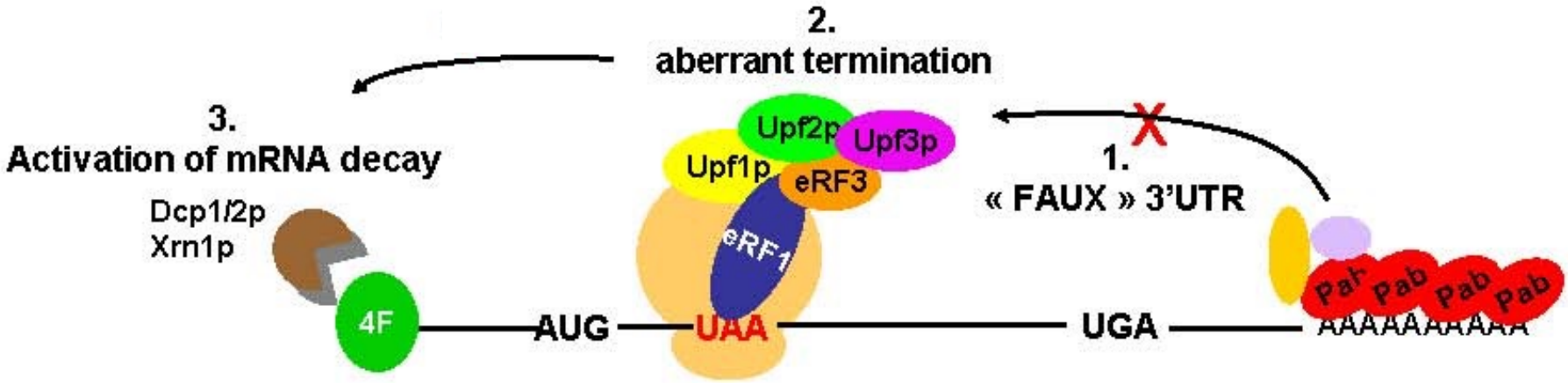
•NMD is inhibited by drugs and mutations that block translation initiation and elongation

•Nonsense-containing mRNAs are associated with polysomes

•NMD can be prevented by nonsense-suppressing tRNAs

•Factors essential for NMD interact with the translation termination release factors eRF1 and eRF3

•NMD is suppressed by Pab1 overexpression



How a PTC can be distinguished from a natural termination codon ????????

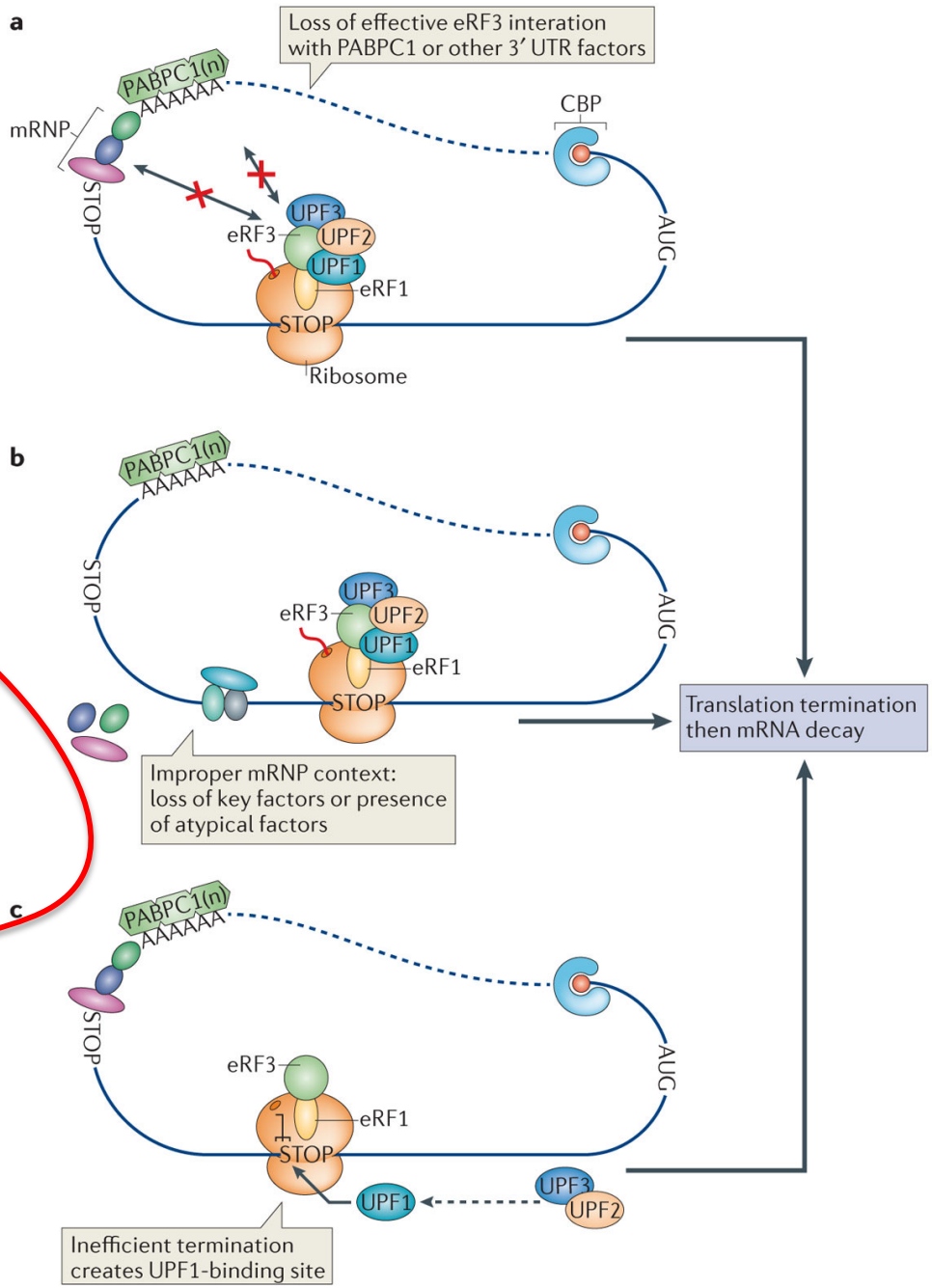
Models for NMD activation in yeast

UPF proteins may associate with a prematurely terminating ribosome because:

a. essential interactions between **poly(A)-binding protein** (**PABPC1**; or other 3′ untranslated region (UTR)-associated proteins) **eRF3** have been disrupted;

b. The mRNP context is not accommodating for termination (that is, crucial proteins have not been added to or removed from the mRNP) or

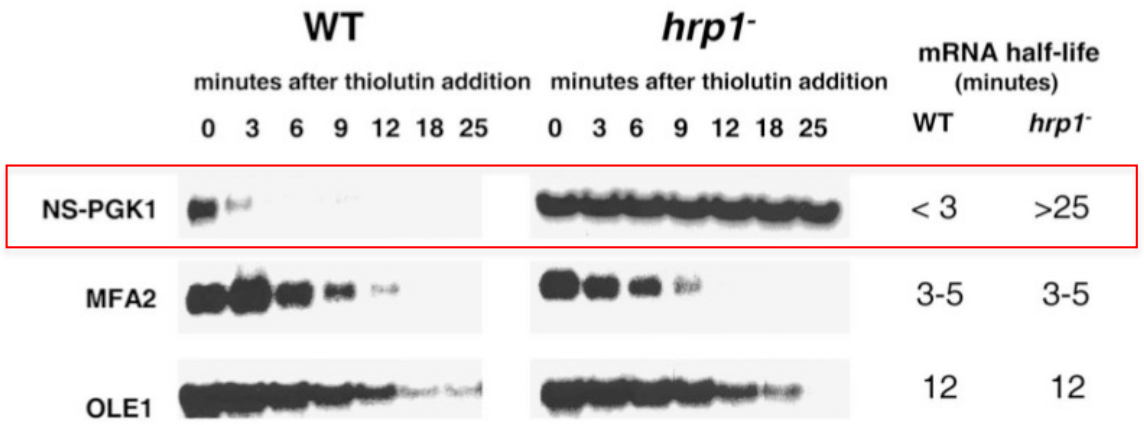
c. the inefficiency of premature termination has left the ribosome in an atypical conformation



Mutations in HRP1 specifically affect the NMD pathway

•**Hrp1p/Nab4p** was originally identified as a poly(A)$^+$ RNA-binding protein structurally related to mammalian heterogenous nuclear ribonucleoproteins

•Hrp1p is essential for growth, shuttles between the nucleus and the cytoplasm, and play a role in 3'-end cleavage and polyadenylation



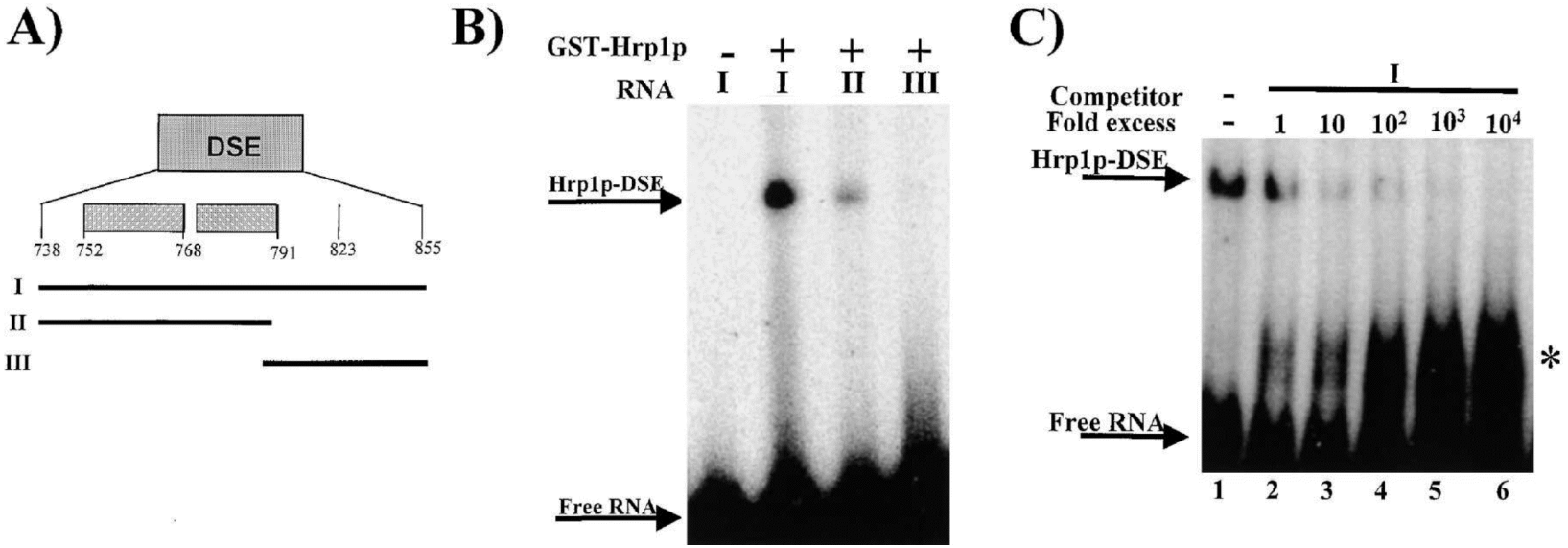
non-sense reporter

the drug thiolutin was added to block transcription

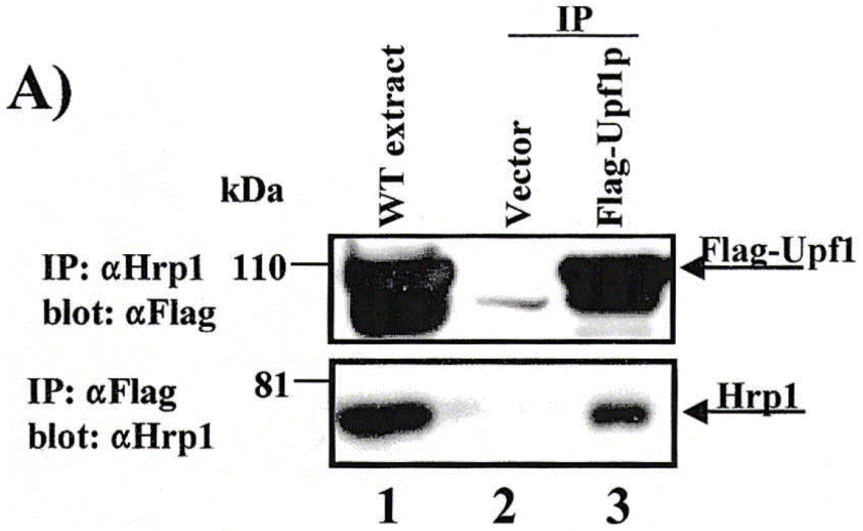
Hrp1p/Nab4p Specifically Interacts with the DSE

•Transcripts containing a premature termination codon, but lacking a **DSE (downstream sequence element)**, are not degraded by the NMD pathway

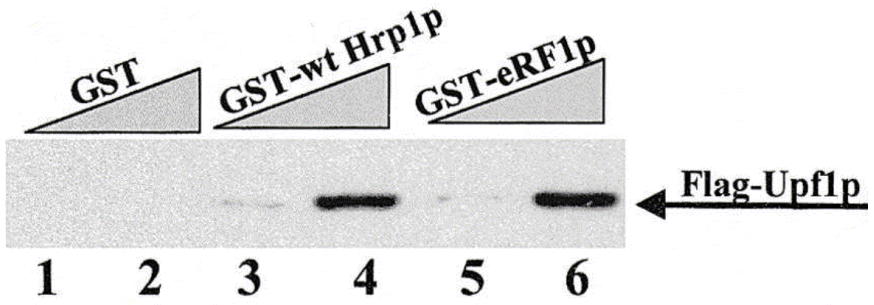
•The sequences in the **DSE** important for activation of the NMD pathway have been characterized in several transcripts



Hrp1p Interacts with Upf1p



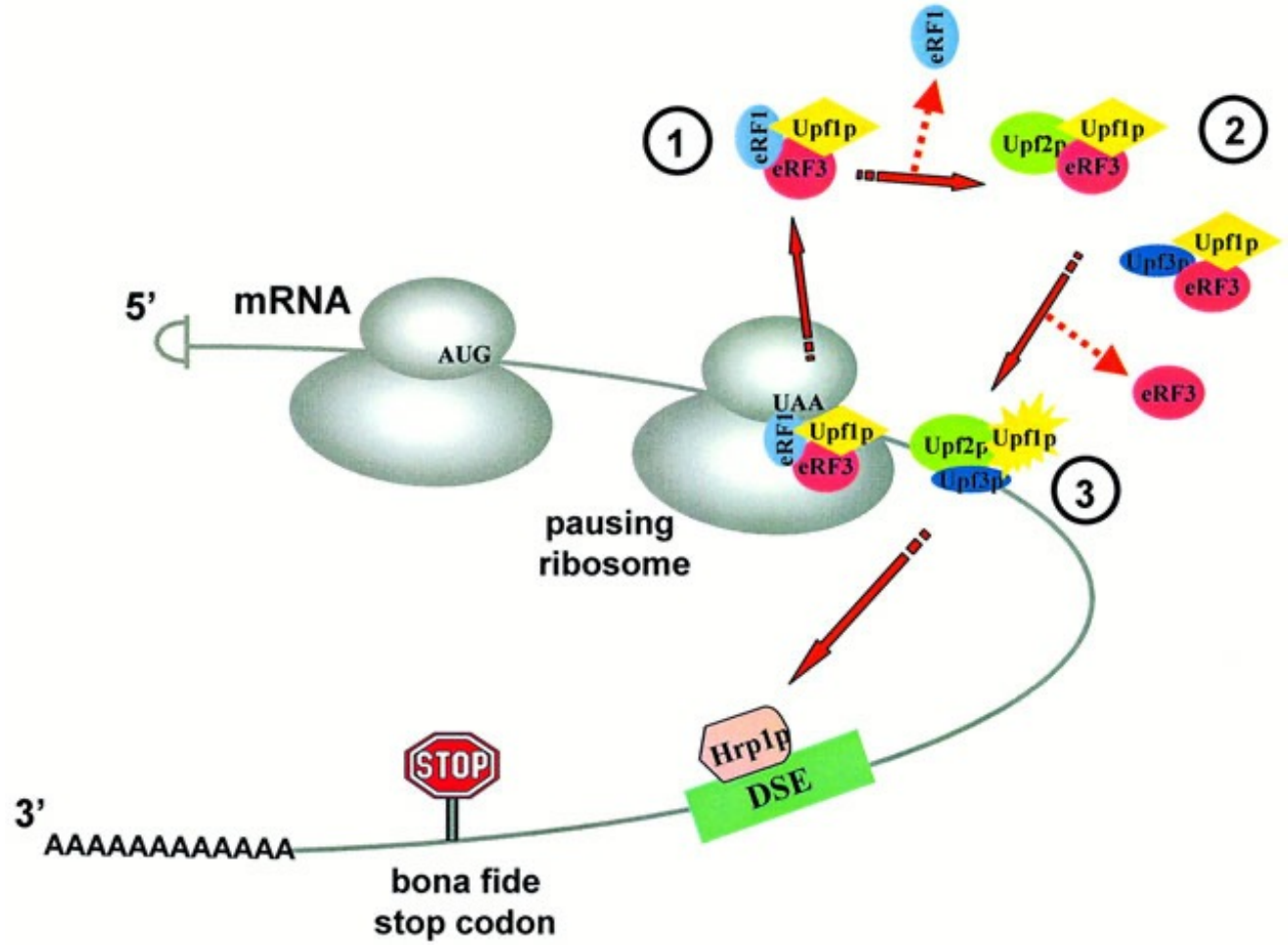
Co-IP



GST Pull-down

The Hrp1 dependent NMD Pathway

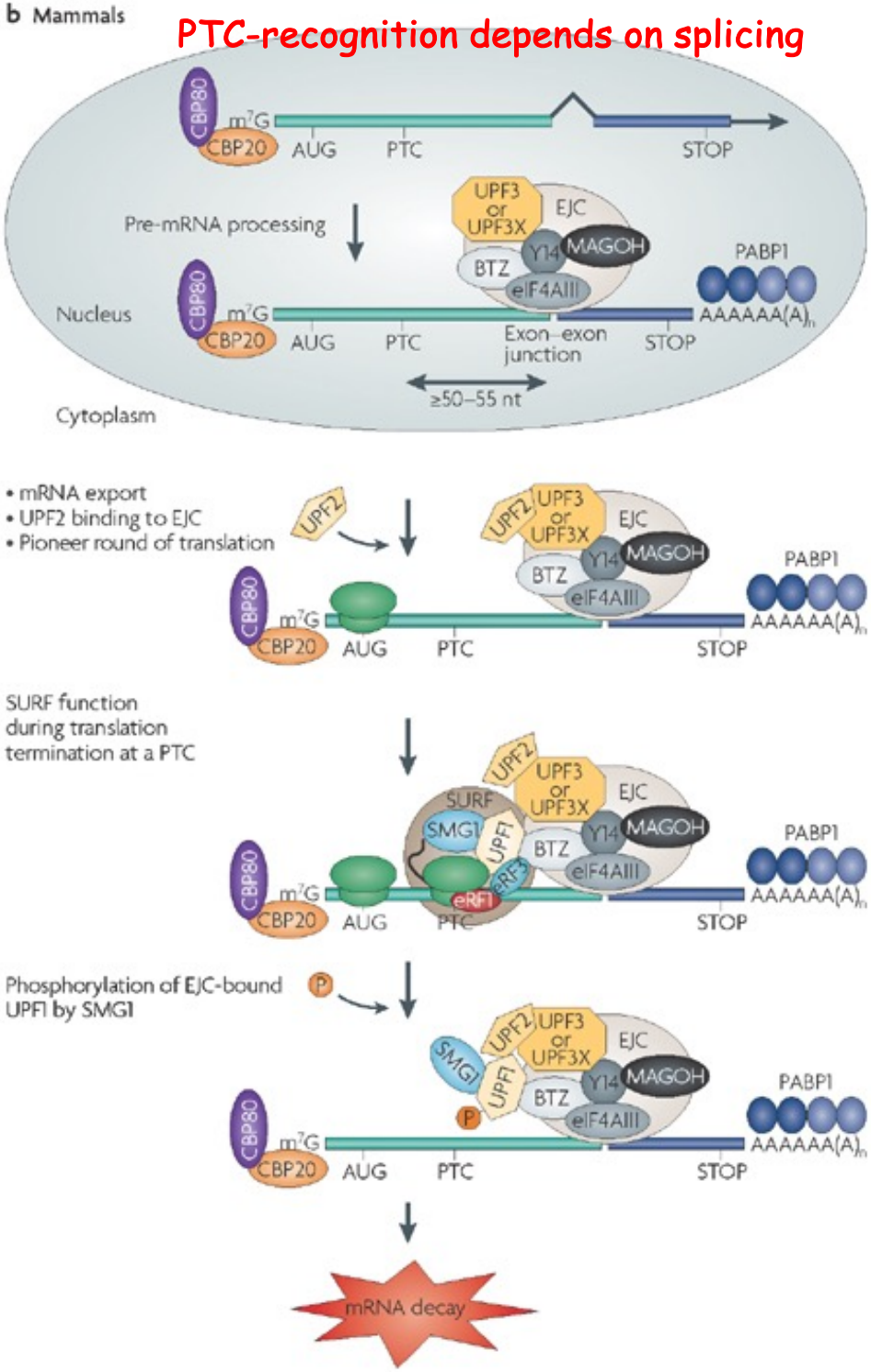
The premature termination codon (**PTC**) prevents the ribosome from displacing **Hrp1** from the **DSE**, and following termination, the surveillance complex (SC) recognizes Hrp1p as a signal that RNP remodeling is incomplete, and subsequently, the mRNA is decapped at the 5- end.



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



NMD effectors

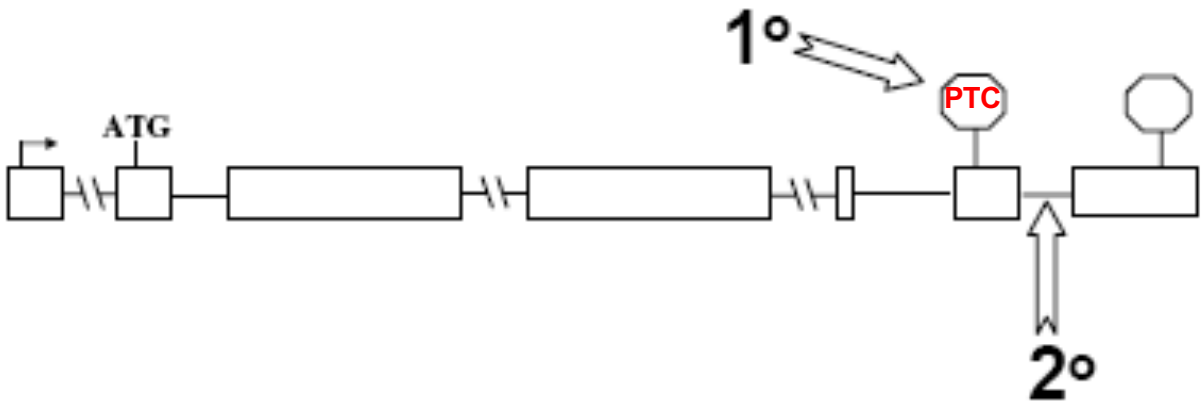
Organism	 Yeast (*Saccharomyces cerevisiae*)	 Nematodes (*Caenorhabditis elegans*)	 Fruitfly (*Drosophila melanogaster*)	 Mammals (*Mus musculus*)
Effectors	Upf1 Upf2 Upf3	SMG-2(UPF1) SMG-3(UPF2) SMG-4(UPF3) SMG-1 SMG-5 SMG-6 SMG-7	UPF1 UPF2 UPF3 SMG1 SMG5 SMG6	UPF1(RENT1) UPF2 UPF3a/b SMG1 SMG5 SMG6 SMG7
Phenotype	Not essential [5]	Viable worms with morphological effects on genitalia [11,15,23,40]	UPF1 and UPF2 required for larval development and cell proliferation [43,57]	UPF1JKO: embryonic lethal required for cell cycle progression UPF3 KD: effects on cell proliferation [7,50,58]

Detection of mRNAs with PTCs in mammals

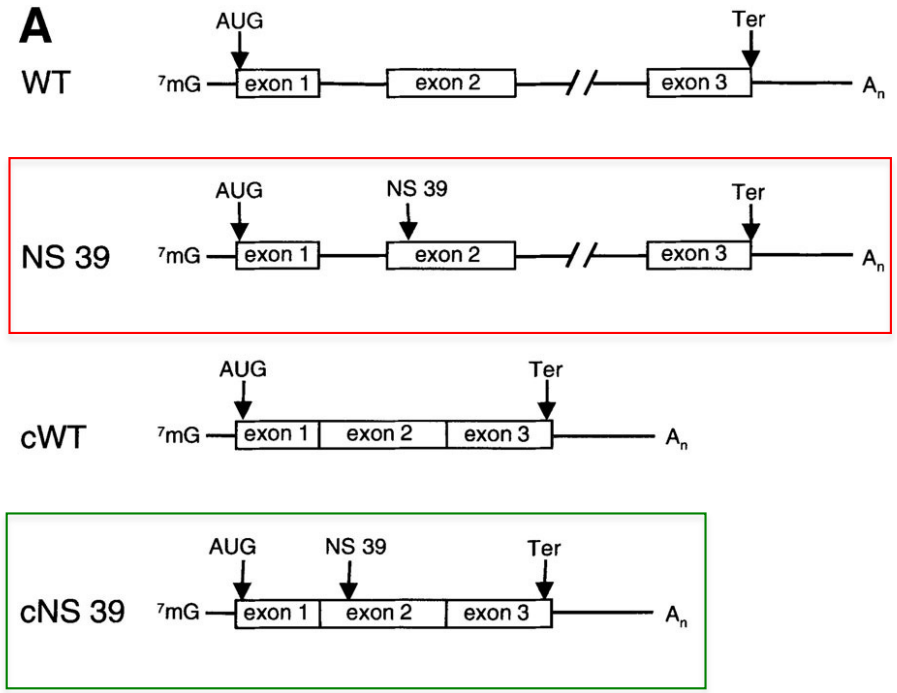
Two signals are required for NMD:

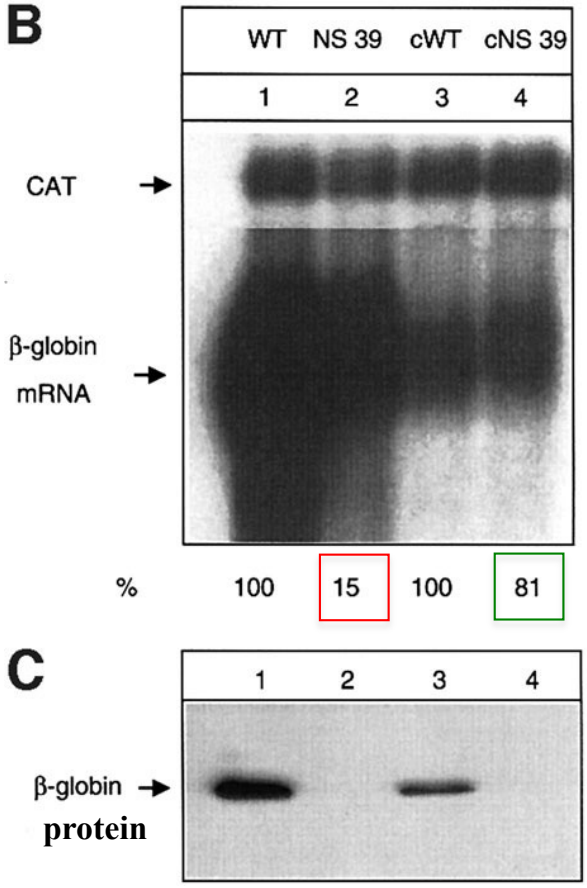
1. Premature Stop Codon (PTC)

2. Intron



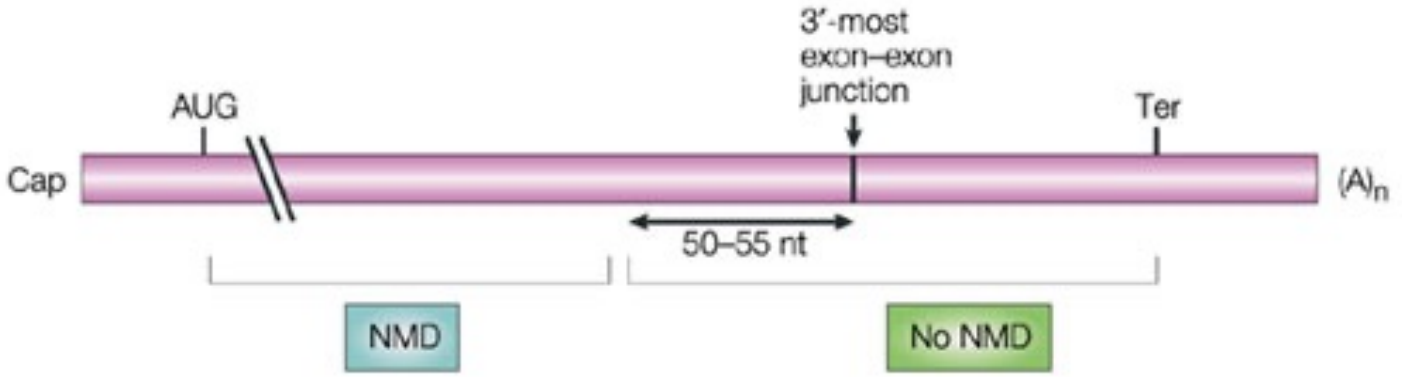
Splicing is indispensable for NMD





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

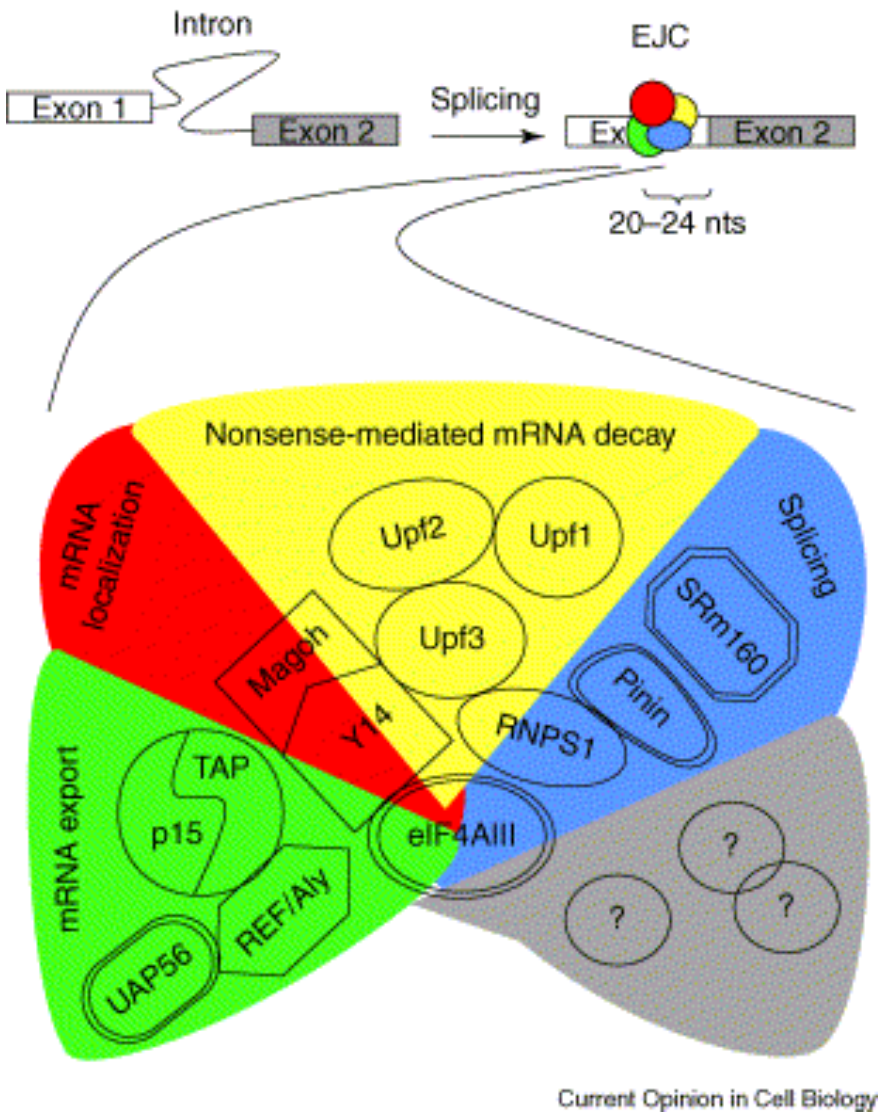
A **premature termination codon (PTC)** that is located in the region indicated in blue, which is followed by an exon–exon junction more than 50–55 nucleotides (nt) downstream, elicits **NMD**, whereas a PTC that is located in the region indicated in green fails to elicit NMD.



The Exon Junction Complex (EJC)

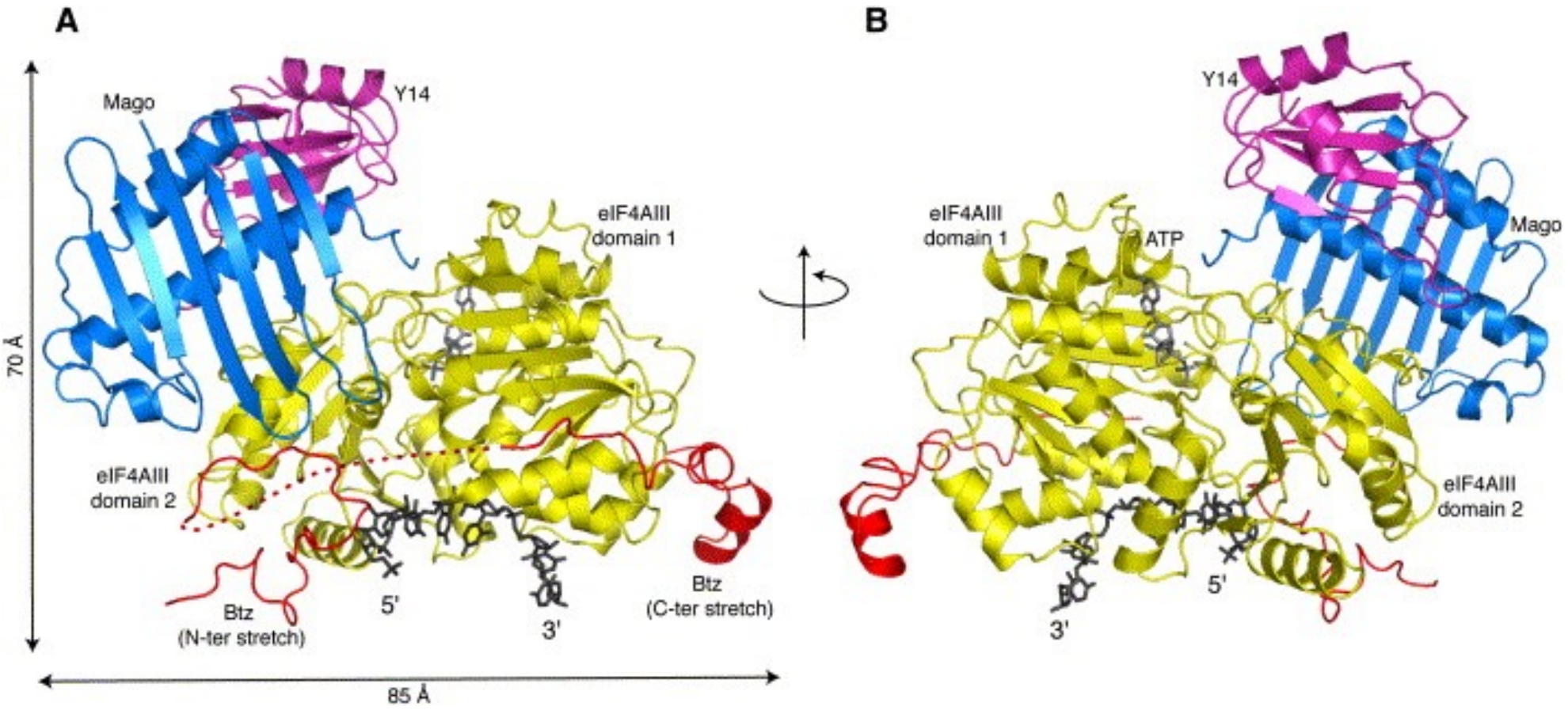
•several proteins that specifically associate with spliced RNAs were identified by crosslinking and immunoprecipitation approaches

•these splicing-dependent proteins are deposited at a specific position approximately 20–24 nts upstream of exon–exon junctions

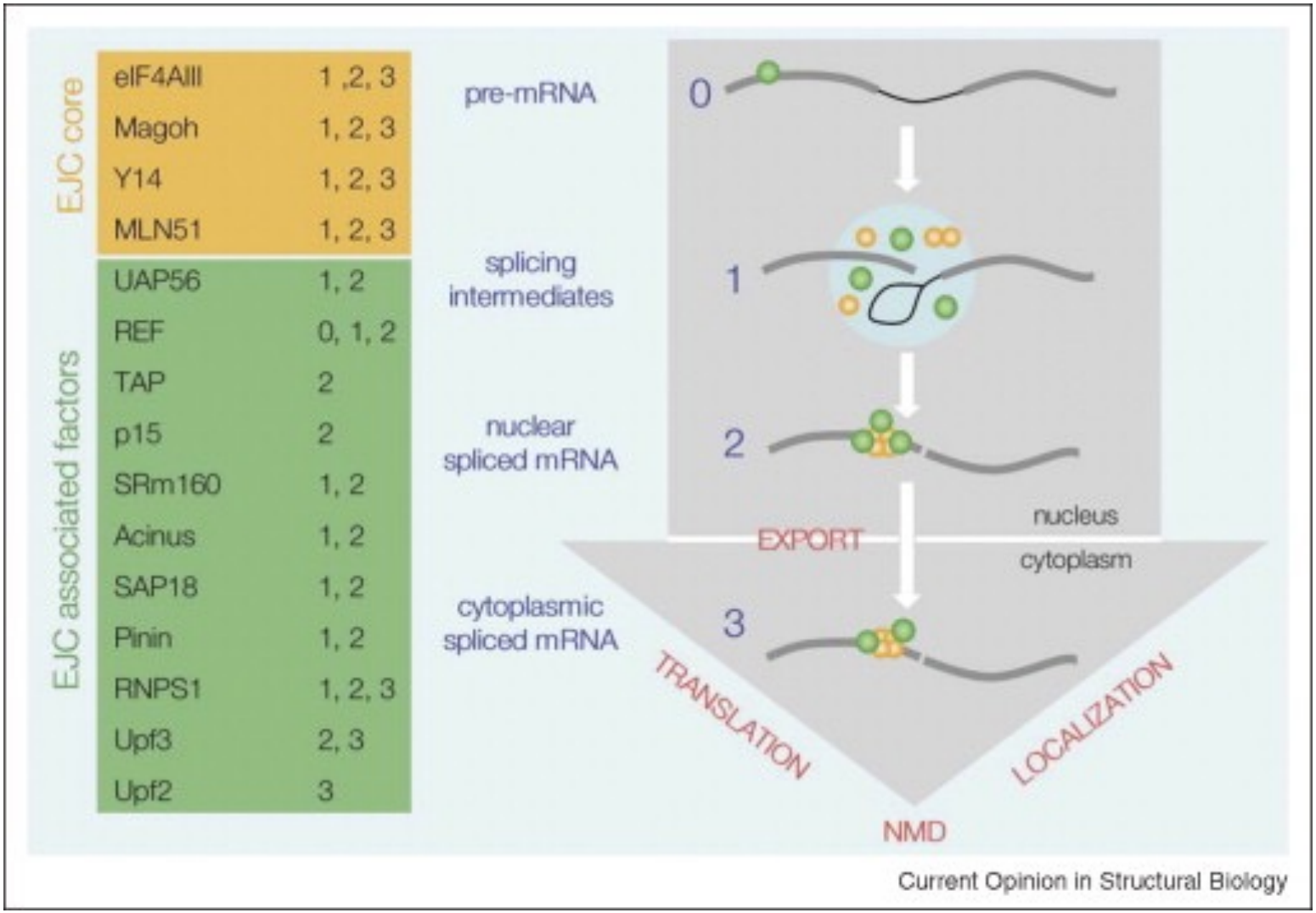


Structure of EJC

EJC core complex, which consists of **eIF4AIII**, **MNL51/BTZ**, **Magoh**, and **Y14**, is locked onto RNA ~20–24 nt upstream of exon–exon junction

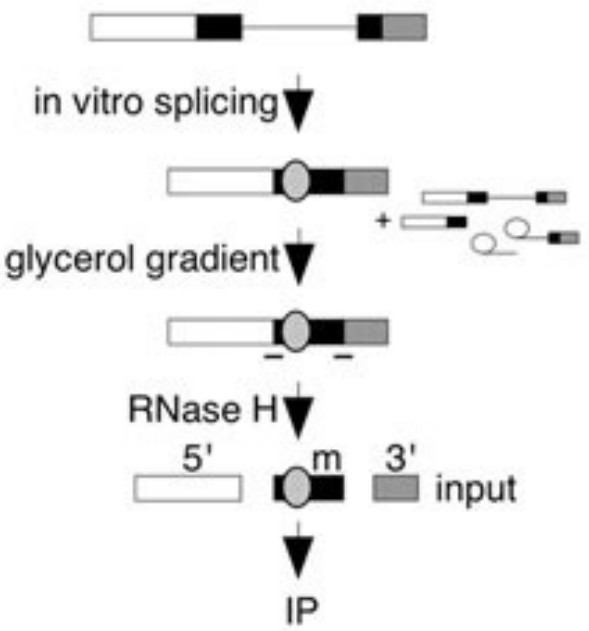


The protein composition of the EJC changes as mRNAs travel from the nucleus to the cytoplasm

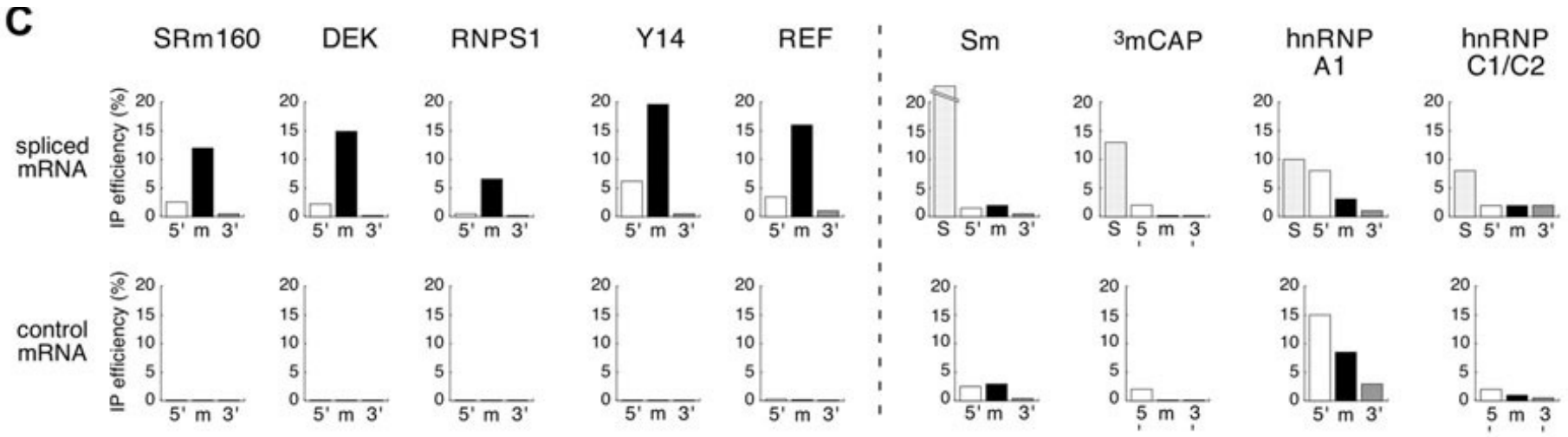


Strategy for EJC components identifications

1.



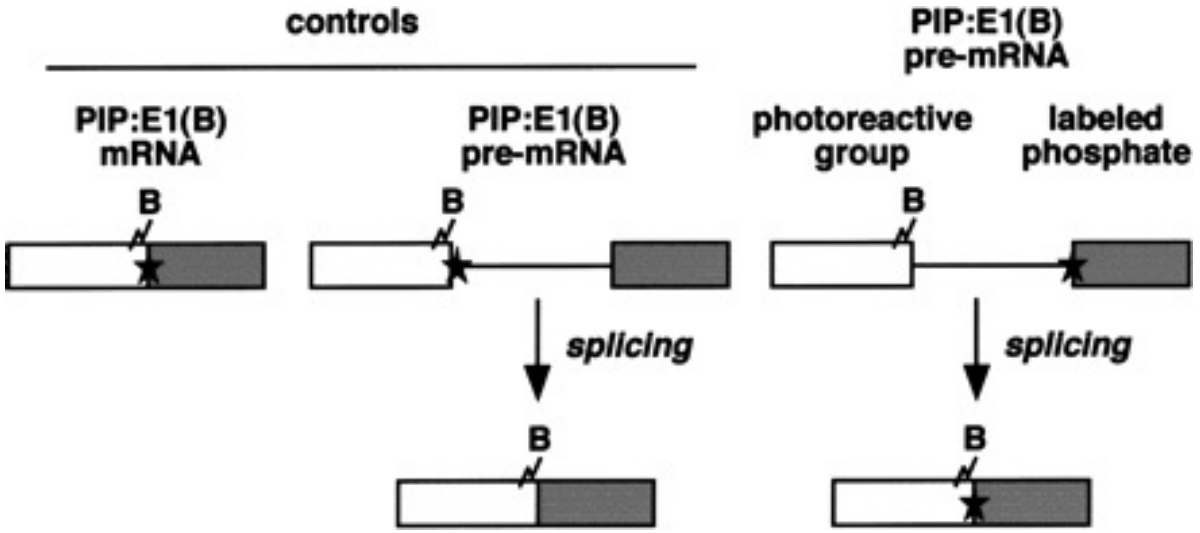
spliced mRNPs were separated from spliceosomes by glycerol gradient fractionation



Strategy for EJC components identifications

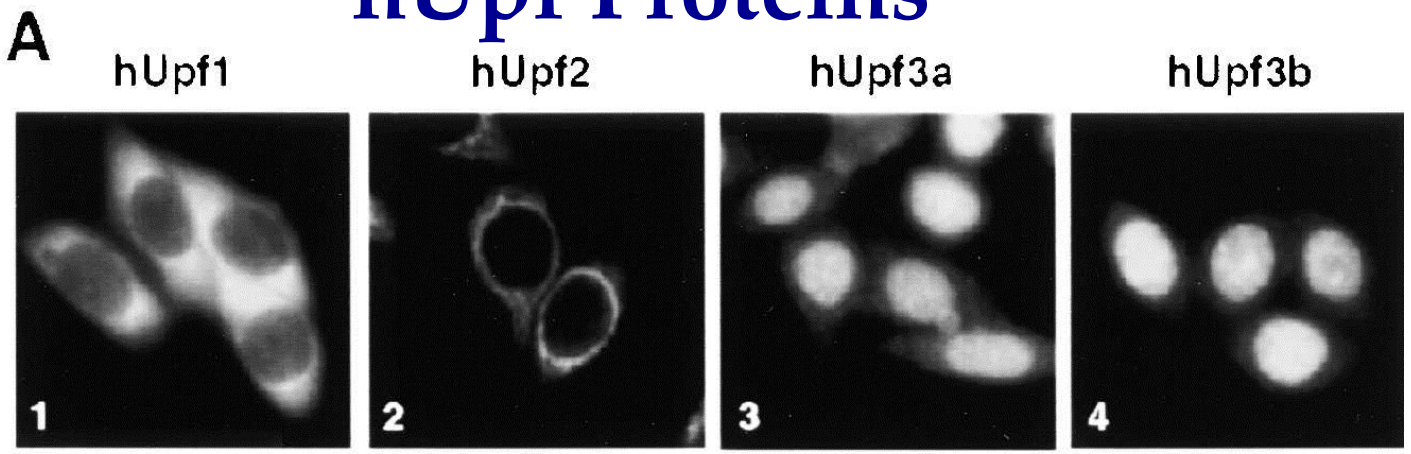
Single intron-containing pre-mRNAs having two site-specific modifications: a photoreactive group near the intron-proximal end of one exon, and a single ^{32}P at or near the opposite intron–exon boundary (star)

2.



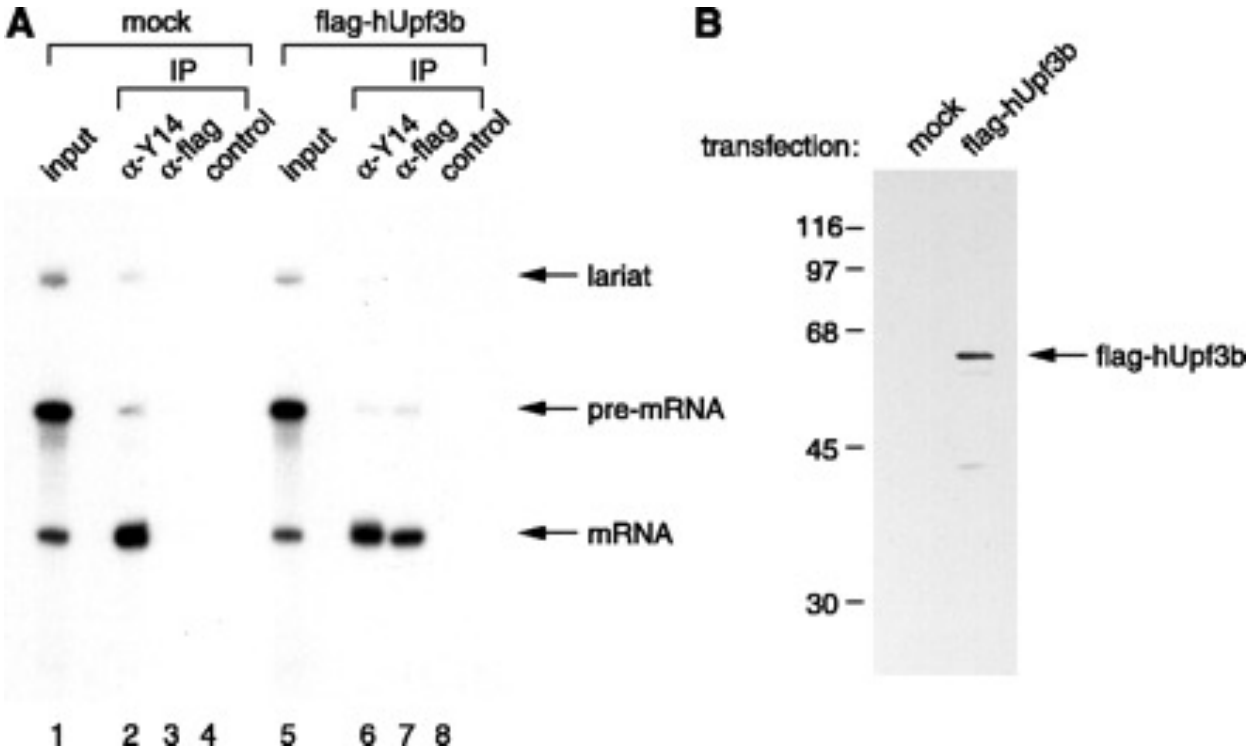
After irradiation at a wavelength appropriate for the photoreactive group followed by ribonuclease treatment and then electrophoresis through a denaturing gel, only proteins attached to the cross-linkable moiety at the exon–exon junction, and therefore associated with the ^{32}P, are detectable by autoradiography.

hUpf Proteins



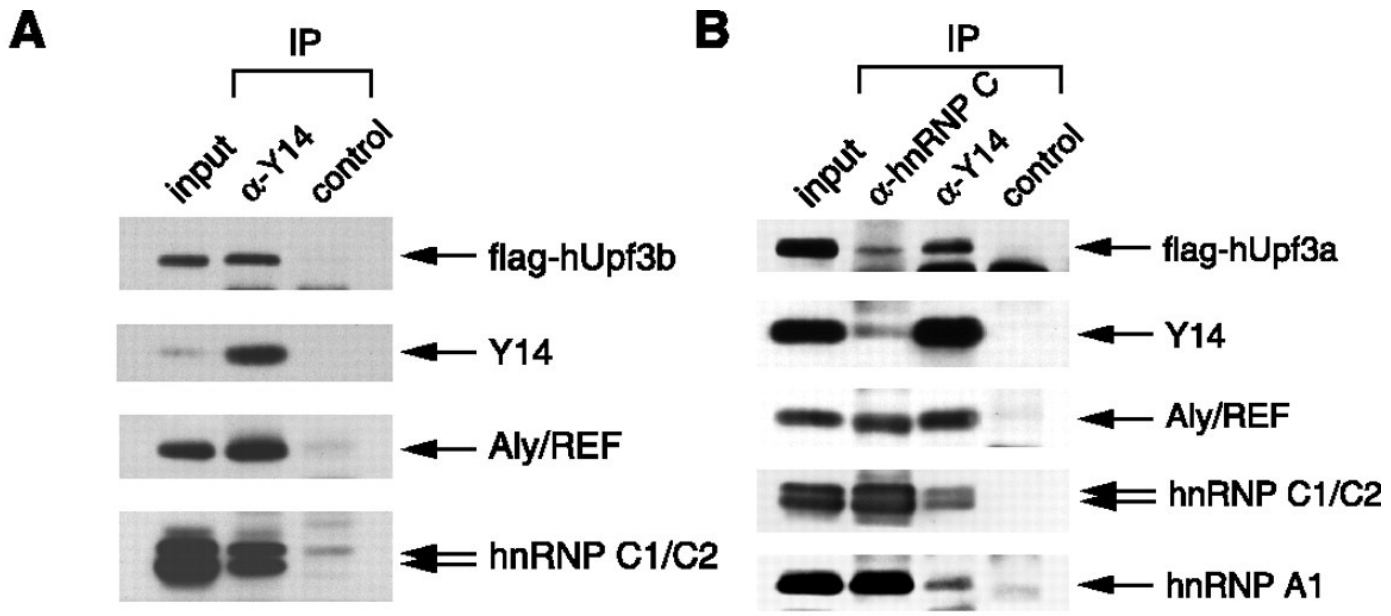
1. **hUpf1** is evenly distributed throughout the cytoplasm
2. **hUpf2** show strong perinuclear staining.
3. **hUpf3a** and **hUpf3b (also known as Upf3X)** appear predominantly nuclear. UPF3 paralogues differentially function in NMD to support embryogenesis, neurogenesis or gametogenesis. UPF3B and UPF3A, which is a poor activator of NMD relative to UPF3B, compete for binding to UPF2, indicating that the abundance of UPF3 serves as a molecular rheostat that fine-tunes NMD. During spermatogenesis, increased ratio of UPF3A to UPF3B upregulates NMD targets that promote spermatogenesis.

hUPF3b is associated with spliced mRNAs



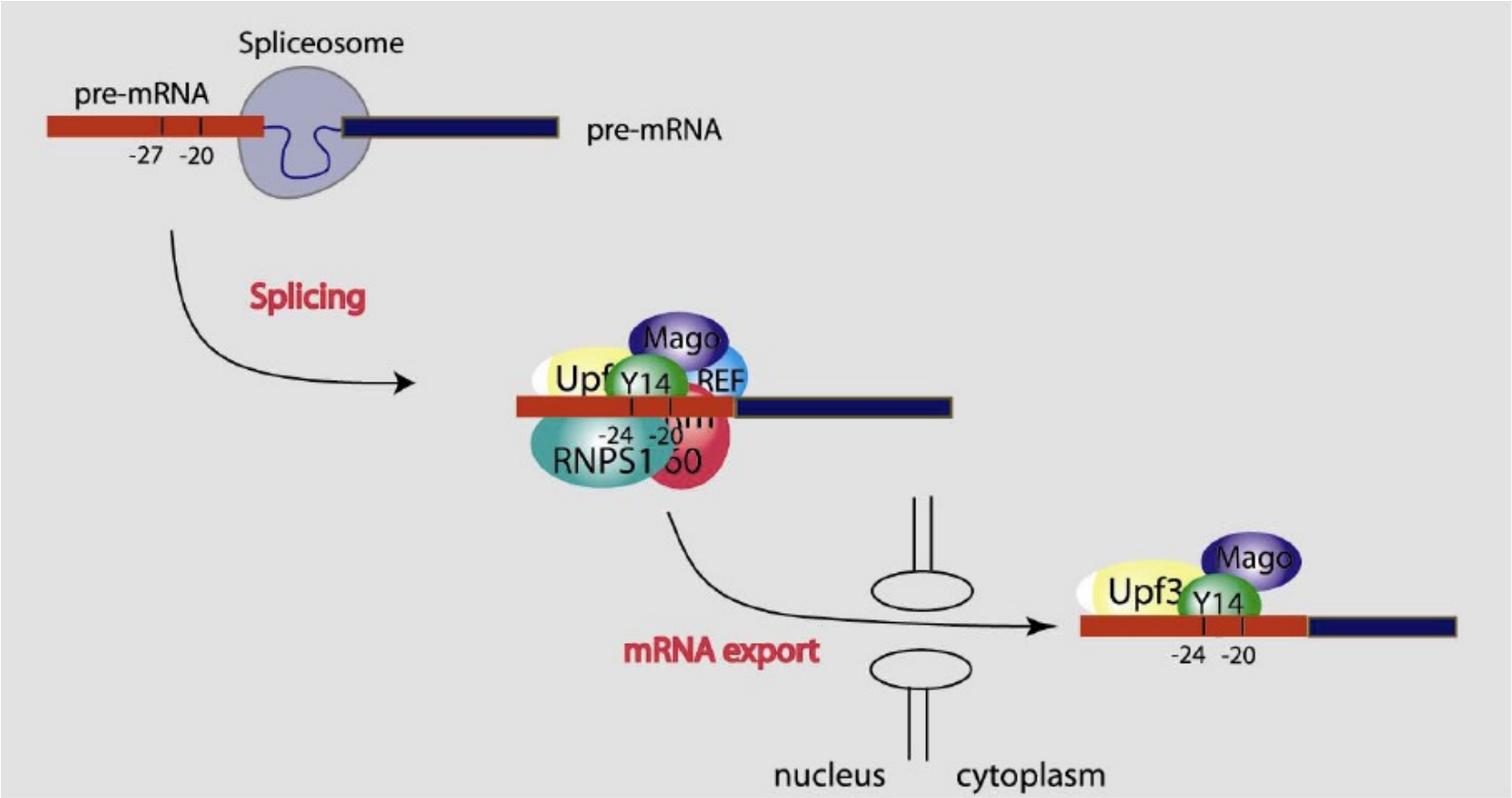
Immunoprecipitations were carried out after *in vitro* splicing of ^{32}P-labeled pre-mRNA after transfection of **Flag-hUpf3b**.

The hUpf3 proteins are associated with Y14-containing mRNP complexes



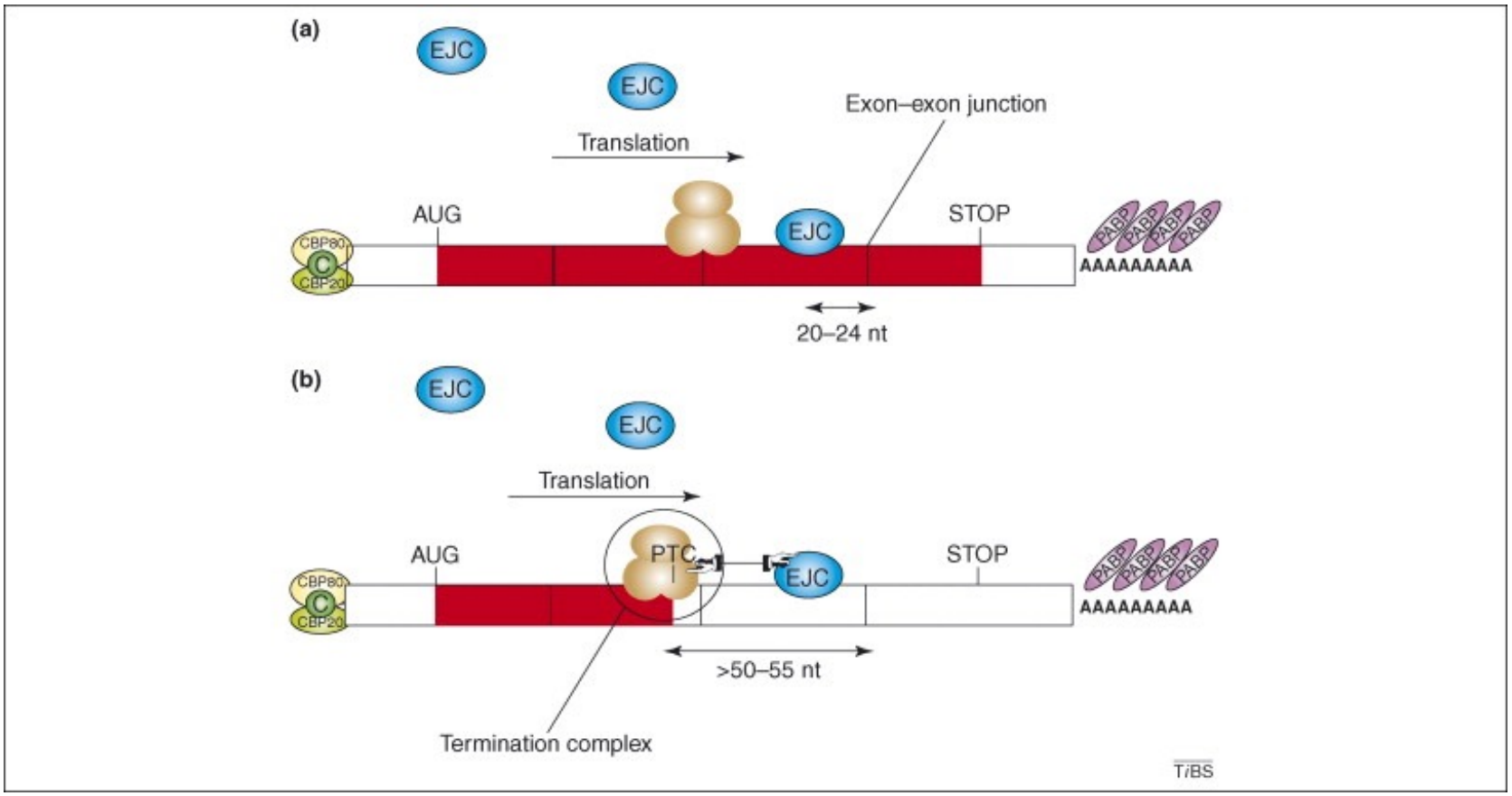
Immunoprecipitations (IP) were performed using the nucleoplasmic fraction of HeLa cells 24 hours after transfection of **Flag-hUpf3b**

EJC: protein complex deposited upstream of splice junctions

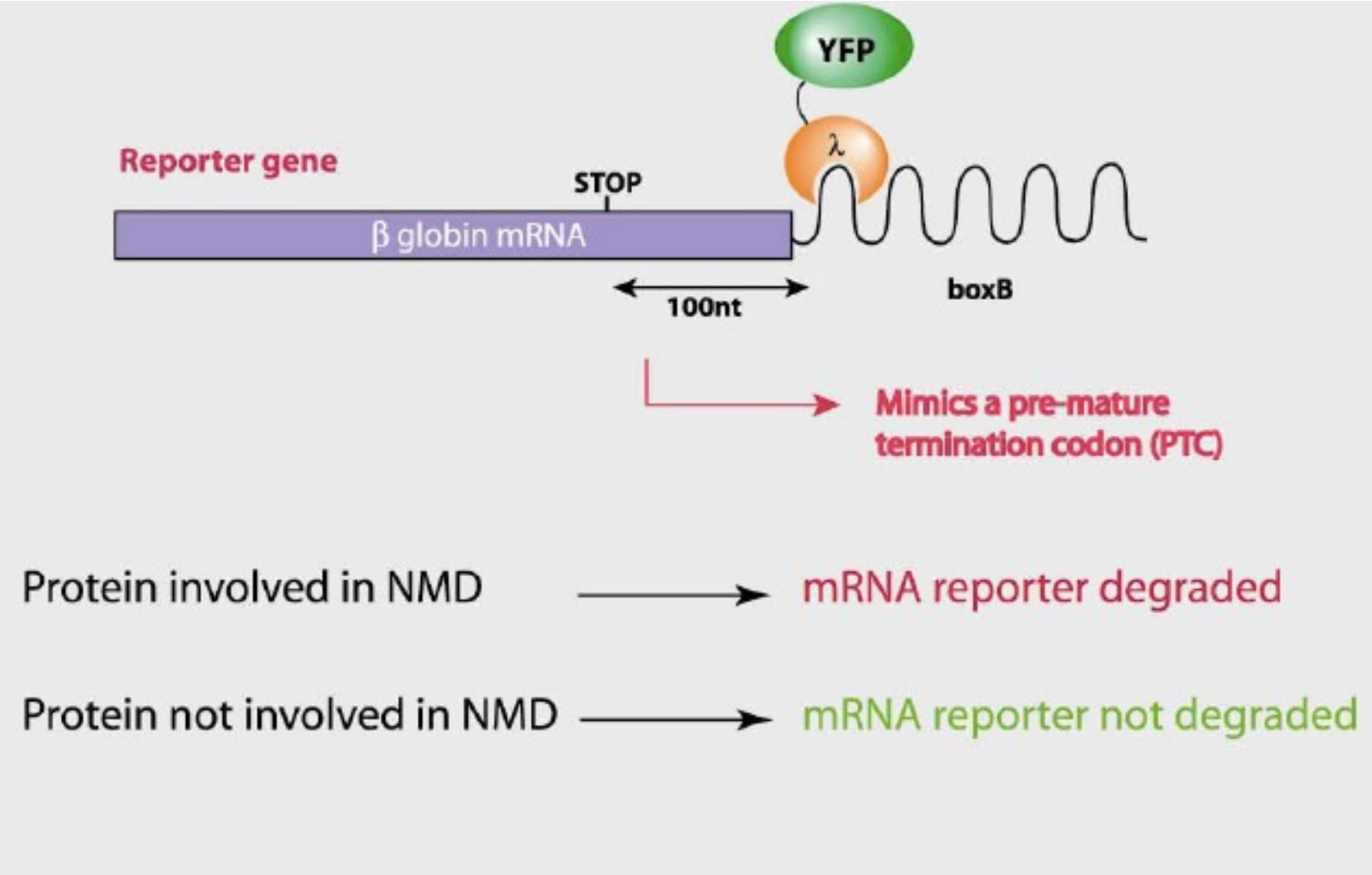


Provide a binding platform for recruitment of the NMD machinery

NMD model in mammals

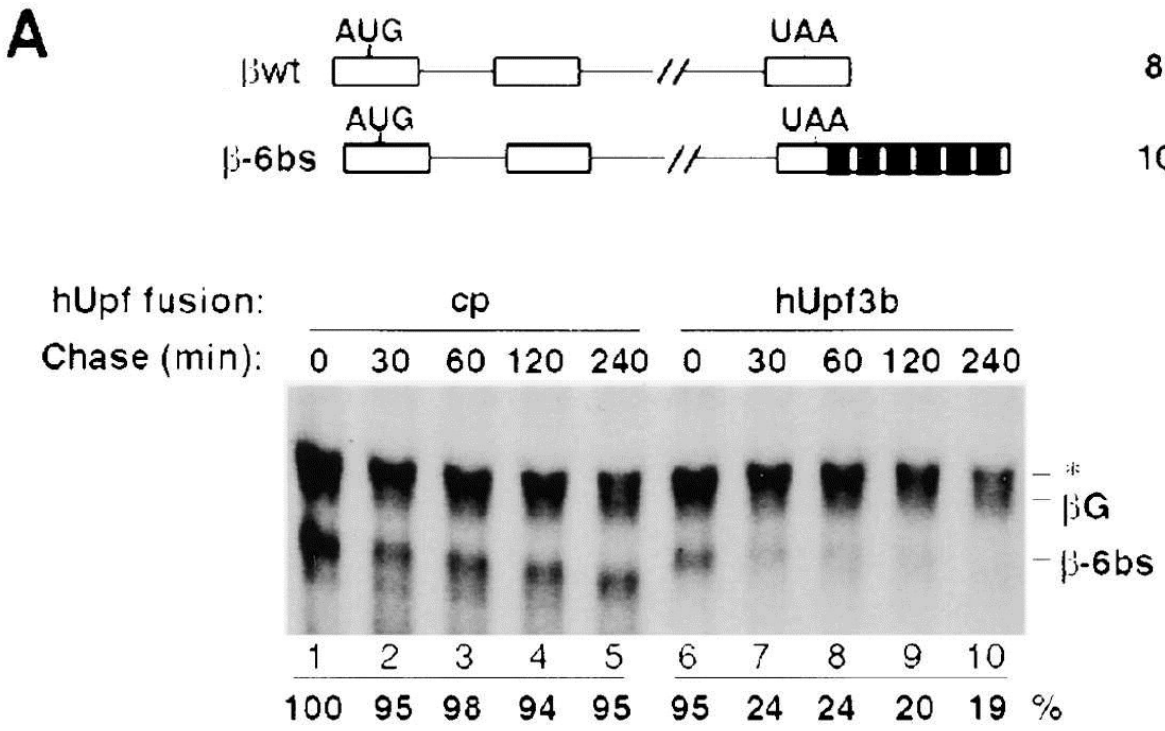


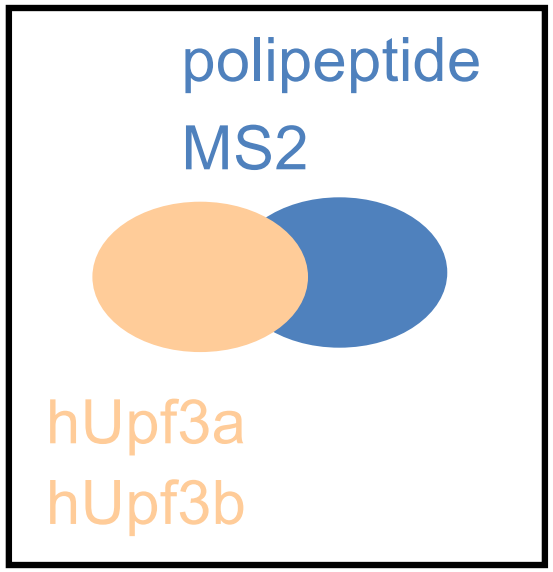
Monitoring NMD by thethering proteins to the 3′-UTR



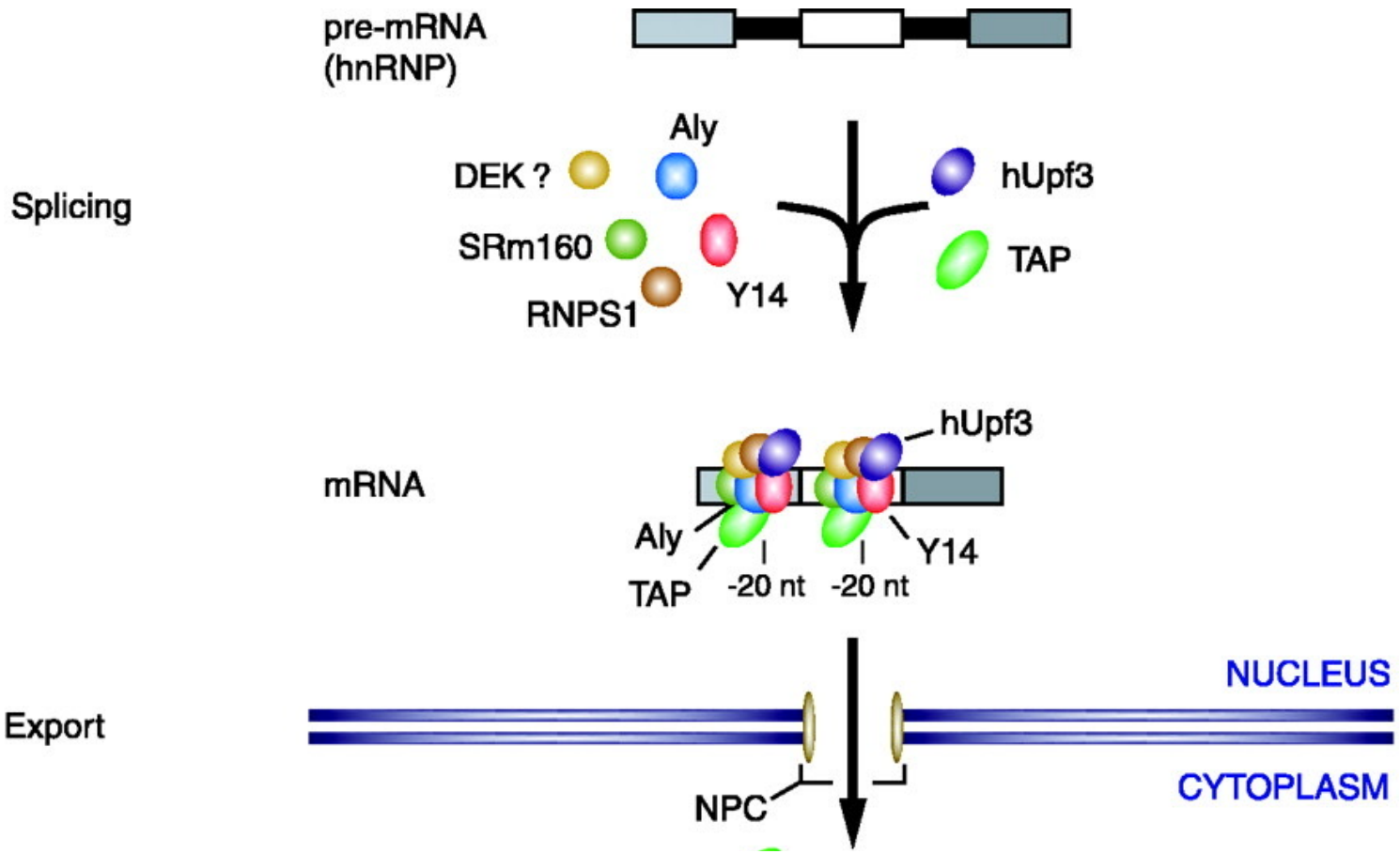
hUpf3 Trigger NMD When Tethered to the 3′UTR of β-Globin mRNA

HeLa "Tet-off" cells were cotransfected with plasmids expressing β-6bs (6x MS2 bs) and βwt from a tetracycline-responsive promoter, as well as plasmids producing MS2 coat protein alone (cp, lanes 1–5) or fused to the N terminus of hUpf3 (lanes 6–10)

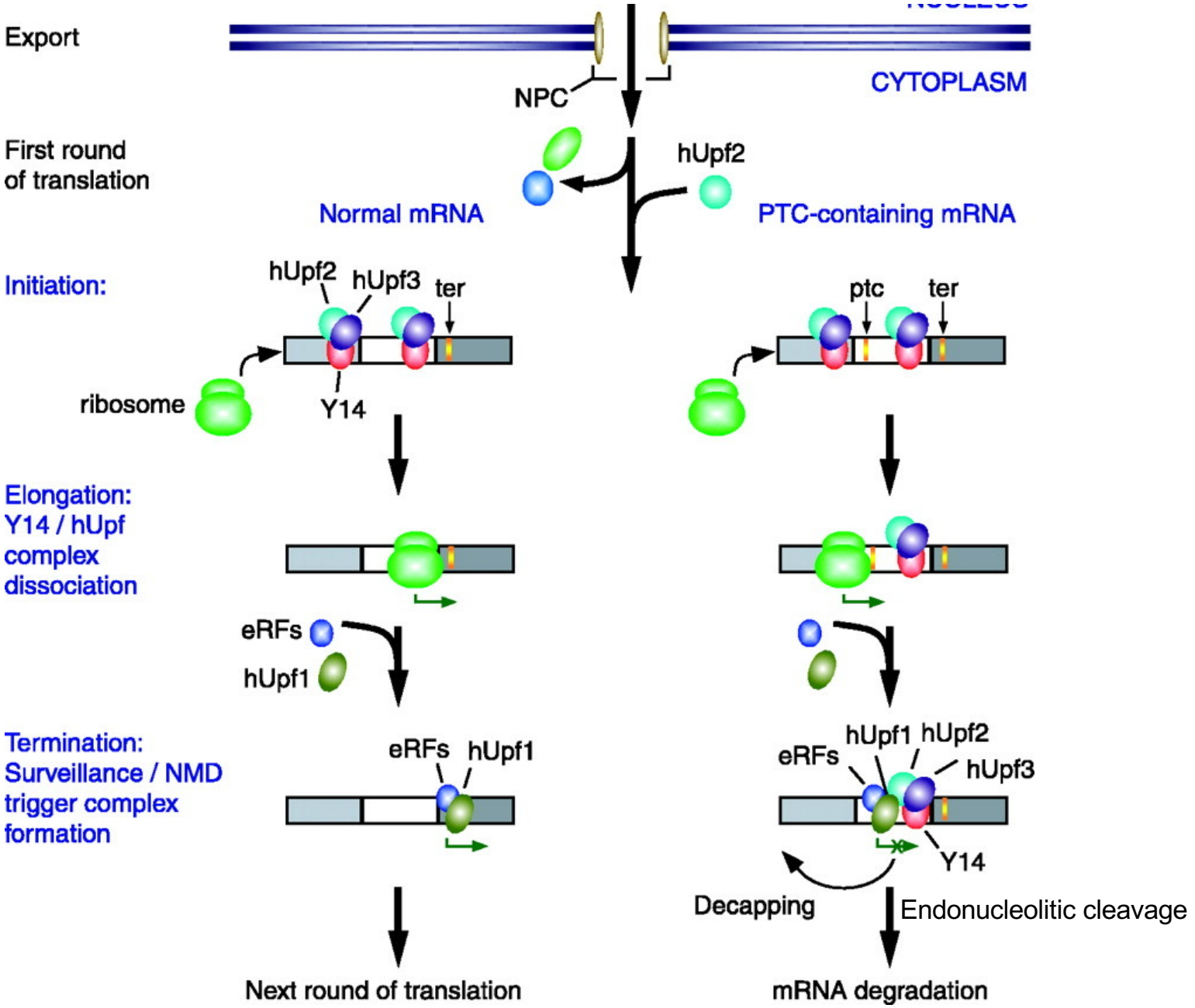




NMD model in mammals



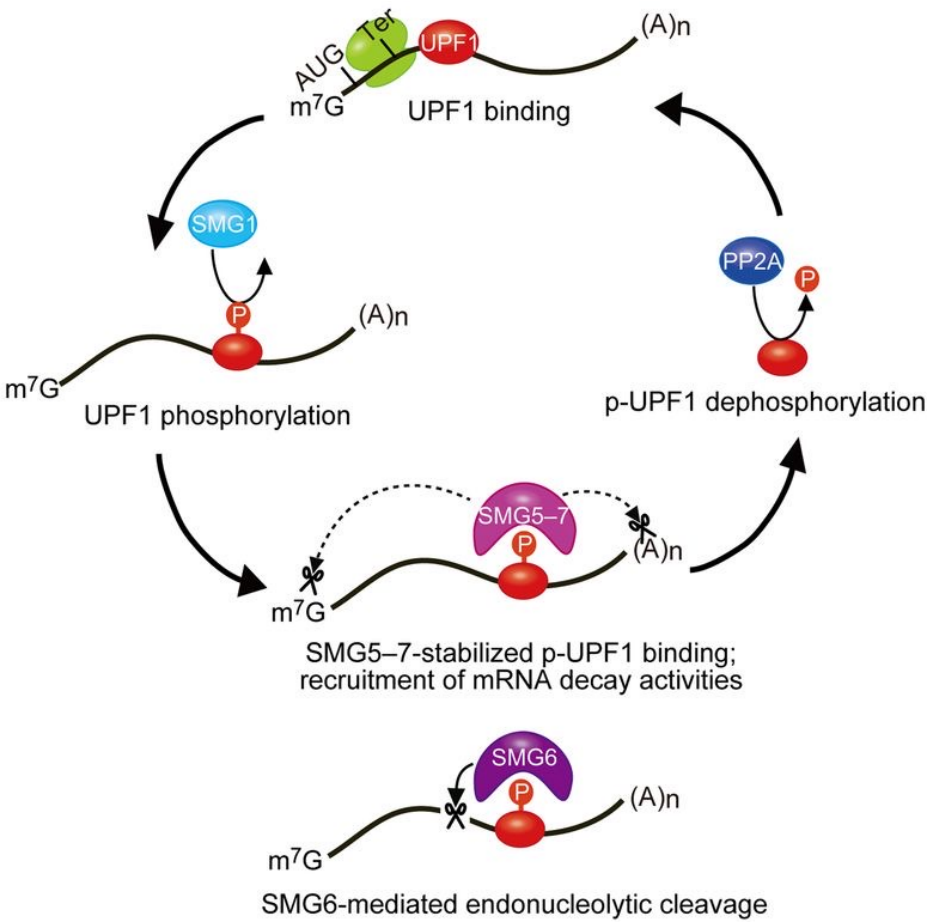
NMD model in mammals



UPF1-P is essential for NMD

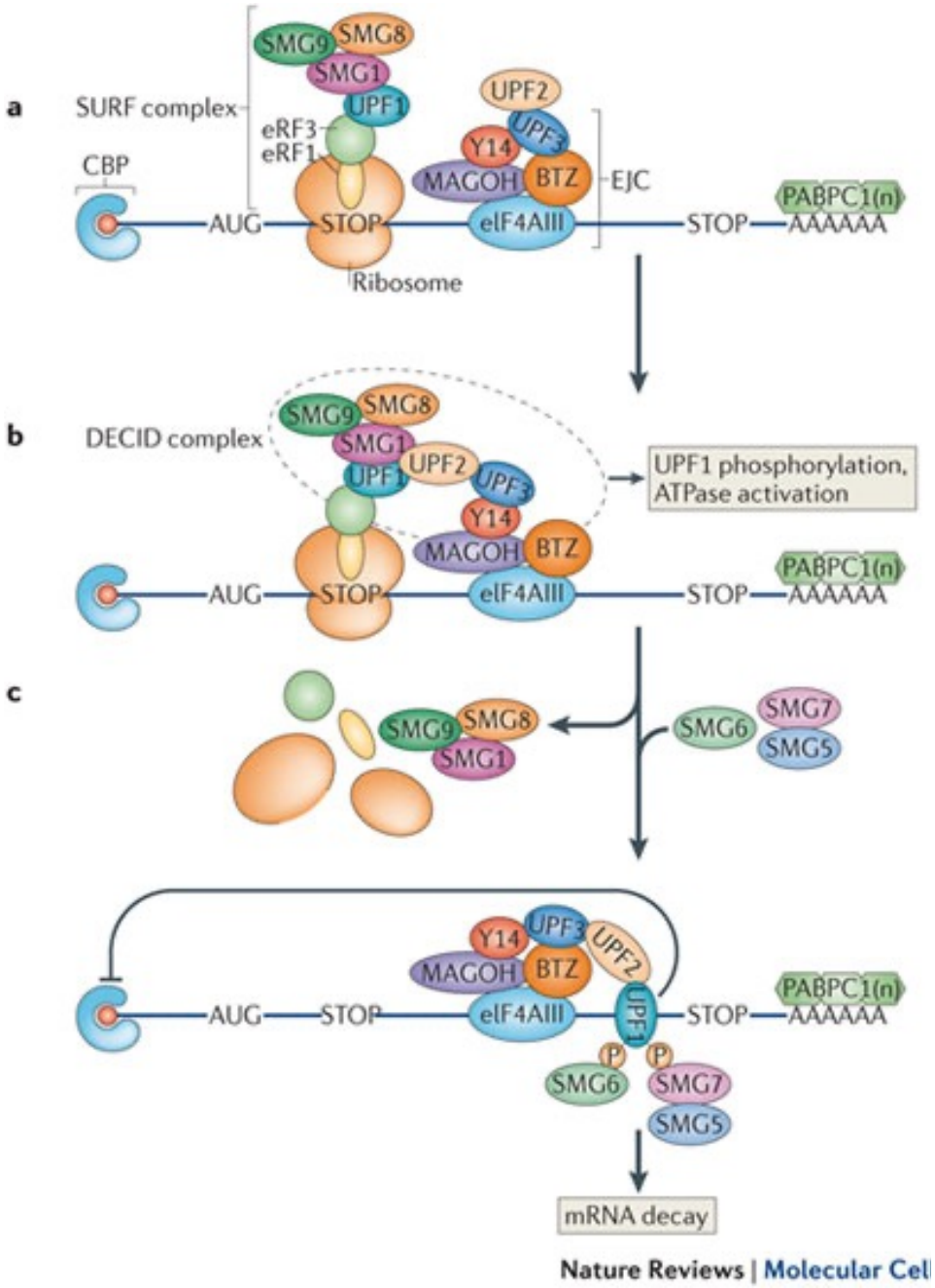
In worms and other multicellular organisms, NMD is also regulated by **SMG factors**, which are proteins that are involved in a cycle of UPF1 phosphorylation and dephosphorylation.

Regulated UPF1 phosphorylation by **SMG1** requires UPF1 recognition of a termination codon as one that triggers NMD. p-UPF1 recruits **SMG6** or **SMG5–SMG7**, which directly or indirectly trigger(s) mRNA decay, respectively. While the binding of SMG6 is sufficiently transient to be undetectable, SMG5–SMG7 stabilize p-UPF1 binding to NMD target 3′ UTRs. PP2A returns p-UPF1 to a dephosphorylated state after mRNA decay is initiated.



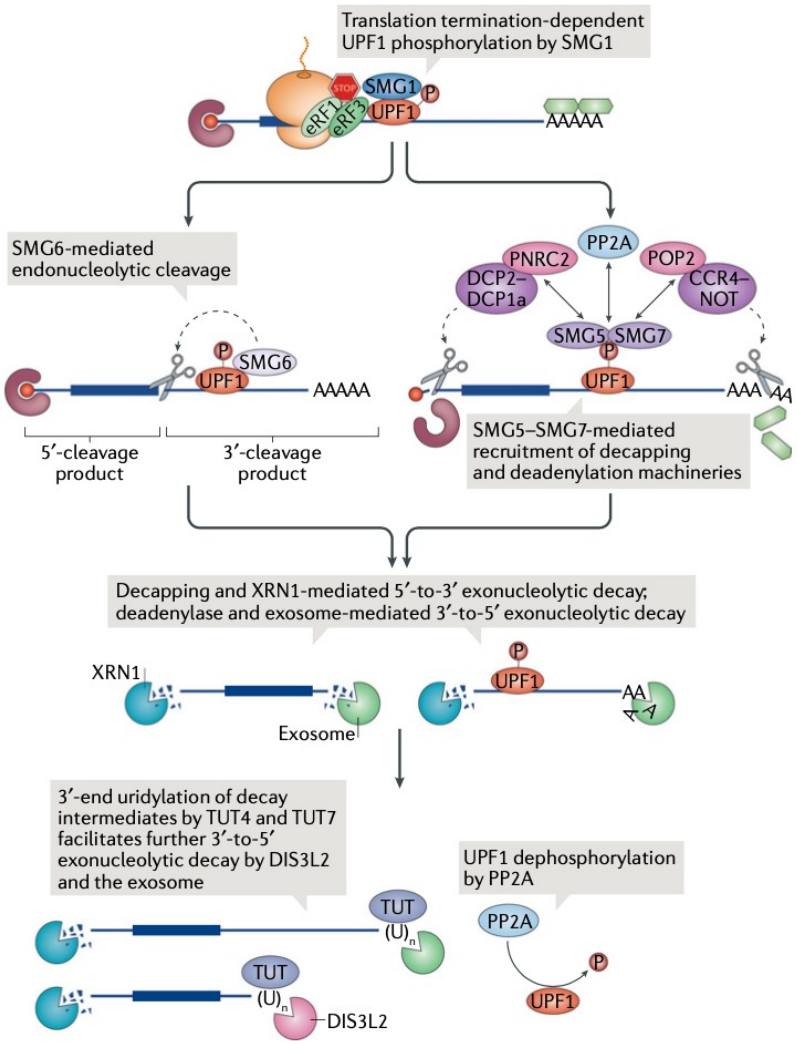
UPF1-P is essential for NMD

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 various mRNA decay activities by recruiting decay factors or adaptor proteins for decay complexes through its N- and C-terminal phospho-sites.



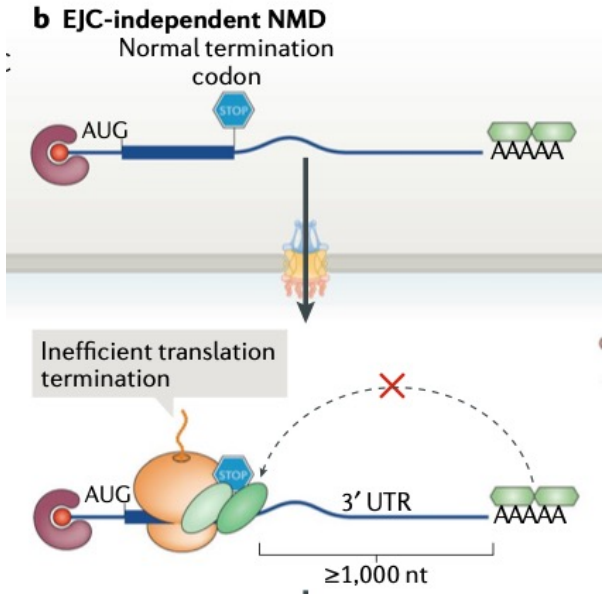
Degradation of the NMD targets

Following SMG1-mediated UPF1 phosphorylation, phosphorylated UPF1 recruits either the endonuclease SMG6 or the SMG5–SMG7 complex. SMG6 cleaves the mRNA near PTC, whereas SMG5–SMG7 recruits the deadenylation complex CCR4–NOT through its subunit POP2 and the decapping complex DCP2–DCP1a through its subunit PNRC2. SMG5–SMG7 also recruits protein phosphatase 2A (PP2A), which dephosphorylates UPF1. NMD intermediates from either pathway are degraded 5′-3′ by the exonuclease XRN1 and 3′-5′ by the exosome or DIS3L2. Terminal uridylyltransferase 4 (TUT4) and TUT7 can append non-templated uridines at the 3′ ends; the uridylated decay intermediates are favoured for degradation by DIS3L2.



EJC-independent NMD

The discovery that not only the initially identified PTC-containing mRNAs but also many PTC-less mRNAs are targeted by NMD posed the question, which features render an RNA susceptible to NMD and pointed out our limited understanding of the mechanism of substrate selection. Besides the presence of an ORF-interrupting PTC, upstream ORFs (uORFs), introns in the 3′-UTR and long 3′ UTRs are empirically identified features that can trigger NMD



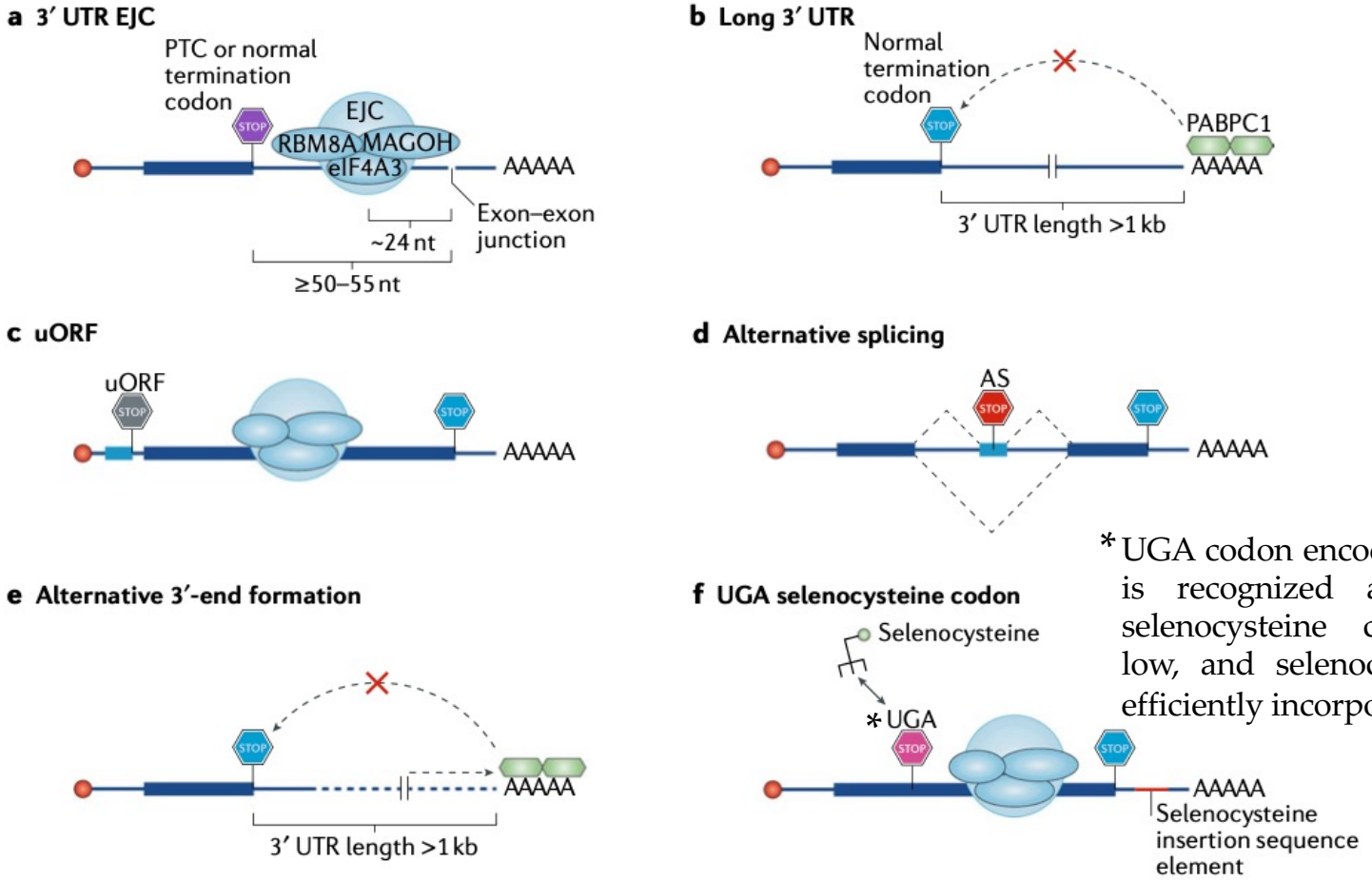
Physiological roles of NMD

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 (~10%) are upregulated and thus affected by NMD.

Features of cellular mRNAs that activate NMD



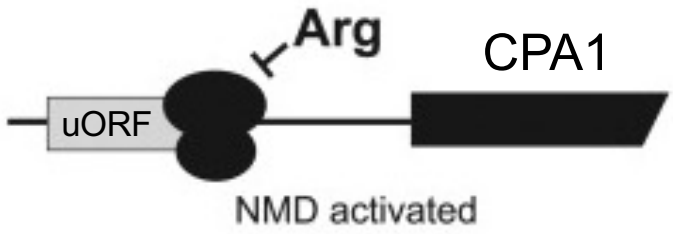
*UGA codon encoding selenocysteine is recognized as a PTC when selenocysteine concentrations are low, and selenocysteine cannot be efficiently incorporate.

Transcripts without a 3′-UTR EJC may also be targeted for NMD, although the molecular mechanism of this process is still not well understood.

Physiological roles of NMD

Nutrient homeostasis

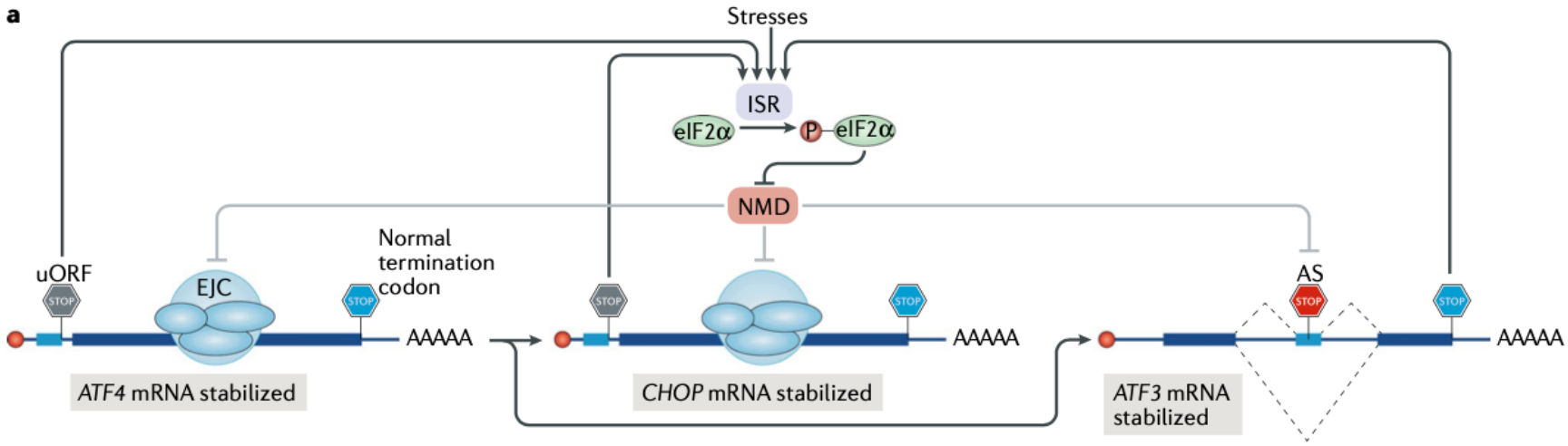
The ***CPA1*** gene, which encodes a small subunit of carbamoyl phosphate synthetase (a protein that is required for arginine biosynthesis), exemplifies the role of NMD in nutrient homeostasis in *S. cerevisiae*. In this case, arginine addition promotes ribosome stalling and translation termination at the termination codon of an upstream ORF of CPA1 mRNA, which activates NMD.



Physiological roles of NMD

Adaptation to stress

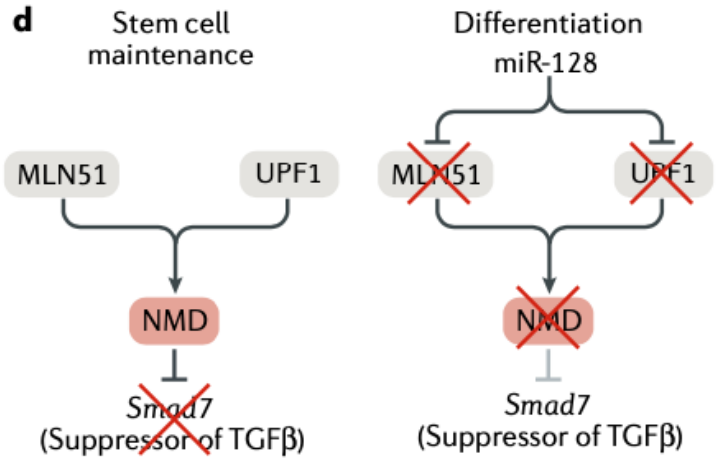
During the integrated stress response (ISR), eIF2α is phosphorylated leading to the suppression of NMD. NMD suppression enables the expression of NMD targets, including those encoding ATF4, CHOP and ATF3, which coordinate the expression of proteins that alleviate the stresses. Following resolution of the ISR, NMD is resumed and suppresses the expression of ATF4 and CHOP, because their mRNAs contain an upstream open reading frame (uORF), and of ATF3, because of a premature termination codon introduced by alternative splicing (AS), thereby ensuring that the ISR is only active during stress



Physiological roles of NMD

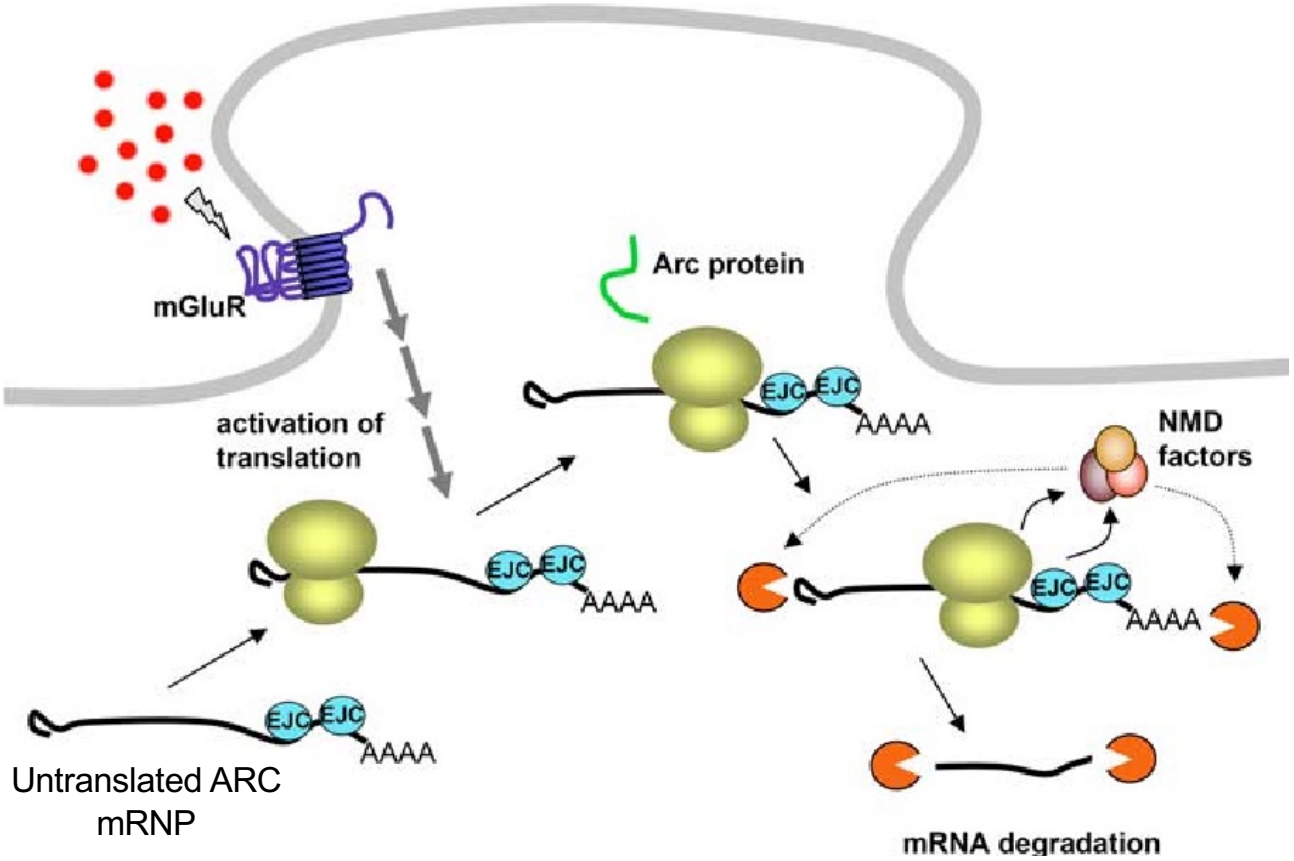
Development and differentiation

NMD maintains the pluripotency of mouse neural stem cells by targeting *Smad7* mRNA, which encodes a negative regulator of TGFβ signalling. During neural differentiation, the brain-specific microRNA miR-128 is expressed and inhibits the exon junction complex (EJC) component metastatic lymph node 51 (MLN51) and UPF1. This suppresses NMD, enables the production of SMAD7 and facilitates differentiation.



Translation-dependent degradation of Arc mRNA via the NMD pathway

Untranslated Arc mRNA is stable and can accumulate in dendrites. However, juxtaposition of the ribosome with two EJCs in the 3′-UTR at the termination of translation leads to activation of NMD and rapid mRNA degradation. This mechanism could potentially limit each Arc mRNA to producing just a single copy of Arc protein.



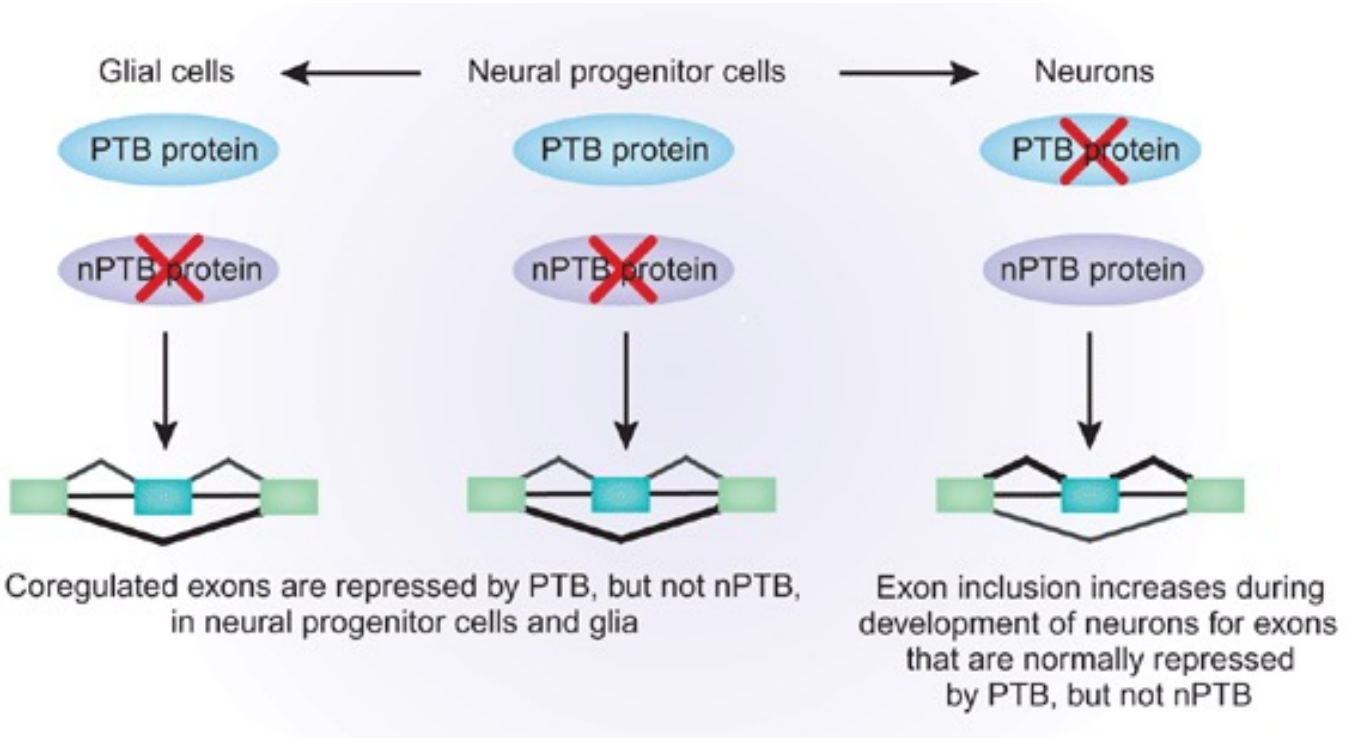
Role of NMD in the production of RNA-binding proteins

NMD functions in the homeostatic control of the expression of genes that encode splicing factors (SR-rich and hnRNP proteins). The splicing-promoted inclusion or exclusion of specific sequences in the mRNA results in the generation of a termination codon that triggers NMD (**autogenous alternative splicing activating NMD**).

Splicing factor gene	Splicing factor	Alternative splicing event
SFRS3	SRp20	SRp20-mediated exon inclusion and/or flanking intron retention that results in PTC-containing SRp20 mRNA; this event is antagonized by ASF/SF2
SFRS7	9G8	9G8-mediated exon inclusion and/or flanking intron retention that results in PTC-containing 9G8 mRNA
SFRS9	SRp30c	Intron retention that results in PTC-containing SRp30c mRNA
FUSIP1	SRp38	Alternative 5′ splice-site selection followed by inclusion of an alternative exon that results in PTC-containing SRp38 mRNA
SFRS5	SRp40	Exon inclusion and/or flanking intron retention that results in PTC-containing SRp40 mRNA
SFRS11	SRp54	Exon inclusion and/or flanking intron retention that results in PTC-containing SRp54 mRNA
SFRS6	SRp55	Exon inclusion and/or flanking intron retention that results in PTC-containing SRp55 mRNA
SFRS4	SRp75	Exon inclusion and/or flanking intron retention that results in PTC-containing SRp75 mRNA
SFRS2	SC35	SC35-mediated alternative splicing that generates exon–exon junctions downstream of the normal termination codon within SC35 mRNA
SFRS2B	SRp46	Alternative splicing that generates an exon–exon junction downstream of normal termination codon within SRp46 mRNA
SFRS1	ASF/SF2	Alternative splicing that generates an exon–exon junction downstream of normal termination codon within ASF/SF2 mRNA
PTBP1	PTB	PTB-mediated exon-skipping that results in PTC-containing PTB mRNA
PTBP2	nPTB	PTB-mediated exon-skipping that results in PTC-containing nPTB mRNA
PTBP3	ROD1	PTB- and nPTB-mediated exon-skipping that results in PTC-containing ROD1 mRNA

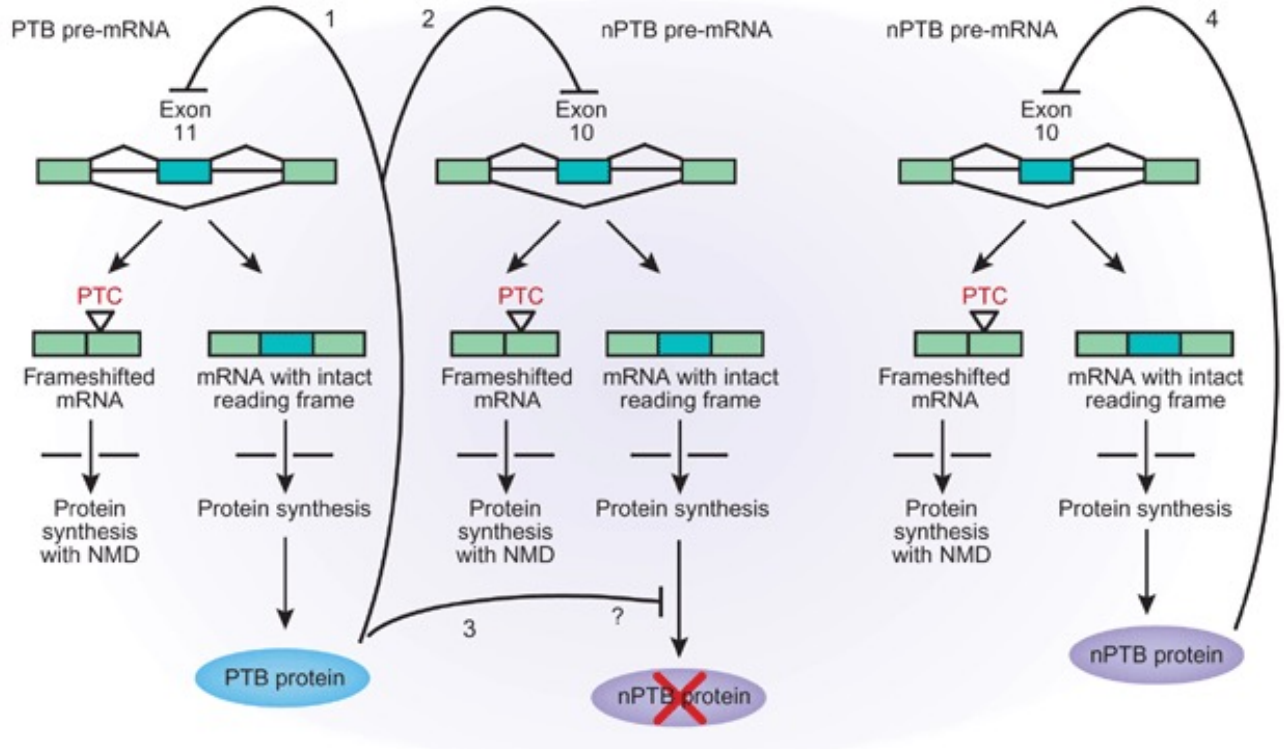
Role of NMD in the production of PTB proteins

Polypyrimdine tract binding proteins (PTBs) and its neurally enriched paralog nPTB are a regulators of alternative splicing. In spite of their highly similar polypeptide sequences, PTB induces exon skipping upon recognition of pyrimidine-rich sequence motifs near target exons, whereas nPTB has weaker activity toward some of the same exons. Moreover, PTB is expressed widely in various types of cells and tissues but is deficient in brain and muscle, whereas nPTB is enriched in neurons and neural tissue. PTB switches to nPTB during neuronal, but not glial cell, development to induce changes in the splicing patterns of brain.



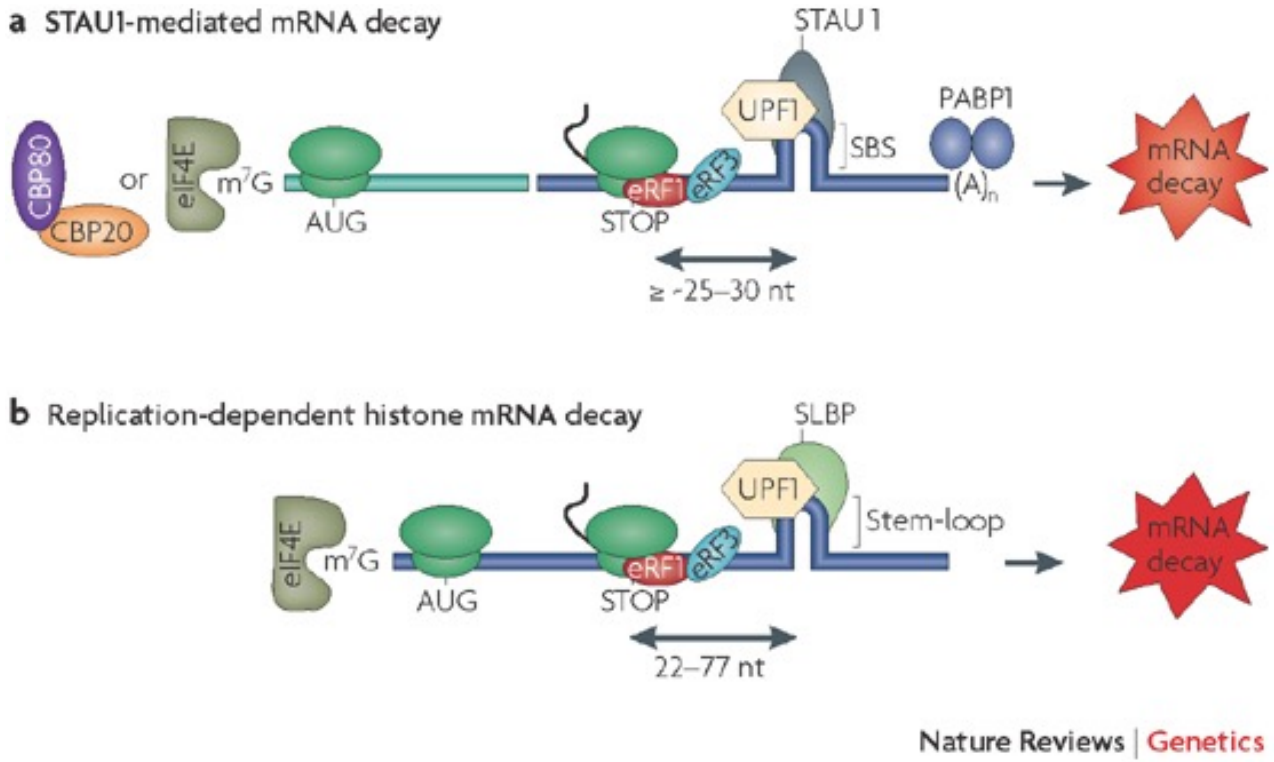
PTB/nPTB regulation

PTB regulates its own expression by inducing the skipping of exon 11 of its pre-mRNA (1). The skipping of exon 11 shifts the reading frame and introduces a **PTC**, which promotes NMD. **PTB** also represses **nPTB** expression at the level of splicing by inducing exon 10 skipping (2). As with PTB autoregulation, the frameshifted mRNA contains a PTC, which targets it for destruction by NMD. In addition, PTB blocks nPTB expression at an unknown post-transcriptional step—possibly translation—even when nPTB mRNA contains an intact reading frame (3). 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 (4).

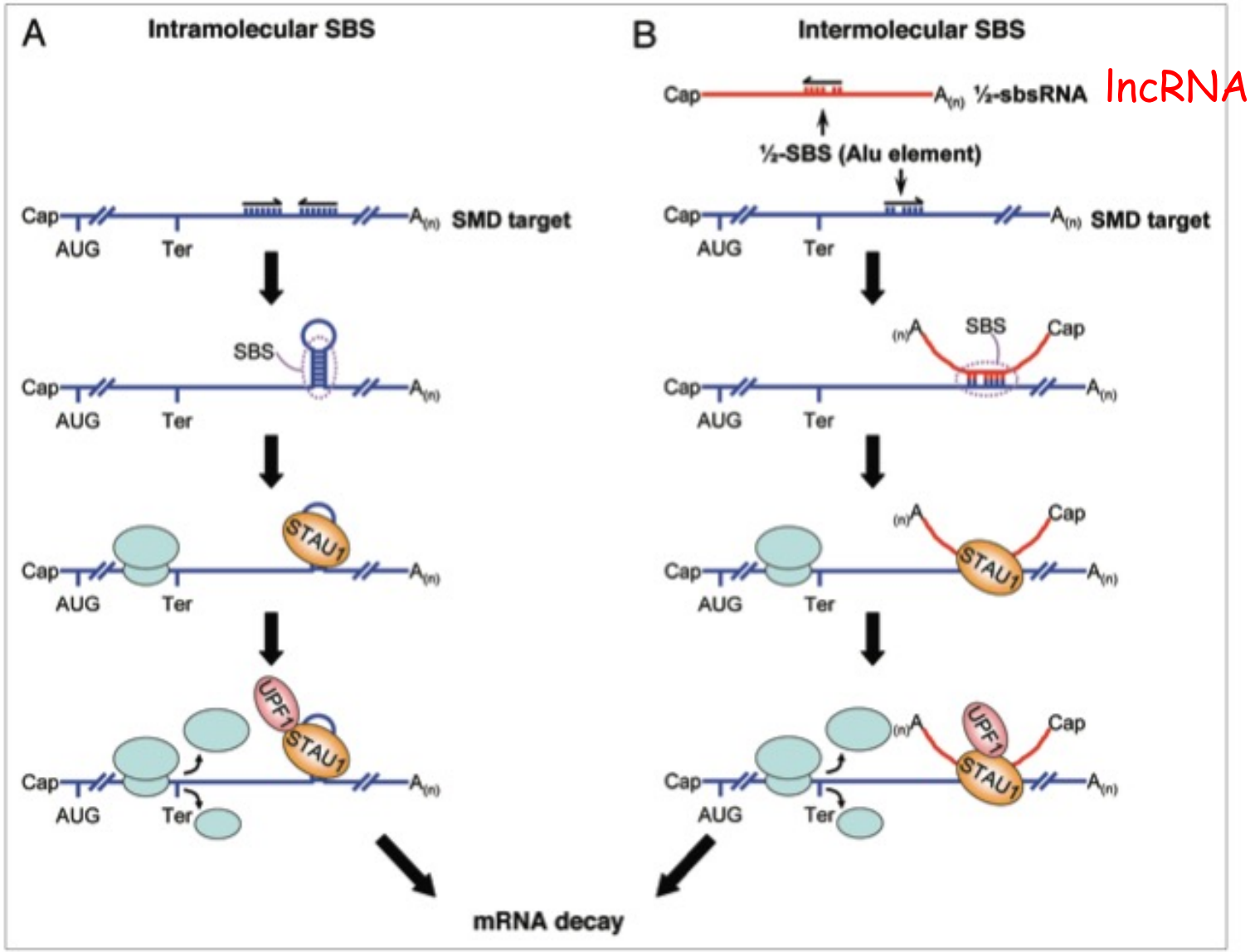


Specialized mRNA-decay pathways

In addition to its role in mRNA decay by NMD, **UPF1** also functions in two specialized non-NMD mRNA-decay pathways in mammalian cells that do not require the mRNA to be derived from splicing. In both pathways, UPF1 is recruited downstream of the normal termination codon by a specific mRNA-binding protein and triggers mRNA decay when translation terminates.



STAU1-mediated mRNA decay (SMD) may be induced by lncRNAs



The multiple lives of NMD factors