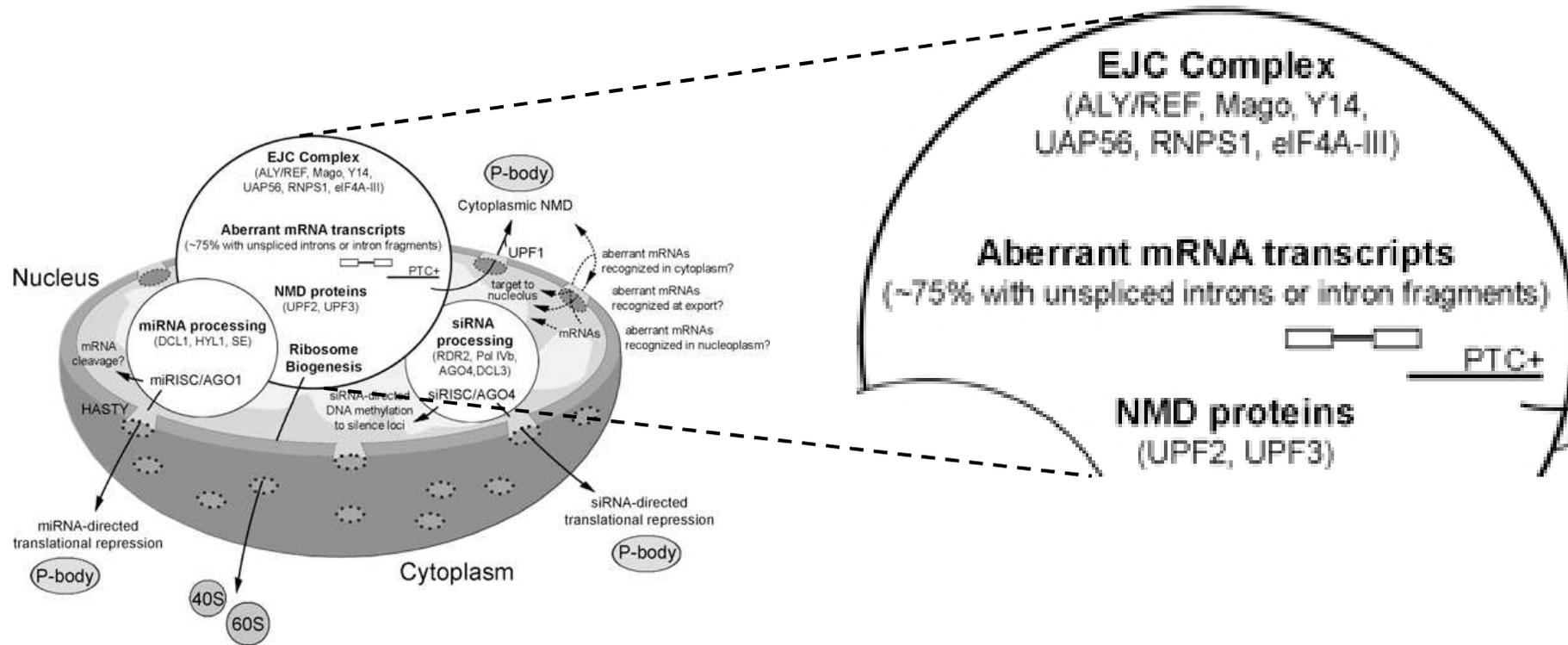
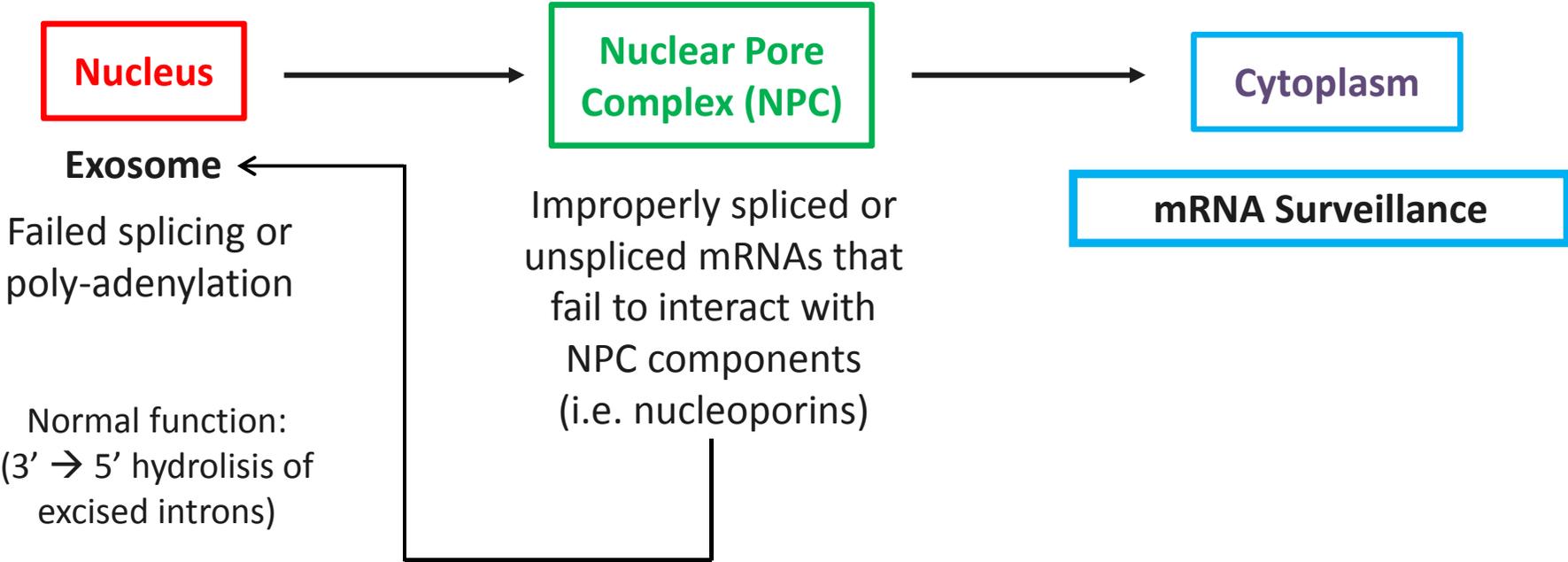
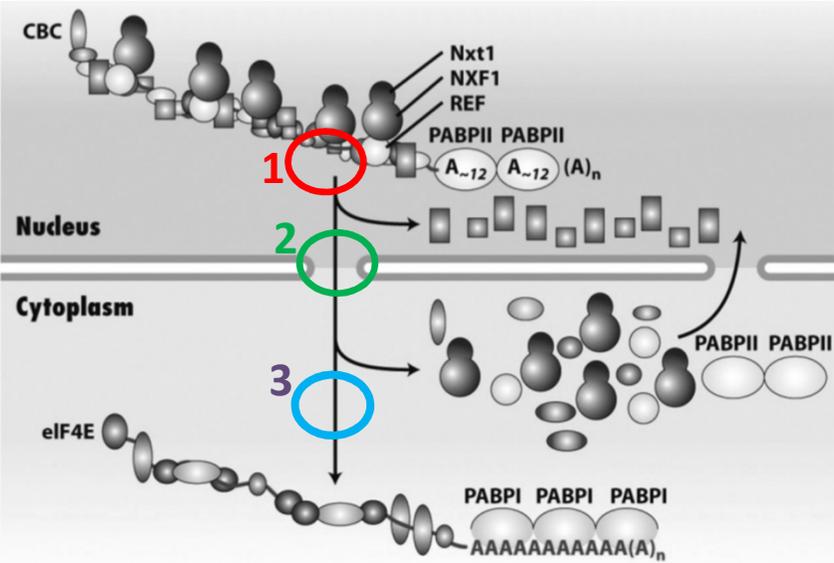


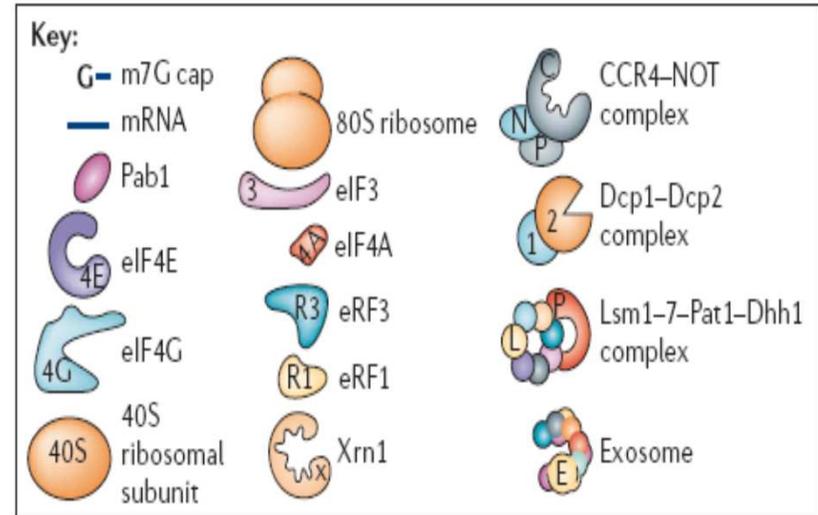
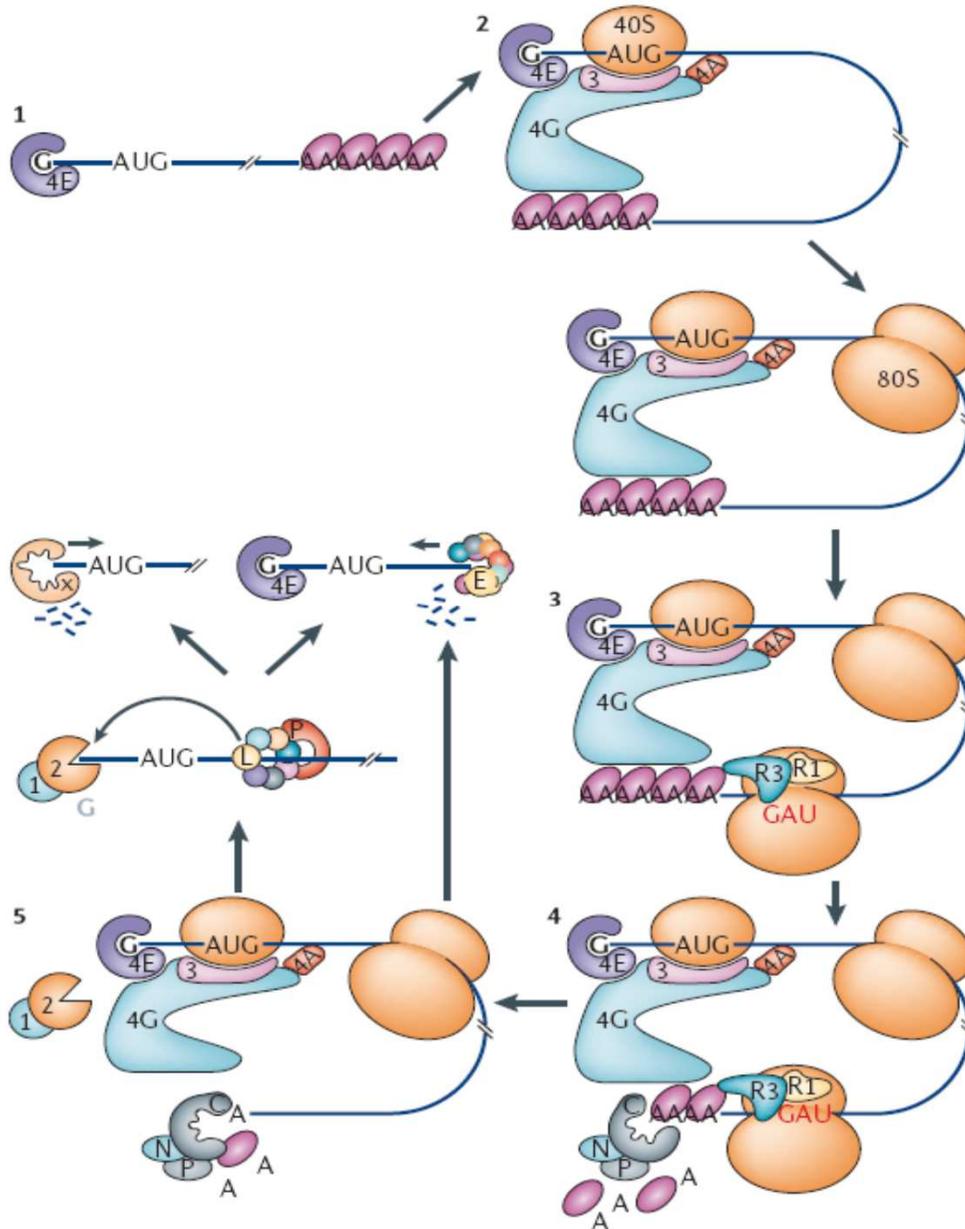
Nonsense-Mediated Decay – Molecular Bases



Quality control mechanisms ensure fidelity to mRNA biogenesis

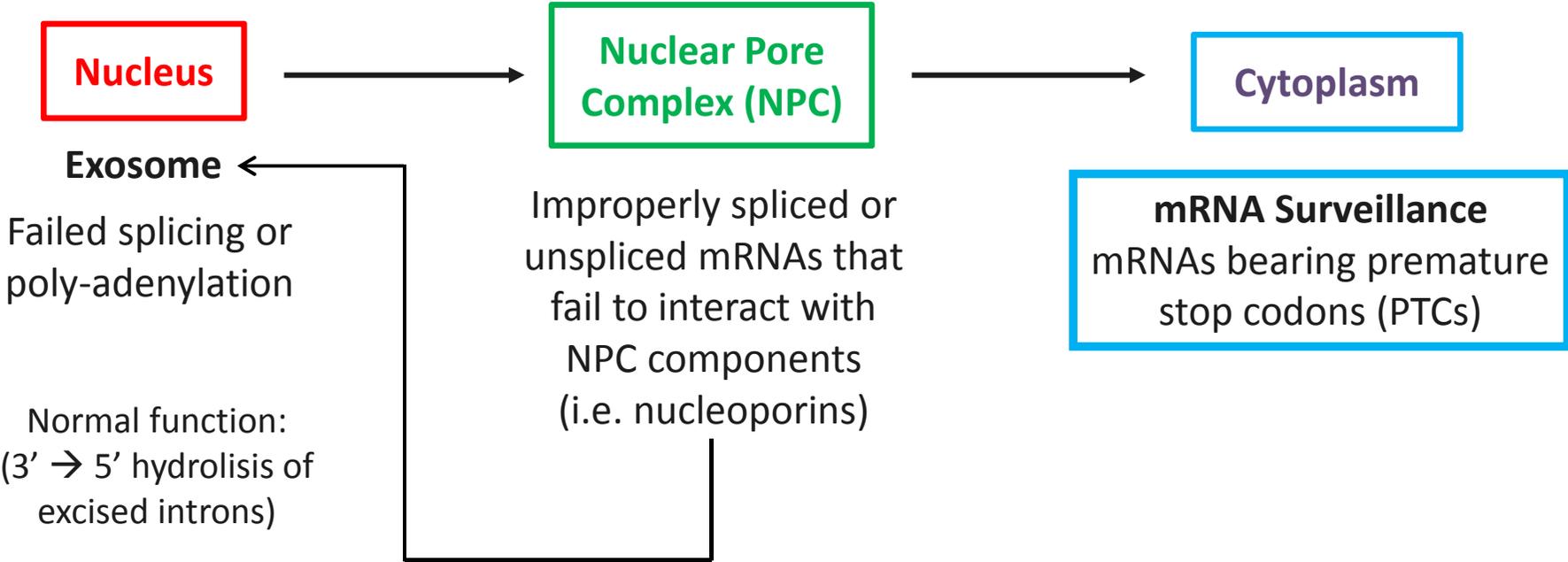
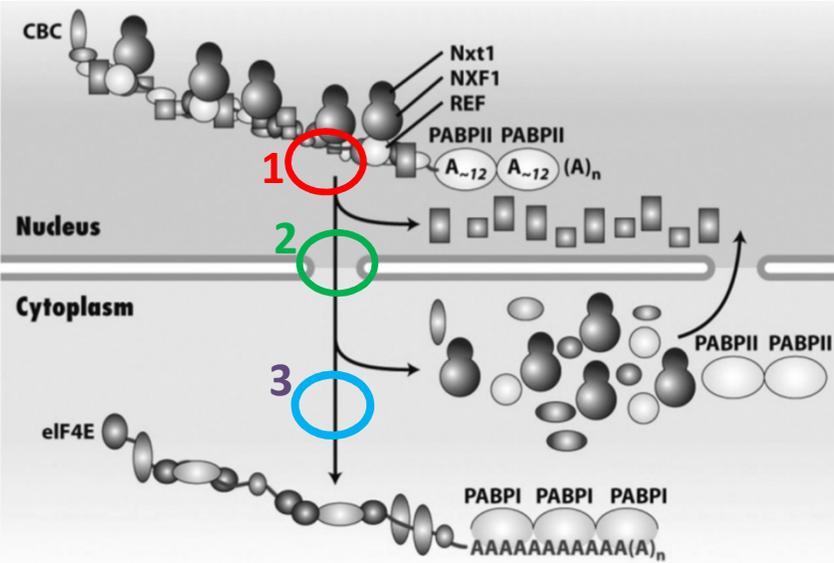


The life cycle of mRNA

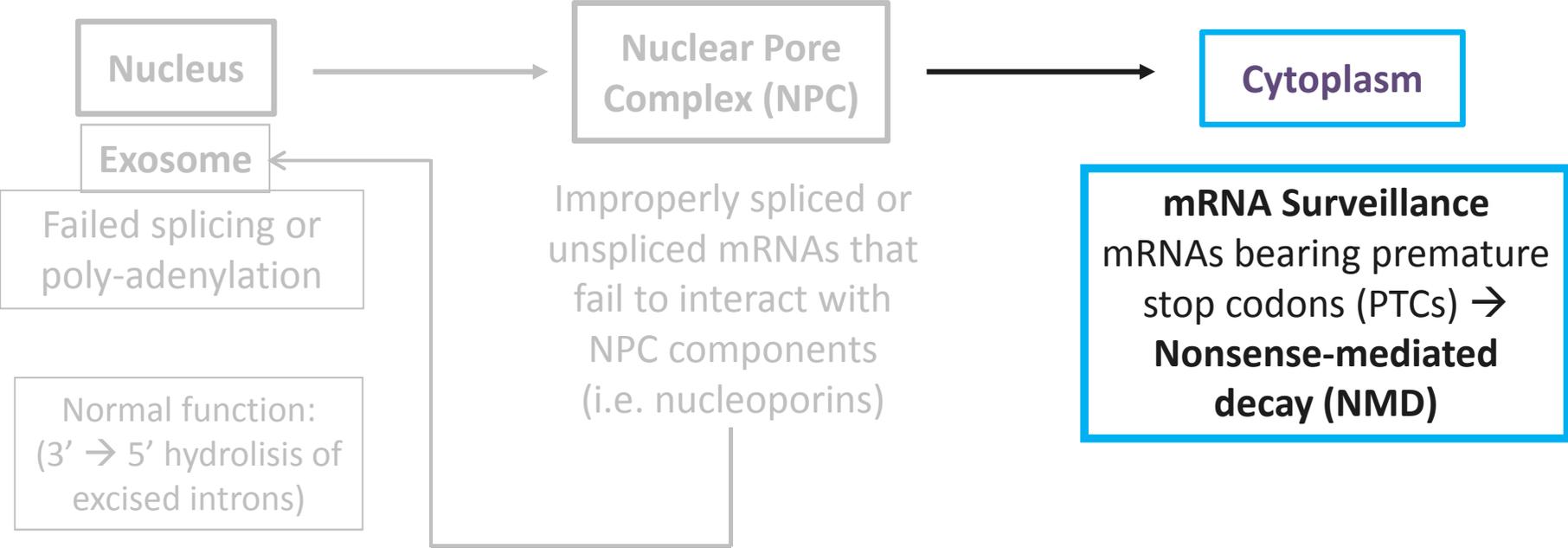
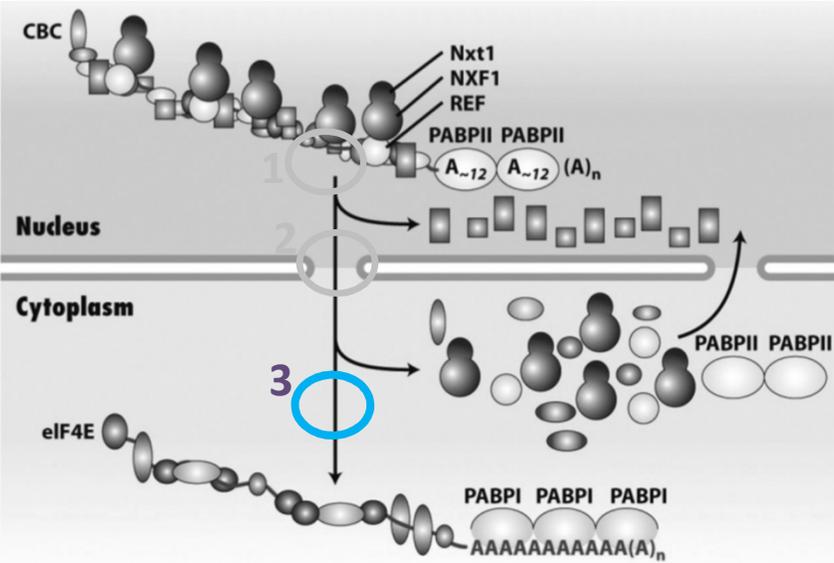


The relative amount of a mRNA is regulated by a balance between properly synthesized (thus exported and translated) and degraded transcripts

Quality control mechanisms ensure fidelity to mRNA biogenesis



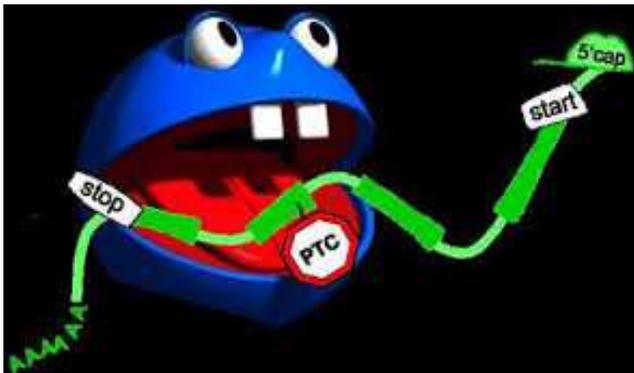
Quality control mechanisms ensure fidelity to mRNA biogenesis



The road to degradation

Premature Stop Codon (PTC)-containing mRNA

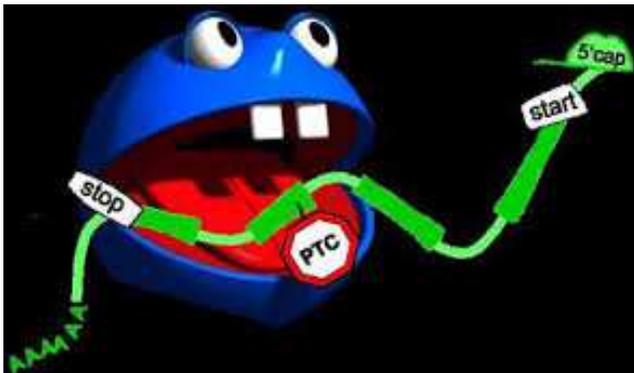
1. Recognition of the stop codon as a PTC
2. Tagging of PTC-containing mRNA
3. Degradation and/or isolation of the tagged mRNA



The road to degradation

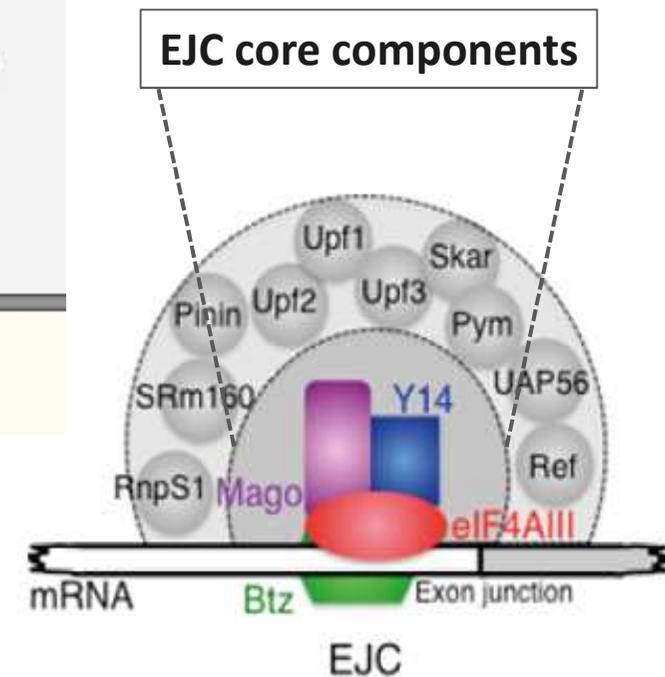
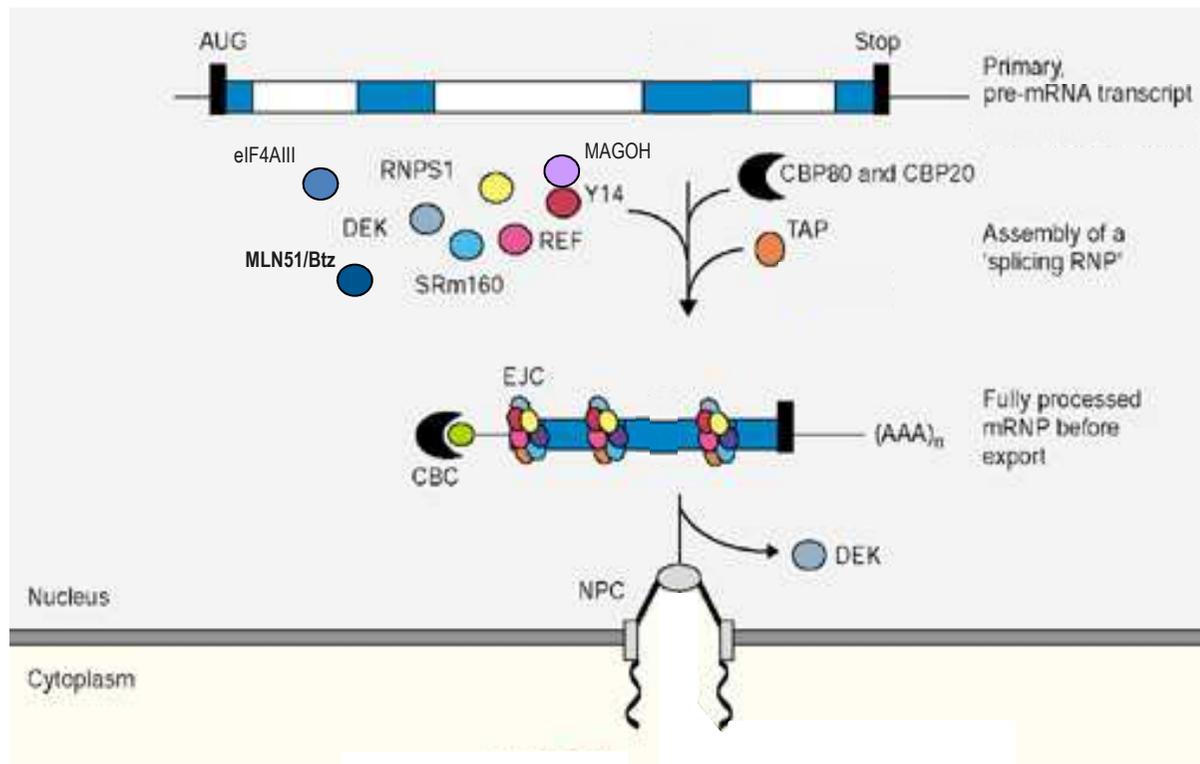
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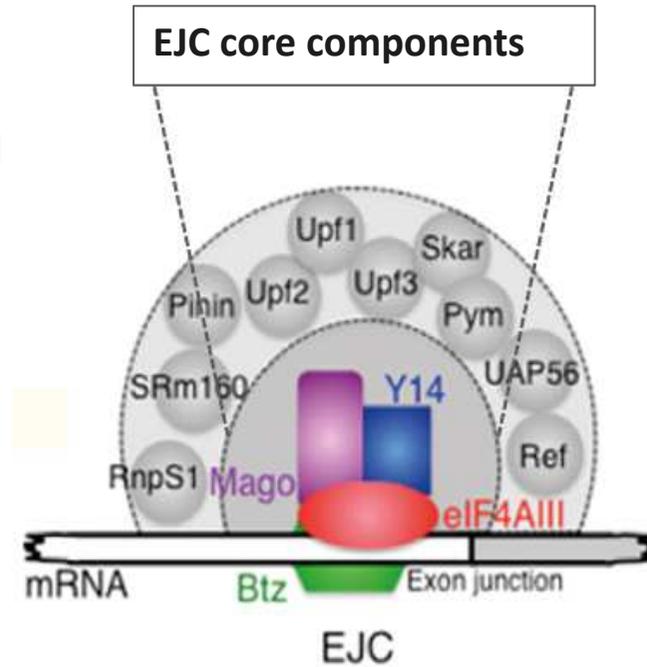
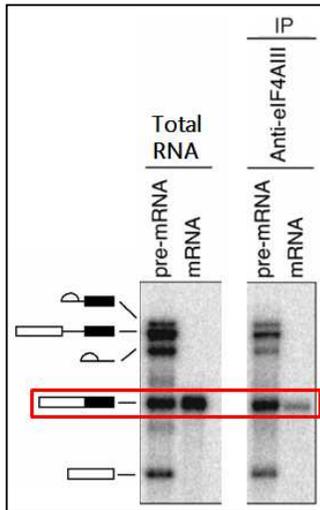
The Exon-Junction Complex (EJC) as a splicing hallmark

Multi-protein complexes deposited during splicing at 20-24 nucleotides upstream of each exon-exon junction

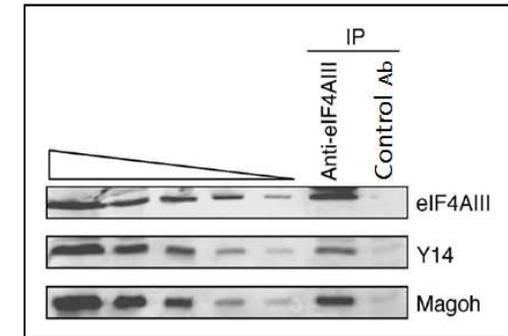


Experimental data indicate that eIF4AIII

**Directly binds pre-mRNA and
Is located on spliced mRNAs**



**Interacts with core
components of the EJCs**

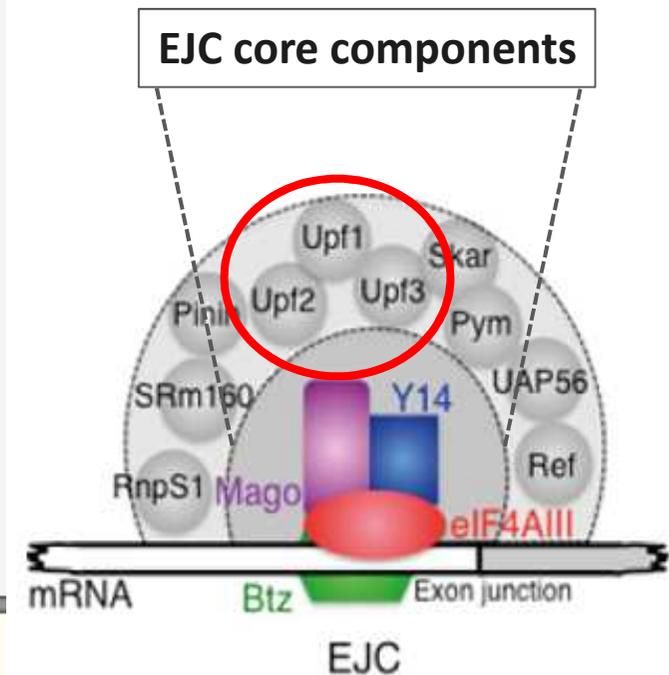
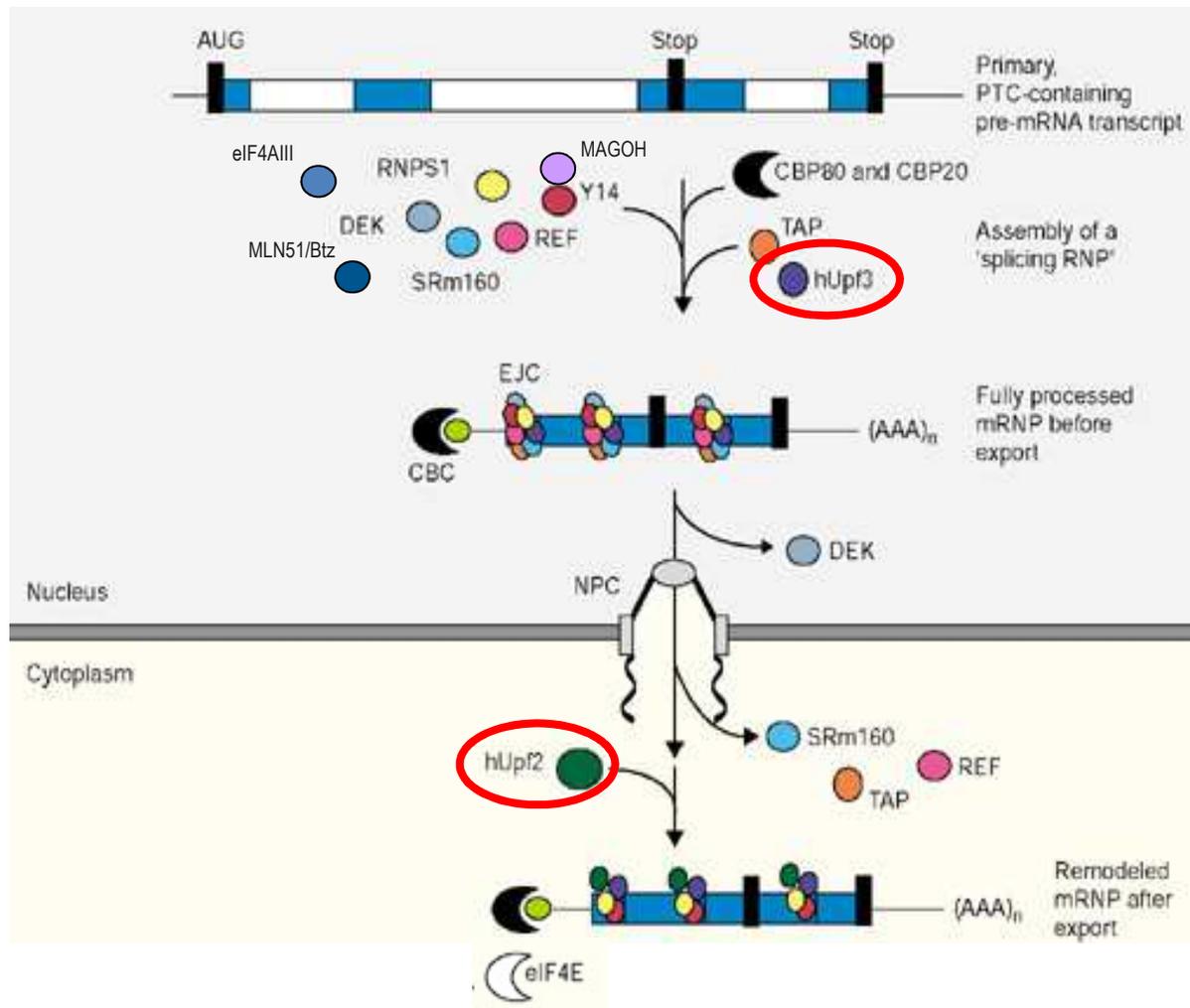


**Represents a scaffold for other proteins
such as those involved in mRNA transport**

EJC core	eIF4AIII	1, 2, 3	pre-mRNA
	Magoh	1, 2, 3	
	Y14	1, 2, 3	
	MLN51	1, 2, 3	
Associated factors	UAP56	1, 2	splicing intermediates
	REF	0, 1, 2	
	TAP	2	nuclear spliced mRNA
	p15	2	
		1, 2	

EJCs as splicing markers and scaffolds for protein effectors

(EJCs are multi-protein complexes deposited during splicing at 20-24 nucleotides upstream of each EJ)



EJCs are *cis*-acting key components → NMD is splicing-dependent

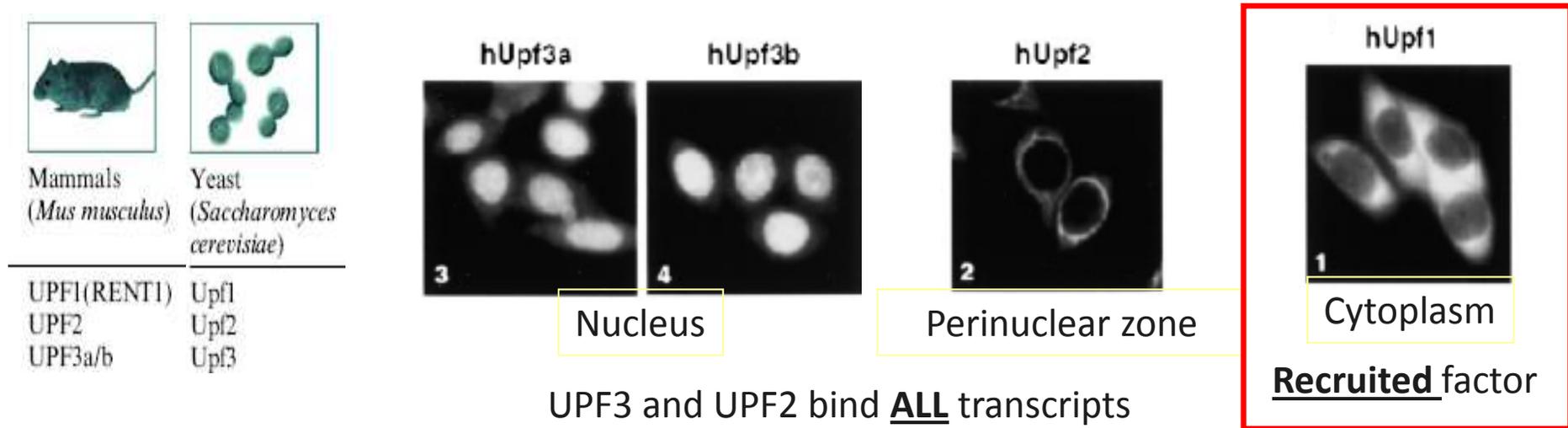
Protein effectors involved in Nonsense-Mediated Decay are conserved

Organism					
	Yeast (<i>Saccharomyces cerevisiae</i>)	Nematodes (<i>Caenorhabditis elegans</i>)	Fruitfly (<i>Drosophila melanogaster</i>)	Mammals (<i>Mus musculus</i>)	Plant (<i>Arabidopsis thaliana</i>)
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(REN1) UPF2 UPF3a/b SMG1 SMG5 SMG6 SMG7	UPF1(IBA1) UPF2 UPF3 nd nd nd nd

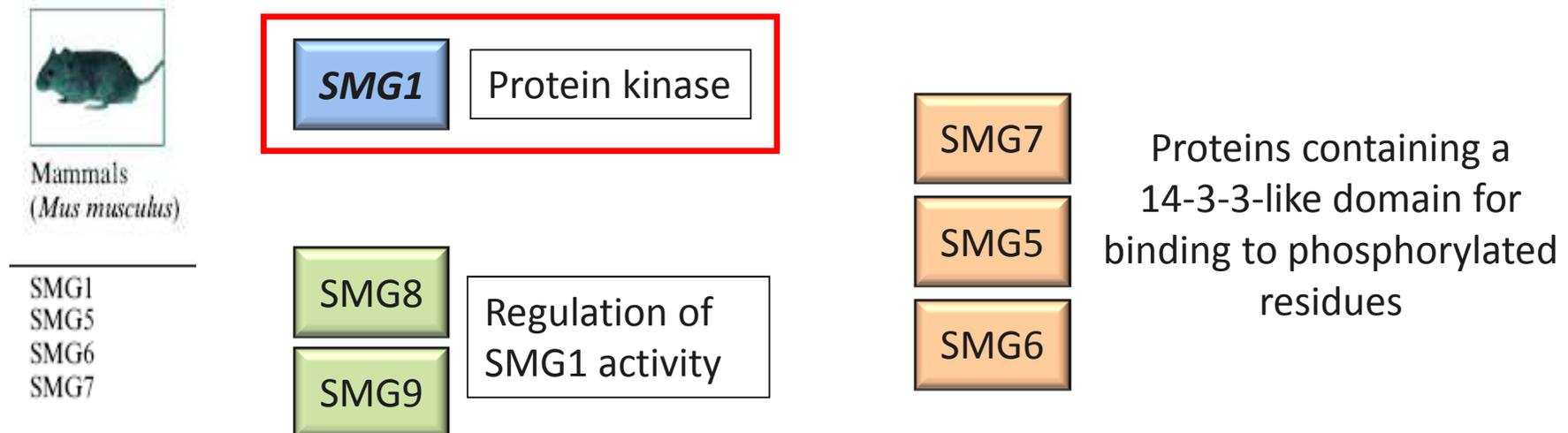
NMD effectors are *trans*-acting proteins able to recognize and bind EJC (cis-acting signals)

Protein effectors involved in Nonsense-Mediated Decay are conserved

hUpf proteins in HeLa cells – immunocytochemical staining

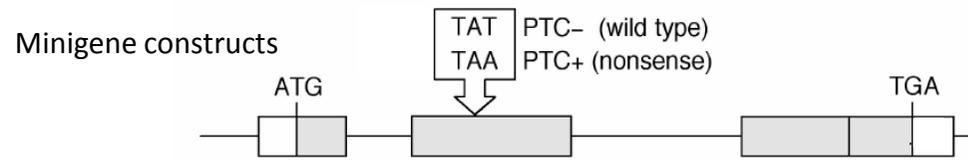
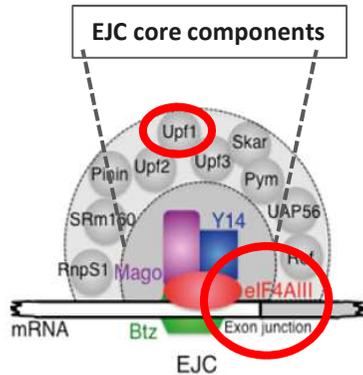


Lykke-Andersen *et al.*, Cell, 2000



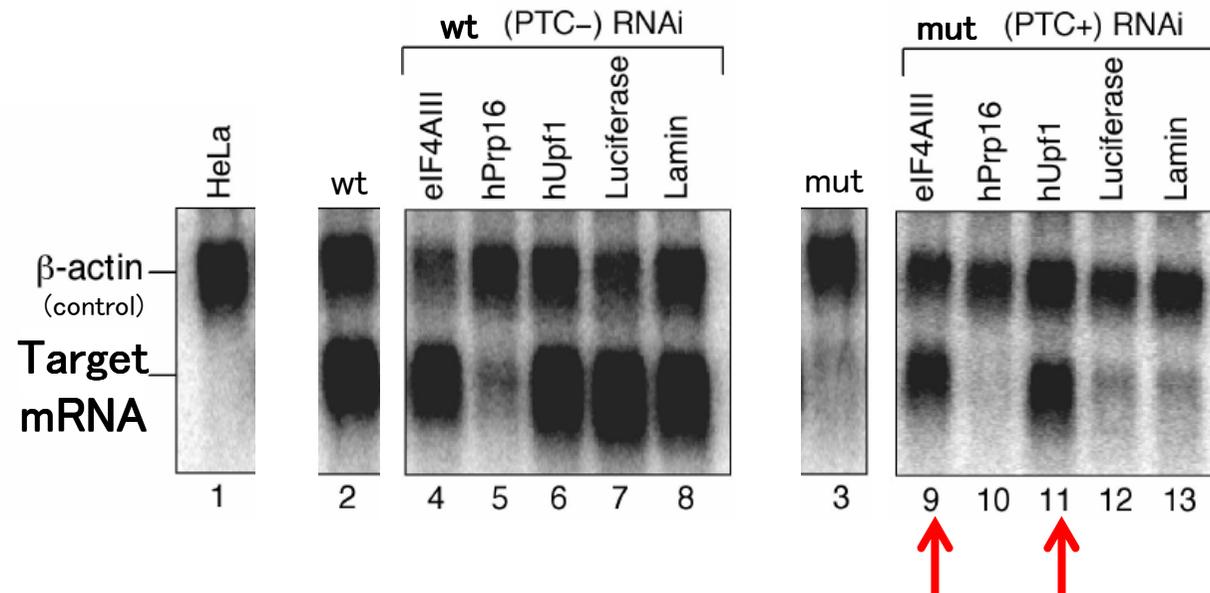
eIF4AIII (part of the EJC core) is required for NMD in mammalian cells

Experimental model: HeLa cells transfected with two different minigene constructs and RNAi technique followed by Northern blot analysis



Northern Blot analysis

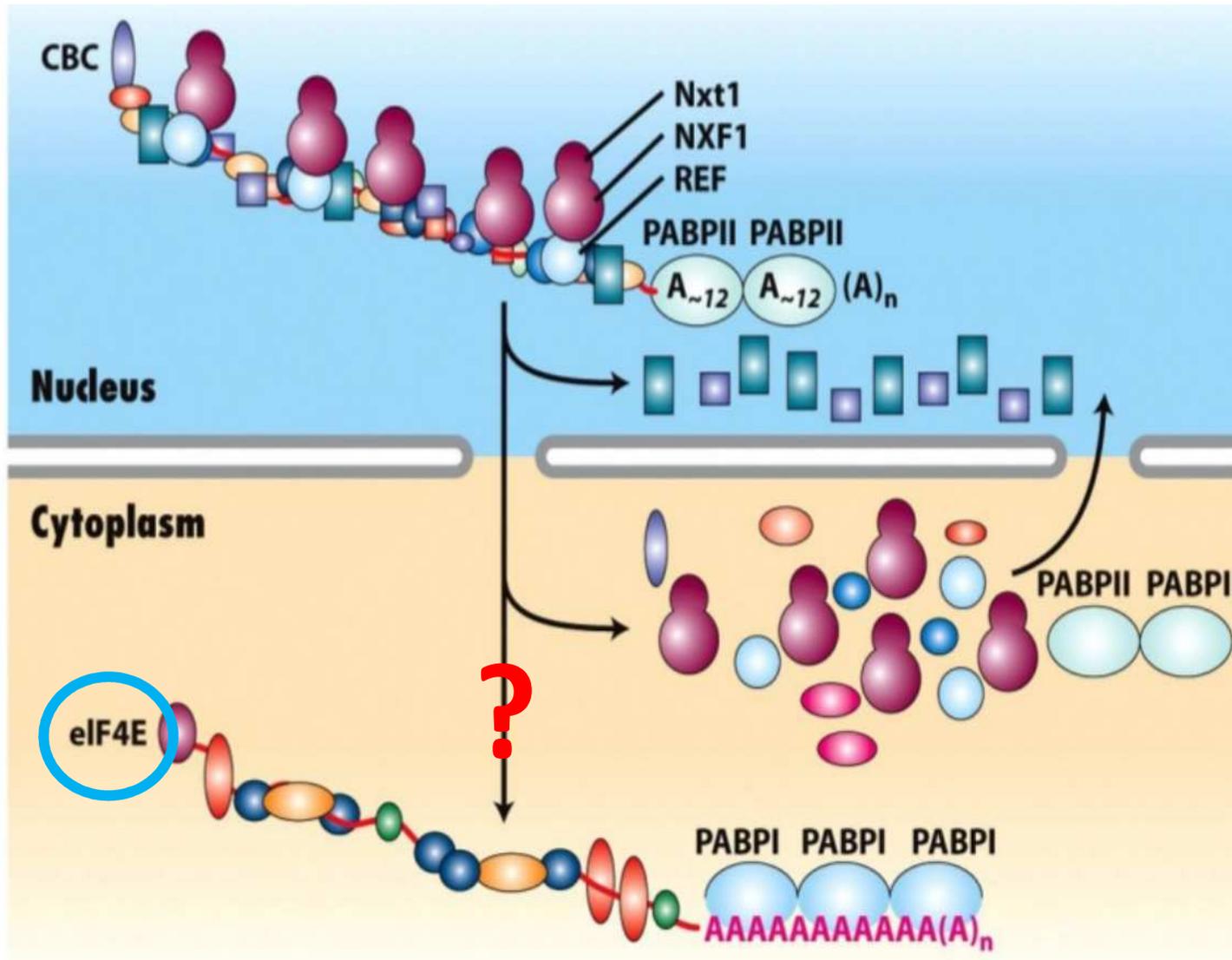
- 1 - "empty" cells
- 2 - PTC- = wt - NO NMD
- 3 - PTC+ = nonsense (NMD)



Result: Depletion of either hUpf1 or eIF4AIII stabilizes PTC+ mRNA to a similar extent

→ NMD is splicing-dependent

Quality control mechanisms ensure fidelity to mRNA biogenesis



eIF4E binds the 5'cap before the steady-state translation of mRNA

Cell, Vol. 106, 607–617, September 7, 2001, Copyright ©2001 by Cell Press

Evidence for a Pioneer Round of mRNA Translation: mRNAs Subject to Nonsense-Mediated Decay in Mammalian Cells Are Bound by CBP80 and CBP20

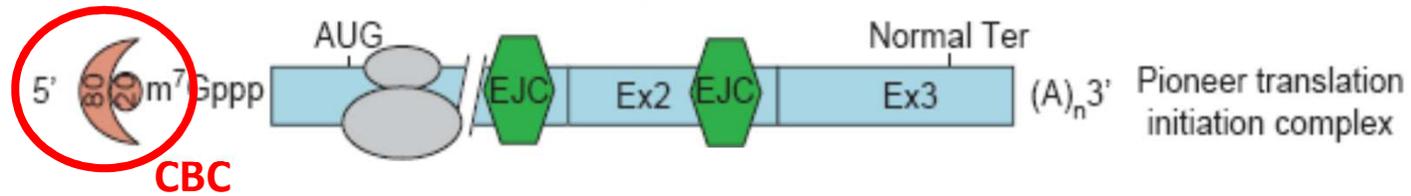
Yasuhito Ishigaki,² Xiaojie Li, Guillaume Serin,
and Lynne E. Maquat¹

The EMBO Journal Vol. 21 No. 13 pp. 3536–3545, 2002

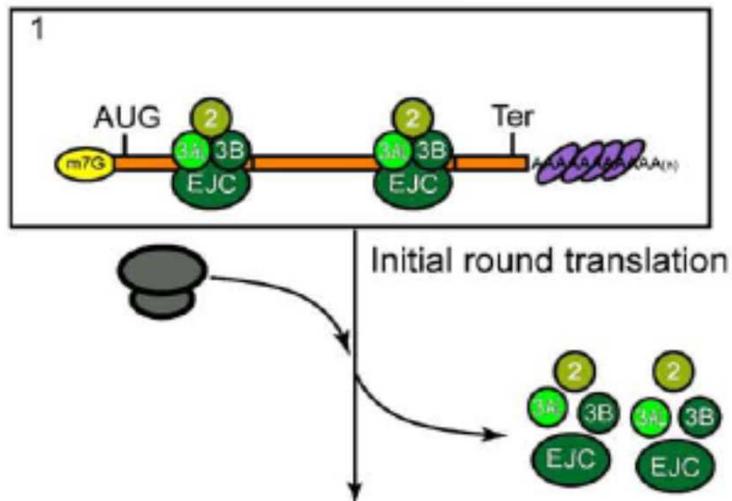
The exon junction complex is detected on CBP80- bound but not eIF4E-bound mRNA in mammalian cells: dynamics of mRNP remodeling

Fabrice Lejeune, Yasuhito Ishigaki¹,
Xiaojie Li and Lynne E. Maquat²

The PIONEER ROUND of translation – Normal Stop Codon



Normal Termination Codon

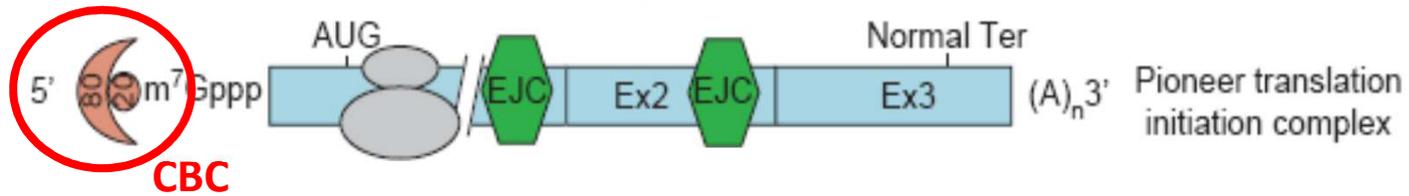


Ribosome scanning of the mRNA in a first round of translation

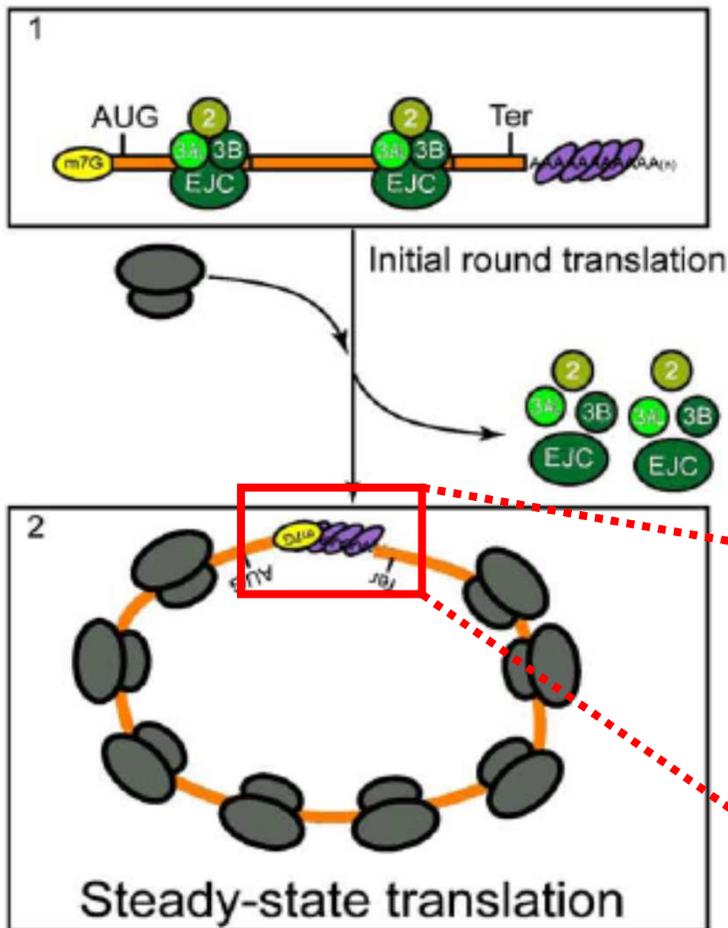


Displacement of EJCs from mRNA

The PIONEER ROUND of translation – Normal Stop Codon



Normal Termination Codon



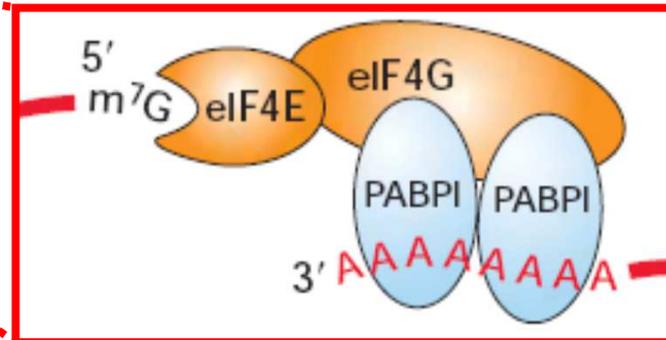
Ribosome scanning of the mRNA in a first round of translation



Displacement of EJCs from mRNA



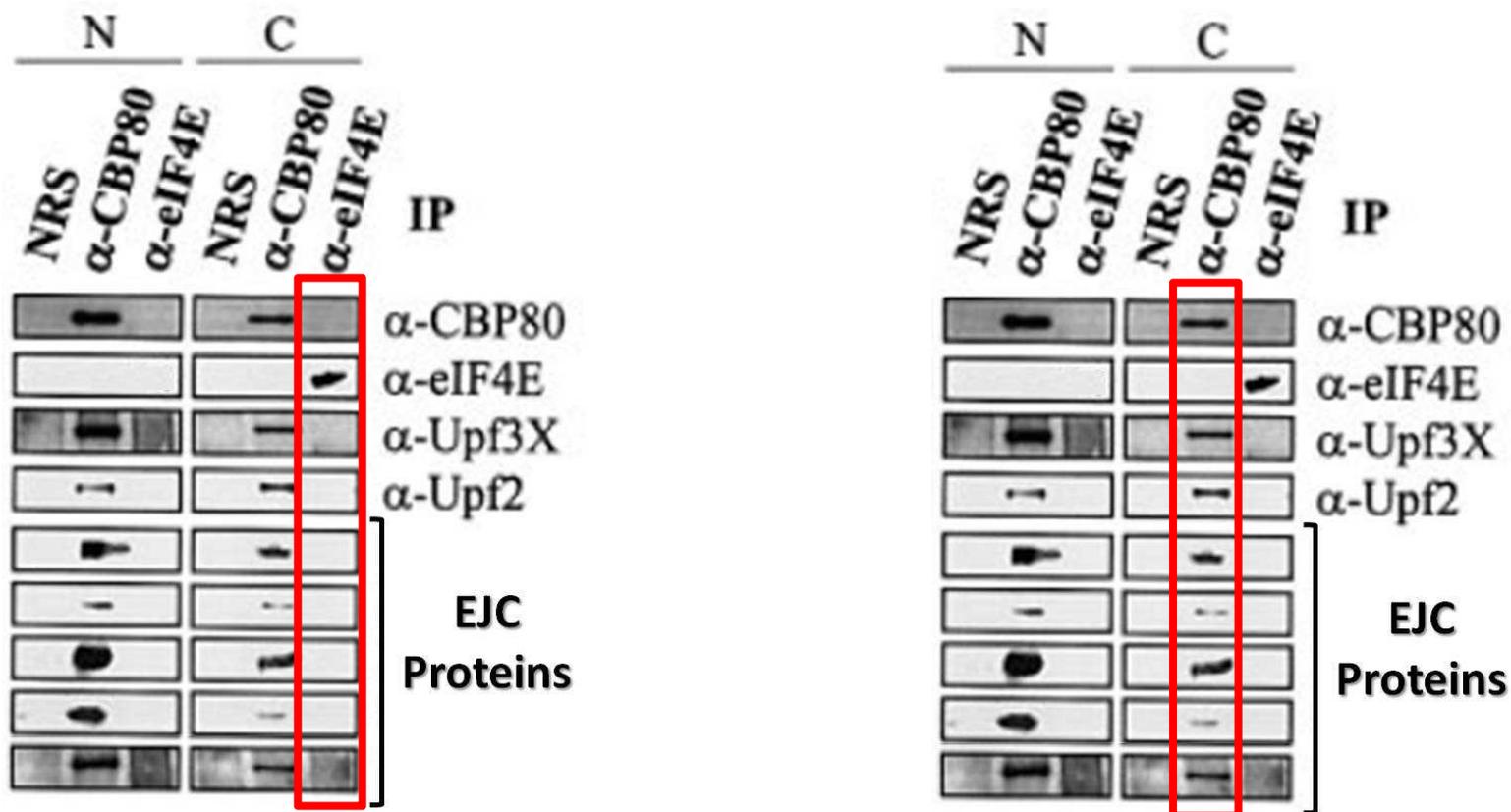
CBC (CBP80/CBP20) → eIF4E



eIF4E-bound mRNAs are immune to NMD

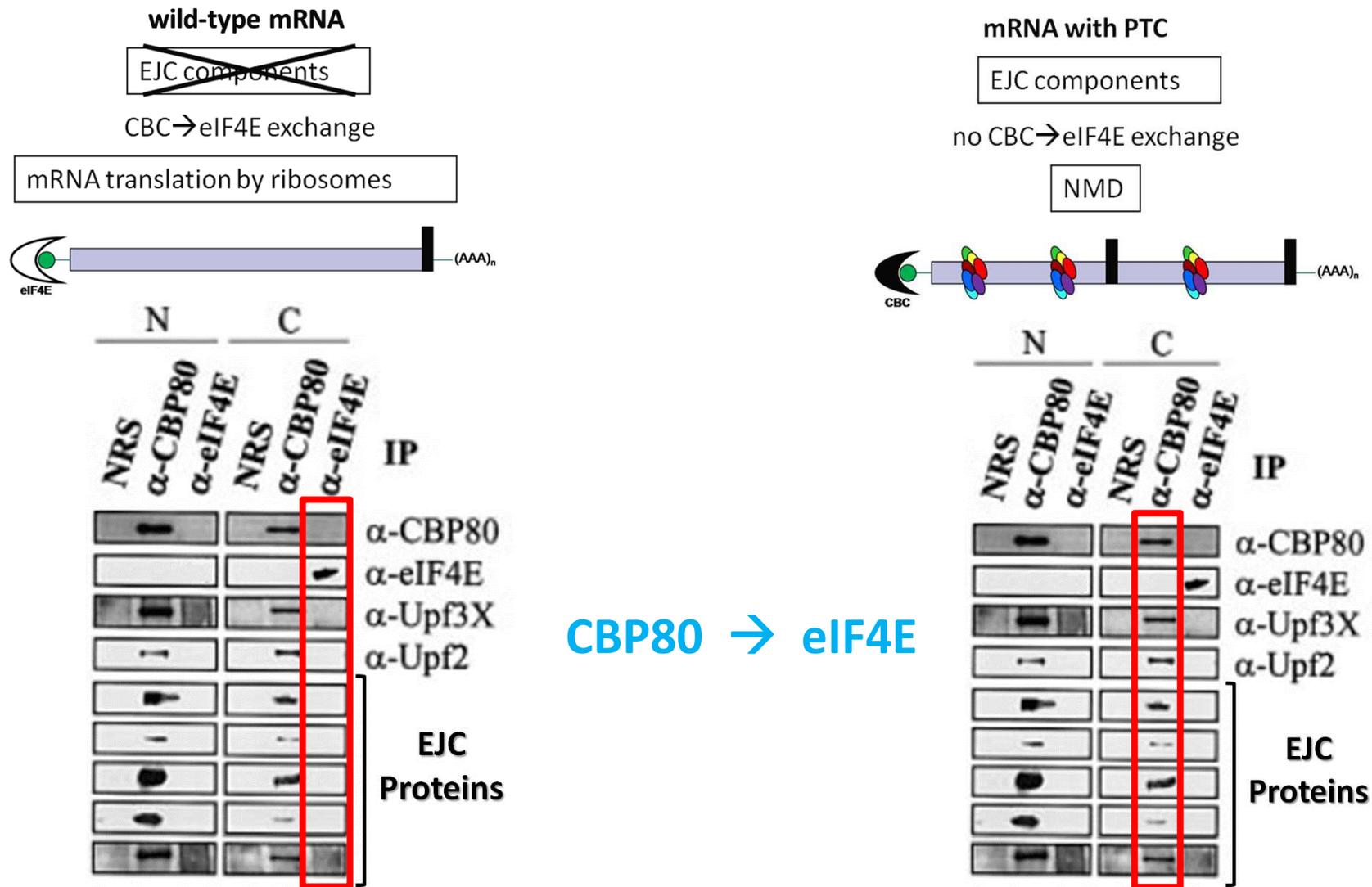
The exon junction complex is detected on CBP80-bound but not eIF4E-bound mRNA in mammalian cells: dynamics of mRNP remodeling

Experimental model: Nuclear (N) and Cytoplasmic (C) CBP80 co-immunoprecipitation and western blotting analysis with antibodies specific for other complexed proteins

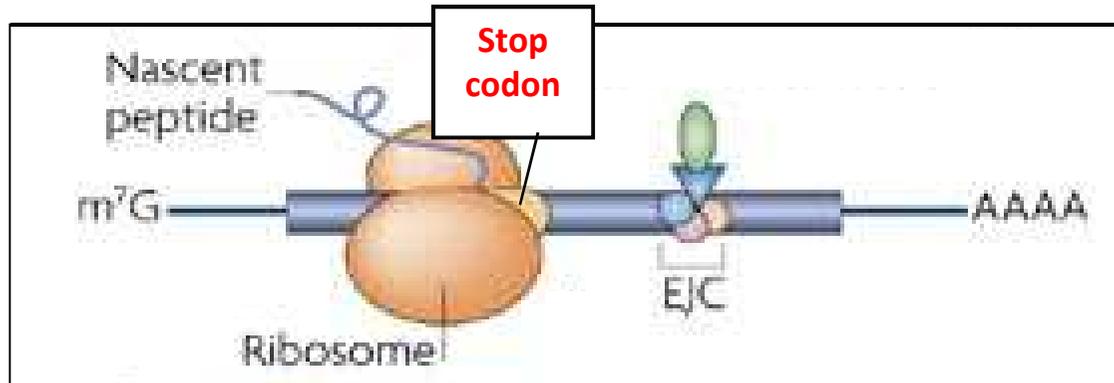


CBP80 but not eIF4E co-immunoprecipitates with EJC components

The exon junction complex is detected on CBP80-bound but not eIF4E-bound mRNA in mammalian cells: dynamics of mRNP remodeling



The PIONEER ROUND of translation – Premature Stop Codon



First round of translation



Premature stop codon

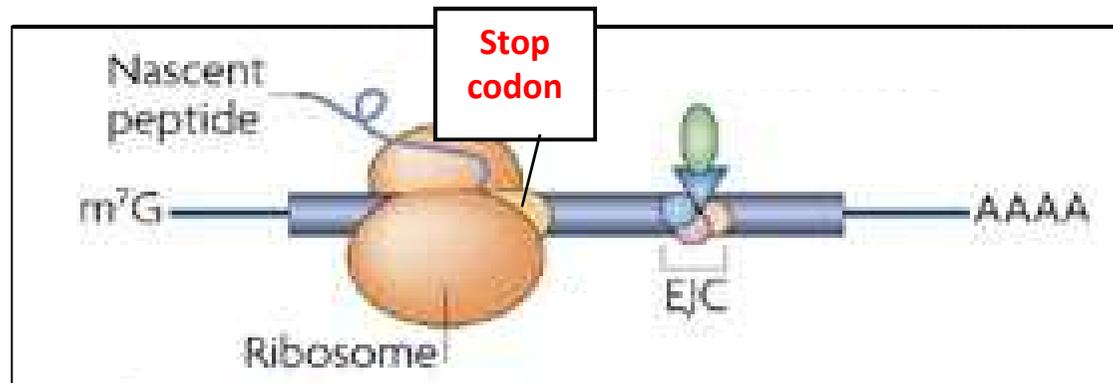


Ribosome stalling at the PTC



~~Displacement of EJCs from mRNA~~

The PIONEER ROUND of translation – Premature Stop Codon



First round of translation



Premature stop codon



Ribosome stalling at the PTC



~~Displacement of EJCs from mRNA~~

The “rule”:

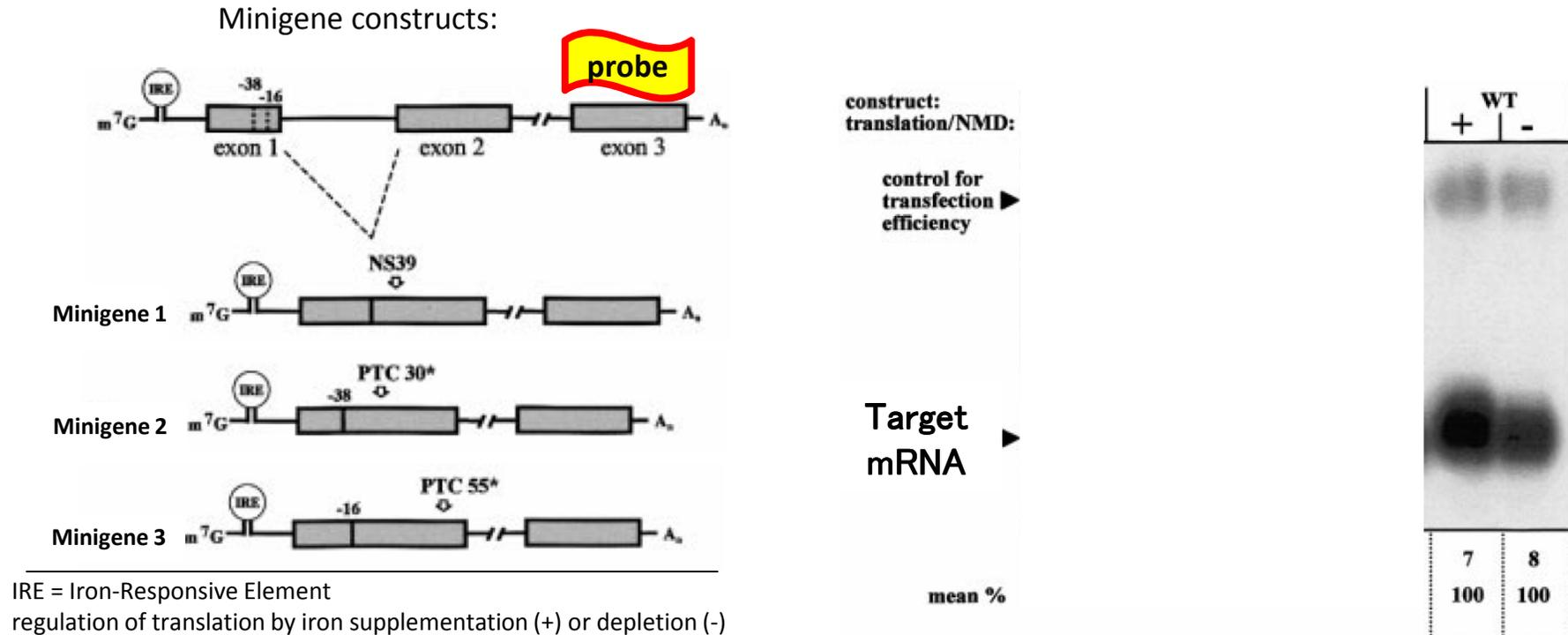
When the distance between a stop codon and the downstream exon-exon junction is more than 55 nt → stop codon = PTC

Interplay between EJCs and ribosomes → **NMD is translation-dependent**

The relative position of a PTC influences the efficiency of NMD

Experimental model:

Minigenes containing PTCs at different positions, transfected in cells displaying normal (+) or suppressed (-) translation and Northern blot analysis with a specific exon 3 probe



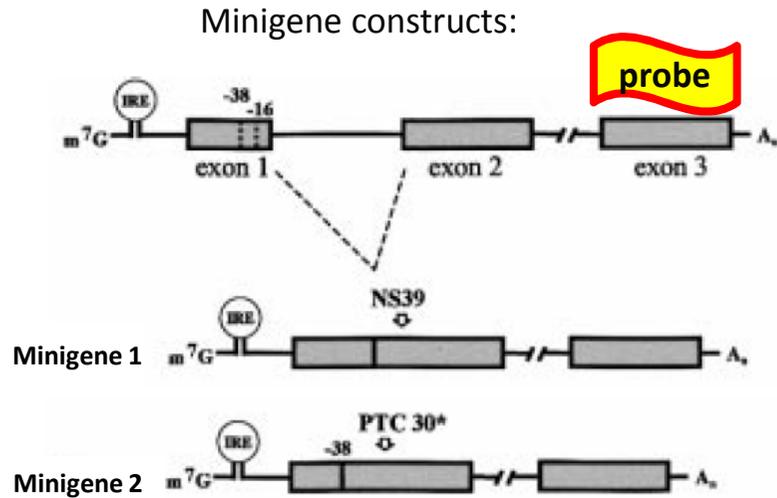
Results:

- i) Translation suppression decreases the NMD → **NMD is translation-dependent**
- ii) **NMD efficiency is influenced by PTC position**

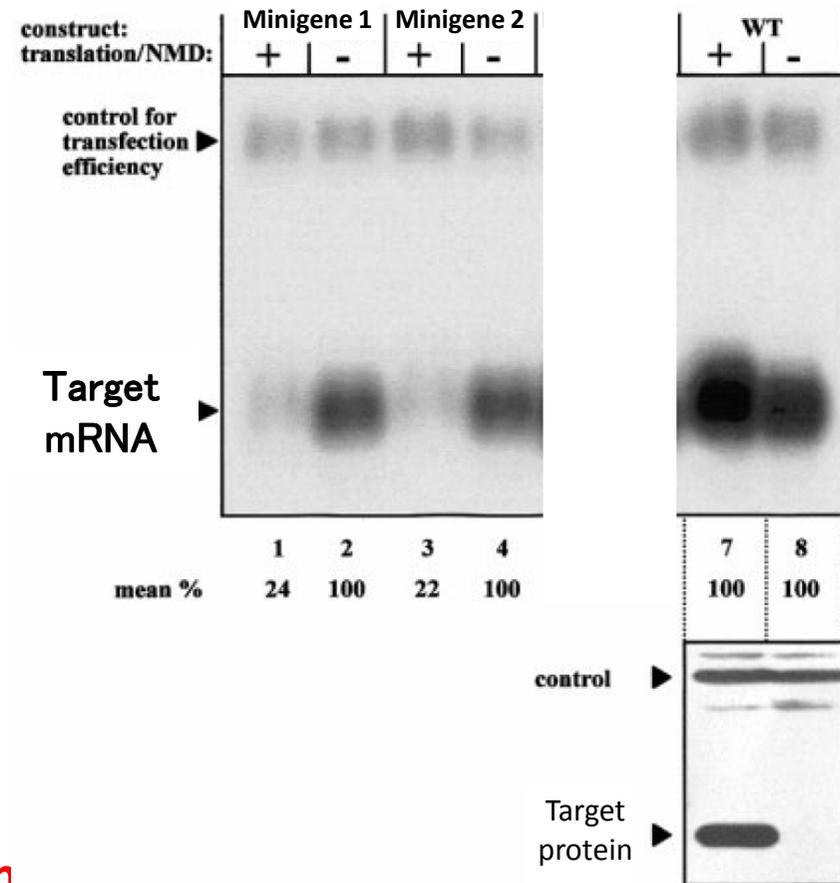
The relative position of a PTC influences the efficiency of NMD

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Minigenes containing PTCs at different positions, transfected in cells displaying normal (+) or suppressed (-) translation and Northern blot analysis with a specific exon 3 probe



IRE = Iron-Responsive Element
regulation of translation by iron supplementation (+) or depletion (-)



Results:

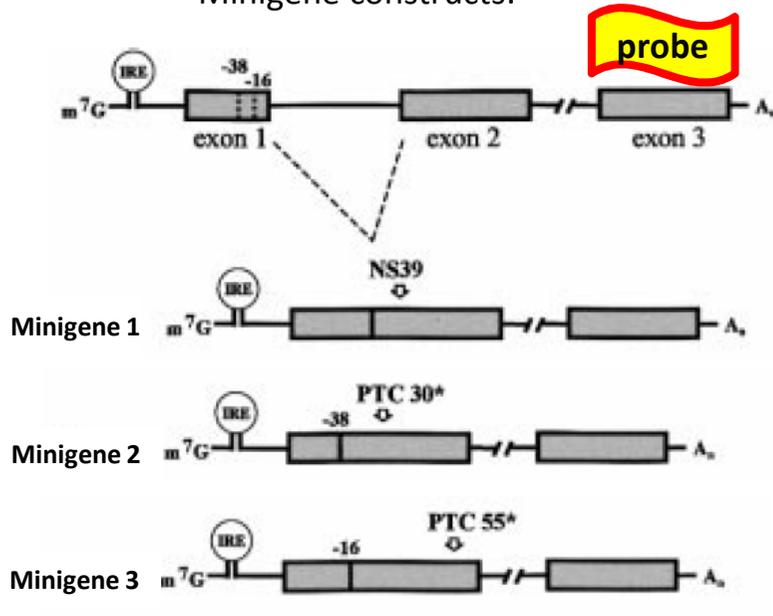
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The relative position of a PTC influences the efficiency of NMD

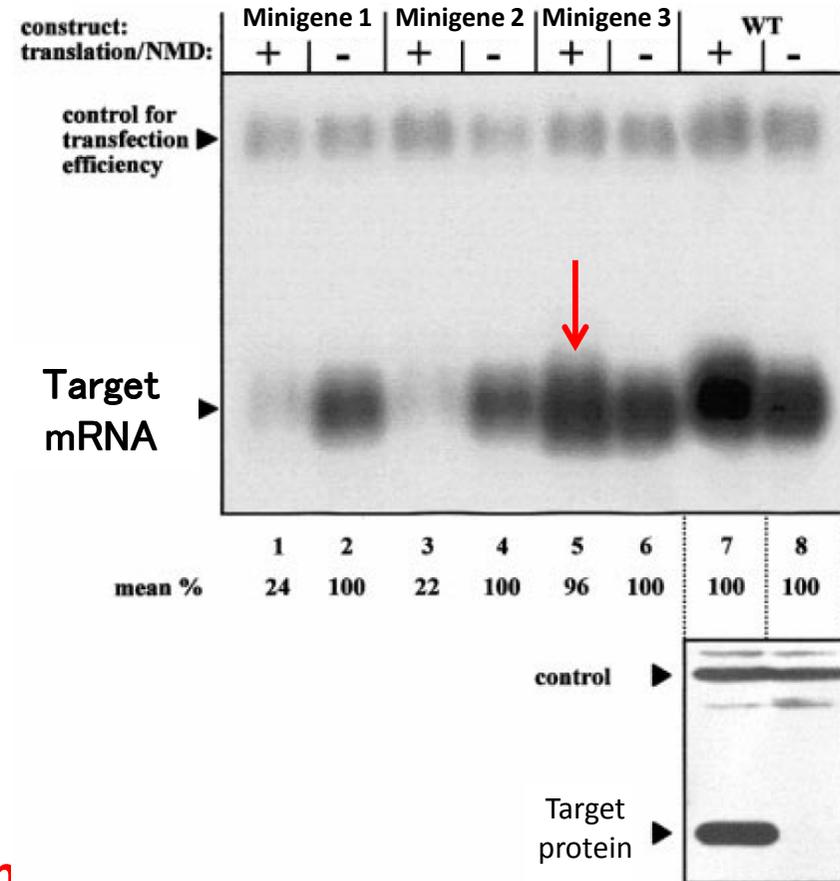
Experimental model:

Minigenes containing PTCs at different positions, transfected in cells displaying normal (+) or suppressed (-) translation and Northern blot analysis with a specific exon 3 probe

Minigene constructs:



IRE = Iron-Responsive Element
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Results:

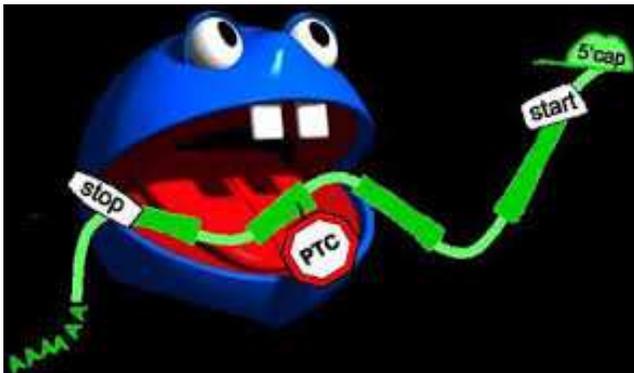
- i) Translation suppression decreases the NMD → **NMD is translation-dependent**
- ii) **NMD efficiency is influenced by PTC position**

The road to degradation

Premature Stop Codon (PTC)-containing mRNA

1. Recognition of the stop codon as a PTC

- EJs
- Protein effectors (UPF3, UPF2, UPF1)
- Splicing-dependent
- Translation-dependent
- Influenced by position of PTCs



Binding of a novel SMG-1–Upf1–eRF1–eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay

Isao Kashima, Akio Yamashita, Natsuko Izumi, Naoyuki Kataoka, Ryo Morishita, Shinichi Hoshino, Mutsuhito Ohno, Gideon Dreyfuss and Shigeo Ohno

Genes & Dev. 2006 20: 355-367

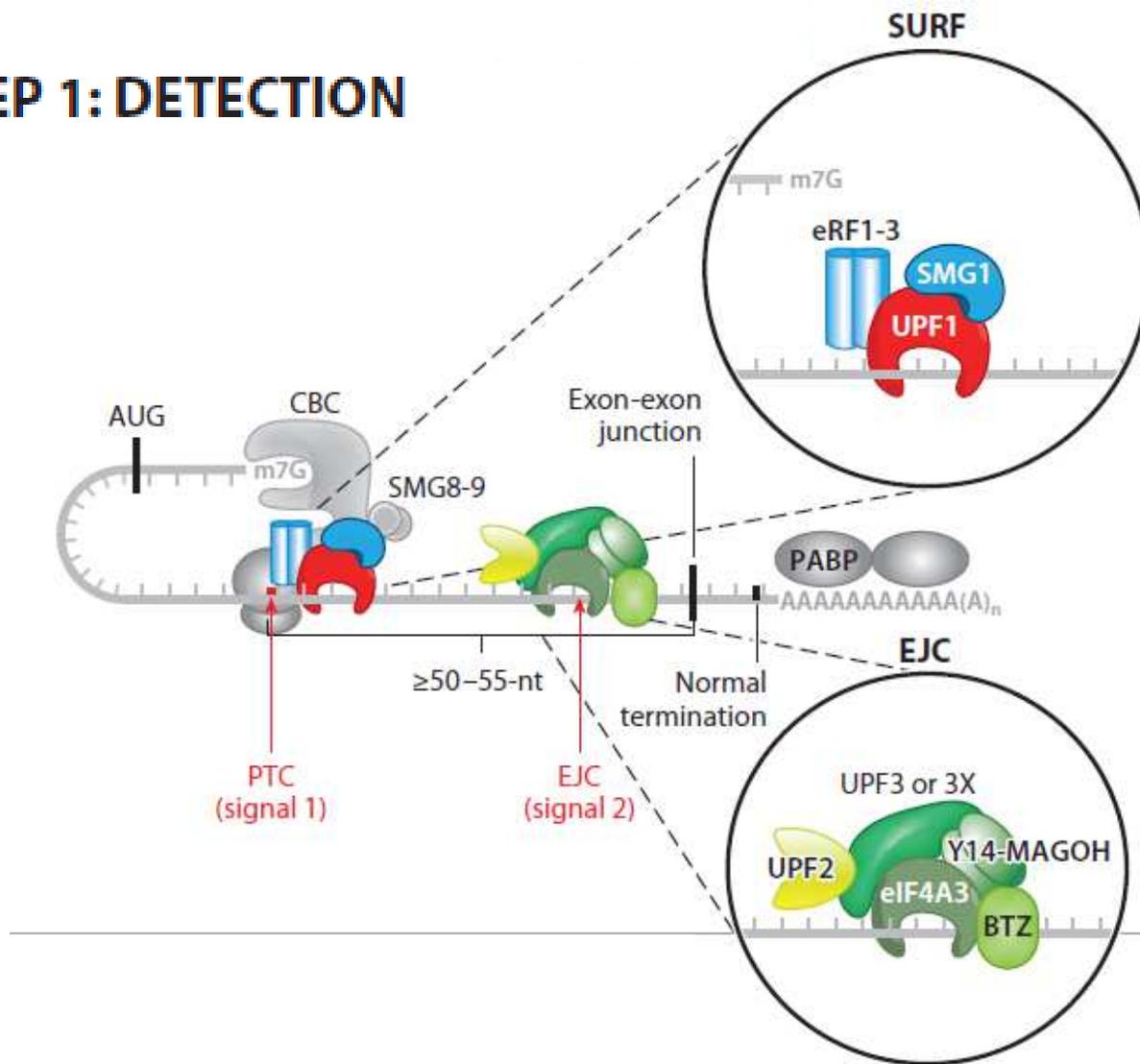


Mammals
(*Mus musculus*)

UPF1(REN1)
UPF2
UPF3a/b
SMG1
SMG5
SMG6
SMG7

Upf1 is recruited within the so-called SURF complex

STEP 1: DETECTION

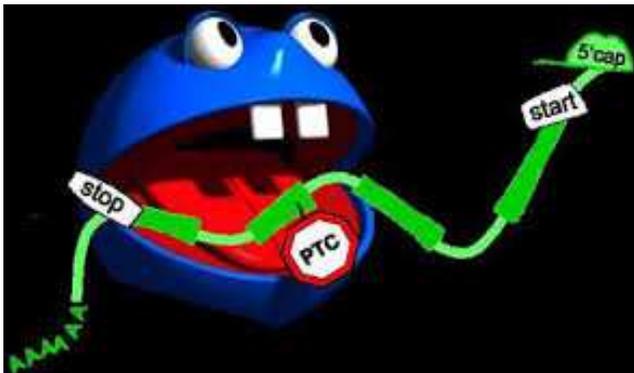


S SMG1
U UPF1
R eRF1
F eRF3
Complex

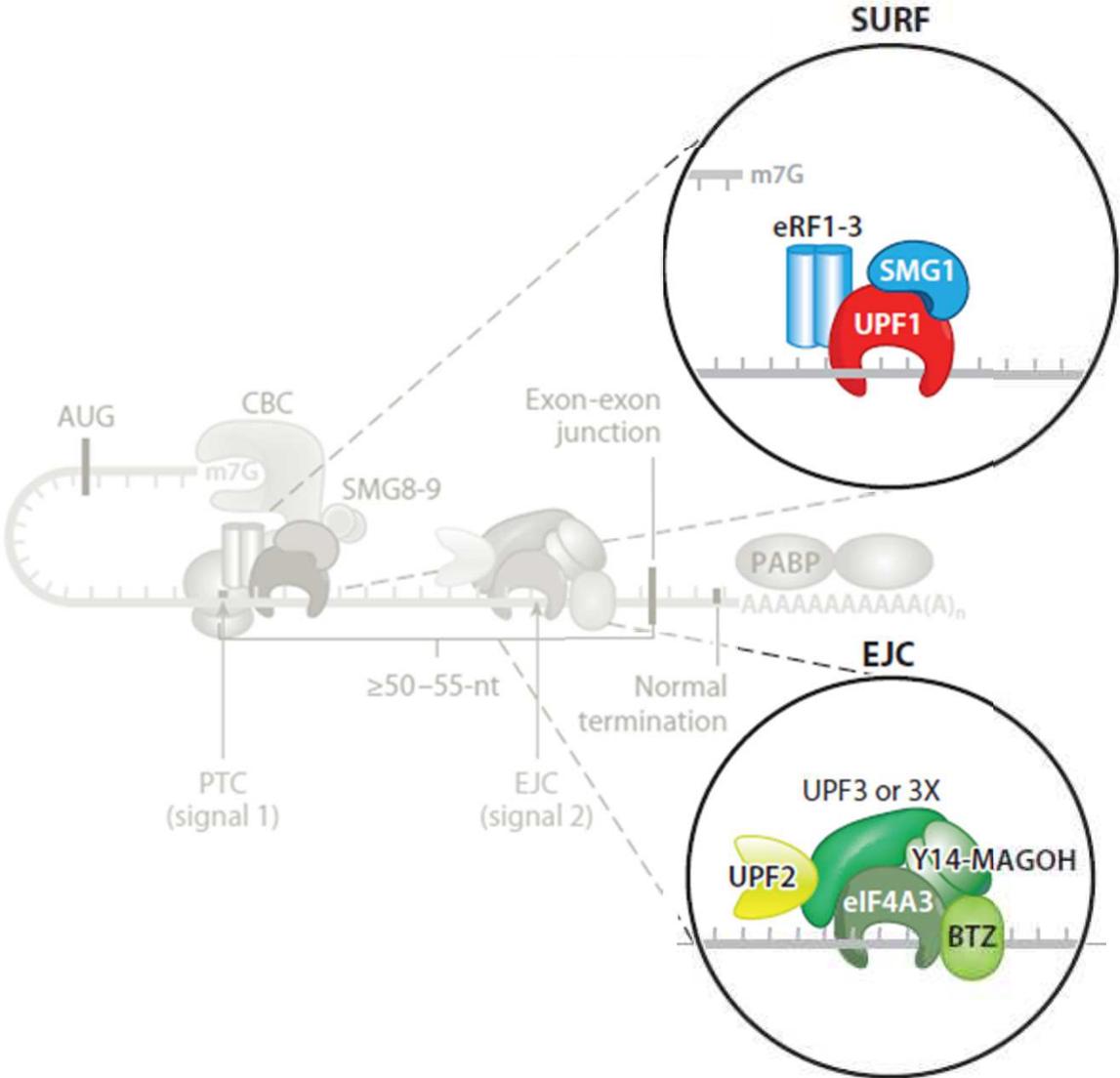
The road to degradation

Premature Stop Codon (PTC)-containing mRNA

1. Recognition of the stop codon as a PTC
2. Tagging of PTC-containing mRNA
3. Degradation and/or isolation of the tagged mRNA

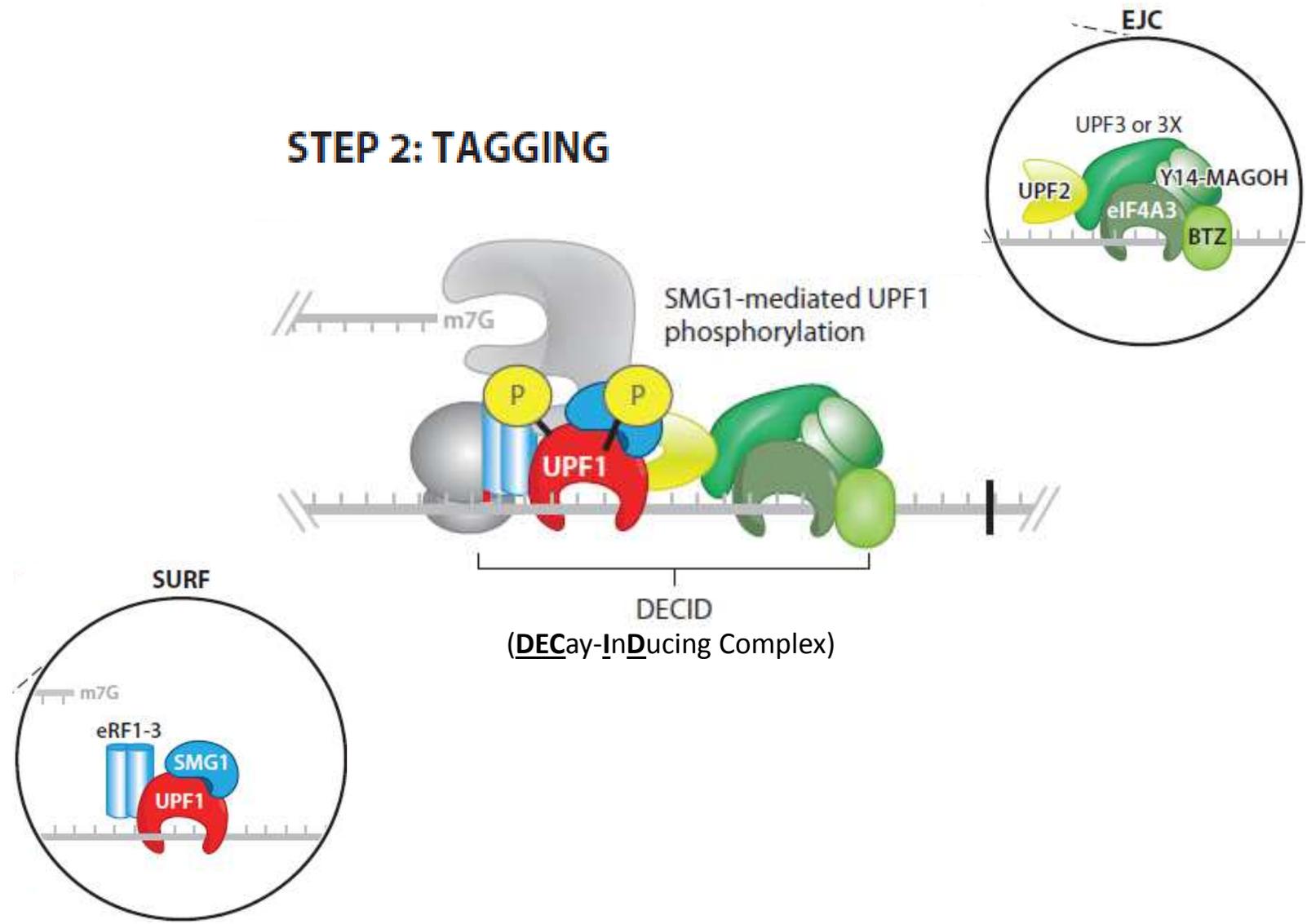


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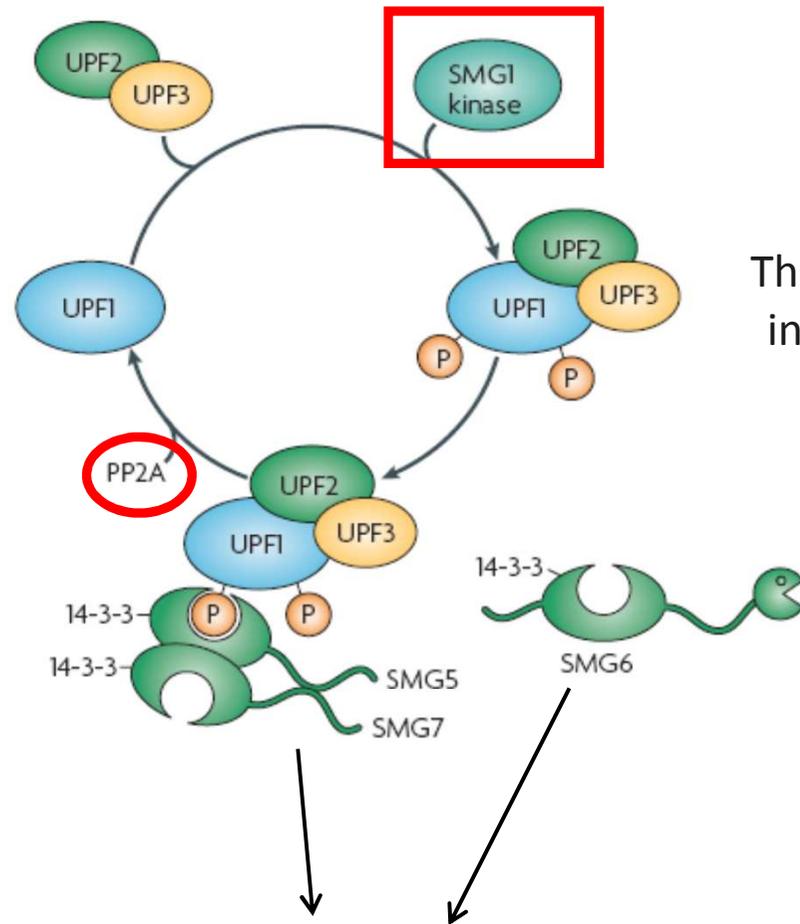


mRNA is committed to decay after phosphorylation of UPF1 by SMG1

STEP 2: TAGGING

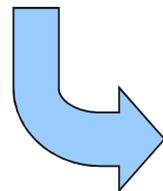


UPF1 is regulated by phosphorylation/dephosphorylation cycles



The UPF3:UPF2:UPF1 complex induces the SMG1-mediated phosphorylation of UPF1

Recruitment of SMG6 and/or SMG5-SMG7 via the phosphate tags on UPF1



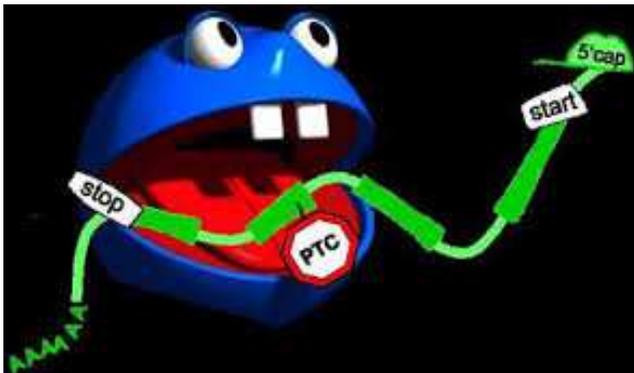
mRNA decay

+ Recycling of UPF1 and other effector proteins involved in NMD

The road to degradation

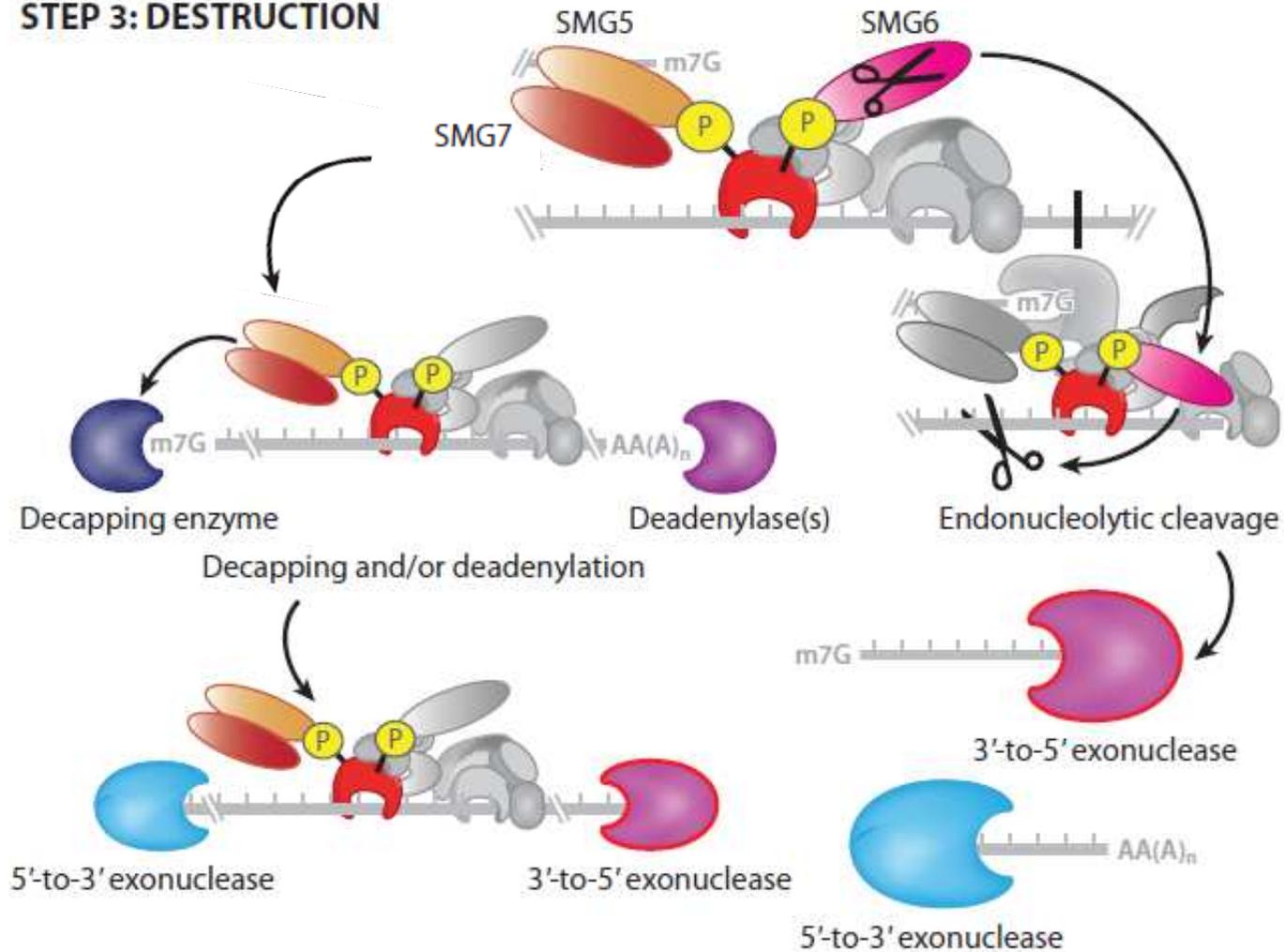
Premature Stop Codon (PTC)-containing mRNA

1. Recognition of the stop codon as a PTC
2. Tagging of PTC-containing mRNA
3. **Degradation and/or isolation of the tagged mRNA**



Degradation of the tagged mRNA committed to NMD

STEP 3: DESTRUCTION

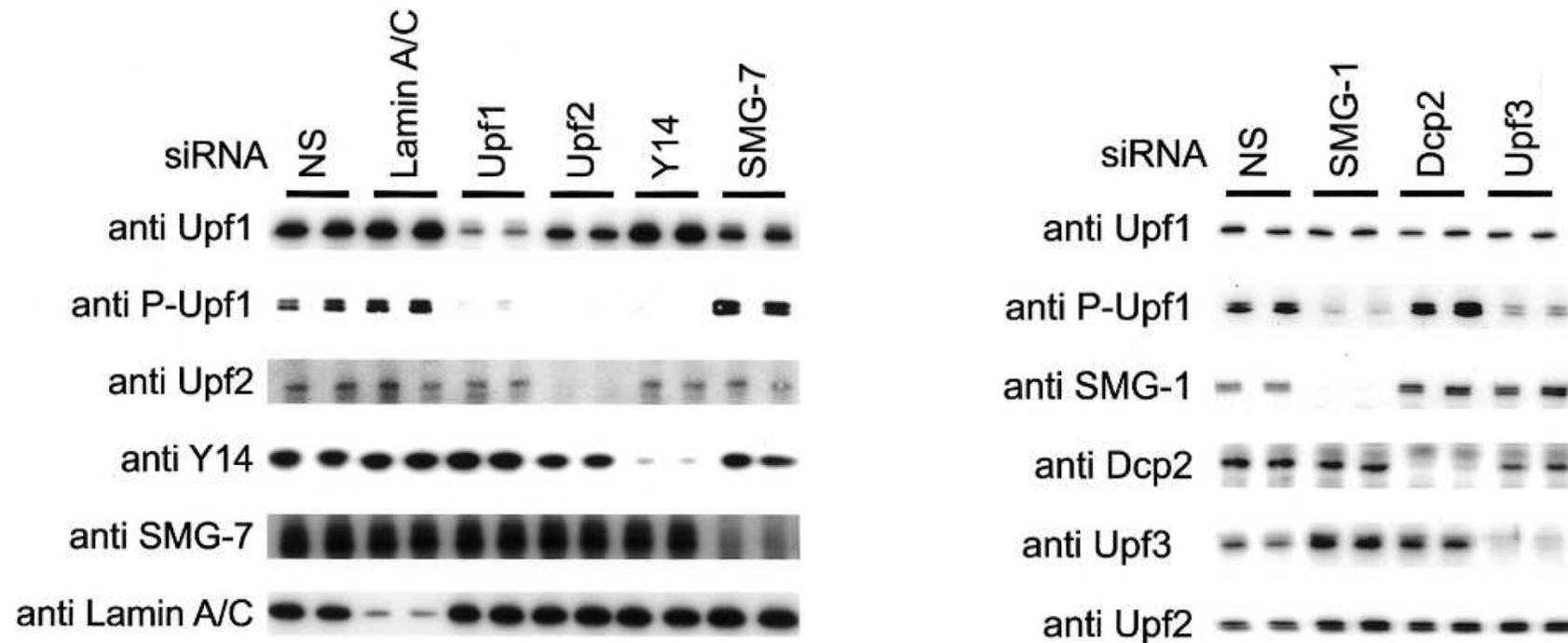


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Experimental model: Lysates of HeLa cells transfected with siRNAs and western blotting analysis on phospho-UPF1 levels after silencing

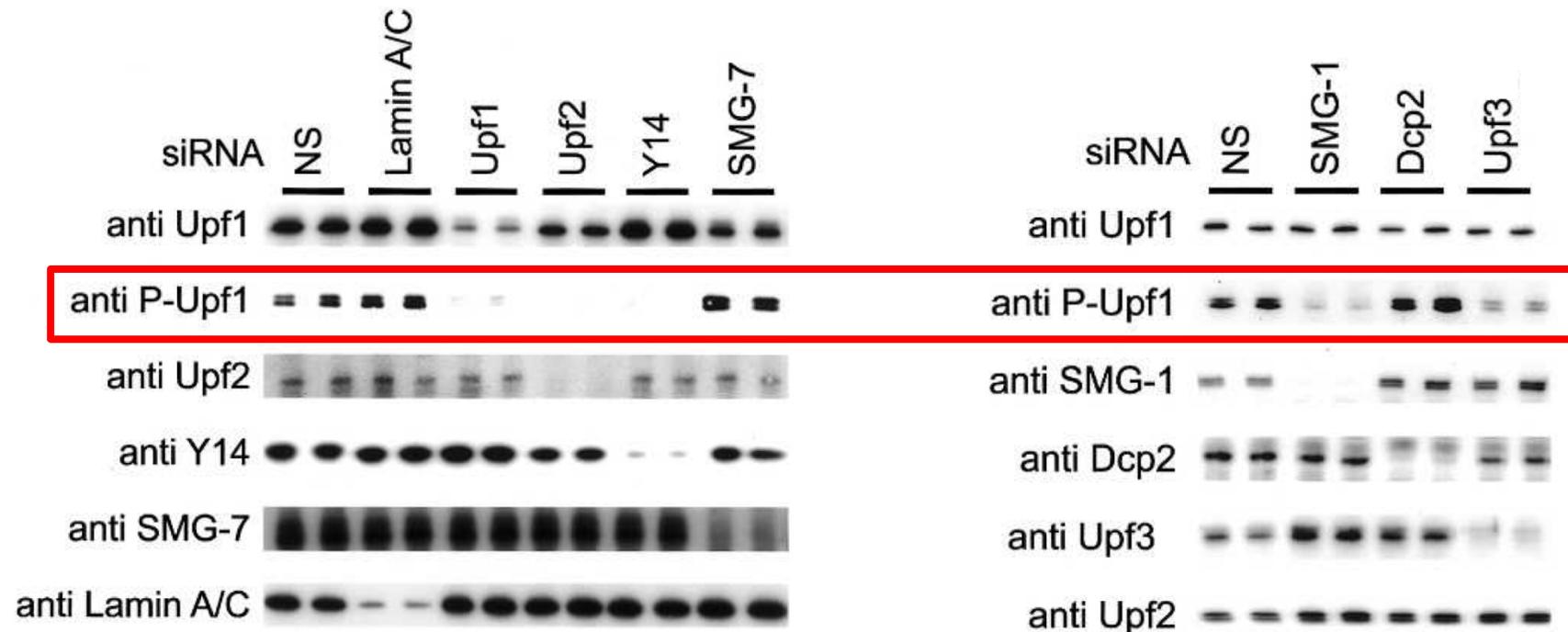


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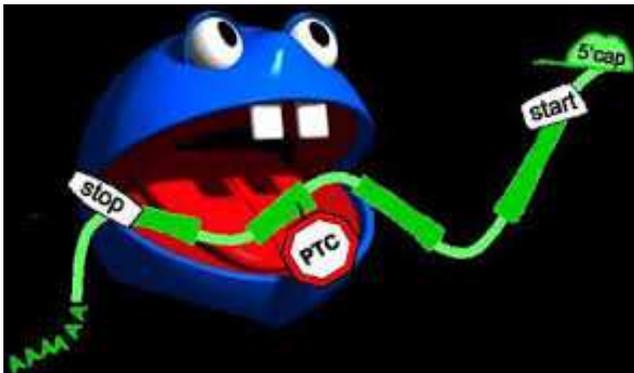


Result: SMG-1-mediated phosphorylation of UPF1 requires UPF2, UPF3, and Y14

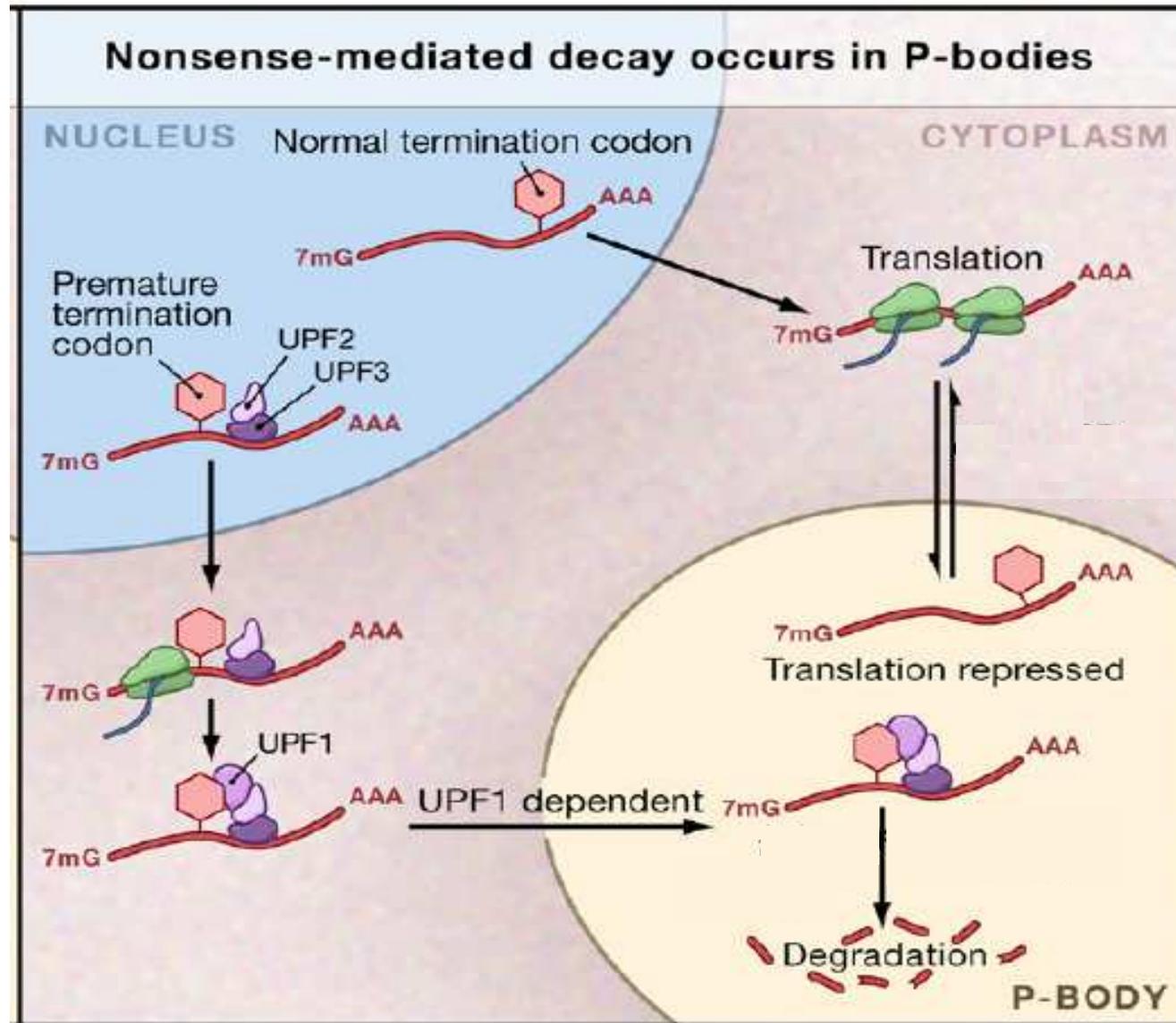
The road to degradation

Premature Stop Codon (PTC)-containing mRNA

1. Recognition of the stop codon as a PTC
2. Tagging of PTC-containing mRNA
3. Degradation and/or **isolation of the tagged mRNA**



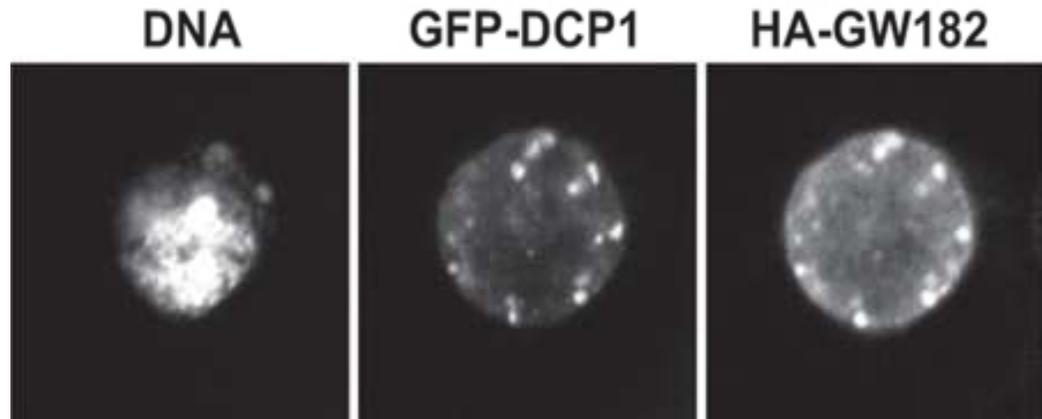
mRNA Processing bodies (P-bodies) contain enzymes involved in mRNA degradation



mRNA Processing bodies (P-bodies) contain enzymes involved in mRNA degradation

Also known as mRNA-decay foci, DCP bodies or GW bodies

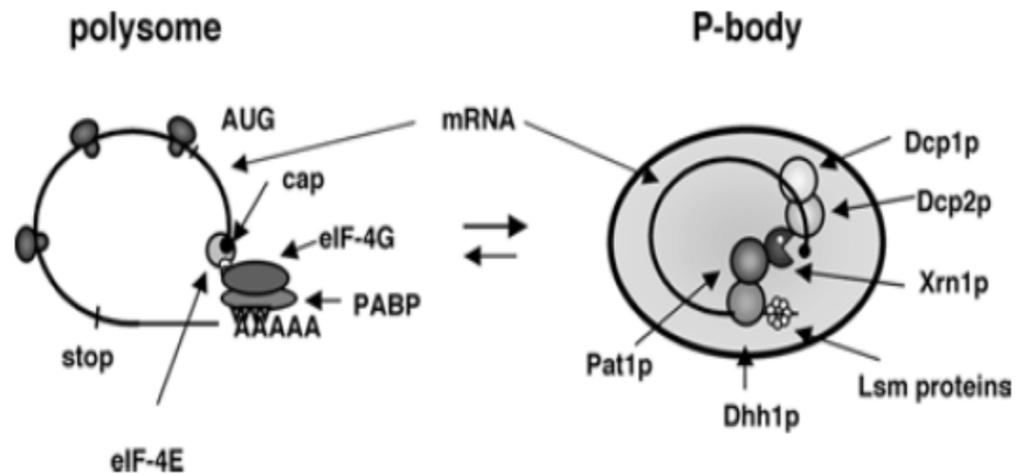
Specialized cytoplasmic regions enriched with degrading enzymes such as XRN1, DCP1, DCP2 e Lsm1-7



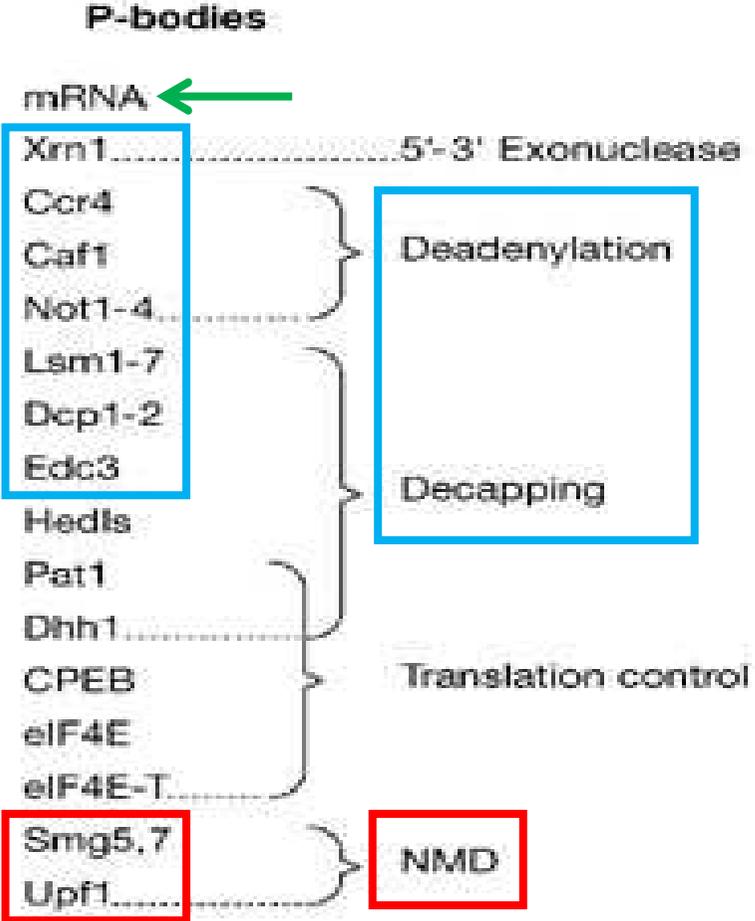
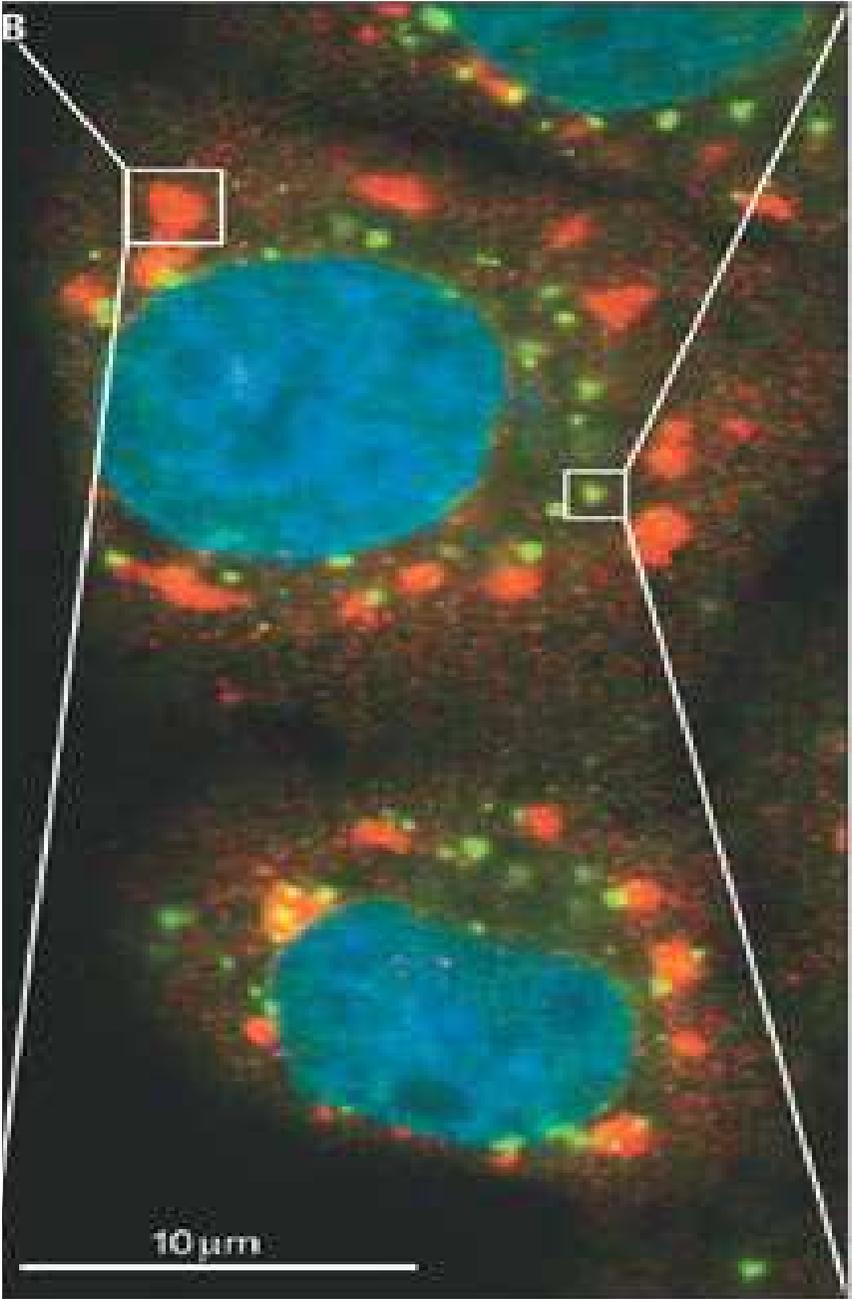
The image shows the co-localisation of the mRNA decapping protein DCP1 with the GW182 antigen, a P-body marker in multicellular organisms.

Involved in storage, repression or degradation of mRNAs

→ mRNAs in P-bodies can move back to the cytoplasmic pool



P-bodies (mRNA-processing bodies)



The road to degradation

Premature Stop Codon (PTC)-containing mRNA

1. Recognition of the stop codon as a PTC
EJCs and effector proteins (Upf and SMG family)
2. Tagging of PTC-containing mRNA
Phosphorylation of the Upf1 effector protein
3. **Degradation** and/or isolation of the tagged mRNA
 - a) **SMG6- and/or SMG5/SMG7-mediated degradation**
 - b) **Isolation and degradation in P-bodies**

