Study of the mammalian nonsense-mediated mRNA decay (NMD)

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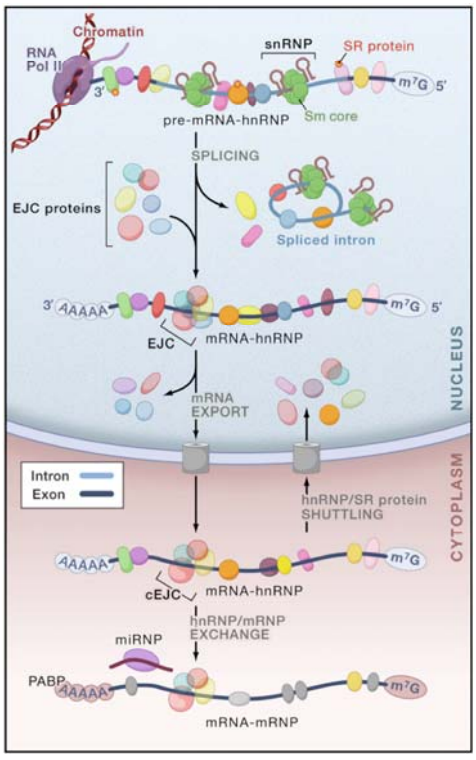
mRNA metabolism

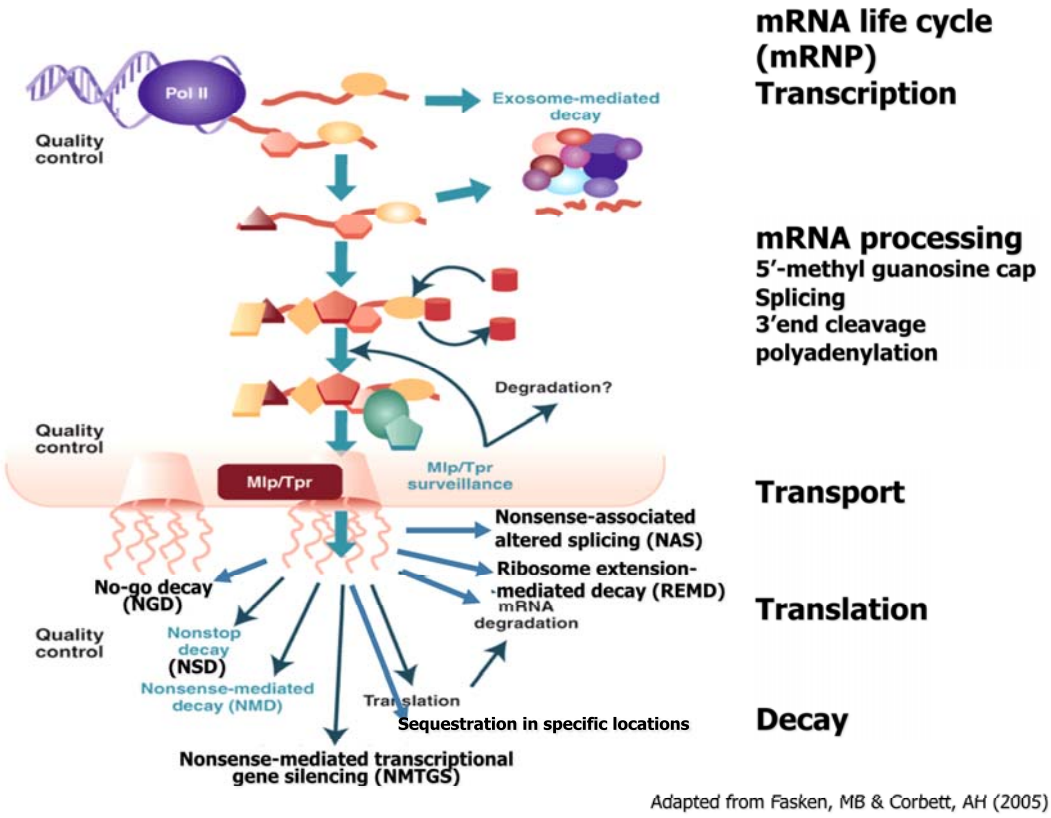
mRNA life cycle:



- **Transcription**
- **5′ Capping**
- **Splicing**
- **3′ Poly(A)**
- **Editing**
- **Export**
- **Translation**
- **Degradation**

Cooper et al (2009) Cell 136: 777

mRNA biogenesis and quality control



Adapted from Fasken, MB & Corbett, AH (2005)

When mRNAs present a premature termination codon (PTC)

PTC = nonsense or stop codon = UAA, UAG, UGA

PTCs can arise in a variety of ways:

- **Nonsense or frameshift mutations in DNA**
- **Errors in RNA splicing (including aberrant alternative splicing)**
- **Alternative translation initiation AUG codons**

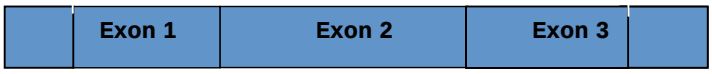
Approximately 1/3 of genetic and acquired diseases are due to PTCs

Nonsense-mediated mRNA decay (NMD)

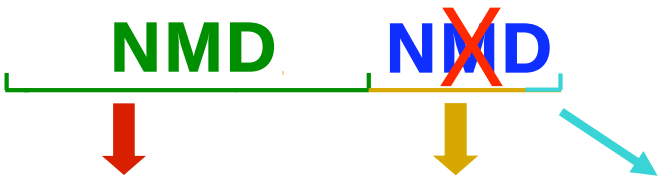
- In mammalian cells, mutations that introduce PTCs into protein coding gene regions, generally, result in decreased steady-state levels of the corresponding mRNA. NMD is a mRNA surveillance mechanism that degrades mRNAs carrying PTCs.

- NMD limits the production of truncated proteins that could have dominant-negative/gain-of-function effects.

NMD modulates disease phenotype



Human β-globin mRNA



No protein

Short protein

Effective proteolysis

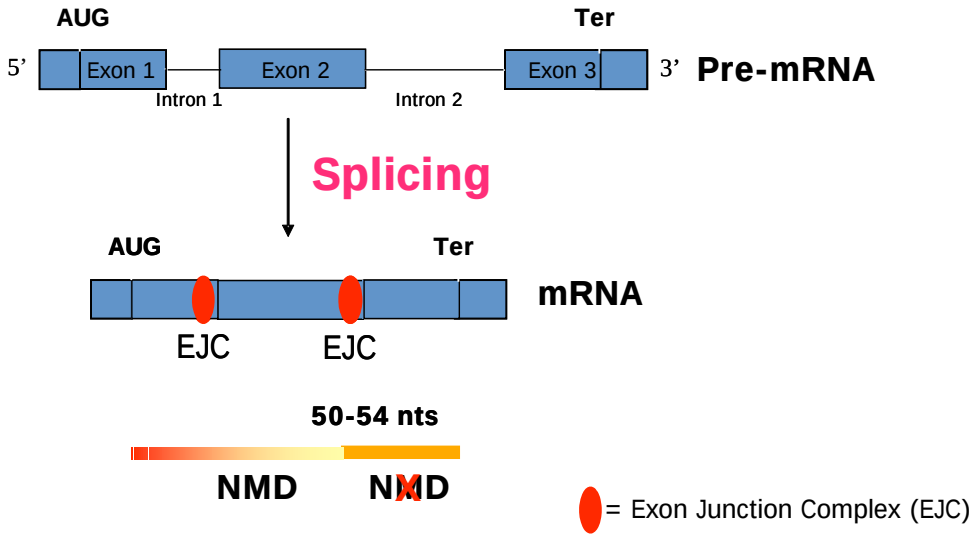
Long truncated protein

Ineffective proteolysis

Thalassemia Phenotype: Recessive (AR) Recessive (AR) Dominant (AD)

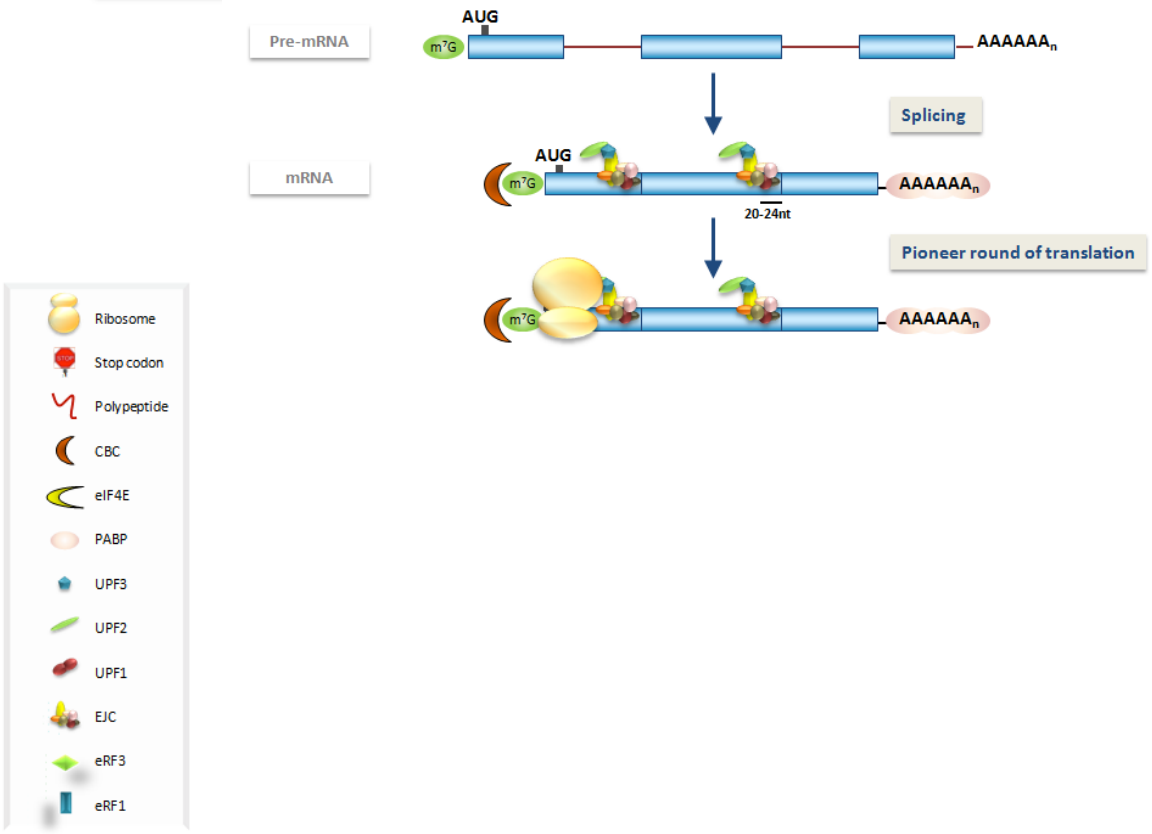
Hall, GW & Thein, S (1994)

A rule for the recognition of PTCs that induce NMD

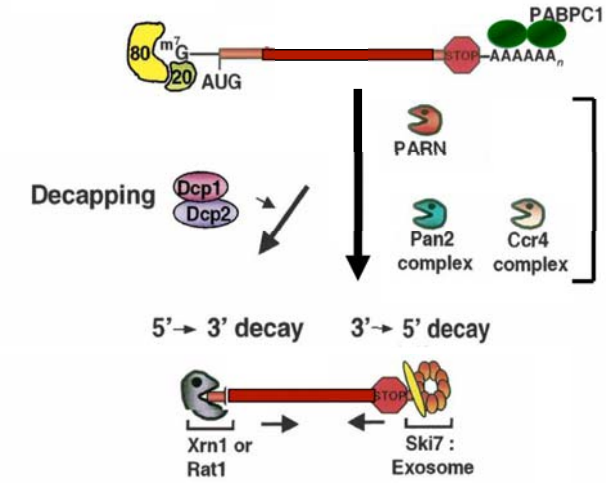


Thermann R, et al. (1998); Zhang J, et al. (1998)

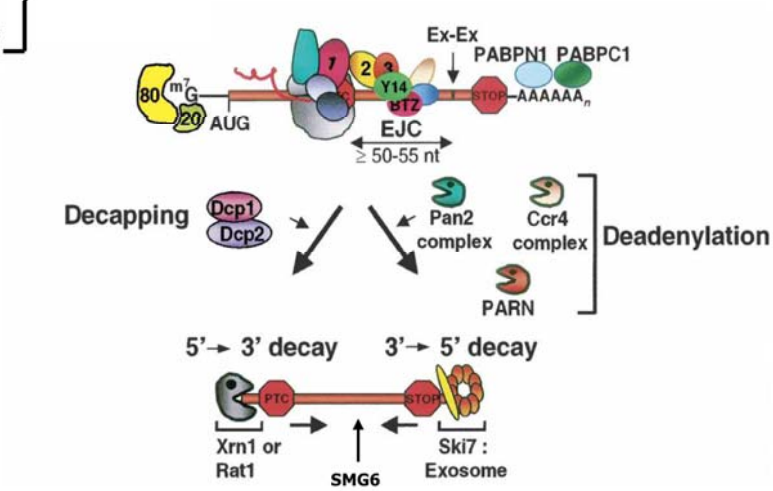
The current mammalian NMD model



Eukaryotic normal mRNA decay versus NMD degradative activities

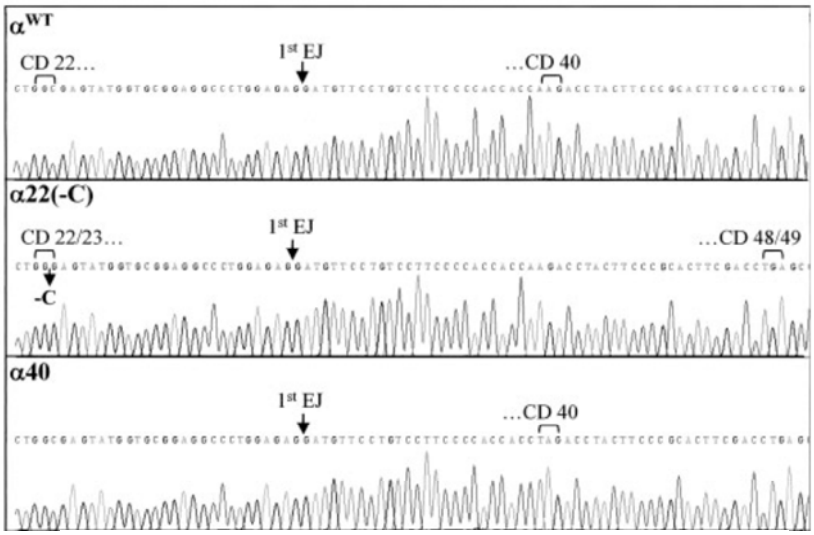


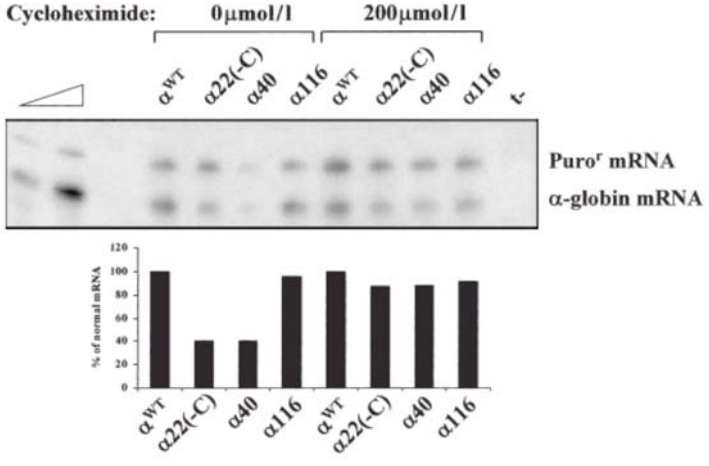
Normal mRNA decay



NMD degradative activities

Adaptado de:
Isken & Maquat (2007) Genes Dev 21: 1833

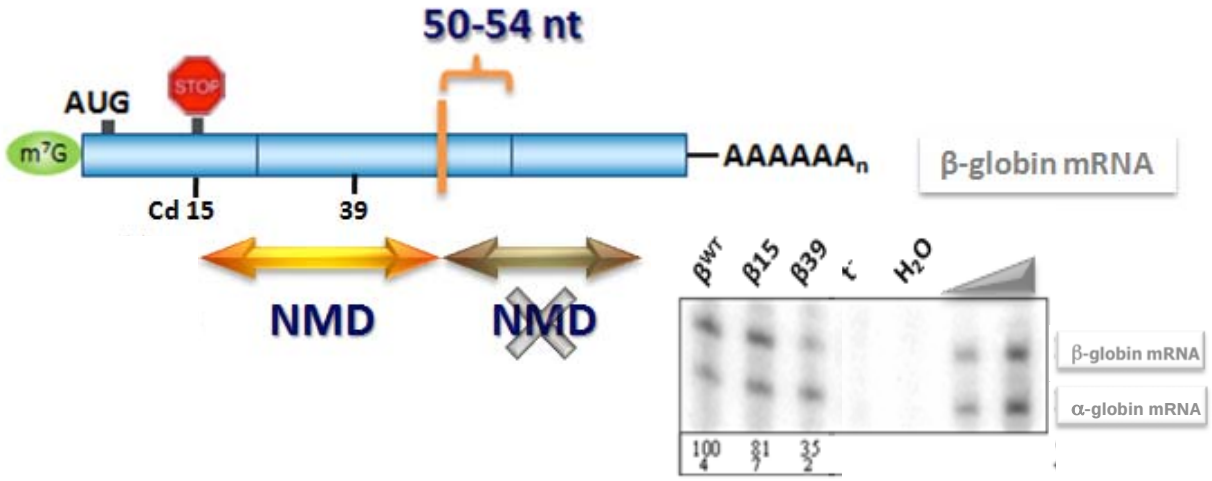




Human α2-globin nonsense-mediated mRNA decay induced by a novel α-thalassemia frameshift mutation at codon 22

FJC Pereira et al. 2006

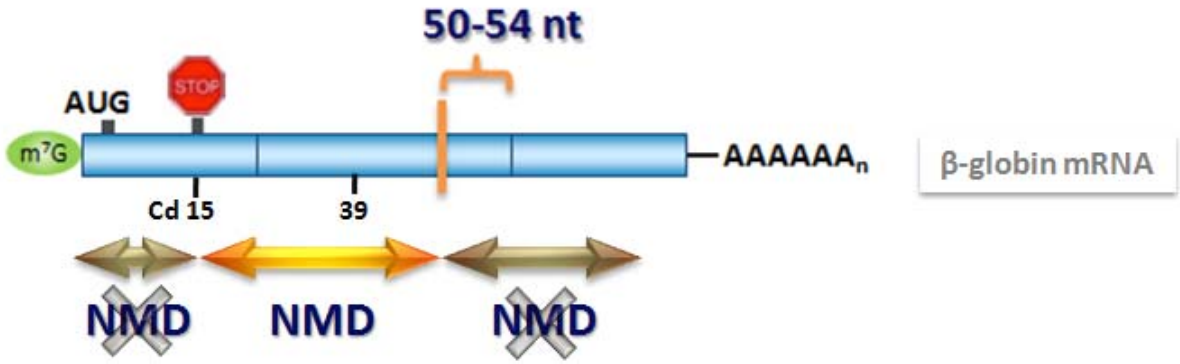
An exception to the NMD rule



Human β-globin mRNAs carrying 5'-proximal PTCs (e.g. β15) accumulate to normal levels, while transcripts carrying a nonsense mutation at codon 39 are classically degraded via the NMD pathway

Romão et al (2000) Blood 96(8):2895

An exception to the NMD rule

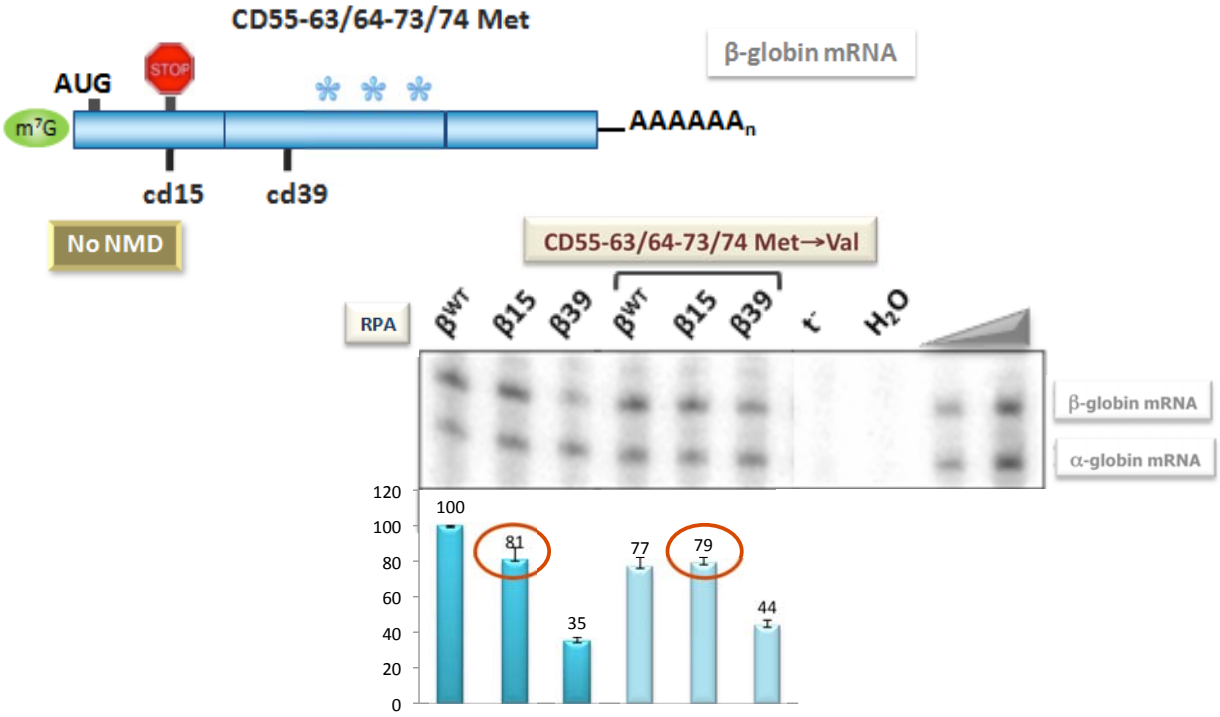


NMD-resistance:

- independent of promoter identity and tissue specificity
- is not a result of abnormal pre-mRNA splicing or impaired translation

Inácio et al (2004) J Biol Chem 279(31):32170

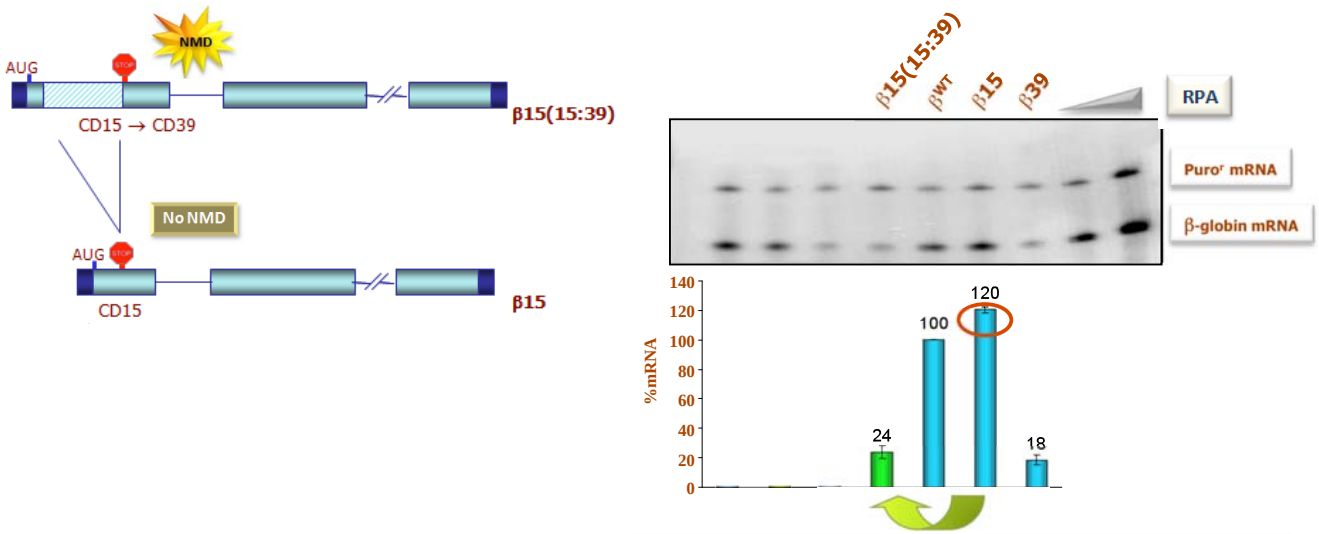
NMD-resistance does not reflect translation reinitiation



Inácio, A et al (2004)

The "AUG proximity effect"

Increasing the distance between the NMD-resistant PTC and the AUG results in mRNA destabilization

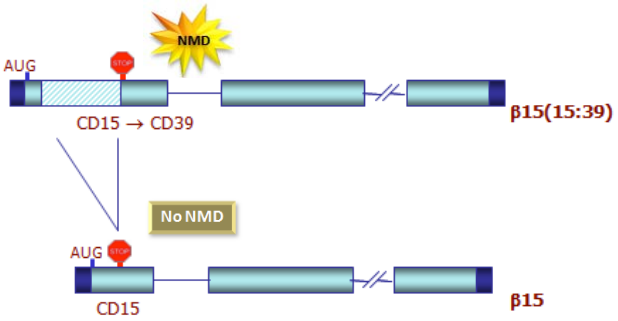


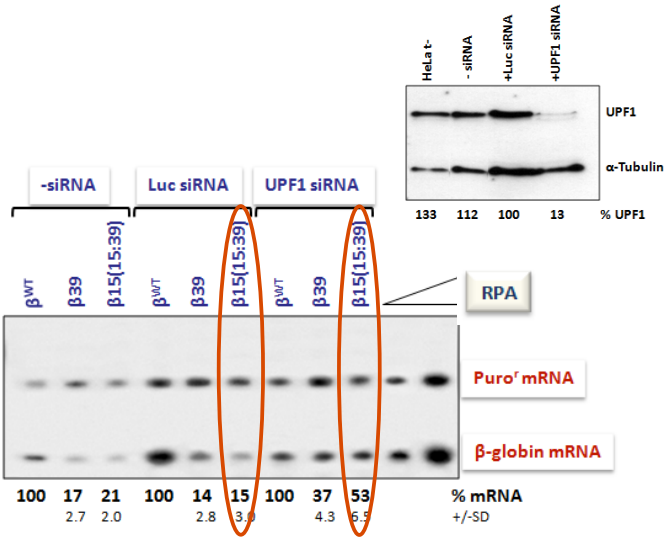
NMD-resistance:

- NMD inhibition is ruled by the proximity of the PTC to the AUG, rather than to a putative 5'UTR determinant

Silva, AL et al (2006)

The "AUG proximity effect"





NMD-resistance:

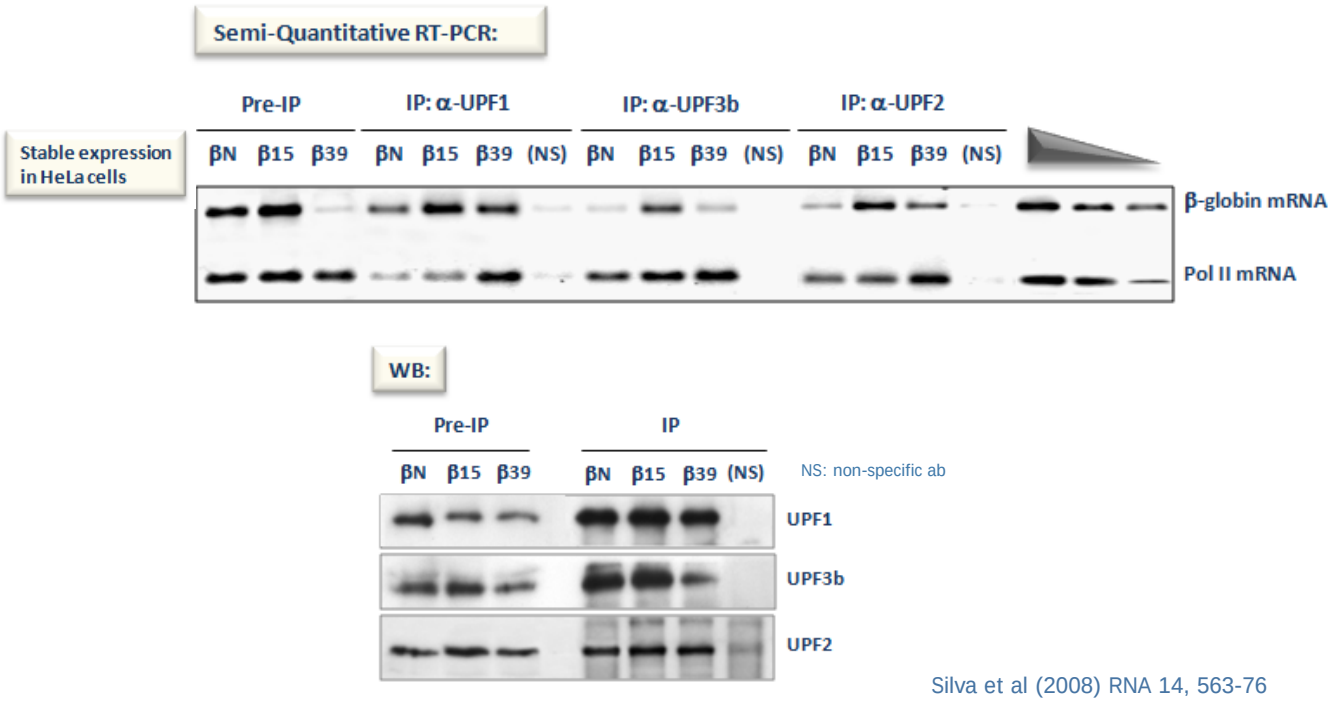
- This novel "AUG-proximity effect" is able to circumvent the full activity of the canonical UPF1-dependent NMD pathway

Silva, AL et al (2006)

What makes mRNAs bearing a small ORF escape NMD?

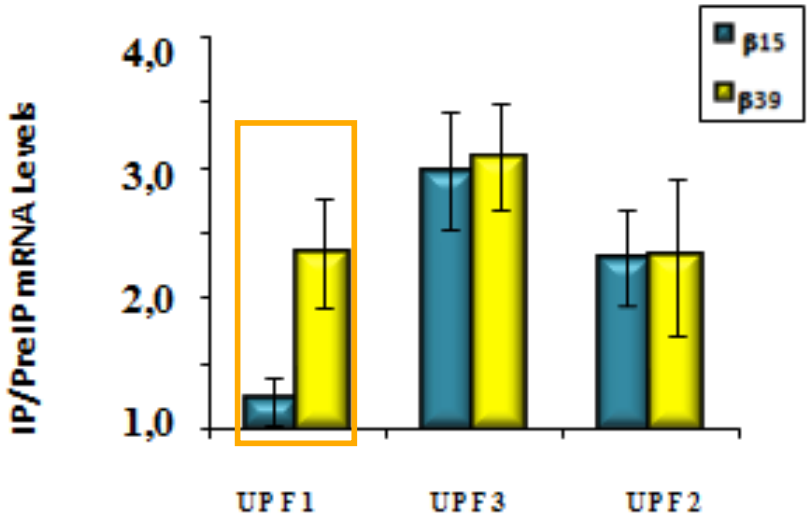
NMD-resistance could reflect an altered association with the UPF factors

Protein / mRNA co-immunoprecipitation assay



Silva et al (2008) RNA 14, 563-76

β15 ability to associate with UPF1 is impaired



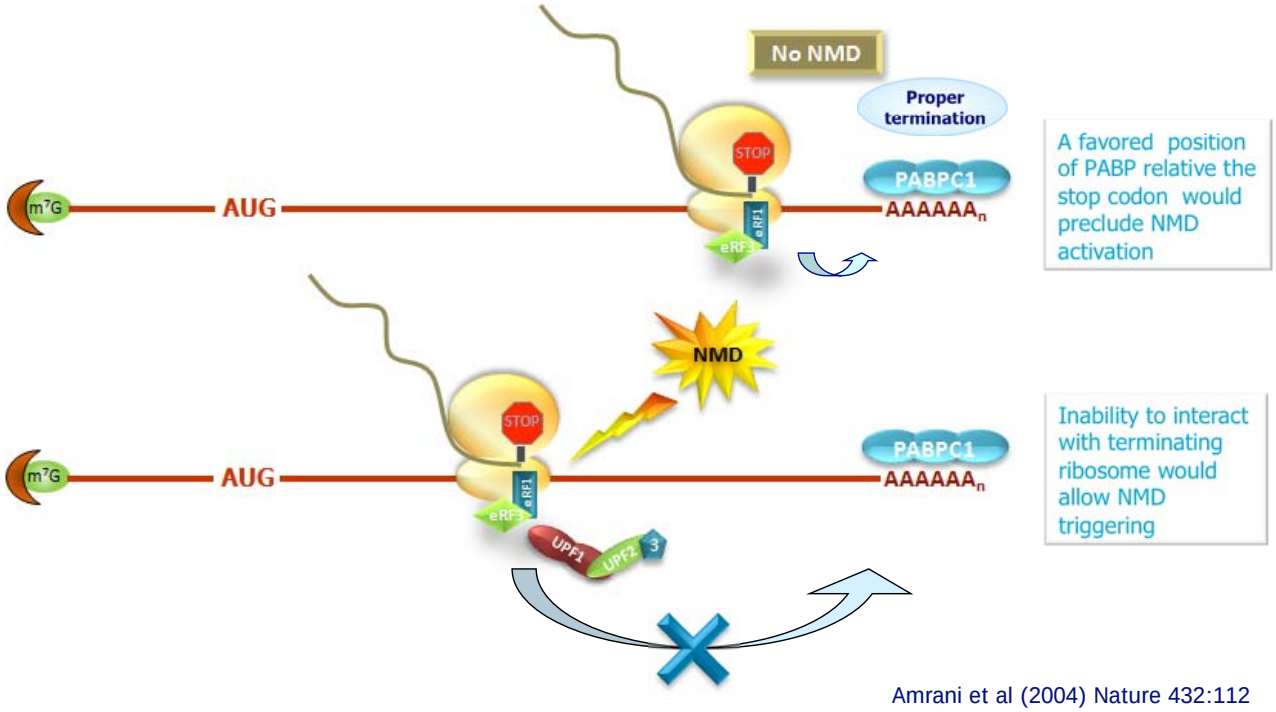
- UPF2 and UPF3b associate predominantly with β15 and β39, when compared to βN
- β39 showed an increased UPF1 enrichment whereas the association of β15 with UPF1 is similar to βN

Silva et al (2008) RNA 14, 563-76

What could be impairing UPF1 association with β15 mRNAs?

The "faux 3'UTR" NMD model in yeast

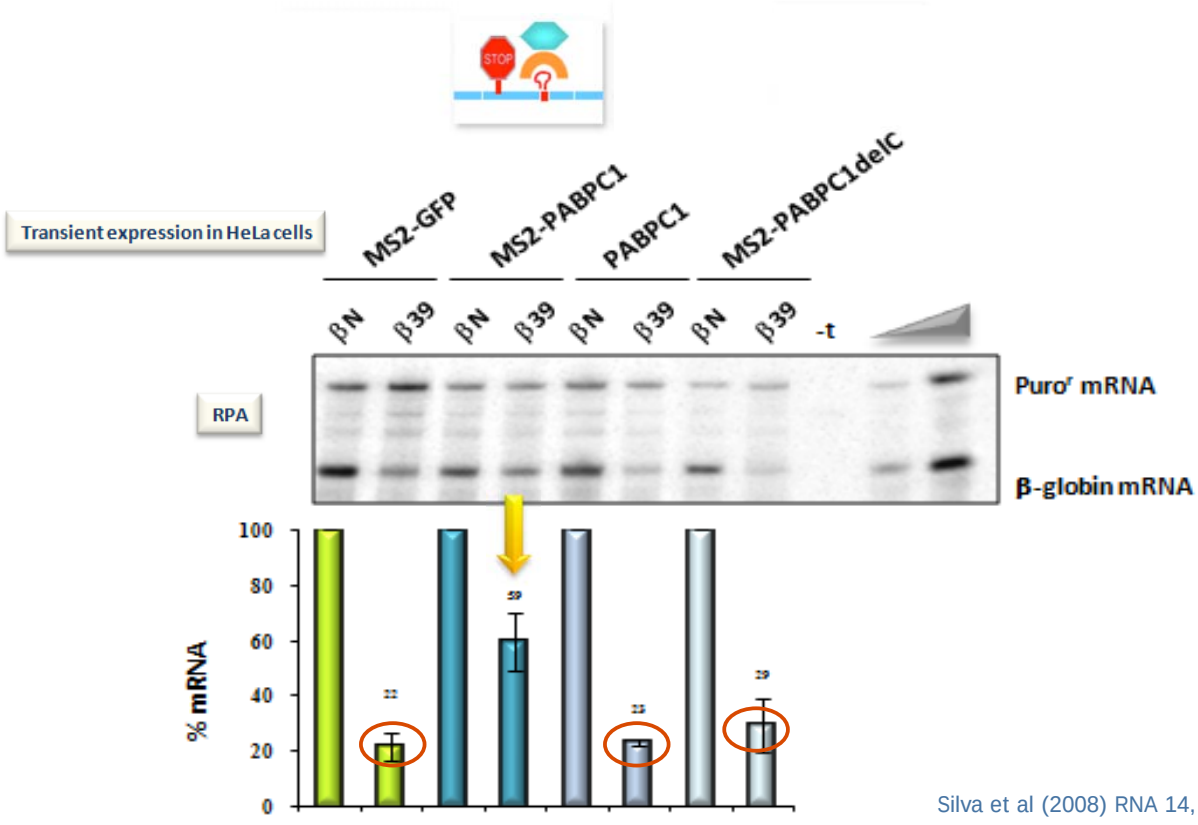
In yeast, it was shown that the proximity of the poly(A)-binding protein (PABP) to a PTC leads to NMD inhibition.



Amrani et al (2004) Nature 432:112

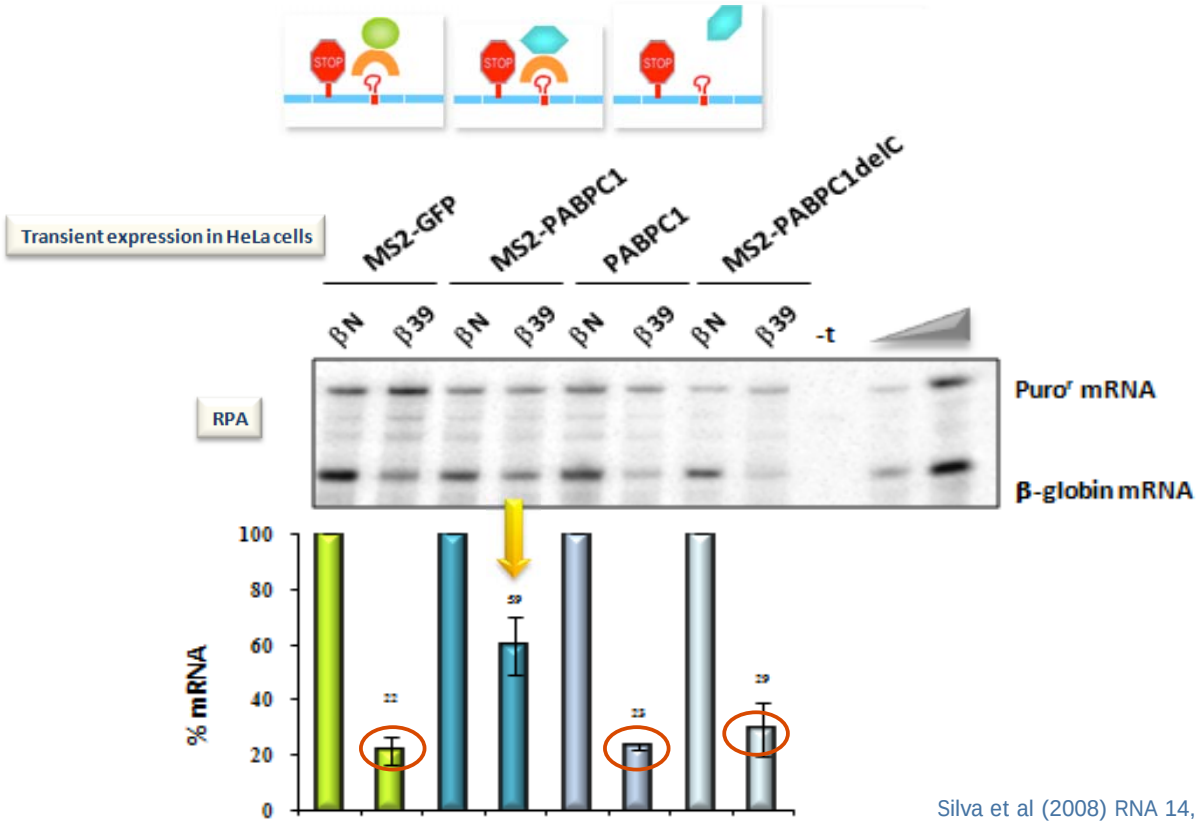
Could the proximity of PABP to a PTC lead also to NMD inhibition in mammals?

NMD-inhibitory effect of PABPC1 requires its C-terminaldomain



Silva et al (2008) RNA 14, 563-76

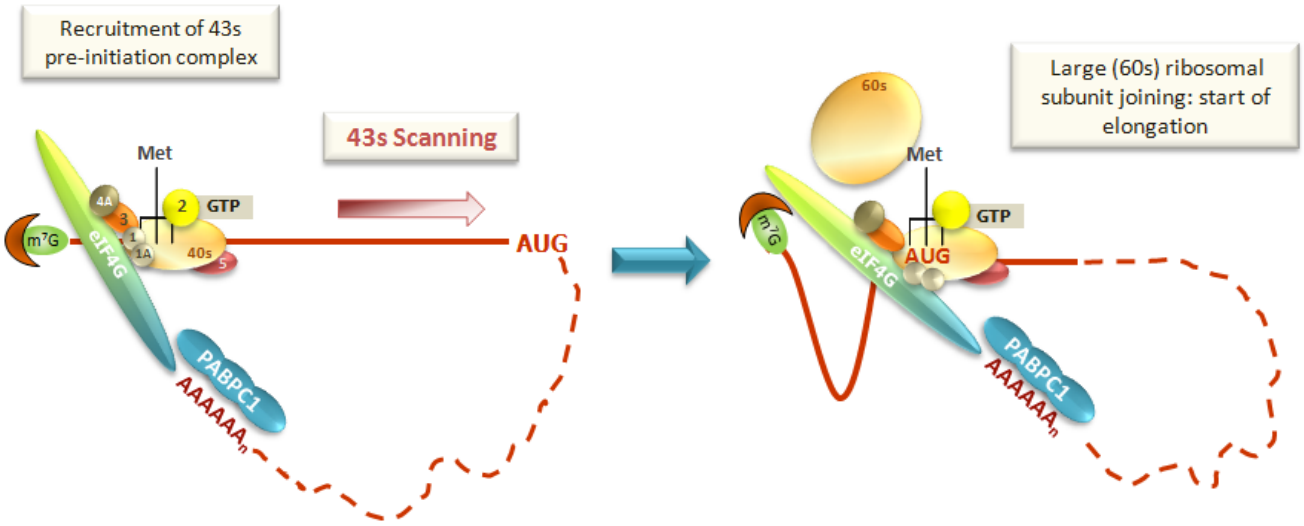
NMD-inhibitory effect of PABPC1 requires its C-terminal domain



Silva et al (2008) RNA 14, 563-76

How could the "AUG-proximity effect" be related with the proximity of PABPC1 to the PTC?

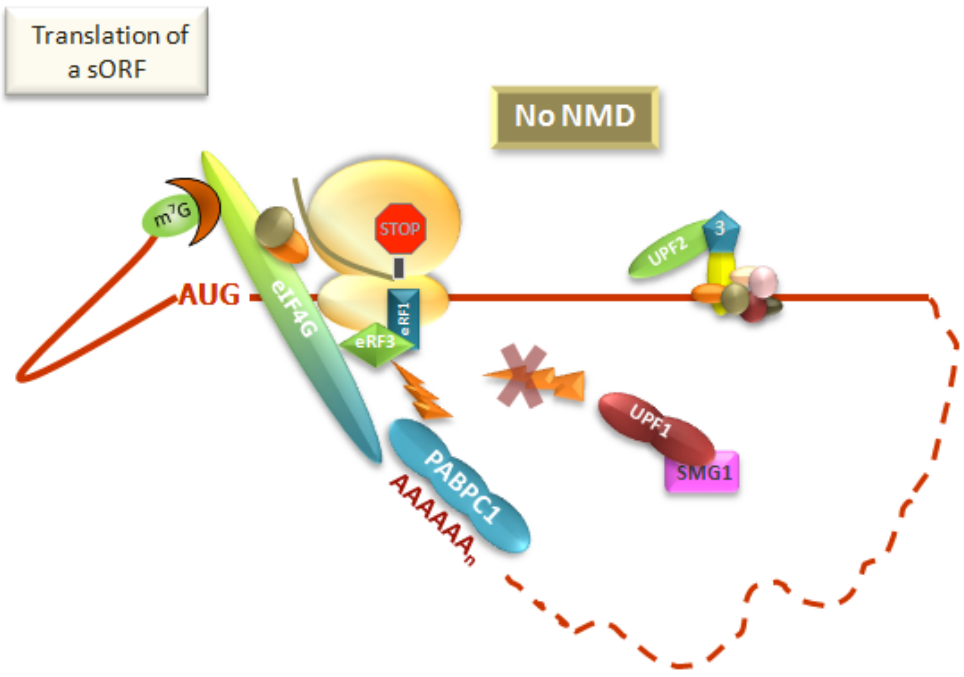
PABPC1/eIF4G associated factors could be brought into the vicinity of the AUG during ribosome scanning on cap-dependent translation



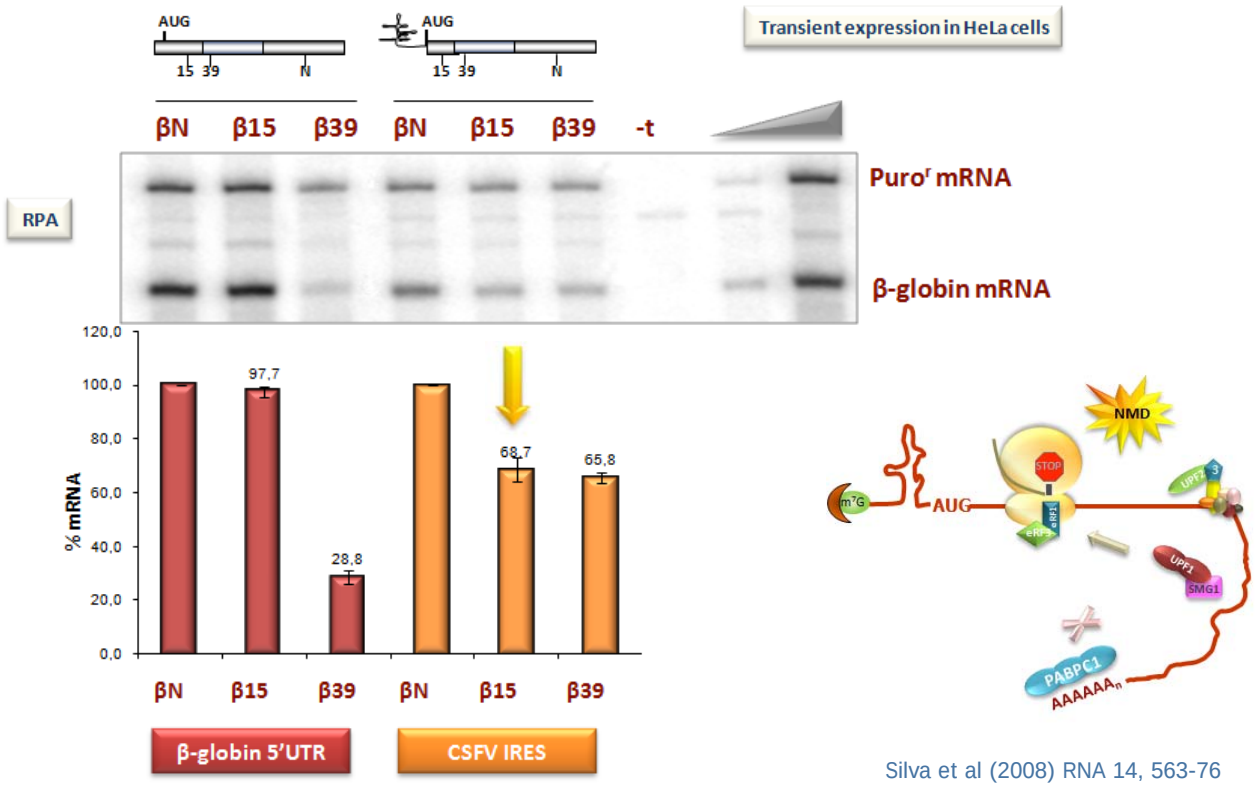
Reviewed by Jackson (2005) Biochem Soc Trans 33:1231

Working model

Inherent nature of the short ORF translation



Translation driven by the CSFV IRES converts the NMD-resistance of β15 mRNA to NMD-sensitivity



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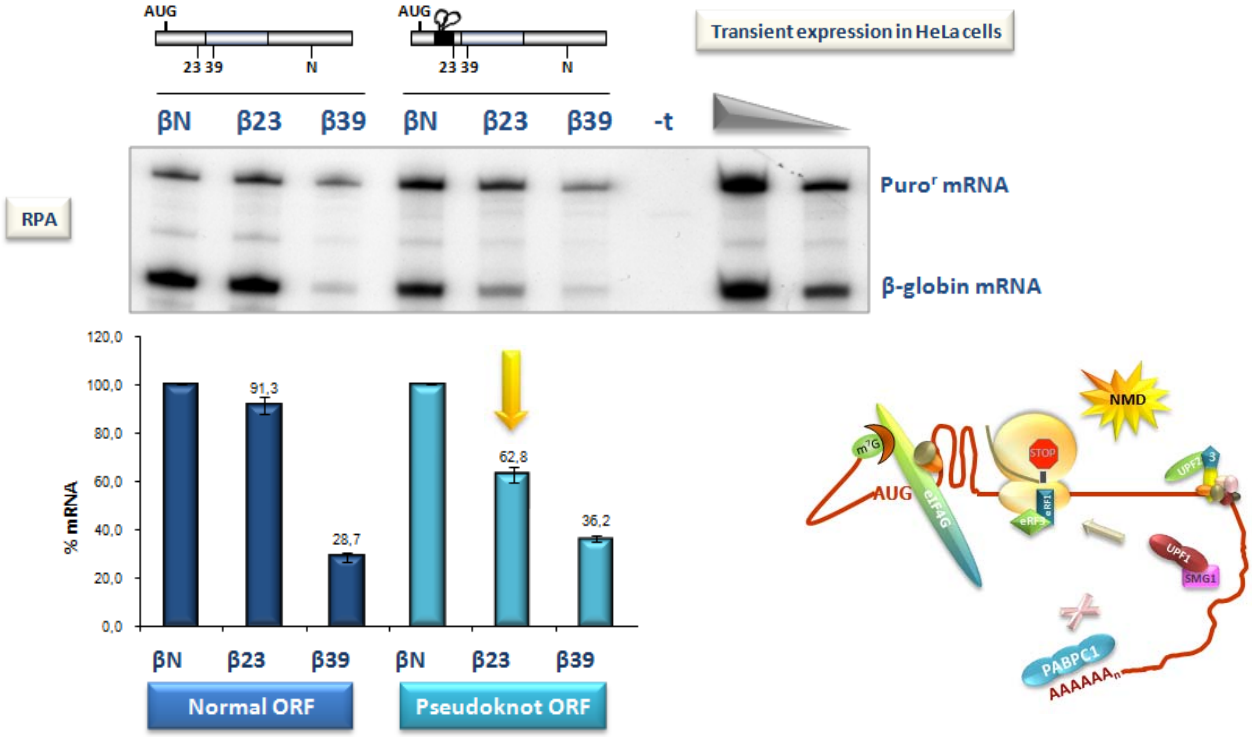
mRNA bearing a AUG-proximal PTC becomes NMD-sensitive when the ORF presents a pseudoknot structure



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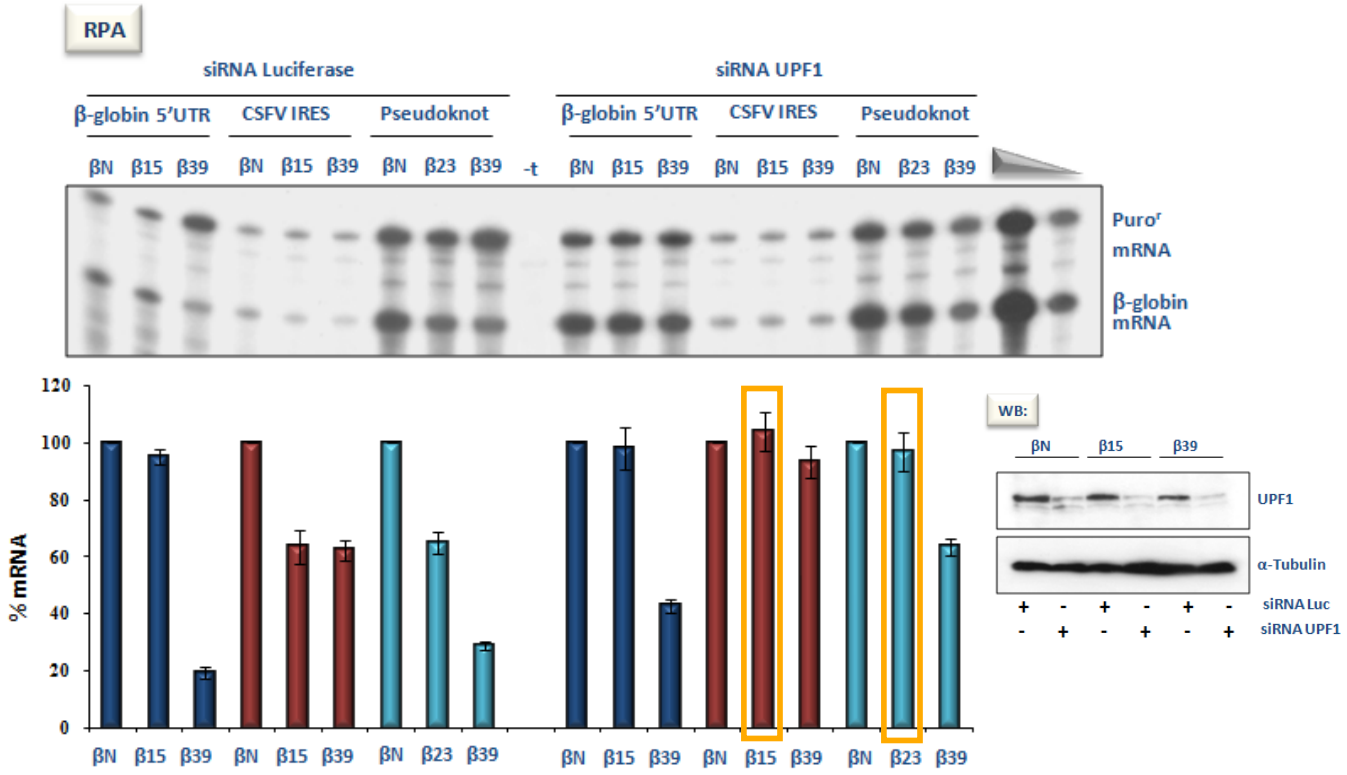
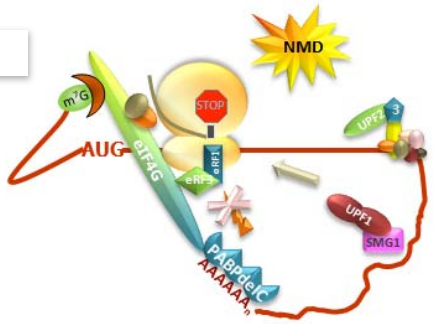
Depletion of UPF1 increases β15-CSFV IRES and β23-Pseudoknot mRNA levels



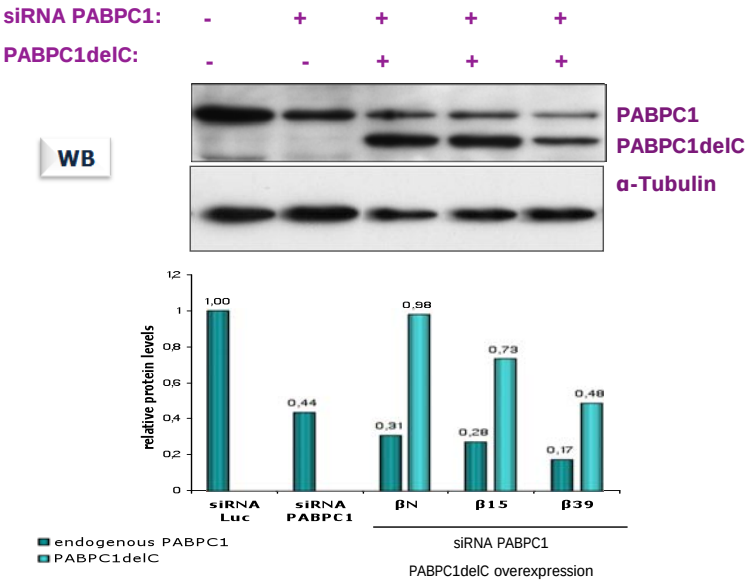
Silva et al (2008) RNA 14, 563-76

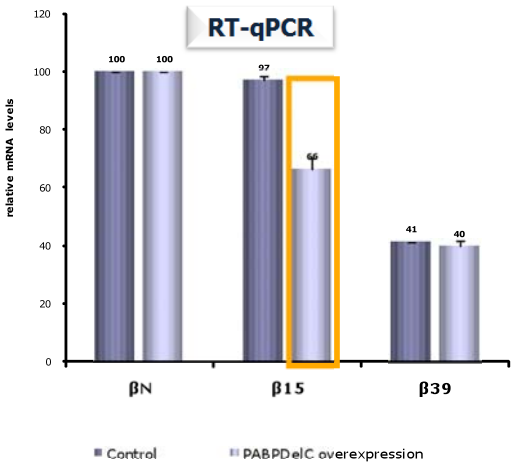
Absence of PABPC1 C-terminal domain destabilizes an AUG-proximal nonsense-mutated transcript

Endogenous PABPC1 knockdown / PABPC1delC overexpression

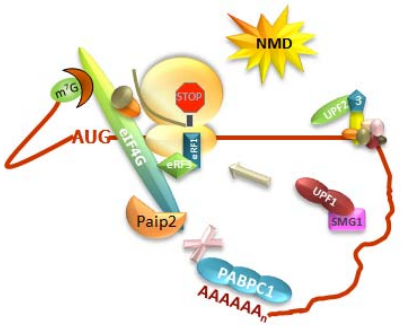


Transient expression in HeLa cells



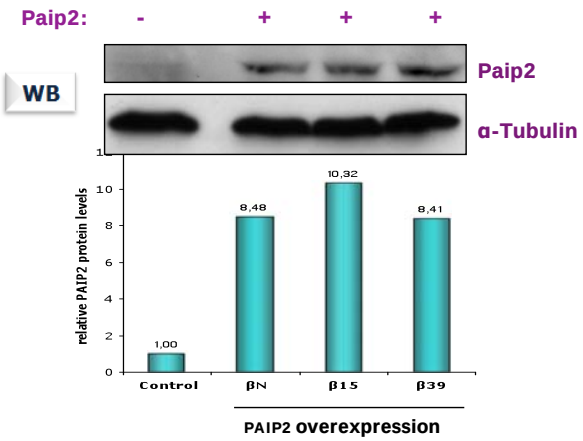


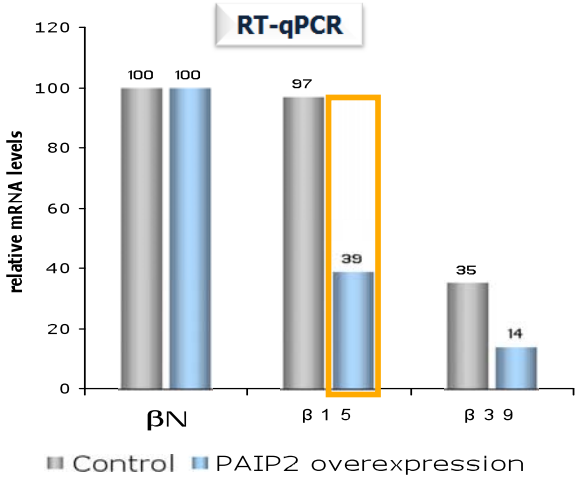
Overexpression of PAIP2 destabilizes an AUG-proximal nonsense-mutated transcript



Paip2 overexpression

Transient expression in HeLa cells





The "AUG-proximity effect" is related with the proximity of PABPC1 to the PTC!

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Financial support :
Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA)
Centro de Investigação em Genética Molecular Humana (CIGMH)
Fundação para a Ciência e a Tecnologia (FCT)