Nonsense-mediated decay-resistance of AUG-proximal nonsense-mutated transcripts: a link between translation initiation and PTC definition

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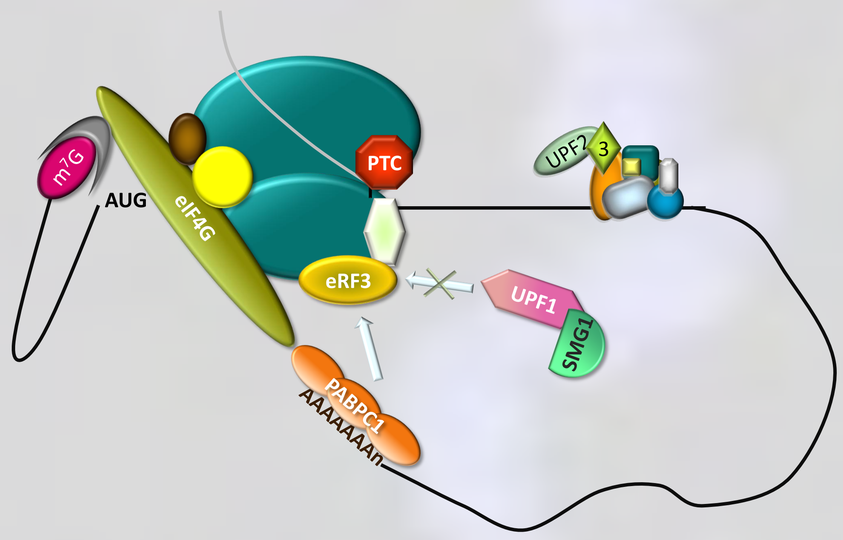
Abstract

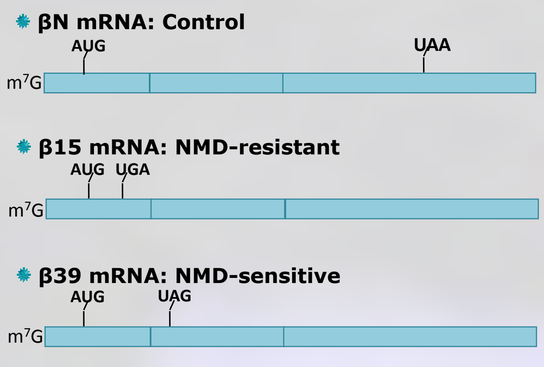
Nonsense-mediated mRNA decay (NMD) is an mRNA surveillance mechanism that rapidly eliminates mRNAs carrying premature translation-termination codons (PTCs). Although in mammals the location of a PTC more than 50 nucleotides upstream of the last exon-exon junction has been pointed as a mark for NMD, it is now know that the physical distance between the PTC and cytoplasmic poly(A)-binding protein 1 (PABPC1) is a crucial determinant ifor PTC definition [3-6]. We have reported that human b-globin mRNAs carrying 5'-proximal PTCs (e.g. b15; PTC at codon 15) can, unexpectedly, evade NMD in mammalian cells [1,2]. The observed NMD-resistance reflects the PTC proximity to the initiation AUG [2,3].

The role of PABPC1 in PTC definition as well as the indication of mRNA circukarization during translation, through 3' end-associated PABP interactions with 5' end-associated eIF4G, which results in mRNA [7], lead us to propose that the NMD-resistance of mRNAs harbouring an AUG-proximal PTC relies in the close proximity of PABPC1 to the PTC, due to the inherent nature of the short open reading frame translation process. Here, we analyse the effect of the inhibition of PABPC1/eRF3 and PABPC1/eIF4G interactions in the NMD commitment of an mRNA harbouring an AUG-proximal PTC. Our findings support a role for PABPC1 and associated translation initiation factors in NMD-resistance of AUG-proximal nonsense-mutated transcripts , providing evidence for a link between translation initiation and PTC definition.

Working Model



β-globin mRNAs



Methods

The plasmid expressing PABPC1 deletional mutant (pDEST_PABPC1delC) was obtained by PCR amplification of PABPC1 sequence excluding its C-terminal domain (T567-A652) and cloned into *Hind*III/*Age*I sites of pDEST26PABPC1 (RZPD). The plasmid expressing eRF3 deletional mutant (pcDNA3_eRF3delN) was constructed by insertion of the eRF3 sequence without the N-terminal domain (M1-S138) into *Eco*RI/*Eco*RV sites of pcDNA3 (Invitrogen).

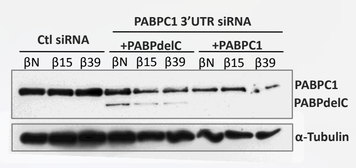
Hela cells treated with PABPC1 siRNAs were cotransfected with pDEST_PABPC1delC and one of the NMD reporter plasmids (pTREβN, pTREβ15 or pTREβ39). For eRF3 knockdown, HeLa cells treated with eRF3 siRNAs were cotransfected with pcDNA3_eRF3delN and one of the NMD reporter plasmids. For overexpression of PAIP2, HeLa cells were cotransfected with pDEST26PAIP2 (RZPD) and one of the NMD reporter plasmids. The same experiment was performed in UPF1-depleted HeLa cells. For the eIF3 subunits knockdowns, for eIF3e-, eIF3h- or eIF3f-depleted HeLa cells (by transfection with siRNAs) were transfected with the NMD reporter plasmids as above.

HeLa cell lysates were used for RNA and protein extraction. Denatured proteins were loaded on a 10% SDS-PAGE gel and subjected to immunoblot analysis for the detection of PABPC1, eRF3, PAIP2, UPF1 and tubulin proteins. Relative mRNA levels were quantified by quantitative reverse transcriptase-coupled real-time PCR (RT-qPCR), using ΔΔCt method [8]. Knockdown efficiency of eIF3e, eIF3h and eIF3f was monitored by semi-quantitative RT-PCR; the mRNA levels of each subunit were normalized to HDAC1 mRNA levels.

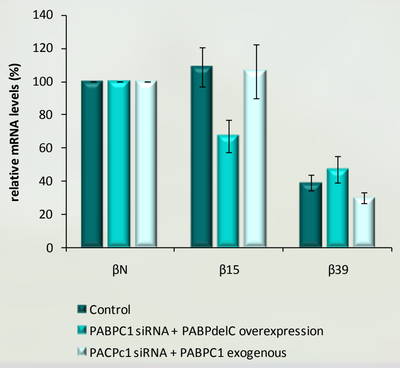
Results

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

Western Blot

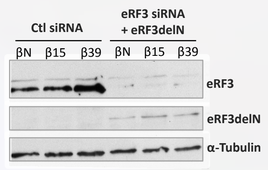


RT-qPCR

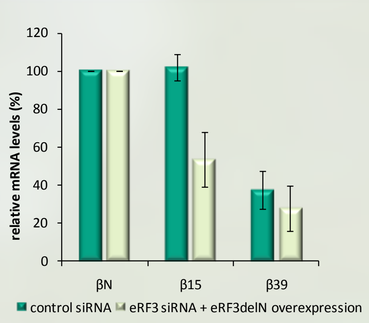


Absence of eRF3 N-terminal domain destabilizes an AUG-proximal nonsense-mutated transcript

Western Blot

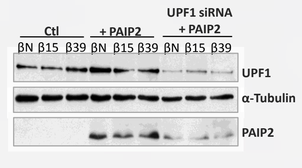


RT-qPCR

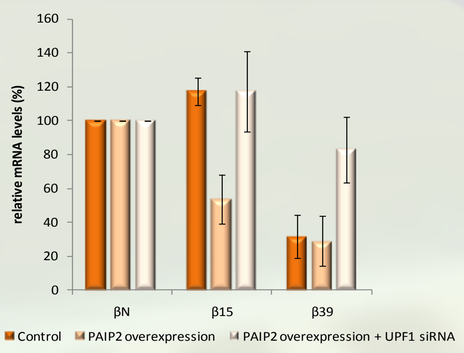


Overexpression of PAIP2 results in decreased levels of a transcript bearing an AUG-proximal PTC

Western Blot

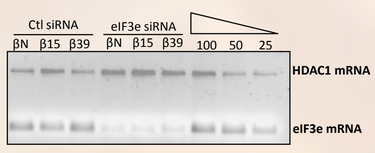


RT-qPCR

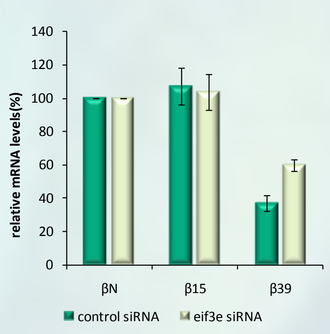


Knockdown of eIF3e subunit does not affect the levels of an AUG-proximal nonsense-mutated transcript and results in stabilization of NMD-sensitive transcritps

RT-PCR

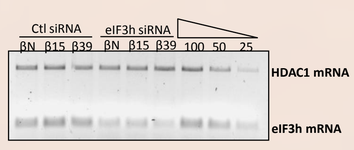


RT-qPCR

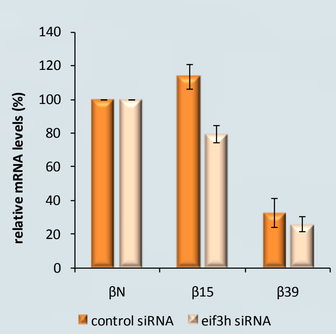


Knockdown of eIF3h subunit destabilizes an AUG-proximal nonsense-mutated transcript

RT-PCR

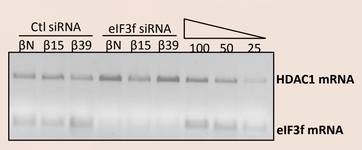


RT-qPCR

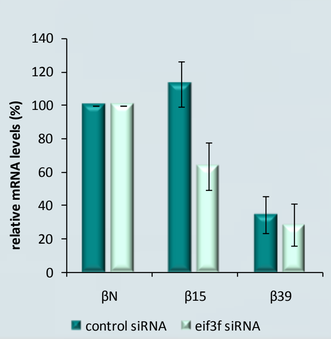


Knockdown of eIF3f subunit results in decreased levels of mRNAs bearing anAUG-proximal PTC

RT-PCR



RT-qPCR



Conclusions

- **Impairing of PABPC1-eRF3 interaction converts NMD-resistance of a transcript with an AUG-proximal PTC into NMD-sensitiveness**
- **PABPC1-eIF4G interaction appears to be required for the inhibitory effect of PABPC1 on NMD**
- **Knockdown of eIF3h and eIF3f subunits destabilizes a transcript carrying an AUG-proximal PTC**

In the context of an AUG-proximal PTC, PABPC1-eIF4G interaction during translation initiation may maintain PABPC1 in the vicinity of the AUG by the time the ribosome reaches the PTC, probably because it travels with the eIF4F/43S complex during scanning. This favoured position would allow PABPC1 to interact with the terminating ribosome via eRF3, which could impair interactions with NMD factors.

References

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