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

I. Peixoto, C. Barbosa, A.L. Silva and L. Romão
Departamento de Genética, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal

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 known that the physical distance between the PTC and cytoplasmic poly(A)-binding protein 1 (PABPC1) is a crucial determinant for PTC definition [14]. 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 [15,16]. The observed NMD-resistance reflects the PTC proximity to the initiation AUG [17,18]. The role of PABPC1 in PTC definition as well as the indication of mRNA circuarization 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, eIF3 and PABP1/eIF4F 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.

Methods

The plasmid expressing PABPC1 deletion mutant (pCIST-PABPC1delC) was obtained by PCR amplification of PABPC1 sequence including its 3’-terminal domain (T527-G1622) and cloned into HindIII/BglII sites of pDESTINO1 (RZPD). The plasmid expressing eIF3f deletion mutant (pDNA-eIF3fΔ396-461) was constructed by insertion of the eIF3f sequence without the N-terminal domain (AA1-138) into EcoRI/BstXI sites of pCDNA3 plasmid (RZPD). HeLa cells treated with pCIST/PABPC1delC and one of the NMD reporter plasmids (pTREB, pTREB15 or pTREB41) for eIF3i knockdown. HeLa cells treated with eIF3f siRNA were cotransfected with pCIST/pPABPC1delC and one of the NMD reporter plasmids. For overexpression of PAIP2, HeLa cells were cotransfected with pCMV/PAIP2 (RZPD) and one of the NMD reporter plasmids. The same experiment was performed in U2OS-depleted HeLa cells. For the eIF3f subunits knockdowns, for eIF3f, eIF3h or eIF3l-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. Derivated proteins were loaded on a 30% SDS-PAGE gel and subjected to immunoblot analysis for the detection of PABPC1, eIF3, PABP, UPF1 and UPF2 proteins. Relative mRNA levels were quantified by quantitative reverse transcriptase-coupled real-time PCR (RT-qPCR), using ΔΔCt method. Knockdown efficiency of eIF3f, eIF3h and eIF3l was monitored by semi-quantitative RT-qPCR; the mRNA levels of each subunit were normalized to H397A mRNA levels.

Results

Impairing of PABPC1-eIF3 interaction converts NMD-resistance of a transcript with an AUG-proximal PTC into NMD-sensitiveness

PABPC1-eIF4F 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-eIF4F 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 eIF4/4G complex during scanning. This favoured position would allow PABPC1 to interact with the terminating ribosome via eIF3, which could impair interactions with NMD factors.

Conclusions

References


ABSTRACT

RESULTS

WORKING MODEL

CONCLUSIONS

β-globin mRNAs

β39 mRNA: NMD-sensitive

β15 mRNA: Control

βN mRNA: Control

βN mRNA: NMD-resistant

β15 mRNA: NMD-resistant

β39 mRNA: Control

METHODS

RESULTS

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

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

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

Knockdown of eIF3e subunit results in decreased levels of transcripts bearing an AUG-proximal PTC

Knockdown of eIF3e subunit destabilizes an AUG-proximal nonsense-mutated transcript

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

Conclusions

In the context of an AUG-proximal PTC, PABPC1-eIF4F 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 eIF4/4G complex during scanning. This favoured position would allow PABPC1 to interact with the terminating ribosome via eIF3, which could impair interactions with NMD factors.