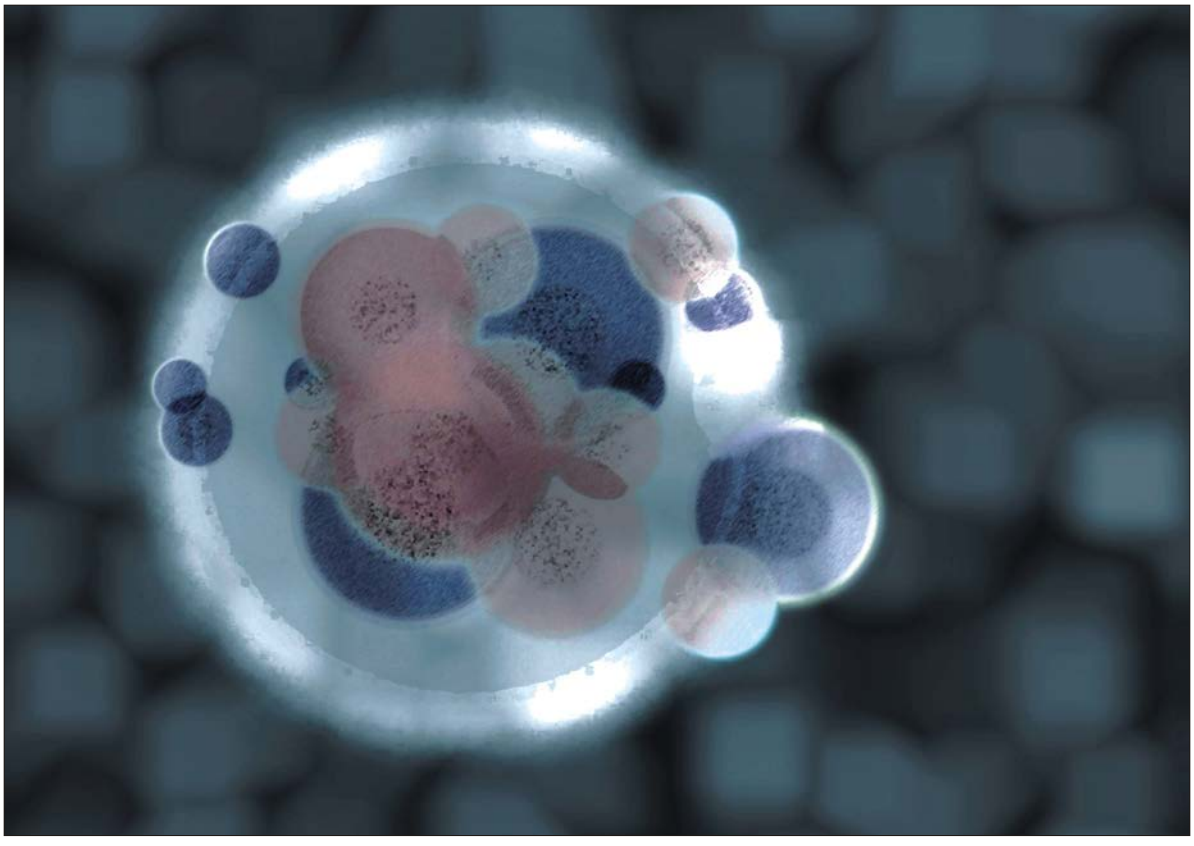

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Mechanisms of the Human β-globin mRNA Stability

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Abstract: Posttranscriptional controls play important roles in the determination of gene expression. A major component of the regulation of gene expression is exerted at the level of mRNA stability. mRNAs can differ dramatically in their intrinsic stabilities. Eukaryotic mRNAs have a considerable range of half-lives, from as short as few minutes to as long as several days. In parallel, specific mRNA decay pathways may also occur to control the quality of mRNA prior to translation. Nonsense-mediated mRNA decay (NMD) is an example of a posttranscriptional mechanism that is used by cells to survey mRNA quality. By degrading abnormal transcripts that prematurely terminate translation, NMD prevents the production of truncated proteins that could have a dominant-negative effect for the cell. Thus, the stability of individual mRNAs reflects the interaction of general determinants with mRNA-specific sequence elements and *trans*-acting proteins that function to dictate mRNA turnover. In this review, we present the major current conceived mechanisms that specify the stability of the normal human β-globin mRNA, as well as the surveillance mechanism of nonsense-mediated mRNA decay, emphasizing aspects specific for this transcript.

Key words: Human β-globin mRNA, mRNA stability, nonsense-mediated mRNA decay

INTRODUCTION

A major component of the regulation of gene expression is exerted at the level of mRNA stability. Ribonucleoprotein (RNP) complexes important to stability control can be considered in two categories, those common to all mRNAs and those that are mRNA specific and define individual decay rates. Two structures shared by almost all eukaryotic mRNAs are a 5' m^7Gppp cap and 3' poly(A) tail. A closed loop structure shared by almost all eukaryotic mRNAs that link the 5' cap structure to the 3' poly(A) tail through the interaction of the cap binding complex with the Poly(A) Binding Protein (PABP) contributes to mRNA stability as it protects the termini from exonucleases activity[1,2]. In addition to these general determinants, a number of *cis*-acting elements and trans-acting factors appear to mediate the stabilities of specific mRNAs. Intrinsic stabilities of mRNA can differ by over a 100-fold, from several minutes to several days[1]. Short-lived mRNAs usually encode proteins that must undergo dramatic changes in expression levels within a brief time frame. Such proteins include cell cycle control factors, proto-oncogenies and cytokines[1,3]. In contrast, mRNAs that encode highly abundant functional protein products tend to be highly stable. Examples of such highly stable mRNAs include crystallins[4], collagens[5] and globins[6,7].

Globin mRNAs are unusually stable. The importance of mRNA globins stability in red blood cell development and function is related to the fact that the final reticulocyte stage of erythroid development lacks the nucleus. However, translation needs to continue for up to 3 days after the cessation of transcription sustaining the synthesis of the globin proteins.

Globin mRNAs half-live determinations have been carried out by *in vivo* (whole animal) studies in anaemic mice and in cell culture studies using Mouse Erythroleukemia (MEL) cells[7-10]. Globin mRNA half-lives determined in most of these studies are in range of 17-24 h. In one particularly careful determination, where artificial lysis of mature cultured cells was avoided by using high osmotic 'stabilizing' media[11], the globin mRNA half-life was found to be greater than 60 h. Direct measurements of globin mRNA in erythroid cells from primary bone marrow preparations confirmed its high-level stability[7].

Mechanism of stabilization of the normal β-globin mRNA: Human α and β-globin genes descend from a common ancestral, share general and specific structural

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features and both encoded mRNAs are extraordinary stable in erythroid cells, which allow α and β-globin proteins to be co-expressed to substantial levels in these cells. Although, the mechanisms involved in regulation of α-globin mRNA stability have been characterized in detail, neither the *cis*-elements nor the *trans*-acting factors that specify the high stability of β-globin mRNA are well known. Different studies have suggested a half-life for the β-globin mRNA in the range of 10 to 20 h[6,7,9,12-15].

Although several naturally occurring mutations are known to affect globin gene expression, few give information about the mechanism underlying the high mRNA stability. An example studied in detail is the α Constant Spring (α^{CS}) mutation. First described in 1971 by Clegg *et al.*[16], α^{CS} mutation consists in a single nucleotide substitution (UAA→CAA) at the major fetal/adult α2-globin mRNA translation termination codon. The α^{CS} mutation allows the ribosome to translate for an additional 31 codons into the 3'UTR, originating the hemoglobin Constant Spring. Since heterozygotes for the mutation express the C-terminally extended globin protein at only 3% of the wild type α-globin level, the α^{CS} mRNA is transcribed at normal rates and the α^{CS} protein is stable[16], the loss of expression reflects a defect in the accumulation of cytoplasmic α^{CS} mRNA[17,18]. Studies in the human α-globin mRNA showed that the lost of stability was contributed by the interference of a determinant within the 3'UTR. This determinant comprises three non-contiguous C-rich sites (Pyrimidine-rich Element; PRE) and binds a 39 kDa RNA binding protein[19-21]. The binding protein, the α-globin Poly(C)-binding protein (αCP), contains 3 KH domain RNA-binding motifs[22]. Three mechanisms have been shown to contribute for the mRNA stability via αCP. First, this α-complex appears to inhibit Poly(A) shortening[18,23]; the protection may be mediated via a direct interaction of the αCP protein with the Poly(A) Binding Protein (PABP)[23]. In addition, the binding of αCP protects mRNA from an erythroid endoribonuclease attack[23]; indeed, the dependence on the α-complex for stabilization of the α-globin mRNA in erythroid cells may reflect cell type-specific mRNA decay pathways[21]. An additional mechanism by which the α-complex can confer stability on an RNA in vitro was also identified and shown to involve inhibition of 3' to 5' exonucleolytic degradation[24].

A frameshift mutation at codon 146 of the human β-globin gene that consists in an insertion of the dinucleotide CA (CAC →CACAC) which abolishes the normal stop codon at position 147, was also described[25]. This insertion results in the elongation of the β-chain by 11 aminoacid residues at the end of the β-chains generating hemoglobin Tak[25]. This hemoglobin has a high oxygen affinity[26,27] that causes secondary polycythemia. Most patients with this condition have asymptomatic erythrocytosis, but a case of homozygosity for the Tak mutation was already reported with plethora, hypoxemia and aggravated respiratory distress[28]. These data indicate that, unlike the α^{CS} mRNA, the Tak mutation in the β-globin mRNA, which permits ribosomes to read 11 codons into the 3'UTR before they encounter an in-frame termination codon, have little impact on the overall β-globin mRNA stability, suggesting that crucial stability elements are located downstream of this region. It has also been suggested that α and β-globin mRNAs might be stabilized through potentially divergent mechanisms.

β-Globin mRNA stability elements: Some preliminary results pointed out for a role of the β-globin 3'UTR in the mRNA stability mechanism; indeed, the replacement of the 3' end of the c-fos mRNA (an unstable mRNA) with the 3' end of the β-globin mRNA increases the half-life of the resultant hybrid mRNA and the reciprocal hybrid transcript shows a decreased half-life relatively to the normal β-globin mRNA[10]. Thus, after PRE was first described in the α-globin mRNA[29], the 3' UTR of the β-globin mRNA was also screened for the presence of a stability element comparable to the α-3'UTR. Here, the destabilizing effect of ribosomal entry into the β-globin mRNA was assessed by introducing two tandem antitermination mutations into the wild-type human β-globin gene that permit ribosomes to translate 37 codons into the 3'UTR of the encoded β-globin mRNA[30]. The mutant β-globin mRNA was destabilized in cultured erythroid cells, indicating that, as in human α-globin mRNA, an unperturbed 3'UTR is crucial to maintaining mRNA stability and that both α and β elements are sensitive to disruption by readthrough ribosomes[30]. However, the extent of readthrough necessary for mRNA destabilization differs for both mRNAs. While the α-globin mRNA is destabilized by ribosomes that readthrough for 4 codons into the 3'UTR, destabilization of the β-globin mRNA needs translation for more than 10 codons[30,31]. Also, the fact that β-globin mRNA stability is destroyed only when two mutations separated by 35 nucleotides are located in *cis* suggests that β-globin mRNA stability motif might be composed of two independent and redundant elements. Consistent with these data is the evidence that the naturally occurring C→G mutation located at 6 nucleotides downstream of the normal termination codon fails to destabilize the β-globin mRNA[32]. In fact, data suggest that the α and β-globin mRNA stability elements are structurally and functionally distinct[30,33].

A mRNP complex that seems to mediate the high stability of human β-globin mRNA: With the aim to characterize *trans*-acting factors involved in the mechanism underlying the high stability of the human β-globin mRNA, Yu and Russell[34] described an mRNP complex that assembles on the 3' UTR of the β-globin mRNA and exhibits some of the properties of the α-complex. The β-globin mRNP complex was shown to contain one or more factors homologous to the αCP. Sequence analysis implicated a specific 14-nucleotide pyrimidine-rich track within the β-globin 3' UTR at 34 nucleotides downstream the normal UAA codon, as the site of the mRNP assembly[34]. According with these data, is the fact that a naturally occurring 13-nucleotides deletion within the β-globin 3' UTR, at 90 nucleotides downstream the native termination codon, does not alter mRNA stability[35]. The existence of a β-globin mRNP complex that is involved in the mechanism by which mRNA is highly stable had been previously suggested. In fact, an independent study had shown that a HeLa whole-cell extract contains a factor that protects β-globin mRNA from attack by RNases in a MEL cell cytoplasmic extract[36]. Indeed, it is easily acceptable that this factor may be a constituent of the β-globin mRNA complex αCP-homologous. Although, this data may suggest that the stabilities of the α and β-globin mRNAs could be coregulated through a related mechanism[34], the fact that the β-globin 3'UTR does not support the assembly of an α-complex[33] possibly indicates that α and β-globin mRNAs achieve high stabilities through potentially different mechanisms.

Mechanism of nonsense-mediated mRNA decay: Contrary to what occurs in the presence of antitermination mutations, which originate elongated protein chains (see above), an mRNA carrying a Premature Translation Termination Codon (PTC) codifies for a C-terminal truncated polypeptide. Such polypeptides can often act in a dominant negative manner, leading to deleterious effects on the cell or organism. However, PTCs usually direct the affected mRNAs to rapid degradation, a surveillance process termed Nonsense-mediated mRNA Decay (NMD)[37]. The physiological importance of NMD is the reduction in the synthesis of C-terminally truncated proteins, thus avoiding dominant-negative effects of non-functional polypeptides. The discovery of the NMD pathway has provided an explanation for the long-lasting observation that the cells degrade PTC-containing mRNAs. In fact, NMD was first discovered in 1979, when the absence of β-globin expression was found to be due to the presence of nonsense codons within the coding sequence[38]. PTCs can be generated by naturally occurring frameshift and nonsense mutations, splicing errors, leaky 40S scanning or utilization of minor AUG initiation sites[39,40]. The deleterious effects that can be caused by the presence of C-terminally truncated proteins and the benefits that can be achieved by the elimination of the mRNAs that encode these proteins, are clearly illustrated by the example of β-thalassemia. Indeed, if a PTC is located at a position that activates NMD, the disease results in a recessive mode of inheritance with asymptomatic heterozygous carriers. In contrast, if the PTC is located at a position that do not activate NMD, the accumulation of truncated translation products can often act in a dominant negative manner, leading to deleterious effects on the cell. This condition is related with a symptomatic form of the disease in heterozygotes called "thalassemia intermedia" and a dominant mode of inheritance[41]. Most of these thalassemias are associated with mutations in the third exon of the β-globin gene. The β-globin mRNA has been widely used as a model system for the study of the NMD mechanism and has been of great value for the establishment of the current NMD models in mammalian systems.

While in most situations, the fate of β-globin nonsense transcripts is ruled by mechanisms that are common to other nonsense containing mRNAs, unusual NMD behaviours have also been described.

General overview of the mammalian NMD mechanism: Immediately upon synthesis, nascent transcripts associate with proteins forming ribonucleoprotein particles, the protein content of which evolves throughout the lifetime of a mRNA[42]. A newly synthesised pre-mRNA, is bound at the 5' cap by the cap-binding protein CBP80-CBP20 heterodimer and at the 3' poly(A) tail, after 3' end formation, by the PABP2 protein. During pre-mRNA splicing "a mark" consisting of a protein complex called the Exon Junction Complex (EJC), is assembled 20-24 nucleotides upstream each of the exon-exon junctions on a mRNA. These marks are used to discriminate premature stop (upstream of the last mark) from natural stop codons (downstream of the last mark).

Although the precise biochemical composition of the EJC remains to be established, at least the following proteins have been identified: RNPS1, the general splicing activator; Aly/REF, a RNA export factor; SRm160, the nuclear matrix localized serine-arginine-containing protein; UAP56, the U2 snRNP auxiliary factor associated protein; TAP, the nuclear mRNA-export factor1/tyrosine-kinase-interacting protein-associated protein; eIF4AIII, the homologous to the eukaryotic translation initiation factors eIF4AI and II; Y14, a shuttling protein; magoh, the Y14

binding protein and, depending on experimental conditions, DEK-an oncoprotein[40,43-53].

The NMD pathway in mammalian cells is a splicing-dependent mechanism and recent studies have shown that some of the EJC proteins are implicated in NMD: RNPS1 couples splicing to nonsense-mediated decay[54,55]; Y14 and magoh form a tight heterodimer in vivo, which is essential for NMD and may have a role in anchoring the NMD-specific factors Upf3 and Upf2 to the mRNA[54,56-58]. Eukaryotic IF4AIII is a member of the eIF4A DEAD-box helicase family of translation initiation factors[59] that binds Y14 and magoh [60] and functions in NMD[50]. Additionally, the human Upf proteins hUpf1, hUpf2 and hUpf3a or hUpf3b, have been identified and shown to be required for NMD. The activation of NMD in the presence of a premature stop codon and a competent downstream EJC, requires a link between the factors involved in translation termination and the EJC complex. That link is provided by the Upf proteins. Data have shown that Upf3 and Upf2 join the EJC in different sub-cellular compartments: Upf3 (Upf3a and Upf3b) is loaded onto mRNAs in the nucleus during splicing via interactions with components of the EJC. In contrast, Upf2 joins the complex soon after cytoplasmic export is initiated[54,61,62]. According to current models, recognition of a nonsense codon as 'premature' involves a direct interaction of EJC-bound Upf2 with the Upf1, a RNA-dependent ATPase and 5'to 3' helicase protein, whose activation by phosphorylation is required for NMD[62-65]. An interaction between Upf1 and Upf3 may also be involved in this process[66].

The activation of the NMD pathway also requires translation of the nonsense-containing mRNA. Indeed, according to present models, translating ribosomes displace EJCs from the open reading frame (ORF) during the 'pioneer' round of translation[67]. However, if the mRNA contains a PTC located more than 50-54 nt 5' of at least one EJC, complex components positioned 3' to the termination mutation will remain on the mRNA. The retention of one or more EJCs on the mRNA triggers the NMD response by an as yet undefined mechanism but that certainly requires a mechanistic link between NMD-specific factors and translation termination at the premature stop codon.

Some factors that are involved in NMD have been suggested to also function in the termination of translation: studies in yeast revealed that Upf1 interacts with release factors eRF1 and eRF3 and appears to influence the translation termination efficiency; Upf2 and Upf3 were also shown to interact with the release factor eRF3[68]. Therefore, the termination event is considered to be critical in the process of distinguishing a premature termination codon from a normal one. The translation termination process consists of a termination codon-dependent hydrolysis of the peptidyl-tRNA bond, resulting in the release of the nascent polypeptide chain from the ribosome[69]. In eukaryotes, this process is under the control of two interacting release factors, eRF1 and eRF3. The eRF1 protein has a structure mimicking that of a tRNA molecule. It recognizes the stop codon in the A site of the ribosome and catalyzes the hydrolysis of the peptidyl-tRNA bond[70]. The eRF3 protein is a ribosome- and eRF1-dependent GTPase and stimulates eRF1 activity in a GTP-dependent manner[71].

Although in mammalian systems the interaction of hUpf1 with the translation release factors eRF1 and eRF3 lacks direct evidence, hUpf1 may be recruited to the mRNA by translation release factors and serves as a bridge between the terminating ribosome and the downstream EJC-associated hUpf2 and hUpf3. This association could form an active NMD-complex that triggers rapid decay of the mRNA[66].

The human β-globin NMD and the "50-54 nt boundary rule" : Studies supporting the "50-54 nt boundary rule" suggest that an exon-exon junction serves as the major *cis*-acting NMD-regulatory element[72-75]. Generally, NMD occurs when translation terminates more than 50-54 nt upstream of the 3′-most exon-exon junction. In this circumstance, the components of the termination complex are able to interact with junction-bound NMD-specific factors, forming the surveillance complex that elicit NMD. In contrast, an mRNA is immune to NMD if translation terminates less than 50-54 nt upstream of the 3'most exon-exon junction or downstream of the junction[76]. In this case, the translating ribosomes will be able to remove the EJCs and the associated NMD-specific factors and, therefore, disable the formation of the surveillance complex.

The human β-globin gene has two intervening non-coding sequences (introns) and three coding regions (exons) that are flanked by 5' and 3' non-coding sequences, the untranslated regions[77]. Thus, after the two introns are spliced out, two EJCs are assembled on the transcript, each one located 20-24 nt from each exon-exon junction[54,78]. A β-globin NMD-behaviour corroborating the "50-54 nt boundary rule" has been broadly described. Indeed, the first indications regarding whether a β-globin PTC elicits NMD, concerning its position relatively to the downstream intron, were obtained by Kulozik and Maquat labs[72,73]. These authors showed that β-globin nonsense mutations located in the 3' region of exon 1 (at codon 26) and within the 5' two-thirds of exon 2 (at codons 36, 60-61, 75 and 82) elicit

NMD. In contrast, mRNAs bearing PTCs towards the 3' end of exon 2 (at codons 88, 91, 95, 98, 101 and 103) and those with PTCs in exon 3 (at codons 106, 107, 114, 121 and 141) are all NMD-resistant. This illustrates a clear boundary between 48 and 66 nucleotides upstream of intron 2. Although the onset of the NMD process usually requires a minimal distance between the nonsense mutation and the final exon-exon junction, on β-globin transcripts, the requirement for a maximal distance appears to be considerably less crucial. Indeed, a β-globin mRNA with a nonsense mutation at codon 39 remains NMD competent, even when the distance between the PTC and the 3' exon-exon junction is increased from the normal 180 nt up to 654 nt[79]. This is a much longer distance than the one required for NMD in yeast or in the TPI transcripts[74,75,80]. On the other hand, an intronless human β-globin gene, carrying a nonsense mutation at codon 39, generates a mRNA that lacks the EJCs and, consequently, is immune to NMD[79]. This is supported by the fact that naturally intronless mRNAs containing PTCs are not usually degraded by NMD[81]. Furthermore, the insertion of an intron more than 50 nt downstream of the native stop codon, redefines this codon as premature and triggers this mRNA to NMD[73]. In fact, it is unusual to find an intron in the 3' untranslated region of an mRNA and when such introns do occur they are almost always positioned less than 50 nt downstream of the stop codon[76].

However, exceptions to the "50-54 nt boundary rule" have also been reported for the human β-globin mRNAs. The transcripts containing naturally occurring nonsense mutations in the 5'-region of exon 1 accumulate to levels similar to those of normal β-globin transcripts, which contradict the "50-54 nt boundary rule"[82]. Furthermore, a functional analysis of these mRNAs with 5'-proximal nonsense mutations demonstrated that their resistance to NMD does not reflect abnormal RNA splicing or translation reinitiation and is independent of promoter identity and erythroid specificity[15]. Instead, the proximity of the nonsense codon to the translation initiation AUG comprises the basis of their NMD resistance. Therefore, the AUG-proximity effect can override the "50-54 nt boundary rule" in establishing the overall efficiency of NMD[15].

In addition, other unconventional behaviour concerning the β-globin NMD was also described. The NMD was found to be triggered in hybrid mouse-human β-globin transcripts (carrying nonsense mutations at codons 21-22, 39 or 60-61) in the absence of the last intron[72] and therefore, in the absence of the downstream EJC. Thus, the existence of a fail-safe mechanism was suggested where, in the absence of intron 2, a *cis*-acting sequence located within the coding region could function in NMD; this effect was also described for TPI mRNA[74]. Nevertheless, these results are in contrast with what was described by Neu-Yilik *et al.*[79]. Here, authors have shown that splicing is an indispensable component of the β-globin NMD pathway. Additionally, a study using the IVS1 +5 G→A thalassemic β-globin gene as a model system, showed that the splicing of these pre-mRNAs at a criptic site, generates a mature transcript carrying a PTC at codon 30 (located more than 55 nt 5' of the final exon-exon junction) that is immune to NMD[83]. Although the mechanism underling this NMD-behaviour was not clarified, it was demonstrated that neither abnormalities of splicing nor translation reinitiation at downstream AUG codons is the cause for the NMD resistance of this transcript[83].

NMD exceptions have also been reported for other transcripts. For example, nonsense codons positioned close to the initiation AUG of the TPI[84] and immunoglobulin μ heavy chain[85], fail to specify NMD. BRCA1 transcripts carrying nonsense codons positioned in the second exon (in a total of 22 exons) also fail to elicit NMD[86]. It was also described for the fibrinogen A α-chain gene, that premature termination codons located in different exons are not associated with the decay of the mutant mRNAs[87]. While for the TPI, it is well established that NMD is circumvented by re-initiation of translation downstream to the nonsense codon[84], which allows the ribosome to disrupt the downstream EJCs, for other exceptions, additional determinants may be involved in establishing the net stability of nonsense-containing mRNAs. Finally, a boundary-independent nonsense-mediated decay was reported in a T-cell receptor gene[88]. Indeed, rather than a definitive boundary position, nonsense codons have a polar effect, such that nonsense codons distant from the terminal downstream intron trigger a robust NMD while proximal nonsense codons induce a modest NMD[88]. Furthermore, this transcript is susceptible to NMD even when the distance between a PTC and the last exon-exon junction is less than 50-54 nt[89]. This boundary-independent polar effect was attributed to the presence of a novel NMD-regulatory sequence element that acts upstream of the nonsense codon[88].

Cellular localization of the β-globin NMD: While in yeast the NMD appears to be a cytoplasmic event, the site of the corresponding decay process in mammalian cells is less clear. Whether the PTC recognition and NMD are nuclear, nucleus-associated or cytoplasmic events remains a controversial issue. The finding that NMD, a translation-dependent mechanism regardless of its cellular

site[40], can occur in the nuclear fraction of mammalian cells[72,73,89-92], raised the intriguing question of how a nuclear event could be associated with a process that is predominantly cytoplasmic. A possible explanation would imply that the NMD process in the nucleus is directly triggered by a nuclear translation event. The possibility of a nuclear translation-dependent recognition of nonsense codons leading to NMD is supported by some studies[89,93-98]. Indeed, the hypothesis that the NMD could be restricted to the nucleus was highlighted by the observation that the inhibition of mRNA export to the cytoplasm did not affect the NMD in the nuclear fraction[99]. However, there is also evidence arguing against this hypothesis[40,73,100].

Indeed, another working model defends that the NMD process is a nucleus-associated event. In this model, the recognition of a nonsense codon as premature would occur while transcripts are still associated with the nucleus, during mRNA transport towards the cytoplasm, at a point when mRNA copurifies with nuclei but is translated by cytoplasmic ribosomes. In fact, the NMD of most mammalian mRNAs is considered to be a nucleus-associated event and those mRNAs that can escape to the cytoplasm, acquire immunity to further NMD degradation[91,92,101].

In the nucleus, a protein called the cap-binding complex (CBC), consisting of the heterodimer formed by CBP20 and CBP80 cap binding proteins, binds the cap structure of the pre-mRNA. CBC plays an important role in splicing, 3'-end formation and mRNA export[102-104]. Data indicate that NMD is targeted while mRNA is still bound to the cap-binding protein CBP80-CBP20 heterodimer, during what has been called "a pioneer round of translation". In this model an mRNA would undergo the pioneer round of translation as it exits the nuclear pore[67] and this would allow the detection of the PTCs by the cytoplasmic translation machinery, while the mRNA was still nucleus-associated. The pioneer round precedes the exchange of CBP80-CBP20 for the eukaryotic initiation factor eIF4E, which supports the cytoplasmatic steady-state translation[105]. The interaction established between eIF4E and the cytoplasmic (PABP) results in the formation of a translationaly competent circular mRNP that is subject to multiple rounds of translation[67]. Thus, since at this step the EJCs and associated Upf proteins would have been already removed by the ribosome, an eIF4E-bound mRNA would have lost its chance of being committed to NMD. Some differences have already been pinpointed between the pioneer translation and the steady-state translation protein complexes: whereas eIF4GI, PAPB1, eIF3, eIF4AI and eIF2 function in both complexes, eIF4AIII co-immunopurifies with CBP80 but not with eIF4E[106].

Several evidences indicate that β-globin NMD, in non-erythroid cells, is nucleus-associated and dependent on cytoplasmatic translation. The insertion of a hairpin-forming Iron-responsive Element (IRE) in the 5'UTR of the β-globin mRNA enabled its translation to be controlled by the binding of the Iron Regulatory Protein (IRP). The binding of the IRP to the IRE specifically inhibits the translation of these transcripts and abrogates the NMD of the β-globin mRNA carrying a PTC at codon 39. Since IRP localization was restricted to the cytoplasm, the NMD of the nonsense containing β-globin transcripts was shown to be dependent on the cytoplasmic translation[73]. Furthermore, the abundance of nonsense β-globin NMD-competent transcripts was shown to be comparable in nuclear and cytoplasmatic fractions of non-erythroid cells, indicating that the decay takes place prior to the release of the mRNA (in association with the nucleus) into the cytoplasm[72,107].

Although most mammalian mRNAs are subject to NMD while they are associated with the nucleus, a few mammalian nonsense-mRNAs are degraded after they are released into the cytoplasm. An example of such is β-globin mRNA expressed in erythroid cells. Indeed, in earlier studies using transgenic animals, β-globin mRNAs, harboring either a nonsense mutation or a frameshift that results in a PTC, were shown to be degraded in the cytoplasm of erythroid cells[108,109].

Furthermore, the cytoplasmic NMD, similarly to what was described to nucleus-associated NMD, occurs while the mRNA is still associated with CBP80-CBP20 and the same lost of susceptibility to NMD is observed when the CBC is replaced by eIF4E[67]. This indicates that transcripts that undergo cytoplasmic NMD are also subject to a pioneer round of translation as observed for nucleus-associated NMD mRNAs.

The decay pathway of nonsense-mutated human β-globin transcripts: The major decay pathways associated with the regular mRNA turnover comprise rate-limiting steps involved in the disruption of the protective 5' cap and 3' poly(A) terminal structures. In most cases, the poly(A) tail shortening is the initial step required for the decay event. Deadenylation is followed by decapping and subsequent 5' to 3' or 3' to 5' exonucleolytic degradation[110,111].

In contrast, the decay pathway of yeast nonsense transcripts is triggered through rapid decapping, mediated by Dcp1p, followed by 5' to 3' exonucleolytic degradation by the Xrn1p, without a requirement for prior deadenylylation[112,113]. Nevertheless, a minor pathway involving accelerated deadenylation has been recently described[114,115].

In higher eukaryotes, degradation pathways of PTC-containing mRNAs have not been fully elucidated.

A study using nonsense β-globin mRNAs transiently expressed in mammalian non-erythroid cells showed that nonsense transcripts were degraded from both 5' and 3' ends through decapping and 5' to 3' exonucleolitic activity as well as deadenylation and 3' to 5' exonucleolitic activity. This was demonstrated by the observation that the down regulation of the Dcp2 decapping protein or the exosomal component PM/Scl 100, increased the abundance of steady-state nonsense-containing transcripts and slowed down the decay rate of nonsense-containing but not nonsense-free mRNA[116]. Additionally, the downregulation of the poly(A) ribonuclease, PARN, also increased the abundance of nonsense-containing mRNA[116]. It was also demonstrated the existence of an accelerated deadenylation process that precedes decay of the β-globin PTC-containing mRNAs[117]. Consistently, hUpf1 protein was shown to co-immunopurify with PABP[118] and with the human decapping enzymes Dcp1 and Dcp2[119]. Furthermore, all three NMD factors Upf1, Upf2 and Upf3b co-immunopurify with Dcp2, with Xrn1 exonuclease and a putative 5'to 3' exonuclease Rat1, with the exosomal components (PM/Scl 100, Rrp4 and Rrp41) and with PARN[116]. Nevertheless, it should be noticed that in the work by Lejeune *et al.*[116], they could not detect partial or completely deadenylated decay intermediates. They proposed two explanations. First, the decay of the mRNA body could be too fast to allow the detection of decay intermediates. Second, the 3' to 5' exonucleolytic activity could occur to a much lesser extent than the 5' to 3' decay. Indeed, a recent study of the β-globin transcriptional decay rate showed that although deadenylation rate is increased in nonsense-containing transcripts, it is not sufficient to account for the decreased half-live on its own, but rather, a PTC enhances the rate of decapping and the decapped products have been deadenylated to a certain extend[120]. These evidences, along with the finding that the NMD of β-globin transcripts does not require the presence of a poly(A) tail[79], suggest that the 3' to 5' decay does not play a dominant role in β-globin NMD.

Although, in non-erythroid cells, β-globin NMD pathway appears to degrade mRNA from both ends involving decapping, deadenylating and exonucleolytic activities, studies of β-globin expression in erythroid cells showed a different decay pathway for nonsense containing mRNAs. Indeed, it was shown that the nonsense β-globin mRNA decay products were polyadenylated but lacked sequences from the 5'-end of the full-length transcript, although the generated intermediates still contained a cap-like structure[108,109]. In a latter study, using β-globin genes stably expressed in MEL cells, the identification of the upstream counterpart sequences of these decay products showed that they were generated through an endonuclease activity with preference for UG dinucleotides, i.e., the cleavage was not continuous[121]. Therefore, the intermediates are probably capped after endonucleolytic clevage, which presumably confers them resistance to 5' to 3' decay. These results suggest that nonsense β-globin mRNAs in erythroid cells are subjected to a specialized decay pathway that may occur simultaneously or in parallel with the general NMD mechanism.

Studies of the NMD mechanism using the human β-globin mRNA as a model system have illuminated many features of this decay pathway. However, further studies will be important to reveal novel aspects of this pathway. For instance, it will be interesting to understand how the mechanism of nonsense-mediated mRNA decay is able to overcome specific mRNA stabilization pathways.

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