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Review Article

New insights into the interplay between the translation machinery and nonsense-mediated mRNA decay factors

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Faulty mRNAs with a premature stop codon (PTC) are recognized and degraded by nonsense-mediated mRNA decay (NMD). Recognition of a nonsense mRNA depends on translation and on the presence of NMD-enhancing or the absence of NMD-inhibiting factors in the 3′-untranslated region. Our review summarizes our current understanding of the molecular function of the conserved NMD factors UPF3B and UPF1, and of the anti-NMD factor Poly(A)-binding protein, and their interactions with ribosomes translating PTC-containing mRNAs. Our recent discovery that UPF3B interferes with human translation termination and enhances ribosome dissociation *in vitro*, whereas UPF1 is inactive in these assays, suggests a re-interpretation of previous experiments and modification of prevalent NMD models. Moreover, we discuss recent work suggesting new functions of the key NMD factor UPF1 in ribosome recycling, inhibition of translation re-initiation and nascent chain ubiquitylation. These new findings suggest that the interplay of UPF proteins with the translation machinery is more intricate than previously appreciated, and that this interplay quality-controls the efficiency of termination, ribosome recycling and translation re-initiation.

Introduction

Nonsense-mediated mRNA decay (NMD) was first discovered in *Saccharomyces cerevisiae* and in men [1,2]. The pathway was initially described as an mRNA surveillance mechanism that recognizes and degrades transcripts containing a premature termination codon (PTC). Subsequent research showed that NMD is an important post-transcriptional regulator of eukaryotic gene expression and essential for cellular homeostasis, cell cycle progression, cellular stress response, development and differentiation, neural activity and immunity [3,4].

Recognition of a PTC-containing mRNA as an NMD substrate requires translation [5–7]. Translation termination occurring at a PTC, i.e. in a suboptimal environment, slows down the termination reaction, and possibly subsequent ribosome recycling and re-initiation, thus triggering NMD [8–10]. Typical PTC-containing NMD substrates are characterized by the presence of specific landmarks such as an exon junction complex (EJC) downstream from a stop codon or a long 3′ UTR (Figure 1). The EJC is deposited during splicing 20–24 nucleotides (nt) upstream of an exon–exon junction and removed during translation [11,12]. 'Normal' stop codons trigger efficient termination, ribosome recycling and translation re-initiation. These stop codons are typically positioned in the last exon (Figure 1A), and thus, the corresponding mRNAs are EJC-free. Recently, a higher rate of out-of-frame translation or low codon optimality in cellular transcripts was also linked to NMD in yeast [13]. How exactly a PTC is recognized by the NMD machinery is still enigmatic despite many years of research.

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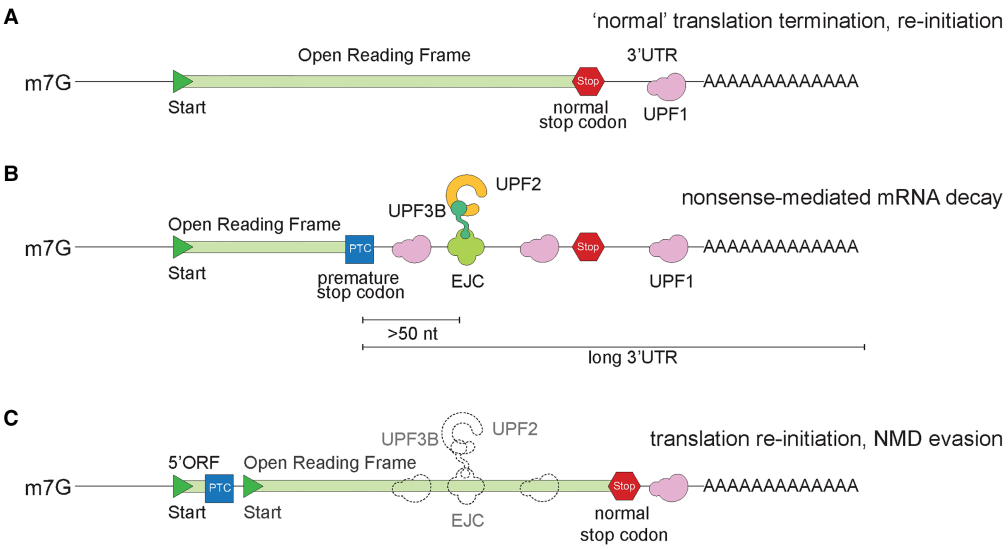

Figure 1. Hallmarks of normal and premature stop codons and their 3′-UTR context.

(**A**) Normal stop codons are positioned in the last exon in proximity of the poly(A) tail. (**B**) Premature stop codons are characterized by an EJC positioned at least 50–55 nt downstream from the PTC (the effect of the EJC is distance-dependent), accumulation of UPF1 next to the PTC and/or in 3′-UTRs which are longer than usual (see the main text for details). (**C**) Transcripts with a PTC close to the start codon can evade NMD by translation re-initiation, despite the presence of downstream EJCs.

Human NMD is mediated by the eukaryotic Release Factors eRF1 and eRF3a, the conserved UP-Frameshift proteins UPF1, UPF2 and UPF3B, the kinase SMG1 and SMG5–SMG9 (Suppressor with Morphological effect on Genitalia). Prevailing NMD models suggest that the ATP-dependent RNA helicase UPF1 is the key factor to recognize the terminating ribosome at the PTC and to nucleate the NMD machinery [14–17]. UPF1 is suggested to interfere with translation termination [16] (see below) and to recruit the SMG1–8–9 kinase complex (SMG1c) [18]. UPF2 and UPF3B, which both are associated with a downstream EJC or recruited by an unknown mechanism, are required to activate UPF1 phosphorylation by SMG1c [18] as well as UPF1's ATPase and helicase activities [19,20], helped by the RNA helicase DHX34 [21]. Phospho-UPF1 then serves as a platform to recruit the endonuclease SMG6 and the SMG5 : 7–PP2A complex for deadenylation of the mRNA [22–24], thus triggering mRNA decay.

In humans, the study of NMD mechanisms is complicated by the existence of different branches of NMD, occurring independent of the presence of UPF2, UPF3B or the EJC [25–27]. Experimentally, the main obstacle, however, for the molecular dissection of the NMD pathway and its branches is the lack of an *in vitro* NMD assay. This is possibly due to the fact that not all factors required for NMD are known; and thus, not all required components for NMD may be present in *in vitro* reactions. Accordingly, current NMD models are mostly based on genetic data in cells, as well as *in vivo* and *in vitro* protein–protein interaction assays. Consequently, the molecular events during translation termination at a PTC, which leads to assembly and activation of the NMD machinery, are still poorly understood.

Several lines of evidence suggest that translation termination at a PTC is mechanistically different and less efficient than normal translation termination: in cells, normal translation termination is too efficient to be followed experimentally, e.g. in primer extension experiments (toe-print assays). Termination at a PTC, however, is slower leading to a toe-print signal of the terminating ribosome [8,28]. Secondly, stop codon read-through assays indicate that PTCs are more susceptible to stop codon suppression, i.e. termination is less efficient [29,30]. Third, UPF1 knockdown reduces stop codon read-through, i.e. UPF1 interferes with translation termination in humans [16]. In contrast, yeast translation experiments indicate that Upf1p is important for termination at a PTC [8,31]. Finally, co-immunoprecipitations indicated that a 'SURF' complex assembles at PTC-stalled ribosomes, comprising SMG1c, UPF1, eRF1 and eRF3a [15,18].

Here, we discuss recent work revealing new functional interactions of NMD factors with the human translation machinery and characteristics of human NMD substrates.

UPF1 accumulates in the 3′-UTR of PTC-containing mRNAs

In human cells, UPF1 is bound to RNAs in a length-dependent, rather than a sequence- or translation-dependent manner [32,33]. UPF1 has been found to be enriched in the 3′-UTR of mRNAs as the result of displacement from the coding region by the translating ribosome [34] (Figure 1). NMD substrate discrimination was suggested to be achieved by ATPase-dependent dissociation of UPF1 from non-target mRNAs, leading to an enrichment of UPF1 on PTC-containing mRNAs next to the stop codon and near the 3′-end of the mRNA (Figure 1B) [35]. However, the accumulation of UPF1 in the 3′-UTR of NMD substrates is not sufficient to commit the mRNA to degradation and a second commitment step is required.

Exon junction complexes enhance NMD

Multiple observations suggest that the EJC — which consists of ATP-bound RNA helicase EIF4A3, Barentsz (MLN51/CASC3) and the heterodimer MAGOH-RBM8A (RNA-binding protein 8A, also known as Y14) as the core components [11,36], is the most important NMD-activating factor: mammalian NMD is enhanced if the PTC is located at least 50–55 nt upstream of an exon–exon junction (Figure 1B) [6,37]. NMD can be artificially triggered by tethering of RBM8A downstream from a stop codon [25,38,39]. *Vice versa*, PTC-containing mRNAs can be stabilized *in vivo* by the elimination of the EJC core component EIF4A3 [40,41]. Co-immunoprecipitations indicated that UPF2 and UPF3B, which are bound to the EJC [11], are part of a larger, decay-inducing complex which also comprises SMG1c, UPF1 and the terminating ribosome [18,21].

Notably, recent bioinformatics studies analyzing genome and transcriptome data corroborated that a downstream EJC is the most important predictor of human NMD [42,43]. Comparison of the matched exome (i.e. the expressed genome) and transcriptome (mRNA) from ∼10 000 human tumours allowed NMD efficiency of nonsense mutations to be determined through measuring the change in expression levels of the mutant mRNA compared with the average 'wild-type' mRNA from the same cancer subtype [43]. NMD efficiency was shown do decrease with the distance between the PTC and the EJC in exceptionally long exons. Moreover, transcripts with a PTC close to the start codon could evade NMD by translation re-initiation (Figure 1C), despite the presence of downstream EJCs [43], as previously reported [28,44,45].

Taken together, all findings agree with a direct cross-talk between the PTC-bound terminating ribosome and EJC-bound factors to induce NMD.

UPF1 and UPF2 are inactive in translation termination *in vitro*

In yeast, all three NMD factors (Upf1-3p) were shown to interact with release factors in pulldown experiments [31]. Human UPF1 could be co-immunoprecipitated with eRF1 and eRF3a [16], and human UPF2 was shown to directly interact with eRF3a in surface plasmon resonance experiments (Figure 2) [46].

More recently, a fully reconstituted human translation system was used to probe the impact of the three human UPF proteins on translation termination [47]. This translation system faithfully monitors each step of mammalian translation, including translation termination, ribosome recycling and re-initiation on an upstream or downstream start codon [48–50]. Using this *in vitro* system, translating ribosomes stalled at a stop codon can be purified. The subsequent addition of release factors followed by toe-printing assays allows observation of stop codon recognition by eRF1–eRF3a [48]. Using limiting amounts of release factors, the system was adapted to test the impact of each NMD factor individually and in combination [47]. Surprisingly, UPF1 and a large fragment of UPF2 had no impact on the efficiency of translation termination. UPF1 was inactive, irrespective of its phosphorylation by the kinase SMG1 or of UPF1's ATPase activity [47]. This suggests that UPF1 does not play a role in translation termination, a finding which contradicts the prevailing NMD models. In agreement with these findings, recent work from Rachel Green's laboratory suggests that yeast Upf1 has no role in translation elongation, termination or ribosome recycling *in vitro* [51].

UPF3B interferes with termination *in vitro*

In contrast, the NMD factor UPF3B, which is a peripheral EJC subunit, interfered with stop codon recognition and peptide release from the ribosome [47]. In agreement with a role of UPF3B in translation termination,

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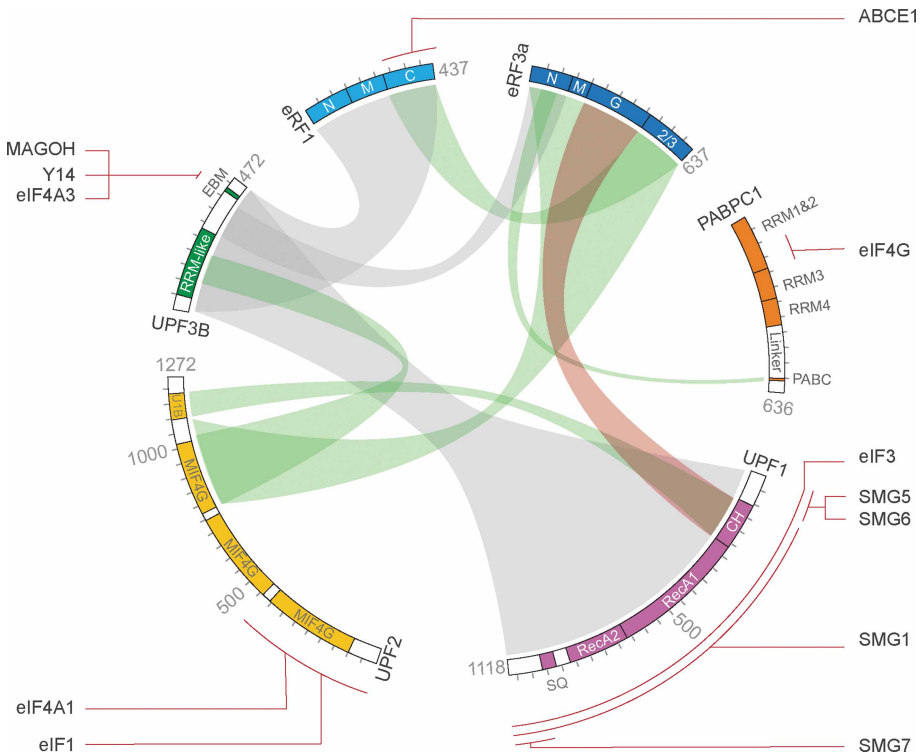

Figure 2. Scheme illustrating the interactome of translation and NMD factors.

A scheme for each factor UPF1, UPF2, UPF3B, eRF1, eRF3a and PABPC1 is represented in the central circle. Graduations indicate 50 amino acids. Domains specific to each factor are mapped to the scheme. Coloured ribbons are indicating known interactions: green for structural data, grey for *in vitro* pulldowns using purified proteins and red for *in vivo* co-immunoprecipitations from cell extracts (adapted from ref. [83]). N: N-terminal domain; M: middle domain; C: C-terminal domain; 2/3: 2 and 3 domains; G: GTPase domain; RRM: RNA-recognition motif; PABC: Poly(A)-binding protein C-terminus. SQ: serine–glutamine-rich domain; CH: cysteine–histidine-rich domain; helicase domain: RecA1 and RecA2; MIF4G: middle domain of eIF4G; U1B: UPF1-binding domain; EBM: exon junction complex-binding motif.

purified UPF3B was shown to interact directly with eRF3a and with the ribosome (Figure 2). Moreover, a direct interaction of UPF3 and UPF1 was discovered (Figure 2) [47]. Previous co-immunoprecipitations were repeated and it was shown that UPF3B is part of the complex between UPF1 and eRF3a, likely mediating this interaction. For UPF3B's inhibitory effect on translation termination, the RNA-recognition motif (RRM) and the middle domain are required, but not the EJC-binding domain, consistent with an association of UPF3B with the EJC in the 3′-UTR of NMD substrates (Figure 1B). The fact that UPF3B binds RNA may be relevant for the EJC-independent branch of NMD [27,47,52]. UPF3B bound to the mRNA 3′-UTR could interact with the PTC-bound ribosome and eRF3a and slow-down translation termination (Figure 3). A transient interaction of UPF3B and UPF1 on the ribosome could contribute to the assembly of the NMD machinery at the PTC, for instance by recruiting UPF2 and SMG1–8–9.

UPF2 addition to the termination reaction interferes with UPF3B's inhibition of translation termination [47]. Similarly, UPF3B was found to disrupt the interaction between UPF2 and eRF3a to form a more stable UPF2–UPF3B complex [46]. UPF2 and UPF3B are required for activation of SMG1 kinase as well as stimulation of UPF1's helicase and ATPase activities. Taken together, UPF2 binding could co-ordinate the switch from PTC-related activities of UPF3B (and of UPF1, see below) to UPF1 phosphorylation and activation of mRNA decay.

Ribosome dissociation by UPF3B

When release factors are added in excess to the translation termination reaction, UPF3B was observed to destabilize post-termination complexes (post-TCs), as evidenced by a diminution of the post-TC bands and an

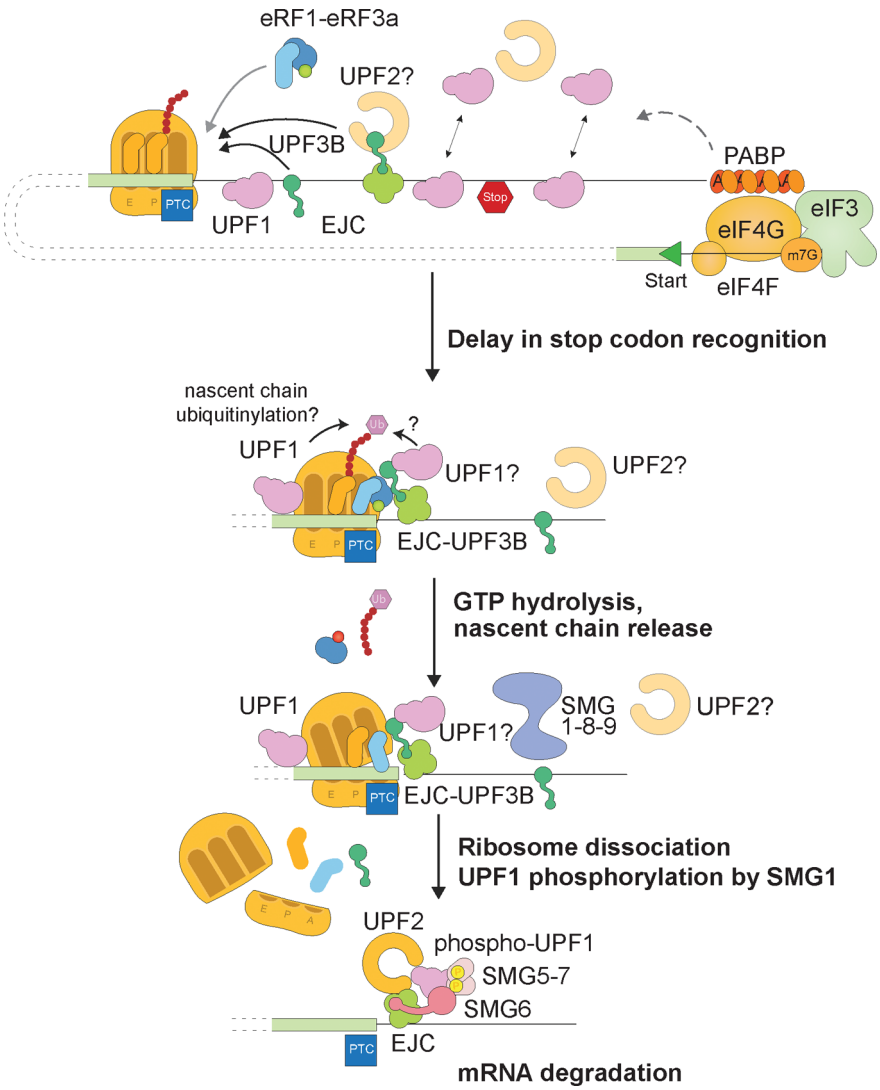

Figure 3. Modified model for initiation of NMD, adapted from ref. [83].When a ribosome encounters a PTC, stop codon recognition can be delayed by the presence of UPF3B bound to the EJC, or by UPF3B bound to the mRNA in EJC-independent NMD [27]. Moreover, the long distance to PABP prevents efficient translation termination and re-initiation. At the PTC, the ribosome, release factors, UPF3B and likely also UPF1 can form a complex. Hydroxyl-radical probing suggests that yeast Upf1 binds to the ribosomal L1 stalk, near to the E-site [51]. UPF1 can also bind UPF3B [47]. UPF1 may ubiquitylate the PTC-encoded nascent chain and target the nascent polypeptide to degradation. After termination, UPF3B and UPF1 both could be involved in ribosome dissociation. UPF1 can recruit other NMD factors, including the SMG1–8–9 complex. UPF2 binding displaces UPF3B from the ribosome-release factor complex [47]. UPF2–UPF3B activates the kinase SMG1 leading to UPF1 phosphorylation. Phospho-UPF1 recruits decay factors such as the SMG6 endonuclease and the SMG5–7 heterodimer. SMG6 displaces UPF3B from the EJC and cuts the mRNA close to the PTC.

increased signal for the full-size mRNA/cDNA bands in toe-printing experiments [47]. UPF3B-induced ribosome dissociation was incomplete and only observed after GTP hydrolysis (and not in the presence of non-hydrolysable GTP analogues) and after nascent chain release [47]. This UPF3B activity is reminiscent to the activity of eIF1-1A-eIF3-eIF3j, which together promotes splitting of post-TCs into 60S subunits, tRNA, mRNA and 40S subunits [53].

Again, UPF2 inhibited UPF3B's capacity to dissolve post-TCs [47]. This could indicate either that UPF2 joins the factors on the PTC-stalled ribosome at a later stage, after translation termination and ribosome

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dissociation (Figure 3), or that UPF3B's effect on translation termination is specific to the UPF2-independent NMD branch [25]. In the former case, UPF1, UPF2 and UPF3B will be engaged in differently composed complexes at different stages of termination and NMD: UPF3B binds ribosome-release factors, interacts with UPF1 on the ribosome and dissociates post-TCs in the absence of UFP2 (Figure 3). Subsequent UPF2 joining would displace release factors from the complexes, in order to form UPF1–UPF2–UPF3B-containing complexes engaging in messenger ribonucleoprotein remodelling [19] and in the recruitment of decay enzymes triggering the decay phase of NMD [54].

Intriguingly, UPF3B has been reported to have a general effect on human translation and stimulate protein synthesis when tethered to the coding region of mRNA [55]. This yet uncharacterized effect depends on the presence of UPF3B's RRM-like domain and is independent of UPF2 and RBM8A (EJC) binding.

Efficient translation termination and re-initiation prevents NMD

Unusually, long 3′ UTRs have been shown to trigger NMD in yeast, *Drosophila* and *Caenorhabditis elegans* [8,56–58] (Figure 1B). NMD can be prevented by tethering Poly(A)-binding protein (PABP) downstream from a PTC or by introducing a secondary structure into the 3′ UTR mRNA to bring PABP closer to the stop codon [17,56,59]. A direct stimulation of translation termination by PABP was demonstrated using a reconstituted mammalian translation system [60]. PABP was shown to interact with the N-terminal part of eRF3a [61] and with the ribosome (Figure 2), and it was suggested to promote the recruitment of eRFs to the ribosome thus facilitating stop codon recognition [60]. Thus, human UPF3B and PABP could compete at the terminating ribosome for the binding of eRF3a (Figure 3), leading to opposing effects on translation termination efficiency [47,60].

Cytoplasmic PABP 1 (PABPC1) also interacts with the initiation factor eIF4G (Figure 2), a subunit of the eIF4F complex which binds the 5′-cap of mRNA. The PABPC1–eIF4G interaction circularizes the mRNA and juxtaposes the stop codon and the start codon, facilitating translation re-initiation on the same mRNA after translation termination. Efficient translation re-initiation was suggested to inhibit NMD [28,44,62]. Consistently, NMD can be prevented by tethering eIF4G to the 3′-UTR downstream from a PTC, suggesting that initiation factors are also anti-NMD factors [9,63]. Similarly, eIF3 is required to prevent NMD of mRNAs with a PTC close to a start codon (Figure 1C), and knockdown of eIF3 subunits renders such mRNAs NMD-sensitive [9,28]. This is explained by the finding that eIF3 can remain bound to elongating ribosomes after initiation [64]. Thus, for relatively short ORFs, PABPC1 could still be close to the ribosome when it reaches the stop codon, thereby preventing NMD [62]. This highlights that at a normal stop codon, a tight link between translation termination, ribosome recycling and translation re-initiation exists, ensuring optimal translation of mRNA.

Interestingly, eIF3 subunits have been shown to interact with UPF1 (Figure 2), which then prevents the formation of elongation-competent 80S initiation complexes and thus represses translation and favours NMD [65–67]. Similarly, in yeast, *in vitro* re-initiation following termination was shown to be less efficient at a PTC compared with a normal stop codon [68]. Moreover, Upf2p has been found to interact with eIF4A (Figure 2), which is part of the cap-binding complex eIF4F [69], establishing a second potential link between the translation machinery and NMD factors to suppress translation of nonsense mRNAs. Finally, dissociation of post-TCs by UPF3B in the absence of initiation factors [47] could prevent translation re-initiation and favour the formation of decay-inducing complexes.

NMD avoidance mechanisms

Long 3′-UTRs could stimulate NMD by length-dependent accumulation of UPF1 (see above) [32]. While model systems established a link between 3′-UTR length and NMD susceptibility [8,17,70,71], a genome-wide correlation of 3′-UTR length and NMD is complicated by the fact that many mRNAs with long 3′-UTRs are protected from NMD mostly by unknown mechanisms [43]. One such evasion mechanism includes an RNA-stability element (RSE) located within 200 nt downstream from an NMD-triggering PTC. This RNA motif recruits the polypyrimidine-tract-binding protein 1 (PTBP1) [72] and together they form a 3′-mRNP structure that limits UPF1 association with the 3′-UTR, possibly by preventing initial UPF1 binding or by blocking 5′–3′ translocation of UPF1 along the mRNA [72].

Ribosome recycling and nascent chain degradation at a prematurely terminating ribosome

Little is known about how the PTC-bound ribosome is recycled after translation termination. UPF3B has been shown to dissociate post-termination ribosome complexes [47]. Yeast experiments indicate that PTC-bound ribosomes require Upf1p to be removed from the mRNA [73]. ATPase-deficient Upf1p was shown to lead to an accumulation of mRNA fragments that comprise the PTC-bound ribosome and the downstream 3′-UTR, implicating Upf1p in ribosome release [73]. These fragments were generated by XRN1, a 5′-to-3′ exonuclease, which is stalled by the presence of ribosomes. Thus, the mRNA with the PTC-bound ribosome and the 3′-UTR fragment remained intact. In fact, Upf1p's ATP-binding and RNA-binding activities, as well as Upf2p and Upf3p, were required for efficient removal of the PTC-bound ribosome from the mRNA. This implicates that Upf1p plays a role in termination or ribosome recycling at the PTC *after* complex formation with Upf2p and Upf3p. In agreement, the deletion of any of the three Upf-encoding genes in yeast causes ribosome-release defects [68].

The mRNA degradation phase in NMD is initiated by UPF1 phosphorylation in higher eukaryotes. Phospho-UPF1 recruits SMG6, which cleaves the mRNA close to the PTC, SMG5-7, which recruits CCR4-Not deadenylase and the decapping enzyme Dcp2 (reviewed in ref. [74]) (Figure 2). However, how the C-terminally truncated proteins, which are encoded by nonsense mRNA and which are potentially harmful for the cell, are recognized and efficiently removed is enigmatic. The Not4 subunit of the CCR–Not complex has E3 ubiquitin ligase activity and has been implicated in co-translational quality control, targeting proteins encoded by defective mRNA to the proteasome [75]. Notably, UPF1's cysteine- and histidine-rich (CH) domain (Figure 2) structurally resembles RING domains frequently found in E3 ubiquitin ligases [76].

Yeast Upf1p has been reported to self-ubiquitylate in an Upf3p-dependent manner *in vitro* [77]. Moreover, Upf1p-stimulated and ubiquitin-dependent degradation of proteins encoded by PTC-containing mRNA (PTC product) has been shown in yeast cells [78–80]. Similarly, depletion of E3 ubiquitin ligase Ubr1 also stabilized PTC products in yeast, suggesting that Upf1p and/or Ubr1's E3 ligase activities contribute to the degradation of PTC-induced truncated peptides [78]. In agreement, proteomics identified interactions of UPF1 with ubiquitin–proteasome components [67,81]. Recently and independent of its NMD activity, human UPF1 was shown to act as an E3 ubiquitin ligase promoting the degradation of the transcription factor MYOD which regulates myogenesis [82]. It is therefore tempting to speculate that UPF3B and UPF1 bound to the PTC-stalled ribosome could promote ubiquitylation of the nascent chain and its subsequent degradation by the proteasome (Figure 3).

Conclusions

Taken together, the interplay of UPF proteins and the translation machinery appear to be much more intricate than previously appreciated. The new findings suggest that the NMD factors quality control the efficiency of translation termination, ribosome recycling *and* translation re-initiation: both, UPF1 and UPF3B are found in the 3′-UTR of 'normal' mRNAs [32,34,35,52], and in conjunction with an EJC, they strongly enhance NMD (Figure 3). If the ribosome fails to terminate and re-initiate translation efficiently on an mRNA, the assembly of NMD complexes and their activation is favoured. Consistently, UPF3B interferes with translation termination *in vitro* [47], and UPF1 is suggested to inhibit translation re-initiation by binding eukaryotic initiation factor 3 [65,66]. While the PTC-stalled ribosome is likely the assembly platform for the NMD machinery [18] or the trigger for NMD machinery activation, the order of factor recruitment, the molecular function of the UPF factors and the regulation of NMD still require further clarification.

Abbreviations

CH, Cysteine–Histidine-rich domain; EJC, Exon Junction Complex; eRF, eukaryotic Release Factor; NMD, Nonsense-Mediated mRNA Decay; nt, nucleotides; PABP, Poly(A)-Binding Protein; PABPC1, Cytoplasmic PABP 1; Post-TC, Post-Termination Complex; PTC, Premature Termination Codon; RRM, RNA-Recognition Motif; SMG, Suppressor with Morphological effect on Genitalia; SMG1c, complex comprising the kinase SMG1, SMG8 and SMG9; SURF, SMG1c, UPF1, eRF1; UPF, UP-Frameshift protein; 3′-UTR, 3′-Un-Translated Region.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Losson, R. and Lacroute, F. (1979) Interference of nonsense mutations with eukaryotic messenger RNA stability. *Proc. Natl Acad. Sci. U.S.A.* **76**, 5134–5137 https://doi.org/10.1073/pnas.76.10.5134
2 Chang, J.C. and Kan, Y.W. (1979) Beta 0 thalassemia, a nonsense mutation in man. *Proc. Natl Acad. Sci. U.S.A.* **76**, 2886–2889 https://doi.org/10.1073/pnas.76.6.2886
3 Lykke-Andersen, S. and Jensen, T.H. (2015) Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. *Nat. Rev. Mol. Cell Biol.* **16**, 665–677 https://doi.org/10.1038/nrm4063
4 Ottens, F. and Gehring, N.H. (2016) Physiological and pathophysiological role of nonsense-mediated mRNA decay. *Pflugers Arch.* **468**, 1013–1028 https://doi.org/10.1007/s00424-016-1826-5
5 Carter, M.S., Li, S. and Wilkinson, M.F. (1996) A splicing-dependent regulatory mechanism that detects translation signals. *EMBO J.* **15**, 5965–5975 PMCID: PMC452383
6 Thermann, R., Neu-Yilik, G., Deters, A., Frede, U., Wehr, K., Hagemeier, C. et al. (1998) Binary specification of nonsense codons by splicing and cytoplasmic translation. *EMBO J.* **17**, 3484–3494 https://doi.org/10.1093/emboj/17.12.3484
7 Karousis, E.D., Nasif, S. and Mühlemann, O. (2016) Nonsense-mediated mRNA decay: novel mechanistic insights and biological impact. *Wiley Interdiscip. Rev. RNA* **7**, 661–682 https://doi.org/10.1002/wrna.1357
8 Amrani, N., Ganesan, R., Kervestin, S., Mangus, D.A., Ghosh, S. and Jacobson, A. (2004) A faux 3′-UTR promotes aberrant termination and triggers nonsense-mediated mRNA decay. *Nature* **432**, 112–118 https://doi.org/10.1038/nature03060
9 Joncourt, R., Eberle, A.B., Rufener, S.C. and Mühlemann, O. (2014) Eukaryotic initiation factor 4G suppresses nonsense-mediated mRNA decay by two genetically separable mechanisms. *PLoS ONE* **9**, e104391 https://doi.org/10.1371/journal.pone.0104391
10 Celik, A., Kervestin, S. and Jacobson, A. (2015) NMD: at the crossroads between translation termination and ribosome recycling. *Biochimie* **114**, 2–9 https://doi.org/10.1016/j.biochi.2014.10.027
11 Le Hir, H., Saulière, J. and Wang, Z. (2016) The exon junction complex as a node of post-transcriptional networks. *Nat. Rev. Mol. Cell Biol.* **17**, 41–54 https://doi.org/10.1038/nrm.2015.7
12 Gehring, N.H., Lamprinaki, S., Kulozik, A.E. and Hentze, M.W. (2009) Disassembly of exon junction complexes by PYM. *Cell* **137**, 536–548 https://doi.org/10.1016/j.cell.2009.02.042
13 Celik, A., He, F. and Jacobson, A. (2017) NMD monitors translational fidelity 24/7. *Curr. Genet.* **63**, 1007–1010 https://doi.org/10.1007/s00294-017-0709-4
14 Czaplinski, K., Ruiz-Echevarria, M.J., Paushkin, S.V., Han, X., Weng, Y., Perlick, H.A. et al. (1998) The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs. *Genes Dev.* **12**, 1665–1677 https://doi.org/10.1101/gad.12.11.1665
15 Kashima, I., Yamashita, A., Izumi, N., Kataoka, N., Morishita, R., Hoshino, S. et al. (2006) Binding of a novel SMG-1-Upf1-eRF1-eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay. *Genes Dev.* **20**, 355–367 https://doi.org/10.1101/gad.1389006
16 Ivanov, P.V., Gehring, N.H., Kunz, J.B., Hentze, M.W. and Kulozik, A.E. (2008) Interactions between UPF1, eRFs, PABP and the exon junction complex suggest an integrated model for mammalian NMD pathways. *EMBO J.* **27**, 736–747 https://doi.org/10.1038/emboj.2008.17
17 Singh, G., Rebbapragada, I. and Lykke-Andersen, J. (2008) A competition between stimulators and antagonists of Upf complex recruitment governs human nonsense-mediated mRNA decay. *PLoS Biol.* **6**, e111 https://doi.org/10.1371/journal.pbio.0060111
18 Yamashita, A., Izumi, N., Kashima, I., Ohnishi, T., Saari, B., Katsuhata, Y. et al. (2009) SMG-8 and SMG-9, two novel subunits of the SMG-1 complex, regulate remodeling of the mRNA surveillance complex during nonsense-mediated mRNA decay. *Genes Dev.* **23**, 1091–1105 https://doi.org/10.1101/gad.1767209
19 Chamieh, H., Ballut, L., Bonneau, F. and Le Hir, H. (2008) NMD factors UPF2 and UPF3 bridge UPF1 to the exon junction complex and stimulate its RNA helicase activity. *Nat. Struct. Mol. Biol.* **15**, 85–93 https://doi.org/10.1038/nsmb1330
20 Chakrabarti, S., Jayachandran, U., Bonneau, F., Fiorini, F., Basquin, C., Domcke, S. et al. (2011) Molecular mechanisms for the RNA-dependent ATPase activity of Upf1 and its regulation by Upf2. *Mol. Cell* **41**, 693–703 https://doi.org/10.1016/j.molcel.2011.02.010
21 Hug, N. and Cáceres, J.F. (2014) The RNA helicase DHX34 activates NMD by promoting a transition from the surveillance to the decay-inducing complex. *Cell Rep.* **8**, 1845–1856 https://doi.org/10.1016/j.celrep.2014.08.020
22 Eberle, A.B., Lykke-Andersen, S., Mühlemann, O. and Jensen, T.H. (2009) SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells. *Nat. Struct. Mol. Biol.* **16**, 49–55 https://doi.org/10.1038/nsmb.1530
23 Boehm, V., Haberman, N., Ottens, F., Ule, J. and Gehring, N.H. (2014) 3′ UTR length and messenger ribonucleoprotein composition determine endocleavage efficiencies at termination codons. *Cell Rep.* **9**, 555–568 https://doi.org/10.1016/j.celrep.2014.09.012
24 Loh, B., Jonas, S. and Izaurralde, E. (2013) The SMG5-SMG7 heterodimer directly recruits the CCR4-NOT deadenylase complex to mRNAs containing nonsense codons via interaction with POP2. *Genes Dev.* **27**, 2125–2138 https://doi.org/10.1101/gad.226951.113
25 Gehring, N.H., Kunz, J.B., Neu-Yilik, G., Breit, S., Viegas, M.H., Hentze, M.W. et al. (2005) Exon-junction complex components specify distinct routes of nonsense-mediated mRNA decay with differential cofactor requirements. *Mol. Cell* **20**, 65–75 https://doi.org/10.1016/j.molcel.2005.08.012

26 Chan, W.-K., Huang, L., Gudikote, J.P., Chang, Y.-F., Imam, J.S., MacLean, J.A, II. et al. (2007) An alternative branch of the nonsense-mediated decay pathway. *EMBO J.* **26**, 1820–1830 https://doi.org/10.1038/sj.emboj.7601628
27 Metze, S., Herzog, V.A., Ruepp, M.-D. and Mühlemann, O. (2013) Comparison of EJC-enhanced and EJC-independent NMD in human cells reveals two partially redundant degradation pathways. *RNA* **19**, 1432–1448 https://doi.org/10.1261/rna.038893.113
28 Peixeiro, I., Inacio, A., Barbosa, C., Silva, A.L., Liebhaber, S.A. and Romao, L. (2012) Interaction of PABPC1 with the translation initiation complex is critical to the NMD resistance of AUG-proximal nonsense mutations. *Nucleic Acids Res.* **40**, 1160–1173 https://doi.org/10.1093/nar/gkr820
29 Welch, E.M., Barton, E.R., Zhuo, J., Tomizawa, Y., Friesen, W.J., Trifillis, P. et al. (2007) PTC124 targets genetic disorders caused by nonsense mutations. *Nature* **447**, 87–91 https://doi.org/10.1038/nature05756
30 Keeling, K.M., Xue, X., Gunn, G. and Bedwell, D.M. (2014) Therapeutics based on stop codon readthrough. *Annu. Rev. Genomics Hum. Genet.* **15**, 371–394 https://doi.org/10.1146/annurev-genom-091212-153527
31 Wang, W., Czaplinski, K., Rao, Y. and Peltz, S.W. (2001) The role of Upf proteins in modulating the translation read-through of nonsense-containing transcripts. *EMBO J.* **20**, 880–890 https://doi.org/10.1093/emboj/20.4.880
32 Hogg, J.R. and Goff, S.P. (2010) Upf1 senses 3'UTR length to potentiate mRNA decay. *Cell* **143**, 379–389 https://doi.org/10.1016/j.cell.2010.10.005
33 Kurosaki, T. and Maquat, L.E. (2013) Rules that govern UPF1 binding to mRNA 3' UTRs. *Proc. Natl Acad. Sci. U.S.A.* **110**, 3357–3362 https://doi.org/10.1073/pnas.1219908110
34 Zünd, D., Gruber, A.R., Zavolan, M. and Mühlemann, O. (2013) Translation-dependent displacement of UPF1 from coding sequences causes its enrichment in 3' UTRs. *Nat. Struct. Mol. Biol.* **20**, 936–943 https://doi.org/10.1038/nsmb.2635
35 Lee, S.R., Pratt, G.A., Martinez, F.J., Yeo, G.W. and Lykke-Andersen, J. (2015) Target discrimination in nonsense-mediated mRNA decay requires Upf1 ATPase activity. *Mol. Cell* **59**, 413–425 https://doi.org/10.1016/j.molcel.2015.06.036
36 Buchwald, G., Ebert, J., Basquin, C., Sauliere, J., Jayachandran, U., Bono, F. et al. (2010) Insights into the recruitment of the NMD machinery from the crystal structure of a core EJC-UPF3b complex. *Proc. Natl Acad. Sci. U.S.A.* **107**, 10050–10055 https://doi.org/10.1073/pnas.1000993107
37 Nagy, E. and Maquat, L.E. (1998) A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends Biochem. Sci.* **23**, 198–199 https://doi.org/10.1016/S0968-0004(98)01208-0
38 Lykke-Andersen, J., Shu, M.D. and Steitz, J.A. (2001) Communication of the position of exon-exon junctions to the mRNA surveillance machinery by the protein RNPS1. *Science* **293**, 1836–1839 https://doi.org/10.1126/science.1062786
39 Gehring, N.H., Neu-Yilik, G., Schell, T., Hentze, M.W. and Kulozik, A.E. (2003) Y14 and hUpf3b form an NMD-activating complex. *Mol. Cell* **11**, 939–949 https://doi.org/10.1016/S1097-2765(03)00142-4
40 Shibuya, T., Tange, T.Ø., Sonenberg, N. and Moore, M.J. (2004) eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. *Nat. Struct. Mol. Biol.* **11**, 346–351 https://doi.org/10.1038/nsmb750
41 Palacios, I.M., Gatfield, D., St Johnston, D. and Izaurralde, E. (2004) An eIF4AIII-containing complex required for mRNA localization and nonsense-mediated mRNA decay. *Nature* **427**, 753–757 https://doi.org/10.1038/nature02351
42 Lappalainen, T., Sammeth, M., Friedländer, M.R., 't Hoen, P.A.C., Monlong, J., Rivas, M.A. et al. (2013) Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* **501**, 506–511 https://doi.org/10.1038/nature12531
43 Lindeboom, R.G., Supek, F. and Lehner, B. (2016) The rules and impact of nonsense-mediated mRNA decay in human cancers. *Nat. Genet.* **48**, 1112–1118 https://doi.org/10.1038/ng.3664
44 Zhang, J. and Maquat, L.E. (1997) Evidence that translation reinitiation abrogates nonsense-mediated mRNA decay in mammalian cells. *EMBO J.* **16**, 826–833 https://doi.org/10.1093/emboj/16.4.826
45 Neu-Yilik, G., Amthor, B., Gehring, N.H., Bahri, S., Paidassi, H., Hentze, M.W. et al. (2011) Mechanism of escape from nonsense-mediated mRNA decay of human beta-globin transcripts with nonsense mutations in the first exon. *RNA* **17**, 843–854. https://doi.org/10.1261/rna.2401811
46 López-Perrote, A., Castaño, R., Melero, R., Zamarro, T., Kurosawa, H., Ohnishi, T. et al. (2016) Human nonsense-mediated mRNA decay factor UPF2 interacts directly with eRF3 and the SURF complex. *Nucleic Acids Res.* **44**, 1909–1923 https://doi.org/10.1093/nar/gkv1527
47 Neu-Yilik, G., Raimondeau, E., Eliseev, B., Yeramala, L., Amthor, B., Deniaud, A. et al. (2017) Dual function of UPF3B in early and late translation termination. *EMBO J.* **36**, 2968–2986 https://doi.org/10.15252/embj.201797079
48 Alkalaeva, E.Z., Pisarev, A.V., Frolova, L.Y., Kisselev, L.L. and Pestova, T.V. (2006) In vitro reconstitution of eukaryotic translation reveals cooperativity between release factors eRF1 and eRF3. *Cell* **125**, 1125–1136 https://doi.org/10.1016/j.cell.2006.04.035
49 Pisarev, A.V., Skabkin, M.A., Pisareva, V.P., Skabkina, O.V., Rakotondrafara, A.M., Hentze, M.W. et al. (2010) The role of ABCE1 in eukaryotic posttermination ribosomal recycling. *Mol. Cell* **37**, 196–210 https://doi.org/10.1016/j.molcel.2009.12.034
50 Skabkin, M.A., Skabkina, O.V., Hellen, C.U. and Pestova, T.V. (2013) Reinitiation and other unconventional posttermination events during eukaryotic translation. *Mol. Cell* **51**, 249–264 https://doi.org/10.1016/j.molcel.2013.05.026
51 Schuller, A.P., Zinshteyn, B., Enam, S.U. and Green, R. (2017) Directed hydroxyl radical probing reveals Upf1 binding to the 80S ribosomal E site rRNA at the L1 stalk. *Nucleic Acids Res.* https://doi.org/10.1093/nar/gkx1263
52 Hauer, C., Sieber, J., Schwarzl, T., Hollerer, I., Curk, T., Alleaume, A.-M. et al. (2016) Exon junction complexes show a distributional bias toward alternatively spliced mRNAs and against mRNAs coding for ribosomal proteins. *Cell Rep.* **16**, 1588–1603 https://doi.org/10.1016/j.celrep.2016.06.096
53 Pisarev, A.V., Hellen, C.U. and Pestova, T.V. (2007) Recycling of eukaryotic posttermination ribosomal complexes. *Cell* **131**, 286–299 https://doi.org/10.1016/j.cell.2007.08.041
54 Okada-Katsuhata, Y., Yamashita, A., Kutsuzawa, K., Izumi, N., Hirahara, F. and Ohno, S. (2012) N- and C-terminal Upf1 phosphorylations create binding platforms for SMG-6 and SMG-5:SMG-7 during NMD. *Nucleic Acids Res.* **40**, 1251–1266 https://doi.org/10.1093/nar/gkr791
55 Kunz, J.B., Neu-Yilik, G., Hentze, M.W., Kulozik, A.E. and Gehring, N.H. (2006) Functions of hUpf3a and hUpf3b in nonsense-mediated mRNA decay and translation. *RNA* **12**, 1015–1022 https://doi.org/10.1261/rna.12506
56 Eberle, A.B., Stalder, L., Mathys, H., Orozco, R.Z. and Mühlemann, O. (2008) Posttranscriptional gene regulation by spatial rearrangement of the 3' untranslated region. *PLoS Biol.* **6**, e92 https://doi.org/10.1371/journal.pbio.0060092
57 Gatfield, D., Unterholzner, L., Ciccarelli, F.D., Bork, P. and Izaurralde, E. (2003) Nonsense-mediated mRNA decay in *Drosophila*: at the intersection of the yeast and mammalian pathways. *EMBO J.* **22**, 3960–3970 https://doi.org/10.1093/emboj/cdg371

Biochemical Society Transactions (2018) **46** 503–512
https://doi.org/10.1042/BST20170427

58 Longman, D., Plasterk, R.H.A., Johnstone, I.L. and Caceres, J.F. (2007) Mechanistic insights and identification of two novel factors in the *C. elegans* NMD pathway. *Genes Dev.* **21**, 1075–1085 https://doi.org/10.1101/gad.417707

59 Silva, A.L., Ribeiro, P., Inacio, A., Liebhaber, S.A. and Romao, L. (2008) Proximity of the poly(A)-binding protein to a premature termination codon inhibits mammalian nonsense-mediated mRNA decay. *RNA* **14**, 563–576 https://doi.org/10.1261/rna.815108

60 Ivanov, A., Mikhailova, T., Eliseev, B., Yeramala, L., Sokolova, E., Susorov, D. et al. (2016) PABP enhances release factor recruitment and stop codon recognition during translation termination. *Nucleic Acids Res.* **44**, 7766–7776 https://doi.org/10.1093/nar/gkw635

61 Kozlov, G. and Gehring, K. (2010) Molecular basis of eRF3 recognition by the MLLE domain of poly(A)-binding protein. *PLoS ONE* **5**, e10169 https://doi.org/10.1371/journal.pone.0010169

62 Pereira, F.J., Teixeira, A., Kong, J., Barbosa, C., Silva, A.L., Marques-Ramos, A. et al. (2015) Resistance of mRNAs with AUG-proximal nonsense mutations to nonsense-mediated decay reflects variables of mRNA structure and translational activity. *Nucleic Acids Res.* **43**, 6528–6544 https://doi.org/10.1093/nar/gkv588

63 Fatscher, T., Boehm, V., Weiche, B. and Gehring, N.H. (2014) The interaction of cytoplasmic poly(A)-binding protein with eukaryotic initiation factor 4G suppresses nonsense-mediated mRNA decay. *RNA* **20**, 1579–1592 https://doi.org/10.1261/rna.044933.114

64 Mohammad, M.P., Munzarova Pondelickova, V., Zeman, J., Gunisova, S. and Valasek, L.S. (2017) In vivo evidence that eIF3 stays bound to ribosomes elongating and terminating on short upstream ORFs to promote reinitiation. *Nucleic Acids Res.* **45**, 2658–2674 https://doi.org/10.1093/nar/gkx049

65 Isken, O., Kim, Y.K., Hosoda, N., Mayeur, G.L., Hershey, J.W. and Maquat, L.E. (2008) Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay. *Cell* **133**, 314–327 https://doi.org/10.1016/j.cell.2008.02.030

66 Morris, C., Wittmann, J., Jäck, H.-M. and Jalinot, P. (2007) Human INT6/eIF3e is required for nonsense-mediated mRNA decay. *EMBO Rep.* **8**, 596–602 https://doi.org/10.1038/sj.embor.7400955

67 Flury, V., Restuccia, U., Bachi, A. and Mühlemann, O. (2014) Characterization of phosphorylation- and RNA-dependent UPF1 interactors by quantitative proteomics. *J. Proteome Res.* **13**, 3038–3053 https://doi.org/10.1021/pr5002143

68 Ghosh, S., Ganesan, R., Amrani, N. and Jacobson, A. (2010) Translational competence of ribosomes released from a premature termination codon is modulated by NMD factors. *RNA* **16**, 1832–1847 https://doi.org/10.1261/rna.1987710

69 Mendell, J.T., Medghalchi, S.M., Lake, R.G., Noensie, E.N. and Dietz, H.C. (2000) Novel Upf2p orthologues suggest a functional link between translation initiation and nonsense surveillance complexes. *Mol. Cell. Biol.* **20**, 8944–8957 https://doi.org/10.1128/MCB.20.23.8944-8957.2000

70 Muhlrad, D. and Parker, R. (1999) Aberrant mRNAs with extended 3' UTRs are substrates for rapid degradation by mRNA surveillance. *RNA* **5**, 1299–1307 https://doi.org/10.1017/S1355838299990829

71 Bühler, M., Steiner, S., Mohn, F., Paillusson, A. and Mühlemann, O. (2006) EJC-independent degradation of nonsense immunoglobulin-mu mRNA depends on 3' UTR length. *Nat. Struct. Mol. Biol.* **13**, 462–464 https://doi.org/10.1038/nsmb1081

72 Ge, Z., Quek, B.L., Beemon, K.L. and Hogg, J.R. (2016) Polypyrimidine tract binding protein 1 protects mRNAs from recognition by the nonsense-mediated mRNA decay pathway. *eLife* **5**, e11155 https://doi.org/10.7554/eLife.11155

73 Serdar, L.D., Whiteside, D.L. and Baker, K.E. (2016) ATP hydrolysis by UPF1 is required for efficient translation termination at premature stop codons. *Nat. Commun.* **7**, 14021 https://doi.org/10.1038/ncomms14021

74 He, F. and Jacobson, A. (2015) Nonsense-mediated mRNA decay: degradation of defective transcripts is only part of the story. *Annu. Rev. Genet.* **49**, 339–366 https://doi.org/10.1146/annurev-genet-112414-054639

75 Lykke-Andersen, J. and Bennett, E.J. (2014) Protecting the proteome: eukaryotic cotranslational quality control pathways. *J. Cell Biol.* **204**, 467–476 https://doi.org/10.1083/jcb.201311103

76 Kadlec, J., Guilligay, D., Ravelli, R.B. and Cusack, S. (2006) Crystal structure of the UPF2-interacting domain of nonsense-mediated mRNA decay factor UPF1. *RNA* **12**, 1817–1824 https://doi.org/10.1261/rna.177606

77 Takahashi, S., Araki, Y., Ohya, Y., Sakuno, T., Hoshino, S., Kontani, K. et al. (2008) Upf1 potentially serves as a RING-related E3 ubiquitin ligase via its association with Upf3 in yeast. *RNA* **14**, 1950–1958 https://doi.org/10.1261/rna.536308

78 Verma, R., Oania, R.S., Kolawa, N.J. and Deshaies, R.J. (2013) Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome. *eLife* **2**, e00308 https://doi.org/10.7554/eLife.00308

79 Kuroha, K., Tatematsu, T. and Inada, T. (2009) Upf1 stimulates degradation of the product derived from aberrant messenger RNA containing a specific nonsense mutation by the proteasome. *EMBO Rep.* **10**, 1265–1271 https://doi.org/10.1038/embor.2009.200

80 Kuroha, K., Ando, K., Nakagawa, R. and Inada, T. (2013) The Upf factor complex interacts with aberrant products derived from mRNAs containing a premature termination codon and facilitates their proteasomal degradation. *J. Biol. Chem.* **288**, 28630–28640 https://doi.org/10.1074/jbc.M113.460691

81 Brannan, K.W., Jin, W., Huelga, S.C., Banks, C.A., Gilmore, J.M., Florens, L. et al. (2016) SONAR discovers RNA-binding proteins from analysis of large-scale protein-protein interactomes. *Mol. Cell* **64**, 282–293 https://doi.org/10.1016/j.molcel.2016.09.003

82 Feng, Q., Jagannathan, S. and Bradley, R.K. (2017) The RNA surveillance factor UPF1 represses myogenesis via its E3 ubiquitin ligase activity. *Mol. Cell* **67**, 239–251.e6 https://doi.org/10.1016/j.molcel.2017.05.034

83 Schweingruber, C., Rufener, S., Zünd, D., Yamashita, A. and Mühlemann, O. (2013) Nonsense-mediated mRNA decay – mechanisms of substrate mRNA recognition and degradation in mammalian cells. *Biochim. Biophys. Acta* 1829, 612–623 https://doi.org/10.1016/j.bbagrm.2013.02.005