The meaning of NMD: translate or perish

Brogna, Saverio; McLeod, Tina; Petric, Marija

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Saverio Brogna, Tina McLeod and Marija Petric

University of Birmingham
School of Biosciences
Edgbaston
Birmingham, B15 2TT
UK

Corresponding author
s.brogna@bham.ac.uk
Tel. +44 121 414 5569

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Premature translation termination leads to a reduced mRNA level in all types of organisms. In eukaryotes, the phenomenon is known as nonsense-mediated mRNA decay (NMD). This is commonly regarded as the output of a specific surveillance and destruction mechanism that is activated by the presence of a premature translation termination codon (PTC) in an atypical sequence context. Despite two decades of research, it is still unclear how NMD discriminates between PTCs and normal stop codons. We suggest that cells do not possess any such mechanism and instead propose a new model in which this mRNA depletion is a consequence of the appearance of long tracts of mRNA that are unprotected by scanning ribosomes.

NMD and gene expression

Gene expression, the process that decodes the DNA sequence into specific RNAs and proteins, is characteristically complex in eukaryotes. In addition to the multitude of mechanisms that regulate transcription and pre-mRNA processing, accurate and robust gene expression depends on mechanisms that link these nuclear processes with translation and mRNA turnover [1]. This interplay is most obvious in nonsense-mediated mRNA decay (NMD), a mechanism believed to degrade mRNAs that harbor a premature translation termination codon (PTC). PTCs can arise from various causes: genetic mutation, inaccurate transcription and, more frequently, unproductive pre-mRNA splicing. The expression of 5-30% of the genome, depending on the organism, is affected by NMD [2]. For example, upon suppression of NMD, the level of alternatively spliced mRNAs predicted to encode a PTC increases in all organisms, from yeast to humans [3-11]. This selective degradation of alternatively spliced (AS) mRNA (referred to as AS-NMD) has been proposed to be an evolutionarily conserved means of regulating gene expression [12, 13]. NMD seems to compensate for the inefficient splicing of introns with weak splice sites; it has therefore been suggested that NMD is an important gene expression quality control mechanism that might have co-evolved with the acquisition of introns early in the Eukarya lineage [14-16], possibly in parallel with the origin of the nucleus [17]. In spite of the consensus that NMD is the function of a specific biochemical pathway that has been selected for by evolution, its mechanisms vary extensively and are not well-understood in any organism. Here we critically review current NMD models and discuss the significance of mRNA surveillance in general. Our conclusion is that cells may not require such an mRNA surveillance mechanism or even possess a PTC recognition mechanism(s). We offer a new model based on the idea that NMD is primarily a passive consequence of either ribosome release following premature translation termination or low ribosome occupancy of NMD-sensitive transcripts.

NMD and the emergence of the mRNA surveillance concept

Nonsense mutations are nucleotide substitutions that change a coding triplet into one of the three translation stop codons, UAG, UAA and UGA. These, and other mutations that indirectly lead to premature translation termination, are thus expected to produce truncated polypeptides. However, this is not always the

case as some alleles encoding PTCs produce only very low mRNA levels. It is perhaps partly for this reason, and partly due to proteasome activity, that truncated proteins are not detectable [18]. This feature of gene expression was first observed in the early days of molecular biology in *Escherichia coli*, when this class of mutation was key to the deciphering of the genetic code [19, 20]. In bacteria, these low mRNA concentrations can be attributed either to a non-specific mechanism, in which mRNAs that are not shielded by translating ribosomes become more susceptible to cleavage by RNase E [21, 22], or to premature transcription termination, possibly due to loss of contact between RNA polymerase and the first ribosome trailing on the nascent transcript [23]. Depletion of mRNA is, therefore, the result of reduced transcription or instability of the transcript in *E. coli*.

Comparable effects of nonsense mutations on mRNA levels in eukaryotes were first observed in the late 1970s in yeast and humans [24-26]. This mRNA reduction was initially attributed to cytoplasmic instability caused by a lack of ribosome shielding [24, 26]. However, the present view is that it is the function of a specialized mRNA surveillance mechanism that distinguishes between PTCs and normal stop codons, and triggers rapid mRNA degradation following premature translation termination. This mRNA surveillance hypothesis was first put forward by studies in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*, which discovered that specific proteins might be required for the accelerated breakdown of mRNAs harboring a PTC [27, 28]. Mutations in these proteins were first identified as either suppressors or co-suppressors of nonsense and certain other mutations that affect translation. These were named *upf* in yeast, since they enhanced the activity of an up frameshift tRNA suppressor [29], reviewed in [30], and *smg* in *C. elegans*, for suppressor with morphogenetic effect on genitalia, due to an additional phenotype observed in mutant worms [31]. Notably, the *C. elegans* study also reported that some recessive nonsense mutations in a myosin heavy chain gene (*unc-54*) become dominant in *smg(-)* mutant genetic backgrounds, causing paralysis of the animal due to muscle abnormalities. This is likely due to the stabilization of the mutant *unc-54* mRNA, resulting in the production of truncated myosin polypeptides that interfere with the formation of a functional myosin dimer [28, 32]. These observations engendered the concept

that eukaryotes have evolved an mRNA surveillance or quality control mechanism in which the coordinated action of a set of specific proteins distinguishes PTCs from regular stop codons. This triggers the destruction of aberrant mRNAs that would produce wasteful and potentially toxic peptides if translated. The mechanism was termed nonsense-mediated mRNA decay by a set of studies in yeast [7, 33], one of which also proposed that unspliced pre-mRNA may represent a major source of endogenous NMD substrates [7]. (Two major reviews were published shortly after [34, 35], while the acronym NMD first appeared in a later study [36].) NMD has since been intensively studied in several model eukaryotic organisms, yet as we discuss below, none of the standard NMD models provides a satisfactory description of the process.

Standard NMD models: the surveillance machinery and the hypotheses of a PTC recognition mechanism

NMD is thought to be the joint function of several conserved proteins that act in the same biochemical pathway, of which UPF1, UPF2 and UPF3 are the most conserved. These proteins interact *in vitro* and are thought to form a trimeric complex upon recognition of NMD substrates [37]. This complex was interpreted to represent the ancestral core of the NMD machinery, which is required for both PTC recognition and activation of rapid mRNA degradation across eukaryotes. Despite there being examples of NMD occurring in the absence of these proteins, and NMD suppression taking place when other proteins with no functional connection to the UPFs are depleted [38, 39], the consensus is that NMD is the output of the coordinated actions of the UPFs, and, in animals and plants, several additional proteins that regulate their function [37, 40, 41]. Below we discuss the different models that have been proposed so far to explain the nature and the function of this putative mRNA surveillance machinery.

The DSE model

The first hypothesis to explain how the NMD machinery could discriminate between PTCs and normal stop codons proposed that they are distinguished post-translation termination by the presence of one or more downstream sequence elements (DSEs). These sequences were predicted to occur more frequently in coding regions than in 3’ untranslated regions (3’UTRs) [42, 43].

This model also explains the observation in *S. cerevisiae* that NMD is most apparent the further upstream a PTC is from the normal stop codon, since the earlier a PTC is in the mRNA, the higher the frequency is of a functional DSE motif occurring by chance in the lengthened mutant 3' UTR. The model proposed that an NMD or surveillance complex, including UPF1, UPF2 and UPF3, assembles following termination and scans for a DSE in *S. cerevisiae*. The RNA binding protein, HRP1, was subsequently shown to interact specifically with both the putative DSE *in vitro* and with UPF1 in *S. cerevisiae* [44]. HRP1 is involved in pre-mRNA 3' end processing and transcription termination, and is known to shuttle between the nucleus and the cytoplasm [45-47]. The initial DSE model therefore envisaged that HRP1 might bind the DSE in the nucleus, remaining bound to the mRNA after its export to the cytoplasm. An interaction between UPF1 and DSE-bound HRP1 was thought to identify the mRNA as an NMD substrate, initiating rapid destruction of the mRNA by promoting the recruitment of the decay factors (Figure 1A). The DSE model was largely abandoned when it became apparent that there is no similarity between putative DSEs identified in different mRNAs [36]. However, the derived concept, that NMD-inducing PTCs are distinguished from normal stop codons by a downstream mark after termination, is the basis of current models, particularly in mammalian cells.

The EJC model

As in the yeast DSE model, the EJC (Exon Junction Complex) model also predicts that PTCs are distinguished from normal stop codons by the presence of a downstream signal, in this case a splice junction. The link between splicing and NMD was first reported in mammalian cells, where PTCs often induce strong NMD only when they are located upstream of at least one splice junction [48-50], specifically, at least 50-55 bases upstream of the last junction (introns are infrequent in 3'UTRs) [51]. As splice junctions are highly degenerate sequences, it was unclear at first how they are recognized. The answer came with the discovery of the EJC, a multiprotein complex that is deposited on the mRNA during splicing in the nucleus, and which remains associated with exon junctions during export to the cytoplasm [52, 53]. A core of three proteins, eIF4AIII, Y14 and MAGO, associates with several additional proteins, including UPF2 and

UPF3, to form the EJC [54, 55]. While the precise mechanism remains undefined, the current model (Figure 1B) predicts that SMG1 (a UPF1 kinase) and UPF1 are recruited to all terminating ribosomes, where they form a complex with the eukaryotic release factors (this complex is termed SURF) [56]. Since the EJC core complex binds UPF3 *in vitro*, the prediction is that UPF2, by binding both UPF1 and UPF3, forms a physical bridge between the SURF complex and the EJC when translation termination occurs upstream of an exon-exon junction; this interaction stimulates the helicase activity of UPF1 and the accelerated destruction of the mRNA [55, 56] (reviewed recently in [57]). It has also been proposed that PTC recognition and NMD occur during the first (pioneer) round of translation, while the mRNA is still associated with EJCs and the nuclear cap binding complex (CBC) [58]; however, others have reported that NMD is not restricted to CBC-bound mRNAs in mammalian cells [59, 60].

The EJC model provides a logical explanation as to how splicing can affect NMD not only in mammalian cells, but also in other organisms [61-63]. However, as we discussed previously, there are several long-standing, as well as some recent, observations that the EJC model cannot explain [39]. One such observation is that UPF2 can interact with the SURF, thereby potentially activating UPF1, independently of UPF3 and the EJC in human cells [64], which strongly contradicts the classic SURF-EJC model [56]. More puzzling is the finding that in *Schizosaccharomyces pombe* an intron enhances NMD regardless of whether it is placed before or after the PTC, and that the proteins that form the core of the EJC are not required for such splicing-dependent NMD [8]. Therefore, the means by which splicing affects translation and NMD remains largely unsatisfactorily understood.

The faux *3'-UTR model*

As demonstrated by the early studies in *S. cerevisiae*, NMD can take place in intronless genes. Moreover, it can also occur in the absence of an intron downstream of the PTC in mammalian cells, as well as in other organisms [8, 65-67]. The presence of a downstream splice junction therefore cannot be an evolutionarily conserved second signal that is essential for distinguishing PTCs from normal stop codons during translation.

Presently, the prevalent NMD model is based on the idea that termination at PTCs is biochemically different from that at normal stop codons, because it takes place far from the normal 3'UTR or 3' end of the mRNA [68]. This so-called "faux 3'-UTR" model (Figure 1C) was initially built on observations in *S. cerevisiae* [65], but appears to also apply to other organisms [66, 67, 69, 70]. Cytoplasmic poly(A) binding protein (PABPC), which binds the mRNA poly (A) tail, was found to interact with eukaryotic release factor 3 (eRF3) which associates with the terminating ribosome [71, 72]. Release factor eRF3 forms a complex with eRF1 (which binds the ribosome A site and recognizes all three stop codons in eukaryotes), triggering the release of the polypeptide [73]. The interaction of PABPC with eRF3 stimulates translation termination [72] and was proposed to couple termination and global mRNA decay [74]. In particular, the faux 3'-UTR model proposed that in the absence of an interaction with PABPC, either termination or the release of the ribosome from the mRNA is delayed. This triggers the recruitment or activation of NMD-inducing factors such as UPF1 in place of PABPC, therefore diverting the mRNA for rapid destruction [65]. Numerous reports that UPF1 interacts with eRF1 and eRF3 (reviewed in [37]) are consistent with this model, and deletion of UPF1 and other UPF proteins promotes translation read-through independently of mRNA levels [43, 75, 76]. However this effect on termination appears in part to be indirect in *S. cerevisiae* [77].

The faux 3'-UTR model rationalizes many features of NMD across organisms, such as NMD being more apparent when the PTC is further away from the 3' end, yet there are numerous observations (which we have previously discussed in detail [39]) that it cannot account for. In direct contrast to one of the model's key predictions, that PTCs closer to the 3' end escape NMD because the terminating ribosome is able to interact with PABPC, recent studies reported that the interaction between eRF3 and PABPC is not the means by which mRNAs are stabilized when PABPC is artificially tethered downstream of NMD-inducing PTCs [78, 79]. This interaction appears to affect translation termination but neither general mRNA decay nor NMD in *S. cerevisiae* [80]. Additionally, depletion of PABPC does not substantially change the pattern of NMD in *S.*

pombe, where PTCs early in the coding region lead to more apparent NMD than PTCs closer to the normal stop codon, regardless of the presence of PABPC [8].

It can be argued that 3'UTR proteins other than PABPC are required for efficient termination. This may be the case, but was not envisaged by the faux 3'-UTR model. Moreover, recent studies also seem to invalidate the model's key prediction that UPF1 should selectively associate with mRNAs subjected to NMD. UPF1 was shown to bind mRNAs regardless of whether or not they are affected by NMD, and more generally, no correlation with the position of the stop codon was identified [81-83]. These latter studies contrast a number of others that concluded that more UPF1 binds NMD-sensitive transcripts [84-87]. The latest of these reported that while UPF1 can dynamically bind any mRNA, its ATP-ase activity is specifically inhibited on NMD substrates [84] slowing its dissociation and therefore activating their decay, however, the mechanism by which this target discrimination is achieved remains vague.

NMD might not require any specific PTC recognition mechanism

The ribosome-release model

As we have outlined above, none of the NMD models proposed to date can explain PTC discrimination satisfactorily in any organism. While this might signify that different eukaryotes have evolved diverse PTC discrimination mechanisms, the alternative is that cells do not have such a mechanism at all. Is NMD simply the passive consequence of translation terminating prematurely and the ribosomal subunits being released from the mRNA? Following stop codon recognition and release of the nascent peptide, the post-termination ribosome is recycled for a new round of translation. Recycling comprises two key steps, splitting of the ribosome into its subunits and release of deacetylated tRNA and mRNA [88, 89]. The process requires the activity of a number of proteins, including translation initiation factor eIF3. Based initially on observations that mutations in eIF3 subunits can suppress NMD, as well as on evidence for a physical interaction between eIF3 and UPF1 [90-92], we have previously proposed that NMD might be caused by the release of ribosomes from the mRNA [39]. Our model predicts that when termination occurs at an early position in the coding region, the mRNA becomes unstable, simply because the downstream

sequence remains unprotected by the standard set of translating ribosomes (Figure 2, Key Figure). Our proposal is that in cells without UPF1, or possibly other NMD factors, the mRNA remains densely loaded with ribosomes or ribosomal subunits. These would passively suppress NMD by shielding the transcript from non-specific nuclease attack, thereby preventing activation of general mRNA destruction pathways, which typically involve 5' end decapping [93, 94].

Consistent with this model, mRNAs which are translated by a single ribosome (monosomes) are more sensitive to NMD than mRNAs that are simultaneously translated by several ribosomes (polysomes) in *S. cerevisiae* [95]. Additionally, it was reported that in fact more ribosomes engage with PTC-containing mRNAs in cells lacking UPF1 [96, 97]. While this could be interpreted as evidence that NMD, similar to general mRNA decay, occurs co-translationally [93, 94, 96], the data are also consistent with our model, that in the absence of UPF1, ribosomes, or ribosomal subunits, remain associated with the 3'UTR. Notably, the possibility that ribosomes might passively stabilize the mRNA by migrating downstream of the PTC in the absence of UPF1, was examined by one of the early UPF1 studies in *S. cerevisiae,* but it was dismissed because the distribution of the mRNA which was tested shifted toward heavier polysomal fractions by only a small extent in a strain lacking UPF1 [33]. However, in view of the recent observations discussed above, this small shift might be significant and consistent with the prediction of our model.

Moreover, a lack of UPF1 inhibits recycling of post-termination ribosomes in *S. cerevisiae* [98]. UPF1 might be recruited to the 3'UTR via its interaction with the terminating ribosome or bind mRNAs directly, as discussed, or associate with the ribosome during translation initiation, via direct association with ribosomal proteins [99]. In any case, once bound to the RNA, its ATP-ase activity could promote release of unstable post-termination ribosomes, and also remove associated proteins and resolve RNA secondary structures [100, 101]. The UPF1-like helicase MOV10 might also contribute to this function in mammalian cells [83]. One function of UPF1 could be to prevent translation re-initiation downstream of the stop codon. Translation re-initiation is a feature that can, at

least partially, suppress NMD of mRNAs with a PTC located upstream of sequences that drive re-initiation [102, 103]. Notably, mutations in UPF1 and UPF3 were also isolated in early genetic screens as suppressors of a mutation that introduces an upstream reading frame (uORF) in a *S. cerevisiae* gene; the suppression mechanism probably consisted of an increased re-initiation rate downstream of the uORF [104, 105]. It is therefore possible that NMD is not the output of a specific mechanism which, either at or post-termination, distinguishes stop codons. Instead, it may be the result of the passive destabilization of an mRNA due to the release of ribosomes, ribosomal subunits, or stably associated RNA binding proteins (RBPs) – the latter may account for why many endogenous mRNAs with long 3'UTRs are not affected by UPF1 depletion [106, 107].

There are observations that seemingly disprove our model. For example, it has long been known in the NMD field that blocking ribosome scanning, by inserting a secondary structure in the 5'UTR, does not destabilize the mRNA, in spite of inhibiting translation [108]. We propose that transcripts are intrinsically stable until translation initiation starts removing the RBPs that shield the RNA. UPF1, as well as other helicases involved in translation initiation [109], might be responsible for the unfolding of the mRNP. However, future experiments may reveal that there are not more ribosomes loaded on the mRNA in UPF1 depleted cells, which would disprove this model.

What is the function of NMD?

Does NMD function as an mRNA surveillance mechanism in order to remove aberrant mRNAs? The initial interpretation, that NMD is a specific mRNA surveillance mechanism that destroys mRNAs of nonsense alleles, is questionable, since nonsense mutations are rare, typically inactivate the gene product, and as such will be subjected to negative natural selection. The proposal that NMD might have evolved to remove unspliced pre-mRNAs that would otherwise produce potentially toxic truncated proteins, is more plausible. However, there is no experimental evidence that the small fraction of mRNAs that fail to be spliced can be efficiently translated into toxic, or even stable, peptides without first inactivating the proteasome [18]. On the contrary, it has been reported that there is no correlation between transcript and protein changes

attributable to NMD in HeLa cells [110]. Additionally, while a lack of NMD increases pre-mRNA levels, it does not affect viability of either *S. cerevisiae* or *S. pombe* in laboratory conditions [8, 37], and, as mentioned, has only a mild specific developmental phenotype in *C. elegans* [31, 111]. Most introns are located only a few nucleotides from the AUG in *S. cerevisiae*; if translated, the pre-mRNA would produce short peptides that are unlikely to form toxic interactions with other proteins. Therefore, producing a defective dimer with the wild-type copy of the protein, as reported in *C. elegans* for some alleles of the myosin heavy chain gene in *smg* mutants [28], would be improbable. It was also envisaged that NMD increases the efficiency of gene processing by avoiding energetically wasteful translation of non-functional transcripts. These can be generated not only by inaccurate pre-mRNA processing, as discussed, but also by inaccurate selection of transcription start sites, as recently proposed in *S. cerevisiae* [112]. However, eukaryotic gene expression does not necessarily follow the most energetically efficient path, as exemplified by the fact that many essential genes remain functionally expressed when their introns are artificially removed in *S. cerevisiae* [113].

NMD factors are essential for the viability of many organisms [61, 63, 114] though it is not yet clear whether these effects are due to the global suppression of NMD. It is possible that lethality is due to the mis-expression of one or more essential genes in some, but not necessarily other, NMD mutants. For example, although mutations that inactivate UPF1, UPF2 and UPF3 all suppress global NMD in *Drosophila* [107, 115], loss of function of UPF1 and UPF2 results in embryonic lethality, but UPF3 is not essential for either development or fly viability [116]. These observations were interpreted as evidence that UPF3 is not required for directing the subset of specific transcripts involved in fly development towards NMD [116]. However, they could indicate that global NMD in itself does not have an important function for the organism, or that these factors have other functions unrelated to NMD. Remarkably, the lethality of *Drosophila* UPF1 and UPF2 mutants may be caused by the overexpression of a single gene [117], out of hundreds of predicted targets [107].

NMD was reported to be important for cellular differentiation [118-121]. Depletion of UPF2 in mouse hematopoietic cells resulted in depletion of hematopoietic stem and progenitor cells but only mildly affected differentiated cell numbers [120]. This phenotype could be attributed to the toxic accumulation of PTC-containing mRNAs of cell receptor and immunoglobulin genes, as, during normal maturation of T- and B-cells, these genes undergo somatic rearrangements that frequently introduce NMD-inducing PTC mutations [122]. The interpretation was that NMD is essential for survival of proliferating cells, however, it remains possible that these cells are primarily depleted due to the lack of a functional T-receptor on the cell membrane [123]. It remains to be investigated whether deletion of UPF1 and other NMD factors produce similar phenotypes. In summary, while UPF1, UPF2 and UPF3 are important, which is also underscored by their conservation across eukaryotes, they do not necessarily function to identify and target specific mRNAs for destruction. Whether the reason for this evolutionary conservation is, as we proposed, their function in promoting efficient release of ribosomes from the mRNA, will need to be investigated by future studies.

Concluding remarks and future perspective

While the consensus is that NMD functions as a specific mRNA decay pathway that targets transcripts which encode a PTC, or possess other NMD inducing features such as long 3'UTRs, uORFa and a downstream splice junction [2], it is becoming increasingly apparent that NMD affects many more transcripts than initially envisaged. There is little overlap between the lists of putative targets identified in different organisms or cell-types, and many have no shared features [106, 107, 124]. We discussed the possibility that NMD is not the output of a specific biochemical process - see also [39], but rather is the passive consequence of ribosome release following translation termination (Figure 2, Key Figure). The fact that NMD is seemingly suppressed by depletion of specific proteins does not necessarily imply that these proteins are the effectors of NMD. NMD-like phenomena clearly occur in bacteria, which have no UPF proteins or other NMD-machinery, and UPF1 is not required for NMD in trypanosomes [125]. Additionally, deletion of UPF1 and UPF2 does not completely suppress NMD in fission yeast [8]; and there are reports of non-specific mRNA stabilization in NMD

mutants of different organisms, in which mRNA levels can rise above those in wild-type cells (discussed in [39].

Whether a transcript is affected by NMD could be mainly a consequence of multiple features, some of which are either acquired fortuitously during gene evolution or arise due to inaccurate transcription or pre-mRNA processing. The result of these may be slow translation initiation [126] leading to the mRNA being translated only by a single ribosome [95]. However, codon usage, which is a global determinant of mRNA levels [127, 128], shows no significant correlation with NMD [95, 126]. In any case, organisms might not need such an mRNA surveillance mechanism: gene features conducive to NMD, such as weak splice signals, if detrimental to gene function, would be expected to be removed through the generations by natural selection.

Despite this, NMD remains an important feature of eukaryotic gene expression. Understanding its different causes will be important for gaining a more accurate understanding of gene expression and developing new treatments for particular genetic disorders arising from PTCs [129-131]. Depletion of UPF1 in particular appears to stabilize transcripts of genes required for resistance to different cellular stresses [117, 132-134]. Understanding the NMD phenomenon therefore might also allow its manipulation in diseases such as cancer, in which tumor progression relies on the regulation of stress-response mechanisms [134]. Many open questions still remain (see Outstanding Questions Box); one key point will be to understand how UPF1 affects ribosome release, and whether other NMD factors have a similar effect on translation. Additionally, UPF1 and the other so-called NMD factors might have other functions independent of translation and NMD in the cytoplasm. There is evidence, for instance, that UPF1 has a role in nuclear processes, which raises the possibility that it is these additional functions that make the proteins necessary for survival in some organisms [135]. Finally, in light of observations that nonsense mutations can impinge on co-transcriptional processes, leading to reduced production of the corresponding mRNA, the issue of whether NMD can occur in the nucleus will need to be re-investigated [70, 136-139]. A recent study concluded that in some instances NMD may wrongly appear to be nuclear in mammalian cells because PTC-containing mRNAs are degraded

within seconds of them reaching the cytoplasm, while still associated with the nucleus [108, 140]. However there is new evidence that the ribosome can translate nuclear RNA in human cells [137, 141], and also nascent transcripts in *Drosophila* [142]. NMD might therefore be the compounded effect of releasing ribosomes from both processed and nascent transcripts, the former leading to instability of the mature mRNA while the latter may reduce its production [143], as in bacteria. The presence of translating ribosomes within the nucleus can potentially change not only our understanding of NMD, since it could explain how NMD is enhanced by pre-mRNA splicing, but also that of eukaryotic molecular biology in general.

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Figure legends

Figure 1. Current NMD models. (A) DSE model. Translation termination occurs normally at a PTC. Following peptide release (in red), a surveillance complex comprising UPF1, UPF2 and UPF3 (depicted by the blue car) assembles downstream and scans the 3'UTR. If a downstream sequence element (DSE) is encountered, the interaction between UPF1 and HRP1 bound to the DSE identifies the stop codon as premature, triggering rapid mRNA decay. (B) EJC model. Translation termination occurs normally at a PTC. A SURF complex, consisting of SMG1, UPF1, eRF1 and eRF3, assembles upon termination. SURF association with a downstream EJC identifies that termination is premature and induces mRNA decay. (C) Faux 3'UTR model. Translation termination occurs abnormally at a PTC. Ribosomes terminating in proximity of the poly(A) tail interact with PABPC via eRF3, which leads to normal termination. Early termination, distant from the 3' end, is abnormal because it precludes this interaction with PABPC (heat map shows its highest concentration in red), instead inducing the recruitment of NMD factors to the terminating ribosome. The dashed line represents a putative interaction between the 5'-cap and PABPC predicted to keep the mRNA in a closed-loop conformation. The E, P and A sites are the tRNA binding sites on the ribosome.

Figure 2. The NMD ribosome release model. Translation termination occurs normally at a PTC. Ribosomal subunits remain joined and associated with mRNA after nascent peptide release (in red). Such post-termination ribosomes can migrate, possibly bidirectionally, towards codons capable of pairing with the anticodon of the deacetylated tRNA, which remained in the P site. UPF1 is recruited to terminating ribosomes from which it moves onto the downstream sequence. It then, possibly by interacting with eIF3, facilitates dissociation of non-translating post-termination ribosomes or ribosomal subunits which have migrated along the 3'UTR. The process might also release otherwise stably associated different RNA binding proteins (depicted by small circles). If translation terminates prematurely, the downstream region of the mRNA remains therefore exposed and accessible to decay factors, which leads to its rapid degradation. In the absence of UPF1 (bottom

diagram) post-termination ribosomes are not efficiently released and continue to migrate downstream, shielding (together with residual RNA-binding proteins) the mRNA from degradation.

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The Trends box

Mutations that interrupt translation reduce mRNA levels in all organisms studied to date.

It has long been thought that in eukaryotes this mRNA depletion is the function of a specific and evolutionarily conserved mRNA surveillance mechanism termed nonsense-mediated mRNA decay (NMD).

On the contrary, we argue that NMD is a passive consequence of ribosomes being prematurely released from the mRNA.

Low ribosome occupancy is the key determinant of NMD.

Outstanding Questions Box

What is the mechanism that drives association of UPF1 with mRNA and not other classes of RNA? Is UPF1 brought to mRNA by a direct interaction with the ribosome?

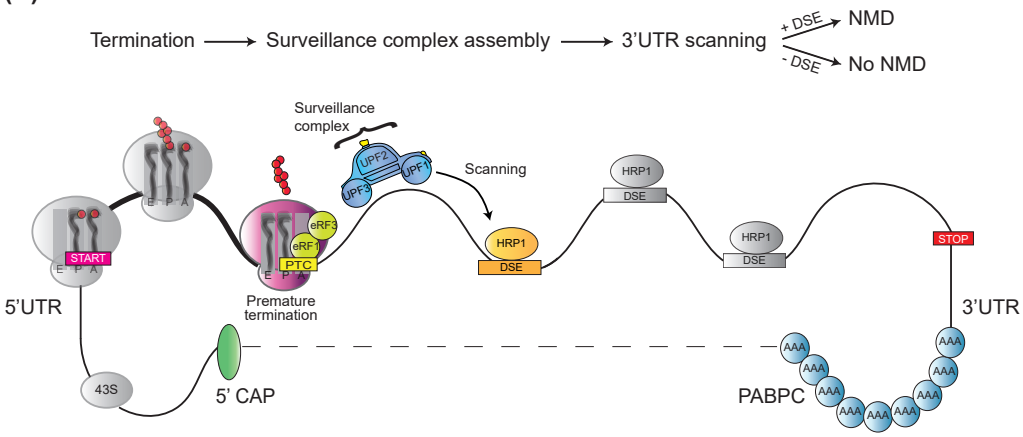
How does UPF1 affect ribosome release from the 3'UTR subsequent to translation termination, as well as ribosome occupancy in general?

Do UPF2 and UPF3 also affect ribosome occupancy? Is the function of UPF1 in ribosome release dependent on UPF2 and UPF3?

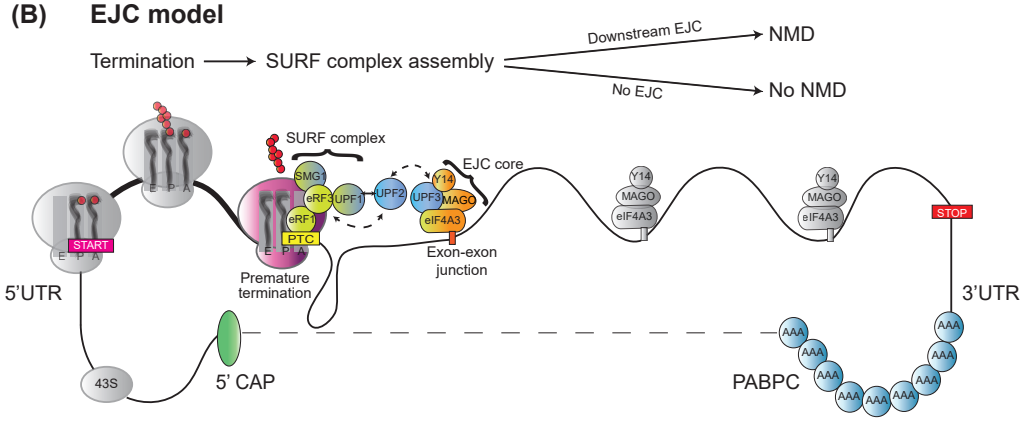
What is the mechanism that links pre-mRNA splicing to translation and NMD in the absence of the EJC?

Are mRNAs affected by NMD while still in the nucleus?

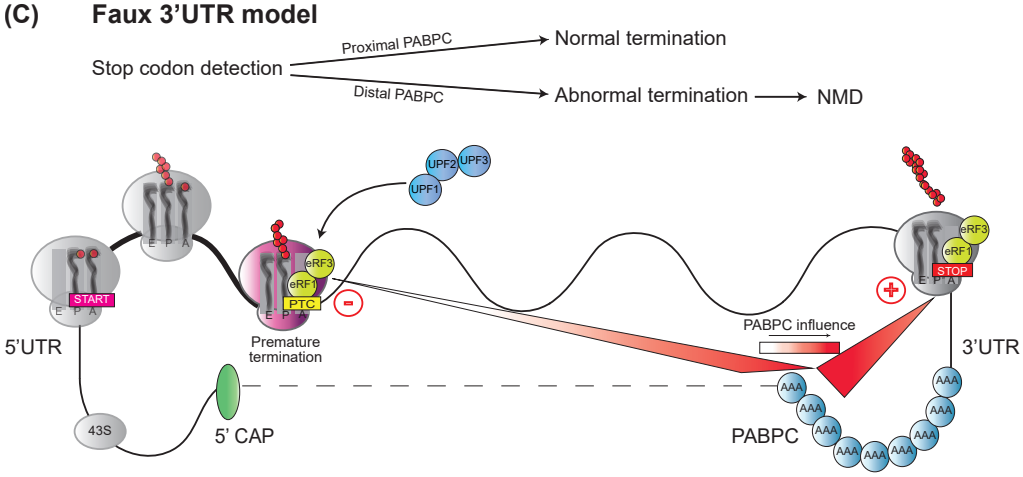
(A) DSE model



(B) EJC model

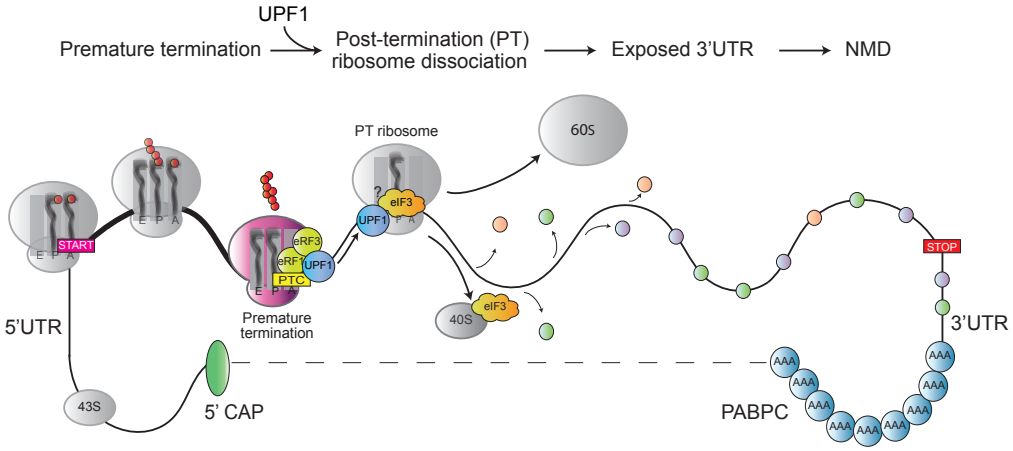


(C) Faux 3'UTR model



Release model

Wild type: *efficient ribosome release*



UPF1 null mutant*: inefficient ribosome release*