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Nonsense-mediated mRNA decay: terminating erroneous gene expression

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Nonsense-mediated mRNA decay is a surveillance pathway that reduces errors in gene expression by eliminating aberrant mRNAs that encode incomplete polypeptides. Recent experiments suggest a working model whereby premature and normal translation termination events are distinct as a consequence of the spatial relationship between the termination codon and mRNA binding proteins, a relationship partially established by nuclear pre-mRNA processing. Aberrant termination then leads to both translational repression and an increased susceptibility of the mRNA to multiple ribonucleases.

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Abbreviations

CBP	cap binding protein
DSE	downstream sequence element
EJC	exon–exon junction complex
mRNP	messenger ribonucleoprotein particle
NMD	nonsense-mediated mRNA decay
ORF	open reading frame
PARN	poly(A) ribonuclease
PTC	premature translation termination codon
RNP	ribonucleoprotein
UTR	untranslated region

Introduction

Eukaryotic cells exhibit quality-control mechanisms that recognize and degrade mRNAs that have not completed nuclear pre-mRNA processing or that fail to encode a polypeptide faithful to the gene sequence. Such aberrant mRNAs are degraded rapidly, presumably before the accumulation of deviant protein products that could have adverse effects on the cell. One such quality-control system is referred to as nonsense-mediated mRNA decay (NMD). Although the first mRNA substrates identified for NMD encoded premature translation termination codons (PTCs; see for example [1,2]), it is now appreciated that NMD degrades a variety of aberrant mRNAs that arise as a result of either mutations or defects in pre-mRNA processing. The common theme is that substrates of NMD manifest alterations in the normal spatial relationship between the termination codon and additional RNA features. For example, NMD substrates include transcripts with an upstream ORF in the 5′ UTR [3], extended 3′ UTRs (which alters the spatial relationship between the termination codon and the 3′ poly(A) tail [4–7]), bicistronic mRNAs [8••], and mRNAs with additional, and utilized, out-of-frame AUG codons, which lead to premature translation termination as a consequence of translational recoding or leaky ribosome scanning [9].

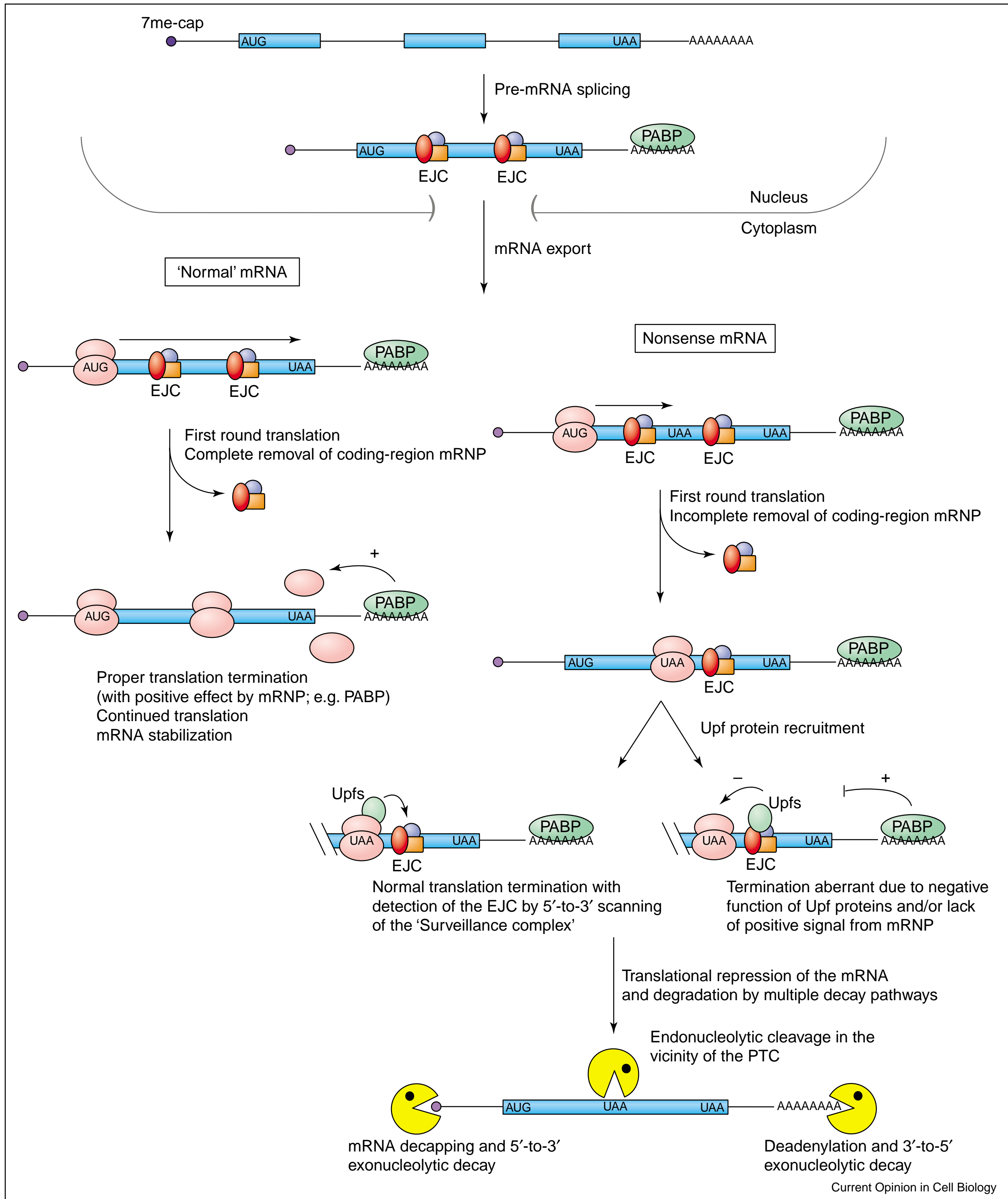
NMD can target a substantial part of the eukaryotic transcriptome and can be biologically important. For example, microarray experiments suggest that the levels of ~10% of yeast transcripts are affected by alterations in NMD [8••,10]. Furthermore, analysis of mammalian splicing suggests that possibly one-third of alternative splicing events produce transcripts that are substrates for NMD [11•,12]. The importance of mRNA surveillance is exemplified by the expression of truncated polypeptides that result in gain-of-function or dominant-negative mutant proteins having deleterious effects [13–15]. Therefore, it is consequential to understand how events surrounding translation termination contribute to the recognition of normal versus aberrant transcripts and how recognition is communicated into rapid degradation of the mRNA. In this review, we will present recent developments which provide insight into these molecular events that contribute to NMD.

Translational termination: distinct events for normal versus aberrant mRNAs

A critical distinction between premature, or aberrant, and normal translation termination appears to be a biochemical difference in the termination reaction itself; this difference then elicits downstream consequences for the fate of the mRNA. The possibility that termination events could be distinct was first suggested from evidence that a ribosome positioned at a termination codon can terminate, read through, or resume scanning after termination, depending on the termination context [16,17].

Evidence that NMD involves alterations in the termination process has come from the analysis of proteins that are essential for NMD, which include Upf1, Upf2 and Upf3 [18]. Upf1 protein has RNA-dependent ATPase and 5′–3′ helicase activities which are required for NMD and presumably promote an unknown RNA–RNA or ribonucleoprotein (RNP) rearrangement. Importantly, Upf

Figure 1



Models for nonsense-mediated decay of mRNA. Nuclear pre-mRNA processing events fashion mRNPs that are exported to the cytoplasm for translation. During the pioneer round of translation, ribosomes displace RNPs from the entire length of the coding region for wild-type mRNAs (left panel). The absence of certain RNPs and/or positive signals from remaining RNPs command proper translational termination, continued

proteins interact directly with the translation termination factors eRF1 and/or eRF3, providing a direct link between the termination complex and the NMD machinery [19,20]. Moreover, this interaction appears to be functionally important: *upf1Δ*, *upf2Δ* or *upf3Δ* strains show an increased frequency of nonsense suppression (i.e. translational readthrough) at termination codons in a variety of yeast genes [21,22]. These data suggest that Upf proteins cycle between a free state and an interaction with terminating ribosomes. Interestingly, in *C. elegans*, *Drosophila* and mammals, additional proteins are required for NMD [23]. These proteins are involved in catalyzing a cycle of Upf1 phosphorylation and dephosphorylation required for NMD [24,25,26•,27•,28••,29•]. It is proposed that brief phosphorylation of Upf1 occurs during translational termination, as PP2a, the Upf1 phosphatase, interacts with eRF1 [30]. In addition, Upf1 is hyperphosphorylated when associated with polysomes [31]. The phosphorylation cycle may modulate the ATPase or helicase activity of Upf1 and/or the remodeling of the ribosome-associated termination event [29•].

Compelling evidence that premature and normal translation termination events are distinct has come from a biochemical analysis of termination. A critical observation is that the ribosome toeprint generated at a normal translation termination codon is distinct from the toeprint of a ribosome terminating at a premature termination codon (Allan Jacobson, personal communication); moreover, this distinction is dependent upon the presence of Upf1p at the premature termination codon. These data suggest that premature translation termination events in the presence of Upf1p will be aberrant, whereas 'normal' termination proceeds in the absence of Upf function. Given this, an important question is what features of a messenger ribonucleoprotein particle (mRNP) define a translation termination event as premature versus normal.

Translation termination and organization of the mRNP

A critical determinant of whether an mRNA is subjected to NMD is the relationship between the translation termination codon and other mRNP features. In mammalian mRNAs, stop codons at distances greater than 50–55 nucleotides upstream from the 3′-most exon–exon junction are perceived as premature and lead to NMD [32–35]. The coupling between RNA splicing and NMD is dictated by the splicing-dependent deposition of a protein complex, referred to as the exon–exon junction complex (EJC), ~20–24 nucleotides upstream of the 5′ splice site following splicing [36–40]. Furthermore, an interaction between the EJC protein Y14 and Upf3 is required to trigger mRNA decay, thus providing a physical and functional link between the EJC and NMD [41••].

An mRNA can also be recognized as aberrant through mRNP features independent of an EJC, as is the case for several mammalian genes [42]. Moreover, in yeast and *Drosophila*, for example, the recognition of a PTC is independent of an intron and RNA splicing [28••,43,44]. In yeast, evidence suggests that specific sequences within the coding region, referred to as downstream sequence elements (DSEs), are required to trigger NMD when found 3′ of a translation termination codon [45]. Such elements have been proposed to bind Hrp1p, which in turn interacts with Upf1p, and which, like the EJC complex, may recruit additional NMD factors to the mRNA and thereby trigger decay [46]. However, there may be additional features of yeast mRNAs that distinguish normal and aberrant transcripts, as a nonsense codon downstream of a well-characterized DSE can still trigger NMD [47•]. An unresolved issue is whether the various mRNP features influence the nature of termination, or function to trigger NMD in a step following termination.

A popular model for NMD suggests that the first ribosome to translate the mRNA removes the EJC or other mRNP complexes from along the entire coding region, and that if such 'coding region' complexes remain on the mRNA following termination, NMD is activated. This is supported by the observation that translating ribosomes can remove the EJC component Y14 [48]. Evidence that EJC remodeling occurs during the first, or pioneer, round of translation includes observations that NMD reduces a pool of mRNAs bound to CBP80 and CBP20, components of the nuclear cap-binding complex [49,50]. Recently, eIF4AIII, a homologue of the translational initiation factor eIF4AI, was identified as a component of mammalian EJCs and as a protein also required for NMD in mammalian cells [51•,52]. Additionally, eIF4AIII is primarily a nuclear protein and interacts with eIF4G and eIF4B. It remains to be established, however, whether eIF4AIII provides a link between the first round of mRNA translation and NMD, or whether its role is more indirect.

Two divergent views have been put forth to explain how protein complexes remaining on the mRNP might trigger NMD (Figure 1). In one, ribosomes terminating at pre-

(Figure 1 Legend Continued) translation and mRNA stability. Failure of the ribosome to remove an RNP as a consequence of a PTC (right panel) may lead to either appropriate translational termination and 'Surveillance complex' scanning of the mRNA, or improper termination as a result of negative signals from the downstream RNP combined with the failure of other positive signals to be communicated to the ribosome. Interactions between an inappropriate RNP and the 'surveillance complex' or ribosome result in translational repression and rapid degradation involving multiple decay pathways.

mature stop codons deposit a Upf-containing protein complex, referred to as the 'Surveillance complex', on the mRNA; this protein complex scans 3′ for EJCs or DSEs and if one is encountered, NMD is triggered [53]. An alternative model suggests that the mRNP features mark the context of the normal termination codon in a manner that leads to proper termination and mRNA stabilization [17]. In the absence of such positive interactions, termination is aberrant and the mRNA is targeted for NMD.

Several observations suggest that the poly(A) tail and its associated binding protein, Pab1p, may constitute a positive factor that affects the nature of termination and the process of NMD. First, in yeast, the poly(A) tail is generally proximal to the termination codon and could provide contextual information. In addition, Pab1p interacts with eRF3, can influence the nature of translation termination, and may even promote ribosome recycling [54]. Similarly, in mammals the poly(A) tail might interact with the last EJC as a result of exon definition and thereby also provide an important mark that enhances the function of the mRNA. Consistent with the EJC and Upf proteins having positive roles in mRNA function, the presence of either an EJC or a Upf protein within the coding region serves to enhance mRNA 3′-end formation and increases translation yield of the mRNA [55,56,57••]. These data hint at the possibility that NMD might arise, at least in part, from a failure of the terminating ribosome to interact with Pab1p.

Recognition of aberrant mRNAs and downstream events

The recognition of an mRNA as aberrant by the NMD machinery leads to a series of changes in the behavior of the mRNA. Primarily, being recognized as nonsense-containing increases the susceptibility of an mRNA to multiple mRNA degradation systems. Two general degradation pathways for normal mRNAs have been identified in eukaryotic cells [58]. In both, decay of the mRNA begins with shortening of the 3′ poly(A) tail followed by 3′-to-5′ degradation and/or decapping and 5′-to-3′ degradation. In yeast, NMD triggers rapid deadenylation, removes the inhibition of decapping by the poly(A) tail, and accelerates the rate of decapping [47•,59]. These events result in the mRNA being degraded primarily by rapid deadenylation-independent decapping. However, when decapping and 5′-to-3′ decay is blocked, NMD promotes faster decay of mRNAs through the 3′-to-5′ decay pathway [47•,59,60•,61•]. Kinetic analysis provides evidence that NMD accelerates 3′-to-5′ decay primarily by a change in deadenylation rates, although there may be a small effect on the rate of 3′-to-5′ decay of the mRNA body [47•,61•].

The decapping and 3′-to-5′ decay pathways in mammals are also stimulated by NMD. For example, mammalian NMD increases the rate of deadenylation and decapping [62,63••,64]. In addition, depletion of the decapping enzyme, Dcp2, or of Rrp6p or PARN, proteins putatively required for 3′-to-5′ degradation of mRNAs, leads to an increase in the abundance of steady-state nonsense-containing mRNAs [63••]. With evidence that both the 5′-to-3′ and 3′-to-5′ degradation pathways can act separately on NMD substrates in yeast or mammals, the question arises of how defects in either pathway can cause a decrease in the decay of NMD substrates.

NMD can also target an mRNA for enhanced rates of endonucleolytic cleavage. For example, in erythrocytes, NMD can trigger faster susceptibility of the β-globin mRNA to cleavage by the PMR1-like endonuclease [65,66]. Thus, even though there exists conservation of the proteins required for NMD, there are several different mechanisms by which NMD accelerates RNA degradation.

The existence of multiple degradation pathways for NMD substrates indicates that NMD leads to dramatic changes in the status of the mRNA. An appealing possibility is that NMD leads to targeting of the mRNA to a non-translating pool of transcripts. Because translation factors and translation itself appear to protect mRNAs from destruction, this would generally increase the instability of the transcript. Consistent with this view, measuring the translation efficiency of nonsense-containing mRNAs demonstrated that Upf1p plays a role in repressing the translation of the mRNA, independent of mRNA degradation [7]. This argues that one consequence of NMD is to render the RNA translationally inactive and thereby susceptible to the various decay pathways.

Physical interactions between the NMD, translation and decay machineries

An emerging concept is that NMD leads to both translational repression and the recruitment of ribonucleases. The reduction in translation could be due to aberrant translation termination *per se*, or may be a more direct result of the function of the Upf proteins. For example, proper termination may enhance the efficiency of subsequent rounds of translation by promoting ribosome recycling to the initiation complex, whereas aberrant termination would fail to complete this function and lead to reduced translation. By contrast, the Upf2 protein has been found to interact physically with eIF4A and eIF1 [43]. Since mutations in the yeast eIF1 protein inhibit NMD [67], this interaction may be part of an active mechanism by which aberrant mRNAs are translationally repressed.

Several observations also provide evidence for a more direct interaction between the Upf proteins and the mRNA degradation machinery. For example, yeast Upf1p shows a strong two-hybrid interaction with Dcp2p,

a subunit of the decapping enzyme [68]. Similarly, mammalian decapping enzyme co-immunoprecipitates with Upf proteins [63••,69]. Biochemical interactions have also been detected between Upf1 and factors involved in 3′-to-5′ degradation of mRNAs. In mammalian cells, Upf1 can co-immunoprecipitate with components of the exosome, which is the nuclease complex responsible for 3′-to-5′ degradation of mRNAs [63••]. In addition, there exists a physical interaction between yeast Upf1p and Ski7p, an exosome-associated protein that may adapt the nuclease complex to different substrates [61•]. These physical interactions suggest that Upf proteins may play an additional, and more direct, role in recruiting nucleases to the mRNA.

Conclusions and future directions

Over the last few years there has been significant progress in understanding substrate recognition and in characterizing the protein players required for NMD. However, an increased comprehension of the molecular interactions between the Upf proteins, translational termination and initiation factors, and the decay machinery is needed to reveal the actual molecular mechanism of NMD. In addition, there are several broader issues that still require consideration. For example, where does NMD occur in the cell and what constraints does the location put on the mechanism? In mammalian cells, NMD can affect both nuclear and cytoplasmic mRNA levels, suggesting either that translation and NMD can occur in the nucleus, or that there is feedback following cytoplasmic NMD. In yeast, and possibly mammals, discrete cytoplasmic foci, referred to as P-bodies, have been identified, in which normal decapping and 5′-to-3′ degradation can occur [70•,71–73]. Does NMD also occur in P-bodies, and if so, what regulates formation or mRNA entrance into P-bodies? Similarly, it remains unclear how, in mammalian cells, the recognition of an mRNA as nonsense-containing can affect events in the nucleus including splicing, abundance of the nuclear mRNA pool, and retention of transcripts at the gene [74]. Understanding these issues should not only lead to insights into the process of NMD, but also reveal general properties of mRNA biology in eukaryotic cells.

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