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Recognition of nonsense mRNA: towards a unified model

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Abstract

Among the different cellular surveillance mechanisms that ensure accurate gene expression, nonsense-mediated mRNA decay rapidly degrades mRNAs harbouring PTCs (premature translation-termination codons) and thereby prevents the accumulation of potentially deleterious proteins with C-terminal truncations. In the present article, I review recent data from yeast, fluitflies, nematode worms and human cells and endeavour to merge these results into a unified model for recognition of nonsense mRNA. According to this model, the distinction between translation termination at PTCs and at 'normal' termination codons relies on the physical distance between the terminating ribosome and PABP [poly(A)-binding protein]. Correct translation termination is promoted by a PABP-mediated signal to the terminating ribosome, whereas the absence of this signal leads to the assembly of an mRNA decay-promoting protein complex including the conserved NMD factors UPF (up-frameshift) 1–3.

Introduction

Eukaryotic cells possess a number of surveillance mechanisms that monitor different steps during gene expression and collectively ensure sufficiently high fidelity in expressing genetic information to allow for complex forms of life. For all these quality-control systems, the central question is how they can distinguish 'correct' from 'wrong'. An emerging commonality among RNA surveillance systems is that this distinction relies on a kinetic competition between steps of normal RNA biogenesis or function and RNA degradation pathways [1]. NMD (nonsense-mediated mRNA decay), the mRNA quality-control process that I discuss here in more detail, also appears to rely on such a kinetic competition between efficient translation termination (the 'correct' event) and assembly of a protein complex that elicits rapid degradation of the mRNA, therewith defining the translation termination event as 'wrong'. Recent data indicate that this basic concept to identify nonsense mRNA has been evolutionarily conserved among eukaryotes, despite the apparent differences in NMD among different organisms.

Biological roles of NMD

The term 'nonsense-mediated mRNA decay' describes a translation-dependent post-transcriptional surveillance mechanism that recognizes and selectively degrades mRNAs of which the ORF (open reading frame) is truncated by the presence of a PTC (premature translation-termination codon) [2,3]. In so doing, NMD prevents or at least substantially reduces the synthesis of aberrant C-terminally truncated proteins, many of which are dangerous for a cell, because they often exert a dominant-negative function by competing with the respective full-length protein [4]. Given that approximately one third of the known disease-associated mutations in humans lead to the production of a nonsense mRNA, NMD serves as an important modulator of the clinical phenotypes of genetic diseases [4], and manipulating NMD therefore represents a promising strategy for future therapies of many genetic disorders [5]. Besides safeguarding the cell from deleterious truncated proteins, recent transcriptome profiling experiments indicate that NMD also contributes to regulating the expression of many normal (i.e. physiological) mRNAs. Genome-wide expression analysis of *Saccharomyces cerevisiae*, *Drosophila melanogaster* and human cells defective in NMD revealed that NMD affects the expression levels of approx. 5–10 % of the transcriptome (reviewed in [6]). It was estimated that about half of the affected mRNAs are direct NMD targets in *S. cerevisiae* [7], whereas the ratio between directly and indirectly regulated mRNAs is not yet known for the other organisms. The mRNAs affected by NMD in the different organisms are associated with a broad range of biological processes, but most of these NMD targets are not encoded by orthologous genes across different species [6]. The biological role of NMD as a post-transcriptional regulator of these different physiological mRNAs remains to be explored. In addition to nonsense and frameshift mutations in the DNA that result in the synthesis of mRNAs with PTCs, a large amount of PTC-containing mRNAs arise from alternative pre-mRNA splicing [8]. With regard to alternative splicing representing a frequent source for NMD substrates, I find it noteworthy that a correlation can be observed between the

Key words: alternative splicing, exon junction complex, gene expression, nonsense-mediated mRNA decay, RNA surveillance, translation-termination codon.

Abbreviations used: CBC, cap-binding complex; eIF4A3, eukaryotic initiation factor 4A3; EJC, exon junction complex; eRF3, eukaryotic release factor 3; MLN51, metastatic lymph node 51; NMD, nonsense-mediated mRNA decay; ORF, open reading frame; PABP, poly(A)-binding protein; PTC, premature translation-termination codon; RRP, rRNA processing 3′–5′ exonuclease; SMG, suppressor with morphogenetic effects on genitalia; TC, translation-termination codon; UPF, up-frameshift; UTR, untranslated region; XRN1, 5′–3′ exoribonuclease 1.

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extent of alternative splicing occurring in an organism and the severity of the phenotype caused by NMD deficiency in that organism. Virtually no alternative splicing occurs in *S. cerevisiae*, and NMD-deficient yeast strains exhibit no detectable phenotype under laboratory conditions [9]. In *Caenorhabditis elegans*, where alternative splicing occurs to a moderate extent, NMD-deficient strains have morphological defects of the genitalia, but are still fertile [10]. *D. melanogaster* shows extensive alternative splicing, and NMD-deficient *D. melanogaster* do not develop beyond the larval stage [11]. In *Arabidopsis thaliana*, alternative splicing is also common, and two NMD factors were shown to be essential for development and survival of the plant [12]. Finally, the majority of mammalian pre-mRNAs are alternatively spliced, and a homozygous knockout of the central NMD factor UPF (up-frameshift) 1 (see below) in mice is embryonic lethal [13]. In mammals, however, elucidation of the biological functions of NMD and their importance for the organism was complicated further by recent reports implicating NMD factors in telomere maintenance, DNA repair and genome stability [14–17]. Thus it is currently not clear whether the embryonic lethality of $UPF^{-/-}$ mice is due to its NMD deficiency or whether it is a consequence of defects in maintaining telomeres and genome stability. It also remains to be seen whether NMD factors indeed have independent functions in these seemingly unrelated processes, or if a yet unknown mechanistic link between NMD, telomere maintenance and DNA repair exists.

NMD factors

Trans-acting factors involved in NMD have initially been identified in genetic screens. Screens for translational suppressors in *S. cerevisiae* identified the three genes *UPF1*, *UPF2*/*NMD2* and *UPF3*, mutations of which were found to decrease decay rates of nonsense mRNAs and to promote read-through of PTCs (reviewed in [9]). In screens with *C. elegans*, loss-of-function mutations in seven genes called *SMG1* to *SMG7* (suppressor with morphogenetic effects on genitalia) were identified, and only several years later, were they recognized to be defective in NMD [10,18,19]. Sequence comparisons revealed that *SMG2* is homologous with yeast *UPF1*, *SMG3* is homologous with *UPF2*, and *SMG4* is homologous with *UPF3*. As genome sequences became available, homologues of these NMD factors were identified in other eukaryotic organisms (reviewed in [20]). All seven factors are present in humans, and *D. melanogaster* has homologues for SMG1, UPF1, UPF2, UPF3, SMG5 and SMG6, but appears to lack a homologue for SMG7. The human genome contains two genes homologous with UPF3, UPF3a on chromosome 13 and UPF3b on the X chromosome (also known as UPF3X), and two alternatively spliced mRNAs from each gene encode in total four UPF3 isoforms. It is likely that additional, as yet unknown, NMD factors exist. Notably, Longman and colleagues recently identified two additional proteins in *C. elegans*, called SMGL-1/hNAG and SMGL-2/hDHX34, which are required for NMD in nematodes and humans [21]. To search systematically for additional mammalian NMD factors, luciferase and GFP (green fluorescent protein)-based NMD reporter systems have been developed and could be used for genome-wide RNAi (RNA interference) library screens [22,23].

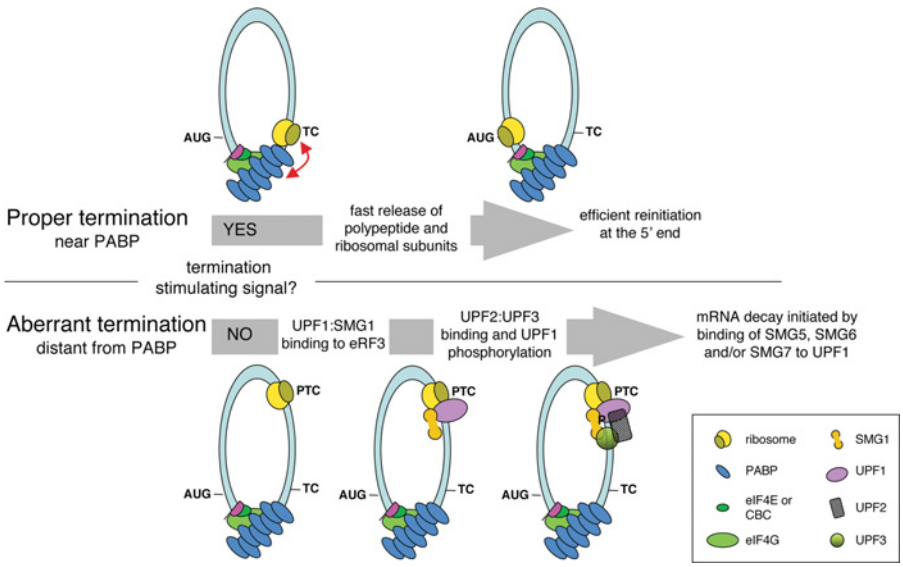
Mechanistic models to distinguish premature from normal translation termination

A key issue for understanding NMD is how a TC (translation-termination codon) is recognized as premature and distinguished from a natural correct TC, and how that distinction leads to differences in mRNA stability. Remarkably different mechanistic NMD models have been proposed from studies of mammalian systems compared with studies in *S. cerevisiae*, *C. elegans*, *D. melanogaster* and plants [24], despite the evolutionary conservation of the core NMD factors UPF1, UPF2 and UPF3. According to the prevailing model for mammalian NMD, TCs are identified as premature when located upstream of the 3′-most EJC (exon junction complex) (see below) in the mRNP (messenger ribonucleoprotein), whereas termination codons located downstream of the last EJC would not elicit NMD [24]. However, several examples of mammalian genes that do not comply with this rule question the generality of this EJC-dependent NMD model [25–31]. Instead, the most recent data from my laboratory [32,33] suggests that the basic mechanism for PTC recognition in human cells might be similar to or identical with that which has been suggested for yeast [9]. In fact, a model for an evolutionarily conserved mechanism for PTC recognition has begun to emerge based on converging results obtained with different model organisms [21,33–37]. The central feature of this unified NMD model, or '*faux* 3′ UTR (untranslated region) model' as it has been called for yeast, is that the mechanism of translation termination at a PTC is intrinsically different from translation termination at a 'correct' TC [9].

In contrast with the EJC-dependent NMD model that defines 'correct' by the removal of an RNA-degradation-promoting feature (the EJC) from the mRNP, correct translation termination depends on a positive signal according to the *faux* 3′ UTR model. It has been demonstrated in yeast that ribosomes do not efficiently dissociate from the mRNA when stalling at a PTC, presumably because in that spatial environment they cannot receive the required termination-stimulating signal [34]. The nature of this termination-stimulating signal is not yet known, but it can be mediated by PABP [poly(A)-binding protein]: artificial tethering of PABP in close proximity of an otherwise NMD-triggering PTC efficiently suppresses NMD in yeast, fruitfly and human cells [32,34,35]. On the basis of reported biochemical interactions [38], the unified NMD model posits that the decision of whether or not NMD is triggered relies on a competition between UPF1 and PABP for binding to eRF3 (eukaryotic release factor 3) bound to the terminating ribosome (Figure 1). According to this model, a translation-termination event is defined as 'correct' if the ribosome stalls close enough to the

Figure 1 | **Schematic view of the order of events typifying proper translation termination on an mRNA (upper) or aberrant termination that triggers NMD (lower)**

Grey arrows represent the time axis. See the text for details.



poly(A) tail to efficiently interact with PABP, which leads to fast/efficient dissociation of the two ribosomal subunits by yet unknown mechanisms. If, however, the spatial distance between the terminating ribosome and the poly(A) tail is too big for this interaction to occur, UPF1 can bind to the ribosome-bound eRF3 instead. I propose that, at this stage, PABP could still displace UPF1 and prevent NMD. The definitive commitment of the UPF1-bound mRNA for degradation by NMD occurs with the SMG1-mediated phosphorylation of UPF1, which requires binding of UPF2 and UPF3 to the SMG1–UPF1–eRF complex. Finally, the phosphorylated UPF1 will be bound by the 14-3-3-like phosphoserine-binding domains of SMG5, SMG6 and/or SMG7, ultimately leading to the degradation of the mRNA. The molecular relationship between UPF1, SMG5–SMG7 and the cellular degradation enzymes is not well understood, and several different degradation pathways can be triggered. Evidence from yeast and mammals suggests that rapid degradation is achieved by shunting nonsense transcripts efficiently into the normal mRNA-turnover pathway that proceeds through deadenylation, followed by decapping and XRN1 (5′–3′ exoribonuclease 1)-mediated 5′–3′ exonucleolytic decay as well as exosome-mediated 3′–5′ exonucleolytic decay [39]. In support of this idea, an interaction between UPF1 and the decapping enzyme subunit DCP2 (decapping protein 2) was found in both yeast and human cells [40,41], and UPF1, UPF2 and UPF3b were reported to co-immunopurify also with the 5′–3′ exonuclease XRN1, exosomal components PM/Scl100 [RRP (rRNA processing 3′–5′ exonuclease) 6], RRP4 and RRP41, and PARN [poly(A) ribonuclease] in African green monkey cells [42]. In contrast with this degradation from the ends, the decay of nonsense mRNAs is initiated by an endonucleolytic cleavage near the PTC in *D. melanogaster*. The resulting fragments are rapidly exonucleolytically degraded in both directions, without undergoing deadenylation or decapping [43].

Mammalian EJC downstream of TC functions as NMD enhancer

A set of proteins, collectively called the EJC, is deposited on mRNAs 20–24 nt upstream of exon–exon junctions as a consequence of splicing [44]. The four core components of the EJC, Y14, MAGOH (mago–nashi homologue), eIF4A3 (eukaryotic initiation factor 4A3) and Barentsz/MLN51 (metastatic lymph node 51) [45,46], escort the mRNA to the cytoplasm, therewith providing information about its nuclear history to the cytoplasm. Additional factors associate with EJCs more dynamically; some already leave before mRNA export or only bind the EJC in the cytoplasm [44]. Identification of UPF2 and UPF3 as EJC components immediately indicated a role for the EJC in NMD, and knockdown of Y14, eIF4A3 and Barentsz/MLN51 in mammalian cells indeed reduced the down-regulation of NMD reporter mRNAs [47–50]. However, the EJC is dispensable for NMD in *D. melanogaster* and *C. elegans* [21,51], and *S. cerevisiae* does not possess an EJC at all. Thus it appears that, in mammals, which produce a large number of nonsense mRNAs by extensive alternative pre-mRNA splicing, the EJC may have evolved to facilitate efficient recognition and degradation of these 'aberrantly spliced' transcripts.

EJCs located within the ORF presumably get stripped off the mRNA by elongating ribosomes [52,53], and only EJCs located downstream of the TC would therefore still be present when the first ribosome terminates. With regard to the unified NMD model, I propose that the presence of an EJC downstream of a terminating ribosome would enhance

NMD by facilitating SMG1–UPF2–UPF3-dependent UPF1 phosphorylation. As part of such a 3′-UTR-bound EJC, UPF2 and UPF3 are positioned ideally for immediate interaction with ribosome-bound UPF1 and SMG1. As a consequence, the time window between UPF1 binding to the terminating ribosome and its SMG1-mediated phosphorylation would be shortened and the balance between PABP action (NMD suppression) and UPF1 action (NMD promotion) would tilt towards NMD.

Restriction of mammalian NMD to pioneer round of translation

In addition to the function of EJCs in NMD, mammalian NMD appears to differ from yeast NMD in another aspect. Several lines of evidence suggest that NMD targets exclusively CBC (cap-binding complex)-bound mRNA in mammals (reviewed in [2,3]). The CBC component CBP80 (cap-binding protein 80) interacts with UPF1, and this interaction was shown to promote NMD by enhancing the binding of UPF1 to UPF2 during the first round of translation [54]. In contrast, NMD targets also eIF4E-bound steady-state mRNA in yeast [55,56]. It remains to be seen whether eIF4E-bound mammalian nonsense mRNAs are immune to NMD under experimental conditions that prevent their previous degradation during the CBC-bound state.

Conclusion

In summary, recent results from studying NMD in yeast, fruitfly, nematode worm and human cells suggest that the basic mechanism for identification of nonsense mRNAs is conserved among these organisms. The molecular understanding of this mechanism is most advanced in yeast (reviewed in [9]), and it remains to be seen how extensive conservation is in metazoans and in which aspects the systems have evolved differently.

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