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A Perfect Message: RNA Surveillance and Nonsense-Mediated Decay

Minireview

Matthias W. Hentze* and Andreas E. Kulozik†
*Gene Expression Programme
EMBL
Meyerhofstrasse 1
D-69117 Heidelberg
Germany
†Department of Pediatrics
Charité-Virchow Medical Center
Augustenburger Platz 1
D-13353 Berlin
Germany

The robustness of biological systems depends on the function of proofreading mechanisms against operating errors. Studies in yeast and of human genetic disorders have lead the way to a conserved surveillance mechanism that identifies faulty open reading frames (ORFs) and eliminates "imperfect messages" that contain premature translation termination codons (PTCs) and code for nonfunctional or even harmful polypeptides; this mechanism has been termed nonsense-mediated mRNA decay (NMD).

The Biological and Medical Importance of NMD

It was noticed in 1979 that PTC mutations of the yeast *ura3* gene destabilized the encoded mRNA (Losson and Lacroute, 1979). In the same year, the level of PTC-mutated β-globin mRNA from a thalassemia patient was reported to be very low (Chang and Kan, 1979), foreshadowing the broad phylogenetic distribution of NMD and its role in human genetic disorders. The vast majority of β-thalassemia heterozygotes are phenotypically healthy, whereas homozygotes suffer from a severe anemia. Normal erythropoiesis requires α/β-globin chain balance. In heterozygotes, the relative excess of insoluble α-globin chains are degraded but accumulate in homozygotes to damage the erythroid precursors. PTC mutations in the two 5′ proximal exons of the β-globin gene are common, and the mRNA is subjected to NMD. In contrast, a dominant form of inheritance is associated with a rare nonsense mutation in the last exon. This PTC-mutated mRNA escapes NMD, is normally abundant, and is translated (Hall and Thein, 1994). This additional burden causes the proteolytic system of the erythroid precursor cells to fail, which results in the precipitation of insoluble globin chains and in severe dyserythropoiesis. Therefore, NMD helps to protect millions of β-thalassemia heterozygotes from significant disease. NMD is not restricted to β-globin mRNAs and has been reported in many other human diseases.

The beneficial effect of NMD has also been documented for the *C. elegans* myosin heavy chain *unc-54* mRNA (Pulak and Anderson, 1993). Heterozygous worms are phenotypically normal, whereas homozygotes display striking abnormalities of muscle architecture and function. However, when NMD is inactivated by mutations of the *smg* genes (suppressor with morphogenetic effects on genitalia), otherwise normal worms that are heterozygous for some critical *unc-54* mutations become immobile. These findings led to the suggestion that NMD serves as an mRNA surveillance mechanism to eliminate mRNAs encoding truncated polypeptides that could act in a dominant-negative fashion (Figure 1).

The role of NMD extends beyond mRNA surveillance in genetic abnormalities. B and T cell maturation are processes with a particular requirement for "perfect messages." The immunoglobulin and T cell receptor genes are subject to extensive recombination and somatic mutation events, and the synthesis of resulting nonfunctional polypeptides is suppressed by NMD (Li and Wilkinson, 1998). This function of NMD as a quality control of gene expression has also been implicated in the disposal of nonfunctional by-products of normal RNA processing.

NMD—An Enigma in RNA Metabolism

NMD has captured much attention as one of the most enigmatic processes in RNA metabolism. Basically, a cell must discriminate the normal stop codon from a premature one (discrimination phase). Subsequently, a PTC-mutated mRNA is committed to a pathway (commitment phase) that ultimately results in its degradation (degradation phase). NMD in yeast is becoming increasingly well understood (Jacobson and Peltz, 1996; Ruiz-Echevarria et al., 1998). By contrast, diverse models are considered for mammalian NMD (Maquat, 1995; Li and Wilkinson, 1998). We will describe some key findings from the mammalian and yeast systems and will suggest that most of the available evidence can be integrated into a common model.

Mammalian NMD: Nuclear Aspects

There is little doubt that mammalian NMD is a posttranscriptional mechanism that involves the nucleus. Two types of alterations have been described: the abnormal splicing of pre-mRNAs, and the degradation of spliced mRNAs. Both alterations have been referred to as NMD. Regarding the first, examples of both nonsense-associated alternative splicing (e.g., fibrillin mRNA) and of intron retention (e.g., Igκ RNA) have been investigated. In these examples, PTCs are associated with an effect on splicing, for which we suggest the term "nonsense-associated altered splicing" (NAS). NAS is intriguing, because it suggests that the sense of a pre-mRNA can be interpreted by a nuclear reading frame scanner before splicing (Li and Wilkinson, 1998). Other alternative,

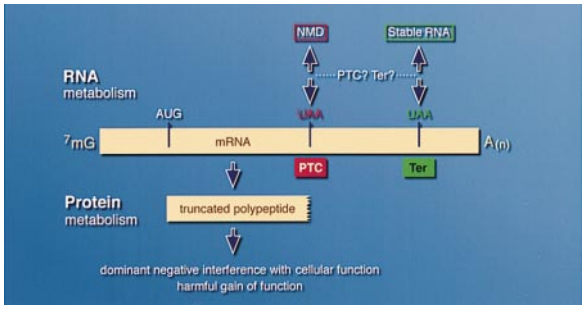

Figure 1. Nonsense-Mediated Decay: An Important Surveillance Mechanism and an Enigma in RNA Metabolism

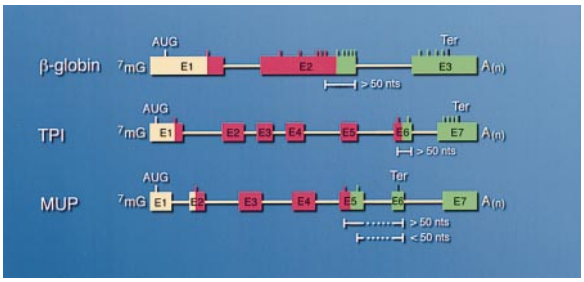

Figure 2. Intron-Related Positional Boundaries for Mammalian NMD

The mapped boundaries for NMD of three mammalian mRNAs are indicated. Red, PTCs in this region cause NMD; green, PTCs in this region do not trigger NMD. Note that the RNAs are not drawn to scale. No positional boundaries similar to those shown here have been found for the TCRβ mRNA.

more conventional explanations for NAS have also been documented in some cases where PTC mutations inactivate exonic splice enhancers to cause exon skipping (Shiga et al, 1997). How might one distinguish between mechanisms for nuclear PTC recognition and other events that cause alterations of splicing? If NAS relied on the nuclear interpretation of the ORF, one would predict that the three nonsense mutations, but not the 60 remaining mutations of an affected triplet, would alter splicing. One might also expect that a NAS-associated nuclear scanning mechanism would not be restricted to a few codons in a given exon. Such critical predictions for the function of a notional "nuclear scanner" in NAS remain to be more fully explored.

By contrast to NAS, no splicing alterations are found in the second type of PTC-induced RNA processing abnormality, nonsense-mediated decay (NMD) *senso strictu*. Here, spliced mRNA is subject to degradation. Even if NMD does not exhibit effects *on* splicing, there is mounting evidence for a role *of* splicing. Early on, it was found that nonsense codons located toward the 3′ end of the ORF rarely elicit NMD. For some cases, this positional bias has now been shown to be defined by the relative position of the PTC to a boundary ≈50 nt 5′ of the final exon/intron junction (Figure 2). PTCs 5′ to this boundary subject the mRNA to NMD, whereas mRNAs with a PTC 3′ to this boundary are usually stable (Thermann et al., 1998; Zhang et al., 1998a, 1998b). The importance of splicing for NMD has been documented by the insertion of an intron downstream from the normal stop codon. This manipulation subjects mRNAs without a PTC to NMD (Carter et al., 1996; Thermann et al., 1998). The hypothesis that cells can distinguish PTCs from normal stop codons by the presence or absence of a downstream intron is reinforced by the observation that 3′ UTR introns are rare (Hawkins, 1988) and usually located closer to the stop codon than the positional boundary mentioned above (Nagy and Maquat, 1998). While these results clearly implicate splicing in NMD, splicing of downstream introns does not seem to always be required (e.g., Zhang et al., 1998b). Also, downstream introns are not always sufficient to specify NMD (e.g., male *sex lethal* mRNA in *Drosophila* embryos). Splicing thus plays a clear role in specifying NMD, even if it is neither absolutely necessary nor always sufficient. Since NMD appears to affect fully spliced mRNAs (Zhang et al., 1998b), the information provided by splicing must affect a postsplicing process.

An intriguing aspect of many cases of NMD in vertebrate cells relates to the observation that the reduction of mRNA levels is already apparent for the nucleus-associated RNA (Maquat, 1995; Li and Wilkinson, 1998). This might suggest that the PTC-mutated mRNA is already degraded in the nucleus. On the other hand, there is no positive evidence to suggest that the postsplicing step(s) involved in NMD occurs within the nucleus except for the apparent reduction of nucleus-associated mRNA.

Mammalian NMD: Cytoplasmic Aspects

To examine whether NMD leads to a reduction of the cytoplasmic- or nucleus-associated forms of PTC-mutated mRNAs, various protocols have been employed to cleanly separate nuclei from cytoplasm. Nucleus-associated RNA can be located within the nucleoplasm, associated with the nuclear pore, or be almost fully exported and not yet released from the nucleus. By contrast, the RNA in the supernatant is considered to be cytoplasmic. Hence, the definition of nuclear association is operational. Alternatively, a functional definition can be applied: mRNA that is translated by ribosomes is considered to be cytoplasmic. However, most experiments employing such a functional definition fail to distinguish traditional cytoplasmic ribosomes from a ribosome-like nuclear scanner as suggested for NAS.

Initially, NMD was thought to be a consequence of the lack of ribosomes that could protect the mRNA downstream from a nonsense codon. The available evidence indicates now that a mechanism with the hallmarks of ribosomal translation is actually required for NMD (Maquat, 1995; Li and Wilkinson, 1998). However, it could not be excluded that this mechanism is mediated by a hypothetical nuclear cousin of the cytoplasmic translation apparatus. The cytoplasmic binding of iron regulatory proteins (IRPs) to iron-responsive elements to regulate the translation of a PTC-mutated β-globin mRNA has been used as an approach to avoid such ambiguities. IRPs are spatially restricted to the cytoplasm (Thermann et al., 1998) and thus served as a specific tool to inhibit cytoplasmic translation without affecting the activity of a notional ribosome-related nuclear scanner. NMD was found to be specifically abrogated by IRP-mediated translational repression, revealing the requirement of cytoplasmic translation for NMD (Thermann et al., 1998). If NMD of nucleus-associated RNA generally requires translation by cytoplasmic ribosomes, one must ask about the kinetic relationship between the first "translational encounter" of an mRNA vis-à-vis its release from the nucleus. mRNAs can be translated while still being exported from the nucleus and do not need to be translated in order to be exported. However, the important general question concerning the kinetic relationship between the release of an mRNA from nuclear association and its first translation is currently unanswered.

Insights from Yeast

Compared to mammalian cells, the understanding of the discrimination, commitment, and degradation steps of NMD in yeast is further advanced. Similar to mammalian

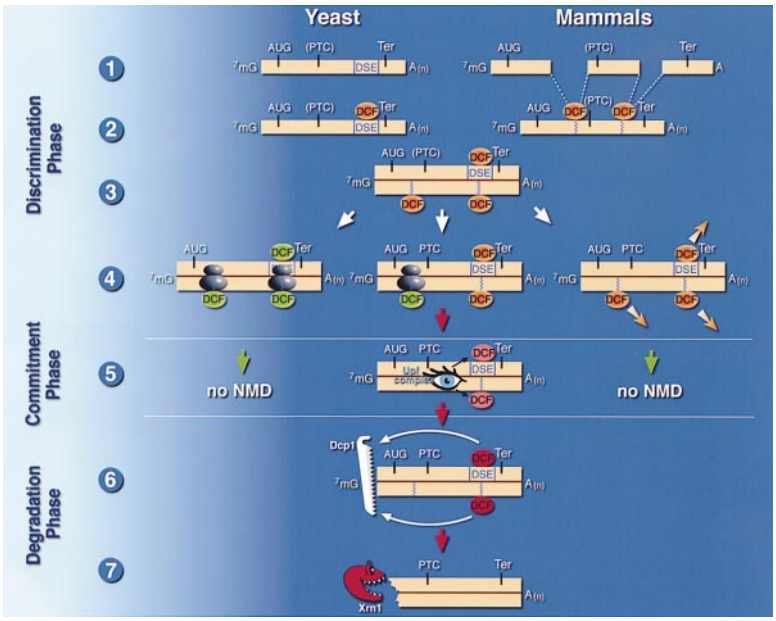

Figure 3. Model for NMD by a Common Posttermination Surveillance Mechanism in Mammals and Yeast

Hypothetical downstream commitment factors (DCFs) are directed to the mRNAs either by the yeast downstream sequence element (DSE) or by mammalian splicing. Subsequent to DCF binding (step 2), both yeast and mammalian mRNAs are suggested to follow similar pathways. Hence, they are depicted on top of each other from step 3 onward. DCF shown in orange symbolizes an uncommited state. Following an encounter with elongating ribosomes, DCF shown in green indicates lack of potential to cause NMD. Commitment to NMD is triggered when DCF (now red) is recognized by the posttermination surveillance complex (shown as an eye). When the mRNA is not translated, DCFs do not trigger NMD (orange) and may eventually dissociate (arrows). PTC, premature termination codon; DCP1, decapping enzyme (saw); Xrn1, 5′→3′ exonuclease (packman).

cells, yeast mRNAs containing PTCs are often degraded faster than their wild-type counterparts (Jacobson and Peltz, 1996). In contrast to mammalian cells, there are no reported effects of PTCs *on* splicing (NAS), and the important role of cytoplasmic translation is well appreciated.

While PTCs in mammalian mRNAs can be identified by the presence of a downstream intron, yeast discriminates premature from normal stop codons by exonic downstream sequence elements (DSE). Stop codons followed by a DSE within approximately 200 nucleotides are interpreted as PTCs if the DSE is encountered by a posttermination surveillance complex and not by elongating ribosomes that have reinitiated translation upstream of the DSE (Ruiz-Echevarria et al., 1998). PTC-mutated yeast mRNAs can also escape degradation when a stabilizer element (STE) is inserted between the PTC and the DSE, or occurs upstream of the PTC. Finally, translation elongation is dispensible for NMD in yeast, because the presence of a cassette consisting of an AUG initiation codon immediately followed by stop codons upstream of a DSE suffices to subject an mRNA to NMD (Ruiz-Echevarria et al., 1998). Two *cis*-acting sequences, a termination codon and a DSE, thus direct mRNAs to NMD in yeast, unless a dominant *cis*-acting stabilizer sequence is positioned between the two (Figure 3) or upstream of the PTC.

Further support for the importance of translation (termination) comes from the analysis of Upf (up frameshift) proteins that are required for NMD but not other mRNA degradation pathways in yeast. These proteins form a complex that promotes efficient translation termination and interacts with the termination factors eRF1 and eRF3 (Czaplinski et al., 1998). Analysis of Upf1p mutants led to the hypothesis that a Upf protein–containing surveillance complex is recruited to mRNAs when the ribosome is complexed with the translation termination machinery. It was also shown that a dominant-negative mutant of Upf2p fails to block NMD when fused to a nuclear localization signal (Jacobson and Peltz, 1996), supporting the overall view that yeast NMD is a cytoplasmic process.

A common pathway for the degradation of wild-type mRNAs in yeast commences with the deadenylation of the mRNAs, followed by decapping by the enzyme Dcp1p and subsequent 5′ to 3′ degradation by the exonuclease Xrn1p (Beelman et al., 1996). The degradation phase of NMD in yeast enters this pathway in a deadenylation-independent way. How the commitment to NMD triggers the activity of Dcp1p is still unknown. Yeast NMD appears to differ from mammalian NMD with regard to the involvement of splicing, the role of DSEs and introns, and the apparent lack of nuclear association of the yeast RNAs undergoing degradation.

A Perfect Model?

Using the information from yeast as a conceptual template to model NMD in mammalian cells, both pathways can be merged. With reference to the findings in yeast, we refer to this proposed pathway as a "posttermination surveillance model" (Ruiz-Echevarria et al., 1998; see also Li and Wilkinson, 1998; Thermann et al., 1998; Zhang et al., 1998b).

Mammalian NMD exploits splicing as a means to introduce a downstream point of reference to discriminate between PTCs and normal stop codons (Figure 3, step 1). Some serine-arginine (SR)-rich proteins bind to pre-mRNAs during splicing and remain associated with the exonic splice product (Caceres et al., 1998). Tagging of exonic splice junctions by SR or other nuclear proteins during and after export of the mRNA into the cytoplasm could represent a functional correlate to the DSE and (still hypothetical) DSE-binding proteins in yeast (Figure 3, step 2). In both systems, such proteins would serve as downstream commitment factors (DCFs) (Figure 3, step 3). DSE-like sequences that bind DCFs in a splicing-independent way may also exist in mammalian cells and explain cases where PTCs cause NMD without the presence of a downstream intron.

DCFs encountered by elongating ribosomes during the first translation of an mRNA before (NMD of nucleus-associated RNA) or after (NMD of cytoplasmic RNA) its release from the nuclear fraction will be interpreted to represent a spliced-out intron or a DSE within the ORF and thus not cause NMD (Figure 3, step 4, left). Likewise, lack of cytoplasmic translation before the dissociation of DCFs would fail to cause commitment to NMD (Figure 3, step 4, right). By contrast, recognition of DCFs downstream of a stop codon by a posttermination surveillance complex commits the mRNA to the NMD pathway (Figure 3, step 4, middle; step 5). A cotransfected dominant-negative mutant of a human UPF1 homolog stabilized NMD reporter constructs in mammalian cells, providing evidence for the functional involvement of a Upf protein in mammalian NMD (Sun et al., 1998). DCF recognition by a posttermination complex might trigger NMD, while the encounter with elongating ribosomes would not. This proposal would explain why translation reinitiation 3′ of a PTC abrogates NMD in mammalian cells (Zhang and Maquat, 1997), as seen in yeast. Furthermore, the existence of a correlate to the yeast STE in animal cells might explain why some mRNAs with introns downstream of a stop codon excape NMD. Little information is currently available for the degradation phase of mammalian NMD (Figure 3, steps 6 and 7). Based on yeast NMD, the posttermination surveillance model suggests the involvement of putative Dcp1p and Xrn1p homologs.

Is the posttermination surveillance model a perfect model to explain NMD as a quality control mechanism for perfect messages in all eukaryotic cells? With regard to mammalian cells, it remains to be determined. The envisaged coupling between mRNA translation and turnover bears provocative implications that reach beyond the understanding of NMD: DCF-binding sequences in the 3′ UTR of mRNAs would be predicted to act as translation-dependent “instability determinants,” able to affect the turnover of wild-type mRNAs. The identification of *trans*-acting factors that mediate mammalian NMD will shed light on the mechanistic similarities and differences with yeast, and it will permit a direct test of whether NMD and the turnover of some wild-type mRNAs share common factors. For yeast, the mechanistic understanding is further advanced, and the time seems ripe to unravel the composition and function of the surveillance complex biochemically and to identify factors at the juncture between DSE recognition and the activation of the decapping enzyme Dcp1p. Once more, yeast may hold valuable insights into mechanisms that operate in the context of human genetic diseases.

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