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Review

Nonsense-mediated mRNA decay — Mechanisms of substrate mRNA recognition and degradation in mammalian cells ☆

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ABSTRACT

The nonsense-mediated mRNA decay (NMD) pathway is well known as a translation-coupled quality control system that recognizes and degrades aberrant mRNAs with truncated open reading frames (ORF) due to the presence of a premature termination codon (PTC). However, a more general role of NMD in posttranscriptional regulation of gene expression is indicated by transcriptome-wide mRNA profilings that identified a plethora of physiological mRNAs as NMD targets. In this review, we focus on mechanistic aspects of target mRNA identification and degradation in mammalian cells, based on the available biochemical and genetic data, and point out knowledge gaps. Translation termination in a messenger ribonucleoprotein particle (mRNP) environment lacking necessary factors for proper translation termination emerges as a key determinant for subjecting an mRNA to NMD, and we therefore review recent structural and mechanistic insight into translation termination. In addition, the central role of UPF1, its crucial phosphorylation/dephosphorylation cycle and dynamic interactions with other NMD factors are discussed. Moreover, we address the role of exon junction complexes (EJCs) in NMD and summarize the functions of SMG5, SMG6 and SMG7 in promoting mRNA decay through different routes. This article is part of a Special Issue entitled: RNA Decay mechanisms.

1. Introduction

The expression of genetic information in eukaryotes depends on the proper timing and spatial order of numerous chemical reactions and molecular interactions [1]. Errors inherent in each step along the elaborate pathway of mRNA synthesis and processing sum up to a considerable fraction of aberrant mRNAs that undermine the accuracy of gene expression. mRNA quality control processes therefore play an important, in vertebrates even an essential role, in recognizing and eliminating such problematic mRNAs [2–4]. So far, three different translation-coupled mRNA surveillance systems have been described in eukaryotes that all recognize and degrade mRNAs that cause problems during the process of translation. Two of these surveillance pathways, no-go mRNA decay and non-stop mRNA decay, act on mRNAs on which the ribosome halts unexpectedly either somewhere within the ORF or at the physical 3' end, respectively. In both cases, there is no stop codon present and hence no release factors engage with the stalled ribosome. No-go mRNA decay and non-stop mRNA decay share common factors and appear to be mechanistically related [4]. These two processes are described by Inada in another review in this issue (ref: this issue) and in other recent reviews [5,6]. In this review, we will focus on mechanistic aspects of NMD.

More than 30 years ago, it was discovered in *Saccharomyces cerevisiae* and in human bone marrow cells of β-thalassemia patients that the half-lives of mRNAs of which the ORFs are truncated by the presence of a PTC are reduced compared to the corresponding PTC-free mRNAs [7,8]. Over the years, destabilization of PTC-containing mRNAs has been reported in many other eukaryotic species, including

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the two highly divergent protists *Giardia lamblia* and *Trypanosoma brucei* [9,10], suggesting that NMD has evolved very early during the development of eukaryotes and exists in essentially all eukaryotes.

Because about 30% of all known disease-associated mutations result in the production of mRNAs with a PTC, NMD plays an important role as a modulator of the clinical manifestations of many genetic diseases (reviewed in [11–13]). NMD can be beneficial by preventing the production of C-terminally truncated proteins with a dominant-negative function, but there are also cases where the truncated protein encoded by the PTC-containing mRNA still has some residual function and the NMD-mediated reduction of mRNA abundance results in more severe clinical problems. A better understanding of the molecular mechanism of NMD will therefore also benefit the development of therapeutic approaches aiming at specifically manipulating NMD efficacy and substrate specificity.

Until the advent of transcriptome-wide identification of endogenous NMD targets, NMD has been merely perceived as a quality control system that evolved to rid cells of aberrant, PTC-containing mRNAs. However, it has meanwhile become clear that in addition to its quality control function, NMD also plays an important role in regulating gene expression by targeting many physiological mRNAs that code for full-length functional proteins, thereby influencing a wide variety of biological processes. In fact, mRNA profiling of NMD defective *S. cerevisiae* [14–16], *Caenorhabditis elegans* [17], *Drosophila melanogaster* [18] and human cells [19–23] revealed that NMD affects the levels of 3–10% of all cellular mRNAs. Moreover, there is new evidence for differential regulation of individual NMD factors and NMD activity in different cell types and tissues, and for an autoregulation of NMD factor abundance [22,24–26]. Together with the accumulating evidence for the existence of multiple branches of the NMD pathway [27,28] (see below), this reveals a so far underappreciated complexity of NMD regulation and suggests that the involvement of NMD in a wide spectrum of biological processes. Consistent with this view, more complex organisms generally are more sensitive to reduced or abolished NMD activity than simpler ones. For example, NMD is an essential process in mammals, zebrafish, and fruit flies, whereas NMD deficient mutants of *S. cerevisiae* and *C. elegans* are viable and have only mild phenotypes. Knockout of the NMD factors UPF1, UPF2 or SMG1 (see below) in mice leads to early embryonic death [29–31], knockdown of NMD factors in zebrafish disrupts brain development [32,33], and there is evidence for a function of NMD in mammalian brain development [25]. Furthermore, mutations in the human NMD factor UPF3B are associated with mental retardation, autism and schizophrenia [34–36]. For a comprehensive review of the emerging roles of NMD in gene regulation, development and cellular responses to environmental cues, we refer the reader to an accompanying review by Karam et al. (ref: this issue). In addition, the role of NMD in animal embryogenesis was recently reviewed by Hwang and Maquat [37].

While the phenomenon of NMD and its impact on gene expression and genetic diseases is well documented, the understanding of the underlying molecular mechanisms is still fragmented, in spite of a fair amount of genetic, structural and biochemical data that has been gathered during the years. Definitively, the lack of a functional in vitro system for NMD is hampering progress in elucidating the mechanism of NMD. In this review, we summarize the available data about the major known NMD factors and discuss current mechanistic models of NMD, thereby focusing on mammalian NMD and pointing out commonalities and differences to models derived from other species.

2. What defines an NMD substrate?

The discovery that not only the initially identified PTC-containing mRNAs but also many PTC-less mRNAs are targeted by NMD (re-) posed the question, which features render an RNA susceptible to NMD and pointed out our limited understanding of the mechanism of substrate selection. Besides the presence of an ORF-interrupting PTC, upstream ORFs (uORFs), introns in the 3′ untranslated region (UTR) and long 3′ UTRs are empirically identified features that can trigger NMD (Fig. 1). Furthermore, poly(A) site mutation or alternative polyadenylation can generate NMD-targeted mRNAs [38–40]. It should be emphasized however that about 28% of the human mRNAs have 3′ UTRs of >1000 nucleotides (C.S., unpublished data), yet only a small percentage of them are NMD substrates.

PTCs can arise at the DNA level by mutations or at the level of RNA by transcription errors or alternative pre-mRNA splicing. In fact, alternative splicing has been detected for at least 75% of human pre-mRNAs [41] and 45% of these alternatively spliced pre-mRNAs produce at least one mRNA isoform that might be degraded by NMD [42]. Consistently, splice isoforms of 30% of all expressed genes were found to be upregulated in mouse tissues ablated for the NMD factor UPF2 [43]. Hence, these unproductively spliced transcripts appear to constitute a substantial fraction of the NMD substrates in human cells.

Given that long 3′ UTRs can elicit NMD, the widespread differential poly(A) site usage observed under stress conditions [44,45], during mouse development [46], in proliferating cells [47], in cancer cells [48], by CF1 [49], and by U1 snRNP levels [50] are all predicted to potentially alter the half-lives of mRNAs and thereby influence gene expression.

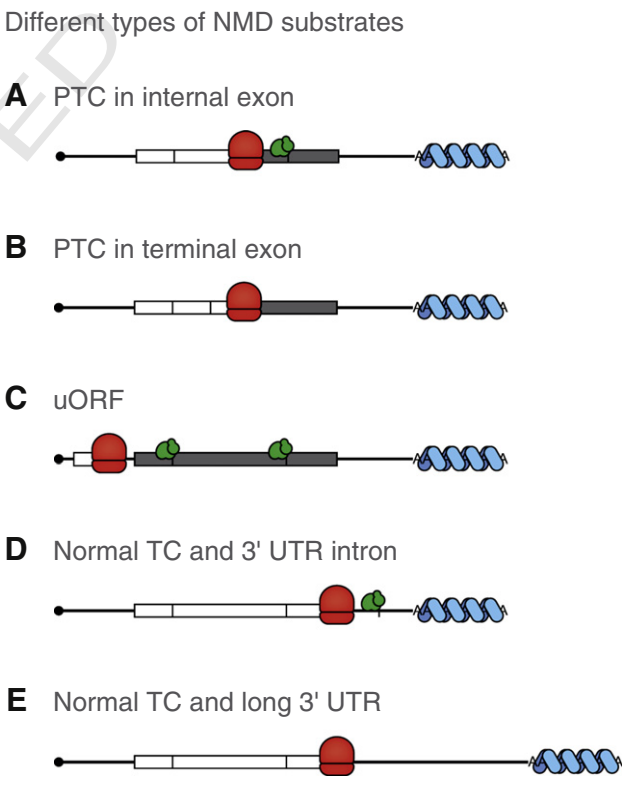

Fig. 1. Different types of mRNAs that can be substrates for NMD. (A) The classical mammalian NMD targets comprise mRNAs with a truncated ORF due to a premature termination codon (PTC) in an internal exon, on which one or several exon junction complexes (EJCs) are expected to remain after translation termination. (B) PTCs in terminal exons are the classical NMD substrates in *S. cerevisiae*, but many examples of this type have also been found in other organisms, including mammals. (C) Upstream ORFs (uORFs) 5′ of the main ORF are well-known NMD-inducers. (D) Introns in the 3′ UTR will in most cases lead to a remaining EJC after translation termination that can trigger NMD. (E) A long 3′ UTR can also function as an NMD-inducing feature in all investigated organisms. mRNAs are illustrated as black lines with a 5′ cap (black dot) and a 3′ poly(A) tail coated by poly(A)-binding protein (PABP, blue). White boxes denote translated coding sequence and gray boxes regions of the coding sequence that is not translated due to the presence of a PTC (A, B) or a uORF (C). Terminating ribosomes are illustrated in red, EJCs in green.

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