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Opinion

The Meaning of NMD: Translate or Perish

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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.

Trends

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.

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 coevolved 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

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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 nonspecific 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 cosuppressors 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 later, 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]. In the following, we discuss the different models that have been proposed so far to explain the nature and the function of this putative mRNA surveillance machinery.