

Aberrant mRNA transcripts and nonsense-mediated decay

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Abstract

Nobody's perfect, and even the cell turns out a certain fraction of erroneous mRNA transcripts. One of the key quality control mechanisms put in place to recognize and eliminate these transcripts before they can be translated into faulty proteins is nonsense-mediated decay. Proteins involved in nonsense-mediated decay are highly conserved across species from plants to humans, and recent studies in *Arabidopsis thaliana* reveal both intriguing similarities and differences in the mechanisms employed to carry it out.

Introduction and context

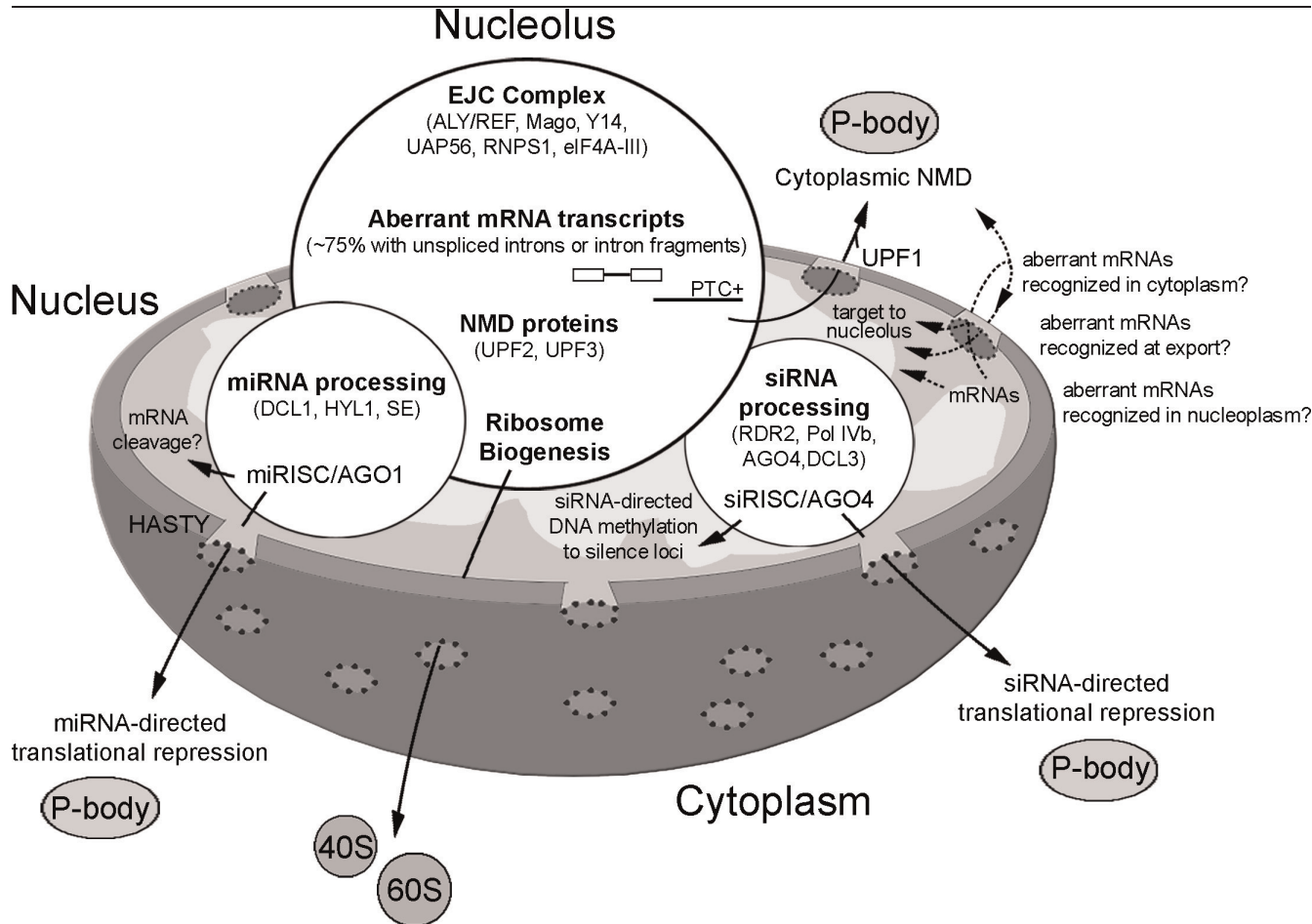
RNA surveillance pathways scan for errors arising during transcription and splicing, targeting aberrant RNAs for degradation [1]. Nonsense-mediated decay (NMD) is one such pathway, detecting mRNAs that harbor premature termination or 'nonsense' codons (PTCs) [2,3]. It has been estimated that NMD regulates approximately 10% of all human mRNAs, and that approximately 30% of all disease-associated mutations generate PTCs. The core machinery comprises three *trans*-acting factors called up-frameshift (UPF) proteins. UPF1 is an RNA helicase recruited to mRNAs upon recognition of stop codons by the translation apparatus. Rapid decay is triggered when UPF1 interacts with UPF2 and UPF3. A key event linking recognition of PTCs to NMD is phosphorylation of UPF1 by the SMG1 kinase and recruitment of the translational termination release factors eRF1 and eRF3 (to form the SURF complex), which promotes transcriptional repression and recruitment of mRNA degradation proteins [4].

In yeast and invertebrates, abnormally long 3' untranslated regions (UTRs) downstream of stop codons can act as signals for NMD, while in mammalian cells it is primarily the location of the exon junction complex (EJC) [2]. This multiprotein complex is deposited 20-24 nucleotides upstream of exon-exon junctions after pre-

mRNA splicing. By marking the location of introns relative to the stop codon, the EJC can signal the presence of a PTC and recruit NMD factors that target the transcript for decay.

Until recently, plant NMD was poorly understood, although many of the protein factors involved in NMD in other eukaryotes were known to be present. Surprisingly, proteomic analysis of purified *Arabidopsis* nucleoli placed six components of the EJC within this subnuclear organelle [5,6]. The nucleolus, best known as the site of ribosome biogenesis, has recently been implicated in a much wider range of functions, including regulation of mitosis, stress response, biogenesis of ribonucleoprotein particles, RNA editing, and processing of non-coding RNAs [7,8]. The presence of EJC proteins in both the *Arabidopsis* [5] and human [9] nucleolar proteome suggests that this structure may have additional functions in mRNA export and NMD-mediated mRNA surveillance. Yeast nucleoli have long been implicated in processing and transport of poly(A)⁺ mRNA [10]. As illustrated in Figure 1, plant nucleoli and nucleolar-associated Cajal and D-bodies have also been implicated as sites of small interfering RNA and microRNA biogenesis [11-15], and several recent studies have detected microRNA-like small non-coding RNAs derived from small nucleolar

Figure 1. RNA processing in the *Arabidopsis* nucleus



In addition to its role in transcribing and processing rRNA into ribosomal subunits for export to the cytoplasm, the *Arabidopsis* nucleolus has also been implicated in other pathways, including nonsense-mediated decay (NMD). Both exon-joining complex (EJC) members and NMD effector proteins were identified by proteomic analysis and shown to localize to this subnuclear organelle, and aberrant mRNAs were shown to be more abundant in nucleolar fractions than in nucleoplasmic fractions. The mechanism of this nucleolar targeting of aberrant mRNA is unclear, and may take place in the nucleus, cytoplasm, or both. Equally unclear is whether NMD takes place in the nucleolus and/or nucleus, or whether transcripts are prepared here for export to the cytoplasm, where UPF1 is recruited to activated degradation. Both pathways may exist, with the latter primarily handling premature termination codon (PTC)+ mRNAs. A similar combination of nuclear and cytoplasmic pathways has been proposed for small interfering RNA (siRNA) and microRNA (miRNA) processing in *Arabidopsis*, which take place in nucleolar-associated bodies (Cajal bodies and D-bodies). AGO, argonaute; DCL, dicer-like; eIF4A-III, eukaryotic initiation factor 4AIII; HYL1, hyponastic leaves 1; P-body, processing body; Pol IVa, RNA polymerase IVa; RDR2, RNA-dependent RNA polymerase 2; RISC, RNA-induced silencing complex; RNPS1, RNA binding protein S1, serine-rich domain; SE, serrate; UPF, up-frameshift.

RNAs in mammals, protozoans, and viruses [16-19]. It is not that far-reaching to suggest that, once the nucleolus evolved as a site enriched in rRNA processing proteins, the cell subsequently exploited it as a convenient site to carry out other RNA processing functions.

Major recent advances

Plant nonsense-mediated decay pathways

When plant NMD was first analyzed to determine whether it uses a system more like that found in yeast or in

mammalian cells, the unexpected answer was 'both' [20-22]. Recently, Kerényi *et al.* [23] undertook a functional dissection of these pathways in *Nicotiana benthamiana* and identified several of the *trans* factors involved. They verified the co-existence of two NMD pathways: one that eliminates mRNAs with long 3' UTRs in a manner similar to yeast NMD and a second, distinct pathway that degrades mRNAs harboring 3' UTR-located introns. The EJC factors Mago and Y14 were shown to only be required for this intron-based pathway, suggesting that it is similar to mammalian NMD. In addition, a protein involved in

both pathways, SMG7, was found to be subject to feedback regulation, suggesting the evolutionary conservation of NMD autoregulation throughout all eukaryotes.

Enrichment of aberrant mRNA and NMD proteins in plant nucleoli

With the exact location(s) of NMD in mammalian cells still under debate, particularly with regard to the identification and labeling of PTC+ transcripts, recent results from *Arabidopsis* studies raise the intriguing possibility that certain steps in the plant NMD pathway may occur, at least in part, within the nucleus. Having previously shown the association of EJC and NMD proteins with the plant nucleolus using a proteomic approach [5], Kim *et al.* [24] next compared the distribution of mRNA classes in whole cell, nuclear, and nucleolar libraries. Not only did they show that mRNAs are present in nucleoli, they demonstrated a clear abundance of aberrantly-spliced mRNAs in this structure compared with the nucleoplasm. Further examination of these transcripts revealed that most contain PTCs and are putative or known targets of NMD, and several were shown to accumulate in NMD-mutant plants, suggesting a correlation between the enrichment of aberrant mRNAs in nucleoli and their turnover by NMD.

In addition to EJC proteins and aberrant mRNAs, NMD factors UPF2 and UPF3 were also shown to localize to the plant nucleolus. However, while plant UPF3 shows a clear accumulation in nucleoli, human UPF3, although detected in the nucleolar proteome [9], is predominantly nucleoplasmic. This difference is surprising, and may indicate a unique function for the nucleolus in plant NMD. UPF1 is primarily cytoplasmic in both species. One possibility put forth by the authors is that plants identify intron-containing transcripts and prepare them for degradation by the NMD pathway in the nucleus and nucleolus, while other PTC+ transcripts without intron fragments are identified upon export by a pioneer round of translation.

Future directions

These studies highlight the importance of considering eukaryotic pathways in their evolutionary context. The two NMD pathways described here to identify and target specific classes of aberrant mRNA for degradation in plant cells bear striking similarities to counterpart systems in yeast and mammals. At the same time, the obligatory role of splicing and the EJC in initiating NMD in mammalian cells is being questioned, with alternative or complementary EJC-independent pathways proposed [25,26]. Taken together, the results suggest that these RNA surveillance pathways may be more evolutionarily conserved than previously thought, despite the apparent simplification in certain lineages.

Although the presence of NMD factors in the nucleolus suggests a role in this pathway, several questions remain. For example, it is still unclear how aberrant transcripts are recognized and targeted to the nucleolus, and whether NMD can occur in the nucleus or relies on the transfer of aberrant mRNPs to the cytoplasm. Indeed, controversy still exists over the location of EJC-mediated NMD in mammalian cells. While it is generally agreed that a pioneering round of translation is required to detect a PTC, and the current consensus is that this initial round of translation occurs in the cytoplasm, in close proximity to the nucleus [27,28], the possibility of some sort of proofreading or downstream processing step in the nucleus cannot be ruled out. It is likely that dissection of NMD pathways in eukaryotic systems will continue to throw up surprises.

Abbreviations

EJC, exon-joining complex; NMD, nonsense-mediated decay; PTC, premature termination codon; UPF, up-frameshift; UTR, untranslated region.

Competing interests

The author declares that she has no competing interests.

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