mRNA surveillance: the perfect persist

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Summary

In eukaryotes, an elaborate set of mechanisms has evolved to ensure that the multistep process of gene expression is accurately executed and adapted to cellular needs. The mRNA surveillance pathway works in this context by assessing the quality of mRNAs to ensure that they are suitable for translation. mRNA surveillance facilitates the detection and destruction of mRNAs that contain premature termination codons by a process called nonsense-mediated decay. Moreover, recent studies have shown that a distinct mRNA surveillance process, called nonstop decay, is responsible for depleting mRNAs that lack in-frame termination codons. mRNA surveillance thereby prevents the synthesis of truncated and otherwise aberrant proteins, which can have dominant-negative and other deleterious effects.

Key words: mRNA surveillance, Nonsense-mediated decay, Exon-junction complex

Introduction

It is estimated that 30% of inherited genetic disorders in humans result from premature termination codon (PTC) mutations (Frischmeyer and Dietz, 1999). Nonsense-mediated decay (NMD) is thought to underlie the recessive nature of many of these diseases. For example, recessive forms of β-thalassemia result from PTC mutations occurring in the first or second exon of the β-globin gene (Kugler et al., 1995). The NMD pathway rapidly destroys the resultant mRNAs, such that heterozygous individuals are healthy. However, when a PTC is present in the third and final exon, it escapes detection by the decay machinery, leading to translation of a truncated protein that heterodimerizes with α-globin to produce a dominant-negative protein. Individuals with this type of mutation suffer from a form of anemia characterized by the presence of insoluble inclusion bodies in erythroid cells (Kugler et al., 1995).

In addition to having a damage-control function, NMD is a critical process in normal cellular development. For example, the immunoglobulin and T-cell receptor genes in mammalian lymphocytes undergo dramatic rearrangement during maturation of the immune system. This somatic recombination results in a high frequency (~66%) of frame-shifted genes containing PTCs. To cope with this, the PTC-containing mRNAs in lymphocytes are downregulated by 90-99% by NMD, which prevents the synthesis of defective proteins (Carter et al., 1995).

mRNA surveillance is an enigmatic process because it requires a cellular machinery that can discriminate normal from aberrant mRNAs. Recent studies have shown that in mammals, detection of PTC-containing mRNAs relies on communication of the nuclear history of an mRNP to the translation apparatus by a protein complex deposited upstream of exon-exon junctions after pre-mRNA splicing. Another aspect of mRNA surveillance has recently been discovered that functions to remove cellular mRNAs that lack in-frame termination codons. This nonstop decay process occurs in the cytoplasm and is mediated by the exosome, a multisubunit complex of 3′→5′ exonucleases. mRNA surveillance mechanisms thus function both to maintain proper levels of normal transcripts and to deplete aberrant transcripts from the cell.

Biology of mRNA turnover

The steady-state level of a given mRNA depends on the balance between its rates of synthesis and degradation. Importantly, the decay rate can be modulated to control the amount of polypeptide the cell produces. The NMD and nonstop decay pathways differ from regulatory mRNA turnover in that their functions are to detect aberrant transcripts and promote their rapid decay. PTCs may result from genomic mutation, inaccurate transcription or improper pre-mRNA processing events, such as splicing and polyadenylation. mRNAs lacking termination codons are believed to arise primarily from premature polyadenylation within the open reading frame (Frischmeyer et al., 2002).

The prevalent route of mRNA degradation in *Saccharomyces cerevisiae* proceeds via removal of the poly-A tail by deadenylation, followed by decapping and 5′→3′ exonucleolytic decay (Fig. 1) (Decker and Parker, 1993). Alternatively, transcripts can be degraded from the 3′ end by the exosome (Jacobs et al., 1998), which may be responsible for the majority of mRNA degradation in mammals (Mukherjee et al., 2002; Wang and Kiledjian, 2001). In addition, mRNAs can be targeted for cleavage by endoribonucleases, followed by exonucleolytic decay from the 5′ and 3′ ends (Binder et al., 1994). By contrast, the NMD pathway in *S. cerevisiae* acts via deadenylation-independent decapping, followed by 5′→3′ exonucleolytic decay (Muhlrad and Parker, 1994), whereas nonstop decay appears to proceed via deadenylation-independent 3′→5′ exonucleolytic decay (Frischmeyer et al., 2002; van Hoof et al., 2002). In bypassing the rate-limiting step of deadenylation, the mRNA surveillance pathways allow the rapid removal of irregular mRNAs from cells.

The NMD pathway is not solely limited to mRNAs containing a PTC within the proper coding region. Other types of aberrant transcripts subject to NMD include pre-mRNAs with retained introns (containing in-frame stop codons) (Mitrovich and Anderson, 2000), a subset of mRNAs with upstream open reading frames in their 5′ UTRs (Ruiz-Echevarria and Peltz, 2000; Welch and Jacobson, 1999) and mRNAs that inherit extended 3′ UTRs owing to improper polyadenylation site usage (Muhlrad and Parker, 1999).

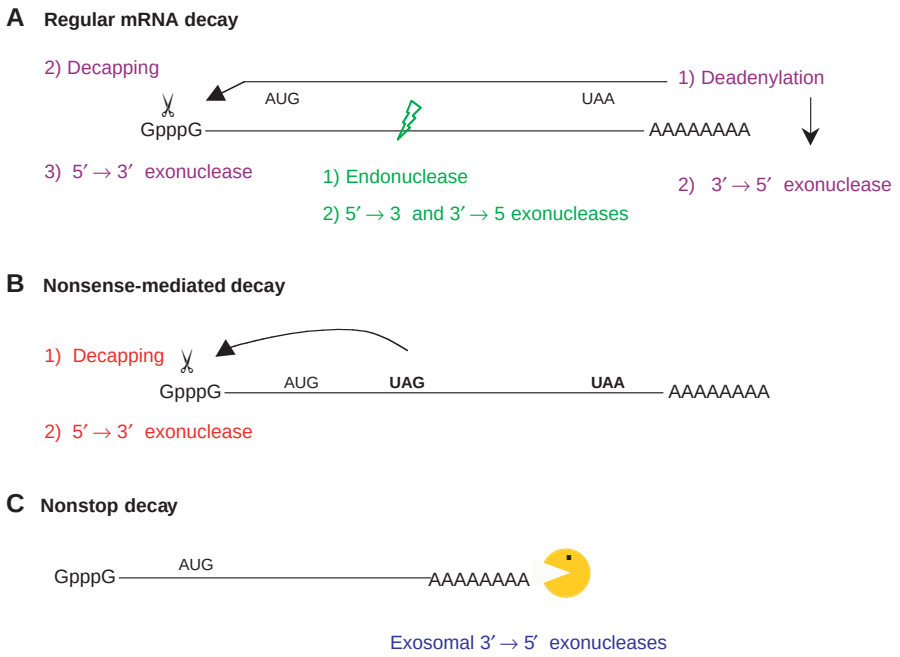

Fig. 1. Mechanisms of mRNA turnover. (A) Normal mRNAs are degraded by different pathways. Following deadenylation, mRNAs can either be degraded from the 3′ end by the exosome or decapped and degraded from the 5′ end (shown in purple). Alternatively, mRNAs can be cleaved by endonucleases and those fragments degraded from both ends (green). (B) A premature stop codon triggers rapid decapping and 5′ to 3′ exonucleolytic digestion. (C) Transcripts lacking a termination codon are degraded by the exosome in a deadenylase-independent manner.

The role of translation in mRNA surveillance

How do cells distinguish mRNAs that have proper, improper and no translation termination codons? The requirement for ongoing translation to detect mRNAs that have PTCs is well documented. The NMD pathway is disabled by (1) insertion of a stem-loop structure or an iron-response element in the 5′UTR to prevent translation initiation (Belgrader et al., 1993; Thermann et al., 1998), (2) inhibition of translation elongation with cycloheximide (Carter et al., 1995; Herrick et al., 1990; Zhang et al., 1997), (3) expression of suppressor tRNAs that prevent premature termination (Belgrader et al., 1993) and (4) mutation of the AUG start codon, which prevents 80S ribosome formation (Naeger et al., 1992; Ruiz-Echevarria et al., 1998). Similarly, nonstop decay also depends on translation, because an mRNA lacking a termination codon is stabilized in the presence of cycloheximide (Frischmeyer et al., 2002). Although both NMD and nonstop decay require translation, the two pathways have significantly different mechanisms.

Upf proteins: factors involved in NMD

Three interacting trans-acting factors, Upf1p, Upf2p, and Upf3p, are required for NMD in *S. cerevisiae* but play no role in nonstop decay. Despite their necessity for NMD, the *UPF* genes are not essential in yeast. Loss-of-function mutations in any of the *UPF* genes result in a slow-growth phenotype and increased frequency of stop-codon readthrough, as well as stabilization of nonsense-containing mRNAs (Leeds et al., 1992; Maderazo et al., 2000; Wang et al., 2001; Weng et al., 1996a; Weng et al., 1996b). Approximately 225 of ~6000 expressed yeast sequences (~4%) are affected by inactivation of the NMD pathway (Lelivelt and Culbertson, 1999). Seven genes are required for NMD in *C. elegans*: *SMG-1* to *SMG-7* (Cali et al., 1999; Mango, 2001; Pulak and Anderson, 1993). *SMG-2*, *SMG-3* and *SMG-4* encode homologs of Upf1p, Upf2p and Upf3p, respectively (Page et al., 1999). *SMG-1* encodes a PI-3-kinase-related kinase (PIKK) that binds to and phosphorylates SMG-2. Less is known about SMG-5 to SMG-7, but they appear to be required for dephosphorylation of SMG-2 (Page et al., 1999). Homologs of SMG-1, SMG-5, SMG-6 and SMG-7 have not been identified in the yeast genome. Human homologs of Upf1p/SMG-2, Upf2p/SMG-3, Upf3p/SMG-4 and SMG-1 have been identified and demonstrated to be involved in NMD (hUpf1, hUpf2, hUpf3, and hSMG-1) (Applequist et al., 1997; Denning et al., 2001; Lykke-Andersen et al., 2000; Mendell et al., 2000; Perlick et al., 1996; Serin et al., 2001; Yamashita et al., 2001). Human homologs of SMG-5, SMG-6 and SMG-7 have not yet been characterized, although apparent homologs of SMG-5 exist (Clissold and Ponting, 2000).

Upf1p is the best-studied factor in NMD. It is a cytoplasmic protein that has a cysteine-histidine-rich region at its N-terminus, reminiscent of a zinc finger (Applequist et al., 1997; Atkin et al., 1997; Bhattacharya et al., 2000). Upf1p is a group I helicase that has RNA-dependent ATPase and ATP-dependent 5′→3′ helicase activities (Bhattacharya et al., 2000; Weng et al., 1996a). It interacts with translation release factors eRF1 and eRF3, which is consistent with its roles in both translation termination and NMD (Czaplinski et al., 1998; Wang et al., 2001). Mutations in the *S. cerevisiae UPF1* gene have shown that the roles of Upf1p in translation termination and NMD are genetically separable. For example, a mutation in the cysteine-histidine-rich domain results in a nonsense-suppression phenotype, but the NMD pathway remains intact. By contrast, mutations in the Upf1p helicase domain abolish NMD function, but translation termination proceeds normally (Weng et al., 1996b).

Upfs 1, 2 and 3 interact with each other in yeast and humans, and they have been collectively termed the Upf complex. However, indirect immunofluorescence indicates that the Upf

complex is highly dynamic, because human Upf proteins accumulate at different cellular locations. hUpf1 is cytoplasmic, hUpf2 is mainly perinuclear, whereas hUpf3 is a predominantly nuclear, nucleocytoplasmic shuttling protein (Lykke-Andersen et al., 2000; Serin et al., 2001).

Recent results demonstrate that phosphorylation of Upf1 by SMG-1 plays an important role in NMD in *C. elegans* and humans (Denning et al., 2001; Page et al., 1999; Yamashita et al., 2001). Phosphorylated hUpf1 preferentially interacts with hUpf3, rather than the unphosphorylated form, which suggests that the activity of hUpf1 is modulated by phosphorylation (Yamashita et al., 2001). Phospho-hUpf1 also copurifies with polysomal cell fractions, which suggests that it functions concomitantly with translation (Pal et al., 2001). Overexpression of human SMG-1 increases decay of an NMD reporter mRNA. By contrast, overexpression of a kinase-inactive hSMG-1 mutant protein resulted in its stabilization (Yamashita et al., 2001). Thus far, phosphorylation of yeast Upf1 has not been reported, which is consistent with the absence of a SMG-1 homolog in yeast.

Detection of mRNAs with premature termination codons

The mechanisms by which PTC-containing mRNAs are recognized in *S. cerevisiae* and mammals may differ significantly. In *S. cerevisiae*, a *cis*-acting element that destabilizes mRNAs when located downstream of a nonsense codon has been described (Zhang et al., 1995). This downstream sequence element (DSE) appears to be required for recognition of a stop codon as premature. The yeast protein Hrp1p has been shown to bind to a DSE as well as Upf proteins (Gonzalez et al., 2000).

In mammals, a PTC is recognized by its position relative to the last exon-exon junction. As a general rule, mammalian transcripts that contain a nonsense codon more than ~50 nucleotides upstream of the last exon-exon junction will be subjected to NMD (Zhang et al., 1998a; Zhang et al., 1998b). Accordingly, the vast majority of mammalian genes contain the termination codon in the last exon or <50 nucleotides upstream of the last intron (Nagy and Maquat, 1998). This suggests that NMD requires an intron in the target mRNA. Supporting this view is the observation that intronless transcripts, such as hsp70 and histone mRNAs, are immune to NMD (Maquat and Li, 2001). How does the mRNA surveillance machinery evaluate the nuclear processing history of a given mRNA? The observation that pre-mRNA splicing results in alteration of the mRNP structure and composition supports the idea that the loci of splicing events in the nucleus are communicated to the translational machinery by the presence of some identifying 'mark' on the mRNA (Le Hir et al., 2000b).

Such a 'mark' has indeed been found to be deposited 20-24 nucleotides upstream of the exon-exon junction as a result of pre-mRNA splicing and is called the exon-junction complex (EJC) (Le Hir et al., 2000a). The EJC is a highly dynamic structure that consists of at least eight proteins (Table 1), some of which leave the nucleus with the mRNA (Kataoka et al., 2001; Kim et al., 2001a; Kim et al., 2001b; Le Hir et al., 2001b; Le Hir et al., 2000a). The EJC stimulates nuclear export of spliced mRNA (Le Hir et al., 2001b), probably as a result of the interaction between the EJC subunit Aly/REF and the nuclear export receptor TAP/p15 (Le Hir et al., 2001a; Luo et al., 2001; Stutz et al., 2000; Zhou et al., 2000). It may also function in mRNA localization, because the EJC subunits magoh and Y14 are homologs of the *Drosophila* proteins Mago nashi and Tsunagi, which may be important for localization of embryonic mRNAs (Kataoka et al., 2001; Mohr et al., 2001).

The importance of the EJC in NMD was demonstrated by its interaction with hUpf3 and the observation that specific subunits, RNPS1 and to a lesser extent Y14, are capable of triggering NMD when tethered downstream of a translation termination codon (Kataoka et al., 2001; Kim et al., 2001b; Lykke-Andersen et al., 2001). These data suggest that every cellular intron-containing mRNA is tested for the position of the termination codon relative to the EJC. In PTC-containing mRNAs, in which translation termination occurs upstream of one or more EJCs, the hUpf proteins will bridge the translation release factors and the EJC and trigger decay (Fig. 2). Normal mRNAs, however, remain stable because all EJCs have been displaced by the time of translation termination (Fig. 2). How the Upf proteins, once assembled on the PTC-containing mRNA, trigger decay is poorly understood.

Trans-acting factors in nonstop decay

In contrast to NMD, nonstop decay does not rely on the Upf proteins. Genetic studies in *S. cerevisiae* have shown that a nonstop mRNA is stabilized in yeast strains lacking exosome

Table 1. Components of the human exon-junction complex (EJC)

Gene	Mass (kDa)	Function	References
Aly/REF	70	mRNA export factor, binds to Y14, recruits TAP/p15 and dissociates from mRNA during nuclear export	Le Hir et al., 2000a; Stutz et al., 2000; Zhou et al., 2000
RNPS1	50	General splicing coactivator; triggers NMD when tethered to mRNA	Le Hir et al., 2000a; Lykke-Andersen et al., 2001; Mayeda et al., 1999
SRm160	160	Ser-Arg-rich splicing coactivator	Blencowe et al., 2000; Le Hir et al., 2000a
Y14	17	Binds to Aly/REF and RNPS1, persists on mRNA in cytoplasm	Kim et al., 2001b; Le Hir et al., 2000a
DEK	45	Phosphoprotein, associates with SRm160, an autoantigen in rheumatic diseases, and is translocated in acute myeloid leukemia	Le Hir et al., 2000a; McGarvey et al., 2000
UAP56	60	General splicing factor, binds to Aly to couple splicing and mRNA export, DEAD box helicase	Luo et al., 2001
hUpf3a hUpf3b	45	Associates with hUpf1 and hUpf2 and is required for nonsense-mediated decay	Kim et al., 2001a; Lykke-Andersen et al., 2000
magoh	17	Binds to Y14 and TAP, persists on mRNA after export, homolog of *Drosophila mago nashi*. It has a possible role in cytoplasmic localization of mRNAs	Kataoka et al., 2001, Le Hir et al., 2001a, Mohr et al., 2001

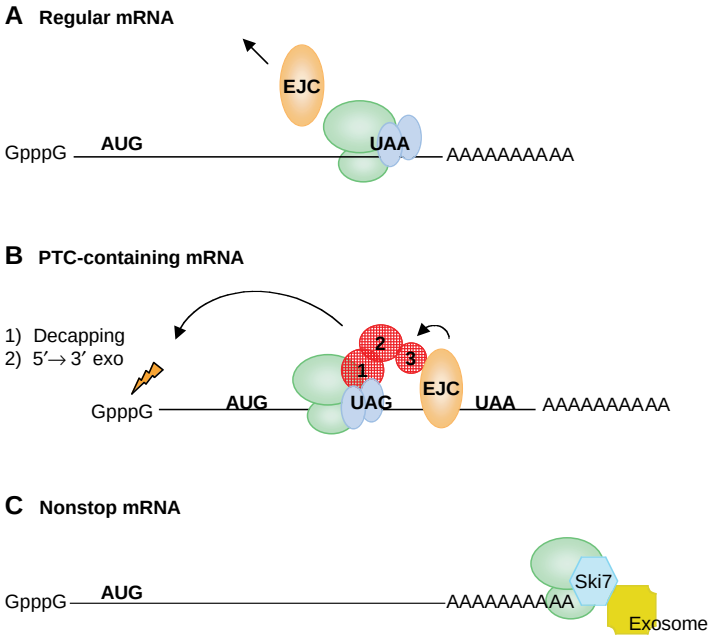

Fig. 2. Factors involved in mRNA surveillance. (A) During translation of a normal mRNA, the EJCs are displaced from the transcript by the translocating ribosome. Absence of a downstream EJC during translation termination results in a stable message. Translation release factors are shown in blue. (B) During translation termination of a PTC-containing transcript, the downstream EJC signals to the terminating ribosome via Upf3. Upf3, together with Upfs 1 and 2, may signal the presence of the PTC to the 5′ end of the transcript, resulting in decapping and rapid exonucleolytic digestion of the mRNA. This model is based on yeast studies, and it is not yet known whether mammalian NMD also occurs by 5′→3′ exonucleolytic decay. (C) The ribosome translates through the poly-A tail in the absence of an in-frame termination codon. Ski7 is thought to bind to the empty ribosomal A site and recruit the exosome, resulting in rapid decay from the 3′ end.

subunit proteins but not in a *upf1* strain (Frischmeyer et al., 2002). The exosome-associated protein Ski7p is required for the degradation of nonstop transcripts. Ski7p is a cytoplasmic protein whose C-terminal GTPase domain exhibits similarity to the translation factors EF1A and eRF3 (Araki et al., 2001; van Hoof et al., 2002). It has been proposed that when the ribosome reaches the 3′ end of an mRNA lacking a termination codon, the GTPase domain of Ski7 binds to the empty ribosomal A site and recruits the cytoplasmic exosome to degrade the transcript from the 3′ end (Fig. 2) (Frischmeyer et al., 2002; van Hoof et al., 2002). Nonstop decay has also been detected in mammals, but very little is known about its mechanism (Frischmeyer et al., 2002).

Nucleus or cytoplasm - where's the action?

Even though NMD depends on translation, several recent reports suggest that mammalian NMD, in contrast to nonstop decay, could be an entirely nuclear process for some transcripts. For example, low levels of translation have been detected in the nucleus (Iborra et al., 2001). Cellular fractionation studies have shown that decreased steady-state levels of many PTC-containing transcripts are associated with isolated nuclei (Belgrader et al., 1993; Zhang and Maquat, 1997). PTCs have also been found to inhibit splicing and cause altered splice-site selection in pre-mRNAs (Brogna, 2001; Carter et al., 1996; Dietz and Kendzior, 1994; Dietz et al., 1993; Gersappe and Pintel, 1999; Qian et al., 1993), although many of these observations may be explained by mutation of splicing enhancers (Liu et al., 2001). In addition, human Upf proteins interact with mRNAs associated with nuclear poly-A- and cap-binding proteins (Ishigaki et al., 2001). However, both of these proteins shuttle between the nucleus and cytoplasm. Although these reports suggest that NMD of some transcripts may be a nuclear process, other data argue against it. For example, inclusion of an alternative exon containing several in-frame PTCs in the *C. elegans* U2AF mRNA results in its nuclear retention without accelerated decay (MacMorris et al., 1999). Also, an iron-response element that depends on the cytoplasmic iron-response-element binding protein to repress translation is capable of blocking NMD of a PTC-containing β-globin mRNA (Thermann et al., 1998).

Evolution of mRNA surveillance

It is clear that the mRNA surveillance pathway is conserved across eukaryotes, from yeast to humans. One may speculate that mRNA surveillance evolved to mitigate the potentially harmful gain-of-function and other dominant-negative effects of truncated proteins. mRNA surveillance may also serve as a safeguarding mechanism that allows organisms to accumulate a reservoir of mutations while masking otherwise deleterious phenotypic consequences. Despite the level of conservation observed in this pathway, intriguing differences exist. The coming years will undoubtedly be exciting as scientists further uncover the mechanisms of mRNA surveillance.

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