

Cutting the nonsense: the degradation of PTC containing mRNAs

Pamela Nicholson and Oliver Mühlemann¹

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland

Abstract

In eukaryotes, mRNAs harbouring PTCs (premature translation-termination codons) are recognized and eliminated by NMD (nonsense-mediated mRNA decay). In addition to its quality-control function, NMD constitutes a translation-dependent post-transcriptional pathway to regulate the expression levels of physiological mRNAs. In contrast with PTC recognition, little is known about the mechanisms that trigger the rapid degradation of mammalian nonsense mRNA. Studies have shown that mammalian NMD targets can be degraded via both an SMG6 (where SMG is suppressor of morphological defects on genitalia)-dependent endonucleolytic pathway and a deadenylation and decapping-dependent exonucleolytic pathway, with the possible involvement of SMG5 and SMG7. In contrast, *Drosophila melanogaster* NMD is confined to the former and *Saccharomyces cerevisiae* NMD to the latter decay pathway. Consistent with this conclusion, mammals possess both SMG6 and SMG7, whereas *D. melanogaster* lacks a SMG7 homologue and yeast have no SMG6 equivalent. In the present paper, we review what is known about the degradation of PTC-containing mRNAs so far, paying particular attention to the properties of the NMD-specific factors SMG5–SMG7 and to what is known about the mechanism of degrading mRNAs after they have been committed to the NMD pathway.

Introduction: NMD (nonsense-mediated mRNA decay)

The cascade of events during gene expression involves a series of complex and tightly linked steps from transcription of the DNA-encoded genetic information to the eventual protein synthesis. Whereas the intricacy of gene expression allows for fine-tuned regulation at many different levels, it also makes each step susceptible to mistakes. Therefore, to ensure the accuracy of gene expression, cells have evolved elaborate quality-control mechanisms both in the nucleus and in the cytoplasm at multiple stages during gene expression (reviewed in [1]). At the level of mRNA, two important features are monitored: first, whether it has the correct set of proteins bound to a particular mRNA and, secondly, whether the coding potential of the mRNA is intact. The NMD pathway deals with the latter; it acts to selectively identify and degrade mRNAs that contain a PTC (premature translation-termination codon), and hence reduce the accumulation of potentially toxic truncated proteins.

PTCs can appear in mRNAs for a variety of reasons. At the DNA level, PTCs arise from erroneous bases in the gene sequence of the genome (mutations) or due to insertions or deletions leading to frameshift mutations in

genes. At the RNA level, PTCs can be produced by alternative splicing (or splicing errors in general) or less frequently by transcriptional errors. It is estimated that, in mammals, approx. one-third of alternatively spliced transcripts contain PTCs and are substrates for NMD [2]. Often PTCs are generated in genes belonging to the Ig superfamily as the result of the programmed recombination of different gene segments. During the rearrangement of the V (variable), D (diversity) and J (joining) segments in these genes, non-templated nucleotides are added or lost at the junctions between the recombination sites and hence two out of three cases result in a frameshift and a PTC downstream [3].

NMD is one of the best-studied mRNA quality-control mechanism in eukaryotes and it has become clear in recent years that many physiological mRNAs are also NMD substrates, signifying a role for NMD beyond mRNA quality control as a translation-dependent post-transcriptional regulator of gene expression (reviewed in [4]). Genome-wide studies in *Saccharomyces cerevisiae*, *Drosophila melanogaster* and human cells showed that 3–10% of all mRNAs are regulated by the NMD factor UPF1 (where UPF is up-frameshift) [5–7]. Thus many endogenous NMD substrates were found to contain no obvious PTCs.

It appears that NMD controls a large and diverse inventory of transcripts which reflects the important influence of NMD on the metabolism of the cell and accordingly in many human diseases.

A third of all known disease-causing mutations are predicted to cause a PTC, and consequently NMD is a significant modulator of genetic disease phenotypes in humans [8]. In this sense, NMD can act either in a beneficial

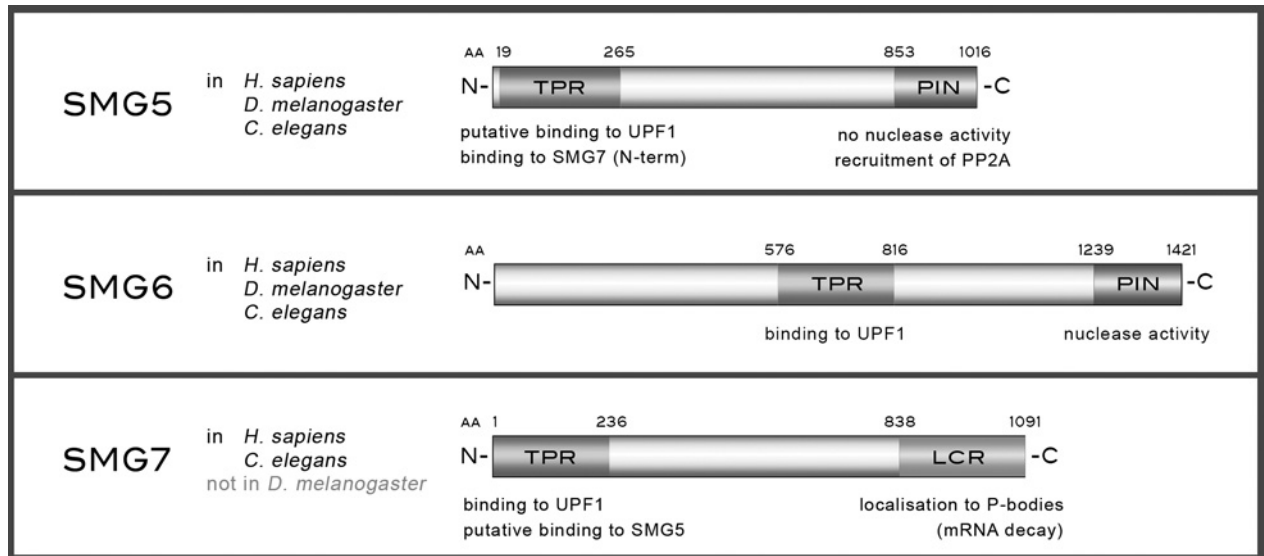
Key words: endonuclease, mRNA degradation, nonsense-mediated mRNA decay, premature translation-termination codon (PTC).

Abbreviations used: CCR4, carbon catabolite repressor 4; DCP2, decapping enzyme 2; NMD, nonsense-mediated mRNA decay; PP2A, protein phosphatase 2A; PNRC2, proline-rich nuclear receptor co-regulatory protein 2; PTC, premature translation-termination codon; TPR, tetratricopeptide repeat; SMG, suppressor of morphological defects on genitalia; UPF, up-frameshift.

¹To whom correspondence should be addressed (email oliver.muehlemann@dcb.unibe.ch).

Figure 1 | Schematic illustration of SMG5, SMG6 and SMG7

SMG5, SMG6 and SMG7 all contain two TPRs, a domain which adopts a 14-3-3-like fold. This 14-3-3-like fold has been demonstrated for SMG7, and is predicted for SMG5 and SMG6 [37]. The C-termini of SMG5 and SMG6 contain a PIN domain; whereas the catalytic centre of SMG5 is mutated, SMG6 has endonuclease activity. AA, amino acid; LCR, low-complexity region.



or in a detrimental way; the latter if it prevents the production of proteins with some residual function and the former if it prevents the synthesis of toxic truncated proteins [9–11].

Despite a great deal of scientific research for over 20 years, the process of NMD is far from being fully understood with regard to its physiological relevance to the cell, the molecular mechanisms that underpin this pathway and all of the factors that are involved. To investigate the process of NMD, two crucial questions need to be addressed: (i) what are the exact molecular mechanisms involved in the recognition of mRNA containing a PTC; and (ii) after identification of a PTC-containing mRNA, how is it degraded? A great deal of effort has gone into answering the former question; how the cell is able to distinguish between a normal translation-termination codon and a PTC in the mRNA. The molecular mechanisms involved in this aspect of NMD are not clearly defined, but it is clear that NMD depends on translation and various models have been proposed, as reviewed in [12–16]. In the present paper, we focus on the research that has aimed at trying to answer the latter question about how exactly PTC containing mRNAs are degraded; first by summarizing the NMD-specific factors that have been shown to be involved and, secondly, by discussing the possible pathways that have been proposed to explain this process.

SMG5, SMG6 and SMG7: the degradation players

The first *trans*-acting factors required for NMD were identified via genetic screens in *S. cerevisiae* (UPF1, UPF2

and UPF3) [17–20] and in *Caenorhabditis elegans* (SMG1, SMG5, SMG6 and SMG7, called SMGs for suppressor of morphological defects on genitalia) [21–23]. The UPF proteins constitute the core NMD machinery where UPF1 is imperative for NMD and hence is the most conserved UPF factor [24,25]. UPF1 is a group 1 RNA helicase and nucleic-acid-dependent ATPase which is central to PTC recognition [26,27].

The SMG proteins determine the phosphorylation status of UPF1 where SMG1 catalyses the phosphorylation of UPF1 at several serine residues [28,29] and SMG5, SMG6 and/or SMG7 are involved in the dephosphorylation of UPF1 [25]. A comprehensive summary and discussion of all known NMD factors and factors associated with NMD has been thoroughly reviewed in [14,16].

For some time, SMG5, SMG6 and SMG7 were thought of as metazoan-specific NMD factors required for the degradation of nonsense mRNAs. However, this is clearly not the case, as a SMG7 yeast orthologue called Ebs1p has been discovered [30]. Although SMG5, SMG6 and SMG7 share similar features as depicted in Figure 1 and discussed below, they do not appear to be functionally redundant as previously assumed [25,31,32]. SMG5–SMG7 have been identified in all metazoans analysed so far, with the exception of *D. melanogaster*, where SMG7 is lacking [33]. On the other hand, in plants, so far only SMG7 has been identified [34]. Co-immunoprecipitation experiments [32,35], yeast two-hybrid interaction studies [32], co-localization data [36] and GST (glutathione transferase) pull-down assays [37] have verified an interaction between SMG5 and SMG7, probably via dimerization of their 14–3–3-protein-like domains [36].

C. elegans mutated for SMG5, SMG6 or SMG7 are deficient in NMD and accumulate hyperphosphorylated SMG2 (the UPF1 orthologue) because UPF1 dephosphorylation is induced by PP2A (protein phosphatase 2A) which is recruited by SMG5, SMG6 and/or SMG7 [25]. PP2A is a heterotrimer composed of a catalytic subunit, a structural A/PR65 subunit and at least one of the three different classes of regulatory B subunit which are known to be encoded by at least 13 genes and various splice variants. It is evident that an important function of these regulatory subunits is to target the PP2A holoenzyme to separate cellular locations and complexes (reviewed in [38]). Indeed, the C-terminal part of SMG5 binds to PP2A, but a direct interaction between PP2A and SMG7 is uncertain [32,35]. This difference might present a possible explanation as to why both SMG5 and SMG7 are required for NMD, although when SMG7 is tethered to a reporter transcript, it can degrade the mRNA independently of SMG5 [36]. Perhaps the dedicated role of SMG5 would be the dephosphorylation of UPF1. However, this is not compatible with the finding that SMG5 is present and essential for NMD in *D. melanogaster* [33], yet SMG7 is lacking and nonsense mRNAs are degraded via a pathway involving SMG6.

Human SMG6 has been shown to co-purify with the catalytic subunit of PP2A, SMG1, UPF1, UPF2 and UPF3B, and also to specifically target dephosphorylation of UPF1, but not of UPF2 [31].

SMG5, SMG6 and SMG7 all contain two TPRs (tetratricopeptide repeats) situated in the middle sector of SMG6 and in the N-terminus of SMG5 and SMG7. TPR-containing domains consist of 34-amino-acid-long TPRs that are usually associated with protein–protein interactions [39]. For SMG7, the TPRs were shown to adopt a similar fold to 14-3-3, which is a signal-transduction protein that binds phosphoserine-containing polypeptides. Sequence similarities suggest that there is conservation of this 14-3-3-like domain structure in SMG5 and SMG6. For SMG6 and SMG7, it has been confirmed experimentally that this domain is able to bind phosphorylated serine residues of UPF1 [37].

The C-termini of SMG5 and SMG6 contain a PIN domain which is named after its homology with the N-terminal domain of pili biogenesis protein (PilT N terminus) [40]. PIN domains are found in all three kingdom of life where they often function as phosphodiesterases and usually exhibit nuclease activity [41]. Despite the fact that the PIN-like domains of SMG5 and SMG6 adopt a similar overall fold that is related to ribonucleases of the RNase H family, SMG6 harbours the canonical triad of aspartic acid residues crucial for RNase H activity, whereas SMG5 lacks two of the three key catalytic residues [42]. These structural differences are apparent at the molecular level, as only the PIN domain of SMG6 has nuclease activity on single-stranded RNA *in vitro* [42], and it was demonstrated recently in human and *D. melanogaster* cells that SMG6 is the endonuclease that can initiate cleavage of nonsense mRNA in close proximity to the PTC [43,44]. Instead of a PIN domain, SMG7 contains a low-complexity structure at its C-terminus and, when it is tethered to a reporter mRNA, it is sufficient to elicit

degradation independently of a PTC, UPF1, SMG5 and SMG6 [36]. Possibly SMG7 functions at a late step in NMD, downstream of PTC recognition or may reflect mRNA degradation independently of NMD.

Evidence for endonucleolytic and exonucleolytic decay pathways

Although good progress has been achieved in the clarification of the PTC-recognition mechanism, little is known about the subsequent degradation of the recognized nonsense mRNA. Current models propose that the factors SMG5, SMG6, and SMG7 are involved in this process as discussed above.

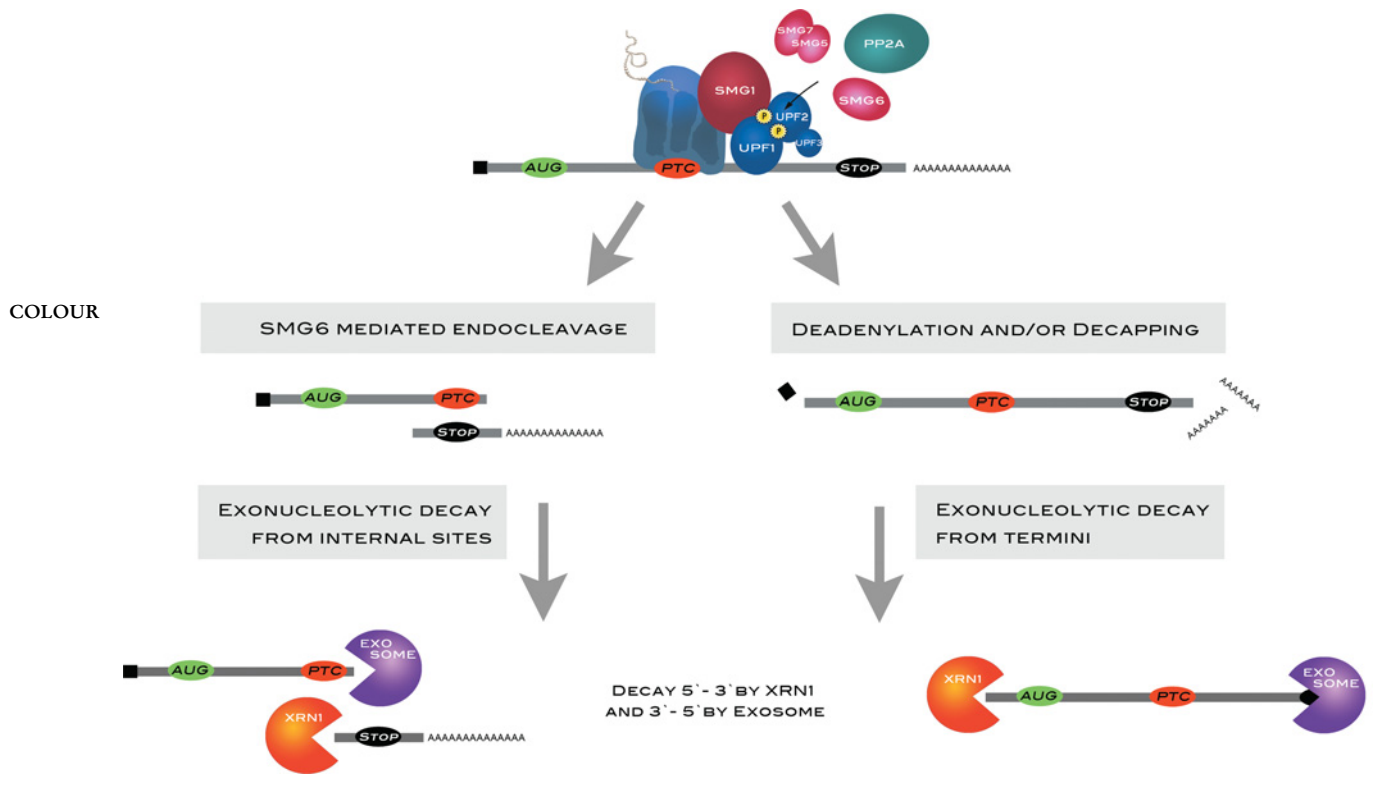
An important question to address is what is the pathway of degradation that NMD follows? For instance, is the general mRNA-degradation pathway utilized after improper translation termination, or is NMD initiated by special nucleases? The major mRNA-turnover pathway generally begins with the removal of the poly(A) tail. In yeast, the deadenylation reaction is catalysed by the Ccr4p–Caf1p complex, whereas in mammals, the poly(A) tail is shortened by the consecutive action of two different complexes: the PAN2–PAN3 [where PAN is poly(A) nuclease] complex first shortens full-length poly(A) tails of approx. 200 bases to approx. 110 bases. These intermediate poly(A) tails are subsequently targeted by the CCR4 (carbon catabolite repressor 4)–CAF1 (CCR4-associated factor 1) complex that removes the remaining adenines. Following the removal of the poly(A) tail, mRNAs are degraded either by decapping [by DCP2 (decapping enzyme 2)] and 5'→3' decay (by exonuclease XRN1) or by 3'→5' decay by the exosome (reviewed in [45,46]). There are special exceptions to the main mRNA-turnover pathway such as the deadenylation-independent decapping pathway and endonucleolytic cleavage degradation pathway [47].

Research carried out in *S. cerevisiae* suggests that nonsense mRNAs are rapidly degraded using the main mRNA-turnover pathway as outlined above [48–51]. Additionally, it has also been reported that the nonsense mRNAs can be degraded by deadenylation-independent decapping and XRN1-mediated decay in yeast [51–53]. In contrast, in *D. melanogaster*, where there is no SMG7, nonsense mRNAs are degraded by endonucleolytic cleavage of the PTC-containing mRNA near the PTC. The resulting 5' and 3' decay intermediates are then rapidly degraded in the 3'→5' direction by the exosome and in the 5'→3' direction by XRN1 respectively [43,54].

The research generated by studies focusing on the degradation of NMD reporter transcripts in mammalian cells is not so straightforward and paints a contradictory picture. Studies have shown that nonsense mRNAs can be degraded via the conventional mRNA-turnover pathway, starting with deadenylation, followed by decapping and XRN1-mediated exonucleolytic decay [49,50,53]. This is quite consistent with the finding that the UPF proteins can form a complex with XRN1, XRN2 and various exosome

Figure 2 | Model for degradation of NMD substrates

After the recognition of the PTC and the SMG1-mediated phosphorylation of UPF1, two independent routes are possible. The right-hand pathway involves SMG6, which binds phosphorylated UPF1, leading to the dephosphorylation of UPF1 through the recruited PP2A. The nonsense mRNA is degraded by endonucleolytic cleavage in close proximity to the PTC by SMG6 followed by the rapid decay of the two remaining fragments. The left-hand pathway involves SMG5 and SMG7, which are thought to bind phosphorylated UPF1, recruit PP2A to dephosphorylate UPF1 and promote the degradation of the nonsense mRNA via the ends of the transcripts. Further work is needed to clarify the roles of SMG5 and SMG7 and to elucidate the factors which determine the fate of a PTC-containing mRNA towards the two pathways.



components [49]. Furthermore, a recent report has shown that PNRC2 (proline-rich nuclear receptor co-regulatory protein 2), a protein that is better known as a transcription factor, seems to play a role in human NMD where its down-regulation abrogates NMD and tethering of this 16 kDa protein to reporter transcripts promotes degradation of the reporter mRNA.

Interestingly, PNRC2 is able to interact with UPF1 and DCP1, displaying a higher affinity for phosphorylated UPF1, suggesting that PNRC2 functions like an adaptor by providing a link between mRNAs bound by UPF1 and the decapping machinery [55]. It has been shown by tethering and RNAi (RNA interference) depletion studies that SMG7-mediated degradation is dependent on DCP2 and XRN1, but it is not clear how SMG7-promoting degradation of nonsense mRNAs depends on DCP2 and XRN1 and whether PNRC2 is involved. In contrast with degrading nonsense mRNA from the ends of the mRNA, it was reported recently that degradation of human nonsense mRNAs can occur by endonucleolytic cleavage near the PTC [44]. Moreover, the PIN domain of SMG6 was shown to possess the

endonuclease activity responsible for initiating NMD in both *D. melanogaster* and human cells [43,44]. This finding led to an adjustment to the model of NMD, and it appears that endonucleolytic cleavage is a conserved feature in metazoan NMD.

Endonucleolytic cleavage is essential for many RNA-degradation and RNA-processing reactions. For instance, it has recently been discovered that the exosome component Rrp44p (Dis3p) has endonucleolytic activity in addition to its 3'→5' exonucleolytic activity. Interestingly, the domain responsible for the endonucleolytic activity in Rrp44p is a PIN domain which is similar to that found in SMG6 and in the ribosomal processing protein Nob1p [56]. Further examples of endonucleases include human Argonaute2 which mediates RNA cleavage targeted by miRNAs (microRNAs) and siRNAs (short interfering RNAs). And in the nucleus, CPSF-73 (cleavage and polyadenylation specificity factor 73 kDa) is the endonuclease involved in 3'-end formation where it cleaves the pre-mRNA in both polyadenylated mRNAs [57,58] and replication-dependent histone mRNAs which do not contain a poly(A) tail [59]. Endonucleolysis is

also found in the mRNA-degradation pathway in bacteria (reviewed in [60]). As mentioned above, SMG6 is absent from yeast. Thus the presence of the fast SMG6-mediated endonucleolytic pathway in metazoans is likely to be a resulting adaptation to the increased amount of defective transcripts generated in higher eukaryotes.

It therefore seems that mammalian NMD targets can be degraded by both a SMG6-dependent endonucleolytic pathway and a deadenylation- and decapping-dependent exonucleolytic pathway, as illustrated in Figure 2. However, this 'branched NMD model' raises several questions that need to be addressed.

Looking to the future: are there really two pathways?

Does mammalian NMD really consist of two independent routes for RNA decay: an endonucleolytic SMG6-dependent route and a decapping-dependent exonucleolytic route? Despite the lack of concrete evidence, several lines of support exist to suggest that the degradation of nonsense mRNA can occur via two different routes. First, two different complexes are proposed containing PP2A, phosphorylated UPF1 and either SMG5/7 or SMG6, although the exclusiveness of the latter component in either complex has not been shown [31,35]. Secondly, it has been proposed that SMG5 and SMG7 co-localize to cytoplasmic P-bodies (processing bodies), possibly while SMG6 forms separate cytoplasmic foci [36]. Such issues regarding where nonsense mRNA degradation occurs have been reviewed recently in [61]. Finally, in *D. melanogaster*, which lacks SMG7, nonsense mRNA seems to be exclusively degraded by endonucleolytic cleavage, as no SMG6 orthologue and no endonucleolysis were observed in yeast, reinforcing the idea of two at least rather different pathways.

If indeed there are two pathways, further work is required to determine the relative contributions of these two decay pathways which would be difficult to calculate experimentally, since they may be redundant and hence could substitute for one another. They might also be linked to each other, and the depletion of one factor might ultimately affect both degradation routes. Furthermore, it is difficult to discriminate experimentally between the fraction of NMD substrates and the fraction of mRNAs that escapes NMD and hence are degraded by the normal decay route. Finally, the ratio between the two different pathways may also vary for different mRNAs, cell types or differential cellular states. Another aspect of this degradation mechanism that needs to be clarified is what determines which route is taken by the different types of mRNAs directed to the NMD pathway. Assuming that SMG5/7 are not part of the pathway involving SMG6, then binding of either SMG5/7 or SMG6 to phosphorylated UPF1 might represent a branching point. Taking this slightly further, what might determine which factor binds phosphorylated UPF1? Perhaps it is substrate-specific and/or dependent upon an upstream process. Clearly

all of these open questions need to be explored as well as a more in-depth characterization of SMG5–SMG7 to gain a better molecular understanding of the process of NMD after the point of PTC recognition.

Acknowledgements

We acknowledge and sincerely thank David Zünd for discussions and Nicole Kleinschmidt for the design of Figures 1 and 2.

Funding

O.M. is a fellow of the Max Cloëtta Foundation and the work in his laboratory is supported by the European Research Council [grant number ERC-StG 207419], the Swiss National Science Foundation [grant number 31003A-127614], the Novartis Foundation for Biomedical Research, the Helmut Horten Foundation and by the Kanton Bern.

References

- 1 Doma, M.K. and Parker, R. (2007) RNA quality control in eukaryotes. *Cell* **131**, 660–668
- 2 Lewis, B.P., Green, R.E. and Brenner, S.E. (2003) Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 189–192
- 3 Li, S. and Wilkinson, M.F. (1998) Nonsense surveillance in lymphocytes? *Immunity* **8**, 135–141
- 4 Rehwinkel, J., Raes, J. and Izaurralde, E. (2006) Nonsense-mediated mRNA decay: target genes and functional diversification of effectors. *Trends Biochem. Sci.* **31**, 639–646
- 5 Rehwinkel, J., Letunic, I., Raes, J., Bork, P. and Izaurralde, E. (2005) Nonsense-mediated mRNA decay factors act in concert to regulate common mRNA targets. *RNA* **11**, 1530–1544
- 6 Mendell, J.T., Sharifi, N.A., Meyers, J.L., Martinez-Murillo, F. and Dietz, H.C. (2004) Nonsense surveillance regulates expression of diverse classes of mammalian transcripts and mutes genomic noise. *Nat. Genet.* **36**, 1073–1078
- 7 He, F., Li, X., Spatrick, P., Casillo, R., Dong, S. and Jacobson, A. (2003) Genome-wide analysis of mRNAs regulated by the nonsense-mediated and 5' to 3' mRNA decay pathways in yeast. *Mol. Cell* **12**, 1439–1452
- 8 Frischmeyer, P.A. and Dietz, H.C. (1999) Nonsense-mediated mRNA decay in health and disease. *Hum. Mol. Genet.* **8**, 1893–1900
- 9 Kuzmiak, H.A. and Maquat, L.E. (2006) Applying nonsense-mediated mRNA decay research to the clinic: progress and challenges. *Trends Mol. Med.* **12**, 306–316
- 10 Khajavi, M., Inoue, K. and Lupski, J.R. (2006) Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *Eur. J. Hum. Genet.* **14**, 1074–1081
- 11 Holbrook, J.A., Neu-Yilik, G., Hentze, M.W. and Kulozik, A.E. (2004) Nonsense-mediated decay approaches the clinic. *Nat. Genet.* **36**, 801–808
- 12 Brogna, S. and Wen, J. (2009) Nonsense-mediated mRNA decay (NMD) mechanisms. *Nat. Struct. Mol. Biol.* **16**, 107–113
- 13 Rebbapragada, I. and Lykke-Andersen, J. (2009) Execution of nonsense-mediated mRNA decay: what defines a substrate? *Curr. Opin. Cell Biol.* **21**, 394–402
- 14 Chang, Y.F., Imam, J.S. and Wilkinson, M.F. (2007) The nonsense-mediated decay RNA surveillance pathway. *Annu. Rev. Biochem.* **76**, 51–74
- 15 Isken, O. and Maquat, L.E. (2007) Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. *Genes Dev.* **21**, 1833–1856

- 16 Nicholson, P., Yepiskoposyan, H., Metzke, S., Zamudio Orozco, R., Kleinschmidt, N. and Mühlemann, O. (2005) Nonsense-mediated mRNA decay in human cells: mechanistic insights, functions beyond quality control and the double-life of NMD factors. *Cell. Mol. Life Sci.* **67**, 677–700
- 17 He, F. and Jacobson, A. (1995) Identification of a novel component of the nonsense-mediated mRNA decay pathway by use of an interacting protein screen. *Genes Dev.* **9**, 437–454
- 18 Leeds, P., Peltz, S.W., Jacobson, A. and Culbertson, M.R. (1991) The product of the yeast *UPF1* gene is required for rapid turnover of mRNAs containing a premature translational termination codon. *Genes Dev.* **5**, 2303–2314
- 19 Leeds, P., Wood, J.M., Lee, B.S. and Culbertson, M.R. (1992) Gene products that promote mRNA turnover in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**, 2165–2177
- 20 Culbertson, M.R., Underbrink, K.M. and Fink, G.R. (1980) Frameshift suppression in *Saccharomyces cerevisiae*. II. Genetic properties of group II suppressors. *Genetics* **95**, 833–853
- 21 Cali, B.M., Kuchma, S.L., Latham, J. and Anderson, P. (1999) *smg-7* is required for mRNA surveillance in *Caenorhabditis elegans*. *Genetics* **151**, 605–616
- 22 Pulak, R. and Anderson, P. (1993) mRNA surveillance by the *Caenorhabditis elegans smg* genes. *Genes Dev.* **7**, 1885–1897
- 23 Hodgkin, J., Papp, A., Pulak, R., Ambros, V. and Anderson, P. (1989) A new kind of informational suppression in the nematode *Caenorhabditis elegans*. *Genetics* **123**, 301–313
- 24 Culbertson, M.R. and Leeds, P.F. (2003) Looking at mRNA decay pathways through the window of molecular evolution. *Curr. Opin. Genet. Dev.* **13**, 207–214
- 25 Page, M.F., Carr, B., Anders, K.R., Grimson, A. and Anderson, P. (1999) SMG-2 is a phosphorylated protein required for mRNA surveillance in *Caenorhabditis elegans* and related to Upf1p of yeast. *Mol. Cell. Biol.* **19**, 5943–5951
- 26 Czaplinski, K., Weng, Y., Hagan, K.W. and Peltz, S.W. (1995) Purification and characterization of the Upf1 protein: a factor involved in translation and mRNA degradation. *RNA* **1**, 610–623
- 27 Bhattacharya, A., Czaplinski, K., Trifillis, P., He, F., Jacobson, A. and Peltz, S.W. (2000) Characterization of the biochemical properties of the human *Upf1* gene product that is involved in nonsense-mediated mRNA decay. *RNA* **6**, 1226–1235
- 28 Yamashita, A., Ohnishi, T., Kashima, I., Taya, Y. and Ohno, S. (2001) Human SMG-1, a novel phosphatidylinositol 3-kinase-related protein kinase, associates with components of the mRNA surveillance complex and is involved in the regulation of nonsense-mediated mRNA decay. *Genes Dev.* **15**, 2215–2228
- 29 Kashima, I., Yamashita, A., Izumi, N., Kataoka, N., Morishita, R., Hoshino, S., Ohno, M., Dreyfuss, G. and Ohno, S. (2006) Binding of a novel SMG-1-Upf1-eRF1-eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay. *Genes Dev.* **20**, 355–367
- 30 Luke, B., Azzalin, C.M., Hug, N., Deplazes, A., Peter, M. and Lingner, J. (2007) *Saccharomyces cerevisiae* Ebs1p is a putative ortholog of human Smg7 and promotes nonsense-mediated mRNA decay. *Nucleic Acids Res.* **35**, 7688–7697
- 31 Chiu, S.Y., Serin, G., Ohara, O. and Maquat, L.E. (2003) Characterization of human Smg5/7a: a protein with similarities to *Caenorhabditis elegans* SMG5 and SMG7 that functions in the dephosphorylation of Upf1. *RNA* **9**, 77–87
- 32 Anders, K.R., Grimson, A. and Anderson, P. (2003) SMG-5, required for *C. elegans* nonsense-mediated mRNA decay, associates with SMG-2 and protein phosphatase 2A. *EMBO J.* **22**, 641–650
- 33 Gatfield, D., Unterholzner, L., Ciccarelli, F.D., Bork, P. and Izaurralde, E. (2003) Nonsense-mediated mRNA decay in *Drosophila*: at the intersection of the yeast and mammalian pathways. *EMBO J.* **22**, 3960–3970
- 34 Kerényi, Z., Merai, Z., Hiripi, L., Benkovics, A., Gyula, P., Lacomme, C., Barta, E., Nagy, F. and Silhavy, D. (2008) Inter-kingdom conservation of mechanism of nonsense-mediated mRNA decay. *EMBO J.* **27**, 1585–1595
- 35 Ohnishi, T., Yamashita, A., Kashima, I., Schell, T., Anders, K.R., Grimson, A., Hachiya, T., Hentze, M.W., Anderson, P. and Ohno, S. (2003) Phosphorylation of hUPF1 induces formation of mRNA surveillance complexes containing hSMG-5 and hSMG-7. *Mol. Cell* **12**, 1187–1200
- 36 Unterholzner, L. and Izaurralde, E. (2004) SMG7 acts as a molecular link between mRNA surveillance and mRNA decay. *Mol. Cell* **16**, 587–596
- 37 Fukuhara, N., Ebert, J., Unterholzner, L., Lindner, D., Izaurralde, E. and Conti, E. (2005) SMG7 is a 14–3–3-like adaptor in the nonsense-mediated mRNA decay pathway. *Mol. Cell* **17**, 537–547
- 38 Virshup, D.M. (2000) Protein phosphatase 2A: a panoply of enzymes. *Curr. Opin. Cell Biol.* **12**, 180–185
- 39 D'Andrea, L.D. and Regan, L. (2003) TPR proteins: the versatile helix. *Trends Biochem. Sci.* **28**, 655–662
- 40 Wall, D. and Kaiser, D. (1999) Type IV pili and cell motility. *Mol. Microbiol.* **32**, 1–10
- 41 Clissold, P.M. and Ponting, C.P. (2000) PIN domains in nonsense-mediated mRNA decay and RNAi. *Curr. Biol.* **10**, R888–R890
- 42 Glavan, F., Behm-Ansmant, I., Izaurralde, E. and Conti, E. (2006) Structures of the PIN domains of SMG6 and SMG5 reveal a nuclease within the mRNA surveillance complex. *EMBO J.* **25**, 5117–5125
- 43 Huntzinger, E., Kashima, I., Fauser, M., Sauliere, J. and Izaurralde, E. (2008) SMG6 is the catalytic endonuclease that cleaves mRNAs containing nonsense codons in metazoan. *RNA* **14**, 2609–2617
- 44 Eberle, A.B., Lykke-Andersen, S., Mühlemann, O. and Jensen, T.H. (2009) SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells. *Nat. Struct. Mol. Biol.* **16**, 49–55
- 45 Meyer, S., Temme, C. and Wahle, E. (2004) Messenger RNA turnover in eukaryotes: pathways and enzymes. *Crit. Rev. Biochem. Mol. Biol.* **39**, 197–216
- 46 Garneau, N.L., Wilusz, J. and Wilusz, C.J. (2007) The highways and byways of mRNA decay. *Nat. Rev. Mol. Cell Biol.* **8**, 113–126
- 47 Schmid, M. and Jensen, T.H. (2008) The exosome: a multipurpose RNA-decay machine. *Trends Biochem. Sci.* **33**, 501–510
- 48 Yamashita, A., Chang, T.C., Yamashita, Y., Zhu, W., Zhong, Z., Chen, C.Y. and Shyu, A.B. (2005) Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover. *Nat. Struct. Mol. Biol.* **12**, 1054–1063
- 49 Lejeune, F., Li, X. and Maquat, L.E. (2003) Nonsense-mediated mRNA decay in mammalian cells involves decapping, deadenylation, and exonucleolytic activities. *Mol. Cell* **12**, 675–687
- 50 Chen, C.Y. and Shyu, A.B. (2003) Rapid deadenylation triggered by a nonsense codon precedes decay of the RNA body in a mammalian cytoplasmic nonsense-mediated decay pathway. *Mol. Cell. Biol.* **23**, 4805–4813
- 51 Cao, D. and Parker, R. (2003) Computational modeling and experimental analysis of nonsense-mediated decay in yeast. *Cell* **113**, 533–545
- 52 Muhlrud, D. and Parker, R. (1994) Premature translational termination triggers mRNA decapping. *Nature* **370**, 578–581
- 53 Couttet, P. and Grange, T. (2004) Premature termination codons enhance mRNA decapping in human cells. *Nucleic Acids Res.* **32**, 488–494
- 54 Gatfield, D. and Izaurralde, E. (2004) Nonsense-mediated messenger RNA decay is initiated by endonucleolytic cleavage in *Drosophila*. *Nature* **429**, 575–578
- 55 Cho, H., Kim, K.M. and Kim, Y.K. (2009) Human proline-rich nuclear receptor coregulatory protein 2 mediates an interaction between mRNA surveillance machinery and decapping complex. *Mol. Cell* **33**, 75–86
- 56 Fatica, A., Tollervey, D. and Dlakic, M. (2004) PIN domain of Nob1p is required for D-site cleavage in 20S pre-rRNA. *RNA* **10**, 1698–1701
- 57 Keller, W., Bienroth, S., Lang, K.M. and Christofori, G. (1991) Cleavage and polyadenylation factor CPF specifically interacts with the pre-mRNA 3' processing signal AAUAAA. *EMBO J.* **10**, 4241–4249
- 58 Mandel, C.R., Kaneko, S., Zhang, H., Gebauer, D., Vethantham, V., Manley, J.L. and Tong, L. (2006) Polyadenylation factor CPSF-73 is the pre-mRNA 3'-end-processing endonuclease. *Nature* **444**, 953–956
- 59 Nicholson, P. and Muller, B. (2008) Post-transcriptional control of animal histone gene expression: not so different after all. *Mol. Biosyst.* **4**, 721–725
- 60 Schoenberg, D.R. (2007) The end defines the means in bacterial mRNA decay. *Nat. Chem. Biol.* **3**, 535–536
- 61 Mühlemann, O. and Lykke-Andersen, J. (2010) How and where are nonsense mRNAs degraded in mammalian cells? *RNA Biol.* **7**, 28–32

Received 30 July 2010
doi:10.1042/BST038xxxx