BJ www.biochemj.org

BJ Disease

Biochem. J. (2010) **430**, 365–377 (Printed in Great Britain) doi:10.1042/BJ20100699

REVIEW ARTICLE

NMD: RNA biology meets human genetic medicine

Madhuri BHUVANAGIRI*†‡, Anna M. SCHLITTER*‡, Matthias W. HENTZE*† and Andreas E. KULOZIK*‡[1]

*Molecular Medicine Partnership Unit, University of Heidelberg and European Molecular Biology Laboratory, 69120 Heidelberg, Germany, †European Molecular Biology Laboratory, Heidelberg 69117, Germany, and ‡Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, 69120 Heidelberg, Germany

NMD (nonsense-mediated mRNA decay) belongs to the best-studied mRNA surveillance systems of the cell, limiting the synthesis of truncated and potentially harmful proteins on the one hand and playing an initially unexpected role in the regulation of global gene expression on the other hand. In the present review, we briefly discuss the factors involved in NMD, the different models proposed for the recognition of PTCs (premature termination codons), the diverse physiological roles of NMD, the involvement of this surveillance pathway in disease and the current strategies for medical treatment of PTC-related diseases.

Key words: disease therapy, nonsense-mediated mRNA decay (NMD), premature termination codon (PTC), PTC-related disease.

INTRODUCTION

The process of eukaryotic gene expression involves a number of interlinked steps from transcription, capping, splicing and polyadenylation, to translation and mRNA degradation. Many different regulatory pathways have evolved to maintain the fidelity and accuracy of the expression of individual genes. Among these regulatory mechanisms, a specialized surveillance mechanism that targets transcripts with PTCs (premature termination codons) is the NMD (nonsense-mediated mRNA decay) pathway [1–3]. PTC-bearing mRNAs need to be eliminated because they encode C-terminally truncated proteins, some of which possess dominant-negative or deleterious gain-of-function activity. It has emerged that in addition to its role in eliminating faulty transcripts, NMD can also modulate the expression of many physiological mRNAs involved in various cellular processes, such as haemopoietic cell differentiation, stress responses or the maintenance of chromosome structure and function [4–6]. These studies indicate a role for NMD in the post-transcriptional regulation of gene expression along with mRNA quality-control activity. Furthermore, the biological and medical significance of the NMD pathway is highlighted by studies which revealed that an escape from NMD can result in severe clinical phenotypes [7,8]. It is estimated that 30 % of known disease-associated mutations are due to PTC-containing mRNAs [7]. In the present review, we describe the factors involved in NMD, mechanisms suggested in the literature for the recognition of PTCs, the diverse physiological substrates of NMD involved in various cellular processes, the involvement of NMD in disease and the current strategies for treatment of PTC-related diseases.

ORIGIN OF PTCs

mRNAs harbouring PTCs comprise the major group of NMD substrates [2–4]. PTCs originate in a variety of ways. Nonsense and frameshift-mutated mRNAs are the most obvious NMD targets [4]. Programmed DNA rearrangements that occur in TCR (T-cell receptor) and Ig (immunoglobulin) genes of lymphocytes generate frameshift mutations and downstream PTCs in two-thirds of all cases, which are also targeted by NMD [9]. At the RNA level, errors in transcription and pre-mRNA splicing also generate mRNAs with PTCs that are substrates for NMD. It is estimated that approx. 60–70 % of human pre-mRNAs are alternatively spliced and, among these, 45 % are predicted to have at least one spliced form that is expected to be targeted by NMD [10]. NMD thus affects a large proportion of the cell's transcriptome, which highlights the importance of this post-transcriptional mechanism in the quality control of gene expression. In addition to PTC-containing RNAs, several studies have suggested that a large set of non-faulty transcripts are also recognized and eliminated by NMD. More details about these physiological mRNAs that are also targeted by NMD are discussed later in the review.

MAJOR NMD COMPONENTS

Activation of NMD first necessitates that a PTC is distinguished from a proper translation termination codon. In higher eukaryotes, this process involves the deposition of landmarks on the mRNA that specify the position of exon junctions. The case where a termination codon is located in the 3′ terminal exon is generally interpreted as proper, whereas termination codons that are located further 5′ are generally interpreted as premature [11,12]. These landmarks consists of multisubunit protein complexes that are recruited to positions approx. 20–25 nt upstream of exon junctions, and that are hence termed EJCs (exon junction complexes). The EJC is composed of at least four core proteins [eIF4A3 (eukaryotic initiation factor 4A3), MAGOH (mago nashi homologue), Y14 and BTZ (Barentsz)] and additional, more peripheral, proteins that probably confer the functionality of the EJC in various post-transcriptional processes, including NMD [13]. When the translation termination complexes, including the

Abbreviations used: BMD, Becker muscular dystrophy; BTZ, Barentsz; CF, cystic fibrosis; CHD1, cadherin-1; DECID, decay-inducing complex; DMD, Duchenne muscular dystrophy; DSE, downstream sequence element; ECM, extracellular matrix; eIF, eukaryotic initiation factor; eRF, eukaryotic release factor; EJC, exon junction complex; HDGC, hereditary diffuse gastric cancer; MAGOH, mago nashi homologue; NLS, nuclear localization signal; NMD, nonsense-mediated mRNA decay; PABPC1, poly(A)-binding protein 1; PIKK, phosphoinositide 3-kinase-related protein kinase; PIN, PilT N-terminus; PP2A, protein phosphatase 2A; PTC, premature termination codon; RBD, RNA-binding domain; RNP, ribonucleoprotein; siRNA, small interfering RNA; SMG, suppressor with morphological defects in the genitalia; TCR, T-cell receptor; TPR, tetratricopeptide; UPF, up-frameshift; UTR, untranslated region.

[1] To whom correspondence should be addressed (email andreas.kulozik@med.uni-heidelberg.de).

Table 1 Cellular localization, biochemical characteristics and functions of NMD factors

Protein	Cellular localization	Biochemical characteristics	Functions	References
UPF1	Shuttling protein, but known to be mainly cytoplasmic	RNA helicase, nucleic acid-dependent ATPase, RNA-binding protein	NMD; promotes translation; histone mRNA decay; role in DNA replication; involved in maintenance of telomere integrity	[14,15,18–24]
UPF2	Cytoplasmic; mainly perinuclear (also has NLS)	Phosphoprotein	NMD; promotes translation; EJC adaptor; involved in maintenance of telomere integrity	[20,23,25–29]
UPF3a	Mainly nuclear, but shuttles	RNA-binding, phosphoprotein	NMD; promotes translation; EJC protein, interacts with UPF2; involved in maintenance of telomere integrity. Probably weaker NMD activity than UPF3b	[20,23,28,30,32]
UPF3b	Mainly nuclear, but shuttles	RNA-binding, phosphoprotein	NMD; promotes translation; EJC protein interacts with UPF2; involved in maintenance of telomere integrity	[20,23,28,30–33]
SMG1	Cytoplasmic and nuclear	serine/threonine kinase of PIKK family, ATP-binding, phosphoprotein	NMD; phosphorylates UPF1; involved in maintenance of genome stability, stress response and DNA repair	[14,19]
SMG5	Mainly cytoplasmic, some nuclear (shuttles)	PINc domains	NMD; interacts with PP2A and promotes UPF1 dephosphorylation	[15,35,38,40,41,71]
SMG6	Mainly cytoplasmic	PINc domains	NMD; interacts with PP2A and promotes UPF1 dephosphorylation. Documented endonuclease function	[16,41,71]
SMG7	Mainly cytoplasmic, some nuclear (shuttles)	Two TPR repeats	NMD; interacts with PP2A and promotes UPF1 dephosphorylation	[15,35–37]
SMG8	Not known	Phosphoprotein	Forms a complex with SMG1 and inhibits its activity	[42]
SMG9	Not known	Phosphoprotein	Forms a complex with SMG1	[42]
Y14	Nuclear and cytoplasmic	RNA-binding	EJC core protein; promotes translation; forms heterodimer with MAGOH, part of pre-EJC complex	[31,46,47,50,58]
MAGOH	Nuclear and cytoplasmic	RNA-binding, acetylation	EJC core protein; promotes translation; forms heterodimer with Y14, part of pre-EJC complex	[47,58]
eIF4A3	Nuclear and cytoplasmic	RNA-binding, RNA helicase	EJC core protein; anchors to other EJC proteins, part of pre-EJC complex	[48,49,58]
BTZ	Nuclear and cytoplasmic	RNA-binding, phosphoprotein	EJC core protein; interacts with eIF4A3	[51,58]
PYM	Nuclear and cytoplasmic	Interacts with Y14/MAGOH	Contributes to EJC disassembly and EJC factor recycling	[54]
hNAG	Not known	WD40 (β-propeller domain) repeats	NMD	[72]
DHX34	Not known	ATP-dependent RNA helicase	NMD	[72]

release factors eRF1 and eRF3, interact with downstream EJCs via the so-called UPF (up-frameshift) factors (see below), it induces the phosphorylation of the NMD protein UPF1 through the kinase SMG1 (suppressor with morphological defects in the genitalia 1), which subsequently triggers exo- and endo-nucleolytic mRNA degradation [14–16]. The NMD proteins UPF2 and UPF3b are thought to bridge the EJC and the post-termination complex including UPF1 [17] (Table 1).

UPF PROTEINS: CORE NMD FACTORS

UPF1 is a complex phosphoprotein with RNA/DNA-dependent ATPase and $5'\rightarrow3'$ RNA helicase activity [18]. The C-terminus of the human UPF1 protein contains four serine/threonine-rich clusters which harbour 14–18 potential phosphorylation sites. These serine-rich sites are of key importance in NMD, because they undergo cyclic rounds of phosphorylation/dephosphorylation which is crucial for the remodelling of the mRNA surveillance complex [15,19]. The N-terminus of human UPF1 contains a proline/glycine-rich region, which has been shown to mediate the interaction with the protein SMG5 that is required for dephosphorylation of UPF1 [15]. Immunofluorescence studies have shown UPF1 to be a predominantly cytoplasmic protein [20]. However, using other experimental approaches it has also been shown to shuttle between the nucleus and the cytoplasm [21]; the functional significance of this shuttling is still being explored. Knock-out of the *upf1* gene in mice was found to be lethal. Embryos died between days 3.5 and 5.5 of gestation and even an attempt to create *upf1*-null fibroblast cell lines was unsuccessful, suggesting that NMD is essential for a basic cell biological process [22]. However, UPF1 is also known to play an important role in other physiological processes, such as telomere maintenance [23] and translation termination [24]. Therefore it is possible that the embryonic lethality of UPF1 deficiency in mammals is unrelated to NMD.

UPF2 is commonly referred to as an adapter molecule that bridges UPF1 and UPF3 to elicit NMD. Structural analysis of UPF2 has revealed at least three conserved eIF4G-like (MIF4G) domains and one putative NLS (nuclear localization signal) in its N-terminus [25,26]. UPF1-binding domains were identified both in its N-terminus and its C-terminus, with the C-terminus contributing more to the UPF1 interactions [27,28]. The crystal structure of the complex between the interacting domains of UPF2 and UPF3 revealed contacts between the negatively charged residues of the MIF4G domains of UPF2 and the positively charged β-sheet surface of the RNP domain [ribonucleoprotein-type RBD (RNA-binding domain)] of UPF3b. *In vitro* analysis showed that either UPF2 alone or the UPF2–UPF3b complex, but not UPF3b alone, were able to bind to RNA [28]. UPF2 is a predominantly cytoplasmic protein that accumulates in perinuclear regions [20]. However, the N-terminal region contains a typical NLS. Furthermore, UPF2 is thought to be recruited to the mRNA as a result of splicing, which suggests a role of UPF2 in the nucleus [25]. UPF2 protein is thought to be required for modulating phosphorylation of UPF1 (possibly via its binding function to the EJC), because UPF1 phosphorylation was down-regulated by silencing of UPF2 in HeLa cells [29]. In support of the role of UPF2 in phosphorylation of UPF1, biochemical data have suggested direct binding between UPF2 and SMG1, the kinase that is known to phosphorylate UPF1 during NMD [14].

Mammals have two *UPF3* isoforms called *UPF3a* and *UPF3b*, whereas *Caenorhabditis elegans* and *Saccharomyces cerevesiae*

have only one *UPF3* isoform [26]. In humans *UPF3*a is located on chromosome 13 and *UPF3b* on the X chromosome. UPF3a and UPF3b proteins directly interact with UPF2 through their N-terminal RBD domain and both are known to be nuclear proteins that can shuttle between the nucleus and the cytoplasm [20,26]. UPF3b elicits a strong NMD response when tethered downstream of a stop codon, whereas UPF3a is only marginally active in this assay system. Furthermore, studies have pointed to a crucial region in the C-terminus of UPF3b that is not conserved in UPF3a and mediates the difference in the NMD activity of the two proteins [30]. The C-terminal domain is responsible for the interaction between UPF3b and the EJC core protein Y14 [31]. Recently, it has been suggested that UPF3a and UPF3b compete for binding to UPF2. At low concentrations of UPF3b, more UPF3a was found to be bound to UPF2. However, when UPF3b levels were high, less UPF3a was bound and the abundance of UPF3a was found to be decreased [32]. A recent SNP (single nucleotide polymorphism) analysis found that mutations in UPF3b in humans cause syndromic and non-syndromic mental retardation. It was intriguing that NMD in the cells of these patients was active, which suggests that UPF3b-independent NMD pathways exist and that the function of UPF3b can be (partially) substituted by other proteins, such as UPF3a [33].

SMG PROTEINS

SMG proteins mediate the phosphorylation and dephosphorylation of UPF proteins. This group of proteins includes four core members termed SMG1, SMG5, SMG6 and SMG7. Depletion of any of these proteins was found to inhibit NMD in mammalian cells [34–36]. SMG1 is the UPF1 kinase and belongs to the family of PIKKs (phosphoinositide 3-kinase-related protein kinases) [19]. Studies in mammalian cells have shown that kinase-deficient mutants of SMG1 act in a dominant-negative fashion to inhibit NMD, whereas overexpression of SMG1 kinase increases the level of human UPF1 phosphorylation and enhances the efficiency of NMD [19].

SMG5, SMG6 and SMG7 are non-redundant proteins that are involved in the dephosphorylation of UPF1 [35,37,38]. Intriguingly, these SMG proteins are not by themselves phosphatases, but they promote the dephosphorylation of UPF proteins by recruiting essential protein phosphatases such as PP2A (protein phosphatase 2A). All three proteins contain two TPR (tetratricopeptide) repeats, that mediate protein–protein interactions [39]. Structure analysis of SMG7 revealed a 14-3-3-like domain in its N-terminus. Residues that bind phosphoserine-containing peptides in 14-3-3 are conserved at equivalent positions in SMG7 [37]. Mutation analysis of these residues showed that this domain is responsible for UPF1 binding and for targeting mRNAs associated with phosphorylated UPF1 for degradation. The 14-3-3 site of SMG7 was also found to be conserved in SMG5 and SMG6 [37]. The C-termini of SMG5 and SMG6 contain PIN-like domains (for PilT N-terminus), which usually function as phosphodiesterases with nuclease activity [40]. SMG6 has a more authentic ribonuclease fold, such as those found in RNase H-type nucleases [41]. Recently, human SMG6 was shown to function as an endonuclease cleaving nonsense-mutated mRNAs near the PTC as a first step of mRNA degradation [16]. Other SMG proteins, called SMG8 and SMG9, have also been reported and were found to regulate the remodelling of mRNA surveillance complex during NMD [42].

EJC COMPLEX

In higher eukaryotes, splicing plays a critical role for the PTC recognition process [11,43]. Consequently, naturally intronless, PTC-containing mRNAs are not subject to NMD [44,45]. Splicing deposits an assembly of proteins, known as the EJC, 20–24 nucleotides upstream of exon–exon junctions [46]. The EJC core is composed of four proteins: Y14, MAGOH, eIF4A3 and MLN51 (also known as BTZ). The EJC core is assembled in a strictly hierarchical order [46,47]. The assembly is initiated by the formation of a pre-EJC complex that consists of eIF4A3, Y14 and MAGOH, which occurs even before exon ligation is completed. This pre-EJC provides a binding platform for other EJC components that join later after the release from the spliceosome, which connects the core EJC with its functions [47]. eIF4A3, a DEAD-box RNA helicase serves as a link between the EJC components and the mRNA substrate. eIF4A3 is mainly a nuclear protein, but also shuttles to the cytoplasm [48]. The binding of the Y14–MAGOH dimer to the C-terminus of eIF4A3 inhibits its ATPase activity and stabilizes the binding of other EJC components to the RNA [49]. MLN51 is a mammalian orthologue of the BTZ protein that is required for the localization of *oskar* mRNA in *Drosophila* [50]. MLN51 contains a conserved N-terminal domain that is involved in the binding to spliced mRNA *in vitro* and in the interaction with MAGOH [49,51]. In addition to the EJC core proteins, other proteins, such as UPF2 and UPF3b, SRm160 and RNSP1 are recruited to the EJC. EJCs are removed from mRNAs during the first round of translation, which may either be cap-dependent [52] or cap-independent [53]. During this process, the ribosome-associated EJC cofactor PYM has recently been identified to mediate efficient EJC disassembly and thus contribute to the recycling of its components [54].

MECHANISM OF RECOGNITION OF PTC

The key mechanistic questions in NMD research are (i) how an mRNA with a normal stop codon is discriminated from an mRNA with a premature termination codon and (ii) how this discrimination leads to differences in mRNA stability. Even though the core NMD factors are conserved among species, different mechanisms by which NMD can be elicited have been proposed on the basis of studies of mammalian, *Drosophila melanogaster*, *C. elegans* or *S. cerevisiae* cells.

Downstream marker model

Earlier studies on *PGK1* mRNA in yeast suggested that the presence of a so-called DSE (downstream sequence element) 3′ of a stop codon renders the mRNA unstable and stimulates NMD (Figure 1). Moreover, deletion of this DSE improved the stability of the mRNA [55]. Later, *in vitro* studies suggested that the binding of the heterogeneous nuclear RNP protein Hrp1p to *PGK1* mRNA triggered NMD by promoting the interaction with Upf1p. Furthermore, mutated Hrp1p, which stabilizes nonsense-containing mRNAs, also abolished its affinity for the DSE and failed to interact with Upf1p [56]. These observations indicated that Hrp1p is an important component of the NMD pathway in yeast. Nonetheless, the DSE–Hrp1p interaction has so far only been shown for the PGK1 mRNA and it is an open question if this mechanism acts more generally.

In higher eukaryotes, the position of a stop codon 5′ of an EJC is considered to induce NMD and to enhance its efficiency (Figure 2) [46]. The recognition of the stop codon by release factors eRF1 and eRF3 triggers translation termination [24,57].

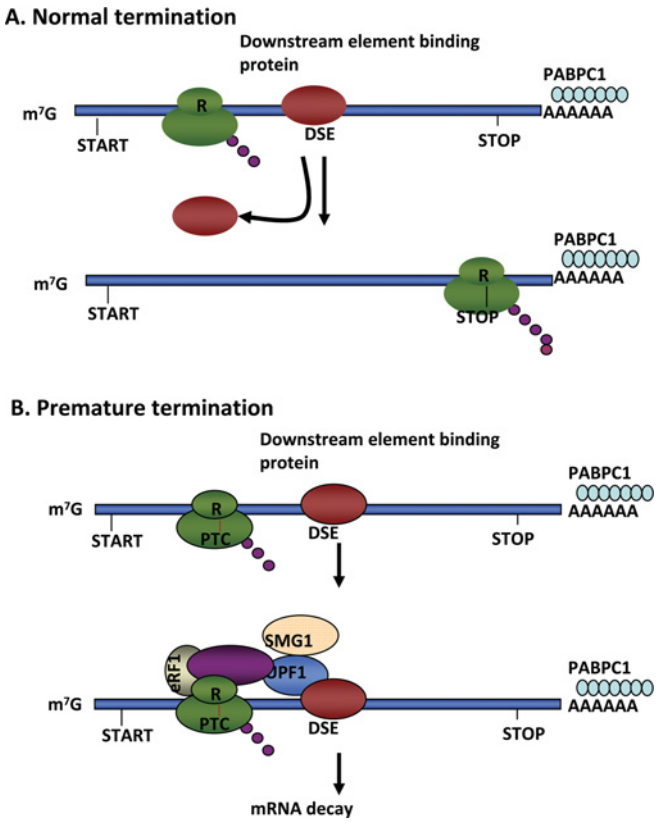

Figure 1 Downstream marker model in yeast

The PTC is defined relative to the position of a downstream *cis*-acting element where a marker protein is bound. (**A**) Under normal circumstances, elongating ribosomes (R) remove the downstream element binding protein which results in proper translation termination. (**B**) In the case of transcripts with a PTC, the ribosome is stalled at the PTC and the downstream marker protein is not removed. Furthermore, an interaction between the downstream element binding protein and core NMD factors, such as UPF1, triggers NMD.

UPF1 is recruited to the site soon after and, together with SMG1, forms the post-termination complex termed SURF. Furthermore, SURF interacts with UPF2, UPF3b and additional EJC proteins bound to the downstream exon–exon boundary. This interaction has been proposed to result in the formation of the DECID (decay-inducing complex) that triggers UPF1 phosphorylation and the dissociation of eRF1 and eRF3 [14]. Subsequently, SMG5/7 or SMG6 mediate dephosphorylation of UPF1. Degradation of the nonsense-mutated mRNA is then thought to be induced by endonucleolytic cleavage by SMG6 [16] and 5′→3′ and 3′→5′ exonucleolytic degradation [36].

It is interesting to note that this linear model of NMD suggested by early genetic and biochemical studies is probably oversimplified. In contrast, many previous studies in mammals suggested the possibility of alternative branches of the NMD pathway, which vary in their dependence on the cofactors UPF2, EJC core components and UPF3b, but converge at the point of UPF1-dependence [58–61].

A combination of tethering assays and the analysis of endogenous NMD targets following siRNA (small interfering RNA)-mediated depletion of NMD proteins provided the primary evidence for the existence of UPF2-independent and UPF2-dependent (or EJC core independent) pathways [58]. Consistently, in some instances, such as the PTCs in the penultimate exon of immunoglobulin-μ, NMD can also be activated in a splicing-independent and EJC-independent fashion [59].

Furthermore, work on TCR-β mRNA indicated that depletion of UPF3b, by itself or in combination with UPF3a, had no effect on NMD [60]. On a similar note, inactivating mutations of UPF3b in patients do not appear to impair NMD efficiency [33]. It is important to note that a large number of endogenous NMD targets that are regulated by UPF1 are not affected by UPF3b depletion, further supporting the existence of an UPF3b-independent pathway. Mapping experiments indicated that a region encompassing the TCR-β VDJ exon and adjacent intron sequences is responsible for UPF3b-independence [60].

The faux 3′-UTR (untranslated region) model

This model posits that NMD is triggered by the intrinsically aberrant nature of an extended 3′-UTR that results from a PTC (Figure 3) [62]. Proper translation termination and the normal rate of mRNA decay requires the interaction between the terminating ribosome and a specific set of factors including the poly(A)-binding protein, PABPC1. Proper spacing between the stop codon and the 3′-UTR is considered as a crucial facet of this model.

In the event of normal termination, once the ribosomes encounter the stop codon, eRF1 recognizes the termination codon in the A-site and forms a complex with eRF3 at the C-terminus. Meanwhile, the N-terminus of eRF3 interacts with the C-terminal domain of PABPC1, which is believed to catalyse proper and efficient ribosome release and translation termination [63–66]. Consistent with this hypothesis, mammalian cells lacking PABPC1 exhibited an increased read-through of termination codons [61]. Furthermore, tethering of PABPC1 downstream of a PTC rescues the stability of the mRNA [62,67]. However, at a PTC, the stop codon is not in the appropriate position and usually does not have a normal 3′-UTR immediately downstream required for translation termination. According to this model, the long 3′-UTR following the PTC is not properly configured to bring PABPC1 to the proximity of the release factors bound at the termination codon. Therefore ribosomes that terminate prematurely are released less efficiently or too slowly compared with those encountering normal terminators, in some way favouring the activation of NMD proteins thus leading to rapid degradation of the mRNA [62,68,69]. In support of this hypothesis, if PABPC1 is absent, UPF1 has been shown to more readily interact with the translation termination factors eRF1 and eRF3 [70]. However, the importance of EJCs separating a PTC from the mRNA 3′ end is not excluded by this model, because the EJC may function as a barrier between the poly(A) RNP and the termination complex.

Studies in *S. cerevisiae*, *D. melanogaster* and *C. elegans* have shown that, in these organisms, PTC recognition occurs irrespective of splicing and thus independent of exon–exon junctions [3,71,72]. However, tethering studies strongly suggest that the faux 3′-UTR model provides a mechanism for PTC recognition in *Drosophila* [62,73].

NMD ALSO REGULATES PHYSIOLOGICAL mRNA

Whole genome transcriptomic profiling of yeast, *Drosophila* and human cells suggests that NMD, initially seen as a ‘vacuum cleaner of the cell’ plays a crucial role in global gene expression [74]. These studies further demonstrated that NMD directly or indirectly regulates the abundance of 3–10 % of all mRNAs in these different cell types [5,75,76]. In some cases, NMD reduces genomic noise by targeting transcripts from non-functional pseudogenes, transcripts encoded by transposable elements or LTRs (long terminal repeats), mRNAs with open

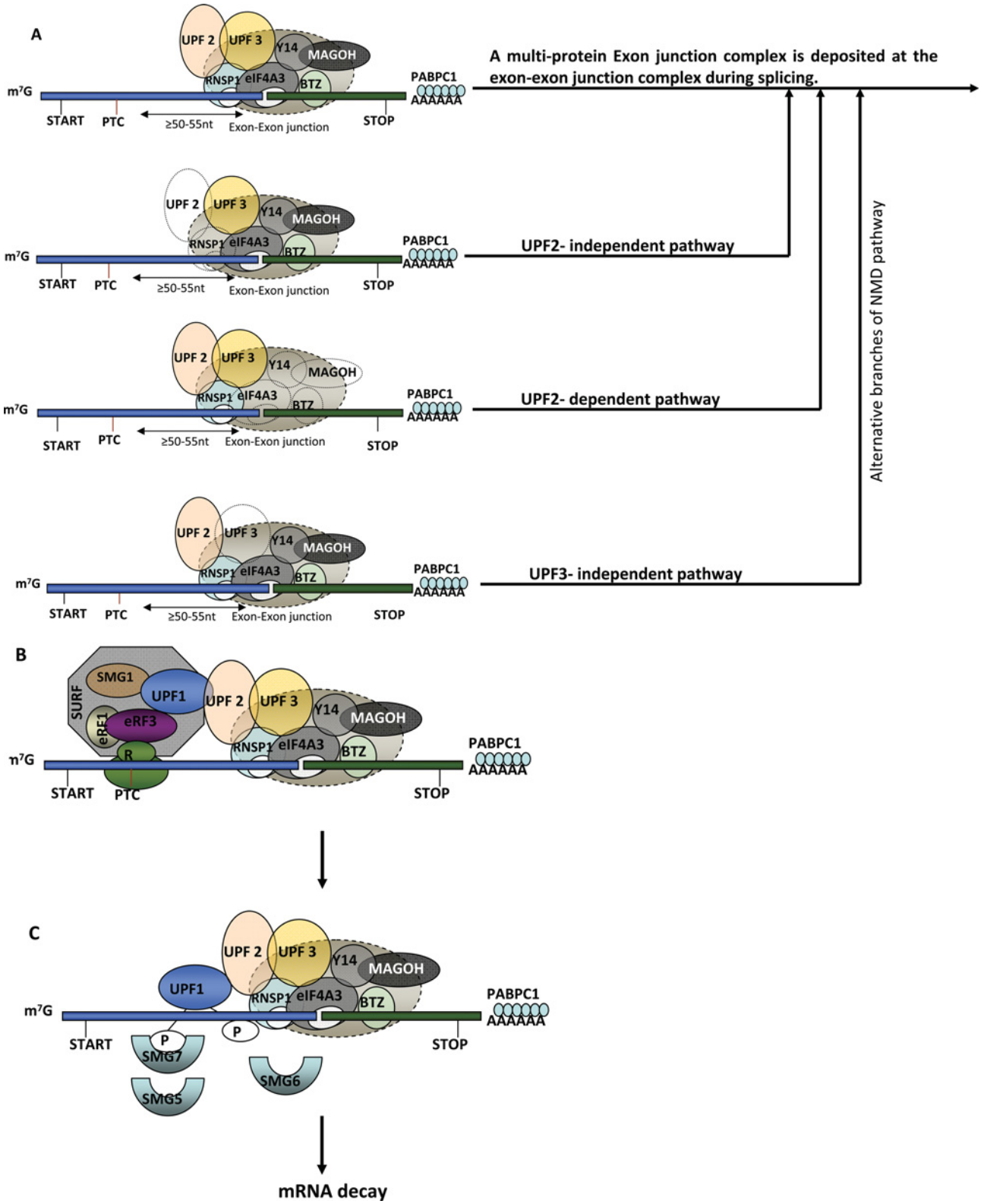

Figure 2 Downstream marker model in mammals

(**A**) In mammals, the EJC serves as a downstream marker. Pre-mRNA splicing results in the deposition of the EJC 20–24 nt upstream of exon junctions. The core EJC components are Y14/MAGOH, BTZ and eIF4A3. Thereafter, NMD factors such as UPF3 and UPF2, along with other accessory proteins such as RNSP1, are recruited at the EJC. Generally, PTCs located at least 50–55 nt upstream of the 3′ terminal exon junction elicit strong NMD responses. Other alternative branches through which NMD can be activated are also illustrated. (**B**) A translation termination event at a PTC upstream of an EJC leads to the formation of the SURF complex, which consists of UPF1, SMG1 kinase and the release factors eRF1 and eRF3. SURF interacts with UPF2, UPF3 and additional EJC proteins. (**C**) The interaction of the SURF complex with the EJC results in the formation of the DECID which triggers UPF1 phosphorylation and the dissociation of eRF1 and eRF3. UPF1 phosphorylation leads to the recruitment of SMG5/SMG7 and SMG6 proteins and the mRNA is degraded by SMG6-mediated endonucleolytic cleavage and by exonucleolytic decay.

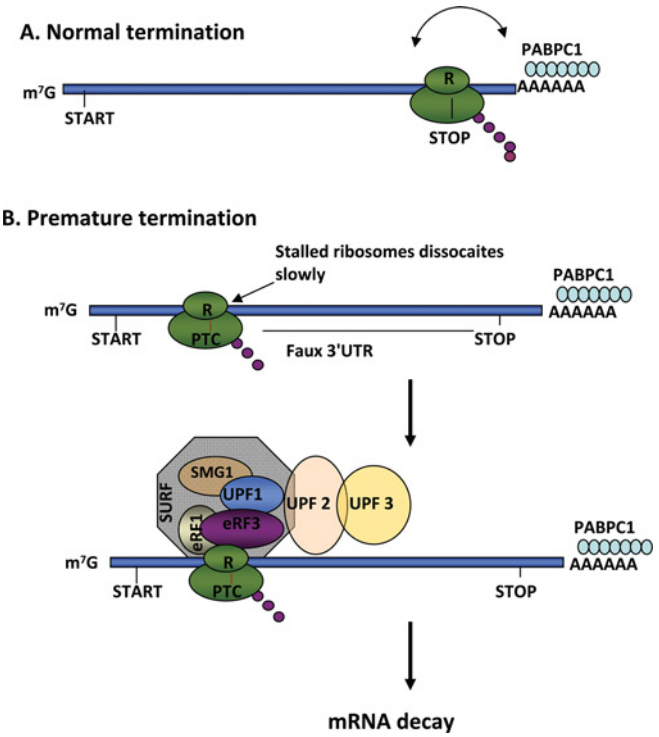

Figure 3 The faux 3′-UTR model

(**A**) Owing to the proximity between the normal stop codon and PABPC1 bound to the poly(A) tail, termination at normal stop codon is promoted by PABPC1 and favours translation re-initiation over induction of mRNA decay. (**B**) At a PTC, the stop codon is not in the appropriate position. The long 3′-UTR following the PTC is not properly configured to bring PABPC1 to the proximity of release factors bound at the termination codon. Therefore ribosomes that terminate prematurely are released less efficient compared with those encountering normal terminators, which favours the assembly of NMD complex proteins, leading to degradation of the mRNA.

reading frames in the 5′-UTR and transcripts that escaped from nuclear retention [69,77]. In other cases, NMD serves regulatory functions. Notably, mRNAs containing UGA triplet(s) that regulate selenocysteine incorporation can also elicit NMD, depending on the levels of selenium in the cell [78]. Furthermore, knockdown experiments of key NMD factors such as UPF proteins and SMG kinases suggested that NMD plays a role in regulating the expression of physiological RNA transcripts involved in stress responses, haemopoietic stem cell development, regulation of alternative splice forms, genomic stability, cell-cycle progression and telomere length maintenance, and embryonic development.

Nutrient homoeostasis and protection during oxidative stress

NMD targets were found to have several regulatory functions. In mammalian cells, amino acid starvation induces a global inhibition of cellular translation. Most often this inhibition is mediated by phosphorylation of translation initiation factor eIF2α [79]. Functional studies of the translation initiation complex suggested that phosphorylation of eIF2A inhibits NMD [80]. Subsequently, microarray studies demonstrated that inhibition of NMD induced by amino acid starvation up-regulated many transcripts involved in amino acid homoeostasis [5].

Similarly, eIF2α is also phosphorylated under hypoxic conditions and NMD is inhibited. Moreover, hypoxia-induced mRNAs that are known to play a predominant role in the integrated stress response were also found to be up-regulated when NMD is inhibited [81]. Although global inhibition of translation would be expected to inhibit NMD, which is known to be translation-dependent, it is also possible that stabilization of NMD targets during stress might contribute to the recovery of cellular metabolism.

Haemopoetic stem and progenitor cells

Conditional UPF2 knockout mice were generated to monitor the role of NMD in the development and maintenance of the haemopoietic system. These mouse models revealed that haemopoietic-cell-specific depletion of mUpf2 led to rapid, complete and lasting cell-autonomous extinction of all haemopoietic stem and progenitor populations [82]. However, in principle, this effect could also be explained by non-NMD related functions of UPF2 [23,83].

Regulation of alternative splice forms

Genome-wide transcriptome analyses have shown that alternative splicing and NMD act together to regulate gene expression, providing an additional layer of post-transcriptional regulation. Large-scale analyses of reliable alternative isoforms of known human genes suggested that one third of the alternative transcripts contained PTCs and are targets of NMD. RUST (regulated unproductive splicing and translation) through the coupling of alternative splicing and NMD was suggested to be required for the autoregulation of many splicing factors, such as SC35, PTB and 9G8 [10]. Notably, the core components of the spliceosome such as the U1 protein, common snRNP (small nuclear ribonucleoproteins) such as Sm proteins, and the SF1 protein are also regulated by alternative splicing and NMD [84].

Chromosome structure and function

Global expression analyses identified a significant fraction of NMD-sensitive genes coding for proteins that affect chromosome structure and behaviour, including telomere replication and maintenance, chromatin silencing, recombination and repair [85]. Consistent with the role of NMD in telomere maintenance, in yeast, mRNAs encoding the regulators of telomerase activity (Est1p, Est3p, Stn1p and Ten1p), catalytic subunit of telomerase (Est2p) and proteins that affect telomeric structure (Sas2p and Orc5p) are all controlled by NMD [86]. Additionally, NMD factors were also demonstrated to play a role in the protection of telomere ends and regulation of telomere length. Human SMG5 and SMG6 were shown to interact with telomerase and to be involved in the maintenance of telomere length. UPF1, UPF2, UPF3, SMG1, SMG5, SMG6 and SMG7 were all found to be enriched at the telomere ends [83]. Depletion of UPF1, SMG1 and SMG6, and to a lesser extent UPF2 and SMG7, generated chromosome and chromatid breaks and telomere loss [23].

DNA repair and cell-cycle regulation

SMG1 kinase and UPF1 were found to play a role in DNA repair and cell-cycle regulation [87]. SMG1 kinase responds to stress induced by DNA-damaging agents such as IR or UV-B light, and phosphorylates cellular proteins involved in DNA repair. Consistently, siRNA-mediated depletion of SMG1 in U2OS cells resulted in the accumulation of dsDNA (double-stranded DNA) breaks and accumulation of cells in G_2–M-phase [87]. On a similar

note, UPF1 was also shown to bind to chromatin and this binding gradually increases as the cell moves from G_1-phase to S-phase in the cell cycle, suggesting a possible role of UPF1 in DNA synthesis [88]. Down-regulation of UPF1 in HeLa cells resulted in cell growth arrest in S-phase.

Embryonic development

Studies on NMD components in *D. melanogaster* suggested that key NMD factors, such as the UPF1 and UPF2, are found to be essential for larval development [89]. Likewise, UPF1 knockdown in mice results in early embryonic lethality. Moreover, attempts to establish embryonic stem cells from UPF1-null mice also failed [22]. Similarly, even in plants, studies on *Arabidopsis thaliana* showed that UPF1 and SMG7 genes are essential for embryonic viability and mutant alleles have pleiotropic effects, suggesting that the NMD pathway in plants regulates transcripts with a broad range of cellular activities [90]. Previously, studies in zebrafish have shown that depletion of orthologues of UPF1, UPF2, SMG5 and SMG6 have a severe impact on zebrafish embryonic development, early patterning and viability [91]. However, it is still an open question whether these effects are caused by an inactivation of the NMD pathway or by other essential cellular functions.

NMD AND DISEASE ASSOCIATION

It is estimated that approximately one-third of all inherited disorders are caused by nonsense or frameshift mutations that introduce PTCs [4,8,92]; a survey of PTC-related diseases suggests that NMD represents a crucial modulator of the clinical phenotype of many genetic diseases (Table 2). NMD has a Janus-face regarding disease manifestation. In most cases NMD can be beneficial and helps in the elimination of transcripts which might encode C-terminally truncated, dominant-negative proteins leading to toxic effects. This beneficial effect of NMD is exemplified by β-thalassaemia. Haemoglobin A is a heterotetramer consisting of two α- and two β-subunits which are non-covalently bound to each other. In the case of heterozygous carriers with NMD-sensitive mutations in the β-globin gene (e.g. nonsense mutation NS39 in exon II), NMD degrades the mutated mRNA and the synthesis of the truncated protein is limited. The decreased amount of β-globin chains results in excess of free α-chains which are proteolytically degraded [93]. However, the haemoglobin function is compensated by the remaining β-globin production from the normal allele. Heterozygotes are thus essentially healthy and only show mostly harmless abnormalities of haemological indices. In contrast, NMD-insensitive mutations lead to the production of truncated β-globin chains. These chains form toxic protein precipitations which are thought to overburden the proteolytic mechanism of red blood precursors. Carriers with these rarer kinds of NMD-insensitive mutations are clinically affected and β-thalassaemia is inherited in an unusual dominant fashion [94]. Thus NMD protects the majority of heterozygous β-thalassaemia carriers from manifestations of the disease. A similar effect has since been documented in many other diseases (Table 2).

Alternatively, NMD can aggrevate disease phenotypes, because degrading truncated proteins that would otherwise retain some normal function produces haploinsufficiency. The dystrophinopathies are important examples in this respect. Nonsense mutations in the dystrophin gene that elicit an NMD response prevent the synthesis of truncated protein either with partial or complete functionality and result in the severe form of the disorder called DMD (Duchenne muscular dystrophy), whereas other nonsense mutations that escape NMD and guide the synthesis of C-terminally truncated dystrophin protein are associated with a milder form of the disorder, termed BMD (Becker muscular dystrophy) [95].

NMD AND CANCER

The potential influence of NMD on cancer has previously been suggested by a study of patients with HDGC (hereditary diffuse gastric cancer) and germline mutations of the CHD1 (cadherin-1) gene [96]. Approx. 80 % of the HDGC-associated mutations within the CDH1 gene create PTCs and putative NMD targets [97,98]. Patients with CDH1-PTCs that were predicted to be NMD-competent showed an earlier age-of-onset of gastric cancer when compared with those patients who carried PTC mutations that were predicted to be NMD-insensitive. These data thus indicated that the elimination of CDH1 mRNA with C-terminally truncated open reading frames by NMD has an unfavourable effect on the clinical progression of HDGC [96]. Similar links between NMD and the expression of tumour suppressor genes have also been suggested in other cases, such as BRCA1 (breast cancer 1, early onset), TP53 and WT1 (Wilms' tumour protein) [4].

In conclusion, the effect of NMD on the clinical phenotype of various diseases suggests that augmentation or inhibition of the efficiency of NMD might offer potential therapeutic strategies with wide applications in medicine.

THERAPEUTIC APPROACHES FOR CORRECTING PTCs

The large number of PTC-related inherited disorders with limited therapeutic options calls for pharmacological modulation of NMD. In principle, both stimulation and inhibition of NMD could be envisaged as a therapeutic strategy depending on the effect of NMD on pathophysiologically relevant proteins [7,99]. However, so far only inhibition of NMD for diseases that could be favourably modulated by mutated proteins with residual function have been developed clinically (Table 3). The development of rational strategies that can modulate NMD demands a much deeper understanding of the pathway and the functional consequences of its perturbation.

Therapies based on translational read-through

In clinical conditions where NMD is detrimental and haploinsufficiency is directly related to the severity of the disease, translational read-through at stop codons generating missense-mutated, but functionally active, proteins would be a useful strategy in these situations.

Aminoglycosides

Aminoglycosides are able to bind the decoding centre of the ribosome and decrease the accuracy of codon–anticodon pairing. The recognition of stop codons is suppressed and, instead of chain termination, an amino acid is incorporated into the polypeptide chain [100–103]. A decade ago this potential of aminoglycosides was first investigated in CF (cystic fibrosis) treatment [104,105] and later on followed in other diseases [106]. After successful trials in animal models, clinical trials in patients with CF or DMD were performed with gentamicin [107,108]. These trials showed that aminoglycosides can promote *in vivo* read-through of nonsense mutations and can lead to the expression of full-length

Table 2 Clinical conditions in which NMD modulates disease phenotypes

Gene name	Phenotype	Reference
β-Globin (*HBB*)	5′ PTC: recessively inherited β-thalassaemia major; heterozygotes healthy controls 3′ PTC: dominantly inherited β-thalassaemia intermedia	[93,94]
Rhodopsin (*RHO*)	5′ PTC: recessively inherited blindness; heterozygotes have abnormalities on retinogram, but no clinical disease 3′ PTC: dominantly inherited blindness	[120,121]
SRY-box 10 (*SOX 10*)	5′ PTC: haploinsufficiency leading to congenital neurosensory deafness and colonic agangliosis 3′ PTC: dominantly inherited neural developmental defect including neurosensory deafness, colonic agangliosis, peripheral neuropathy and central dysmyelinating leukodystrophy	[122]
Interferon-γ receptor 1 (*IFNGR1*)	5′ PTC: recessively inherited susceptibility to mycobacterial infection; heterozygotes healthy 3′ PTC: dominantly inherited susceptibility to mycobacterial infection	[123,124]
Cone-rod homeobox containing gene (*CRX*)	5′ PTC: no homozygotes to date; heterozygotes healthy 3′ PTC: dominantly inherited Leber congenital amaurosis	[125]
Receptor tyrosine kinase-like orphan receptor 2 (*ROR2*)	5′ PTC: recessively inherited Robinow syndrome (orodental abnormalities, hypoplastic genitalia, multiple rib/vertebral anomalies); heterozygotes healthy 3′ PTC: dominantly inherited brachydactyly type B (shortening of digits and metacarpals)	[126]
Chloride channel 1, skeletal muscle (*CLCN1*)	5′ PTC: recessively inherited Becker disease 3′ PTC: dominantly inherited Thomsen disease (muscular disorder characterized by muscle stiffness and an inability of the muscle to relax)	[127]
Von Willebrand factor (*VWF*)	5′ PTC: recessively inherited type 3 von Willebrand disease; heterozygotes healthy 3′ PTC: dominantly inherited type 2A 3 von Willebrand disease	von Willebrand database (http://www.vwf.group.shef.ac.uk/), [128]
Coagulation factor X (*F10*)	5′ PTC: recessively inherited bleeding tendency; heterozygotes healthy 3′ PTC: dominantly inherited bleeding tendency	[129]
Myelin protein zero (*MPN*)	5′ PTC: haploinsufficiency, Charcot–Marie–Tooth disease (neuropathy with loss of muscle tissue and touch sensation) 3′ PTC: dominant-negative or gain-of-function, congenital hypomyelinating neuropathy	[122]
Elastin (*ELN*)	5′ PTC: haploinsufficiency, supravalvular aortic stenosis, valvular heart disease 3′ PTC: dominant-negative or gain-of-function, congenital cutis laxa, connective tissue disorder	[130]
Collagen type I, α1 (*COL1A1*)	5′ PTC: dominantly inherited, osteogenesis imperfecta (OI) type I (mild form) 3′ PTC: OI type II–IV (severe form)	[131,132]
Ataxia-telangiectsia mutated gene (*ATM*)	5′ PTC: mild form of ataxia 3′ PTC: severe form with short survival time	[133]
Survival motor neuron gene (*SMN1*)	5′ PTC: spinal muscular atrophy (SMA) type III (mild form) 3′ PTC: spinal muscular atrophy (SMA) type I (severe form)	[134,135]
Dystrophin (*DMD*)	PTCs at different positions can cause mild to severe phenotype. However, most commonly 5′ PTCs cause severe forms of muscular dystrophy (DMD) 3′ PTCs cause milder forms of muscular dystrophy (BMD)	[95]
Cystic fibrosis transmembrane conductance regulator gene (*CFTR*)	PTCs at different positions can cause mild to severe phenotype. However, most commonly 5′ PTC: severe form of CF 3′ PTC: milder form of CF. Patients with less efficient NMD respond better to nonsense suppression treatment.	[136]
Paired box gene 6 (*PAX 6*)	5′ PTC: aniridia, congenital absence of the iris 3′ PTC: not detected, however, dominant-negative protein is predicted to show severe phenotype	[137]
Ectodysplasin-A receptor gene (*EDAR*)	5′ PTC: autosomal recessive hypohidoritc ectodermal dysplasia (HED) 3′ PTC: autosomal dominant hypohidoritc ectodermal dysplasia (HED)	[138,139]
Beta subunit of sodium channel (*SCNN1B*)	5′ PTC: autosomal recessive pseudohypoaldosteronism I (PHA1) 3′ PTC: dominant Liddle syndrome	[140,141]

proteins and/or the correction of protein function. However, it is interesting to note that although a number of studies pointed to the clinical significance of aminoglycides, variability in the response to aminoglycoside treatment was one of the major concerns in many of these studies [99]. Moreover, the requirement of high concentrations for a prolonged effect, the intravenous administration mode and known side effects, such as kidney damage and hearing loss, limited the usefulness of its systemic application.

PTC124

PTC124, a 1,2,4-oxidiazole compound, is a new drug in development for mutation-specific treatment of inherited diseases such as DMD and CF. PTC124 was initially identified in a high-throughput screen for its ability to promote read-through of nonsense codons. Furthermore, cultured muscle cells from DMD patients and *mdx* mice (a mouse model for DMD) produced full-length dystrophin after PTC124 treatment [109]. PTC124

Table 3 Available therapies for treating PTC-associated diseases

Therapeutic approach	Effect on NMD	Models tested	Remarks	References
Aminoglycoside	Readthrough effect: translational misreading and incorporation of an amino acid at PTCs.	Patients with CF and cultured cells from BMD and DMD patients were tested	Variability in response, high doses needed intravenous administration, kidney damage and hearing loss	[99–106,142]
PTC124	Readthrough effect: mechanism unknown.	Patients with CF and DMD, phase II trials and animal models tested	80000 compounds were screened, safety and clinical effectiveness not yet proven	[109–111]
Suppressor tRNA	Chimaeric tRNA recognizes a termination codon and introduces an amino acid into the nascent polypeptide	Cultured cells from DMD patients	Lack of efficient method of delivery, potential immune reaction, effect of suppressor tRNAs on physiological targets with same termination codon	[112–114]
Antisense oligonucleotide	Short nucleotide sequence binds a specific region of an mRNA to suppress or redirect splicing to inhibit incorporation of PTC into the mRNA	DMD mouse models and cultured cells from DMD patients	Lack of efficient method of delivery, potential immune reaction, issues of transfection efficiency, unknown safety profile	[115–118]
Inhibitors of NMD	Use of inhibitors of NMD cofactors	Cultured fibroblasts of patients with Ulrich's disease	Unfavourable safety profile of existing compounds	[119]

was reported to promote selective read-through of PTCs without affecting normal stop codons and inducing the production of abnormally long proteins. These promising results led to its evaluation in patients. PTC124 is being investigated in phase II clinical studies in CF and DMD patients. Its oral bioavailability and favourable safety profile make PTC124 an attractive drug for further investigation [110].

However, reports have challenged the validity of the high-throughput screens that were used for the identification of PTC124 [111]. Future prospects for this compound will largely depend on its efficacy and safety in clinical trials.

Suppressor tRNA

In the suppressor tRNA approach, chimaeric tRNAs are used that can specially recognize one of the three termination codon triplets and introduce an amino acid instead of termination. Studies in β-thalassaemia and DMD have shown that the expression of PTC-containing β-globin or PTC-containing dystrophin alleles have been partially corrected in cultured cells by ectopic expression of suppression tRNAs [112,113].

The major drawback of this therapy method is the lack of efficient methods of delivery and stable retention of the expression of the suppressor tRNA in the correct cell types in patients. Moreover, the immune reaction against suppressor tRNA and the required vectors for proper delivery raises additional concerns regarding the clinical use of this approach [114].

Therapies based on the elimination of the PTC-carrying portion of a mutated transcript

In this approach, repair strategies aim at the elimination of the PTC-carrying portion of a mutated transcript. One such approach is to use antisense oligonucleotides to restore normal splicing in cases where splicing abnormalities result in PTCs [115]. This approach was first implemented for correcting aberrant splicing of the β-globin gene [116]. Later a modified version of this approach was used to restore splicing of a PTC-containing dystrophin mRNA [117].

In spite of the potential therapeutic opportunity and the advantage to use it in variable ways, the major setback to this oligonucleotide approach is the current lack of availability of a proper delivery system, issues of transfection efficiency, potential immune responses and undesired side effects [118].

Therapies based on inhibitors of NMD

Both stimulation and an inhibition of NMD might, in principle, offer a promising perspective in treating a variety of inherited and acquired genetic disorders (Table 2). It would thus be helpful to identify compounds that can directly modulate NMD itself.

In Ulrich's disease, a frameshift mutation with a PTC in the collagen VI α2 gene causes loss of collagen VI and functional defects in ECM (extracellular matrix). SMG1 kinase inhibitors, such as wortmannin and caffeine, that block NMD were reported to increase the expression of the mutant collagen VI α2 subunit, resulting in collagen VI assembly and partially functional ECM formation [119]. These results suggested that NMD inhibitors might open a perspective as therapeutic tools to correct human genetic diseases that are aggravated by NMD.

Notwithstanding this beneficial role of inhibiting NMD by wortmannin and caffeine treatment in cell culture experiments, the high toxicity of both of these compounds at the required doses limits their therapeutic potential in patients. Considering the variety of physiological functions of NMD and its cofactors, it will be a challenge to identify and develop compounds that are both effective and safe.

CONCLUSIONS

NMD represents one of the key gene expression mechanisms that safeguards the fidelity of mRNA expression and is intricately involved in quality control at a post-transcriptional level. Mechanistically, NMD highlights the co-operation between nuclear and cytoplasmic events of the gene expression machinery. NMD also holds exciting promise both to explain the phenotypic variability of many inherited and acquired genetic diseases and to provide perspectives for developing novel therapeutic strategies.

ACKNOWLEDGEMENTS

We thank G. Neu-Yilik for sharing her knowledge on NMD and S. Duggimpudi for literature support.

FUNDING

M.B is supported by an EIPOD postdoctoral fellowship from EMBL. The experimental work of the authors is supported by grants from the Deutsche Forschungsgemeinschaft (DFG) to A.E.K and M.W.H.

REFERENCES

1 Hentze, M. W. and Kulozik, A. E. (1999) A perfect message: RNA surveillance and nonsense-mediated decay. Cell **96**, 307–310
2 Maquat, L. E. (2004) Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat. Rev. Mol. Cell Biol. **5**, 89–99
3 Behm-Ansmant, I., Kashima, I., Rehwinkel, J., Sauliere, J., Wittkopp, N. and Izaurralde, E. (2007) mRNA quality control: an ancient machinery recognizes and degrades mRNAs with nonsense codons. FEBS Lett. **581**, 2845–2853
4 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
5 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
6 Isken, O. and Maquat, L. E. (2008) The multiple lives of NMD factors: balancing roles in gene and genome regulation. Nat. Rev. Mol. Cell Biol. **9**, 699–712
7 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
8 Frischmeyer, P. A. and Dietz, H. C. (1999) Nonsense-mediated mRNA decay in health and disease. Hum. Mol. Genet. **8**, 1893–1900
9 Li, S. and Wilkinson, M. F. (1998) Nonsense surveillance in lymphocytes? Immunity **8**, 135–141
10 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
11 Thermann, R., Neu-Yilik, G., Deters, A., Frede, U., Wehr, K., Hagemeier, C., Hentze, M. W. and Kulozik, A. E. (1998) Binary specification of nonsense codons by splicing and cytoplasmic translation. EMBO J. **17**, 3484–3494
12 Maquat, L. E. (1995) When cells stop making sense: effects of nonsense codons on RNA metabolism in vertebrate cells. RNA **1**, 453–465
13 Tange, T. O., Nott, A. and Moore, M. J. (2004) The ever-increasing complexities of the exon junction complex. Curr. Opin. Cell Biol. **16**, 279–284
14 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
15 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
16 Eberle, A. B., Lykke-Andersen, S., Muhlemann, O. and Jensen, T. H. (2009) SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells. Nat. Struct. Mol. Biol. **16**, 49–55
17 Chamieh, H., Ballut, L., Bonneau, F. and Le Hir, H. (2008) NMD factors UPF2 and UPF3 bridge UPF1 to the exon junction complex and stimulate its RNA helicase activity. Nat. Struct. Mol. Biol. **15**, 85–93
18 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
19 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
20 Lykke-Andersen, J., Shu, M. D. and Steitz, J. A. (2000) Human Upf proteins target an mRNA for nonsense-mediated decay when bound downstream of a termination codon. Cell **103**, 1121–1131
21 Mendell, J. T., ap Rhys, C. M. and Dietz, H. C. (2002) Separable roles for rent1/hUpf1 in altered splicing and decay of nonsense transcripts. Science **298**, 419–422
22 Medghalchi, S. M., Frischmeyer, P. A., Mendell, J. T., Kelly, A. G., Lawler, A. M. and Dietz, H. C. (2001) Rent1, a trans-effector of nonsense-mediated mRNA decay, is essential for mammalian embryonic viability. Hum. Mol. Genet. **10**, 99–105
23 Azzalin, C. M., Reichenbach, P., Khoriauli, L., Giulotto, E. and Lingner, J. (2007) Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. Science **318**, 798–801
24 Czaplinski, K., Ruiz-Echevarria, M. J., Paushkin, S. V., Han, X., Weng, Y., Perlick, H. A., Dietz, H. C., Ter-Avanesyan, M. D. and Peltz, S. W. (1998) The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs. Genes Dev. **12**, 1665–1677
25 Mendell, J. T., Medghalchi, S. M., Lake, R. G., Noensie, E. N. and Dietz, H. C. (2000) Novel Upf2p orthologues suggest a functional link between translation initiation and nonsense surveillance complexes. Mol. Cell. Biol. **20**, 8944–8957
26 Serin, G., Gersappe, A., Black, J. D., Aronoff, R. and Maquat, L. E. (2001) Identification and characterization of human orthologues to *Saccharomyces cerevisiae* Upf2 protein and Upf3 protein (*Caenorhabditis elegans* SMG-4). Mol. Cell. Biol. **21**, 209–223
27 He, F., Brown, A. H. and Jacobson, A. (1996) Interaction between Nmd2p and Upf1p is required for activity but not for dominant-negative inhibition of the nonsense-mediated mRNA decay pathway in yeast. RNA **2**, 153–170
28 Kadlec, J., Izaurralde, E. and Cusack, S. (2004) The structural basis for the interaction between nonsense-mediated mRNA decay factors UPF2 and UPF3. Nat. Struct. Mol. Biol. **11**, 330–337
29 Wittmann, J., Hol, E. M. and Jack, H. M. (2006) hUPF2 silencing identifies physiologic substrates of mammalian nonsense-mediated mRNA decay. Mol. Cell. Biol. **26**, 1272–1287
30 Kunz, J. B., Neu-Yilik, G., Hentze, M. W., Kulozik, A. E. and Gehring, N. H. (2006) Functions of hUpf3a and hUpf3b in nonsense-mediated mRNA decay and translation. RNA **12**, 1015–1022
31 Gehring, N. H., Neu-Yilik, G., Schell, T., Hentze, M. W. and Kulozik, A. E. (2003) Y14 and hUpf3b form an NMD-activating complex. Mol. Cell **11**, 939–949
32 Chan, W. K., Bhalla, A. D., Le Hir, H., Nguyen, L. S., Huang, L., Gecz, J. and Wilkinson, M. F. (2009) A UPF3-mediated regulatory switch that maintains RNA surveillance. Nat. Struct. Mol. Biol. **16**, 747–753
33 Tarpey, P. S., Raymond, F. L., Nguyen, L. S., Rodriguez, J., Hackett, A., Vandeleur, L., Smith, R., Shoubridge, C., Edkins, S., Stevens, C. et al. (2007) Mutations in UPF3B, a member of the nonsense-mediated mRNA decay complex, cause syndromic and nonsyndromic mental retardation. Nat. Genet. **39**, 1127–1133
34 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
35 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
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 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
39 D'Andrea, L. D. and Regan, L. (2003) TPR proteins: the versatile helix. Trends Biochem. Sci. **28**, 655–662
40 Clissold, P. M. and Ponting, C. P. (2000) PIN domains in nonsense-mediated mRNA decay and RNAi. Curr. Biol. **10**, R888–R890
41 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
42 Yamashita, A., Izumi, N., Kashima, I., Ohnishi, T., Saari, B., Katsuhata, Y., Muramatsu, R., Morita, T., Iwamatsu, A., Hachiya, T. et al. (2009) SMG-8 and SMG-9, two novel subunits of the SMG-1 complex, regulate remodeling of the mRNA surveillance complex during nonsense-mediated mRNA decay. Genes Dev. **23**, 1091–1105
43 Carter, M. S., Li, S. and Wilkinson, M. F. (1996) A splicing-dependent regulatory mechanism that detects translation signals. EMBO J. **15**, 5965–5975
44 Maquat, L. E. and Li, X. (2001) Mammalian heat shock p70 and histone H4 transcripts, which derive from naturally intronless genes, are immune to nonsense-mediated decay. RNA **7**, 445–456
45 Brocke, K. S., Neu-Yilik, G., Gehring, N. H., Hentze, M. W. and Kulozik, A. E. (2002) The human intronless melanocortin 4-receptor gene is NMD insensitive. Hum. Mol. Genet. **11**, 331–335
46 Le Hir, H., Gatfield, D., Izaurralde, E. and Moore, M. J. (2001) The exon-exon junction complex provides a binding platform for factors involved in mRNA export and nonsense-mediated mRNA decay. EMBO J. **20**, 4987–4997

47 Gehring, N. H., Lamprinaki, S., Hentze, M. W. and Kulozik, A. E. (2009) The hierarchy of exon-junction complex assembly by the spliceosome explains key features of mammalian nonsense-mediated mRNA decay. PLoS Biol. **7**, e1000120

48 Shibuya, T., Tange, T. O., Sonenberg, N. and Moore, M. J. (2004) eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. Nat. Struct. Mol. Biol. **11**, 346–351

49 Ballut, L., Marchadier, B., Baguet, A., Tomasetto, C., Seraphin, B. and Le Hir, H. (2005) The exon junction core complex is locked onto RNA by inhibition of eIF4AIII ATPase activity. Nat. Struct. Mol. Biol. **12**, 861–869

50 Hachet, O. and Ephrussi, A. (2001) *Drosophila* Y14 shuttles to the posterior of the oocyte and is required for oskar mRNA transport. Curr. Biol. **11**, 1666–1674

51 Degot, S., Le Hir, H., Alpy, F., Kedinger, V., Stoll, I., Wendling, C., Seraphin, B., Rio, M. C. and Tomasetto, C. (2004) Association of the breast cancer protein MLN51 with the exon junction complex via its speckle localizer and RNA binding module. J. Biol. Chem. **279**, 33702–33715

52 Ishigaki, Y., Li, X., Serin, G. and Maquat, L. E. (2001) Evidence for a pioneer round of mRNA translation: mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20. Cell **106**, 607–617

53 Holbrook, J. A., Neu-Yilik, G., Gehring, N. H., Kulozik, A. E. and Hentze, M. W. (2006) Internal ribosome entry sequence-mediated translation initiation triggers nonsense-mediated decay. EMBO Rep. **7**, 722–726

54 Gehring, N. H., Lamprinaki, S., Kulozik, A. E. and Hentze, M. W. (2009) Disassembly of exon junction complexes by PYM. Cell **137**, 536–548

55 Peltz, S. W., Brown, A. H. and Jacobson, A. (1993) mRNA destabilization triggered by premature translational termination depends on at least three cis-acting sequence elements and one trans-acting factor. Genes Dev. **7**, 1737–1754

56 Gonzalez, C. I., Ruiz-Echevarria, M. J., Vasudevan, S., Henry, M. F. and Peltz, S. W. (2000) The yeast hnRNP-like protein Hrp1/Nab4 marks a transcript for nonsense-mediated mRNA decay. Mol. Cell **5**, 489–499

57 Wang, W., Czaplinski, K., Rao, Y. and Peltz, S. W. (2001) The role of Upf proteins in modulating the translation read-through of nonsense-containing transcripts. EMBO J. **20**, 880–890

58 Gehring, N. H., Kunz, J. B., Neu-Yilik, G., Breit, S., Viegas, M. H., Hentze, M. W. and Kulozik, A. E. (2005) Exon-junction complex components specify distinct routes of nonsense-mediated mRNA decay with differential cofactor requirements. Mol. Cell **20**, 65–75

59 Buhler, M., Steiner, S., Mohn, F., Paillusson, A. and Muhlemann, O. (2006) EJC-independent degradation of nonsense immunoglobulin-mu mRNA depends on 3′ UTR length. Nat. Struct. Mol. Biol. **13**, 462–464

60 Chan, W. K., Huang, L., Gudikote, J. P., Chang, Y. F., Imam, J. S., MacLean, II, J. A. and Wilkinson, M. F. (2007) An alternative branch of the nonsense-mediated decay pathway. EMBO J. **26**, 1820–1830

61 Ivanov, P. V., Gehring, N. H., Kunz, J. B., Hentze, M. W. and Kulozik, A. E. (2008) Interactions between UPF1, eRFs, PABP and the exon junction complex suggest an integrated model for mammalian NMD pathways. EMBO J. **27**, 736–747

62 Amrani, N., Ganesan, R., Kervestin, S., Mangus, D. A., Ghosh, S. and Jacobson, A. (2004) A faux 3′-UTR promotes aberrant termination and triggers nonsense-mediated mRNA decay. Nature **432**, 112–118

63 Mangus, D. A., Evans, M. C. and Jacobson, A. (2003) Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. Genome Biol. **4**, 223

64 Hoshino, S., Imai, M., Kobayashi, T., Uchida, N. and Katada, T. (1999) The eukaryotic polypeptide chain releasing factor (eRF3/GSPT) carrying the translation termination signal to the 3′-Poly(A) tail of mRNA. Direct association of erf3/GSPT with polyadenylate-binding protein. J. Biol. Chem. **274**, 16677–16680

65 Cosson, B., Berkova, N., Couturier, A., Chabelskaya, S., Philippe, M. and Zhouravleva, G. (2002) Poly(A)-binding protein and eRF3 are associated *in vivo* in human and *Xenopus* cells. Biol. Cell **94**, 205–216

66 Zhouravleva, G., Frolova, L., Le Goff, X., Le Guellec, R., Inge-Vechtomov, S., Kisselev, L. and Philippe, M. (1995) Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. EMBO J. **14**, 4065–4072

67 Behm-Ansmant, I., Gatfield, D., Rehwinkel, J., Hilgers, V. and Izaurralde, E. (2007) A conserved role for cytoplasmic poly(A)-binding protein 1 (PABPC1) in nonsense-mediated mRNA decay. EMBO J. **26**, 1591–1601

68 Amrani, N., Sachs, M. S. and Jacobson, A. (2006) Early nonsense: mRNA decay solves a translational problem. Nat. Rev. Mol. Cell Biol. **7**, 415–425

69 Nicholson, P., Yepiskoposyan, H., Metze, S., Zamudio Orozco, R., Kleinschmidt, N. and Muhlemann, O. (2009) 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

70 Singh, G., Rebbapragada, I. and Lykke-Andersen, J. (2008) A competition between stimulators and antagonists of Upf complex recruitment governs human nonsense-mediated mRNA decay. PLoS Biol. **6**, e111

71 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

72 Longman, D., Plasterk, R. H., Johnstone, I. L. and Caceres, J. F. (2007) Mechanistic insights and identification of two novel factors in the *C. elegans* NMD pathway. Genes Dev. **21**, 1075–1085

73 Behm-Ansmant, I., Gatfield, D., Rehwinkel, J., Hilgers, V. and Izaurralde, E. (2007) A conserved role for cytoplasmic poly(A)-binding protein 1 (PABPC1) in nonsense-mediated mRNA decay. EMBO J. **26**, 1591–1601

74 Neu-Yilik, G. and Kulozik, A. E. (2008) NMD: multitasking between mRNA surveillance and modulation of gene expression. Adv. Genet. **62**, 185–243

75 Mitrovich, Q. M. and Anderson, P. (2005) mRNA surveillance of expressed pseudogenes in *C. elegans*. Curr. Biol. **15**, 963–967

76 Lelivelt, M. J. and Culbertson, M. R. (1999) Yeast Upf proteins required for RNA surveillance affect global expression of the yeast transcriptome. Mol. Cell. Biol. **19**, 6710–6719

77 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

78 Moriarty, P. M., Reddy, C. C. and Maquat, L. E. (1998) Selenium deficiency reduces the abundance of mRNA for Se-dependent glutathione peroxidase 1 by a UGA-dependent mechanism likely to be nonsense codon-mediated decay of cytoplasmic mRNA. Mol. Cell. Biol. **18**, 2932–2939

79 Pain, V. M. (1994) Translational control during amino acid starvation. Biochimie **76**, 718–728

80 Chiu, S. Y., Lejeune, F., Ranganathan, A. C. and Maquat, L. E. (2004) The pioneer translation initiation complex is functionally distinct from but structurally overlaps with the steady-state translation initiation complex. Genes Dev. **18**, 745–754

81 Gardner, L. B. (2008) Hypoxic inhibition of nonsense-mediated RNA decay regulates gene expression and the integrated stress response. Mol. Cell. Biol. **28**, 3729–3741

82 Weischenfeldt, J., Damgaard, I., Bryder, D., Theilgaard-Monch, K., Thoren, L. A., Nielsen, F. C., Jacobsen, S. E., Nerlov, C. and Porse, B. T. (2008) NMD is essential for hematopoietic stem and progenitor cells and for eliminating by-products of programmed DNA rearrangements. Genes Dev. **22**, 1381–1396

83 Azzalin, C. M. and Lingner, J. (2006) The double life of UPF1 in RNA and DNA stability pathways. Cell Cycle **5**, 1496–1498

84 Saltzman, A. L., Kim, Y. K., Pan, Q., Fagnani, M. M., Maquat, L. E. and Blencowe, B. J. (2008) Regulation of multiple core spliceosomal proteins by alternative splicing-coupled nonsense-mediated mRNA decay. Mol. Cell. Biol. **28**, 4320–4330

85 Guan, Q., Zheng, W., Tang, S., Liu, X., Zinkel, R. A., Tsui, K. W., Yandell, B. S. and Culbertson, M. R. (2006) Impact of nonsense-mediated mRNA decay on the global expression profile of budding yeast. PLoS Genet. **2**, e203

86 Dahlseid, J. N., Lew-Smith, J., Lelivelt, M. J., Enomoto, S., Ford, A., Desruisseaux, M., McClellan, M., Lue, N., Culbertson, M. R. and Berman, J. (2003) mRNAs encoding telomerase components and regulators are controlled by UPF genes in *Saccharomyces cerevisiae*. Eukaryotic Cell **2**, 134–142

87 Brumbaugh, K. M., Otterness, D. M., Geisen, C., Oliveira, V., Brognard, J., Li, X., Lejeune, F., Tibbetts, R. S., Maquat, L. E. and Abraham, R. T. (2004) The mRNA surveillance protein hSMG-1 functions in genotoxic stress response pathways in mammalian cells. Mol. Cell **14**, 585–598

88 Muller, B., Blackburn, J., Feijoo, C., Zhao, X. and Smythe, C. (2007) DNA-activated protein kinase functions in a newly observed S phase checkpoint that links histone mRNA abundance with DNA replication. J. Cell Biol. **179**, 1385–1398

89 Metzstein, M. M. and Krasnow, M. A. (2006) Functions of the nonsense-mediated mRNA decay pathway in *Drosophila* development. PLoS Genet. **2**, e180

90 Yoine, M., Nishii, T. and Nakamura, K. (2006) *Arabidopsis* UPF1 RNA helicase for nonsense-mediated mRNA decay is involved in seed size control and is essential for growth. Plant Cell Physiol. **47**, 572–580

91 Wittkopp, N., Huntzinger, E., Weiler, C., Sauliere, J., Schmidt, S., Sonawane, M. and Izaurralde, E. (2009) Nonsense-mediated mRNA decay effectors are essential for zebrafish embryonic development and survival. Mol. Cell. Biol. **29**, 3517–3528

92 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

93 Thein, S. L., Hesketh, C., Taylor, P., Temperley, I. J., Hutchinson, R. M., Old, J. M., Wood, W. G., Clegg, J. B. and Weatherall, D. J. (1990) Molecular basis for dominantly inherited inclusion body β-thalassemia. Proc. Natl. Acad. Sci. U.S.A. **87**, 3924–3928

94 Hall, G. W. and Thein, S. (1994) Nonsense codon mutations in the terminal exon of the β-globin gene are not associated with a reduction in β-mRNA accumulation: a mechanism for the phenotype of dominant β-thalassemia. Blood **83**, 2031–2037

95 Kerr, T. P., Sewry, C. A., Robb, S. A. and Roberts, R. G. (2001) Long mutant dystrophins and variable phenotypes: evasion of nonsense-mediated decay? Hum. Genet. **109**, 402–407

96 Karam, R., Carvalho, J., Bruno, I., Graziadio, C., Senz, J., Huntsman, D., Carneiro, F., Seruca, R., Wilkinson, M. F. and Oliveira, C. (2008) The NMD mRNA surveillance pathway downregulates aberrant E-cadherin transcripts in gastric cancer cells and in CDH1 mutation carriers. Oncogene **27**, 4255–4260

97 Kaurah, P., MacMillan, A., Boyd, N., Senz, J., De Luca, A., Chun, N., Suriano, G., Zaor, S., Van Manen, L., Gilpin, C. et al. (2007) Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA, J. Am. Med. Assoc. **297**, 2360–2372

98 Oliveira, C., Seruca, R. and Carneiro, F. (2006) Genetics, pathology, and clinics of familial gastric cancer. Int. J. Surg. Pathol. **14**, 21–33

99 Linde, L. and Kerem, B. (2008) Introducing sense into nonsense in treatments of human genetic diseases. Trends Genet. **24**, 552–563

100 Davies, J., Gorini, L. and Davis, B. D. (1965) Misreading of RNA codewords induced by aminoglycoside antibiotics. Mol. Pharmacol. **1**, 93–106

101 Palmer, E., Wilhelm, J. M. and Sherman, F. (1979) Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics. Nature **277**, 148–150

102 Lynch, S. R., Gonzalez, R. L. and Puglisi, J. D. (2003) Comparison of X-ray crystal structure of the 30S subunit-antibiotic complex with NMR structure of decoding site oligonucleotide-paromomycin complex. Structure **11**, 43–53

103 Vicens, Q. and Westhof, E. (2001) Crystal structure of paromomycin docked into the eubacterial ribosomal decoding A site. Structure **9**, 647–658

104 Zsembery, A., Jessner, W., Sitter, G., Spirli, C., Strazzabosco, M. and Graf, J. (2002) Correction of CFTR malfunction and stimulation of Ca-activated Cl channels restore HCO3- secretion in cystic fibrosis bile ductular cells. Hepatology **35**, 95–104

105 Bedwell, D. M., Kaenjak, A., Benos, D. J., Bebok, Z., Bubien, J. K., Hong, J., Tousson, A., Clancy, J. P. and Sorscher, E. J. (1997) Suppression of a CFTR premature stop mutation in a bronchial epithelial cell line. Nat. Med. **3**, 1280–1284

106 Keeling, K. M., Du, M. and Bedwell, D. (2006) Therapies of nonsense-associated diseases. In Nonsense-Mediated mRNA Decay (Maquat, L.E., ed.), pp. 121–136, Landes Biosciences, Austin

107 Wilschanski, M., Famini, C., Blau, H., Rivlin, J., Augarten, A., Avital, A., Kerem, B. and Kerem, E. (2000) A pilot study of the effect of gentamicin on nasal potential difference measurements in cystic fibrosis patients carrying stop mutations. Am. J. Respir. Crit. Care Med. **161**, 860–865

108 Wagner, K. R., Hamed, S., Hadley, D. W., Gropman, A. L., Burstein, A. H., Escolar, D. M., Hoffman, E. P. and Fischbeck, K. H. (2001) Gentamicin treatment of Duchenne and Becker muscular dystrophy due to nonsense mutations. Ann. Neurol. **49**, 706–711

109 Welch, E. M., Barton, E. R., Zhuo, J., Tomizawa, Y., Friesen, W. J., Trifillis, P., Paushkin, S., Patel, M., Trotta, C. R., Hwang, S. et al. (2007) PTC124 targets genetic disorders caused by nonsense mutations. Nature **447**, 87–91

110 Du, M., Liu, X., Welch, E. M., Hirawat, S., Peltz, S. W. and Bedwell, D. M. (2008) PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. Proc. Natl. Acad. Sci. U.S.A. **105**, 2064–2069

111 Auld, D. S., Thorne, N., Maguire, W. F. and Inglese, J. (2009) Mechanism of PTC124 activity in cell-based luciferase assays of nonsense codon suppression. Proc. Natl. Acad. Sci. U.S.A. **106**, 3585–3590

112 Temple, G. F., Dozy, A. M., Roy, K. L. and Kan, Y. W. (1982) Construction of a functional human suppressor tRNA gene: an approach to gene therapy for β-thalassaemia. Nature **296**, 537–540

113 Kiselev, A. V., Ostapenko, O. V., Rogozhkina, E. V., Kholod, N. S., Seit Nebi, A. S., Baranov, A. N., Lesina, E. A., Ivashchenko, T. E., Sabetskii, V. A., Shavlovskii, M. M. et al. (2002) Suppression of nonsense mutations in the Dystrophin gene by a suppressor tRNA gene. Mol. Biol. **36**, 43–47

114 Atkinson, J. and Martin, R. (1994) Mutations to nonsense codons in human genetic disease: implications for gene therapy by nonsense suppressor tRNAs. Nucleic Acids Res. **22**, 1327–1334

115 Alter, J., Lou, F., Rabinowitz, A., Yin, H., Rosenfeld, J., Wilton, S. D., Partridge, T. A. and Lu, Q. L. (2006) Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. Nat. Med. **12**, 175–177

116 Dominski, Z. and Kole, R. (1993) Restoration of correct splicing in thalassemic pre-mRNA by antisense oligonucleotides. Proc. Natl. Acad. Sci. U.S.A. **90**, 8673–8677

117 Mann, C. J., Honeyman, K., Cheng, A. J., Ly, T., Lloyd, F., Fletcher, S., Morgan, J. E., Partridge, T. A. and Wilton, S. D. (2001) Antisense-induced exon skipping and synthesis of dystrophin in the mdx mouse. Proc. Natl. Acad. Sci. U.S.A. **98**, 42–47

118 Hu, H. and Gatti, R. A. (2008) New approaches to treatment of primary immunodeficiencies: fixing mutations with chemicals. Curr. Opin. Allergy Clin. Immunol. **8**, 540–546

119 Usuki, F., Yamashita, A., Higuchi, I., Ohnishi, T., Shiraishi, T., Osame, M. and Ohno, S. (2004) Inhibition of nonsense-mediated mRNA decay rescues the phenotype in Ullrich's disease. Ann. Neurol. **55**, 740–744

120 Rosenfeld, P. J., Cowley, G. S., McGee, T. L., Sandberg, M. A., Berson, E. L. and Dryja, T. P. (1992) A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. Nat. Genet. **1**, 209–213

121 Sung, C. H., Davenport, C. M., Hennessey, J. C., Maumenee, I. H., Jacobson, S. G., Heckenlively, J. R., Nowakowski, R., Fishman, G., Gouras, P. and Nathans, J. (1991) Rhodopsin mutations in autosomal dominant retinitis pigmentosa. Proc. Natl. Acad. Sci. U.S.A. **88**, 6481–6485

122 Inoue, K., Khajavi, M., Ohyama, T., Hirabayashi, S., Wilson, J., Reggin, J. D., Mancias, P., Butler, I. J., Wilkinson, M. F., Wegner, M. and Lupski, J. R. (2004) Molecular mechanism for distinct neurological phenotypes conveyed by allelic truncating mutations. Nat. Genet. **36**, 361–369

123 Jouanguy, E., Altare, F., Lamhamedi, S., Revy, P., Emile, J. F., Newport, M., Levin, M., Blanche, S., Seboun, E., Fischer, A. and Casanova, J. L. (1996) Interferon-γ-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. N. Engl. J. Med. **335**, 1956–1961

124 Jouanguy, E., Lamhamedi-Cherradi, S., Lammas, D., Dorman, S. E., Fondaneche, M. C., Dupuis, S., Doffinger, R., Altare, F., Girdlestone, J., Emile, J. F. et al. (1999) A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nat. Genet. **21**, 370–378

125 Rivolta, C., Berson, E. L. and Dryja, T. P. (2001) Dominant Leber congenital amaurosis, cone-rod degeneration, and retinitis pigmentosa caused by mutant versions of the transcription factor CRX. Hum. Mutat. **18**, 488–498

126 Schwabe, G. C., Tinschert, S., Buschow, C., Meinecke, P., Wolff, G., Gillessen-Kaesbach, G., Oldridge, M., Wilkie, A. O., Komec, R. and Mundlos, S. (2000) Distinct mutations in the receptor tyrosine kinase gene ROR2 cause brachydactyly type B. Am. J. Hum. Genet. **67**, 822–831

127 Pusch, M. (2002) Myotonia caused by mutations in the muscle chloride channel gene CLCN1. Hum. Mutat. **19**, 423–434

128 Schneppenheim, R., Budde, U., Obser, T., Brassard, J., Mainusch, K., Ruggeri, Z. M., Schneppenheim, S., Schwaab, R. and Oldenburg, J. (2001) Expression and characterization of von Willebrand factor dimerization defects in different types of von Willebrand disease. Blood **97**, 2059–2066

129 Millar, D. S., Elliston, L., Deex, P., Krawczak, M., Wacey, A. I., Reynaud, J., Nieuwenhuis, H. K., Bolton-Maggs, P., Mannucci, P. M., Reverter, J. C. et al. (2000) Molecular analysis of the genotype-phenotype relationship in factor X deficiency. Hum. Genet. **106**, 249–257

130 Tassabehji, M., Metcalfe, K., Donnai, D., Hurst, J., Reardon, W., Burch, M. and Read, A. P. (1997) Elastin: genomic structure and point mutations in patients with supravalvular aortic stenosis. Hum. Mol. Genet. **6**, 1029–1036

131 Korkko, J., Ala-Kokko, L., De Paepe, A., Nuytinck, L., Earley, J. and Prockop, D. J. (1998) Analysis of the COL1A1 and COL1A2 genes by PCR amplification and scanning by conformation-sensitive gel electrophoresis identifies only COL1A1 mutations in 15 patients with osteogenesis imperfecta type I: identification of common sequences of null-allele mutations. Am. J. Hum. Genet. **62**, 98–110

132 Willing, M. C., Deschenes, S. P., Slayton, R. L. and Roberts, E. J. (1996) Premature chain termination is a unifying mechanism for COL1A1 null alleles in osteogenesis imperfecta type I cell strains. Am. J. Hum. Genet. **59**, 799–809

133 Li, A. and Swift, M. (2000) Mutations at the ataxia-telangiectasia locus and clinical phenotypes of A-T patients. Am. J. Med. Genet. **92**, 170–177

134 Parsons, D. W., McAndrew, P. E., Monani, U. R., Mendell, J. R., Burghes, A. H. and Prior, T. W. (1996) An 11 base pair duplication in exon 6 of the SMN gene produces a type I spinal muscular atrophy (SMA) phenotype: further evidence for SMN as the primary SMA-determining gene. Hum. Mol. Genet. **5**, 1727–1732

135 Sossi, V., Giuli, A., Vitali, T., Tiziano, F., Mirabella, M., Antonelli, A., Neri, G. and Brahe, C. (2001) Premature termination mutations in exon 3 of the SMN1 gene are associated with exon skipping and a relatively mild SMA phenotype. Eur. J. Hum. Genet. **9**, 113–120

136 Rowntree, R. K. and Harris, A. (2003) The phenotypic consequences of CFTR mutations. Ann. Hum. Genet. **67**, 471–485
137 Tzoulaki, I., White, I. M. and Hanson, I. M. (2005) PAX6 mutations: genotype-phenotype correlations. BMC Genet. **6**, 27
138 Monreal, A. W., Ferguson, B. M., Headon, D. J., Street, S. L., Overbeek, P. A. and Zonana, J. (1999) Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. Nat. Genet. **22**, 366–369
139 Chassaing, N., Bourthoumieu, S., Cossee, M., Calvas, P. and Vincent, M. C. (2006) Mutations in EDAR account for one-quarter of non-ED1-related hypohidrotic ectodermal dysplasia. Hum. Mutat. **27**, 255–259

Received 6 May 2010/10 June 2010; accepted 14 June 2010
Published on the Internet 27 August 2010, doi:10.1042/BJ20100699

140 Jackson, S. N., Williams, B., Houtman, P. and Trembath, R. C. (1998) The diagnosis of Liddle syndrome by identification of a mutation in the beta subunit of the epithelial sodium channel. J. Med. Genet. **35**, 510–512
141 Kerem, E., Bistritzer, T., Hanukoglu, A., Hofmann, T., Zhou, Z., Bennett, W., MacLaughlin, E., Barker, P., Nash, M., Quittell, L. et al. (1999) Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. N. Engl. J. Med. **341**, 156–162
142 Wilschanski, M., Famini, C., Blau, H., Rivlin, J., Augarten, A., Avital, A., Kerem, B. and Kerem, E. (2000) A pilot study of the effect of gentamicin on nasal potential difference measurements in cystic fibrosis patients carrying stop mutations. Am. J. Respir. Crit. Care Med. **161**, 860–865