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Citation for published version:

Hug, N, Longman, D & Caceres, JF 2016, 'Mechanism and regulation of the nonsense-mediated decay pathway' *Nucleic Acids Research*, pp. 1483-1495. DOI: 10.1093/nar/gkw010

Digital Object Identifier (DOI):

[10.1093/nar/gkw010](https://doi.org/10.1093/nar/gkw010)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Nucleic Acids Research

Publisher Rights Statement:

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SURVEY AND SUMMARY

Mechanism and regulation of the nonsense-mediated decay pathway

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Received October 28, 2015; Revised December 28, 2015; Accepted December 31, 2015

ABSTRACT

The Nonsense-mediated mRNA decay (NMD) pathway selectively degrades mRNAs harboring premature termination codons (PTCs) but also regulates the abundance of a large number of cellular RNAs. The central role of NMD in the control of gene expression requires the existence of buffering mechanisms that tightly regulate the magnitude of this pathway. Here, we will focus on the mechanism of NMD with an emphasis on the role of RNA helicases in the transition from NMD complexes that recognize a PTC to those that promote mRNA decay. We will also review recent strategies aimed at uncovering novel transacting factors and their functional role in the NMD pathway. Finally, we will describe recent progress in the study of the physiological role of the NMD response.

INTRODUCTION

Cells have evolved different surveillance mechanisms to target messenger RNAs (mRNAs) with mutations that would otherwise lead to errors in the synthesis of proteins, and also to eliminate other incorrectly processed cellular RNAs. These mechanisms operate both in the cell nucleus and cytoplasm (1). One of the best-studied RNA surveillance pathways is the Nonsense-mediated decay (NMD) pathway, which targets mRNAs harboring premature termination codons (PTC) for degradation. This mechanism operates in the cytoplasm and is intimately linked to translation termination (2,3).

Initially, NMD was described as a post-transcriptional mRNA quality control mechanism responsible for the removal of PTC-containing mRNAs, which if left intact, would lead to production of truncated proteins with pre-

dicted deleterious effects for the organism. From a medical perspective, this suggests that the NMD pathway has a role in the modulation of the phenotypic outcome of genetic disorders that are caused by the presence of a PTC (4,5). However, it has become evident in recent years that this pathway is not solely dedicated to the destruction of PTC-containing transcripts, but that it also has an important role in controlling the expression of naturally occurring transcripts (6–8) (Figure 1). This general role of the NMD pathway on gene expression requires the existence of buffering mechanisms to tightly regulate the magnitude of the NMD response upon environmental and/or genetic insults. Accordingly, a negative feedback regulatory network that controls the levels of core NMD factors operates in mammals (9,10), in nematodes and zebrafish (11), and also in plants (12). Interestingly, the magnitude of the NMD response has also been shown to be variable among individuals (13). The NMD pathway is not exclusively dedicated to mRNAs, as shown by the substantial number of long non-coding RNAs (lncRNAs) that are substrates of NMD in *Arabidopsis*, *S. cerevisiae* and mouse ES cells (14–16). This is not entirely surprising, taking into account the recently revealed association of the translation machinery with lncRNAs (17).

In this article, we will cover recent advances regarding the NMD mechanism, building on the roles of core NMD factors, the functions of RNA helicases in NMD complex assembly, as well as recent strategies used to identify novel NMD factors. Finally, we will review the crucial role of the NMD mechanism in buffering gene expression and its impact on cell physiology.

PTC definition

A crucial aspect of the NMD pathway is the ability to distinguish normal termination codons from PTCs. Despite the relatively high conservation of NMD core factors across evolution, the mechanism of PTC definition varies across

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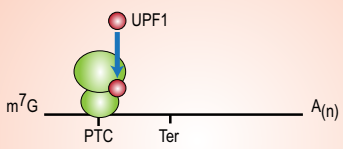
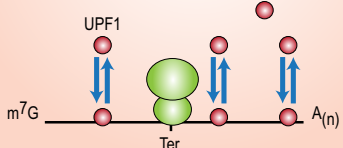
Target	Signal	NMD Function	Example
'Faulty' transcripts 	PTC	Quality control strong NMD	NMD 'on' removal of deleterious transcripts NMD 'off' disease phenotype
'Normal' transcripts 	Combination of weaker features: uORF, long 3'UTR, introns after stop codon; some sequence elements prevent NMD	Fine-tuning of gene expression variable NMD	Regulation of endogenous transcripts in: Development Stress response Immune response

Figure 1. Dual role of the Nonsense-mediated decay (NMD) pathway. NMD degrades PTC-containing transcripts ('faulty' transcripts) (top panel) and also regulates the expression of naturally occurring transcripts ('normal transcripts'), acting as a fine-tuning mechanism of gene expression (bottom panel). Green spheres represent both ribosomal subunits, whereas red circles depict the core NMD factor, UPF1.

different species. In mammals, NMD is intimately linked to pre-mRNA splicing, and mRNAs harboring a PTC 50 to 55 nucleotides upstream of the final exon–exon junction are efficiently degraded (18–21). This is signaled by the presence of the exon junction complex (EJC), a multi-subunit protein complex, which is deposited 20 to 24 nucleotides upstream of an exon–exon junction during pre-mRNA splicing (22,23). The EJC remains associated to the mRNA until it is displaced by the translation machinery with the help of the ribosome-associated protein PYM acting as an EJC disassembly factor (24,25,26). Recent transcriptome-wide analysis of EJC deposition established that EJCs are not equally assembled at every exon junction, as previously hypothesized (27,28); indeed, approximately half of all EJCs are present at non-canonical positions (29). A remaining challenge is to understand whether the observed variation on EJC loading affects NMD efficiency. By contrast, PTC definition occurs independently of exon boundaries in *S. cerevisiae*, where the distance between the PTC and the 3' end defines NMD, as proposed by the faux 3' UTR model (30,31). In *Schizosaccharomyces pombe* splicing enhances NMD; however, EJC components are not required for NMD and what seems to enhance NMD is the proximity of the intron to the PTC (32). The presence of introns is also not required to define PTCs in *Drosophila* or in *C. elegans*, exposing a significant level of mechanistic diversity in this critical step of the NMD process (33,34).

NMD mechanism and the role of RNA helicases in NMD progression

The initial identification of factors with roles in the NMD pathway was achieved by means of unbiased genetic screens in *Caenorhabditis elegans* and *Saccharomyces cerevisiae*. This led to the identification of seven genes in nematodes, termed *smg-1–7* (suppressor with morphological effect on genitalia), given that mutations of these genes led to abnormal morphogenesis of the male bursa and

the hermaphrodite vulva (35,36). Importantly, *smg* mutant worms are viable, indicating that NMD is not essential in nematodes. Similarly, three genes, termed *UPF1–3* (for up-frameshift), that are orthologues of *C. elegans smg-2*, *smg-3* and *smg-4* genes, were identified in *S. cerevisiae* (37,38). Homology searches led to the identification of orthologous genes in other species, including *Arabidopsis*, *Drosophila* and mammals (39).

RNA helicases have a central role in the mechanism of NMD progression. In general, these enzymes can use adenosine triphosphate (ATP) to translocate along nucleic acids, potentially unwinding secondary structure and acting to remodel RNA-protein complexes. Alternatively, they might act as "place markers" remaining temporarily fixed in a defined position while signaling to, or directly recruiting, the degradation machinery (40,41). In the latter case, RNA helicases clamp the RNA in an ATP-dependent fashion to provide nucleation centers to assemble larger RNA-protein complexes. The central component of the NMD pathway in all organisms studied is the protein UPF1/SMG2, an ATP-dependent RNA helicase of the SF1 superfamily, which undergoes cycles of phosphorylation and dephosphorylation that are essential for NMD progression. Phosphorylation of UPF1 is carried out by the SMG1c complex, comprised of the protein kinase SMG1, a phosphoinositide 3-kinase (PI3K)-like kinase and two additional subunits, SMG8 and SMG9 (42–44). Initially, UPF1 associates with SMG1 and acts as a clamp, interacting directly with the eukaryotic release factors eRF1 and eRF3 to form the so-called surveillance complex (SURF) in the vicinity of the PTC (Figure 2). Two subunits of the SMG1c complex, SMG8 and the NTPase SMG9 associate tightly with SMG1 and regulate its activity through the induction of conformational changes, with SMG8 binding to the preformed SMG9-SMG1 complex and maintaining the kinase in its inactive state (45–47). Subsequently, the SURF complex interacts with UPF2, UPF3b and an EJC downstream of the

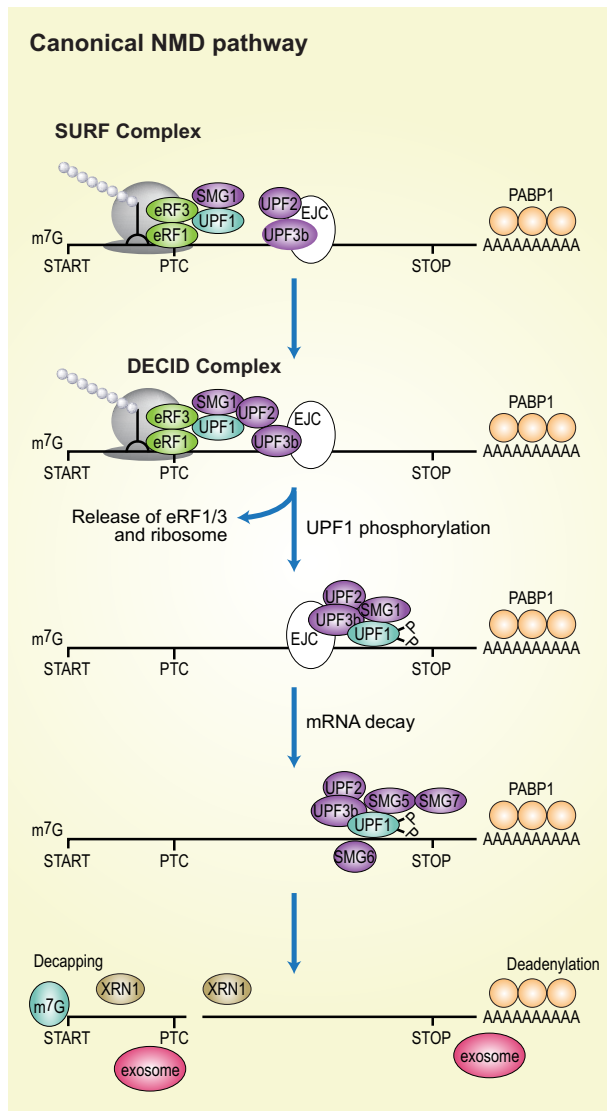


Figure 2. Mechanism of NMD activation in mammalian cells, indicating the transition from the surveillance complex (SURF) to the decay-inducing complex (DECID). UPF1 and its associated kinase, SMG1, bind to the eukaryotic release factors eRF1 and eRF3 to form the SURF complex in the vicinity of a premature termination codon (PTC). A subsequent interaction of this complex with UPF2, UPF3b and an exon junction complex (EJC) downstream of the PTC triggers the formation of the DECID complex, resulting in UPF1 phosphorylation and release of eRF1 and eRF3. Phosphorylated UPF1 acts to recruit SMG5, SMG6 and SMG7, and general mRNA degradation factors that lead to mRNA degradation.

PTC to form the decay-inducing complex (DECID) that triggers UPF1 phosphorylation and dissociation of eRF1 and eRF3 (48–50) (Figure 2). As a consequence of the remodeling of NMD complexes, UPF1 adopts its active helicase conformation due to the reorganization of its inhibitory domains through association with UPF2 (51–54). The active UPF1 helicase functions as RNPase translocating along the mRNA, resolving secondary structure and clearing the mRNA from proteins allowing access to nucleases (55,56). The activated NMD complex consisting of UPF1, UPF2 and UPF3b is translocated from its position

upstream of the EJC toward the 3' of the EJC (57) (Figure 2). Subsequently, phosphorylated UPF1 associates with the phospho-binding proteins SMG5, SMG6 and SMG7, and general mRNA degradation factors and further rearrangements of this complex lead to mRNA degradation (58). SMG6 itself is an endonuclease, which can form both phospho-dependent and phospho-independent interactions with UPF1 (59–62). SMG6 cleaves NMD targets in the vicinity of the PTC (59,63), leading to the initiation of NMD-mediated RNA degradation (64–66). SMG5 and SMG7 bind as a heterodimer to phosphorylated UPF1. The SMG7 subunit recruits directly POP2, the catalytic subunit of the Deadenylation complex (67) and additionally initiates decapping and XRN1-catalyzed 5'-3' degradation (68). This canonical mammalian NMD pathway is not universal, since alternative NMD branches that are independent of UPF2, UPF3b or the EJC have been described (69–71).

Another RNA helicase with a role in EJC-mediated NMD progression is eIF4AIII, a core component of the EJC, that binds RNA in an ATP-dependent fashion and recruits additional EJC factors, namely the heterodimer Y14/MAGOH and Barentz (BTZ, MLN51 or CACSC3) (72,73) (Figure 3A). The crystal structure analysis of this complex revealed that eIF4AIII binds RNA in an ATP-dependent fashion and recruits the other EJC core factors (72,73). The heterodimer Y14/MAGOH stabilizes the high affinity of eIF4AIII for RNA by preventing the ATP hydrolysis of eIF4AIII and further primes it to bind to Barentz (74,75). This arrangement allows eIF4AIII to clamp several proteins onto RNA in a stable and sequence-independent manner (76), which in turn is used by the NMD machinery to recognize an aberrant PTC (Figure 3A). Thereby, a short motif of the core NMD factor UPF3b binds to the EJC core factors and serves as a platform to assemble an active NMD complex (52,77). Two other DEAD box proteins DDX5 and DDX17 have also recently been described to interact with UPF3b and this interaction seems crucial for the degradation of a limited subset of NMD substrates (78).

Additional RNA helicases also function as auxiliary factors in the NMD pathway. Through a process involving their ATPase activities, the RNA helicases RUVBL1 and RUVBL2 associate with the SURF complex and promote the transition to the DECID complex (Figure 3B, upper panel) (79). Given their tight association to the SMG1 kinase, it can be speculated that RUVBL1, 2 contribute to the regulation of the SMG1–8–9 complex by promoting the activation of the large SMG1 kinase molecule. It was recently shown that the RNA helicase DHX34, a member of the DExH/D box family of proteins, associates with the SURF complex and promotes the transition to the DECID complex (80). Whereas members of this family have been originally described as RNA helicases that unwind RNA, it has also been found that other family members function as RNPases that remodel RNA-protein interactions (81,82). Thereby, DHX34 probably affects the remodeling of the SURF complex by promoting the dissociation of the eukaryotic release factors eRF1 and eRF3 in an ATP-dependent manner from the SURF complex at the PTC (80) (Figure 3B, lower panel). It was recently shown that another

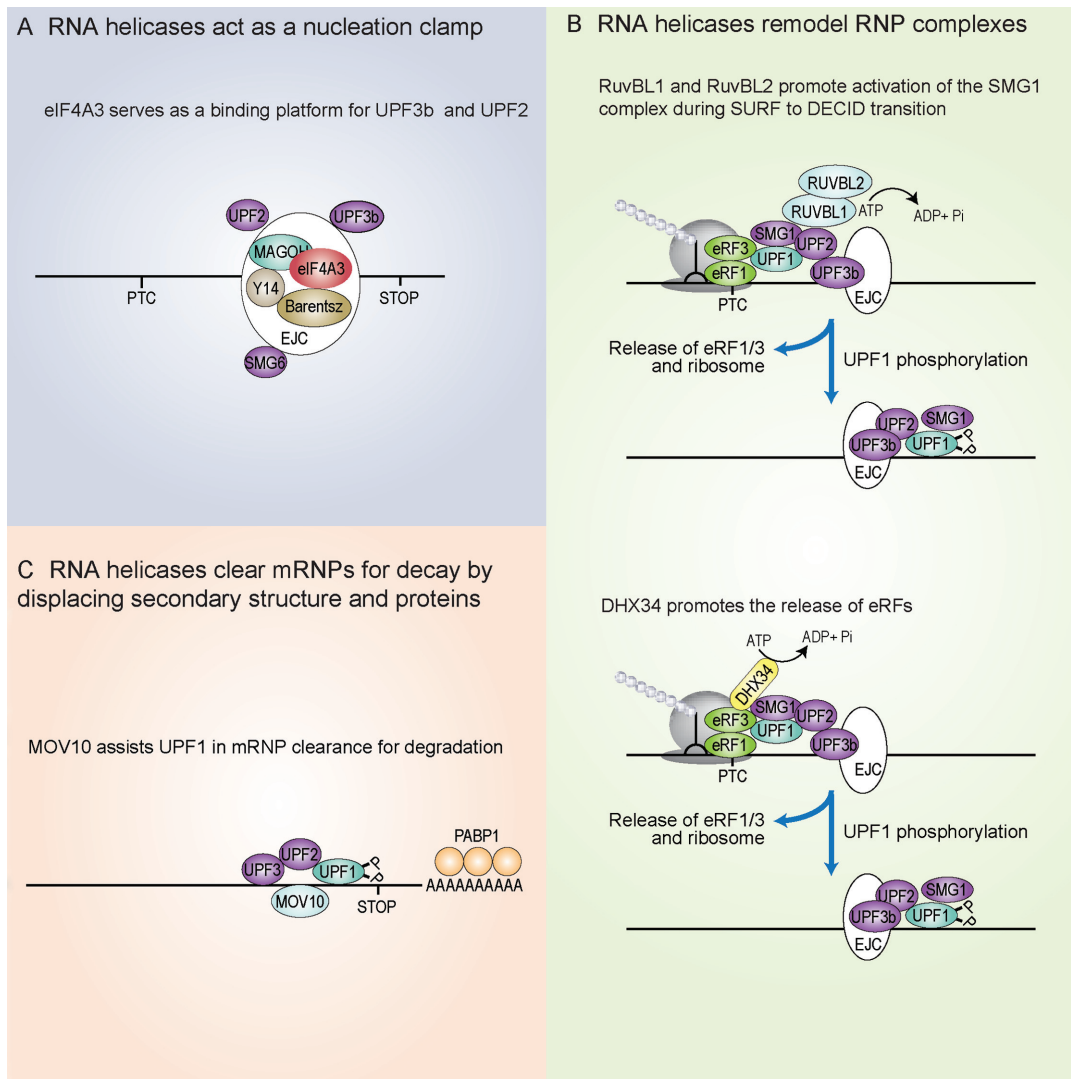


Figure 3. Role of RNA helicases in NMD. (A) The exon junction complex (EJC) component, eIF4AIII, nucleates NMD factors, promoting interactions with UPF2 and UPF3b. (B) RuvBL proteins promote activation of the SMG1 kinase during initial stages of NMD (upper panel). DHX34 promotes the transition from the SURF to DECID complex (lower panel). (C) Function of the RNA helicases UPF1 and MOV10 in mRNA clearance.

UPF1-like RNA helicase, MOV10, preferentially binds to 3' UTR regions, as is the case with UPF1 (83,84), and contributes to degradation of UPF1-regulated transcripts (85,86) (Figure 3C). Whether MOV10 helicase acts in a redundant way with the UPF1 helicase as an RNA clearance factor to resolve secondary structures and displace proteins or if the two proteins perform distinct actions remains to be clarified.

Non-EJC dependent models of NMD

Although it has been clearly established that the presence of an EJC downstream of a PTC promotes NMD in mammalian cells, there is also increasing evidence of an active NMD response in its absence. Therefore, NMD activation can rely on both EJC-dependent and EJC-independent pathways (87). Alternative models for NMD activation that do not require the presence of an EJC have been described in other organisms, such as yeast and nematodes, but could

also operate in human cells (88). In particular, mRNAs harboring long 3' UTRs have been shown to be sensitive to NMD, irrespective of the presence of an EJC (83). A central issue in NMD concerns the mechanism by which UPF1 is recruited to an NMD target. Recent studies showed that UPF1 binds target RNAs before mRNA translation and subsequently translating ribosomes displace it from coding sequences leading to the accumulation of UPF1 at 3' UTRs (86,89). This observation challenges the assumption that UPF1 recruitment marks mRNA for degradation. Moreover, UPF1 binding is not enriched on endogenous transcripts that are upregulated in the absence of UPF1 (89,90). A recent study proposed that binding of phosphorylated UPF1 (P-UPF1) marks mRNAs for degradation. It was shown that P-UPF1 is enriched on endogenous transcripts degraded by NMD and predominantly unphosphorylated UPF1 is released from non-targeted transcripts in an ATP-dependent manner (91). However, P-UPF1 binding is not exclusive to NMD targets, and it is also not apparent what

makes UPF1 'stick' preferentially to NMD targets and get phosphorylated. Further clues about how UPF1 discriminates NMD targets come from a recently published RIP-seq data of wild-type or ATPase-deficient UPF1 cells, which showed that UPF1 release from non-target mRNAs rather than UPF1 binding itself was more important for NMD target selection. Faster dissociation of UPF1 from non-target mRNAs requires correct ATPase activity of UPF1 and its dissociation is enhanced by translation and PABC1 binding, also in an ATPase-dependent manner (92). Prematurely terminated translation associated with a PTC leads to mRNA degradation and it has been proposed that this is due to a competition between UPF1 and the poly (A) binding protein, PABPC1, for binding to the translation release factor, eRF3 (93). As such, the distance from the PTC to the poly (A) tail is a determining factor on whether UPF1 will bind near the PTC (30,71,94,95).

How are NMD targets recognized?

Even though our understanding of the NMD process is rapidly increasing, the important question of how NMD targets are selected in a global scale still remains unresolved. So what rules govern NMD target selection? Although both EJC-dependent or independent models for target recognition can be applicable to a selected number of targets, the situation is less clear when analyzing high-throughput NMD targets. For instance, profiling of transcripts regulated by UPF1 revealed that many transcripts that are up-regulated in the absence of UPF1 lack recognizable NMD features, whereas many transcripts that would be predicted to be NMD targets are unaffected by UPF1 depletion (96–98).

The emerging picture suggests that likely no single NMD feature will be globally sufficient to elicit NMD. Instead, a combination of NMD-targeting and NMD-antagonizing features contributes to determine NMD susceptibility of any given mRNA. For some mRNAs, a canonical model will apply, where a PTC situated 50–55 nucleotides upstream of an EJC elicits robust mRNA downregulation (99). This is certainly the case for 'faulty' PTC-containing transcripts for which NMD was originally described (see Figure 1). In cells, these transcripts are usually rare, and only produced as a result of genomic mutation or incorrect RNA processing, and are often associated with diseases. They are very efficiently degraded by NMD, thus preventing the production of truncated proteins. For other mRNAs, a distinct combination of NMD features will determine if, and how efficiently, transcripts are degraded. This mode of target determination is likely used for a vast majority of endogenous NMD targets, where NMD is an essential tool for the fine-tuning of gene expression.

Additional NMD trans-acting factors and their role in the regulation of NMD

A variety of strategies have been used in an attempt to uncover novel factors with a role in the NMD response, including forward and reverse genetic screens and interactome studies (Figure 4). A limitation of forward genetic screens lays in their inability to identify genes that are essential for

viability. Additionally, the search of novel NMD factors using mutagenesis in *C. elegans* was somewhat undermined by the fact that almost 90% of *smg* mutations identified were alleles of *smg-1*, *smg-2* or *smg-5* (35,36,100). This limitation was overcome in reverse genetic screens using RNAi that allows the identification of NMD factors independently of their abundance or whether they are essential for cell survival.

In this context, the Interactome-Mass spectrometry approach emerges as a promising avenue for the identification of abundant proteins that form part of NMD complexes. A study using a yeast two-hybrid approach with UPF1 as bait identified human proline-rich nuclear receptor coregulatory protein 2 (PNRC2), as an interactor. This factor was also found to interact with the decapping activator, DCP1a, which led to a model whereby phosphorylated UPF1 acts as a platform to recruit PNRC2, providing a link with mRNA degradation (101,102). A comprehensive search using stable isotope labeling by amino acids in cell culture (SILAC) was designed to distinguish between proteins binding to either the hypo- or hyper-phosphorylated form of UPF1. This resulted in the identification of several RNA-binding proteins that preferentially associate with hyper-phosphorylated UPF1 in the nucleus. It also confirmed the eukaryotic translation initiation factor 3 (eIF3) as a UPF1 interactor (103). An interactome search for the RNA helicase MOV10, which belongs to the UPF1-like group of the helicase superfamily 1 (SF1)(104), identified UPF1 as the major interactor (86). Finally, use of SMG1 as bait identified RuVB-like 1 (RUVBL1) and RuVB-like 2 (RUVBL2) proteins, two adenosine triphosphatases that are part of (AAA+) family of proteins (Figures 3 and 4). These proteins have been reported to be involved in several cellular functions, such as transcription, DNA repair, telomere maintenance and RNA modification, and also shown to act in the initial stages of the NMD pathway. Other SMG1 interactors identified in the same experiment were RPB5, a subunit of RNA polymerases I, II and III (105), as well as the homolog of a nematode NMD factor, termed SMG10, both of which also form part of the RUVBL1/2 complex (79).

Use of a GFP-reporter-based RNAi screen in *C. elegans* resulted in the identification of two novel NMD factors, termed *smgl-1* and *smgl-2*, that unlike *smg* genes are essential for viability, strongly suggesting that these two genes have roles in NMD but also fulfill other essential functions in nematodes (33) (Figure 4). Both genes are conserved throughout evolution with clear orthologues in mouse, human and fugu. The *C. elegans* gene *smgl-1* corresponds to human NBAS (Neuroblastoma amplified sequence), also known as NAG (for Neuroblastoma amplified gene). The *C. elegans smgl-2* gene corresponds to human DHX34 (DEAH box protein 34), a DExH/D box protein (Figure 3B, lower panel). Both NBAS and DHX34 are *bona fide* NMD factors both in human cells, as well as in zebrafish (33,106). Identification of target genes regulated by DHX34 and NBAS, in human, zebrafish and also in *C. elegans* revealed a large co-regulation of targets between DHX34, NBAS and the core NMD factor UPF1 in all species (11). The *NBAS* gene was initially identified as a gene amplified in human neuroblastomas (107,108), and later found to encode

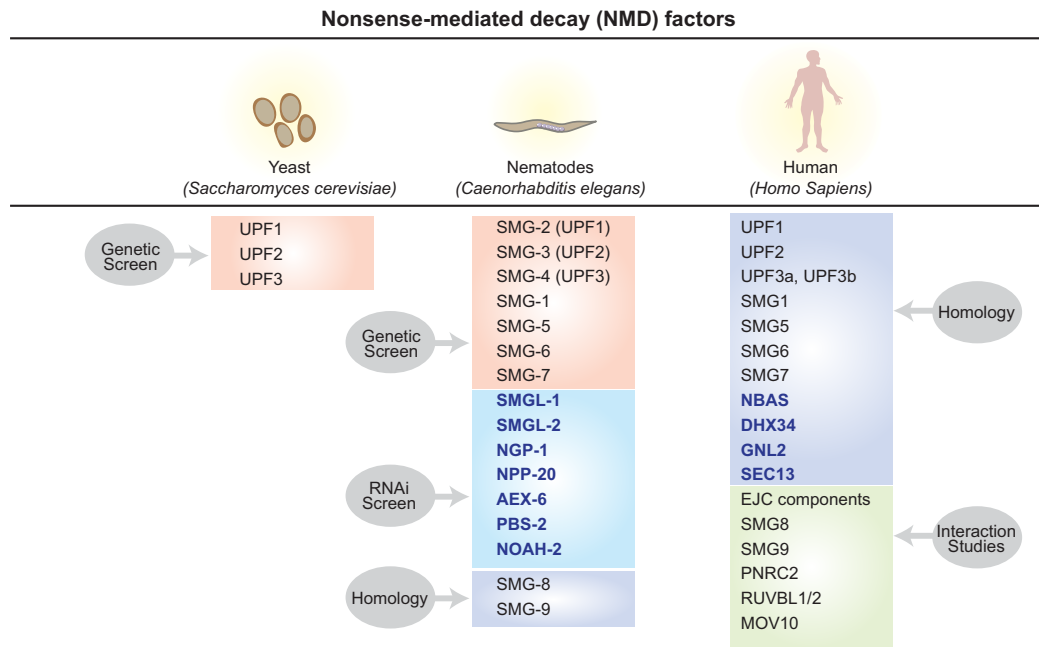


Figure 4. NMD factors in yeast, nematodes and humans, indicating the method by which they were first identified.

a peripheral membrane protein that is a component of the Syntaxin 18 complex, with a role in Golgi-to-endoplasmic reticulum retrograde transport (109). Interestingly, mutations in *NBAS* have been found in several human conditions, including a hereditary short stature syndrome in the Siberian Yakut population, characterized by optic nerve atrophy and Pelger–Huët anomaly of granulocytes (SOPH syndrome)(110), as well as in patients with a multisystem disease involving liver, eye, immune system, connective tissue and bone (111). Another recent report identified compound heterozygous *NBAS* mutations with recurrent acute liver failure in infancy in a group of patients of European descent (112). It remains to be seen whether the phenotypes of *NBAS* mutants are due to compromised NMD response and/or defects in retrograde transport between the ER and Golgi. Currently it is unclear, how *NBAS* contributes to NMD in mechanistic terms; however, we have recently found that *NBAS* interacts with the core NMD factor UPF1 (Longman and Caceres, unpublished data). Combined with a reported role in ER-Golgi vesicular trafficking (109), we speculate that *NBAS* might represent a connecting link between NMD and transcripts entering the secretory pathway. Interestingly, profiling of transcripts regulated by *NBAS* showed significant enrichment for genes involved in the cellular stress response (11).

The original NMD RNAi screen in *C. elegans* was revisited; using a novel RNAi library that included many previously untested genes (113,114). This new screen resulted in the identification of five novel nematode NMD genes that are highly conserved throughout evolution and are required for NMD in nematodes (Figure 4) (115). Two of their human homologs, *GNL2* (*ngp-1*) and *SEC13* (*npp-20*), were also found to act in the NMD pathway in human cells (115). Moreover, expression profiling showed that

a significant proportion of transcripts that are regulated by *GNL2* and *SEC13* were also largely upregulated when canonical NMD factors UPF1 and UPF2 were depleted (Casadio, Longman and Caceres, unpublished). The *GNL2* gene encodes a putative GTPase, whose yeast homolog, *Nog2p*, is involved in the processing of the pre-60S ribosomal particles (116), whereas human *SEC13* is a constituent of the endoplasmic reticulum and the nuclear pore complex (NPC) (117). Whether these previously described roles of *GNL2* and *SEC13* are related to their role in NMD will require additional investigation. This screen also identified the *C. elegans* gene, *noah-2*, which is present in *Drosophila melanogaster* (*nompA*) but absent in humans, and has a role as an NMD factor in fruitflies (115). Interestingly, the expression of *nompA* is restricted to the type I sensory organs of the peripheral nervous system (PNS), suggesting the possibility that NMD can act in a tissue-specific manner.

Physiological role of the NMD response

The NMD response has been shown to be variable among different cell-types and tissues (118,119), and even among individuals where this can be correlated with clinical presentations of human diseases caused by the presence of a PTC (13). What are the mechanisms that lead to a variable NMD response? One such mechanism could be the variation in the relative abundance of RNPS1, a peripheral component of the EJC, observed in different HeLa cells subtypes that correlates well with the magnitude of the NMD response (120). The relatively recent realization that NMD acts to regulate endogenous gene expression of mRNAs lacking a PTC strongly suggested that the NMD response itself has to be precisely regulated in order to avoid undesirable alterations to the gene expression program of cells and tissues. One way to regulate the NMD activity is via a negative

feedback regulatory network whereby a large proportion of core NMD factors are negatively regulated by NMD. This feedback loop was initially observed in mammalian cells, and was later also found in nematodes, zebrafish and plants (118).

The NMD pathway is not essential in nematodes where mutations in the core NMD factors (*smg1-7*) lead to discrete phenotypes (35). A similar scenario is found in yeast, where the loss of the Upf proteins has no obvious effect on growth (37). By contrast, targeted disruption of mammalian core NMD factors results in embryonic lethality in the mouse, as observed for *Upf1* (121), *Upf2* (98) or *Smg6* (122). Along the same lines, *Smg1* is required for embryogenesis and was shown to regulate target genes via alternative splicing coupled to NMD (123). The phenotypes observed with inactivation of mouse core NMD genes could be interpreted as indicating an essential role for the NMD pathway in mammals. An alternative explanation is related to the existence of additional cellular functions for UPF proteins (124). These include a reported role for UPF1 in genome stability in human cells (125), as well as its role in the SMD pathway (Staufen-mediated decay), whereby UPF1 is recruited to the 3' UTRs of mRNAs bound by the RNA-binding protein STAUFEN 1 and induces mRNA degradation (126,127). UPF1 has also been shown to be involved in regulating the degradation of histone mRNAs, where it is recruited by the stem-loop binding protein (128). The *Smg1* gene has also an additional role in genotoxic stress (129). As in the mouse system, *Drosophila* *Upf1* and *Upf2* are also required for animal development and viability (130). By contrast, *Drosophila* *Upf3* plays a peripheral role in the degradation of most NMD targets and is not required for development or viability (131). The role of the NMD response and/or of individual NMD factors during development and also during neuronal development has recently been reviewed (93,119). Below, we will focus on a few examples on how the NMD pathway impacts on several physiological processes, including the stress response, the immune response and on viral replication.

Stress response

In response to stress, cells initiate a complex cascade of events leading to dynamic changes in gene expression that are designed to alleviate stress and restore homeostasis, or trigger apoptosis. It was observed that NMD is repressed by a variety of stress conditions, such as hypoxia, nutrient deprivation or infection (132,133). This inhibition is, at least partially, mediated by phosphorylation of the translation initiation factor eIF2 α , which leads to inhibition of mRNA translation and is a common step for the initiation of many stress pathways (134). This results in many transcripts that are normally degraded by NMD being upregulated, including those encoding factors that are required during stress response, helping to establish a more robust stress response and increasing cell survival (135). NMD also modulates the unfolded protein response (UPR), which is triggered by endoplasmic reticulum (ER) stress. A chronic activation of the UPR contributes to the pathogenesis of a wide variety of human disorders; thus, UPR activation must be tightly regulated. It was shown that NMD directly tar-

gets mRNAs encoding several essential UPR components, including many UPR sensors (136–138). In this way, NMD controls the threshold of UPR activation, and prevents the inappropriate response to harmless levels of stress (136). Additionally, NMD inhibition itself, can also induce UPR (137,138). It is possible that inhibition of NMD overloads the ER with truncated misfolded proteins that themselves could generate sufficient stress signal. However, the extent of this NMD-mediated effect on UPR activation remains to be determined. Moreover, NMD inhibition leads to the activation of autophagy that decreases accumulation of detrimental proteins in the cell. Autophagy activation is partially due to the stabilization of the NMD target ATF4 mRNA (139). Conversely, hyperactivation of the NMD response blocks the induction of autophagy in response to cellular stresses. In summary, under normal conditions, NMD activity is required for protecting cells from inappropriate activation of UPR by innocuous stimuli. In response to stress, NMD inhibition augments UPR response by increased expression of transcripts that encode UPR sensors such as IRE1 α . NMD re-activation then helps terminate the stress response and restore homeostasis. Therefore, NMD helps to fine-tune the threshold of UPR response, and the activity of NMD is in turn regulated by the UPR.

Immune response

A robust immune response is needed for preventing or limiting infection; however, if left uncontrolled it can lead to pathologies or death. It is therefore crucial that organisms are able to maintain immune homeostasis by suppressing or switching off the immune response. It was recently proposed that NMD plays an active role in the regulation of the immune response. Cytokines are signaling molecules (e.g. IL-6 or TNF) that modulate the inflammatory immune response. In response to infection, cytokines are rapidly upregulated and initiate an immune response cascade via binding to their receptors at the surface of the cells. It was recently shown that UPF1, together with the RNA-binding protein, Regnase-1, regulates the early (acute) phase of inflammation response by degrading cytokine mRNAs. Regnase-1 binds to a stem-loop in 3' UTRs of translationally active cytokine mRNAs and acts together with UPF1 to downregulate these targets, whereas the role of another RNA-binding protein Roquin, that controls the late (chronic) phase of inflammation, is independent of UPF1 (140). Although it is not entirely clear that the action of Regnase-1 together with UPF1 represents NMD per se, the helicase activity of UPF1 is required. Another way that NMD could affect the inflammation response is via controlling the stability of the cytokine receptor mRNAs. One recently documented example is the complex regulation of the human CCR5 cytokine receptor mRNA stability (141). CCR5 mRNA harbors a programmed -1 ribosomal frameshift (-1 PRF) signal directed by an mRNA pseudoknot that is formed by mRNA-miRNA interactions. This -1 PRF directs the translating ribosome to a PTC, leading to mRNA downregulation.

A better understanding of NMD modulation during immune response can be useful for the development of new therapies for autoimmune diseases or cancer. For example, upregulation of NMD during chronic inflammation can

help to restore immune homeostasis. On the other hand, blocking NMD in cancer may result in the synthesis of tumor-specific proteins that can increase natural immune response directed against the tumor (142). This could indeed be a feasible approach, as it was recently demonstrated that the increase of intracellular calcium by commonly used cardiac glycosides inhibits NMD (143). A role for NMD in the control of the immune response is also seen in plants, where this pathway contributes to innate immunity in *Arabidopsis* (144–146). NMD acts to downregulate numerous TIR domain-containing, nucleotide-binding, leucine-rich repeat (TNL) immune receptor-encoding mRNAs. Bacterial infection of plants leads to host-programmed inhibition of NMD, resulting in the upregulation of those naturally NMD-regulated TNL transcripts. By contrast, constitutive NMD activity prevents accumulation of TNL receptors and impairs plant defense. Thus, a mechanism of host-regulated NMD contributes to disease resistance by controlling the threshold for activation of TNL resistance pathways (145).

Viral replication

Apart from its role in immune response regulation, NMD also serves as a natural barrier to virus replication. A genome-wide RNAi screen in HeLa cells looking for host factors that restrict virus replication identified several components of the NMD pathway (147). Downregulation of UPF1, SMG5 and SMG7 led to an increase in the level of viral proteins and higher viral infection. Viruses have also evolved mechanisms to evade NMD-mediated degradation. For example, Rous sarcoma virus harbors a stability element in its 3' UTR (RSE, for Rous sarcoma virus stability element) that protects the viral RNA from NMD. It was hypothesized that this viral stability element may prevent UPF1 function (148,149). The HTLV-1 virus Tax and Rex proteins also inhibit NMD (150,151); however, their mode of action is not entirely clear. It has been shown that the Tax protein interacts with UPF1 and with a component of eIF3, INT6 that also has a proposed role in NMD. Tax increases the accumulation of phosphorylated UPF1 in P-bodies and this leads to enhanced stability of HTLV-1 mRNAs (150). Similarly, plants also employ NMD to restrict viral replication by destabilizing viral transcripts containing internal stop codons or long 3' UTRs. As expected, plant viruses also evolved mechanisms that either evade NMD, or modify host endogenous NMD activity (152). Therefore, the host NMD response that reduces viral infection and is in turn counteracted by viruses modulating NMD seems to be an evolutionarily conserved relationship.

NMD inhibitors and therapeutic possibilities

NMD activity can be modulated by many cellular perturbations. Treatment of human cells with chemotherapeutic compounds results in a pronounced decline in NMD activity. This is partly due to the proteolytic production of a dominant-negative form of the key NMD factor UPF1 and results in the upregulation of genes involved in the response to apoptotic stresses, leading to cell death (153). Furthermore, caspases cleave both UPF1 and UPF2 during apoptosis, impairing the NMD response (154). In an interesting

link with a human disease, it has been recently reported that increased expression of the double homeobox transcription factor DUX4, which is observed in patients with the muscular dystrophy, Facioscapulohumeral muscular dystrophy (FSHD), triggers proteolytic degradation of UPF1, leading to pronounced NMD inhibition. DUX4 mRNA is itself an NMD target, thus, inhibition of NMD by DUX4 protein stabilizes DUX4 mRNA through a double-negative feedback loop in FSHD muscle cells (155).

The development of inhibitors of NMD could be important to gain mechanistic insights into NMD function but could also prove to be important for therapeutic purposes. The search for NMD inhibitors is currently approached by means of several strategies. A screen in HeLa cells stably expressing an NMD reporter that used a library of clinically licensed compounds identified 5-azacytidine, an analog of the naturally occurring nucleoside cytidine, which has been previously approved for the treatment of myelodysplastic syndrome and myeloid leukemia (156). This inhibitory effect of 5-azacytidine on NMD, depends on the induction of MYC expression, which was previously shown to inhibit NMD activity (157). A small molecule inhibitor of NMD, termed NNMDI 1, was shown to stabilize hyperphosphorylated isoforms of UPF1 and to compromise its interaction with SMG5 (158). Compounds that disrupt the SMG7-UPF1 complex and inhibit NMD have also been recently identified. These compounds when combined with a PTC 'read-through' drug led to restoration of full-length p53 protein in cells harboring a PTC-mutated p53 (159). Pateamine A (PatA), a natural product first isolated from marine sponges, was also shown to inhibit NMD through a direct interaction with the EJC component, eIF4AIII. Importantly, this PatA-mediated inhibition of NMD is independent of the previously reported role of this compound in inhibition of translation initiation (160). The dietary compound curcumin has also been shown to inhibit the NMD pathway by downregulating the expression of core NMD factors at the transcriptional level (161). Finally, pyrimidine derivatives have been identified as hSMG-1 kinase inhibitors (162). In many cases, expression of a PTC-containing mRNA can be advantageous for the cell, when the production of a truncated protein is less harmful than elimination of its encoding mRNA by NMD. This is particularly important in the case of certain human diseases caused by mutations that introduce PTCs. A number of drugs have been identified that induce suppression of translation termination at PTCs in mammalian cells (163,164). This PTC suppression therapy, which is currently in clinical trials for treatment of several genetic diseases caused by PTCs, can be combined with strategies to inhibit NMD. One such strategy of NMD inhibition combined with PTC read-through was recently reported (165). Here, antisense oligonucleotides (ASOs) designed to block assembly of an EJC downstream of PTCs and inhibit NMD in a gene-specific fashion were combined with read-through compounds. This approach restored expression of full length protein from a nonsense-mutant allele (165). Thus, these complementary strategies could have an important role in alleviating the phenotypic consequences of a wide range of genetic diseases caused by the presence of a PTC.

CONCLUSION

In the last few years, there has been a significant increase in our understanding on how the NMD mechanism operates, how the different sub-complexes are assembled and the role of some of the NMD trans-acting factors. The realization of a more general role for NMD in regulating gene expression raised some new questions, such as how is the NMD response buffered, how are non-PTC-containing endogenous transcripts targeted by the NMD machinery and whether there are more trans-acting factors yet to be identified. A full understanding of the role of the NMD response in the physiology of cells represents both an interesting mechanistic challenge but also an opportunity for future therapeutic interventions.

ACKNOWLEDGEMENTS

We thank Claudio Alonso (University of Sussex) for comments and critical reading of the manuscript and Craig Nicol (MRC HGU) for drawing the figure illustrations.

FUNDING

Unit program grant from the UK Medical Research Council (MRC) and a grant from the Wellcome Trust [09518/Z/11/Z]. Funding for open access charge: Wellcome Trust [09518/Z/11/Z].

Conflict of interest statement. None declared.

REFERENCES

- Isken, O. and Maquat, L.E. (2007) Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. *Genes Dev.*, **21**, 1833–1856.
- Kervestin, S. and Jacobson, A. (2012) NMD: a multifaceted response to premature translational termination. *Nat. Rev. Mol. Cell Biol.*, **13**, 700–712.
- Schweingruber, C., Rufener, S.C., Zünd, D., Yamashita, A. and Mühlemann, O. (2013) Nonsense-mediated mRNA decay - mechanisms of substrate mRNA recognition and degradation in mammalian cells. *Biochim. Biophys. Acta*, **1829**, 612–623.
- Bhuvanagiri, M., Schlitter, A.M., Hentze, M.W. and Kulozik, A.E. (2010) NMD: RNA biology meets human genetic medicine. *Biochem. J.*, **430**, 365–377.
- Miller, J.N. and Pearce, D.A. (2014) Nonsense-mediated decay in genetic disease: friend or foe? *Mutat. Res. Rev. Mutat. Res.*, **762**, 52–64.
- Alonso, C.R. (2005) Nonsense-mediated RNA decay: a molecular system micromanaging individual gene activities and suppressing genomic noise. *Bioessays*, **27**, 463–466.
- Chang, Y.-F., Imam, J.S. and Wilkinson, M.F. (2007) The nonsense-mediated decay RNA surveillance pathway. *Annu. Rev. Biochem.*, **76**, 51–74.
- Palacios, I.M. (2013) Nonsense-mediated mRNA decay: from mechanistic insights to impacts on human health. *Brief. Funct. Genomics*, **12**, 25–36.
- Huang, L., Lou, C.-H., Chan, W., Shum, E.Y., Shao, A., Stone, E., Karam, R., Song, H.-W. and Wilkinson, M.F. (2011) RNA homeostasis governed by cell type-specific and branched feedback loops acting on NMD. *Mol. Cell*, **43**, 950–961.
- Yepiskoposyan, H., Aeschimann, F., Nilsson, D., Okoniewski, M. and Mühlemann, O. (2011) Autoregulation of the nonsense-mediated mRNA decay pathway in human cells. *RNA*, **17**, 2108–2118.
- Longman, D., Hug, N., Keith, M., Anastasaki, C., Patton, E.E., Grimes, G. and Cáceres, J.F. (2013) DHX34 and NBAS form part of an autoregulatory NMD circuit that regulates endogenous RNA targets in human cells, zebrafish and *Caenorhabditis elegans*. *Nucleic Acids Res.*, **41**, 8319–8331.
- Degtjar, E., Fridman, A., Gottlieb, D., Vexler, K., Berezin, I., Farhi, R., Golani, L. and Shaul, O. (2015) The feedback control of UPF3 is crucial for RNA surveillance in plants. *Nucleic Acids Res.*, **43**, 4219–4235.
- Nguyen, L.S., Wilkinson, M.F. and Geetz, J. (2014) Nonsense-mediated mRNA decay: inter-individual variability and human disease. *Neurosci. Biobehav. Rev.*, **46 Pt 2**, 175–186.
- Hurt, J.A., Robertson, A.D. and Burge, C.B. (2013) Global analyses of UPF1 binding and function reveal expanded scope of nonsense-mediated mRNA decay. *Genome Res.*, **23**, 1636–1650.
- Kurihara, Y., Matsui, A., Hanada, K., Kawashima, M., Ishida, J., Morosawa, T., Tanaka, M., Kaminuma, E., Mochizuki, Y., Matsushima, A. et al. (2009) Genome-wide suppression of aberrant mRNA-like noncoding RNAs by NMD in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.*, **106**, 2453–2458.
- Smith, J.E., Alvarez-Dominguez, J.R., Kline, N., Huynh, N.J., Geisler, S., Hu, W., Collier, J. and Baker, K.E. (2014) Translation of small open reading frames within unannotated RNA transcripts in *Saccharomyces cerevisiae*. *Cell Rep.*, **7**, 1858–1866.
- Smith, J.E. and Baker, K.E. (2015) Nonsense-mediated RNA decay—a switch and dial for regulating gene expression. *Bioessays*, **37**, 612–623.
- Nagy, E. and Maquat, L.E. (1998) A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends Biochem. Sci.*, **23**, 198–199.
- Thermann, R., Neu-Yilik, G., Deters, A., Frede, U., Wehr, K., Hagemeyer, C., Hentze, M.W. and Kulozik, A.E. (1998) Binary specification of nonsense codons by splicing and cytoplasmic translation. *EMBO J.*, **17**, 3484–3494.
- Zhang, J., Sun, X., Qian, Y., LaDuca, J.P. and Maquat, L.E. (1998) At least one intron is required for the nonsense-mediated decay of triosephosphate isomerase mRNA: a possible link between nuclear splicing and cytoplasmic translation. *Mol. Cell. Biol.*, **18**, 5272–5283.
- Zhang, J., Sun, X., Qian, Y. and Maquat, L.E. (1998) Intron function in the nonsense-mediated decay of beta-globin mRNA: indications that pre-mRNA splicing in the nucleus can influence mRNA translation in the cytoplasm. *RNA*, **4**, 801–815.
- 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.
- Lykke-Andersen, J., Shu, M.D. and Steitz, J.A. (2001) Communication of the position of exon-exon junctions to the mRNA surveillance machinery by the protein RNPS1. *Science*, **293**, 1836–1839.
- Gehring, N.H., Lamprinaki, S., Kulozik, A.E. and Hentze, M.W. (2009) Disassembly of exon junction complexes by PYM. *Cell*, **137**, 536–548.
- Le Hir, H. and Séraphin, B. (2008) EJC at the heart of translational control. *Cell*, **133**, 213–216.
- Bono, F. and Gehring, N.H. (2011) Assembly, disassembly and recycling: the dynamics of exon junction complexes. *RNA Biol.*, **8**, 24–29.
- Singh, G., Kucukural, A., Cenik, C., Leszyk, J.D., Shaffer, S.A., Weng, Z. and Moore, M.J. (2012) The cellular EJC interactome reveals higher-order mRNP structure and an EJC-SR protein nexus. *Cell*, **151**, 750–764.
- Saulière, J., Murigneux, V., Wang, Z., Marquet, E., Barbosa, I., Le Tonquèze, O., Audic, Y., Paillard, L., Roest Crolius, H. and Le Hir, H. (2012) CLIP-seq of eIF4AIII reveals transcriptome-wide mapping of the human exon junction complex. *Nat. Struct. Mol. Biol.*, **19**, 1124–1131.
- Le Hir, H., Saulière, J. and Wang, Z. (2016) The exon junction complex as a node of post-transcriptional networks. *Nat. Rev. Mol. Cell Biol.*, **17**, 41–54.
- 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.
- 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.

32. Wen, J. and Brogna, S. (2010) Splicing-dependent NMD does not require the EJC in *Schizosaccharomyces pombe*. *EMBO J.*, **29**, 1537–1551.
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. Longman, D., Plasterk, R.H.A., Johnstone, I.L. and Cáceres, J.F. (2007) Mechanistic insights and identification of two novel factors in the *C. elegans* NMD pathway. *Genes Dev.*, **21**, 1075–1085.
35. 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.
36. Pulak, R. and Anderson, P. (1993) mRNA surveillance by the *Caenorhabditis elegans* smg genes. *Genes Dev.*, **7**, 1885–1897.
37. 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.
38. 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.
39. Mühlemann, O., Eberle, A.B., Stalder, L. and Zamudio Orozco, R. (2008) Recognition and elimination of nonsense mRNA. *Biochim. Biophys. Acta*, **1779**, 538–549.
40. Cordin, O., Banroques, J., Tanner, N.K. and Linder, P. (2006) The DEAD-box protein family of RNA helicases. *Gene*, **367**, 17–37.
41. Rajkowsch, L., Chen, D., Stampfl, S., Semrad, K., Waldsich, C., Mayer, O., Jantsch, M.F., Konrat, R., Bläsi, U. and Schroeder, R. (2007) RNA chaperones, RNA annealers and RNA helicases. *RNA Biol.*, **4**, 118–130.
42. Denning, G., Jamieson, L., Maquat, L.E., Thompson, E.A. and Fields, A.P. (2001) Cloning of a novel phosphatidylinositol kinase-related kinase: characterization of the human SMG-1 RNA surveillance protein. *J. Biol. Chem.*, **276**, 22709–22714.
43. Grimson, A., O'Connor, S., Newman, C.L. and Anderson, P. (2004) SMG-1 is a phosphatidylinositol kinase-related protein kinase required for nonsense-mediated mRNA Decay in *Caenorhabditis elegans*. *Mol. Cell Biol.*, **24**, 7483–7490.
44. 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.
45. 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.
46. Fernández, I.S., Yamashita, A., Arias-Palomo, E., Bamba, Y., Bartolomé, R.A., Canales, M.A., Teixidó, J., Ohno, S. and Llorca, O. (2011) Characterization of SMG-9, an essential component of the nonsense-mediated mRNA decay SMG1C complex. *Nucleic Acids Res.*, **39**, 347–358.
47. Arias-Palomo, E., Yamashita, A., Fernández, I.S., Núñez-Ramírez, R., Bamba, Y., Izumi, N., Ohno, S. and Llorca, O. (2011) The nonsense-mediated mRNA decay SMG-1 kinase is regulated by large-scale conformational changes controlled by SMG-8. *Genes Dev.*, **25**, 153–164.
48. 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.
49. Melero, R., Uchiyama, A., Castaño, R., Kataoka, N., Kurosawa, H., Ohno, S., Yamashita, A. and Llorca, O. (2014) Structures of SMG1-UPFs complexes: SMG1 contributes to regulate UPF2-dependent activation of UPF1 in NMD. *Structure*, **22**, 1105–1119.
50. Deniaud, A., Karuppusamy, M., Bock, T., Masiulis, S., Huard, K., Garzoni, F., Kerschgens, K., Hentze, M.W., Kulozik, A.E., Beck, M. et al. (2015) A network of SMG-8, SMG-9 and SMG-1 C-terminal insertion domain regulates UPF1 substrate recruitment and phosphorylation. *Nucleic Acids Res.*, **43**, 7600–7611.
51. Chakrabarti, S., Jayachandran, U., Bonneau, F., Fiorini, F., Basquin, C., Domcke, S., Le Hir, H. and Conti, E. (2011) Molecular mechanisms for the RNA-dependent ATPase activity of Upf1 and its regulation by Upf2. *Mol. Cell*, **41**, 693–703.
52. 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.
53. Fiorini, F., Boudvillain, M. and Le Hir, H. (2013) Tight intramolecular regulation of the human Upf1 helicase by its N- and C-terminal domains. *Nucleic Acids Res.*, **41**, 2404–2415.
54. Clerici, M., Mourão, A., Gutsche, I., Gehring, N.H., Hentze, M.W., Kulozik, A., Kadlec, J., Sattler, M. and Cusack, S. (2009) Unusual bipartite mode of interaction between the nonsense-mediated decay factors, UPF1 and UPF2. *EMBO J.*, **28**, 2293–22306.
55. Franks, T.M., Singh, G. and Lykke-Andersen, J. (2010) Upf1 ATPase-dependent mRNP disassembly is required for completion of nonsense-mediated mRNA decay. *Cell*, **143**, 938–950.
56. Fiorini, F., Bagchi, D., Le Hir, H. and Croquette, V. (2015) Human Upf1 is a highly processive RNA helicase and translocase with RNP remodelling activities. *Nat. Commun.*, **6**, 7581. DOI: 10.1038/ncomms8581.
57. Melero, R., Buchwald, G., Castaño, R., Raabe, M., Gil, D., Lázaro, M., Urlaub, H., Conti, E. and Llorca, O. (2012) The cryo-EM structure of the UPF-EJC complex shows UPF1 poised toward the RNA 3' end. *Nat. Struct. Mol. Biol.*, **19**, 498–505.
58. Schoenberg, D.R. and Maquat, L.E. (2012) Regulation of cytoplasmic mRNA decay. *Nat. Rev. Genet.*, **13**, 246–259.
59. 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.
60. Okada-Katsuhata, Y., Yamashita, A., Kutsuzawa, K., Izumi, N., Hirahara, F. and Ohno, S. (2012) N- and C-terminal Upf1 phosphorylations create binding platforms for SMG-6 and SMG-5:SMG-7 during NMD. *Nucleic Acids Res.*, **40**, 1251–1266.
61. Chakrabarti, S., Bonneau, F., Schüssler, S., Eppinger, E. and Conti, E. (2014) Phospho-dependent and phospho-independent interactions of the helicase UPF1 with the NMD factors SMG5-SMG7 and SMG6. *Nucleic Acids Res.*, **42**, 9447–9460.
62. Nicholson, P., Josi, C., Kurosawa, H., Yamashita, A. and Mühlemann, O. (2014) A novel phosphorylation-independent interaction between SMG6 and UPF1 is essential for human NMD. *Nucleic Acids Res.*, **42**, 9217–9235.
63. Huntzinger, E., Kashima, I., Fauser, M., Saulière, J. and Izaurralde, E. (2008) SMG6 is the catalytic endonuclease that cleaves mRNAs containing nonsense codons in metazoan. *RNA*, **14**, 2609–2617.
64. Schmidt, S.A., Foley, P.L., Jeong, D.-H., Rymarquis, L.A., Doyle, F., Tenenbaum, S.A., Belasco, J.G. and Green, P.J. (2014) Identification of SMG6 cleavage sites and a preferred RNA cleavage motif by global analysis of endogenous NMD targets in human cells. *Nucleic Acids Res.*, **43**, 309–323.
65. Lykke-Andersen, S., Chen, Y., Ardal, B.R., Lilje, B., Waage, J., Sandelin, A. and Jensen, T.H. (2014) Human nonsense-mediated RNA decay initiates widely by endonucleolysis and targets snoRNA host genes. *Genes Dev.*, **28**, 2498–2517.
66. Lykke-Andersen, J. and Bennett, E.J. (2014) Protecting the proteome: eukaryotic cotranslational quality control pathways. *J. Cell Biol.*, **204**, 467–476.
67. Loh, B., Jonas, S. and Izaurralde, E. (2013) The SMG5-SMG7 heterodimer directly recruits the CCR4-NOT deadenylase complex to mRNAs containing nonsense codons via interaction with POP2. *Genes Dev.*, **27**, 2125–2138.
68. Unterholzner, L. and Izaurralde, E. (2004) SMG7 acts as a molecular link between mRNA surveillance and mRNA decay. *Mol. Cell*, **16**, 587–596.
69. 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.
70. Chan, W.-K., Huang, L., Gudikote, J.P., Chang, Y.-F., Imam, J.S., MacLean, J.A. and Wilkinson, M.F. (2007) An alternative branch of the nonsense-mediated decay pathway. *EMBO J.*, **26**, 1820–1830.
71. 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.
72. Andersen, C.B.F., Ballut, L., Johansen, J.S., Chamieh, H., Nielsen, K.H., Oliveira, C.L.P., Pedersen, J.S., Séraphin, B., Le Hir, H. and Andersen, G.R. (2006) Structure of the exon junction core complex with a trapped DEAD-box ATPase bound to RNA. *Science*, **313**, 1968–1972.
 73. Bono, F., Ebert, J., Lorentzen, E. and Conti, E. (2006) The crystal structure of the exon junction complex reveals how it maintains a stable grip on mRNA. *Cell*, **126**, 713–725.
 74. Ballut, L., Marchadier, B., Baguet, A., Tomasetto, C., Séraphin, 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.
 75. Noble, C.G. and Song, H. (2007) MLN51 stimulates the RNA-helicase activity of eIF4AIII. *PLoS One*, **2**, e303.
 76. Le Hir, H., Moore, M.J. and Maquat, L.E. (2000) Pre-mRNA splicing alters mRNP composition: evidence for stable association of proteins at exon-exon junctions. *Genes Dev.*, **14**, 1098–1108.
 77. 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.
 78. Geißler, V., Altmeyer, S., Stein, B., Uhlmann-Schiffler, H. and Stahl, H. (2013) The RNA helicase Ddx5/p68 binds to hUpf3 and enhances NMD of Ddx17/p72 and Smg5 mRNA. *Nucleic Acids Res.*, **41**, 7875–7888.
 79. Izumi, N., Yamashita, A., Iwamatsu, A., Kurata, R., Nakamura, H., Saari, B., Hirano, H., Anderson, P. and Ohno, S. (2010) AAA+ proteins RUVBL1 and RUVBL2 coordinate PIKK activity and function in nonsense-mediated mRNA decay. *Sci. Signaling*, **3**, ra27.
 80. Hug, N. and Cáceres, J.F. (2014) The RNA helicase DHX34 activates NMD by promoting a transition from the surveillance to the decay-inducing complex. *Cell Rep.*, **8**, 1845–1856.
 81. Schwer, B. (2001) A new twist on RNA helicases: DEXH/D box proteins as RNPsases. *Nat. Struct. Biol.*, **8**, 113–116.
 82. Jankowsky, E. and Bowers, H. (2006) Remodeling of ribonucleoprotein complexes with DEXH/D RNA helicases. *Nucleic Acids Res.*, **34**, 4181–4188.
 83. Hogg, J.R. and Goff, S.P. (2010) Upf1 senses 3' UTR length to potentiate mRNA decay. *Cell*, **143**, 379–389.
 84. Kurosaki, T. and Maquat, L.E. (2013) Rules that govern UPF1 binding to mRNA 3' UTRs. *Proc. Natl. Acad. Sci. U.S.A.*, **110**, 3357–3362.
 85. Sievers, C., Schlumpf, T., Sawarkar, R., Comoglio, F. and Paro, R. (2012) Mixture models and wavelet transforms reveal high confidence RNA-protein interaction sites in MOV10 PAR-CLIP data. *Nucleic Acids Res.*, **40**, e160.
 86. Gregersen, L.H., Schueler, M., Munschauer, M., Mastrobuoni, G., Chen, W., Kempa, S., Dieterich, C. and Landthaler, M. (2014) MOV10 is a 5' to 3' RNA helicase contributing to UPF1 mRNA target degradation by translocation along 3' UTRs. *Mol. Cell*, **54**, 573–585.
 87. Bühler, M., Steiner, S., Mohn, F., Paillusson, A. and Mühlemann, O. (2006) EJC-independent degradation of nonsense immunoglobulin-mu mRNA depends on 3' UTR length. *Nat. Struct. Mol. Biol.*, **13**, 462–464.
 88. He, F. and Jacobson, A. (2015) Nonsense-mediated mRNA decay: degradation of defective transcripts is only part of the story. *Annu. Rev. Genet.*, **49**, 339–366.
 89. Zünd, D., Gruber, A.R., Zavolan, M. and Mühlemann, O. (2013) Translation-dependent displacement of UPF1 from coding sequences causes its enrichment in 3' UTRs. *Nat. Struct. Mol. Biol.*, **20**, 936–943.
 90. Tani, H., Imamachi, N., Salam, K.A., Mizutani, R., Ijiri, K., Irie, T., Yada, T., Suzuki, Y. and Akimitsu, N. (2014) Identification of hundreds of novel UPF1 target transcripts by direct determination of whole transcriptome stability. *RNA Biol.*, **6**, 1370–1379.
 91. Kurosaki, T., Li, W., Hoque, M., Popp, M.W.-L., Ermolenko, D.N., Tian, B. and Maquat, L.E. (2014) A post-translational regulatory switch on UPF1 controls targeted mRNA degradation. *Genes Dev.*, **28**, 1900–1916.
 92. Lee, S.R., Pratt, G.A., Martinez, F.J., Yeo, G.W. and Lykke-Andersen, J. (2015) Target discrimination in nonsense-mediated mRNA decay requires Upf1 ATPase activity. *Mol. Cell*, **59**, 413–425.
 93. Fatscher, T., Boehm, V. and Gehring, N.H. (2015) Mechanism, factors, and physiological role of nonsense-mediated mRNA decay. *Cell. Mol. Life Sci.*, **72**, 4523–4544.
 94. Eberle, A.B., Stalder, L., Mathys, H., Orozco, R.Z. and Mühlemann, O. (2008) Posttranscriptional gene regulation by spatial rearrangement of the 3' untranslated region. *PLoS Biol.*, **6**, e92.
 95. 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.
 96. 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 mutates genomic noise. *Nat. Genet.*, **36**, 1073–1078.
 97. Wittmann, J., Hol, E.M. and Jäck, H.-M. (2006) hUPF2 silencing identifies physiologic substrates of mammalian nonsense-mediated mRNA decay. *Mol. Cell Biol.*, **26**, 1272–1287.
 98. Weischenfeldt, J., Damgaard, I., Bryder, D., Theilgaard-Mönch, K., Thoren, L. a, Nielsen, F.C., Jacobsen, S.E.W., 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.
 99. Nicholson, P., Yepiskoposyan, H., Metz, S., Zamudio Orozco, R., Kleinschmidt, N. and Mühlemann, O. (2010) 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.
 100. Zarkower, D., De Bono, M., Aronoff, R. and Hodgkin, J. (1994) Regulatory rearrangements and smg-sensitive alleles of the *C. elegans* sex-determining gene *tra-1*. *Dev. Genet.*, **15**, 240–250.
 101. 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.
 102. Lai, T., Cho, H., Liu, Z., Bowler, M.W., Piao, S., Parker, R., Kim, Y.K. and Song, H. (2012) Structural basis of the PNRC2-mediated link between mrna surveillance and decapping. *Structure*, **20**, 2025–2037.
 103. Flury, V., Restuccia, U., Bachi, A. and Mühlemann, O. (2014) Characterization of phosphorylation- and RNA-dependent UPF1 interactors by quantitative proteomics. *J. Proteome Res.*, **13**, 3038–3053.
 104. Fairman-Williams, M.E., Guenther, U.-P. and Jankowsky, E. (2010) SF1 and SF2 helicases: family matters. *Curr. Opin. Struct. Biol.*, **20**, 313–324.
 105. Woychik, N.A., Liao, S.M., Kolodziej, P.A. and Young, R.A. (1990) Subunits shared by eukaryotic nuclear RNA polymerases. *Genes Dev.*, **4**, 313–323.
 106. Anastasaki, C., Longman, D., Capper, A., Patton, E.E. and Cáceres, J.F. (2011) Dhx34 and Nbas function in the NMD pathway and are required for embryonic development in zebrafish. *Nucleic Acids Res.*, **39**, 3686–3694.
 107. Wimmer, K., Zhu, X.X., Lamb, B.J., Kuick, R., Ambros, P.F., Kovar, H., Thoraval, D., Motyka, S., Alberts, J.R. and Hanash, S.M. (1999) Co-amplification of a novel gene, NAG, with the N-myc gene in neuroblastoma. *Oncogene*, **18**, 233–238.
 108. Scott, D.K., Board, J.R., Lu, X., Pearson, A.D., Kenyon, R.M. and Lunec, J. (2003) The neuroblastoma amplified gene, NAG: genomic structure and characterisation of the 7.3 kb transcript predominantly expressed in neuroblastoma. *Gene*, **307**, 1–11.
 109. Aoki, T., Ichimura, S., Itoh, A., Kuramoto, M., Shinkawa, T., Isobe, T. and Tagaya, M. (2009) Identification of the neuroblastoma-amplified gene product as a component of the syntaxin 18 complex implicated in Golgi-to-endoplasmic reticulum retrograde transport. *Mol. Biol. Cell*, **20**, 2639–2649.
 110. Maksimova, N., Hara, K., Nikolaeva, I., Chun-Feng, T., Usui, T., Takagi, M., Nishihira, Y., Miyashita, A., Fujiwara, H., Oyama, T. et al. (2010) Neuroblastoma amplified sequence gene is associated with a novel short stature syndrome characterised by optic nerve atrophy and Pelger-Huët anomaly. *J. Med. Genet.*, **47**, 538–548.
 111. Garcia Segarra, N., Ballhausen, D., Crawford, H., Perreau, M., Campos-Xavier, B., van Spaendonck-Zwarts, K., Vermeer, C., Russo, M., Zambelli, P.-Y., Stevenson, B. et al. (2015) NBAS mutations cause a multisystem disorder involving bone, connective

- tissue, liver, immune system, and retina. *Am. J. Med. Genet. A*, **167A**, 2902–2912.
112. Haack, T.B., Staufner, C., Köpke, M.G., Straub, B.K., Kölker, S., Thiel, C., Freisinger, P., Baric, I., McKiernan, P.J., Dikow, N. *et al.* (2015) Biallelic mutations in NBAS cause recurrent acute liver failure with onset in infancy. *Am. J. Hum. Genet.*, **97**, 163–169.
 113. Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M. *et al.* (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature*, **421**, 231–237.
 114. Rual, J.-F., Ceron, J., Koreth, J., Hao, T., Nicot, A.-S., Hirozane-Kishikawa, T., Vandenhaute, J., Orkin, S.H., Hill, D.E., van den Heuvel, S. *et al.* (2004) Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. *Genome Res.*, **14**, 2162–2168.
 115. Casadio, A., Longman, D., Hug, N., Delavaine, L., Vallejos Baier, R., Alonso, C.R. and Cáceres, J.F. (2015) Identification and characterization of novel factors that act in the nonsense-mediated mRNA decay pathway in nematodes, flies and mammals. *EMBO Rep.*, **16**, 71–78.
 116. Saveanu, C., Bienvenu, D., Namane, A., Gleizes, P.E., Gas, N., Jacquier, A. and Fromont-Racine, M. (2001) Nog2p, a putative GTPase associated with pre-60S subunits and required for late 60S maturation steps. *EMBO J.*, **20**, 6475–6484.
 117. Shaywitz, D.A., Orci, L., Ravazzola, M., Swaroop, A. and Kaiser, C.A. (1995) Human SEC13Rp functions in yeast and is located on transport vesicles budding from the endoplasmic reticulum. *J. Cell Biol.*, **128**, 769–777.
 118. Huang, L. and Wilkinson, M.F. (2012) Regulation of nonsense-mediated mRNA decay. *Wiley Interdiscip. Rev.*, **3**, 807–828.
 119. Lykke-Andersen, S. and Jensen, T.H. (2015) Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. *Nat. Rev. Mol. Cell Biol.*, **16**, 665–677.
 120. Viegas, M.H., Gehring, N.H., Breit, S., Hentze, M.W. and Kulozik, A.E. (2007) The abundance of RNPS1, a protein component of the exon junction complex, can determine the variability in efficiency of the Nonsense Mediated Decay pathway. *Nucleic Acids Res.*, **35**, 4542–4551.
 121. 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.
 122. Li, T., Shi, Y., Wang, P., Guachalla, L.M., Sun, B., Joerres, T., Chen, Y.-S., Groth, M., Krueger, A., Platzer, M. *et al.* (2015) Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay. *EMBO J.*, **34**, 1630–1647.
 123. McIlwain, D.R., Pan, Q., Reilly, P.T., Elia, A.J., McCracken, S., Wakeham, A.C., Itie-Youten, A., Blencowe, B.J. and Mak, T.W. (2010) Smg1 is required for embryogenesis and regulates diverse genes via alternative splicing coupled to nonsense-mediated mRNA decay. *Proc. Natl. Acad. Sci. U.S.A.*, **107**, 12186–12191.
 124. Varsally, W. and Brogna, S. (2012) UPF1 involvement in nuclear functions. *Biochem. Soc. Trans.*, **40**, 778–783.
 125. Azzalin, C.M. and Lingner, J. (2006) The human RNA surveillance factor UPF1 is required for S phase progression and genome stability. *Curr. Biol.*, **16**, 433–439.
 126. Kim, Y.K., Furic, L., Desgroseillers, L. and Maquat, L.E. (2005) Mammalian Staufen1 recruits Upf1 to specific mRNA 3' UTRs so as to elicit mRNA decay. *Cell*, **120**, 195–208.
 127. Park, E. and Maquat, L.E. (2013) Staufen-mediated mRNA decay. *Wiley Interdiscip. Rev.*, **4**, 423–435.
 128. Kaygun, H. and Marzluff, W.F. (2005) Regulated degradation of replication-dependent histone mRNAs requires both ATR and Upf1. *Nat. Struct. Mol. Biol.*, **12**, 794–800.
 129. 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.
 130. Metzstein, M.M. and Krasnow, M.A. (2006) Functions of the nonsense-mediated mRNA decay pathway in *Drosophila* development. *PLoS Genet.*, **2**, e180.
 131. Avery, P., Vicente-Crespo, M., Francis, D., Nashchekina, O., Alonso, C.R. and Palacios, I.M. (2011) *Drosophila* Upf1 and Upf2 loss of function inhibits cell growth and causes animal death in a Upf3-independent manner. *RNA*, **17**, 624–638.
 132. Gardner, L.B. (2010) Nonsense-mediated RNA decay regulation by cellular stress: implications for tumorigenesis. *Mol. Cancer Res.*, **8**, 295–308.
 133. Karam, R., Wengrod, J., Gardner, L.B. and Wilkinson, M.F. (2013) Regulation of nonsense-mediated mRNA decay: implications for physiology and disease. *Biochim. Biophys. Acta*, **1829**, 624–633.
 134. Wang, D., Zavadil, J., Martin, L., Parisi, F., Friedman, E., Levy, D., Harding, H., Ron, D. and Gardner, L.B. (2011) Inhibition of nonsense-mediated RNA decay by the tumor microenvironment promotes tumorigenesis. *Mol. Cell Biol.*, **31**, 3670–3680.
 135. 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.
 136. Karam, R., Lou, C.-H., Kroeger, H., Huang, L., Lin, J.H. and Wilkinson, M.F. (2015) The unfolded protein response is shaped by the NMD pathway. *EMBO Rep.*, **16**, 599–609.
 137. Sakaki, K., Yoshina, S., Shen, X., Han, J., DeSantis, M.R., Xiong, M., Mitani, S. and Kaufman, R.J. (2012) RNA surveillance is required for endoplasmic reticulum homeostasis. *Proc. Natl. Acad. Sci. U.S.A.*, **109**, 8079–8084.
 138. Oren, Y.S., McClure, M.L., Rowe, S.M., Sorscher, E.J., Bester, A.C., Manor, M., Kerem, E., Rivlin, J., Zahdeh, F., Mann, M. *et al.* (2014) The unfolded protein response affects readthrough of premature termination codons. *EMBO Mol. Med.*, **6**, 685–701.
 139. Wengrod, J., Martin, L., Wang, D., Frischmeyer-Guerrero, P., Dietz, H.C. and Gardner, L.B. (2013) Inhibition of nonsense-mediated RNA decay activates autophagy. *Mol. Cell Biol.*, **33**, 2128–2135.
 140. Mino, T., Murakawa, Y., Fukao, A., Vandenbon, A., Wessels, H.-H., Ori, D., Uehata, T., Tartey, S., Akira, S., Suzuki, Y. *et al.* (2015) Regnase-1 and Roquin regulate a common element in inflammatory mRNAs by spatiotemporally distinct mechanisms. *Cell*, **161**, 1058–1073.
 141. Belew, A.T., Meskauskas, A., Musalgaonkar, S., Advani, V.M., Sulima, S.O., Kasprzak, W.K., Shapiro, B.A. and Dinman, J.D. (2014) Ribosomal frameshifting in the CCR5 mRNA is regulated by miRNAs and the NMD pathway. *Nature*, **512**, 265–269.
 142. Pastor, F., Kolonias, D., Giangrande, P.H. and Gilboa, E. (2010) Induction of tumour immunity by targeted inhibition of nonsense-mediated mRNA decay. *Nature*, **465**, 227–230.
 143. Nickless, A., Jackson, E., Marasa, J., Nugent, P., Mercer, R.W., Piwnica-Worms, D. and You, Z. (2014) Intracellular calcium regulates nonsense-mediated mRNA decay. *Nat. Med.*, **20**, 961–966.
 144. Riehs-Kearnan, N., Gloggnitzer, J., Dekrout, B., Jonak, C. and Riha, K. (2012) Aberrant growth and lethality of Arabidopsis deficient in nonsense-mediated RNA decay factors is caused by autoimmune-like response. *Nucleic Acids Res.*, **40**, 5615–5624.
 145. Gloggnitzer, J., Akimcheva, S., Srinivasan, A., Kusenda, B., Riehs, N., Stampfl, H., Bautor, J., Dekrout, B., Jonak, C., Jiménez-Gómez, J.M. *et al.* (2014) Nonsense-mediated mRNA decay modulates immune receptor levels to regulate plant antibacterial defense. *Cell Host Microbe*, **16**, 376–390.
 146. Rigby, R.E. and Rehwinkel, J. (2015) RNA degradation in antiviral immunity and autoimmunity. *Trends Immunol.*, **36**, 179–188.
 147. Balistreri, G., Horvath, P., Schweingruber, C., Zünd, D., McInerney, G., Merits, A., Mühlemann, O., Azzalin, C. and Helenius, A. (2014) The host nonsense-mediated mRNA decay pathway restricts mammalian RNA virus replication. *Cell Host Microbe*, **16**, 403–411.
 148. Weil, J.E. and Beemon, K.L. (2006) A 3' UTR sequence stabilizes termination codons in the unspliced RNA of Rous sarcoma virus. *RNA*, **12**, 102–110.
 149. Quek, B.L. and Beemon, K. (2014) Retroviral strategy to stabilize viral RNA. *Curr. Opin. Microbiol.*, **18**, 78–82.
 150. Mocquet, V., Neusiedler, J., Rende, F., Cluet, D., Robin, J.-P., Terme, J.-M., Duc Dodon, M., Wittmann, J., Morris, C., Le Hir, H. *et al.* (2012) The human T-lymphotropic virus type 1 tax protein inhibits nonsense-mediated mRNA decay by interacting with INT6/EIF3E and UPF1. *J. Virol.*, **86**, 7530–7543.

151. Nakano, K., Ando, T., Yamagishi, M., Yokoyama, K., Ishida, T., Ohsugi, T., Tanaka, Y., Brighty, D.W. and Watanabe, T. (2013) Viral interference with host mRNA surveillance, the nonsense-mediated mRNA decay (NMD) pathway, through a new function of HTLV-1 Rex: implications for retroviral replication. *Microbes Infect.*, **15**, 491–505.
152. Garcia, D., Garcia, S. and Voinnet, O. (2014) Nonsense-mediated decay serves as a general viral restriction mechanism in plants. *Cell Host Microbe*, **16**, 391–402.
153. Popp, M.W. and Maquat, L.E. (2015) Attenuation of nonsense-mediated mRNA decay facilitates the response to chemotherapeutics. *Nat. Commun.*, **6**, 6632. DOI:10.1038/ncomms7632.
154. Jia, J., Furlan, A., Gonzalez-Hilarion, S., Leroy, C., Gruenert, D.C., Tulasne, D. and Lejeune, F. (2015) Caspases shutdown nonsense-mediated mRNA decay during apoptosis. *Cell Death Differ.*, **22**, 1754–1763.
155. Feng, Q., Snider, L., Jagannathan, S., Tawil, R., van der Maarel, S.M., Tapscott, S.J. and Bradley, R.K. (2015) A feedback loop between nonsense-mediated decay and the retrogene DUX4 in facioscapulohumeral muscular dystrophy. *Elife*, **4**, e04996.
156. Bhuvanagiri, M., Lewis, J., Putzker, K., Becker, J.P., Leicht, S., Krijgsveld, J., Batra, R., Turnwald, B., Jovanovic, B., Hauer, C. *et al.* (2014) 5-azacytidine inhibits nonsense-mediated decay in a MYC-dependent fashion. *EMBO Mol. Med.*, **6**, 1593–1609.
157. Wang, D., Wengrod, J. and Gardner, L.B. (2011) Overexpression of the c-myc oncogene inhibits nonsense-mediated RNA decay in B lymphocytes. *J. Biol. Chem.*, **286**, 40038–40043.
158. Durand, S., Cougot, N., Mahuteau-Betzer, F., Nguyen, C.-H., Grierson, D.S., Bertrand, E., Tazi, J. and Lejeune, F. (2007) Inhibition of nonsense-mediated mRNA decay (NMD) by a new chemical molecule reveals the dynamic of NMD factors in P-bodies. *J. Cell Biol.*, **178**, 1145–1160.
159. Martin, L., Grigoryan, A., Wang, D., Wang, J., Breda, L., Rivella, S., Cardozo, T. and Gardner, L.B. (2014) Identification and characterization of small molecules that inhibit nonsense-mediated RNA decay and suppress nonsense p53 mutations. *Cancer Res.*, **74**, 3104–3113.
160. Dang, Y., Low, W.-K., Xu, J., Gehring, N.H., Dietz, H.C., Romo, D. and Liu, J.O. (2009) Inhibition of nonsense-mediated mRNA decay by the natural product pateamine A through eukaryotic initiation factor 4AIII. *J. Biol. Chem.*, **284**, 23613–23621.
161. Feng, D., Su, R.-C., Zou, L., Triggs-Raine, B., Huang, S. and Xie, J. (2015) Increase of a group of PTC(+) transcripts by curcumin through inhibition of the NMD pathway. *Biochim. Biophys. Acta*, **1849**, 1104–1115.
162. Gopalsamy, A., Bennett, E.M., Shi, M., Zhang, W.-G., Bard, J. and Yu, K. (2012) Identification of pyrimidine derivatives as hSMG-1 inhibitors. *Bioorg. Med. Chem. Lett.*, **22**, 6636–6641.
163. Keeling, K.M., Wang, D., Conard, S.E. and Bedwell, D.M. (2012) Suppression of premature termination codons as a therapeutic approach. *Crit. Rev. Biochem. Mol. Biol.*, **47**, 444–463.
164. Keeling, K.M., Xue, X., Gunn, G. and Bedwell, D.M. (2014) Therapeutics based on stop codon readthrough. *Annu. Rev. Genomics Hum. Genet.*, **15**, 371–394.
165. Nomakuchi, T.T., Rigo, F., Aznarez, I. and Krainer, A.R. (2015) Antisense oligonucleotide-directed inhibition of nonsense-mediated mRNA decay. *Nat. Biotechnol.*, **10.1038/nbt.3427**.