Trends in
Neurosciences

CellPress
OPEN ACCESS

Review

Nonsense-mediated mRNA decay in neuronal physiology and neurodegeneration

Marija Petrić Howe[1,2,*] and Rickie Patani[1,2,*]

The processes of mRNA export from the nucleus and subsequent mRNA translation in the cytoplasm are of particular relevance in eukaryotic cells. In highly polarised cells such as neurons, finely-tuned molecular regulation of these processes serves to safeguard the spatiotemporal fidelity of gene expression. Nonsense-mediated mRNA decay (NMD) is a cytoplasmic translation-dependent quality control process that regulates gene expression in a wide range of scenarios in the nervous system, including neurodevelopment, learning, and memory formation. Moreover, NMD dysregulation has been implicated in a broad range of neurodevelopmental and neurodegenerative disorders. We discuss how NMD and related aspects of mRNA translation regulate key neuronal functions and, in particular, we focus on evidence implicating these processes in the molecular pathogenesis of neurodegeneration. Finally, we discuss the therapeutic potential and challenges of targeting mRNA translation and NMD across the spectrum of largely untreatable neurological diseases.

Highlights

Nonsense-mediated mRNA decay (NMD) is a fundamental surveillance and gene regulatory pathway in eukaryotic cells.

NMD is an important regulator of neuronal homeostasis by modulating processes such as development, learning, and memory.

Mutations in key NMD factors have been identified in a range of neurodevelopmental disorders, and NMD dysregulation has been described in several neurodegenerative diseases.

NMD modulation exhibits potential therapeutic benefits in some animal disease models. Translating these paradigms, however, to neurodegeneration in clinical settings is complex because of (i) limited understanding of the full scope of roles of NMD factors, (ii) limited knowledge of the disease stage at which NMD is dysregulated, and (iii) the difficulty of optimally targeting such a broad cellular process.

The aforementioned concepts highlight the importance of further mechanistic understanding of the roles of NMD in acute and chronic adverse contexts.

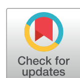
NMD in the nervous system

NMD is a eukaryotic mRNA surveillance mechanism and regulator of mRNA stability that broadly serves to downregulate premature translation termination codon (PTC)-containing mRNAs [1,2]. It can degrade transcripts that contain genetic nonsense mutations that occur in ~30% of all human diseases, and/or transcripts resulting from RNA processing errors. Ultimately, NMD can mitigate the harmful effects of such phenomena by limiting the synthesis of the resulting and potentially deleterious C-terminally truncated proteins [2,3]. Beyond restricting the level of aberrant transcripts, NMD has a well-established role in fine-tuning the expression of physiologically occurring endogenous mRNAs, some of which encode full-length proteins [4]. Altogether, NMD modulates crucial cellular processes, including cellular response to stress. In the context of the nervous system, NMD is involved in neurodevelopment, including neurogenesis and cellular differentiation [5,6].

Owing to their polarised structure, neurons rely heavily on post-transcriptional control of gene expression, localising mRNAs to specific sub-cytoplasmic areas, and triggering their stimulus-dependent translation in a tightly regulated manner [7]. Such mRNA regulation is primarily mediated by RNA-binding proteins (RBPs) and their respective protein and RNA interactomes [7]. It follows that localised mRNA translation in neurons can trigger NMD in a time- and location-dependent manner, thereby modulating key neuronal processes, particularly those that occur remotely from the cell body [8,9]. Indeed, NMD has galvanised considerable attention in neuroscience not only because it regulates a variety of processes, from axonal guidance during neurodevelopment through to synaptic potentiation [5,8,10], but also because NMD dysregulation has been observed in several neurodevelopmental and neurodegenerative diseases [11–13].

[1]The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK
[2]Department of Neuromuscular Diseases, Queen Square Institute of Neurology, University College London (UCL), Queen Square, WC1N 3BG London, UK

*Correspondence:
marija.petric-howe@crick.ac.uk
(M. Petrić Howe) and
rickie.patani@crick.ac.uk
(R. Patani).



© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

In this review we provide an overview of NMD as an mRNA regulatory mechanism before discussing its role in regulating key neuronal functions and its involvement in neurodegeneration, with an emphasis on amyotrophic lateral sclerosis (ALS). We discuss findings from human studies, as well as from a range of experimental model organisms. Finally, we highlight the prospects for targeting NMD as a potential therapeutic strategy and discuss outstanding questions in the field.

Factors underlying NMD

Defining true NMD targets has proved to be challenging owing to the multistep nature of the process that exhibits a degree of redundancy, while involving several factors that play roles in broader cellular processes, including other types of mRNA degradation [14–17]. Moreover, the magnitude of NMD differs substantially between transcripts, depending on their characteristics, the composition of their messenger ribonucleoprotein (mRNP) complex [14], and the cell/tissue-specific concentration of NMD factors and enhancers. This gives the concept of localised NMD responses such as endoplasmic reticulum (ER) NMD considerable traction [18]. Despite the aforementioned challenges, some features are considered to be prominent predictors of NMD, as described in Box 1.

Key factors involved in NMD include up-frameshift proteins 1, 2, and 3 (UPF1, UPF2, and UPF3), – evolutionarily conserved proteins that comprise the core NMD machinery in all eukaryotes [19]. Auxiliary factors involved in the process in higher eukaryotic organisms mainly modulate the functions of core NMD factors by recruiting components of the degradation machinery and/or triggering mRNA degradation [20,21]. These were termed SMG (suppressor with morphogenetic effect on genitalia) proteins owing to the phenotypes observed in a mutagenesis screen in *Caenorhabditis elegans* where most of these factors were identified (see Table 1). In human cells, UPF3 exists as paralogue proteins UPF3A and UPF3B [22]. UPF3B exhibits a stimulating effect on NMD, whereas the action of UPF3A is complex. UPF3A was initially reported to exhibit

Box 1. NMD target determination

Alternative splicing-coupled NMD is common in mammalian cells where it is typically caused by inclusion of an exon that contains a PTC (poison exon) or by exclusion of an exon which gives rise to a PTC in the new reading frame (essential exon) [88]. Of human multi-exon genes, ~95% undergo alternative splicing and up to ~35% of mammalian alternative splicing events are predicted to contain a PTC [89,90]. However, not every PTC triggers NMD to the same extent, and a transcript cannot be considered to be a *bona fide* NMD target merely by the presence of a PTC [90]. NMD inhibition does not seem to affect the expression of weakly expressed and poorly conserved alternative splicing PTC-containing isoforms [90]. Instead, NMD-specific regulation primarily targets a small proportion of PTC-carrying alternative splicing events, often in relatively conserved genes and those that encode RNA-binding proteins and splicing factors [88,91,92]. These genes often direct splicing to NMD-targeting isoforms when the protein level is high, allowing the splicing factor to modulate its splicing patterns and regulate the level of its own protein production [88]. Alternative splicing-coupled NMD is also established as a regulatory mechanism for cell type- and tissue-specific gene expression in the absence of such clear autoregulatory feedback loops. In these cases, splicing modulation can change the splicing pattern of a gene in favour of either NMD or productive translation. Importantly, many of these regulatory events have been shown to occur specifically in the nervous system [25,26,33,93].

The development of NMD reporters, based on genes that possess NMD-inducing features, was crucial to advance our understanding of the characteristics of transcripts targeted by NMD and to provide key mechanistic insights into this process in a range of cell- and tissue-specific NMD responses across diverse models [94–96]. A PTC positioned at least 50 nt upstream of an exon–exon junction is broadly considered to be a predictor of efficient NMD in mammalian cells [87]. Additional NMD-inducing *cis* features include upstream open reading frames (uORFs), retained introns, and long 3'-untranslated regions (UTRs) [97–100]. Interestingly, a combination of long-read and short-read sequencing reported no difference in 3′-UTR mean length between NMD-sensitive and insensitive transcripts, disputing long 3′-UTRs as a characteristic of NMD-targeted transcripts [101]. The presence of an intron in the 3′-UTR was found to be a more accurate predictor of efficient NMD [101]. Altogether, long 3′-UTRs remain an NMD-inducing characteristic in lower eukaryotes, whereas NMD is primarily determined by splicing-dependent signals in mammalian systems [87].

very low NMD activity, but was later characterised as an NMD inhibitor that antagonises UPF3B function [22]. Further complicating the picture, UPF3A has more recently been reported to be an NMD enhancer that is somewhat functionally redundant with UPF3B, and becomes upregulated when UPF3B expression decreases [23,24]. Table 1 provides a list of proteins involved in NMD in mammalian cells and their respective functions.

NMD was typically considered to occur during the pioneer round of translation shortly after or during mRNA entry into the cytoplasm. However, mRNAs are often transported to particular sub-cytoplasmic locations in a translationally repressed state, and the PTC is only 'recognised' and the mRNA targeted for degradation when translation is triggered by a specific stimulus, thus enabling NMD to act in a spatiotemporally regulated manner [10,18]. The extent of mRNA degradation by NMD is context-dependent, and several models have been formulated to describe the mechanism of NMD, of which the exon junction complex (EJC) model and the *faux* 3′-untranslated region (UTR) model are the most prominent (Figure 1).

Roles of NMD in the nervous system

NMD in neurodevelopment

Temporal changes in NMD activity can have differential effects on neuronal development [5,11]. In neural stem cells, a key pro-differentiation factor, SMAD7, which negatively regulates proliferative TGF-β signalling, is continually degraded by NMD, thus maintaining the stem cell state [5]. Analyses primarily in mouse neural cell lines and mouse brain, as well as explorations in human neuronal lines and *Xenopus laevis*, indicate that a neuronal-specific miRNA, miR-128, is dramatically upregulated during development and binds to and suppresses UPF1, UPF3B, and an EJC factor, MLN1. As a consequence of NMD suppression, SMAD7 is upregulated, which in turn inhibits TGF-β signalling and ultimately triggers cellular differentiation. Additional miRNAs involved in NMD suppression in neurons that were identified in the study include

Table 1. List of proteins involved in the NMD process in mammalian cells (i.e., NMD factors) and their respective functions

Protein	Function	Refs
UPF1	ATP-dependent helicase Central NMD factor	[82]
UPF2	Regulates UPF1 activity Bridges UPF1 and UPF3	[83]
UPF3A	Initially identified as an NMD suppressor	[22]
	Its recently discovered NMD-activating function can compensate for UPF3B function	[23,24]
UPF3B	Part of the EJC complex Brings the EJC, UPF2, and UPF1 together	[19]
SMG1	Kinase that phosphorylates and activates UPF1	[84]
SMG5	Forms a heterodimer with SMG7 Deadenylation, decapping, and exonucleolytic degradation of NMD targets Phosphatase that dephosphorylates UPF1	[20]
SMG6	Endonucleolytic cleavage of mRNA	[21]
SMG7	Forms a heterodimer with SMG5 Deadenylation, decapping, and exonucleolytic degradation of NMD targets	[20]
SMG8	Subunit of the SMG1 complex	[85]
	Suppressor of SMG1 kinase activity	[86]
SMG9	Subunit of the SMG1 complex	[85]
	Suppressor of SMG1 kinase activity	[86]

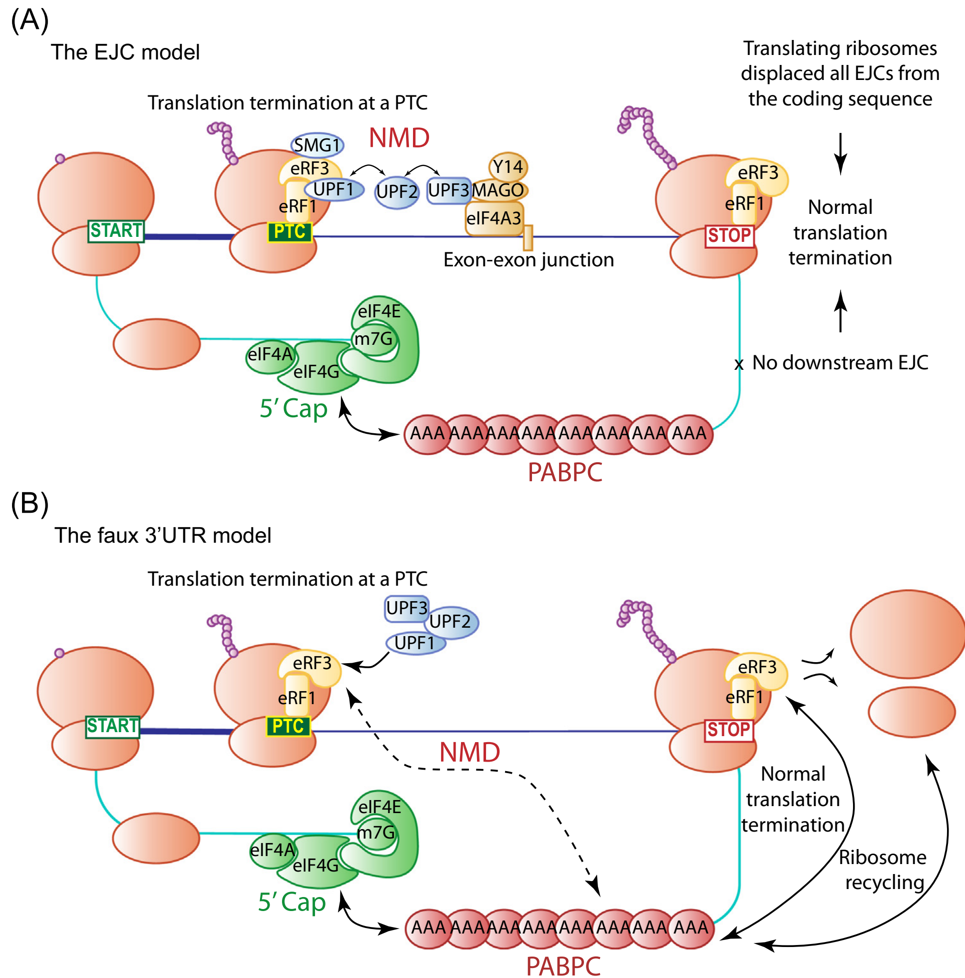


Figure 1. Models of nonsense-mediated mRNA decay (NMD). (A) The exon junction complex (EJC) model is a prevalent conceptualisation of mammalian NMD. Eukaryotic release factors 1 and 3 (eRF1 and eRF3) associate with the ribosome once it terminates translation at a stop codon, followed by UPF1 and SMG1 association, which together form the SURF (SMG1–UPF1–eRF1–eRF3) complex. A ribosome terminating at a premature translation termination codon (PTC) undergoes NMD if there is at least one EJC downstream, in which case UPF2 serves as a connection between UPF1 bound to the terminating ribosome and UPF3 bound to the EJC. Once this interaction is established, SMG1 phosphorylates and activates UPF1, triggering mRNA degradation. Introns are rare in the 3′-untranslated region (3′-UTR), therefore, when a ribosome terminates at a normal stop codon and no EJCs are located downstream, protein synthesis can be completed. (B) The *faux* 3′UTR model. Upon termination at a normal stop codon, interaction between eRFs and PABPCs stimulates release of the newly synthesised protein as well as of ribosomes, enabling ribosome recycling for another round of translation and stabilising the translation circuit. When termination occurs at a PTC, no interaction between eRFs and PABPCs is achieved. Ribosome stalled at a PTC leads to NMD factors binding the terminating ribosome instead, causing mRNA degradation. Ribosomes are depicted in the figure in orange, and nascent peptides in purple. Abbreviations: eIF, eukaryotic initiation factor; eIF4A3, MAGO, Y14, components of the exon junction complex; m7G, 7-methylguanosine; PABPC, cytoplasmic poly(A)-binding protein; START, start codon; STOP, termination codon; UPF, up-frameshift protein. Panels inspired by Figure 1 in [87].

miR-9 and miR-124. UPF3B and UPF1 are not only modulated by miR-128 and miR-9 but also negatively regulate these miRNAs [5]. Such bidirectional control suggests that NMD factors and these miRNAs form a negative feedback loop that directs the fate of a neural stem cell

into either stemness or a terminally differentiated state, depending on the input signal (Figure 2A).

NMD has recently been shown to regulate neuronal survival and homeostasis in a study on mouse brain development that provided *in vivo* genetic evidence for the physiological significance of NMD coupled to alternative splicing [25]. Notably, neuron-specific inclusion of the evolutionarily conserved *Bak1* microexon 5 was reported to trigger NMD of *Bak1* transcripts, thus limiting BAK1 protein production. Given that BAK1 represents a major checkpoint for apoptosis, its suppression by NMD provides a mechanism for neurons to reduce apoptosis, which is essential for organismal survival. Moreover, by analysing *Bak1/BAK1* splicing across human tissues and between mouse and human neuronal differentiation, the study found that this developmental regulation of BAK1 is indeed conserved from mouse to human tissues [25]. Addressing the impact of acute and chronic adverse contexts on this regulatory mechanism remains an important goal for

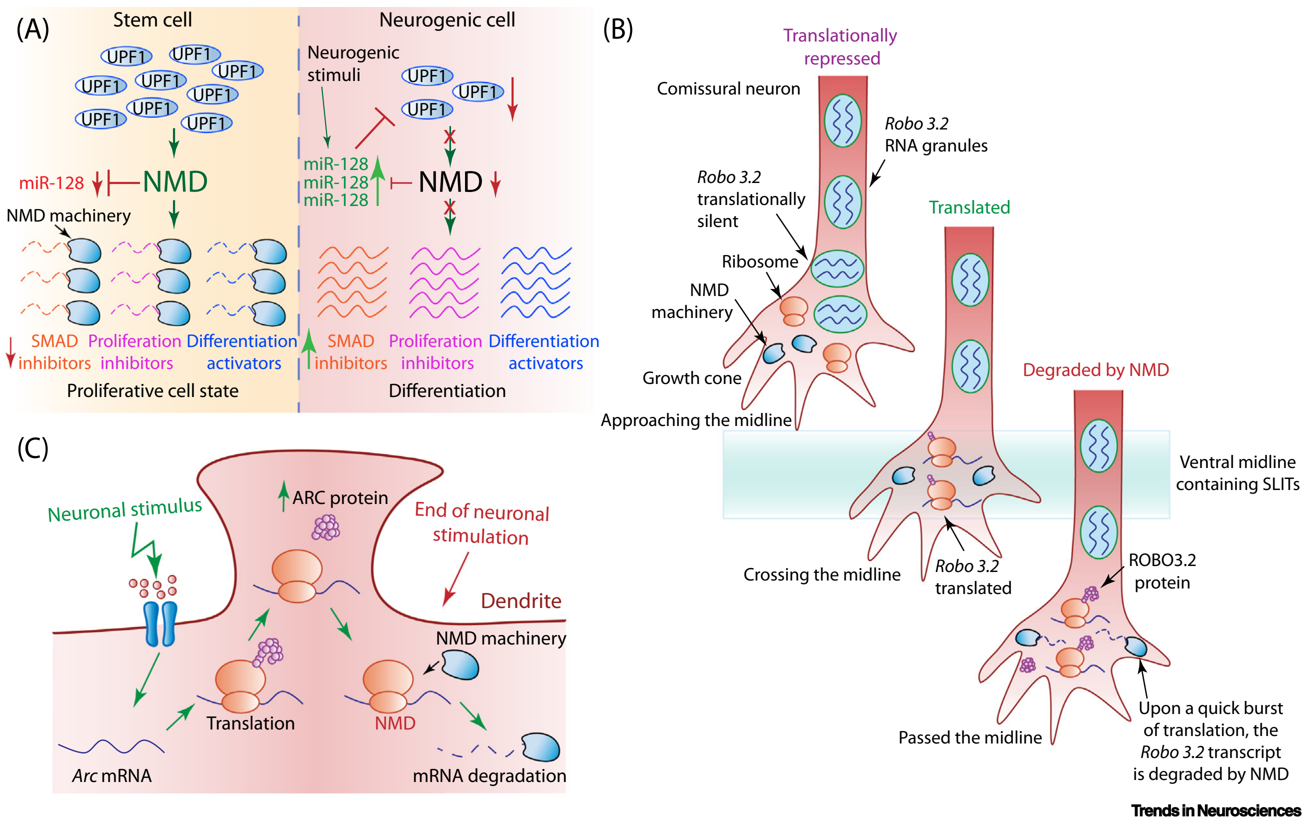


Figure 2. Overview of roles of nonsense-mediated mRNA decay (NMD) in the nervous system. (A) NMD regulates neural cell fate. (Left) In neuronal stem cells, NMD factors are highly expressed, and NMD efficiently downregulates its targets, including cell proliferation inhibitors, differentiation activators, SMAD inhibitors, and miR-128, all of which maintain the proliferative cell state. (Right) In response to neurogenic stimuli, miR-128 is upregulated, reducing the expression of up-frameshift protein 1 (UPF1) and reducing NMD efficiency, thus causing NMD targets that control stem cell state to be upregulated, and directing the cell to commit to a neural lineage. The NMD machinery is depicted in blue. Inspired by the graphical abstract in [5]. (B) NMD regulates axon guidance. Axon positioning relies on the interaction between surface membrane receptors, (roundabout proteins, ROBOs) and proteins of the extracellular matrix. In commissural axons, *Robo3.2* is in a translationally repressed state before crossing the ventral midline (left). Once the midline is reached, *Robo3.2* (middle) is translated, thus enabling other ROBOs to interact with the extracellular matrix, and repelling the axon from this area. When the axon crosses the midline, *Robo3.2* transcript is degraded by NMD (right), ensuring that ROBO3.2 protein is only produced in a tight temporal and spatial manner. Inspired by the model depicted in Figure S7 of [8]. (C) NMD modulates synaptic plasticity. *Arc* expression is regulated at the translational and NMD levels in response to neuronal stimulation. Neuronal stimulus causes rapid *Arc* mRNA synthesis, translocation to the dendrites and quick burst of translation. Once the stimulation ends, the mRNA is rapidly degraded by NMD, preventing further protein synthesis.

future studies. NMD has also been shown to be involved in mouse axon formation via temporal regulation of TRIM46 [26]. An intriguing concurrent but independent regulation of two alternative exons was revealed, where inclusion of exon 8 leads to NMD of *Trim46* and exclusion of exon 10 leads to an unstable TRIM46 protein isoform. During axonogenesis, transcriptional activation and enhanced exon 8 exclusion/exon 10 inclusion in turn increase stable TRIM46 protein production. Together, these regulatory mechanisms coordinate the spatiotemporal expression of TRIM46, which is among the earliest markers of axon specification [26].

Depletion of UPF3B at a late neural stem cell stage reduces the ability of these cells to differentiate, which suggests that NMD activity promotes the later stages of neuronal differentiation [11]. Furthermore, mouse hippocampal neurons deficient in UPF3B exhibit reduced axonal growth and increased arborisation of both axons and dendrites [27]. Cumulatively, these studies suggest temporal regulation of NMD whereby it is suppressed at early stages of differentiation but becomes reactivated once precursors commit to a neuronal lineage, after which NMD persists as an important regulator of neuron-specific homeostatic functions.

NMD in neuronal homeostasis and axon guidance

NMD also plays a role in directing axon growth by guiding it in the required direction towards another cell/tissue (e.g., neuron or muscle) with which it will establish a synapse [28,29] (Figure 2B). Axon guidance depends in part on the interaction between neuronal surface membrane receptors (roundabout proteins, ROBOs) and proteins of the extracellular matrix (SLIT proteins) [30]. The expression of ROBO proteins 1, 2, and 3 is restricted to specific neuronal subtypes in a temporally regulated manner, and the process of axon guidance is particularly well studied in commissural axons that cross the ventral midline [30]. ROBO1 and ROBO2 perform key roles in commissural axon growth because they interact with SLITs located in the midline area where this interaction is regulated by ROBO3. *Robo3* expresses two transcript isoforms in mouse commissural neurons – *Robo3.1* and *Robo3.2* – giving rise to two protein isoforms with distinct C-terminal domains [8,9]. *Robo3.1* is the only isoform translated as the axons approach the midline area, while *Robo3.2* transcript is still in its translationally repressed state. Once the axon passes the midline, ROBO3.2 protein is synthesised, increasing the ability of ROBO1 and ROBO2 to bind to SLITs, which in turn repels the axon from the midline area, allowing appropriate axon positioning [9].

The *Robo3.2* transcript in mice contains a retained intron which introduces an NMD-inducing PTC into the new reading frame [8], but the transcript can also lead to the production of a ROBO3.2 protein isoform with an alternative C-terminal end. Because *Robo3.2* is translationally repressed until it reaches the midline, it escapes NMD. Once the axon has crossed the midline, local cues trigger rapid translation of *Robo3.2* mRNA but only brief upregulation of the protein because the transcript is also degraded by NMD. This allows tight temporal and spatial control of the expression of the protein. Moreover, neurons can exhibit varied magnitudes of NMD, which could modulate ROBO3.2 protein expression differently in different types of neurons, resulting in varied axonal trajectories in the brain and spinal cord [8].

NMD and synaptic function

NMD modulates synaptic plasticity, a process that enables fine-tuning of synaptic strength in response to patterns of neural activity, and that is considered to be crucial for learning and memory (Figure 2C) [31,32]. A key transcript that plays a role in synaptic plasticity, *Arc* mRNA, has also been identified as an NMD target [10]. Upon activation, the *Arc* gene exhibits fast transcriptional activation and mRNA localisation to the dendrites, followed by its translation. Upon protein synthesis and the end of neuronal stimulation, the transcript that harbours two introns in its 3′-UTR

CellPress
OPEN ACCESS

undergoes degradation by NMD [10]. Hence, the protein is synthesised only during neuronal activation, which in turn enhances synaptic strength.

NMD regulates the expression of the most abundant synaptic protein PSD-95, and thereby is an important determinant in synaptogenesis. Specifically, in early mouse brain PTBP1 and PTBP2 repress *Psd-95* exon 18 splicing, generating an isoform with a PTC in exon 19 that is targeted by NMD [33]. During embryonic development, the sequential downregulation of PTBP1 and PTBP2 permits splicing of exon 18 and alleviates post-transcriptional NMD-mediated repression of *Psd95*, allowing its expression late in neuronal maturation. Importantly, this study also showed that the PTC in exon 19 is conserved across mammalian species including humans. Hence, it is conceivable that similar regulation of this alternative splicing event is also present in humans [33]. It would be of considerable interest to examine in future studies how acute and chronic adverse contexts could impact on the fidelity of this process.

NMD and neurological disorders

Mutations of key factors involved in NMD have been associated with a range of neurodevelopmental disorders. In this context, copy-number variants of most NMD and EJC genes were found to contribute to disease pathology [34]. In addition, NMD is implicated in a range of neurological diseases which have a profound impact on patients, carers, and society. These range from intellectual disabilities that impair daily functions to progressive neurodegenerative disorders such as ALS [12,35–37]. NMD involvement in the pathogenesis of neurodevelopmental disorders has been reviewed elsewhere [38,39], and some of the key studies are summarised in Box 2. In the following we focus primarily on neurodegenerative disorders, where several NMD targets have been implicated in ALS, and frontotemporal dementia (FTD) (depicted in Figure 3, Key figure) [12,37].

Box 2. NMD and neurodevelopmental disorders

Copy-number variants of *UPF2* have been linked to autism spectrum disorder and other forms of intellectual disability (ID), and other protein-coding variants were identified in disorders linked to speech and language deficiencies [34]. In mice, selective and conditional removal of *Upf2* in the forebrain results in memory, communication, and social deficits [36]. In this model, UPF2 loss resulted in elevated neuroinflammation, a phenotype alleviated by anti-inflammatory agents that also improved the behavioural deficiencies [36].

Dysfunction of UPF3B can lead to ID, autism, attention deficit hyperactivity disorder (ADHD), and schizophrenia [11,102,103]. Disease-causing mutations typically reside within the middle region of UPF3B that is important for its role in mRNA translation termination and ribosome recycling, as evidenced by *in vitro* studies [104,105], as well as for UPF3B interaction with UPF2 [106]. Other mutations were identified in the region that encodes an amino acid residue, Y160D, that is crucial for stabilising the UPF2–UPF3B interaction. These mutations lead to a greatly reduced affinity of UPF3B for UPF2 and reduced NMD efficiency. UPF3A, which is greatly upregulated in response to UPF3B downregulation and by the Y160D mutation, binds to UPF2 instead, but seemingly cannot fully compensate for UPF3B function [106]. Some of the mutations in UPF3B identified in X-linked intellectual disability disorders (XLID) are found within the eRF3 interacting domain, whereas others introduce a PTC [103], resulting in reduced transcript levels.

Upf3b null mice exhibit deficiencies in fear-conditioned learning and prepulse inhibition [107], the latter being often observed in schizophrenia and related disorders. In *Upf3b* null mice, cortical pyramidal neurons also manifest reduced dendritic spine maturation, and neural stem cells exhibit impaired differentiation with delayed electrical maturation. Many dysregulated transcripts within the frontal cortex of *Upf3b* null mice were identified as direct NMD targets with established roles in neural differentiation and disease [107]. Transcriptome-wide effects of UPF3B deficiency were further explored using lymphoblastoid cell lines derived from people with ID and loss of function mutations in *UPF3B* [108]. Affected upregulated genes include Rho GTPase activating protein 24 (*ARHGAP24*) that is involved in axon and dendrite growth and branching, as well as *ROBO1* that is involved in axon guidance. Interestingly, UPF3A protein was shown to be stabilised in such patients, and this correlated with decreased symptoms and a reduced extent of transcriptome deregulation [108]. Cumulatively, this suggests that UPF3A might partly compensate for and modulate UPF3B function in a dose- (and indeed context-) dependent manner.

Key figure

Translation and nonsense-mediated mRNA decay (NMD) defects in neurodegenerative diseases

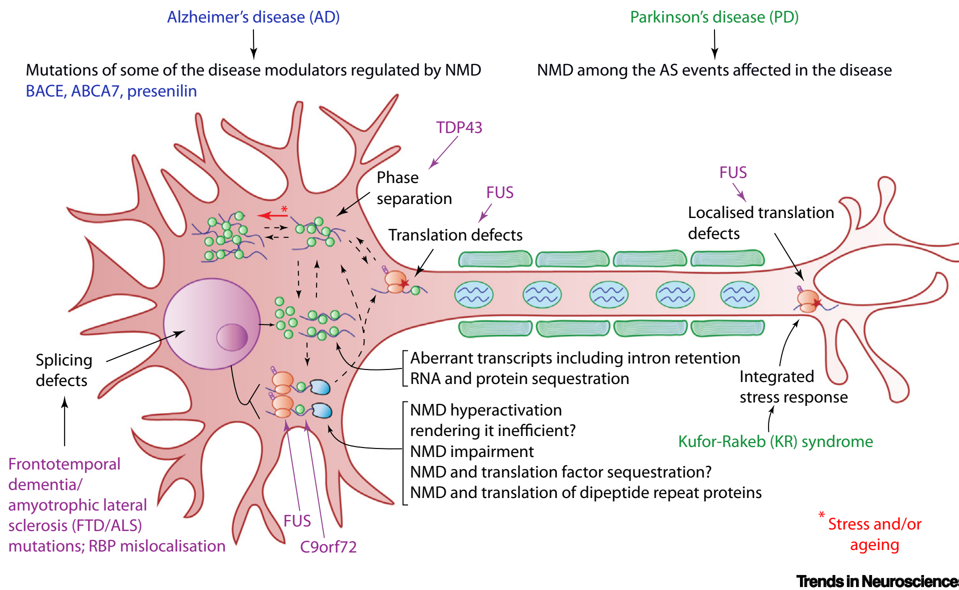


Figure 3. Alzheimer's disease (AD) is highlighted in blue. Parkinson's disease (PD) and Kufor Rakeb (KR) syndrome, a rare form of Parkinsonism, are highlighted in green. The details of how NMD is implicated in the pathogenesis of these diseases are illustrated and highlighted in text. Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are highlighted in purple. FTD/ALS mutations often reside in genes encoding splicing factors and affect their function and localisation, thus causing splicing defects that give rise to aberrant transcripts. This results in RNA and protein sequestration/phase separation, possibly stimulated by factors such as stress and ageing, ultimately leading to disease manifestation. For several mutations, defects in NMD and translation have also been observed at both a localised and global level. In addition, the aberrant transcripts sequester translation factors, which may impair translation elsewhere. This has been observed in ALS, either as a result of impaired translation of faulty transcripts and/or in response to unfolded protein and integrated stress responses. Abbreviations: AS, alternative splicing; RBP, RNA-binding protein.

NMD and neurodegeneration

FTD and ALS

FTD is a progressive neurodegenerative disorder characterised by changes in personality, behaviour, and language function owing to loss of neurons in the frontal and temporal lobes [40]. ALS in turn is a progressive neurodegenerative disease where loss of upper and lower motor neurons leads to paralysis, swallowing and speaking difficulties, and eventually respiratory failure [41–44]. Relative to ALS/FTD studies, examination of NMD in other forms of neurodegeneration, including Alzheimer's disease and Parkinson's disease, is still in its infancy, and is primarily limited to nonsense mutations and aberrant mRNA processing observed in some forms of the disease, as highlighted in Figure 3. For this reason we focus here on ALS/FTD.

Both familial and sporadic forms of ALS have been linked to dysregulation of RNA metabolism in motor neurons and nuclear-to-cytoplasmic mislocalisation of specific RBPs, either with or without their aggregation [45,46]. ALS is an age-related disease that might be triggered – at least in part – by cellular ageing in already vulnerable cells [47]. ALS motor neurons may already have a

predisposition for aberrant RNA and protein phase separation but exist in a state of compensated dysfunction. Stress and/or cellular ageing could tip the cells into decompensated dysfunction which may result in clinical manifestation and indeed progression [48,49]. If the cells are already prone to deregulated phase-separation dynamics, a stress response could promote further pathological aggregation, which in turn might hinder normal mRNA transport, localisation, and translation in axons and dendrites [48,50,51]. The cells may ultimately become unable to undergo appropriate activation upon extrinsic stimuli. Global repression of translation and in particular NMD may cause the accumulation of aberrant transcripts, natural NMD targets, and faulty transcripts that arise due to RBP mislocalisation which could also contribute to aberrant phase transitions. Alternatively, NMD might become hyperactivated in a compensatory manner to combat the accumulation of faulty transcripts. Such homeostatic dysregulation could progress over time and ultimately induce toxicity and initiate cell death, highlighting the importance of exploring the link between NMD and disease further [12].

Familial FTD, ALS, and ALS with FTD have variable genetic backgrounds, but a hexanucleotide repeat expansion (HRE) of a GGGGCC (G_4C_2) sequence in the first intron of the *C9orf72* gene is the most common mutation underlying these diseases [52–54]. Healthy individuals typically contain up to 30 repeats within the gene, whereas individuals who develop these diseases exhibit between 700 and 1600 repeats [55]. mRNA translation and NMD are heavily implicated in these types of neurodegeneration, as detailed below, and the best-characteristic examples are schematised in Figure 3. At least in part, *C9orf72* pathogenesis occurs as a consequence of a non-canonical form of mRNA translation, termed repeat-associated non-AUG (RAN) translation, that leads to the production of dipeptide repeat proteins (DPR proteins) [56,57]. These proteins can interfere with nucleocytoplasmic mRNA and protein trafficking, leading to pathological protein aggregation and further exacerbating defects in transcript localisation and metabolism [12,58]. Indeed, global mislocalisation of proteins towards a more cytoplasmic proteome was observed in HEK cells expressing a C9orf72 pathogenic repeat expansion [12]. The key proteins identified are involved in mRNA processing and translation, in particular eRF1 (a release factor and regulator of translation termination, peptide release, and ribosome recycling) that is also important for triggering NMD [59]. In cells with a pathogenic number of repeats, this protein appears to reside on the cytoplasmic side of nuclear membrane invaginations. An increased presence of UPF1 was also observed in these structures [12]. It is possible that UPF1 is pooled to HRE transcripts to eliminate them by NMD. Alternatively, UPF1 might exhibit NMD-independent roles in relation to these transcripts. Nonetheless, if UPF1 and eRF1 are possibly being sequestered within cytoplasmic invaginations of the nuclear membrane, this raises the question of whether their function is impaired elsewhere in the cell. One might also speculate that NMD integrity could become impaired by UPF1 sequestration to stress granules, as observed in some cases of repetitive expansions. However, in discord with this possibility, it appears that stress granule formation and NMD inhibition are independent consequences of DPRs and that the former does not determine the latter [60]. Instead, it seems that the NMD deficit observed in the *C9orf72* post-mortem brain by assessing a panel of putative NMD targets is more likely caused by DPR-mediated translational repression. Moreover, UPF1 exhibited protective effects on the survival of primary cortical neurons treated with PR20 ($ProArg_{20}$) DPR proteins, but its NMD-deficient mutants did not, suggesting that any UPF1 therapeutic benefit could be driven by its function in NMD. By contrast, by assessing a panel of five endogenous NMD targets, NMD seemed not to be affected in an induced pluripotent stem cell (iPSC)-derived neuronal model of *C9orf72* mutation [61], although it remains to be determined whether these findings would be generalised in systematic assessment of NMD status by looking at more targets across the genome. In a *Drosophila* model of this mutation, UPF1 overexpression reduced neurotoxicity, whereas its knockdown was deleterious, suggesting that promoting UPF1 function could have therapeutic benefits, which the authors of

this study argue are driven by UPF1 modulation of DPR levels rather than via effects on the transcripts themselves [61]. Altogether, even though the link between UPF1 and *C9orf72* -related ALS remains incompletely resolved, potential benefits observed in the aforementioned studies argue in favour of assessing this connection further in the hope of designing more informed therapeutic strategies. To reconcile seemingly divergent findings with regard to the role of NMD in *C9orf72* -mediated neurodegeneration with fidelity and precision, future research should consider that its role may be (i) disease stage-specific, (ii) developmental stage-specific, (iii) species-specific, and (iv) determined, at least in part, by heterologous cell–cell (e.g., neuron–glia) interactions. Beyond these studies, it will be crucial to determine the non-canonical roles of UPF1 in physiological and acute/chronic adverse contexts. Specifically, *Upf1* knockout is embryonic lethal [62], and its knockdown/overexpression will fundamentally alter cellular homeostasis. Therefore, although the aforementioned interventions may be of therapeutic benefit in some models of *C9orf72* -mediated neurodegeneration, the broader canonical and non-canonical actions of UPF1 on cellular physiology are of crucial importance to consider before determining its candidacy as a viable therapeutic target.

Nonsense mutations in the progranulin (*GRN*) gene can cause FTD. Knock-in mice for the most common *Grn* mutation, which introduces a PTC at position 493, exhibit reduced *Grn* mRNA levels, lack progranulin, and have several neurological defects [63]. These mice match *Grn* knockout mice, and exhibit TDP-43 accumulation in the cytoplasm and reduced synaptic activity [64]. The mutation-containing *Grn* mRNA isoform is an NMD target and is stabilised upon NMD inhibition. Furthermore, the truncated protein derived from the mutant transcript isoform is functional [63]; however, NMD inhibition as a potential therapeutic strategy remains to be explored in this disease context.

TDP-43 is an RBP involved in mRNA transport and localisation as well as in localised mRNA translation control, primarily of G-quadruplex-containing mRNAs [65]. Mutations in the *TARDBP* gene (which encodes TDP-43) have been demonstrated to cause ALS [66]. Moreover, ubiquitination, abnormal phosphorylation, cleavage, and aggregation of wild-type TDP-43 in the cytoplasm is the key hallmark of >95% of all ALS cases, with the exceptions of *FUS* and *SOD1* familial ALS. Notably, TDP-43 proteinopathy is also a pathological hallmark of ~45% of all FTD cases [41]. The *TARDBP* gene (which encodes TDP-43 protein) contains three alternative polyadenylation signals (PASs) as well as three alternative introns within the last exon, making it another RBP that can autoregulate its expression via alternative splicing-coupled NMD [67]. The protein switches to distal alternative PASs that trigger NMD once the canonical protein-coding transcript and protein levels are satisfactory. Upon reduction in transcript and protein level as a result of NMD activation, the proximal PAS is selected, and this increases the level of functional protein [67]. In healthy cells, *TARDBP* mRNA and TDP-43 protein levels in nuclear and cytoplasmic compartments depend on the balance between protein synthesis and NMD. However, *TARDBP* mutations identified in ALS switch this balance towards synthesising the protein that accumulates in the cytoplasm, potentially because of splicing and/or NMD defects [67]. Whether this balance could feasibly be restored via NMD manipulation is of particular interest, considering how widespread TDP-43 pathology is in ALS and beyond.

FUS is a DNA-binding protein and RBP, and *FUS* mutations have been identified in a subset of familial ALS cases [68,69]. Importantly, in addition to the relevance of FUS pathology in ALS, FUS pathology also characterises ~10% of all FTD cases [42]. This protein is predominantly nuclear; however, it also localises to neuronal dendrites, axon terminals, and neuromuscular junctions [70]. FUS can form ribonucleoprotein granules and plays key roles in splicing, mRNA processing, and localised translation [37]. In normal physiology, FUS regulates localised translation in axons [71,72] and modulates the activity and expression of ion channels [73], transporters,

and other proteins required for synaptic function [74]. Mutant FUS is mislocalised to the cytoplasm in motor neurons where it forms stable aggregates that are thought to contribute to pathogenesis [73]. It has also been shown that wild-type FUS can be mislocalised from the nucleus in sporadic ALS cases, but FUS inclusions do not form [46]. Mutant FUS accumulates within synaptic ends, triggering a local integrated stress response (ISR) which suppresses local translation and impairs synaptic transmission, thus reducing neuronal survival [72] (Figure 3). NMD factors reside within FUS inclusions [37]. In addition, UPF1, the phosphorylated active form of UPF1 (p-UPF1), UPF3B, and XRN1 are all upregulated in *FUS* mutant cells. By contrast, UPF3A was found to be downregulated. Even though the role of UPF3A in NMD is incompletely resolved, the cumulative data implicate hyperactivation of NMD in *FUS* mutant cells. In addition, UPF1 in *FUS* mutant cells coprecipitates more with mutant FUS and considerably less with its own mRNA and *UPF3B* mRNA, suggesting a potential NMD autoregulatory impairment [37]. NMD autoregulation is typically achieved through NMD factors binding to their own transcripts, thus modulating their levels in response to the NMD requirement [75,76], and mutant FUS could impair this process. Moreover, the levels of endogenous NMD targets are decreased in *FUS* mutant cells, further suggesting that NMD is activated to a higher degree [37]. How much the observed NMD impairment contributes to disease remains a salient issue to address.

UPF1, a key NMD regulator, was identified in a yeast genetic screen as an attenuator of TDP-43- and FUS-mediated cell toxicity [77]. UPF1 overexpression was also found to promote cell survival in primary rodent cortical neuron models of ALS [78]. Notably, overexpression of either wild-type or mutant TDP-43 or FUS significantly reduced neuronal survival of the mutant cells, whereas human UPF1 (hUPF1) overexpression led to a significant increase in survival. Overexpression of UPF1, however, did not rescue survival phenotypes in either the *SOD1* or Huntingtin (*HTT*) mutant cells used in the study [78]. This is possibly due to divergent disease mechanisms involving different pathways that are independent of UPF1. UPF1 seems to exert its protective effect on TDP-43 and FUS at least in part via NMD because NMD suppression via a small-molecule inhibitor followed by UPF1 overexpression had an attenuated, albeit still positive, effect on cell survival [78]. In addition, human UPF2 appeared to have beneficial effects on cellular survival in these disease models [78]. Overexpression of MOV10, which (much like UPF1) is a helicase of superfamily 1 (SF1) and has a recently described role in NMD, was also found to abrogate ALS phenotypes [78,79]. The therapeutic potential of UPF1 has been further explored in an *in vivo* study that used a rodent spinal cord TDP-43 overexpression model which results in progressive paralysis of the limbs [13]. Simultaneous overexpression of UPF1 appeared to abrogate some of the disease phenotypes. From a therapeutic perspective, it should be noted that UPF1 is a broad regulator of RNA metabolism, and its manipulation would not be straightforward because it would probably cause several off-target effects. An added complication is that our mechanistic understanding of how this protein and its roles are affected in different types of diseases is limited. With that, the observed beneficial effects of UPF1 modulation in preclinical studies argue in favour of further exploration.

Concluding remarks and future perspectives

NMD is a complex, spatiotemporally regulated, and context-specific process. It operates in cell type- and tissue-specific manners and comprises different pathways (canonical and non-canonical) which can work cooperatively or competitively within or between cells. This complex interplay of NMD pathways determines cellular and tissue homeostasis. The departure from homeostasis that often accompanies disease states may affect NMD and its inherent complexity in different ways at different stages of the disease. These points notwithstanding, NMD modulation as a therapeutic strategy could be particularly beneficial when an underlying cause or modulator of neuronal pathology is a mutation-derived PTC-harbouring mRNA. The appropriate strategy would be case-specific and would depend on the functional outcome of the mutation in question.

Outstanding questions

Components of the NMD machinery subserve different cellular functions, and understanding the relationship between these functions and the 'molecular logic' of their regulation is a pertinent question to address. This will advance our understanding of how NMD factors become deregulated in neurodegeneration and provide insights into therapeutically tractable targets. What is the full functional scope of NMD components?

Functional redundancy appears to be among the design principles of NMD activity. Layers of redundancy may allow a shift in the threshold for NMD activation in different states such as acute versus chronic adverse contexts and/or in a stimulus-specific fashion. Does NMD exhibit multi-level functional thresholding?

NMD appears to regulate functionally coherent subsets ('regulons') of RNAs in a context-dependent manner. This role of NMD and the impact on the RBPs bound to target RNAs requires further investigation. Could NMD serve to release RBPs bound to cytoplasmic transcripts, which could then relocalise to the nucleus to facilitate splicing? Might NMD have a further role in fine-tuning biomolecular ribonuclear condensates? Could these processes be perturbed in neurodegeneration?

A low-resolution view of NMD may assume that the process is spatially homogeneous in the cell. However, emerging evidence implicates heterogeneity in the subcellular localisation of NMD activity. How does a departure from NMD spatiotemporal homeostasis contribute to acute injury and neurodegeneration? Relatedly, is NMD differentially activated in different stages of a disease, between diseases, and in various cell types?

What are the potential side effects of NMD manipulation at the cell, tissue, organ, and organism levels, and how could these inform therapeutic candidacy?

Beyond disease-inducing nonsense mutations that could be directly targeted by NMD, NMD itself is affected in a range of disease models, and NMD modulation had beneficial outcomes for some of the phenotypes [13,78].

Localised NMD, such as ER-NMD as well as NMD limited to synaptic ends, is of increasingly recognised importance for cellular functions. However, the exact effects that localised NMD has on neuronal function as well as on disease onset and progression remain to be fully explored (see Outstanding questions). Beyond RNA quality control, NMD has recently been suggested to contribute to a form of protective adaptation through a mechanism known as 'transcriptional compensation' [80,81]. This notion proposes a compensatory mechanism to adapt to the harmful effects of a mutation by increasing the expression of a related gene (or possibly even a set of genes) with the capacity to counteract the otherwise negative consequences of the mutated gene. In the context of NMD, comprehensive understanding of such potential non-canonical functions would be important when considering the viability of this pathway as a target for therapeutic intervention. It is clear that merely activating or inhibiting NMD is an over-simplistic approach to disease therapy. A more nuanced approach, for example, targeting specific downstream factors in NMD, would probably be more tractable. Our review highlights the complexity of NMD and argues for careful investigation of the true granularity of its spatiotemporal regulation across different neurological diseases.

Acknowledgments

This work was supported by the Francis Crick Institute which receives its core funding from Cancer Research UK (FC010110), the UK Medical Research Council (MRC; FC010110), and the Wellcome Trust (FC010110). M.P.H. is funded through an MRC grant (MR/S006591/1) and R.P. holds an MRC Senior Clinical Fellowship (MR/S006591/1) and a Lister Research Prize Fellowship. Figures were prepared using Adobe Illustrator.

Declaration of interests

R.P. serves on the scientific advisory board of AstronauTx. M.P.H. declares no competing interests.

References

1. Kurosaki, T. *et al.* (2019) Quality and quantity control of gene expression by nonsense-mediated mRNA decay. *Nat. Rev. Mol. Cell Biol.* 20, 406–420
2. 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
3. Lloyd, J.P.B. (2018) The evolution and diversity of the nonsense-mediated mRNA decay pathway. *F1000Res.* 7, 1299
4. Nasif, S. *et al.* (2018) Beyond quality control: the role of nonsense-mediated mRNA decay (NMD) in regulating gene expression. *Semin. Cell Dev. Biol.* 75, 78–87
5. Lou, C.H. *et al.* (2014) Posttranscriptional control of the stem cell and neurogenic programs by the nonsense-mediated RNA decay pathway. *Cell Rep.* 6, 748–764
6. Goetz, A.E. and Wilkinson, M. (2017) Stress and the nonsense-mediated RNA decay pathway. *Cell. Mol. Life Sci.* 74, 3509–3531
7. Lennox, A.L. *et al.* (2018) RNA on the brain: emerging layers of post-transcriptional regulation in cerebral cortex development. *WIREs Dev. Biol.* 7, e290
8. Colak, D. *et al.* (2013) Regulation of axon guidance by compartmentalized nonsense-mediated mRNA decay. *Cell* 153, 1252–1265
9. Chen, Z. *et al.* (2008) Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. *Neuron* 58, 325–332
10. Farris, S. *et al.* (2014) Selective localization of arc mRNA in dendrites involves activity- and translation-dependent mRNA degradation. *J. Neurosci.* 34, 4481–4493
11. Jolly, L.A. *et al.* (2013) The UPF3B gene, implicated in intellectual disability, autism, ADHD and childhood onset schizophrenia regulates neural progenitor cell behaviour and neuronal outgrowth. *Hum. Mol. Genet.* 22, 4673–4687
12. Ortega, J.A. *et al.* (2020) Nucleocytoplasmic proteomic analysis uncovers eRF1 and nonsense-mediated decay as modifiers of ALS/FTD C9orf72 toxicity. *Neuron* 106, 90–107
13. Jackson, K.L. *et al.* (2015) Preservation of forelimb function by UPF1 gene therapy in a rat model of TDP-43-induced motor paralysis. *Gene Ther.* 22, 20–28
14. Kishor, A. *et al.* (2019) Nonsense-mediated mRNA decay: the challenge of telling right from wrong in a complex transcriptome. *Wiley Interdiscip. Rev. RNA* 10, e1548
15. Yi, Z. *et al.* (2021) The branched nature of the nonsense-mediated mRNA decay pathway. *Trends Genet.* 37, 143–159
16. Karousis, E.D. and Mühlemann, O. (2022) The broader sense of nonsense. *Trends Biochem. Sci.* 47, 921–935
17. Lavysh, D. and Neu-Yilik, G. (2020) UPF1-mediated RNA decay – danse macabre in a cloud. *Biomolecules* 10, 999
18. Longman, D. *et al.* (2020) Identification of a localized nonsense-mediated decay pathway at the endoplasmic reticulum. *Genes Dev.* 34, 1075–1088
19. Chamieh, H. *et al.* (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
20. Loh, B. *et al.* (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
21. Eberle, A.B. *et al.* (2009) SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells. *Nat. Struct. Mol. Biol.* 16, 49–55
22. Shum, E.Y. *et al.* (2016) The antagonistic gene paralogs Upf3a and Upf3b govern nonsense-mediated RNA decay. *Cell* 165, 382–395

23. Yi, Z. *et al.* (2022) Mammalian UPF3A and UPF3B can activate nonsense-mediated mRNA decay independently of their exon junction complex binding. *EMBO J.* 41, e109202
24. Wallmeroth, D. *et al.* (2022) Human UPF3A and UPF3B enable fault-tolerant activation of nonsense-mediated mRNA decay. *EMBO J.* 41, e109191
25. Lin, L. *et al.* (2020) Developmental attenuation of neuronal apoptosis by neural-specific splicing of Bak1 microexon. *Neuron* 107, 1180–1196
26. Vuong, J.K. *et al.* (2022) Multilayered regulations of alternative splicing, NMD, and protein stability control temporal induction and tissue-specific expression of TRIM46 during axon formation. *Nat. Commun.* 13, 2081
27. Laumonnier, F. *et al.* (2010) Mutations of the UPF3B gene, which encodes a protein widely expressed in neurons, are associated with nonspecific mental retardation with or without autism. *Mol. Psychiatry* 15, 767–776
28. Zou, D. *et al.* (2015) A critical role of RBM8a in proliferation and differentiation of embryonic neural progenitors. *Neural Dev.* 10, 18
29. Mao, H. *et al.* (2015) Rbm8a haploinsufficiency disrupts embryonic cortical development resulting in microcephaly. *J. Neurosci.* 35, 7003–7018
30. Jaworski, A. *et al.* (2010) Collaborative and specialized functions of Robo1 and Robo2 in spinal commissural axon guidance. *J. Neurosci.* 30, 9445–9453
31. Notaras, M. *et al.* (2020) UPF2 leads to degradation of dendritically targeted mRNAs to regulate synaptic plasticity and cognitive function. *Mol. Psychiatry* 25, 3360–3379
32. Magee, J.C. and Grienberger, C. (2020) Synaptic plasticity forms and functions. *Annu. Rev. Neurosci.* 43, 95–117
33. Zheng, S. *et al.* (2012) PSD-95 is post-transcriptionally repressed during early neural development by PTBP1 and PTBP2. *Nat. Neurosci.* 15, 381–388
34. Nguyen, L.S. *et al.* (2013) Contribution of copy number variants involving nonsense-mediated mRNA decay pathway genes to neuro-developmental disorders. *Hum. Mol. Genet.* 22, 1816–1825
35. Deka, B. *et al.* (2021) Functional roles of human up-frameshift suppressor 3 (UPF3) proteins: from nonsense-mediated mRNA decay to neurodevelopmental disorders. *Biochimie* 180, 10–22
36. Johnson, J.L. *et al.* (2019) Inhibition of Upf2-dependent nonsense-mediated decay leads to behavioral and neurophysiological abnormalities by activating the immune response. *Neuron* 104, 665–679
37. Kamelgarn, M. *et al.* (2018) ALS mutations of FUS suppress protein translation and disrupt the regulation of nonsense-mediated decay. *Proc. Natl. Acad. Sci. U. S. A.* 115, E11904–E11913
38. Lee, P.J. *et al.* (2021) Regulation of nonsense-mediated mRNA decay in neural development and disease. *J. Mol. Cell Biol.* 13, 269–281
39. Jaffrey, S.R. and Wilkinson, M.F. (2018) Nonsense-mediated RNA decay in the brain: emerging modulator of neural development and disease. *Nat. Rev. Neurosci.* 19, 715–728
40. Chung, D.-E.C. *et al.* (2017) *Neurobiology of FTD*, Oxford Medicine Online
41. Ling, S.-C. *et al.* (2013) Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* 79, 416–438
42. Ferrari, R. *et al.* (2011) FTD and ALS: a tale of two diseases. *Curr. Alzheimer Res.* 8, 273–294
43. van den Bos, M.A.J. *et al.* (2019) Pathophysiology and diagnosis of ALS: insights from advances in neurophysiological techniques. *Int. J. Mol. Sci.* 20, 2818
44. Mejzini, R. *et al.* (2019) ALS genetics, mechanisms, and therapeutics: where are we now? *Front. Neurosci.* 13, 1310
45. Luisier, R. *et al.* (2018) Intron retention and nuclear loss of SFPQ are molecular hallmarks of ALS. *Nat. Commun.* 9, 2010
46. Tyzack, G.E. *et al.* (2019) Widespread FUS mislocalization is a molecular hallmark of amyotrophic lateral sclerosis. *Brain* 142, 2572–2580
47. Pandya, V.A. and Patani, R. (2020) Decoding the relationship between ageing and amyotrophic lateral sclerosis: a cellular perspective. *Brain* 143, 1057–1072
48. Khalfallah, Y. *et al.* (2018) TDP-43 regulation of stress granule dynamics in neurodegenerative disease-relevant cell types. *Sci. Rep.* 8, 7551
49. Patel, A. *et al.* (2015) A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162, 1066–1077
50. Kapur, M. *et al.* (2017) Regulation of mRNA translation in neurons – a matter of life and death. *Neuron* 96, 616–637
51. Alberti, S. *et al.* (2017) Granulostasis: protein quality control of RNP granules. *Front. Mol. Neurosci.* 10, 84
52. DeJesus-Hernandez, M. *et al.* (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256
53. Renton, A.E. *et al.* (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72, 257–268
54. Daoud, H. *et al.* (2012) C9orf72 hexanucleotide repeat expansions as the causative mutation for chromosome 9p21-linked amyotrophic lateral sclerosis and frontotemporal dementia. *Arch. Neurol.* 69, 1159–1163
55. Lee, Y.-B. *et al.* (2013) Hexanucleotide repeats in ALS/FTD form length-dependent RNA foci, sequester RNA binding proteins, and are neurotoxic. *Cell Rep.* 5, 1178–1186
56. Mori, K. *et al.* (2013) The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science* 339, 1335–1338
57. Zu, T. *et al.* (2013) RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. *Proc. Natl. Acad. Sci. U. S. A.* 110, E4968–E4977
58. Jovičić, A. *et al.* (2015) Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat. Neurosci.* 18, 1226–1229
59. Zhouravleva, G. *et al.* (1995) Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. *EMBO J.* 14, 4065–4072
60. Sun, Y. *et al.* (2020) C9orf72 arginine-rich dipeptide repeats inhibit UPF1-mediated RNA decay via translational repression. *Nat. Commun.* 11, 3354
61. Zaepfel, B.L. *et al.* (2021) UPF1 reduces C9orf72 HRE-induced neurotoxicity in the absence of nonsense-mediated decay dysfunction. *Cell Rep.* 34, 108925
62. Medghalchi, S.M. *et al.* (2001) Rent1, a trans-effector of nonsense-mediated mRNA decay, is essential for mammalian embryonic viability. *Hum. Mol. Genet.* 10, 99–105
63. Nguyen, A.D. *et al.* (2018) Murine knockin model for progranulin-deficient frontotemporal dementia with nonsense-mediated mRNA decay. *Proc. Natl. Acad. Sci. U. S. A.* 115, E2849–E2858
64. Wils, H. *et al.* (2012) Cellular ageing, increased mortality and FTLD-TDP-associated neuropathology in progranulin knockout mice. *J. Pathol.* 228, 67–76
65. Ishiguro, A. *et al.* (2016) TDP-43 binds and transports G-quadruplex-containing mRNAs into neurites for local translation. *Genes Cells* 21, 466–481
66. Sreedharan, J. *et al.* (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319, 1668–1672
67. Koyama, A. *et al.* (2016) Increased cytoplasmic TARDBP mRNA in affected spinal motor neurons in ALS caused by abnormal autoregulation of TDP-43. *Nucleic Acids Res.* 44, 5820–5836
68. Vance, C. *et al.* (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323, 1208–1211
69. Lagier-Tourenne, C. *et al.* (2010) TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum. Mol. Genet.* 19, R46–R64
70. Birsa, N. *et al.* (2020) Cytoplasmic functions of TDP-43 and FUS and their role in ALS. *Semin. Cell Dev. Biol.* 99, 193–201
71. Sévigny, M. *et al.* (2020) FUS contributes to mTOR-dependent inhibition of translation. *J. Biol. Chem.* 295, 18459–18473
72. López-Erauskin, J. *et al.* (2020) ALS/FTD-linked mutation in FUS suppresses intra-axonal protein synthesis and drives disease without nuclear loss-of-function of FUS. *Neuron* 106, 354
73. Udagawa, T. *et al.* (2015) FUS regulates AMPA receptor function and FTLD/ALS-associated behaviour via GluA1 mRNA stabilization. *Nat. Commun.* 6, 7098

74. Wang, J.-Y. *et al.* (2023) Brain region-specific synaptic function of FUS underlies the FTLD-linked behavioural disinhibition. *Brain* 146, 2107–2119
75. Huang, L. *et al.* (2011) RNA homeostasis governed by cell type-specific and branched feedback loops acting on NMD. *Mol. Cell* 43, 950–961
76. Yepiskoposyan, H. *et al.* (2011) Autoregulation of the nonsense-mediated mRNA decay pathway in human cells. *RNA* 17, 2108–2118
77. Ju, S. *et al.* (2011) A yeast model of FUS/TLS-dependent cytotoxicity. *PLoS Biol.* 9, e1001052
78. Barmada, S.J. *et al.* (2015) Amelioration of toxicity in neuronal models of amyotrophic lateral sclerosis by hUPF1. *Proc. Natl. Acad. Sci. U. S. A.* 112, 7821–7826
79. Gregersen, L.H. *et al.* (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
80. El-Brolosy, M.A. *et al.* (2019) Genetic compensation triggered by mutant mRNA degradation. *Nature* 568, 193–197
81. Ma, Z. *et al.* (2019) PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components. *Nature* 568, 259–263
82. Applequist, S.E. *et al.* (1997) Cloning and characterization of HUPF1, a human homolog of the *Saccharomyces cerevisiae* nonsense mRNA-reducing UPF1 protein. *Nucleic Acids Res.* 25, 814–821
83. Chakrabarti, S. *et al.* (2011) Molecular mechanisms for the RNA-dependent ATPase activity of Upf1 and its regulation by Upf2. *Mol. Cell* 41, 693–703
84. Lloyd, J.P.B. and Davies, B. (2013) SMG1 is an ancient nonsense-mediated mRNA decay effector. *Plant J.* 76, 800–810
85. Yamashita, A. *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
86. Zhu, L. *et al.* (2019) Cryo-EM structure of SMG1–SMG8–SMG9 complex. *Cell Res.* 29, 1027–1034
87. Brogna, S. *et al.* (2016) The meaning of NMD: translate or perish. *Trends Genet.* 32, 395–407
88. Pervouchine, D. *et al.* (2019) Integrative transcriptomic analysis suggests new autoregulatory splicing events coupled with nonsense-mediated mRNA decay. *Nucleic Acids Res.* 47, 5293–5306
89. Pan, Q. *et al.* (2008) Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat. Genet.* 40, 1413–1415
90. Pan, Q. (2006) Quantitative microarray profiling provides evidence against widespread coupling of alternative splicing with nonsense-mediated mRNA decay to control gene expression. *Genes Dev.* 20, 153–158
91. Lareau, L.F. *et al.* (2007) Unproductive splicing of SR genes associated with highly conserved and ultraconserved DNA elements. *Nature* 446, 926–929
92. Saltzman, A.L. *et al.* (2008) Regulation of multiple core spliceosomal proteins by alternative splicing-coupled nonsense-mediated mRNA decay. *Mol. Cell. Biol.* 28, 4320–4330
93. Zheng, S. (2016) Alternative splicing and nonsense-mediated mRNA decay enforce neural specific gene expression. *Int. J. Dev. Neurosci.* 55, 102–108
94. Paillusson, A. *et al.* (2005) A GFP-based reporter system to monitor nonsense-mediated mRNA decay. *Nucleic Acids Res.* 33, e54
95. Boelz, S. *et al.* (2006) A chemiluminescence-based reporter system to monitor nonsense-mediated mRNA decay. *Biochem. Biophys. Res. Commun.* 349, 186–191
96. Hoek, T.A. *et al.* (2019) Single-molecule imaging uncovers rules governing nonsense-mediated mRNA decay. *Mol. Cell* 75, 324–339
97. Boehm, V. *et al.* (2014) 3′UTR length and messenger ribonucleoprotein composition determine endocleavage efficiencies at termination codons. *Cell Rep.* 9, 555–568
98. Hamid, F.M. and Makeyev, E.V. (2014) Emerging functions of alternative splicing coupled with nonsense-mediated decay. *Biochem. Soc. Trans.* 42, 1168–1173
99. Hurt, J.A. *et al.* (2013) Global analyses of UPF1 binding and function reveal expanded scope of nonsense-mediated mRNA decay. *Genome Res.* 23, 1636–1650
100. Heyer, E.E. and Moore, M.J. (2016) Redefining the translational status of 80S ,onosomes. *Cell* 164, 757–769
101. Karousis, E.D. *et al.* (2021) Nanopore sequencing reveals endogenous NMD-targeted isoforms in human cells. *Genome Biol.* 22, 223
102. Addington, A.M. *et al.* (2011) A novel frameshift mutation in UPF3B identified in brothers affected with childhood onset schizophrenia and autism spectrum disorders. *Mol. Psychiatry* 16, 238–239
103. Tarpey, P.S. *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
104. Neu-Yilik, G. *et al.* (2017) Dual function of UPF3B in early and late translation termination. *EMBO J.* 36, 2968–2986
105. Mühlemann, O. and Karousis, E.D. (2017) New functions in translation termination uncovered for NMD factor UPF3B. *EMBO J.* 36, 2928–2930
106. Bufton, J.C. *et al.* (2022) Structures of nonsense-mediated mRNA decay factors UPF3B and UPF3A in complex with UPF2 reveal molecular basis for competitive binding and for neurodevelopmental disorder-causing mutation. *Nucleic Acids Res.* 50, 5934–5947
107. Huang, L. *et al.* (2018) A Upf3b-mutant mouse model with behavioral and neurogenesis defects. *Mol. Psychiatry* 23, 1773–1786
108. Nguyen, L.S. *et al.* (2012) Transcriptome profiling of UPF3B/NMD-deficient lymphoblastoid cells from patients with various forms of intellectual disability. *Mol. Psychiatry* 17, 1103–1115