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.

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].

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.
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
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

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

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

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**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
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.
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-cyttoplasmic 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\_C\_G\_C\_G\_C) 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 (ProArg20) 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 terminals, 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.
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.

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