1038–1051 *Nucleic Acids Research, 2018, Vol. 46, No. 3* *Published online 20 December 2017*
doi: 10.1093/nar/gkx1272

SURVEY AND SUMMARY

Nonsense-mediated mRNA decay: a 'nonsense' pathway makes sense in stem cell biology

Xin Han†, Yanling Wei†, Hua Wang†, Feilong Wang, Zhenyu Ju and Tangliang Li*

Institute of Aging Research, School of Medicine, Hangzhou Normal University, Hangzhou, Zhejiang 310036, China

Received August 14, 2017; Revised December 05, 2017; Editorial Decision December 07, 2017; Accepted December 09, 2017

ABSTRACT

Nonsense-mediated mRNA decay (NMD) is a highly conserved post-transcriptional regulatory mechanism of gene expression in eukaryotes. Originally, NMD was identified as an RNA surveillance machinery in degrading 'aberrant' mRNA species with premature termination codons. Recent studies indicate that NMD regulates the stability of natural gene transcripts that play significant roles in cell functions. Although components and action modes of the NMD machinery in degrading its RNA targets have been extensively studied with biochemical and structural approaches, the biological roles of NMD remain to be defined. Stem cells are rare cell populations, which play essential roles in tissue homeostasis and hold great promises in regenerative medicine. Stem cells self-renew to maintain the cellular identity and differentiate into somatic lineages with specialized functions to sustain tissue integrity. Transcriptional regulations and epigenetic modulations have been extensively implicated in stem cell biology. However, post-transcriptional regulatory mechanisms, such as NMD, in stem cell regulation are largely unknown. In this paper, we summarize the recent findings on biological roles of NMD factors in embryonic and tissue-specific stem cells. Furthermore, we discuss the possible mechanisms of NMD in regulating stem cell fates.

INTRODUCTION

Nonsense-mediated mRNA decay (NMD) is an evolutionarily conserved post-transcriptional mechanism in regulating the gene expression in eukaryotic cells (1–11). Classically, NMD degrades mRNA species with premature termination codons (PTCs) or nonsense mutations to quench transcriptome noises (1,12). Around 12% of single nucleotide mutations found in human gene mutation database will generate mRNAs with the PTCs (13), which are occasionally associated to human diseases, such as β-thalassemia and Duchenne muscular dystrophy (14). Furthermore, genetic mutations in components of the NMD machinery are implicated in human neurological disorders, immune diseases and cancers (5,15). Thus, understanding the biological functions and mechanisms of NMD would be beneficial for designing strategies to treat PTC-generated human diseases by manipulating NMD activity, and to cure human genetic disorders arising from mutations in NMD factors.

Extensive biochemical and structural studies have identified key components of the NMD machinery and revealed how these NMD factors are orchestrated to degrade mRNA targets (1,6–8,16,17). In mammals, the NMD machinery includes a key phosphoinositide 3-kinase (PI3K) complex (SMG1, SMG8 and SMG9), UPF proteins (UPF1, UPF2, UPF3A and UPF3B), eukaryotic release factors (eRF1 and eRF3), exon junction complex (EJC) members (eIF4A3, RBM8A, MAGOH and MLN51) and SMG proteins (SMG5, SMG6 and SMG7), which trigger the degradation of mRNA targets (2,5,6,10,18). The major roles of these NMD components in the mRNA decay machinery are summarized in Table 1. In this review, we will not emphasize on initiation and execution mechanisms of NMD machinery since recently Schweingruber *et al.* (2), Karam *et al.* (3), Popp and Maquat (4), Lykke-Andersen and Jensen (5), He and Jacobson (6), Fatscher *et al.* (7), Hug *et al.* (8), Ottens and Gehring (9) and Karousis *et al.* (10) extensively reviewed how the NMD machinery is assembled onto its mRNA targets and mRNA decay is executed in the mammalian cells. In this review, we will first give a short introduction on features of NMD-targeted RNAs and roles of NMD factors as revealed by cellular studies. Further, we will mainly focus on discussions of NMD functions in mam-

*To whom correspondence should be addressed. Tel: +86 571 2886 1743; Fax: +86 571 2886 1695; Email: li.tangliang@hznu.edu.cn
†These authors contributed equally to the paper as first authors.

malian embryonic and tissue-specific stem cells and biological roles of NMD in mammals.

Diversity of NMD targets in a cell transcriptome

mRNAs with PTCs are classical targets of the NMD machinery. Recent findings suggest that transcripts of genes with physiological significances in cell functions are regulated by the NMD machinery (5). Since NMD affects the mRNA half-life, inhibition of NMD results in high levels of NMD target gene transcripts in a cell. In this regard, identification of highly represented DEGs (Differential Expression Genes) in NMD deficiency conditions is one of the major strategies in defining NMD targets (12,19–21). Recently, transcriptome-based strategies, such as microarray and RNA-Seq, have enabled the identifications of new groups of NMD targets with features of upstream open reading frame (uORF), long 3′ UTR, introns in 3′ UTR, etc. (2,5). Combing gene knockdown and microarray/RNA-Seq, Mendell *et al.,* Yepiskoposyan *et al.* and others found that NMD targets are enriched in mRNAs with features of PTC, 5′ uORFs, long 3′ UTRs and introns in 3′ UTRs (12,22). An integrated bioinformatic analysis on the RNA-Seq data generated from human cells with NMD factors UPF1, SMG6 or SMG7 gene knockdowns and rescue experiments further showed that SMG6 mediated endonucleolytic decay route and SMG5/7 mediated exonucleolytic decay route are largely redundant in degrading mRNAs with the feature of introns in 3′ UTR regions (21). This study further proposed that long non-coding RNAs, and transcripts from miRNA and snoRNA host genes could be regulated by the NMD machinery (21,23).

Although studies with DEG based bioinformatic analysis from microarray and RNA-Seq data have identified a series of NMD targets, it is difficult to distinguish whether an upregulated gene transcript upon NMD deficiency is a direct NMD target. To overcome this obstacle, Tani *et al.* developed BRIC-Seq (5′-bromo-uridine immunoprecipitation chase—deep sequencing analysis), a transcriptome wide approach to directly determine NMD targets with prolonged RNA half-lives in NMD factor depleted cells (24). By conducting BRIC-Seq in UPF1 knockdown human cells, new UPF1 targets with increased half-lives have been identified and experimentally validated (24). In recent years, PAR-CLIP (photoactivatable ribonucleotide enhanced crosslinking and immunoprecipitation) has been shown to be advantageous in the large scale and unbiased identification of RNA targets of RNA binding proteins (RBPs) (25). PAR-CLIP allows for a direct capture of RNA species bound to an RBP. With this method, serial studies have identified UPF1 specific RNA targets in mouse ESCs (25), HEK293 cells (26) and HeLa cells (27). Of note, the efficiency of PAR-CLIP relies largely on the specificity of antibody against RBPs. Thus, abundances of RNA targets as revealed by PAR-CLIP could be underestimated. Taken together, studies with these new techniques further confirm that NMD targets are beyond PTC containing mRNAs.

NMD is not a linear pathway, but rather with branches mediated by different NMD factors (5,8). For example, NMD branches mediated by SMG6 and SMG5/7 have preferences in their RNA targets (28), indicating NMD factors may selectively bind and degrade RNA targets through recognizing unique sequence motifs on RNAs. XRN1 degrades RNAs with its 5′-3′ exoribonuclease activity (29). Knockdown of XRN1 in mammalian cells leaves intact 3′ fragments of decay intermediates, which allows for the direct capture of sequence features of NMD targets. Ottens *et al.* employed a '3′ fragment capture and degradome sequencing' technique on human cell samples with XRN1 single and XRN1/SMG6 double knockdown and found that the SMG6-mediated endonucleolytic route degrades mRNAs with features of PTC, 5′ uORFs and long 3′ UTRs, while the SMG5/7-mediated exonucleolytic route mainly targets mRNAs with features of 5′ uORFs and long 3′ UTRs (30). Schmidt *et al.* used a similar method, namely 'Parallel Analysis of RNA Ends' MaxSeq (PARE MaxSeq), and identified the 5′ termini sequence composition of SMG6 mediated decay intermediates (31). More than 60% of SMG6 targets harbor a degenerate pentameric sequence motif [(U/A)-(G/A)↓(A/C)-N-(C/U)] at 5′ termini. Recently, by datamining on published datasets, including the RNA footprinting profile of phosphorylated UPF1, two sets of UPF1 PAR-CLIP data and the UPF1 target dataset generated with BIRC-Seq (24,32–34), Imamachi *et al.* found that UPF1 preferably binds RNAs with high GC-rich motifs embedded in 3′ UTRs (35).

The identifications of NMD targets from different cell lines extend our understandings on how NMD shapes the transcriptome. These studies with different sequencing and analyzing strategies show that NMD functions beyond an RNA surveillance machinery in degrading PTC containing mRNAs, but plays essential roles in post-transcriptional gene expression regulation in degrading mRNAs implicated in various cellular functions. Of note, NMD targeted mRNAs with 5′ uORFs and long 3′ UTRs are enriched in the categories of oncogenes, transcriptional factors and stress response genes (5,36). Thus, NMD could coordinate the activation or repression of different signaling pathways and function in every level of cell dynamics (37).

Functional complexity of NMD factors: hints from cellular studies

In line with the diversity of NMD targets in cells, RNAi-based knockdowns on NMD factors in human cells cause severe defects in cell viability (5,38,39). These studies reveal that NMD functions in cell cycle regulation (40,41), cell viability (42,43) and amino acid starvation response (44), through modulating RNA stability. Furthermore, NMD factors possess NMD-independent roles (Table 1 and Figure 1), which include DNA damage response (41,45,46), telomere integrity maintenance (38,47), intracellular calcium dynamics (11,48), virus infection and replication (49–52) and tumorigenesis (Figure 1 and Table 1). SMG1, UPF1, UPF2, SMG5, SMG6 and SMG7 are involved in DNA damage response and telomere integrity maintenance (20,38,40,41,45,46,53–55), both of which are essential for cell viability. SMG1, together with ATM and ATR, belongs to the family of PI3Ks (56). Upon DNA damage induction, SMG1 phosphorylates p53 and induces p53-dependent cell cycle arrest (41,45,55). Accordingly, knockdown of SMG1 renders human cells sensitive to ionizing radiation (55).

Table 1. Functions of NMD factors

NMD factors	NMD functions	NMD independent functions
SMG1	• Phosphorylate UPF1 • Promote the binding of SMG5/7 and SMG6 to UPF1	• Member of the PI3K family • Telomere integrity maintenance • Participate in DNA damage response by phosphorylating p53 and activating p53 dependent cell cycle arrest
SMG5	• Form complex with SMG7 and recruit deadenylase complex • Interact with PP2A and play a role in UPF1 dephosphorylation	• Telomere integrity maintenance
SMG6	• Possess the endoribonuclease activity and cleave NMD targets near PTCs • Interact with PP2A and play a role in UPF1 dephosphorylation	• Telomere integrity maintenance • Participate in DNA damage response in human immortal cells (but not in murine cells)
SMG7	• Form complex with SMG5 and recruit deadenylase complex to NMD targets • Interact with PP2A and play a role in UPF1 dephosphorylation	• Telomere integrity maintenance • Participate in DNA damage response by regulating p53 protein stability
UPF1	• Recognize the stalled ribosomes on PTC containing mRNAs • Interact with UPF2/UPF3B to initiate the assembly of decay complex • Bind to mRNAs with long 3' UTRs and initiate mRNA decay	• DNA damage response • Bind to chromatin and regulate cell cycle S phase progression • Histone mRNA decay • Telomere integrity maintenance • UPF1 is an E3 ligase of the ubiquitin system • Staufen1-mediated mRNA decay
UPF2	• Bind to UPF1 and UPF3B • Facilitate the UPF1 phosphorylation by SMG1	• Telomere integrity maintenance • DNA damage response
UPF3B	• Minimum NMD activity • Interact with UPF2 and stimulate the ATPase and helicase activity of UPF1	• N.D.
UPF3A	• Compete with UPF3B in binding to UPF2 • Repress NMD	• N.D.

Note: N.D., not defined.

SMG5, SMG6 and SMG7 are mammalian homologs of yeast telomere length regulator Est1 (Ever shorter telomere 1) (57). The yeast *Est1* mutant shows a progressive loss of telomere length and the premature ageing phenotype (57). In human immortal cells, knockdown or overexpression of SMG5 and SMG6 compromises telomere integrity and cell viability (38,58,59). UPF1, with our current understanding, is an NMD factor with the most distinct functions reported. UPF1, as a core NMD factor, is involved in the degradation of PTC containing mRNAs and RNA species with other NMD features (12). UPF1 also participates in a variety of NMD independent processes, such as: (i) UPF1 is a downstream factor of activated PI3K signaling and could be phosphorylated by SMG1, ATM and ATR, indicating that UPF1 may directly take part in DNA damage response signaling (38,60); (ii) UPF1 is loaded onto chromatin in cell cycle S-phase and regulates S-phase progression (40,46); (iii) ATR-UPF1 axis is responsible for the degradation of replication-dependent histone mRNAs (61,62), indicating a role of UPF1 in balancing the DNA synthesis and histone mRNA translation during S-phase progression; (iv) UPF1 is associated with telomeres and maintains telomere integrity (54,63); (v) UPF1 is a component of ubiquitin system and has E3 ubiquitin ligase activity (64); (vi) UPF1 sits at the intercross of Staufen1-mediated mRNA decay (SMD) and NMD through a competitive binding by Staufen 1 or UPF2 (65). Therefore, UPF1 may selectively participate in SMD or NMD, depending on cellular states (66). Taken together, NMD factors function beyond NMD machinery. Thus, it is not clear whether the severe cellular phenotypes as shown with RNAi-based strategies are mediated by NMD.

Due to the functional complexity of NMD factors (Table 1), defining the biological roles of NMD *per se* with cell and animal models could be challenging (67,68). For example, DNA damage response, one of the NMD factors' common functions, has been proposed in regulating stem cell dynamics, i.e. self-renewal and differentiation (56,69,70). Genomic instability imposed by defective DNA damage repair machinery occasionally results in early embryonic lethality of mammals (71). Thus, using an NMD factor with minimum NMD-independent functions could be optimal for elucidating roles of NMD. Among these NMD factors that have been reported with established mouse models, SMG6, UPF2 and UPF3A could be ideal for dissecting *in vivo* roles of NMD *per se* since these NMD factors possess minimum NMD-independent functions (Table 1). For example, SMG6 has been implicated in the NMD and telomere maintenance through stimulating telomerase activity to elongate telomeres (38). Since mouse chromosomes have longer telomere reservoirs as compared with humans, telomere dysfunctions do not generate any developmental

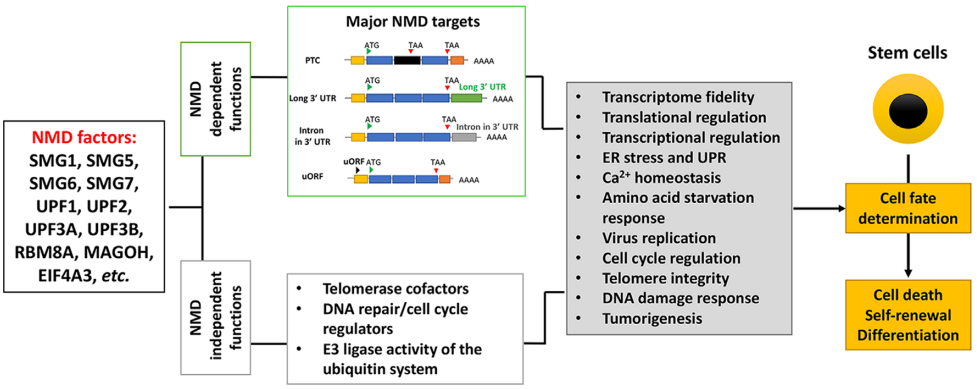

Figure 1. NMD machinery regulates stem cell fates though NMD-dependent and NMD-independent functions. NMD machinery degrades PTC containing mRNAs to maintain transcriptome fidelity, and regulates the stability of mRNAs with features of 5′ uORFs, long 3′ UTRs, introns in 3′ UTRs, etc. to modulate gene expression. In addition, NMD factors play important roles through their mRNA decay-independent functions, such as acting as telomerase cofactors, DNA repair/cell cycle regulators or an E3 ligase of the ubiquitin system. Through these NMD-dependent and -independent functions, NMD factors regulate a series of cellular processes (summarized in gray box) and consequentially control stem cell fates, such as self-renewal, differentiation and even cell death.

defect or pathological abnormality in the first generation of telomerase deficient mice (72). Thus, the biological defects as revealed with Smg6 deficient cells and mice could be largely independent of telomere maintenance function of Smg6, but represent Smg6-NMD roles (20).

The essential role of NMD in animal development

The development of an animal starts from a fertilized totipotent egg. In mouse, a fertilized egg undergoes at least four rounds of symmetric cell divisions to form a blastocyst at embryonic stage 3.5 (E3.5) (73), which contains an outer layer, the trophectoderm and an inner cell mass (ICM) with around 32 pluripotent cells. The pluripotent cells in the ICM divide quickly to reach around 100 cells, namely epiblast, at E4.5. During this period, the multipotent primitive endoderm cells are formed to cover the blastocoelic surface of the epiblast. The epiblast cells still maintain pluripotency, and could give rise to embryo proper by differentiating into three germ layers, i.e. ectoderm, endoderm and mesoderm (74). After the implantation of mouse embryos in the uterus finishing around E6.5, the gastrulation starts (73). During gastrulation (E6.5–E7.5), precursors of different germ layers are formed from the epiblast, and experience complicated patterning steps in the embryo to form a framework for further mouse organogenesis (75). From E8.0, mouse organogenesis starts at different ontological sites of the embryo and finally forms different tissues with specific functions (76). The progression of developmental stages is strictly controlled by temporal and spatial regulations in gene expression, abnormalities of which could generate developmental defects and embryo lethality.

The activity of NMD is a dynamic process during animal development (77–79). Accordingly, in zebrafish embryos, knockdown of any of the NMD factors, such as Upf1, Upf2, Smg1, Smg5 and Smg6, results in massive cell death in embryonic tissues and animal lethality (80). In mice, genetic ablations of NMD factors, such as Smg1 (19), Smg6 (20), Upf1 (81), Upf2 (82) and Upf3a (83), as well as EJC component Rbm8a (84) and Magoh (85), cause early embryonic lethality (Figure 2). The Upf3b knockout mouse generated with gene trap strategy is a viable NMD-deficient mouse model, possibly due to the fact that Upf3b deletion does not completely abolish the NMD activity (86,87). Of note, although NMD factors have different roles as mentioned above (Table 1), NMD factor knockout mice die around a similar developmental stage (Figure 2). Smg1 gene-trapped mouse embryos have normal Mendelian ratios before E10.5, but all die before E12.5 (19); Upf1 knockout mouse embryos are viable at E3.5, but die before E7.5 (81); The relative frequency of Upf2 knockout mouse embryos shows a great reduction at E3.5, and no Upf2 knockout embryos could be detected at E9.5 (82). Furthermore, Smg6 knockout embryos are viable at E3.5, but no viable mutant embryos could be isolated around E12.5. Most of Smg6 knockout embryos die around E7.5–E9.5 (20). Recently, Shum *et al.* found that Upf3a (an NMD repressor) knockout mice die between E4.5 and E8.5 (83). Taken together, these studies reveal that embryos of NMD factor mutant mice could successfully develop to E4.5 before the start of the primitive streak, and die between E5.5 and E9.5, during which gastrulation and early stages of organogenesis occur. These data strongly suggest that NMD *per se* is not essential for the maintenance or proliferation of murine pluripotent stem cells (PSCs) from the ICM and epiblast. The fact that all NMD factor mutant embryos die during gastrulation or early stages of organogenesis indicates that NMD is essential for the early development of mice, when the pluripotent cells start to specify into three different germ layers. In the future, in-depth analysis by dissecting NMD mutant mice embryos at different developmental stages and immunocytochemistry investigations with lineage-specific markers will resolve the biological roles of NMD in early mammalian development.

NMD in embryonic stem cell self-renewal and differentiation

The embryonic stem cell (ESC) is an important model to study the fundamental mechanisms of developmental biology (88,89). The establishment of ESC lines from ICMs of blastocysts greatly facilitates our investigation on the mechanisms of ESC identity maintenance during self-renewal

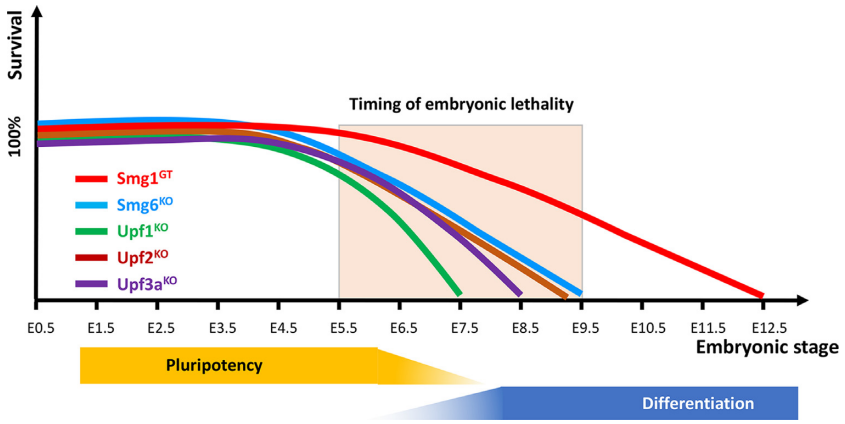

Figure 2. Embryonic lethality in knockout mice of NMD factors. *Smg1* mutant mice generated with gene trap strategy ($Smg1^{GT}$), as well as *Smg6*, *Upf1*, *Upf2* and *Upf3a* conventional knockout (KO) mice, could develop until embryonic stage E4.5 and die within a narrow development window (E5.5–E9.5). During E5.5–E9.5, pluripotent epiblast cells commit to three germ layers (ectoderm, endoderm and mesoderm) and mouse organogenesis starts. Current studies suggest that NMD *per se* may be dispensable for the proliferation of mouse pluripotent cells, but essential for the transition from pluripotency toward differentiation.

and cell fate commitment to ectoderm, endoderm and mesoderm (89). The successful reprograming of human patient derived somatic cells to PSCs enlightens the promise for applications of ESCs and PSCs in regenerative medicine (16,90). Transcriptional regulation and epigenetic modulation mechanisms are implicated in ESC self-renewal and differentiation (91–95). Recently, NMD has emerged as an important regulatory mechanism in ESC biology.

Although mouse embryos with NMD factor deficiency are viable at E3.5 (96), none succeeds in establishing a stable NMD deficient mouse ESC (mESC) line by outgrowing ICMs from blastocysts. Gene knockdown approaches with siRNAs or shRNAs are widely used to dissect NMD roles in mESCs (78,86). By analyzing the function of Upf1 in a mouse embryonic carcinoma cell line (P19), Lou *et al.* found that overexpression of Upf1 promotes self-renewal of P19 cells (78) (Table 2 and Figure 3A, upper panel). Knockdown of Upf1 reduces the expression of stemness markers (Nanog and Oct4), and simultaneously enhances the expression of neuronal lineage markers (Sox2 and Nestin), indicating that Upf1 knockdown accelerates P19 embryonic carcinoma cell differentiation toward ectoderm (78). Mechanistically, Lou *et al.* showed that gene transcripts of TGF-β signaling pathway members, such as Smad6 and Smad7, are targeted by Upf1-NMD (78). Intriguingly, using the E14.1 mESC line, which is widely used in gene targeting studies (97), Li *et al.* found that NMD safeguards the proper differentiation of mESCs (20). Stable knockdowns of NMD factors, such as Smg1, Smg5, Smg6, Upf1, and Upf2, in E14.1 mESCs generate variable mESC clones with different extents of NMD defects, indicating that NMD is dispensable for the cellular viability of mESCs (20) (Table 2). Furthermore, by transient expression of Cre recombinase in mESCs with two conditional alleles of *Smg6* gene ($Smg6^{F/F}$ mESCs), Li *et al.* generated several lines of Smg6 knockout mESCs. Smg6 knockout mESCs behave indistinguishably from wild-type mESCs in cellular proliferation, cell cycle distribution and cell death index (20). The major defect of Smg6 knockout mESCs and Smg1, Smg5, Upf1 and Upf2 stable knockdown mESCs is the differentiation failure. Mechanistically, Smg6-NMD, by targeting 3′ UTR of *c-Myc* mRNA, destabilizes pluripotent gene *c-Myc* mRNAs and thus promotes mESCs differentiation (Figure 3A, upper panel). Furthermore, ectopic expressions of NMD proficient Smg6 truncated proteins in Smg6 knockout mESCs could rescue differentiation defects. These findings strongly suggest that: (i) Smg6-NMD activity is dispensable for the cell viability of mESCs, but essential for the pluripotency exit; (ii) The embryonic lethality of NMD factor knockout mice is most likely due to an essential role of NMD in mESC differentiation.

Compared to mESCs, NMD plays distinct roles in human ESCs (20,78,98). Lou *et al.* employed a human ESC line (H9) and found that NMD factors are highly expressed in human PSCs, indicating that NMD promotes pluripotency in human ESCs (98). Knockdown of UPF1 drives H9 cells differentiation toward endoderm lineage by promoting the TGF-β signaling, and suppresses mesoderm lineage through inhibiting the BMP/WNT signaling (Table 2 and Figure 3A, lower panel). Thus, UPF1 mediated NMD may be functionally involved in human ESC fate commitment toward different germ layers (98). Taken together, current studies in human and mouse ESCs reveal a dynamic requirement of NMD during ESC differentiation, but NMD functions in stem cell self-renewal and differentiation remain controversial, which could be attributed to the following facts. First, human and mouse ESCs in different culture condition may require distinct maintenance and differentiation programs, which could generate distinct responses and biological outputs upon NMD deficiencies (98–100). Second, different gene manipulation strategies are used in these studies. Li *et al.* used the complete gene knockout strategy in mESCs; Lou *et al.* applied an RNAi knockdown strategy, which may not completely deplete NMD factors in ESCs. Thirdly, different NMD factors are investigated in these studies and the NMD-independent functions of these NMD factors could be partially responsible for these discrepancies (20,78,98). Since gene editing tools, such as TALEN and CRISPR-Cas9, are routinely used to generate gene knockout human and mouse ESCs

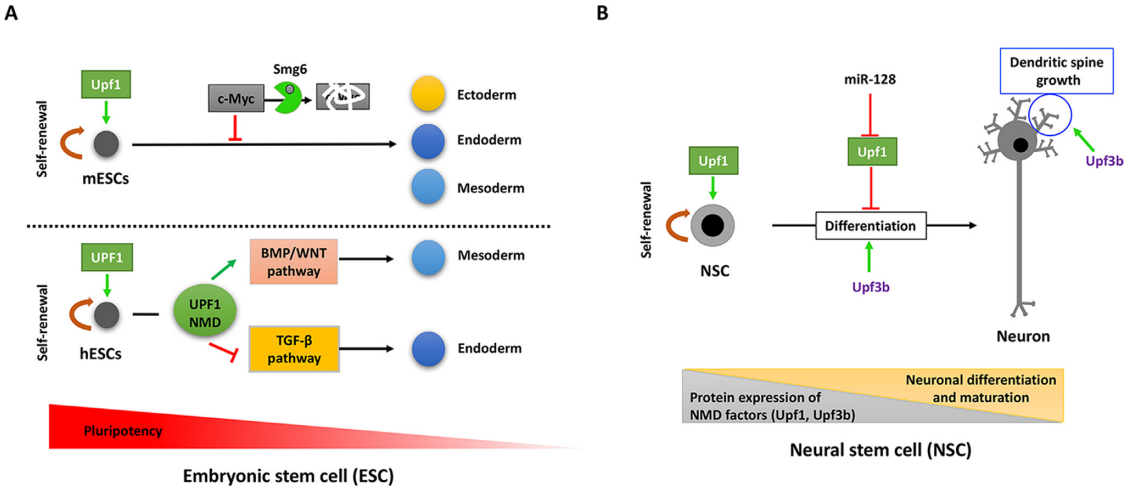

Figure 3. NMD function in ESCs (**A**) and NSCs (**B**). (A) Essential roles of NMD factors in the self-renewal and differentiation of mouse (upper panel) and human (lower panel) ESCs. Upper panel: NMD factor Upf1 promotes the self-renewal of mouse ESCs; During differentiation, Smg6 mediated NMD destabilizes mRNA transcripts of pluripotency gene *c-Myc*, and thus safeguards mouse ESC differentiation into three germ layers. Lower panel: by destabilizing mRNA transcripts of key components in BMP/WNT and TGF-β pathways, UPF1 regulates human ESC fate commitment between mesoderm and endoderm. (B) NMD function in mouse NSC self-renewal and differentiation. RNA transcripts and protein levels of NMD factors (Upf1 and Upf3a) decrease during NSC differentiation and neuronal maturation. NMD factor Upf1 promotes NSC self-renewal and is repressed by miR128 during NSC differentiation; However, Upf3a-NMD is required for NSC differentiation and neuronal maturation, such as dendritic spine outgrowth.

(101–104), direct comparisons on different NMD factor deficient ESCs within same species could efficiently elucidate roles of NMD in ESC self-renewal and differentiation.

NMD in neural stem cell self-renewal and differentiation

Neural stem cells (NSCs) are originated from mammalian neuroepithelium (105). During the early development of mammalian brain, NSCs proliferate at the ventricular zone of the neocortex and differentiate into different types of functional neurons to build up the mammalian brain and establish proper brain functions (105,106). The mammalian neurogenesis relies on transcriptional regulation, epigenetic modulation, genomic stability maintenance and tight cell cycle control of NSCs (107–111). Abnormalities in neurogenesis may generate neurological diseases in humans. UPF3B is the first NMD factor found to be associated with human neurodevelopmental disorders (112). Patients with UPF3B mutations manifest neurological symptoms, such as intellectual disability, schizophrenia and autism (112,113). Recent studies by genomic sequencing in human patients with neurodevelopmental disorders identify mutations in UPF2, UPF3A, SMG6, SMG9 and EJC component RBM8A, indicating that NMD is essential for mammalian brain development (15,114,115). Furthermore, since NMD participates in synapse architecture maintenance and synaptic vesicle recycling and is thus required for functions of mature neurons, the neurological symptoms in patients with NMD factor mutations could be due to synergistic effects of prenatal brain development abnormalities and postnatal neuronal dysfunctions (87,116,117).

How does NMD safeguard the neurogenesis? Recent studies from mammalian NSCs reveal some mechanistic clues. mRNA transcripts of *Upf1*, *Upf2*, *Smg1* and *Smg6* were reduced during the differentiation process of mouse NSCs and human neural progenitors (78) (Figure 3B). Furthermore, protein levels of NMD factors, such as Upf1 and Upf3b, decrease during differentiation of cultured mouse and rat NSCs (78,118,119), indicating NMD may promote NSC self-renewal (Figure 3B). In accordance with this hypothesis, using a siRNA approach, Lou *et al.* found that Upf1 knockdown or Upf3b deficiency in NSCs could stimulate mouse NSC differentiation with upregulations of neuronal markers (78). Of note, in Lou *et al.*'s study, Upf1 knockdown enhances the expression of NSC markers Sox2 and Nestin as well as the mature neuron marker DCX (78). These data are paradoxical since mammalian neurogenesis is accompanied with downregulation of NSC markers and upregulation of neuronal markers. Since Sox2 and Nestin have been widely used as stem cell markers for NSCs as shown by many groups (107,120,121), the co-upregulations of NSC markers (Sox2 and Nestin) and neuronal maker (DCX) in NSCs with Upf1 knockdown may generate a transcriptome chaotic state in NSCs and consequentially compromise NSCs fate toward mature neurons. In line with this hypothesis, Alrahbeni *et al.* found that NMD inhibition by Amlexanox or overexpression of UPF3B missense mutation compromises the differentiation of NSC toward mature neuron (118). With mouse NSCs, Jolly *et al.* and Huang *et al.* further showed that Upf3b deficient NSCs are reluctant to differentiation (87,119). Upf3b deficient NSCs are hyper-proliferative and show higher self-renewal capacity in differentiation culture conditions (87,119). These data indicate that NMD promotes NSC differentiation *in vitro* and *in vivo*. Taken together, these data suggest that: (i) abundances of mRNAs and protein expression levels of NMD factors in cells may not reflect the magnitude of NMD activity; (ii) functions of NMD in NSC self-renewal and differentiation are under debate. Thus, future studies on NSCs with deficiencies in other NMD factors, such as Upf1, Smg5 and Smg6 will solve the discrepancies of current knowledge on NMD functions in NSCs. Since NSC differentiation is a

Table 2. Biological roles of NMD factors in embryonic and tissue specific stem cells

Cell type	Targeted gene	Stem cell and mouse phenotypes	Mechanisms	Ref.
Embryonic stem cell (ESC)	Upf1, knockdown in P19 cells (*in vitro*)	Upf1 promotes pluripotency of P19 cells; Upf1 knockdown promotes neuronal differentiation of P19 cells.	Upf1-NMD promotes pluripotency by destabilizing mRNAs of G1/S transition inhibitors, neuronal differentiation factors, and TGF-β inhibitors.	[78]
	Smg6, KO in mESC (*in vitro + in vivo*)	Smg6 KO mESCs have telomere defect, but no proliferation defect; Differentiation block is found in Smg6 KO mESCs.	Smg6-NMD destabilizes *c-Myc* mRNA and promotes mESC differentiation.	[20]
	Smg1/5/6, Upf1/2 knockdown in mESC (*in vitro*)	NMD factor knockdowns render differentiation block of mESCs.	N.D.	[20]
	Upf1, knockdown in H9 human ESC (*in vitro*)	Upf1 knockdown promotes endoderm lineage differentiation of hESC.	NMD inhibits TGF-β signaling, while facilitates BMP/WNT signaling.	[98]
Neural stem cell (NSC)	Upf3b KO in mNSCs (*in vitro + in vivo*)	Upf3b null promotes NSC proliferation, while inhibits differentiation.	N.D.	[119]
	Upf3b KO in mNSCs (*in vitro + in vivo*)	Upf3b null promotes NSC proliferation, while inhibits differentiation and neuronal maturation.	RNA-Seq has been conducted; no specific target was found to be responsible for the phenotype.	[87]
	Upf1, knockdown in mNSCs (*in vitro*)	Upf1 knockdown promotes NSC differentiation and neuronal maturation.	UPF1-NMD inhibits neuronal differentiation by destabilizing the mRNAs of G1/S transition inhibitors, neuronal differentiation factors, and TGF-β inhibitors.	[78]
	Expression of truncated hUPF3B in rat hippocampal NSCs (*in vitro*)	Expression of truncated hUPF3B compromises neuronal differentiation and neurite outgrowth.	N.D.	[118]
Spermatogonial stem cell (SSC)	Upf2 cKO in prospermatogonia (*in vivo*)	"Sertoli-only syndrome", testicular atrophy and dramatic germ cell depletion.	Accumulation of gene transcripts with long 3' UTRs; no specific target was found to be responsible for the phenotype.	[129]
	Upf2 cKO in spermatogonia (*in vivo*)	Testicular atrophy, delayed meiotic entry and massive depletion of spermatocytes and spermatids.	Accumulation of gene transcripts with long 3' UTRs; no specific target was found to be responsible for the phenotype.	[129]
	Upf3a cKO in spermatogonia (*in vivo*)	Defective spermatogenesis in Upf3a cKO males; dramatic reduction in sperm counts; a differentiation block of spermatocytes.	More that 10 Upf3a targets in pachytene spermatocytes have been identified with RNA-Seq.	[83]
Hematopoietic stem cell (HSC)	Upf2 cKO in adult HSCs (*in vivo*)	Upf2 deficiency causes the death of hematopoietic stem cells and progenitors.	N.D.	[82]
Hepatoblast	Upf2, cKO in hepatoblasts (*in vivo*)	Upf2 deficiency causes mitotic arrest, and terminal differentiation failure of hepatoblasts.	N.D.	[137]

Note: KO, knockout; cKO, conditional knockout; N.D., not defined.

step-wise process controlled with temporal gene expression regulation, NMD may play distinct roles at different stages of NSC differentiation through targeting specific RNA targets.

The biological roles of NMD factors in brain development are enlightened with mouse models for the EJC components Rbm8a, Magoh and Eif4a3 (84,85,122). Rbm8a is upregulated at the onset of neurogenesis in neocortex, regulates NSC proliferation and prevents premature neuronal differentiation (84). Haploinsufficiency of *Rbm8a* in mouse developing neocortex results in microcephaly with reduced cortex size, which is due to defective neurogenesis upon the loss of one *Rbm8a* allele in NSCs (84). Furthermore, *Magoh* haploinsufficient mice show microcephaly (85). The neocortex tissues from *Magoh* haploinsufficient mice display depletions of intermediate neural progenitors expressing Tbr2 and mature neurons due to high frequencies of apoptosis. *Magoh* haploinsufficient NSCs are prone to differentiate rather than self-renew at early stages of neocortex development. This phenomenon could be explained by the roles of Magoh in mitosis and genome integrity maintenance (85). *Eif4a3* haploinsufficient mice phenotypically mimic *Rbm8a* and *Magoh* mutant mice, indicating that these three EJC factors regulate brain development in the same pathway. Interestingly, loss of *p53* could partially rescue the microcephaly phenotype conferred by *Eif4a3* haploinsufficiency

(122), suggesting that EJC haploinsufficiency in developing brain compromises neurogenesis through activating the p53 dependent cell death pathway. Of note, in all the studies conducted in mouse models with EJC component haploinsufficiencies, NMD activities in NSCs have not been assayed in the brain samples from mutants (122). Thus, a mechanistic link between NMD and neurological pathogenesis could not be established. Furthermore, EJC components play NMD independent roles in gene expression regulation (123–125), which potentially could contribute to the defective neurogenesis as seen in these EJC mutant mice. Since UPF2, UPF3A, SMG6, SMG9 and RBM8A are implicated in human neurodevelopmental diseases, establishments and analysis of respective mouse models for these NMD factors specifically deleted in embryonic and adults NSCs could advance our knowledge on NMD roles in mammalian brain development and adult brain function. Furthermore, identification of RNA targets of these NMD factors in NSCs will be helpful for elucidating NMD mediated post-transcriptional gene regulatory mechanisms in NSC self-renewal and differentiation.

NMD in spermatogonial stem cell and spermatogenesis

The emergence and development of spermatogonial stem cells (SSCs) and spermatogenesis are tightly regulated during embryonic and postnatal development of mammals (126). In mouse, the SSC development starts as early as embryonic day 6.25 (E6.25), when primordial germ cells (PGCs, the ancestors of SSCs) evolve within the proximal epiblast (126). During E10–11, PGCs migrate to the gonad ridge. At this development stage, the bi-potential gonad acquires sex specificity and forms the male germ cell niche, the embryonic sertoli cells (SCs). Once PGCs interact with embryonic SCs and other niche components, sex determination will be completed. The PGCs further proliferate to expand the PGC pool (namely, prospermatogonia) and finally develop into the spermatogonia or SSCs after birth. SSC is the only stem cell population which sustains the reproductive capacity of adult males. During puberty and adulthood, SSCs self-renew to maintain the stem cell pool and differentiate to progenitors, which undergo further meiosis to generate functional spermatids.

Large scale RNA-Seq analysis in human and mouse shows that testis tissues have the highest expression of NMD factors, such as Smg1, Upf1, Upf2, Smg5, Smg6 and Smg7, indicating that NMD is essential for spermatogenesis. Recently, conditional ablation of NMD factor Upf2 or Upf3a in developing germ cells and embryonic SCs suggested an indispensable role of NMD in regulating spermatogenesis (83,127–131) (Table 2). Bao *et al.* conducted several studies to investigate Upf2-NMD in spermatogenesis (128,129). SCs, together with Leydig cells, build up the developmental niche for spermatogenesis during embryonic stages and adulthood. By conditional deletion of *Upf2* in embryonic SCs starting from E14.5, Bao *et al.* found that loss of Upf2 in developing SCs leads to severe testicular atrophy and mouse sterility (128). *Upf2* deficient developing SCs cannot differentiate into mature SCs to support spermatogenesis. Specific deletion of *Upf2* in prospermatogonia by the Ddx4-Cre mouse line (*Upf2* deletion starts at E15) results in infertile mice with germ cell free seminiferous tubules in testis, phenotypically mimicking the 'sertoli-only' phenotype (129). The *Upf2* deficient prospermatogonia fails to develop into spermatogonia. This finding indicates that Upf2-NMD is essential for the early male germ cell development (128,129). Furthermore, postnatal deletion of *Upf2* in Type A spermatogonia with Stra8-Cre line (*Upf2* deletion starts around postnatal day 3, P3) causes reduced testis weight and mice infertility in adult. Although, at postnatal day 12 (P12), zygotene spermatocytes are presented in wild-type and Upf2 deficient testis, Upf2 mutant mice, starting from P14, showed a dramatic reduction of spermatocytes and spermatids inside seminiferous tubules in testis (129). These data suggest that Upf2-NMD is essential for cell fate transition during each developmental stage of testis. Transcriptomic analysis shows that Upf2-NMD has differential regulation modes in SCs and germ cells (128,129). In SCs, Upf2-NMD seems to degrade the PTC containing mRNAs, while in germ cells, Upf2-NMD specifically targets mRNAs with long 3′UTR. This cell type specificity of NMD targets was further confirmed by the study on a mouse model with testis-specific knockout of Tdrd6, a component of the chromatoid body, where Upf2 executes its NMD function (131,132).

Loss of NMD compromises SSC development and spermatogenesis. On the other hand, hyper-activation of NMD results in spermatogenesis defect as well. Upf3a, a paralog of Upf3b in mice, has been recently identified as an NMD repressor through competing with NMD activator Upf3b for the binding to Upf2 (83). Overexpression of Upf3a represses Upf3b expression, and thus upregulates a subset of NMD targets; Upf3a deficiency could activate the NMD machinery. Deletion of *Upf3a* at postnatal day3 with Stra8-Cre mouse line causes spermatocyte progression defect and further depletion of spermatogonia, indicating that hyper-activation of NMD could partially block the differentiation and maturation of spermatids in mice (83). It seems that Upf3a also targets mRNA species with long 3′ UTRs, suggesting a cell type-specific requirement of NMD in degrading mRNAs with long 3′ UTRs in spematogenonia and its progeny (83,129,132). It is interesting to note that testis tissues in wild type mice, mainly composed of mature spermatids, are enriched with gene transcripts with short 3′ UTRs (133), which are supposed to be resistant to mRNA decay. The short 3′ UTR setting in testis could preserve transcriptome stability of cells and maintain the cell identity.

These studies on NMD function in different developmental stages of testis indicate that fine-tuning of NMD activity is essential for the differentiation of embryonic SCs, prospermatogonia and SSCs (83,128,129). Current studies employed whole testis tissues with the mixed cell populations, including SCs, spermatogonia as well as cells at different developmental stages of spermatogenesis, to conduct the RNA-Seq analysis and identified transcriptional changes in NMD factor deficient testis tissues (83,128,129,132). Further investigation on specific mRNAs or pathways targeted by NMD in purified SCs or spermatogonia will disclose more in-depth mechanisms of NMD in SSC maintenance, differentiation and spermatogenesis.

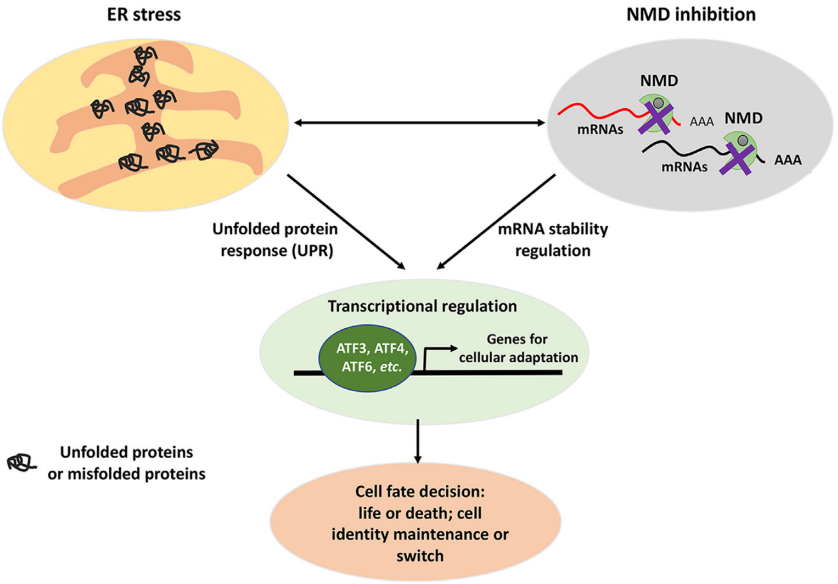

Figure 4. ER stress, UPR and NMD in cell fate determination. ER is an organelle for protein-folding. Accumulations of unfolded/misfolded proteins in ER will trigger the activation of adaptive UPR. UPR-related transcriptional factors, such as ATF3, ATF4 and ATF6, are unregulated, and further bind to their target gene promoters and initiate cellular programs for ER homeostasis restoration and cellular adaptation to ER stress. Furthermore, NMD inhibition could induce ER stress and UPR activation possibly through productions of truncated and unfolded/misfolded proteins. At least 10 UPR components, including ATF3, ATF4 and ATF6, are direct targets of NMD. Thus, NMD, together with ER stress-UPR signaling, maintains stem cell identity and safeguards cell identify transition, such as stem cell self-renewal and differentiation.

NMD in hematopoietic stem cell and hepatoblast

The hematopoietic stem cell (HSC) is considered as a prototype to study the function of tissue specific stem cells in development, tissue homeostasis, ageing and regeneration (56,134,135). Many studies show that factors participating in transcription regulations, epigenetic modifications, metabolic pathways and genomic stability maintenance are essential for HSC self-renewal, differentiation and hematopoiesis (56,136). Weischenfeldt *et al.* conducted a pioneer study on whether NMD could affect adult hematopoiesis (82) (Table 2). Using an inducible Cre line (Mx1-cre), Weischenfeldt *et al.* showed that deletion of Upf2 in HSC and its progeny results in mice lethality due to severe bone marrow atrophy (82). Upf2 deficient HSCs fail to expand *in vitro*. Intriguingly, knocking out Upf2 specifically in the myeloid lineage with the LysM-Cre mouse line is compatible with cellular viability and terminal differentiation of myeloid lineage cells. In this regard, Upf2 is essential for the cellular fitness of highly proliferative cell populations such as HSCs, common lymphoid progenitors and B cell progenitors since Upf2 knockout results in fast depletion of these cells (82). However, the mechanism underlying the death of Upf2 deficient HSCs is unknown. Detailed analysis on the purified HSCs with deficiencies of Upf2 or other NMD factors could shed more light on the biological roles and mechanisms of NMD in HSC maintenance and differentiation.

In addition to aforementioned studies on functions of NMD in ESC, SSC and HSC, Upf2 conditional mice have been employed to study the role of NMD in embryonic liver development and adult liver regeneration (137). The development of liver begins around E8.5. Hepatoblasts proliferate during the embryonic stages, and further exit the cell cycle and form functional hepatocytes and cholangiocytes through the actions of multiple transcriptional factors, such as HNF4α (138). In adulthood, hepatocytes are quiescent, but will reenter cell cycle to proliferate and compensate for the loss of liver mass upon liver damages (139). Deletion of Upf2 in fetal liver around E10 with the Alfp-cre mouse line causes perinatal lethality of mutant mice (137) (Table 2). Upf2 deficiency does not alter the proliferation and viability of fetal liver cells, but confers a terminal differentiation defect of hepatoblasts toward mature hepatocytes (137). Furthermore, the liver regeneration assay (partial hepatectomy) shows that Upf2 deficient hepatocytes fail to be activated, and reenter into cell cycle to proliferate and compensate the loss of liver mass (137). These data indicate that NMD, during development, may facilitate the cell identity transition, i.e. hepatoblasts toward differentiated hepatocytes, and during stress conditions, activate quiescent hepatocytes toward cycling hepatocytes (20).

Identification of NMD-mediated regulatory mechanisms in stem cell biology: a perspective

Through degrading PTC containing mRNAs and natural gene transcripts that play significant roles in cell functions, NMD serves as a general mRNA surveillance mechanism in regulating transcriptome stability and fine-tuning gene expression (1,5,7,9). Since the first description of the NMD phenomenon in eukaryotic cells in the late 1970s, there has been a booming of findings in understanding the biochemistry and structure of this conserved pathway (5,6,8,17). However, only until recently the biological roles of NMD in mammals start to emerge (5,36). Studies in mouse ESCs, NSCs, SSCs, HSCs and hepatoblasts indicate that NMD may not be required for the cellular viability of stem cells,

but essential for stem cell maintenance and differentiation (20,78,82,83,98,118,119,128,129,137).

How does NMD regulate the stem cell maintenance and differentiation programs and consequently safeguard embryonic development and maintain tissue homeostasis? The answer resides in the mRNA targets of the NMD machinery. NMD targets PTC containing mRNAs for elimination. Failure in degrading PTC containing mRNAs will allow the translational machinery to produce a large amount of truncated proteins, which may generate a stress condition in endoplasmic reticulum (ER), where truncated proteins fold and maturate (140). Defects in protein folding and maturation will generate ER stress and activate the unfolded protein response (UPR) (Figure 4). In line with this hypothesis, Sakaki *et al.* showed that knockdown of SMG6 in mammalian cells generates ER stress and UPR signaling activation (141). Thus, through degrading the aberrant mRNAs and reducing the accumulation of truncated or misfolded proteins, NMD could play an important role in protecting cells from the deleterious effect of ER stress and UPR activation. Surprisingly, Karam *et al.* found that gene transcripts from 10 UPR components, such as transcriptional factors ATF3, ATF4 and ATF6, are *bona fide* NMD targets (140). Thus, NMD plays an important role in shaping the UPR pathway to combat ER stress (36) (Figure 4). Of note, ER stress and its induced UPR signaling are implicated in the maintenance and differentiation of various types of stem cells (142–146). The magnitudes of ER stress and UPR signaling mediate distinct cell fates (147). Upon high ER stress, UPR activation will confer cell death (147). In low ER stress conditions, cells may adapt their transcriptional programs to sustain their viability in trade-off with other cellular functions, such as differentiation (147). It would be interesting to investigate the magnitudes of ER stress in NMD deficient cells and to test whether the genetic manipulation of UPR signaling could alleviate the defects as shown in NMD factor deficient stem cells and mouse models.

Since NMD targets natural gene transcripts with roles in cell functions, fine-tuning of mRNA stability and protein expression could be another NMD-mediated mechanism in regulating stem cell dynamics. For example, working with Smg6 deficient ESCs, Li *et al.* found that Smg6-NMD regulates the mRNA stability of a key pluripotency regulator (*c-Myc*) and safeguards ESC differentiation; Lou *et al.* demonstrated that Upf1-NMD destabilizes mRNA transcripts of TGF-β signaling repressors Smad6 and Smad7 to promote pluripotency (20,78,98,148). The NMD targets determining cell fates were mainly identified through analyzing the DEGs generated from RNA-Seq data. The integrated analysis of RNA-Seq and PAR-CLIP sequencing for direct determination of NMD factor binding RNAs will accelerate the findings of key genes in the maintenance and differentiation of distinct stem cell population.

Manipulation of NMD for the treatment of human diseases

A large proportion of human genetic diseases are caused by the in-frame PTCs generated by nonsense mutations or splicing abnormalities in genes (149). During mRNA translation, the recognition of a PTC will prematurely terminate translation process, consequentially generating a truncated polypeptide with functional abnormalities; Furthermore, a PTC in mRNA will trigger NMD activation and mRNA degradation. Thus, reduction of normal protein expression is considered as a major cause for nonsense mutation-generated human genetic diseases. The read-through chemicals, including Gentamicin, could simulate the insertion of an amino acid at the PTC site during mRNA translation and thus allow the translation machinery to produce full length polypeptide (150). Treatment of read-through chemicals shows certain improvements on cellular defects in human diseases models. Furthermore, NMD inhibition has been shown to be effective in increasing expression levels of functional truncated proteins in cellular models of human diseases, such as hereditary elliptocytosis and Ullrich's disease (150–152). Consequentially, NMD inhibition partially alleviates cellular defects in patients derived cell lines. Recently, combinational therapeutic strategies by applying read-through chemicals and NMD inhibitors show great promises in treatments of human diseases caused by nonsense mutations in genes (150,153–156). However, NMD inhibition in disease therapies should be applied with caution since knockdowns or knockouts of NMD factors in animal models indicate that NMD deficiency generates strong toxicities to cells as well as to tissues. Furthermore, deficiencies of different NMD factors in humans cause severe pathological symptoms in the central nervous system, as well as in the immune system (15,51). In this way, at current stage, a transcript-specific inhibition of NMD will have less adverse effects than general NMD inhibition (157). Furthermore, since NMD-mediated mechanisms contributing to these severe symptoms as shown in existing NMD factor mutants remain unsolved, understanding NMD biology and its mediated molecular pathways in mammalian stem cells is a prerequisite for future designing NMD inhibitors with minimal effects on disturbing stem cell maintenance and tissue homeostasis.

ACKNOWLEDGEMENTS

We are grateful to Dr Tao Tan and Dr Zhihui Huang for their critical reading and comments of the manuscript and to all other members of the Li Laboratory for helpful discussions. We are also grateful to four anonymous reviewers for their constructive suggestions on the manuscript.

FUNDING

National Key Research and Development Program of China, Stem Cell and Translational Research [2016YFA0100603]; National Natural Science Foundation of China [81571380, 31770871]; Natural Science Foundation of Zhejiang Province, P. R. China [LY16H080009]; Zhejiang Provincial Key Lab of Geriatrics, P. R. China. Funding for open access charge: National Key Research and Development Program of China, Stem Cell and Translational Research [2016YFA0100603]; National Natural Science Foundation of China [81571380].
Conflict of interest statement. None declared.

REFERENCES

1. Frischmeyer,P.A. and Dietz,H.C. (1999) Nonsense-mediated mRNA decay in health and disease. *Hum. Mol. Genet.*, **8**, 1893–1900.
2. Schweingruber,C., Rufener,S.C., Zund,D., Yamashita,A. and Muhlemann,O. (2013) Nonsense-mediated mRNA decay—mechanisms of substrate mRNA recognition and degradation in mammalian cells. *Biochim. Biophys. Acta*, **1829**, 612–623.
3. Karam,R., Wengrod,J., Gardner,L.B. and Wilkinson,M.F. (2013) Regulation of nonsense-mediated mRNA decay: implications for physiology and disease. *Biochim. Biophys. Acta*, **1829**, 624–633.
4. Popp,M.W. and Maquat,L.E. (2013) Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu. Rev. Genet.*, **47**, 139–165.
5. 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.
6. He,F. and Jacobson,A. (2015) Nonsense-mediated mRNA decay: degradation of defective transcripts is only part of the story. *Annu. Rev. Genet.*, **49**, 339–366.
7. Fatscher,T., Boehm,V. and Gehring,N.H. (2015) Mechanism, factors, and physiological role of nonsense-mediated mRNA decay. *Cell Mol. Life Sci.*, **72**, 4523–4544.
8. Hug,N., Longman,D. and Caceres,J.F. (2016) Mechanism and regulation of the nonsense-mediated decay pathway. *Nucleic Acids Res.*, **44**, 1483–1495.
9. Ottens,F. and Gehring,N.H. (2016) Physiological and pathophysiological role of nonsense-mediated mRNA decay. *Pflugers Arch.*, **468**, 1013–1028.
10. Karousis,E.D., Nasif,S. and Muhlemann,O. (2016) Nonsense-mediated mRNA decay: novel mechanistic insights and biological impact. *Wiley Interdiscip. Rev. RNA*, **7**, 661–682.
11. Nickless,A., Bailis,J.M. and You,Z. (2017) Control of gene expression through the nonsense-mediated RNA decay pathway. *Cell Biosci.*, **7**, 26.
12. Mendell,J.T., Sharifi,N.A., Meyers,J.L., Martinez-Murillo,F. and Dietz,H.C. (2004) Nonsense surveillance regulates expression of diverse classes of mammalian transcripts and mutes genomic noise. *Nat. Genet.*, **36**, 1073–1078.
13. Krawczak,M., Ball,E.V., Fenton,I., Stenson,P.D., Abeysinghe,S., Thomas,N. and Cooper,D.N. (2000) Human gene mutation database-a biomedical information and research resource. *Hum. Mutat.*, **15**, 45–51.
14. Khajavi,M., Inoue,K. and Lupski,J.R. (2006) Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *Eur. J. Hum. Genet.*, **14**, 1074–1081.
15. Sartor,F., Anderson,J., McCaig,C., Miedzybrodzka,Z. and Muller,B. (2015) Mutation of genes controlling mRNA metabolism and protein synthesis predisposes to neurodevelopmental disorders. *Biochem Soc. Trans.*, **43**, 1259–1265.
16. Shi,Y., Inoue,H., Wu,J.C. and Yamanaka,S. (2017) Induced pluripotent stem cell technology: a decade of progress. *Nat. Rev. Drug. Discov.*, **16**, 115–130.
17. Conti,E. and Izaurralde,E. (2005) Nonsense-mediated mRNA decay: molecular insights and mechanistic variations across species. *Curr. Opin. Cell Biol.*, **17**, 316–325.
18. Boehm,V. and Gehring,N.H. (2016) Exon junction complexes: supervising the gene expression assembly line. *Trends Genet.*, **32**, 724–735.
19. McIlwain,D.R., Pan,Q., Reilly,P.T., Elia,A.J., McCracken,S., Wakeham,A.C., Itie-Youten,A., Blencowe,B.J. and Mak,T.W. (2010) Smg1 is required for embryogenesis and regulates diverse genes via alternative splicing coupled to nonsense-mediated mRNA decay. *Proc. Natl. Acad. Sci. U.S.A.*, **107**, 12186–12191.
20. Li,T., Shi,Y., Wang,P., Guachalla,L.M., Sun,B., Joerss,T., Chen,Y.S., Groth,M., Krueger,A., Platzer,M. *et al.* (2015) Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay. *EMBO J.*, **34**, 1630–1647.
21. Colombo,M., Karousis,E.D., Bourquin,J., Bruggmann,R. and Muhlemann,O. (2017) Transcriptome-wide identification of NMD-targeted human mRNAs reveals extensive redundancy between SMG6- and SMG7-mediated degradation pathways. *RNA*, **23**, 189–201.
22. Yepiskoposyan,H., Aeschimann,F., Nilsson,D., Okoniewski,M. and Muhlemann,O. (2011) Autoregulation of the nonsense-mediated mRNA decay pathway in human cells. *RNA*, **17**, 2108–2118.
23. Lykke-Andersen,S., Chen,Y., Ardal,B.R., Lilje,B., Waage,J., Sandelin,A. and Jensen,T.H. (2014) Human nonsense-mediated RNA decay initiates widely by endonucleolysis and targets snoRNA host genes. *Genes Dev.*, **28**, 2498–2517.
24. Tani,H., Imamachi,N., Salam,K.A., Mizutani,R., Ijiri,K., Irie,T., Yada,T., Suzuki,Y. and Akimitsu,N. (2012) Identification of hundreds of novel UPF1 target transcripts by direct determination of whole transcriptome stability. *RNA Biol.*, **9**, 1370–1379.
25. Hurt,J.A., Robertson,A.D. and Burge,C.B. (2013) Global analyses of UPF1 binding and function reveal expanded scope of nonsense-mediated mRNA decay. *Genome Res.*, **23**, 1636–1650.
26. Gregersen,L.H., Schueler,M., Munschauer,M., Mastrobuoni,G., Chen,W., Kempa,S., Dieterich,C. and Landthaler,M. (2014) MOV10 is a 5′ to 3′ RNA helicase contributing to UPF1 mRNA target degradation by translocation along 3′ UTRs. *Mol. Cell*, **54**, 573–585.
27. Zund,D., Gruber,A.R., Zavolan,M. and Muhlemann,O. (2013) Translation-dependent displacement of UPF1 from coding sequences causes its enrichment in 3′ UTRs. *Nat. Struct. Mol. Biol.*, **20**, 936–943.
28. Metze,S., Herzog,V.A., Ruepp,M.D. and Muhlemann,O. (2013) Comparison of EJC-enhanced and EJC-independent NMD in human cells reveals two partially redundant degradation pathways. *RNA*, **19**, 1432–1448.
29. Labno,A., Tomecki,R. and Dziembowski,A. (2016) Cytoplasmic RNA decay pathways—enzymes and mechanisms. *Biochim. Biophys. Acta*, **1863**, 3125–3147.
30. Ottens,F., Boehm,V., Sibley,C.R., Ule,J. and Gehring,N.H. (2017) Transcript-specific characteristics determine the contribution of endo- and exonucleolytic decay pathways during the degradation of nonsense-mediated decay substrates. *RNA*, **23**, 1224–1236.
31. Schmidt,S.A., Foley,P.L., Jeong,D.H., Rymarquis,L.A., Doyle,F., Tenenbaum,S.A., Belasco,J.G. and Green,P.J. (2015) Identification of SMG6 cleavage sites and a preferred RNA cleavage motif by global analysis of endogenous NMD targets in human cells. *Nucleic Acids Res.*, **43**, 309–323.
32. Kurosaki,T., Li,W., Hoque,M., Popp,M.W., Ermolenko,D.N., Tian,B. and Maquat,L.E. (2014) A post-translational regulatory switch on UPF1 controls targeted mRNA degradation. *Genes Dev.*, **28**, 1900–1916.
33. Lee,S.R., Pratt,G.A., Martinez,F.J., Yeo,G.W. and Lykke-Andersen,J. (2015) Target discrimination in nonsense-mediated mRNA decay requires Upf1 ATPase activity. *Mol. Cell*, **59**, 413–425.
34. Van Nostrand,E.L., Pratt,G.A., Shishkin,A.A., Gelboin-Burkhart,C., Fang,M.Y., Sundararaman,B., Blue,S.M., Nguyen,T.B., Surka,C., Elkins,K. *et al.* (2016) Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). *Nat. Methods*, **13**, 508–514.
35. Imamachi,N., Salam,K.A., Suzuki,Y. and Akimitsu,N. (2017) A GC-rich sequence feature in the 3′ UTR directs UPF1-dependent mRNA decay in mammalian cells. *Genome Res.*, **27**, 407–418.
36. Goetz,A.E. and Wilkinson,M. (2017) Stress and the nonsense-mediated RNA decay pathway. *Cell Mol. Life Sci.* **74**, 3509-3531.
37. Neu-Yilik,G., Gehring,N.H., Hentze,M.W. and Kulozik,A.E. (2004) Nonsense-mediated mRNA decay: from vacuum cleaner to Swiss army knife. *Genome Biol.*, **5**, 218.
38. Chawla,R. and Azzalin,C.M. (2008) The telomeric transcriptome and SMG proteins at the crossroads. *Cytogenet. Genome Res.*, **122**, 194–201.
39. Nicholson,P., Yepiskoposyan,H., Metze,S., Zamudio Orozco,R., Kleinschmidt,N. and Muhlemann,O. (2010) Nonsense-mediated mRNA decay in human cells: mechanistic insights, functions beyond quality control and the double-life of NMD factors. *Cell Mol. Life Sci.*, **67**, 677–700.
40. Azzalin,C.M. and Lingner,J. (2006) The human RNA surveillance factor UPF1 is required for S phase progression and genome stability. *Curr. Biol.*, **16**, 433–439.
41. Gehen,S.C., Staversky,R.J., Bambara,R.A., Keng,P.C. and O'Reilly,M.A. (2008) hSMG-1 and ATM sequentially and

independently regulate the G1 checkpoint during oxidative stress. *Oncogene*, **27**, 4065–4074.
42. Jia,J., Furlan,A., Gonzalez-Hilarion,S., Leroy,C., Gruenert,D.C., Tulasne,D. and Lejeune,F. (2015) Caspases shutdown nonsense-mediated mRNA decay during apoptosis. *Cell Death Differ.*, **22**, 1754–1763.
43. Nelson,J.O., Moore,K.A., Chapin,A., Hollien,J. and Metzstein,M.M. (2016) Degradation of Gadd45 mRNA by nonsense-mediated decay is essential for viability. *Elife*, **5**, e12876.
44. Martin,L. and Gardner,L.B. (2015) Stress-induced inhibition of nonsense-mediated RNA decay regulates intracellular cystine transport and intracellular glutathione through regulation of the cystine/glutamate exchanger SLC7A11. *Oncogene*, **34**, 4211–4218.
45. Gewandter,J.S., Bambara,R.A. and O'Reilly,M.A. (2011) The RNA surveillance protein SMG1 activates p53 in response to DNA double-strand breaks but not exogenously oxidized mRNA. *Cell Cycle*, **10**, 2561–2567.
46. Azzalin,C.M. and Lingner,J. (2006) The double life of UPF1 in RNA and DNA stability pathways. *Cell Cycle*, **5**, 1496–1498.
47. Azzalin,C.M., Reichenbach,P., Khoriauli,L., Giulotto,E. and Lingner,J. (2007) Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. *Science*, **318**, 798–801.
48. Nickless,A., Jackson,E., Marasa,J., Nugent,P., Mercer,R.W., Piwnica-Worms,D. and You,Z. (2014) Intracellular calcium regulates nonsense-mediated mRNA decay. *Nat. Med.*, **20**, 961–966.
49. Balistreri,G., Horvath,P., Schweingruber,C., Zund,D., McInerney,G., Merits,A., Muhlemann,O., Azzalin,C. and Helenius,A. (2014) The host nonsense-mediated mRNA decay pathway restricts mammalian RNA virus replication. *Cell Host Microbe*, **16**, 403–411.
50. Ramage,H.R., Kumar,G.R., Verschueren,E., Johnson,J.R., Von Dollen,J., Johnson,T., Newton,B., Shah,P., Horner,J., Krogan,N.J. *et al.* (2015) A combined proteomics/genomics approach links hepatitis C virus infection with nonsense-mediated mRNA decay. *Mol. Cell*, **57**, 329–340.
51. Balistreri,G., Bognanni,C. and Muhlemann,O. (2017) Virus escape and manipulation of cellular nonsense-mediated mRNA decay. *Viruses*, **9**, 24.
52. Ajamian,L., Abel,K., Rao,S., Vyboh,K., Garcia-de-Gracia,F., Soto-Rifo,R., Kulozik,A.E., Gehring,N.H. and Mouland,A.J. (2015) HIV-1 recruits UPF1 but excludes UPF2 to promote nucleocytoplasmic export of the genomic RNA. *Biomolecules*, **5**, 2808–2839.
53. Luo,H., Cowen,L., Yu,G., Jiang,W. and Tang,Y. (2016) SMG7 is a critical regulator of p53 stability and function in DNA damage stress response. *Cell Discov.*, **2**, 15042.
54. Chawla,R., Redon,S., Raftopoulou,C., Wischnewski,H., Gagos,S. and Azzalin,C.M. (2011) Human UPF1 interacts with TPP1 and telomerase and sustains telomere leading-strand replication. *EMBO J.*, **30**, 4047–4058.
55. Brumbaugh,K.M., Otterness,D.M., Geisen,C., Oliveira,V., Brognard,J., Li,X., Lejeune,F., Tibbetts,R.S., Maquat,L.E. and Abraham,R.T. (2004) The mRNA surveillance protein hSMG-1 functions in genotoxic stress response pathways in mammalian cells. *Mol. Cell*, **14**, 585–598.
56. Li,T., Zhou,Z.W., Ju,Z. and Wang,Z.Q. (2016) DNA damage response in hematopoietic stem cell ageing. *Genomics Proteomics Bioinformatics*, **14**, 147–154.
57. Lundblad,V. and Szostak,J.W. (1989) A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell*, **57**, 633–643.
58. Reichenbach,P., Hoss,M., Azzalin,C.M., Nabholz,M., Bucher,P. and Lingner,J. (2003) A human homolog of yeast Est1 associates with telomerase and uncaps chromosome ends when overexpressed. *Curr. Biol.*, **13**, 568–574.
59. Snow,B.E., Erdmann,N., Cruickshank,J., Goldman,H., Gill,R.M., Robinson,M.O. and Harrington,L. (2003) Functional conservation of the telomerase protein Est1p in humans. *Curr. Biol.*, **13**, 698–704.
60. Matsuoka,S., Ballif,B.A., Smogorzewska,A., McDonald,E.R. 3rd, Hurov,K.E., Luo,J., Bakalarski,C.E., Zhao,Z., Solimini,N., Lerenthal,Y. *et al.* (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science*, **316**, 1160–1166.
61. Kaygun,H. and Marzluff,W.F. (2005) Regulated degradation of replication-dependent histone mRNAs requires both ATR and Upf1. *Nat. Struct. Mol. Biol.*, **12**, 794–800.
62. Choe,J., Ahn,S.H. and Kim,Y.K. (2014) The mRNP remodeling mediated by UPF1 promotes rapid degradation of replication-dependent histone mRNA. *Nucleic Acids Res.*, **42**, 9334–9349.
63. Azzalin,C.M. (2012) UPF1: a leader at the end of chromosomes. *Nucleus*, **3**, 16–21.
64. Feng,Q., Jagannathan,S. and Bradley,R.K. (2017) The RNA surveillance factor UPF1 represses myogenesis via its E3 ubiquitin ligase activity. *Mol. Cell*, **67**, 239–251.
65. Park,E. and Maquat,L.E. (2013) Staufen-mediated mRNA decay. *Wiley Interdiscip. Rev. RNA*, **4**, 423–435.
66. Gong,C., Kim,Y.K., Woeller,C.F., Tang,Y. and Maquat,L.E. (2009) SMD and NMD are competitive pathways that contribute to myogenesis: effects on PAX3 and myogenin mRNAs. *Genes Dev.*, **23**, 54–66.
67. Lou,C.H., Shum,E.Y. and Wilkinson,M.F. (2015) RNA degradation drives stem cell differentiation. *EMBO J.*, **34**, 1606–1608.
68. Brogna,S., McLeod,T. and Petric,M. (2016) The meaning of NMD: translate or perish. *Trends Genet.*, **32**, 395–407.
69. Behrens,A., van Deursen,J.M., Rudolph,K.L. and Schumacher,B. (2014) Impact of genomic damage and ageing on stem cell function. *Nat. Cell Biol.*, **16**, 201–207.
70. Vitale,I., Manic,G., De Maria,R., Kroemer,G. and Galluzzi,L. (2017) DNA damage in stem cells. *Mol. Cell*, **66**, 306–319.
71. Park,Y. and Gerson,S.L. (2005) DNA repair defects in stem cell function and aging. *Annu. Rev. Med.*, **56**, 495–508.
72. Strong,M.A., Vidal-Cardenas,S.L., Karim,B., Yu,H., Guo,N. and Greider,C.W. (2011) Phenotypes in mTERT(+)/(-) and mTERT(-)/(-) mice are due to short telomeres, not telomere-independent functions of telomerase reverse transcriptase. *Mol. Cell. Biol.*, **31**, 2369–2379.
73. Kojima,Y., Tam,O.H. and Tam,P.P. (2014) Timing of developmental events in the early mouse embryo. *Semin. Cell Dev. Biol.*, **34**, 65–75.
74. Tam,P.P. and Loebel,D.A. (2007) Gene function in mouse embryogenesis: get set for gastrulation. *Nat. Rev. Genet.*, **8**, 368–381.
75. Tam,P.P. and Behringer,R.R. (1997) Mouse gastrulation: the formation of a mammalian body plan. *Mech. Dev.*, **68**, 3–25.
76. Mitiku,N. and Baker,J.C. (2007) Genomic analysis of gastrulation and organogenesis in the mouse. *Dev. Cell*, **13**, 897–907.
77. Bruno,I.G., Karam,R., Huang,L., Bhardwaj,A., Lou,C.H., Shum,E.Y., Song,H.W., Corbett,M.A., Gifford,W.D., Gecz,J. *et al.* (2011) Identification of a microRNA that activates gene expression by repressing nonsense-mediated RNA decay. *Mol. Cell*, **42**, 500–510.
78. Lou,C.H., Shao,A., Shum,E.Y., Espinoza,J.L., Huang,L., Karam,R. and Wilkinson,M.F. (2014) Posttranscriptional control of the stem cell and neurogenic programs by the nonsense-mediated RNA decay pathway. *Cell Rep.*, **6**, 748–764.
79. Pereverzev,A.P., Gurskaya,N.G., Ermakova,G.V., Kudryavtseva,E.I., Markina,N.M., Kotlobay,A.A., Lukyanov,S.A., Zaraisky,A.G. and Lukyanov,K.A. (2015) Method for quantitative analysis of nonsense-mediated mRNA decay at the single cell level. *Sci. Rep.*, **5**, 7729.
80. Wittkopp,N., Huntzinger,E., Weiler,C., Sauliere,J., Schmidt,S., Sonawane,M. and Izaurralde,E. (2009) Nonsense-mediated mRNA decay effectors are essential for zebrafish embryonic development and survival. *Mol. Cell. Biol.*, **29**, 3517–3528.
81. Medghalchi,S.M., Frischmeyer,P.A., Mendell,J.T., Kelly,A.G., Lawler,A.M. and Dietz,H.C. (2001) Rent1, a trans-effector of nonsense-mediated mRNA decay, is essential for mammalian embryonic viability. *Hum. Mol. Genet.*, **10**, 99–105.
82. Weischenfeldt,J., Damgaard,I., Bryder,D., Theilgaard-Monch,K., Thoren,L.A., Nielsen,F.C., Jacobsen,S.E., Nerlov,C. and Porse,B.T. (2008) NMD is essential for hematopoietic stem and progenitor cells and for eliminating by-products of programmed DNA rearrangements. *Genes Dev.*, **22**, 1381–1396.
83. Shum,E.Y., Jones,S.H., Shao,A., Dumdie,J., Krause,M.D., Chan,W.K., Lou,C.H., Espinoza,J.L., Song,H.W., Phan,M.H. *et al.* (2016) The antagonistic gene paralogs Upf3a and Upf3b govern nonsense-mediated RNA decay. *Cell*, **165**, 382–395.

84. Mao,H., Pilaz,L.J., McMahon,J.J., Golzio,C., Wu,D., Shi,L., Katsanis,N. and Silver,D.L. (2015) Rbm8a haploinsufficiency disrupts embryonic cortical development resulting in microcephaly. *J. Neurosci.*, **35**, 7003–7018.
85. Silver,D.L., Watkins-Chow,D.E., Schreck,K.C., Pierfelice,T.J., Larson,D.M., Burnetti,A.J., Liaw,H.J., Myung,K., Walsh,C.A., Gaiano,N. *et al.* (2010) The exon junction complex component Magoh controls brain size by regulating neural stem cell division. *Nat. Neurosci.*, **13**, 551–558.
86. Huang,L., Lou,C.H., Chan,W., Shum,E.Y., Shao,A., Stone,E., Karam,R., Song,H.W. and Wilkinson,M.F. (2011) RNA homeostasis governed by cell type-specific and branched feedback loops acting on NMD. *Mol. Cell*, **43**, 950–961.
87. Huang,L., Shum,E.Y., Jones,S.H., Lou,C.H., Dumdie,J., Kim,H., Roberts,A.J., Jolly,L.A., Espinoza,J.L., Skarbrevik,D.M. *et al.* (2017) A Upf3b-mutant mouse model with behavioral and neurogenesis defects. *Mol Psychiatry*, doi:10.1038/mp.2017.173.
88. Kim,J. and Orkin,S.H. (2011) Embryonic stem cell-specific signatures in cancer: insights into genomic regulatory networks and implications for medicine. *Genome Med.*, **3**, 75.
89. Keller,G. (2005) Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev.*, **19**, 1129–1155.
90. Stadtfeld,M. and Hochedlinger,K. (2010) Induced pluripotency: history, mechanisms, and applications. *Genes Dev.*, **24**, 2239–2263.
91. Huang,G., Ye,S., Zhou,X., Liu,D. and Ying,Q.L. (2015) Molecular basis of embryonic stem cell self-renewal: from signaling pathways to pluripotency network. *Cell Mol. Life Sci.*, **72**, 1741–1757.
92. Yeo,J.C. and Ng,H.H. (2013) The transcriptional regulation of pluripotency. *Cell Res.*, **23**, 20–32.
93. Liang,G. and Zhang,Y. (2013) Embryonic stem cell and induced pluripotent stem cell: an epigenetic perspective. *Cell Res.*, **23**, 49–69.
94. He,S., Nakada,D. and Morrison,S.J. (2009) Mechanisms of stem cell self-renewal. *Annu. Rev. Cell Dev. Biol.*, **25**, 377–406.
95. Betschinger,J. (2017) Charting developmental dissolution of pluripotency. *J. Mol. Biol.*, **429**, 1441–1458.
96. Hwang,J. and Maquat,L.E. (2011) Nonsense-mediated mRNA decay (NMD) in animal embryogenesis: to die or not to die, that is the question. *Curr. Opin. Genet. Dev.*, **21**, 422–430.
97. Li,T. and Wang,Z.Q. (2011) Point mutation at the Nbs1 Threonine 278 site does not affect mouse development, but compromises the Chk2 and Smc1 phosphorylation after DNA damage. *Mech. Ageing Dev.*, **132**, 382–388.
98. Lou,C.H., Dumdie,J., Goetz,A., Shum,E.Y., Brafman,D., Liao,X., Mora-Castilla,S., Ramaiah,M., Cook-Andersen,H., Laurent,L. *et al.* (2016) Nonsense-mediated RNA decay influences human embryonic stem cell fate. *Stem Cell Rep.*, **6**, 844–857.
99. Moon,S.Y., Park,Y.B., Kim,D.S., Oh,S.K. and Kim,D.W. (2006) Generation, culture, and differentiation of human embryonic stem cells for therapeutic applications. *Mol. Ther.*, **13**, 5–14.
100. Blair,K., Wray,J. and Smith,A. (2011) The liberation of embryonic stem cells. *PLoS Genet.*, **7**, e1002019.
101. Hockemeyer,D., Wang,H., Kiani,S., Lai,C.S., Gao,Q., Cassady,J.P., Cost,G.J., Zhang,L., Santiago,Y., Miller,J.C. *et al.* (2011) Genetic engineering of human pluripotent cells using TALE nucleases. *Nat. Biotechnol.*, **29**, 731–734.
102. Takata,N., Sakakura,E., Sakuma,T. and Yamamoto,T. (2017) Genetic tools for self-organizing culture of mouse embryonic stem cells via small regulatory RNA-mediated technologies, CRISPR/Cas9, and inducible RNAi. *Methods Mol. Biol.*, **1622**, 269–292.
103. Yao,X., Wang,X., Hu,X., Liu,Z., Liu,J., Zhou,H., Shen,X., Wei,Y., Huang,Z., Ying,W. *et al.* (2017) Homology-mediated end joining-based targeted integration using CRISPR/Cas9. *Cell Res.*, **27**, 801–814.
104. Wu,X., Scott,D.A., Kriz,A.J., Chiu,A.C., Hsu,P.D., Dadon,D.B., Cheng,A.W., Trevino,A.E., Konermann,S., Chen,S. *et al.* (2014) Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells. *Nat. Biotechnol.*, **32**, 670–676.
105. Namba,T. and Huttner,W.B. (2017) Neural progenitor cells and their role in the development and evolutionary expansion of the neocortex. *Wiley Interdiscip. Rev. Dev. Biol.*, **6**, e256.
106. Taverna,E., Gotz,M. and Huttner,W.B. (2014) The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. *Annu. Rev. Cell Dev. Biol.*, **30**, 465–502.
107. Tapias,A., Zhou,Z.W., Shi,Y., Chong,Z., Wang,P., Groth,M., Platzer,M., Huttner,W., Herceg,Z., Yang,Y.G. *et al.* (2014) Trrap-dependent histone acetylation specifically regulates cell-cycle gene transcription to control neural progenitor fate decisions. *Cell Stem Cell*, **14**, 632–643.
108. Frappart,P.O., Tong,W.M., Demuth,I., Radovanovic,I., Herceg,Z., Aguzzi,A., Digweed,M. and Wang,Z.Q. (2005) An essential function for NBS1 in the prevention of ataxia and cerebellar defects. *Nat. Med.*, **11**, 538–544.
109. Renault,V.M., Rafalski,V.A., Morgan,A.A., Salih,D.A., Brett,J.O., Webb,A.E., Villeda,S.A., Thekkat,P.U., Guillerey,C., Denko,N.C. *et al.* (2009) FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell*, **5**, 527–539.
110. Tapias,A. and Wang,Z.Q. (2017) Lysine acetylation and deacetylation in brain development and neuropathies. *Genomics Proteomics Bioinformatics*, **15**, 19–36.
111. Hoch,N.C., Hanzlikova,H., Rulten,S.L., Tetreault,M., Komulainen,E., Ju,L., Hornyak,P., Zeng,Z., Gittens,W., Rey,S.A. *et al.* (2017) XRCC1 mutation is associated with PARP1 hyperactivation and cerebellar ataxia. *Nature*, **541**, 87–91.
112. Tarpey,P.S., Raymond,F.L., Nguyen,L.S., Rodriguez,J., Hackett,A., Vandeleur,L., Smith,R., Shoubridge,C., Edkins,S., Stevens,C. *et al.* (2007) Mutations in UPF3B, a member of the nonsense-mediated mRNA decay complex, cause syndromic and nonsyndromic mental retardation. *Nat. Genet.*, **39**, 1127–1133.
113. Addington,A.M., Gauthier,J., Piton,A., Hamdan,F.F., Raymond,A., Gogtay,N., Miller,R., Tossell,J., Bakalar,J., Inoff-Germain,G. *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.
114. Nguyen,L.S., Kim,H.G., Rosenfeld,J.A., Shen,Y., Gusella,J.F., Lacassie,Y., Layman,L.C., Shaffer,L.G. and Gecz,J. (2013) Contribution of copy number variants involving nonsense-mediated mRNA decay pathway genes to neuro-developmental disorders. *Hum. Mol. Genet.*, **22**, 1816–1825.
115. Shaheen,R., Anazi,S., Ben-Omran,T., Seidahmed,M.Z., Caddle,L.B., Palmer,K., Ali,R., Alshidi,T., Hagos,S., Goodwin,L. *et al.* (2016) Mutations in SMG9, encoding an essential component of nonsense-mediated decay machinery, cause a multiple congenital anomaly syndrome in humans and mice. *Am. J. Hum. Genet.*, **98**, 643–652.
116. Long,A.A., Mahapatra,C.T., Woodruff,E.A. 3rd, Rohrbough,J., Leung,H.T., Shino,S., An,L., Doerge,R.W., Metzstein,M.M., Pak,W.L. *et al.* (2010) The nonsense-mediated decay pathway maintains synapse architecture and synaptic vesicle cycle efficacy. *J. Cell Sci.*, **123**, 3303–3315.
117. Colak,D., Ji,S.J., Porse,B.T. and Jaffrey,S.R. (2013) Regulation of axon guidance by compartmentalized nonsense-mediated mRNA decay. *Cell*, **153**, 1252–1265.
118. Alrahbeni,T., Sartor,F., Anderson,J., Miedzybrodzka,Z., McCaig,C. and Muller,B. (2015) Full UPF3B function is critical for neuronal differentiation of neural stem cells. *Mol. Brain*, **8**, 33.
119. Jolly,L.A., Homan,C.C., Jacob,R., Barry,S. and Gecz,J. (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.
120. Ellis,P., Fagan,B.M., Magness,S.T., Hutton,S., Taranova,O., Hayashi,S., McMahon,A., Rao,M. and Pevny,L. (2004) SOX2, a persistent marker for multipotential neural stem cells derived from embryonic stem cells, the embryo or the adult. *Dev. Neurosci.*, **26**, 148–165.
121. Park,D., Xiang,A.P., Mao,F.F., Zhang,L., Di,C.G., Liu,X.M., Shao,Y., Ma,B.F., Lee,J.H., Ha,K.S. *et al.* (2010) Nestin is required for the proper self-renewal of neural stem cells. *Stem Cells*, **28**, 2162–2171.
122. Mao,H., McMahon,J.J., Tsai,Y.H., Wang,Z. and Silver,D.L. (2016) Haploinsufficiency for core exon junction complex components disrupts embryonic neurogenesis and causes p53-mediated microcephaly. *PLoS Genet.*, **12**, e1006282.
123. Choudhury,S.R., Singh,A.K., McLeod,T., Blanchette,M., Jang,B., Badenhorst,P., Kanhere,A. and Brogna,S. (2016) Exon junction complex proteins bind nascent transcripts independently of pre-mRNA splicing in Drosophila melanogaster. *Elife*, **5**, e19881.

124. Le Hir,H., Sauliere,J. and Wang,Z. (2016) The exon junction complex as a node of post-transcriptional networks. *Nat. Rev. Mol. Cell Biol.*, **17**, 41–54.
125. Woodward,L.A., Mabin,J.W., Gangras,P. and Singh,G. (2017) The exon junction complex: a lifelong guardian of mRNA fate. *Wiley Interdiscip. Rev. RNA*, **8**, e1411.
126. Griswold,M.D. (2016) Spermatogenesis: the commitment to meiosis. *Physiol. Rev.*, **96**, 1–17.
127. Jones,S.H. and Wilkinson,M. (2017) RNA decay, evolution, and the testis. *RNA Biol.*, **14**, 146–155.
128. Bao,J., Tang,C., Yuan,S., Porse,B.T. and Yan,W. (2015) UPF2, a nonsense-mediated mRNA decay factor, is required for prepubertal Sertoli cell development and male fertility by ensuring fidelity of the transcriptome. *Development*, **142**, 352–362.
129. Bao,J., Vitting-Seerup,K., Waage,J., Tang,C., Ge,Y., Porse,B.T. and Yan,W. (2016) UPF2-dependent nonsense-mediated mRNA Decay pathway is essential for spermatogenesis by selectively eliminating longer 3′UTR transcripts. *PLoS Genet.*, **12**, e1005863.
130. Muhlemann,O. (2016) Spermatogenesis studies reveal a distinct nonsense-mediated mRNA decay (NMD) mechanism for mRNAs with long 3′UTRs. *PLoS Genet.*, **12**, e1005979.
131. MacDonald,C.C. and Grozdanov,P.N. (2017) Nonsense in the testis: multiple roles for nonsense-mediated decay revealed in male reproduction. *Biol. Reprod.*, **96**, 939–947.
132. Fanourgakis,G., Lesche,M., Akpinar,M., Dahl,A. and Jessberger,R. (2016) Chromatoid body protein TDRD6 supports long 3′ UTR triggered nonsense mediated mRNA decay. *PLoS Genet.*, **12**, e1005857.
133. MacDonald,C.C. and McMahon,K.W. (2010) Tissue-specific mechanisms of alternative polyadenylation: testis, brain, and beyond. *Wiley Interdiscip. Rev. RNA*, **1**, 494–501.
134. Orkin,S.H. and Zon,L.I. (2008) Hematopoiesis: an evolving paradigm for stem cell biology. *Cell*, **132**, 631–644.
135. Mikkola,H.K. and Orkin,S.H. (2006) The journey of developing hematopoietic stem cells. *Development*, **133**, 3733–3744.
136. Gottgens,B. (2015) Regulatory network control of blood stem cells. *Blood*, **125**, 2614–2620.
137. Thoren,L.A., Norgaard,G.A., Weischenfeldt,J., Waage,J., Jakobsen,J.S., Damgaard,I., Bergstrom,F.C., Blom,A.M., Borup,R., Bisgaard,H.C. *et al.* (2010) UPF2 is a critical regulator of liver development, function and regeneration. *PLoS One*, **5**, e11650.
138. Zaret,K.S. and Grompe,M. (2008) Generation and regeneration of cells of the liver and pancreas. *Science*, **322**, 1490–1494.
139. Zhang,L., Liu,L., He,Z., Li,G., Liu,J., Song,Z., Jin,H., Rudolph,K.L., Yang,H., Mao,Y. *et al.* (2015) Inhibition of wild-type p53-induced phosphatase 1 promotes liver regeneration in mice by direct activation of mammalian target of rapamycin. *Hepatology*, **61**, 2030–2041.
140. Karam,R., Lou,C.H., Kroeger,H., Huang,L., Lin,J.H. and Wilkinson,M.F. (2015) The unfolded protein response is shaped by the NMD pathway. *EMBO Rep.*, **16**, 599–609.
141. Sakaki,K., Yoshina,S., Shen,X., Han,J., DeSantis,M.R., Xiong,M., Mitani,S. and Kaufman,R.J. (2012) RNA surveillance is required for endoplasmic reticulum homeostasis. *Proc. Natl. Acad. Sci. U.S.A.*, **109**, 8079–8084.
142. van Galen,P., Kreso,A., Mbong,N., Kent,D.G., Fitzmaurice,T., Chambers,J.E., Xie,S., Laurenti,E., Hermans,K., Eppert,K. *et al.* (2014) The unfolded protein response governs integrity of the haematopoietic stem-cell pool during stress. *Nature*, **510**, 268–272.
143. Yang,Y., Cheung,H.H., Tu,J., Miu,K.K. and Chan,W.Y. (2016) New insights into the unfolded protein response in stem cells. *Oncotarget*, **7**, 54010–54027.
144. van Lidth de Jeude,J.F., Meijer,B.J., Wielenga,M.C.B., Spaan,C.N., Baan,B., Rosekrans,S.L., Meisner,S., Shen,Y.H., Lee,A.S., Paton,J.C. *et al.* (2017) Induction of endoplasmic reticulum stress by deletion of Grp78 depletes Apc mutant intestinal epithelial stem cells. *Oncogene*, **36**, 3397–3405.
145. Blanco-Gelaz,M.A., Suarez-Alvarez,B., Ligero,G., Sanchez,L., Vidal-Castineira,J.R., Coto,E., Moore,H., Menendez,P. and Lopez-Larrea,C. (2010) Endoplasmic reticulum stress signals in defined human embryonic stem cell lines and culture conditions. *Stem Cell Rev.*, **6**, 462–472.
146. Xiong,G., Hindi,S.M., Mann,A.K., Gallot,Y.S., Bohnert,K.R., Cavener,D.R., Whittemore,S.R. and Kumar,A. (2017) The PERK arm of the unfolded protein response regulates satellite cell-mediated skeletal muscle regeneration. *Elife*, **6**, e22871.
147. Hetz,C. (2012) The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.*, **13**, 89–102.
148. Shadle,S.C., Zhong,J.W., Campbell,A.E., Conerly,M.L., Jagannathan,S., Wong,C.J., Morello,T.D., van der Maarel,S.M. and Tapscott,S.J. (2017) DUX4-induced dsRNA and MYC mRNA stabilization activate apoptotic pathways in human cell models of facioscapulohumeral dystrophy. *PLoS Genet.*, **13**, e1006658.
149. Linde,L. and Kerem,B. (2008) Introducing sense into nonsense in treatments of human genetic diseases. *Trends Genet.*, **24**, 552–563.
150. Keeling,K.M. (2016) Nonsense suppression as an approach to treat lysosomal storage diseases. *Diseases*, **4**, 32.
151. Usuki,F., Yamashita,A., Higuchi,I., Ohnishi,T., Shiraishi,T., Osame,M. and Ohno,S. (2004) Inhibition of nonsense-mediated mRNA decay rescues the phenotype in Ullrich's disease. *Ann. Neurol.*, **55**, 740–744.
152. Moriniere,M., Delhommeau,F., Calender,A., Ribeiro,L., Delaunay,J. and Baklouti,F. (2010) Nonsense-mediated mRNA decay (NMD) blockage promotes nonsense mRNA stabilization in protein 4.1R deficient cells carrying the 4.1R Coimbra variant of hereditary elliptocytosis. *Blood Cells Mol. Dis.*, **45**, 284–288.
153. Usuki,F., Yamashita,A., Kashima,I., Higuchi,I., Osame,M. and Ohno,S. (2006) Specific inhibition of nonsense-mediated mRNA decay components, SMG-1 or Upf1, rescues the phenotype of Ullrich disease fibroblasts. *Mol. Ther.*, **14**, 351–360.
154. Bhuvanagiri,M., Lewis,J., Putzker,K., Becker,J.P., Leicht,S., Krijgsveld,J., Batra,R., Turnwald,B., Jovanovic,B., Hauer,C. *et al.* (2014) 5-azacytidine inhibits nonsense-mediated decay in a MYC-dependent fashion. *EMBO Mol. Med.*, **6**, 1593–1609.
155. Shao,A. and Wilkinson,M.F. (2014) Suppressing nonsense—a surprising function for 5-azacytidine. *EMBO Mol. Med.*, **6**, 1518–1520.
156. Keeling,K.M., Wang,D., Conard,S.E. and Bedwell,D.M. (2012) Suppression of premature termination codons as a therapeutic approach. *Crit. Rev. Biochem. Mol. Biol.*, **47**, 444–463.
157. Nomakuchi,T.T., Rigo,F., Aznarez,I. and Krainer,A.R. (2016) Antisense oligonucleotide-directed inhibition of nonsense-mediated mRNA decay. *Nat. Biotechnol.*, **34**, 164–166.