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CELL SCIENCE AT A GLANCE

Nonsense-mediated mRNA decay in humans at a glance

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ABSTRACT

Nonsense-mediated mRNA decay (NMD) is an mRNA quality-control mechanism that typifies all eukaryotes examined to date. NMD surveys newly synthesized mRNAs and degrades those that harbor a premature termination codon (PTC), thereby preventing the production of truncated proteins that could result in disease in humans. This is evident from dominantly inherited diseases that are due to PTC-containing mRNAs that escape NMD. Although many cellular NMD targets derive from mistakes made during, for example, pre-mRNA splicing and, possibly, transcription initiation, NMD also targets ~10% of normal physiological mRNAs so as to promote an appropriate cellular response to changing environmental milieus, including those that induce apoptosis, maturation or differentiation. Over the past ~35 years, a central goal in the NMD field has been to understand how cells discriminate mRNAs that are targeted by NMD from those that are not. In this Cell Science at a Glance and the accompanying poster, we review progress made towards this goal, focusing on human studies and the role of the key NMD factor up-frameshift protein 1 (UPF1).

KEY WORDS: NMD, RNA quality control, mRNA surveillance, Superfamily 1 ATP-dependent RNA helicase, UPF1 protein

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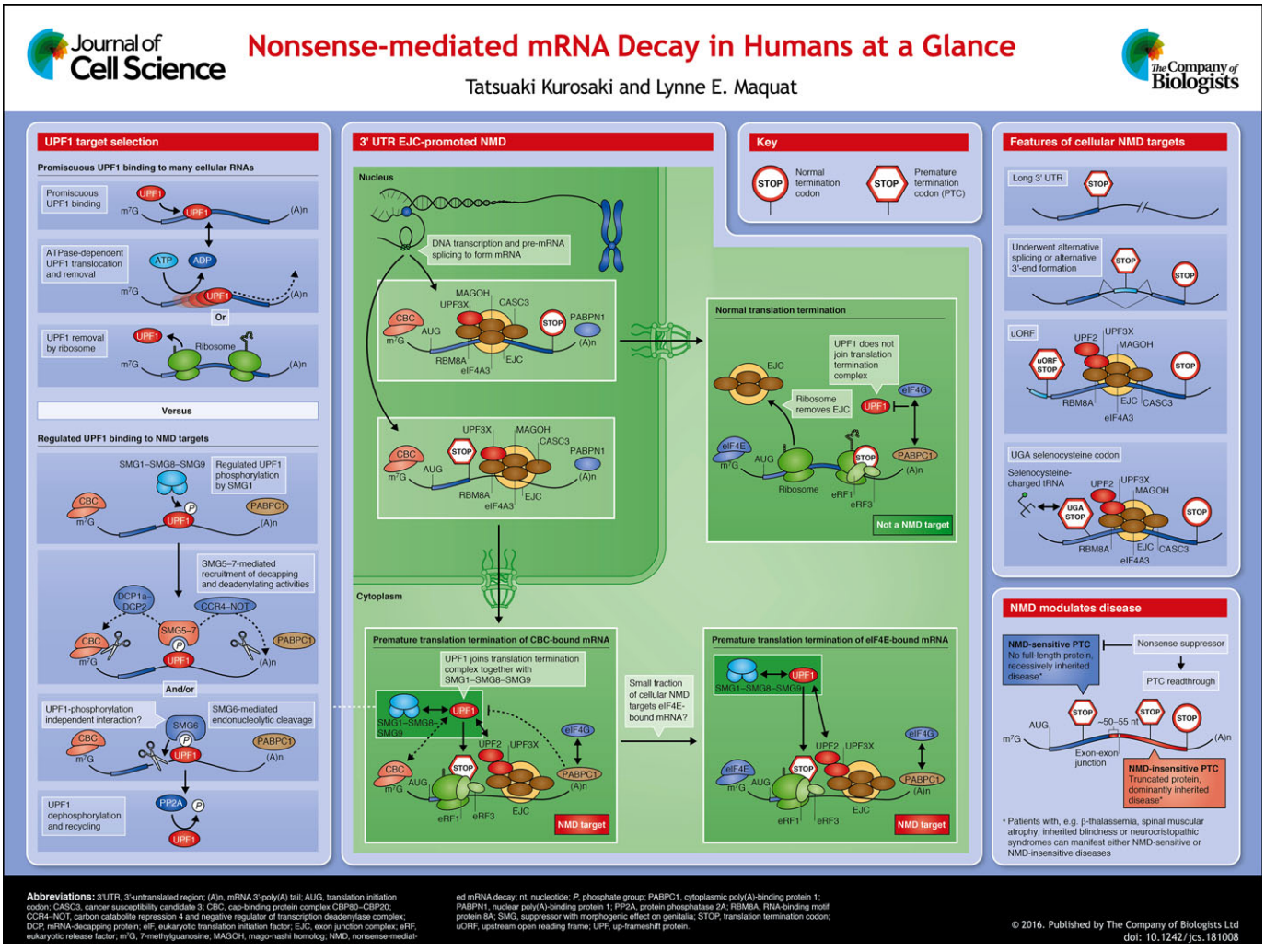
Introduction

Nonsense-mediated mRNA decay (NMD) is a translation-dependent mRNA surveillance mechanism in eukaryotes that helps to maintain the quality of gene expression. NMD accelerates the degradation of aberrant mRNAs harboring a premature termination codon (PTC) and, in this capacity, is estimated to downregulate one-third of



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disease-causing mRNAs (Frischmeyer and Dietz, 1999; Mort et al., 2008). In humans, PTCs were first reported to reduce mRNA abundance in 1979 (Chang et al., 1979) and, shortly after, to reduce mRNA stability (Maquat et al., 1981) through studies of the β^0-thalassemias. PTCs that trigger NMD were also observed in patients with other diseases, including triose phosphate isomerase (TPI) deficiency (Daar and Maquat, 1988) and Marfan syndrome (Caputi et al., 2002), as well as in naturally occurring transcripts that encode certain selenoproteins, such as glutathione peroxidase 1 (GPx1), providing the first evidence that NMD sustains normal cellular metabolism (Moriarty et al., 1997, 1998).

It is now widely accepted that NMD functions in at least two distinct cellular processes: (1) to downregulate abnormal transcripts that are generated as a consequence of routine errors in gene expression, and (2) to maintain an appropriate level of gene expression by downregulating physiological mRNAs in response to cellular needs. Transcriptome-wide analyses have revealed that NMD modulates ∼10% of human cell mRNAs that, for example, harbor an upstream open reading frame (uORF) or an intron downstream of the normal termination codon (see, for example, Mendell et al., 2004). NMD also downregulates many alternatively spliced mRNAs that are largely mistakes (Lewis et al., 2003; Pan et al., 2006), but in some cases provide a means for RNA-binding proteins or ribosomal proteins to autoregulate their own levels (Cuccurese et al., 2005; Lareau and Brenner, 2015; Saltzman et al., 2008; Sureau et al., 2001; Tani et al., 2012). It is also likely that NMD might be used to eliminate aberrant transcripts that escape nuclear quality control, as was found to typify transcripts from 47% of expressed genes in *Saccharomyces cerevisiae*, largely because of non-specific transcription initiation (Malabat et al., 2015). Here, we present progress made towards the goal of understanding how human cells identify NMD targets by reviewing the roles of known NMD factors and features of mRNAs that are NMD targets. Outstanding questions in the field include: what yet-to-be-identified proteins contribute to the NMD mechanism? To what extent do different 5′-cap-binding proteins typify mRNAs undergoing NMD? How can we predict 3′-untranslated region (3′UTR) structures in 3D and define 3′UTR sequences that inhibit NMD so as to develop rules for which mRNA isoforms are NMD targets? And how is the efficiency of NMD regulated during cellular adaptation to changing environmental conditions?

Central NMD factors in human cells

The key human NMD factor, up-frameshift protein 1 (UPF1), was named after its ortholog in *S. cerevisiae* (Leeds et al., 1991). UPF1 is a ∼123–124 kDa RNA-dependent ATPase and ATP-dependent RNA helicase that hydrolyzes ATP and unwinds RNA in the 5′-to-3′ direction, one purpose of which is presumably to remove proteins that are associated with an NMD target during the process of mRNA decay (Bhattacharya et al., 2000; Chakrabarti et al., 2011; Chamieh et al., 2008; Fiorini et al., 2013; Franks et al., 2010; Shigeoka et al., 2012). At least *in vitro*, human UPF1 manifests high processivity, but slow unwinding and translocation rates, which are estimated to be no more than one nucleotide per second (Fiorini et al., 2015).

During NMD, UPF1 associates with a translation termination codon through the translation termination complex, which consists of eukaryotic release factor 1 (eRF1, also known as ETF1) and eRF3 (for which there are two isoforms, eRF3a and eRF3b, also known as GSPT1 and GSPT2) (Ivanov et al., 2008; Kashima et al., 2006; Singh et al., 2008), together with the NMD factors suppressor with morphogenic effect on genitalia 1 (SMG1), which is a phosphatidylinositol 3-kinase-related protein kinase, SMG8 and SMG9 (Yamashita et al., 2009) (see poster). In humans, two UPF3 paralogs, UPF3 and UPF3X (also called UPF3A and UPF3B, respectively), differently modulate NMD activity (Chan et al., 2009; Kunz et al., 2006; Lykke-Andersen et al., 2000; Serin et al., 2001) in ways that, like UPF1 and another NMD factor, UPF2, have been shown to be crucial for, for example, normal neuronal maturation and development (Colak et al., 2013; Jolly et al., 2013; Laumonnier et al., 2010; Lou et al., 2014; Nguyen et al., 2012, 2013; Tarpey et al., 2007). UPF3 or UPF3X are generally associated with exon junction complexes (EJCs; see below) that are deposited in the nucleus upstream of newly spliced exon–exon junctions, whereas UPF2 is generally associated with EJCs after newly synthesized mRNAs are exported to the cytoplasm (Kim et al., 2001; Lejeune et al., 2002; Lykke-Andersen et al., 2001). NMD is triggered upon the translation-dependent and highly regulated association of UPF1 with an EJC when translation terminates sufficiently upstream of an EJC so that the terminating ribosome does not physically displace the EJC (see below). UPF2 bound by UPF3 or UPF3X interacts directly with the cysteine- and histidine-rich domain of UPF1, so as to open and activate the UPF1 helicase domain, facilitating mRNA unwinding and protein remodeling, activities that appear to be crucial for mRNA decay (Chamieh et al., 2008; Chakrabarti et al., 2011); Franks et al., 2010; Lykke-Andersen et al., 2000; Mendell et al., 2000; Serin et al., 2001; Kurosaki et al., 2014).

Activation of NMD by EJCs

The idea that pre-mRNA splicing deposits a ‘mark’ on newly synthesized mRNA in the nucleoplasm and that this mark persists until at least the first round of translation was derived from data indicating that PTCs situated more than ∼50–55 nucleotides upstream of an exon–exon junction generally trigger NMD (Nagy and Maquat, 1998). This mark was renamed the EJC, which is deposited ∼20–24 nucleotides upstream of ∼80% of exon–exon junctions (Le Hir et al., 2000a,b, 2001; Saulière et al., 2012; Singh et al., 2012). EJCs consist of four core components: eukaryotic translation initiation factor 4A3 (eIF4A3), cancer susceptibility candidate 3 (CASC3), RNA-binding motif protein 8A (RBM8A or Y14), and either mago-nashi homolog (MAGOH) or MAGOHB (see poster). EJCs upstream of and within mRNA coding regions are removed by ribosomes during translation in the cytoplasm (Dostie and Dreyfuss, 2002; Gehring et al., 2009; Sato and Maquat, 2009). However, because PTCs shorten the length of the coding region, any downstream EJCs that normally reside within the coding region would fail to be removed from what becomes the 3′UTR.

After UPF1, together with SMG1, SMG8 and SMG9, forms a complex with eRF1 and eRF3 at a termination codon, a single 3′ UTR EJC that is deposited at an exon–exon junction residing more than ∼50–55 nucleotides downstream of the termination codon (see poster) is sufficient to interact with the UPF1 complex, through EJC-bound UPF2 and UPF3 or UPF3X, and trigger NMD (Gehring et al., 2003; Hwang et al., 2010; Kim et al., 2001; Shibuya et al., 2004; Yamashita et al., 2009). mRNA decay requires SMG1-mediated UPF1 phosphorylation, which is tightly regulated by a conformational change in SMG1 that is mediated by SMG8 and SMG9 (Arias-Palomo et al., 2011; Deniaud et al., 2015; Melero et al., 2014; Yamashita et al., 2009) (see poster). SMG1 recognizes and phosphorylates serine and threonine residues that are situated adjacent to a glutamine residue (S/TQ motifs), which are enriched within the C-terminal end of human UPF1 (Kashima et al., 2006; Okada-Katsuhata et al., 2012; Yamashita et al., 2001). The 3′UTR EJC-promoted NMD pathway controls various cellular processes, given that it is triggered by a number of events, such as the

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introduction of a PTC due to a translational frameshift because of alternative splicing, the introduction of a 3′UTR EJC because of alternative splicing and/or alternative 3′-end formation, translation termination at an uORF or translation termination at some UGA selenocysteine codons (Mendell et al., 2004) (see poster).

There are also several variations of NMD. For example, some cellular NMD targets are less sensitive to a downregulation of UPF2 or UPF3X, suggesting that one of these two factors is sufficient for NMD in some instances (Chan et al., 2007; Gehring et al., 2005; Huang et al., 2011; Ivanov et al., 2008). Consistent with there being alternative or 'branched' NMD mechanisms, RBM8A, MAGOH, CASC3 and eIF4A3 have been reported to activate NMD in a UPF2-independent pathway, whereas NMD that involves the RNA-binding protein with serine-rich domain 1 (RNPS1) requires UPF2 (Gehring et al., 2005; Lykke-Andersen et al., 2001). Another alternative mechanism, which has been called 'failsafe NMD' or '3′UTR EJC-independent NMD', has the general characteristic of a long 3′UTR (estimated to be ≥420 nucleotides, but exceptions exist) and appears to be less efficient than 3′UTR EJC-promoted NMD (Bühler et al., 2006; Eberle et al., 2008; Matsuda et al., 2007; Singh et al., 2008; Zhang et al., 1998a,b).

3′UTR EJC-independent NMD

The activation of NMD by a long mRNA 3′UTR presumably reflects the physical distance required between a termination codon and the mRNA 3′ poly(A) tail through its association with the cytoplasmic poly(A)-binding protein 1 (PABPC1), which can modulate NMD in the absence of a 3′UTR EJC (see below). However, genome-wide analyses of transcript levels or their half-lives suggest that 3′UTR length, as measured linearly by the number of its constituent nucleotides, does not strongly correlate with NMD activity (Hurt et al., 2013; Tani et al., 2012).

There are several reasons why predicting whether an mRNA is an NMD target cannot be made based on its 3′UTR length alone. For one, tethering PABPC1 or eIF4G, which interacts with PABPC1, to a position that resides between a termination codon and the 3′ poly(A) tail can inhibit NMD (Eberle et al., 2008; Fatscher et al., 2014; Joncourt et al., 2014; Peixeiro et al., 2012; Silva et al., 2008; Singh et al., 2008). Thus, NMD can be inhibited if PABPC1 is brought sufficiently close to the 3D environment of a termination codon, either by direct binding or through eIF4G-mediated recruitment, effectively shortening the 3′UTR length regardless of how many nucleotides constitute the 3′UTR. Another indication that the proximity between PABPC1 and a termination codon can inhibit NMD derives from the finding that most 5′ PTCs only inefficiently trigger 3′UTR EJC-promoted NMD, presumably because mRNAs form a 'closed-loop' that brings PABPC1 in proximity to the 5′-end of the mRNA through its eIF4G-mediated interaction with 5′-cap-binding proteins (Lejeune et al., 2004; Peixeiro et al., 2012; Silva et al., 2008) (see poster).

A second indication that mRNA 3′UTR length per se cannot be used to predict NMD targets derives from the discovery of naturally occurring but yet-to-be well characterized 3′UTR-stabilizing elements (Toma et al., 2015). These elements might recruit complexes that inhibit NMD, such as the RNA-binding cytidine deaminase (APOBEC1) and complementing specificity factor (ACF) complex that inhibits the NMD of apolipoprotein B (apoB) mRNA after a CAA codon has been edited to a UAA PTC (Chester et al., 2003). Notably, the 3′UTR RNA stability element in Rous sarcoma virus (Weil and Beemon, 2006; Weil et al., 2009) might represent a good system for characterizing host proteins and salient cis-acting features that a viral RNA has co-opted for defeating NMD in the host cell. The positioning of these stabilizing elements close to, but downstream of, the termination codon appears to be important for their function in inhibiting NMD.

Translation modulates binding of human UPF1 to cellular mRNAs

How does translation intersect with the activation of UPF1? Although the premature termination of translation enhances the binding of steady-state UPF1, which is largely hypo-phosphorylated (i.e. yet to be activated by SMG1-mediated phosphorylation), to the downstream 3′UTR (Kurosaki and Maquat, 2013; Lee et al., 2015), recent biochemical and transcriptome analyses have revealed that steady-state UPF1 associates with regions of cellular RNAs that are physically accessible to its binding, independently of translation. These RNAs include canonically defined NMD targets and also non-NMD targets, including long non-coding RNAs (Gregersen et al., 2014; Hogg and Goff, 2010; Hurt et al., 2013; Kurosaki and Maquat, 2013; Lee et al., 2015; Zünd et al., 2013). This promiscuous UPF1 binding is prevented or removed from 5′UTRs and coding regions by scanning and translationally active ribosomes, respectively, but is possible in 3′UTR regions and has been found to correlate with 3′UTR length (Gregersen et al., 2014; Hogg and Goff, 2010; Kurosaki and Maquat, 2013; Lee et al., 2015; Zünd et al., 2013). Steady-state UPF1 undergoes a transient interaction with any available RNA, from which it is released by its ATP-dependent RNA helicase activity (Kurosaki et al., 2014; Lee et al., 2015), and does not detectably form a complex with UPF2 or UPF3X (Kurosaki et al., 2014) (see poster). Importantly, because steady-state UPF1 binds to cellular RNAs promiscuously, and independently of NMD, cellular NMD targets cannot be predicted based on their co-immunoprecipitation with steady-state UPF1 (Gregersen et al., 2014; Zünd et al., 2013). Instead, cellular NMD targets can be identified based on their binding to phosphorylated UPF1 as discussed below (Kurosaki et al., 2014).

Role of human UPF1 phosphorylation in triggering NMD

The above-mentioned step of SMG1-mediated phosphorylation and thus activation of UPF1 represses any additional translation initiation events on an NMD target, and repression appears to be crucial for mRNA decay (Isken et al., 2008). Phosphorylation of UPF1 also enhances its interaction with SMG6 and/or the SMG5–SMG7 heterodimer (Franks et al., 2010; Kashima et al., 2006; Kurosaki et al., 2014; Ohnishi et al., 2003), although a phosphorylation-independent interaction between UPF1 and SMG6 has also been reported (Chakarabarti et al., 2014; Nicholson et al., 2014) (see poster). SMG5 and SMG6, which possess a PilT-N-terminal (PIN) domain that typifies some ribonucleases, bind to phosphorylated UPF1 and promote its dephosphorylation through the recruitment of protein phosphatase 2A, apparently after mRNA decay initiates (Anders et al., 2003; Boehm et al., 2014; Chiu et al., 2003; Kurosaki et al., 2014; Lee et al., 2015; Ohnishi et al., 2003; Schmidt et al., 2015) (see poster). Additionally, SMG6 is an active endonuclease that transiently associates with and cleaves NMD targets in the vicinity of the PTC, approximately where the interaction between phosphorylated UPF1 and the 3′UTR of mRNA occurs (Eberle et al., 2009; Gatfield and Izaurralde, 2004; Huntzinger et al., 2008; Kurosaki et al., 2014; Lykke-Andersen et al., 2014). In contrast, SMG5 and SMG7 lack any intrinsic nuclease activity and form a stable heterodimer on a NMD target (Jonas et al., 2013; Kurosaki et al., 2014; Loh et al., 2013; Ohnishi et al., 2003). This results in the recruitment of the CCR4–NOT

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deadenylase complex by SMG7 (Loh et al., 2013), whereas SMG5 recruits the mRNA-decapping protein DCP2 and its binding partner DCP1a (Cho et al., 2009, 2013). Given that mRNA decay intermediates lacking a 5′ cap or 3′ poly(A) tail are intrinsically unstable, the ultimate outcome is the exonucleolytic decay of the NMD target (see poster).

The cellular site of NMD and pioneer round of translation

For the majority of the mRNAs analyzed, NMD is associated with the nucleus, meaning that the observed reduction in mRNA abundance is found in nuclear fractions and is not attributable to their contamination with cytoplasmic content; early explanations posited that NMD degrades newly synthesized mRNAs as they exit the nuclear pore complex because these mRNAs could be co-purified with nuclei and simultaneously translated (Belgrader et al., 1994; Cheng and Maquat, 1993; Humphries et al., 1984; Urlaub et al., 1989). Consistent with this idea, a recent study has shown that NMD of PTC-containing β-globin mRNA occurs within 5–56 s of entering the cytoplasm and within ∼430 nm of the nuclear boundary, and there is no evidence for NMD taking place in the nucleoplasm or after newly synthesized PTC-containing β-globin mRNA is released into the cytoplasm (Trcek et al., 2013). Indeed, the decay of PTC-containing β-globin and TPI mRNAs is biphasic, with NMD occurring first, before the fraction of mRNA that escapes NMD is degraded with a half-life that is the same as that of its PTC-free counterpart (Belgrader et al., 1994; Cheng and Maquat, 1993; Trcek et al., 2013). Consistent with these findings, NMD appears to occur largely during the first or pioneer round of translation, which utilizes mRNAs that have yet to lose both the cap-binding protein (CBP) heterodimer CBP80–CBP20, which is deposited prior to its being replaced by eIF4E (Ishigaki et al., 2001), and the EJCs (Lejeune et al., 2002). Notably, this round can involve more than one ribosome, depending on the length of the coding region and efficiency of translation initiation (Isken et al., 2008). Although it has been shown that a proportion of the human PTC-containing mRNAs encoding β-globin, T-cell receptor β and immunoglobulin μ are targeted for NMD in their eIF4E-bound form (Durand and Lykke-Andersen, 2013; Rufener and Mühlemann, 2013), as is the case in yeast (Gao et al., 2005), it remains to be determined how much the decay of human eIF4E-bound mRNAs contributes to cellular NMD (see poster). Given that translation does not promote the replacement of CBP80–CBP20 by eIF4E on an mRNA (Sato and Maquat, 2009), it is possible that some newly synthesized eIF4E-bound mRNA could be targeted for NMD. However, EJC constituents are not detectable in immunoprecipitations of eIF4E-bound mRNAs (Kashima et al., 2006; Lejeune et al., 2002), raising uncertainty about the extent to which the NMD of eIF4E-bound mRNAs generally contributes to cellular metabolism, although, possibly, circumstances exist under which it does (Durand and Lykke-Andersen, 2013).

An exception to nucleus-associated NMD is provided by the selenoprotein-encoding GPx1 mRNA, which is targeted for NMD after it has been released from nuclei into the cytoplasm, but while it remains bound by CBP80–CBP20 (Ishigaki et al., 2001; Moriarty et al., 1998). In fact, CBP80 has been shown to activate NMD through its transient and/or weak interaction with UPF1, which promotes the regulated binding of the SMG1–UPF1 complex to eRF1–eRF3 and, subsequently, to a downstream EJC (Hosoda et al., 2005; Hwang et al., 2010) (see poster). It should be noted, however, that others have failed to detect an interaction between CBP80 and UPF1 (Rufener and Mühlemann, 2013).

Therapeutic approaches for PTC-associated diseases

One-third of inherited human diseases are due to PTCs that are introduced by nonsense mutations, frameshift mutations or splicing errors (Frischmeyer and Dietz, 1999; Linde and Kerem, 2008), and nonsense mutations account for ∼20% of the around 43,000 disease-associated single-base pair substitutions (Mort et al., 2008) and for 5 to 70% of genetic disorders (Lee and Dougherty, 2012). NMD functions to eliminate transcripts that harbor nonsense codons that would otherwise result in the production of truncated proteins that have the potential to increase disease severity. Indeed, PTCs that are unable to trigger NMD because they reside less than 50 nucleotides upstream of the last exon–exon junction or within the last exon cause dominantly inherited forms of, for example, β-thalassemia, spinal muscular atrophy, inherited blindness and neurocristopathic syndromes (Bhuvanagiri et al., 2010; Holbrook et al., 2004) (see poster).

One potential therapeutic treatment of diseases that are due to in-frame nonsense (but not frameshift) mutations is to suppress translation termination at a PTC and, therefore, allow some full-length protein to be produced. This can been achieved with the use of the nonsense-codon suppressors Ataluren, read-through compound (RTC)13, Amlexanox, synthetic aminoglycosides and nonaminoglycosides (Du et al., 2008, 2009; Gonzalez-Hilarion et al., 2012; Nakamura et al., 2012; Shalev and Baasov, 2014; Welch et al., 2007), although their lack of drug-target specificity is a great concern, given the large number of physiological cellular mRNAs that are NMD targets. Among these compounds, Ataluren (Translarna) is farthest along the drug-development pipeline, as it has shown the greatest effectiveness in treating Duchenne muscular dystrophy caused by nonsense mutations, but at doses that do not detectably inhibit NMD, consistent with its mild effects on cellular metabolism (Finkel et al., 2013; Welch et al., 2007). Even though results from the recent phase 3 clinical trial, which employed the 6-minute walk test for the treatment of Duchenne muscular dystrophy caused by nonsense mutations, failed to meet the pre-determined endpoint with statistical significance, the drug might be useful for a sub-group of patients (see, for example, http://www.raredr.com/news/ptc-therapeutics-phase-3-duchenne-study-fails-to-meet-primary-endpoint). In contrast to Ataluren, cardiac glycosides, which elevate the level of intracellular Ca^{2+}, are capable of inhibiting NMD at concentrations that reportedly do not affect cellular viability (Nickless et al., 2014). It might be possible to concomitantly use Ataluren and cardiac glycosides, or possibly a clinically effective nonsense suppressor with either another small reagent that has been reported to inhibit NMD (Bhuvanagiri et al., 2014; Martin et al., 2014; Dang et al., 2009; Feng et al., 2015; Gopalsamy et al., 2012; Usuki et al., 2004) or one or more antisense oligonucleotides that occlude deposition of the one or more EJCs that would normally reside downstream of a particular PTC (Nomakuchi et al., in press). Another approach that might be worthwhile for obtaining full-length protein from disease-associated mRNAs is site-directed pseudouridylation of in-frame PTCs (Karijolich and Yu, 2011); by providing a gene-specific therapeutic strategy, such a strategy would be expected to have only minimal toxic side effects.

Perspectives

NMD is a fundamental cellular mechanism utilized by eukaryotes to downregulate PTC-containing mRNAs that are routinely generated by cells through errors made during gene expression and that also naturally exist or are generated as part of autoregulatory mechanisms to maintain cellular homeostasis. Furthermore, NMD is intimately

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connected to human health and disease; PTCs that trigger NMD can give rise to disease by precluding the production of full-length protein, and PTCs that escape NMD can generate toxic levels of truncated proteins that can be detrimental. In fact, deficiencies in NMD activity lead to intellectual disabilities and developmental defects.

Although extensive studies on NMD over three decades have revealed details of the molecular mechanisms through which NMD occurs, developing molecular therapies for nonsense mutations that cause various inherited diseases remains challenging. The payoff, however, is that many different diseases with diverse physical symptoms can potentially be affected by modulating the activity of a single pathway, NMD. Revealing the full picture of how NMD operates will provide important insights into the treatment of associated diseases and aid in the development of effective and safe therapeutic strategies.

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Competing interests
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