



Nonsense-mediated decay approaches the clinic

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Nonsense-mediated decay (NMD) eliminates mRNAs containing premature termination codons and thus helps limit the synthesis of abnormal proteins. New results uncover a broader role of NMD as a pathway that also affects the expression of wild-type genes and alternative-splice products. Because the mechanisms by which NMD operates have received much attention, we discuss here the emerging awareness of the impact of NMD on the manifestation of human genetic diseases. We explore how an understanding of NMD accounts for phenotypic differences in diseases caused by premature termination codons. Specifically, we consider how the protective function of NMD sometimes benefits heterozygous carriers and, in contrast, sometimes contributes to a clinical picture of protein deficiency by inhibiting expression of partially functional proteins. Potential 'NMD therapeutics' will therefore need to strike a balance between the general physiological benefits of NMD and its detrimental effects in cases of specific genetic mutations.

In 1979, two research groups discovered that nonsense mutations result in decreased abundance of affected mRNA transcripts, rather than in production of truncated proteins. The destabilizing effect of nonsense mutations on mRNA was initially observed in human β -globin¹ and in yeast orotidine-5'-phosphate decarboxylase², and later in genes from *Drosophila melanogaster*³ and *Caenorhabditis elegans*⁴. These results demonstrated broad phylogenetic conservation of a cellular mechanism, now known as NMD, that reduces the abundance of transcripts containing a premature termination codon (PTC). Although important aspects of the NMD mechanism can differ between yeast, worms, flies and humans, the core process and proteins seem to be conserved. In addition to its role in eliminating 'faulty' transcripts encoding potentially dominant negative proteins, NMD also has a role in controlling expression of 'normal' genes and is implicated in several essential physiological processes. NMD is not always beneficial: in some instances, truncated proteins encoded by mRNAs carrying PTCs are fully or partially functional but are not synthesized because of NMD. For such cases, treatment strategies to reduce the effects of NMD are under evaluation. In this review, we focus on mammalian NMD and its expanding physiological role and medical implications.

The molecular mechanism of NMD

NMD is one of the most enigmatic and complex processes in mammalian mRNA metabolism. It must distinguish a normal stop codon from a premature one. For this discrimination, both splicing and

translation are crucial in mammals. A translation termination codon is generally interpreted as normal if no intron follows more than 50 nucleotides downstream⁵. Thus, most normal stop codons are located in the last exon, whereas NMD-activating PTCs are usually located further upstream. The importance of splicing is demonstrated by the NMD-insensitivity of naturally intronless genes^{6,7} and of intronless cDNA versions of genes encoding PTCs that are normally subject to NMD⁸. Because the PTC is probably identified during ribosomal translation, the position of (excised) introns must be communicated from the splicing apparatus to the translational apparatus. This seems to occur through splicing-mediated marking of exon-exon boundaries, in which a dynamic assembly of proteins, known as the exon junction complex (EJC; **Table 1**), is deposited in a sequence-nonspecific manner ~20–24 nucleotides 5' to every exon-exon junction⁹ (**Fig. 1**). Consistent with their role in indicating the positions of excised introns, several EJC proteins remain associated with exon-exon junctions after mRNAs exit the nucleus^{10,11}.

The degradation of mRNAs containing PTCs seems to require the key protein UPF1, which associates with both ribosomes¹² and release factors¹³ and undergoes phosphorylation^{14,15} and dephosphorylation^{15,16} in a cycle essential for NMD¹⁷. In humans, this cycle is probably mediated by orthologs of the *C. elegans* smg proteins SMG-1, SMG-5 and SMG-7 (**Fig. 1** and **Table 1**), but its exact role in triggering transcript decay is not yet well elucidated. A detailed mechanistic description of NMD as it is currently understood is available in recent reviews^{5,18–20} and in the references in **Table 1**.

NMD targets and exceptions

The current mechanistic understanding of NMD leads to the prediction that transcripts containing PTCs 5' to the last 50 nucleotides of the penultimate exon should be targeted by NMD²¹. But many transcripts containing PTCs do not obey this prediction, indicating that the current understanding of NMD is incomplete. Such exceptions

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include, most notably, transcripts from the T-cell receptor- β locus, in which NMD is triggered by PTCs residing in the last 50 nucleotides of the penultimate exon²². Other transcripts seem to undergo NMD despite having a PTC in the last exon²³. In contrast, other expected NMD substrates have been reported to be insensitive to NMD, including pathological transcripts^{24–26} and also normal splice variants^{27,28}. It is generally not known how these transcripts escape NMD, although in one case (a form of ApoB that contains a PTC), a protein complex involved in the generation of the PTC by C→U RNA editing remains associated with the mRNA, concealing the PTC from NMD²⁹. In addition to these exceptions, there seem to be intertissue³⁰ and interindividual³¹ variations in NMD efficiency. In sum, because NMD does not degrade some eligible transcripts, seems to operate with variable efficiency and does not completely degrade all transcripts containing PTCs, even for NMD-competent mRNAs, translation of residual mRNAs containing PTCs can potentially lead to functionally important expression of truncated proteins. For instance, a PTC-containing alternative-splice product of the high-affinity IgE receptor- β subunit is expressed at low levels, typical for NMD, but nevertheless is translated into a truncated protein that seems to compete with the full-length

isoform in a physiologically relevant way³². A similar example is the *unc-49* locus of *C. elegans*, which produces a PTC-containing splice form that not only is expressed at substantial levels, but also encodes a GABA-receptor isoform that is essential for worm locomotion²⁸.

Given these uncertainties, it is important to appreciate that mRNAs containing PTCs cannot be assumed *a priori* either to result in synthesis of considerable amounts of truncated proteins or to be degraded by NMD. Thus, the functional consequences of a PTC mutation must be established by experimentation (**Supplementary Fig. 1** online). In this context, a quantitative method of mRNA analysis (*i.e.*, northern blotting, RNase protection analysis or quantitative RT-PCR) is required to determine the extent of transcript downregulation. Reducing UPF1 expression by treatment with specific small interfering RNA or overexpression of a dominant negative mutant are currently used as functional assays to assess the NMD-sensitivity of a transcript containing PTCs. Pleiotropic inhibitors of translation (*e.g.*, cycloheximide) may also be used, although the effects are less specific. To determine whether substantial translation of truncated protein occurs, the presence and degree of protein expression should be established.

Table 1 Protein components of the human NMD machinery

Protein	Step in NMD	Cellular localization	Functional characteristics	<i>S. cerevisiae</i> / <i>C. elegans</i> orthologs
UPF1 (RENT1)	NMD factor thought to function late in NMD and to bridge between premature termination event and EJC	Shuttles; primarily cytoplasmic	ATPase, helicase; involved in translational termination (yeast); dominant-negative variant and UPF1 siRNAs diminish NMD; phosphorylation cycle necessary for NMD; additional functions in nonsense-mediated altered splicing and in RNAi (<i>C. elegans</i>)	Upf1/smg2
UPF2 (RENT2)	NMD factor suggested to be recruited to EJC by UPF3 and perinuclear then to recruit UPF1	Mainly cytoplasmic,	Binds to UPF1 and both forms of UPF3; not clear at which stage the assembly with an NMD-active ribonucleoprotein particle occurs	Upf2/smg3
UPF3B (UPF3X)	Probably first NMD factor that associates with EJC; can bind complex containing UPF2 and Y14/MAGOH	Mainly nuclear; shuttles	Deletion of a domain that mediates interaction with a complex containing Y14 abolishes NMD in tethering assay; UPF3B can elicit NMD in a tethered function assay without direct binding to UPF2	Upf3/smg4
UPF3A (UPF3)	Unclear; associated with EJC	Mainly nuclear, shuttles	Shorter (UPF3A-S) and longer (UPF3A-L) splice variants differentially distribute into distinct complexes with UPF1, probably related to UPF1's phosphorylation status	
Y14/MAGOH heterodimer	EJC component; proposed to help recruit UPF proteins; functionally interacts with UPF3A/B	shuttling	Stable heterodimer; accompanies mRNA into polysomes; ultimately removed by translation; forms an NMD-activating complex with UPF3B	
RNPS1	EJC component; proposed	Shuttles	Splicing coactivator	
eIF4AIII (refs. 97–99)	EJC component; thought to bind to the exon-exon junction directly and then to anchor Y14/MAGOH and BTZ to the EJC	Nuclear and cytoplasmic; shuttles	Found in splicing complexes and spliceosomes containing pre-mRNA; closely related to the general translation initiation factors eIF4AI and eIF4AII; role in translation unclear	
BARENTSZ (BTZ ⁹⁸ , MLN51 and CASC3)	Forms a complex with eIF4AIII and Y14/MAGOH, perhaps in the cytoplasm	Cytoplasmic	Dual role in NMD and in mRNA localization; interacts with eIF4AIII	
P29 (ref. 100; PYM)	Forms a ternary complex with Y14/MAGOH, perhaps in the cytoplasm	Cytoplasmic owing to active export from nucleus by CRM1	RNA binding protein; the amino acids in MAGOH that have a role in NMD are the same as those used to bind to P29; P29 triggers NMD in tethering assay	
SMG-1	UPF1 kinase	Predominantly cytoplasmic	Related to phosphatidylinositol 3-kinase	NA/smg1
SMG-5 (SMG5/7b), SMG-6 (SMG5/7a) and SMG-7	Promote UPF1 dephosphorylation	Predominantly cytoplasmic; shuttle	SMG-5 and SMG-7 probably interact with PP2CA (Fig. 1); all seem to be involved in dephosphorylation of UPF1; SMG-5 and SMG-6 are identical to EST1B and EST1A, respectively, which are involved in telomere maintenance	NA/smg5, smg6 and smg7

'NMD factors' indicates factors that are required for NMD in all organisms investigated. All EJC components listed here, as well as P29 and BTZ, seem to be important for NMD in human cells, as shown by RNAi or by their ability to trigger NMD when artificially tethered downstream of a normal termination codon. The EJC contains several additional components that do not seem to have a role in NMD. More detailed information on the NMD mechanism and the factors listed here is available in refs. 5,19,20 and references therein. References concerning proteins whose involvement in NMD has been discovered too recently to be included in the most recent reviews are cited in the table.

The role of NMD in regulation of normal gene expression

Although PTCs are often pathological, normal mRNAs may also contain PTCs as a result of somatic rearrangements in DNA^{33,34}, the presence of upstream open reading frames, the use of alternative open reading frames³⁵ or the presence of UGA codons encoding selenocysteine^{36,37}. Faulty or alternative splicing also creates PTCs, and NMD probably limits the expression of such transcripts. The number of such NMD-modulated splice forms could be very large: a recent analysis predicted that ~3,100 of 16,000 human genes examined should produce at least one alternative-splice product, one-third of which would contain PTCs. Approximately 75% of these alternative transcripts would be expected to undergo NMD due to the position of the PTC³⁸. NMD may therefore serve as a molecular 'vacuum cleaner', ridding the cell of many alternatively spliced mRNAs. Yet, as NMD does not completely ablate expression of mRNAs containing PTCs, and as residual expression levels vary considerably between transcripts, cell types and even individuals, NMD can contribute to balancing the expression of protein or RNA isoforms, with respect to both each other and other gene products. To date, a physiologic role for NMD in controlling the amounts or proportions of splice products has been demonstrated for a handful of transcripts, including calpain 10 (ref. 39); ATP-binding cassette, sub-family C, member 4 (ref. 40); ferrochelatase⁴¹, arginine/serine-rich splicing factor 2 (ref. 42); and polypyrimidine

tract binding protein 1 (ref. 43; Table 2). Arginine/serine-rich splicing factor 2 and polypyrimidine tract binding protein 1 seem to regulate their own expression through splicing-directed NMD.

Further contributions from NMD to physiological gene expression are evident in the maturation of the immune system. In B and T cells, the immunoglobulin and T-cell receptor genes, respectively, are rearranged extensively, introducing PTCs in two-thirds of cases, and transcripts from these genes are degraded by NMD. Presumably, at the level of the entire B- or T-cell population, permitting expression of only successfully rearranged genes results in a large number of fully functional variant immunoglobulin or T-cell receptor proteins, greatly increasing the ability of the immune system to respond to a variety of antigens (reviewed in refs. 33 and 34). Furthermore, NMD components seem to be involved in telomere maintenance. A regulator of telomerase in yeast, Est1p, shows homology to *C. elegans* NMD proteins smg-5, smg-6 and smg-7, as well as human EST1A (also named SMG-6 (ref. 15) and SMG5/7a (ref. 16)). EST1A copurifies with the UPF proteins involved in NMD¹⁶, associates with telomerase⁴⁴ and aids in telomere capping⁴⁵, suggesting that it, as well as other components of the NMD machinery, has a role in telomere maintenance in humans. The functional importance of the complete NMD pathway in this respect has been demonstrated in *Saccharomyces cerevisiae*, where transcripts encoding various telomere maintenance components are

Figure 1 Simplified model of NMD. During splicing in the nucleus, a protein complex (EJC) is deposited 5' of exon-exon junctions. Upon transport through the nuclear pore, the EJC (red) is remodeled, several proteins are released and others persist and accompany the mRNP into the polysomes. During translation of a PTC-free mRNA (left), the EJCs (green) are stripped off the mRNA by the translating ribosome (blue). This first round of translation, which may occur either while the mRNA is still associated with the nuclear membrane or when it is completely translocated, validates the mRNA as error-free and, hence, it remains stable. If the translating ribosome encounters a PTC upstream of at least one EJC (right), however, NMD is triggered. How the presence of downstream EJC proteins is communicated to the ribosome is not known. The EJC proteins are thought to recruit NMD factors to the transcript, although the details and sequence of events that ultimately leads to the assembly of an NMD-active complex have yet to be determined (reviewed in ref. 5). Gray shading on the mutated transcript indicates the altered 3' RNP domain in comparison with the error-free mRNA. The phosphoprotein UPF1 (yellow) is a key player in mammalian NMD and is involved in translational termination, as indicated by its interaction with release factors (eRF1 and eRF3b; pentagonal symbols at the ribosome). Degradation may be possible from either the 5' or the 3' end, probably depending on the type of transcript. The inset shows the essential phosphorylation cycle of UPF1. This cycle is necessary for NMD to occur and is mediated through SMG factors: protein SMG-1 phosphorylates UPF1, and then a complex consisting of SMG-5, SMG-7 and the catalytic subunit of protein phosphatase 2A (PP2CA) dephosphorylates UPF1.

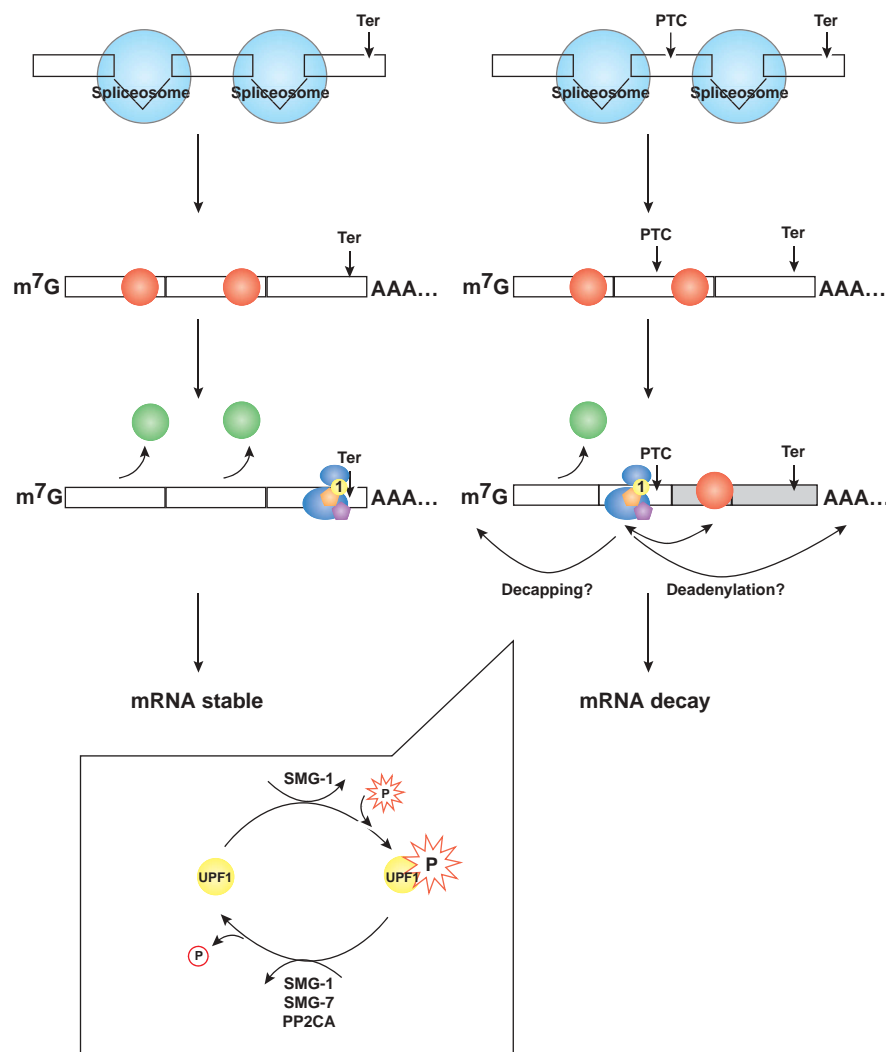


Table 2 Putative physiological roles of NMD

Role	Gene or protein regulated	Function of regulated gene or protein	Reference(s)
Regulation of alternative splice forms through degradation of transcripts containing PTC	Calpain 10	Linked to type 2 diabetes	39
	ATP-binding cassette, sub-family C, member 4	Multidrug resistance transporter	40
	Arginine/serine-rich splicing factor 2	Splicing factor; autoregulates through splicing-induced NMD	42
	Polypyrimidine tract binding protein 1	RNA binding protein; autoregulates through splicing-induced NMD	43
	Ferrochelatase	Heme biosynthesis	41
Embryonic development (UPF1 protein)	Specific UPF1 targets are unknown, but homozygous null mice die <i>in utero</i>	Unknown whether embryonic lethality is related to the NMD function of UPF1 or is a separate function	47
Immune maturation through degradation of transcripts from nonproductive gene rearrangements	Immunoglobulin (B cell)	Humoral immune response	33,34
	T-cell receptor (T cell)	Cellular immune response	33,34
Regulation of selenoprotein synthesis (UGA codes for selenocysteine when selenium is abundant and acts as a PTC when selenium levels are low)	Glutathione peroxidase 1	Antioxidant enzyme functioning in many tissues	36
	Phospholipid hydroperoxide glutathione peroxidase	Protects against toxic lipid hydroperoxides	37
Degradation of exonic sequences containing PTC after splicing out small nucleolar RNAs from intronic sequence	Fibrillarin-associated, snoRNAs U25-U31	Ribosomal RNA processing and modification	48,49,50
	U17a and U17b snoRNAs		
Telomere capping (EST1A (identical to SMG6 or SMG5/7a) and EST1B (identical to SMG5 or SMG5/7b))	Telomerase catalytic subunit	SMG proteins are key components in the phosphorylation cycle of NMD (Fig. 1) and also regulate access of telomerase to the telomere; in yeast, transcripts coding for telomere maintenance factors are NMD substrates	16,44,45,46

themselves direct or indirect NMD targets, and where disruption of NMD leads to telomere defects (reviewed in ref. 46). The functional involvement of NMD in a crucial genetic process such as telomere maintenance could be an additional reason for its broad phylogenetic conservation. Moreover, NMD or its components also affect several other important biological processes, including growth and development⁴⁷, production of small nucleolar mRNAs^{48,49} (reviewed in ref. 50) and regulation of selenoprotein mRNAs^{36,37} (Table 2).

NMD-mediated protection against hereditary disorders

The medical significance of NMD in this respect was first appreciated in β -thalassemia, and this disorder demonstrates the protective effects of NMD against the production of faulty proteins. Normal erythroid cells are loaded with hemoglobin A, which is composed of two α - and two β -globin subunits that interact to form the quaternary complex necessary for oxygen transport. In the common recessive form of β -thalassemia caused by NMD-sensitive PTC mutations, defective β -globin mRNA is degraded by NMD and, therefore, synthesis of truncated β -globin is limited⁵¹. The resultant excess of free α -globin, which is harmful to the cell, is degraded proteolytically. In contrast to homozygotes, heterozygotes with these mutations generally synthesize enough β -globin from the remaining normal allele to support near-normal hemoglobin levels and are therefore healthy. Rare NMD-

insensitive last-exon PTC mutations, however, give rise to truncated, nonfunctional β -globin that overwhelms the cell's proteolytic system and causes toxic precipitation of insoluble globin chains⁵². This stark contrast between asymptomatic heterozygotes with NMD-competent mutations and affected heterozygotes with NMD-incompetent mutations (Fig. 2) shows that, for PTC mutations in the β -globin gene, NMD protects most heterozygous carriers from dominant disease⁵³.

Similar disease-modulating effects of NMD can explain genotype-phenotype relationships in a number of other conditions, including susceptibility to mycobacterial infections^{54,55}, brachydactyly type B⁵⁶, von Willebrand disease⁵⁷, factor X deficiency⁵⁸ and retinal degeneration⁵⁹⁻⁶¹. Even when disease results from NMD-induced protein deficiency, this phenotype may be milder than and different from that caused by an expressed mutant protein. In a recently investigated form of neural developmental anomaly, NMD-competent mutations led to a haploinsufficient phenotype, whereas NMD-incompetent mutations resulted in a more complex condition with additional features⁶² (Table 3). Taken together, the pattern of these genetic conditions supports the contention that NMD protects many heterozygous carriers of genes with PTC mutations from manifesting disease phenotypes that would result from expression of truncated proteins. Owing to the limited amount of data available, however, it is difficult to estimate the overall number of genetic diseases in which NMD

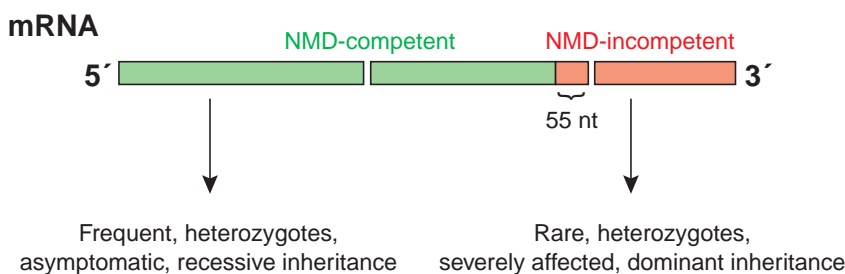


Figure 2 Position-dependent effects of nonsense mutations of NMD correlate with inheritance pattern and clinical severity of disease. For the β -globin gene, the common PTCs in the 5' portion of the gene are targeted by NMD, protecting heterozygotes from thalassemia. In contrast, mRNAs with PTCs in the 3' portion of the gene are translated into truncated proteins that result in symptomatic, dominantly inherited β -thalassemia. Other conditions listed in Table 3 have similar characteristics.



exerts a protective effect. Although the number of mutations leading to PTCs (and therefore the number of potentially 'NMD-eligible' transcripts) could be estimated with reasonable accuracy, at least some portion of these messages, if translated, would probably produce inactive proteins (rather than dominant-negative-acting proteins). Therefore, at this point, it is not possible to determine the total number of conditions in which NMD affects disease phenotype.

NMD in acquired genetic conditions

Mutations in tumor-suppressor genes are common steps in the development and progression of cancer. NMD degrades transcripts containing PTCs from the gene *BRCA1* (ref. 63) and reduces abundance of mRNAs containing PTCs from the *TP53* and Wilms tumor (*WT1*) loci^{64–68}. Evidence that NMD protects against dominant truncated forms of these tumor-suppressor proteins stems from experiments in which intronless cDNA constructs, whose unspliced mRNAs are NMD-incompetent, were expressed in cell lines or animal models. These C-terminally truncated proteins exerted dominant detrimental effects, such as increased chemoresistance, decreased apoptosis, increased tumorigenicity^{69–71} or interference with transcription-activating ability and subcellular localization^{72,73} (Table 4). These studies indicate that if abnormal transcripts containing PTCs were not degraded by NMD, clinically recessive tumor-suppressor mutations could result in the synthesis of truncated, dominant oncoproteins. NMD may thus protect heterozygous carriers of tumor-suppressor genes containing PTC mutations from developing cancer, at least for as long as the other tumor-suppressor allele remains intact.

NMD may also affect expression of tumor-suppressor genes by modulating the proportions of splice variants. This seems to be the case for *TP53*, which expresses a splice form containing a PTC at low levels in normal tissues⁷⁴. The amount of this variant is much greater in a leukemia cell line carrying a splice-site mutation, accounting for about one-half of the total amount of *TP53* transcript⁷⁵. Although a causal relationship has not yet been established, such a splice form could plausibly promote leukemogenesis, prevented under normal conditions by NMD-induced degradation of the transcript. Transcripts from *WT1* also undergo alternative splicing. The more

abundant splice form (+KTS) encodes three additional amino acids at the 3' end of the penultimate exon. A mutation found in acute myelogenous leukemia⁷⁶, Wilms tumor⁷⁷ and Frasier syndrome (male pseudohermaphroditism and progressive glomerulopathy)⁷⁸ is also located in the penultimate exon and introduces an NMD-eligible PTC, but only for the +KTS form. In contrast, for the –KTS form, the PTC is within the terminal 50 bp of the penultimate exon, and so transcripts probably escape NMD and are translated into a truncated protein. NMD could thus limit expression of the primary transcript. This is important because, in general, Frasier syndrome is attributable to mutations that selectively reduce the abundance of the +KTS isoform^{79,80}. Therefore, Frasier syndrome in individuals carrying this particular PTC mutation could be caused by NMD-induced degradation of the +KTS isoform. In addition, the truncated form of the minor splice variant might act in a dominant fashion to promote tumorigenesis.

Medical therapies for PTC-related disease

In contrast to its role in preventing dominant disease, NMD can also eliminate mRNAs that would otherwise result in the production of partly or fully functional truncated protein. In such instances, interventions to prevent degradation of transcripts containing PTCs may be therapeutically useful.

Approaches to protect a mRNA containing a PTC from NMD, thereby promoting the synthesis of a (partially) functional protein, have been explored for cystic fibrosis, Duchenne muscular dystrophy, Hurler syndrome and X-linked nephrogenic diabetes insipidus. Aminoglycoside antibiotics have been tested in this context, because they bind to the decoding center of the ribosome and decrease the accuracy requirements for codon-anticodon pairing⁸¹. Recognition of stop codons is suppressed, and, instead of chain termination, an amino acid is incorporated into the polypeptide chain. Thus, transcripts containing PTCs are not recognized by the NMD machinery, and full-length, albeit missense-mutated, proteins are synthesized. Aminoglycoside treatment induced PTC suppression and resulted in functional improvement^{82–84} in a cystic fibrosis bronchial epithelial cell line⁸³, in muscle cells from *mdx* mice (a model for muscular dystrophy)⁸⁵ and in

Table 3 Genetic conditions in which NMD can modulate phenotype

Gene	Mutation location	Effect of mutation	Reference(s)
β-globin (<i>HBB</i>)	5' to NMD boundary	Recessively inherited β-thalassemia major; heterozygotes healthy	51,52
	3' to NMD boundary	Dominantly inherited β-thalassemia intermedia	51,52
Interferon gamma receptor 1 (<i>IFNGR1</i>)	5' to NMD boundary	Recessively inherited susceptibility to mycobacterial infection; heterozygotes healthy	54
	3' to NMD boundary	Dominantly inherited susceptibility to mycobacterial infection	55
Receptor tyrosine kinase-like orphan receptor 2 (<i>ROR2</i>)	5' to NMD boundary	Recessively inherited Robinow syndrome (orodental abnormalities, hypoplastic genitalia, multiple rib/vertebral anomalies); heterozygotes healthy	56
	3' to NMD boundary	Dominantly inherited brachydactyly type B (shortening of digits and metacarpals)	56
Cone-rod homeobox (<i>CRX</i>)	5' to NMD boundary	No homozygotes to date; mutation found in unaffected heterozygote	59
	3' to NMD boundary	Dominantly inherited retinal disease	59
von Willebrand factor (<i>VWF</i>)	5' to NMD boundary	Recessively inherited type 3 von Willebrand disease; heterozygotes healthy	von Willebrand database
	3' to NMD boundary	Dominantly inherited type 2A disease	57
Coagulation factor X (<i>F10</i>)	5' to NMD boundary	Recessively inherited bleeding tendency; heterozygotes healthy	58
	3' to NMD boundary	Dominantly inherited bleeding tendency	58
Rhodopsin (<i>RHO</i>)	5' to NMD boundary	Recessively inherited blindness; heterozygotes have abnormalities on retinogram but no clinical disease	60
	3' to NMD boundary	Dominantly inherited blindness	61
SRY-Box 10 (<i>SOX10</i>)	5' to NMD boundary	Haploinsufficiency leading to congenital neurosensory deafness and colonic agangliosis	62
	3' to NMD boundary	Dominantly inherited neural developmental defect including neurosensory deafness, colonic agangliosis, peripheral neuropathy and central dysmyelinating leukodystrophy	

Table 4 Effect of NMD on expression of tumor-suppressor genes

Gene	Experimental data	Reference(s)
<i>BRCA1</i>	NMD demonstrated for many transcripts containing PTCs Expression of NMD-insensitive C-terminal truncated mutants leads to increased chemoresistance, decreased apoptosis, increased tumorigenicity and decreased survival time	63 69,70
<i>TP53</i>	PTCs associated with decreased or absent mRNA and protein Expression of NMD-insensitive C-terminal truncated mutant leads to tumorigenesis	64–67 71
<i>WT1</i>	PTCs associated with absent mRNA NMD-insensitive PTC-mutated mRNAs encode truncated protein that inhibits ability of wild-type protein to activate transcription and alters subnuclear localization of wild-type protein	68 72,73

fibroblasts from individuals with Hurler syndrome⁸⁴. Protein expression levels in these experiments were estimated to be up to 15% of normal, although some proteins may have been functionally compromised because of missense amino acids incorporated during stop-codon suppression. Protein produced in this manner has been shown to be at least somewhat functional in animal models. For instance, after treatment with gentamicin, chloride conductance was observed in intestinal glands of cystic fibrosis transmembrane conductance regulator (CFTR)-deficient mice⁸⁶. In a mouse model of X-linked nephrogenic diabetes insipidus, treatment of cultured kidney collecting duct cells with geneticin resulted in production of arginine vasopressin receptor 2 and increased cAMP responses⁸⁷. Additionally, expression of dystrophin-positive myotubes and apparent protection against muscle injury were observed in *mdx* mice treated with gentamicin⁸⁵, although these benefits were not reproduced in a second study⁸⁸.

Of direct relevance to clinicians, some trials of aminoglycoside therapy have been carried out in humans with PTC mutations. The most promising results come from a double-blind, placebo-controlled crossover trial of topically administered gentamicin in a group of 32 individuals with cystic fibrosis. After treatment, nasal potential difference measurements, including response to isoproterenol, improved considerably in individuals with PTC mutations. Full-length CFTR protein was also detected in nasal epithelial cells of two treated individuals⁸⁹. Similar results were observed in a pair of small open studies of individuals with cystic fibrosis^{90,91}. In contrast, clinical studies in individuals with muscular dystrophy have been less encouraging. In one study, four individuals with PTC-caused Duchenne or Becker muscular dystrophy were given intravenous gentamicin for two weeks, resulting in no histologic or functional improvement⁹². In another study, dystrophin production was reported in three of four individuals with Duchenne muscular dystrophy who were given two courses of gentamicin over six weeks. No substantial improvement was observed in functional tests⁹³.

Overall, results from human trials indicate that treatment with aminoglycoside antibiotics leads to some protein production from mRNAs containing PTCs, and that some of this protein may be functional, but a therapeutic benefit has not been shown. The effect of prolonged treatment with aminoglycosides is also a concern, although toxicity problems might be overcome by more selective targeting of NMD. Treatment with a series of small molecules that also promotes translational read-through of PTCs seemed to be more efficacious than treatment with gentamicin in the *mdx* mouse (Investigational Drugs database); these molecules have a structure unrelated to aminoglycoside antibiotics, perhaps thereby avoiding some of their toxicity. A possibly more intractable issue is whether general, long-term suppression of PTCs, as well as the suppression of physiological termination codons and potential translation of pseudogenes, will cause buildup of abnormal mRNAs and abnormal translation of normal

mRNAs, resulting in the production of mutant proteins that could trigger other cellular problems.

A different potential therapeutic approach to PTC-related disease is the use of antisense oligoribonucleotides to redirect splicing, thereby avoiding the production of PTCs in the first place. Initially described for correcting *in vitro* aberrant splicing of the β -globin gene⁹⁴, this strategy uses antisense 2'-O-methylribonucleotides (2OMeAO) that hybridize to splice sites or branch-point junctions of aberrantly spliced pre-mRNA, restoring normal splicing in a substantial fraction of molecules. This approach was modified⁹⁵ for use with a dystrophin mRNA containing a PTC. In this case, the targeted PTC is located in an exon encoding a dispensable protein region. Antisense 2OMeAO directed towards splice sites flanking the mutation promote in-frame skipping of the affected exon, effectively removing the PTC. Treatment of *mdx* mice with these antisense oligos resulted in low-level expression of a shortened but functional dystrophin. In a further step towards the clinic, the efficiency of oligonucleotide delivery to tissues has been enhanced by the use of vehicles such as block copolymer⁹⁶. Before trials of 2OMeAO are feasible in humans, however, a systemic delivery method needs to be developed, and, as with all forms of gene therapy, the issues of transfection efficiency, potential immune responses and side effects must be addressed. Unfortunately, this sort of treatment would be feasible only for mutations in which manipulation of splicing maintains in-frame translation and, if exon skipping results, it does not remove essential protein regions or result in protein misfolding. Therefore, this treatment would probably be limited to specific cases rather than be a general therapy for PTC-related disease.

A further potential approach—currently far from realization—is to modulate NMD itself rather than modulating recognition of PTCs. Selective targeting of the central NMD protein UPF1 using RNA interference has been shown to be useful in disrupting NMD in cell culture; this might be a starting point for development of therapeutics. In addition, some evidence suggests that individuals with identical genetic mutations may have phenotypes of varying severity as a result of variability in NMD efficiency³¹. Although no upstream factors acting on NMD have yet been discovered, identification of such regulatory effectors could permit development of therapies to fine-tune the NMD mechanism, potentially allowing more targeted interventions in individuals with PTC-related disease.

Conclusions

NMD is increasingly appreciated as one of the central mechanisms of RNA surveillance, with an important role both in the physiological control of gene expression and in modulating hereditary and acquired genetic diseases. Therefore, the action of NMD must be considered when the functional effect of PTC mutations and alternatively spliced transcripts is analyzed. Otherwise, genotype-phenotype relationships may be confounded or missed altogether. Although therapies aimed at

mRNAs containing PTCs are under development, and although the mechanistic underpinnings of NMD are being elucidated at a rapid rate, treatments aimed specifically at components of the NMD machinery are not yet on the horizon. With broadened insight into the mechanism of NMD, however, continued development of new strategies to treat PTC-related disease is a realistic goal.

URLs. The Investigational Drugs database is available at <http://www.iddb3.com/>. The von Willebrand database is available at <http://www.shef.ac.uk/vwvf/>.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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