REVIEW

Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function

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Cells routinely make mistakes. Some mistakes are encoded by the genome and may manifest as inherited or acquired diseases. Other mistakes occur because metabolic processes can be intrinsically inefficient or inaccurate. Consequently, cells have developed mechanisms to minimize the damage that would result if mistakes went unchecked. Here, we provide an overview of three quality control mechanisms—nonsense-mediated mRNA decay, nonstop mRNA decay, and no-go mRNA decay. Each surveys mRNAs during translation and degrades those mRNAs that direct aberrant protein synthesis. Along with other types of quality control that occur during the complex processes of mRNA biogenesis, these mRNA surveillance mechanisms help to ensure the integrity of protein-encoding gene expression.

Cellular RNAs are generally subject to quality control or surveillance pathways that guard against defects in gene expression (for recent reviews, see Dimaano and Ullman 2004; Parker and Song 2004; Vinciguerra and Stutz 2004; Conti and Izaurralde 2005; Fasken and Corbett 2005; Maquat 2005; Moore 2005; Saguez et al. 2005; Behm-Ansmant and Izaurralde 2006; Houseley et al. 2006; Behm-Ansmant et al. 2007b). The expression of genes that encode protein is carried out by a complicated series of coordinately regulated reactions (Reed 2003; Reed and Cheng 2005). These reactions include pre-mRNA synthesis and processing in the nucleus, mRNA transport across the nuclear pore complex with the subsequent possibility of localization to a particular cytoplasmic compartment that facilitates proper function, mRNA translation, and, ultimately, mRNA degradation. At each step of these reactions, RNA is in dynamic association with RNA-binding proteins, which in turn can complex either directly or indirectly with other proteins (Dreyfuss et al. 2002). RNA-associated proteins not only reflect the history of the RNA but may also influence future steps of RNA metabolism (Giorgi and Moore 2007).

Cells have evolved pathways to eliminate RNAs that are incorrectly processed or improperly function either because of mutations within the genes that encode them or because of mistakes made during their metabolism and/or function in the absence of mutations within their genes. This review will focus on pathways that eliminate defective mRNAs as a consequence of their inability to properly direct protein synthesis. These pathways encompass the translation-dependent mechanisms of cytoplasmic surveillance. Considering that earlier steps of mRNA maturation, including pre-mRNAs splicing within nuclei and mRNA transport across the nuclear pore complex, are also subject to quality control (Dimaano and Ullman 2004; Vinciguerra and Stutz 2004; Fasken and Corbett 2005; Saguez et al. 2005; Houseley et al. 2006), protein-encoding RNAs are likely to be scrutinized at every stage of their biogenesis and function.

Nonsense-mediated mRNA decay (NMD): when mRNAs harbor a premature termination codon (PTC)

NMD has been studied in a wide variety of organisms and is the best characterized of the pathways that ensure the quality of gene expression by degrading translationally abnormal RNAs. For supplemental reading, there is an entire book devoted to many aspects of NMD (Maquat 2006).

Purpose of NMD

NMD generally eliminates the production of mRNAs that prematurely terminate translation and occurs, although by varying mechanisms, in every eukaryotic cell that has been examined (for recent reviews, see Baker and Parker 2004; Maquat 2004a, 2005; Neu-Yilik et al. 2004; Conti and Izaurralde 2005; Lejeune and Maquat

2005; Yamashita et al. 2005; Amrani et al. 2006; Behm-Ansmant and Izaurralde 2006; Kuzmiak and Maquat 2006; Rehwinkel et al. 2006; Stroupe et al. 2006; Chang et al. 2007; Behm-Ansmant et al. 2007b). NMD appears to have evolved to protect cells from the potentially deleterious effects of routine abnormalities in gene expression that result in the premature termination of translation. For example, transcription initiation upstream of the proper site could generate an mRNA harboring a nonsense codon upstream of or within the usual translational reading frame if translation initiates upstream of the normal site. As another example, inefficient or inaccurate pre-mRNA splicing could result in an mRNA harboring an intron-derived nonsense codon or a nonsense codon downstream from the site of missplicing. Additionally, the programmed DNA rearrangements of *T-cell receptor* (*TCR*) and *immunoglobulin* (*Ig*) genes that augment the diversity of antigen receptors generate a nonsense codon approximately two out of every three occurrences (Li and Wilkinson 1998).

It is important for cells to eliminate mRNAs that prematurely terminate translation since the resulting truncated proteins have the potential to be nonfunctional or acquire dominant-negative or gain-of-function activities. In fact, mutations within NMD factors were discovered in *Caenorhabditis elegans* as suppressors of the abnormal phenotype caused by one of several different defective alleles that only later were found to encode mRNAs that terminate translation abnormally (Hodgkin et al. 1989; Pulak and Anderson 1993). Therefore, NMD provides an important means by which cells ensure the quality of mRNA function and, by so doing, the quality of gene expression.

NMD also targets anywhere from ~3% to 10% of the natural transcriptome in those organisms that have been studied (Lelivelt and Culbertson 1999; Mitrovich and Anderson 2000; He et al. 2003; Mendell et al. 2004; Rehwinkel et al. 2005; Weischenfeldt et al. 2005; Guan et al. 2006; Wittmann et al. 2006; Chan et al. 2007; Ni et al. 2007). This range may be an underestimate due to technical limitations, including the use of arrays that monitor only a fraction of cellular transcripts, and the analysis of transcripts from cells in which an NMD factor has been only incompletely down-regulated. In mammals, natural NMD targets fall into several classes. The best-characterized classes include (1) selenoprotein mRNAs, in which specialized UGA selenocysteine codons intrinsically direct translation termination a fraction of the time (Moriarty et al. 1998; Sun et al. 2001; Mendell et al. 2004; Wittmann et al. 2006); (2) mRNAs characterized by upstream open translational reading frames (ORFs), introns within their 3′ untranslated regions (UTRs), or nonsense-containing transposon or retroviral sequences within their coding regions (Mendell et al. 2004; Wittmann et al. 2006; Chan et al. 2007); and (3) alternatively spliced mRNAs, an estimated one-third of which in humans are thought to be slated for NMD (Lewis et al. 2003; Lareau et al. 2004). All told, natural NMD targets in mammals function in a broad range of cellular processes that include transcription, telomere maintenance,

DNA repair, cell growth, intracellular transport (Rehwinkel et al. 2006), and NMD itself (Mendell et al. 2004; Rehwinkel et al. 2005; Wittmann et al. 2006; Chan et al. 2007). Regulation of the Smg5 NMD factor by NMD in both humans and *Drosophila melanogaster* indicates the evolution of a conserved autoregulatory loop (Mendell et al. 2004; Rehwinkel et al. 2005).

A percentage of alternatively spliced transcripts that are targeted for NMD has been proposed to encode functional protein isoforms (Baek and Green 2005), which is conceivable since NMD is not 100% efficient but generally reduces the abundance of nonsense-containing mRNAs to ~5%–25% of the nonsense-free level. Other alternatively spliced NMD targets are generated by autoregulatory circuits and are nonproductive. These autoregulatory circuits are mediated by RNA-binding proteins that influence the splicing of their own pre-mRNAs so as to inhibit inappropriately high levels of protein production (Jumaa and Nielsen 1997; Sureau et al. 2001; Stoilov et al. 2004; Wollerton et al. 2004; Cuccurese et al. 2005; Ni et al. 2007). For example, NMD degrades mRNA for the polypyrimidine tract-binding protein (PTB) splicing activator when PTB levels are high enough to result in exon skipping within PTB pre-mRNA, which generates a PTC (Wollerton et al. 2004). In fact, the pre-mRNA of every known member of the human SR family of splicing regulators contains ultra-conserved elements that are alternatively spliced as either exonic cassettes that contain in-frame nonsense codons or introns within the 3′ UTR (Lareau et al. 2007). As might be expected, the NMD of splicing activator mRNAs results from the activation of a splicing event, whereas the NMD of splicing repressor mRNAs results from the inhibition of a splicing event (Ni et al. 2007).

Generally, however, most alternatively spliced transcripts that are targeted for NMD are likely to be mistakes that have no biological relevance. In support of this idea, they appear to be too low in abundance to affect cellular metabolism (Pan et al. 2006). Moreover, they are not generated in a tissue-specific way so as to contribute to tissue-specific diversity, which is an important hallmark of functional alternatively spliced transcripts (Pan et al. 2006). The capacity of NMD to eliminate transcripts that are generated in error possibly evolved to maximize the genetic potential of mammalian cell DNA, which harbors remarkably few (~21,561 in human and ~21,839 in mouse) protein-encoding genes (McPherson et al. 2001; Venter et al. 2001; Mouse Genome Sequencing Consortium 2002; http://www.ensembl.org).

Features of an NMD target

Mammalian cells There are at least two distinct mechanisms utilized by mammalian cells to identify an NMD target. Generally, NMD targets are recognized depending on a post-splicing exon junction complex (EJC) of proteins that is deposited ~20–24 nucleotides (nt) upstream of exon–exon junctions (Le Hir et al. 2000, 2001b). Recognition occurs regardless of whether the EJC derives from U2 snRNP-type splicing or U12 snRNP-

type splicing (Hirose et al. 2004). The role of the EJC in NMD explains early observations that intron position within pre-mRNA is an important determinant of NMD (Cheng et al. 1994; Carter et al. 1996; Thermann et al. 1998; Zhang et al. 1998a,b; Sun et al. 2000). As a rule, nonsense codons located >50–55 nt upstream of an exon–exon junction elicit NMD (Nagy and Maquat 1998), although there are exceptions to this rule (see below). The 50- to 55-nt metric makes sense considering that a translationally active ribosome poised at a nonsense codon situated more than ~50–55 nt upstream of an exon–exon junction will not have progressed sufficiently far along the mRNA to remove the EJC deposited ~20–24 nt upstream of that exon–exon junction (Alkalaeva et al. 2006). In contrast, it is thought that a ribosome poised at a nonsense codon located either less than ~50–55 nt upstream of an EJC or downstream from the EJC will have removed the EJC (Dostie and Dreyfuss 2002).

The EJC consists of many factors (Fig. 1A). These factors include (1) REF/Aly, which recruits the mRNA export factor TAP that interacts with UAP56 and Y14; (2) Y14, which forms a stable heterodimer with the mRNA export factor Magoh and also interacts with the NMD factor Upf3 (also called Upf3a) or Upf3X (also called Upf3b); (3) Magoh, which interacts with TAP; (4) SRm160, which functions in splicing and enhances mRNA export; (5) RNPS1, which functions in splicing and mRNA export and may also recruit Upf3 or Upf3X; (6) UAP56, a DEAD-box RNA helicase that interacts with REF/Aly; (7) eukaryotic translation initiation factor 4AIII (eIF4AIII), another RNA helicase that interacts with Y14 and forms the EJC platform; (8) PYM, which forms a trimeric complex with Y14:Magoh; (9) MNL51 (also called Barentsz, BTZ), which associates with and stimulates the RNA-helicase activity of eIF4AIII; (10) Acinus, which forms a stable heterodimer with RNPS1; and (11) SAP18 (Katahira et al. 1999; Luo and Reed 1999; Mayeda et al. 1999; Bachi et al. 2000; Kataoka et al. 2000; Lykke-Andersen et al. 2000, 2001; Stutz et al. 2000; Zhou et al. 2000; Ishigaki et al. 2001; Kim et al. 2001a,b; Le Hir et al. 2001a; Rodrigues et al. 2001; Lejeune et al. 2002; Gehring et al. 2003; Bono et al. 2004, 2006; Chan et al. 2004; Chiu et al. 2004; Degot et al. 2004; Ferraiuolo et al. 2004; Palacios et al. 2004; Shibuya et al. 2004, 2006; Ballut et al. 2005; Tange et al. 2005; Andersen et al. 2006; Stroupe et al. 2006; Noble and Song 2007).

In vitro reconstitution studies indicate that the EJC core complex, which consists of eIF4AIII, MNL51/BTZ, Magoh, and Y14, is locked onto RNA ~20–24 nt upstream of exon–exon junctions by the Magoh–Y14-mediated inhibition of eIF4AIII ATPase activity (Ballut et al. 2005; Tange et al. 2005). Recently reported crystal structures (Andersen et al. 2006; Bono et al. 2006) and a three-dimensional electron microscopic structure (Stroupe et al. 2006) of this core have offered important insights into many previous biochemical findings, including why the EJC binds RNA but not DNA and how the EJC protects 6–8 nt independently of their constitution from RNase A + T1-directed cleavage.

Down-regulating a number of EJC constituents, in-

cluding Y14, MNL51/BTZ, RNPS1, and eIF4AIII, inhibits NMD (Gehring et al. 2003, 2005; Ferraiuolo et al. 2004; Shibuya et al. 2004; Palacios et al. 2004). It is likely that the EJC functions in NMD by serving as a loading platform for the Upf NMD factors. Consistent with this view, tethering any Upf factor to an mRNA >50–55 nt downstream from a normal termination codon is sufficient to trigger NMD (Lykke-Andersen et al. 2000; Gehring et al. 2003, 2005; Kim et al. 2005). The findings that tethering Upf3X elicits NMD in a Upf2-dependent manner and tethering Upf2 elicits NMD in a Upf1-dependent but Upf3X-independent manner support the notion that the order of Upf factor association with EJCs is first Upf3X (or, presumably, Upf3), then Upf2 and finally Upf1 (Kim et al. 2005), the latter in what appears to be a transient interaction (see below). Consistent with Upf1 being the last of the Upf factors to join the EJC, tethered Upf1 still triggers NMD when a dominant-negative nontetherable form of Upf1 is overexpressed (Lykke-Andersen et al. 2001).

Notably, while the poly(A)-binding protein PABPC1 augments the efficiency of NMD in mammalian cells (see below), a PTC situated sufficiently upstream of a splicing-generated exon–exon junction still triggers NMD when the cleavage and polyadenylation signal has been replaced by the 3′ stem–loop structure of histone H1.3 mRNA, which lacks a poly(A) tail (Neu-Yilik et al. 2001). Therefore, since the 50- to 55-nt rule still holds in the absence of PABPC1, a normal termination codon can be distinguished from a PTC in a PABPC1-independent mechanism.

The 50- to 55-nt rule specifies that PTCs situated either <50–55 nt upstream of the 3′-most exon–exon junction or downstream from this junction generally fail to trigger NMD. For a case in point, PTCs within the last exon of the human β-globin gene result in a dominantly inherited form of the hemolytic anemia thalassemia, thereby illustrating the importance of down-regulating mRNAs that encode truncated proteins (Holbrook et al. 2004). However, as with every rule, there are exceptions to the 50- to 55-nt rule. For example, 5′ PTCs within β-globin exon 1 break the rule because they fail to efficiently elicit NMD despite residing >50–55 nt upstream of the exon 1–exon 2 junction (Romao et al. 2000; Danckwardt et al. 2002; Silva et al. 2006). Proximity to the AUG initiation codon, rather than the presence of a specific downstream stabilizing element such as those typifying GCN4 and YAP1 mRNAs of *Saccharoymces cerevisiae* (Ruiz-Echevarría and Peltz 2000), appears to explain why early PTCs within β-globin mRNA fail to efficiently elicit NMD. However, early PTCs within other mRNAs are capable of eliciting a modest level of NMD even independently of peptide bond formation, as evidenced by a PTC that is situated immediately downstream from the AUG initiation codon (Zhang and Maquat 1997; Silva et al. 2006). While not understood, a PTC within β-globin mRNA exon 2 also breaks the rule by failing to efficiently elicit NMD despite residing >50–55 nt upstream of the exon 2–exon 3 junction (Danckwardt et al. 2002).
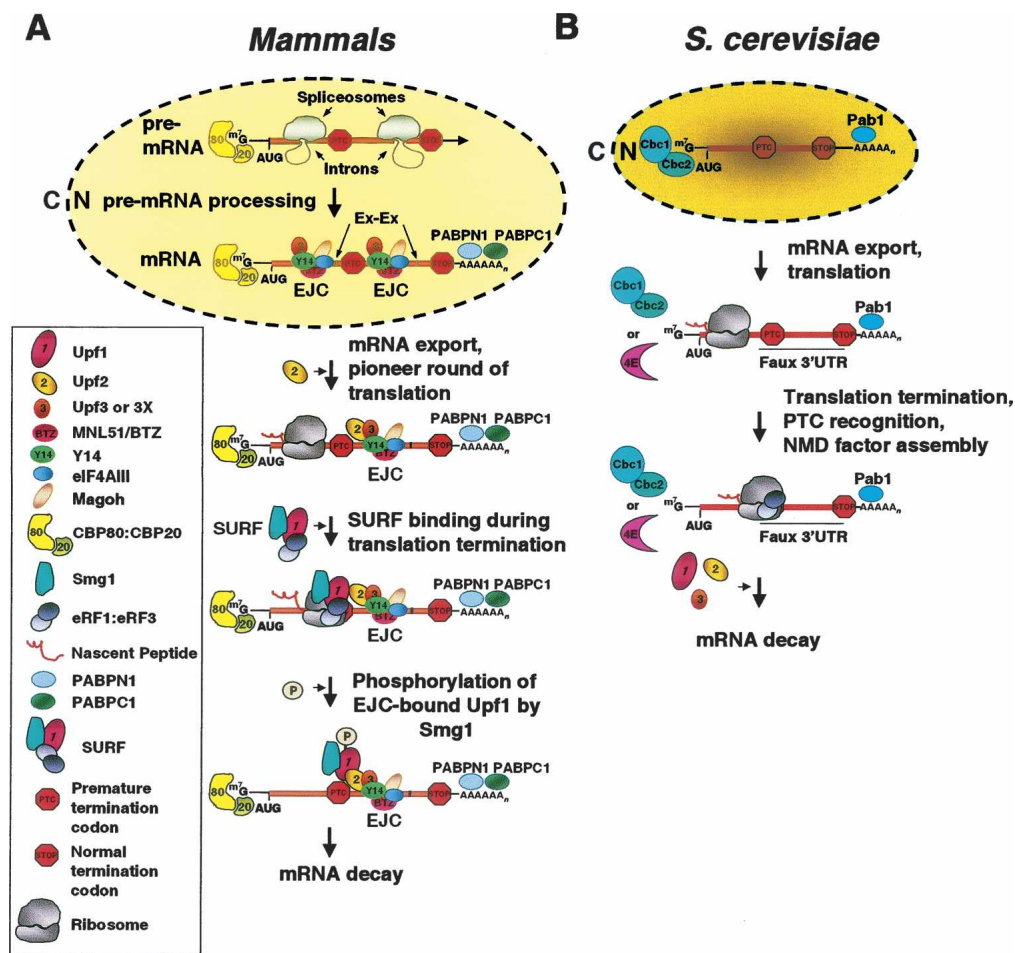
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Figure 1. Models for NMD. (*A*) In mammals, CPB80 and CBP20 begin to associate with the 5′ caps of newly synthesized transcripts prior to pre-mRNA splicing. The Upf1 NMD factor subsequently associates with caps by binding directly to CBP80. The Upf3 or Upf3X NMD factor, which is mostly in the nucleus (N) but shuttles to the cytoplasm (C), is recruited to the EJCs of newly spliced mRNAs in the nucleus and is exported with mRNA to the cytoplasm. In the cytoplasm, Upf3 or Upf3X recruits the Upf2 NMD factor, which is primarily cytoplasmic and can occasionally be seen enriched along the cytoplasmic side of the nuclear envelope. The translation of CBP80:CBP20-bound mRNA defines the pioneer round. Not shown are the complete cadre of EJC constituents or the ability of PABPC1 to enhance the pioneer round of translation by binding to eIF4G, which in turn binds CBP80. Translation termination involves the tetrameric complex SURF, which consists of the Upf1 kinase Smg1, Upf1, and the eRF1 and eRF3 translation termination factors. Generally, if translation terminates >50–55 nt upstream of an exon–exon (Ex–Ex) junction—e.g., at a PTC—then NMD will occur. Subsequently, Upf1 in complex with Smg1 binds EJC-associated Upf2 in a way that is promoted by CBP80. It is unclear whether the Upf1 in SURF derives from CBP80-bound Upf1 at the cap. Upf1 binding to the EJC triggers Upf1 phosphorylation and NMD, presumably by recruiting and/or activating mRNA degradative activities. Additional rounds of decay depend on Smg5, Smg6, and Smg7, which appear to recruit PP2A for the dephosphorylation and, thus, recycling of Upf1. Alternatively, if translation terminates either <50–55 nt upstream of the 3′-most exon–exon junction or downstream from this junction, then the mRNA will be immune to NMD. mRNA that is immune to NMD or otherwise escapes NMD undergoes remodeling. Remodeling includes the replacement of CBP80:CBP20 at the cap by eIF4E, loss of PABPN1, and removal of EJCs, including the associated Upf factors, presumably by translating ribosomes. (*B*) In *S. cerevisiae*, newly synthesized and steady-state mRNAs are targeted for NMD if they harbor a faux 3′ UTR. In at least one mechanism, the abnormally long distance between the termination site and poly(A)-bound Pab1 is thought to result in inefficient translation termination. Under these circumstances, termination involves not only eRF1 and eRF3, which fail to effectively mediate the release of the nascent polypeptide, but probably also Upf1, Upf2, and Upf3. The Upf factors recruit and/or activate mRNA degradative activities. In contrast, when translation occurs normally, termination is presumed to be highly efficient, possibly because interactions between Pab1 and ribosome-bound eRF3 enhance eRF3-stimulated eRF1 activity so the mRNA is not targeted for NMD. Analogously to *A*, the ability of Pab1 to promote translation by mediating interactions between the mRNA 3′ and 5′ ends are not shown.

Apolipoprotein (apo) B mRNA provides another exception to the 50- to 55-nt rule. PTCs at all positions tested, except for the PTC created by the editing complex APOBEC1–ACF1 within exon 26, elicit NMD (Kim et al. 1998; Chester et al. 2003). Immunity of edited apoB mRNA to NMD depends on this editing complex, which forms around the editing site and includes the so-called mooring sequence (Chester et al. 2003). Formation of the

editing complex may preclude interactions between the translation termination complex at the PTC and the downstream EJC. It could be useful for therapeutic purposes to determine whether a higher-order RNA structure or a stably associated protein complex situated between a PTC and the downstream EJCs generally severs a functional PTC–EJC connection. Drug-mediated severing may provide a useful means to impair the NMD of particular disease-associated transcripts (Kuzmiak and Maquat 2006).

For reasons that are unknown, PTCs as close as 8–10 nt upstream of the last exon–exon junction of Ig μ and TCR-β transcripts also trigger NMD and exemplify exceptions to the rule (Carter et al. 1996; Wang et al. 2002b; Bühler et al. 2004). In fact, PTCs within Ig μ and TCR-β transcripts that reside closer to the 5′ end of the penultimate exon elicit NMD more efficiently than PTCs that reside near the 3′ end of the exon (Wang et al. 2002b), making Ig μ and TCR-β transcripts the only known mammalian mRNAs to manifest a type of polar NMD. Ig μ and TCR-β transcripts are also remarkable for undergoing unusually robust NMD, which presumably offers a selective advantage since *Ig μ* and *TCR-β* genes naturally acquire PTCs during the programmed DNA rearrangements and hypermutagenesis that typify lymphocyte development. Whereas NMD in mammalian cells generally reduces mRNA abundance to ~5%–25% of the normal level, the NMD of Ig μ and TCR-β transcripts reduces mRNA abundance to ~1%–5% of normal (Gudikote and Wilkinson 2002; Bühler et al. 2004). Robust down-regulation correlates with the presence of *cis*-acting sequences that promote efficient splicing downstream from the PTC and, as a consequence, efficient translation even in nonlymphocytic cells (Gudikote et al. 2005).

mRNAs that are subject to "fail-safe" NMD, which may provide a backup mechanism should EJCs fail to trigger NMD (see below), are also exceptional NMD targets. The existence of a fail-safe pathway was uncovered in studies of artificially generated genes that (1) lack what is normally the 3′-most intron and (2) harbor PTCs in what is normally the penultimate exon. Demonstrated fail-safe targets consist of mRNAs for triose phosphate isomerase (TPI) (Cheng et al. 1994; D. Matsuda, N. Hosoda, Y.K. Kim, and L.E. Maquat, in prep.), β-globin (Zhang et al. 1998b; D. Matsuda, N. Hosoda, Y.K. Kim, and L.E. Maquat, in prep.), β-hexosaminidase α subunit (Rajavel and Neufeld 2001), and Ig μ (Bühler et al. 2006), although they probably also include mRNAs for numerous other proteins should functional EJCs fail to form downstream from a PTC.

Given that fail-safe NMD requires the encoding gene to harbor an intron upstream of the PTC, we suggest that referring to this pathway as EJC-independent NMD (Bühler et al. 2006) is inappropriate (D. Matsuda, N. Hosoda, Y.K. Kim, and L.E. Maquat, in prep.). Considering that splicing is known to enhance steady-state translation (Le Hir et al. 2003), an intron upstream of a PTC may be required for an efficient pioneer round of mRNA translation; i.e., efficient PTC recognition during the

time mRNA is bound by the requisite NMD factors (see below).

Studies are just beginning to reveal how a PTC triggers fail-safe NMD in the absence of a splicing event downstream from the PTC. For example, it was recently shown that a PTC-free Ig μ minigene can be converted to an NMD substrate by extending the 3′ UTR, suggesting that a normal termination codon can trigger NMD in the absence of a downstream EJC, provided the 3′ UTR is abnormally long (Bühler et al. 2006). In the view that 3′ UTR lengths in mammalian cells are considerably heterogenous, it is difficult to imagine that the fail-safe pathway is specified simply by the number of nucleotides residing downstream from a nonsense codon. Probably, not only 3′ UTR length but also 3′ UTR higher-order structure must be considered when predicting which nonsense codons trigger NMD.

Nonmammalian cells Considerable data indicate that NMD in *S. cerevisiae* (Amrani et al. 2004), *D. melanogaster* (Behm-Ansmant et al. 2007a), plants (Kertesz et al. 2006; Schwartz et al. 2006), *Caenorhabditis elegans* (Longman et al. 2007), and probably *Saccharomyces pombe* (Maquat 2004b; Conti and Izaurralde 2005) can also and may generally be triggered by an abnormally long distance between the site of translation termination and the position of the downstream poly(A) tail as demarcated by the poly(A)-binding protein (Pab1 in yeast; PABPC1 in other organisms). This abnormally long distance, which has been called a "faux" 3′ UTR (Amrani et al. 2004), apparently allows recruitment of the Upf NMD factors by a mechanism that has yet to be determined (Fig. 1B). In support of this conclusion, nonsense codons within a particular mRNA trigger NMD to a degree that depends on their distance upstream of the 3′ UTR in *S. cerevisiae* (Muhlrad and Parker 1999), *C. elegans* (Pulak and Anderson 1993; Longman et al. 2007), and *D. melanogaster* (Behm-Ansmant et al. 2007a). Second, tethering Pab1 in *S. cerevisiae* (Coller et al. 1998; Amrani et al. 2004) or PABPC1 in *D. melanogaster* (Behm-Ansmant et al. 2007a) downstream from a PTC, which effectively eliminates faux 3′ UTR function, abolishes NMD. Third, down-regulating the cellular abundance of PABPC1 in *D. melanogaster* abrogates NMD (Behm-Ansmant et al. 2007a). The role of Pab1 and PABPC1 in NMD may at least in part reflect their function in promoting efficient nonsense codon recognition. For example, Pab1 and PABPC1 support the mechanism by which the mRNA 3′ poly(A) tail and 5′ cap interact so as to synergistically enhance translation initiation (Tarun and Sachs 1996; Gray et al. 2000; Kahvejian et al. 2005; Karim et al. 2006). Furthermore, tethering Pab1 downstream from a PTC in *S. cerevisiae* has been shown to increase the efficiency of translation termination and ribosome release (Amrani et al. 2004).

Data demonstrating that tethering Pab1 or PABPC1 downstream from a PTC in, respectively, *S. cerevisiae* or *D. melanogaster* abrogates NMD suggests that normal termination can be distinguished from abnormal termination by the proximity of a translation termination

event to the poly(A)-binding protein independently of the processes of 3′ end cleavage and polyadenylation. In support of this conclusion, mRNAs in *S. cerevisiae* that prematurely terminate translation and end in a DNA-encoded poly(A) tract immediately upstream of a self-cleaving hammerhead ribozyme are abnormally short-lived relative to their nonsense-free counterparts (Baker and Parker 2006). Furthermore, NMD in *D. melanogaster* occurs when the cleavage and polyadenylation signal is replaced by $(A)_{45}$ followed by either a self-cleaving hammerhead ribozyme or the 3′ stem–loop structure of histone H4 mRNA (Behm-Ansmant et al. 2007a).

Pab1 and PABPC1 may increase the efficiency of translation termination by recruiting eRF3, with which it interacts directly (Uchida et al. 2002; Amrani et al. 2004; Kashima et al. 2006), since tethering eRF3 downstream from a PTC also abolishes NMD, although not as effectively as tethering Pab1 of PABC1 (Amrani et al. 2004; Behm-Ansmant et al. 2007b). At least for *S. cerevisiae*, data indicating that the poly(A)-binding protein is instrumental to NMD must be tempered with the finding that a poly(A) tail and, thus, Pab1 are not required to differentiate normal termination codons from PTCs. For example, PTC-containing mRNAs that undergo 3′-end formation by hammerhead ribozyme-mediated cleavage and, thus, are unadenylated, can be subject to NMD, although NMD is detectable only if the rapid default decay pathway of unadenylated mRNA is prevented (S. Meaux, A. van Hoof, and K. Baker, pers. comm.). Data indicating that neither Pab1 nor PABPC1 is required either to define normal termination codons or to distinguish normal termination codons from PTCs raises the possibility that there may be multiple ways to classify a termination event as either normal or abnormal.

As noted above for fail-safe NMD targets in mammalian cells, measurements of 3′ UTR lengths strictly using the number of constituent nucleotides are also insufficient indicators of NMD targets in nonmammalian cells. In *D. melanogaster*, for example, the average 3′ UTR is 520 nt, whereas the average 3′ UTR of an NMD target is 450 nt, and many 3′ UTRs that are longer than average are not regulated by NMD (Rehwinkel et al. 2005; Behm-Ansmant et al. 2007a). Predictions of NMD targets may be more straightforward for *S. cerevisiae*, since the average 3′ UTR is ~100 nt and less variable than in *C. elegans* (Graber et al. 1999), and *S. cerevisiae* transcripts with the longest 3′ UTRs are generally regulated by NMD (Muhlrad and Parker 1999). Nevertheless, formulaic predictions of NMD targets in any organism must consider that 3′ UTRs may fold into a structure that brings the poly(A)-binding protein or another determinant into sufficiently close proximity to a translation termination codon to preclude NMD. Additionally, stabilizing elements within 3′ UTRs, such as those found within the 3′ UTR of unspliced Rous sarcoma virus RNA (Weil and Beemon 2006) or the coding regions of *S. cerevisiae* GCN4 and YAP1 mRNAs (Ruiz-Echevarría and Peltz 2000), could also inhibit NMD. To confound predictability further, mRNAs in *D. melanogaster* with long 3′ UTRs have been shown to escape NMD if they are in-

herently unstable (Behm-Ansmant et al. 2007a). It is clear from these and other studies (see below) that establishing rules to define NMD targets that do not depend on an EJC situated downstream from a PTC may be complicated.

An additional NMD mechanism that appears to function in *S. cerevisiae* involves loosely defined downstream elements (DSEs) (Amrani et al. 2006). Like EJCs that have the potential to trigger the NMD of PTC-bearing transcripts in mammals, DSEs reside within what is normally the translational reading frame. However, DSE-mediated NMD is also similar to NMD that is triggered by a faux 3′ UTR because it appears to involve inefficient translation termination, although due to an aberrant mRNP structure that features bound Upf NMD factors. Data indicate that the Upf NMD factors are recruited by a DSE-binding protein, the only characterized of which is Hrp1 (Gonzalez et al. 2000; Wang et al. 2006). It is possible that DSEs typify a subclass of mRNAs and are not generally NMD determinants. Alternatively, their true nature may have yet to be revealed.

The finding that NMD in *S. cerevisiae* (Zhang et al. 1997; Gonzalez et al. 2001), *S. pombe* (Mendell et al. 2000), *D. melanogaster* (Gatfield et al. 2003), and plants (Voelker et al. 1990; Dickey et al. 1994; van Hoof and Green 1996; Petracek et al. 2000; Arciga-Reyes et al. 2006) can target transcripts that derive from intronless genes has been interpreted to indicate that NMD in these organisms does not require splicing. Furthermore, *S. cerevisiae* lacks all known constituents of mammalian EJCs except for REF/Aly. Additionally, down-regulating Y14, REF/Aly, or RNPS1 in *D. melanogaster* (Gatfield et al. 2003), or Y14, REF/Aly, or eIF4AIII in *C. elegans* (Longman et al. 2007)—i.e., proteins that constitute mammalian EJCs—or removing all introns downstream from a PTC in *C. elegans* (Longman et al. 2007) fails to inhibit NMD. Finally, inserting an intron 80 nt downstream from the GUS transcript stop codon in *Arabidopsis thaliana* does not reduce mRNA abundance to a greater extent than is observed by inserting the intron within the coding region (Rose 2004).

Nevertheless, there is recent evidence that not only a faux 3′ UTR but also splicing can function in NMD in *Nicotinium tabacum* (Kertesz et al. 2006). First, 3′ UTRs of 700, 500, or 300 nt that derive from bacterial sequences reduce the abundance of mRNAs produced by a *PHA* minigene in a Upf1-dependent mechanism. Second, inserting an intron 99 base pairs (bp) but not 28 bp downstream from the translation termination codon of the *PHA* minigene decreases PHA mRNA abundance in a Upf1-dependent mechanism. In further support of the view that splicing functions in plant NMD, tethering Upf1 to the 3′ UTR of the *PHA* minigene reduces mRNA abundance (Kertesz et al. 2006), possibly analogously to how tethering Upf1 to a 3′ UTR recapitulates EJC function in mammalian cell NMD (Lykke-Andersen et al. 2001; Kim et al. 2005). However, tethering Upf1 to the 5′ UTR of the *PHA* minigene also reduces mRNA abundance (Kertesz et al. 2006). At this point, it is unclear whether data demonstrating that introns can influence

the efficiency of NMD in nonmammalian organisms reflects a role in NMD per se or a more general role in mRNA translation or decay. For example, the finding that an intron situated upstream of a PTC within the rice waxy transcript augments the efficiency of NMD (Isshiki et al. 2001) could reflect the established role of introns in promoting translation. While EJCs may exist in current-day plants, as evidenced by detectable Magoh–Y14–PYM complexes (Park and Muench 2007), additional studies are required to determine whether EJC-dependent NMD exists in organisms other than mammals.

Temporal aspects of NMD

Mammalian cells Depending on the particular mRNA, NMD in mammalian cells occurs in one of two places: in association with nuclei, or in the cytoplasm. Regardless of the cellular site, half-life studies indicate that NMD degrades newly synthesized, fully spliced, and polyadenylated mRNA (Cheng and Maquat 1993; Belgrader et al. 1994; Sun et al. 2000). In support of the idea that NMD is the consequence of nonsense codon recognition after and not before splicing, spliceable introns that have been engineered to interrupt nonsense codons have no effect on the efficiency of NMD (Zhang and Maquat 1996; Wang et al. 2002c). Furthermore, nonsense codons generally do not affect the rate of intron removal (e.g., see Cheng and Maquat 1993; Lytle and Steitz 2004).

It is unclear exactly where in the cell nucleus-associated NMD takes place. While we and others favor the idea that it occurs during or immediately after mRNA export from the nucleus to the cytoplasm so as to involve translation by cytoplasmic ribosomes (see Dahlberg and Lund 2004 and references therein), others have proposed that it occurs within the nucleoplasm so as to involve translation by nuclear ribosomes (see Iborra et al. 2004 and references therein).

The restriction of mammalian NMD to newly synthesized mRNA (Fig. 1A) was first evident with the observation that the decay rate of PTC-containing nucleus-associated TPI mRNA was abnormally fast, whereas the decay rate of PTC-containing cytoplasmic TPI mRNA was like that of PTC-free TPI mRNA (Cheng and Maquat 1993; Belgrader et al. 1994). Additional insight derived from studies of the two cap-binding protein complexes. CBP80 along with CBP20 constitute the mostly nuclear cap-binding complex (Izaurralde et al. 1994), whereas eukaryotic translation initiation factor 4E (eIF4E) comprises the mostly cytoplasmic cap-binding complex (Gingras et al. 1999). Initial data revealed that PTC-containing CBP80:CBP20-bound β-globin mRNA and PTC-containing eIF4E-bound β-globin mRNA are reduced to the same percentage of, respectively, PTC-free CBP80:CBP20-bound β-globin mRNA and PTC-free eIF4E-bound β-globin mRNA (Ishigaki et al. 2001). This result also typfied other mRNAs. Since eIF4E-bound mRNA is a product of CBP80:CBP20-bound mRNA (Lejeune et al. 2002), it follows that eIF4E-bound mRNA is not detectably targeted for NMD. In support of this

conclusion, eIF4E-binding protein 1 (4E-BP1), which inhibits steady-state translation by competing with eIF4GI for binding to eIF4E, fails to inhibit NMD (Chiu et al. 2004).

The restriction of NMD to CBP80:CBP20-bound mRNA can be explained by at least three findings. First, EJCs are detected on mRNA that is bound by CBP80:CBP20 but not mRNA that has undergone remodeling so that eIF4E has replaced CBP80:CBP20 at the cap (Ishigaki et al. 2001; Lejeune et al. 2002). The importance of EJCs to NMD is illustrated by reports that down-regulating the EJC constituents Y14, MNL51/BTZ, eIF4AIII, Upf2, or Upf3X inhibits NMD (Wang et al. 2002c; Gehring et al. 2003, 2005; Ferraiuolo et al. 2004; Mendell et al. 2004; Palacios et al. 2004; Shibuya et al. 2004; Kim et al. 2005; Wittmann et al. 2006; Chan et al. 2007). Second, CBP80:CBP20-bound mRNA can be translated during what has been called a "pioneer" round of translation, similarly to how eIF4E-bound mRNA is translated during subsequent rounds of steady-state translation (Ishigaki et al. 2001; Chiu et al. 2004). Third, CBP80 is not a passive component of the pioneer translation initiation complex, but instead interacts directly with the Upf1 NMD factor and promotes NMD during the pioneer round of translation by augmenting the binding of Upf1 to the Upf2 NMD factor (Hosoda et al. 2005). Consistent with this view, down-regulating CBP80 inhibits NMD (Hosoda et al. 2005). Furthermore, tethering Upf1, which is the last of the Upf proteins to join the EJC (Lykke-Andersen et al. 2001; Hosoda et al. 2005; Kim et al. 2005), >50–55 nt downstream from a nonsense codon bypasses the need for CBP80, so that mRNA decay is extended to eIF4E-bound mRNA as evidenced by its sensitivity to 4E-BP1 (Hosoda et al. 2005). These findings indicate that mammalian cells utilize the pioneer round of translation for quality control and use subsequent steady-state rounds of translation for the bulk of protein production. The binding of Upf1 to the poineer translation initiation complex at the mRNA 5′ end, and the presence of Upf2 and Upf3X or Upf3 at post-splicing EJC of this complex, suggests that CBP80:CBP20-bound mRNA is effectively poised for NMD-mediated surveillance.

It was recently proposed that CBP80:CBP20 may not be required for NMD since nonsense-containing mRNAs initiating translation using an encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES)-dependent mechanism rather than a cap-dependent mechanism undergo NMD (Holbrook et al. 2006). However, CBP80:CBP20 is still a constituent of newly synthesized mRNP regardless of how translation initiates. Thus, for reasons listed above, it remains likely that NMD resulting from EMCV IRES-dependent translation initiation is also restricted to CBP80:CBP20-bound mRNA.

Interestingly, fail-safe NMD, like natural NMD, also targets CBP80:CBP20-bound mRNA, but not detectably eIF4E-bound mRNA (D. Matsuda, N. Hosoda, Y.K. Kim, and L.E. Maquat, in prep.). Since fail-safe NMD requires Upf1 (Bühler et al. 2006; D. Matsuda, N. Hosoda, Y.K.

Kim, and L.E. Maquat, in prep.), CBP80 may provide an efficient delivery system for Upf1 during fail-safe NMD as it does during NMD that depends on an EJC situated downstream from the nonsense codon.

The initially unexpected existence of the pioneer translation initiation complex has been validated not only by demonstrating that 4E-BP1 fails to inhibit NMD and, therefore, the pioneer round of translation (Chiu et al. 2004) but also by characterizing functional components. For example, a constitutively inactive phosphomimetic version of eIF2α inhibits steady-state translation to the same extent as 4E-BP1 but, unlike 4E-BP1, also inhibits NMD (Chiu et al. 2004). Therefore, eIF2α, unlike eIF4E, is an integral component of the pioneer translation initiation complex. As noted earlier, PABPC1 is another component of the complex as evidenced by the inhibition of NMD using Paip2 (Chiu et al. 2004), which destabilizes the interaction of PABPC1 with poly(A) and competes with eIF4G for binding to PABPC1 so as to prevent mRNA circularization (Khaleghpour et al. 2001; Karim et al. 2006). Remarkably, PABPC1 begins to associate with poly(A) much earlier in mRNA biogenesis than previously thought by binding to unspliced pre-mRNAs (Hosoda et al. 2006), fueling the interesting possibility that PABPN1 may function primarily in poly(A) biogenesis rather than as an integral poly(A)-binding protein (Meyer et al. 2004). eIF4G, which interacts directly with CBP80, also functions during the pioneer round of translation, as demonstrated by the inhibition of NMD using protease-mediated eIF4G cleavage (Lejeune et al. 2004).

Nonmammalian cells In contrast to NMD in mammalian cells, NMD in *S. cerevisiae* targets both newly synthesized and steady-state mRNA (Fig. 1B). First, NMD degrades not only mRNAs that are bound by Cbc1:Cbc2, a complex that is orthologous to mammalian CBP80:CBP20, but also mRNAs that are bound by eIF4E (Gao et al. 2005). Consistent with this conclusion, *S. cerevisiae* lacking Cbc1 are viable and able to support NMD (Das et al. 2000) by targeting eIF4E-bound mRNA (Gao et al. 2005). Second, NMD in *S. cerevisiae* has been shown to take place without significant shortening of the mRNA poly(A) tail (Muhlrad and Parker 1994; Cao and Parker 2003), indicating that it targets newly synthesized mRNA. Third, nonsense-containing transcripts in *S. cerevisiae* harboring a temperature-sensitive RNA polymerase II that has been thermally inactivated accumulate on polysomes in the presence of the translational inhibitor cycloheximide, and these transcripts are lost from polysomes (i.e., continue to be degraded) once cycloheximide has been washed away (Zhang et al. 1997). Since polysomes contain mostly steady-state mRNA, the resumed disappearance of polysome-associated mRNA after the removal of cycloheximide suggests that NMD targets steady-state mRNA. Fourth, NMD occurs in *S. cerevisiae*, in which each of the three Upf NMD factors, under the control of a galactose-responsive promoter, has been induced after a period of repression (Maderazo et al. 2003). The resumed disappearance of

cytoplasmic mRNA after the induction of these factors suggests that NMD targets steady-state mRNA, since the repression of Upf NMD factors far exceeds the time it takes to synthesize and export nuclear mRNAs. Fifth, inserting variants of a programmed -1 ribosomal frameshift signal into a reporter mRNA in *S. cerevisiae* so that ribosomes encounter a downstream nonsense codon at low frequencies (i.e., 1%–12%) reveals that more efficient frameshifting, which occurs largely after the pioneer round of translation, results in decreased mRNA stability (Plant et al. 2004). Sixth, leaky translation termination at PTCs in *S. cerevisiae* that is sufficiently inefficient to occur primarily after the pioneer round antagonizes NMD (Keeling et al. 2004). Taken together, these findings indicate that mRNAs in *S. cerevisiae*, like mRNAs in mammalian cells, undergo a Cbc1:Cbc2-mediated pioneer round of translation and that nonsense codon recognition during this round of translation leads to NMD. However, mRNAs in *S. cerevisiae* also undergo NMD when bound by eIF4E, which does not appear to be true of mammalian mRNAs.

While the kinetics of NMD in organisms other than mammals or *S. cerevisiae* has yet to be examined, independence from EJCs may indicate that NMD in these organisms targets both newly synthesized and steady-state mRNA.

NMD factors

Overview NMD factors were first identified in *S. cerevisiae* and *C. elegans* using genetic screens (Table 1; Hodgkin et al. 1989; Leeds et al. 1991). Up-frameshift (Upf)1, Upf2, and Upf3 [referred to, respectively, as suppressor with morphological effect on genitalia 2 (Smg2), Smg3, and Smg4 in *C. elegans*] are conserved from *S. cerevisiae* to humans, whereas Smg1, Smg5, and Smg6 are present in all metazoans that have been examined except for *S. cerevisiae* and *S. pombe* (Maquat 2004b; Conti and Izaurralde 2005), and Smg7 is present in all metazoans that have been examined except for *S. cerevisiae*, *S. pombe*, and *D. melanogaster* (Maquat 2004b; Conti and Izaurralde 2005). Smg lethal 1 (Smgl1) and Smgl2 comprise a new class of NMD factors in *C. elegans* that are essential for *C. elegans* embryonic viability, have orthologs in fugu, mouse, and humans, but not *S. cerevisiae*, and have been demonstrated to be functionally conserved in humans (Longman et al. 2007).

Upf1 is a group I RNA helicase and ATPase (Weng et al. 1996; Bhattacharya et al. 2000). In metazoans, Upf1 is regulated by cycles of Smg1-mediated phosphorylation that, at least in mammals and *C. elegans*, depend on (1) Upf2/Smg3 and Upf3 or Upf3X/Smg4 (Page et al. 1999; Denning et al. 2001; Pal et al. 2001; Yamashita et al. 2001; Ohnishi et al. 2003; Brumbaugh et al. 2004; Grimson et al. 2004) and (2) Smg5-, Smg6-, and Smg7-mediated dephosphorylation (Cali et al. 1999; Anders et al. 2003; Chiu et al. 2003; Ohnishi et al. 2003; Grimson et al. 2004; Fukuhara et al. 2005). Smg1 is a phosphoinositol-3-kinase (PIK)-related protein kinase. Smg5, Smg6, and Smg7 are not phosphatases, but may recruit protein

Table 1. *NMD factors*

Factor	Function
Upf1 (Smg2 in *C. elegans*)	ATPase; RNA helicase; binds Upf2/Smg3, eRF1, and eRF3; in mammals, binds CBP80 and, transiently, the EJC by interacting with Upf2, promotes histone mRNA decay at the end of S phase by interacting with the stem–loop-binding protein, and promotes Staufen1-mediated mRNA decay by interacting with Staufen1
Upf2 (Smg3 in *C. elegans*)	Binds Upf1 and Upf3 or Upf3X/Smg4; in mammals, recruits Upf1 to the EJC by interacting with Upf3 or Upf3X; shown to be required for Upf1 phosphorylation in mammals and *C. elegans*
Upf3, also called Upf3a	Binds Upf2/Smg3; in mammals, recruits Upf2 to the EJC by interacting weakly with Y14
Upf3X, also called Upf3b (Smg4 in *C. elegans*)	Binds Upf2/Smg3; in mammals, recruits Upf2 to the EJC by interacting more strongly with Y14 than does Upf3
Smg1	In mammals and *C. elegans*, shown to be a PIK-related protein kinase that phosphorylates Upf1; component of the SURF complex together with Upf1, eRF1, and eRF3 in mammals
Smg5	Forms complex with Smg7, phosphorylated Upf1, and PP2A; shown to promote Upf1 dephosphorylation in mammals and *C. elegans*
Smg6	Forms complex with phosphorylated Upf1 and PP2A; shown to promote Upf1 dephosphorylation in mammals and *C. elegans*
Smg7	Forms complex with Smg5, phosphorylated Upf1, and PP2A; shown to promote Upf1 dephosphorylation in mammals and *C. elegans*
Smgl-1	Essential for embryonic development in *C. elegans*
Smgl-2	Essential for embryonic development in *C. elegans*; DEAD/DEAH-box RNA helicase in mammals

phosphatase 2A (PP2A) to phosphorylated Upf1/Smg2. In support of this idea, Smg6 is part of a complex consisting of PP2A and phosphorylated Upf1 (Chiu et al. 2003; Ohnishi et al. 2003). Furthermore, Smg5 and Smg7 interact with each other and also comprise a complex consisting of PP2A and phosphorylated Upf1 (Anders et al. 2003; Ohnishi et al. 2003). Additionally, inhibiting the interaction of Smg5 with the Upf1–Smg6–Smg7 complex using the small hydrophobic tetracyclic indole derivative NMDI1 results in the accumulation of phosphorylated Upf1 (Durand et al. 2007).

Smg7 contains an N-terminal 14–3–3-like phosphoserine-binding site that, when mutated, impairs its binding to phosphorylated Upf1 in vitro (Fukuhara et al. 2005). The finding that the 14–3–3-like domain of Smg7 contains several tetratricopeptide repeats that typify Smg5 and Smg6 suggests that Smg5 and Smg6 may also bind phosphoserine residues, although with unknown consequences (Fukuhara et al. 2005). In addition to binding phosphorylated Upf1 and Smg5 within its N-terminal domain, Smg7 appears to target mRNAs for decay within its C-terminal domain (Unterholzner and Izaurralde 2004). In fact, overexpressing Smg7 in human cells results in its accumulation in cytoplasmic P-bodies, which are foci rich in degradative factors of the 5′-to-3′ decay pathway (see below), together with Smg5 and Upf1 in a way that requires both N- and C-terminal domains of Smg7 (Unterholzner and Izaurralde 2004). The NMDI1-mediated inhibition of the Smg5–Upf1 interaction also results in the accumulation of phosphorylated Upf1 together with Smg6, Smg7, Upf3, Upf3X, and PTC-containing mRNAs in P-bodies (Durand et al. 2007). Smg7 is thought to provide the molecular link between translation termination that occurs sufficiently upstream of an EJC to trigger NMD, and the decay machinery. In support of this idea, tethering Smg7 to an mRNA results in mRNA decay even in the absence of a termination codon

upstream of the tethering site (Unterholzner and Izaurralde 2004). In fact, tethering Smg7 also bypasses the need for Upf1, Smg5, and Smg6 (Unterholzner and Izaurralde 2004). Additionally, tethering C-terminal fragments of Smg7 that do not interact with Smg5 or Upf1 reduces mRNA abundance (Unterholzner and Izaurralde 2004). Thus, the C-terminal domain of Smg7 appears to recruit mRNA degradative activities either directly or indirectly (see below).

Even in *C. elegans* and mammals, where it has been most studied, the role of Upf protein phosphorylation is only poorly understood. Smg1 is essential for NMD in *C. elegans* and mammals (Pulak and Anderson 1993; Yamashita et al. 2001). However, down-regulating or mutating Smg1 in *D. melanogaster* does not inhibit the NMD of PTC-containing alcohol dehydrogenase mRNA (Chen et al. 2005). In support of Upf1 phosphorylation playing a more significant role in mammals and *C. elegans* than in *D. melanogaster*, Smg1 functions as a nonessential potentiator of NMD and larval viability (Metzstein and Krasnow 2006). In contrast, Upf1 and Upf2 are required for NMD, larval viability, and the proper expression of dozens of normal genes during *D. melanogaster* development (Metzstein and Krasnow 2006).

There are additional indications that cycles of Upf1 phosphorylation and dephosphorylation may be less important or absent in organisms other than mammals and *C. elegans*. For example, a possible Smg1 ortholog in *Oryza sativa* is only 15% identical (32% similar) to human Smg1 (Templeton and Moorhead 2005), and no Smg1 ortholog has been found for *Arabidopsis thaliana* (Maquat 2004b). As another example, even though two proteins have been identified in *A. thaliana* using the tetratricopeptide repeat and flanking amino acids of human Smg5, Smg6, and Smg7 (Maquat 2004b), neither contains a PIN domain or has been demonstrated to function in NMD. Thus, whether Upf1 undergoes cycles

of phosphorylation and dephosphorylation in plants as it does in metazoans will require future studies.

The importance of Upf1 phosphorylation and dephosphorylation in *S. cerevisiae* is also uncertain. For example, while Upf1 is a phosphoprotein, orthologs to Smg1, Smg5, Smg6, and Smg7 have yet to be identified (Wang et al. 2006), raising the possibility that Upf1 phosphorylation may not impact NMD in *S. cerevisiae*. Studies of *S. cerevisiae* suggest that phosphorylation within the N-terminal domain of Upf2 is critical for efficient NMD and affects the interaction of Upf2 with Hrp1 (Wang et al. 2006). Interestingly, human Upf2 is also phosphorylated, but with unknown consequences to its function (Chiu et al. 2003).

PTC recognition in mammalian cells   In mammals, Upf2, Upf3, and Upf3X are stable constituents of EJCs (Lykke-Andersen et al. 2000, 2001; Ishigaki et al. 2001; Kim et al. 2001a; Le Hir et al. 2001b; Lejeune et al. 2002; Gehring et al. 2003, 2005). Upf3X is recruited to the EJC by interacting with Y14 (Gehring et al. 2003). Upf3 differs from Upf3X by an important amino acid that weakens its interaction with Y14 and makes Upf3 less important to NMD than Upf3X (Gehring et al. 2003; Kunz et al. 2006). Upf3 and Upf3X, both of which are primarily nuclear (Lykke-Andersen et al. 2000; Serin et al. 2001), interact directly with Upf2 (Serin et al. 2001; Kadlec et al. 2004, 2006), which is primarily cytoplasmic and enriched along the nuclear envelope (Lykke-Andersen et al. 2000; Serin et al. 2001). As noted above, Upf1 is a stable constituent of the CBP80:CBP20 cap-binding complex (Hosoda et al. 2005), although it shuttles between nuclei and cytoplasm and is primarily cytoplasmic (Lykke-Andersen et al. 2000; Serin et al. 2001; Mendell et al. 2002).

According to current models of mammalian NMD (Fig. 1A), translation termination during the pioneer round of translation involves the SURF complex, which consists of Smg1, Upf1, and the eRF1 and eRF3 translation termination factors (Kashima et al. 2006). It would make sense, but is unproven, that Upf1 of SURF derives from CBP80-bound Upf1. As indicated earlier, Upf1 is known to be the last of the Upf proteins to associate with the EJC. Accordingly, if translation terminates sufficiently upstream of an EJC to elicit NMD, then the Upf1 and Smg1 constituents of SURF bind to the EJC (Kashima et al. 2006). eRF1 and eRF3 of SURF are not likely to accompany Smg1 and Upf1 to the EJC, since data indicate that Upf1 binding to EJC-bound Upf2 is in competition with Upf1 binding to eRF1 (Kashima et al. 2006). While steady-state Upf1 is primarily hypophosphorylated (Pal et al. 2001; Yamashita et al. 2001), Upf1 binding to the EJC results in the Smg1-mediated phosphorylation of Upf1 (Kashima et al. 2006; Wittmann et al. 2006). Since the Upf factors interact with mRNA degradative activities known to function in NMD (see below), it is reasonable to propose that Upf1 phosphorylation triggers steps that are required for mRNA decay, including the recruitment of degradative activities to mRNA. Less is known about how and when Smg5,

Smg6, and Smg7 mediate the dephosphorylation of Upf1. However, it appears to occur after NMD substrates move to P-bodies, since down-regulating Upf2, which inhibits Upf1 phosphorylation, inhibits the accumulation of NMD factors and substrates in P-bodies (Durand et al. 2007). Furthermore, inhibiting Upf1 dephosphorylation using NMDI1 results in the accumulation of NMD factors and substrates in P bodies (Durand et al. 2007).

Interestingly, recent data suggest that not all NMD events in mammalian cells depend to the same extent on either Upf2 or Upf3X. For example, some natural NMD targets are less sensitive to Upf2 depletion than others (Gehring et al. 2005; Wittmann et al. 2006). Additionally, depletion of Upf3X, either by itself or together with Upf3, has no effect on the NMD of TCR-β mRNA or the abundance of some, but not all natural NMD targets that were identified using microarrays (Chan et al. 2007). The TCR-β sequence responsible for Upf3X insensitivity appears to consist of functionally redundant segments and includes the same region that has been shown to promote efficient TCR-β and Ig μ NMD (Gudikote and Wilkinson 2002; Bühler et al. 2004; Chan et al. 2007). Taken together, these findings suggest that not all EJCs are functionally identical. Some EJCs may be devoid of Upf2 or Upf3X, contain either protein in a nonfunctional state, or otherwise function in a way that is less sensitive to Upf2 or Upf3X down-regulation.

PTC recognition in nonmammalian cells   The molecular gymnastics of Upf1, Upf2, and Upf3 as mRNP proteins in organisms other than mammals is less well understood (Fig. 1B). The fact that steady-state mRNA is targeted for NMD indicates that NMD factors may not be stable constituents of mRNP, but associate with mRNA only when translation terminates abnormally. Alternatively, and possibly less likely, NMD factors may stably associate with mRNP and are either not removed by the process of translation or are removed but can reassociate with mRNA to function in subsequent rounds of translation.

Except for the recruitment of Upf1 and Upf2 to the DSE of PGK1 mRNA by Hrp1 in *S. cerevisiae* (Gonzalez et al. 2000; Wang et al. 2006), it is generally unclear how and when Upf factors associate with mRNAs in yeast and other nonmammalian organisms. Furthermore, tethering a Upf factor downstream from a termination codon has never been reported to trigger NMD in yeast. A popular model that has been put forth to mechanistically explain how a PTC leads to abnormally inefficient translation termination and, therefore, NMD posits that Upf1 and the translation termination factor eRF3 are in competition for direct binding to Pab1 (Amrani et al. 2006). According to this model, a termination event is recognized as normal when Pab1 is sufficiently close to eRF3, so that it out-competes Upf1 for binding to eRF3. Alternatively, a termination event is deemed abnormal when Pab1 is not sufficiently close to eRF3 to compete with Upf1 for binding to eRF3 (Wang et al. 2001; Amrani et al. 2004). However, whether eRF3 binding to Pab1 excludes Upf1 binding to Pab1 and vice versa, and how Pab1-in-

dependent translation termination events are classified as either normal or abnormal, remain to be clarified. Additionally, the significance of the finding that eRF1, which is generally complexed to eRF3, competes with Upf2 for binding to Upf1 (Wang et al. 2001) is uncertain.

Upf protein function after PTC recognition The contributions of Upf proteins to NMD after an mRNA has been slated for NMD are best demonstrated for *S. cerevisiae*. Cytoplasmic P-bodies typify all eukaryotes that have been examined, although one cell may have one or more P-bodies, and an adjacent cell may have none that is detectable. P-bodies contain the Dcp1:Dcp2 decapping complex, the Dhh1/Me31B/RCK/p54, Scd6/CAR-1/RAP55, Edc3, Pat1, and Lsm1–7 activators of decapping, and the Xrn1 5′-to-3′ exonuclease (Parker and Sheth 2007). Data suggest that mRNAs present in P-bodies are translationally repressed if not undergoing 5′-to-3′ decay (Parker and Sheth 2007). For example, Upf1, Upf2, and Upf3 localize to P-bodies in strains lacking Dcp1, Dcp2, or Xrn1, each of which is known to contribute to NMD (see below). The localization of Upf1 to P-bodies does not require Upf2 or Upf3, but Upf1 is needed for the localization of Upf2 or Upf3 to P-bodies, and the localization of Upf2 and Upf3 is interdependent (Sheth and Parker 2006). Moreover, a reporter mRNA that efficiently undergoes NMD accumulates in P-bodies in a Upf1-dependent manner (Sheth and Parker 2006). Consistent with the view that Upf1 acts upstream of Upf2 and Upf3, mRNA localization itself is insufficient for NMD, which subsequently requires Upf2 and Upf3. Mutating the ATP hydrolytic activity of Upf1 results in increased Upf1 accumulation in P-bodies without detectable accumulation of Upf2 or Upf3, suggesting that ATP hydrolysis functions at a step after P-body localization and is required for the recruitment of Upf2 and Upf3 (Sheth and Parker 2006). The finding that overexpressing ATPase-defective Upf1 or, to a lesser extent, wild-type Upf1, reduces polysome size and increases P-body formation (Sheth and Parker 2006) suggests that Upf1 contributes to the translational repression that precedes NMD (Muhlrad and Parker 1999). Upf1 ATPase activity is not required for Upf1 binding to RNA, indicating that Upf1 binding to mRNA precedes translational repression (Cheng et al. 2007). However, expressing an ATPase-defective Upf1 that fails to bind RNA results in P-body formation (Cheng et al. 2007). Therefore, Upf1 binding to mRNA appears to occur after mRNA is targeted to P-bodies. This is in distinct contrast to what occurs in mammalian cells, where Upf1 binds to mRNA prior to mRNA translation (Hosoda et al. 2005).

Degradative activities of NMD

S. cerevisiae In *S. cerevisiae*, Upf1 interacts with the Dcp2 constituent of the decapping complex in two-hybrid analyses (He and Jacobson 1995). In support of this interaction being functionally significant, characterizations that include analyses of mRNA decay intermediates indicate that NMD degrades mRNAs primarily by deadenylation-independent decapping via the Dcp1:Dcp2 complex, followed by 5′-to-3′ decay of the transcript body by the Xrn1 exonuclease (Fig. 2A; Muhlrad and Parker 1994; He and Jacobson 2001; Cao and Parker 2003; He et al. 2003). Intermediates were assessed in the absence of individual or combined degradative activities using genes engineered to express full-length mRNAs containing an exonucleolytic barrier in the form of a G-tract. That NMD in *S. cerevisiae* involves decapping, followed by decay of the mRNA body from the 5′ end is compatible with the above-described localization of the Upf proteins to P-bodies when either Dcp1:Dcp2-mediated decapping or Xrn1-mediated 5′-to-3′ decay has been inhibited (Sheth and Parker 2006). A minor NMD pathway relies on accelerated deadenylation and, subsequently, 3′-to-5′ decay of the transcript body by the exosome (Cao and Parker 2003; Mitchell and Tollervey 2003). Notably, both NMD pathways are distinct from conventional mRNA decay in *S. cerevisiae*, which occurs primarily by deadenylation-dependent decapping (Muhlrad and Parker 1994).

Mammalian cells Since it is not possible to engineer an exonucleolytic barrier into mammalian transcripts, presumably due to the countering effects of RNA helicases, different experimental approaches have been developed to analyze the enzymology and polarity of mRNA decay during mammalian cell NMD. Results indicate that NMD in mammals, like NMD in *S. cerevisiae*, also degrades mRNAs from both 5′ and 3′ ends by recruiting decapping and 5′-to-3′ exonuclease activities as well as deadenylating and 3′-to-5′ exonuclease activities (Fig. 2B). First, the half-lives and/or levels of PTC-containing mRNAs are increased by down-regulating the Dcp2 decapping protein, poly(A) ribonuclease (PARN), or the PM/Scl100 component of the exosome (Lejeune et al. 2003), the latter of which is not confined to nuclei (Lejeune et al. 2003) as once thought (Brouwer et al. 2001; Chen et al. 2001). Second, down-regulating Dcp2 or Xrn1 inhibits the reduction in mRNA abundance that results from tethering Smg7 downstream from a termination codon (Unterholzner and Izaurralde 2004). Third, NMD factors Upf1, Upf2, and Upf3X coimmunopurify with decapping complex constituents Dcp1 and Dcp2, 5′-to-3′ exonucleases Rat1 and Xrn1, exosomal components PM/Scl100 and Rrp41, and PARN (Lykke-Andersen 2002; Lejeune et al. 2003). Fourth, mammalian Upf proteins interact in yeast two-hybrid analyses with decay factors of the 5′-to-3′ and 3′-to-5′ decay pathways (Lehner and Sanderson 2004). Additionally, Smg5 and Smg7 localize to cytoplasmic P-bodies that also contain Lsm4, indicating that they colocalize with decapping and 5′-to-3′ degradative activities (Unterholzner and Izaurralde 2004).

It is unclear which of the two decay pathways contributes more to NMD in mammalian cells. Some data indicate that NMD involves primarily deadenylation, although via Pan2 followed by Ccr4 rather than PARN, and only subsequently Dcp2-mediated decapping as a back-up mechanism (Yamashita et al. 2005). However,
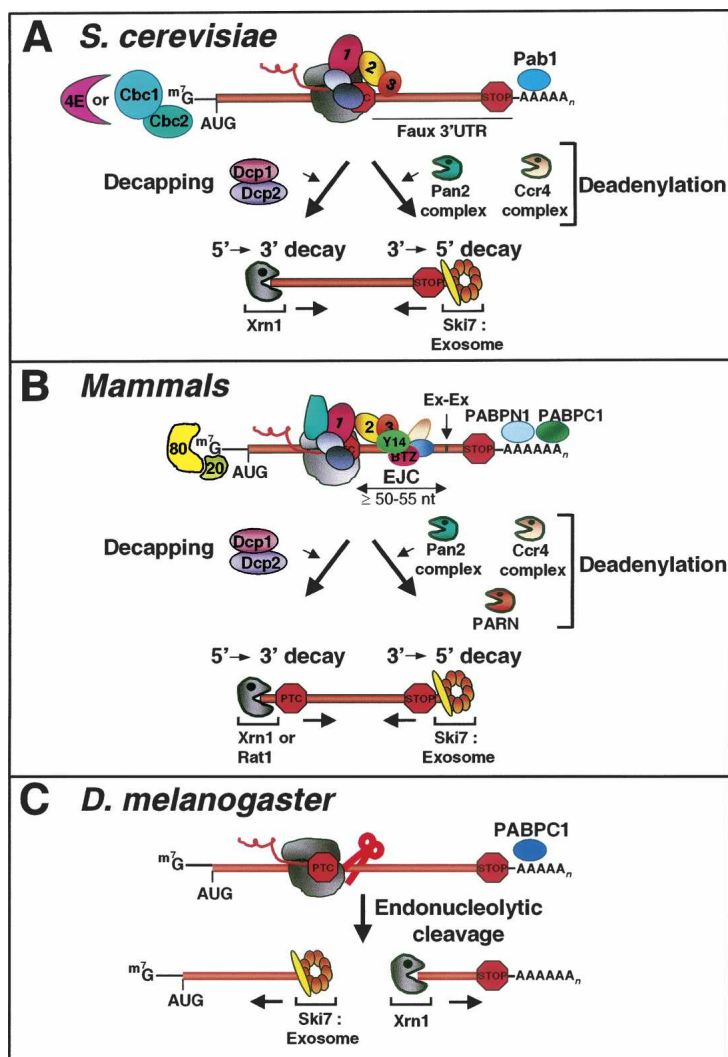
Figure 2. NMD degradative activities. (*A*) NMD in *S. cerevisiae* proceeds primarily by (1) deadenylation-independent Dcp1:Dcp2-mediated decapping, followed by Xrn1-mediated activity, and (2) to a lesser extent by Pan2- and Ccr4-mediated deadenylation and exosome-mediated decay. Here and elsewhere, factors that promote either decapping complex activity, such as the Lsm proteins, or exosome activity, such as the Ski2:Ski3:Ski8 complex, are not specified, although Ski7 is shown. (*B*) NMD in mammals generally involves (1) decapping by the Dcp1:Dcp2 complex followed by 5′-to-3′ decay of the body of the transcript by either Xrn1 or Rat1, and (2) deadenylation by PARN or Pan2 and Ccr4, followed by 3′-to-5′ decay of the body of the transcript by the exosome. It is unclear which pathway typifies the majority of NMD. (*C*) NMD in *D. melanogaster* entails one or more endonucleolytic cleavages that occur in the vicinity of the PTC. The resulting 5′ decay product is degraded from the 3′ end by the exosome, whereas the resulting 3′ decay product is degraded from the 5′ end by the Dcp1:Dcp2 decapping complex and Xrn1.

other data indicate that the PTC-mediated increase in the rate of deadenylation is insufficient to account for the decrease in mRNA half-life, which is supported by the detection of decapped products that are only partially deadenylated (Couttet and Grange 2004).

For reasons unknown, the NMD of β-globin mRNA in mouse erythroid tissues appears to be unique. The erythroid cells of mice that are transgenic for one of several β⁰-thalassemic β-globin alleles, as well as mouse erythroleukemic cells that stably express one of several of these alleles, generate readily detectable β-globin mRNA decay intermediates. These intermediates are polyadenylated and, irrespective of PTC position, missing roughly the same regions from the mRNA 5′ end (Lim et al. 1989, 1992; Lim and Maquat 1992; Stevens et al. 2002). Remarkably, the 5′ ends of the decay intermediates are generated primarily at UG dinucleotides by a polysome-associated endonucleolytic activity similar to polysome ribonuclease 1 (PMR1) of *Xenopus laevis* (Stevens et al. 2002; Bremer et al. 2003). These ends are subsequently capped and relatively resistant to further 5′-to-3′ decay (Lim and Maquat 1992). The resulting 5′-

cleavage products, while detectable (Stevens et al. 2002), are more susceptible to decay, presumably by the exosome. The PMR1-like activity that degrades nonsense-containing β-globin mRNA in erythroid cells may be superimposed on or may supercede the typical exonucleolytic pathways of NMD. Furthermore, considering the kinetics of decay (Lim et al. 1992), the PMR1-like activity is likely not restricted to CBP80:CBP20-bound mRNA and probably also targets eIF4E-bound mRNA. Since microRNA-directed mRNA cleavage involves the addition of uridines or adenosines to the 3′ ends of the 5′-cleavage products as a means to enhance exosome-mediated decay (Shen and Goodman 2004), the 3′ ends of the 5′-cleavage products of β-globin NMD in mouse erythroid cells may likewise be modified.

D. melanogaster   The general NMD pathway in *D. melanogaster* is also initiated primarily by endonucleolytic cleavage rather than from either mRNA end (Fig. 2C; Gatfield and Izaurralde 2004). While cleavage may be influenced by nucleotide sequence, it occurs in the general vicinity of the PTC. However, product heterogeneity

has precluded determining the exact site(s) of cleavage. The 5′-cleavage product is degraded from the 3′ end by the exosome, and the 3′-cleavage product is degraded from the 5′ end by Xrn1 (Gatfield and Izaurralde 2004). Therefore, neither decapping nor deadenylation is required for 5′-to-3′ exonucleolytic decay. It remains to be determined whether the 3′ ends of the 5′-cleavage products of NMD in *D. melanogaster* are modified prior to 3′-to-5′ decay.

NMD-mediated pressures on the evolution of gene structure

It is reasonable to propose that NMD may have provided a selective pressure for where introns colonize within mammalian genes given the role of the post-splicing EJC in NMD. However, compared with 5′ UTRs and coding regions, there is a paucity of introns within the 3′ UTRs of not only human and mouse genes, as would be expected of genes regulated by EJC-dependent NMD, but also *A. thaliana* and *D. melanogaster* genes (Hong et al. 2006). Furthermore, for humans, mice, *A. thaliana*, and *D. melanogaster*, the distribution of introns within 3′ UTRs is not uniform, becoming sharply reduced beginning with the normal termination codon rather than 50–55 nt downstream from the normal termination codon (Scofield et al. 2007). Notably, though, human and mouse genes have roughly one-third the number of introns situated 55 bp or more downstream from the normal termination codon than do plants and flies (Scofield et al. 2007).

Taken together, these data indicate that NMD-imposed restrictions to intron colonization within 3′ UTRs have contributed to the paucity of introns within the 3′ UTRs of mammalian genes. Furthermore, another selective mechanism has likely contributed to the paucity of introns within the 3′ UTRs of all eukaryotic genes. The suggestion that 3′ UTRs are more amenable than other gene regions to the conversion of spliceable introns into nonspliceable introns (Scofield et al. 2007) may apply to those organisms in which an abnormally long 3′ UTR does not generally trigger NMD or to instances where the 3′ UTR that contains a nonspliceable intron folds into a configuration that does not trigger NMD.

Nonsense-associated altered splicing (NAS)

NAS, which to date been studied only in mammalian cells, can be divided into two categories. One category occurs independently of translation and does not have the potential to serve in the capacity of quality control. It is exemplified by nonsense mutations that, like other single point mutations, disrupt the binding of an exonic splicing enhancer (ESE) by its cognate SR protein so as to alter splice-site selection (e.g., see Caputi et al. 2002; Cartegni and Krainer 2002).

The other category appears to be translation dependent, is not attributable to an altered ESE or another type of *cis*-acting effector of splicing, and has the potential to

provide a means of quality control (Gersappe and Pintel 1999; Li et al. 2002; Mendell et al. 2002; Wang et al. 2002a,c). For example, in-frame nonsense codons residing between a bona fide 5′ splice site and a downstream intronic 5′ splice site have been reported to promote selection of the proper 5′ splice site during the splicing of CAD and IDUA pre-mRNAs so as to result in the suppression of splicing (SOS) at the intronic splice site (Li et al. 2002). In support of the importance of SOS to proper splice site choice as a means of quality control, >90% of human genes examined were found to have at least one in-frame nonsense codon between the utilized 5′ splice site and a downstream intronic 5′ splice site (Miriami et al. 2004). However, according to a different set of criteria, there appeared to be no significant enrichment of in-frame nonsense codons between used and downstream intronic 5′ splice sites (Zhang et al. 2003; Zhang and Chasin 2004). Studies using β-globin pre-mRNAs containing two copies of a 5′ splice site with or without an in-frame nonsense codon in between revealed that the nonsense codon does reduce the level of transcripts that derived from use of the upstream splice site (Zhang and Krainer 2004). Nevertheless, it does so by triggering NMD rather than NAS (Zhang and Krainer 2004). The recent report that SOS occurs independently of translation and is unaffected by down-regulating Upf1 or Upf2 (Wachtel et al. 2004) suggests a mechanism that does not involve the surveillance of mRNA but, instead, perhaps pre-mRNA.

Possibly, TCR-β transcripts present another example of NAS that has the potential to provide quality control in a way that appears to be distinct from NMD but does depend on translation. TCR-β NAS can occur when Upf2 has been down-regulated so as to preclude TCR-β NMD (Mendell et al. 2002; Wang et al. 2002a). However, NAS and NMD are not mutually exclusive, and NAS is not triggered by all PTCs that trigger NMD. PTC recognition that leads to NAS occurs after splicing, since a PTC that is split by an intron triggers NAS (Wang et al. 2002c). The favored model is that PTC recognition during the process of cytoplasmic translation acts in *trans* to increase production of the PTC-free alternatively spliced mRNA that derives from newly synthesized pre-mRNA (Wang et al. 2002a), possibly as a means to down-regulate TCR-β gene expression. However, how PTC recognition in the cytoplasm can feed back to the nucleus in a manner that is not only allele-specific but also splicing isoform-specific remains to be clarified.

Nonsense-mediated pathways that do not control the quality of gene expression

Mammalian cells utilize the Upf1 NMD factor for at least two conditionally regulated mRNA decay pathways as exemplified by Staufen 1-mediated mRNA decay (SMD) (Kim et al. 2005, 2007) and the decay of cell cycle-regulated histone mRNAs at the end of S phase (Kaygun and Marzluff 2005). These pathways depend on an mRNA-specific binding protein—Staufen1 in the case of SMD targets, and stem–loop-binding protein in the case

of histone mRNAs—that recruits Upf1 to a position that resides sufficiently downstream from the normal termination codon to trigger mRNA decay when translation terminates normally. Consistent with these pathways serving in a conditionally regulated rather than quality-control capacity, SMD degrades not only CBP80:CBP20-bound mRNA but also eIF4E-bound mRNA, the latter of which constitutes the primary template for protein synthesis, and thus is the logical target for a regulatory circuit (Hosoda et al. 2005).

There are likely to be many more nonsense-mediated and Upf1-dependent regulatory mechanisms that have yet to be discovered, each of which would conceivably target a coordinately controlled group of mRNAs that have in common the potential to associate via their 3′ UTR sequences with the same Upf1–RNA-binding protein complex. While unproven, one mRNA that could exemplify another Upf1-dependent target is Col10a1 mRNA, the abundance of which appears to be down-regulated by PTCs, even when they reside within the 3′-most exon, in a cell type-specific manner (Bateman et al. 2003).

Mechanisms in mammalian cells that involve the recruitment of Upf1 to specific mRNAs by an RNA-binding protein or a complex of proteins should not be confused with NMD. Generally, if a nonsense-dependent mRNA decay pathway manifests cell type specificity and typifies mRNA that derives from a gene (or experimentally generated cDNA) that does not direct pre-mRNA splicing, then the pathway is not apt to function to control mRNA quality by surveillance. Rather, it is more likely to conditionally regulate gene expression.

Nonsense-mediated transcriptional gene silencing (NMTGS)

There appears to be an additional and more specialized mechanism by which nonsense codons can down-regulate gene expression. Particular to *Ig μ* and *Ig γ* minigenes, if not all *Ig* heavy-chain-encoding genes, nonsense-mediated quality control is not limited to transcript metabolism but also encompasses transcript synthesis via transcriptional gene silencing (Bühler et al. 2005; Stalder and Mühlemann 2007). NMTGS is the result of chromatin remodeling that appears to be induced by small interfering RNA (siRNA), since NMTGS is inhibited by overexpressing the exonuclease 3′hExo (Bühler et al. 2005) that degrades double-stranded siRNA (Kennedy et al. 2004). How PTC recognition leads to siRNA activation is unknown, although translation and Upf1 are required (Stalder and Mühlemann 2007). NMTGS was thought to explain the observation that mature PTC-containing TCR-β mRNA is reduced in abundance even when it is trapped within nuclei (Bühler et al. 2002). However, there is no mechanism comparable to NMTGS that has been observed to down-regulate the synthesis of other types of nonsense-bearing transcripts (e.g., see Urlaub et al. 1989; Cheng and Maquat 1993), including those that derive from a *TCR-β* minigene (Stalder and Mühlemann 2007). Furthermore,

there is no detectable difference in the levels of nonproductive PTC-containing and productive PTC-free Ig pre-mRNAs in a pro-B cell line (O. Mühlemann, pers. comm.). Therefore, the idea that NMTGS specifically down-regulates PTC-containing gene expression is unsubstantiated, at least for the stage of B-cell differentiation that was examined.

Nonstop mRNA decay (NSD): when mRNAs lack a termination codon

NSD has been studied in *S. cerevisiae* and humans. In contrast to NMD, NSD degrades transcripts that lack a termination codon (for review, see Maquat 2002; Vasudevan et al. 2002; Wagner and Lykke-Andersen 2002). NSD targets can arise when polyadenylation occurs prematurely within a coding region (Edwalds-Gilbert et al. 1997; Sparks and Dieckmann 1998; Graber et al. 1999; Frischmeyer et al. 2002), when transcription aborts (Cui and Denis 2003) or, at least in theory, upon incomplete 3′-to-5′ decay of ribosome-associated mRNAs. NSD targets are not likely to arise when normal termination codons are mutated or otherwise fail to be recognized by translating ribosomes, since mRNA 3′ UTRs usually contain many other in-frame termination codons.

The purpose of NSD in eukaryotes is probably twofold: to degrade translationally dead-end templates, and to release the associated ribosomes for the translation of other mRNAs. During NSD in *S. cerevisiae*, the empty A site of a stalled 80S ribosome at the extreme 3′ end of an mRNA is recognized by the C-terminal domain of the exosome-associated factor Ski7 (Fig. 3; Frischmeyer et al.
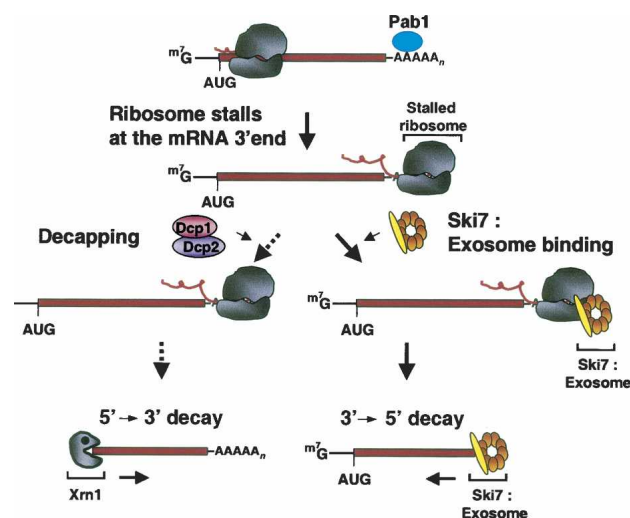

Figure 3. NSD. In *S. cerevisiae*, and probably also in mammals, transcripts lacking a termination codon are degraded by the exosome independently of deadenylation. The exosome is recruited to the empty A site of the ribosome by Ski7, which is a molecular mimic of EF1A and eRF3. NSD targets are also thought to be degraded by decapping followed by Xrn1-mediated degradation, although less efficiently than by exosome-mediated decay.

2002; van Hoof et al. 2002). This Ski7 domain structurally mimics the GTPase domain of (1) eukaryotic elongation factor 1A (eEF1A), which recognizes the ribosomal A site when it is occupied by a sense codon, and (2) eRF3, which recognizes the ribosomal A site when it is occupied by a nonsense codon. Since an exosome mutation that precludes its interaction with Ski7 inhibits NSD, the N-terminal domain of Ski7 is thought not only to interact with other constituents of the Ski complex but additionally to recruit the exosome (van Hoof et al. 2002). NSD also occurs by 5′-to-3′ decay, at least in the absence of Ski7 and, possibly, in its presence, considering that NSD templates lack Pab1 (Inada and Aiba 2005) and the loss of Pab1 promotes decapping (Coller and Parker 2004).

mRNAs that are targeted for NSD can be detected on polysomes in complex with nascent peptide (Inada and Aiba 2005; Akimitsu et al. 2007). The encoded protein is barely detectable, indicating that the mRNAs are also translationally repressed post-initiation (Inada and Aiba 2005; Akimitsu et al. 2007). Generally, mRNAs are translationally repressed if they lack a 3′ UTR, as typifies NSD substrates, contain an abnormally short 3′ UTR (Inada and Aiba 2005), or contain an abnormally long 3′ UTR, as typifies NMD substrates (Amrani et al. 2004). Additionally, translation of a poly(A) tract results in ribosome stalling, partial translational repression, and proteosome-mediated protein degradation (Ito-Harashima et al. 2007). A tract of >10 consecutive lysine residues is adequate to be recognized as abnormal and trigger translational repression (Ito-Harashima et al. 2007).

Notably, NSD is distinct from the exosome-mediated decay of 5′-cleavage products that derive from hammerhead ribozyme-mediated cleavage within the coding region of a translationally active reporter transcript (Meaux and Van Hoof 2006). For one, while the 3′-to-5′ pathway of NSD requires both N- and C-terminal domains of Ski7, decay following ribozyme-mediated cleavage, like the decay of normal mRNAs, does not require the C-terminal domain of Ski7 (Araki et al. 2001; van Hoof et al. 2002; Meaux and Van Hoof 2006). Additionally, decay of 5′-cleavage products that derive from hammerhead ribozyme-mediated cleavage is not inhibited in a strain lacking the C-terminal domain of Ski7 when a PTC is generated upstream of the ribozyme (Meaux and Van Hoof 2006). However, decay may involve NSD at a level that is difficult to detect because of the PTC-promoted pathway. Notably, the NSD-independent decay pathway has yet to be shown to require translation of the target mRNA.

No-go mRNA decay (NGD): when mRNA translation elongation stalls

NGD has been studied only in *S. cerevisiae*. It was discovered when an artificial stem–loop structure was inserted into each of two *S. cerevisiae* mRNAs, creating a block to translation elongation (Doma and Parker 2006). Unlike NMD and NSD pathways in *S. cerevisiae*, NGD

involves endonucleolytic cleavage(s) near to where the translationally active ribosome stalls (Fig. 4). In this regard, NGD resembles NMD in *D. melanogaster*. Also like NMD in *D. melanogaster*, the resulting free end of the 5′-cleavage product is degraded by the exosome, and the resulting free end of the 3′-cleavage product is degraded by Xrn1.

NGD requires Dom34, which is related to eRF1 (Carr-Schmid et al. 2002), since a strain lacking Dom34 also lacks detectable cleavage products in the absence of the exosome accessory factor Ski7 or the 5′-to-3′ exonuclease Xrn1 (Doma and Parker 2006). Additionally, NGD involves Hbs1, which interacts with Dom34 (van Hoof 2005). Hbs1 is a member of the family of GTPases that includes eEF1A and is also related to Ski7 (van Hoof 2005). However, the 3′ decay product is detected in a strain lacking Hbs1 and Xrn1, and the 5′ decay product is detected, although at a drastically reduced level, in a strain lacking Hbs1 and either Ski2 or Ski7 (Doma and Parker 2006). Therefore, Ski7 may function on behalf of Hbs1 when Hbs1 is absent, or Hbs1 may function only when exosome function is debilitated.

Thus, it appears that Dom34 recognizes stalled ribosomes by binding to the available ribosomal A site as a molecular mimic of charged tRNA. While it is formally possible that Dom34 is the endonuclease, it does not share sequence similarities to known endonucleases. Instead, Dom34 together with Hbs1 may help to recruit
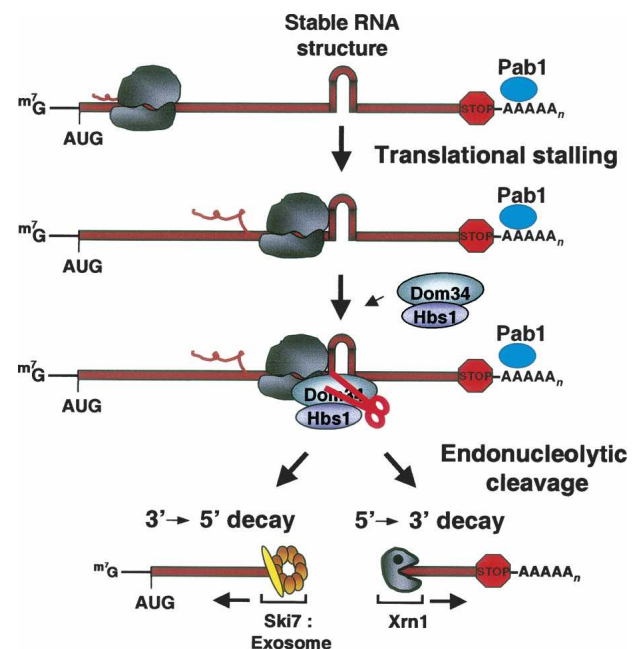

Figure 4. NGD. In *S. cerevisiae*, translationally active mRNAs that harbor a barrier to ribosome progression are targeted for endonucleolytic decay in a mechanism that requires Dom34 and involves Hbs1, the latter of which may be functionally substituted by Ski7. The resulting 5′ decay product is degraded from the 3′ end by the exosome, and the resulting 3′ decay product is degraded from the 5′ end by the Dcp1:Dcp2 decapping complex followed by Xrn1.

the endonuclease. It has also been proposed that the ribosome may itself cleave the mRNA (Tollervey 2006), which happens in *Escherichia coli* when ribosomes stall (Hayes and Sauer 2003; Sunohara et al. 2004). As typifies endonucleolytic cleavage products generated during NMD in *D. melanogaster*, the 3′ ends of the resulting 5′-cleavage NGD products and 5′ ends of the resulting 3′-cleavage NGD products are heterogenous. Thus, it is currently unknown whether NGD involves a single endonucleolytic cleavage at the ribosomal A site, followed by limited exonucleolytic decay or multiple sites of endonucleolytic cleavage.

Pauses in the elongation process resulting from the presence of a pseudoknot, rare codons, or PTCs lead to significantly less endonucleolytic cleavage than a stem–loop structure (Ougland et al. 2004). Therefore, it appears that NGD evolved to degrade mRNAs that are physically altered in ways that cause a complete block in translation elongation rather than just a slowing of elongation that can be resolved by amino acid incorporation or translation termination. NGD may also be triggered by defective ribosomes that have initiated translation but are incapable of catalyzing a subsequent elongation step. NGD presumably evolved as a way to clear mRNAs and their associated ribosomes that are stuck in the process of translation elongation. The possibility that NGD may additionally regulate the metabolism of particular mRNAs is brought to mind by studies of *A. thaliana*, demonstrating that sequences within CGS1 mRNA induce ribosome stalling and endonucleolytic cleavage (Onouchi et al. 2005).

Summary

Cells require flexibility for adaptability. However, one cost of greater flexibility is the increased probability of making mistakes. Cells are most fit when they continue to evolve in constructive ways while exercising an ability to either correct or eliminate the products of error-prone pathways. The dependence of different RNA processes on one another and, in some cases, the same RNA nucleotides narrows the number of possible evolutionary paths but also permits exquisite regulatory coordination.

As reviewed here, NMD in mammals requires a splicing event either downstream from a PTC, in the case of classical NMD, or upstream of a PTC, in the case of fail-safe NMD. In contrast, NMD that typifies modern-day *S. cerevisiae*, *D. melanogaster*, *C. elegans*, and probably modern-day *S. pombe* appears to involve a faux 3′ UTR and not splicing. Whether NMD in modern-day land plants involves not only a faux 3′ UTR but also splicing is unclear. The evolutionary relationship between NMD in mammals and other organisms is unclear. First, whether introns characterized the common ancestor of current-day eukaryotes is uncertain (e.g., see Roy and Gilbert 2006; Hong et al. 2006). If they did, then an argument could be made for a primordial NMD pathway that required an intron upstream of if not downstream from a PTC. In support of this idea, EJC constituents are found throughout the eukaryotic tree (Lynch et

al. 2006). For example, orthologs to eIF4AIII, Magoh, Y14, REF/Aly, and RNPS1 are found in Trichomonads, Oomycetes, and Apicomplexans, which have on average 2.6, 2.8, and 0.8 introns per gene, respectively (Lynch et al. 2006). Furthermore, the common ancestor of all extant eukaryotes has been characterized by a sophisticated spliceosome (Collins and Penny 2005). Therefore, the possibility exists that RNA processing events in ancestors to *S. cerevisiae* included the deposition of EJCs on spliced transcripts. Even if introns were largely acquired during mammalian evolution, data indicating that classical as well as fail-safe NMD in mammals targets newly synthesized CBP80:CBP20-bound mRNA and not detectably steady-state eIF4E-bound mRNA, whereas NMD in *S. cerevisiae* and other nonmammalian eukaryotes targets both newly synthesized and steady-state mRNA, make it difficult to conclude that NMD in mammals is simply a remnant of an ancestral faux 3′ UTR pathway that has been augmented by the evolution EJCs.

This review has focused not only on NMD but also and two other mRNA quality control pathways, NSD and NGD, each of which is triggered by the inability of an mRNA to properly direct protein synthesis. However, it is important to note that mRNAs also undergo various types of surveillance prior to their translation. For example, gatekeeping at the nuclear pore complex occurs during the process of mRNA export from the nucleus to the cytoplasm (for recent reviews, see Dimaano and Ullman 2004; Vinciguerra and Stutz 2004; Fasken and Corbett 2005; Saguez et al. 2005). Gatekeeping works in at least two ways. First, it restricts the export of mRNAs that are incompletely matured. Second, it promotes the export of mRNAs that are associated with the appropriate proteins. Another type of quality control prior to translation appears to target mRNAs that require localization in the cytoplasm for proper function (Kloc and Etkin 2005; St Johnston 2005; Parker and Sheth 2007). mRNA localization provides an important means for the spatial regulation of genes during processes such as protein secretion, synaptic plasticity, cell motility, and embryonic axis formation. mRNAs that are not properly localized are subject to translational repression, if not degradation. In mammals, whether or not translational repression occurs before or after the translation of CBP80:CBP20-bound mRNA depends on the mechanism of repression, which in some instances targets eIF4E-bound mRNA (e.g., see Lasko et al. 2005) and in other instances must target both CBP80:CBP20-bound and eIF4E-bound mRNA (e.g., see Lloyd 2006).

All mRNA surveillance mechanisms, whether they depend on translation or not, require the stepwise assembly of RNA–protein complexes, in which some proteins bind to chromatin-associated transcripts or later-stage forms of pre-mRNA in the nucleus, and others bind to mRNA in either the nucleus or the cytoplasm. Future studies of RNP structure, which is constantly rearranging according to the needs of the cell, will surely lend additional insight into how RNAs are subject to quality control and appropriately regulated throughout their lifetimes.

Acknowledgments

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Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function

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