Nonsense-mediated mRNA decay: molecular insights and mechanistic variations across species

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Nonsense-mediated mRNA decay (NMD) is an mRNA surveillance pathway that ensures the rapid degradation of mRNAs containing premature translation termination codons (PTCs), thereby preventing the synthesis of truncated and potentially harmful proteins. In addition, this pathway regulates the expression of ~10% of the transcriptome and is essential in mice. Although NMD is conserved in eukaryotes, recent studies in several organisms have revealed that different mechanisms have evolved to discriminate natural from premature stop codons and to degrade the targeted mRNAs. With the elucidation of the first crystal structures of components of the NMD machinery, the way is paved towards a molecular understanding of the protein interaction network underlying this process.

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Introduction

Nonsense-mediated mRNA decay (NMD) is an evolutionarily conserved mRNA surveillance pathway that detects and eliminates mRNAs harboring premature translation termination codons (PTCs) in eukaryotes [1,2] (see review by Lejeune and Maquat in this issue). Recently, it has become clear that the NMD pathway not only degrades aberrant mRNAs containing PTCs as a result of mutations or errors during transcription or RNA processing, but is also implicated in regulating the expression of wild-type transcripts [3] (see review by Lejeune and Maquat in this issue). Indeed, gene expression profiling of yeast, *Drosophila* or human cells defective in NMD has revealed that NMD regulates the expression of ~10–20% of the transcriptome [4•,5••,6].

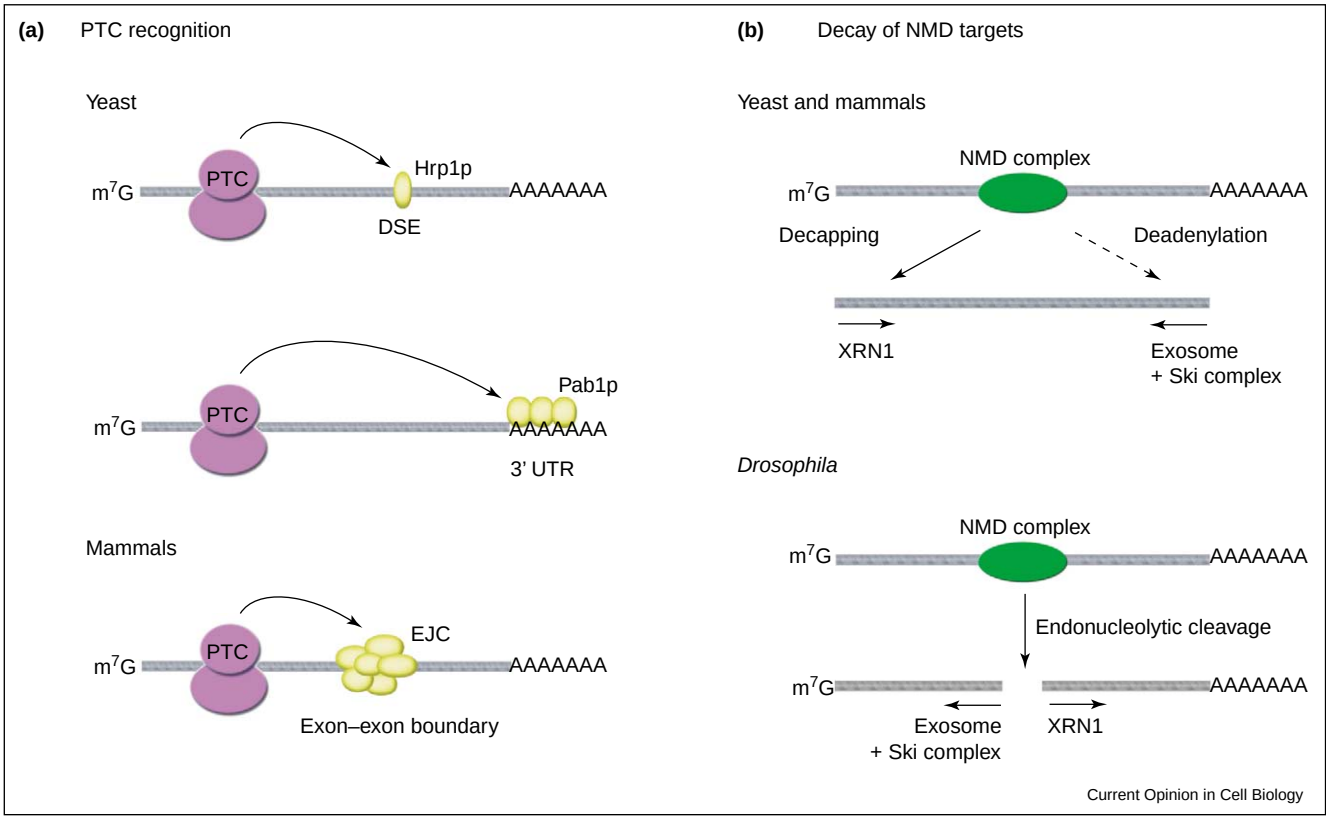
Two critical steps in the NMD pathway have attracted much attention in recent years: the mechanism by which premature stop codons are recognized and discriminated from natural stops (PTC definition), and the mechanism by which PTC-containing mRNAs are targeted for fast degradation. PTC definition is a translation-dependent step involving cross-talk between the ribosome stalled at a stop codon and a downstream *cis*-acting signal on the mRNA. This cross-talk leads to the recruitment of *trans*-acting NMD factors, the assembly of the surveillance complex and ultimately the degradation of the mRNA. Despite conservation of the NMD pathway, the nature of the cis-acting signals and the decay pathway of targeted mRNAs vary across species (Figure 1, see below). In this review, we discuss these mechanistic variations and the molecular insights to which recent structural studies have contributed by visualizing some of the interactions that lead to PTC definition and decay of targeted transcripts.

The conserved core of the surveillance complex consists of UPF1, UPF2 and UPF3

The key players in the NMD pathway were initially identified in genetic screens in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* [7–10]. These screens led to the identification of three yeast (*UPF1–3*) and seven *C. elegans* (*smg-1–7*) genes that play an essential role in NMD. The UPF1, UPF2 and UPF3 proteins (known as SMG-2, SMG-3 and SMG-4 in *C. elegans*) are core components of the surveillance complex whose basic function is conserved in eukaryotes [7–13]. Deletion or silencing of genes encoding UPF1 and UPF2 results in the stabilization of PTC-containing mRNAs in all organisms in which NMD has been investigated [7–15]. UPF3 is also essential for NMD in *S. cerevisiae*, *C. elegans* and *Drosophila*; its role in human cells has been more difficult to assess as two UPF3 paralogs are expressed (UPF3a and UPF3b; also known as UPF3 and UPF3X) [2,10,12–14] (see review by Lejeune and Maquat in this issue).

UPF3 is a predominantly nuclear protein and in human cells associates with spliced mRNAs through interactions with components of the exon-junction complex (EJC), a multimeric protein complex deposited by the spliceosome ~20–24 nucleotides upstream of exon–exon junctions [14,16,17] (see review by Lejeune and Maquat in this issue). UPF2 is perinuclear and might associate with exported mRNAs via interactions with UPF3 and the EJC [14,16]. The RNA helicase UPF1 is a key component of the NMD pathway. It has a predominantly cytoplasmic localization and associates with translation release factors (i.e. eRF1 and eRF3) and with UPF2 and UPF3, providing a link between the surveillance complex and the translation machinery [8,10,11,14,15,18].

Figure 1



Mechanisms of premature translation termination codon (PTC) definition and decay of PTC-containing mRNAs across species. **(a)** Recognition of a PTC results from the cross-talk between the terminating ribosome and a downstream *cis*-acting signal that varies across species [1,2] (see also review by Lejeune and Maquat in this issue). In yeast, the *cis*-acting signal could be a downstream sequence element (DSE) or the 3′ UTR. In mammals, the *cis*-acting signal is an exon–exon boundary. The position of a *cis*-acting signal is communicated to translating ribosomes by the proteins that bind to these signals, such as the DSE-binding protein Hrp1p [48], the poly(A)-binding protein (Pab1p) [50••] or components of the EJC [16,17,32,33•,34•,35–40]. The mechanism by which PTCs are recognized in *Drosophila* remains to be established, but it does not depend on exon–exon boundaries. **(b)** Decay of NMD targets in yeast is initiated by deadenylation-independent decapping followed by 5′-to-3′ exonucleolytic digestion by Xrn1p. An alternative pathway involves accelerated deadenylation and exosome-mediated 3′-to-5′ decay [53•,54•]. Available evidence indicates that this mechanism is conserved in human cells, although the relative contribution of each pathway has not been evaluated [55–57]. In *Drosophila*, decay of NMD targets is initiated by endonucleolytic cleavage in the proximity of the PTC [58•]. The resulting mRNA fragments are degraded from the newly generated ends by the exosome and XRN1, respectively. This decay pathway is independent of deadenylation and decapping. In all species, exosome-mediated decay requires the Ski complex.

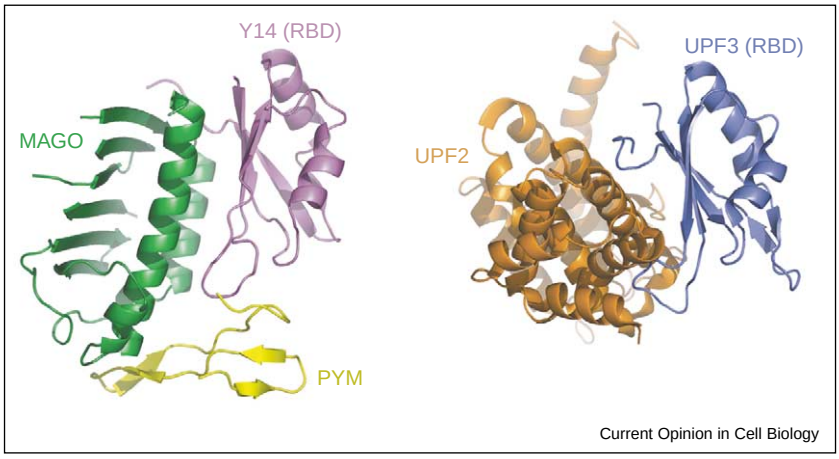
UPF2 interacts directly with UPF3. The interaction between human UPF2 and UPF3b has been recently visualized at the atomic level [19•]. UPF2 consists of three MIF4G (middle portion of eIF4G) domains, whereas UPF3 is characterized by a canonical RNP-type RNA-binding domain (RBD) [19•]. The structure of the complex between the interacting domains of human UPF2 and UPF3b shows an unusual mode of protein–protein interaction involving the third MIF4G domain of UPF2 and the β-sheet surface of the UPF3 RBD (Figure 2) [19•]. This β-sheet surface is normally used by RBD domains to bind nucleic acids. Consistent with this, the RNA-binding activity of the UPF2–UPF3 complex does not reside within UPF3, but within a conserved patch of residues on the surface of UPF2 [19•]. The conservation of the interactions and surfaces implies that the UPF2–UPF3 complex has similar features in other species.

UPF2 also interacts with UPF1. The UPF1-binding site has been mapped to the N- and C-terminal regions of UPF2 [19•,20]. In the UPF2–UPF3 complex, these regions are likely to be accessible for the interaction with UPF1, allowing the assembly of the trimeric core of the surveillance complex. The molecular details of these interactions await further structural characterization.

Phosphorylation/dephosphorylation cycles of UPF1 in multicellular organisms

In metazoans, UPF1 has N- and C-terminal extensions with multiple serine residues that are targets for phosphorylation [11,21]. Regulation of the phosphorylation state of

Figure 2



A novel mode of protein–protein recognition in nonsense-mediated decay (NMD). Crystal structures of the complex between the interacting domains of UPF3 and UPF2 dimers [19•], and of the trimeric Y14–MAGO–PYM complex [33•]. UPF3 and Y14 have a canonical RNP-type RBD found in many proteins involved in RNA metabolism. In both complexes, a novel mode of RBD-protein interaction is observed in which the RBDs engage their β-sheet surface to bind a protein partner instead of binding RNA.

UPF1 involves four additional proteins (SMG1,5,6,7) that were identified as essential NMD factors in *C. elegans* [21–26,27•,28•,29]. With the exception of SMG7, these proteins are conserved in metazoa [13,29].

Phosphorylation of UPF1 is catalyzed by SMG1, a phosphoinositide-3-kinase-related protein kinase [11,21,24–26]. The dephosphorylation of UPF1 is mediated by SMG5, SMG6 and SMG7, three similar but non-redundant proteins [11,23,27•,28•,29]. SMG5, SMG6 and SMG7 are not phosphatases themselves, but are thought to trigger UPF1 dephosphorylation by recruiting protein phosphatase 2A (PP2A). This model arises from the observation that SMG5 and SMG7 interact with each other and are part of a larger complex comprising phosphatase PP2A and phosphorylated UPF1 [27•,28•]. Similarly, SMG6 has been shown to be part of a protein complex comprising PP2A and phosphorylated UPF1 [29], although it is unclear whether this complex also contains SMG5 and SMG7.

Recently, it has been shown that the structure of the N-terminal domain of SMG7 resembles that of 14-3-3, a phosphoserine-binding protein involved in signal transduction pathways (Figure 3) [30••]. The similarity between SMG7 and 14-3-3 is not limited to the fold of the polypeptide backbone, but residues that line the phosphoserine binding pocket of 14-3-3 are conserved at the corresponding pocket of SMG7, suggesting that SMG7 functions as a phosphoserine-binding protein. Indeed, SMG7 binds UPF1 in a phosphorylation-dependent manner and mutation of residues in its 14-3-3-like phosphoserine-binding site impairs UPF1 binding [30••]. These studies suggest a molecular mechanism by which SMG7 specifically interacts with the phosphorylated form of UPF1. The relevance of this observation in the context of NMD is discussed below.

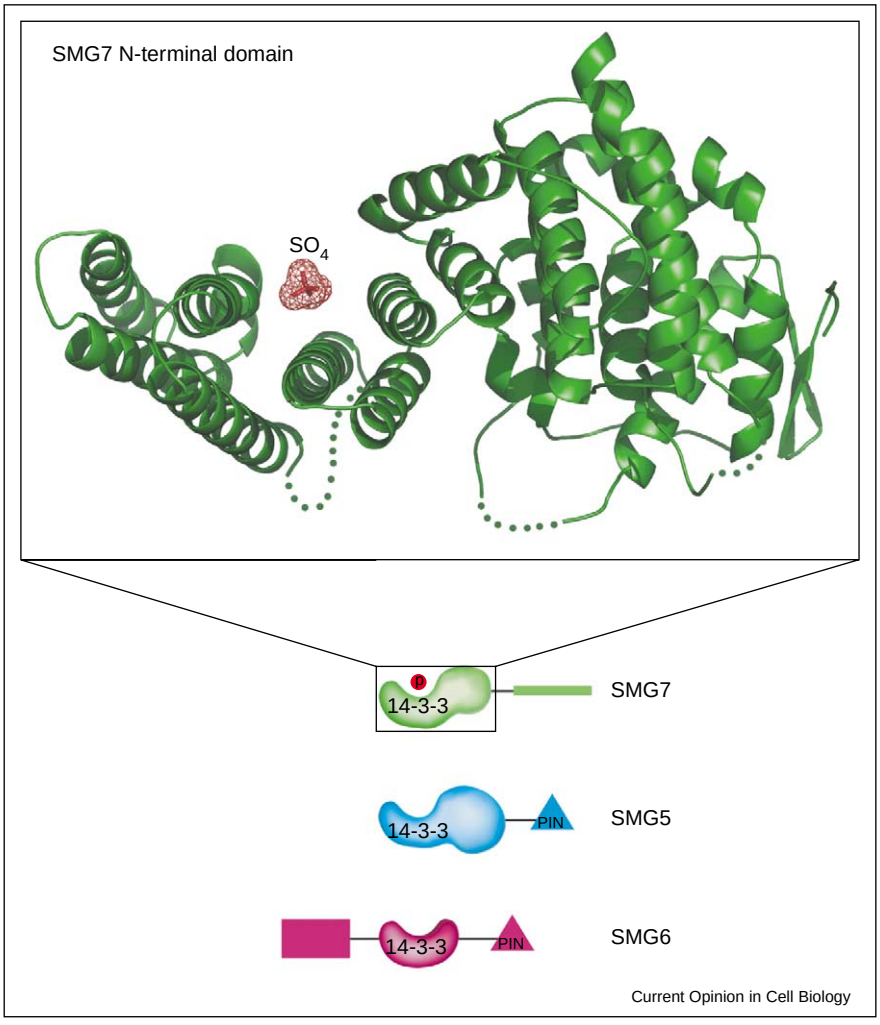
The 14-3-3-like domain of SMG7 comprises several TPR repeats that had been previously identified on the basis of sequence alignment as the hallmark for SMG5, SMG6 and SMG7 [13,23,27•,28•,29,30••,31]. The high level of conservation suggests that SMG5 and SMG6 also contain a 14-3-3-like domain with a conserved binding site for phosphoserine residues. SMG5 and SMG6 also share an additional similar C-terminal domain, which in both cases is predicted to structurally resemble a PIN domain [31]. Although PIN domains are often present in proteins with nuclease activity [31], the role of this domain in the context of the SMG5 and SMG6 proteins remains rather enigmatic.

PTC definition in mammals: the exon junction complex

In mammals, recognition of premature stop codons results from the cross-talk between terminating ribosomes and a downstream EJC comprising UPF3 and UPF2 [1,2] (see review by Lejeune and Maquat in this issue). According to the current model, if translating ribosomes encounter a stop codon upstream of an EJC, UPF1 is recruited by translation release factors and interacts with the UPF2 and UPF3 proteins bound to the downstream EJC. This event would create an opportunity for the assembly of an active surveillance complex consisting of UPF1, UPF2 and UPF3 and possibly other proteins [1,2] (see review by Lejeune and Maquat in this issue).

In addition to UPF3, other components of the EJC have been implicated in PTC definition. These include

Figure 3



SMG5–7 have a 14-3-3-like phosphoserine-binding site. The crystal structure of the N-terminal domain of human SMG7 shows an almost completely α-helical molecule with an unexpected structural homology to 14-3-3, a signal transduction protein that binds phosphoserine-containing polypeptides [30••]. In red, a sulfate ion from the crystallization medium is shown. Coordination of the sulfate ion occurs at the equivalent structural position of phosphate groups in 14-3-3. Below is a schematic drawing of SMG7, SMG5 and SMG6, with their 14-3-3-like domains and PIN domains highlighted [30••,31]. The unique SMG7 C-terminal domain and SMG6 N-terminal domain are represented as rectangles.

RNPS1, Y14 and MAGO, all of which have been shown to elicit mRNA decay when bound downstream of a stop codon [32,33•,34•]. Y14 and MAGO form a heterodimeric complex that acts as a single functional unit in NMD [33•]. The structure of the Y14–MAGO heterodimer has shown that the interaction with MAGO is mediated by the RNA-binding domain (RBD) of Y14, with an overall mode of protein–protein recognition similar to that described above for the UPF2–UPF3 complex (Figure 2) [33•]. Analogous to the UPF3 RBD, the RBD of Y14 does not bind RNA *in vitro* [33•]. Assembly of the Y14–MAGO binary complex leads to the formation of an extensive surface that is lined by conserved residues belonging to both components of the heterodimer. This surface functions as a platform for additional protein–protein interactions [33•].

The search for Y14–MAGO interaction partners has led to the identification of the proteins PYM (partner of Y14 and MAGO), eIF4AIII and Barentsz (Btz, also known as MLN51) as additional components of the NMD machinery [35–40]. In human cells, eIF4AIII is a component of the EJC and, similarly to Y14-MAGO, associates with spliced mRNAs in the nucleus [36–38]. By contrast, both Barentsz and PYM are shuttling proteins with a predominantly cytoplasmic localization [35,39,40], possibly joining the complex only after nuclear export.

PYM interacts directly with Y14–MAGO by contacting both components of the heterodimer (Figure 2) [35]. From the structure of the ternary PYM–Y14–MAGO complex, it is clear that PYM binds to a relatively small portion of the Y14–MAGO surface (Figure 2), suggesting that additional binding partners such as Barentsz and eIF4AIII might be able to access the heterodimer simultaneously. The precise mechanisms by which PYM, eIF4AIII and Barentsz participate in NMD remain to be established.

Interestingly, despite conservation of their molecular interactions, the *Drosophila* orthologs of Barentsz, eIF4AIII, Y14 and MAGO are dispensable for NMD [13,39,41]. Instead, these proteins are required for the proper localization of *oskar* mRNA to the posterior pole of the *Drosophila* embryo [42–46]. Thus, these proteins and the additional components of the EJC might have been co-opted by the NMD machinery during evolution. Some components of the EJC may have acquired a role similar to that of UPF2 or UPF3, providing an explanation for the observation that the requirement for UPF2–UPF3 interaction is bypassed under some experimental conditions in human cells [34•].

PTC definition in yeast and *Drosophila* occurs independently of exon boundaries

As mentioned above, although several components of the human EJC are conserved in *Drosophila* and are involved in post-transcriptional mRNA metabolism, they do not play a role in PTC definition or NMD [13]. Indeed, in both *Drosophila* and *S. cerevisiae*, PTC definition occurs independently of exon–exon boundaries [1,2,13] (see review by Lejeune and Maquat in this issue). This is consistent with the observation that PTC-containing mRNAs transcribed from intronless genes are subjected to NMD in yeast and *Drosophila* [1,2,13]. What differentiates a premature termination codon from a natural one in these organisms? In yeast, some mRNAs have been shown to harbor loosely defined downstream sequence elements (DSEs) with a function analogous to that of mammalian exon–exon junctions (Figure 1) [1,2,47] (see review by Lejeune and Maquat in this issue). The protein Hrp1p has been shown to bind this element [48]. However, the lack of a strong consensus among yeast DSEs suggests that multiple *cis*-acting sequence elements and *trans*-acting binding factors may exist.

Alternatively, it is possible that a generic feature of the mRNA, such as the poly(A)-tail or a mark deposited during the cleavage and polyadenylation reaction, provides the positional information needed to discriminate premature from natural stop codons in yeast and *Drosophila*. This possibility is consistent with the proposed model that the process of premature translation termination is intrinsically aberrant, because the stop codon is not in the appropriate context [49,50••]. According to this model, 3′ untranslated regions (UTRs) would be marked by a specific set of proteins. If a terminating ribosome is able to interact with these 3′ UTR-bound proteins, proper termination can occur. If the termination process is impaired or too slow, as a result of the inability of the terminating ribosome to establish these interactions, the NMD complex may be assembled, leading to the rapid degradation of the mRNA.

This model has recently received strong support in yeast [50••]. It was shown that translation termination is aberrant at premature stop codons and that prematurely terminating ribosomes fail to release efficiently. This effect is abolished in strains lacking Upf1p or if the nonsense codon is flanked with a normal 3′ UTR. Moreover, tethering the poly(A)-binding protein (Pab1p) downstream of the PTC, which mimics a normal 3′UTR, leads to efficient translation termination and abolishes NMD [50••]. It would be of interest to determine whether this model also accounts for PTC recognition in *Drosophila* and mammals. In the latter case, the presence of EJC proteins bound downstream of a stop codon may interfere with proper translation termination.

Degradation of PTC-containing mRNAs: XRN1, the Ski complex and the exosome

Independent of the mechanism by which PTCs are defined, once the mRNA is recognized as being aberrant, its degradation is mediated by the enzymes that are involved in general mRNA decay. In eukaryotic cells, general mRNA degradation is initiated by shortening of the poly(A)-tail by deadenylases [51]. Following this first rate-limiting step, mRNAs can be degraded via one of two pathways. In one pathway, deadenylation triggers decapping, and this exposes the mRNA body for digestion by the major cytoplasmic 5′-to-3′ exonuclease XRN1. Decay of mRNAs through this pathway is thought to occur in specialized cytoplasmic bodies or mRNA decay foci (also known as P-bodies or GW-bodies) that are enriched in XRN1, the decapping enzymes DCP1 and DCP2 and decapping co-activators such as the LSm1–7 complex (see review by Fillman and Lykke-Anderson in this issue). In the second mRNA decay pathway, deadenylation is followed by 3′-to-5′ degradation of the transcript. This requires the exosome (a multimeric assembly of 3′-to-5′ exonucleases) and the Ski complex, a trimeric protein complex that regulates exosome activity [51].

The enzymes and co-activators involved in general mRNA decay also function in NMD. In *S. cerevisiae*, the major decay pathway for NMD substrates involves removal of the cap structure by the decapping enzymes Dcp1p/Dcp2p and decapping co-activators such as the LSm1–7 complex. Following decapping, the body of the transcript is exposed to 5′-to-3′ degradation by Xrn1p (Figure 1) [52,53•,54•]. Thus, one function of the

surveillance complex is to bypass deadenylation, the rate-limiting step in mRNA decay, and to directly promote decapping. An alternative pathway, which also contributes to the decay of PTC-containing mRNAs, relies on the accelerated deadenylation and 3′-to-5′ degradation by the exosome and the Ski complex (Figure 1) [53•,54•]. Decay of NMD substrates in mammals has been shown to occur by a similar exonucleolytic mechanism [55–57].

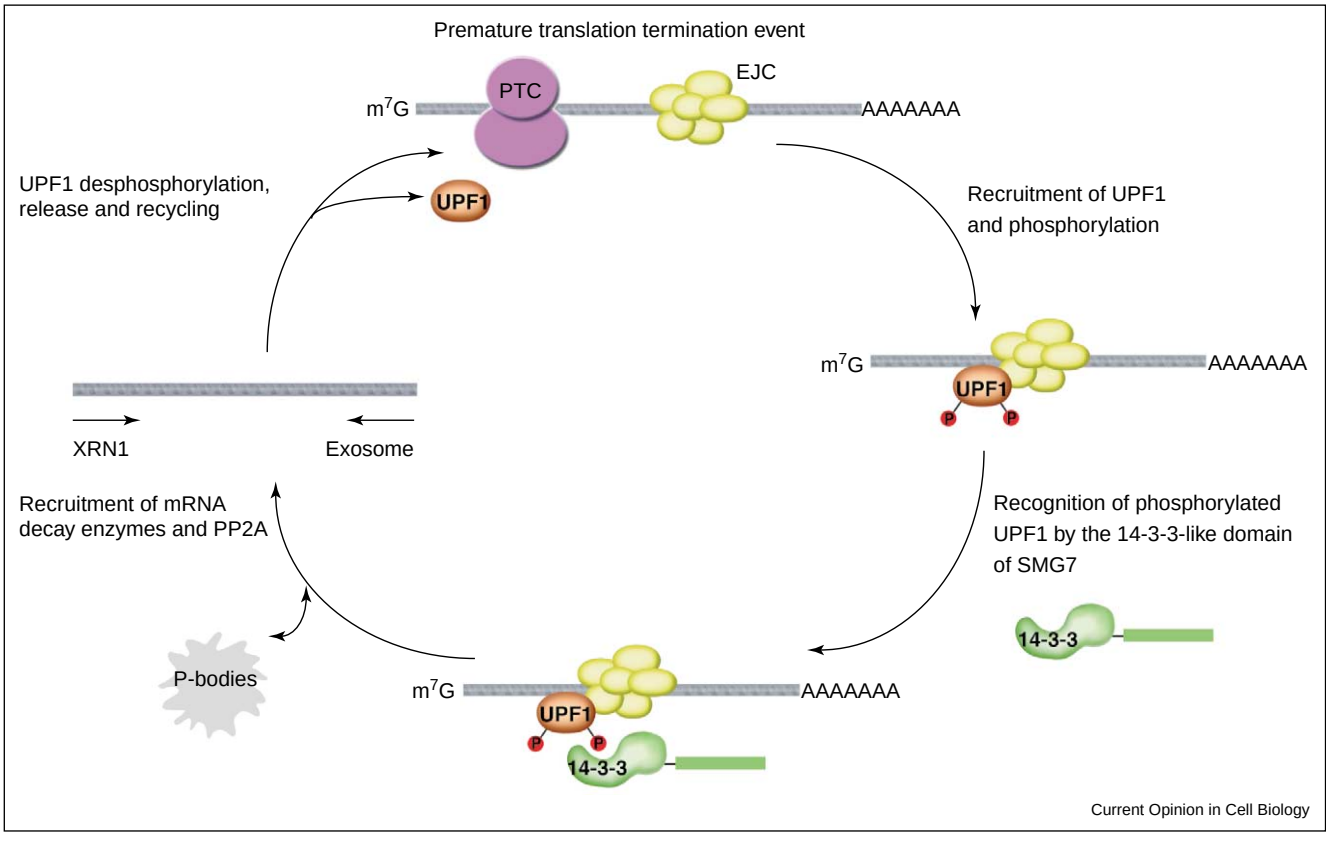
Unexpectedly, degradation of nonsense transcripts in *Drosophila* was found to be initiated by endonucleolytic cleavage in the vicinity of the PTC (Figure 1). The resulting 5′ fragment is rapidly degraded from its 3′-end by the exosome in a process that also requires components of the Ski complex. The 3′ fragment is degraded from its free 5′ end by XRN1 [58•]. Thus, the mRNA fragments are degraded from the newly generated ends without undergoing decapping or deadenylation, suggesting that, in contrast to yeast and mammals, the decapping enzymes, the LSm1–7 complex and deadenylases are not required for NMD in *Drosophila* [58•].

From PTC recognition to mRNA degradation in metazoa

What is the molecular mechanism that leads from the recognition of a PTC and assembly of the surveillance complex to the recruitment of mRNA decay enzymes? A hint on how the decay enzymes are recruited to NMD targets in mammals comes from studies on the cellular localization and function of the SMG5–7 proteins [59]. When overexpressed, SMG7 accumulates in cytoplasmic foci corresponding to endogenous P-bodies. Overexpression of SMG7 also causes the accumulation of SMG5 or UPF1 in P-bodies, and this requires both the N-terminal and C-terminal domains of SMG7.

Consistent with its localization in P-bodies, full-length SMG7 is able to elicit mRNA decay when tethered to a reporter transcript. This activity resides within the C-terminal domain of SMG7. The 14-3-3-like domain of SMG7, by contrast, is not required to elicit mRNA decay when SMG7 is artificially tethered to the transcript [59]. These observations indicate that SMG7 has two functional domains: an N-terminal domain with a 14-3-3-like

Figure 4



Model for the role of human SMG7 in NMD. Phosphorylation of UPF1 as a result of a premature translation termination event leads to the recruitment of SMG7 via a specific interaction with its 14-3-3-like domain. SMG7 then targets the bound mRNA for decay via its C-terminal domain. Decay may occur in the cytoplasm or in P-bodies. SMG7 also recruits PP2A, resulting in UPF1 dephosphorylation and dissociation from the 14-3-3-like binding site. This enables the recycling of these proteins for a new round of NMD [27•,28•,30••,59].

fold that interacts with phosphorylated UPF1 (and has also been shown to interact with SMG5), and a C-terminal domain that targets bound mRNAs for decay [27•,28•,59]. Thus, the modular domain organization of SMG7 provides a molecular link between the NMD and the mRNA degradation machineries.

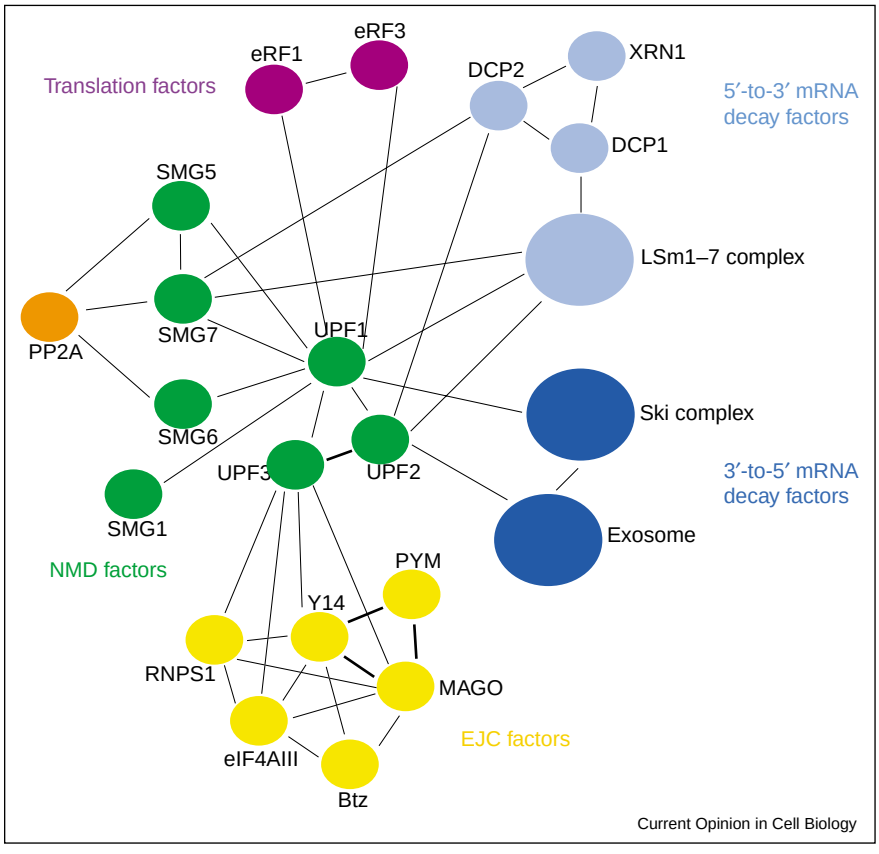
The combination of the structural and functional studies described above suggests a model for how changes in the phosphorylation state of UPF1 are coupled to the degradation of PTC-containing transcripts (Figure 4). In this model, recognition of a PTC leads to the assembly of the surveillance complex on the aberrant mRNA and the phosphorylation of UPF1. Phosphorylated UPF1 recruits SMG7 (most likely in a complex with SMG5 and PP2A) [27•,28•,30••]. SMG7 then targets the PTC-containing transcript for decay [59]. The association of SMG7, SMG5 and PP2A would also trigger the dephosphorylation of UPF1, and this event might be involved in recycling of the NMD factors for another round of targeting. It is currently unclear whether decay of targeted transcripts, UPF1-dephosphorylation and dissociation of the surveillance complex from the mRNA occurs in the cytoplasm or whether the entire surveillance complex escorts the mRNA to P-bodies, where it undergoes decapping and rapid 5′-to-3′ decay.

Although this model is consistent with available data in human cells, it represents an oversimplification of the NMD pathway, as there are many observations that remain unexplained. For instance, what is the role of SMG6 in this pathway? Are P-bodies sites of decay for NMD substrates? What are the mechanisms connecting the surveillance complex to the mRNA decay enzymes in yeast cells, which lack SMG1 and SMG5–7 orthologs? How are the decay enzymes recruited to NMD substrates in *Drosophila* cells, which appear to lack a SMG7 ortholog? What is the identity of the *Drosophila* endonuclease and how is it recruited to nonsense transcripts?

Conclusions and perspectives

NMD factors have been identified by genetic screens in *S. cerevisiae* or *C. elegans* and more recently by biochemical approaches in human cells. Although there are certainly

Figure 5



Protein interaction network involved in NMD in mammals. Only the proteins for which experimental evidence for a role in NMD is available are shown. Large nodes represent multimeric protein complexes (i.e. the exosome, the Ski complex and the LSm1-7 complex). Note that links do not necessarily represent direct interactions. Bold lines indicate that structural details of these interactions are known. The EJC proteins are linked to this network in mammals, but not in *Drosophila*.

more factors to be discovered, the complexity of the protein interaction network involved in NMD is already emerging (Figure 5). Moreover, investigations of the NMD pathway across species suggest that the complexity of the network increases from simpler organisms such as *S. cerevisiae* to humans by the introduction of additional nodes and links. To dissect the molecular interactions underlying this network and to decipher how it is connected to other cellular processes and how it evolved remains a challenge.

Gene expression profiles in cells deficient for NMD have demonstrated that this pathway regulates the expression of various classes of wild-type transcripts. The identification of endogenous targets provides relevant information on the cellular processes that are likely to be regulated by NMD. An important goal for the future is to understand how the regulation of these endogenous targets leads to the complex phenotypes observed at the cellular level. For example, *Drosophila* cells depleted of UPF1 arrest at the G_2/M-phase of the cell cycle (Rehwinkel and Izaurralde, unpublished). How does the NMD pathway impinge on the cell cycle? The ultimate challenge will be to apply the insights gained at the molecular and cellular levels to the organism level to understand, for instance, why UPF1 is essential in mice, but not in *S. cerevisiae or C. elegans* [60].

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- •• of outstanding interest

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