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Translation drives mRNA quality control

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Abstract

There are three predominant forms of co-translational mRNA surveillance: nonsense-mediated decay (NMD), no-go decay (NGD) and non-stop decay (NSD). While discussion of these pathways often focuses on mRNA fate, there is growing consensus that there are other important outcomes of these processes that must be simultaneously considered. Here, we seek to highlight similarities between NMD, NGD and NSD and their likely origins on the ribosome during translation.

In eukaryotes, grossly aberrant mRNAs, such as those lacking a 5′ cap or 3′ poly(A) tail, are unlikely to effectively engage in the translational cycle. However, mRNAs containing more subtle errors cannot be as easily discriminated against. Some of these mRNAs, if translated, can produce aberrant protein products that are detrimental to the cell. To minimize these errors, cells have evolved mechanisms to monitor mRNAs as they are translated and to degrade troublesome transcripts – these processes are broadly referred to as “mRNA surveillance”. Most of the recognition events occur on the ribosome, thus directly implicating translation in these processes. It follows as no surprise that increasing evidence shows that the effects of these surveillance pathways are not restricted to the mRNA, but rather have broad consequences for the translational output of a cell. Studies on mRNA surveillance have traditionally focused on mRNA fate and many excellent reviews cover this area of interest (e.g. ref. [1,2]). In this review, we focus on exploring mRNA surveillance from the perspective of its origins on the ribosome. We hope that this approach provides a new perspective from which to consider mRNA surveillance and will lead to new and unanticipated insights that inform future experiments.

mRNA surveillance: what defines a substrate?

There are three classically identified mRNA surveillance pathways in eukaryotes: nonsense-mediated decay (NMD), non-stop decay (NSD), and no-go decay (NGD). Historically, the hallmark activity of each process is the selective degradation of a class of aberrant mRNAs: NMD specifically targets mRNAs containing a premature termination codon (PTC), NSD targets mRNAs lacking a termination codon and NGD targets mRNAs containing a range of potential stall-inducing sequences. In this section, we discuss in more specific terms our current understanding of the molecular features that define these three classes of targeted mRNA.

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NMD

All stop codons must initially be recognized by the canonical translation termination factors eRF1 and eRF3 (Fig. 1a). What then distinguishes a "premature" stop codon from an authentic one? In higher eukaryotes, premature termination codons are generally thought to be recognized by their proximity to protein complexes (called exon-junction complexes, or EJCs) deposited near exon junctions during pre-mRNA splicing in the nucleus[3,4]. As authentic stop codons are typically located in the 3′ exon of spliced mRNAs, the presence of an EJC downstream of a stop codon immediately marks an mRNA as suspect (Fig 1b). Given that translating ribosomes likely displace such bound protein complexes, EJCs effectively define mRNA status during an initial, or "pioneer," round of translation[5]. We note however that NMD does not strictly depend on the presence of an EJC even in higher eukaryotes[6].

Broad applicability of this model is further compromised by the fact that there are few introns in some organisms, including the model yeast *S. cerevisiae*, and yet NMD in these organisms is robust. In these organisms, and perhaps elsewhere, NMD is proposed to be induced by recognition of a stop codon upstream of an extended 3′ untranslated region (UTR)[7–9] (Fig 1b). Whether this feature defines a PTC because of the increased binding of Upf1 to the extended 3′ UTR[8] or because of increased separation of positive termination effectors, such as poly(A)-binding protein (PABP), from the site of termination[6] is not clear. And, while the presence of a poly(A) tail and PABP are non-essential for recognition of NMD substrates in yeast[10], such studies still leave room for models where Upf1 is a positive effector of NMD that competes with PABP as a positive effector of normal termination[11]. Regardless of the trigger, a unifying theme is that RNA elements downstream of the PTC must interface with the translational apparatus to define stop codons as authentic or premature.

NGD and NSD: are all stalls equal?

NGD, as its name suggests, is a blanket term for a process that targets mRNAs with sequence features that cause translating ribosomes to "not go" or stall (typically at sense codons). The most effective NGD-targeting sequences to be studied are those induced by inhibitory mRNA structures such as stable stem-loops, pseudoknots, GC-rich sequences or damaged RNA bases[12,13] (Fig. 2a, top). It is suspected that more subtle perturbations in the mRNA, such as strings of certain codons[14] or certain peptide sequences[15], may also stimulate an NGD response (Fig 2b). Evidence suggests that, at least in the case of peptide-mediated arrest, these stalls are dependent on the conserved ribosomal protein RACK1[15], though a complete analysis of the role of RACK1 in NGD has yet to be presented. As we will discuss below, such mRNA stalling features typically result in endonucleolytic cleavage of the mRNA, which in turn likely identifies these ribosomes complexes as prime targets for surveillance.

As a technical note, while the term "stalling" is broadly used in the literature, kinetically distinguishing between transient pauses and stable stalls is difficult with currently available techniques (e.g. toeprinting and ribosomal profiling). So, for example, while these techniques identify high ribosome density at certain proline-rich sequence motifs[16,17], the

duration of such a pause in the cell remains to be determined. To maintain consistency with the literature, translational pauses that are sufficient to induce NGD will be referred to as stalls throughout this review. Implicit in this characterization, however, is the understanding that in most cases the kinetic features of these 'stalling' determinants remain to be thoroughly evaluated.

NSD was similarly discovered as a mechanism to resolve ribosome complexes stalled on defective mRNA. As the name implies, nonstop decay is broadly interpreted as a process for eliminating mRNAs that lack a stop codon[18,19]. mRNAs not carrying an in-frame stop signal can be of two types: the first class includes truncated mRNAs (Fig 2a, bottom) where the ribosome simply runs to the end of the template; the second class includes mRNAs lacking a stop codon but with a poly(A) tail (Fig 2c). In the latter case, it was initially assumed that ribosomes would translate through the poly(A) tail, reach the 3′ end of the mRNA and stall at the end of the template. If this were true, NGD and NSD substrates would differ based on whether the inducing stall occurs mid-message (NGD) or at the end of the message (NSD).

Recently, however, these distinctions between NGD and NSD have become blurred. It is now thought that translation of poly(A) sequences into poly-lysine can cause ribosome stalling through interactions between the positively charged peptide and the overwhelmingly negatively charged exit channel of the ribosome[20,21]. The potency of these stalls increases with length, but can be observed after incorporating as few as 6 lysine residues (corresponding to the translation of as few as 18 adenosine nucleotides)[20]. Since the typical length of a poly(A) tail is ~70 nucleotides in yeast and ~200 in human cells[22], a ribosome that translates into the poly(A) region likely will stall long before reaching the 3′ end of the mRNA. Therefore, poly(A) read-through, which was previously referred to as an end-of-message stall, or NSD, may also involve peptide-mediated internal stalling, reminiscent of NGD. Regardless of how they are classified, what ultimately appears to unify all NGD and NSD substrates is the formation, following endonucleolytic cleavage, of a secondary stall formed by the upstream translating ribosome reaching the cleavage site (Fig 2d). This secondary stall is a clear target for Dom34:Hbs1 or, in yeast, Ski7.

Independent of the cause, all stalls require similar resolution of the ribosome complex. In the end, 'unnatural' stalls and stochastic translational pausing must be distinguished from one another. While the mechanism of this discrimination is unknown, it seems likely that the kinetics of these events play a critical role such that the surveillance machinery efficiently recognizes only sufficiently long-lasting stalls. Such models have been well supported in other systems; for example, in protein quality control, specifically during protein folding, cells rely on the length of time a misfolded species exists to distinguish between transient folding intermediates and terminally misfolded protein products[23].

Ribosome recognition by key mRNA surveillance factors

If a stalled ribosome complex is a substrate for surveillance, what are the specialized cellular factors that recognize these ribosome complexes and target them for resolution?

NMD

Some of the key factors involved in NMD – the UPF (for 'UPstream Frameshifting') genes *Upf1*, *Upf2* and *Upf3* - were identified in early genetic screens in yeast[24–27]. Each of the three factors is highly conserved in eukaryotes and implicated in NMD in a broad range of organisms[28]. Upf1 is an enzyme containing both ATPase and helicase activities[29]; inhibition of either of these activities impedes NMD[30]. Upf1 interacts with both eRF1 and eRF3 and is likely present during initial recognition of a premature stop codon[31,32] (Fig 1b). Upf1 also interacts directly with Upf2 and Upf3[33]. Upf2 and Upf3 modulate Upf1 activity and are thought to function as protein scaffolds[34–36]; any direct catalytic function for Upf2 and Upf3 is unknown. Further studies in higher eukaryotes have implicated numerous other critical and conserved factors involved in NMD[37]. While several of these factors will be discussed in this perspective, in particular those that directly engage the translational machinery, for a more extensive review of the role of these other factors in NMD we direct the reader to ref. [28].

While some is known about the core Upf factors and what they can do in isolation, less is known about how these factors help to identify premature stop codons or begin to specify the downstream events of NMD. As described in the previous section, during NMD, something about the mRNA sequence downstream of a stop codon informs the ribosome termination complex that termination is occurring too early. The Upfs are proposed, for example, to bridge the interaction between the ribosome and this downstream mRNA signal[35,38]. In the EJC model, this signal includes the EJC proteins, MAGOH, Y14, and eIF4AIII, which directly interact with the Upfs and, in turn, the terminating ribosome (Fig. 1b)[35]. Another model (the 3′ UTR model) suggests that Upf1 directly interacts with (and even coats) the 3′ UTR of an mRNA; as such, a PTC will be associated with a longer 3′ UTR sequence than an authentic termination codon, and thus with a larger target for Upf1 binding[8]. An additional consequence of a long 3′ UTR is that the poly(A) tail is less proximal to the stop codon. Some studies have argued that proximity to the poly(A) tail lowers the likelihood of a stop codon being recognized as premature[6] while other studies argue that the polyA tail plays little if any role in NMD[10]. Finally, still other models argue that Upf1 is directly associated with the small ribosomal subunit, evaluating encounters with termination codons as they appear in the decoding center[39].

In all of the models, Upf1 is involved as a critical effector molecule, though little is known about the actual mechanism of PTC recognition or the effects of Upf recognition on downstream ribosome function. It will be important to make these connections in moving forward.

NGD and NSD

Two protein factors, Dom34 (or Pelota) and Hbs1, were originally implicated in NGD through genetic approaches showing that the decay of mRNAs on which ribosomes stalled depended on their presence[12]. Strikingly, Dom34 and Hbs1 are structurally related to the canonical termination factors eRF1 and eRF3[40–45], immediately suggesting that NGD, like NMD, involves a modified termination event. Indeed, Dom34 and Hbs1 interact directly with the A site of the ribosome, like the canonical termination factors, but promote an event

akin to ribosome recycling[46,47]. That Dom34 and Hbs1 function directly on the ribosome suggests that the effects of these surveillance pathways may have broad consequences for ribosome function and translational output.

Also characteristic of the initiation of NGD is an early endonucleolytic cleavage event. It is not fully understood how the endonucleolytic cleavage is triggered, but cleavage increases in the presence of Dom34, suggesting that Dom34 could play a stimulatory role[12]. While Dom34 was originally proposed to act directly as the endonuclease[44], many biochemical and genetic studies have since argued against such an activity[15,41,48].

Critical insight into the specificity of recognition by the Dom34:Hbs1 complex was recently obtained in *in vitro* studies showing that these factors operate most efficiently on ribosome complexes with very little mRNA sequence extending 3′ of the site of stalling[49] (Fig 2d); subsequent studies further established that this limitation is imposed by Hbs1 and not by Dom34[50]. Consistent with this idea, structural studies position the N-terminus of Hbs1 at the mRNA entry channel[46], poised to monitor mRNA length. In this way, mRNA length detection by Hbs1 drives the events of NGD specifically on ribosome complexes stalled proximal to the 3′ end of an mRNA[50]. The central importance of the endonucleolytic cleavage event becomes clear in the light of this biochemical result; cleavage of the mRNA generates a strong inducing signal for Dom34:Hbs1 activity. As a caveat, it is important to reiterate that genetic experiments suggest a role for Dom34 binding prior to cleavage. The interplay between Dom34 and the unknown endonuclease, and which signal ultimately initiates NGD, is poorly understood. It is interesting to speculate that recognition of stalled ribosomes in bacterial systems as mediated by tmRNA:SmpB and/or YaeJ relies on similar clues. Recent X-ray structures of bacterial ribosomes bound to these different “rescue” factors reveal specific protein moieties located near the decoding center where mRNA length could be directly monitored[51,52].

Ski7 is a factor that has specifically been implicated in recognition of nonstop-stalled ribosome complexes during NSD[19] (Fig. 2). Ski7 is a translational GTPase, closely related to the NGD factor Hbs1 (and thus to eRF3), having arisen through the duplication of a common ancestral gene[53]. Ski7 is known to interact with the exosome, placing it at the interface of ribosome recognition and mRNA degradation. Yet Ski7 is rare, found only in a small subset of yeasts[40]; organisms lacking this gene likely rely on the related Hbs1 to function in both NGD and NSD. Consistent with this prediction, Hbs1 from a yeast lacking Ski7 (*S. kluyveri*) can complement both Hbs1 and Ski7 deletions in *S. cerevisiae*[53]. On a mechanistic level, many questions remain concerning the initial recognition of NSD-targeted ribosome complexes. For example, no binding partner, such as Dom34 for Hbs1 or eRF1 for eRF3, has been identified for Ski7 in yeast. Moreover, the interaction of Ski7 with the ribosome remains wholly uncharacterized. As Dom34 and Hbs1 are seen to be active on ribosomes stalled at the 3′ end of messages, Ski7 may play a redundant role with Dom34/Hbs1 as suggested by recent studies from Inada’s group[54]. That said, if NGD and NSD are essentially equivalent processes, it is unclear why Ski7 would be preserved in yeast. Appreciation for significant overlap between NGD and NSD is newly developing and will require further experiments to deconvolute.

What are the consequences of aberrant translation?

Despite their several differences, what ultimately unites all three surveillance pathways – NMD, NSD and NGD – is the presence in the cell of a problematic ribosome complex that must be resolved on multiple levels (Fig. 3). First, the unproductive mRNA must be eliminated. Second, the incomplete protein product may have dominant or toxic effects, and so again elimination makes sense. Third, and perhaps most significantly, ribosomes are energetically costly to replace and so the cell will ideally seek to recover stalled subunits for subsequent rounds of translation. Each of these events is discussed individually below.

mRNA decay

The hallmark of mRNA surveillance pathways has long been the selective degradation of aberrant mRNAs. Canonical mRNA degradation occurs in both the 5′-3′ direction, by the exonuclease Xrn1, and the 3′-5′ direction, by the exosome, Ski7 and the Ski complex[55,56] (Box 1). In NMD, targeted mRNAs undergo accelerated decay from both directions[57,58]. *In vivo*, Upf1 associates with multiple factors implicated in mRNA degradation, which suggests plausible mechanisms for this acceleration[59,60]. For example, the tethering of human Smg7, a Upf1-interacting protein, to the 3′ UTR of a reporter gene bypasses the requirement for Upf1 function in NMD in mammalian cells[61]; as such, these studies argue that Smg7 may be directly involved in recruitment of mRNA decay components that act downstream of Upf1. Additionally, endonucleolytic cleavage of PTC-containing mRNAs has been observed in several higher eukaryotes[62,63]. In both *Drosophila* and humans, this endonucleolytic cleavage event is catalyzed by the PilT N-terminus (PIN) domain of Smg6, an NMD factor conserved in metazoans[62,64]. However, no PIN domain containing proteins have been implicated in NMD in yeast nor has endonucleolytic activity been observed in this organism during NMD.

Box 1

Classical mRNA degradation mechanisms in yeast

Turnover of stable messenger RNA occurs through two general mechanisms: 5′-3′ and 3′-5′ degradation (for reviews, see refs [92,93]). In both cases, decay initiates via deadenylation[56] catalyzed by the CCR4-POP2-NOT complex[94,95]. Substantial deadenylation (leaving behind fewer than ~10 adenosines) is required for mRNA degradation to further progress. After this, 5′-3′ degradation is thought to be the primary direction of mRNA degradation in yeast[56]. This process begins with removal of the mRNA cap structure by the decapping enzyme Dcp2[96]. Removal of the 5′ cap sensitizes mRNA to degradation by the 5′ exonuclease Xrn1[97]. Recent evidence suggests that 5′-3′ degradation by Xrn1 occurs co-translationally[98], allowing ribosomes to complete a round of translation while the trailing mRNA is degraded. Prior to this recent report, Xrn1-mediated degradation had been observed only on non-translating mRNPs that appear to accumulate in discrete cytoplasmic foci, called P bodies (reviewed in ref. [99]).

As mentioned above, an alternative (3′-5′) degradation pathway also exists. This process also follows deadenylation and is catalyzed by a multifactor ring complex termed the exosome[55]. The core exoribonuclease activity of the yeast exosome resides in one

subunit, Dis3[100]. The remaining subunits, while catalytically inactive, form a pore-like structure through which the RNA is threaded[101]. While the exosome has both nuclear and cytoplasmic RNA processing functions, the cytoplasmic activities seem to be primarily responsible for 3′-5′ degradation of bulk mRNA[102]. The cytoplasmic exosome further requires Ski7 and the Ski complex – composed of Ski2, Ski3, and Ski8 – which tether the exosome to mRNA targets[55,103,104]. We recall that an additional role for Ski7 in NSD has also been proposed, as discussed in the main text. The differential roles of Ski7 in NSD and basal exosome function are of ongoing interest.

The rate of decay is ultimately influenced by multiple features of an mRNA. The most well characterized features are protein binding sites including, for example, AU-rich elements (AREs) or PUF protein binding sites. These features are typically found in the 3′ UTR and can have either positive or negative effects on mRNA half-life. The complex determinants (including the binding sites for various trans acting factors) that control half-life for the majority of mRNAs are poorly understood, and likely vary by organism. There is currently no generalized, predictive model for mRNA half-life.

NSD-targeted messages have recently been shown to be endonucleolytically cleaved upstream of stalled ribosomes[52,63]. The catalytic subunit of the exosome, Rrp44p (alternatively called Dis3p), can promote both endonucleolytic and exonucleolytic activities, both of which appear to be involved in degradation of nonstop messages[65,66]. In yeast, recruitment of the exosome to these messages is promoted by Ski7[19]. A favored model is that Ski7 fulfills a bridging function: the C-terminal domain of Ski7, which resembles a translational GTPase, binds to NSD-targeted ribosome complexes while the N-terminal domain recruits the exosome[19].

NGD-targeted mRNAs are also generally subject to endonucleolytic cleavage, as discussed above (Fig 2). Following endonucleolytic cleavage, the 3′ and 5′ mRNA fragments are subsequently degraded by Xrn1 and the exosome, respectively[12] (Fig 3a). Endonucleolytic cleavage during NGD occurs upstream of the stalling site in the mRNA and results in a 5′ mRNA fragment lacking a poly(A) tail[40,52]. This fragment, if translated by another ribosome, results in another stalled complex – a conspicuous target for additional rounds of mRNA surveillance (Fig 2d). If secondary stalls induce additional cleavages, multiple cleavage events should occur with ribosome-sized spacing upstream of the initial stall site. In fact, several groups have confirmed such a prediction and reported regularly spaced cleavage events positioned just upstream of mRNA stall sequences of interest[52,65].

Endonucleolytic cleavage has been implicated in all three mRNA surveillance pathways though the cellular factor responsible for the cleavage and the actual inducing stimuli are incompletely defined (with the exception of the cleavage factor, Smg6, involved in NMD in higher eukaryotes). That said, endonucleolytic cleavage is a potent mechanism for triggering mRNA decay. A single endonucleolytic event circumvents the need for the normal initial steps in mRNA decay, decapping and deadenylation, which are typically slow and tightly regulated (Box 1). As such, cleavage is likely to be an irreversible process that commits a stalled ribosome (and its mRNA) to the surveillance pathway. The extent to which there is overlap between NMD-, NGD- and NSD-based endonucleolytic cleavage mechanisms is yet

to be resolved. Deciphering how cleavage occurs, including how complexes are selected for cleavage and the identification of the endonuclease(s) involved in NGD and NSD, will greatly advance our understanding of these processes *in vivo*.

Degrading the peptide

The partial peptide derived from the stall-inducing mRNA is not likely to play a positive physiological role in the cell and so these peptides are typically targeted for degradation. Various studies have identified NMD-, NSD- and NGD-derived protein products as readily processed substrates for the proteasome[20,67,68]. These data suggest that quality control pathways can accelerate the degradation of stalled or incomplete protein products. In the case of both NGD and NSD, the terminated protein product likely originates from peptidyl-tRNA that is directly produced by the actions of Dom34 and Hbs1[54].

Two E3 ligases, Not4 and Ltn1 (also known as either YMR247C or Rkr1 in yeast), have been shown to target NSD protein products for polyubiquitylation and subsequent degradation[68–70]. It is unknown whether these same E3 ligases are similarly involved in the destabilization of NGD- or NMD-derived proteins. One intriguing alternative candidate for this role is the N-terminus of Upf1, which itself has an E3 ligase motif that is known to contribute to NMD[36]. Additional work will be required to fully elucidate the mechanisms by which these different classes of stalled peptides are recognized, as well as the extent to which these degradative events occur co-translationally as a function of mRNA surveillance or post-translationally through more canonical pathways.

Recovering the ribosomes

Ribosomes are large cellular machines that are energetically costly to synthesize and thus are worth preserving if their malfunction is not the source of the problem. To recover ribosomes, some form of ribosome recycling must take place to allow for the dissociated ribosomal subunits to engage in re-initiation.

For NGD and NSD, as anticipated from similarities to the translation termination factors eRF1 and eRF3, Dom34 and Hbs1 were shown to directly bind to the A site of the ribosome, in a codon-independent manner, and to dissociate ribosome complexes[47]. *In vitro*, this subunit splitting activity is further stimulated by Rli1[49,50], an essential ATPase known to be required for canonical ribosome dissociation[71,72]. Structural data suggest that Rli1 forces Dom34 – or in the case of canonical recycling, eRF1 – through the ribosomal subunit interface, disrupting critical intersubunit bridges in the process and leading directly to subunit dissociation[73]. I*n vivo*, genetic studies demonstrate that Dom34, Hbs1 and, presumably, Rli1 are required for subunit dissociation during both NSD and NGD[54]; because Rli1 is an essential gene, it is has been difficult to establish its specific roles *in vivo*. The splitting of ribosome complexes by Dom34 facilitates subsequent rounds of translation initiation[49,74]. As for NMD, given the essential role of Upf1, a known ATPase, in the process, and the typical energetic demands of a ribosome splitting reaction, Upf1 is a viable candidate for filling this role. While there are some data consistent with such a model in yeast [7,39], it is also possible that the NMD factors simply serve to recruit more canonical recycling factors in the cell such as Rli1, and even Dom34/Hbs1.

Eliminating ribosomes when they are faulty

An interesting twist in this survey of mRNA surveillance is the apparently related process of nonfunctional ribosome decay (NRD). Dom34, the eRF1 homolog that is intimately involved in NGD and NSD, is critical for the rapid turnover of demonstrably faulty small ribosomal subunits[75]. This was clearly demonstrated when small ribosomal subunits carrying debilitating mutations in key residues involved in tRNA selection were specifically targeted for rapid turnover[76]. Given the dependence of this process on Dom34[75], NRD likely initiates like NGD with a stalled ribosome complex, except that the stall-inducing signal in this case is located within the ribosome rather than within the mRNA. While it seems unlikely that Dom34 specifically targets ribosomes for degradation, ribosomes that are repeatedly dissociated by Dom34 will be repeatedly exposed to the degradative machinery, thus resulting in an acceleration in the rate of their degradation. These data very clearly define a specific role for the surveillance machinery in ribosome fate.

Broad surveillance mechanism or specific gene regulator?

Some of the most interesting questions surrounding mRNA surveillance pathways revolve around their effects at an organismal level. To what extent are these surveillance mechanisms quality control pathways and to what extent do cells exploit these pathways to selectively modulate broader translational events? We know that the ubiquitin-proteasome system, for example, is involved in both basal and selective protein turnover.

NMD

There are numerous studies establishing that NMD modulates the stability of a variety of specific transcripts including alternatively spliced messages, messages containing upstream ORFs, and transcripts that derive from transposons, pseudogenes, or out-of-frame gene rearrangements (as in T cell receptor and immunoglobulin genes)[37]. In all, NMD regulates a high number and broad range of transcripts *in vivo* with estimates indicating as many as 10% of all eukaryotic genes[77–80]. While each of the above mRNAs could contain a premature stop codon, there is evidence to suggest this is not always the case[9]. The mechanism by which non-PTC containing genes might be targeted by the NMD machinery is unclear though it is certainly possible that additional factors might be involved.

There has long been interest in NMD because of its strong connection to human disease; indeed, some 30% of inherited genetic disorders are thought to involve gene mutations which result in premature stop codons[81]. The ability to modulate NMD and selectively increase read-through of these stop codons[82,83] has shown promise as a therapeutic strategy for diseases such as cystic fibrosis[84,85]. Further insights into NMD and stop codon read-through should aid in identifying additional drug targets and advancing these therapies.

NSD/NGD

While genome-wide efforts at characterizing NSD and NGD targets have not been published, genome-wide analysis has revealed that alternative polyadenylation sites are common in both higher and lower eukaryotes[18]. Premature polyadenylation occurring within coding sequences is likely to elicit NGD/NSD in response to translation of poly-lysine

tracts. NGD is also involved in responding to chemically damaged mRNAs, as depurinated mRNA appears to stall translation, leading to mRNA degradation in a Dom34-dependent manner[13]. Oxidative mRNA damage is similarly likely to cause ribosome stalling[86]; a role for Dom34 in responding to oxidative mRNA damage has not directly been explored although deletion of *DOM34* sensitizes yeast to a variety of oxidative stressors[87]. Intriguingly, oxidative mRNA damage may be clinically relevant as it is involved in the early pathogenesis of many neurological diseases such as Alzheimer's and amyotrophic lateral sclerosis (ALS)[88,89].

Another intriguing case of potential NGD targeting is found in work done on the *CGS1* coding sequence in *Arabidopsis*. A region within *CGS1*, *MTO1*, arrests translation and induces mRNA degradation in the presence of S-adenosyl-*L*-methionine[90]. Ribosomal stalling in this context is peptide-mediated, caused by compaction of the nascent chain in the exit tunnel, and results in subsequent mRNA cleavage[91] – both characteristic of NGD. At present, involvement of Dom34 and Hbs1 in this process is merely speculative.

Conclusions

In this review we hope to have emphasized the interconnectedness of translation and mRNA surveillance, as well as the multifaceted response of the cell to translation of aberrant transcripts. NMD, NGD and NSD effect strikingly similar fates for aberrant mRNAs, the ribosomes translating them and the altered protein products they encode. Indeed, broadly overlapping strategies are similarly used to resolve translational stalls in bacteria, indicative of the broad utility of such a three-pronged approach (Box 2). The term 'mRNA surveillance' inadvertently downplays the capacity of surveillance systems to broadly address translation of aberrant mRNAs, and it is not clear that mRNA degradation is even the most critical outcome. The majority of factors implicated in these processes either interact directly with known translation factors or are themselves translation factor homologs. We suggest that it will ultimately be informative to consider these surveillance events from the perspective of their origins on the ribosome.

Box 2

tmRNA: universality of surveillance outcomes

Bacterial mRNAs are significantly less stable than their eukaryotic counterparts. Furthermore, the coupling of transcription and translation in bacteria limits the opportunity to assess mRNA quality prior to translation. As such, one might anticipate there would be a larger number of aberrant mRNAs actively being translated and so the need for surveillance systems to deal with such transcripts seems great.

Not surprisingly, parallel systems do appear to be found in bacteria. Stalled bacterial ribosome complexes result in 1) the degradation (with possible endonucleolytic cleavage) of the stalled mRNA[105,106], 2) the tagging of the peptide for proteolysis[107], and 3) the rescue of ribosomes through canonical recycling processes[108]. Indeed, these effects are strikingly reminiscent of the outcomes observed during eukaryotic ribosome rescue. However, none of the factors required for these activities in eukaryotes are conserved in

bacteria. Instead, there seem to be several different systems in place to deal with ‘stalled’ ribosome complexes, some better characterized than others.

The best characterized of these ribosome rescue events depends on an intriguing functional RNA referred to as tmRNA (for a review, see ref. [109]). tmRNA is an RNA that contains regions resembling a charged alanyl-tRNA (the t for transfer) and a short open reading frame ending in a stop codon (the m for mRNA). tmRNA requires two protein factors, SmpB and EFTu, for its function. Like Dom34:Hbs1, tmRNA:SmpB:EFTu:GTP binds to the A site of ribosome complexes, and is most efficient on ribosome complexes carrying only a short 3′ mRNA extension[110]; a recent structure of ribosome-bound tmRNA quaternary complex reveals how mRNA length may be directly monitored[52]. Upon tmRNA binding, the alanine residue is directly incorporated into the growing peptide chain, and the ORF-containing region of the tmRNA substitutes for the problematic stalled mRNA sequence. Translation resumes on the tmRNA ORF, resulting in a hybrid protein product that is ultimately tagged for degradation by bacterial proteases, such as the ClpXP system. tmRNA-rescued ribosomes are competent for subsequent recycling through canonical means since translational termination is effectively routine.

Other less well-characterized ribosome rescue events may depend on release factor homologs (typically missing the codon recognition domain) that survey the cell for stalled ribosomal complexes. While the molecular requirements of such rescue events have not been characterized, a recent X-ray structure of YaeJ bound to the ribosome reveals the presence of a protein domain that appears to engage, and likely monitor, the mRNA channel[51]. Thus, while the factors involved and the molecular mechanisms of ribosome rescue in bacteria and eukaryotes are quite distinct, the outcomes of the pathways are markedly conserved.

The molecular mechanisms by which these surveillance pathways are initiated on the ribosome remain a significant question in the field. Also of considerable interest are questions concerning the cell’s ability to exploit these surveillance processes to selectively regulate translation of non-aberrant transcripts. As our molecular understanding of these processes grows, and our ability to analyze translation in vivo increases, our ability to interpret biological relevance should similarly expand.

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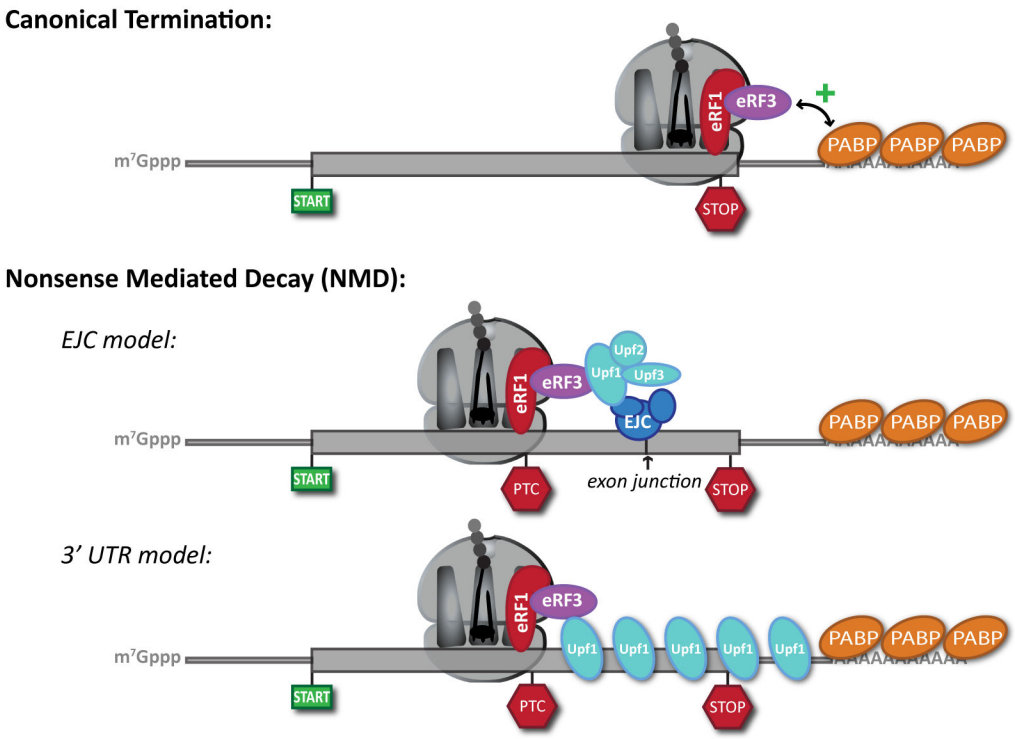

Figure 1. Recognition of NMD surveillance targets

(a) Canonical termination. Capped and polyadenylated messages are translated through the open reading frame until recognition of a stop codon by the eukaryotic release factors, eRF1 and eRF3. Close proximity of authentic stop codons with the poly(A) tail is proposed to facilitate interactions between eRF3 and poly(A)-binding proteins (PABP) that positively contribute to peptide release. **(b)** Nonsense-mediated decay (NMD). In the case of a premature stop codon (PTC), lack of proximity is proposed to disrupt interaction between eRF3 and PABP. Canonical termination is further modified by the presence of NMD factors. In the EJC model of higher eukaryotes, this results from encountering a stop codon upstream of an exon-junction complex (EJC). In this model, communication between the termination factors and the EJC is effectively bridged by Upf1 in coordination with Upf2 and Upf3. In the 3′ UTR model, a PTC effectively extends the de facto 3′ untranslated region (UTR) of the message. This provides a larger binding platform for Upf1, which drives the termination event towards NMD rather than classical termination. Thick line, open reading frame; thin line, 5′ and 3′ UTR.

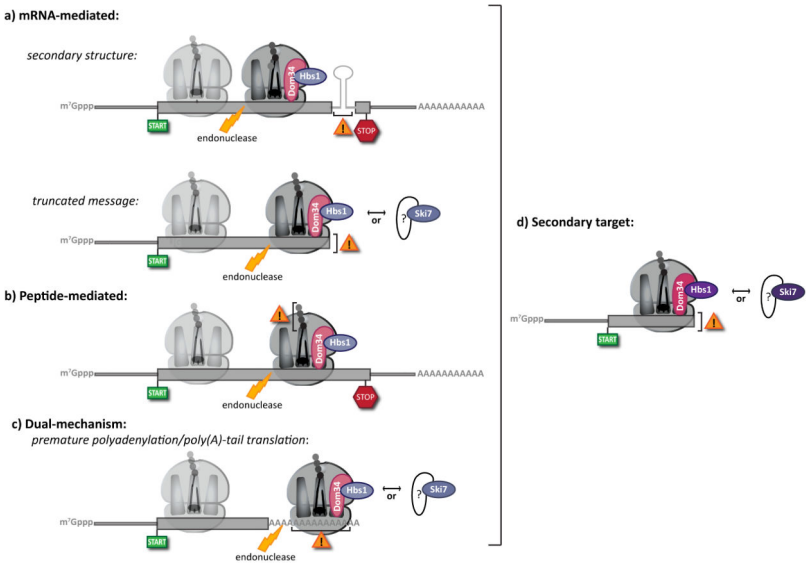

Figure 2. Recognition and initiation of NGD and NSD complexes

No-go decay (NGD) and non-stop decay (NSD) both involve the recognition of stalled ribosome complexes. These stalls can arise through multiple mechanisms. **(a)** mRNA-mediated targets. Inhibitory mRNA secondary structures stall ribosomes at internal loci (top), while truncated mRNAs result in terminal stalls (bottom). While classically distinguished as NGD and NSD targets, respectively, increasing evidence suggests that such distinctions belie common mechanistic features. **(b)** Peptide-mediated targets. Inhibitory peptide sequences lead to internally stalled ribosomes, classically defined as NGD substrates. **(c)** Dual-mechanism targets. Translation of the poly(A) tail, originally considered to mimic a truncated message and invoke NSD, likely induces ribosome stalling prior to its arrival at the end of the message. As such, the distinction between NGD and NSD under these conditions is ambiguous in the absence of further experimentation. In all cases, **(a)**, **(b)** and **(c)**, endonucleolytic cleavage occurs upstream of the stalled ribosome, potentially stimulated by Dom34 and Hbs1. This tentative role for Dom34 and Hbs1 prior to cleavage is indicated by the increased transparency of these factors in **(a–c).** Following cleavage, the trailing ribosome (shown transparently) elongates to the point of cleavage, generating an ideal target for Dom34/Hbs1 (or Ski7) recognition **(d)**. At present, no Dom34-like factor has been identified that interacts with Ski7.

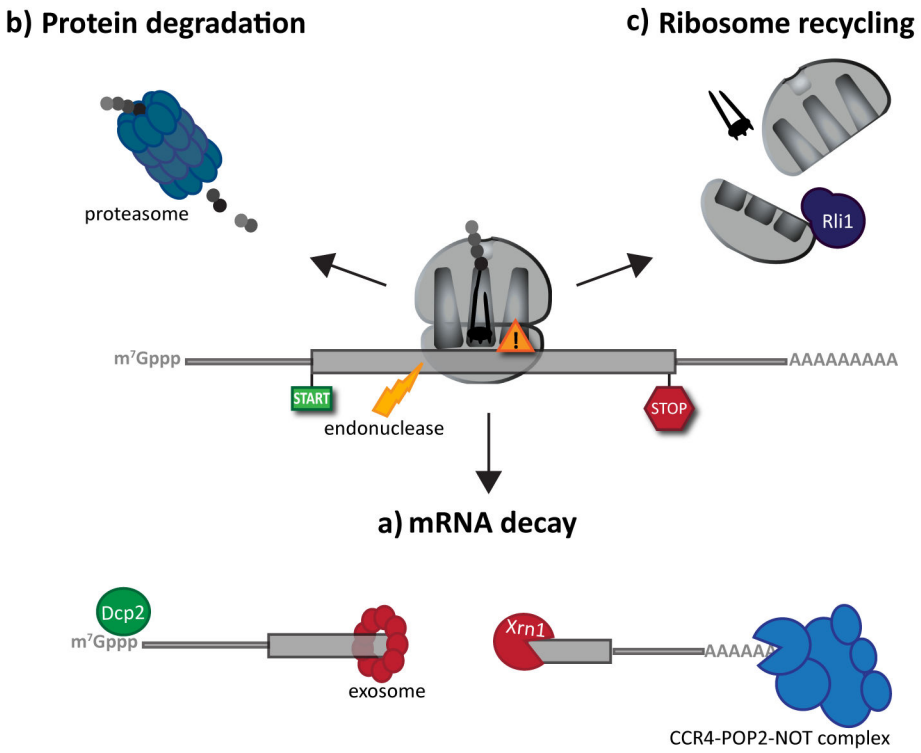

Figure 3. mRNA surveillance pathway outcomes

Following the recognition of NMD, NGD or NSD ribosome complexes, at least three discrete salvage pathways are invoked: mRNA decay, protein degradation and ribosome recycling. **(a)** mRNA decay. Endonucleolytic cleavage subverts the need for deadenylation, by the CCR4-POP2-NOT complex, and decapping, by Dcp2, prior to mRNA decay. Rapid mRNA degradation then proceeds through canonical means, including 5′-3′ degradation by Xrn1 and 3′-5′ degradation by the exosome. **(b)** Protein degradation. Targeted degradation of aberrant peptides occurs via the ubiquitin-proteasome system. Several E3 ligases have been implicated in this process, but the molecular features of substrate recognition remain to be determined. **(c)** Ribosome recycling. Dom34:Hbs1 are known to exploit the canonical recycling activities of Rli1 to effect ribosome recycling during NGD and NSD. Recycling of ribosome complexes during NMD are less well-characterized, but may involve Upf1.