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Quality control

Protecting the proteome: Eukaryotic cotranslational quality control pathways

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The correct decoding of messenger RNAs (mRNAs) into proteins is an essential cellular task. The translational process is monitored by several quality control (QC) mechanisms that recognize defective translation complexes in which ribosomes are stalled on substrate mRNAs. Stalled translation complexes occur when defects in the mRNA template, the translation machinery, or the nascent polypeptide arrest the ribosome during translation elongation or termination. These QC events promote the disassembly of the stalled translation complex and the recycling and/or degradation of the individual mRNA, ribosomal, and/or nascent polypeptide components, thereby clearing the cell of improper translation products and defective components of the translation machinery.

Quality control pathways monitor protein synthesis

The correct translation of messenger RNAs (mRNAs) into functional proteins requires the precise coordination of staggeringly complex molecular factors that govern protein biogenesis. Defects in protein synthesis can lead to the production of potentially toxic defective translation products whose unregulated accumulation can negatively impact nearly every cellular pathway. In addition, RNAs and proteins involved in translation are subject to damage by irradiation and chemical modification that can negatively impact protein biogenesis. An important set of quality control (QC) pathways specializes in cotranslationally monitoring protein synthesis to prevent deleterious production of erroneous translation products (Fig. 1). Translational stalling provides an opportunity for the QC machinery to engage the translational machinery and subvert the normal translational process. Once recruited to stalled translation complexes, the QC pathways disassemble the defective translation complex and recycle and/or degrade its individual components. A subset of these pathways acts to degrade defective nascent polypeptides that fail to undergo proper cotranslational folding. Another subset resolves stalls in translation elongation or termination that arise from defects in the mRNA template, translation machinery, or nascent polypeptide. Collectively, these cotranslational QC pathways protect the proteome by clearing defective mRNAs, ribosomes, and polypeptides.

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Abbreviations used in this paper: mRNA, messenger RNA; mRNP, mRNA–protein; NGD, no-go decay; NMD, nonsense-mediated decay; NSD, non-stop decay; PTC, premature termination codon; QC, quality control; RQC, ribosome QC complex; rRNA, ribosomal RNA.

When mRNA defects stall the ribosome: Cotranslational mRNA QC pathways

Nonsense-mediated decay. There are at least three types of defects in mRNAs that stall the translating ribosome and activate cotranslational QC pathways in eukaryotes. The first type to be discovered was defects in the mRNA that lead to the generation of premature termination codons (PTCs), which cause premature translation termination and activate the nonsense-mediated mRNA decay (NMD) pathway (Fig. 2; Losson and Lacroute, 1979; Maquat et al., 1981). There are multiple mechanisms by which PTCs can arise in mRNAs. The most common in organisms with high intron frequencies is thought to be cryptic or alternative splicing events. These splicing changes give rise to PTCs by either causing frame shifting within the coding region, or exposing stop codons within retained introns or alternative exons (Lareau et al., 2007; McGlincy and Smith, 2008; Sayani et al., 2008). In addition, PTCs can arise from transcription errors, genetic mutations, or recombination events (Chang et al., 2007; Isken and Maquat, 2007; Rebbapragada and Lykke-Andersen, 2009; Schweingruber et al., 2013). Moreover, a subset of seemingly normal endogenous mRNAs is targeted by the NMD pathway (Mendell et al., 2004; Guan et al., 2006; Johansson et al., 2007). When a ribosome terminates translation at a PTC, a set of NMD factors—the Upf and Smg proteins (Leeds et al., 1991, 1992; Pulak and Anderson, 1993)—associate with the PTC-containing mRNA and target it for degradation by RNA decay enzymes. Degradation of NMD substrates is initiated by endonucleolytic cleavage, decapping,

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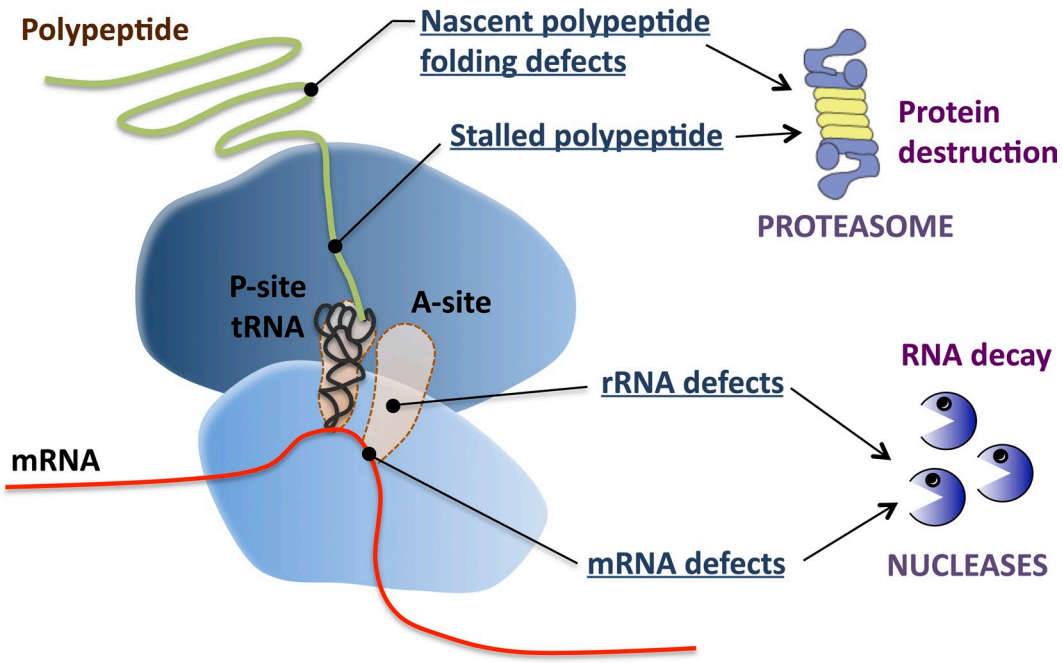

Figure 1. **Multiple cotranslational quality control (QC) pathways monitor the translation process.** QC pathways cotranslationally detect and degrade defective nascent polypeptides, ribosomes, and mRNAs.

or deadenylation depending on the specific mRNA and organism (Muhlrad and Parker, 1994; Chen and Shyu, 2003; Mitchell and Tollervey, 2003; Gatfield and Izaurralde, 2004). The subsequent degradation of the mRNA body involves disassembly of the mRNA–protein (mRNP) complex, which is dependent on the ATPase activity of the central NMD factor Upf1 (Franks et al., 2010).

The specific mechanism by which Upf/Smg proteins distinguish premature from normal termination events remains under investigation. Current evidence suggests that the translation termination event performed by the eRF1–eRF3 termination complex at a PTC is rendered inefficient due to the absence of a normal 3′UTR, which at regular termination codons stimulates the termination process (Amrani et al., 2004). One factor that is associated with 3′UTRs and is thought to stimulate efficient translation termination on normal mRNAs is cytoplasmic poly(A)-binding protein (PABPC; Amrani et al., 2004). When PABPC is positioned in proximity to the translation termination event it antagonizes NMD, whereas the same protein when located distal to translation termination fails to do so and allows assembly of NMD factors (Amrani et al., 2004; Behm-Ansmant et al., 2007; Eberle et al., 2008; Ivanov et al., 2008; Singh et al., 2008). In metazoans, NMD is further stimulated when termination occurs upstream of exon–exon junctions, which are marked by the exon–junction complex that is deposited during pre-mRNA splicing in the nucleus and interacts with Upf proteins in the cytoplasm (Kim et al., 2001; Le Hir et al., 2001; Lykke-Andersen et al., 2001; Gehring et al., 2003; Singh et al., 2007). Although PABPC and the exon–junction complex are well-established factors in NMD, evidence suggests the existence of additional mRNP components that serve to distinguish normal mRNAs from those targeted for NMD (González et al., 2000; Meaux et al., 2008; Singh et al., 2008). In addition to mRNA degradation, there is evidence that the NMD pathway actively represses recruitment of new ribosomes to the target mRNA (Muhlrad and Parker, 1999; Isken et al., 2008). Moreover, as discussed in more detail later, there is evidence suggesting that the native polypeptide produced from a PTC-containing mRNA might be subjected to proteolysis in a manner stimulated by the central NMD factor Upf1 (Kuroha et al., 2009, 2013; Verma et al., 2013).

Non-stop decay. Another type of defect that subjects mRNAs to cotranslational QC is a defect that results in the absence of a termination codon. The resulting "non-stop" mRNAs are subjected to the non-stop decay (NSD) pathway once the translating ribosome reaches the mRNA 3′ end without encountering a termination codon (Fig. 3; Frischmeyer et al., 2002; van Hoof et al., 2002). Non-stop mRNAs likely arise primarily by processing errors where polyadenylation occurs prematurely within the mRNA coding region (Frischmeyer et al., 2002; van Hoof et al., 2002). Another possible source of NSD substrates are truncated mRNAs resulting from endonucleolytic cleavage events within the protein coding region (Tsuboi et al., 2012; Matsuda et al., 2014). However, an mRNA endonucleolytically cleaved by a hammerhead ribozyme is efficiently degraded by cellular exonucleases and does not require the NSD machinery for rapid degradation, suggesting that general exonucleolytic mRNA decay pathways may dominate over the NSD pathway on such substrates (Meaux and Van Hoof, 2006).

NSD has been most intensely investigated in *Saccharomyces cerevisiae*, where studies have revealed a critical role for the GTPase Ski7, a homologue of the translation termination factor eRF3 (van Hoof et al., 2002). In addition to a C-terminal eRF3-like GTPase domain, Ski7 contains an N-terminal domain important for the catalytic activity of the cytoplasmic form of the *S. cerevisiae* 3′-to-5′ exonuclease exosome complex (van Hoof

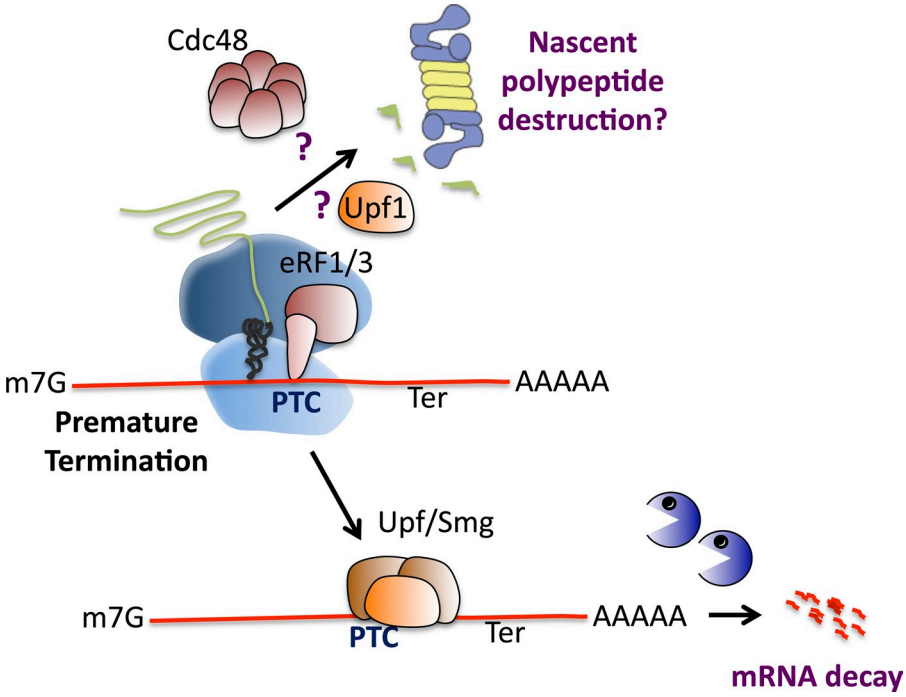

Figure 2. **Nonsense-mediated mRNA decay (NMD).** Premature termination codon (PTC)–containing mRNAs are degraded when a stall in the eRF1/eRF3-dependent translation termination process is detected by Upf and Smg proteins. Some evidence suggests that the resulting truncated protein product can be targeted for ubiquitylation and destroyed by the proteasome in a Upf1- and Cdc48-dependent fashion. m7G refers to the mRNA 7-methyl guanosine cap; AAAAA refers to the poly(A)-tail; Ter indicates a normal termination codon.

et al., 2000, 2002). Deletion of any one of these Ski7 domains, as well as inactivation of exosome components, stabilizes mRNAs targeted for NSD (van Hoof et al., 2002; Schaeffer and van Hoof, 2011). Based on these observations, and the similarity with eRF3, Ski7 is hypothesized to enter the empty A-site of ribosomes that are stalled at the 3′ end of nonstop mRNAs and recruit the exosome to activate mRNA degradation (van Hoof et al., 2002). Recent studies also suggest a potential role for another eRF3-homologous factor, Hbs1, and its associated eRF1-related factor Dom34, in *S. cerevisiae* NSD. Deletion of these factors, when combined with deletions in 5′-to-3′ or 3′-to-5′ exonucleases, lead to increased stability of NSD substrates and accumulation of degradation intermediates (Tsuboi et al., 2012). Moreover, the Hbs1–Dom34 complex, along with the ribosome-recycling ATPase Rli1 (called ABCE1 in human), promote disassembly of ribosomes when stalled at, or near, the 3′ end of mRNA in vitro (Pisareva et al., 2011; Shoemaker and Green, 2011). Taken together, these observations indicate that active release of the ribosome by Hbs1–Dom34 and Rli1 might be a prerequisite for efficient degradation of an mRNA targeted for NSD. However, the exact nature of the interplay between Ski7 and Hbs1–Dom34 in NSD remains to be fully understood.

In addition to degradation of the mRNA, evidence suggests that NSD substrates are subjected to translational repression (Inada and Aiba, 2005; Akimitsu et al., 2007), but the mechanism remains uncharacterized. Beyond *S. cerevisiae*, NSD has also been observed in human tissue culture cells (Frischmeyer et al., 2002; Saito et al., 2013), although some studies observed no effect of the absence of a stop codon on the decay rate of specific mRNAs in human cells (Akimitsu et al., 2007; Torres-Torronteras et al., 2011). One possible explanation that could reconcile these observations is that the relative contribution of translation repression and mRNA decay to the silencing of nonstop mRNAs is substrate dependent. The *SKI7* gene is unique to a subset of *Saccharomycetaceae* including all *Saccharomyces* species (van Hoof, 2005; Atkinson et al., 2008; Marshall et al., 2013), whereas other fungi—and possibly other eukaryotes—produce Ski7 and Hbs1 proteins by alternative splicing from a single *HBS1* gene (Marshall et al., 2013). Correspondingly, evidence suggests that siRNA-mediated depletion of Hbs1 or Pelota (the human orthologue of Dom34) in human tissue culture cells results in impaired NSD (Saito et al., 2013).

No-go decay. A third type of mRNA defect that activates cotranslational QC causes ribosomes to stall during translation elongation and activates the no-go decay (NGD) pathway (Fig. 4). This pathway was first discovered with the observation that insertion of a stable RNA hairpin structure that inhibits translation elongation in the *S. cerevisiae PGK1* mRNA causes endonucleolytic cleavage and accelerated degradation of the mRNA (Doma and Parker, 2006). In addition to RNA structural elements, rare codons and polylysine–codon tracts (Tsuboi et al., 2012), as well as mRNA depurination by a viral enzyme (Gandhi et al., 2008), have been shown to active the NGD pathway. A possible important role of the NGD pathway is to clear the cell of mRNAs that have been subjected to chemical or irradiation-mediated RNA damage (Doma and Parker, 2006; Harigaya and Parker, 2010). Interestingly, the observation that translation of a polylysine tract within the nascent polypeptide activates the NGD pathway (Tsuboi et al., 2012) raises the question of whether a non-stop mRNA produced by premature polyadenylation within the coding region, thereby producing a polypeptide with a terminal polylysine tract, can be a target of either NGD or NSD pathways depending on whether the ribosome stalls while translating the poly(A)-tail or after reaching the mRNA 3′ end.

NGD is initiated by endonucleolytic cleavage near the ribosome stall site as evidenced by the size of mRNA degradation intermediates that accumulate when 5′-to-3′ or 3′-to-5′ exonucleases are inactivated in *S. cerevisiae* (Doma and Parker, 2006;

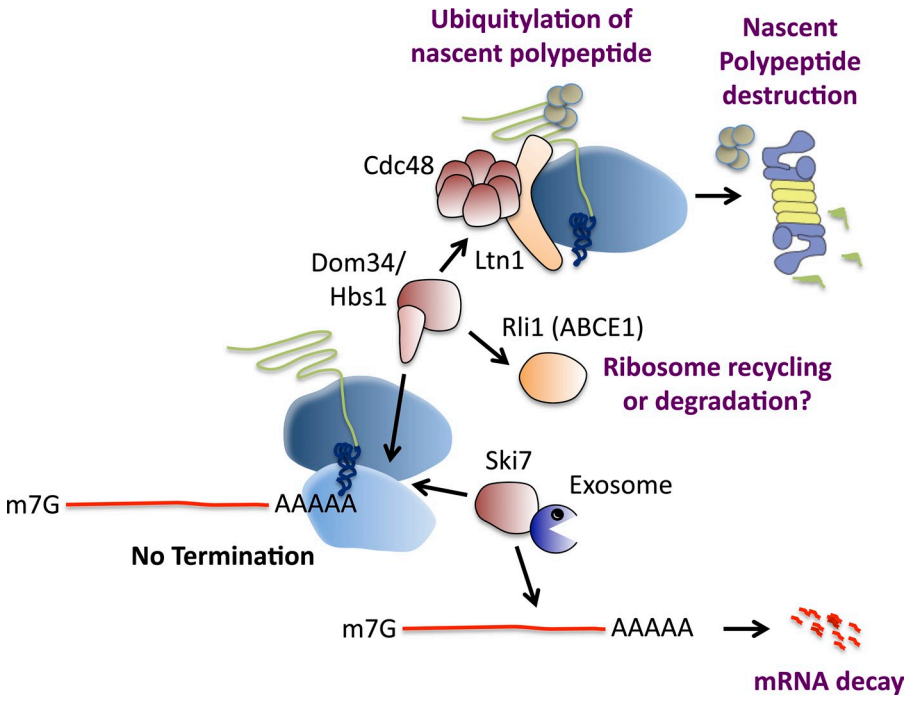

Figure 3. **Non-stop mRNA decay (NSD) and protein destruction.** mRNAs on which the ribosome reaches the 3′ end without encountering a stop codon are detected by the eRF3-like factor Ski7 and targeted for mRNA decay by the exosome. The stalled ribosome is thought to be released by the Hbs1–Dom34–Rli1 complex. Extraction and destruction of the defective protein products arising from NSD mRNA substrates is mediated by ubiquitin pathway components Ltn1 and Cdc48.

Tsuboi et al., 2012). The Hbs1–Dom34 complex plays an important role in the NGD pathway as inactivation of either of these factors stabilizes NGD substrates (Doma and Parker, 2006; Passos et al., 2009). Genetic deletion analyses suggest that Hbs1 and Dom34 stimulate an initial endonucleolytic cleavage event within the NGD substrate (Doma and Parker, 2006; Passos et al., 2009; Tsuboi et al., 2012), as well as the subsequent degradation of the mRNA 5′ fragment by the exosome (Tsuboi et al., 2012). It remains unknown whether any of these effects are a consequence of direct activation of nucleases by Hbs1–Dom34, analogous to Ski7 in NSD, or instead a secondary consequence of ribosome disassembly by the Hbs1–Dom34 complex. One report presented evidence for in vitro endonucleolytic activity of Dom34 (Lee et al., 2007); however, a later report saw no evidence for such an activity (Passos et al., 2009). Moreover, genetic and in vitro evidence demonstrated that cleavage of the NGD mRNA can be triggered by the stalled ribosome even in the absence of Dom34 or Hbs1, suggesting that these factors are not essential for endonucleolytic cleavage of NGD substrates (Passos et al., 2009; Tsuboi et al., 2012). The observation that the Hbs1–Dom34 complex stimulates separation of stalled ribosomes in vitro only when the 3′ end of the mRNA extending from the stalled ribosome is short (Pisareva et al., 2011; Shoemaker and Green, 2011) suggests that endonucleolytic cleavage must precede ribosome recycling by Hbs1–Dom34. Establishing the identity of the endonuclease involved in NGD is an important goal for future study. Beyond *S. cerevisiae*, NGD has been detected in *Drosophila* S2 cells (Passos et al., 2009), but little is currently known about the NGD pathway in metazoans.

When ribosomal RNA defects impair the ribosome: Ribosome QC pathways

Ribosomal RNAs (rRNAs) make up more than three quarters of the total RNA in cells and are likely a major target for damage mediated by irradiation and chemical modification (Brégeon and Sarasin, 2005; Feyzi et al., 2007; Kong et al., 2008). A number of pathways for ribosomal QC have been described, some of which occur during ribosome biogenesis in the nucleus (Houseley et al., 2006; Reinisch and Wolin, 2007) and others after transport of ribosomal subunits to the cytoplasm (LaRiviere et al., 2006; Cole et al., 2009; Fujii et al., 2009, 2012; Lebaron et al., 2012; Strunk et al., 2012). One ribosome QC pathway that occurs cotranslationally was discovered when the effect of inserting point mutations into the decoding center of the small ribosomal subunit 18S rRNA was monitored in *S. cerevisiae* (LaRiviere et al., 2006; Cole et al., 2009). Such mutant 18S rRNAs are subject to rapid degradation by a pathway that has been named 18S nonfunctional rRNA decay (18S NRD). This occurs cotranslationally, as evidenced by the inhibition of the pathway by chemicals that block translation initiation or elongation (Cole et al., 2009). Interestingly, 18S NRD is impaired upon depletion of Hbs1 or Dom34 (Cole et al., 2009). This has led to the hypothesis that 18S NRD is related to NGD and raises the interesting question of whether the 18S NRD and NGD pathways are capable of distinguishing which component is responsible for a stall in translation elongation (Fig. 4; Cole et al., 2009; Swisher and Parker, 2009). Alternatively, fully functional components of the stalled translation complexes might be degraded as collateral damage with those nonfunctional components that cause the stall. However, a study of point mutations in Hbs1 has demonstrated that at least some aspects of these pathways are separable, suggesting that NGD and 18S NRD might not fully overlap (van den Elzen et al., 2010).

Another cytoplasmic QC pathway that monitors defective ribosomes is the 25S NRD pathway, which targets large ribosomal subunits with inactivating nucleotide substitutions in the peptidyl transferase center of the large rRNA (LaRiviere et al., 2006; Cole et al., 2009; Fujii et al., 2009, 2012). This pathway

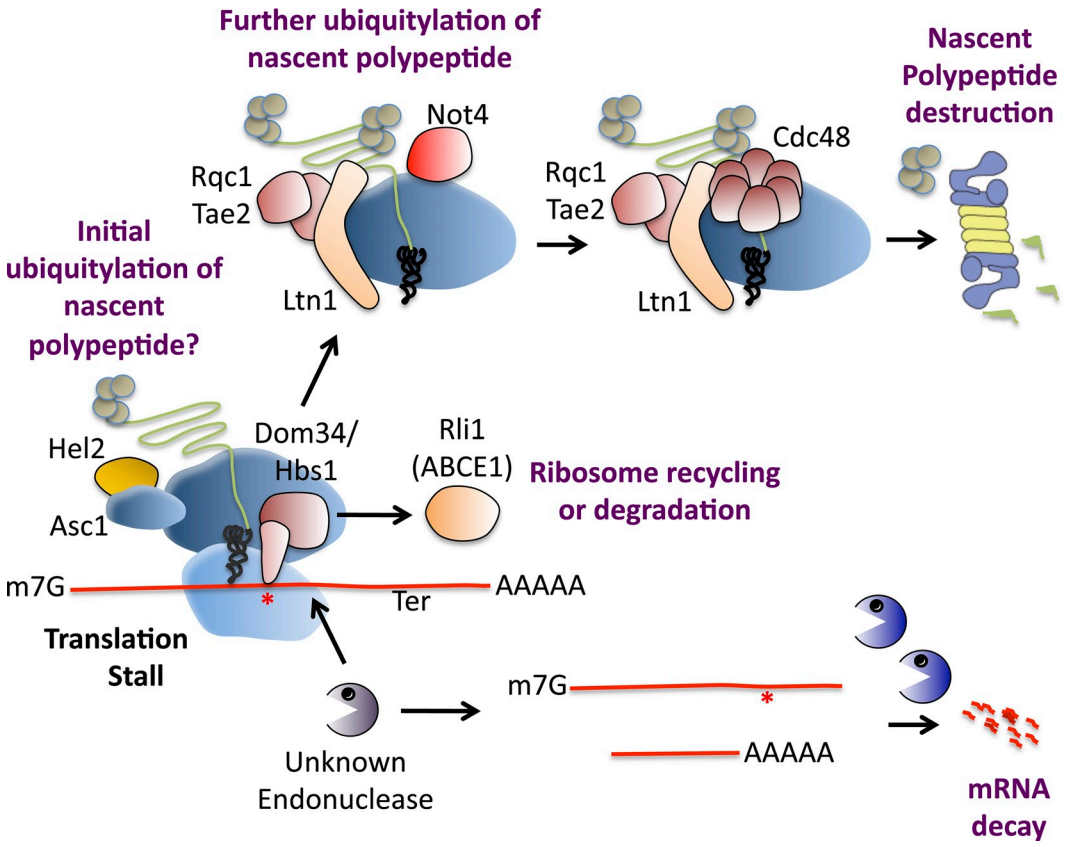

Figure 4. **No-go mRNA decay (NGD) and protein destruction.** Diverse mRNA or nascent chain features (*) that result in terminally stalled translation elongation complexes are resolved by a host of mRNA and protein destruction factors. Initial mRNA cleavage by unknown endonuclease(s) allows the alternative ribosome release factors, Hbs1 and Dom34 to mediate ribosomal splitting via Rli1 (ABCE1) and subsequent mRNA decay by RNA exonucleases. The nascent polypeptide is targeted for ubiquitylation by the Ltn1-containing ribosome quality control complex (RQC) and Not4. Hel2 and Asc1 putatively target nascent chains for ubiquitylation before and independent of the RQC.

activates degradation of the large rRNA in a process that involves ubiquitylation of large ribosomal subunit proteins and requires the activity of the proteasome (Fujii et al., 2009, 2012). Rtt101 was identified as a cullin family ubiquitin ligase involved in this process together with a cofactor Mms1 (Fujii et al., 2009) and the AAA ATPase Cdc48–Npl4–Ufd1 complex, which was found to promote the release of the targeted large ribosomal subunit from the small subunit (Fujii et al., 2012). Interestingly, the ubiquitin ligase components, the Cdc48 complex, and the proteasome are all required for degradation of the defective large rRNA, suggesting that removal of protein components from the rRNP is required for rRNA degradation (Fujii et al., 2012), reminiscent of the role of mRNP disassembly in NMD (Franks et al., 2010). Similar to 18S NRD substrates, 25S NRD substrates can be observed in the cytoplasm by in situ hybridization, but unlike 18S NRD, 25S NRD is not inhibited by chemicals that block translation initiation or elongation (Cole et al., 2009). This indicates that 25S NRD occurs in the cytoplasm before the large subunit engages in active translation, which raises the interesting question of how the activity of the peptidyl transferase center can be monitored pre-translation. Another pre-translation cytoplasmic ribosome QC pathway was recently discovered, which involves eIF5B-dependent assembly of an 80S-like ribosome followed by Dom34- and Rli1-dependent disassembly, to monitor maturation of small ribosomal subunits before translation (Lebaron et al., 2012; Strunk et al., 2012). Future studies should reveal the fate of 40S subunits that fail this QC step, and whether the large ribosomal subunit is also monitored in such a step, for example, as part of the 25S NRD pathway.

When the nascent polypeptide is defective: Cotranslational protein QC pathways

Despite the presence of a variety of proofreading steps that serve to monitor the correct charging of tRNAs and delivery of cognate tRNAs during translation elongation (Zaher and Green, 2009; Yadavalli and Ibba, 2012), compared with the low mutation rates associated with DNA replication processes, mRNA translation is many orders of magnitude more error-prone, resulting in the continual production of incorrectly synthesized proteins (Drummond and Wilke, 2009). These defective translation products can arise from a plethora of sources including amino acid misincorporation by noncognate aminoacylated tRNAs, defective cotranslational protein folding, stop codon read-through, and ribosome elongation stalling, as well as from mRNA defects discussed earlier. The vectorial nature of protein biogenesis requires the activity of various protein homeostasis factors associated with actively translating ribosomes to facilitate the proper production of folded and active protein products (Kramer et al., 2009; Pechmann et al., 2013). For example,

N-terminal acetyltransferases, N-terminal aminopeptidases, and nascent chain–binding complexes physically interact with the emerging nascent chain and the ribosomal exit tunnel to both modify the potentially labile N terminus of the protein and shield the nascent chain from premature degradation before translation termination, protein folding, and assembly into protein complexes (Pechmann et al., 2013). Defective protein products can either be targeted for degradation as they arise from the ribosome, or after the emergence of an entire protein product that is otherwise unable to acquire a natively folded state. The combined activity of ribosome-associated chaperones and ubiquitin ligases play important roles in the targeting of both cotranslational and post-translational substrates for ubiquitylation followed by proteolysis, and as such, the principles governing post-translational protein QC pathways will likely apply to cotranslational degradation pathways.

Quantifying cotranslational ubiquitylation and degradation. The selective recognition and removal of defective translation products presents a potentially demanding task for protein homeostasis pathways. Initial studies examining the fate of newly translated proteins in mammalian cells suggested that as much as 30% of all nascent chains are targeted for ubiquitin-dependent proteasomal degradation (Schubert et al., 2000). Subsequent studies have suggested that a smaller portion of nascent chains are targeted for degradation and it is likely that the extent of nascent chain degradation is highly dependent upon the cellular and organismal context (Vabulas and Hartl, 2005; Yewdell and Nicchitta, 2006). Further, it is unknown what fraction of these degraded newly synthesized proteins is targeted cotranslationally. To begin to address this question, two recent studies directly quantified the extent of nascent chain ubiquitylation associated with ribosomes in *S. cerevisiae* and mammalian cells (Duttler et al., 2013; Wang et al., 2013). Studies in mammalian cells using puromycin labeling of nascent chains followed by capture of exogenously expressed ubiquitin indicated that 12–15% of nascent chains are ubiquitylated (Wang et al., 2013). Interestingly, the amount of cotranslational ubiquitylation was stimulated by addition of translation elongation inhibitors (Wang et al., 2013), suggesting that ribosome stalling in itself is a signal for nascent chain ubiquitylation. The extent of cotranslational ubiquitylation and the production of defective translation products are likely to vary between tissues and organisms depending on both the translational fidelity rates and the capacity of the recognition and degradation systems. Indeed, the extent of cotranslational ubiquitylation was lower in *S. cerevisiae* than in mammalian cells (Duttler et al., 2013). Utilization of genomic methods to identify transcripts that were enriched with ubiquitin-associated ribosomal complexes from *S. cerevisiae* extracts enabled the bioinformatic analysis of transcript and protein features that may direct ubiquitylation of defective nascent chains (Duttler et al., 2013). One finding from this analysis was that mRNA transcripts coding for longer proteins were enriched within ubiquitin-associated ribosomal complexes compared with the average protein length within the proteome. In agreement with this, early studies using synthetic substrates with destabilizing N-terminal amino acids (termed N-end rule substrates) demonstrated that large protein substrates based on β-galactosidase were targeted for cotranslational degradation to a greater extent than small substrates based on Ura3p (Turner and Varshavsky, 2000). Interestingly, in wild-type *S. cerevisiae* strains, 40% of the N-end rule β-galactosidase substrate was subject to cotranslational degradation, indicating that the extent of cotranslational ubiquitylation will likely largely depend upon the individual substrate being interrogated (Turner and Varshavsky, 2000).

Ribosome-associated protein QC pathways: Targeting polypeptides within stalled translation complexes. Whereas the study of mRNA QC pathways has historically focused on mechanisms of mRNA turnover, the fate of the protein products arising from defective mRNAs has been the subject of several recent studies. As discussed earlier, a subset of mRNA substrates for the NSD pathway are generated from premature polyadenylation within the protein coding region. On such mRNAs, the absence of a termination codon causes translation to continue into the poly(A)-tail and the addition of a polylysine tract to the nascent polypeptide. Recent studies have demonstrated that polybasic sequences cause ribosome stalling and cotranslational ubiquitylation and turnover of the native polypeptide (Ito-Harashima et al., 2007; Wilson et al., 2007; Kuroha et al., 2009; Bengtson and Joazeiro, 2010; Matsuda et al., 2014). This pathway is often referred to as NSD; however, evidence has shown that proteins containing polybasic tracts are targeted for degradation independently of whether they are produced from mRNAs that are substrates of the NSD pathway, or from non-NSD mRNAs that encode internal or terminal polybasic tracts regardless of whether the polybasic tract consists of lysines or arginines (Ito-Harashima et al., 2007; Dimitrova et al., 2009; Bengtson and Joazeiro, 2010; Kuroha et al., 2010). Moreover, recent evidence suggests that this pathway targets truncated polypeptides produced from mRNAs targeted for NSD or NGD, regardless of the presence of polybasic tracts (Tsuboi et al., 2012; Shao et al., 2013; Matsuda et al., 2014). Thus, an important question for future study is what is the specificity of this QC pathway? Does it target polypeptides associated with stalled translation complexes in general, or is it specific to a specific subset of polypeptides containing specific biochemical properties that lead to translational stalling, such as those containing polybasic tracts?

The Not4 component of the Ccr4–Not deadenylase complex was originally identified as an E3 ubiquitin ligase that targets polybasic-containing nascent chains (Dimitrova et al., 2009). Subsequent studies suggest that Not4 might play a role in the targeting of polypeptides produced from a subset of NGD substrates containing translation stall–inducing elements distant from the 3′ end, but not polypeptides produced from NSD substrates (Matsuda et al., 2014). Further, Not4 is likely involved in the ubiquitylation of other ribosome-associated substrates (Panasenko et al., 2009; Panasenko and Collart, 2012). Recently, the ubiquitin ligase Ltn1 was demonstrated to cotranslationally ubiquitylate and activate degradation of proteins containing polybasic tracts (Wilson et al., 2007; Bengtson and Joazeiro, 2010), as well as other polypeptides produced from NGD or NSD substrate mRNAs (Figs. 3 and 4; Shao et al., 2013; Matsuda et al., 2014). Ltn1 was shown to physically associate

with both the targeted polypeptide and 80S or 60S ribosomal particles in density gradient centrifugation experiments (Bengtson and Joazeiro, 2010; Brandman et al., 2012). This suggests that polysomal collapse into monosomes may precede ubiquitylation and extraction of defective translational products. Recent studies have described a larger Ltn1-containing ribosome QC complex (RQC) comprised of Ltn1, two scaffolding proteins, Tae2 and Rqc1, and the AAA ATPase Cdc48 (Fig. 4; Brandman et al., 2012; Defenouillère et al., 2013). Although the RQC was subsequently demonstrated to be required for the cotranslational degradation of model polybasic-containing proteins, it is worth noting that the complex was originally identified in a yeast genetic screen aimed at identifying novel modulators regulating the activity of the thermal stress-inducible transcription factor, Hsf1 (Brandman et al., 2012). This suggests that the RQC may play a more general role in sensing protein homeostasis stress at the level of the ribosome and communicating the ribosomal stress signal to Hsf1. The observation that the Rqc1 component of the RQC contains its own polybasic sequence suggests an interesting feedback loop where RQC activity controls its own abundance through Rqc1 targeting. Indeed, Rqc1 levels are elevated in Ltn1- and Tae2-deficient strains (Brandman et al., 2012). The same genetic screen identified a second ubiquitin ligase, Hel2, as well as the previously implicated Asc1 (Kuroha et al., 2010) as proteins that participate in polybasic-containing polypeptide destruction. Hel2 was also identified in a separate study as a ubiquitin ligase that, when deleted, reduced the amount of ribosome-associated polyubiquitylated polypeptides (Duttler et al., 2013). Deletion of Hel2 or Asc1 results in the stabilization of polybasic-containing polypeptides despite the presence of wild-type RQC function (Brandman et al., 2012). Other studies have demonstrated that loss of Asc1 results in the increased accumulation of full-length protein from NGD reporter mRNAs (Kuroha et al., 2010; Matsuda et al., 2014). This observation is in contrast to the accumulation of shorter stalled polypeptide products observed upon loss of Ltn1 or Not4 that occurs without accumulation of the full-length protein. This suggests that Hel2 and Asc1 promote ribosome stalling and may be initially recruited to stalled ribosomes before monosome collapse and recruitment of the RQC (Fig. 4). However, complete definition of this model will require further experimentation.

The ubiquitylated nascent chain must be extracted from the ribosome to allow for ribosome recycling. The presence of the AAA ATPase Cdc48 (VCP/p97 in mammals) within the RQC immediately suggested that Cdc48 might provide the force necessary to extract defective translation products from stalled ribosomal complexes, allowing for ribosome recycling. Consistent with this, loss of Cdc48, or its associated proteins Npl4 and Ufd1, results in the accumulation of ubiquitylated polypeptides associated with ribosomes in excess to what was observed with loss of proteasome activity (Verma et al., 2013), although given the role of Cdc48 in the 25S NRD pathway (Fujii et al., 2012) a portion of the ubiquitylated material could have originated from the ubiquitylation of ribosomal subunits. Interestingly, inactivation of Cdc48 also results in the accumulation of both full-length polybasic proteins and a peptidyl-tRNA linked product (Verma et al., 2013). This suggests that Cdc48 activity is needed to extract the peptidyl-tRNA product from stalled ribosomal complexes before hydrolysis of the peptidyl-tRNA bond and subsequent degradation of the defective translational product. It is possible that Cdc48 plays a more general role in targeting diverse defective nascent chain substrates, in addition to polybasic substrates. In agreement with this, loss of Cdc48 or Ufd1, but not Ltn1, results in stabilization of a truncated protein produced from an NMD mRNA substrate (Verma et al., 2013). Interestingly, the ubiquitin ligase Ubr1 was also demonstrated to target the truncated protein product from an NMD mRNA substrate, arguing that a combination of ligases likely act to remove diverse defective nascent chain substrates (Inada, 2013). Although studies suggest that Cdc48 recruitment into the RQC requires Ltn1 and Rqc1, a separate Cdc48-containing complex may target non-NSD and -NGD substrates.

The order of events leading to eventual defective translation product removal is not fully understood. Early experiments indicated that the RQC associated with both 80S and 60S subunits in density gradient experiments (Bengtson and Joazeiro, 2010; Brandman et al., 2012; Defenouillère et al., 2013; Verma et al., 2013). Recent in vitro studies suggest that Ltn1 primarily associates with the 60S ribosomal subunit in a manner that is stimulated by ribosomal stalling (Shao et al., 2013). Further, Ltn1 recruitment requires the activity of Hbs1 (Shao et al., 2013), and biochemical studies have revealed that ribosome release by the Hbs1–Dom34–Rli1 complex occurs in the absence of cleavage of the peptidyl-tRNA linkage and results in the retention of the peptidyl-tRNA product with the released 60S ribosome (Shoemaker et al., 2010; Pisareva et al., 2011; Shoemaker and Green, 2011, 2012). Taken together, these observations suggest a model in which ribosomal stalling leads to monosome collapse and subsequent ribosome splitting by Hbs1–Dom34–Rli1, which acts to recruit Ltn1, and presumably the RQC, to free 60S particles containing a peptidyl tRNA-linked defective nascent chain (Fig. 4). Structural studies revealed that Ltn1 adopts an extended flexible structure similar to other HEAT repeat–containing proteins (Lyumkis et al., 2013). This type of structure could allow Ltn1 to make contacts with the ribosomal region surrounding the nascent chain exit tunnel and distant 60S regions within an individual 60S or 80S ribosomal complex. Detailed structural analysis of an Ltn1-bound ribosome should shed more light on possible RQC recruitment mechanisms.

Conclusions and perspectives

The emerging picture is that of a complex set of QC pathways dedicated to monitor the translation process in eukaryotes (Figs. 1–4). Given the number of QC pathways that have evolved to monitor this process, there appears to be high selective pressure on the ability to resolve protein synthesis defects. This point is further supported by the existence of related pathways in bacteria (Moore and Sauer, 2007). Most of the eukaryotic cotranslational QC pathways have been studied by introducing mutations or specific elements into RNAs that cause stalls in the translation process. Important areas of future studies include measuring the extent to which stalled translation complexes occur as a result of naturally occurring RNA damage, for example from irradiation or chemical modification, such as by reactive

oxygen species, and the importance of QC pathways for resolving such events. Another important question is whether defects in components of the translation machinery other than mRNAs and the 18S rRNA decoding center activate cotranslational QC. For example, decay pathways that target defective tRNAs before their entry into the translation cycle have been described (Alexandrov et al., 2006; Wilusz et al., 2011); could defects in tRNAs occur that stall translation complexes and cause cotranslational QC? Similarly, could defects in translation initiation, elongation, or termination factors, or in ribosomal proteins or rRNA regions additional to the 18S rRNA decoding region be targets of cotranslational QC pathways?

In the case of cotranslational native polypeptide QC pathways, it is worth noting that the majority of studies have relied upon synthetic model substrates containing a defined lesion within the mRNA. These synthetic substrates have been instrumental in the identification of a subset of the machinery targeting defective translational products in *S. cerevisiae*. However, these same substrates have been infrequently used in mammalian studies and it is unclear if this small subset of defined model substrates faithfully represents all the features that typify endogenous defective translation products. In support of the idea that more pathways have yet to be discovered, knockdown or genetic loss of known ribosome-associated ubiquitin ligases did not result in a significant reduction in ubiquitylated nascent chains associated with ribosomes in *S. cerevisiae* or mammalian cells (Duttler et al., 2013; Wang et al., 2013). This result suggests that a combination of ubiquitin ligases, both known and unknown, likely contribute to defective nascent chain ubiquitylation. Systematic studies using both diverse model substrates and endogenous defective nascent chain substrates are needed to clearly define the breadth of substrates targeted by individual ribosome-associated QC components.

Another key topic for future study is the specific mechanisms by which defective nascent polypeptides are detected by QC pathways, and whether there are central components that tie together RNA and protein QC pathways. The selective recognition of mRNA decay substrates, the recruitment of mRNA decay factors, and the subsequent cleavage and destruction of the target mRNAs are central features of all mRNA QC pathways. Analogous events must also occur for the selective destruction of defective protein products. One possibility is that defective nascent chain–targeting machineries may use mRNA QC factors to identify terminally stalled ribosomal complexes and activate subsequent defective translation product removal. Conversely, defective features of the nascent chain may serve as the recognition event to recruit mRNA decay factors. An example of this was recently reported for nascent chains that contain mutations within their N-terminal signal sequences rendering them incapable of binding to the signal receptor particle (SRP). The mutated signal sequence generated a signal to degrade the mRNA in a manner dependent on the RNA silencing protein Ago2 (Karamyshev at al. 2014). This example suggests that careful determination of the codependence of mRNA and defective nascent polypeptide QC factors for proper targeting of the mRNA or nascent polypeptide is needed to understand the mechanisms used to recruit QC factors to terminally stalled ribosomal complexes. Finally, an important question is, to what extent are fully functional components of stalled translation complexes recycled? Does a stall in translation generally lead to degradation of all components of the translation complex, or are QC pathways capable of identifying and degrading specifically those components that are defective while recycling those components that remain functional? Future research should establish the responsible factors and detailed mechanisms of the cotranslational mRNA, ribosome, and native polypeptide QC pathways and to what extent they act cooperatively to ensure faithful clearance of potentially harmful defective translational products and machinery without compromising the performance of fully functional factors.

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