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Ubiquitination of Newly Synthesized Proteins at the Ribosome

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Abstract

Newly synthesized proteins can be misfolded or damaged because of errors during synthesis or environmental insults (e.g., heat shock), placing a significant burden on protein quality control systems. In addition, numerous human diseases are associated with a deficiency in eliminating aberrant proteins or accumulation of aggregated proteins. Understanding the mechanisms of protein quality control and disposal pathways for misfolded proteins is therefore crucial for therapeutic intervention in these diseases. Quality control processes function at many points in the life cycle of proteins, and a subset act at the actual site of protein synthesis, the ribosome. Here we summarize recent advances in the role of the ubiquitin proteasome system in protein quality control during the process of translation.

1. Introduction

Maintaining an intact and functional proteome is a crucial and challenging task for a cell. Every human cell contains ~2 billion protein molecules, ranging in size from 3 kDa to 3800 kDa [1]. To synthesize and maintain a proteome at this scale, approximately three million ribosomes work constantly at a translation rate of six amino acids per second [1–3]. Protein synthesis and maturation is highly complicated, requiring over 400 proteins and consuming up to 75% of the total cellular energy budget [4–6]. In order to obtain their functional states, nascent proteins must attain specific three-dimensional conformations. In many cases successful protein folding may require co- or post-translational protein modifications, binding partners, or specific intracellular localization [7].

Protein synthesis is an error prone process [8, 9]. The rate of amino acid mis-incorporation during protein translation is one in every 5,000–10,000 amino acids in eukaryotic cells [10–14]. Given that the average length of human proteins is approximately 550 amino acids, roughly 5%–10% of proteins of average length would be expected to contain at least one mis-incorporated amino acid [1]. In addition to these errors during protein synthesis, newly synthesized proteins are more sensitive to environmental changes such as elevated

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temperature and increased reactive oxygen species (ROS) [15]. As a result, a significant fraction of newly synthesized proteins never attain their functional state, even with the help of molecular chaperones. This generates a continuous stream of misfolded proteins to be dealt with by quality control monitoring and disposal systems. One observation that suggests that newly synthesized proteins represent a major burden on these systems is that inhibition of protein synthesis prevents the accumulation of ubiquitinated proteins one normally sees when cells are treated with proteasome inhibitors [16]. This further suggests that quality control pathways for newly synthesized proteins may be crucial for therapeutic intervention in protein misfolding-related diseases, including neurodegenerative diseases, type 2 diabetes, cystic fibrosis, peripheral amyloidosis, cancer, and cardiovascular disease [17–19].

Eukaryotes have evolved two major pathways to eliminate aberrant proteins: the ubiquitin-proteasome system (UPS) and autophagy. The UPS is the major pathway for elimination of misfolded proteins in eukaryotic cells [20, 21]. Substrates of the UPS are marked with ubiquitin and subsequently delivered to the 26S proteasome for degradation [22]. Conjugation of ubiquitin to the substrate requires three types of enzymes: ubiquitin activating enzymes (E1s), ubiquitin conjugating enzymes (E2s), and ubiquitin ligases (E3s) (Figure 1) [23, 24]. Ubiquitin is first activated by E1 enzyme in an ATP-dependent manner, and then transferred to E2 enzyme. Following the binding of the ubiquitin-charged E2 to an E3 enzyme, the ubiquitin on the E2 is used for ubiquitination of E3-bound substrates [24]. E3 enzymes of the UPS are responsible for substrate recognition and can selectively recognize (with or without chaperones or adaptors) and target soluble misfolded proteins for degradation, before they have the potential to form aggregates. Alternatively, proteins can be degraded via autophagy, a process that occurs within the lumen of lysosomes. Several types of autophagy exist and are distinguishable by the manner in which proteins are delivered to the lysosome: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Although autophagy was originally thought to be a nonspecific process, selective degradation by autophagy has been shown for both macroautophagy and CMA (via the chaperone Hsc70) [25]. In the case of CMA, the chaperone Hsc70 identifies and delivers proteins containing a distinct motif (KFERQ-like) [25].

2. Co-translational protein quality control pathways

Up to 30% of total newly synthesized proteins in eukaryotic cells have been reported to be very rapidly degraded in a proteasome-dependent manner [26]. Although a subsequent study argued that the percentage is much lower (~15%), there is general agreement that the rapid degradation of newly synthesized proteins is UPS-dependent [27]. Since polypeptides normally cannot complete folding until they are fully synthesized and released from ribosomes, the rapid degradation of newly synthesized proteins has generally been assumed to occur post-translationally (i.e., after release from ribosome), following, for example, failure of chaperone-assisted folding mechanisms (Figure 2) [18]. However, increasing evidence suggests that protein synthesis can in some cases be tightly coupled with ubiquitination and degradation of nascent polypeptides.

Two studies observed co-translational ubiquitination in an *in vitro* rabbit reticulocyte translation system. The substrates were the cystic fibrosis transmembrane conductance

regulator (CFTR), a very large protein prone to misfolding, and the secretory protein ApoB (Apolipoprotein B100) [28, 29]. Proteasomes have also been reported to be associated with the translation machinery, suggesting that both ubiquitination and proteasomal degradation may occur on the surface of the ribosome [30]. Furthermore, a proof-of-principle study using the “ubiquitin sandwich” technique showed that an engineered protein bearing an amino-terminal (N-end) degradation signal could be degraded co-translationally in *S. cerevisiae* [31]. The extent, specificity, and biologic significance of co-translational ubiquitination and degradation, however, remained largely unknown.

Two studies established that a significant amount of ubiquitination of ribosome-associated nascent chains occurs in cells in both yeast and mammalian cells. An estimation of the fraction of ribosome-associated nascent chains that are ubiquitinated was made in both cases [32, 33]. Using puromycin labeling of nascent chains, 12–15% of nascent chains were determined to be ubiquitinated co-translationally in mammalian cells [33]. This and another study showed that the extent of co-translational ubiquitination (CTU) is lower in *S. cerevisiae* cells, with 1–6% of nascent chains being ubiquitinated [32, 33]. Further analyses of these nascent chains indicated that they were primarily modified with K48-linked ubiquitin and they can be subject to degradation by the proteasome. Interestingly, the CTU level was enhanced approximately 50% under conditions that promoted protein misfolding or translational errors, consistent with the hypothesis that CTU reflects a quality control pathway that monitors the state of nascent polypeptides [32, 33].

As mentioned above, errors during protein synthesis or translation of defective mRNAs can lead to the generation of aberrant protein products [8, 9]. A subset of these errors may result in translational stalling, while others, such as nascent chain misfolding, have no effect on protein translation [34]. Thus, aberrant translation products can be present in both stalled and active translation complexes. Utilization of pactamycin, an inhibitor of translation initiation that results in run-off of active translation complexes, confirmed that CTU occurs on stalled complexes (referred to as CTU^S), as previously proposed [35–37]. However, these results also indicated that the majority of CTU (approximately 2/3) occurs in active translation complexes (referred to as CTU^A) (Figure 3).

3. Co-translational ubiquitination on stalled translation complexes (CTU^S)

Recycling of stalled translation complexes has been widely investigated in the context of mRNA quality control pathways [34, 38]. Several recent studies have addressed the more specific question of how aberrant nascent proteins within stalled translation complexes are cleared and degraded [35–37, 39, 40]. There are at least three forms of co-translational mRNA quality control pathways: nonsense-mediated decay (NMD), no-go decay (NGD), and nonstop-decay (NSD). NMD, the first discovered co-translational mRNA quality control pathway in eukaryotic cells, is activated by the translation of mRNAs containing premature termination codons (PTCs) [41, 42]. NSD and NGD pathways were identified as necessary for degradation of “non-stop codon” mRNAs (i.e., mRNAs lacking stop codons) and damaged mRNAs that cause translational stalling, respectively [43–45].

mRNA features that can lead to translational stalling and activate NGD or NSD pathways include inhibitory secondary structures, rare codons, and mRNA degradation or truncation [34]. Stalled translation complexes arising in NGD and NSD pathways will not contain a termination codon at the A site of ribosomes. In this case, the translation releasing factors eRF1 and eRF3 cannot be recruited to recycle the stalled translation machinery. Instead, two homologous factors, Hbs1 and Pelota (Dom34 in yeast), recognize these types of stalled complexes following mRNA cleavage and resolve them with the cooperation of the ATPase ABCE1 (Rli1 in yeast) [46, 47]. ATP hydrolysis by ABCE1 drives the separation of 80S into 40S and 60S subunits [46, 48]. Importantly, Pelota/Dom34 cannot catalyze hydrolysis of the tRNA ester bond to release the nascent peptide [47, 48]. As a result, a 60S-peptidyl-tRNA complex is generated after Hbs1-Pelota mediated disassembly of a stalled ribosome complex. As discussed in the next paragraph, this 60S-peptidyl-tRNA complex provides a unique marker for recruiting a ubiquitin ligase to target the nascent chain for degradation.

Ltn1 has been shown to promote the degradation of ribosome-associated nascent peptides generated from NSD and NGD pathways [35, 36]. Ltn1 associates only with the 60S subunit of the ribosome, and not 80S ribosomes or polysomes [35, 36, 49]. Consistent with this, Ltn1-dependent nascent protein ubiquitination is reliant on ribosome subunit dissociation [50]. In an elegant study, the Hegde group recently successfully reconstituted ribosome-associated ubiquitination with purified factors *in vitro* [51]. They found that the primary role of the ribosome splitting factors Hbs1, Pelota, and ABCE1 is to allow Ltn1 access to the nascent chain, and these splitting factors can be bypassed by artificially removing the 40S subunit. This suggests that steric hindrance impedes Ltn1 recruitment to 80S ribosomes [51], and this was consistent with the Ltn1-60S ribosome cryo-EM structure (discussed below). In addition, this study confirmed that Ltn1 is the E3 ligase that ubiquitinates nascent chains within the 60S-peptidyl-tRNA complex by using two independent and unbiased strategies: an activity-based purification strategy and a substrate-based co-purification strategy [51].

Cryo-EM structural studies revealed that Ltn1 adopts a long and flexible structure similar to other HEAT repeat-containing proteins, such as exportin and the cullins [51–53]. Two of the initial studies determined that the structure of Ltn1 allows one end (the C-terminus) to bind the region of the ribosome near the nascent chain exit tunnel, and the other end (the N-terminus) to contact the region of the 60S that interfaces with the 40S subunit [51, 53]. This structure simultaneously explains the restriction of Ltn1 to the 60S ribosome (because the 40S subunit would block its binding), as well as why Ltn1 functions only in the CTU^S pathway (because it functions downstream of ribosome splitting, which only occurs after recognition of stalled translation complexes). In addition, the orientation of the C-terminal RING domain of Ltn1 near the exit tunnel puts it in the ideal place to ubiquitinate the trapped nascent chain, as the RING domain is responsible for recruiting a ubiquitin-charged E2 [52, 53].

More recent studies revealed that Ltn1 is a component of a ribosome-associated quality control complex called the RQC, which is composed of two additional scaffolding proteins, Tae2 and Rqc1, and the AAA ATPase Cdc48/p97 and its co-factors [36, 37]. Ltn1, Tae2 and Rqc1 are able to associate with the 60S subunit independently, whereas recruitment of CDC48/p97 requires Tae2, Rqc1, and Ltn1-mediated ubiquitination of nascent chains,

suggesting that the CDC48/p97 is recruited downstream of the ubiquitination of nascent chains [36]. Given the ‘segregase’ activity of CDC48/p97 in the UPS [54], CDC48/p97 has been proposed to extract ubiquitinated nascent chains from 60S-peptidyl-tRNA complex and deliver them for proteasomal degradation [36, 37]. Although independent of each other, Tae2 has been found to stabilize the binding of Ltn1 to ribosomes [55]. In agreement with this, ubiquitination and degradation of nonstop substrates is reduced in the absence of Tae2 [36, 55]. The structure of a native 60S-RQC complex revealed an additional density bridging the N terminus of Ltn1 with the tRNA at the P site of the ribosome that disappeared upon Tae2 deletion. This suggested that Tae2 facilitates the selective binding of Ltn1 to the 60S-peptidyl-tRNA complex, but not to empty 60S subunits [53]. This was confirmed by recent structural analysis of purified nascent chain-containing 60S-Listerin-NEMF complexes [56]. NEMF (the mammalian Tae2 homolog) was found to make multiple contacts with the 60S and peptidyl-tRNA allowing it to both sense stalled 60S and form an optimal binding site for the N terminus of Ltn1.

In summary, these recent studies sketch a working framework for elimination of stalled nascent chains by the Ltn1-dependent RQC pathway. This process is initiated by translational stalling, leading to the recruitment of nuclease(s) and the digestion of the mRNA. The resulting 80S complex is then targeted by the Hbs1/Pelota/ABCE1 recycling system to produce a 60S-peptidyl-tRNA complex that first recruits Tae2 followed by Ltn1 to ubiquitinate the nascent chain. The ubiquitinated nascent chain is then extracted from the 60S subunit and delivered for proteasomal degradation by the CDC48/p97 complex (Figure 4). It is worth noting that while we have referred to this pathway as the CTUS^S pathway, it is not in fact co-translational in the sense that the nascent chains are being ubiquitinated downstream of disassembly of the ribosome. Nevertheless, the importance of this Ltn1-dependent pathway is highlighted by the phenotype observed in Ltn1 mutant mice. Mutation of mouse Ltn1 homolog, LISTERIN, resulted in early-onset and progressive neurological and motor dysfunction, suggesting that these phenotypes are the result of a loss in turnover of defective ribosome-associated polypeptides [57].

While Ltn1 is the only E3 shown to directly ubiquitinate stalled nascent chains, several other E3 ligases including Hel2, Upf1, Ubr1, and Not4 have been proposed to play a role in the quality control of stalled complexes [36, 37, 39, 58, 59]. Hel2 is a polysome-associated ubiquitin E3 ligase that, when deleted, reduced the level of ribosome-associated ubiquitinated nascent chains [32]. In addition, the *hel2* mutant was found to stabilize a full-length GFP/RFP reporter protein in the presence of a functioning RQC complex [36]. This observation suggests that Hel2 functions in promoting translation stalling at codons of polybasic residues, and may perform its function upstream of 80S ribosome disassembly. The functions of the remaining E3s are less clear. Both Upf1 and Ubr1 have been shown to stimulate degradation of stalled nascent chains arising from mRNAs containing a PTC [37, 58, 60]. In addition, deletion of Ubr1 reduces accumulation of endogenous, tRNA-linked ubiquitinated nascent polypeptides on ribosomes in *cdc48* mutant cells [37]. Not4 is a component of the multi-functional CC4-Not complex, a complex with both ubiquitination and deadenylation activities that has been linked to transcriptional regulation, mRNA degradation, and proteasome assembly [61]. Deletion of Not4 by various groups has had

conflicting results: one group demonstrated stabilization of polybasic reporter proteins in *not4* mutant yeast cells [39] whereas another group demonstrated an increase in co-translational ubiquitination and proposed this was due to a decrease in mRNA quality control [32]. Alternatively, deletion of Not4 could be impacting proteasome function leading to increased co-translational ubiquitination [62, 63].

4. Co-translational ubiquitination on active translation complexes (CTU^A)

As described above, the pathway for degradation of nascent chains on stalled translation complexes is well on the way to being elucidated. In contrast, enzymes and factors that influence the recently described process of CTU within active translation complexes (CTU^A) remain largely uncharacterized. Translation of mRNAs containing some subtle changes or damage, such as single base substitutions, could have significant effects on protein folding without affecting translation elongation. In addition, even if a polypeptide is translated faithfully, there is no guarantee that folding will proceed properly. Therefore, it is expected that, like stalled translation complexes, active translation complexes will also contain aberrant protein products.

There are several lines of evidence indicating that CTU can occur on translating ribosomes. First, nascent chains that were ubiquitinated *in vivo* could be translated to completion *in vitro* when added to a reticulocyte lysate system [33]. Second, a short treatment with pactamycin, a translation initiation inhibitor, was used to run off active translation complexes *in vivo*; if all CTU occurred on stalled ribosomes, pactamycin would be expected to have little effect on the level of ubiquitinated nascent chains. However, a brief pactamycin treatment resulted in the loss of approximately two-thirds of all ubiquitinated nascent chains from polysomes [33], suggesting that the majority of ubiquitinated nascent chains are in active translation complexes. Third, CTU levels were enhanced by treatments expected to enhance protein misfolding without affecting translation elongation, including 1) an amino acid analog (L-azetidine-2-carboxylic acid, a proline analog with a four-carbon ring), 2) Hsp70 inhibitors, and 3) depletion of nascent polypeptide-associated complex proteins (NAC), a ribosome associated chaperone important for co-translational protein folding [32, 33]. These results strongly suggest that the CTU pathway can target misfolded nascent chains while translation is occurring. Interestingly, the translation elongation rate can impact the generation of correctly folded proteins. A recent study reported that a reduced elongation rate actually increases translation fidelity and therefore the quality of nascent chains [64]. This may be because 1) the reduced translation rate gives a nascent chain more time to attain a proper configuration during translation, and/or 2) because the total translational load is reduced thus allowing the protein folding machinery (chaperones) to more fully engage the proteins that are being translated. Our unpublished data (F.W., J. M. H.) has shown that CTU levels are also decreased when translation elongation rates are reduced, further supporting the idea that the CTU pathway can target misfolded nascent chains on translating ribosomes.

Protein quality control systems often recognize misfolded proteins on the basis of properties such as exposure of long stretches of hydrophobic residues. Since nascent chains must share this property, to some extent, with misfolded proteins, it remains unclear how a misfolded

nascent chain is distinguished from a “normal” nascent chain. Normally polypeptides cannot complete folding until they are fully synthesized and released from the ribosome, whereas protein domains can be relatively independent folding units, and can often form stable and compact three-dimensional structures. One model is that polypeptides undergo “domain-wise” co-translational quality surveillance, corresponding to individually folding domains [65, 66]. In this model, a protein domain, rather than the full length protein, is the minimal quality control unit. As a result, the nascent polypeptide containing at least one misfolded domain may make that protein the target of the CTU^A pathway. Consistent with this hypothesis, large proteins with multiple domains were highly enriched among identified total CTU targets, whereas proteins shorter than 300 amino acids were largely excluded (the length of a domain is between 50–300 amino acids) [32, 33].

The ligases for CTU^A have not yet been identified. Several E3 ligases, which are known to function in elimination of misfolded proteins, have been tested for their involvement in CTU^A in human cells. These include CHIP, an Hsp70-associated RING E3 [67], Ubr1, a ligase that is involved in ER-associated protein degradation (ERAD) [68] and co-translational degradation of artificial proteins bearing destabilizing N-terminal residues [31], and Ube3C/Hul5, which is suggested to target misfolded cytosolic proteins [69]. Deletion of any of these individual ligases did not lead to a significant reduction in total CTU levels, suggesting that none of them plays a predominant (or perhaps any) role in the CTU^A pathway. Duttler and colleagues tested a panel of candidate CTU ligases in yeast in single gene deletion strains, and also found that no single E3 deletion caused a dramatic reduction in the total CTU level. However, small reductions (5%–10%) were observed in multiple single deletion strains [32]. It is possible that there are multiple E3 ligases that function in the CTU^A pathway, with each recognizing a specific subset of CTU^A substrates, or that some of them may function redundantly.

The putative CTU^A E3 ligase(s) may recognize misfolded nascent chains in at least two broad ways: either via an interaction with ribosomes (akin to Ltn1), or by direct recognition of nascent chains. In the first model, ligase(s) associated with actively translating ribosomes might evaluate nascent chains at early stages of folding as they emerge from the exit tunnel. This model is similar to that proposed for the co-translational modification of newly synthesized proteins by ISG15, an interferon-induced ubiquitin like protein [70]. In this case, the ISG15 ligase, Herc5, co-fractionates with polysomes by interaction with the 60S subunit, with nascent chains being ISGylated stochastically as they emerge from the exit tunnel. In the second model, the ligase(s) might monitor fully exposed ribosome-associated nascent chains, either with or without the cooperation of chaperones, without ever directly interacting with the ribosome. It has been reported that some E3 ligases dedicated to protein quality control, such as CHIP, Ubr1 and two ER-anchored ligases, Hrd1 and Doa10, employ chaperones to recognize misfolded substrates. This might suggest that CTU^A ligases also employ accessory folding factors to distinguish misfolded from properly folded nascent chains [71, 72]. Alternatively, a recent study revealed that the nuclear ligase, San1, is capable of recognizing a broad range of distinctly misfolded proteins on its own [73]. San1 contains intrinsically disordered N- and C-terminal domains with discontinuous highly ordered and conserved small segments, and these serve as its substrate recognition motifs [73].

Perspectives

While much progress has been made in characterizing the Ltn1-dependent CTU^S pathway, some questions remain. For example, while total CTU products have been analyzed, the endogenous and predominant substrates of the CTU^S pathway remain unknown. It is also worth noting that while approximately 20% of all ribosomes appear to be in stalled complexes (including both transiently and permanently stalled complexes), only a fraction of these stalled complexes (15%–18%) contained ubiquitinated nascent chains [33], suggesting there are multiple types of stalled complexes and possibly multiple responses to stalled complexes. This is consistent with the model that a portion of stalled complexes may provide a regulatory role in protein translation at a post-initiation level [74]. This observation raises an important question: how does the quality control system distinguish ‘unnaturally’ stalled nascent chains needing to be disposed, from transient translation pausing with potential regulatory functions? This question has been partially answered by a recent study that profiled *in vivo* substrates of Dom34 with a ribosome profiling technique [75]. This study revealed that Dom34 targets ribosomes stalled on truncated mRNAs, rather than ribosomes that are transiently paused, for example, at polyproline stretches [75].

There are many remaining questions to be addressed in the CTU^A pathway. Similar to the CTU^S pathway, the endogenous substrates of CTU^A pathway remain unknown. In addition, the E3 ligases are currently unknown. Successful identification of CTU^A E3 ligases is a requirement to define the molecular mechanism of recognizing misfolded nascent chains in the CTU^A pathway. In addition to E3 ligases, it remains unclear whether other unidentified factors are required for the CTU^A pathway. Unlike the RQC pathway, which requires ribosome splitting to permit the ubiquitination machinery to efficiently access the nascent chain, the CTU^A pathway has the potential to target nascent chains without restructuring of the ribosome. This further suggests that CTU^A involves factors distinct from those involved in the RQC pathway.

The existence of ubiquitin-mediated degradation events occurring on ribosome-associated nascent chains is a fascinating example of a highly intertwined relationship between two highly complex enzymatic systems. In the case of CTU^A, the timing of both events – translation elongation and polyubiquitination – raises important questions regarding the kinetics of translation elongation versus the kinetics of recognition and ubiquitination of misfolded proteins. Characterization of this pathway has the potential to greatly influence our understanding of the generation and clearance of misfolded proteins, as well as the influence of newly synthesized proteins in protein folding diseases.

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Highlights

- Newly synthesized proteins place a significant burden on quality control systems
- Quality control mechanisms exist that engage ribosome-associated nascent polypeptides
- Ltn1 mediates ubiquitination of nascent chains downstream of ribosome stalling
- Ubiquitination of nascent chains also occurs in active translation complexes

Figure 1. The ubiquitin conjugation pathway

Ubiquitin is conjugated to substrate via E1–E2–E3 enzyme cascades. In human cells, there are two E1s, approximately 60 E2s, and over 600 E3s [76]. This large pool of E3 ligases defines the substrate specificity of ubiquitin conjugation pathway. E3 ligases are classified into two major groups, the RING E3s and HECT (Homologous to E6AP C-Terminus) E3s. The RING E3s function, minimally, as scaffold proteins, facilitating the transfer of ubiquitin directly from E2 to substrate. In the case of HECT E3 ligases, ubiquitin is transferred from the charged E2 to the active site cysteine of HECT E3, and from the E3 to the substrate.

Figure 2. Classic post-translational model for quality control of newly synthesized proteins

This model represents quality control of newly synthesized proteins initiating post-translationally, after nascent polypeptides have been released from ribosomes. If polypeptides cannot fold into their native structures, even with the help of chaperones, E3 ligase(s) will be recruited to target them for proteasomal degradation. Alternatively, excess misfolded/unfolded proteins may form protein aggregates, which may be eliminated by autophagy.

Figure 3. CTU occurs in both active (CTU^A) and stalled (CTU^S) translation complexes
 CTU has been reported to occur in both active and stalled translation complexes, with the majority of CTU (approximate 2/3) occurring in active complexes. CTU^A has been proposed to target misfolded nascent chains that are being actively translated, whereas CTU^S is proposed to ubiquitinate nascent chains arising from irreversibly stalled translation complexes.

Figure 4. Model for elimination of stalled nascent chain by the Ltn1-dependent RQC pathway
 Various types of mRNA defects and features may lead to a stalled translation complex. The NSD and NGD pathways can recognize the stalled complex and degrade the mRNA fragment downstream of the stalled ribosome. The stalled 80S ribosome-nascent chain complex is targeted by the recycling factors (Hbs1, Pelota, ABCE1) to generate a 60S-peptidyl-tRNA complex. Tae2 (NEMF) recognizes and binds the 60S-peptidyl-tRNA complex allowing Ltn1 to form a stable interaction and ubiquitinate the stalled nascent polypeptide. Proteasomal degradation of ubiquitinated nascent chains is dependent on the activity of Cdc48/p97 complex.