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Review

Design Principles of Protein Biosynthesis-Coupled Quality Control

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The protein biosynthetic machinery, composed of ribosomes, chaperones, and localization factors, is increasingly found to interact directly with factors dedicated to protein degradation. The coupling of these two opposing processes facilitates quality control of nascent polypeptides at each stage of their maturation. Sequential checkpoints maximize the overall fidelity of protein maturation, minimize the exposure of defective products to the bulk cellular environment, and protect organisms from protein misfolding diseases.

Cells have extensive surveillance systems to detect errors during the biosynthesis of essentially all of its major macromolecules. This includes DNA replication (Reha-Krantz, 2010), transcription (Sydow and Cramer, 2009), translation (Zaher and Green, 2009), and maturation of mRNAs (van Hoof and Wagner, 2011), tRNAs (Yadavalli and Ibba, 2012), and proteins (Buchberger et al., 2010). Each of these biosynthetic processes has intrinsic limits on overall fidelity, resulting in a low but tangible rate of errors. In addition to biosynthetic errors, environmental insults such as ionizing radiation, reactive oxygen species, and temperature fluctuations result in damage to cellular macromolecules. Cells therefore face a constant barrage of defective or damaged macromolecules that, if left unresolved, have the potential to disrupt cellular homeostasis, reduce fitness, cause disease, and contribute to aging. Thus, there is a strong selective pressure to detect defective macromolecules and either correct or dispose of them.

The potentially disruptive nature of defective macromolecules places a premium on early detection and rapid resolution. This explains why many quality control processes have evolved to act at the site of biosynthesis, before an erroneous product is released and can engage downstream cellular pathways. The most obvious examples include internal proofreading by DNA polymerases (Reha-Krantz, 2010) and kinetic proofreading during translation (Zaher and Green, 2009). Less obvious is the exploitation of compartments, such as the nucleus and endoplasmic reticulum (ER), for maturation of nascent RNAs and proteins in a protected environment before their regulated trafficking to their site of function.

These early-acting quality control systems coexist with partially redundant downstream mechanisms. For example, proofreading mechanisms during replication are complementary to DNA mismatch repair that deal with errors after they have occurred. Similarly, fidelity of decoding during translation is combined with postsynthesis quality control of misfolded proteins to avoid defects. Thus, numerous quality control mechanisms leading from DNA to functional protein each make unique, overlapping contributions to minimize the error rate of this complex process. The physiologic relevance of each contribution is evidenced by the numerous protein misfolding and neurodegenerative diseases that result when these quality control processes fail (Balch et al., 2008).

In this Review, we examine the concept of biosynthesis-coupled quality control during protein maturation. After a brief historical perspective on the initial development of this field, we discuss several examples of interactions between various biosynthetic machinery and degradation factors. This includes interactions between ribosomes, targeting factors, and chaperones with the ubiquitin-proteasome system. Although the relevance of some of these interactions for quality control is not fully established, their consideration at this juncture is nevertheless worthwhile for providing a conceptual framework for this emerging area and highlighting key questions for future research.

Evolving Concepts of Protein Quality Control

Nascent proteins must fold into their final three-dimensional form and in many cases must be modified, assembled with partners, and localized to specific locations in order to function. If these processes fail, the affected protein must be recognized and degraded. How this critical triage decision is made has been a central question in the field of quality control for over 20 years.

One of the earliest mechanisms of triage, kinetic partitioning, emerged from studies in bacterial systems (Wickner et al., 1999). In this view, newly synthesized polypeptides released from the ribosome partition between chaperones and proteases, both capable of recognizing nonnative proteins. Cycles of chaperone binding and release would provide an opportunity to fold, while partitioning to proteases leads to the irreversible fate of degradation (Figure 1A). Given that the major classes of chaperones and proteases in bacteria do not interact with each other, partitioning seems to be the primary strategy for quality control in bacteria.

Although partitioning was initially postulated to apply to eukaryotes as well, two sets of observations led to a qualitatively different concept for quality control. The first concerned the nature of the degradation system in eukaryotes (Hershko and Ciechanover, 1998). Quality control in both the cytosol and ER were found to typically culminate at the proteasome (Buchberger et al., 2010). Commitment to proteasomal degradation normally relies on ubiquitin ligases to tag clients with a polyubiquitin chain. However, the ligases often do not recognize their clients per se, but rely on adaptors that bring a subset of cellular proteins in proximity to the ligase. Specificity of degradation is therefore

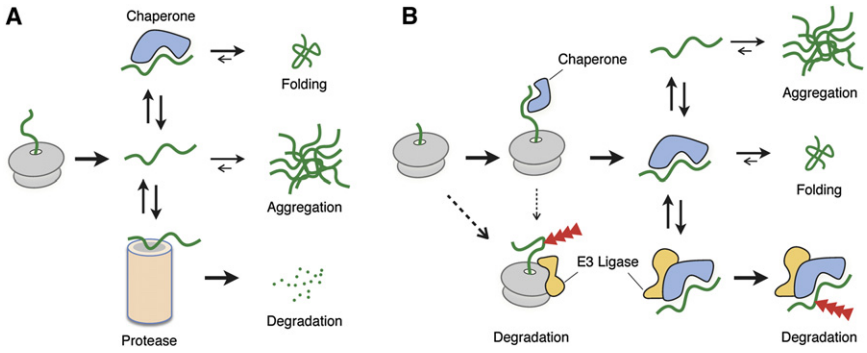

Figure 1. Passive versus Coupled Mechanisms of Quality Control

(A) The passive partitioning strategy. Newly synthesized polypeptides released from the ribosome partition reversibly between chaperones (blue) and proteases (orange). Upon release from chaperones, the polypeptide can either fold or reenter another round of partitioning. Degradation or aggregation are alternative fates for folding-incompetent polypeptides.

(B) The coupled strategy. Chaperones engage polypeptides during synthesis and continue to aid folding posttranslationally by cycles of binding and release. Ubiquitin ligases (orange) can be reversibly recruited to the biosynthetic machinery (such as ribosomes or chaperones) to target nascent polypeptides for degradation via attachment of polyubiquitin (red triangles).

imparted by the ligase in conjunction with any associated adaptor(s) (Deshaies and Joazeiro, 2009).

The second key observation was the discovery that the major cytosolic chaperone Hsp70 can interact directly with a ubiquitin ligase (Ballinger et al., 1999). This immediately suggested that chaperones might serve as adaptors that permit certain ubiquitin ligases to recognize nonnative proteins (Connell et al., 2001; Höhfeld et al., 2001; Meacham et al., 2001). Some time later, chaperones in the ER such as BiP, PDI family members, and GRP94 were also found to interact with components of ubiquitin ligase complexes involved in ER-associated degradation (Bernardi et al., 2008; Christianson et al., 2008; Denic et al., 2006; Hosokawa et al., 2008). These observations implied that nascent eukaryotic proteins did not passively partition between the folding and degradation machinery as seen in bacteria. Instead, the two pathways seemed to be more intimately linked, with quality control relying on an active role for chaperones in delivering nonnative proteins to degradation factors (Figure 1B).

Chaperone-Associated Quality Control

The best-studied class of chaperones that links protein folding to degradation is the Hsp70 family of ATPases (Mayer and Bukau, 2005). The ATP-bound state of Hsp70s has a low affinity for substrate, favoring dynamic binding and release. By contrast, the ADP-bound state has a high affinity for substrates, thereby binding and shielding the client. A wide range of interacting partners (often termed cochaperones) have been described that regulate the activity of Hsp70s (Kampinga and Craig, 2010).

These include J-domain family members that typically stimulate ATPase activity, nucleotide exchange factors that drive ADP replacement by ATP, factors that recruit Hsp70 to specific cellular locations, and ubiquitin ligases. Furthermore, the Hsp70 system can function together with other chaperones via their linking by organizing factors. For example, Hop is a two TPR domain protein that juxtaposes Hsp70 and Hsp90 via their C-terminal tails to facilitate folding of certain substrates. Thus, based on the associated factors, the basic Hsp70 module can be co-opted for a wide range of functions ranging from protein folding, protein complex assembly and dissociation, protein targeting, protein translocation, and protein degradation.

A key advance in understanding the role of Hsp70 in degradation came with the discovery that its C terminus associates with the ubiquitin ligase CHIP (Ballinger et al., 1999). This suggested that clients with prolonged Hsp70 interaction would eventually be ubiquitinated, thereby effecting quality control of folding-defective proteins (Connell et al., 2001; Meacham et al., 2001). Subsequent studies of Hsp70 interaction partners identified additional links to degradation pathways including other ubiquitin ligases, the proteasome, and autophagy factors (Esser et al., 2004; Arndt et al., 2007; Gamerdinger et al., 2011).

Two illustrative examples are the J-domain protein HSJ1 and the nucleotide exchange factor Bag1. HSJ1, like many J-proteins, stimulates the ATPase activity of Hsp70 to favor substrate binding (Cheetham et al., 1994). Importantly, however, it also contains ubiquitin interacting motifs (UIM) that bind to mono- and polyubiquitin (Chapple et al., 2004; Howarth et al., 2007; Westhoff et al., 2005). This suggests that ubiquitinated clients on Hsp70 would preferentially recruit HSJ1 via a bipartite interaction with both the chaperone (via the J-domain) and ubiquitin (via the UIM domain). ATP hydrolysis stimulated by the J-domain would then stabilize this complex.

Bag1, on the other hand, is an exchange factor (Briknarová et al., 2001; Höhfeld and Jentsch, 1997; Takayama et al., 1997) that can associate with the proteasome via a ubiquitin-like (Ubl) domain (Lüders et al., 2000). It is therefore plausible that proteasome-bound Bag1 would recruit Hsp70 complexed with a ubiquitinated client. Because proteasomes also contain ubiquitin receptors (Finley, 2009), recruitment could involve a bipartite interaction, with both Hsp70 and ubiquitin contributing to the avidity. Once recruited, the Bag domain would stimulate nucleotide exchange to induce release of the substrate, which could then be captured in an unfolded state by the proteasome. Thus, via the sequential actions of CHIP, HSJ1, Bag1, and the proteasome, an Hsp70-bound client could be routed for degradation in a highly regulated manner without release from the chaperone (Figure 2).

Although this is an attractive scheme and the individual activities of the factors have been documented, their concerted sequential action as described above remains to be firmly established. Furthermore, the situation is considerably more complex because of the concurrent presence of dozens of competing factors (Arndt et al., 2007; Esser et al., 2004; Gamerdinger et al., 2011) including other J-proteins and Bag proteins (Kampinga and Craig, 2010; Takayama et al., 1999), factors like HOP that would compete with CHIP for Hsp70 binding (Muller et al., 2012), and the fact that CHIP can ubiquitinate not only clients (Murata et al., 2001; Younger et al., 2004), but also Hsp70 and Bag proteins (Alberti et al., 2002; Jiang et al., 2001).

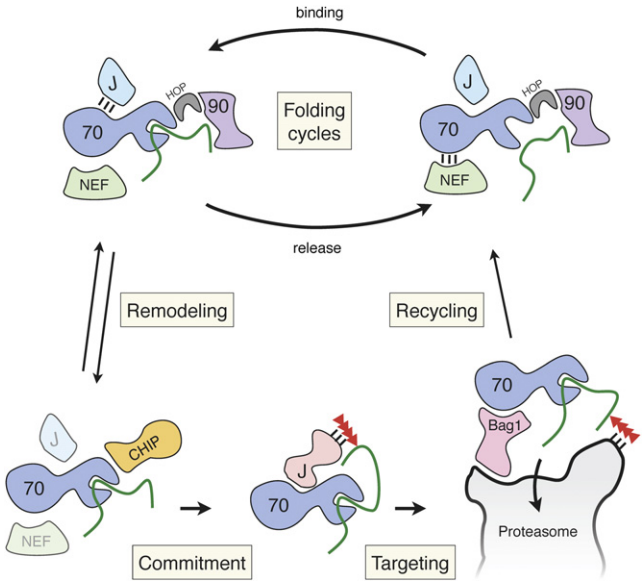

Figure 2. Speculative Model of Chaperone-Coupled Quality Control
A chaperone complex facilitates protein folding by cycles of binding and release. These complexes typically contain one or more chaperones (such as Hsp70 and Hsp90, as depicted), cochaperones (such as a J-domain protein and NEF, as depicted), and organizing factors like Hop that bridge Hsp70 and Hsp90 via their C-terminal tails. Nascent chain binding is favored by the action of the J-domain, while the NEF drives release. The chaperone complex can be reversibly remodeled and recruit a ubiquitin ligase (such as CHIP). Ubiquitination might facilitate further remodeling by recruiting yet other proteins (such as HDJ1, a J-domain protein that has the capacity to interact with ubiquitin) to generate a targeting complex. This can target to the proteasome (via adaptors, such as Bag1), where substrate is induced to release and enter the proteasome for degradation. The chaperone is recycled for further rounds of quality control.

Thus, the actual events that determine when a folding attempt should be aborted, and how the ubiquitinated product is delivered to the proteasome, remain to be elucidated.

Nevertheless, the above possible scenario highlights the key events that need to occur to efficiently target a chaperone-bound protein for degradation (Figure 2). First, a profolding complex composed of Hsp70, a J-protein, a nucleotide exchange factor (NEF), and perhaps other chaperones, must be remodeled into a prodegradation complex that includes a ubiquitin ligase (Arndt et al., 2007; Esser et al., 2004; McClellan et al., 2005; Muller et al., 2012). Due to the dynamic nature of the various cofactor interactions with Hsp70, remodeling is presumably a reversible process. Whether the timing of remodeling is stochastic, regulated, or in some manner coupled to the number of prior folding attempts remains unknown. However, the precise timing of the switch from a folding to a degradation pathway is critical to determining overall efficiency of protein maturation, risk of aggregation, and half-life. Mechanistic insight into this key step remains a vexing problem that merits scrutiny.

Once the ubiquitin ligase becomes part of the chaperone complex, the client is polyubiquitinated (Connell et al., 2001; Meacham et al., 2001; Stankiewicz et al., 2010) in what is presumably the committed step in degradation. The complex of Hsp70 with ubiquitinated substrate therefore represents a "targeting complex" with the destination being the proteasome. In most protein targeting reactions, a committed targeting complex is typically stabilized until its regulated disassembly at the destination (Shan and Walter, 2005). It is therefore logical to posit that commitment to degradation is accompanied by a shift in Hsp70 activity from dynamic to stable client binding. This would ensure sequestration of the misfolded substrate until its delivery to the proteasome and avoid trying to "fold" or otherwise release a committed degradation substrate. How complex stabilization is achieved is unclear, but could be via recruitment of a specific J-domain (as postulated above), exploitation of the peptide binding capacity of CHIP itself (Rosser et al., 2007), or some other mechanism.

Targeting of chaperone-bound clients to the proteasome could be mediated by ubiquitin receptors on the proteasome, potentially with the aid of factors (such as Bag1) that bridge the chaperone and proteasome. Following delivery, the targeting complex should be disassembled to release the substrate for degradation and recycle the factors for another round. By analogy to other targeting reactions, complex disassembly should be selectively stimulated at the destination. Thus, it is attractive to posit that dissociation of chaperone-client complexes is coupled in some manner to proteasome delivery. This key reaction remains to be studied.

Analogous reactions would presumably need to occur in the ER lumen during quality control. The most directly related from a mechanistic point of view is presumably BiP, the ER-lumenal Hsp70 family member. Like its cytosolic counterpart, BiP engages nascent proteins early in their biogenesis at the translocon (Zimmermann, 1998) and subsequently participates in their folding (Simons et al., 1995). If folding is unsuccessful, BiP-associated clients are triaged for degradation via targeting to a ubiquitin ligase-containing dislocation apparatus (Mehnert et al., 2010) that exports proteins from the ER to the cytosol (Hampton and Sommer, 2012).

BiP's role in these processes is regulated by its interacting partners that include various J-domain proteins, NEFs, chaperones, and adaptors (Otero et al., 2010). Thus, as in the cytosol, BiP-mediated quality control in the ER is likely to involve a number of cofactors that enable BiP to be used during both biosynthetic and degradation pathways (Otero et al., 2010; Vembar et al., 2010). However, the coordination of these various cofactor activities to properly regulate these two opposing activities remains unclear.

Although less well studied, other classes of chaperones are also similarly linked to degradation machinery. These include Hsp90 in the cytosol (Connell et al., 2001) and PDI (Bernardi et al., 2008), GRP94 (Christianson et al., 2008), and calnexin (Oda et al., 2003) in the ER lumen. Although a detailed discussion of their known interacting partners is beyond the scope of this review, it suffices to note that each of them can interact with factors whose main function is to effect degradation. The repeated emergence of this theme in otherwise unrelated chaperones suggests a fundamental advantage to coupling biosynthesis and degradation.

One such advantage is that the rather formidable task of recognizing nonnative proteins is relegated to factors already optimized for this job. Furthermore, coupling permits degradation to proceed while the misfolded client remains in the chaperone's protective confines, preventing inappropriate interactions with other cytosolic components. These benefits, however,

come with the need to now regulate chaperone activity toward two opposing fates via modulation of its interaction partners. It is therefore not surprising that the interaction partners, such as the J-domain family (Kampinga and Craig, 2010), have diversified considerably more than the chaperones themselves during evolution.

Achieving the correct balance between biosynthesis and degradation is critical to maintaining protein homeostasis. Excessive folding attempts would increase the risk of misfolded protein aggregation with each cycle that fails. By contrast, overaggressive degradation may allow insufficient time for many proteins to mature. Indeed, relaxing the activity of the chaperone-associated ligase CHIP allows a greater proportion of the difficult-to-fold CFTR to mature (Grove et al., 2009). This presumably comes at a cost, and the optimal setpoint is a fine balance between many competing factors (Balch et al., 2008).

For this reason, it is likely that different cells whose priorities vary widely will have different setpoints as dictated by the expression patterns and abundances of the various chaperones and cofactors. For example, postmitotic cells such as neurons may be more aggressive in their quality control given their particular sensitivity to protein misfolding and disease. The relevance of these cell-type-specific differences will only be revealed after the roles of each component is clearly defined and placed in context with its partners. This remains a major challenge in understanding chaperone-associated quality control.

Although our discussion has focused on chaperone-mediated recognition of potential degradation substrates, it is worth noting that some quality control ubiquitin ligases such as San1 and Hrd1 can directly recognize nonnative features of their clients (Rosenbaum et al., 2011; Fredrickson et al., 2011; Sato et al., 2009). Thus, chaperone-associated quality control operates within a broader framework that includes ubiquitin ligases that may use different principles of recognition. For a more general discussion of these other quality control pathways, the reader is directed to several other papers (Buchberger et al., 2010; Mehnert et al., 2010; Taylor and Rutter, 2011; Theodoraki et al., 2012; Varshavsky, 2011).

Quality Control during Protein Localization

A prerequisite for initiating the folding process for 30%–50% of all newly synthesized proteins is proper localization into a compartment such as the ER, mitochondria, peroxisomes, or plastids (Inaba and Schnell, 2008; Ma et al., 2011; Chacinska et al., 2009; Shao and Hegde, 2011). Failure of localization results in an immature precursor in the wrong compartment that can be detrimental for several reasons. The mislocalized protein (MLP) would typically lack appropriate cofactors and chaperones, thereby engaging folding machinery in futile cycles. MLPs are often precursors that contain hydrophobic domains, such as signal peptides or transmembrane domains (TMDs), that make them particularly aggregation prone. MLPs that contain partial activity could disrupt cellular homeostasis (e.g., a mislocalized protease or nuclease). Thus, prompt degradation of MLPs is crucial for avoiding these adverse fates.

A priori, one might assume that MLPs are handled no differently than a cytosolic protein that fails to fold. Although this would seem the simplest solution, it comes at the expense of occupying the folding machinery for prolonged periods given that the nascent chain is unlikely to ever acquire a stable conformation. Furthermore, most chaperones involved in folding are typically designed to bind short (approximately three- to five-residue) hydrophobic stretches (Rüdiger et al., 1997), and may not be especially suitable for highly hydrophobic elements like TMDs and signal peptides. Given that mislocalization seems to be a relatively frequent event (Kang et al., 2006; Levine et al., 2005; Rane et al., 2004), particularly under certain conditions like ER or mitochondrial stress (Kang et al., 2006; Nargund et al., 2012), relying solely on cytosolic quality control may not be a particularly suitable solution.

An alternative remedy to this problem is if the targeting machinery dedicated to localizing clients were linked to degradation such that any delay or failure in targeting results in immediate destruction. Such a system has the advantage that the localization factors are customized to recognize and shield their respective clients. Furthermore, the clients are presegregated from the cytosolic folding machinery, avoiding its futile engagement. Recent studies suggest that, indeed, targeting machinery to different organelles might interact with ubiquitin ligases for the purposes of MLP degradation.

This principle is best highlighted with the targeting system for mammalian tail-anchored (TA) membrane proteins (Hegde and Keenan, 2011). These single-spanning membrane proteins are posttranslationally targeted to the ER by an ATPase termed TRC40 (Favaloro et al., 2008; Stefanovic and Hegde, 2007) or Get3 in yeast (Schuldiner et al., 2008). This targeting factor recognizes and shields the TMD of TA proteins in the cytosol and releases it for insertion upon encountering an ER-localized receptor. The initial loading of TA proteins onto TRC40 involves several additional cytosolic factors (Jonikas et al., 2009; Mariappan et al., 2010; Wang et al., 2010).

Biochemical studies in mammalian and yeast systems have shown that TRC40 is transiently part of a larger TMD-recognition complex (TRC) that captures, sorts, and loads TA proteins onto TRC40. The TRC in mammals is composed of a Bag6 subcomplex (containing Bag6, TRC35, and Ubl4A), TRC40, and probably SGTA (Mariappan et al., 2010; Winnefeld et al., 2006). Of these proteins, Bag6, TRC40, and SGTA each are capable of directly interacting with and shielding the hydrophobic TMD of TA proteins (Leznicki et al., 2010; Mariappan et al., 2010; Mateja et al., 2009; Stefanovic and Hegde, 2007; Wang et al., 2010). Potential clients that engage the TRC are sorted among these binding proteins, each of which may confer a different fate (Figure 3A).

Initial engagement with this complex is via Bag6 (Mariappan et al., 2010) or SGTA (Wang et al., 2010) and may be facilitated by the ability of certain TRC component(s) to interact with the ribosome (Mariappan et al., 2010). Once substrates engage the TRC, targeting to the ER can only be achieved if they are loaded onto TRC40 (Figure 3A). This loading appears to be highly specific for the TMDs of ER-destined TA proteins (Hessa et al., 2011; Mariappan et al., 2010; Wang et al., 2010), although the basis of this specificity remains unclear. Clients that do not load onto TRC40 remain associated with TRC via direct binding to Bag6 (Hessa et al., 2011; Mariappan et al., 2010). This outcome results in substrate ubiquitination via a yet-unidentified ubiquitin ligase recruited by a ubiquitin-like (Ubl) domain in Bag6 (Hessa et al., 2011) (Figure 3A).

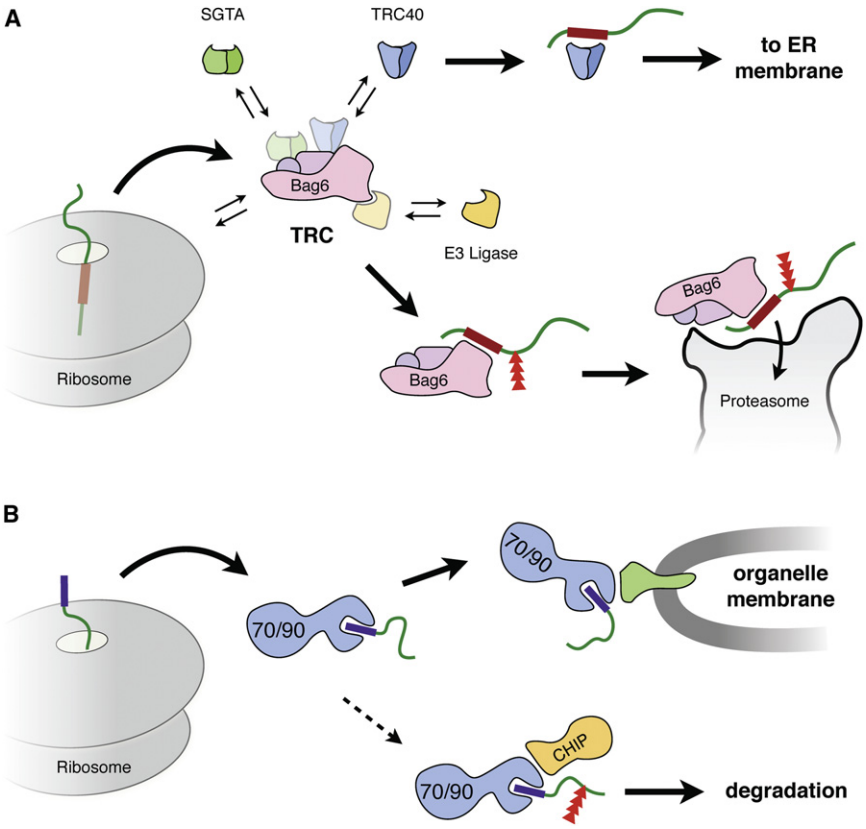

Figure 3. Quality Control during Protein Localization

(A) Tail-anchored protein targeting and quality control. Newly synthesized proteins containing a TMD (red) can engage a TRC after release from the ribosome. Initial capture by the TRC might be facilitated by its transient interaction with the ribosome. The TRC is a dynamic complex composed of a core Bag6-subcomplex (pink), SGTA (green), TRC40 (blue), and a yet-unidentified E3 ubiquitin ligase (orange). Bag6, SGTA, and TRC40 all have the capacity to bind the TMD, with the latter strongly favoring only substrates with a single TMD close to the C terminus (i.e., tail-anchored proteins). Loading of substrate onto TRC40 results in targeting and insertion into the ER membrane, while prolonged engagement with the TRC results in substrate ubiquitination and targeting to the proteasome.

(B) Quality control during targeting to chloroplasts and mitochondria. Proteins containing a targeting sequence (purple) contain as part of their targeting complex the Hsp70 and/or Hsp90 chaperones. These chaperones have receptors at the destination membrane that bind to the same region as the ubiquitin ligase CHIP. Thus, any failure of targeting would result in recruitment of CHIP, substrate ubiquitination, and degradation.

Thus, TA protein targeting in mammals involves an obligate engagement with factors that directly link to ubiquitination machinery. This means that if TRC40 is not immediately available, then the prolonged interaction with Bag6 would favor degradation. Indeed, in vitro studies show that depletion or saturation of TRC40 results in increased TA protein ubiquitination (Hessa et al., 2011). Even if substrates are successfully loaded onto TRC40, it is conceivable that delayed targeting might permit re-engagement with the TRC and eventual ubiquitination via Bag6. In this manner, any failures in targeting would permit rapid degradation without release from the machinery designed specifically to bind and shield highly hydrophobic TMDs.

Avoiding the release of TMD-containing proteins free into the cytosol is probably important for avoiding aggregation and fruitlessly engaging general chaperones such as Hsp70. Indeed, in yeast (which do not have an obvious Bag6 homolog), deletion of TA targeting machinery leads to substantial TA protein aggregation (Jonikas et al., 2009; Schuldiner et al., 2008). Aggregation in yeast, while probably undesirable, can nevertheless be resolved by either disaggregases such as Hsp104 (Winkler et al., 2012) or selective partitioning to mother cells during cell division (Zhou et al., 2011). Thus, the evolution of direct links between the targeting and degradation machinery in complex organisms may have been favored by their lower tolerance for protein misfolding stress and absence of robust disaggregation systems.

How are failures in other targeting pathways handled by the cell? In the case of cotranslational targeting of secretory and membrane proteins to the ER, the answer appears to also involve the Bag6 protein. Analysis of failed translocation products in vitro suggested the existence of a pathway dedicated to ubiquitination of proteins with highly hydrophobic domains such as signal peptides and TMDs (Iwamuro et al., 1999; Hessa et al., 2011). Subsequent crosslinking experiments combined with ubiquitination assays identified Bag6 as a key player in this pathway (Hessa et al., 2011).

Bag6 proved capable of interacting not only with TA proteins (Leznicki et al., 2010; Mariappan et al., 2010), but also with any protein containing long linear hydrophobic domains such as signal peptides, TMDs, and GPI-anchor signal (Hessa et al., 2011). Furthermore, unlike TRC40, which seems to favor proteins with only one TMD near the C terminus, Bag6 seems nonselective with respect to either the number or position of hydrophobic elements (Hessa et al., 2011). Thus, the substrate specificity of Bag6 matches well with the features of secretory and membrane protein precursors.

Bag6 would ordinarily never have the opportunity to interact with these proteins during biosynthesis because they are typically recognized cotranslationally by SRP as they emerge from the ribosome (Shan and Walter, 2005). SRP would enjoy considerable advantage in recognizing these proteins because its substrate-binding domain is positioned precisely at the exit tunnel on the ribosome (Halic et al., 2004). Thus, secretory and membrane proteins are normally targeted early in their synthesis to the ER translocon (Shan and Walter, 2005), where they can complete synthesis in the protected environment afforded by the ribosome-translocon complex (Becker et al., 2009; Ménétret et al., 2005). Only when SRP-dependent targeting fails would the Bag6 complex have an opportunity to capture the nascent chain.

This capture may be facilitated by the ability of Bag6 complex to interact with ribosomes containing TMDs within the exit tunnel

(Mariappan et al., 2010). How the complex is recruited to these ribosomes is entirely unclear; however, analogous "signaling" from inside the tunnel to influence ribosome functions such as elongation (Lu and Deutsch, 2008), termination (Cao and Geballe, 1996), SRP interaction (Berndt et al., 2009), and translocon interaction (Liao et al., 1997) has been described. Regardless of the mechanism, this observation suggests that the cotranslational targeting machinery (ribosome-nascent chain-SRP complexes) may include a factor (Bag6) that links to the ubiquitination machinery. This link (as well as substrate specificity of Bag6 for particularly hydrophobic linear domains) presumably affords Bag6 an advantage over other chaperones normally dedicated to folding cytosolic proteins and whose interactions typically occur via much shorter hydrophobic patches. Thus, Bag6, in addition to being an integral component of the TA targeting pathway, is also loosely coupled to cotranslational targeting to facilitate quality control in the case of failure.

Similar principles may apply to targeting to other organelles (Figure 3B). For both chloroplast transport and mitochondrial transport, the cytosolic targeting complex contains Hsp70 and/or Hsp90 (May and Soll, 2000; Qbadou et al., 2006; Young et al., 2003). These chaperones serve to not only maintain the unfolded state of their clients, but also interact with receptors at the target membrane to facilitate targeting. These receptors contain TPR domains that interact with the C-terminal peptide on the chaperones (Schlegel et al., 2007). Remarkably, the same peptide domain interacts with CHIP (Ballinger et al., 1999).

This suggests that failed or delayed targeting would allow the exposed TPR binding motif of the targeting complex to interact with CHIP rather than its receptor, thereby triggering client degradation (Figure 3B). Indeed, CHIP has been implicated in chloroplast precursor degradation when import is blocked (Lee et al., 2009). A major advantage of this mechanism would be that triage for degradation would not necessitate release of the polypeptide from its chaperone-protected state. This may be especially important for highly hydrophobic clients like membrane proteins of the chloroplast or mitochondria. Interestingly, a mechanism for coupled degradation does not seem to exist within the "prokaryotic-like" stroma compartment of the chloroplast. Instead, an unusual variant of the signal recognition particle has evolved disaggregation activity to deal with aggregated mislocalized membrane proteins (Jaru-Ampornpan et al., 2010). This nicely illustrates within a single system the contrasting strategies employed to deal with mislocalization.

Finally, after targeting has occurred to a translocon, translocation and membrane insertion are not necessarily assured (Levine et al., 2005). Failures during these late stages in localization are handled efficiently by the ubiquitin-proteasome system (Garrison et al., 2005; McKibbin et al., 2012), suggesting this step is subject to surveillance and quality control. Very little is known about how this might occur, but in the case of the ER, some evidence exists for cotranslational ubiquitination of translocon-engaged substrates. Notable clients include the multispanning membrane protein CFTR (Sato et al., 1998), whose biogenesis may be particularly inefficient, and the very large apolipoprotein B (Zhou et al., 1998), which needs to associate cotranslationally with lipids for correct maturation. In both cases, nascent chains may get targeted for degradation during translocation by machinery that remains poorly understood.

A recent study in yeast suggested that clients displaying prolonged interaction with the ER translocon are ubiquitinated by the ER-resident ubiquitin ligase Hrd1 (Rubenstein et al., 2012). Whether Hrd1 can interact with Sec61 to mediate this activity is not clear. Nevertheless, the findings do lend credence to the notion that surveillance mechanisms exist to monitor catastrophic failures at essentially all steps of ER protein localization from targeting to translocation. These surveillance mechanisms not only protect the substrate from aggregation, but may also buffer the cytosolic and ER folding machinery from excess nonproductive clients.

Quality Control at the Ribosome

Protein quality control has traditionally been viewed as a post-translational process. After all, how can a protein be evaluated for its ability to fold before its synthesis is even complete? Recent studies examining quality control during translation have started to address this question, revealing that polypeptides of inappropriate length are deemed defective and routed for degradation directly from the ribosome.

The best-studied example of protein quality control linked to protein synthesis is the tmRNA system of prokaryotes (reviewed by Janssen and Hayes, 2012; Felden and Gillet, 2011). This system resolves the problem of a translating ribosome that reaches the end of an mRNA without encountering an in frame stop codon. This stalled ribosome recruits a hybrid transfer-messenger RNA (i.e., tmRNA) that together with associated proteins serves to tag the nascent protein for degradation, recycle the ribosome, and degrade the associated mRNA. This is accomplished by a "trans-translation" mechanism in which the stalled ribosome uses tmRNA as a template to complete synthesis of the protein. The sequence encoded by the tmRNA serves as a degradation tag for the released protein, and the termination codon in the tmRNA allows ribosome recycling. Thus, a defect in the normal translation cycle is directly communicated to the truncated protein product to effect its quality control. Although the tmRNA system is not conserved in eukaryotes, it has long been appreciated that the same problems of ribosome recycling, protein degradation, and mRNA degradation must be solved.

Resolution of problems during the eukaryotic translation cycle has been studied most extensively in the context of mRNA surveillance (van Hoof and Wagner, 2011). Only recently has it been appreciated that protein quality control is probably an integral part of these pathways (Bengtson and Joazeiro, 2010; Dimitrova et al., 2009; Ito-Harashima et al., 2007). Early studies of mRNA stability recognized that messages containing premature stop codons were often rapidly degraded by a process termed nonsense-mediated decay (NMD) (Losson and Lacroute, 1979; Maquat et al., 1981). Subsequent studies defined additional mRNA surveillance pathways termed no-go decay for stalled ribosomes (Doma and Parker, 2006) and nonstop decay (NSD) for messages lacking an in-frame stop codon (Frischmeyer et al., 2002; van Hoof et al., 2002). These pathways appear to be conceptually and mechanistically related, most notably by their requirement of at least one round of translation to trigger degradation of the defective mRNA (Maquat et al., 2010; Shoemaker and Green, 2012; van Hoof and Wagner, 2011).

Investigating the basis for this translation requirement helped explain how very subtle problems in an mRNA, such as

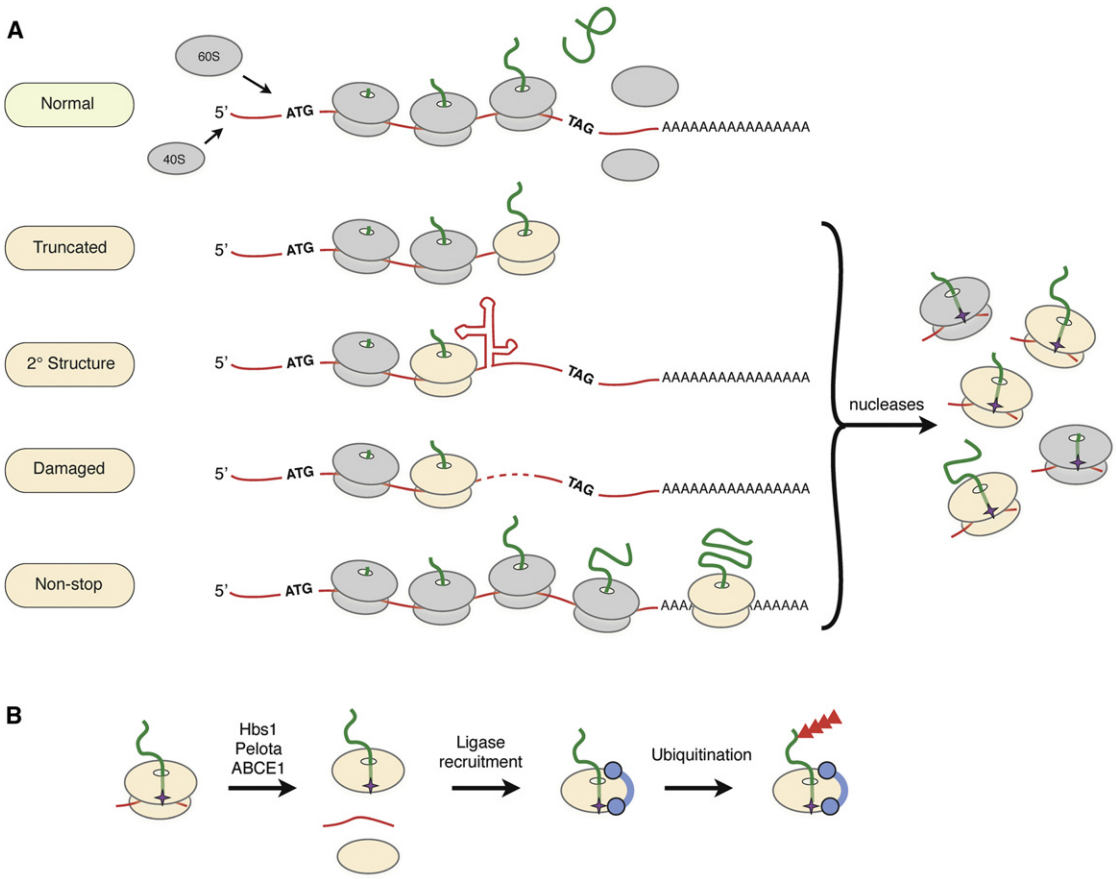

Figure 4. Quality Control at the Ribosome during mRNA Surveillance

(A) The normal translation cycle of initiation, elongation, termination, and recycling (top panel) can be disrupted by various types of mRNA defects that lead to a stalled ribosome (depicted in tan). The mRNA surveillance machinery (not depicted) recognizes the stalled ribosome and digests the mRNA to leave behind unresolved 80S ribosome-nascent chain complexes.

(B) Hypothetical series of events that lead to resolution of the stalled 80S ribosome-nascent chain complex. Recycling factors (Hbs1, Pelota, ABCE1) split the 80S ribosome into subunits. A ubiquitin ligase (such as Listerin, blue) is recruited to the large subunit and catalyzes polyubiquitination of the nascent chain to commit it for degradation.

a frameshift or single nonsense mutation, can be recognized. In essence, a pioneer round of translation "test-drives" the mRNA to confirm its fidelity. Thus, encountering a termination codon far from the poly-A tail, reading into the poly-A tail, reaching the end of a message without encountering a stop codon, or stalling within the coding region all signal the recruitment of endonucleases and exonucleases that degrade the presumably defective mRNA. Degradation ensures that potentially detrimental defective protein products are not produced from repeated use of defective mRNAs. However, the issue of how the defective protein product (and in some cases, the stalled ribosome) from the pioneer round of translation is handled remained to be explained.

Two sets of studies, one studying nascent polypeptide degradation and another analyzing ribosome recycling (discussed below), have shed light on this problem. When considered together, they are beginning to sketch an initial framework for quality control at the ribosome (Figure 4). Studies on the fate of nascent chains generated during NSD suggested that translating ribosomes stall when they decode the poly-A tail and the nascent polypeptide synthesized to that point is degraded via the ubiquitin-proteasome system (Ito-Harashima et al., 2007). This is an attractive model because poly-A translation would be a unique feature of nonstop mRNAs, and therefore could serve as a specific signal for nascent chain ubiquitination. The mechanism of stalling remains unclear (Wilson and Beckmann, 2011), although it has been speculated to involve interactions between polylysine (encoded by poly-A) and the ribosomal exit tunnel. Consistent with this idea, similar results were observed with polyarginine coding segments (Dimitrova et al., 2009). Thus, stalled ribosome-nascent chain complexes containing a polybasic peptide of sufficient length within the exit tunnel trigger nascent chain degradation.

Two ubiquitin ligases, Not4 and Ltn1, have been implicated in this process (Bengtson and Joazeiro, 2010; Dimitrova et al., 2009). Both ligases are capable of associating with ribosomes, and deletion of either gene in yeast results in stabilization of the polypeptide fragment preceding a polybasic sequence. The reason for the discrepancy in the two studies implicating different ligases is presently unclear, and it has not been possible yet to fully resolve direct from indirect effects on nascent chain degradation. Future in vitro reconstitution studies will be needed

to define the ligase(s) that directly ubiquitinate the nascent chain and rigorously determine if ubiquitination occurs on peptidyl-tRNA products on the ribosome.

These unresolved issues notwithstanding, the implication from these studies is that ligase(s) associated with or recruited to the translation machinery mediate ubiquitination of nascent polypeptides and commit them for degradation before they are released into the bulk cytosol. This would spare the cytosolic folding and quality control machinery from handling these defective proteins and minimize the risk of inappropriate interactions or dominant-negative effects. Thus, in the case of NSD, mRNA surveillance and nascent chain quality control seem to be intimately linked via the translation apparatus (Shoemaker and Green, 2012).

Because translating ribosomes on normal mRNAs should be refractory to these mechanisms, it is imperative that the mRNA and protein degradation pathways are specific for defective complexes. One possibility is that a stalled ribosome is the initial signal for recruitment of nucleases (Schaeffer and van Hoof, 2011), ribosome recycling factors (Pisareva et al., 2011; Shoemaker et al., 2010), and ubiquitin ligases (Bengtson and Joazeiro, 2010; Dimitrova et al., 2009) that together resolve the stalled complex. However, the specific factors involved, the mechanism of recruitment, and the order of events all remain to be clearly delineated. Furthermore, how the cell would distinguish between a normal pausing event during translation and that resulting from defective substrates is not well understood. Nevertheless, some insight has indirectly come from studies of ribosome recycling of stalled translation complexes.

Recycling is the process by which 80S ribosomes are split into 60S and 40S subunits that can re-enter the translation cycle following translation termination at a stop codon (Jackson et al., 2012). Here, GTP-bound eRF3 in complex with eRF1 targets to a translating ribosome containing a stop codon in the A site. Once bound, GTP hydrolysis by eRF3 triggers a conformational change in eRF1 such that its highly conserved GGQ motif can catalyze hydrolysis of the ester bond between the peptidyl-tRNA and nascent polypeptide. This releases the peptide into the cytosol. After eRF3 dissociation, the ATPase ABCE1 (Rli1 in yeast) is then recruited to the A site, where it uses ATP hydrolysis to drive 80S separation into 60S and 40S subunits (Pisarev et al., 2010).

In the case of stalled translation complexes, the A site would not contain a stop codon, and therefore cannot recruit the eRF1-eRF3 complex. Instead, two homologous factors termed Hbs1 and Pelota (Dom34 in yeast) act in conjunction with ABCE1 to serve a similar function (Pisarev et al., 2010; Shoemaker et al., 2010; Tsuboi et al., 2012), but with two key differences. First, the recycling reaction can only occur on translation complexes stalled very close (within ~12 nucleotides in the mammalian system) to the end of an mRNA. This length is notable because it is precisely the number of mRNA residues protected by the ribosome from the A site to the cytosol. This may be used as a cue to distinguish stalled from translating ribosomes, analogous to the mechanism used by ribosome rescue factors in prokaryotes (Gagnon et al., 2012; Neubauer et al., 2012). Second, Pelota does not contain a GGQ motif, and hence cannot catalyze hydrolysis of the tRNA ester bond. Thus, dissociation of 80S complexes containing a short (four-residue) nascent chain resulted in "drop-off" of an intact peptidyl tRNA (Pisarev et al., 2010; Shoemaker et al., 2010).

Both of these observations have implications for how stalled translation complexes are resolved. The requirement for minimal mRNA protrusion outside the ribosome implies that ribosome recycling cannot occur until nuclease(s) have first digested the mRNA downstream of the ribosome. The nuclease that performs this task remains to be elucidated, but may be recruited by or act in conjunction with Dom34 (Doma and Parker, 2006). The fact that the tRNA is not hydrolyzed to release the peptide implies that ribosome splitting would produce one of two rather unusual products. Either a peptidyl-tRNA would be released free into the cytosol (provided the nascent chain was very short), or a 60S-peptidyl-tRNA complex would be generated. Because each of these species is never part of the normal translation cycle, they could provide unique targets for recruitment of ubiquitin ligases that polyubiquitinate the nascent peptide for degradation. Consistent with this idea, Ltn1 was observed to cofractionate with 60S subunits, but not 80S or polysomes (Bengtson and Joazeiro, 2010).

Taking the specificity of the ribosome recycling factors and current knowledge of mRNA surveillance pathways into account, one can envision a plausible working framework for ribosome-associated quality control (Figure 4). Any of several events could initiate the process including (1) encountering a premature termination codon during the pioneer translation cycle, (2) ribosome stalling due to mRNA secondary structure, rare codons, amino acid insufficiency, or mRNA damage, (3) reading into the poly-A tail, or (4) reaching the end of a message. In each case, the ribosome would stop elongating, albeit for different reasons, leading to the recruitment of endonucleases and/or exonucleases. Digestion of the mRNA 3′ to the translating ribosome (or perhaps in the A site) would generate a RNC species that is the target for the Hbs1/Pelota/ABCE1 recycling system. Splitting of the subunits produces a 60S-peptidyl-tRNA that could recruit Ltn1 to ubiquitinate the nascent chain. The ubiquitinated nascent chain could then be extracted from the 60S subunit, perhaps via the p97 ATPase complex or proteasome. Thus, the mRNA would be destroyed, the ribosomal subunits recycled, and the nascent chain targeted for degradation without accessing the bulk cytosol.

This framework is appealing for a number of reasons. First, multiple different and seemingly unrelated situations could converge on a single initiating species: the nucleolytically processed RNC that is the target for recycling factors (Figure 4A). The pathway for the generation of this species may differ for NSD, NMD, and NGD, each of which seem to involve different factors and requirements (Shoemaker and Green, 2012; van Hoof and Wagner, 2011). Nevertheless, nucleolytic digestion of mRNAs engaged in translation seems to be a universal theme, thereby producing essentially the same stalled RNC species.

Second, the unique nature of the 60S-peptidyl-tRNA would markedly aid recognition by the ubiquitin ligase (Figure 4B). Specific recognition is a critical issue because the abundance of ubiquitin ligases such as Ltn1 is between two and three orders of magnitude lower than that of ribosomes (Ghaemmaghami et al., 2003). Although Ltn1 could potentially recognize the same cues that recruit nucleases to stalled ribosomes, this mechanism is less appealing because the factors may compete

rather than act sequentially in a defined order. Furthermore, even a weak affinity for translating ribosomes would risk ubiquitinating normal nascent chains, particularly those that take a long time to synthesize. By exploiting recycling factors to provide the requisite specificity, the ligase would not pose a risk of interfering with normal protein maturation.

Third, committing the nascent chain for degradation would occur without ever releasing it from the ribosome. In this manner, exposure to the cytosol, engagement of protein folding machinery, and risk of inappropriate interactions are all markedly minimized. Thus, mRNA quality control and protein quality control likely intersect at the ribosome to ensure that defective messages and their aberrant protein products are recognized extremely early and each routed for degradation before they can pose significant harm.

Similar mechanisms of protein quality control may be employed to deal with incomplete nascent chains on mRNAs that are turned over as a part of normal degradation. In this situation, the timing of mRNA digestion by endonucleases and exonucleases relative to the translation cycle is not completely understood (Shoemaker and Green, 2012). However, the only way partially synthesized products could be entirely avoided is if all engaged ribosomes are allowed to complete translation before mRNA degradation is initiated (Hu et al., 2009). In the absence of such coordination, translating ribosomes and their associated incomplete nascent chains would need to be resolved in conjunction with mRNA downregulation.

Thus, protein quality control of nascent chains at the ribosome may be a common phenomenon in higher eukaryotes where mRNA remodeling is extensive. Furthermore, there are other situations where large amounts of defective mRNAs are generated. For example, DNA recombination in generating T cell receptor diversity results in a large proportion of out of frame mRNAs that are degraded by NMD in a translation-dependent manner (Wang et al., 2002). Ribosome-associated quality control may therefore be of special importance in particular cell types or during differentiation.

What about quality control on the ribosome on the basis of nascent chain folding? Although certainly a possibility, it is difficult at present to see how deviations from normal could be detected. This is because with few exceptions, polypeptides in the process of being synthesized are normally nonnative. Thus, the logic of triaging for degradation a polypeptide that has yet to be given a chance to fold is not immediately apparent, at least from the standpoint of protein maturation. Nevertheless, this possibility should not be entirely discounted because other beneficial outcomes such as antigen presentation (Dolan et al., 2011) or amino acid recycling (Vabulas and Hartl, 2005) might have driven its evolution.

Advantages of Coupled Biosynthesis and Degradation

As illustrated by the foregoing examples, linking quality control to each step in protein biosynthesis has three major advantages that are worth underscoring. First, the defective product in question is recognized at the earliest stage possible, limiting the burden on downstream biosynthetic and quality control pathways and minimizing the potential for disruption of cellular homeostasis. Second, multiple checkpoints on quality provides a measure of redundancy that likely increases overall fidelity of the final product. Third, each stage of quality control uses different parameters to evaluate the nascent polypeptide. These parameters could be different structural features of the polypeptide as distinguished by chaperones combined with polypeptide-independent cues such as the state of the ribosome. Using multiple parameters provides a more complete assessment than could be achieved by any single quality control mechanism.

From a mechanistic standpoint, linking biosynthesis with quality control greatly facilitates defective substrate recognition. In essence, quality control can piggyback on biosynthetic complexes that are in an ideal position to evaluate maturation status. For example, an abnormal translation cycle giving rise to truncated products is most easily indicated by ribosome position along the mRNA relative to fiduciary markers such as the poly-A tail, stop codons, and the 3′ end. Similarly, localization factors are optimally evolved to recognize localization elements, and their prolonged interaction is therefore a sure sign that localization has failed. Finally, chaperones are customized to recognize nonnative states of proteins, making them the ideal sensor of protein misfolding. Thus, a repeated theme is the exploitation by quality control machinery of unique reactions surrounding individual biosynthetic events to reroute nascent chains toward degradation. An understanding of the timing mechanism used to shift nascent chain fate remains a major challenge in all quality control pathways.

The distribution of quality control tasks across multiple steps means that deficiencies in any one mechanism would not necessarily be crippling. For example, an inability to detect and degrade truncated products at the ribosome would release them to the downstream folding and/or localization machinery. In most cases, these products would still be degraded because they would not fold correctly. Only in instances where the truncation was near a domain boundary might the product be completely stabilized by failure of ribosome-associated quality control. From a physiologic standpoint, this robustness built into the system likely provides protection from fluctuations that might temporarily saturate one or another pathway.

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