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Review

Ubiquitination of newly synthesized proteins at the ribosome

Feng Wang [1], Larissa A. Canadeo [1], Jon M. Huibregtse*

Department of Molecular Biosciences and Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712, USA

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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 5000–10,000 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 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) (Fig. 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 adapters) and target soluble misfolded proteins for degradation, before they have the potential to form aggregates. Alternatively, proteins can be degraded via autophagy, a

* Corresponding author. Tel.: +1 512 232 7700; fax: +1 512 232 3432.
E-mail address: huibregtse@austin.utexas.edu (J.M. Huibregtse).
[1] These authors contributed equally to this work.

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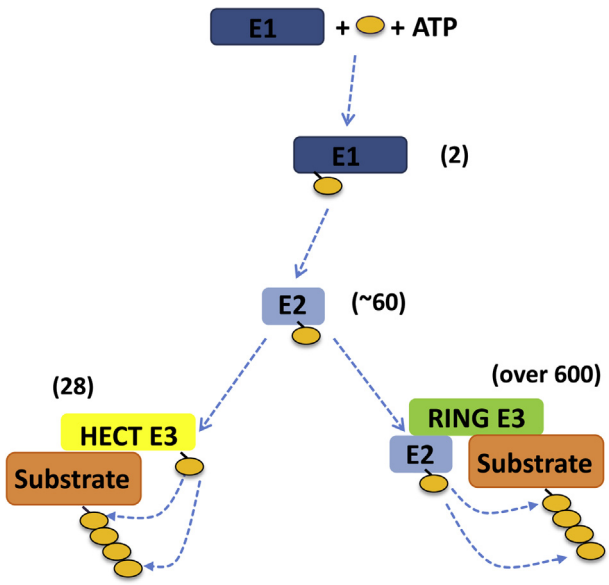

Fig. 1. The ubiquitin conjugation pathway. Ubiquitin is conjugated to substrate via E1-E2-E3 enzyme cascades. In human cells, there are two E1s, approximately 60 E2s, and over 600 E3s [76]. This large pool of E3 ligases defines the substrate specificity of ubiquitin conjugation pathway. E3 ligases are 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.

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 (Fig. 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}) (Fig. 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)

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