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PROTEIN METABOLISM

Quality control at the ribosome

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To avoid the burden of misfolded or aggregated proteins, cells have evolved quality control systems, comprising chaperones, folding factors and the degradation machinery, which promote the correct folding or mediate the removal of incorrectly folded species. Two studies now further our understanding of how multilayered protein quality control can operate concurrently with translation of nascent peptides at the ribosome.

Both groups first confirmed that a proportion of nascent peptides are ubiquitylated and degraded as they emerge from the ribosome. Wang *et al.* found that 12–15% of nascent peptides are modified co-translationally in human cell lines, and Duttler *et al.* detected ~1.1% peptide ubiquitylation in yeast. Furthermore, Wang *et al.* showed that the peptides were targeted primarily by Lys48-linked ubiquitylation, as mutation of ubiquitin at this residue greatly reduced the chain length of the ubiquitylated nascent peptides. Importantly, both groups found that use of a proteasome inhibitor led to the accumulation of ubiquitylated nascent peptides, highlighting that this modification targets them for proteasomal degradation. Duttler *et al.* observed that several E3 ubiquitin ligases, including both ribosome-bound and unbound ones, contribute to targeting nascent peptides. Of note, yeast lacking both of the ribosome-bound ligases Hel2 and Rkr1 had significantly reduced levels of ubiquitylated nascent peptides compared with wild-type cells.

Previously, co-translational ubiquitylation has been observed specifically for truncated peptides, the translation of which has been stalled, for example, by non-stop codons. Wang *et al.* confirmed that one-third of the total co-translationally ubiquitylated peptides were associated with stalled ribosomes, and treatment with compounds that affect translational fidelity or block translation significantly increased the numbers of ubiquitylated peptides arising from stalled complexes. Notably, however, they found that two-thirds of peptides were part of actively translating complexes, which indicates that they may be targeted for degradation because they are misfolded. Indeed, use of inhibitors blocking the chaperone heat shock protein 70 (HSP70; which promotes co-translational folding) or of the chemical AZC (which promotes misfolding) led to accumulation of modified peptides arising from active, but not stalled, complexes.

In their analysis, Duttler *et al.* asked whether co-translational ubiquitylation targets all nascent peptides or just a subset that share common features. They found that co-translational ubiquitylation preferentially targets peptides that are derived from highly expressed and rapidly translated mRNAs, peptides that are longer or peptides that are prone to aggregation. This further highlights that co-translational ubiquitylation primarily targets misfolded nascent peptides. Interestingly, a systems-level analysis revealed that impairment of co-translational folding (through AZC treatment) led to widespread co-translational ubiquitylation of nascent peptides irrespective of their specific features.

Finally, Duttler *et al.* reasoned that cells are likely to have a system that protects susceptible nascent peptides from ubiquitylation and degradation and promotes their correct folding. As such, they examined a potential role for the ribosome-bound chaperone NAC (nascent polypeptide-associated complex), which is known to bind most nascent chains and promote their sorting and trafficking. Loss of NAC resulted in enhanced ubiquitylation of susceptible nascent peptides (those carrying the features described above), which also displayed increased aggregation propensity. This indicates that NAC, and probably other ribosome-bound chaperones, protect susceptible peptides and promote their folding. Similarly, Wang *et al.* observed a ~50% increase in co-translational ubiquitylation following knockdown of a NAC subunit in human cells lines.

The two studies confirm that multilayered control is in place in which nascent peptides that are prone to misfolding can either be protected by chaperones such as NAC or targeted for co-translational ubiquitylation and proteasomal degradation. Together, these mechanisms may offer some protection from the deleterious effects that arise from the accumulation of misfolded proteins and aggregates, and ensure cellular and organismal homeostasis.

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> “multilayered protein quality control can operate concurrently with translation”

ORIGINAL RESEARCH PAPERS Wang, F., Durfee, L. A. & Huibregtse, J. M. A cotranslational ubiquitination pathway for quality control of misfolded proteins. *Mol. Cell* 11 Apr 2013 (doi:10.1016/j.molcel.2013.03.009) | Duttler, S., Pechmann, S. & Frydman, J. Principles of cotranslational ubiquitination and quality control at the ribosome. *Mol. Cell* 11 Apr 2013 (doi:10.1016/j.molcel.2013.03.010)