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RESEARCH HIGHLIGHT

Cleaning up stalled ribosome-translocon complexes with ufmylation

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Ufmylation, a metazoan-specific ubiquitin-like post-translational modification, is implicated in numerous cellular processes and mammalian phenotypes. Here, Wang et al. uncover an important function of ribosome ufmylation in protein quality control at ribosomes that have stalled during co-translational translocation of endoplasmic reticulum (ER)-targeted proteins.

Post-translational modifications (PTMs) diversify the proteome and modulate protein function on faster time scales compared to transcriptional or translational regulation. While ubiquitination is one of the most common PTMs, several ubiquitin-like proteins have also been described. One intriguing example first described in 2004 is ufmylation,[1] in which the ubiquitin-like small protein Ubiquitin-Fold Modifier 1 (UFM1) is reversibly conjugated to lysine residues on substrates via a cascade of UFM1-specific ligases and proteases. Interestingly, this modification is found only in metazoans and can occur in plants, but not yeast, suggesting that it may have a specific role in multicellular organisms. Indeed, embryonic lethal hematopoietic defects in *Ufm1*-knockout mice reveal a significant physiologic function. Furthermore, abnormalities in ufmylation are implicated in a wide range of conditions such as leukodystrophy, schizophrenia, hip dysplasia, diabetes, heart failure, and breast cancer (reviewed in refs. [2,3]). How, then, does this fascinating PTM influence fundamental aspects of cellular physiology?

While several ufmylation substrates have been reported, including those involved in estrogen signaling and DNA damage repair,[4,5] an unexpected connection to ribosomes has recently emerged. Recent findings suggest that the ribosome's central function in mRNA translation can be modulated by additional enzymes or other proteins that associate with and potentially modify the ribosome. In particular, UFL1, which is the only known enzyme that determines the substrate specificity of ufmylation, was found to associate with ribosomes in an mRNA-independent manner.[6] Moreover, both UFL1 and UFM1 were enriched in fractions corresponding to the large ribosome subunit, suggesting a possibly novel function for ufmylation in translation control. Indeed, Walczak et al.[7] subsequently showed that several C-terminal lysines of the large ribosomal subunit protein RPL26 are primary substrates for ufmylation, and noted that these residues are where the ribosome's peptide exit tunnel interfaces with the translocon at the ER. Interestingly, UFM1 knockout or ablation of the RPL26 ufmylation sites caused a mild ER stress phenotype, consistent with previously reported associations between UFM1 and ER homeostasis (reviewed in refs. [2,3]). However, it remained unclear how ufmylation altered ribosome function and what regulated this modification.

In a recent paper published in *Cell Research*, Wang et al.[8] reported that RPL26 ufmylation increases upon stalling of ER translocon-associated ribosomes. During synthesis of most membrane and secreted proteins, nascent peptides are co-translationally translocated from translocon-docked ribosomes into the ER lumen, where they are folded, post-translationally modified, and trafficked elsewhere. Within the ER lumen, failed protein processing triggers ER-associated degradation (ERAD), in which the defective proteins are exported to the cytoplasm, ubiquitinated, and proteasomally degraded (reviewed in ref. [9]). Less is known, however, about the cellular response to ribosome stalling during co-translational translocation. Ribosomes can stall for several reasons: for example, mRNAs that lack a stop codon or that are prematurely adenylated cause ribosomes to stall over polyadenylation tracts. In the cytoplasm, stalling is resolved by the ribosome-associated protein quality control (RQC) response, in which the large and small ribosomal subunits are dissociated, the nascent peptide is ubiquitinated and extracted from the large subunit for proteasomal degradation, and the mRNA is degraded. Importantly, RQC ensures that ribosomes are salvaged from unproductive translation complexes, and that potentially defective mRNAs and nascent peptides are eliminated (reviewed in ref. [10]). Interestingly, Wang et al. suggest that ufmylation of ER-associated ribosomes represents a distinct, ER-specific ribosome stalling response (Fig. 1). The authors designed a transcript that encodes an ER-targeted reporter protein and contains a polyadenylation tract. Expression of this reporter transcript induced RPL26 ufmylation, while expression of a similar transcript lacking the ER signal sequence did not. In contrast to proteasome-mediated ERAD, nascent peptides from translocon-associated stalled ribosomes are trafficked to lysosomes for degradation. This process is ablated by lysosome-inhibiting drugs, and also by deleting RPL26 ufmylation sites, suggesting that RPL26 ufmylation is part of a specific response to translocon-associated ribosome stalling.

Discovering a novel ER-specific RQC pathway, especially one that involves a metazoan-specific PTM, suggests that maintaining ER proteostasis involves more players than previously understood and has important roles in multicellular organism physiology. Interestingly, Wang et al. show that degradation of their reporter is inhibited but still occurs gradually when RPL26 ufmylation is completely ablated, suggesting that some ufmylation-independent RQC is also active at the ER.[8] The division of labor between distinct RQC pathways at the ER, and the types of stalling that ribosomal ufmylation can resolve, remain to be explored. Future work may also identify additional factors necessary for processing ufmylated ER-associated ribosomes. While core

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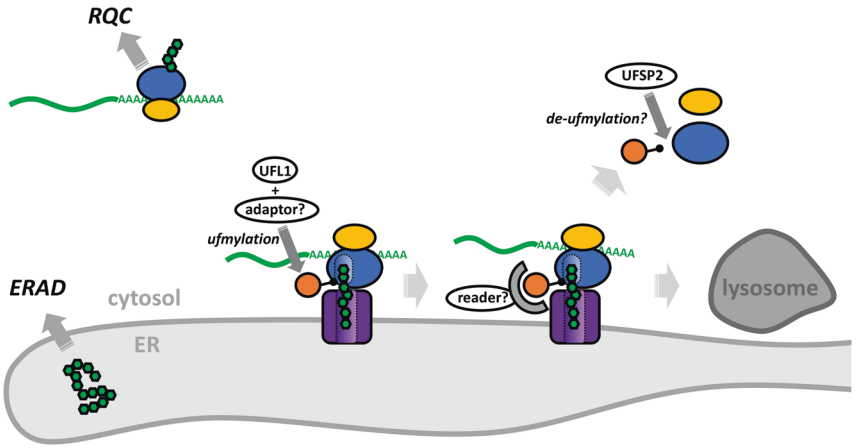

Fig. 1 A schematic diagram showing the role of ribosomal ufmylation in quality control at the translocon-associated stalled ribosomes, a process that is distinct from ribosome-associated protein quality control (RQC) and ER-associated degradation (ERAD)

ufmylation enzymes have been identified,[2] other proteins may specify their activity in the ER context. For example, an adaptor protein may sense stalled ribosomes and recruit UFM1 ligases. Once conjugated to RPL26, UFM1 may interact with reader and effector proteins that mediate ribosome dissociation and lysosomal trafficking of stalled peptide. Finally, rescued ribosomes are likely de-ufmylated by the protease UFSP2. While experimental and clinical data show that impaired de-ufmylation is also pathogenic,[2,7] the regulation of deufmylation remains unexplored.

Considering the growing list of ufmylation substrates, it bears asking how much each of their respective pathways contributes to observed loss-of-function phenotypes, and why certain tissues are more severely affected. Across tissues, ufmylated substrates of distinct molecular weights have different tissue-dependent abundance patterns.[1] In both studies by Walczak et al. and Wang et al., RPL26 is the predominant ufmylation substrate in the studied cell types, which are mainly immortalized cell lines.[7,8] Since ufmylation-deficient mice have striking hematopoietic defects, Wang et al. proposed that hematopoiesis increases ER burden and sensitizes cells to ER-RQC failure. In their in vitro models, RPL26 ufmylation increased during erythropoiesis. Furthermore, UFM1 depletion impaired secretion of specific proteins such as Clusterin, and UFM1 depletion or RPL26 ufmylation site ablation reduced hemoglobin expression.[8] Several underlying mechanisms are possible. Could compromised ER-RQC selectively deplete proteins that are sensitive to suboptimal co-translational translocation? Accordingly, are some cell types more susceptible due to smaller reserves of ribosomes or translocons, or greater abundance of mRNAs prone to stalling at the ER? Future studies, such as ribosome profiling of cells with genetically ablated RPL26 ufmylation sites or phenotyping of similarly edited mice, should enable a deeper understanding of how ER-specific RQC, or potentially other yet unknown roles of ribosome ufmylation, may contribute to the intriguing tissue-specific phenotypes associated with loss of function of UFM1. While more questions remain, identifying a role in translocon-specific protein quality control brings UFM1 into a new light. More broadly, these studies highlight the growing appreciation that ribosome modifications orchestrate ribosome function and protein quality control, processes that are vital to our understanding of normal cellular physiology and disease states.

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