

REVIEW ARTICLE

UFMylation: A Key Role in Maintaining Endoplasmic Reticulum Homeostasis

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ABSTRACT

UFMylation, a ubiquitin-like post-translational modification system, plays an essential role in regulating endoplasmic reticulum (ER) function. This process involves a cascade of biochemical reactions mediated by several core molecular components, including UFM1, the E1 enzyme UBA5, the E2 enzyme UFC1, the E3 enzyme UFL1, as well as the accessory proteins DDRGK1 and CDK5RAP3. During UFMylation, UFM1 undergoes maturation, activation, conjugation, and deconjugation in a dynamic process, whereas the ER-localized UFL1-DDRGK1 complex governs substrate selection and modification efficiency. The major function of UFMylation is to modulate the ER stress response, thereby balancing adaptive cellular remodeling and apoptotic signaling. Through the modification of key substrate proteins, UFMylation activates a synergistic clearance mechanism that coordinates ER-phagy with ribosome-associated quality control, thereby facilitating the removal of stalled ribosomes and damaged ER to maintain ER proteostasis and structural integrity. Dysregulation of UFMylation is frequently associated with various diseases characterized by abnormal ER function. In this review, we will describe the molecular pathways associated with ER-associated UFMylation process and then discuss its core regulatory functions within the ER and its possible involvements in several congenital human diseases.

1 | Introduction

The endoplasmic reticulum (ER), as a central organelle in eukaryotic cells, plays a critical role in cellular survival by mediating key functions such as protein synthesis and folding, lipid metabolism, and the regulation of calcium homeostasis [1–4]. Challenges such as protein folding overload, oxidative stress, or metabolic disturbances lead to abnormal accumulation of unfolded proteins in the ER lumen, triggering ER stress. This subsequently activates adaptive response mechanisms, including the unfolded protein response (UPR). If the imbalance in

homeostasis persists or intensifies, it may trigger apoptosis, thereby causing tissue damage and playing a key role in the development of major pathological conditions, including neurodegenerative diseases, metabolic syndrome, and cardiovascular disorders [5–9]. To preserve ER functional integrity, cells have evolved a multi-tiered quality control network, including the stress-responsive UPR, ribosome-associated quality control (RQC) for clearing translation-stalled aberrant peptide chains, and ER-phagy for selectively degrading damaged ER structures [10, 11]. These three processes synergistically form the ER homeostasis defense framework.

Abbreviations: CAT-tail, carboxy-terminal alanine and threonine tail; CDK5RAP3, CDK5 regulatory subunit associated protein 3; cryo-EM, cryo-electron microscopy; EBV, Epstein–Barr virus; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated protein degradation; ER-AP, endoplasmic reticulum-arrested protein; ER-phagy, endoplasmic reticulum autophagy; GC, Granulosa cell; GIMs, GABARAP-interacting motifs; LIRs, LC3-interacting regions; MAM, mitochondria-associated ER membrane; NEMF, nuclear export mediator factor; OST, oligosaccharyltransferase; PROTAC, proteolysis-targeting chimeras; PTM, posttranslational modification; RPL26, 60S ribosomal protein L26; RQC, ribosome-associated quality control; RTJ, ribosome-translocon junction; sAImS, shuffled ATG8-interacting motifs; TAQC, translocon-associated quality control; Tg, thapsigargin; Tm, tunicamycin; TRAPP, transport protein particle; UB, ubiquitin; UBA5, UBL activating enzyme-5; UBL, ubiquitin-like protein; UBS, UFC1-binding sequence; UFBP1, UFM1 binding protein-1; UFC1, UFM conjugating enzyme-1; UFM, UFM1-interacting motif; UFL1, UFM1 specific ligase-1; UFM1, ubiquitin fold modifier-1; UFSP1, UFM1 specific protease-1; UFSP2, UFM1 specific protease-2; UPR, unfolded protein response; UREL, UFM1 ribosome E3 ligase; vDUB, viral deubiquitinase.

In recent years, the critical role of ubiquitin-like protein (UBL) modifications in regulating target protein activity, stability, subcellular localization, and macromolecular interactions has become increasingly prominent. Multiple UBL pathways—including SUMO, NEDD8, ATG8, ATG12, URM1, UFM1, FAT10, and ISG15—have been identified. Similar to ubiquitination, these modifications are achieved through an enzymatic cascade comprising E1 activating enzymes, E2 conjugating enzymes, and E3 ligases [12, 13]. Among these, UFMylation, an evolutionarily conserved ubiquitin-like modification discovered in 2004, has attracted widespread attention due to its unique ER-localized function [14]. UFMylation utilizes UFM1 as the modifier molecule and is carried out through a cascade involving UBA5 (E1-activating enzyme), UFC1 (E2-conjugating enzyme), UFL1 (E3 ligase), and the ER-anchored auxiliary complex DDRGK1–CDK5RAP3 [15–17]. Unlike the ubiquitination system, only one E1, one E2, and one E3 enzyme have been identified for UFMylation to date, underscoring the specificity of this modification pathway. Consequently, mutations in these core components lead directly to ER structural disruption, dysregulation of stress response pathways, and cell death, highlighting the irreplaceable and central role of UFMylation in maintaining ER homeostasis [18].

This review will systematically dissect the molecular mechanisms by which UFMylation regulates ER function. We will first elaborate the molecular basis of this specific enzymatic reaction cascade, and then focus on how this pathway integrates and modulates three key processes: the dynamic equilibrium of UPR, the precise activation of ER-phagy, and the efficient execution of ER-RQC. Building upon this foundation, we will explore the pathological links between UFMylation dysregulation and major diseases such as metabolic disorders, cardiovascular dysfunction, and neurodegenerative pathologies, and eventually discuss the potential of targeting this pathway for therapeutic strategies. By clarifying the multifaceted roles of UFMylation as the “guardian of ER homeostasis,” this review aims to provide novel perspectives for research on organelle quality control mechanisms and the possible strategies for prevention/treatment of related diseases.

2 | Molecular Mechanism of UFMylation

UFMylation is an important ubiquitin-like modification that relies on the synergistic action of several core molecules for an orchestrated cascade of biochemical reactions. As mentioned earlier, the core components of this modification system include the modifier UFM1, catalytic enzymes (UBA5, UFC1, and UFL1) and key accessory proteins (DDRGK1 and CDK5RAP3). The spatiotemporal expression, subcellular localization, and interacting networks of these molecules collectively determine the specificity of UFMylation modification and its regulated biological functions [16].

2.1 | The Main Participants in UFMylation

UFM1 is a small protein consisting of only 85 amino acids. Although it shares approximately 15% sequence homology

with ubiquitin, it adopts a conserved β -grasp fold, which serves as a critical structural foundation for its interactions with enzymes and target proteins [14]. UFM1 contains 6 lysine residues, among which Lys69 can serve as a linkage site to form a poly-UFM1 chain which enhances its binding to target proteins or acts as a signaling hub to recruit downstream effectors such as the autophagy adaptor proteins for autophagic degradation [19, 20]. The E1 activating enzyme UBA5 is primarily cytoplasmic and acts as the key rate-limiting enzyme in the pathway, and its deficiency causes embryonic lethality and hematopoietic defects in mice, highlighting its essential role in development [21]. From a mechanistic perspective, UBA5 plays an essential role in facilitating the efficient transfer of UFM1 to the E2 enzyme UFC1 and subsequent downstream substrates [22]. The UFC1 is distributed in the cytoplasm and nucleus. It forms a thioester bond with activated UFM1 through its active site, Cys116. UBA5 interacts with UFC1 through the UFC1-binding sequence (UBS) containing several conserved hydrophobic residues, and mutation of this sequence completely blocks the transfer of UFM1 from UBA5 to UFC1 [23, 24]. The E3 ligase UFL1 forms a stable complex with the accessory protein DDRGK1. This complex is primarily localized to the endoplasmic reticulum (ER) and serves as the core regulatory factor for ER-associated UFMylation [25]. The N-terminus of UFL1 exhibits a significantly higher affinity for UFC1 than for UBA5 (approximately 10-fold higher), ensuring efficient UFM1 transfer, while its C-terminus directly binds substrates (e.g., ribosomal protein RPL26, ER membrane protein HRD1, etc.) and determines substrate specificity [26–28].

Among the accessory proteins, DDRGK1 and CDK5RAP3 are the core components of the UFM1 ribosome E3 ligase complex (UREL complex) in the UFMylation system. Specifically, DDRGK1 serves as the anchor point of the complex on the ER membrane, providing a structural basis for E3 ligase activity. It can form the minimal E3 catalytic unit with UFL1. Under conditions of starvation or ER stress, it assists UFL1 in moving to the ER surface and activating. Besides, DDRGK1 is the earliest identified UFMylation substrate [29]. CDK5RAP3 stabilizes the UFL1-DDRGK1 complex and acts as a substrate adapter to help recognize and recruit specific substrates, ensuring precise recognition by the complex [30]. At the same time, it can participate in signal transduction. In ER-autophagy, after being de-ufmylated, CDK5RAP3 exposes the motif for binding to the autophagic membrane and recruits the autophagic machinery. In summary, DDRGK1 and CDK5RAP3 are the “steering wheel” and “sights” of the UFMylation system, with the former dominating the positioning and assembly of the complex and the latter ensuring the precision and specificity of the modification. Together, they form the molecular basis for cells to respond to ER stress, recycle ribosomal resources, and maintain protein homeostasis [25, 31]; and their dysfunction is directly related to various developmental defects and diseases.

In addition, it is noted that many key protein molecules involved in Ufmylation can be localized to different cellular compartments, relating to their nuclear or cytoplasmic functions. For instance, DDRGK1 (containing NLS), UFL1, UBA5, UFC1, etc., are involved in DNA damage repair, transcriptional regulation, or cell cycle progression in the nucleus [32–34]. This couples UFMylation with the nuclear signaling network, expanding the

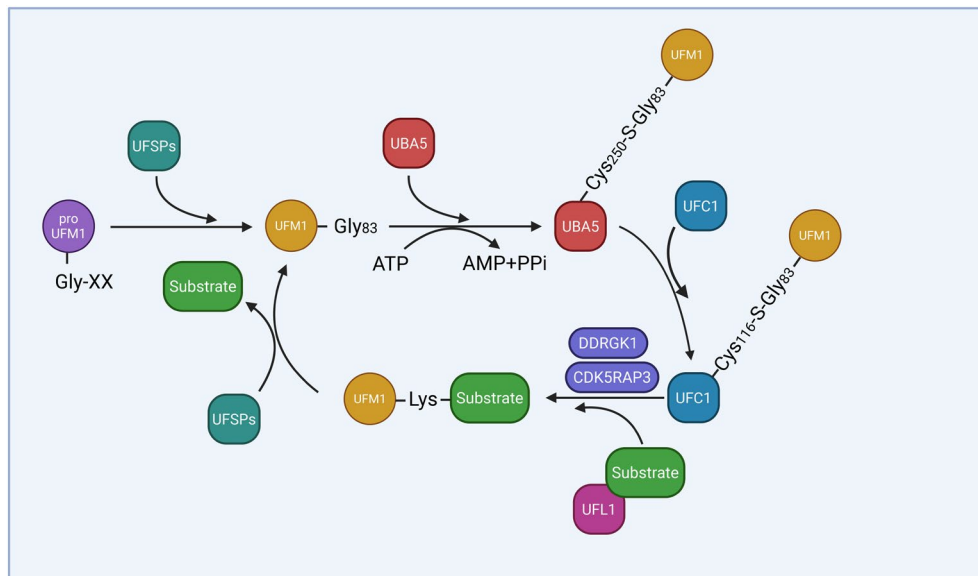


FIGURE 1 | The UFMylation Pathway The UFMylation pathway involves a series of enzymatic reactions regulating the binding and dissociation of UFM1 to target substrates. (1) Maturation: Precursor pro-UFM1 is cleaved at its C-terminus by UFSP1/2 proteases to generate mature UFM1, exposing Gly83. (2) Activation: Mature UFM1 is activated by E1 enzyme UBA5 in an ATP-dependent manner, forming a UBA5-Cys250~UFM1-Gly83 thioester bond. (3) Conjugation: Activated UFM1 is transferred to E2 enzyme UFC1, forming a UFC1~UFM1 thioester intermediate. This intermediate, with the E3 ligase complex UFL1-DDRGK1, transfers UFM1 to target substrates (e.g., DDRGK1, RPL26) located on the ER membrane, forming an isopeptide bond. (4) Deconjugation (deUFMylation): UFSP1/2 proteases cleave the isopeptide bond, releasing UFM1 for recycling. S: Thioester bond.

dimension of protein modifications. Therefore, it is reasonable to believe that non-ER ubiquitin-like modifications will be an important and growing field of future research.

2.2 | The Process of UFMylation

UFMylation involves a series of highly ordered enzymatic reactions encompassing four core steps: maturation, activation, conjugation, and deconjugation (deUFMylation) of UFM1 (Figure 1). This cascade ensures the precision and dynamics of this modification process [16, 35]. The first step involves the maturation of UFM1, which begins with the cleavage of its C-terminal Ser84-Cys85 dipeptide in the precursor pro-UFM1 precursor protein by the proteases UFSP1 or UFSP2, exposing the critical glycine residue (Gly83) [36, 37]. Unlike other ubiquitin-like proteins, UFM1 uses a single glycine residue as its conjugation site. UFSP2 plays the dominant role in UFM1 maturation, and knockout of UFSP2 leads to the accumulation of pro-UFM1 and a significant decrease in mature UFM1 levels. In contrast, UFSP1 has a relatively mild role in UFM1 maturation and appears to be more cell-type specific [38, 39]. The next step is the activation process of UFM1, which involves the formation of a high-energy thioester bond between UBA5 and mature UFM1 (UBA5-Cys250~UFM1-Gly83) through an ATP-dependent mechanism. This process requires UBA5 homodimerization, which enhances its ATP-binding capacity. UBA5 binds to UFM1 through the UFM1-interacting motif (UFIM) in its N-terminal region [40]. Structural studies reveal that UFM1 binding stabilizes the UBA5 dimer and enhances its ATP hydrolysis activity [41]. The activated UFM1 is then transferred via a thioester bond to the E2 conjugating enzyme UFC1, forming

the UFC1~UFM1 intermediate. Subsequently, UFM1 is covalently attached to lysine residues on target proteins through the concerted action of UFL1 and its accessory proteins DDRGK1 and CDK5RAP3 [23, 25]. UFL1 functions as a scaffold-type E3 ligase, mediating the UFM1 transfer between UFC1 and the substrate protein through physical interactions while lacking intrinsic catalytic activity itself [42]. Finally, deconjugation (deUFMylation) of UFM1 ensures the reversibility of the modification. This reaction is mediated by the proteases UFSP2 or UFSP1, which cleave the isopeptide bond linking UFM1 to the target protein, thereby releasing UFM1 for recycling. UFSP2 exhibits significantly higher cleavage efficiency for UFM1 conjugates than UFSP1, playing a dominant role in the maintenance of UFMylation dynamic balance. Moreover, UFSP2 subcellular localization is regulated by cellular stress signals; for example, it can translocate to damage sites upon DNA damage, mediating the deUFMylation of histone H4 and participating in DNA damage repair [37, 43].

3 | Role of UFMylation in the Regulation of ER Stress

It is well established that defects in UFMylation are prominent at the cellular level as endoplasmic reticulum (ER) stress [15]. The absence or mutation of any core component within the UFMylation cascade—including UFM1, its dedicated E1, E2, or E3 enzymes, or essential auxiliary factors—invariably induces ER stress, although the severity varies [44]. ER stress refers to the cellular adaptive response triggered by disturbances in endoplasmic reticulum function, resulting from factors such as oxidative damage, calcium ion imbalance, defective protein

glycosylation, mitochondrial dysfunction, as well as protein misfolding and aggregation. ER stress is transmitted through discrete yet interconnected unfolded protein response (UPR) signaling cascades initiated by the sensors IRE1 α , PERK, and ATF6, leading to the activation of transcriptional programs and translational control [7].

UPR stress sensors are activated either by releasing their inhibitory interactions with the ER chaperone HSPA5 (GRP78/BiP) or through direct binding of misfolded proteins to IRE1 α and PERK. IRE1 α possesses kinase and RNase domains within its cytosolic region. Upon activation, IRE1 α homodimerizes and autophosphorylates, subsequently catalyzing the unconventional splicing of XBP1 mRNA by removing a short intron harboring a stop codon. The spliced form of XBP1 (XBP1s) functions as a highly active transcription factor that governs the transcription of genes encoding chaperones and quality control proteins. PERK attenuates protein translation by phosphorylating the α subunit of the eIF2 α complex. The mRNA of the transcription factor ATF4 contains alternative open reading frames that enhance its translation under conditions of eIF2 α inhibition. ATF4 regulates the expression of genes involved in folding, redox control, amino acid metabolism, autophagy, and apoptosis. Specifically, ATF4 controls the expression of CHOP and members of the BCL-2 family, which serve as mediators of apoptosis during ER stress. Under ER stress, ATF6 dissociates from HSPA5 and translocates to the Golgi apparatus. Consecutive cleavage of ATF6 by proteases S1P and S2P releases an active transcription factor that regulates the expression of UPR target genes.

Although these three UPR pathways are interconnected and exhibit distinct yet complementary functions, the intensity and duration of the stress ultimately determine whether the cell undergoes an adaptive response or engages apoptotic pathways [7].

3.1 | UFMylation Deficiency Triggers ER Stress

Accumulating evidence demonstrates that UFMylation plays a central role in embryonic development. Specifically, the deletion of most core UFMylation components, such as Uba5 [21], Ufl1 [45], and Ddrk1 [46], results in embryonic lethality in mice. Due to the timing of embryonic death, which generally occurs before embryonic day 12.5–13.5 (E12.5–E13.5), UFMylation appears to be crucial for organogenesis and the development of specific organs and tissues, including erythrocytes, the skeletal system, and the brain [47]. Furthermore, with the application of conditional knockout mouse models, an increasing number of cell types and tissues have been found to exhibit developmental and functional abnormalities upon loss of UFMylation components. Notably, the most prominent physiological abnormality associated with UFMylation deficiency is increased ER stress and activation of the UPR pathway (Table 1).

As previously noted during erythroid development, deficiencies in various UFMylation components consistently cause hematopoietic defects in mice. This occurs because UFMylation disruption compromises cellular homeostasis, triggering and exacerbating ER stress, ultimately leading to hematopoietic stem cell death. These findings demonstrate that intact UFMylation is essential for normal ER function. Similar phenotypes manifest

in hepatocytes [52, 53], intestinal endothelial cells [55, 56], pancreatic acinar cells [51], follicular granulosa cells [59], renal tubular epithelial cells [49], and other cell types upon UFMylation component interference, revealing the universality of this mechanism. Furthermore, analogous UFMylation systems have been identified in *Bombyx mori* ovarian cells [67] and *Arabidopsis* protoplasts [68]. Disrupting UFMylation components in these models similarly intensifies ER stress and activates the UPR pathway, indicating evolutionary conservation and functional significance across species.

The significant correlation between UFMylation and ER function is further evidenced by the upregulation of UFMylation components under conditions of heightened ER stress. Researchers induced ischemic heart disease in model mice by specifically expressing MCP-1 in the mouse heart. Microarray analysis revealed changes in the gene expression profile during cardiac disease progression in this model, demonstrating transcriptional activation of numerous ER stress-related genes. Concurrently, significant upregulation of the Ufm1 gene was observed. This represents the first reported association between UFMylation and ER stress [69]. Granulosa cell (GC) proliferation and differentiation within follicles regulate oocyte maturation; consequently, severe ER stress in GCs may lead to ovarian dysfunction [7]. Treatment of follicular GCs with ER stress inducers tunicamycin (Tm) or thapsigargin (Tg) significantly upregulated both mRNA and protein levels of UFMylation pathway components and induced apoptosis [58]. Similarly, treatment of primary cultured GCs with cisplatin induced ER stress, increased expression of UFMylation pathway components, and promoted apoptosis. Notably, concomitant Ufl1 knockdown exacerbated these effects, whereas Ufl1 overexpression alleviated ER stress and suppressed apoptosis [59]. Collectively, these findings indicate that UFMylation functions as a protective mechanism intrinsically designed to mitigate ER stress. Its deficiency exacerbates the severity of ER stress, subsequently inducing cellular developmental arrest or apoptosis.

3.2 | UFMylation in Regulation of the UPR

Defective UFMylation consistently induces ER stress, although the magnitude of this effect varies considerably across different cell types and tissues. This variability is primarily evident in the distinct activation patterns and downstream outputs of the UPR signaling pathway (Table 1). While these differences may reflect inherent cellular tolerances to UFMylation defects, our understanding of this aspect remains limited.

DDRKG1 is a critical factor for maintaining ER homeostasis. Its inactivation directly induces ER stress and disrupts UPR balance [61, 70]. Mechanistically, UFMylation of DDRKG1 enables it to specifically interact with unphosphorylated IRE1 α , suppressing IRE1 α phosphorylation. This suppression reduces the ubiquitination-mediated degradation of IRE1 α , thereby stabilizing the IRE1 α protein and sustaining the activation of the adaptive IRE1 α -XBP1 signaling pathway. Consequently, loss of DDRKG1 or mutation of its key UFMylation site (e.g., K267R, mimicking a non-ufmylatable state) decreases IRE1 α stability. This instability not only inhibits the IRE1 α -XBP1 pathway but also aberrantly activates the pro-apoptotic PERK-eIF2 α -CHOP pathway. Although

TABLE 1 | ER stress and UPR activation by Ufmylation mutants.

Cell or organ type	Disease model	Evidence of elevated ER stress and dysregulated UPR pathway	References
Brain	CDK5RAP3 ^{f/f} ; Nestin-Cre	Elevated ER stress and induction of the IRE1a signaling pathway	[48]
Kidney	Ufl1 ^{f/f} ; Pax8-Cre	Elevated ER stress and induction of the PERK signaling pathway	[49]
Heart	Ufl1 ^{f/f} ; αMHC-Cre	Elevated ER stress	[50]
Cardiomyocytes	si-Ufl1	Inhibition PERK signaling pathway	
Pancreatic acinar cells	Ufl1 ^{f/f} ; ROSA-CreERT2	Mild elevation of ER stress	[51]
Bone marrow cells	Ufl1 ^{f/f} ; Cre ^{ERT}	Elevated ER stress and UPR signaling pathways	[45]
Hepatocytes	Cdk5rap3 ^{tm1d/tm1d} ; Alb-Cre	Elevated ER stress and induction of the IRE1a and PERK signaling pathways after partial hepatectomy	[52]
	Cdk5rap3 ^{tm1d/tm1d} ; Foxa3-Cre	Elevated ER stress and induction of the IRE1a and PERK signaling pathways	[53]
L-02 cells	shDDRGK1 under FFA treatment	Elevated ER stress and induction of the IRE1a, ATF6 and PERK signaling pathways	[54]
Hematopoietic stem cells	Ddrgk1 ^{f/f} ; Cre ^{ERT}	Elevated ER stress and evoked UPR signaling pathways	[46]
Intestinal epithelial cells	Cdk5rap3 ^{ΔAIEC}	Elevated ER stress and induction of the IRE1a signaling pathway	[55]
	Ddrgk1 ^{f/f} ; Villin-Cre	Elevated ER stress and induction of the IRE1a signaling pathway	[56]
	Ddrgk1 ^{f/f} ; ROSA-CreERT2	Elevated ER stress and induction of the IRE1a and PERK signaling pathways	
Plasma cells	Ddrgk1 ^{f/f} ; Cd19-Cre	Elevated ER stress and induction of the IRE1a and PERK signaling pathways	[57]
Granulosa cells	si-Ufm1 under Tm or Tg treatment	Elevated ER stress and UPR signaling pathways	[58]
	shUFL1 under cisplatin treatment	Elevated ER stress and induction of the IRE1a signaling pathway	[59]
	oe-UFL1 under cisplatin treatment	Alleviated ER stress and inhibition of the IRE1a signaling pathway	

(Continues)

TABLE 1 | (Continued)

Cell or organ type	Disease model	Evidence of elevated ER stress and dysregulated UPR pathway	References
Cortical organoids from proband-derived induced pluripotent stem cells	UBA5 ^{A371T/R55H} , UBA5 ^{A371T/F292*}	Elevated ER stress, inhibition of the IRE1a signaling pathway, and induction of the ATF6 and PERK signaling pathways	[60]
MCF7, HepG2 cells, hematopoietic stem cells	shDDRKG1	Elevated ER stress, inhibition of the IRE1a signaling pathway and induction of the PERK signaling pathway.	[61]
HepG2, MCF7, HeLa cells	sgDDRKG1, sgUFL1, sgUFM1	Elevated ER stress and induction of the IRE1a and PERK signaling pathways	[62]
U2OS cells	shUba5, shUfc1, shUfl1, shCdk5rap3	Elevated ER stress	[63]
	sgUFL1	Elevated ER stress and induction of the IRE1a, ATF6 and PERK signaling pathways	[64]
	sgCdk5rap3	Elevated ER stress and induction of the IRE1a signaling pathway	
KGN cells	si-Ufl1 under LPS treatment	Elevated ER stress	[65]
	oe-Ufl1 under LPS treatment	Alleviated ER stress	
HK2 cells	oe-Ufl1, oe-Ddrkg1 (CI-AKI)	Alleviated ER stress	[66]
<i>Bombyx mori</i> ovarian cells	shBmUFM1	Elevated ER stress and induction of the IRE1a and PERK signaling pathways	[67]
<i>Arabidopsis</i> protoplasts	Ufl1 T-DNA insertional mutant	Elevated ER stress under salt stress condition	[68]

Note: *, nonsense variant.
Abbreviations: CI-AKI, contrast-induced Acute kidney injury; oe, over express; Tg, thapsigargin; Tm, tunicamycin.

the precise mechanism by which UFMylated DDRGK1 suppresses PERK activation remains unclear, these disruptions ultimately lead to cell death in various cell types (Figure 2) [61, 70].

UFMylation regulates different UPR branches with high selectivity and forms a dynamic bidirectional feedback network with the UPR, enabling precise maintenance of proteostasis. For instance, during plasma cell differentiation, DDRGK1 promotes ER expansion and immunoglobulin secretion by suppressing the PERK pathway. Simultaneously, the IRE1α/XBP1 axis upregulates the expression of DDRGK1 and its pathway-associated genes, establishing a “UFMylation-UPR adaptive feedback loop” that supports cell-specific functions. However, this regulation exhibits cell-type specificity. Unlike its effects in tumor cells and hematopoietic stem cells, DDRGK1 deficiency during plasma cell development does not reduce IRE1α levels but instead paradoxically increases expression of both IRE1α and its downstream genes. Further investigation revealed that

DDRGK1 promotes plasma cell development independently of its UFMylation at K267, suggesting distinct functional regulation of DDRGK1 depending on the cellular context. This adds complexity to our understanding of DDRGK1-mediated UFMylation-UPR regulation (Figure 3) [57].

The interdependence between UFMylation and the UPR is further highlighted by the fact that ER stress or inhibition of vesicular transport activates the Ufm1 promoter through the transcription factor XBP1. This induction leads to increased expression of UFMylation-related proteins, including Ufm1, Uba5, and Ufl1. Consistent with their dynamic equilibrium, UFMylation deficiency itself triggers the UPR and causes abnormal expansion of the ER network [63].

Beyond regulating the UPR core machinery, UFMylation also maintains organelle homeostasis by modifying specific ER functional proteins and mediates cross-organelle

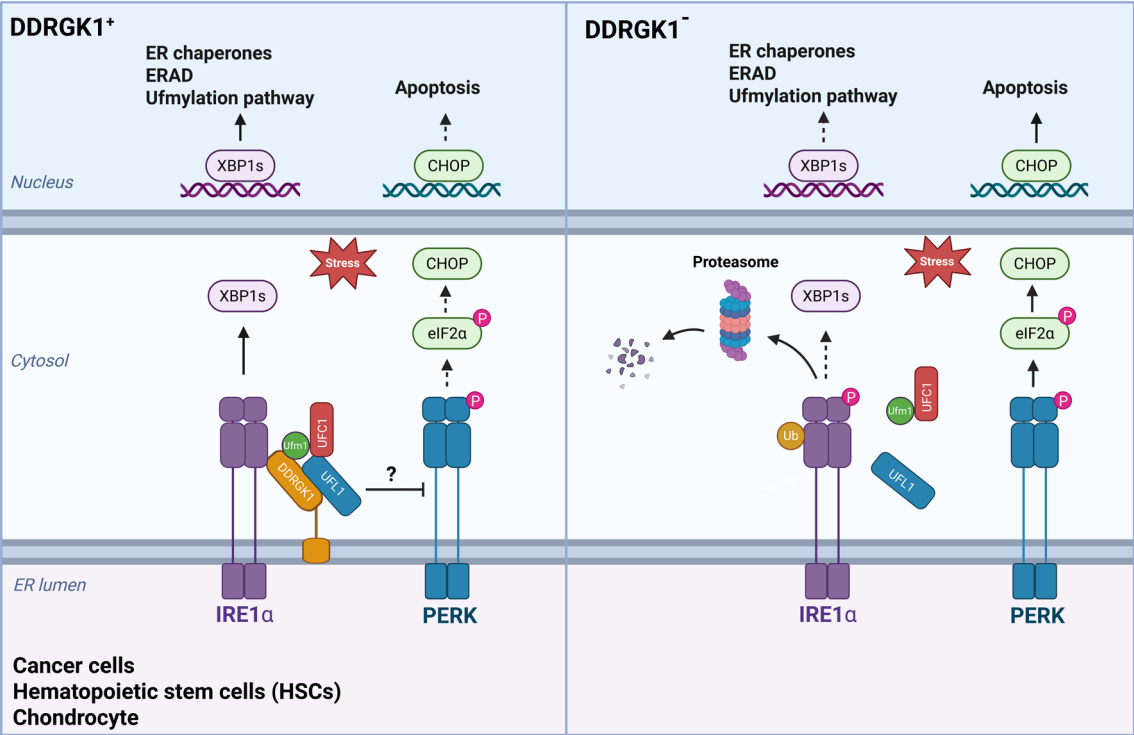


FIGURE 2 | DDRGK1 Maintains Cellular Homeostasis: Balancing IRE1α Activation and PERK Suppression UFMylation-modified DDRGK1 specifically interacts with unphosphorylated IRE1α, inhibiting its phosphorylation and subsequent ubiquitin-mediated degradation. This stabilizes IRE1α, sustaining adaptive IRE1α-XBP1 signaling. Concurrently, UFMylation-modified DDRGK1 suppresses activation of the pro-apoptotic PERK-eIF2α-CHOP pathway (dashed arrows denote suppression). Loss of DDRGK1 reduces IRE1α stability, impairing the IRE1α-XBP1 pathway and derepressing the PERK-CHOP pathway, ultimately triggering cell death.

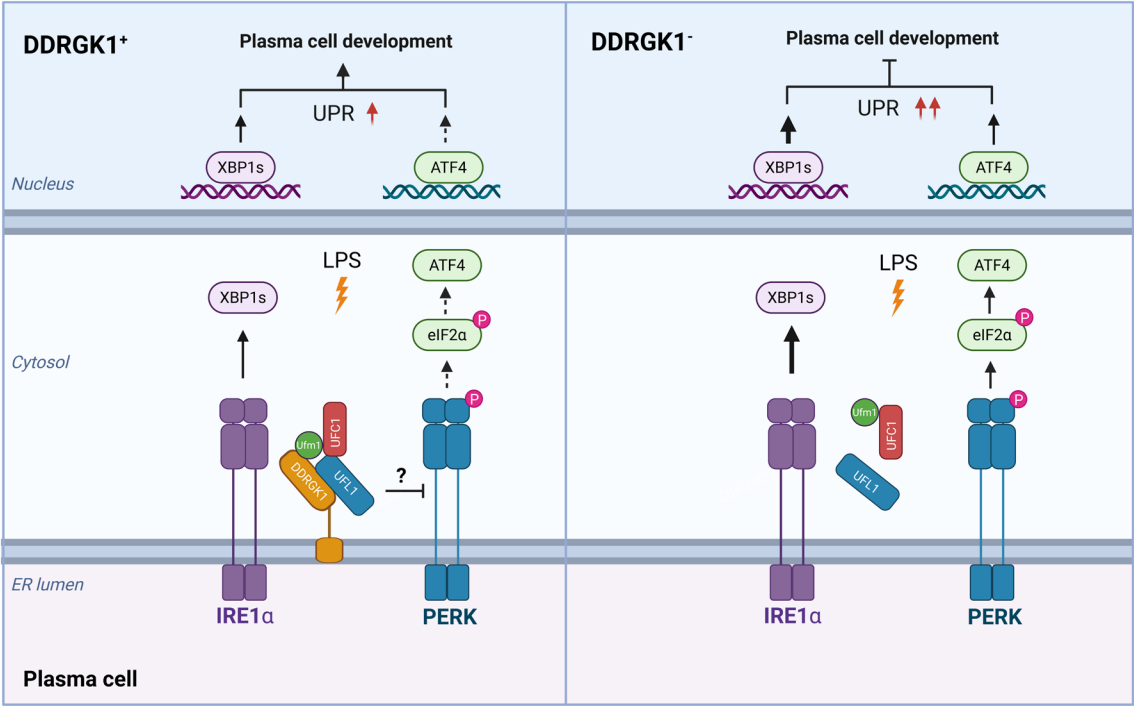


FIGURE 3 | DDRGK1 Ensures Proper Plasma Cell Differentiation by Regulating UPR Balance During LPS-induced plasma cell differentiation, DDRGK1 promotes appropriate activation of the adaptive IRE1α-XBP1 pathway while suppressing the PERK-eIF2α-ATF4 pathway. Conversely, loss of DDRGK1 triggers excessive activation of IRE1α-XBP1 (bold arrows indicate significant augmentation) and derepression/activation of PERK-eIF2α-ATF4, leading to aberrant plasma cell development.

communication. For example, the ER chaperone P4HB is a novel UFMylation substrate. Its mono-UFMylation at lysine sites prevents degradation via the ubiquitin-proteasome pathway. Defective UFMylation of P4HB not only triggers ER stress but also leads to mitochondrial dysfunction and oxidative stress, exacerbating metabolic disorders in palmitate-induced fatty liver models [71]. In pancreatic acinar cells, the UFMylation enzymes UFL1 and DDRGK1 contribute to maintaining normal pancreatic enzyme synthesis and secretion by regulating the expression of the ER chaperone BiP. UFL1 deficiency causes BiP downregulation, leading to ER stress, apoptosis, and trypsin activation. Upregulation of UFL1 and DDRGK1 upon alcohol exposure can alleviate pancreatic injury by modulating BiP levels [51].

In summary, the UFMylation system constitutes a core mechanism for the fine-tuned regulation of the UPR. UFMylation and the UPR form an essential, dynamic, bidirectional feedback network crucial for proteostasis, characterized by significant cell-type-specific complexities. Critically, dysregulation of both the UFMylation pathway and the UPR is implicated in the pathogenesis of diverse diseases. Emerging insights into the underlying molecular mechanisms are now revealing promising avenues for potential therapeutic interventions.

4 | Synergistic Action of ER-Phagy and RQC via UFMylation

ER-phagy selectively degrades damaged or excess ER [10], while RQC handles ribosomal translation stalling and aberrant nascent chains on the ER membrane [72]. These two distinct pathways are tightly coupled together through the post-translational UFMylation system which acts as a central signaling hub to coordinate their synergistic action in maintaining ER functional homeostasis.

4.1 | Ribosome Stalling: The Common Trigger and UFMylation as the Master Regulator

During the translational translocation process, ribosome stalling during cotranslational translocation at the ER serves as the common initiation signal for both ER-phagy and RQC. This signal is transduced through UFMylation of the 60S ribosomal subunit protein RPL26 (uL24). The heterotrimeric E3 ligase complex UREL (composed of UFL1, DDRGK1, and CDK5RAP3) selectively recognizes stalled ribosomes via a C-shaped clamp structure, binding the ribosomal region ranging from the tRNA site to the peptide exit channel. Cryo-electron microscopy reveals that UREL binding alters the conformation of the peptidyl transferase center, enabling precise UFMylation of a conserved C-terminal lysine on RPL26 [27, 73, 74].

Functioning as the integrative hub, RPL26 UFMylation dissociates the stalled 60S ribosome from the SEC61 translocon complex [74], the essential protein-conducting channel in the ER membrane. This critical step disrupts the ribosome-translocon junction (RTJ), exposing the stalled nascent chain (termed ER-arrested protein (ER-Aps)) to the cytoplasmic degradation

machinery. UFMylation thereby synchronously activates two distinct clearance pathways: (1) the ER-phagy pathway, triggering lysosomal degradation of ER fragments harboring the stalled ribosomal complex (Figure 4) [73, 75]; and (2) the ER-associated RQC (ER-RQC) pathway, mediated by LTN1-dependent ubiquitination and subsequent proteasomal degradation of the aberrant nascent chain (Figure 5) [75]. Therefore, the UFMylation-dependent ER-phagy is distinct from classical ER-associated degradation (ERAD) and cytosolic RQC pathways, establishing UFMylation as an ER-specific quality control signal [75]. This dual, synergistic mechanism ensures efficient clearance of stalled ribosomal complexes and associated defective proteins.

4.2 | UFMylation-Dependent Regulation of ER-Phagy

ER-phagy refers to the selective autophagic degradation of the ER, which is mediated by specific receptor proteins. Each receptor is localized to a specific ER subdomain [10, 76] and contains conserved motifs including LC3-interacting regions (LIRs), GABARAP-interacting motifs (GIMs), or shuffled ATG8-interacting motifs (sAIMs) that facilitate the receptor binding to LC3 family proteins on autophagosomes. The association between the UFM1 system and ER-phagy was established through the identification of the UFM1 E3 ligase complex [62]. UFMylation facilitates the precise degradation of specific ER subdomains via ER-phagy by modifying key substrates. For instance, UFMylation of RPL26 (a ribosomal protein) and RPN1 (a subunit of the oligosaccharyl-transferase (OST) complex) upon ribosome stalling, along with UFMylation of other ER proteins like CYB5R3 (NADH-cytochrome b5 reductase 3), has been implicated in inducing ER-phagy (Figure 4) [62, 77]. Notably, CDK5RAP3, which contains multiple sAIM domains capable of binding both UFM1 and ATG8 family proteins, functions as a key ER-phagy receptor [78, 79].

Upon ribosome stalling (Ribosome stalling-induced pathway): The UFM1 E3 complex (comprising UFL1, DDRGK1, and CDK5RAP3) catalyzes the UFMylation of RPL26 and RPN1. Subsequently, CDK5RAP3 acts as a dual adaptor: one of its sAIM domains binds UFMylated RPL26 and RPN1, while the other simultaneously binds LC3 on autophagosomal membranes. This dual interaction facilitates the recruitment of autophagosomal membranes to the ER subdomains that harbor UFMylated ribosomal and OST components, thereby promoting their degradation through ER-phagy (Figure 4) [62, 79].

An alternative, mechanistically distinct pathway for UFM1-mediated ER-phagy involves the UFMylation of the ER-localized reductase CYB5R3. UFMylation of CYB5R3 is enhanced by prior UFMylation of DDRGK1 at Lys267 and is catalyzed by the UFL1-DDRGK1 complex. The UFMylated CYB5R3 is then recognized by an sAIM domain in CDK5RAP3 which, in turn, binds LC3 through another sAIM domain and initiates the autophagic degradation of this ER subdomain. Defects in this specific pathway result in microcephaly in mice, highlighting its critical role in neurodevelopment (Figure 4) [77].

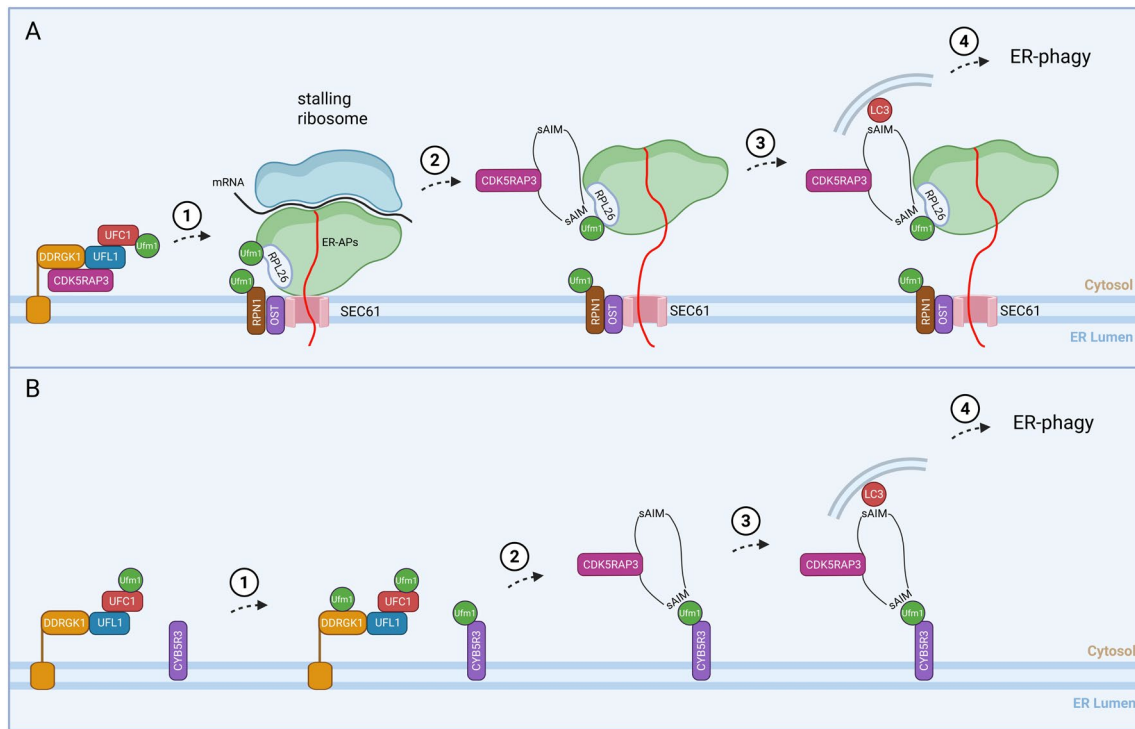


FIGURE 4 | ER-phagy Mediated by UFMylation (A) Ribosome Stalling-Induced ER-phagy: ① Ribosome stalling triggers the UFM1 E3 complex (UFL1, DDRGK1, CDK5RAP3) to catalyze UFMylation of RPL26 and RPN1. ② CDK5RAP3 binds UFMylated RPL26/RPN1 via one SAIM domain. ③ CDK5RAP3 simultaneously binds LC3 on autophagosomal membranes via another SAIM domain. ④ This dual interaction recruits autophagosomes, leading to lysosomal degradation of the ER subdomain containing the stalled ribosomal complex/OST components. (B) CYB5R3-Mediated ER-phagy: ① The UFL1-DDRGK1 complex catalyzes UFMylation of DDRGK1, then UFMylated DDRGK1 facilitates UFMylation of CYB5R3 by the UFL1-DDRGK1 complex. ② UFMylated CYB5R3 is recognized by an SAIM domain in CDK5RAP3. ③ CDK5RAP3 simultaneously binds LC3 via another SAIM domain, ④ recruiting autophagosomes for lysosomal degradation of the ER subdomain containing UFMylated CYB5R3.

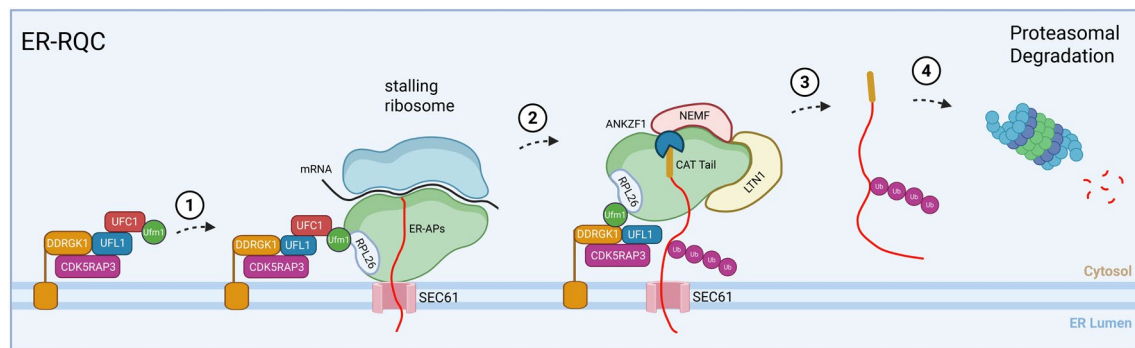


FIGURE 5 | ER-RQC Mediated by UFMylation Ribosome stalling during ER protein synthesis leads to nascent chain accumulation. ① The UFL1-DDRGK1 complex (with UFC1 and CDK5RAP3) catalyzes UFMylation of RPL26 on the 60S subunit. ② UFMylated RPL26 recruits the ER-RQC factors LTN1 (Listerin) and NEMF. NEMF mediates CAT-tail addition to the nascent chain, exposing lysine residues. LTN1 ubiquitinates (Ub) the nascent chain. The tRNA endonuclease ANKZF1 (Vms1) cleaves the tRNA and ubiquitinated nascent chain. ③ The cleaved, ubiquitinated nascent chain is extracted from the ribosome. ④ The ubiquitinated nascent chain is targeted for degradation by the proteasome.

The UFMylation/ER-phagy pathway is evolutionarily conserved, evidenced by its presence in unicellular algae like *Chlamydomonas reinhardtii* [79]. It exhibits a universal response pattern to environmental stress across species. Under salt stress, plants significantly elevate the expression of UFL1, UFM1, and UFC1 [68, 80]. UFL1 directly interacts with ATG8 and cooperates with the ATG1-ATG6 kinase complex to clear damaged ER structures. Loss of UFL1 results in abnormal accumulation of ER

sheets and persistent stress responses, with the salt-hypersensitive phenotype closely resembling that of autophagy-deficient ATG5 mutants [68, 80]. In mammals, UFMylation regulates immune homeostasis; for instance, UFL1 inhibits autophagic degradation of the NLRP3 inflammasome by blocking its K63 ubiquitination via UFMylation. Myeloid cell-specific deletion of UFL1 significantly alleviates endotoxemia and peritonitis [20], underscoring its important role in innate immunity.

UFMylation regulates autophagic flux at multiple levels. At the initiation stage of autophagy, UFMylation of VCP/p97 at lysine residue K109 stabilizes the core autophagy factor BECN1 via ATXN3-mediated deubiquitination, thereby facilitating the assembly of the phosphatidylinositol 3-kinase (PI3K) complex. Pathogenic mutations that affect VCP/p97 UFMylation are associated with the development of neurological disorders and various types of cancer [19]. At the subcellular localization level, the lipid droplet biogenesis factor ACSL3, functioning as a binding partner of GABARAPL2, anchors the UFM1-activating enzyme UBA5 to the endoplasmic reticulum membrane [81]. At the degradation level, DDRGK1 deficiency disrupts autophagic flux by blocking autophagosome-lysosome fusion and thus impairing lysosomal acidification and cathepsin D function [82]. Furthermore, in neuroblastoma, CDK5RAP3 suppresses ER-phagy and apoptosis by maintaining UFMylation function, and its high expression correlates with tumor progression and poor prognosis [83].

4.3 | UFMylation-Dependent Regulation of Endoplasmic Reticulum-Associated RQC

During endoplasmic reticulum-mediated synthesis of secretory and membrane proteins, ribosome stalling results in the accumulation of nascent chains (stalled peptides or ER-APs). These nascent chains are physically shielded from cytoplasmic degradation machinery by the RTJ complex [75]. As described in section 4.1, the key event that initiates ER-associated ribosome quality control (ER-RQC) is the UFMylation of RPL26 on the 60S ribosomal subunit. This post-translational modification is catalyzed by the UFL1-DDRGK1 complex, in cooperation with UFC1 and CDK5RAP3 [73, 75, 84–86]. Importantly, the UFM1 E3 complex associates with ufmylated RPL26 on the 60S ribosomal subunit through the UFM domain of DDRGK1, independent of its canonical E3 ligase activity. CDK5RAP3 helps to direct the complex's specificity. Subsequently, the RQC factors LTN1 and NEMF (nuclear export mediator factor) are recruited to the 60S subunit. While the RING domain of LTN1 plays a critical role in mediating the ubiquitination of the nascent polypeptide chain, NEMF enables the exposure of buried lysine residues via the carboxy-terminal alanine and threonine tail (CAT-tail) addition [87]. The ubiquitinated nascent chain is subsequently cleaved by the tRNA endonuclease ANKZF1, thereby facilitating its release from the ribosome and subsequent targeting for proteasomal degradation through K48-linked polyubiquitination [88] (Figure 5).

In addition, RPL26 UFMylation also initiates the lysosomal degradation of stalled nascent chains through a distinct mechanism, independent of ER-RQC and ERAD [84]. This process involves translocon-associated quality control (TAQC), a mechanism in which ubiquitin-like modification of ribosomes facilitates the targeting of translocation-arrested proteins to lysosomes. Within the TAQC pathway, SAYSVFN domain-containing protein 1 (SAYSD1) interacts with the Sec61 translocon and directly recognizes stalled ribosomes in conjunction with ufmylated RPL26. SAYSD1 captures stalled nascent peptide chains, ensuring their delivery to lysosomes

for degradation with the aid of the Transport Protein Particle (TRAPP) complex [89].

4.4 | Physiological Functions and Pathological Implications of the UFMylation-ER-Phagy-RQC Axis

The UFMylation-dependent synergistic pathway is dynamically regulated under physiological stress. A prime example occurs during peak hemoglobin synthesis in erythroid differentiation, where increased ribosome stalling elevates RPL26 UFMylation levels [75]. The synchronous activation of lysosomal degradation (ER-phagy) and proteasomal clearance (ER-RQC) ensures the timely removal of translocon-blocked ribosomal complexes, which is essential for maintaining protein secretion flux and mature hemoglobin production [75].

Dysregulation of UFMylation leads to dual dysfunction of ER-phagy and ER-RQC, causing significant pathological consequences. UFMylation deficiency results in the abnormal accumulation of SEC61-60S complexes on the ER membrane [74], in addition to reduced lysosomal clearance efficiency and impaired proteasomal degradation [75]. This manifests in various developmental disorders, including anemia and neurodevelopmental defects [75]. Specific defects, such as CYB5R3 modification impairment, can directly cause developmental disorders [77]. For instance, neuronal UFMylation is crucial for maintaining mitochondrial homeostasis in aging neurons by stabilizing ATG9 and coordinating the autophagy-mTORC1 signaling axis [90]. Pathogenic mutations affecting UFMylation targets, like VCP/p97, are linked to neurological diseases and cancers [19]. Furthermore, dysfunction of key components like DDRGK1 [82] or CDK5RAP3 (associated with neuroblastoma progression and poor prognosis) [83] contributes to disease pathogenesis.

Pathogens exploit this critical cellular quality control axis. The Epstein-Barr virus (EBV) encodes the viral deubiquitinase (vDUB), which binds stalled ribosomes and blocks a specific, non-canonical ubiquitination event that is a prerequisite for RPL26 UFMylation [73]. This intervention simultaneously dismantles both UFMylation-dependent lysosomal targeting (for ER-phagy) and proteasomal degradation capability (for ER-RQC) [73, 75]. Consequently, stalled nascent chains accumulate abnormally in the ER, creating an environment optimized for viral protein synthesis and infectious virion production [73]. This pathological hijacking provides compelling evidence for the biological necessity of UFMylation as a common switch for both pathways.

In summary, ribosome stalling-induced RPL26 UFMylation functions as the central integrative signaling hub for the synergistic quality control mediated by ER-phagy and ER-RQC. By uncoupling the ribosome-translocon complex [74] and activating dual degradation programs executed by lysosomes and proteasomes [73, 75], the UFMylation-dependent mechanism is essential for maintaining ER proteostasis and functional homeostasis. Its high degree of evolutionary conservation and involvement in diverse physiological processes, such as erythropoiesis, neurodevelopment, environmental stress response,

and immunity, manifest its fundamental biological importance. Not surprisingly, UFMylation dysfunction underlies a spectrum of developmental disorders, neurodegenerative conditions, and cancers. The susceptibility of this pathway to hijacking by pathogens like EBV further highlights its cellular importance and positions it as a promising target for therapeutic intervention [73, 75, 85, 89].

5 | Conclusion and Perspectives

UFMylation, frequently referred to as a ubiquitin-like modification in recent years, is emerging as a “central processor” regulating endoplasmic reticulum functional homeostasis. Utilizing the UBA5-UFC1-UFL1 core enzymatic cascade and auxiliary scaffolds such as DDRGK1 and CDK5RAP3, UFMylation accurately executes the spatiotemporal “modification-deconjugation” cycle. It orchestrates a three-dimensional regulatory network encompassing the UPR, ER-phagy, and ER-RQC. UFMylation is now known to possess several important biological functions. Firstly, it determines the stress response and ultimate fate of a cell by either initiating adaptive repair or proceeding toward apoptosis. This process is accomplished by three steps: selectively stabilizing IRE1 α , inhibiting the PERK-CHOP signaling axis, and forming a positive feedback loop with XBP1. Secondly, through mono- or poly-UFM1 chain modification of key substrates such as CYB5R3 and RPL26, UFMylation marks damaged ER or stalled ribosomes for recruiting effector molecules such as the ATG8 family proteins and LTN1/NEMF in order to achieve synergistic clearance via dual lysosomal-proteasomal pathways. Finally, this pathway is highly conserved during evolution. It has been established as a core mechanism for cellular homeostasis across species ranging from salt-stressed plants, virus-infected animals, to human erythroid differentiation.

Nevertheless, significant gaps remain in our understanding of UFMylation and its regulation of ER function at the molecular level. First, how is the substrate specificity determined spatiotemporally? Beyond known membrane and ribosomal protein substrates, it is crucial to investigate whether secreted proteins, lipid droplet surface factors, and proteins at mitochondria-associated ER membrane (MAM) contact sites are also UFM1 targets. This urgently requires comprehensive mapping using lysine-UFM1-specific probes coupled with mass spectrometry-based crosslinking proteomics. Second, how do cells dynamically reprogram the conformation of the E3 complex and the topology of UFM1 chains (mono-UFM1 vs. poly-UFM1 chains) in response to specific signals such as oxidative stress, calcium oscillations, or viral infection? Answering this important question requires the development of “millisecond-level” conformational tracking methodologies integrating time-resolved cryo-EM and advanced live-cell imaging. Third, to develop therapeutic interventions for UFMylation-related human diseases, it is essential to elucidate the underlying molecular mechanisms. Conditions such as anemia linked to DDRGK1 mutations, cardiomyopathy due to Ufl1 deficiency, and viral hijacking of DDRGK1 by EBV highlight the therapeutic relevance of modulating the UFMylation pathway. Currently, however, there is a notable lack of reversible, organelle-targeted small-molecule modulators or proteolysis-targeting chimera (PROTAC) strategies [91]. Despite this, some small-molecule inhibitors targeting

UBA5 have shown promising potential in anti-tumor clinical trials [92, 93]. Such inhibitors can enhance anti-proliferative effects by interfering with the UFMylation system in cancer cells, thereby inducing autophagy and endoplasmic reticulum stress. Furthermore, inhibitors targeting UFSP2 can suppress de-UFMylation, helping to maintain UFMylation levels associated with endoplasmic reticulum homeostasis and protect normal cellular functions [15]. Therefore, developing targeted therapeutic strategies against the UFMylation system may represent a feasible and innovative treatment approach.

Looking ahead, research efforts should focus on the following key issues: (1) Structural dimension: it is important to utilize cryo-EM to solve the structures of three distinct conformational states (pre-modification, modification-in-progress, post-modification) of the UREL (UFL1-DDRGK1-CDK5RAP3)-60S ribosomal complex. This will reveal the molecular switch governing geometric matching of the RPL26 lysine residue and UFM1 chain elongation. (2) Physiological dimension: it is necessary to generate conditional knockout animal models (e.g., in neurons, pancreatic β -cells, hematopoietic stem cells) and combine them with single-cell UFMylomics. This integrated approach will elucidate how different cell types utilize UFMylation to integrate metabolic, immunological, and secretory programs. (3) Organelle crosstalk dimension: We need to systematically track the interorganellar transduction of P4HB-UFM1-mediated oxidative stress signals using techniques such as split-APEX2 proximity labeling coupled with MAM purification [94]. (4) Translational dimension: it is also crucial to develop blood-brain barrier-penetrant UFSP2 agonists to accelerate DNA damage repair and design competitive peptide inhibitors targeting the EBV DDRGK1 interaction interface to block viral hijacking. Meanwhile, we need to leverage the Salubrin-eIF2 α regulatory paradigm which inhibits eIF2 α dephosphorylation and develop precise drug combinations that synergistically activate both the UPR and UFMylation pathways. This strategy holds promise for treating UFMylation deficiency-related pathologies, including anemia, cardiomyopathy, and neurodegenerative diseases.

In conclusion, the UFMylation system provides a “molecular Rosetta Stone” for deciphering ER homeostasis and offers novel, targetable nodes for treating diseases spanning metabolism, immunity, infection, and beyond. As integrated, closed-loop research encompassing structure, function, pathology, and therapeutic intervention continues to converge, UFMylation is poised to join the ubiquitin-proteasome system as a fundamental pathway reshaping our understanding of cell biology and the practice of precision medicine.

Author Contributions

All authors participated in the design and discussion of manuscript conception and outline, contributed to editing of the draft manuscript. All authors approved the final version of the manuscript.

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Consent

The authors declare their agreement to participate and publish.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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