Science Bulletin 68 (2023) 1600–1602

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Research Highlight

Deciphering the ER remodeling dynamics: ubiquitination of reticulon homology domain proteins fuels ER-phagy and impacts neurodegeneration

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The endoplasmic reticulum (ER), the most expansive membrane-bound organelle within cells, acts as a cellular hub interacting with the plasma membrane and other intracellular organelles, constituting an intricate organelle-interaction network. Fundamental to cellular functions, the ER governs protein and lipid synthesis, transport, calcium ion exchange, and inter-organelle communication [1]. To maintain cellular equilibrium, the ER continuously modulates its morphology, aligning with processes such as vesicle formation, collagen degradation, and the unfolded protein response (UPR). This morphological versatility is primarily enabled by ER-phagy, the ER's selective autophagy, where the microtubule associated protein 1 light chain 3 (LC3)-interacting region (LIR) of ER-phagy receptors latch onto lipidated mammalian ATG8 proteins on autophagosomal membranes. This mechanism of selective autophagy facilitates the lysosomal degradation of specific ER components under basal and non-stress conditions, ensuring a balanced ER size [2].

Mammalian family with sequence similarity 134, member B (FAM134B) and yeast autophagy related 40 (Atg40), the best characterized reticulon homology domain (RHD)-containing ER-phagy receptor, has the ability to sense and trigger ER membrane curvature [1,3,4]. The phosphorylation and acetylation modifications present in FAM134B-RHD relay a signal cascade that initiates its oligomerization, effectively segmenting the ER into suitable sizes [5,6]. Furthermore, FAM134B-mediated ER-phagy contributes to ER quality control by eliminating ER subdomains that harbor ER-associated degradation (ERAD)-resistant misfolded proteins, a capability evidenced in alpha1-antitrypsin Z (ATZ) polymers, disease-associated intracellular cholesterol transporter 1 (NPC1) mutant, and endogenous pro-collagen [7–9]. Despite its non-binding nature with LC3 proteins, a trait shared with numerous ER-resident membrane-shaping proteins possessing RHDs, ADP ribosylation factor like GTPase 6 interacting protein 1 (ARL6IP1) has been implicated in similar neurodegenerative disorders [10]. This association hints at ARL6IP1 potentially collaborating with other RHD-containing ER-phagy receptors in the remodeling of the ER.

While essential in ER-phagy, understanding the regulatory mechanisms of RHD-containing receptors remains incomplete. González et al. [11] and Foronda et al. [12] provided the latest insights into this process by showing that ubiquitination plays a critical role in stimulating RHD-bearing protein-mediated ER-phagy (Fig. 1). In their study, González et al. [11] employed mass spectrometry to identify four primary ubiquitination sites (K90, K160, K247, and K264) within the cytosolic segments of FAM134B-RHD. Likewise, Foronda et al. [12] located the primary ubiquitination site (K96) and potential sites (K114 and K130) near ARL6IP1's RHDs. Using computational simulations, they demonstrated that the ubiquitination of FAM134B and ARL6IP1's RHDs optimizes the membrane remodeling functions of ER-shaping clusters. Using the bimolecular complementation affinity purification (BiCAP) assay, the endogenous ER-anchored protein autocrine motility factor receptor (AMFR), also known as gp78, was identified as the E3 ligase responsible for the ubiquitination of FAM134B-RHD and ARL6IP1-RHD. This ubiquitination escalates the size of ER-phagy clusters, catalyzes extensive membrane deformations, and promotes larger autophagosomal structures. Importantly, these clusters contain ubiquitination ligases and deubiquitinases, capable of dynamically altering RHD-containing protein ubiquitination, thus influencing these multivalent clusters' formation and expansion. Lastly, the teams confirmed that FAM134B-RHD and ARL6IP1-RHD ubiquitination enhances the FAM134B's LC3B binding, adjusting the dynamic ER-phagy flux. Consistently, the disruption of ARL6IP1 leads to defects in ER structure, increases the number of spike-like ER protrusions, expands ER sheets, fragments ER tubules, and decreases resistance to ER stress. Conversely, deletion of E3 ligase AMFR and other proteins containing RHDs (especially ARL6IP1) inhibits the formation of ER-phagy clusters, thereby impairing the flux of FAM134B-mediated ER-phagy. Collectively, these data demonstrate the existence of specific ubiquitination of RHDs that induce ER-phagy by composing clusters of ER-shaping proteins. Since the RHD of FAM134B can be modified by phosphorylation, acetylation, and ubiquitination for clustering and membrane fragmentation to facilitate ER-phagy, it would be interesting to investigate how these posttranslational modification events were coordinated.

FAM134B loss-of-function mutations, implicated in the pathogenesis of hereditary sensory and autonomic neuropathy

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https://doi.org/10.1016/j.scib.2023.07.004

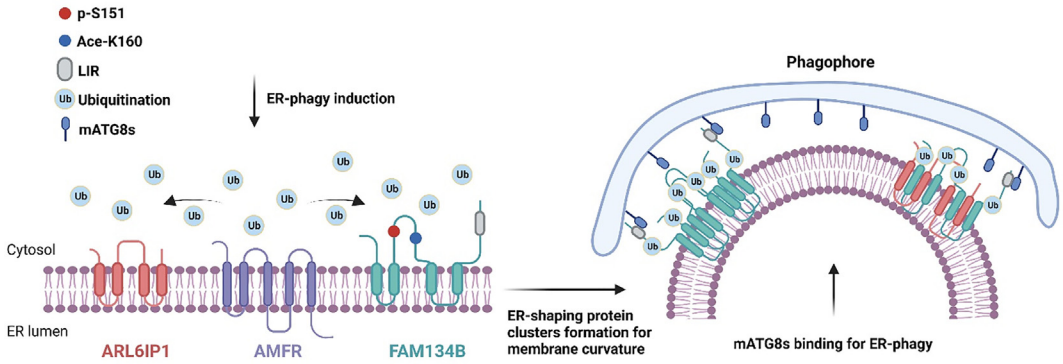

Fig. 1. Clusters of ubiquitinated ER-shaping proteins drive ER-fragmentation for ER-phagy AMFR-mediated ubiquitination of RHD proteins boosts their oligomerization and growth of clusters, thus promoting ER membrane curvature. The clustering of ubiquitinated ER membrane-shaping protein enhances the interaction of mATG8 and FAM134B, adjusting the dynamics of ER-phagy flux. p-S151: phosphorylation at serine 151, Ace-K160: acetylation at lysine 160, LIR: LC3-interacting region.

(HSAN-II), have been discovered in patients [13]. Intriguingly, an HSAN-II-associated FAM134B missense variant (FAM134BG216R) exhibited gain-of-function activity by inducing FAM134B oligomerization, ER scission, and ER-phagy more efficiently than the wild-type form, provoking sensory neuronal cell death [5] These findings underscore FAM134B-mediated ER-phagy's dual role in sensory neuropathy, thereby emphasizing the importance of studying RHD-containing clusters' regulatory mechanisms for understanding and treating neurodegeneration.

Foronda et al. [12] analyzed a disease-linked ARL6IP1 variant (ARL6IP1 K193Ffs) in fibroblasts from spastic paraplegia 61 (SPG61) disease patients. SPG61 is a neurodegenerative disorder typified by progressive leg spasticity (hereditary spastic paraplegia, HSP), and loss of sensory and pain perception, mirroring hereditary sensory autonomic neuropathy II (HSAN-II) symptoms [14]. Remarkably, *Arl6ip1* knockout mice, simulating the ARL6IP1-related disorder, presented symptoms similar to SPG61 patients. Notably, in *Arl6ip1* knockout mice, ER sheets in dorsal root ganglion (DRG) neurons expanded, leading to time-dependent degeneration and sensory loss. These findings collectively imply that ARL6IP1 loss significantly disrupts ER-phagy and compromises cellular fitness. Interestingly, ARL6IP1 binds to FAM134A and FAM134C, FAM134B's close homologs and ER-phagy receptors, potentially explaining why ARL6IP1 mutations result in more severe disease and suggesting that targeting ARL6IP1-ER-phagy clusters could benefit patients with SPG61 or HSAN-II [15].

In conclusion, these elegant studies advanced our understanding of dynamic regulatory mechanisms in ER-phagy pathway. They unveil potential research avenues to investigate the intracellular signals forming ubiquitinated ER membrane-shaping protein clusters for ER remodeling, which contributes to a deeper comprehension of the dysfunctional cellular mechanisms driving sensory neuropathy's pathogenesis.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (3202501232025012, 92254307, and 31970695) and the Ministry of Science and Technology of the People's Republic of China (2021YFC2700901).

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