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Opinion

ER-Phagy, ER Homeostasis, and ER Quality Control: Implications for Disease

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Lysosomal degradation of endoplasmic reticulum (ER) fragments by autophagy, termed ER-phagy or reticulophagy, occurs under normal as well as stress conditions. The recent discovery of multiple ER-phagy receptors has stimulated studies on the roles of ER-phagy. We discuss how the ER-phagy receptors and the cellular components that work with these receptors mediate two important functions: ER homeostasis and ER quality control. We highlight that ER-phagy plays an important role in alleviating ER expansion induced by ER stress, and acts as an alternative disposal pathway for misfolded proteins. We suggest that the latter function explains the emerging connection between ER-phagy and disease. Additional ER-phagy-associated functions and important unanswered questions are also discussed.

Highlights

The selective degradation of the endoplasmic reticulum (ER), termed ER-phagy or reticulophagy, has multiple physiological functions.

Many different types of cell stress can induce ER-phagy, including starvation and misfolded protein accumulation.

ER-phagy pathways participate in ER homeostasis and ER quality control.

ER-phagy performs functions that are independent of the unfolded protein response (UPR) and ER-associated degradation (ERAD).

ER-phagy pathways, that play a role in ER quality control, are cytoprotective.

Autophagic Turnover of ER Fragments Is Mediated by ER-Phagy Receptors

The endoplasmic reticulum (ER), that forms a dynamic contiguous network of interconnected flat sheets and curved tubules, serves as a protein biogenesis hub, and accommodates approximately one-third of the proteome of eukaryotes [1]. However, errors in protein folding, post-translational modifications, and in the assembly of protein complexes can occur and impair ER homeostasis, or 'ER proteostasis' [2]. Under these conditions, the **unfolded protein response** (**UPR**; see Glossary) is induced, and aberrant proteins are retrotranslocated into the cytoplasm via the **ER-associated degradation (ERAD)** machinery and disposed of by the proteasome [2]. Nevertheless, not every damaged and potentially toxic protein is degraded by ERAD, and not every misfolded protein induces the UPR [3,4]. Some proteins are targeted for degradation via alternative ER disposal pathways, such as ER-phagy, that degrade specific parts of the ER by **autophagy** [5–7]. Although ER-phagy is one component of a starvation response, recent studies have linked ER-phagy to ER quality control and other functions [5–7]. The growing links between ER-phagy and homeostasis have prompted us to propose that ER-phagy pathways play a crucial role in human disease. In support, mutations in genes encoding ER-phagy components have been linked to diabetes and some neurological disorders [6,8].

ER-phagy employs autophagy receptors that link ER domains to the autophagy machinery [5–7]. ER-phagy can be non-autophagosome-mediated (micro-ER-phagy) or autophagosome-mediated (macro-ER-phagy) [5–7]. Micro-ER-phagy is characterized by direct piecemeal engulfment of ER fragments by endosomes and/or lysosomes (Figure 1). A role for the membrane-remodeling complex, ESCRT-III, was recently described in this process [9]. By contrast, macro-ER-phagy involves the sequestration of ER fragments into double-membrane vesicles, termed autophagosomes, that arise from a precursor cisterna known as the phagophore. Autophagosomes then deliver their contents to lysosomes/vacuoles for degradation (Figure 1).

In this opinion article, we largely focus on macro-ER-phagy and how it functions in the context of ER homeostasis and quality control. We discuss how some ER-phagy receptors act on several

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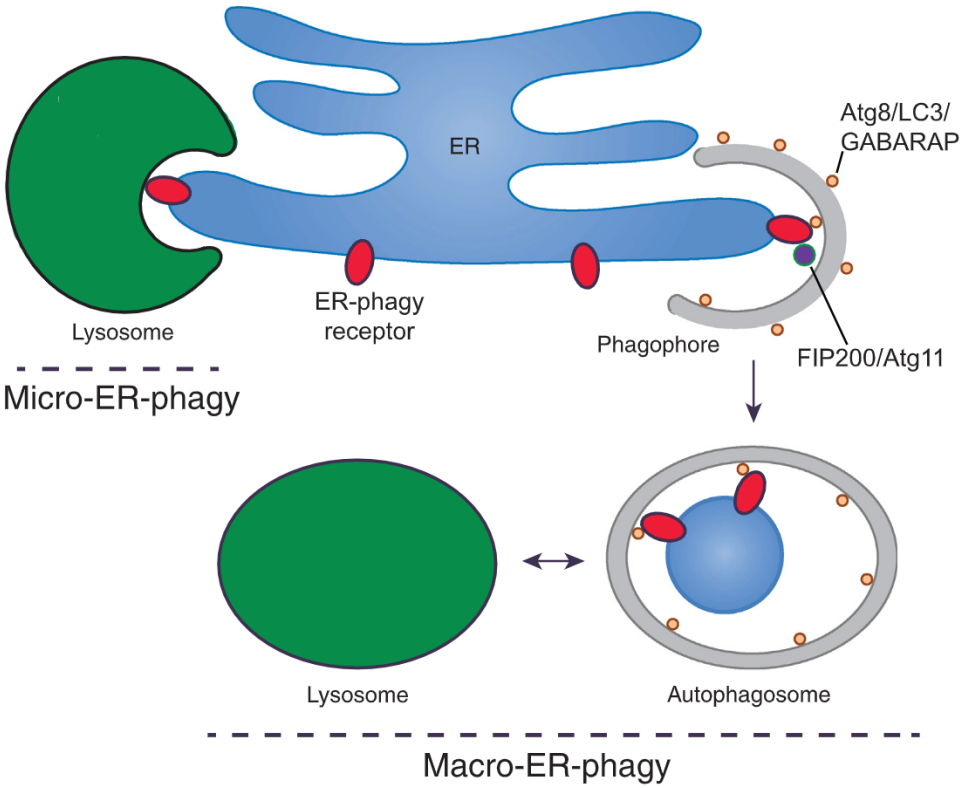

Figure 1. Endoplasmic Reticulum (ER)-Phagy Uses Autophagy Receptors to Package the ER into Autophagosomes. During macro-ER-phagy, ER-phagy receptors bind to Atg8 in yeast, or LC3 and GABARAP proteins in mammals (tan spheres), to package ER fragments into a phagophore (gray). Some of the ER-phagy receptors also bind to the autophagy-inducing ULK complex via its subunit FIP200 (or Atg11 in yeast) to coordinate the autophagy machinery with cargo sequestration. The phagophore expands and then seals to form an autophagosome that delivers ER fragments to the vacuole (yeast) or lysosome (mammals; green) for degradation. During micro-ER-phagy, ER fragments are directly engulfed by lysosomes/vacuoles. Although in some cases this process involves ER-phagy receptors and LC3 or GABARAP proteins (e.g., during RecovER-phagy [9]), micro-ER-phagy in general does not appear to require these factors (e.g., during micro-ER-phagy induced by tunicamycin treatment in yeast [39]).

different pathways, and suggest that some of these pathways connect the ER-phagy machinery to homeostasis and human disease. We propose that ER-phagy degrades ERAD-resistant forms of ERAD substrates. This may require a multiplicity of receptors that recognize a spectrum of misfolded proteins. Examples of ERAD-resistant misfolded proteins that are degraded by ER-phagy include mutant forms of procollagen and proinsulin [8,10], as discussed later. Recent studies have also revealed that an ER-phagy receptor may act on more than one pathway [5–7]. We also propose that the interaction of an ER-phagy receptor with a specific binding partner may define the pathway in which the receptor functions. The contribution of ER-phagy to ER function, and other open questions in the field, are also discussed.

ER-Phagy Receptors

The identification of ER-phagy receptors has accelerated progress in uncovering the many roles of ER-phagy. ER-phagy receptors are either resident ER membrane proteins or, less commonly, cytosolic proteins. ER-phagy receptors connect domains of ER sheets or tubules to the autophagosome biogenesis machinery by binding to members of the ubiquitin-like Atg8 protein family, which consists of the LC3 and GABARAP proteins in mammals (Figure 1) [5–7].

Glossary

Autophagy: a 'self-eating' process in which cellular content is degraded after delivery to the vacuole (in yeasts/fungi) or to the lysosome in (higher organisms); the autophagy pathway can remove malfunctioning or aggregated proteins, aberrant or unwanted protein complexes, damaged or excess organelles, and pathogens to maintain homeostasis. Macro-autophagy, micro-autophagy, and chaperone-mediated autophagy are three mechanistically distinct types of autophagy (only macro-autophagy and micro-autophagy are discussed in this article).

Cortical ER: a term used to describe peripheral and cytoplasmic endoplasmic reticulum (ER) in yeast; cortical ER contains ER sheets and tubules, but not nuclear ER membranes

ER-associated degradation (ERAD): a pathway that rids the ER of misfolded proteins, unassembled subunits of multimeric complexes, or proteins that fail to acquire proper post-translational modifications. ERAD also regulates the steady-state levels of metabolic enzymes in the ER. After selection, ERAD substrates are 'retrotranslocated' or 'dislocated' into the cytoplasm, ubiquitinated, and degraded by the proteasome.

ER expansion: an event that accompanies the application of select compounds or stresses that either need to be metabolized (e.g., barbiturates) or that induce the UPR; expansion requires an increase in lipid synthesis, and in some cases the expression of ER-resident proteins also rises.

ER-to-lysosome-associated degradation (ERLAD): the spectrum of pathways by which ER-to-lysosome transport degrades macromolecules.

N-degron ubiquitin-dependent ER-phagy: a pathway in which ER-resident cargo proteins containing destabilizing N-terminal amino acids are delivered into lysosomes; this pathway requires recognition of the N-terminal amino acid by an autophagy receptor, as well as its ubiquitination.

Nucleophagy: a selective type of autophagy in which a part of the nucleus is targeted for degradation.

ULK complex: a protein complex that contains the ULK1 or ULK2 kinase as well as FIP200, ATG13, and ATG101; the complex functions as the most upstream component of the autophagy machinery and responds to various

ER-phagy receptors bind to Atg8 proteins via an LC3-interacting region (LIR) motif, also known as an Atg8-interacting motif (AIM) in yeast [5–7]. Some ER-phagy receptors also bind to FIP200 (Atg11 in yeast), a component of the autophagy-inducing **ULK complex** [11–14]. In this section we introduce the known ER-phagy receptors and then discuss their contribution to ER homeostasis and ER quality control. We suggest that compromised ER homeostasis in individuals with mutations in genes encoding receptors or other components of the ER-phagy machinery contributes to disease.

To date, there are two known ER-resident membrane receptors in *Saccharomyces cerevisiae* (Atg39 and Atg40) and six in mammalian cells (FAM134B, RTN3L, SEC62, CCPG1, ATL3, and TEX264) (Figures 2–4) [14–21]. Each ER-phagy receptor principally resides in a unique domain of the ER (Figure 2) and/or tissue, and responds to a variety of cell stresses that include starvation, misfolded protein accumulation, and imbalances in lumenal calcium [5–7]. For example, SEC62 triggers ER-phagy in flat ER sheets, whereas FAM134B functions on the curved edges of the sheets (Figure 2). In addition, RTN3L and ATL3 carry out ER-phagy on ER tubules (Figure 2). RTN3L localizes to tubules, whereas ATL3 is found at tubule junctions [16–18,21]. Some receptors, such as FAM134B, have isoforms (FAM134A and FAM134C) that also bind to LC3 [16]. Moreover, RTN3L and ATL3 may possess redundant functions because RTN3L overexpression was reported to suppress the ER-phagy defect in ATL3-depleted cells [21]. ATL1, ATL2, and ATL3 were all reported to substitute for each other during ER-phagy [22]. The ATL proteins have been proposed to remodel the ER to facilitate fragmentation during autophagosomal engulfment [22], but this role appears to be unrelated to the adaptor function of ATL3 [21].

In addition to the membrane ER-phagy receptors, two soluble mammalian proteins, p62 and CALCOCO1, were identified as ER-phagy receptors, and one soluble receptor, Epr1, was recently reported in *Schizosaccharomyces pombe* [23–26]. Another soluble receptor, C53, was also recently identified in plants and mammals [27]. Interestingly, C53 is recruited to autophagosomes when the ER is stressed. It binds to Atg8 family members via a 'shuffled' recognition motif, and is a component of an ER membrane complex that includes an enzyme required for substrate UFMylation, a ubiquitin-like modification that precludes Atg8 binding. Recent reports have confirmed that UFMylation may be a key regulator of some ER-phagy

cellular stimuli to repress or initiate autophagy by acting on downstream components of the autophagy machinery.

Unfolded protein response (UPR): an inducible response that is initiated by ER membrane proteins in response to an increase in misfolded protein levels in the ER, lipid disequilibrium, and alterations in the ER lumenal ion composition and protein glycosylation. Downstream effects of the UPR include induction of ER-phagy/autophagy, ERAD, secretory pathway functions, and the ER protein folding machinery, as well as reduced protein translation and in specific cases apoptosis.

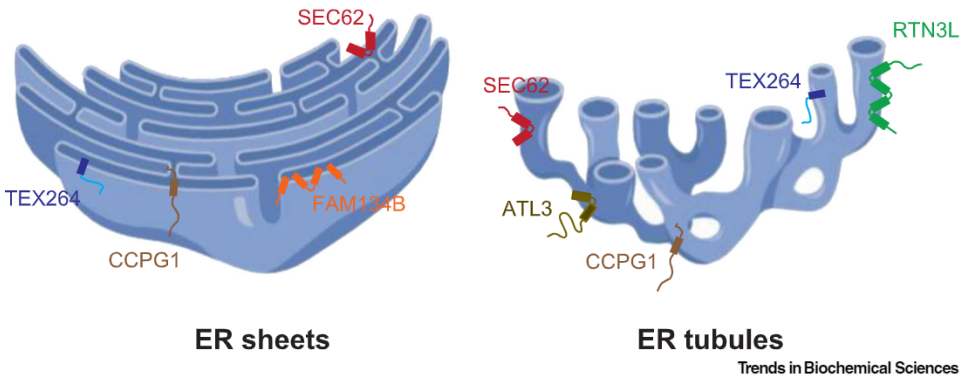

Figure 2. Endoplasmic Reticulum (ER)-Phagy Receptors Reside in Different ER Subdomains. A cross-section of an area of sheets (left) and tubules (right) is shown to illustrate the localization of the six known mammalian ER membrane ER-phagy receptors. RTN3L localizes to tubules, ATL3 is present at the three-way junctions of the ER tubules, and FAM134B resides on the curved edges of the sheets. The other receptors, namely SEC62, CCPG1 and TEX264, are found on flat ER sheets and tubules. Figure created with Biorender.

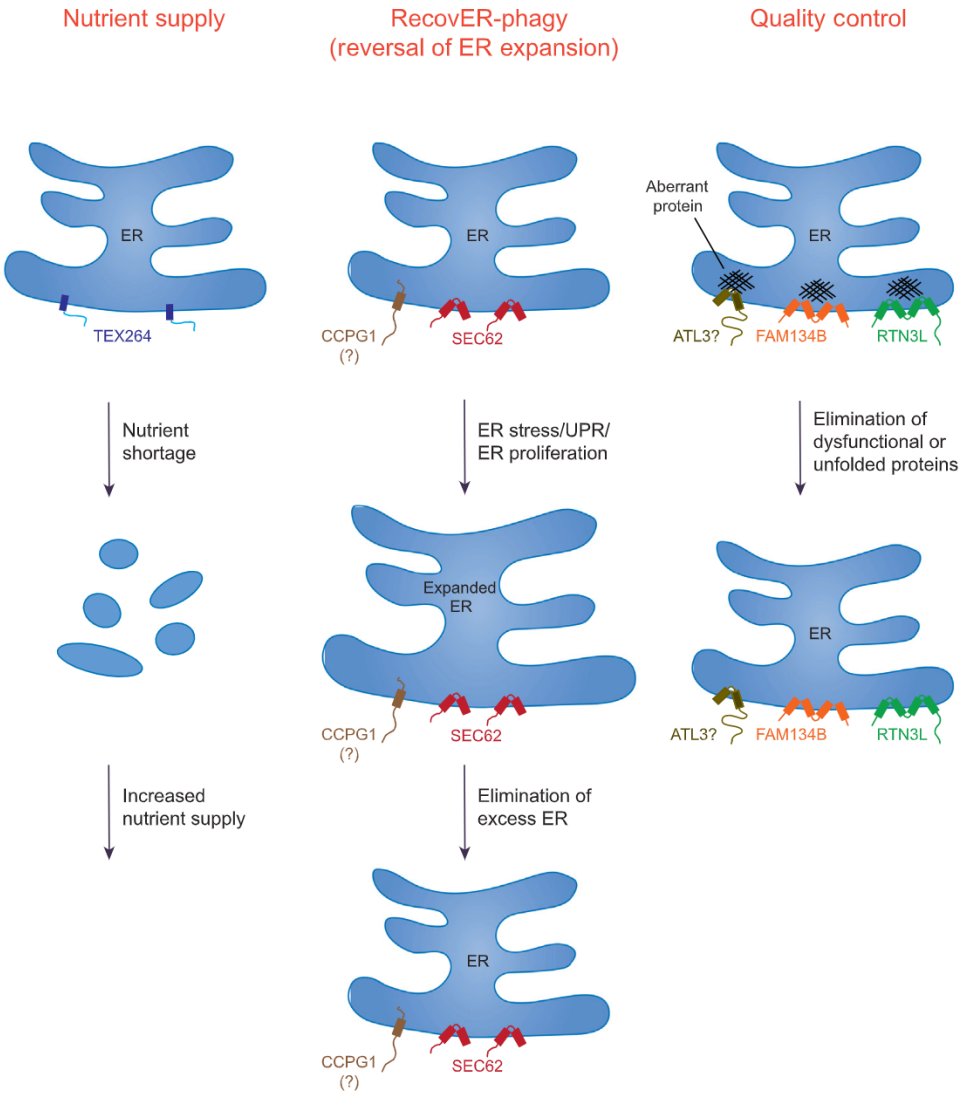

Figure 3. Cellular Roles of Endoplasmic Reticulum (ER)-Phagy and the Contributions of Metazoan ER-Phagy Receptors. The general pathways in which ER-phagy is utilized and the contributing receptors are shown. These pathways include: nutrient supply (TEX264), RecovER-phagy ('reversal of ER expansion') (SEC62 and possibly CCPG1), and quality control (FAM134B, RTN3L, and possibly ATL3). Not all the listed pathways are limited to the indicated ER-phagy receptors. For example, although TEX264 contributes to ~50% of autophagic flux during amino acid deprivation, other ER receptors, such as FAM134B, can also participate in starvation-induced ER-phagy, albeit far less efficiently. Note that a significant fraction of the ER is fragmented in the nutrient supply pathway to illustrate that TEX264 promotes the degradation of a large portion of this organelle during amino acid starvation. SEC62 mediates RecovER-phagy. Although CCPG1 function appears to be regulated by the unfolded protein response (UPR), it remains to be determined whether it also definitively participates in RecovER-phagy. In quality control, FAM134B facilitates the removal and degradation of misfolded procollagen (shown) and disease-causing mutant NPC1, whereas misfolded pro-insulin (Akita), proopiomelanocortin (POMC), and arginine vasopressin (pro)-AVP utilize RTN3L. There are no known misfolded proteins that specifically utilize ATL3. Also note that FAM134B and RTN3L are inserted into the lipid bilayer via reticulon domains, whereas the other receptors shown possess *bona fide* transmembrane segments.

pathways [28]. By contrast, p62 appears to promote the degradation of damaged ER subdomains marked by ubiquitin because it acts with the E3 ligase, TRIM13, to elicit **N-degron ubiquitin-dependent ER-phagy** [23] (discussed further later).

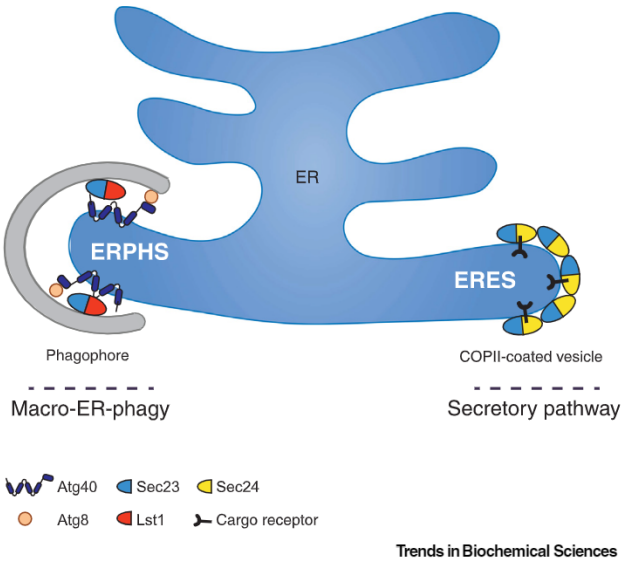

Figure 4. Macro-Endoplasmic Reticulum (ER)-Phagy in Yeast. Yeast Atg40 has a domain structure similar to mammalian FAM134B, but it localizes to the tubular ER, like RTN3L. In yeast, the sheets and tubules in the cytoplasm, and at the cell cortex, are referred to as the cortical ER. Atg40 is largely present on the cortical ER, whereas Atg39 is principally localized in the nuclear envelope. Atg40, which contains a reticulon-like domain, recruits Atg8 via a LIR motif to initiate cortical macro-ER-phagy. Autophagosome-driven fragmentation of the cortical ER occurs at ER-phagy sites (ERPHS) with the aid of the Lst1–Sec23 complex. By contrast, transport vesicles bud from the ER at ER exit sites (ERES) where the coat protein complex II (COPII) coat protein complex is assembled. Soluble secretory cargo proteins are loaded into vesicles via a receptor-mediated process, and transmembrane cargo proteins interact with the COPII coat subunits or specific adaptors.

As mentioned earlier, although ER-phagy is commonly induced by amino acid starvation or drugs such as rapamycin or Torin that mimic starvation, ER-phagy can also be induced by other cellular and environmental cues such as the UPR [5–7]. For example, some receptors including TEX264 largely induce macro-ER-phagy by acting in response to nutrient deprivation, whereas SEC62 (see later) only triggers micro-ER-phagy in response to resolution of the UPR [18–20]. Interestingly, the compound loperamide, that upregulates the transcription factor ATF4 and other ER stress markers in glioblastoma cells, induces FAM134B-dependent macro-ER-phagy, and to a lesser extent TEX264-dependent ER-phagy [29]. Although the precise mechanism by which ER-phagy receptors are activated is largely unknown, their enhanced expression and oligomerization appears to be part of the mechanism by which they are activated [15,19,30,31] (discussed further later).

ER-Phagy, Nutrient Deprivation, and ER-Fragmenting Activity

Amino acid starvation inhibits the TORC1 kinase, which induces autophagosome biogenesis by derepressing the ULK complex [32]. The response of FAM134B, RTN3L, ATL3, CCPG1, and TEX264 to starvation is consistent with the observation that these receptors support macro-ER-phagy pathways that employ autophagosomes [5–7].

TEX264, which resides in ER sheets and tubules (Figure 2), facilitates ~50% of the observed autophagic flux during amino acid starvation (Figure 3) [19,20]. Under nutrient-rich conditions, TEX264 is found on punctate structures on the ER membrane that enlarge during starvation. These enlarged structures colocalize with LC3 and other ATG marker proteins. TEX264 associates with LC3 at three-way junctions of ER tubules, suggesting that it is packaged into autophagosomes at these locations. Interestingly, TEX264 contains a gyrase inhibitor-like domain and a long intrinsically disordered region (IDR) that is required for its function as an ER-phagy receptor. The IDR, which bridges the ER to the growing autophagosomal membrane, is present in most ER-phagy receptors [5]. Unexpectedly, recent studies revealed a role for

TEX264 that appears to be independent of its activity as an LC3-binding protein and ER-phagy receptor [33]. In combination with the ATPase p97 and SPRTN, TEX264 localizes to the nuclear envelope to repair DNA [33].

During ER-phagy, a portion of the ER must fragment. Although TEX264 lacks ER-fragmenting activity, the overexpression of FAM134B and RTN3L drives ER fragmentation, as does the multimerization of RTN3L [16,17]. FAM134B and RTN3L, as well as the closely related yeast Atg40 protein, contain reticulon homology domains (RHDs) [15–17]. RHDs consist of two hairpin-like domains that each possess 28–36 hydrophobic amino acids separated by a stretch of 60–70 hydrophilic amino acids [1]. The RHDs generate membrane curvature by inserting into the cytoplasmic surface of the membrane. FAM134B also contains two amphipathic helices that may contribute to membrane curvature [34]. The other mammalian ER membrane receptors lack RHDs. Instead, these receptors could use another mechanism to fragment the ER, or they might work with FAM134B or RTN3L [5].

ER-Phagy Restores ER Homeostasis

If, as we suggest earlier, ER-phagy acts as a general regulator of ER homeostasis, then the UPR and ER-phagy should be linked. Consistent with this hypothesis, the accumulation of misfolded proteins in the ER induces the UPR, **ER expansion**, and ER-phagy [35]. UPR-generated ER expansion dilutes the concentration of misfolded proteins, although the concentration of chaperones in the ER increases [35]. Autophagy can re-establish pre-stress levels of the ER [36,37]. This notion is consistent with an earlier observation that autophagy destroys excess ER after administered barbiturates are removed [38]. The translocon component, SEC62, plays a crucial role in the removal of UPR-generated excess ER by virtue of a C-terminal LIR domain that recruits LC3 [18]. Because overexpressed SEC62, which is not associated with the translocon, is sufficient to drive this process – known as 'RecovER-phagy' – a signal that releases SEC62 from the translocon must be generated after the UPR has been resolved (Figure 3]. The nature of this signal is unknown. In a study where ER-phagy was induced by inhibiting the ER Ca^{2+} pump, the turnover of excess ER via RecovER-phagy was found to be via micro-ER-phagy [9]. This further distinguishes SEC62-mediated RecovER-phagy from macro-ER-phagy, which employs the other five ER-phagy membrane receptors.

CCPG1 function is also linked to the UPR, but, unlike SEC62, CCPG1 is induced by the UPR. In addition, CCPG1-mediated ER-phagy utilizes the macroautophagy machinery. Indeed, CCPG1 binds through independent motifs to both GABARAP and FIP200 [14]. In the exocrine pancreas of CCPG1 hypomorphic mice, insoluble secreted proteins accumulate in the ER, the ER becomes distended, and ER stress and apoptotic markers are induced. The global increase in insoluble ER proteins may be an indirect secondary consequence of the accumulation of unfolded proteins, or due to the loss of a specific ER function that occurs when CCPG1 activity is compromised. An interesting aspect to explore in the future will be to determine whether CCPG1 also contributes to RecovER-phagy and, if so, whether it participates through a micro- or a macro-ER-phagy process. We suggest that this analysis will permit an understanding of whether the UPR, depending on a specific cue, can engage different ER-phagy programs. In this context, yeast appears to use both Atg40-dependent macro-ER-phagy and ESCRT-dependent micro-autophagy when the UPR is triggered with tunicamycin [39]. Notably, the UPR can also be induced by lipid bilayer stress [40–44], but it is unclear whether SEC62- and CCPG1-dependent lysosomal delivery of the ER is associated with this stress. How excess ER is ultimately turned over under these stress conditions, and whether SEC62 or CCPG1 are directly involved, requires further investigation.

ER-Phagy and ER Quality Control

ER-phagy also prevents disease manifestations and maintains cellular homeostasis by degrading deleterious proteins that are not removed by other ER disposal pathways (Figure 3). Examples include the disease-causing form of human α1-antitrypsin, the Z variant (ATZ), which fails to induce the UPR; ERAD-resistant misfolded procollagen; and the ERAD-resistant mutant prohormone Akita, discussed later [3,23,45]. ER-phagy receptors that regulate ER quality control likely work in concert with chaperones [3,46,47] (also see later), and possibly additional machinery, to mark the site on the ER that is targeted for degradation. For example, the N-degron ER-phagy pathway, that contributes to the clearance of ATZ aggregates [23] (Figure 3), may target sites on the ER that are marked with ubiquitin. ATZ can also be degraded by an LC3-mediated **ER-to-lysosome-associated degradation (ERLAD)** pathway that is proposed to be vesicle-mediated [46], suggesting redundant action between different pathways to detoxify the ER. It is worth noting that the term ERLAD has also been used to group all ER-lysosome transport pathways [46].

In contrast to what has been observed in mammalian cells, when ATZ is heterologously expressed in yeast, ATZ turnover is enhanced by Atg40-dependent ER-phagy [4,48]. ATZ overexpression enhances Atg40 expression and upregulates macro-ER-phagy in the absence of the UPR [4]. ATZ degradation also requires Lst1 [4], a coat protein complex II (COPII) coat protein subunit that complexes with Sec23. On the secretory pathway, Lst1–Sec23 packages correctly folded proteins into ER-to-Golgi COPII-coated transport vesicles [4,49]. In either *atg40Δ* or *lst1Δ* mutant yeast cells, ATZ aggregates in the ER [4]. When ER-phagy is induced, Lst1–Sec23 binds to Atg40 to mark specific subdomains on the ER, named ER-phagy sites (ERPHS) [4] (Figure 4). ERPHS are targeted to and sequestered into autophagosomes. ERPHS appear to be distinct from the ER exit sites (ERES) that bud ER-derived COPII-coated vesicles that traffic correctly folded proteins to the secretory pathway. The formation of the ERPHS may require ER network rearrangements. For example, ERPHS do not form in cells lacking *lnp1*, the gene that encodes the yeast homolog of LUNAPARK [4,50]. Lnp1 stabilizes nascent ER junctions that arise when two tubules fuse with one another [51]. The role of Lnp1 in ERPHS formation may be medically important because mutations in human LNPK lead to a complex neurodevelopmental syndrome [52], although it is unclear whether this syndrome arises directly from a defect in ER-phagy. In addition to the components mentioned earlier, the lipid transporter, Vps13, and Atg40 oligomerization may participate in the formation and/or function of ERPHS [53,54]. Exactly how ERPHS form on the ER is currently unknown. It also remains to be addressed whether misfolded proteins accumulate in the ERPHS during ER-stress. However, ERPHS formation may be conserved in mammalian cells because the Lst1 homolog, SEC24C, is required for the degradation of ER sheets and tubules in Torin-treated U2OS cells [4]. Interestingly, recent studies have implicated SEC24C in the maintenance of neuronal homeostasis [55].

As discussed earlier, some receptors that contribute to ER quality control also bind to molecular chaperones. FAM134B binds to calnexin, and RTN3L copurifies with two J domain proteins, DNAJB12 and DNAJB14 [3,46,47]. Recent studies have shown that RTN3L facilitates the degradation of several aggregation-prone mutant prohormones, including variants of proinsulin (Akita), pro-opiomelanocortin (POMC), and pro-arginine-vasopressin (Pro-AVP) (Figure 3) [8]. In the case of Akita, it was shown that low molecular weight oligomers are removed from the ER by ERAD, whereas larger high molecular weight oligomers are only cleared by RTN3-mediated ER-phagy [8]. Interestingly, mutations in FAM134B and ATL3 are associated with hereditary sensory and autonomic neuropathy (HSAN), which leads to the loss of myelinated and unmyelinated fibers [56–59]. These disorders primarily affect the

peripheral nervous system [56–59]. RTN3L, which is abundant in neurons, has also been linked to Alzheimer's disease [60]. Together, these observations suggest that the ER-phagy pathways participating in ER quality control may be particularly important in cells, such as neurons, that do not actively divide.

FAM134B is also required for the degradation of the transmembrane Niemann–Pick disease type C1 protein (NPC1), as well as misfolded and mutant forms of procollagen, by ER-phagy [3,61]. Like Akita, procollagen is degraded by ERAD, but some forms of procollagen are resistant to degradation by ERAD and are degraded by ER-phagy instead [10]. Procollagen is an abundant, large fibrillar trimeric protein that traffics through the secretory pathway in enlarged COPII-coated secretory vesicles [49]. A significant fraction of procollagen is unable to fold correctly, and ER-phagy prevents ER accumulation. Two distinct mechanisms have been described for the degradation of misfolded and mutant procollagen by ER-phagy [3,45]. One report showed that FAM134B delivers procollagen to lysosomes via macro-ER-phagy [3]. In this context, procollagen binds to calnexin, which in turn interacts with FAM134B to trigger lysosomal delivery by ER-phagy [3]. In another study, procollagen accumulated in COPII-coated ER exit sites that were directly engulfed into lysosomes via micro-ER-phagy in a process that appears to employ p62 and ubiquitin [45]. It is currently unclear why procollagen can be degraded by both macro-ER-phagy and micro-ER-phagy, although these two mechanisms may function redundantly to avoid procollagen accumulation in the ER, which would be cytotoxic.

Concluding Remarks

Since the identification of the first ER-phagy receptors 5 years ago, many questions have emerged about the contribution of ER-phagy to cellular homeostasis and the roles that the different ER-phagy receptors play (see Outstanding Questions). We have outlined the major functions of ER-phagy and discussed the growing connection between these processes and disease. Examples include mutations in ER-phagy receptors linked to neurodegenerative disorders that have also been shown to disrupt ER-phagy [16,21]. In addition, some mutant proteins that are known ER-phagy substrates, such as proinsulin Akita and vasopressin, lead to autosomal dominant forms of diabetes [8]. ER-phagy has also recently been implicated in the regulation of metabolism as well as cellular differentiation and development. Specifically, the induction of macro-ER-phagy in chondrocytes was shown to be triggered by fibroblast growth factor (FGF) signaling, which in turn increased the levels of FAM134B and macro-ER-phagy via the TFEB transcriptional factor [31].

Although we have outlined three main roles for ER-phagy (Figure 3), some autophagy receptors, such as FAM134B, exhibit other ER-phagy-associated functions. For example, an ER stress-dependent increase in cytosolic calcium initiates FAM134B phosphorylation and macro-ER-phagy [30]. FAM134B has also been implicated in degradation at ER–mitochondria contact sites [62], and it limits viral replication by favoring the turnover of specific viral glycoproteins [63,64]. These observations raise an important question. How can one autophagy receptor affect so many pathways? Studies in yeast provide a potential answer to this question [4]. Atg40 largely resides on and degrades the **cortical ER**, but a minor fraction of Atg40 resides on the nuclear membrane where it also participates in **nucleophagy** [15]. However, when Atg40 functions with Lst1–Sec23, it acts exclusively in cortical ER-phagy, and not in nucleophagy [4]. Likewise, FAM134B may only contribute to ER quality control when bound to calnexin [3,46]. We propose that the interaction of an ER-phagy receptor with a specific binding partner defines the pathway in which it functions. Identifying and understanding how these binding partners interact with ER-phagy receptors is an important goal for future studies.

Outstanding Questions

What is the spectrum of ER-phagy receptors and binding partners, and what are their molecular roles?

What is the role of post-translational modifications in ER-phagy?

How are different ER-phagy pathways selected for different substrates?

Do ER-phagy receptors function together, synergistically, or play redundant roles?

As noted in this article, a growing body of evidence indicates that ER-phagy degrades ERAD-resistant forms of ERAD substrates. As a component of ER proteostasis, the relative roles of ER-phagy versus ERAD are unclear because direct measurements of substrate flux through these pathways have not been studied. The contribution of each pathway is possibly controlled by a myriad of ER stress-response transducers, or simply the levels of different aggregation-prone or ERAD-resistant proteins. Similarly, we only have a rough understanding of the substrate-specific decisions that direct a protein to a specific pathway. In ERAD, glycan- and chaperone-based selection plays a crucial role [65]. In turn, although the chaperone-like protein calnexin has been implicated in the selection of macro-ER-phagy substrates (see earlier), we suggest that other chaperone-like proteins function similarly.

These and many other questions remain unanswered, including how specific domains of the ER are targeted for ER-phagy. We propose that post-translational modifications, such as ubiquitinylation and UFMylation, play a central role in this process. Based on the findings discussed earlier, we also suggest that multiple second messengers regulate ER-phagy. Currently, it is also unclear how the decision to utilize different ER-phagy pathways is made, and whether multiple receptors work with one another in these processes. In addition to shedding light on the multifaceted tasks of ER-phagy, future studies could provide insight into the development of therapeutic approaches that may delay the onset or cure the growing number of diseases associated with ER-phagy.

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Declaration of Interests

The authors declare no conflicts of interest.

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