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ER-phagy: shaping up and de-stressing the endoplasmic reticulum

Author: Simon Wilkinson

Affiliation: Edinburgh Cancer Research UK Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, EH4 2XR, United Kingdom

Email: simon.wilkinson@igmm.ed.ac.uk

Telephone: +44(0) 131 651 8592

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Abbreviations

ER: endoplasmic reticulum

pER: peripheral ER

rER: rough ER

sER: smooth ER

UPR: unfolded protein response

Grp78: 78 KDa glucose-regulated protein

PERK: protein kinase R (PKR)-like endoplasmic reticulum kinase

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IRE1α: inositol-requiring enzyme 1 alpha

ATF6: activating transcription factor 6

eIF2α : eukaryotic initiation factor 2 alpha

ERAD: ER-associated degradation

EGF: Epidermal Growth Factor

RTN3: Reticulon 3

XBP1: X-box Binding Protein 1

ATG: Autophagy-related

FIP200: FAK-interacting Protein 200kDa

ULK: Unc51-like Kinase

mTORC1: Mammalian Target of Rapamycin Complex 1

AMPK: Adenosine Monophosphate-activated Kinase

PI3P: Phosphatidylinositol-3'-phosphate

PI3KC3 complex I: class III Phosphatidylinositol 3'-Kinase (VPS34)

WIPI2: WD Repeat Domain, Phosphoinositide Interacting 2 protein

LAP: LC3-associated phagocytosis

SQSTM1: Sequestosome 1

OPTN: Optineurin

NDP52: Nuclear Dot Protein 52

TAX1BP1: TAX1-binding Protein 1

NBR1: (Neighbour of BRCA1

LIR: LC3-interacting region

GIM: GABARAP-interacting motif

ESCRT: Endosomal Sorting Complexes Required for Transport

MEFs: mouse embryonic fibroblasts

FAM134B: Family With Sequence Similarity 134 Member B

RETREG1: Reticulophagy Regulator 1

RHD: Reticulon Homology Domain

ATL1-3: Atlastins 1-3

PC: procollagen

COPII: Coat Protein Complex II

ERES: ER exit site

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NPC1: Niemman-Pick type C disease protein 1

ATZ: mutant α-1-antitrypsin

STX17: Syntaxin 17

VAMP8: Vesicle-Associated Membrane Protein 8

ERLAD: ER-to-lysosome-associated degradation

HSAN: hereditary sensory and autonomic neuropathy

HSP: hereditary spastic paraparesis

Atg11BR: Atg11-binding region

FIR: FIP200-binding region sequences

IRE1α: Inositol-requiring Enzyme 1α

MAMs: mitochondria-associated membranes, ER-mitochondrion contact sites

UBAN: Ub-binding domain in ABIN proteins and NEMO

GnRHR: gonadotrophin-releasing hormone receptor

TBK1: TANK-binding kinase 1

STING: Stimulator of Interferon Genes

Abstract

The endoplasmic reticulum (ER) network has central roles in metabolism and cellular organisation. The ER undergoes dynamic alterations in morphology, molecular composition and functional specification. Remodelling of the network under fluctuating conditions enables the continual performance of ER functions and minimises stress. Recent data have revealed that selective autophagy-mediated degradation of ER fragments, or ER-phagy, fundamentally contributes to this remodelling. This review provides a perspective on established views of selective autophagy, comparing these with emerging mechanisms of ER-phagy and related processes. The text discusses the impact of ER-phagy on the function of the ER and the cell, both in normal physiology and when dysregulated within disease settings. Finally, unanswered questions regarding the mechanisms and significance of ER-phagy are highlighted.

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Introduction

The endoplasmic reticulum (ER) is a ubiquitous subcellular compartment of eukaryotic cells. Mammalian ER is a continuous, lipid bilayer-bound lumen. It is divisible into the nuclear envelope (NE) and a cytoplasmic peripheral ER (pER) composed of flat, sac-like sheets and a reticulated, tubular network [1]. The ER is a key organelle in support of metabolism and in control of subcellular organisation and signalling (Figure 1). Ribosome studded sheets (rough ER, rER) serve in the biosynthesis of transmembrane and secreted proteins. The oxidising rER lumen facilitates disulphide bond formation within nascent polypeptides and contains enzymes that catalyse glycosylation. The tubular smooth ER (sER) functions in lipid and steroid hormone synthesis, and detoxification. The ER acts as a dynamic intracellular calcium (Ca^{2+}) reservoir, controlling cytosolic calcium levels. Finally, the ER membrane houses junctional complexes at contacts with organelles including peroxisomes, lipid droplets, the Golgi apparatus, mitochondria, endosomes, and the plasma membrane. These contacts regulate organellar function, Ca^{2+} homeostasis, lipid composition, fission, trafficking, and participation in signal transduction events [2].

The ER undergoes dynamic alterations in morphology, molecular composition and functional specification. For instance, the ER is remodelled downstream of acute stimuli, such as compromise of ER protein or lipid metabolism. Indeed, the best-described ER remodelling network is the unfolded protein response (UPR). In the UPR, lumenal unfolded protein binds the chaperone Grp78 (78 KDa glucose-regulated protein), titrating this away from the transmembrane sensors PERK (protein kinase R (PKR)-like endoplasmic reticulum kinase), IRE1α (inositol-requiring enzyme 1 alpha) and ATF6 (activating transcription factor 6). Loss of Grp78 binding activates these sensors. The consequent cytosolic signalling cascades mediate restoration of ER status by cessation of general protein translation, via PERK-mediated phosphorylation of the translation factor eIF2α (eukaryotic initiation factor 2 alpha), and by transcriptional upregulation of lumenal oxidoreductases and

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chaperones, and ERAD (ER-associated degradation) factors [3]. ERAD is the major proteasomal pathway for degradation of unwanted ER membrane or lumenal proteins [4]. The ERAD machinery drives retrotranslocation of polypeptides to the cytosol, whereupon proteasomal-mediated proteolysis occurs. However, acute ER remodelling is not always homeostatic. For example, upon EGF (Epidermal Growth Factor) stimulation, RTN3 (Reticulon 3) protein drives tubulation of pER and consequent ingress into the juxta-plasma membrane region. This in turn facilitates EGF receptor endocytosis [5]. ER remodelling can also fail or be overwhelmed in disease settings. Consequentially, loss of vital ER functions leads directly to deterioration in cell health or, alternatively, unresolved UPR signalling promotes pathologic inflammation, cell death and even tumourigenicity [6-8]. ER status is also specified by differentiation programs. For example, the sarcoplasmic reticulum has a prominent role in regulating cytosolic Ca^{2+} fluxes within skeletal muscle. Some highly secretory cells, such as pancreatic acinar cells, are majority composed of abundant, polarised rER; conversely, steroid hormone producing adrenal cells contain an extensive, specialised sER. ER status associated with differentiation state is also dependent upon gene expression and signalling networks. For example, the UPR transcription factor XBP1 (X-box Binding Protein 1) drives expansion of rER during differentiation of antibody-secreting plasma cells [9] and gastric acinar cells [10].

Recent findings have revealed that autophagy, the transport of cytoplasmic components into the lysosome for degradation, is a key ER remodelling process [11]. Two main forms of autophagy regulate ER status. In microautophagy, sequestration of cytoplasmic material occurs via engulfment into endosomes or lysosomes [12]. Conversely, macroautophagy sequesters cytoplasm in nascent phagocytic vesicles, called autophagosomes, which fuse with lysosomes [13]. In some systems, ER perturbations upregulate macroautophagy in order to alleviate ER stress [14]. Mechanistically, autophagy might indirectly regulate ER status. However, this review focuses on mechanisms by which the ER remodelling occurs by *direct* and *selective* degradation of ER in the lysosome. Most

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notably, this occurs via macroautophagy (macroER-phagy) or microautophagy (microER-phagy) of ER fragments, collectively ER-phagy (Figure 2). ER-phagy related processes, such as lysosomal degradation of ER-derived vesicles packed with ER lumenal content, also play a role; they will be compared with ER-phagy herein (Figure 2). Importantly, ER-phagy responses - also termed reticulophagies [15] – and related processes are emerging as mechanistically diverse and important players in ER remodelling; ER-phagy has been observed in insect [16], plant [17], yeast and mammalian cells (Smith and Wilkinson 2017). This review will also demonstrate that such ER-phagy also plays a key role in normal physiology and may be overwhelmed or aberrant in a number of disease conditions, including neurodegenerative disorders or cancer.

Overview of autophagy

In order to frame our current knowledge of ER-phagy, key general autophagy principles are outlined below. More can be found in dedicated review articles [12, 13, 18, 19]. This review focusses on mammals. However, the text highlights other examples where informative.

The core macroautophagy machinery

Macroautophagy is initiated via the co-ordinated action of complexes of evolutionarily-conserved ATG (Autophagy-related) proteins, which results in the generation and expansion of nascent double lipid-bilayer structures (phagophores or isolation membranes), which close around cytoplasmic material to form double-membraned autophagosomes. Dynamic signal transduction regulates localisation and activity of many ATG proteins in response to stimuli such as nutrient, ER or hypoxic stress. Basal macroautophagy also occurs in most systems, reflecting the autophagy activity permitted by tonic signalling in unchallenged cells or animals.

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Autophagy protein complexes act in a temporal hierarchy (Figure 3). The ULK complex is an early-acting assembly, comprising the scaffolding ATG proteins FIP200 (FAK-interacting Protein 200kDa, alias RB1CC1), ATG13, ATG101, and the serine-threonine protein kinases ULK1/2 (Unc51-like Kinases 1/2) [20]. The enzymatic activities of ULK1/2 promote autophagy and are key signal integrators; phosphorylations of ULK1 by mTORC1 (Mammalian Target of Rapamycin Complex 1) and AMPK (Adenosine Monophosphate-activated Kinase) inhibit and activate kinase activity, respectively [21]. Upon ULK1/2 activation, the ULK and VPS34 complexes (discussed below) recruit to nascent phagophores, which are generated via deformation, budding and fusion of mixed membrane sources, including endosomes, plasma membrane and the ER [19]. Indeed, the phagophore membrane may be contiguous with the ER (Figure 3), although this does not prove that the lipids therein are derived predominantly from the ER [22, 23]. In either case, the relatively small lipid and protein mass that could potentially exit the ER via this route is not considered selective ER-phagy.

Phosphatidylinositol-3'-phosphate (PI3P) lipid is generated from phosphatidylinositol (PI) at the phagophore by the action of the Class III Phosphatidylinositol 3'-Kinase (VPS34) complex (PI3KC3 complex I). ULK1/2 can phosphorylate two components of this VPS34 complex, the VPS34 lipid kinase and BECLIN1 (ATG6). Other complex members include VPS15 and ATG14L, the latter of which targets the complex to the phagophore. PI3P generated thusly at the phagophore recruits the lipid-binding protein WIPI2 (WD Repeat Domain, Phosphoinositide Interacting 2 protein) [24]. ULK1/2 may also stimulate ATG9L1/2 to deliver vesicular membrane to growing phagophores [25-27]. Both WIPI2 and FIP200 interact with ATG16L1 to promote recruitment of the ATG5-12 (ATG16L1-ATG5-ATG12) complex [28, 29]. ATG5 is covalently modified by ATG12 in a ubiquitin-like conjugation reaction, catalysed by ATG7 and ATG10. The ATG5-12 complex acts as an E3-like enzyme in a second ubiquitin-like conjugation reaction called lipidation, in partnership with ATG7 and ATG3 (E1 and E2-like activities). In this reaction, ubiquitin-like proteins of the mammalian LC3/GABARAP (ATG8)

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family covalently modify phosphatidylethanolamine in the phagophore, enhancing expansion and closure. Note that, in humans, LC3/GABARAP proteins are divided into the MAP1LC3 (LC3A, LCB and LC3C) and GABARAP (GABARAP, GABARAPL1 and GABARAPL2/GATE16) subfamilies, whereas in yeast a single orthologue termed Atg8 exists.

Some stimuli engage non-canonical forms of macroautophagy that are independent of some core ATG proteins, such as BECLIN1 or ULK1/2 [30]. Outwith autophagy *per se,* there are macroautophagy-related membrane trafficking processes that similarly depend upon a subset of ATG proteins. For example, LC3-associated phagocytosis (LAP) is the ULK-complex independent modification of plasma membrane-derived phagosomes with LC3/GABARAP [31, 32]. There is evidence for the existence of ER-phagy related ER degradation pathways that may exhibit similarly unconventional features, as described later.

Selective macroautophagy is defined by cargo recognition

Autophagosome generation from the ER can result in adjacent ER fragment capture via simple spatial proximity [22, 23]. However, additional molecular determinants, other than ATG proteins, are required for efficient selective sequestration of ER [33, 34]. Instructively, mature research on other selective macroautophagy processes such as mitophagy (mitochondrial cargo), aggrephagy (protein aggregates) and xenophagy (cytosolic pathogens), has revealed a ubiquitous requirement of cargo receptor proteins (Figure 3), which molecularly bridge the autophagosome and the cargo [35]. In mammals, this frequently involves direct recognition of both polyubiquitin modifications of cargo and of LC3/GABARAP, via discrete regions of the receptor. This is exemplified by the receptors p62/SQSTM1 (Sequestosome 1), OPTN (Optineurin), NDP52/CALCOCO2 (Nuclear Dot Protein 52), TAX1BP1/CALCOCO3 (TAX1-binding Protein 1) and NBR1 (Neighbour of BRCA1). Notably, linear

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peptide motif(s) with the minimal consensus sequence $(W/Y/F)_1$-X_2-X_3-$(L/I/V)_4$, known as LC3-interacting regions (LIRs) [36] or, a subset of the former, GABARAP-interacting motifs (GIMs) [37], mediate receptor interaction with LC3/GABARAP. Yeast cargo receptors may also bind Atg11, which has no mammalian orthologue [38]. Cargo receptors may also integrate signals to moderate selective autophagy. For example, phosphorylation of OPTN stimulates ATG8 and ubiquitin binding [39, 40].

Selective microautophagy processes

In microautophagy, endosomes or lysosomes (the vacuole in yeast), can invaginate to subsume cargo. Alternatively, lysosome or vacuole membranes can protrude to enwrap cargo [12, 41]. Microautophagy pathways employ diverse molecular mechanisms. Nonetheless, the dual mechanistic principles of selective macroautophagy - membrane remodelling and recognition of cargo – apply to selective microautophagy. Microautophagy of peroxisomes in the yeast *Pichia pastoris* exemplifies this. Proteins such as Atg18 [42] and Vac8 [43, 44] drive vacuolar membrane protrusion while the core Atg proteins adjacently build an Atg8-labelled phagophore-like structure that donates membrane to the protrusions [45]. Peroxisomal Atg30 acts as a receptor, linking peroxisomes to Atg11 on the vacuole and phagophore [46]. There are few molecular details on mammalian microautophagy, with the partial exception of the endosomal invagination pathway [47]. This process is characterised by use of ESCRT (Endosomal Sorting Complexes Required for Transport)-family proteins for membrane remodelling, as are some yeast microautophagy pathways [48]. Recognition of cargo for internalisation is mediated by the chaperone Hsc70 [47]; in fission yeast, a similar process may involve Nbr1 [49]. Intriguingly, the mammalian orthologue NBR1 is a macroautophagy receptor that can also be degraded by endosomal microautophagy [50].

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ER-phagy pathways: mechanisms and importance

Macroautophagy of the ER (macroER-phagy) was first identified ultrastructurally [16, 51, 52]. For instance, autophagosomes packed with ER fragments were seen in cultured hepatocytes recovering from phenobarbital-induced sER expansion *in vitro* [51] or in guinea pig pancreata after subcutaneous cobalt injection [52]. In the first description of microER-phagy, induction of the UPR in the yeast *Saccharomyces cerevisiae* was seen to drive ER expansion, resulting in a counter-balancing expulsion of concentric whorls of ER membrane, which were then engulfed by vacuolar invagination, all of this occurring independently of Atg proteins [53, 54] (Figure 2). However, no ER-phagy specific molecular players were identified in the above systems, thereby limiting investigation of mechanism and of functional importance. Conversely, core macroautophagy proteins have also been shown to regulate ER size and function. For example, ER stress triggers pro-survival macroautophagy in mouse embryonic fibroblasts (MEFs) (Ogata et al. 2006). Tissue-specific deletion of murine *Atg5* in terminally differentiated B-lymphocytes (plasma cells) [55], or *Atg5* or *Atg7* in pancreatic acinar cells, drives ER expansion, UPR and cell death [56, 57]. *Atg5* deletion in mature T-lymphocytes results in ER accumulation and defective Ca^{2+} signalling [58]. Finally, *Atg7* is required for secretion of collagen from mouse chondrocytes, an important process in bone growth. In the absence of this, procollagen II accumulates within a distended ER [59]. It is possible that some of these phenomena involve selective ER degradation, but the lack of known ER-phagy specific genes available to test during the execution of these studies precluded determination of this. However, recent breakthroughs have identified several ER membrane-resident cargo receptors that specifically facilitate ER-phagy, enabling rapid progress in establishing mechanistic and functional principles. Thusly, the following exploration of ER-phagy is structured around a discussion of individual receptors, highlighting the following principles: selectivity determinants for autophagy-mediated recognition of the ER *per se* and of particular subcomponents thereof; fragmentation of ER to facilitate sequestration; co-ordination of ER-phagy via cell signalling.

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FAM134B and Atlastins in sheet turnover and proteostasis

FAM134B (Family With Sequence Similarity 134 Member B), also known as RETREG1 (Reticulophagy Regulator 1), is an ER membrane protein that preferentially localises to ER sheets [33] (Figure 4). Post-translational insertion into the lipid bilayer is mediated by an RHD (Reticulon Homology Domain), structurally defined by two hydrophobic hairpin helices that do not intrude into the ER lumen. The sequences N-terminal and C-terminal to the RHD are cytosolic, enabling a C-terminal LIR motif (FELL) to mediate LC3/GABARAP recognition [33]. FAM134B expression in human U2OS osteosarcoma cells causes ER fragmentation and coalescence into FAM134B and LC3/GABARAP-enriched autophagosomes, dependent upon the LIR motif. Conversely, RNA interference (RNAi) against *FAM134B* in U2OS, or *Fam134b* knockout in MEFs, promotes ER expansion [33]. Thus, FAM134B mediates basal macroER-phagy. Nutrient starvation upregulates FAM134B-dependent macroER-phagy further. This occurs along with FAM134B-independent LC3/GABARAP lipidation and turnover of p62/SQSTM1, highlighting FAM134B's ER-phagy selectivity. Consistent with its sub-ER localisation, FAM134B predominantly acts upon ER sheets but not tubules [34].

How does FAM134B expression drive fragmentation of the ER and is this required for ER-phagy? The asymmetric insertion of RHD domains into lipid bilayers causes membrane curvature, potentially facilitating scission [60]. However, the extent to which the RHD domain of FAM134B contributes to fragmentation remains to be formally tested. Recently, ATL1-3 (Atlastins 1-3) were identified as requirements for macroER-phagy [61]. Atlastins are dynamin-superfamily GTPases that are anchored in the ER via two transmembrane helices (Figure 4). The cytosolic GTPase activity drives homotypic ER membrane fusion and thus ER branching or scission. ATL2 binds to FAM134B, localises with FAM134B at autophagosome biogenesis sites, and is required for FAM134B-driven ER-phagy. This observation strongly supports a role for receptor-coordinated membrane fragmentation in ER-phagy [61].

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How does FAM134B determine ER status? FAM134B might indiscriminately target ER, merely to control organellar volume. Alternatively, ER-phagy could also have more finely tuned actions upon the ER. One clear functional role emerging for FAM134B is in proteostasis [62, 63]. ER lumenal procollagen (PC) transits to the Golgi apparatus via COPII (Coat Protein Complex II)-dependent transport from ER exit sites (ERES). However, data from human Saos-2 osteosarcoma cells and MEFs indicate that some newly synthesised PC misfolds and is eliminated by FAM134B-driven ER-phagy (Figure 4). Mechanistically, FAM134B binds the transmembrane protein calnexin, which recognises unfolded PC via its lumenal chaperone domain [62]. Whether FAM134B also assists in nucleation of PC aggregates or if FAM134B is recruited to ER subregions where PC-calnexin has already clustered is unclear. Not just lumenal misfolded proteins, but also ER transmembrane proteins such as mutant NPC1 (Niemman-Pick type C disease protein 1), may be subject to FAM134B-driven, ER-phagy mediated sequestration. Mutant NPC1 degradation by ER-phagy may be a compensatory pathway for ERAD [64]. Taken together, these studies show that subregions of ER may be targeted by ER-phagy receptors via recognition of specific ER moieties.

Roles for FAM134B in proteostasis may extend beyond canonical selective macroautophagy. For example, calnexin cooperates with FAM134B in ER removal of a polymerisation-prone, hereditary mutant of α-1-antitrypsin (ATZ) [63]. Single ER membrane-delimited vesicles form from sites of lumenal calnexin-ATZ clustering. However, while FAM134B is incorporated into vesicles, the vesiculation process itself is FAM134B- and ATG-independent. Nonetheless, partially reminiscent of LC3-associated phagocytosis (LAP), LC3/GABARAP lipidation is required for vesicle fusion with endolysosomes. Fusion also relies upon the interaction between the ER SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein STX17 (Syntaxin 17) and the lysosomal SNARE protein VAMP8 (Vesicle-Associated Membrane Protein 8) [63]. FAM134B-

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LC3/GABARAP interaction at vesicle-lysosome contact sites drives fusion, suggesting that lysosomal LC3/GABARAP recognises vesicular receptor. However, this concept requires experimental confirmation (Figure 4). This ER-phagy related process was termed a form of autophagy-related ERLAD (ER-to-lysosome-associated degradation) [63]. Interestingly, an independent study showed that PC aggregates enter ER buds at ERESs [65]. This occurs concomitant with LC3/GABARAP labelling of buds, and engulfment by lysosomal invagination and microautophagy. The molecular dependencies of this are unclear but, speculatively, they might have overlap with the autophagy-related mechanism of ERLAD described for ATZ. Importantly, ERLAD was more latterly suggested to be a useful overarching term for all processes that result in lysosomal clearance of faulty ER gene products that are proteasome-resistant and escape ERAD, including some forms of *bona fide* ER-phagy [62]. For clarity, this is how the term shall be used in this review (Figure 2).

Atg40, an ER-phagy receptor in *Saccharomyces cerevisiae*, is similarly organised to FAM134B, with an RHD and a single, C-terminal Atg8-interacting motif [66]. Such cross-species conservation of ER-phagy illustrates its fundamental importance. Indeed, FAM134B function is important for cellular health. In MEFs and human A549 lung cancer cells, FAM134B protects against ER stressors; murine *Fam134b* knockout leads to ER dilation and cell death of peripheral sensory neurones [33]. This reflects a form of hereditary sensory and autonomic neuropathy (HSAN type II) in humans caused by *FAM134B* nonsense mutations [67]. However, it remains to be determined whether the primary cause of these neuropathies is defective FAM134B-mediated procollagen (PC) quality control within the ER lumen, as might be suggested by the *in vitro* study described above [62]. No effect of *Fam134b* knockout was reported in other organs, albeit in unchallenged mice. However, FAM134B's paralogues, FAM134A and FAM134C, also bind LC3/GABARAP [33]. Their roles require investigation.

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RTN3L in tubular remodelling

RTN1-4 (Reticulons 1-4) are RHD-containing ER reshaping proteins. RTN3 drives ER tubulation, sheet edge curvature and sheet fenestration [68, 69]. However, the RTN3L splice isoform of RTN3 also has an extended cytosolic N-terminus containing six LIR motifs [34]. Using similar experimental approaches to the FAM134B study [33], it was demonstrated that RTN3L was a *bona fide*, LC3/GABARAP-family binding macroER-phagy receptor in nutrient-starved MEFs (Figure 5). In this context, RTN3L mediates degradation of tubular but not sheet ER. Importantly, as with FAM134B, RTN3L does not mediate LC3 lipidation or p62/SQSTM1 degradation, indicating a selective role in ER degradation and not in general autophagy responses. GABARAP interaction is required for fragmentation of the ER by focal clustering of multimerised RTN3L molecules [34]. A requirement of the FAM134B LIR for fragmentation of ER was similarly seen, but explored in less depth, in a prior study [33]. These observations underscore that the LC3/GABARAP lipidation machinery in ER-phagy not only promotes phagophore growth and recognition of cargo, but may also recruit activities required for ER membrane dynamics. It is unknown if Atlastins co-operate with RTN3L in driving fragmentation and ER-phagy. Functionally, initial RNAi data highlight a potential role for RTN3 in PC proteostasis [62]. However, *Rtn3* null mice have no ER dysfunction phenotype [70]. Given that heterodimers of RTN3L with shorter RTN3 isoform(s) are impaired in ER fragmentation [34], an *Rtn3l* specific loss-of-function mouse might yet reveal its physiological function.

ATL3 as a GABARAP-binding receptor

Although ATL2 cooperates with FAM134B in ER remodelling, the Atlastin ATL3 additionally contains two GABARAP-selective LIR motifs (GIM motif) [71]. These mediate ATL3 degradation by autophagy, and ER-phagy of tubular ER (Figure 4). The situation with ATL3 thus parallels RTN3L, where a known ER reshaping factor also acts as a receptor, potentially combining ER recognition and membrane reshaping principles. A point mutation within the GIM of *ATL3* that precludes GABARAP-binding was

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found in the neuropathy HSAN type I [71], suggesting that dysfunctional ER-phagy is part of the disease mechanism. Interestingly, loss-of-function of human *ATL1*, which also participates in ER-phagy (discussed above), underlies a related degenerative condition of the central nervous system termed hereditary spastic paraparesis (HSP) [72].

SEC62 and the UPR

SEC62 and SEC63 are ER transmembrane proteins that bind the SEC61 translocon to promote post-translational entry of polypeptides into the rER. Mammalian (not yeast) SEC62 contains a cytosolic LIR motif at the C-terminus, which plays no role in translocation [73]. SEC62 specifically appears to mediate macroER-phagy during cellular recovery from an acute UPR response (Figure 5). Indeed, SEC62 does not participate in FAM134B-driven PC proteostasis [62]. SEC62-dependent autophagosomes contain selected UPR-upregulated proteins, including chaperones such as calnexins, but largely exclude other ER components, for example ERAD proteins. This observation highlights once again the emerging theme of ER-phagy mediated recognition of specific subregions or subcompositions of ER. The molecular mechanism by which SEC62 facilitates ER-phagy of specific intralumenal cargo requires investigation. It is not known how ER fragmentation occurs during SEC62-mediated ER-phagy. It is also unclear how this pathway is stimulated by the UPR. However, some evidence supports a model where SEC63 competes with mammalian LC3/GABARAP for SEC62 binding and thus inhibits ER-phagy [73]; the SEC62-SEC63 interaction might be lost during recovery from ER stress. The physiological function of SEC62-mediated ER-phagy at organismal level also requires investigation.

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CCPG1 in exocrine secretory cells

CCPG1 is a type II, single pass transmembrane protein [74]. In contrast to FAM134B and RTN3L, CCPG1 contains a lumenal region of undefined function. The N-terminal cytosolic region contains a LIR motif that promotes incorporation into autophagosomes. CCPG1 stimulates ER-phagy upon overexpression in HeLa cells, dependent upon the LIR motif. [75, 76] (Figure 5). Showing that endogenous CCPG1 is required for ER-phagy, it was observed that *CCPG1* deletion blocked ER-phagy in response to nutrient starvation, as seen previously for FAM134B or RTN3 deletion (see above). It is unclear to what extent complete nutrient starvation models physiological stimuli for ER-phagy. It is a useful experimental tool to stimulate ER-phagy in cultured cells and co-opt the function of ER-phagy receptors for mechanistic studies; however, these data should not be taken as suggesting that CCPG1 co-operates with FAM134B or RTN3L in degradation of sheet-like or tubular ER under physiologic conditions. This remains to be addressed. In this regard, endogenous CCPG1 loss also blocked ER-phagy induced by the ER stressor DTT (dithiothreitol, an inhibitor of disulphide bond formation and protein folding), suggesting a link with acute ER stress responses (rather than recovery, as seen with SEC62). *CCPG1* has no sequence orthologue outside vertebrates. However, several features of CCPG1 function highlight similarities with *Saccharomyces cerevisiae* Atg39, a receptor for autophagy of the perinuclear ER (equivalent to the mammalian nuclear envelope). Atg39 is a single pass transmembrane protein with a cytosolic, N-terminal Atg8-interacting motif [66]. Atg39-driven ER-phagy also requires an Atg11-binding region (Atg11BR). Intriguingly, CCPG1 action in ER-phagy also requires two FIP200-binding region (FIR) sequences, similar to the yeast Atg11BR consensus, which bind the C-terminal region of FIP200. This region of FIP200 is itself homologous to the C-terminal coiled-coil of yeast Atg11 that recognises Atg11BRs [38]. Finally, *CCPG1* transcriptional upregulation is triggered by the UPR [76], consistent with its role in DTT-driven ER-phagy. This provides an example of how ER-phagy may be regulated by signal transduction, in this instance coordinating CCPG1-dependent events with other transcriptionally-induced ER remodelling activities.

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The following questions arise regarding CCPG1-driven autophagy mechanisms. What molecules provides ER membrane fragmentation activity? When does FIP200 binding occur? Prior to LC3/GABARAP lipidation and attachment to the phagophore? And for what purpose? One hypothesis states that FIP200 clusters with CCPG1 on the ER to mark sites of autophagosome biogenesis, prior to lipidation of LC3/GABARAP on the phagophore and "handover" of CCPG1 (Figure 5). Are there determinants for subER-selectivity in CCPG1-mediated ER degradation? RNAi data from Saos-2 cells do show that CCPG1 may have minor roles in PC clearance [62]. Indeed, when *Ccpg1* function is ablated in mice, exocrine pancreatic acinar and gastric chief cells display ER expansion [76]. In particular, CCPG1-deficient ER in the pancreas harbours numerous lumenal protein inclusions, resulting in UPR elevation. Mice remain viable under unchallenged conditions, but may be sensitive to pro-inflammatory stimuli during ageing. This requires further investigation. It has been speculated that CCPG1 and ER-phagy act to directly remove these lumenal protein aggregates from the pancreas, but this idea also requires testing [77]. Overall, CCPG1 exemplifies how ER-phagy might have specific roles in determination of ER status in cell types that have specialised ER function.

TEX264 and nutrient starvation

Very recently, TEX264 was identified as a single pass, transmembrane receptor for nutrient starvation-induced ER-phagy. It has a C-terminal cytosolic region with a single LIR motif [78, 79]. TEX264 is ubiquitously turned over by autophagy *in vivo* and is responsible for more than half of the nutrient starvation-induced autophagic flux from the ER in cultured cells. Interestingly, not all ER proteins are equally sensitive to the presence of TEX264 for degradation by ER-phagy, again suggesting mechanisms of selectivity related to the site of initiation of ER-phagy via a particular receptor, or intrinsic recognition of select ER species via molecular interactions with the receptor. Time-resolved imaging of TEX264 incorporation into ER foci within autophagosomes showed that LC3 recruitment preceded TEX264 recruitment at three-way junction sites in the tubular ER.

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Interestingly, rings of TEX264 colocalising with LC3 were produced upon recruitment of the former, suggesting that fragmentation of ER might be a late event, only occurring once loops of tubular ER are bound in close apposition to the membrane of an unclosed, but otherwise fairly complete, autophagosome.

Other potential ER-phagy receptors

The cytosolic cargo receptor p62/SQSTM1 recruits to ER-containing autophagosomes and facilitates excess ER turnover in mouse liver [80]. The ER transmembrane protein and UPR transducer IRE1α (Inositol-requiring Enzyme 1α) binds p62/SQSTM1, as well as the other cytosolic ubiquitin-binding receptors Optineurin and NBR1. This observation has led to the suggestion that ER-phagy could sequester active IRE1α-enriched ER subdomains in order to terminate UPR signalling [81]. The proposed involvement of these ubiquitin-binding receptors in ER-phagy highlights a need to explore potential cytosolic ubiquitylation of ER membrane proteins in marking sites of ER-phagy. Interestingly, ERES-derived buds that are targeted by mammalian microER-phagy were found to be labelled with ubiquitin [65].

The lumenal chaperone calreticulin contains a LIR motif [82]. However, it is unclear if this participates in ER-phagy. Calreticulin might need to be cytosolic in order to bind LC3/GABARAP and, in this event, it is uncertain how it could target the ER. Finally, overexpression of an ER-targeted form of the mitophagy receptor Bnip3 may drive LIR-dependent ER-phagy, but it is not known whether this occurs endogenously [83].

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Unanswered questions in ER-phagy

As highlighted above, several aspects of ER-phagy mechanism and function are not yet resolved. Additional important questions are discussed further below. Addressing these areas will be important for the progress of the field. Such is the open nature of this field, and the diversity and abundance of potential viewpoints and questions arising, that the interested reader is also referred to several recent opinion articles [84, 85].

Molecular mechanisms of ER-phagy

Selectivity and receptors

The known repertoire of ER-phagy receptors is likely incomplete and requires further elucidation, as suggested by the apparent tissue-restricted effects of *Fam134b*, *Rtn3* and *Ccpg1* knockout [33, 70, 76]. Novel receptor(s) will have to fulfil potentially three functions, via direct activity or recruitment of other players. By definition, the receptor itself will directly mediate the recognition of the ER membrane by phagophores or lysosomes. Secondly, an ER lumenal facing or membrane-embedded activity will function in imparting specificity for subregions of ER, or subER content. Thirdly, in the case of macroER-phagy, deformation and scissioning of the ER membrane must be localised at site of autophagosome biogenesis; this may be coordinated by receptors. It remains to be determined how frequently these activities are encoded by separate polypeptides. While gain-of-function experiments, such as overexpression, have shown that receptors such as FAM134B, RTN3L and CCPG1 can drive ER-phagy, this does not mean that all of the activities in this list are directly supplied by that molecule. For example, RHD-containing proteins such as FAM134B and RTN3L do not span the membrane, but have intrinsic ability to curve the ER membrane. In the case of FAM134B, cargo selection can occur via interaction of the RHD with the membrane-embedded region of the ER chaperone calnexin. This observation also underscores that membrane-embedded

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regions of receptors are not simply anchors, but can also participate in scaffolding the multimolecular complexes required for ER-phagy. Contrastingly, CCPG1 is a transmembrane protein with both an extensive lumenal domain and a cytosolic domain, unique thus far among known cargo receptors. Unlike FAM134B and RTN3L therefore, CCPG1 could potentially recognise ER content via lumenal interactions, while simultaneously linking to the cytosolic autophagy apparatus. Similar to CCPG1, TEX264 has no intrinsic membrane reshaping activity [78, 79]. TEX264 does have a selective effect on turnover of different ER protein species [79]. It is unclear whether this is mediated via interaction of TEX264, directly or indirectly, with such proteins, or whether TEX264 is restricted to activity at particular ER subregions enriched in these proteins.

Whereas RHD proteins such as FAM134B and RTN3L have membrane-reshaping abilities, other receptors may not have such intrinsic activity. For instance, might CCPG1 and TEX264 have to interact directly or indirectly with ER membrane reshaping proteins in order to drive ER-phagy? If so, would it cluster depending upon its LC3/GABARAP or FIP200 interactions in order to fragment the ER? This is likely the case with FAM134B and RTN3L, where overexpression-mediated fragmentation is strictly dependent upon LC3/GABARAP-mediated clustering via intact LIR motifs [33, 34]. Even proteins with intrinsic reshaping ability, such as FAM134B and RTN3L, may interact with other RHD-family proteins [34] or, in the case of FAM134B, other reshaping proteins such as ATL2 [61]. Heterotypic interactions of ER-phagy receptors with other receptors could also be necessary for optimal ER-phagy. It might be hypothesised that coincident activation or localisation of co-operating species of receptors and/or reshaping proteins at particular ER subdomains would impart a layer of regulation on engagement of ER-phagy in response to specific stresses. In this regard, FAM134B and TEX264 were shown to target to the same autophagosomes in response to nutrient stress; however, preliminary evidence suggests the ER-phagy mediated by either receptor may be at least partially independent of the other [79]. Uncovering determinants of clustering of membrane-embedded receptors and ancillary proteins at autophagy initiation sites may also give deeper mechanistic insight into the triggers of ER-phagy (and thus also cellular functions of ER-phagy). In addition to

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interaction with ATG proteins, such factors could include formation of complexes with lumenal species such as unfolded proteins, or sensitivity to local ER membrane phospholipid composition or shape, lumenal redox potential or disturbances in ER lumenal flow [86].

For those homotypic interactors amongst the macroER-phagy receptors, particularly those that have multiple ATG protein interaction sites, such as RTN3L or CCPG1, initial ATG protein-mediated recruitment and microclustering might also promote feedforward engagement of the autophagy machinery. This would putatively occur via receptor-mediated recruitment of further receptor and ATG proteins. For RTN3L, homotypic interaction also enhances membrane fragmentation. Furthermore, CCPG1 is a special case wherein the receptor binds two distinct proteins in the ATG hierarchy, LC3/GABARAP and FIP200. This marks out CCPG1 as unusual amongst mammalian receptors. Could this binding of ATG proteins in addition to LC3/GABARAP, FIP200 or otherwise, be a mode of action of other mammalian ER-phagy receptors? Indeed, in this respect, two additional LC3/GABARAP-binding autophagy receptors in mammals, NDP52 and p62/SQSTM1, were recently discovered to interact with the C-terminus of FIP200. Recognition of mitochondria or bacteria by NDP52 results in FIP200 recruitment. In this instance, recruitment of the ULK complex via FIP200 stimulates local macroautophagy activity [87, 88]. p62/SQSTM1 in protein aggregates binds FIP200 via a polypeptide sequence overlapping the LIR motif, resulting in sequential, mutually exclusive binding of FIP200 then LC3/GABARAP. This imparts directionality on the clearance of ubiqutinated protein cargo [89]. It is conceivable that the dual FIP200 and LC3/GABARAP binding of CCPG1 might be involved in similar mechanism(s) in ER-phagy.

Finally, outwith macroER-phagy, for example in microER-phagy mediated clearance of PC or the ERLAD process for ATZ clearance, molecular factors that drive budding at particular sites, and subsequent lysosomal fusion or engulfment, await complete identification. The role that ER-

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lysosome contact site proteins might have in facilitating ERES-localised microautophagy should also be considered.

Sites of ER-phagy initiation

There may exist "hotspots" with high potential for ER-phagy initiation within the ER network, based upon known propensities for involvement in macroautophagy, for example MAMs (mitochondria-associated membranes, ER-mitochondrion contact sites) [90, 91] or ERESs [92]. Indeed, Rab-family GTPases, such as Ypt1/Rab1, which mediate ERES-dependent anterograde transport from the ER, are known to play a role in yeast macroER-phagy [93]. Identifying such molecularly distinct hotspots might give further insight into the mechanisms and functions of ER-phagy. Consideration of localised cytoskeletal dynamics in ER-phagy may also be important. The ER is shaped by the microtubule cytoskeleton, and the role of this in ER-phagy remains to be investigated. Yeast Lnp1 promotes ER-phagy via the actin-dependent encounter of Atg40 with the core Atg machinery [94]. In mammalian systems, CCPG1 may have a role in regulating the RHO and CDC42 GTPases, which are master determinants of actin dynamics [74].

Signalling in ER-phagy initiation

Signalling regulation of ER-phagy also requires deeper exploration. While it is known that the canonical UPR transcriptionally regulates *CCPG1*, it is likely that other events are also involved in co-ordination of ER-phagy with cellular responses in different settings. Discovery of these may also give further insight into the cellular functions of ER-phagy. For example, are ER-phagy receptors or other ER membrane proteins post-translationally modified? This is a highly attractive option given that the cytosolic surface of the ER acts as a scaffold for cell signalling pathways. Ubiquitination is a prime candidate, as ubiquitin-dependent and -independent modes of selective autophagy have been

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described within the pantheon of other selective ER-phagy pathways [95]. This diversity might also exist between different forms of ER-phagy. Phosphorylation and acetylation of cargo or receptors is also prevalent in other selective autophagy paradigms but remains to be addressed for ER-phagy. For example, the binding affinity of Optineurin for LC3/GABARAP and polyubiquitinated cargo is modulated via phosphorylation near the core LIR motif and in the UBAN (Ub-binding domain in ABIN proteins and NEMO) domain, respectively [39, 40]. Finally, are other ER status-sensing relays implicated in ER-phagy? Examples of the latter that might be tested include non-canonical UPR-driven gene sets activated by lipid bilayer abnormalities [96], or Ca^{2+} and NF-κB (Nuclear Factor-κB) driven signalling resulting from ER protein overload [97].

Functions of ER-phagy

Proteostasis

Investigations of the cellular functions of ER-phagy have uncovered roles in proteostasis and UPR regulation, as outlined above, including specific targeting of PC by ER-phagy and ATZ by ER-phagy related ERLAD. Furthermore, mutant GnRHR (gonadotrophin-releasing hormone receptor) is degraded by autophagy and thus potentially by ER-phagy [98]. However, mutant GnRHR may be incorporated from the ER membrane into the delimiting membrane of autophagosomes in a form of ERLAD for which mechanistic details are unclear. Similarly, another ERAD-resistant aberrant lumenal protein species for which there is evidence of lysosomal degradation, but where the role of macroER-phagy is unclear, is the lumenal granule of beta subunits of thyrotrophic hormone in the secretory cells of the stimulated pituitary gland. In fact, the existing morphological evidence suggests a similar pathway to the ER-phagy related ERLAD process that removes ATZ [99]. Mutant dysferlin in muscle cells is another potential target of ERLAD [100]. In another example of putative proteostatic roles, antibody-secreting plasma cells require core ATG proteins to manage immunoglobulin synthesis [55]. In the absence of ATG proteins, an expanded ER is observed, concomitant with excess

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immunoglobulin synthesis and secretion. *Ccpg1* is highly upregulated during the differentiation of these cells, suggesting a potential role for proteostatic ER-phagy [101]. Indeed, this would represent an important role for ER-phagy in health, given the critical role of immunoglobulin secretion in immune surveillance. Potentially, ER-phagy might be optimal at a "sweet spot" level where ER volume and immunoglobulin secretion would be at the maximal level tolerated without cellular toxicity. This is an area of ER-phagy biology that warrants urgent investigation.

Other potential roles including innate immunity

Notwithstanding its clear involvement in proteostasis, other potential roles for ER-phagy should be addressed. For example, does ER-phagy remodel the ER in order to: determine the capacity for steroid hormone or phospholipid synthesis; resolve topological perturbations of the network; regulate calcium homeostasis; regulate platforming of cellular signalling; or regulate organelle contact site-dependent processes? ER-phagy is induced by lipotoxic stress in cultured HepG2 hepatocytes but its relevance is unclear [102]. Perhaps most strikingly, macroER-phagy has been implicated in generating "signalling" phagophores that scaffold activation of TBK1 (TANK-binding kinase 1) by its upstream regulator STING (Stimulator of Interferon Genes) in order to co-ordinate the cellular response to bacterial infection [103]. It appears that molecular patterns associated with some live bacteria are detected by STING, resulting in UPR signalling and induction of ER-phagy. The generation of autophagy structures containing a variety of ER components and STING seems to be required for TBK1 activation and interferon responses. It is presumed that early, unsealed autophagosomes provide a signalling scaffold for STING-TBK1 signalling out into the cytosol. Indeed, regardless of the former mechanism, a wider ER-phagy involvement in innate immune responses to infection is currently emerging, in addition to the aforementioned potential role of ER-phagy during immunoglobulin production in adaptive immunity. For example, FAM134B suppresses proliferation of Ebolavirus in MEFs [104], and Flaviviruses in human brain microvascular endothelial cells [105].

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The Flavivirus protease NS2B3 mediates cleavage of FAM134B to prevent virion sequestration in ER-derived autophagosomes. *Ccpg1* is induced in intestinal Paneth cells in response to Norovirus infection, also suggestive of a role in host defence [106]. HSV-1 (herpes simplex virus type I)-infected macrophages may sequester virions into nuclear membrane-derived vesicles that incorporate pieces of nuclear membrane within. However, it is unclear if the efficiency of membrane fragment sequestration is sufficient to class this as selective ER-phagy [107]. Interestingly, micronuclei are degraded by macroautophagy, suggesting that the ER-derived membranes around these organelles may be involved in this process [108].

ER-phagy in cancer and ageing

Cancers should be assessed for changes in ER-phagy molecule expression and function. Already, mutations and alterations in FAM134B expression have been observed in various malignancies [109]. Functionally, FAM134B loss may promote colorectal cancer cell tumourigenicity [110]. Conversely, glioma cells bearing *IDH1* mutations may require FAM134B-driven ER-phagy to survive proteotoxic stress, framing this as a synthetic lethal therapeutic target [111]. The SEC62 gene is amplified in a number of cancers, including lung adenocarcinomas, prostate, thyroid, and head and neck squamous cell carcinoma; accordingly it has been hypothesised that excess SEC62 may not be incorporated into SEC61 complexes and instead lead to sensitised ER-phagy responses and, consequently, resistance to anti-cancer ER stress [112, 113]. Finally, defective ER-phagy may also be involved in ageing, as suggested by a mouse model of progeria driven by *Slc33a1* overexpression, but further investigation is required [114].

To finish, a caveat should be noted in regard of the above questions and observations; once an ER-phagy protein is implicated in a given phenomenon or disease, it is important to mechanistically

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ascertain whether this is due to defective ER-phagy or whether the protein serves to regulate the ER via other, co-ordinated functions. This requires more sophisticated experimentation than simple gene knockout. Nonetheless, overall, ER-phagy is clearly of importance in health and disease, and its roles will be clarified by future studies.

Conclusions

Identification of molecularly distinct pathways for ER degradation by ER-phagy and related processes has allowed elucidation of key principles of mechanism. As well as cargo receptors, a rich mix of other players participates, including chaperones and membrane reshaping molecules. Distinct ER-phagy pathways play diverse roles in different cell types and are implicated in disease aetiologies. Excitingly, the field has only just begun to uncover the full complement of ER-phagy mechanisms and functions.

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Conflict of interest

The author has no conflict of interest to declare.

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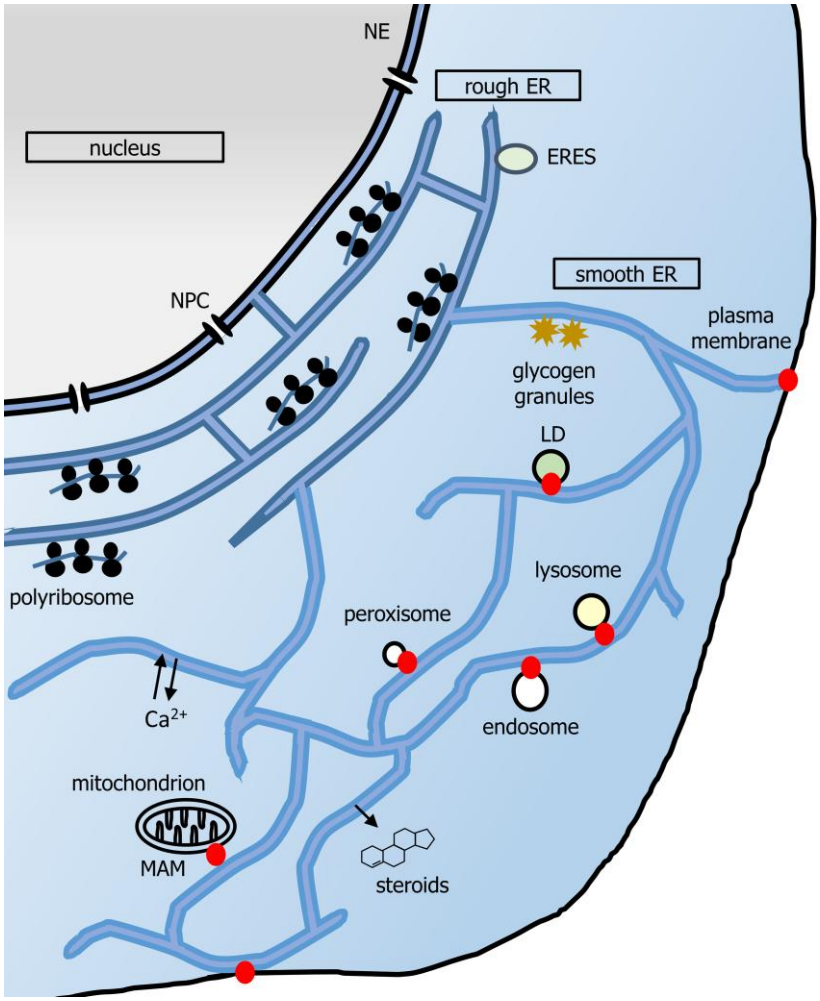

Figure 1 **A schematic of mammalian endoplasmic reticulum (ER)**

Nuclear pore complexes (NPCs) gate nucleocytoplasmic transport at the nuclear envelope (NE). The peripheral ER (pER) is composed of the rough ER (rER) and smooth ER (sER). rER (darker blue) is composed of flattened, stacked, frequently fenestrated, sheets, connected by helicoidal junctions (shown in cross-section here). rER is studded with polyribosomes synthesising secretory protein and functions in import, folding, glycosylation and onward secretion of such protein. Onward transport originates from ribosome-free subdomains of rER (ER-exit sites, ERESs). Smooth ER (sER, lighter blue) extends in a reticular network throughout the cell, characterised by three-way junctions. ER tubules also exist in dense arrays in the perinuclear region (not shown for simplicity). The smooth ER functions in detoxification reactions, and lipid and steroid synthesis. Lipid synthesis contributes to organellar membrane generation, for example during formation of lipid droplets (LDs). Organelle

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contact sites (red dots) may also regulate signalling, for example immune signalling and transfer of calcium to mitochondria both occur at MAMs (mitochondrial-associated membranes). Contact sites may also regulate membrane dynamics, for example endosome budding and mitochondrial fission. Although contact sites may be at the rER or sER, depending upon the organelle (for example, both in the case of MAMs), for clarity they are depicted herein at the cell periphery. Note that the yeast ER has a different morphology; extensive cortical ER runs parallel to the plasma membrane, and is connected by tubules to the perinuclear ER, which delimits the nucleoplasm.

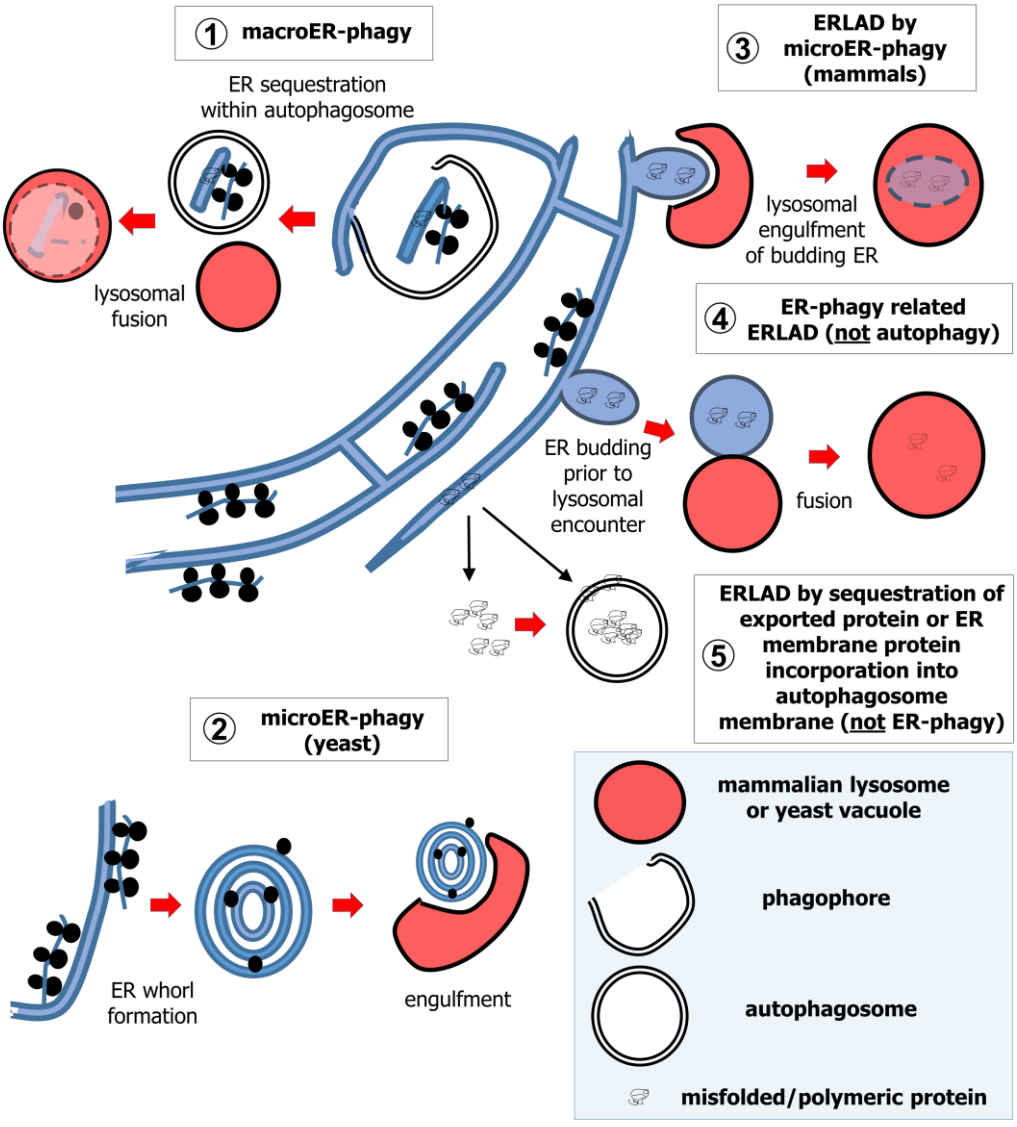

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Figure 2 Pathways by which ER material transits to the lysosome

This review will reference five distinct routes via which ER fragments or lumenal material may be delivered to the lysosome. These include processes that are either *bona fide* ER-phagy pathways, or related processes. Firstly, in macroautophagy (macroER-phagy [1]), fragments of ER are sequestered by the growth of an encircling double-membraned phagophore, which then forms an enclosed autophagosome and fuses with lysosomes. MacroER-phagy can participate in ERLAD (ER-to-lysosome-associated degradation) if particular proteasome-resistant ER proteins are concentrated within the cargo fragment of ER. MicroER-phagy is said to occur when lysosomal invagination or protrusion engulfs portions of ER. In yeast microER-phagy [2], the ER expels whorls of membrane prior to vacuolar invagination. In mammals [3], procollagen-enriched buds of ER forming from ER-exit sites (ERESs) may be targeted in a microautophagy-mediated ERLAD pathway. In contrast, non ER-phagy processes that involve some or all of the core autophagy machinery are [4] an ER-phagy related ERLAD pathway in which single membrane ER-derived vesicles packed with misfolded lumenal protein species, such as mutant α-1-antitryspin, fuse with lysosomes and [5] hypothetic autophagy-dependent but non ER-phagy ERLAD pathways, wherein aggregated or mutant protein would be expelled from the ER prior to cytosolic sequestration by autophagy or be incorporated directly from the ER membrane into the delimiting membrane of the autophagosome.

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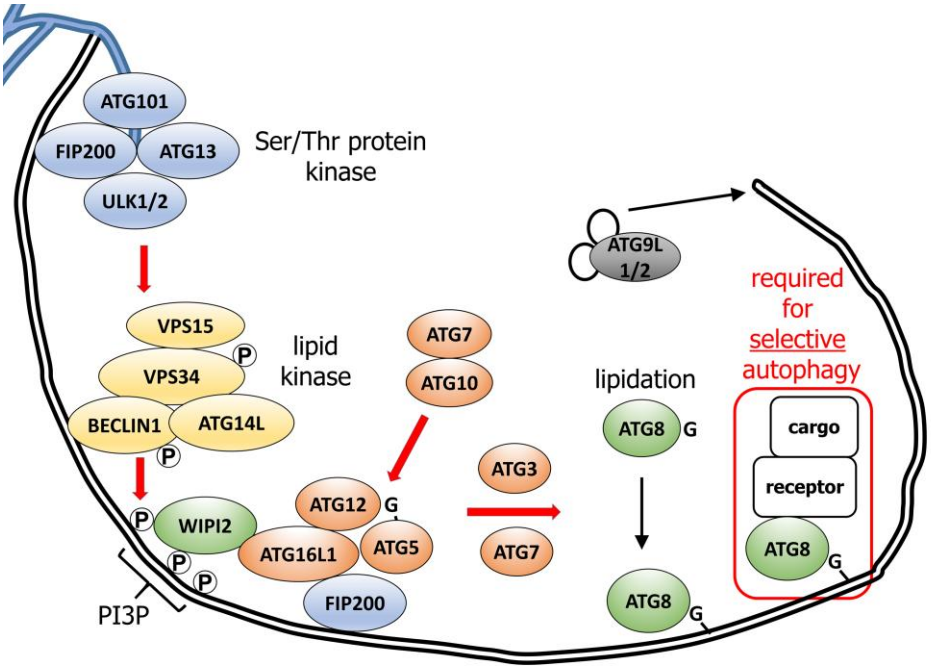

Figure 3 Essential mechanism of autophagosome generation in mammals

A phagophore is shown here (double black lines represent the dual lipid bilayer), notionally extending from an ER cradle (blue tubules). The hierarchy of ATG protein action that initiates and matures the phagophore is depicted as described in the text. Briefly, the ULK1/2 complex activity drives VPS34-complex mediated phosphorylation of phosphatidylinositol to phosphatidyl-3'-inositolphosphate, which in turn recruits WIPI2. WIPI2 and FIP200 recruit the ATG5 complex. The ATG5 complex acts with ATG3 and ATG7 to attach phosphatidylethanolamine in the phagophore to the exposed C-terminal glycine of proteolytically processed LC3/GABARAP. Further lipid is delivered from various sources, such as tubular endosomes; the transmembrane ATG proteins ATG9L1/2 co-ordinate this. Note that while LC3/GABARAP plays a role in accelerating expansion and closure of phagophores, it is also required for selection of cargo via interaction with cargo receptors.

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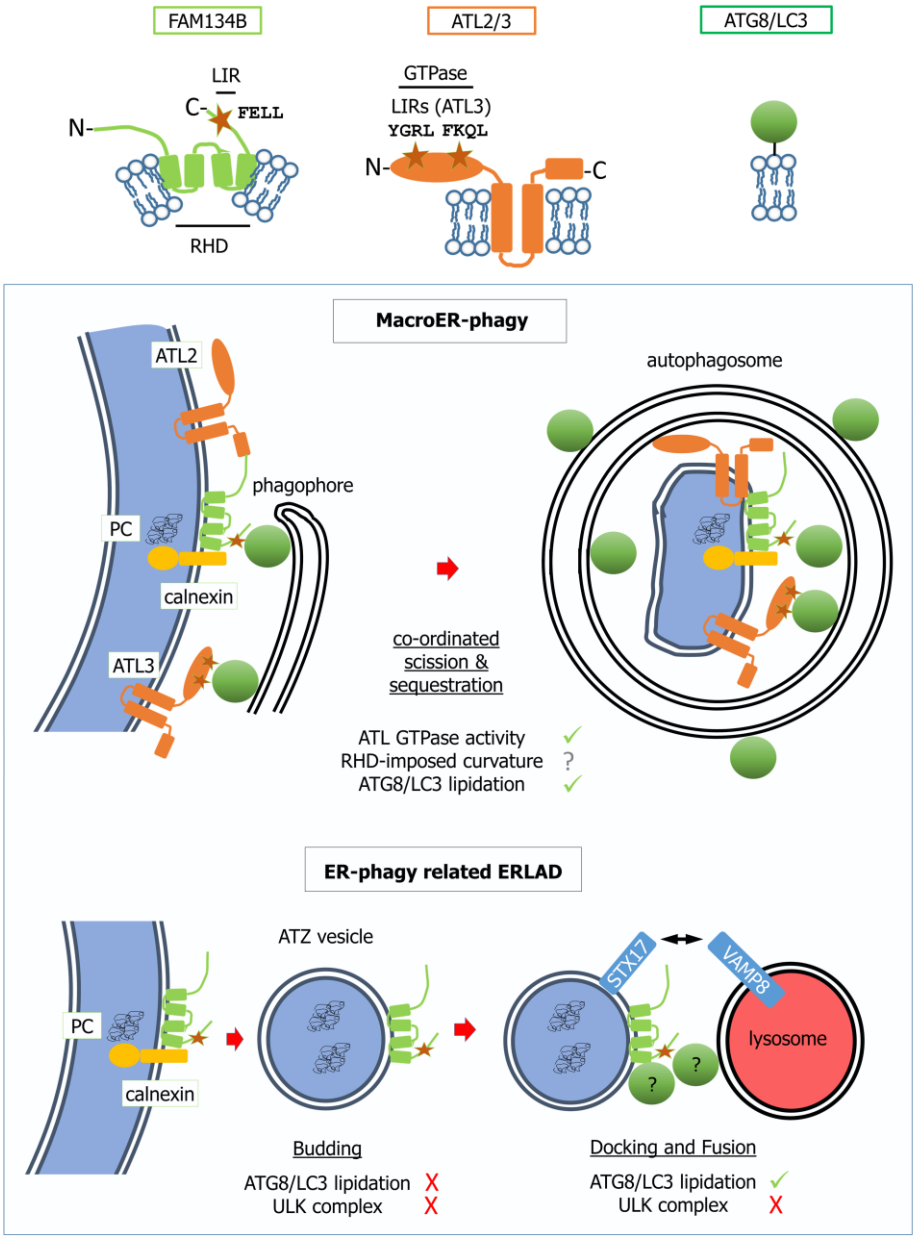

Figure 4 **A model of FAM134B and Atlastin function in delivery of ER content to lysosomes**

In the key at the top of the diagram, the core sequence of each LC3-interacting region (LIR) motif is shown for each receptor (RHD = reticulon homology domain, GTPase = dynamin-like GTPase domain). The box provides a schematic overview of FAM134B- and Atlastin-dependent macroER-phagy and of ER-phagy related ER-to-lysosome associated degradation (ERLAD) of mutant α-1-antitrypsin (ATZ). Note that the cartoon of macroER-phagy is shown particularly in the context of clearance of specific ER lumenal moieties (procollagen, PC), to illustrate the full breadth of our knowledge of this process, but macroER-phagy likely operates in other contexts to functionally

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remodel the ER in different ways. In macroER-phagy, the LIR motif of FAM134B drives clustering at sites of autophagosome genesis, probably aided by initial phagophore generation and recruitment of lipidated LC3/GABARAP. RHD-mediated curvature in conjunction with the GTPase activity of the Atlastins (ATL2 and ATL3 depicted here), results in ER fragmentation. For tubular ER degradation (which is largely FAM134B-independent, but RTN3L-dependent) fragmentation may also be promoted by LC3/GABARAP-mediated recruitment of ATL3 via LIR motifs (also known GIM motifs due to selectivity for GABARAP subfamily proteins). LC3/GABARAP-mediated recognition of FAM134B (and ATL3 for tubular ER) also ensures that the ER fragment is incorporated into the mature autophagosome. In contrast, in ER-phagy related ERLAD, single-membraned vesicles derive from the ER, incorporating FAM134B. However, interaction of FAM134B with LC3/GABARAP is only required for lysosomal fusion, along with the SNARE pairing of STX17 and VAMP8. This delivers the ER lumenal contents into the lysosome, although the membrane is donated to the lysosome, whereas in macroautophagy the entire fragment of ER, membrane and lumen, is internalised and degraded. A minimal LC3/GABARAP lipidation machinery, excluding ATG proteins such as the ULK complex, is required for ER-phagy related ERLAD. Selectivity for lumenal content in macroER-phagy or ER-phagy related ERLAD is at least partly mediated via binding of FAM134B to the chaperone calnexin, which can in turn bind to misfolded or polymerised PC or ATZ.

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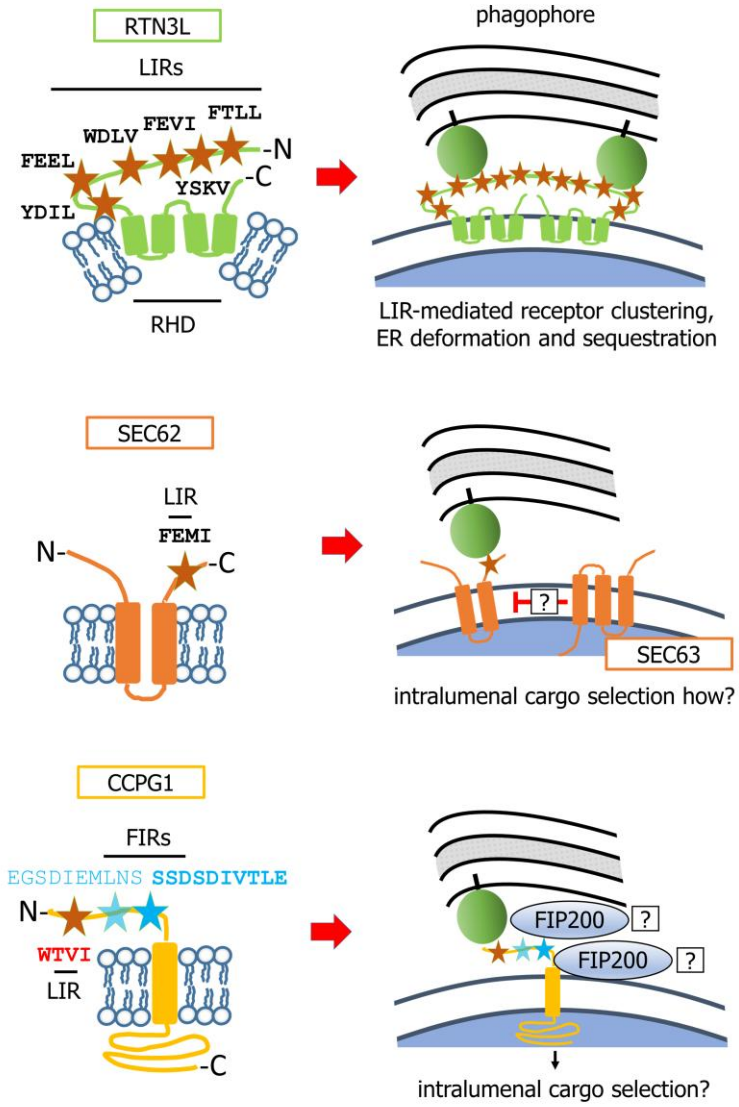

Figure 5 Models of action of RTN3L, SEC62 and CCPG1 in macroER-phagy

Schematics of receptors RTN3L, SEC62 and CCPG1 are depicted on the left-hand side of the diagram. Core LC3-interacting region (LIR) motif sequences are shown. Additionally, in CCPG1, minor (light blue) and major (bold blue) sequences supporting FIP200-binding activity are shown (FIR = FIP200 interacting region). LC3/GABARAP molecules (green circles) bind LIR motifs in RTN3L to mediate focal recruitment at the nascent phagophore and oligomer formation, promoting ER curvature and incorporation of the eventual ER fragment into the mature autophagosome. The LIR motif of SEC62 mediates binding of ER fragments containing UPR-upregulated proteins to the nascent phagophore. How these subregions of ER are generated or how SEC62 recognises specific lumenal cargoes are unknown. When the UPR is at basal levels, SEC63 may bind SEC62 and compete for LC3/GABARAP

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interaction. Finally, CCPG1 uses a LIR to bind LC3/GABARAP on the phagophore and FIR regions to bind FIP200 on either the ER or the phagophore. Both interactions are required for sequestration of CCPG1-enriched ER into autophagosomes. CCPG1 has a substantial (>450 amino acid) lumenal domain that could hypothetically participate in recognition of specific lumenal cargoes.

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