The various shades of ER-phagy
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Introduction

The endoplasmic reticulum (ER) forms a continuous membrane network composed of sheets, tubules and matrices, which originate at the nuclear membrane and spread throughout the cytosol [1–3]. Novel super-resolution microscopy assays revealed that the ER, classically thought to be composed of sheets and tubules, actually consists of tubules of different densities. The apparent flat sheets are the result of very dense tubular matrices, which were described as sheets because conventional optical microscopy could not reach a high enough resolution to distinguish the tight clustering of tubular junctions [2]. The various ER subdomains mediate a large number of vital cellular functions including Ca<sup>2+</sup> homeostasis, protein synthesis, glycosylation, secretion, transport as well as lipid synthesis [4,5]. The ER is also a pivotal transportation hub for large number of intracellular and extracellular proteins: proteins destined for the plasma membrane, Golgi, extracellular matrix and many others travel to or through the ER to their destination. The abundance of individual ER structures within a cell correlates with cell type and tissue-specific functions. Selective turnover of the ER by autophagy is termed ER-phagy and plays a vital role in ER health and homeostasis. The impact of ER-phagy on cellular homeostasis, its relationship to ER stress and unfolded protein response (UPR), its crosstalk with the other ER degradative mechanisms [ER-associated protein degradation (ERAD) and ER to lysosome-associated degradation], as well as its implications in human pathologies have been reviewed in depth in recent manuscripts [6–13]. In the mammalian systems, the term ER-phagy is associated with the selective elimination of discrete fragments of the ER, enwrapped inside the autophagosomes and degraded via the lysosomal machinery. ER-phagy has also different key functions

Abbreviations
ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; GABARAP, gamma aminobutyric acid receptor-associated protein; IM, intramembrane; LC3, MAP1LC3 microtubule-associated proteins 1A/1B light chain 3; LIR, LC3-interacting region; mATG8, mammalian ATG8; TM, transmembrane; UIM, ubiquitin interacting motifs; UPR, unfolded protein response.
The history of ER-phagy

It is now well accepted that macroautophagy (often synonymously termed autophagy) is not just a bulk degradative process, but is rather a selective mechanism able to isolate and eliminate specific cargo [14]. In the case of ER-phagy, autophagy cargo is defined as a discrete fragment of ER content (e.g., ER-derived membranes with protein content). However, this view was not prevalent in the past decades. ER membranes were first documented inside a vesicular structure by Locke et al., in 1965 [15]. At that time, selective autophagy was still unknown, and the word ‘autophagy’ had just been introduced in 1963 by Christian de Duve. ER fragments inside autophagosomes were subsequently observed in rat hepatocytes after treatment with phenobarbital [16] and in guinea pig pancreatic cells [17]. Over decades, alterations and dysfunctions in the ER were observed in cellular and animal models where the autophagy machinery was impaired [18–20]. These phenotypes have mostly been attributed to a general impairment in macroautophagy. Moreover, in yeast, ER membranes were detected inside autophagosomes under starvation conditions. Even though this event was mainly attributed to a nonselective autophagic process, this marks the first time a selective engulfment of the ER was described [21]. Immediately following this observation, microautophagy of ER whorls was found to contribute to the rescue of ER morphology following UPR-induced ER membrane expansion in yeast. It is in this context that the term ER-phagy arose for the first time [22–24], even though the underpinnings of this process were still unknown. Originally, interest in the ER, from the perspective of the autophagy field, could be mainly attributed to its role as a reservoir for autophagic membranes rather than itself being considered an autophagy target. Only in recent years, after the characterization of the first ER-phagy receptors (see next paragraph), FAM134B in mammals and ATG39 and ATG40 in yeast [25,26], the molecular basis of ER-phagy was revealed and its full biological importance began to be recognized [10].

Several mammalian ER-phagy receptors have been identified so far: SEC62, RTN3 (its long isoform RTN3L), CCPG1, ATL3, and TEX264 [27–32] (Fig. 1A,B). The sheer number of ER-phagy receptors, which will certainly only continue to rise, reflects the complexity of this selective form of autophagy; while, at the same time, unveiling the many shades and flavors of ER-phagy, in different cell and tissue types as well as under a multitude of conditions.

ER-phagy receptors: different players, different substrates spectra, same purpose

Autophagy receptors, including the specific ER-phagy ones, are defined as linkers between autophagic cargo and the MAP1LC3 microtubule-associated proteins 1A/1B light chain 3 (LC3)/gamma aminobutyric acid receptor-associated protein (GABARAP) family on autophagic membranes. By this, they facilitate cargo engulfment and its subsequent degradation via lysosome [33]. As such, all ER-phagy receptors harbor a functional LC3-interacting region (LIR) and mediate ER degradation via lysosomes. These two common features aside, they have distinct structural domains, localize to different ER subdomains, act under different conditions (basal vs stresses), and focus on specific subsets of ER substrates (i.e. ER-phagy receptors interact directly or indirectly, with protein aggregates inside the ER lumen to facilitate their degradation). This is because many of the identified receptors have a precise and well-established (ER-phagy independent) biological function at the ER, such as shaping ER membranes (i.e. FAM134B, RTN3L, ATL3). Taking a closer look at their structural characteristics, bona fide receptors are divided into intramembrane (IM) and transmembrane (TM) proteins. IM receptors are anchored to the ER membranes via a specific domain, (i.e. reticulum homology domain) inserted in the ER lipid bilayers. These receptors only face the cytosol, and do not have any structural domains in the intraluminal space of the ER. On the other hand, TM receptors span the entirety of the ER membrane with a portion of the protein facing the intraluminal space. IM proteins include: ATL3, FAM134B, RTN3L; and TM proteins include: CCPG1, SEC62, TEX264, respectively [3,25,27,29,30,34,35]. Members of the IM group have well known functions in shaping ER subdomains, modulating tubule/sheet ratios as well as tubule branching [3,35]. In the case of FAM134B,
biophysical and mathematical modeling indicates that this receptor can induce budding of membranes. As a consequence, the edges of ER sheets are enriched with FAM134B protein clusters and are hot spots for ER-phagy [36]. All three IM autophagy receptors are devoid of an ER luminal domain; therefore, one may argue that this limits their spectrum of protein substrates to ER trans- or intramembrane proteins. However, it is important to consider that ER membrane proteins with a luminal domain could function as autophagy adaptors. By simultaneously binding to protein aggregates, inside the ER lumen, and ER-phagy receptors, such adaptors could be utilized as coreceptors. These proteins can serve as a bridge allowing the inclusion of ER luminal protein aggregates in the substrate’s spectrum of IM ER-phagy receptors, which themselves cannot directly interact with proteins inside the ER lumen. Indeed, such an adaptor has been identified in the case of procollagen, where the ER-resident chaperone calnexin acts as a co-receptor for FAM134B-mediated ER-phagy to prevent the accumulation of misfolded procollagen [37] (Fig. 2A).

Thus far, no intrinsic function (such as shaping of ER membranes) has been reported for any of the TM receptors. However, the TM receptors: CCPG1, SEC62 and TEX264, harbor cytosolic, ER-membrane and ER-intraluminal domains (a very small one for TEX264). Therefore, they are in principle capable of directly interacting with various ER-localized proteins: ER luminal, membrane and even ER-associated cytosolic proteins. This could potentially further divide ER-phagy receptors into those facilitating bulk ER-phagy, with a potential broad spectrum of ER elimination (entire portions of ER and a wide range of...

Fig. 1. Schematic representation of ER-phagy receptors in mammals and yeast. Abbreviations are (A) LIR (mammalian); FIR, FIP200-interacting region (mammalian); (B) AIM, Atg8-interacting region (yeast); Atg11BR, Atg11-binding region (yeast). C, C-terminal domain; N, N-terminal domain.
intraluminal ER protein aggregates), and those with higher selectivity for specific type ER domains or protein aggregates (Fig. 2C). Current knowledge partly supports this hypothesis. CCPG1, SEC62, and TEX264 all act under stress/stress-resolving conditions such as protein aggregation, ER inflation, and nutrient deprivation [27,29,30,32]. Clearance of an overload of misfolded proteins, large amounts of membranes or ER contents could indeed be called bulk ER-phagy and presumably depends on a receptor with a broad substrate spectrum. In contrast, dependency on co-receptors, like in the case of FAM134B and misfolded procollagen [37], might speak to a more targeted and specific ER-phagy receptor with a narrow substrate spectrum. Of note, RTN3 specifically regulates the elimination of prohormone aggregates, in particular Akita proinsulin aggregates, from the ER lumen [38]. The molecular mechanisms are not completely clear; nevertheless, the involvement of a co-receptor is a concrete possibility in this case too.

In yeast, only two ER-phagy receptors have been identified so far: Atg39 and Atg40 [26]. Atg39 is
How is ER-phagy activated and what kind of regulation levels do exist?

Identifying the signaling events which regulate ER-phagy remains a major hurdle in the field due to the complexity of the ER in terms of its proteome, morphology and function in different cells and tissues. Thus far, efforts to identify a precise stressor or stimulus that ubiquitously induces ER-phagy have been unsuccessful. Similarly, there are no common molecular pathways regulating ER-phagy or at least they are still unexplored. Indeed, direct regulation of gene transcription by the UPR has, so far, only been observed for CCPG1 [30].

So, how are ER-phagy receptors ‘activated’? These receptors have no clear enzymatic activity; however, the binding of intraluminal ER protein aggregates or autophagic membranes to these receptors as well as their ability to cluster (as further explained below) could serve as surrogate measures for their activity. As mentioned above, in the case of CCPG1, regulation may occur on the level of transcription and/or translation [30]. However, for most autophagy receptors expression does not seem to be the major regulatory step and therefore regulation must occur further downstream. Moreover, constant binding to LC3 and continuous turnover via autophagy, under basal conditions, has been shown for some autophagy receptors, such as cytosolic p62. It is unclear if this is accompanied by low-level turnover of p62 substrates as well. It has also been shown that the binding affinity of p62 can be modulated by phosphorylation, so p62 activity depends on post-transcriptional modifications (PTMs) [33]. Considering that ER-phagy can be triggered by starvation, some PTMs of receptors could change (i.e. phosphorylation state) and likely affect their activity.

Another feature of the ‘active’ state of an ER-phagy receptor seems to be clustering. Clustering likely helps to maximize recruitment/tethering of adjacent autophagic membranes via mATG8s. Moreover, increasing the local density of ER-phagy receptors, which are also ER morphogens (i.e. FAM134B and RTN3L) promotes ER membrane shaping to allow budding and fragmentation [31,36]. ER-phagy receptors appear to work independently from one another and primarily form homodimers/multimers. The only exception, thus far, was reported for RTN3L and ATL3, which were found to interact with one another after protein overexpression in HEK293T cells [28]. Although the atlantins family member, ATL2, has not been officially defined as an ER-phagy receptor, ATL2 interacts with FAM134B. This interaction works to separate the ER buds marked by FAM134B oligomers from the rest of the ER body [40]. The interaction between RTN3L and ATL3 could have a similar function and at the same time help to tether RTN3L to tubular junctions (Fig. 2A,B). In yeast, Lnp1, a protein found in three-way junctions, is required to relocalize Atg40 from the cortex to the cell interior in order to promote the interaction between Atg40 and the autophagic machinery [41].

In summary, the field of ER-phagy is still largely in its infancy and the underpinnings of this vital pathway are yet to be fully defined. The precise modifications,
necessary interactions, cascades of events preceding the activation of the ER-phagy receptors are all open questions, which remain to be answered. In principle, the following possibilities exist and, most probably, a combination of those takes place:

1. Activation via PTMs: PTMs can reveal or mask binding interfaces, which are necessary for the oligomerization, and modulation of receptor binding affinity to its substrates and to autophagic membranes.

2. Co-receptors, auxiliary and scaffold proteins (i.e. ER chaperones or ER resident proteins): clustering as well as substrate binding could be facilitated via chaperones, this is likely the case with Calnexin in misfolded procollagen elimination [37]. Multiple chaperones could serve the same receptor or recognize the same intraluminal ER protein aggregates. It is also conceivable that individual chaperones could bind to several ER-phagy receptors. Both possible scenarios will increase the efficiency of ER cargo elimination. The regulation of such adapters may occur at several levels: gene expression, PTMs and protein–protein interaction. This is also relevant considering that not all of the receptors are equally expressed among different cell types and tissues. Of note, scaffold proteins, like Lnp1, can also anchor receptors to certain ER subdomains [42].

3. Local changes in the ER lipid bilayer (composition or modification of lipids) could allow and/or cause local enrichment of receptors. Changes in the lipid bilayer are also able to cause changes in protein structure [43,44], which may influence oligomerization and binding interfaces for substrates/autophagic membranes. In addition, with the same principle, substrates could be relocalized to ER membrane crafts containing ER-phagy receptors.

Conclusion

The complexity of the ER is reflected by the intricacy of ER-phagy processes. This field is relatively new and the mechanisms and roles of ER-phagy have only recently begun to be unraveled. The number of ER-phagy receptors is destined to continue to grow. New receptors will be identified by investigating ER proteins harboring functional LIR domains or ubiquitin-interacting motifs, which are sequences that are able to bind mATG8s and recruit the initiation membranes [45]. Uncovering the distinct molecular pathways that regulate wholesale ER degradation, versus the degradation of potions of the ER and/or ER protein aggregates selection is of great importance. Identifying and characterizing any auxiliary players, which may assist ER-phagy receptors in ER turnover including chaperones and membrane shaping molecules, will be equally important. It is likely that ER-phagy pathways are diverse and vary based on cell and tissue type. Finally, these pathways are implicated in the etiology of ER-phagy–related diseases (i.e. sensory neuropathies or infectious diseases [6,7,10]). Therefore, the study of the molecular mechanisms of ER-phagy is likely holds the key to identify therapeutically relevant molecules that could act as potential druggable targets for the treatment of ER-related disease.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

AS and PG jointly wrote the manuscript.

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