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Proteasomal and lysosomal clearance of faulty secretory proteins: ER-associated degradation (ERAD) and ER-to-lysosome associated degradation (ERLAD) pathways

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3405 WORDS

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About 40% of the eukaryotic cell's proteins are inserted co- or post-translationally in the endoplasmic reticulum (ER), where they attain the native structure under the assistance of resident molecular chaperones and folding enzymes. Subsequently, these proteins are secreted from cells or are transported to their sites of function at the plasma membrane or in organelles of the secretory and endocytic compartments. Polypeptides that are not delivered within the ER (mis-localized proteins, MLPs) are rapidly destroyed by cytosolic proteasomes, with intervention of the membrane protease ZMPSTE24 if they remained trapped in the SEC61 translocation machinery. Proteins that enter the ER, but fail to attain the native structure are rapidly degraded to prevent toxic accumulation of aberrant gene products. The ER does not contain degradative devices and the majority of misfolded proteins generated in this biosynthetic compartment are dislocated across the membrane for degradation by cytosolic 26S proteasomes by mechanisms and pathways collectively defined as ER-associated degradation (ERAD). Proteins that do not engage ERAD factors, that enter aggregates or polymers, are too large, display chimico/physical features that prevent dislocation across the ER membrane (ERAD-resistant misfolded proteins) are delivered to endolysosome for clearance, by mechanisms and pathways collectively defined as ER-to-lysosomes associated degradation (ERLAD). Emerging evidences lead us to propose ERLAD as an umbrella term that includes the autophagic and non-autophagic pathways activated and engaged by ERAD-resistant misfolded proteins generated in the ER for delivery to degradative endo-lysosomes.

Keywords: autophagy; endo-lysosomes; endoplasmic reticulum (ER); ER-associated degradation (ERAD); ER-phagy and ER-phagy receptors; ER-to-lysosome-associated degradation (ERLAD); protein folding and quality control, recovER-phagy.

1. Clearance of mis-localized secretory proteins

Secretory proteins and proteins destined to the plasma membrane or to organelles of the secretory and endocytic compartments must be inserted co- or post-translationally in the ER lumen or membrane. This is ensured by hydrophobic signal sequences and transmembrane domains that direct nascent polypeptide chains to the SEC61 polypeptide-conducting channel (Rapoport, 2007; Casson, McKenna, and High, 2016). Targeting of nascent polypeptides to the ER may fail and the failure rate may depend on the efficiency of the hydrophobic signal sequence and on particular physiologic or pathologic conditions (Hessa et al., 2011; Kang et al., 2006; Oyadomari et al., 2006; Yamamoto et al., 2017; Levine et al., 2005). For example, mis-localization of polypeptides can be enhanced during ER stress, by mutations within the signal peptide sequence, by ablation of signal recognition particle subunits or by defective function of the translocation machinery (Kang et al., 2006; Orsi et al., 2006; Arnold et al., 1990; Hussain et al., 2013; Zimmermann, Muller, and Wullich, 2006; Suzuki and Kawahara, 2016). Cytosolic accumulation and aggregation of mis-localized proteins (MLPs) upon defective clearance is linked to disease conditions such as spongiform neurodegeneration and severe ataxia (Chakrabarti and Hegde, 2009; Ma, Wollmann, and Lindquist, 2002; Rane et al., 2008). To prevent perturbation of cytosolic homeostasis by MLPs, hydrophobic domains exposed by the latter engage the ribosomal-associated complex BAG6 (Hessa et al., 2011; Leznicki and High, 2012) and the BAG6-associated ubiquitin ligase RNF126 that ensure efficient degradation of MLPs by 26S proteasomes (**Figure 1A**) (Casson, McKenna, and High, 2016; Hessa et al., 2011; Rodrigo-Brenni, Gutierrez, and Hegde, 2014; Minami et al., 2010; Leznicki et al., 2013; Wunderley et al., 2014).

2. Clearance of proteins trapped in the translocon complex

Nascent polypeptides enter the ER through the SEC61 polypeptide-conducting channel (Aviram and Schuldiner, 2017; Lang et al., 2017). This process is somewhat inefficient and polypeptides may remain stuck in the translocation machinery (**Figure 1B**). This happens more frequently for post-translational protein translocation, where polypeptides are targeted to the ER through the signal recognition particle (SRP)-independent translocation pathway (Ast and Schuldiner, 2013). These proteins rapidly disengage from cytosolic chaperones and may prematurely start folding their cytosolic domain before completion of translocation across the ER membrane (**Figure 1B**) (Plath and Rapoport, 2000)). In these situations, translocation may stall. Translocons are unclogged on proteolytic intervention of zinc

metallopeptidase STE24 (ZMPSTE24, **Figure 1B**) (Ast, Michaelis, and Schuldiner, 2016), an ER membrane-bound zinc metalloprotease originally shown to process yeast mating factor and human lamin A (Bergo et al., 2002; Michaelis and Hrycyna, 2013; Pendas et al., 2002; Michaelis and Barrowman, 2012). ZMPSTE24 mutations affecting biogenesis of nuclear lamin A are linked to metabolic disorders and progeroid diseases (Barrowman et al., 2012). However, the same mutations are also preventing the de-clogging activity of the metalloprotease. At least in some cases, it is this second function of ZMPSTE24 that, when defective, is linked to disease. For example, in type-II diabetes patients, pancreatic β cells are dysfunctional upon accumulation of islet amyloid polypeptide (IAPP) aggregates. Genetic studies reveal a 2-fold enrichment of loss-of-function ZMPSTE24 variants in these patients. In a yeast model of the disease, IAPP oligomers induce ER stress and a concomitant translocation defect. The de-clogging activity of ZMPSTE24 has been correlated with strong suppression of IAPP cytotoxicity (Kayatekin et al., 2018).

3. Protein misfolding in the ER

Protein folding is error-prone. Inherited or spontaneous mutations in the protein coding sequence may dramatically reduce folding efficiency. Terminally misfolded polypeptides must be removed from the ER lumen in order to prevent secretion or toxic accumulation of faulty gene products. The ER welcomes about 40% of the gene products in Eukarya. Hence, ER folding and quality control machineries must face the uninterrupted arrival of a vast variety of polypeptide chains. These may be inserted in the membrane or be soluble in the ER lumen; they may display or lack oligosaccharides and other co- and post-translational modifications; they may contain or lack intra- or inter-molecular disulphide bonds and peptidyl-prolyl bonds in *cis*, whose formation needs enzymatic assistance; they may fold individually, or must wait for partners because they participate in oligomeric complexes. *General* chaperone systems assist a large variety of newly synthesized polypeptides (e.g., the glycoprotein-dedicated calnexin-calreticulin cycle). *Client*-specific chaperones such as the collagen-specific heat shock protein 47 (HSP47) (Ito and Nagata, 2019) or *tissue*-specific chaperones such as the testis-specific ER lectin calmegin (Watanabe et al., 1995) have a more restrict set of substrates (Ellgaard, Molinari, and Helenius, 1999). Likewise, cells deploy multiple solutions to sort-out and destroy polypeptides that have aborted the folding program. The ER does not contain a degradation machinery and its membrane must not represent an obstacle to deliver proteins to be degraded to the appropriate catabolic device, being it the

cytosolic proteasome (via ERAD) or the lysosome (via ERLAD). ERAD is an umbrella term coined by Jeff Brodsky that includes all the general and client-specific pathways ensuring delivery of misfolded proteins across the ER membrane for proteasomal degradation (McCracken and Brodsky, 1996) (**Figure 1C**). The molecular mechanisms and the client-specificity of ERAD pathways are still a matter of intense study, have thoroughly been reviewed elsewhere (e.g., (Brodsky and Wojcikiewicz, 2009; Ismail and Ng, 2006; Needham, Guerriero, and Brodsky, 2019; Pisoni and Molinari, 2016; Hebert and Molinari, 2007; Smith, Ploegh, and Weissman, 2011)) and will be briefly covered in section 3.1. ERLAD is an umbrella term defining autophagic and non-autophagic pathways that eventually deliver ERAD-resistant misfolded proteins to degradative endo-lysosomes for clearance (Fregno et al., 2018; Forrester et al., 2019) (**Figure 1D**). The characterization of ERLAD pathways is still in its infancy, is schematically summarized in **Figure 2A**, and is covered in section 3.2. The concerted action of ERAD and ERLAD maintains the function of the ER by ensuring rapid clearance of faulty gene products.

3.1 The cytosolic connection: ER-Associated Degradation (ERAD)

Nascent polypeptide chains and not-yet-native intermediates of folding programs must be preserved and allocated a sufficient time to complete maturation (Molinari, 2007). The slow and progressive dismantling of protein-bound oligosaccharides by ER-resident α1,2-mannosidases of the ER degradation-enhancing α-mannosidase-like (EDEM) protein family is the best characterized trick that allows the quality control machinery to distinguish freshly synthesized proteins to which more time has to be allocated to conclude folding (they display oligosaccharides with 9 mannose residues) from polypeptides that failed to achieve a native structure despite long retention in the biosynthetic compartment (they display 5 to 7 mannose residues) (Helenius, 1994; Olivari and Molinari, 2007; Lederkremer, 2009). De-mannosylated oligosaccharides displayed by terminally misfolded polypeptides engage the mannose-binding lectins Osteosarcoma Amplified 9 (OS-9) and XTP3-Transactivated Gene B (XTP3-B), which participate in multimeric complexes built around one of the 20, and counting, E3 ubiquitin ligases embedded in the ER membrane (**Figure 1C**) (Bernasconi et al., 2010; Ninagawa et al., 2011; Groisman et al., 2011; Christianson et al., 2008; Hosokawa et al., 2008; Neutzner et al., 2011; Bernasconi et al., 2008). These complexes are named "dislocons" or "retrotranslocons" because they are involved in ill-defined transport pathways that transfer misfolded polypeptides across the ER membrane into the cytosol for degradation

by 26S proteasomes (**Figure 1C**) (Mehrtash and Hochstrasser, 2018). The selection of one of the many ERAD pathways operating to remove misfolded polypeptides from the ER lumen depends on various characteristics of the protein-to-be-destroyed. These include: i) the position of the folding defect (Ismail and Ng, 2006; Denic, Quan, and Weissman, 2006; Carvalho, Goder, and Rapoport, 2006); ii) the topology of the misfolded protein (Bernasconi et al., 2010; Ninagawa et al., 2011; Guerra et al., 2018); iii) the presence of N-linked oligosaccharides (Christianson et al., 2008; Bernasconi et al., 2008; Liu et al., 1997; Oda et al., 2003; Molinari et al., 2003), disulfide bonds (Guerra et al., 2018; He et al., 2015; Molinari et al., 2002; Ushioda et al., 2008; Grubb et al., 2012; Timms et al., 2016; Gorasia et al., 2016) or peptidyl-prolyl bonds in the *cis* configuration (Bernasconi et al., 2010).

3.2 The lysosomal connection: ER-to-Lysosome-Associated Degradation (ERLAD)

Despite the large variety of options offered to misfolded proteins generated in the ER in their way to cytosolic 26S proteasomes (**Figure 1C**), some of them fail to enter ERAD pathways (**Figure 1D**). Reasons behind ERAD-resistance include i) the failure to alert the luminal quality control machinery and to engage ERAD factors such as BiP, α1,2-mannosidases and mannose-binding proteins, ii) a large size and/or iii) the tendency to form aggregates or polymers that cannot be dislocated across the ER membrane.

The list of folding-defective gene products that cannot, or can only very inefficiently be cleared from the ER via ERAD is slowly growing. It comprises the β subunits of thyrotrophic hormone (Noda and Farquhar, 1992), various mutant forms of serpins (Fregno et al., 2018; Teckman and Perlmutter, 2000; Kamimoto et al., 2006; Kroeger et al., 2009), gonadotropin-releasing hormone receptor (GnRHR) (Houck et al., 2014), collagen (Forrester et al., 2019; Ishida et al., 2009; Omari et al., 2018), dysferlin (Fujita et al., 2007), Niemann–Pick type C protein 1 (NPC1) (Schultz et al., 2018), thyroglobulin (Faustino et al., 2018), Bestrophin-1 (BEST1) (Milenkovic et al., 2018), prion (Marzo et al., 2013; Campana et al., 2006), pancreatic enzymes (Smith et al., 2018), progressive ankylosis protein (Kanaujiya et al., 2018) and many others (for yeast, please refer to (Sun and Brodsky, 2018)). All these proteasome-resistant, faulty gene products are delivered from the ER lumen or membrane to degradative endo-lysosomal compartments by autophagic and non-autophagic pathways that only very recently started to be understood at the molecular level and that we collectively define as ER-to-lysosome-associated degradation (ERLAD) pathways (Fregno et al., 2018; Forrester et al., 2019)) (**Figures 1D, 2A**).

3.2.1 The involvement of macro-autophagic and non- macro-autophagic pathways in clearance of ERAD-resistant misfolded proteins from the ER

*Macro*autophagy is regulated by more than 40 autophagy-related (ATG) gene products and ensures removal of proteasome-resistant misfolded proteins (or other aberrant material including fragments of organelles) from the cytosol (Dikic and Elazar, 2018; Bento et al., 2016; Hurley and Young, 2017; Mizushima, 2018). Shortly, cytosolic protein aggregates are decorated with bi-functional *autophagic receptors* that associate with the cargo-to-be-degraded on one side, and engage the ubiquitin-like protein microtubule associated protein 1 light chain 3 (LC3), which is covalently associated to a lipid of the phagophore membrane on the other side. The phagophore seals itself in a double-membrane autophagosome that eventually fuses with endo-lysosomes thereby releasing the cargo in the degradative organelles (**Figure 2A**, arrow 1).

Studies performed in the last two decades established the involvement of *autophagy-related gene products* such as ATG4B, ATG5 or ATG7 in disposal from the ER of various proteasome-resistant defective gene products (Fregno et al., 2018; Forrester et al., 2019; Teckman and Perlmutter, 2000; Kamimoto et al., 2006; Kroeger et al., 2009; Houck et al., 2014; Ishida et al., 2009; Fujita et al., 2007; Faustino et al., 2018). How an "*eat-me*" signal originating in the ER lumen is transduced across the ER membrane to alert the autophagy machinery (or individual components thereof) is unclear. Notably, select autophagy genes participate in non-autophagic pathways (Levine and Kroemer, 2019; Martinez et al., 2015; Gluschko et al., 2018). Moreover, pharmacologic activation of *macro*autophagy by the mammalian target of rapamycin (mTOR) inhibitor rapamycin enhances lysosomal clearance from the ER of mutant dysferlin (Fujita et al., 2007), proalpha1 (I) chains of type 1 collagen (Ishida et al., 2009) and NPC1 (Schultz et al., 2018), but has no effect and in some cases it interfere with disposal from the ER of other faulty gene products such as the Z variant of α1-antitrypsin (ATZ) (Hidvegi et al., 2010; Teckman et al., 2002), GnRHR (Houck et al., 2014) or prion protein (Marzo et al., 2013). As such, an involvement of autophagy gene products in clearance of proteasome-resistant misfolded proteins from the ER should not be considered sufficient to ascribe these catabolic events to *macro*autophagy (see 3.2.2). The current view is that both *macro*autophagic and non-*macro*autophagic pathways are engaged for delivery of ERAD-resistant misfolded polypeptides to endo-lysosomes for clearance. These pathways are collectively referred to as ERLAD, and their characterization awaits thorough genetic, pharmacologic and morphologic analyses.

3.2.2 Establishing ERLAD mechanisms by genetic and morphologic analyses

Our lab recently compared the mechanisms for clearance from the ER of a proteasome-resistant ERLAD-client whose degradation is enhanced by rapamycin (i.e., procollagen (Ishida et al., 2009)) and of a proteasome-resistant ERLAD-client whose degradation is not enhanced by rapamycin (i.e., ATZ (Hidvegi et al., 2010; Teckman et al., 2002)).

3.2.2.a The case of pro-collagen

Collagens are the most abundant proteins in Metazoan and are major components of bones and cartilages. They are synthesized in the ER, where they form triple-helixes before secretion in the extracellular space. Despite assistance by general and collagen-specific molecular chaperones and enzymes (Ito and Nagata, 2019), these large polypeptide chains are prone to misfolding (Ishida et al., 2009; Bienkowski, Curran, and Berg, 1986). Inherited or sporadic mutations in their sequence further reduce folding efficiency and are linked to rare diseases such as osteogenesis imperfecta, Ehlers-Danlos syndrome, Alport disease and many others (Myllyharju and Kivirikko, 2001; Mirigian et al., 2016). It has recently been established that collagen producing cells such as osteoblasts chondrosarcoma cells and fibroblasts maintain collagen production by ensuring the efficient removal from the ER of collagen molecules that fail to attain the native structure (Forrester et al., 2019). The study shows that endogenous procollagen that fails attainment of the native structure binds a functional complex composed of the lectin chaperone calnexin and the LC3-binding protein FAM134B to be segregated in ER subdomains. These are eventually captured by the autophagic machinery and are delivered to lysosomal compartments for clearance (**Figure 2A**, arrow 2). Detailed genetic and morphometric analyses identified the ER resident factors calreticulin, ERp57, UDP-glucose:glycoprotein glucosyltransferase and the autophagy regulators FIP200, ATG7 and ATG16 as additional components of the procollagen quality control machinery (Forrester et al., 2019). Other studies confirm that mutant procollagen engages the *macro*autophagic machinery and that *macro*autophagy induction rescues the disease phenotype (Ishida et al., 2009; Mirigian et al., 2016). A recent study reported on an alternative clearance pathway, where ER exit sites (ERES) containing procollagen are engulfed by endo-lysosomes in processes resembling *micro*autophagy that remain poorly understood mechanistically (**Figure 2A**, arrow 3) (Omari et al., 2018). The major difference between the studies is that the first one describes the fate of endogenous procollagen (Forrester et al., 2019), the second of ectopically expressed mutant procollagen (Omari et al.,

2018).

3.2.2.b The case of α1-antitrypsin Z (ATZ)

The case of the ERLAD substrate ATZ is emblematic. ATZ is a mutant member of the serine protease inhibitor (serpin) superfamily (Roussel et al., 2011) linked to the most common form of α1-antitrypsin (AT) deficiency (PiZZ genotype). ATZ is mainly degraded from the ER by ERAD and is poorly secreted, thus causing loss-of-function lung pathology (Kroeger et al., 2009; Qu et al., 1996). In addition, the glutamate to lysine substitution at position 342 of the polypeptide chain predisposes ATZ to form proteasome-resistant ordered polymers in the ER lumen. In about 10% of the PiZZ patients, polymers accumulate intracellularly resulting in clinically significant hepatotoxicity that determines the most prevalent inherited form of pediatric liver disease, which requires liver transplantation (Hidvegi et al., 2010; Roussel et al., 2011; Eriksson, Carlson, and Velez, 1986; Sharp et al., 1969; Marciniak et al., 2016; Perlmutter, 2011; Wu et al., 1994). It is possible that hepatotoxicity in these patients occurs on insufficient polymers clearance and this ill-defined, lysosome-regulated, catabolic pathway is a target for therapeutic intervention (Lomas, 2018). Molecular dissection of the lysosomal pathway that removes ERAD-resistant ATZ polymers from the ER lumen reveals intervention of the LC3 lipidation gene products ATG4B, ATG5 and ATG7 (Fregno et al., 2018; Kamimoto et al., 2006; Kroeger et al., 2009). A thorough genetic analysis surprisingly showed, however, the dispensability of the gene products required for biogenesis of double membrane autophagosomes (i.e., ULK1, ULK2, FIP200, ATG13, ATG9A). That ERLAD of polymeric ATZ does not rely on conventional *macro*autophagy was confirmed by morphometric analyses that failed to detect ATZ polymers within autophagosomes (Fregno et al., 2018). Rather, proteasome-resistant ATZ segregates, under the assistance of the ER chaperone calnexin, in ER subdomains that display the LC3-binding protein FAM134B at the limiting membrane and that eventually undergo scission to ER-derived vesicles. FAM134B engages membrane-bound LC3 to eventually dock to RAB7/LAMP1-positive degradative organelles. The luminal content of the ER-derived vesicles (i.e., polymeric ATZ and luminal ER marker proteins) is delivered within endo-lysosomes on a fusion event controlled by the ER-resident SNARE protein syntaxin-17 (STX17) and the lysosomal SNARE vesicle-associated membrane protein 8 (VAMP8) (**Figure 2A**, arrow 4) (Fregno et al., 2018).

Conclusive Remarks

ERAD and ERLAD maintain ER homeostasis-Proteins that fail translocation in the ER or that enter the compartment but do not complete folding in due time, are rapidly degraded. Failure to do so results in their intracellular accumulation, perturbation of ER homeostasis and impairment of cell viability. Cells deploy multiple catabolic routes to cope with the error proneness of the protein targeting and folding pathways and with the multitude of sequences, modifications of the polypeptide chains, conformations and sizes of the gene products entering the secretory pathway. For misfolded proteins generated in the ER, clearance relies on delivery to cytosolic proteasomes via ERAD (thoroughly covered elsewhere), or to endo-lysosomes via ERLAD.

Misfolded proteins-induced delivery of ER subdomains to degradative endo-lysosomes-ERLAD clients such as endogenous procollagen (Forrester et al., 2019) and aggregated pancreatic enzymes (Smith et al., 2018) activate membrane-embedded LC3-binding proteins (FAM134B for procollagen, CCPG1 for pancreatic enzymes) that alert the cytosolic *macro*autophagic machinery (**Figure 2A**, arrow 2). Consistently, at least for endogenous procollagen, clearance from the ER is enhanced on pharmacologic inhibition of mTOR (**Figure 2A**, arrow 2). The extent of similarity between this type of misfolded protein-induced ERLAD and ER turnover induced by nutrient deprivation as part of a general organello-phagy triggered by cells to rapidly gain nutrients (**Figure 2B**, arrow 5) awaits precise characterization of the two catabolic pathways by genetic and morphologic investigations.

Other ERLAD clients activate ER-to-endolysosome delivery pathways that rely on autophagy genes controlling LC3 lipidation, but do not require ER capture by double membrane autophagosomes. Rather, they occur via micro-autophagy-like events or via misfolded protein-induced vesicular transport (**Figure 2A**, arrows 3 and 4). Their disposal is not enhanced (and in some cases is inhibited) on pharmacologic inhibition of mTOR.

Membrane-embedded LC3-binding proteins as signal transducers triggering ER-to-lysosome delivery-To date, five ER-resident LC3-binding proteins have been identified that regulate delivery of ER sub-domains to endolysosomes for clearance: family with sequence similarity 134 member B (FAM134B) (Khaminets et al., 2015), SEC62 (Fumagalli et al., 2016), reticulon-3 (RTN3) (Grumati et al., 2017), cell cycle progression 1 (CCPG1) (Smith et al.,

2018) and atlastin-3 (ATL3) (Chen et al., 2019). Initially, these membrane-bound proteins have been involved in turnover of specific ER subdomains: ER sheet by FAM134B (Khaminets et al., 2015), ER tubuli by RTN3 and ATL3 (Grumati et al., 2017; Chen et al., 2019), ER subdomains containing molecular chaperones and folding enzymes, but excluding ERAD factors, by SEC62 (Fumagalli et al., 2016) (**Figure 2B**). FAM134B, RTN3 and ATL3 promote the turnover of ER subdomains on nutrient deprivation (Smith et al., 2018; Khaminets et al., 2015; Chen et al., 2019). CCPG1 is activated on nutrient deprivation and on chemical induction of ER stress (Smith et al., 2018). SEC62 intervenes in ER size collapse after resolution of an ER stress, when volume and content of the biosynthetic compartment must return to pre-stress, physiologic condition to re-establish proteostasis (Fumagalli et al., 2016). As summarized above, FAM134B has been involved in clearance from the ER of polymeric ATZ, misfolded procollagen and mutant NPC1 (Fregno et al., 2018; Forrester et al., 2019; Schultz et al., 2018) (**Figure 2A**, arrows 2 and 4) and CCPG1 ablation causes accumulation of insoluble protein aggregates in highly secretory acinar cells implying the engagement of this LC3-binding protein in removal of protein aggregates from pancreatic cells (**Figure 2A**, arrow 2) (Smith et al., 2018). Whether SEC62, RTN3, ATL3 are also engaged by specific classes of misfolded polypeptides or in organ-specific clearance of misfolded ERAD-resistant proteins is not yet known.

Emerging evidence reveals that ER-resident LC3-binding proteins are activated in response to *exogenous* (e.g., nutrient deprivation, stress induction or stress recovery) and *endogenous* stimuli (i.e., local accumulation of ERAD-resistant misfolded proteins). Future studies will establish the mechanisms regulating the segregation of ERLAD clients within ER subdomains decorated with select LC3-binding proteins, and how the “eat me” signal is transduced across the ER membrane to engage cytosolic factors including autophagy gene products and proteins regulating vesicular budding, transport and fusion with, or engulfment by, endo-lysosomes.

All in all, understanding the catabolic pathways described in this review is crucial for their pivotal role in maintenance of proteostasis (i.e., the capacity to produce the proteome in appropriate quantity and quality), for their link with several human disorders and because their regulators are promising targets for therapeutic interventions.

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Disclosure of interest

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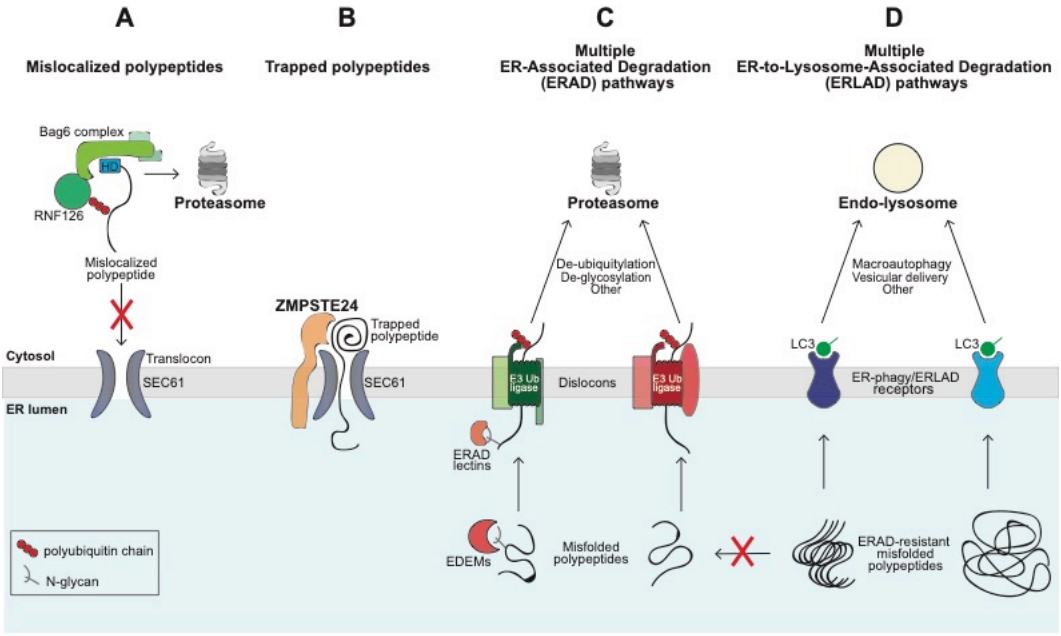

Figure 1. Degradation pathways for gene products destined to the ER.

The figure illustrates the pathways for proteasome- or lysosome-mediated disposal of mis-localized polypeptides (A), of polypeptides trapped in the translocation machinery (B), as well as the multiple pathways ensuring removal of ERAD clients from the ER lumen (C) or of proteins that cannot enter the ERAD pathways and are cleared from the ER by ERLAD (D). HD: Hydrophobic Domain. A color version of the figure is available online.

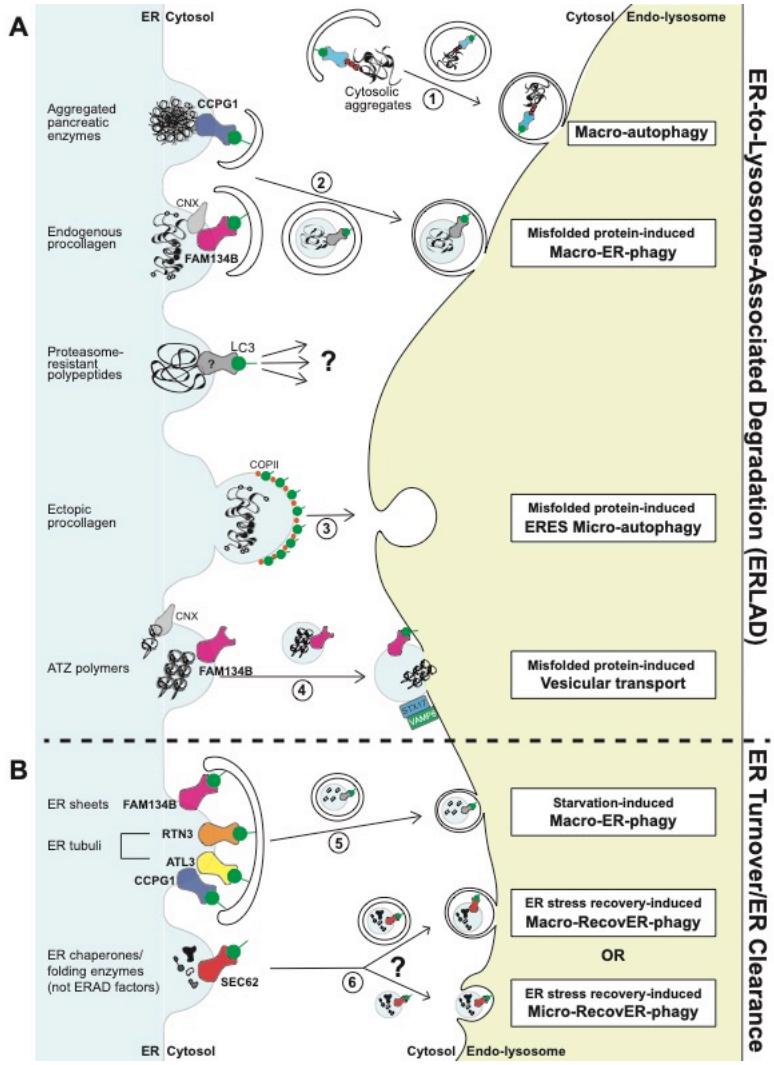

Figure 2. The galaxy of pathways for lysosomal delivery of proteasome-resistant misfolded proteins and for ER turnover.

(A) Arrow 1. The macro-autophagy pathway for degradation of cytosolic protein aggregates. Arrow 2. ER subdomains containing aggregated pancreatic enzymes and decorated with CCPG1 (Smith et al., 2018), or endogenous collagen that failed to attain the native structure and decorated with FAM134B (Forrester et al., 2019) are engulfed by double membrane autophagosomes that eventually deliver their content within endo-lysosomes. Arrow 3. It has been postulated (but not thoroughly characterized mechanistically) that ER exit sites (ERES) are directly engulfed by endo-lysosomes. Arrow 4. Thorough morphologic and genetic analysis revealed that the lectin chaperone calnexin segregates proteasome-resistant ATZ polymers in ER subdomains decorated with FAM134B. The SNARE proteins STX17 and VAMP8 regulate fusion of ATZ-containing ER-derived vesicles with endo-lysosomes.

(B) Arrow 5. FAM134B (Khaminets et al., 2015), RTN3 (Grumati et al., 2017), ATL3 (Chen et al., 2019) and CCPG1 (Smith et al., 2018) have been reported so far to act as ER-phagy receptors during ER turnover elicited on nutrient deprivation. Arrow 6. The translocon component SEC62 decorates excess ER subdomains that are cleared from cells on recovery from ER stress. The topology of ER subdomains accumulated within BafA1-inactivated endo-lysosomes is consistent with engulfment of ER-derived vesicles by double membrane autophagosomes (macro-RecovER-phagy) or by their direct engulfment by endo-lysosomes (micro-RecovER-phagy) (Fumagalli et al., 2016). A color version of the figure is available online.