Size Matters: ERphagy in control of ER size.

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Abstract

The endoplasmic reticulum (ER) performs a myriad of diverse functions in eukaryotic cells. The spatio-temporal demands of ER processes (protein and lipid synthesis, calcium storage and signaling), mean that the ER must be able to enlarge and shrink along with changing demands. Although ER expansion is well understood, much less is known about reduction and maintenance of size. Selective ER autophagy, also known as ERphagy, has recently been observed to recycle unneeded ER membrane. Receptor driven signaling results in packaging of surplus ER membrane into an autophagosome and subsequent recycling through lysosomal fusion. In addition to ER size regulation, ERphagy has been implicated as crucial for the unfolded protein response and even viral infection. In this review we will highlight the broad relevance ER autophagy could have in basic cellular biology. We will discuss the limited advances that have been made towards understanding the signaling networks that regulate ER autophagy, and what parts of this emerging field need reinforcement and further exploration.

Endoplasmic Reticulum, Selective Autophagy, ERphagy, Reticulophagy

Introduction

The endoplasmic reticulum performs many disparate roles in the eukaryotic cell. The ER surrounds the nucleus forming the nuclear envelope. The ER extends out from the nuclear envelope into the cytoplasm in a continuous, membrane bound network. This network consists of two distinct domains; smooth tubular ER which form a spider web like network in the cell periphery, and rough (ribosome studded) flat sheet ER which generally occupy the center of the cell. The distinct morphology of these domains are thought to represent a division of labor. The smooth tubular ER is responsible for Ca2+ signaling, lipid synthesis, and membrane contact site formation(Phillips and Voeltz, 2016). The relatively ribosome rich sheet ER is responsible for the majority of the membrane bound and secreted protein synthesis as well as their folding and insertion into membranes.

The ER membrane provides an ideal scaffold in which the proteins ultimately responsible for all of these essential functions can operate efficiently(Phillips and Voeltz, 2016; Voeltz et al., 2006). The demands the cell places on the ER are spatiotemporally dynamic requiring that the scaffold membrane be equally dynamic(Federovitch et al., 2005). In times of increased demand or stress the ER membrane is able to expand, increasing its available surface area to provide more scaffolding space(Yorimitsu et al., 2006; Cebollero et al., 2012). For example, if a cell is stimulated to begin secreting a protein in large quantities the sheet ER would expand to accommodate more ribosomes and increase the production of the secreted protein(Cebollero et al., 2012). Equally important to these dynamics and ER function is the ER's ability to reduce membrane after the need for increased production has passed. While ER expansions is relatively well understood comparatively little work has been done to understand ER shrinkage.

Autophagy is a process by which the cell is able to recycle organelles and other cellular components by degradation in the vacuole/lysosome. This occurs either by internalizing cargos into a double membraned autophagosome which eventually fuse with the vacuole/lysosome or through direct fusion of cargo with the vacuole/lysosome(Glick et al., 2010). Selective autophagy is distinct from the nonspecific autophagy that occurs during starvation where the cell recycles cellular components indiscriminately. It is a process where specific cellular components are marked for recycling either because they are obsolete or nonfunctional(Zaffagnini and Martens, 2016). ER autophagy or ERphagy, has been proposed as the method through which the ER is able to rid itself of unneeded and dam-

aged membrane and protein aggregates. While selective autophagy of other organelles such as the mitochondrion are thoroughly studied ERphagy remains a poorly characterized process(Ding and Yin, 2012). In this review we will detail the limited advances that have been made in demonstrating the existence of ERphagy and understanding the signaling involved in its regulation. We will discuss the important implications of this work and some of the many questions that remain unanswered.

Autophagy: Intro to the concepts.

Autophagy is divided into three theoretical frame works, macroautophagy, microautophagy, and chaperone mediated autophagy. Autophagocytosis of the ER is thought to occur through all of these methods. Macroautophagy comes in two flavors, the first being a nonspecific autophagy that occurs in response to starvation where cytoplasm is indiscriminately internalized into autophagosomes so that it can be recycled to feed the cell. Selective macroautophagy involves autophagosomal internalization of organelles and cellular components that have been marked for destruction and occurs even during nutrient rich conditions. Unlike macroautophagy, microautophagy and chaperone mediated autophagy do not involve the autophagosome. Instead, both occur by direct engulfment of cellular material by the lysosome. Microautophagy is a random sampling of cellular cytoplasm by the lysosome, whereas chaperone mediated autophagocytosis involves a more complex signaling network centering around the hsc70 complex with binds targets to the lysosomal network(Agarraberes et al., 1997). This review will discuss selective macroautophagy in the greatest depth as this field has seen many interesting advances, with respect to the ER, in recent years. We will also discuss some micro and chaperone mediated autophagocytosis.

Selective autophagy is akin to cellular housekeeping where organelles and other cellular components that have become damaged, toxic, or are no longer required by the cell are recycled. A diverse set of organelles and cellular components have been shown to be recycled by selective autophagocytosis ranging from old mitochondria to toxic protein aggregates. For each recycled component there are autophagy receptors which bind to specific cargo linking it to the autophagosomal machinery(Zaffagnini and Martens, 2016). The autophagosomal machinery (proteins involved) in turn helps recruit the isolation membrane and form the autophagophore. This membrane fuses with itself, internalizing the marked cargo and forming the double membrane autophagosome. The autophagosome then fuses with the lysosome in the case of mammals or the vacuole in the case of yeast, delivering the cargo where upon it is broken down into its molecular constituents.

ERphagy In Yeast:

ERphagy and ERAD:

Selective ERphagy is tightly linked to ER stress and in particular the unfolded protein response (UPR)(Yorimitsu et al., 2006; Kruse et al., 2006; Kamimoto et al., 2006; Kouroku et al., 2007). ER stress causes a significant ER expansion, and is also associated with an increase in autophagosome numbers. While this connection was made in the early 1970's only recently has the mechanism behind these observations been studied. UPR is initiated by an overabundance of misfolded proteins in the ER lumen. This occurs when cellular demand for membrane/secreted proteins exceeds the ER's ability to facilitate protein folding. Bernales et al. asked what changes in ER morphology happened upon UPR induction in yeast by dithiothreitol(Bernales et al., 2007). Using thin section electron microcopy, they observed a large expansion of ER volume and a change in morphology biasing the ER towards large sheet domains. Upon further examination, Bernales et al. noted that in a subset of cells UPR resulted in a marked increase in double membrane autophagosome like structures packed with membrane cisternae. They hypothesized that these were autophagosomes which had specifically internalized ER membrane in response to UPR. Bernales et al. went on to show that these structures did indeed contain ER membrane and that the formation of these structures was dependent on autophagosomal machinery. When the autophagosomal machinery was knocked out the cells were no longer able to tolerate dithiothreitol induced UPR suggesting that ERphagy is an essential component of UPR. Interestingly, the degradation of the membrane contents seemed to be unnecessary for the survival of cells. As long as the ER membrane was packed in autophagosomes the cells were able to tolerate the dithiothreitol induced UPR.

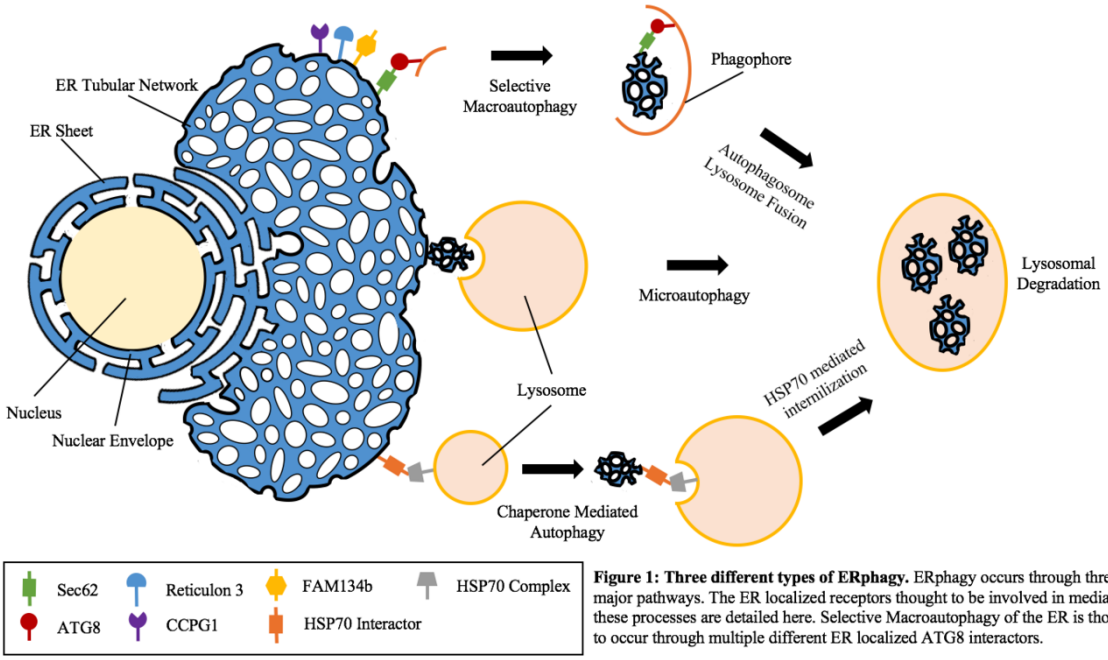

Figure 1: Three different types of ERphagy. ERphagy occurs through three major pathways. The ER localized receptors thought to be involved in mediating these processes are detailed here. Selective Macroautophagy of the ER is thought to occur through multiple different ER localized ATG8 interactors.

ERphagy and ER Stress:

ERphagy has also been connected with the clearance of large protein aggregates in the ER. Kruse et al. asked what cellular processes were implicated in a liver disease associated with a mutant aggregate prone (AP) fibrinogen(Kruse et al., 2006). They showed that upon high expression of AP fibrinogen proteasomal degradation was no longer sufficient to clear aggregates. In these high expression conditions ERphagy was required to clear the AP fibrinogen. This implicates ERphagy not only in the liver disease but also basic protein quality control and ER homeostasis.

Further investigation of the role of ERphagy in maintaining ER homeostasis by Schuck et al. highlighted a role for ERphagy in basic ER stress response(Schuck et al., 2014). They showed that, tunicamycin induced ER stress is associated with an increase in not only ER size but also abnormal ER structures they term ER whorls. They observed by electron microscopy that ER whorls were internalized directly by the vacuole. Through knockout they discovered that this form of microautophagy of the ER is not dependent on any of the canonical autophagy genes (atg1, atg6, atg7, atg8, atg14, atg16). Linking Pho8D60, a marker of vacuole internalization, to ER and cytoplasmic proteins Schuck et al. were able to demonstrate that ER stress results in the selective increase in ERphagy and not a general increase in autophagy. This indicates that ER stress was able to activate an alternative ER specific microautophagy and not other forms of generally autophagy. The signaling cascade involved in the recognition of ER whorls, their separation from the rest of the ER membrane, binding to the vacuole, and internalization has not yet been elucidated.

ERphagy in mammalian systems:

One of the major distinctions between selective and non-selective autophagy is the inclusion of receptor proteins in the signaling pathway. In contrast to non-selective autophagy which is driven primarily in response to serum starvation, functions such as the removal of damaged organelles, elimination of pathogens and the unfolded protein response (UPR), require a more tightly regulated and directed process. Autophagy receptors serve to direct different types of selective autophagy by bridging the cargo to core autophagy machinery. Specifically, these receptors characteristically have an LC3 interacting motif domain (LIR) domains that allows for ATG8 protein binding in addition to other motifs that drive localization of the receptor.(Ichimura et al., 2008) ATG8 proteins of the LC3 and GABARAP family proteins are autophagy ubiquitin-like modifiers that are cru-

cial for autophagosome biogenesis and directing selective autophagy.(Rozenknop et al., 2011) To this end, four primary autophagic receptors for ERphagy have be identified in mammalian cells: FAM134b, RTN3, SEC62 and CCDG.(Khaminets et al., 2015; Grumati et al., 2017; Fumagalli et al., 2016; Smith et al., 2018) While each of these receptors have a LIR domain for canonical ATG8 bridging, there are stark differences between the function and structure of these proteins.

FAM134b and RTN3 are the human homologues of atg39 and atg40 proteins that were first discovered in yeast.21 While both of these proteins contain four reticulon homology domains and at least one LIR motif, they perform non-redundant roles in the turnover of specific ER subdomains.(Mochida et al., 2015) FAM134b localizes to ER sheets, while RTN3 localizes to ER tubules.(Grumati et al., 2017) Each receptor will subsequently mediate an interaction between autophagosomes and allow for turnover of distinct portions of the ER. Further, RTN3 contains an additional layer of regulation in degradation of the ER, in that only the long isoform of RTN3 is able to mediate the ER-autophagosome interaction. Short isoforms of this protein lack the 6 LIR domains required for this function. Grumati et al. 2017 show that clustering of RTN3L protein can fulfill requirements for ERphagy, while hetero-oligomerization of the long and short isoforms actually cause stabilization of the tubules. This postulates a major question: Under which cellular stimulations/ conditions would you expect to a change in equilibrium between the seven RTN3 splice isoforms.

ERphagy and ERAD:

One of the primary functions of the ER is to maintain proteostasis within the cell. Upon insult to the native ER environment, the fidelity of protein folding and biogenesis can become compromised. To fulfill the quality control function of the ER, the conglomerate of signaling events that make up the UPR will be activated. Following UPR activation, upregulation of ER-associated protein degradation (ERAD) protein expression drives the degradation of terminally unfolded protein products. This removal of defective proteins is concomitant to the upregulation of chaperone proteins to correct protein folding where possible. Following resolution of this protein folding perturbation, the ER enters a recovery phase in which general protein expression returns to homeostatic conditions. During this time, increased autophagic flux removes excess UPR related machinery through a method of selective ER autophagy termed recovERphagy. RecovERphagy presents yet another challenge of unknown mechanisms and signaling proteins.

Transmembrane ER protein Sec62 has been identified as an ERphagy temporally specific to this recovery step. (Fumagalli et al., 2016) Similarly to other autophagic receptors, Sec62 mediates interactions between the ER and the autophagosome through a single LIR domain. However, this protein has other known roles as a member of the sec61 translocon complex that delivers nascent proteins across the ER membrane(Jung et al., 2014; Conti et al., 2015), and how Sec62's function is mediated between these roles is not yet known. Additionally, it is not well established how Sec62 selects specific protein cargo to deliver to the autolysosomal pathway. This suggests that there are yet to be identified regulators of protein recruitment during this process. Further, in models where Sec62 is dysregulated, what are the cellular and phenotypic outcomes of interrupted ER stress recovery?

A second ER resident autophagy receptor CCPG1 has been identified to mediate ER turnover following the UPR. CCPG1 is currently the only known ERphagy receptor to be transcriptionally regulated in response to ER stress. The UPR transcriptionally upregulates CCPG1 which then directly interacts with both ATG8 proteins through a LIR domain and FIP200 through a FIR domain. Both of these protein-protein interactions are required for recruitment to sites of autophagosome biogenesis to help maintain ER luminal proteostasis. However, the interaction between CCPG1 and FIP200 is not yet understood.

ERphagy receptors and Ubiquitin Signaling:

Common to other types of selective autophagy, k63-linked ubiquitin modifiers are used for recognition and targeting of protein turnover. Proteins destined for the autophagosome will be poly or mono ubiquitinated and recognized by an autophagic receptor.(Grumati and Dikic) Well established selective autophagy receptor p62 explifies a critical role in both recognizing these ubiquitin sites and binding directly to LC3 through a LIR domain.(Rozenknop et al., 2011) ER associated autophagy receptors have been implicated in LC3 binding, however it is yet to be determined if ubiquitin plays a role in this type of degradation selectivity. Determining the role of ubiquitin in regulated

ER luminal homeostasis will be a key point of interest in future studies.

ERphagy driven phenotypes and implications on human health:

While the list of ERphagy signaling proteins is sure to grow, the few recently described players have already been implicated in human health and disease. Prior to uncovering this pathway, it was found that mutations in FAM134b causes severe sensory and autonomic neuropathy in humans.(Kurth et al., 2009) These FAM134b mutants cause cis-golgi alterations and induce an increased rate of apoptosis in primary dorsal root ganglion neurons. A loss of these specific neurons could explain the early loss of nociception seen in individuals with this genetic disorder (Levi-Montalcini, 1987), although this has not yet been explored. Further, in mice, FAM134b null strains show reduced number of sensory axon numbers in peripheral neurons, suggesting a conserved role for this protein across mammalian species. The cell type specific phenotypes seen in vivo suggest that FAM134b may be a cell type specific ER receptor.

Indeed, a second ERphagy receptor, CCPG1 has been shown to have cell type specific phenotypes as well. CCPG1 deficiency causes degeneration of pancreatic acinar cells in mice while all other tissues remain relatively unaffected. MIST1, a tissue-specific transcription factor expressed in professional secretory cells, binds to the promoter region of CCPG1 and may be responsible for this pancreatic secretory cell phenotype.(Tian et al., 2010) Moving forward, it will be critical to identify other tissue specific transcription factors that bind to promoters of ERphagy receptors. As ER homeostasis is crucial in the vast majority of cell types, it is likely that there are both ERphagy receptors and related transcriptional regulators yet to be identified in mammals.

On the pathogenic front, the ER is utilized by flaviviruses during host infection and replication. Accordingly, there are ER related proteins that serve as restriction factors to inhibit viral replication as first line of defense. FAM134b has been identified as a host cell restriction factor for both dengue virus and zika virus drastically deepening the implication on ERphagy regulation on human health(Lennemann and Coyne). Depletion of FAM134b enhances the replication of both dengue and zika virus and presumably, humans with genetic mutations in this gene would be more susceptible to this virus. Zika virus has seen major expansion in recent years to new and naive populations driving serious developmental defects including microcephaly.(Kraemer et al., 2015; Cofré, 2016) The saliency of this public health issue may serve to drive a more rapid investigation of the ERphagy pathway and could result in valuable therapeutics.

This connection between and ERphagy receptor and viral replication poses several questions: Do other ERphagy receptors have a similar effect on viral replication? Is ERphagy necessary in regulating viral infection? And if so, what other proteins yet to be identified to could be at play?

Future Directions and Concluding Remarks:

Mechanistically, ERphagy presents a unique challenge to the general understanding of selective autophagy due of the ER's large size and continuous nature. In order to be internalized into autophagophore the membrane must first separate from the rest of the ER. This raises several questions about the signals involved in ERphagy as well as the order of events. Does the ER membrane bud off before interacting with autophagy receptors? How do the receptors regulating this process distinguish ER that is to be recycled from the rest of the organelle? What machinery is involved in spatiotemporally regulating these budding events? All of these basic questions are not yet well understood but are certainly interesting and relevant to basic cellular biology.

The rapid influx of literature in this field over the past 4 years has only scraped the surface on the mechanisms and signaling involved in selective autophagy of the ER. While exciting and quickly progressing, more questions have been posed about ERphagy than have been answered. As the field matures, it will be crucial to define golden standards for quantifying ERphagy. This development of phenotypic assays will open up the world of functional screening and lend to more rapid uncovering of this signaling pathway. As genome-wide screening and unbiased omics scale approaches will surely provide new insight to ER autophagy me-

chanisms, the full scope of these biomolecular interactions remains untouched at current.

References

Phillips, M.J., and G.K. Voeltz. 2016. Structure and function of ER membrane contact sites with other organelles. *Nature Reviews Molecular Cell Biology*. 17:69–82. doi:10.1038/nrm.2015.8.

Voeltz, G.K., W.A. Prinz, Y. Shibata, J.M. Rist, and T.A. Rapoport. 2006. A Class of Membrane Proteins Shaping the Tubular Endoplasmic Reticulum. *Cell*. 124:573–586. doi:10.1016/j.cell.2005.11.047.

Federovitch, C.M., D. Ron, and R.Y. Hampton. 2005. The dynamic ER: experimental approaches and current questions.. *Current opinion in cell biology*. 17:409–14. doi:10.1016/j.ceb.2005.06.010.

Yorimitsu, T., U. Nair, Z. Yang, and D.J. Klionsky. 2006. Endoplasmic reticulum stress triggers autophagy.. *The Journal of biological chemistry*. 281:30299–304. doi:10.1074/jbc.M607007200.

Cebollero, E., F. Reggiori, and C. Kraft. 2012. Reticulophagy and Ribophagy: Regulated Degradation of Protein Production Factories. *International Journal of Cell Biology*. 2012:1–9. doi:10.1155/2012/182834.

Glick, D., S. Barth, and K.F. Macleod. 2010. Autophagy: cellular and molecular mechanisms. *The Journal of Pathology*. 221:3–12. doi:10.1002/path.2697.

Zaffagnini, G., and S. Martens. 2016. Mechanisms of Selective Autophagy.. *Journal of molecular biology*. 428:1714–24. doi:10.1016/j.jmb.2016.02.004.

Ding, W.X., and X.M. Yin. 2012. Mitophagy: mechanisms, pathophysiological roles, and analysis.. *Biol Chem*. 393:547–64.

Agarraberes, F.A., S.R. Terlecky, and J.F. Dice. 1997. An intralysosomal hsp70 is required for a selective pathway of lysosomal protein degradation.. *The Journal of cell biology*. 137:825–34.

Kruse, K.B., A. Dear, E.R. Kaltenbrun, B.E. Crum, P.M. George, S.O. Brennan, and A.A. McCracken. 2006. Mutant fibrinogen cleared from the endoplasmic reticulum via endoplasmic reticulum-associated protein degradation and autophagy: an explanation for liver disease.. *The American journal of pathology*. 168:1299–308; quiz 1404–5. doi:10.2353/ajpath.2006.051097.

Kamimoto, T., S. Shoji, T. Hidvegi, N. Mizushima, K. Umebayashi, D.H. Perlmutter, and T. Yoshimori. 2006. Intracellular inclusions containing mutant alpha1-antitrypsin Z are propagated in the absence of autophagic activity.. *The Journal of biological chemistry*. 281:4467–76. doi:10.1074/jbc.M509409200.

Kouroku, Y., E. Fujita, I. Tanida, T. Ueno, A. Isoai, H. Kumagai, S. Ogawa, R.J. Kaufman, E. Kominami, and T. Momoi. 2007. ER stress (PERK/eIF2α phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. *Cell Death and Differentiation*. 14:230–239. doi:10.1038/sj.cdd.4401984.

Bernales, S., S. Schuck, and P. Walter. 2007. ER-Phagy: Selective Autophagy of the Endoplasmic Reticulum. *Autophagy*. 3:285–287. doi:10.4161/auto.3930.

Schuck, S., C.M. Gallagher, and P. Walter. 2014. ER-phagy mediates selective degradation of endoplasmic reticulum independently of the core autophagy machinery. *Journal of Cell Science*. 127:4078–4088. doi:10.1242/jcs.154716.

Ichimura, Y., E. Kominami, K. Tanaka, and M. Komatsu. 2008. Selective turnover of p62/A170/SQSTM1 by autophagy.. *Autophagy*. 4:1063–6.

Rozenknop, A., V.V. Rogov, N.Y. Rogova, F. Löhr, P. Güntert, I. Dikic, and V. Dötsch. 2011. Characterization of the Interaction of GABARAPL-1 with the LIR Motif of NBR1. *Journal of Molecular Biology*. 410:477–487. doi:10.1016/J.JMB.2011.05.003.

Khaminets, A., T. Heinrich, M. Mari, P. Grumati, A.K. Huebner, M. Akutsu, L. Liebmann, A. Stolz, S. Nietzsche, N. Koch, M. Mauthe, I. Katona, B. Qualmann, J. Weis, F. Reggiori, I. Kurth, C.A. Hübner, and I. Dikic. 2015. Regulation of endoplasmic reticulum turnover by selective autophagy. *Nature*. 522:354–358. doi:10.1038/nature14498.

Grumati, P., G. Morozzi, S. Hölper, M. Mari, M.-L.I.E. Harwardt, R. Yan, S. Müller, F. Reggiori, M. Heilemann, and I. Dikic. 2017. Full length RTN3 regulates turnover of tubular endoplasmic reticulum via selective autophagy. *eLife*. 6. doi:10.7554/eLife.25555.

Fumagalli, F., J. Noack, T.J. Bergmann, E. Cebollero, G.B. Pisoni, E. Fasana, I. Fregno, C. Galli, M. Loi, T. Soldà, R. D'Antuono, A. Raimondi, M. Jung, A. Melnyk, S. Schorr, A. Schreiber, L. Simonelli, L. Varani, C. Wilson-Zbinden, O. Zerbe, K. Hofmann, M. Peter, M. Quadroni, R. Zimmermann, and M. Molinari. 2016. Translocon component Sec62 acts in endoplasmic reticulum turnover during stress recovery. *Nature Cell Biology*. 18:1173–1184. doi:10.1038/ncb3423.

Smith, M.D., M.E. Harley, A.J. Kemp, J. Wills, M. Lee,

M. Arends, A. von Kriegsheim, C. Behrends, and S. Wilkinson. 2018. CCPG1 Is a Non-canonical Autophagy Cargo Receptor Essential for ER-Phagy and Pancreatic ER Proteostasis.. *Developmental cell*. 44:217–232.e11. doi:10.1016/j.devcel.2017.11.024.

Mochida, K., Y. Oikawa, Y. Kimura, H. Kirisako, H. Hirano, Y. Ohsumi, and H. Nakatogawa. 2015. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature*. 522:359–362. doi:10.1038/nature14506.

Jung, S.J., J.E. Kim, J.H. Reithinger, and H. Kim. 2014. The Sec62-Sec63 translocon facilitates translocation of the C-terminus of membrane proteins.. *J Cell Sci*. 127:4270–8.

Conti, B.J., P.K. Devaraneni, Z. Yang, L.L. David, and W.R. Skach. 2015. Cotranslational stabilization of Sec62/63 within the ER Sec61 translocon is controlled by distinct substrate-driven translocation events.. *Mol Cell*. 58:269–83.

Grumati, P., and I. Dikic. Ubiquitin signaling and autophagy. doi:10.1074/jbc.TM117.000117.

Kurth, I., T. Pamminger, J.C. Hennings, D. Soehendra, A.K. Huebner, A. Rotthier, J. Baets, J. Senderek, H. Topaloglu, S.A. Farrell, G. Nürnberg, P. Nürnberg, P. De Jonghe, A. Gal, C. Kaether, V. Timmerman, and C.A. Hübner. 2009. Mutations in FAM134B, encoding a newly identified Golgi protein, cause severe sensory and autonomic neuropathy. *Nature Genetics*. 41:1179–1181. doi:10.1038/ng.464.

Levi-Montalcini, R. 1987. The nerve growth factor 35 years later.. *Science (New York, N.Y.)*. 237:1154–62.

Tian, X., R.U. Jin, A.J. Bredemeyer, E.J. Oates, K.M. Błazewska, C.E. McKenna, and J.C. Mills. 2010. RAB26 and RAB3D are direct transcriptional targets of MIST1 that regulate exocrine granule maturation.. *Molecular and cellular biology*. 30:1269–84. doi:10.1128/MCB.01328-09.

Lennemann, N.J., and C.B. Coyne. Dengue and Zika viruses subvert reticulophagy by NS2B3-mediated cleavage of FAM134B. doi:10.1080/15548627.2016.1265192.

Kraemer, M.U.G., M.E. Sinka, K.A. Duda, A.Q.N. Mylne, F.M. Shearer, C.M. Barker, C.G. Moore, R.G. Carvalho, G.E. Coelho, W. Van Bortel, G. Hendrickx, F. Schaffner, I.R.F. Elyazar, H.-J. Teng, O.J. Brady, J.P. Messina, D.M. Pigott, T.W. Scott, D.L. Smith, G.R.W. Wint, N. Golding, and S.I. Hay. 2015. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. *eLife*. 4:e08347. doi:10.7554/eLife.08347.

Cofré, F. 2016. [Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?].. *Rev Chilena Infectol*. 33:96.