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Review

Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium

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Eukaryotic cells respond to the accumulation of unfolded proteins in the endoplasmic reticulum (ER) either by unfolded protein response that leads to an increase in the capacity of the ER to fold its client proteins or by apoptosis when the function of ER cannot be restored. Emerging data now indicate that ER stress is also a potent inducer of macroautophagy, a process whereby eukaryotic cells recycle their macromolecules and organelles. Depending on the context, autophagy counterbalances ER stress-induced ER expansion, enhances cell survival or commits the cell to non-apoptotic death. Here, we discuss the signaling pathways linking ER stress to autophagy and possibilities for their clinical exploitation.
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Macroautophagy (hereafter referred to as autophagy) is a lysosomal pathway involved in the turnover of cellular macromolecules and organelles. The first step of autophagy is the envelopment of cytosol and/or organelles in the isolating membrane, which wraps around the cargo forming an autophagosome, a vesicle surrounded by a double-membrane[1–3] (Figure 1). Autophagosome then undergoes a progressive maturation by fusion with endolysosomal vesicles creating an autolysosome, where the cargo is degraded. Autophagy is controlled by a set of evolutionarily conserved autophagy-related proteins (Atg proteins). The initial nucleation and assembly of the primary autophagosomal membrane requires a kinase complex that consists of class III phosphatidylinositol 3-kinase (PI3K), p150 myristylated protein kinase and beclin 1 (also known as Atg6). The further elongation of the isolation membrane is mediated by two ubiquitin-like conjugation systems one of them resulting in the conversion of microtubule-associated protein 1 light chain 3 (LC3; also know as Atg8) from free form (LC3-I) to a lipid-conjugated membrane-bound form (LC3-II). The accumulation of LC3-II and its localization to vesicular structures are commonly used as markers of autophagy. The expression and/or activity of several Atg proteins have been suggested to be inhibited by the mammalian target of rapamycin complex 1 (mTORC1) protein complex that consists of a serine/threonine kinase called mammalian target of rapamycin (mTOR), regulatory associated partner of mTOR (raptor) and mLST8 (lethal with sec thirteen).[3–5] The inhibition of mTORC1 is sufficient for autophagy induction, indicating that it serves as a major gatekeeper of autophagy induction in unstressed conditions.[6,7] Cells lacking nutrients suppress mTORC1 activity via a pathway involving AMP-activated protein kinase (AMPK) and activate the autophagy machinery that provides the starved cells with an alternative source of intracellular building blocks and energy thereby enhancing the cell survival during the stress.[8–10] Autophagy is also involved in removing damaged or excess organelles. For example, mitochondria that have lost their membrane potential and peroxisomes in response to changing environmental cues can be selectively removed by autophagy.[11–14] Notably, autophagy does not always promote cell survival, but can also mediate non-apoptotic cell death for example in experimental conditions where apoptosis pathways are blocked, or in response to treatments that specifically trigger caspase-independent autophagic cell death.[2]

The endoplasmic reticulum (ER) serves two major functions in the cell. It facilitates the proper folding of newly synthesized proteins destined for secretion, cell surface or intracellular organelles, and it provides the cell with a Ca^{2+} reservoir.[15–17] ER stress occurs in various physiological and pathological conditions where the capacity of the ER to fold proteins becomes saturated, for example, in response to the expression of folding incompetent or aggregation prone proteins, Ca^{2+} flux across the ER membrane, glucose starvation

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Abbreviations: AMPK, AMP-activated protein kinase; ATF6, activating transcription factor 6; Atg, Autophagy-related; Bcl-2, B-cell lymphoma/leukemia 2; Bip, ER-specific member of heat shock protein 70 family; CaMKKβ, Ca^{2+}/calmodulin-dependent kinase kinase-β; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmatic reticulum; GCN2, general control non-derepressible-2; Hac1, homologous to ATF/CREB 1; HRI, heme-regulated inhibitor; IP_3, inositol-1,4,5-triphosphate; IP_3R, Inositol-1,4,5-triphosphate receptor; Ire1, inositol-requiring kinase 1; JNK, c-Jun N-terminal kinase; LC3, microtubule-associated protein 1 light chain 3; LKB1, a serine/threonine kinase gene defective in Peutz–Jeghers syndrome; MEFs, murine embryonic fibroblasts; mLST8, lethal with sec thirteen; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; PERK, RNA-dependent protein kinase-like ER kinase; PI3K, phosphatidylinositol 3-kinase; PKR, RNA-dependent protein kinase; PolyQ, polyglutamine repeat; raptor, regulatory associated partner of mTOR; TAK-1, transforming growth factor-β-activated kinase 1; TRAF-2, tumor necrosis factor receptor-associated factor 2
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(reduced protein glycosylation), hypoxia (reduced formation of disulfide bonds) or defective protein secretion or degradation (Figure 2). ER stress caused by the accumulation of unfolded proteins threatens cell survival and triggers the evolutionarily conserved ER to nucleus signaling pathway called the unfolded protein response (UPR), which reduces global protein synthesis and induces the synthesis of chaperones and other proteins that increase the capacity of the ER to fold its client proteins. When the ER stress is extensive or sustained, and the function of ER cannot be restored, it leads to the removal of the affected cells by apoptosis. Accumulating data now indicate that ER stress is also a potent trigger of autophagy.[18–26] Whereas the induction of autophagy by ER stress is conserved from yeast to

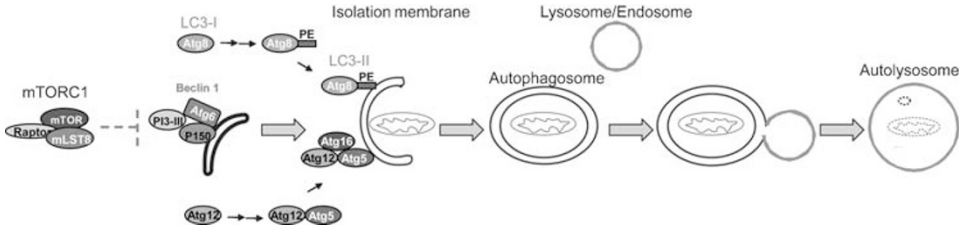

Figure 1 Schematic presentation of the autophagic process and its regulation. The formation of the initial membrane nucleation requires a kinase complex consisting of beclin 1 (Atg6), p150 myristylated kinase and class III PI3K. The isolation membrane chooses its cargo (in this figure a mitochondria) and elongates until the edges fuse forming a double-membrane structure called an autophagosome. Two ubiquitin-like conjugation systems forming Atg8-PE (LC3-II) and Atg5-Atg12 are necessary for the elongation of the isolation membrane. The autophagosome matures by fusing with endosomes and lysosomes, finally forming the autolysosome where the degradation of the cargo occurs. The autophagic process is inhibited by mTORC1 complex that consists of mTOR, raptor and mLST8

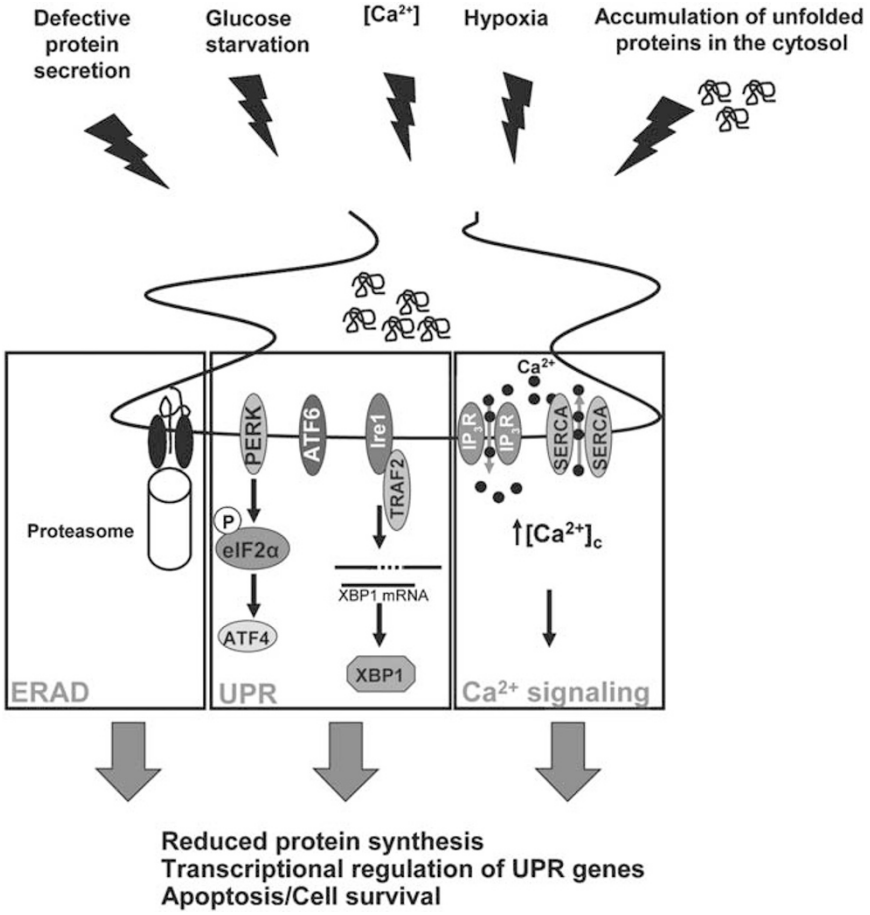

Figure 2 ER stress activates UPR, ERAD and calcium signaling. ER stress is triggered when unfolded proteins accumulate in the ER due to increased input of proteins (e.g. when protein synthesis is increased or the protein secretion from the ER is defective) or decreased capacity of the ER to fold proteins (e.g. upon changes in the Ca^{2+}, oxygen and glucose homeostasis). Notably, ER stress is also induced when unfolded proteins accumulate in the cytosol. ER stress leads to the induction of the UPR, ER-associated degradation (ERAD) and Ca^{2+} signaling to cope with the ER stress (each of these responses are surrounded by a box). These responses reduce the unfolded protein load in the ER by reducing the global protein synthesis, by increasing the folding capacity of the ER and by removing malfolded proteins from the ER by retrotranslocation of the proteins across the ER and degradation by the proteasome. If the stress is extreme or sustained, these signaling pathways can lead to cell death

mammals, the signaling pathways responsible for autophagy induction and its cellular consequences appear to vary according to the cell type and the stimulus. A better understanding of the signaling pathways controlling autophagy and the cellular fate in response to ER stress will hopefully open new possibilities for the treatment of the numerous diseases related to ER stress.

ER Stress Induces Cytoprotective Autophagy in Yeast

Already in the 1980's, ultrastructural studies revealed that cells with autophagic vacuoles often had dilated ER.[27] The first link between protein aggregation in the ER and autophagy derives from data in mammalian cells showing that accumulation of a mutant form of a cell surface protein, decay accelerating factor, was associated with autophagy.[28] Data suggesting that autophagy actually mediates removal of accumulated proteins in the ER come from studies in mammalian cells that express a mutant form of a secretory protein α1-antitrypsin.[29] Subsequently, the direct link between ER stress and autophagy was established in yeast *Saccharomyces cerevisiae* treated with distinct ER stressors, dithiothreitol that inhibits disulfide bond formation or tunicamycin that inhibits N-glycosylation.[19] The signaling pathway that mediates the UPR in yeast consists of a transmembrane signaling protein Ire1 (inositol-requiring kinase 1) that contains an ER luminal stress-sensing domain and a cytosolic endoribonuclease domain.[16] Ire1 is activated by the dissociation of Grp78/BiP (ER-specific member of heat shock protein 70 family) from its ER-sensing domain in response to accumulation of unfolded proteins.[30,31] This allows the cytosolic endonuclease component of Ire1 to splice an intron out of the messenger RNA coding for Hac1 (homologous to activating transcription factor (ATF)/CREB 1) (ortholog to XBP1 in mammalian cells). Translation of this mRNA produces Hac1, a transcription factor, that transmits the signal to the nucleus.[16,31] The transcriptional targets that strictly depend on Hac1 include genes encoding proteins involved in protein folding and modification in the ER, vesicular transport in the secretory pathway downstream of the ER, and proteasome-mediated ER-associated degradation of unfolded proteins that are returned to the cytosol.[32] Hac1 can also activate several genes encoding for Atg5, 7, 8 and 19.[20] Accordingly, dithiothreitol and tunicamycin at concentrations that trigger UPR, upregulate the expression of *ATG* genes as well as the activation of Atg1 kinase and *ATG*-dependent autophagy.[19,20] Even though the ectopic *HAC1* is capable of inducing the expression of several *ATG* genes, it is not sufficient to trigger autophagy in the absence of ER stress.[20] Moreover, *IRE1* and *HAC1* are dispensable for the induction of *ATG* genes in response to ER stress.[20,32] Thus, an Ire1-Hac1-independent signaling pathway appears to be required for the ER stress-induced autophagy in yeast. It remains to be determined whether ER stress affects the activity of Tor kinases, yeast homologues of mTOR or whether Atg1 is activated in a Tor-independent manner.

Autophagy is required for *S. cerevisiae* growth in the presence of high concentrations of tunicamycin, indicating that the autophagy response serves a cytoprotective function in yeast challenged with strong ER stress.[20] The data showing that ER itself is the major autophagosomal cargo during ER stress suggest that the pro-survival effect of autophagy in this model system could be due to augmented removal of unfolded proteins.[20] Additionally, the autophagy response may serve as a negative feedback mechanism removing parts of the expanded ER during the recovery period.

Coupling Unfolded Protein Response to Autophagy in Mammalian Cells

In mammalian cells, the UPR signaling is more complicated than in yeast.[16] At least three mechanistically different ER stress transducers—RNA-dependent protein kinase (PKR)-like ER kinase (PERK), ATF6 and IRE1—operate in parallel to mediate the UPR (Figure 2). They all sense the level of unfolded proteins in the lumen of ER and activate transcription of numerous target genes. In addition to the UPR, ER-stress leads to a release of Ca^{2+} from the ER and an increase in cytosolic free Ca^{2+} $[Ca^{2+}]_C$.[15] Depending on the state of the cell and the type of ER stress encountered, the outcome can be an increase in the capacity of the ER folding machinery, reduction of the amount of proteins entering the ER, enhanced clearance of the proteins from the ER, apoptosis or autophagy.[16,18,19,33]

PERK, IRE1 and increased $[Ca^{2+}]_C$ have been implicated as mediators of ER stress-induced autophagy in mammalian cells[18,24–26] (Figure 3). Polyglutamine repeats (PolyQ) and other misfolded proteins that aggregate in the cytoplasm have been suggested to induce ER stress via a global reduction in proteasome activity, which leads to the accumulation of misfolded and unfolded proteins in the ER.[17] Kouroku *et al*[25] has demonstrated that ER stress in response to ectopic expression of polyQ72 upregulates Atg12 expression and induces autophagy as analyzed by the conversion of LC3-I to LC3-II and an increase in LC3-postive vesicles in mouse embryonic carcinoma cells and murine embryonic fibroblasts (MEFs).[25] Data obtained using murine cells expressing dominant-negative PERK or its downstream target (eukaryotic initiation factor 2α; eIF2α) mutated in the PERK phosphorylation site (eIF2αA/A) demonstrate that the PERK-eIF2α signaling pathway is required for the polyQ72-induced Atg12 upregulation and LC3 conversion.[25] Autophagy induced by a mutant form of a type-II transmembrane protein dysferlin that aggregates and accumulates in the ER is also inhibited by the nonphosphorylatable mutant of eIF2α in eIF2αA/A knockin MEFs, suggesting that also in this model system PERK-eIF2α signaling pathway links ER stress to autophagy.[26] Interestingly, eIF2α mediates autophagy in model systems not associated with ER stress. In addition to PERK, mammalian cells have at least three other eIF2α kinases, that is PKR, general control nonderepressible-2 (GCN2) and heme-regulated inhibitor (HRI), which are activated by viral infection, amino acid starvation and heme depletion, respectively.[34] PKR and PKR-dependent phosphorylation of eIF2α is essential for autophagy in MEFs infected with herpes simplex virus, and eIF2α phosphorylation is required for starvation-induced autophagy in MEFs.[35] Thus, ER stress, starvation and viral infection appear signal autophagy by pathways that all depend on eIF2α. How eIF2α regulates autophagy is presently unknown, but the induction

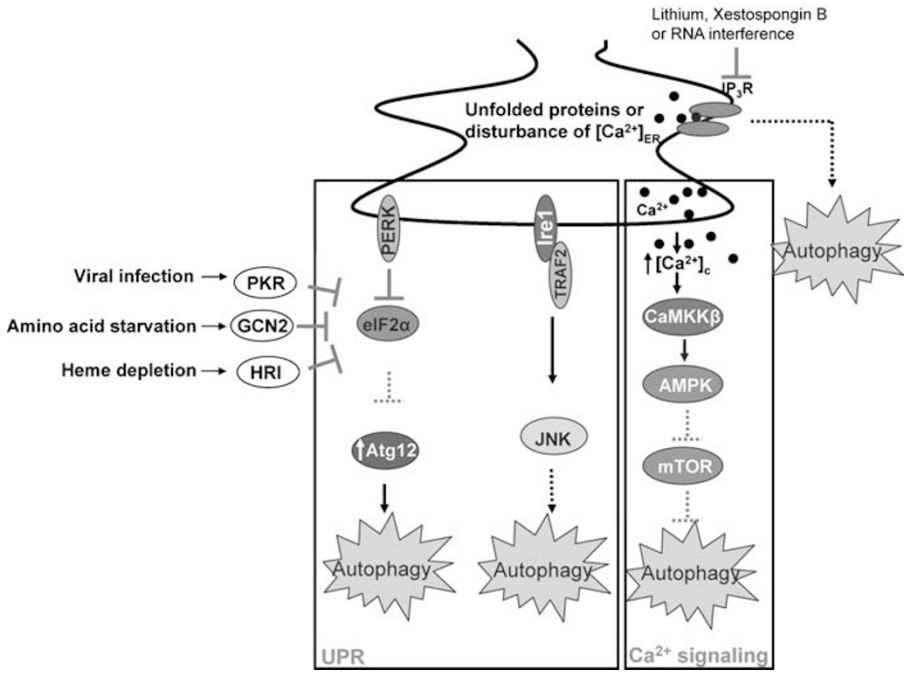

Figure 3 Schematic presentation of four signaling pathways implicated in ER stress-induced autophagy. Two of the branches of the mammalian UPR response, PERK/eIF2α and Ire1/TRAF2/JNK, have been implicated in signaling of ER stress-induced autophagy. Notably, eIF2α has also been suggested as a mediator of autophagy in response to other stresses that activate PERK-related kinases (PKR, GCN2 and HRI). Furthermore, the ER stress-associated increase in $[Ca^{2+}]_C$ can activate autophagy via a pathway involving CaMKK-β, AMPK and mTOR. Finally, blocking of the IP3R either by pharmacological approaches (lithium or xestospongin B) or by RNA interference triggers autophagy in a Ca^{2+}-independent manner. Dashed arrows indicate that the effect is not direct or the mechanism is unknown and boxes separate the UPR and calcium response

of Atg12 expression via ATF4 is likely to participate in this process.[25]

Contradictory to the above, Imaizumi and co-workers have suggested that IRE1 rather than PERK links UPR to autophagy.[18] Using MEFs deficient for IRE1 α or ATF6 and embryonic stem cells deficient for PERK, they have shown that accumulation of LC3-positive vesicles triggered by tunicamycin or thapsigargin (an inhibitor of ER Ca^{2+}-ATPase) fully depends on IRE1, but not PERK or ATF6. Thapsigargin-induced accumulation of LC3-positive vesicles is also completely inhibited in MEFs deficient for tumor necrosis factor receptor-associated factor 2 (TRAF-2), a cytosolic adaptor molecule that links active IRE1 to the activation of c-Jun N-terminal kinase (JNK). And finally, a pharmacological inhibitor of JNK effectively inhibits the LC3 translocation in this model system, suggesting that IRE1-TRAF2-JNK pathway is essential for the induction of autophagy in MEFs challenged with ER stressors. Interestingly, JNK has also been suggested as a mediator of autophagy induced by caspase inhibition and growth factor deprivation in fibrosarcoma cells and $CD4^+$ T cells, respectively [36,37]

It should be noted that the conclusions that either the PERK-eIF2α pathway or the IRE-TRAF2-JNK pathway is the crucial mediator of ER stress-induced autophagy mainly rely on data showing a reduction in the steady-state number of autophagic vesicles on disruption of the relevant pathway.[18,25] These data could reflect enhanced turnover of autophagosomes as well as a decrease in autophagosome formation as suggested by the authors. Additional experiments assessing functional autophagy, for example, by analysis of the degradation rate of long-lived proteins or turnover of autophagosomes, will be needed to establish the role of these pathways in ER stress-induced autophagy.

Coupling Ca^{2+} Signaling to Autophagy

In addition to activating the UPR by the pathways discussed above, ER-stress leads to a release of Ca^{2+} from the ER into the cytosol, which, in turn, can activate various kinases and proteases possibly involved in autophagy signaling.[24,38,39] Thapsigargin that induces 'classical' ER stress as well as other compounds that increase $[Ca^{2+}]_C$, that is, the active form of vitamin D and its pharmacological analog EB1089, ATP and ionomycin, induce Ca^{2+}-dependent autophagy as analyzed by LC3 translocation, electron microscopy and degradation rate of long-lived proteins.[24] Data obtained by employing RNA interference and pharmacological inhibitors demonstrate that Ca^{2+}-mediated autophagy depends on Ca^{2+}/calmodulin-dependent kinase kinase-β (CaMKKβ)-dependent activation of AMPK that ultimately leads to the inhibition of mTORC1 as judged by decreased phosphorylation of the mTORC1 substrate p70S6K1. As mentioned above, AMPK serves as a negative regulator of mTORC1 also in starved cells. This is accomplished by the LKB1-mediated activation of AMPK in response to an increase in cellular AMP/ATP ratio.[40] Furthermore, transforming growth factor-β-activated kinase 1 (TAK-1) has been recently added to the list of putative AMPK kinases.[41,42] TAK-1 is involved in signaling pathways initiated by transforming growth factor-β

and inflammatory cytokines.[43] Whether TAK-1 is required for cytokine-induced autophagy is still unknown, but it serves as a good candidate for yet another AMPK activating kinase that may mediate autophagy.[44] It is not clear whether activation of AMPK is enough to trigger autophagy or whether other independent signals are required in parallel. It should, however, be noted that other UPR-related signaling pathways appear dispensable for Ca^{2+}-induced autophagy, because vitamin D compounds induce CaMKKβ- and AMPK-dependent autophagy in the absence of detectable UPR as analyzed by C/EBP homologous protein (CHOP) and Bip expression levels (M Høyer-Hansen and M Jäättelä, unpublished data).

In addition to ER stress, hypoxia also activates the PERK-eIF2α-ATF4 and AMPK-mTORC1 pathways.[45,46] It is still unknown whether hypoxia also triggers autophagy, if so the above mentioned pathways might be involved.

Inositol-1,4,5-triphosphate (IP_3) is a second messenger that couples receptor activation (e.g. G-protein-coupled receptors and receptor tyrosine kinases) to Ca^{2+} signaling via its receptor (IP_3R) that functions as an ER Ca^{2+} release channel on ligand binding.[47] Inhibition of IP_3R by a pharmacological inhibitor (Xestospogin B), siRNA depletion or lithium-mediated inhibition of IP_3 synthesis triggers autophagosome formation, suggesting that IP_3R activity inhibits autophagy.[22,48] This appears contradictory to data showing that ATP that induces IP_3 synthesis also triggers autophagy.[24] Whereas ATP-induced autophagy has slow kinetics (maximal effects after 2 days) and depends on Ca^{2+} signaling leading to mTORC1 inhibition, autophagy induced by the inhibition of IP_3R is evident already after 2 h, and it occurs in the absence of detectable changes in the Ca^{2+} homeostasis and independently of mTORC1. Thus, IP_3R activation and inhibition are likely to activate autophagy via distinct signaling pathways. It remains to be studied whether inhibition of IP_3R induces ER stress and UPR, or whether it induces autophagy by a both Ca^{2+}- and UPR-independent mechanism.

An increase in the $[Ca^{2+}]_C$ can also activate death-associated protein kinases and calpain proteases, both Ca^{2+}-dependent enzymes that have recently been linked to autophagy regulation.[38,49] MEFs and human osteosarcoma cells deficient or depleted for calpain 4, the small regulatory subunit required for the function of the ubiquitously expressed μ- and m-calpains, fail to form autophagic vacuoles and increase the degradation of long-lived proteins in response to various autophagic stimuli. The failure of rapamycin to induce autophagy in calpain 4-deficient cells indicates that calpain 4 regulates autophagy downstream of mTORC1. Calpain 4-deficient cells have high levels of both LC3-I and LC3-II, and they accumulate LC3 in endosome-like vesicles, suggesting that these cells either fail to recruit LC3 into the initiating membrane or that the LC3 containing membranes fail to mature to autophagosomes. It remains to be studied whether the autophagy-promoting effect of calpain 4 is due to a defective activation of μ- and m-calpains or to a yet unidentified function of calpain 4. The latter possibility is supported by data showing that the inhibition of calpain-like protease activity by various pharmacological inhibitors promotes autophagy.[39,50]

Inhibition of Autophagy by ER Localized B-cell lymphoma/leukemia 2

B-cell lymphoma/leukemia 2 (Bcl-2) is an anti-apoptotic protein located to mitochondrial, ER and nuclear membranes and to a minor extent to the cytoplasm.[51] Interestingly, Bcl-2 expression is downregulated by CHOP, a transcriptional regulator that is induced by all three arms of the UPR.[52] Accumulating evidence suggests, however, that Bcl-2 can both inhibit and enhance autophagy depending on the model system used.[2] The opposite effects of Bcl-2 may be attributed to its post-translational modifications or different subcellular localizations. Supporting the latter hypothesis, the autophagy inhibition by Bcl-2 is evident only when Bcl-2 resides in the ER, where it blocks autophagosome accumulation induced by starvation, vitamin D analog EB1089, ATP and Xestospogin B.[22,24,53]

At least two mechanisms have been proposed for Bcl-2-mediated inhibition of autophagy, a direct interaction with beclin 1 and regulation of ER Ca^{2+} stores possibly via its binding to IP_3R[24,53] (Figure 4). Beclin 1 is a Bcl-2-interacting protein that promotes autophagosome formation when in complex with class III phosphatidylinositol-3-kinase and p150

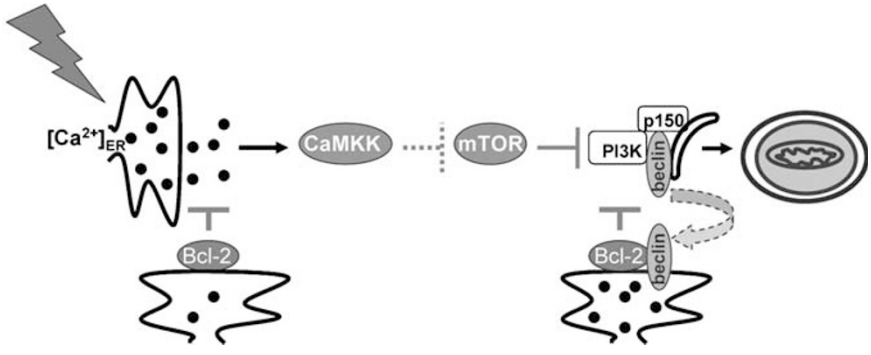

Figure 4 How does ER-localized Bcl-2 block autophagy? Two mechanisms by which ER-localized Bcl-2 inhibits autophagy have been proposed. Bcl-2 at the ER decreases the steady state level of the $[Ca^{2+}]_{ER}$ and thereby the amount of Ca^{2+} that is released after ER stress. This reduces autophagy by abrogating the activation of CaMKK-β, which mediates the Ca^{2+}-induced mTOR inhibition. Alternatively, Bcl-2 at the ER can prevent autophagy by binding beclin 1 and thereby removing it from the PI3K complex that is required for the initial membrane nucleation

myristylated kinase, and Bcl-2 has been suggested to function as an autophagy brake by inhibiting the formation of this autophagy-promoting protein complex.[53] To interact with Bcl-2, beclin 1 ought to colocalize with Bcl-2. The subcellular localization of beclin 1 is, however, controversial. Whereas Levine and coworkers have found ectopically expressed beclin 1 mainly in the ER and mitochondria in colon cancer cells,[53] analysis of endogenous beclin in other cell types suggests that it resides in the trans-golgi network or in as yet uncharacterized cytosolic structures.[24,54]

ER-localized Bcl-2 lowers the steady-state level of Ca^{2+} in the ER ($[Ca^{2+}]_{ER}$) and thereby reduces stimulus-induced Ca^{2+} fluxes from the ER.[24] Thus, it may inhibit Ca^{2+}-dependent autophagy by reducing the increase in $[Ca^{2+}]_C$. This hypothesis is supported by data showing that ER-localized Bcl-2 effectively inhibits autophagy induced by Ca^{2+} mobilizing agents that depend on ER Ca^{2+} stores (EB1089 and ATP), but not that induced by ionomycin, which induces Ca^{2+} entry also via the plasma membrane. Contradictory to this hypothesis, ER-localized Bcl-2 also inhibits Xestospogin B-induced autophagy that is presumably independent of ER Ca^{2+} release.[22] It remains to be studied whether starvation-induced autophagy that is sensitive to ER-targeted Bcl-2 also depends on the flux of Ca^{2+} from the ER.

The two models for the action of ER-targeted Bcl-2 are not necessarily exclusive. Bcl-2 at the ER could depend on beclin 1 binding to decrease the amount of Ca^{2+} released from the ER after agonist stimulation. The lack of detectable colocalization of ER-targeted Bcl-2 and beclin 1 talks, however, against this model.[24] Moreover, ectopic expression of beclin 1 has no effect on either $[Ca^{2+}]_{ER}$ or ATP-induced increase in $[Ca^{2+}]_C$ (G Szabadkai and M Høyer-Hansen, unpublished results). Alternatively, ER-targeted Bcl-2 may be able to inhibit autophagy by different means depending on the signaling pathway involved in autophagy induction. And finally, it should be noted that all three studies suggesting that Bcl-2 has to localize to the ER to block autophagy are based on a Bcl-2 construct, in which the transmembrane domain of Bcl-2 has been exchanged with that of an ER-specific protein cytochrome b5. Thus, the causative role of the transmembrane domain of cytochrome b5 cannot be excluded.

What is ER Stress-Induced Autophagy Good For?

The physiological and pathological relevance of ER stress-induced autophagy is largely obscure. One could speculate that when the amount of unfolded or misfolded proteins exceeds the capacity of the proteasome-mediated degradation system, autophagy would be triggered to remove these proteins. This fits with the observations in yeast showing that ER stress-induced autophagy counterbalances the ER expansion, removes aggregated proteins from the ER and in the case of an intense and persistent stress, serves a cytoprotective function.[20,55] Similarly, autophagy inhibits tunicamycin- or thapsigargin-induced cell death in mammalian cells.[18] In this model system, it has not been investigated whether autophagy assists the degradation of the unfolded proteins in the ER. However, data from other models suggest that autophagy can serve as an ER-associated degradation system also in mammalian cells, and it may play a fundamental role in preventing toxic accumulation of disease-associated mutant proteins in the ER. Ectopic expression of a mutant form of a type-II transmembrane protein dysferlin, which is causative of human muscle dystrophy, accumulates and forms aggregates in the ER and eventually leads to apoptotic cell death.[26] Mutant dysferlin colocalizes with LC3, and the inhibition of functional autophagy in Atg5-deficient MEFs enhances, whereas enhanced autophagy in rapamycin-treated cells reduces the accumulation of the mutant protein in the ER. Likewise, ER aggregates of mutant α1-antitrypsin Z, which is associated with the development of chronic liver injury and hepatocellular carcinoma, induce autophagy-mediated removal of the aggregated proteins.[21,29] These studies do not directly assess the effect of autophagy on cell survival, but as the protein aggregates in the ER are the likely cause of the cell death, autophagy capable of degrading them is envisaged to be cytoprotective. Similarly, experimental models for diseases caused by protein aggregates in the cytosol (e.g. Huntington's disease with polyQ aggregates and Parkinson's disease with α-synuclein aggregates) suggest that the ER stress-induced autophagy enhances the removal of aggregates (in this case cytosolic aggregates) and enhances cell survival.[25,56] Accordingly, pharmacological TOR inhibitors protect against aggregate formation and neurodegenration in fly and mice models of Huntington's disease.[57]

The data presented above encourage the development of autophagy promoting therapies for diseases associated with protein aggregates either in the ER or the cytosol. This note should, however, be taken with caution, because excessive autophagy may also promote cell death, and non-transformed cells may be especially sensitive to ER stress-induced autophagy.[23] This hypothesis is based on data showing that the inhibition of autophagy by Atg5 deficiency in MEFs or by 3-methyladenine in colon epithelial cells inhibits cell death induced by ER stressors (Ca^{2+} ionophore, thapsigargin and tunicamycin), whereas 3-methyladenine or depletion of beclin 1 in colon carcinoma cells sensitizes them to the same treatments. If this difference is really due to the transformation status of the cells remains to be studied. If this proves to be the case, combination therapies with ER stressors and autophagy inhibitors may prove useful in cancer therapy. In contrast, some ER disturbing treatments (e.g. photodynamic therapy and EB1089) have been reported to kill cancer cells by a mechanism that depends on autophagy, and such treatments are more likely to benefit from a combination with another autophagy promoting agent.[58,59] This is supported by the ability of ectopic beclin 1 to enhance autophagy and cell death in EB1089-treated breast cancer cells.[59]

Future Perspectives

The direct link between ER stress and autophagy was reported for less than one year ago. Thus, it is natural that many burning questions concerning the signaling pathways linking ER stress to autophagy, the mechanisms by which ER is selected as autophagic cargo, the crosstalk between ER stress-induced autophagy and cell death pathways, and the impact of autophagy in diseases associated with ER stress remain largely unanswered. The future research will hopefully

clarify these points and pave the way for pharmacological exploitation of the signaling pathways involved.

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