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Functional role of TRP channels in modulating ER stress and Autophagy

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Abstract

Intracellular calcium (Ca^{2+}) levels play a vital role in regulating cellular fate. The coordination and interrelation among the cellular organelles, mainly the intracellular Ca^{2+} stores in endoplasmic reticulum (ER), are crucial in maintaining cytosolic Ca^{2+} levels and in general cellular homeostasis. Moreover, maintaining Ca^{2+} homeostasis is essential for regulating diverse and sometimes opposing processes such as cell survival and cell death in disease conditions such as, neurodegeneration, cancer and aging. Ca^{2+} is able to regulate opposing functions by either regulating the cellular "self-eating" phenomenon of autophagy to promote cell survival or by regulating the programmed cell death process of apoptosis. Autophagy is also important for cell survival especially after induction of ER stress and association between ER stress and autophagy may have relevance to numerous diseases. Moreover, a multitude of evidence is emerging that the functional regulation of TRP channels, their unique localization, and their interaction with other Ca^{2+}-sensing elements define these diverse regulatory pathways. It is this unique function which allows individual TRP channels to contribute differently in the regulation of cell fate and, in turn, determines the precise effect of modulating Ca^{2+} signaling via the particular channel. Thus, in this review we have focused on the aspects of TRP channel localization and function (Ca^{2+} signaling) that affects the ER stress and autophagic process.

Keywords

Ca^{2+}; TRPC1 TRPML1; TRPML3; TRPV; autophagy; ER stress

Introduction

Disease conditions, such as cancer progression, are closely related to dysregulations of the cell cycle and are accompanied by enhanced cell proliferation and/or suppression of apoptosis leading to cell death [1-3]. In contrast, degenerative processes are initiated by either enhanced cell death and/or suppression of cell proliferation. The key factors between

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these opposing biological processes often lead to a perturbed balance between the processes of proliferation, autophagy, and apoptosis [1, 4]. Ca^{2+} is one of the most important regulators of cell survival/death processes. As a second messenger, Ca^{2+} is able to activate or inactivate various regulatory proteins such as enzymes, transcriptional factors, or molecular chaperones. Importantly, Ca^{2+} signaling has been shown to regulate cellular processes such as cell proliferation, survival, migration, invasion, motility, autophagy and apoptosis [5-7]. The adaptability of Ca^{2+} signaling in regulating these diverse functions derives from the multitude of components that can be divided into several distinct processes. First, the response to a stimulus creates Ca^{2+}-mobilizing signals that in turn releases Ca^{2+} from internal stores, such as the endoplasmic reticulum (ER). Second, ER Ca^{2+} release initiates Ca^{2+} entry, via numerous membrane channels like the transient receptor potential (TRP) channels [8, 9]. Third, Ca^{2+} subsequently functions as a second messenger to activate a cascade of Ca^{2+}-sensitive processes, before a series of mechanisms relying on Ca^{2+} pumps and ion exchangers that removes Ca^{2+} from the cytoplasm thereby restoring the resting state (Figure 1A). Each of the above described stages can be accurately controlled to create Ca^{2+} gradients which vary considerably in their spatial and temporal patterns.

Modulation of Ca^{2+} permeable channels expression/function also affects intracellular Ca^{2+} concentrations and consequently Ca^{2+} dependent processes, such as cell proliferation, ER stress, apoptosis, and autophagy [1, 10, 11]. Ca^{2+} permeable channels, including families of transient receptor potential (TRP) channels, Orai's, voltage-gated Ca^{2+} channels, two-pore Ca^{2+} channels, mitochondrial Ca^{2+} uniporter, IP_3 and ryanodine receptors have all been identified to contribute towards changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) [7, 8, 12, 13]. Among all the proteins that modulate Ca^{2+} signaling, the TRP family are the most diverse and have been shown to regulate various Ca^{2+}-dependent physiological processes in different cell types [8] (Figure 1B).

The ER is the main source of intracellular Ca^{2+} and is involved in the synthesis of many macromolecules such as proteins, unsaturated fatty acids, and sterols. The most important function for ER is to regulate protein synthesis, their translocation, proper folding and modulating post-translational modifications, which are all dependent on intracellular Ca^{2+} concentrations. The ER then transports newly synthesized proteins to the Golgi apparatus and ultimately to the vesicles for secretion or display on the plasma membrane surface, a process that is also dependent on intracellular Ca^{2+} concentrations. Stress to the ER triggered by the disruption of Ca^{2+} homeostasis, disturbs folding of proteins and causes a buildup of unfolded/misfolded protein in the ER lumen, thus activating the unfolded protein response (UPR) pathway [14]. The purpose of the UPR pathway is to facilitate protein folding and return the ER to homeostasis. However, if homeostasis is not achieved the UPR pathway may eliminate the cell through apoptosis [15], but recent research suggest that this is not the only known cell fate.

UPR is not the only triggered action by ER stress, induction of autophagy can work in conjunction or independently of UPR to help the cell cope with ER stress by removal of misfolded proteins; however if UPR persists it can also assist in cellular death [16]. Studies have also shown that autophagy is necessary for cell survival especially after ER stress [17]. Ca^{2+} plays a vital role in regulating ER stress and autophagy, and also within the cross-talk

between these two processes. Members of the Ca^{2+}-permeable non-selective cation TRP family have been shown to mediate cellular Ca^{2+} homeostasis and inhibit ER stress through mechanisms which may also lead to autophagy. Importantly, Ca^{2+} is likely to have different regulatory effects on autophagy, depending on the spatial and sequential parameters of Ca^{2+} signaling proteins, nutrient and growth factor availability, as well as in various pathological conditions like cancer, inflammation, neurodegenerative disorders etc. [18, 19]. The role of transient potential (TRP) channel for cell survival and apoptosis is widely recognized, whereas the information about the significance of these channels in regulating autophagy and ER stress is still limited. In the present review we will provide an overview of the literature on the role of the Ca^{2+} permeable TRP channels in the regulation of ER stress and autophagy.

1. Autophagy

Autophagy is a cellular process responsible for the delivery of proteins or organelles to lysosomes for its degradation. When cells encounter stressful situations, they can either try to survive under these conditions via a very beneficial process called autophagy, or by activating a programmed cell death program through the process of apoptosis [11, 18, 20]. The word *autophagy* is derived from the Greek roots “auto” (self) and “phagy” (eating) and broadly refers to the cellular catabolic processes in which target material is transported to lysosomes for degradation. Autophagy is also an important pathway for the clearance of pathogens thereby also indirectly regulating cell survival [21]. Till date, three types of autophagy exist; macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) (Figure 2) [18, 22]. Cellular stress conditions including nutrient starvation, hypoxia conditions, invading microbes, and tumor formation, have been shown to induce autophagy and allows cell survival in these stressful or pathological situations [23]. In addition, autophagy also recycles existing cytoplasmic components that are required to sustain vital cellular functions [24]. Although the precise mechanism as to how autophagy is initiated is not well understood, many of the genes first identified in yeast that are involved in autophagy have orthologues in other eukaryotes including human homologues [22, 25]. Presence of similar genes in all organisms suggests that autophagy might be a phenomenon that is evolutionally conserved and is essential for cell survival. Additionally, since autophagy delivers a fresh pool of amino acids and other essential molecules to the cell, initiation of autophagy is highly beneficial. In particular, autophagy is advantageous during nutritional stress situations or tissue remodeling during development and embryogenesis [22]. Although autophagy and apoptosis are mechanistically different cellular processes, there are some common regulatory proteins that intervene in both of them, such as the anti-apoptotic/anti-autophagy regulators Bcl-2 and Bcl-X_L and Ca^{2+} signaling.

1.1 Molecular types of autophagy

Macroautophagy is the best studied form of autophagy and is characterized by the formation of double membrane vesicles called autophagosomes. The process of autophagosomes formation consists of several stages, namely initiation, elongation and maturation, and fusion [22] (Figure 2). Microautophagy implies direct delivery through the invagination and fission of the lysosomal membrane while CMA delivers material to lysosomes with help of

chaperones. However, unlike macroautophagy, microautophagy does not appear to be related to the cellular adaptation to nutrient deprivation. [18, 24, 26, 27]. In chaperone-mediated autophagy the substrates have a pentapeptide lysosome-targeting motif (KFERQ) that is recognized by a complex of chaperone proteins and target the complex to lysosomal membrane. Given that autophagy has been linked to inhibit several diseases, like neurodegeneration, diabetes and infectious diseases as well as promotion of some cancer especially chemoresistance (as reviewed in [28], [29] and [30]); the linkages between ER stress and autophagy may also have relevance to several of these diseases.

1.2 Calcium and its role in autophagy

Intracellular Ca^{2+} plays an important role of both basal [31] and induced [32] autophagy. The first evidence on Ca^{2+} dependent regulation of autophagy was shown by Gordon et al., 1993 in which the authors suggest a complex role for Ca^{2+}, since chelation of either intra- and extracellular Ca^{2+} as well as elevating cytosolic Ca^{2+} suppressed autophagy [33]. Since then the mechanism by which Ca^{2+} controls the autophagy remains controversial [18]. Previous studies have shown an inhibitory action of Ca^{2+} on autophagy [11, 31] while many recent studies showed a positive role of Ca^{2+} that activate autophagy [18, 34-36]. The majority of studies showing Ca^{2+} permeable channels as autophagy regulators are focused on the inositol trisphosphate receptor (IP_3R) which is the main intracellular Ca^{2+} release channel [37].

1.3 IP3R as autophagic regulator

The stimulatory role of IP_3R on starvation-induced autophagy was initially studied by Decuypere et al., 2011 where they showed that the Ca^{2+} chelator BAPTA-AM as well as the IP_3R inhibitor xestospongin B abolished the starvation induced increase in the autophagy marker LC3 lipidation and GFP-LC3-puncta formation. Furthermore, starvation leads to IP_3R sensitization through increased Beclin1 binding to the IP_3R and a consequent decrease in Bcl2-Beclin1 interaction [18, 38, 39]. The autophagy protein Beclin1 promotes autophagosome formation by interacting with class III PI3-K, p150myristoylated kinase, when it is not bound with Bcl2 [40]. Moreover, autophagy might be inhibited by overexpression of Bcl2 and increased proliferation is observed upon Bcl2 overexpression. Consistent with these findings, Wang et al., 2008 reported that cadmium induces autophagy through elevation of cytosolic Ca^{2+} via the IP_3R and subsequent extracellular signal-regulated kinase (ERK) activation [41]. In contrast, IP_3R inhibitor xestospongin or IP_3R knockdown also induced autophagy in HeLa cells [42, 43]. Importantly, triple IP_3R-deficient DT40 cells demonstrated higher basal autophagy levels as compared to wild-type [44, 45]. Remarkably, the expression of functional IP_3R3, but not Ca^{2+} impermeable mutant IP_3R3^{D2550A}, was able to rescue elevated autophagy in these cells [44]. The authors proposed the mechanism in which constitutive IP_3R mediated Ca^{2+} release is taken up by mitochondria and this uptake is fundamentally required to maintain mitochondrial bioenergetics and ATP production in resting cells thereby suppressing autophagy [44]. Together, these findings indicate a bimodal role for Ca^{2+} release channels in the induction of autophagy, where basal autophagy is independent of IP_3R, whereas induced autophagy may require Ca^{2+} release from IP_3R.

To further complicate the role of IP_3R, studies by Sarkar et al., 2005 showed decreases in IP_3 levels by lithium induced mTOR independent autophagy [46]. Interestingly, mTOR is a regulator of autophagy, but also functions as ATP sensor. Further, TORC1 (protein involved in mTOR) has been shown to maintain macroautophagy at low basal levels [47], whereas, TORC1 inhibition by nutrient starvation or rapamycin (a macrolide that scavenges mTOR through FKBP12) unleashes massive macroautophagy [45] that might explain some of these discrepancies. In contrast, inhibition of inositol monophosphatase, which also decreases IP_3 levels, showed an increase in autophagy [46]. Collectively these reports suggest a complex role for IP_3R, since both stimulatory as well as inhibitory functions for IP_3R toward autophagy has been reported [11, 18, 19]. There could be several reasons for these different results; first, different cells and their growth conditions might regulate these processes differently. Second, phosphorylation of Beclin1 has also been shown to promote its dissociation from Bcl-xL that could explain the differential effects of IP_3R on cell survival after induction of autophagy. Third, Ca^{2+} has also been shown to regulate phosphorylation of many proteins including Bcl2 and Bcl-xL that might contribute to a different outcome. Fourth, evidence also suggests that Bcl2 inhibits autophagy by lowering ER Ca^{2+} levels that is independent of IP_3R. Fifth, multiplicity and cross reactivity among these pathways can also explains the lack of simple generalization of the role of individual components, such as Ca^{2+} signaling and finally, release of ER Ca^{2+} also activates various Ca^{2+} entry channels that could further stimulate or inhibit autophagy (Figure 3).

2. TRP channels and Ca^{2+} signaling

The transient receptor potential (TRP) channel superfamily is one of the largest families of cation channels [48]. The TRP family is divided into subfamilies which are TRPC (canonical), TRPM (melastatin), TRPP (polycystin), TRPV (vanilloid), TRPML (mucolipin), TRPA (ankyrin) and TRPN (NOMPC-like); the latter is found only in invertebrates and fish [49] (Figure 1B). Importantly, all members of TRP family are moderately conserved and share significant homology among them [8, 50, 51]. The phylogenetic tree of the mammalian (human) TRP channel superfamily and the channels shown to involve in autophagy and ER stress is shown in figure 1B. Currently there are more than 21 TRP gene identified in various animals. TRP channels are involved in regulating various cellular functions, ranging from pure sensory function to molecular regulation, hence they serve as gatekeepers for transcellular transport of sodium and calcium ions [48, 52, 53]. The TRP protein in general is a six putative transmembrane protein with a pore forming reentrant loop between S5 and S6 [55]. Most of the TRPs, especially TRPCs, function as homotetramers, though the formation of heteromultimeric channels has also been reported [48]. TRPC is a subfamily of transient receptor potential channels that have the highest degree of homology to the first discovered Drosophila photoreceptors' TRP channels [54].

2.1 TRP channels as regulators for autophagy

A relationship between IP_3R and autophagy has been discussed above; however there are other Ca^{2+} permeable channels that are shown to be involved in the regulation of autophagy. In addition, release of ER Ca^{2+} (via the IP_3R) activates plasma membrane Ca^{2+} channels

that could further enhance these processes. Among them are the transient receptor potential (TRP) channel mainly transient receptor potential mucolipin-1 (TRPML1), also known as mucolipin-1, TRPML3, transient receptor potential vanilloid channel 1 (TRPV1), transient receptor potential canonical 1 (TRPC1) and recently the transient receptor potential melastatin 7 (TRPM7) [55-60] (Figure 1B and 3).

2.2 TRPML channels and autophagy

Several studies have proposed TRPML1 as an autophagic regulator [20]. It has been shown to be accompanied by the impairment of lysosomal pH, accumulation of autophagosomes and abnormal mitochondria, accumulation and aggregation of p62, and ubiquitin proteins, all of which are proposed to be a part of defective autophagy [61-64]. Vergarajauregui et al., 2008 demonstrated that TRPML1 is necessary for efficient fusion of both autophagosomes and late endosomes with lysosomes. They also showed that accumulation of autophagosomes in TRPML1-deficient fibroblasts obtained from mucolipidosis type IV patients was due to increased Beclin-1 dependent autophagosome formation and delayed fusion of autophagosomes with late endosomes/lysosomes [63]. In another study they showed that CMA is attenuated in mucolipidosis type IV fibroblasts and TRPML1 directly interacts with Hsp70 and Hsp40, members of molecular chaperone complex required for CMA. They postulated that this interaction may be required for intra lysosomal Hsp70 which facilitates the translocation of CMA substrate proteins across the lysosomal membrane [65]. Later in 2010, the same group investigated macroautophagy in neurons isolated from cerebellum of TRPML1$^{-/-}$ mouse embryos [66]. These cells showed higher levels of basal autophagy markers compared to wild-type ones. In addition, the autophagy marker LC3 clearance was affected in these cells, suggesting weakening of lysosomal function. Recent studies also showed that lysosomal Ca^{2+} signaling regulates autophagy through calcineurin and its substrate TFEB [26]. Lysosomal Ca^{2+} release through mucolipin 1 stimulates calcineurin which binds and dephosphorylates its substrate TFEB and thereby promoting its nuclear translocation to initiate autophagy [26].

In addition to TRPML1, other members of mucolipin family, TRPML2 and TRPML3, are also involved in autophagy regulation [67, 68]. In contrast to TRPML1, TRPML3 exhibits more limited tissue distribution and is mostly localized to early as well as late endosomes/lysosomes and less to the PM [59, 69]. Recent studies by Kim et al., 2009 showed that overexpression of TRPML3 leads to increased autophagy in HeLa cells and that TRPML3 channels are engaged to autophagosomes upon induction of autophagy [70]. Furthermore, expression of dominant negative mutant TRPML3 (D458K) or knockdown of endogenous TRPML3 by siRNA reduces autophagy. Thus, they proposed that TRPML3 provides the Ca^{2+} that is required for fusion and fission events in autophagy [67]. Hetero-multimerization of TRPML channels also have been shown to have a role in autophagy [69]. A recent study showed that TRPML3 interacts with mammalian ATG8 homologue GATE16 to regulate autophagy, thereby suggesting a vital role of TRPML3 in autophagasome maturation through the interaction with GATE16 [71].

2.3 TRPV channels modulate autophagy

Other TRP channels, mainly the transient receptor potential vanilloid channel 1 (TRPV1), have been proposed to regulate autophagy in thymocytes through reactive oxygen species (ROS)-regulated AMPK and Atg4C pathways [57]. It has been shown that capsaicin, an activator of TRPV1, persuades Beclin-1 dependent accumulation of LC3-II protein. This LC3-II accumulation can be antagonized by capsazepine, a blocker of TRPV1, and compound C, an AMPK inhibitor, suggesting AMPK involvement. Later the same group showed that the loss of TRPV2 homeostatic controls the proliferation and tumor progression in glioblastomas [72]. The study also showed that prostate cancer cells had a Ca^{2+} dependent activation of TRPV2 which increased the invasiveness of tumor cells. Capsaicin induced autophagy is Ca^{2+} dependent, as co-treatment with EDTA markedly reduced LC3-II accumulation. Moreover, capsaicin induces accumulation of ATG4C and triggers its oxidation in a ROS-dependent manner, thus regulating LC3 lipidation levels [57]. However, capsaicin has also showed to have TRPV1-independent effects, such as inhibition of voltage-gated Ca^{2+} channels [73]. Additionally, upon prolonged exposure to capsaicin, TRPV1 desensitization occurs and its activity decreases [74]. Thus, additional experiments using more specific agonists and antagonists as well as siRNA knockdown are needed to confirm the role of TRPV1 in autophagy regulation. It would be interesting as well to compare the effect of capsaicin on autophagy in TRPV1-expressing and TRPV1-null cells. Recent studies showed that the degradation of TRPV1 in HeLa cells are mediated by autophagy and that this pathway can be amended by cortisol [58].TRPV6 channel translocate to the PM through Orai1 mediated mechanism and control cancer cell survival and thereby could also potentially modulate autophagy [75]. TRPML3 and TRPV5 associate to form a novel heteromeric ion channel in dermal melanocytes and possibly involved in TRPML3 mediated regulation of autophagy [76]

2.4 TRPC channels as autophagic regulators

Recent studies from our lab showed the transient potential canonical channel 1 (TRPC1) as a key regulator in hypoxia and nutrient depletion dependent autophagy [60]. We demonstrated that an increase in intracellular Ca^{2+} via TRPC1 regulates autophagy, thereby preventing cell death in two morphologically distinct cells lines. Silencing of TRPC1 or inhibition of autophagy by 3-Methyladenine, attenuated hypoxia-induced increase in intracellular Ca^{2+} influx, decreased autophagy, and increased cell death [60]. Kim et al also showed that TRPC3 depletion reduced SOC and the severity of acute pancreatitis in mice. The authors also demostrated that all stressors that increase SOC activity and induce pancreatitis activate the ER stress response and induced autophagy in pancreatic acini. Deletion of TRPC3 reduced the rate of PERK phosphorylation and also reduced the rate of activation of autophagy in response to supramaximal CCK8 and to bile acids. This reduced the Ca^{2+} influx in *Trpc3*$^{-/-}$ cells and protected them by reducing ER stress and autophagy [77]. However, knockdown of TRPC3 did not affect the hypoxia induce autophagy in our studies [60].

2.5 TRPM7 channels as a regulator of basal autophagy

Recent studies by Chung's lab showed the role of TRPM7 channel in regulation of basal autophagy. Knockdown of endogenous TRPM7 channel in SH-SY5Y neuronal cells resulted in decreased basal autophagy. Further, when TRPM7 channels were expressed in HEK293 cells in a nutrient rich condition, the LC3-II level expression increased indicating a significant role of TRPM7 channels in basal autophagy [56].

2.6 TRPM2 channels and autophagy

TRPM2 ion channel has been shown to involve in H_2O_2-induced autophagy [20, 78, 79]. TRPM2 works both as ion channel and an enzyme [80] and has been shown to be activated and regulated by a variety of stimuli like H_2O_2 and cytosolic Ca^{2+}. TRPM2-mediated Ca^{2+} regulates the interplay between ROS and autophagy [79]. ROS activates TRPM2 via another calcium mobilizing agent ADR-ribose (ADPR) inhibits early autophagy. TRPM2 also activates the calmodulin-dependent protein kinase II (CaMKII) to phosphorylate Ser295 on Beclin1 [78, 79].

2.7 Autophagy via voltage gated channels

Some ion channels, which do not belong to the family of TRP channels, are also proposed to regulate autophagy. Williams et al. found that L-type Ca^{2+} channels antagonists, namely verapamil, loperamide, nimodipine, nitrendipine and amiodarone, induce mTOR-independent autophagy [81]. The study also demonstrated that elevated cytosolic Ca^{2+}, presumably due to activity of L-type Ca^{2+} channels on the PM, can activate calpains, which cleave and activate the α-subunit of heterotrimeric G proteins $G_s\alpha$. $G_s\alpha$ activation, in turn, increases adenylyl cyclase activity leading to increase in cAMP levels which enhance the IP_3 production. Hence, elevated intracellular cAMP levels negatively regulate autophagy by promoting IP_3 production via cAMP-Epac-Rap2B-PLC-ε pathway. In addition, it has been well established that Ca^{2+} entry through these channels activate calmodulin, which in turn activates calmodulin-dependent serine/threonine kinases. These kinases play an important role in autophagy by facilitating the formation of autophagosomes and stimulating vesicular traffic [82]

3. Interplay between ER stress and autophagy

Autophagy is induced by protein aggregation and oxidative stress, which is dependent on the production of reactive oxygen species (ROS). Thus, ER stress and autophagy are often activated in parallel, share signaling pathways (particularly the Ca^{2+} signaling machinery), and team up to remove toxic byproducts of protein misfolding. Morphological changes of cells under ER stress were observed using electron microscopy and showed that autophagosome formation is accelerated when cells are under ER stress. Furthermore, the disturbance of autophagy rendered cells vulnerable to ER stress, suggesting that autophagy plays important roles in cell survival after ER stress [17]. The molecular mechanisms that link ER stress to autophagy may vary and various groups have proposed different hypotheses by which these two pathways cross-talk [30, 83] (Figure 3 and 4). For instance, Ca^{2+} mobilizing agents such as thapsigargin (an irreversible inhibitor of the ER Ca^{2+} ATPase), ionomycin, and ATP (via purinergic receptors) reportedly inhibit the activity of mTOR, a

negative regulator of autophagy, and induce colossal accumulation of autophagosomes in a Beclin1- and Atg7-dependent manner [84]. In this regard, it has been proposed that Ca^{2+} release from the ER stimulates a CaMKKβ/AMPK-dependent pathway leading to the phosphorylation of the tumor suppressor tuberous sclerosis proteins 1/2 (TSC1/TSC2) complex and the downstream repression of mammalian target of *rapamycin* (mTOR) with the subsequent induction of autophagy [32]. Furthermore, Ogata et al. demonstrated that ER stress-induced autophagy is regulated by IRE1α interaction with TRAF2 to regulate jun amino-terminal kinases (JNK) activation [17]. Recent studies showed that JNK-mediated phosphorylation of Bcl2 caused its release of Beclin1, thereby allowing autophagy to proceed [85]. In addition, ER stress also lead to an increase in the expression of transcription factor CHOP, which may also contribute towards autophagy as it is known to down regulate Bcl2 [86] and activate the transcription of ATG5 [87].

3.1 ER stress

ER stress is a condition that disrupts the redox balance and luminal Ca^{2+} homeostasis, thereby resulting in the accumulation of unfolded/misfolded proteins. Activation of ER stress is an evolutionary conserved adaptive response named unfolded protein response (UPR), which is initiated by three major signal transducers of the ER membrane: the protein kinase-like ER kinase (PERK), the inositol requiring enzyme (IRE1) and the activating transcription factor 6 (ATF-6) (Figure 4). ER stress leads to the activation of two protein degradation pathways. First, the ubiquitin-proteasome pathway that is via the endoplasmicreticulum-associated protein degradation (ERAD) protein. Second, UPR and lysosome-mediated protein degradation pathway that is via the autophagy pathway [83]. ERAD involves retro-translocation of unfolded ER proteins to the cytosol where they are ubiquitinated and degraded by the proteasome. When the buildup of misfolded or unfolded proteins exceeds the ER capacity, autophagy can be induced as a secondary response to UPR and degrade accumulated proteins and thus alleviate ER stress [30]. Although ER stress-induced cell death can proceed in both Ca^{2+}-independent and Ca^{2+}-dependent ways, here we have only focused on Ca^{2+}-dependent pathways.

3.2 Mechanism of ER stress induced autophagy

In autophagy, the formation of the autophagosome membrane requires the sequential action of numerous proteins involved in vesicle nucleation, fusion, elongation, with lysosomes, and final degradation of engulfed substrates. The first regulatory process involves attenuation of the mTOR Ser/Thr kinase, which blocks autophagy by phosphorylation of Atg13, and the dissociation of this protein forms a complex formed by Atg1 (a protein kinase) and Atg17. The primary step of vesicle nucleation is the activation of Vps34, a class III phosphatidylinositol 3-kinase, which associates with a complex formed by Beclin-1, UV irradiation resistance-associated tumor suppressor gene (UVRAG), and the kinase Vps15/p150. Beclin1 can also interrelate with the anti-apoptotic protein Bcl2 at the ER, with Bcl2 inhibiting starvation-induced autophagy [85]. The next phase of elongation involves two ubiquitin-like conjugation steps. First, the proteins Atg12 and Atg5 are covalently conjugated together with the cooperation of Atg7 (E1-like) and Atg10 (E2-like). Second, conjugation of Atg8 with phosphatidylethanolamine (PE) in the membranes of autophagic vesicles occurs following its proteolytic cleavage by Atg4, a cysteine protease [88]. The

subsequent recruitment of Atg8 and other autophagy-related proteins is believed to trigger vesicle expansion in a concerted manner, presumably by providing the driving force for membrane curvature [89]. The transient conjugation of Atg8 to the membrane lipid PE is essential for phagophore expansion as its mutation leads to defects in autophagosome formation [90]. It is distributed symmetrically on both sides of the autophagosome and it is assumed that there is a quantitative correlation between the amount of Atg8 and the vesicle size [91]. After finishing vesicle expansion, the autophagosome is ready for fusion with the lysosome and Atg8 can either be released from the membrane for recycling or be degraded in the autolysosome. Ogata et al in 2006 studied neuronal cell survival after ER stress and revealed autophagy was induced after ER stress in a manner similar to the wild-type cells when UPR protein ATF6 was knocked down. This suggests that autophagy plays an important role in cell survival after ER stress [17].

3.3 ROS mediates autophagy induction in response to ER stress

Pérez-Martín et al, 2014 showed that in Chlamydomonas the accumulation of misfolded proteins in the ER under tunicamycin or DTT treatment increased the expression of the ATG8 gene [92], which is important for the induction of autophagy. Pronounced accumulation of the ATG8 protein and its modified forms were also detected in ER stressed cells. They also showed that the ATG8 induction and ER stress caused by DTT is attenuated by exogenous glutathione (GSH). Glutathione plays a vital role in inhibiting reactive oxygen species (ROS) and in the formation of disulfide bonds in the ER associated with oxidative-induced protein folding that are linked to the generation of H_2O_2 by the activity of the ERO1 oxidoreductase [93, 94]. Hence it could be suggested that downregulation of autophagy might be due to the partial subdual of ER stress or to GSH-dependent ROS scavenging. Like tunicamycin treatment, SERCA inhibitor, thapsigargin also led to ER stress and autophagy activation in Chlamydomonas [92]. Suggesting a significant role of store operated Ca^{2+} entry in ROS and ER stress induced autophagy in Chlamydomonas. Moreover, recently it was shown that the proautophagic and antioxidant functions of the ER resident protein kinase, PERK that operate during normal mammary acinus development are challenged in breast tumor cells for them to survive oxidative stress and resist anoikis [95]. Importantly, PERK also regulated cell redox homeostasis via buffering ROS accumulation. Similarly, recent studies by Shen *et al* in human choriocarcinoma showed that the switch from ER-stress induced apoptosis to autophagy is via ROS and is mediated through the activation of JNK/p62 pathway [96]. Redox regulation of protein by moderate levels of ROS is also observed in autophagy. Accumulation of ROS induces autophagy, which in turn serves to attenuate the ROS levels have been also shown [97]. Many studies hypothesize that ROS are crucial for autophagy execution as treatment with antioxidants reverts the process [98]. Ca^{2+} play a vital role in ROS-mediated autophagy process and extensive literature in regulation of autophagy by ROS and role of calcium in ROS is illustrated in recent articles [19, 99]. Thus, it could be anticipated that TRP channels that are main regulators for calcium entry will contribute to these processes; however more research is needed to verify their role in various conditions.

4. ER stress and TRP channels

As we've discussed, disruption of Ca^{2+} homeostasis in the ER is considered an important trigger of ER stress. Members of the TRP family have been shown to mediate cellular Ca^{2+} homeostasis and initiate ER stress through different mechanisms. For example the channels TRPC1[100, 101], TRPV1 [102-109], and TRPC6[110] are expressed at both the ER membrane as well as in the plasma membrane and have been linked to ER Ca^{2+} homeostasis. Further, the channels TRPC3 and TRPC6 have each been identified as having a role in ER stress-induced apoptosis [111, 112]. Together it can be acknowledged that TRP channels have a role in maintaining ER Ca^{2+} homeostasis and disruption of channel function leads to ER stress.

4.1 TRPC channels as inhibitors of ER stress

Transient receptor potential channel 1 (TRPC1) plays a vital role in maintaining ER Ca^{2+} homeostasis and reduction in its function leads to prolonged activation of the UPR pathway and impairs AKT activation, which subsequently leads to neurodegeneration [113]. Our lab has uncovered a direct correlation between TRPC1 and ER stress in dopaminergic neurons of the substantia nigra, where endogenous store-operated Ca^{2+} entry (SOCE), which is critical for maintaining ER Ca^{2+} levels, is dependent on TRPC1 activity [101]. In this study, a neurotoxin-induced mouse model for Parkinson's disease showed decreased TRPC1 expression, TRPC1 interaction with the SOCE modulator stromal interaction molecule 1 (STIM1), and Ca^{2+} entry into the cells. However, the overexpression of functional TRPC1 protected against neurotoxin-induced ER stress and UPR by restoring AKT/mTOR signaling and increasing DA neuron survival. Although the role of autophagy has not been directly assessed in these instances, it is sensible to propose that activation of cell death pathways due to prolonged ER stress sets off a protective response mediated at least partly by autophagy as shown above.

Another TRP channel to play a role in ER stress is TRPC3. Within mouse epithelial cells from pancreatic and parotid acini, the loss of TRPC3 function ameliorates ER stressed induced UPR via PERK signaling [114]. ER stress can cause the activation of Ca^{2+} / calmodulin-dependent protein kinase II (CAMKII) and work done in HCAECs has shown Ca^{2+} influx by TRPC3 is required for the activation of CAMKII within UPR and the eventual ER-stress induced apoptosis [111, 115, 116]. It has also been shown that canonical transient receptor potential-6 (TRPC6) is expressed in the ER membrane blood platelets [110] and in podocytes and the channel can be activated by albumin [112]. Overloading the cell with albumin causes an excess of Ca^{2+} entry resulting in the expression ER stress protein GFP78 and eventual apoptosis. Knocking down TRPC6 abolishes the ER stress and subsequent apoptosis indicating a clear linkage between TRPC6 and ER stress induced apoptosis [112].

4.2 TRPV channels and ER stress

The channel Transient receptor potential vanilloid 1 (TRPV1) has a strong correlation to ER stress due to its expression in the ER membrane. It has been shown in human lung cells, that agonists for this subpopulation of TRPV1 in the ER disrupts ER Ca^{2+} homeostasis and

activates EIF2αK3-dependent ER stress responses [117]. In the same study, the use of a TRPV1 agonist caused a Ca^{2+} release from the ER along with subsequent increased expression of stress-response genes GADD153, GADD45α, ATF3, CCNG2, and BiP/GRP78 mRNA and a decrease in CCND indicating stress to the ER similar to that of ER stress inducing-agents thapsigargin and DTT [14]. Further work indicated inflammation produced endogenous TRPV1 agonists activating TRPV1 in lung cells, thus causing ER stress, GADD153 expression, and lung injury [118]. Within dorsal root ganglion neurons it was found that these ER bound TRPV1 channels have a low sensitivity for agonists such as capsaicin as compared to plasma membrane bound TRPV1 channels, possibly indicating a critical safety mechanism to protect the neurons from Ca^{2+} depletion of ER, leading to ER stress, unfolding protein response, and cell death [104].

5. Conclusion and future directions

The cellular “self-eating” phenomenon of autophagy and ER stress and their cross-talk mechanisms are involved in the maintenance of cellular energetics and cell survival. Over the last few years Ca^{2+}-permeable ion channels have emerged as an important regulator for these two vital processes. The effect of such regulation mostly depends on the Ca^{2+} signals in a spatially restricted subcellular domain that is achieved by many proteins and ion channels as discussed in this review; however more research is needed to fully dissect these intricate relationships. The interactions among the ER, mitochondria and lysosomes are crucial for cell survival, but a comprehensive signaling pathway for activation of the autophagy induced by ER stress awaits further analysis as the role of Ca^{2+} signaling is still controversial. Importantly, most of the studies that have shown this important relationship are performed in cell culture models and future studies using animal models will be necessary to resolve some of these issues. Nevertheless, both autophagy and ER stress have been associated in certain human diseases, such as Cancer progression (loss of autophagy), Parkinson's disease, Alzheimer, ALS, and Huntington's disease, and exploration of the novel signaling pathways relevant to ER stress and autophagy could lead to the development of new therapeutic strategies for these diseases. Deregulation of Ca^{2+} homeostasis, ER stress and autophagy also impairs mitochondrial function, leading to a decrease in ATP production that can make these cells vulnerable to insults. Thus, further studies are still needed to understand the variety of mechanisms, by which Ca^{2+} channels can influence these processes and could also have a broad impact on understanding and developing potential clinical drugs against these diseases.

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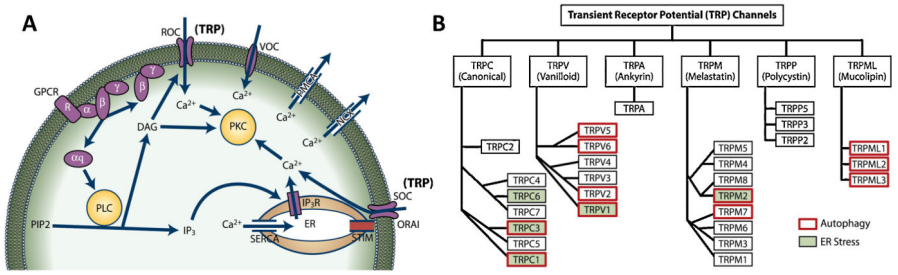

Figure 1. Ca^{2+} signaling and TRP channel classification

Schematic diagram for the common Ca^{2+} signaling pathways are shown in (**A**). Cells have several mechanisms for regulating cytosolic Ca^{2+} concentration, but not necessarily all of the mechanism shown here are present in one single cell type. The activation of G coupled protein receptor complex dissociates and activates the enzyme PLC which catalyzes the dissociation of PIP2 to form DAG and IP_3. DAG activates receptor-operated channels (ROC). IP_3 binds to its receptors in the ER (IP_3R) resulting in the release of the stored Ca^{2+} from the ER. Emptying of the ER Ca^{2+} activates the STIM protein to translocate to the PM and binds to Ca^{2+} release-activated Ca^{2+} channel protein (ORAI), thereby activating store-operated channels (SOC). TRP channels can act as a ROC and SOC. Increased Ca^{2+} in the cytosol is pumped out by PMCA and NCX. SERCA refills the stores. DAG and Ca^{2+} activate PKC which results in various downstream effects. Summary of the mammalian (human) TRP channel superfamily. TRPML (mucolipin), TRPP (polycystin), TRPM (melastatin), TRPA (ankyrin), TRPV (vanilloid) and TRPC (canonical) are shown in (**B**)

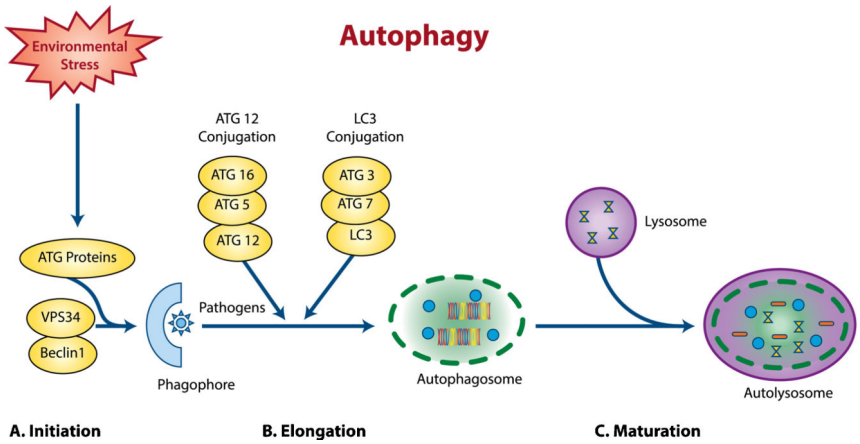

Figure 2. Basic macroautophagy process

Shows a schematic diagram of the basic autophagy process. This process is divided into three processes of a) Initiation b) Elongation and c) Maturation. On stimulation with external environmental stimulus like cells stress, nutrient depletion, hypoxia, etc. the initiation process of autophagy starts with the activation of autophagy-related proteins (ATG) and activation of a complex of class III phoshoinositide 3-kinase VPS34 and beclin 1. The elongation and shape of the autophagosome are controlled by two protein conjugation system, the ATG12 conjugation and LC3 conjugation. During maturation the lysosomes associated autophagosomes are degraded into autolysosomes.

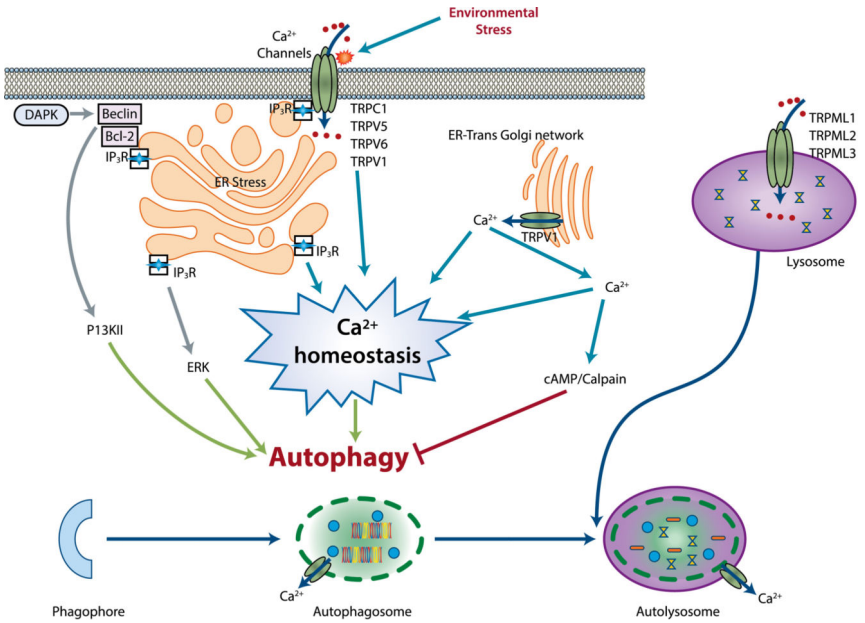

Figure 3. Role of intracellular Ca^{2+} and Ca^{2+} permeable channels in autophagy

Schematic diagram showing intracellular Ca^{2+} and Ca^{2+} permeable channels in the control of autophagy. Stimulation of Ca^{2+} permeable channels by various environmental stresses like nutrient depression and serum starvation activates both excited and non-excited Ca^{2+} channels in the neuronal and non-neuronal cells. Stimulation of the channels also results in release of the store Ca^{2+} from the ER and Golgi bodies. These results in ER stress and disturbed Ca^{2+} homeostasis in the cells; which via various Ca^{2+} regulated proteins like ERK, calpains, cAMP regulates the autophagy processes.

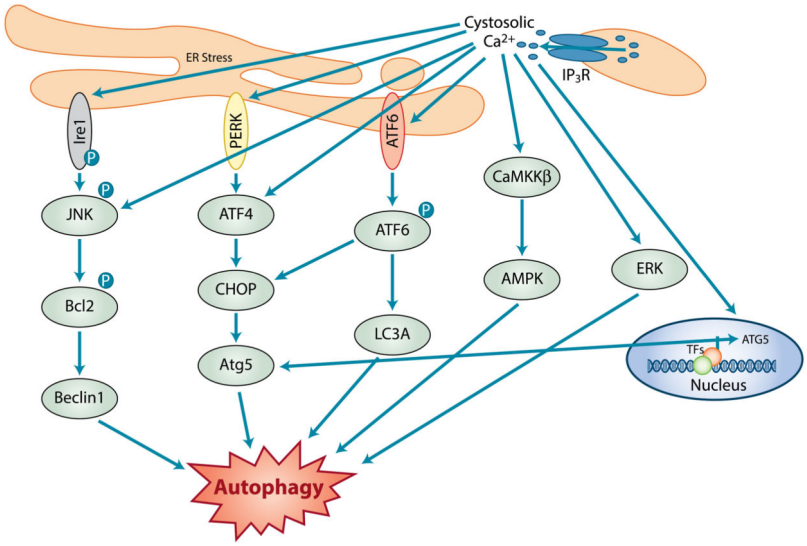

Figure 4. Ca^{2+}, ER stress and autophagy

Schematic diagram showing the three known signaling pathways which implicates ER stress- induced autophagy. Three of the UPR responses include the Ire1/JNK/Bcl2/Beclin1 PERK/ATF4/CHOP/Atg5 and ATF6/LC3A which have been implicated in signaling of ER stress-induced autophagy. The ER stress- associated increase intracellular Ca^{2+} is mainly through the activation of IP_3R which activates the CAMKK-β and ERK pathways which also leads to autophagy.