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Review

Ca^{2+}, autophagy and protein degradation: Thrown off balance in neurodegenerative disease

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ABSTRACT

Substantial progress has been made throughout the last decades in the elucidation of the key players and mechanisms responsible for Ca^{2+} signal generation in both excitable and non-excitable cells. Importantly, these studies led also to the recognition that a close correlation exists between the deregulation of cellular Ca^{2+} homeostasis and the development of several human pathologies, including neurodegenerative disease. Notwithstanding this advances, much less is certain about the targets and mechanisms by which compromised Ca^{2+} signaling exerts its effects on cell function and survival. Recently it has been proposed that deregulation of cellular energy metabolism and protein turnover (synthesis, folding and degradation) are also fundamental pathomechanisms of neurodegenerative disease, pointing to the pivotal role of autophagy, a major cellular pathway controlling metabolic homeostasis. Indeed, activation of autophagy has been shown to represent a highly successful strategy to restore normal neuronal function in a variety of models of neurodegenerative disease. Here we review recent advances in elucidating Ca^{2+} regulation of autophagy and will highlight its relationship to neurodegeneration.

1. Introduction

Proper cellular Ca^{2+} homeostasis plays a fundamental role in neurons, maintaining cell viability and contributing to the highly controlled spatiotemporal pattern of neuronal activity. Neuronal Ca^{2+} signals are generated either by influx through voltage dependent Ca^{2+} channels (VDCCs), or following direct activation of ligand activated influx channels such as the ionotropic glutamate receptors [1,2]. In addition, Ca^{2+} mobilization from endoplasmic reticulum (ER) Ca^{2+} stores through inositol 1,4,5-trisphosphate and ryanodine receptors (IP_3R and RyR) contributes to the generation of local Ca^{2+} signals, but usually represents a slower signaling modality and thus might also be involved in additional cellular homeostatic circuits, including the regulation of ER Ca^{2+} content and protein folding [3]. Removal of Ca^{2+} from the cytosol is ensured by the activity of plasmamembrane and endoplasmic reticulum Ca^{2+}-ATPases (PMCAs and SERCAs, respectively). These pumps extrude Ca^{2+} from the cytosol through active transport, in expense of a substantial fraction of the cellular ATP pool. Additionally, Ca^{2+} extrusion is mediated by the Na^+/Ca^{2+} exchanger (NCX) of the plasmamembrane, also coupled to the function of the Na^+/K^+-ATPase [4,5]. Mitochondrial Ca^{2+} uptake acts as an additional mechanism that buffers intracellular Ca^{2+}, but primarily represents a bimodal signal that on one hand adapts mitochondrial metabolism to the cellular demands; and on the other hand leads to cell death under different stress conditions [6,7]. The early recognition of the importance of Ca^{2+} handling in neuronal function gave the initial boost to the detailed characterization of the mechanisms involved in Ca^{2+} signal generation; but the broad interest in neuronal Ca^{2+} signaling was also due to the now generally accepted concept that if not properly balanced, intracellular Ca^{2+} signals represent a threat to neuronal function and survival. Indeed, glutamate-induced neurotoxicity – excitotoxicity in broader terms – has been attributed to abnormal cellular Na^+ and Ca^{2+} handling as early as in 1981 [8]. Along these lines, it has been well established that the sustained activation of the glutamate controlled Ca^{2+} influx pathway leads to the activation of a *vicious circle* resulting in the disintegration of global cellular Ca^{2+} homeostasis. Ultimately it manifests in an irreversible, delayed increase of cytosolic $[Ca^{2+}]$ (often termed cellular Ca^{2+} overload), and activation of both necrotic and apoptotic cell death pathways [9–11]. The main factors responsible for this

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derangement are (i) the activation of the Ca^{2+} activated family of cystein proteases called calpains [12] that degrade NCX and PMCA, the major transporters responsible for Ca^{2+} extrusion [13,14] and (ii) metabolic insufficiency due to the increased demand of ATP used for Ca^{2+} extrusion in face of a compromised mitochondrial function. The latter is also a Ca^{2+} dependent process, manifested by the loss of mitochondrial membrane potential and ATP production [11,15]. In addition, depletion of intracellular Ca^{2+} stores such as the ER has also been linked to Ca^{2+} dependent neuronal death through compromised protein folding in the ER, and concomitant chronic activation of the ER stress response, a process linked to activation of both Ca^{2+} dependent and independent cell death pathways [16–19]. Importantly, the concept of excitotoxicity has been recently applied to neurodegenerative processes as well, in order to provide the mechanism of cell death in these pathologies. Data from patients, animal and cellular models pointed to the pivotal role of impaired mitochondrial function, deregulation of Ca^{2+} homeostasis and subsequent activation of calpains not only during stroke, but also in motoneuron disease, as well as in Parkinson's, Alzheimer's and Huntington's diseases (for reviews see [20–23]).

While the mechanisms and molecules mediating the deregulation of Ca^{2+} homeostasis have been extensively studied, leading to the establishment of successful strategies in limiting the disease processes, much less is known about the downstream targets of cellular Ca^{2+} overload. Ca^{2+}-induced mitochondrial depolarization, depletion of the cellular ATP pool, deficiency of Ca^{2+} dependent protein folding in the ER and perturbation of cellular redox balance all point to a wide-ranging metabolic disorder underlying neurodegeneration. Importantly, in the recent years, autophagy has been (re-) discovered, and defined as a cluster of fundamental cellular processes aimed at maintaining cellular metabolic homeostasis, balancing the energy and substrate levels according to the actual cellular activity, growth, proliferation and environmental stress conditions. In addition, impaired protein turnover has been shown to underlie neurodegenerative disease, and there is compelling evidence that the process can be rescued by the concerted action of the autophagy-lysosome (ALS) and the ubiquitin-proteasome (UPS) systems (for recent reviews see [24–27]). Finally, intimate connections between cellular Ca^{2+} homeostasis, ALS and UPS have been found in several instances. Here we will briefly review the basic concepts and key players of cellular protein and energy homeostasis, and summarize recent data linking their deregulation to Ca^{2+} signaling and ultimately to neurodegenerative disease.

2. Autophagy and the proteasome—the basics

The term 'autophagy' was first introduced by the Belgian biochemist Christian de Duve, who received the Nobel Prize in Physiology or Medicine in 1974 for leading the discovery of the lysosome and his work on this organelle [28]. De Duve chose this term to distinguish the lysosomal degradation of the cell's own components from the lysosomal degradation of extracellular material, a process that he called heterophagy [29]. Etymologically, autophagy derives from the Greek words *auto* (self) and *phagy* (eating). This term reflected observations made by electron microscopy, which showed novel single- and double-membraned vesicles that contained organelles and parts of the cytoplasm at different stages of degradation [30]. During the following decades, a combination of morphological and biochemical studies identified the presence of hydrolases within the lumen of these vesicles, and provided insight into the early formation of a double-membrane sequestering organelle identified as the phagophore, which develops into a double-membrane vacuole (autophagosome) and delivers portions of the cytosol to lysosomal degradation [31–33]. At present, we are certain that autophagosomes do not contain hydrolytic enzymes, and that it is the fusion between the autophagosome and the lysosome that forms terminal single-membrane autophagic vacuoles (autolysosomes), in which hydrolytic degradation takes place and the resulting elements are returned into the cytosol for anabolic reactions [34]. This process is currently classified as *macroautophagy* (Fig. 1). Importantly, macroautophagy appears to operate in an organelle-selective manner, e.g. towards mitochondria (macromitophagy), endoplasmic reticulum (reticulophagy), portions of the nucleus (nucleophagy), peroxisomes (macropexophagy), microorganisms (xenophagy), ribosomes (ribophagy) or protein aggregates (aggrephagy) [35]. Recent studies revealed new possibilities regarding this selectivity, as poly-ubiquitinated structures (such as organelles, pathogens or aggregate-prone proteins) can be degraded by macroautophagy via p62/SQTSM- and NBR1-mediated recognition [36–38]. Thus, selective ubiquitination might provide a common trigger for the above variety of processes, representing a currently dynamically expanding area of research.

At least two further forms of autophagy have also been described, differing in the mechanism by which substrate material is taken up by the lysosome [35,39]. For instance, during *microautophagy*, the lysosomal membrane directly engulfs the substrate structures. This mechanism constitutes a degradation

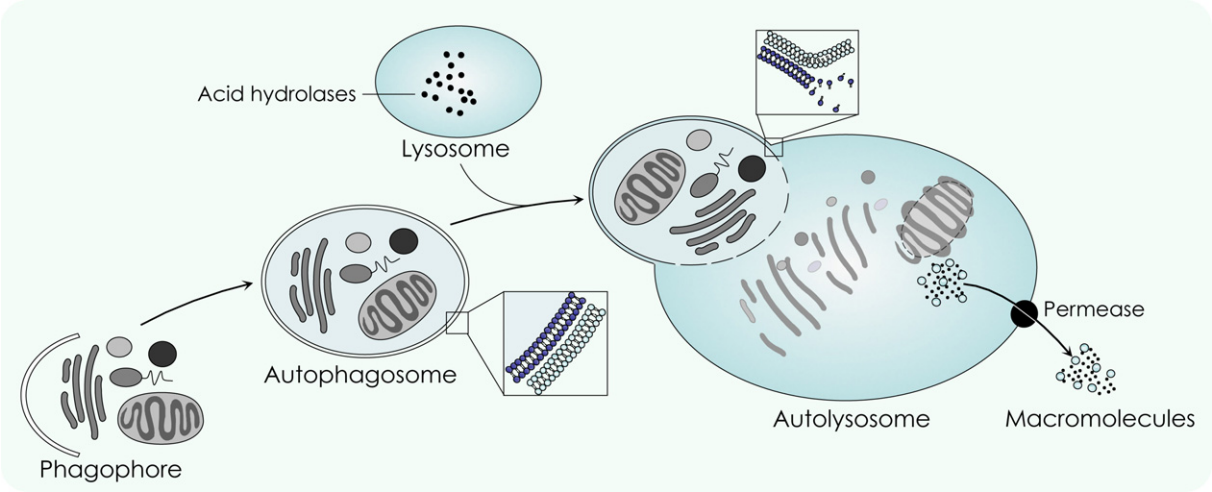

Fig. 1. Current view of macroautophagy.
During the process of macroautophagy, portions of the cytosol including complete organelles are surrounded by phagophores, which expand and develop into double-membrane autophagosomes. The sequestered cargo and inner membrane of the autophagosome are degraded by hydrolases present in the lysosomal lumen. Resulting macromolecules are recycled into the cytosol.

pathway both for organelles and long-lived proteins, but unlike macroautophagy, it does not appear to be related to the cellular adaptation to nutrient deprivation. One particular form of microautophagy is the highly selective degradation of peroxisomes (micropexophagy), mediating adaptation to oxidative stress in yeast [40]. Other subclasses of this pathway include the lysosomal capture of secretion vesicles (crinophagy), mitochondria (micromitophagy) and portions of the nucleus (piecemeal microautophagy of the nucleus) [35]. Finally, a third form of self-eating is named *chaperone-mediated autophagy* (CMA), which is responsive to nutrient deprivation. CMA also operates by selective recognition of substrates, but this pathway does not include bulk engulfment by membranes. Instead, cytosolic proteins that contain a specific pentapeptide lysosome-targeting motif (consensus sequence KFERQ) are recognized by a complex of chaperone proteins (including heat shock 73 kDa protein, hsc73) and targeted to the lysosomal membrane where they bind to the lysosome-associated membrane protein Lamp-2a that operates as a lysosomal receptor. The substrate protein is subsequently unfolded by the extra-lysosomal chaperone complex and transported into the lysosomal lumen for degradation [39]. A KFERQ-related motif is present in about 30% of cytosolic proteins, including α-synuclein [41] and the amyloid β precursor protein (APP) [42]. It is still not clear how the chaperone complex recognizes the KFERQ-related motif. Some post-transcriptional modifications (e.g. oxidation, cleavage or denaturation) of the substrates may render the motif more accessible to chaperones, increasing their lysosomal uptake by CMA. Interestingly, APP can be bound by hsc73 (and hence feed into CMA) when the default pathway for its degradation is inhibited, yet this interaction does not involve the APP KFFEQ sequence [43].

Meticulous research in the past 20 years led to the identification of a phylogenetically conserved family of autophagic (*Atg*) genes, heralding the molecular era in autophagic history. To date, more than 30 *Atg* genes have been described in yeast [44], many of them having orthologs in mammals [34]. A significant connection between the genetic basis of autophagy and its implications in medicine was established in 1999 by the group of Beth Levine, who demonstrated that human cells lacking one allele of *Becn1*/*Atg6* display increased tumor formation [45,46]. *Becn1* was subsequently identified as a haploinsufficient tumor suppressor gene and hence autophagy began to be viewed as a candidate for *in vivo* modulation of tumorigenicity [47]. Further evidence related autophagy to a wide range of human diseases including neurodegeneration, muscle disease, cardiac failure, infection, inflammation and even to the study of lifespan extension [48]. This highlights the crucial role of autophagy in the maintenance of metabolic homeostasis by removing aged or damaged intracellular structures and rendering them available when adaptation to dwindling nutrient resources is necessary [49,50]. Accordingly, current theories propose that neurodegeneration is a result of impaired processing and degradation of proteins, leading to impaired metabolic adaptation to cellular stress and thus compromised cellular survival [27,51,52], a concept strongly supported by *in vivo* genetic models of a series of fundamental genes involved in autophagy [53]. In order to understand the complex relationship between Ca^{2+} mediated cellular stress, autophagy and protein degradation in degenerating neurons in the following section we put across the basic mechanisms by which autophagy and autophagy-linked protein degradation systems are regulated.

2.1. Molecular regulation of autophagy

Macroautophagy (hereafter referred to as autophagy) is centrally regulated by the mammalian target of rapamycin (mTOR), a serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, ATP and stress. It is found in two structurally and functionally distinct multiprotein complexes, TORC1 and TORC2. TORC1 (formed by mTOR, raptor and mLST8) is rapamycin-sensitive and mediates the temporal control of cell growth by transcription, translation and very importantly, autophagy. TORC2 (formed by mTOR, rictor, mLST8 and protor) is rapamycin-insensitive and mediates the spatial control of cell growth by regulating the actin cytoskeleton [54]. High ATP and growth factor levels are stimulatory for TORC1 [55], which under these conditions maintains macroautophagy at low basal levels. On the contrary, TORC1 inhibition by nutrient starvation or rapamycin (a macrolide that scavenges mTOR through FKBP12) unleashes massive macroautophagy [56].

Upstream of TORC1, a regulated balance between protein phosphatases and kinases controls its activation. The insulin/IGF-1 (insulin-like growth factor 1) receptor pathway inhibits autophagy through activation of the class I phosphatidylinositol 3-kinase (PI3K). This class-I PI3K produces phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3], a molecular signal for the activation of protein kinase B (Akt/PKB) that indirectly activates mTOR, thereby allowing for the inhibition of autophagy [57]. Conversely, the tumor suppressor PTEN (phosphatase and tensin homologue) stimulates autophagy through its PtdIns(3,4,5)P_3-phosphatase activity, antagonizing the insulin/IGF-1 pathway [58]. In addition, the role of a nutrient/AMP sensitive protein kinase (AMPK) upstream of TORC1, results crucial for adapting mTOR activity to nutrient starvation conditions [59]. The implications of this kinase in a neurodegeneration-Ca^{2+} context will be discussed further below.

Better studied in yeast models, the pathway downstream of TOR relies on its kinase activity over Atg13. Active TOR maintains Atg13 at a hyperphosphorylated, inactive state. Upon mTOR inhibition, Atg13 becomes dephosphorylated and is able to bind Atg1, a serine/threonine kinase that triggers the cascade of Atg proteins, which operate as two parallel ubiquitin-like conjugation systems [60]. One involves the conjugation of Atg12 to Atg15 by the E1-like enzyme Atg7 and the E2-like enzyme Atg10. This cascade leads to the assembly of a heterotrimeric complex composed of Atg5-Atg12-Atg16, currently considered to promote the elongation of the phagophore [61]. The second ubiquitin-like system involves the conjugation of phosphatidylethanolamine (PE) to Atg8 (LC3 in mammals) by the sequential action of the protease Atg4, the E1-like enzyme Atg7 and the E2-like enzyme Atg3 [62]. Atg8 is thought to serve as a microtubule-anchoring point at the membrane of the autophagosome, allowing for its trafficking to lysosomes.

A second crucial step in phagophore elongation is the activation of a multiprotein complex with class-III PI3K activity. In contrast to the class-I, the class-III PI3K is autophagy-stimulatory through the production of phosphatidylinositol-3-phosphate [PtdIns(3)P], which is thought to serve as a signal for the recruitment of the Atg5-Atg12-Atg16 heterotrimeric complex to the phagophore membrane. The class-III PI3K complex is composed of the catalytic subunit Vps34 and the regulatory proteins Atg6/Vps30 and Vps15 [63]. When the class-III PI3K binds to Vps 14 (complex I), it regulates autophagy. In contrast, when it binds to Vps38 (complex II) it regulates vacuolar trafficking [64]. The mammalian ortholog of Atg6/Vps30 is the tumor suppressor protein Beclin 1, which was first identified as a Bcl-2 interacting protein, and is mono-allelically deleted in 40–75% of sporadic human breast and ovarian cancers [45,46]. Beclin itself does not have any enzymatic activity but acts as a platform, by recruiting several activators or repressors of the class-III PI3K complex I that leads to autophagy. Beclin 1 partners such as UVRAG (UV-resistance associated gene) [65], endophilin B/Bif-1 [66] and Ambra (activating molecule in Beclin-regulated autophagy) [67], can promote the activation of the class-III PI3K, thereby increasing autophagy; on the other hand Bcl-2/X_L [65,68], and the inositol-1,4,5-trisphosphate recep-

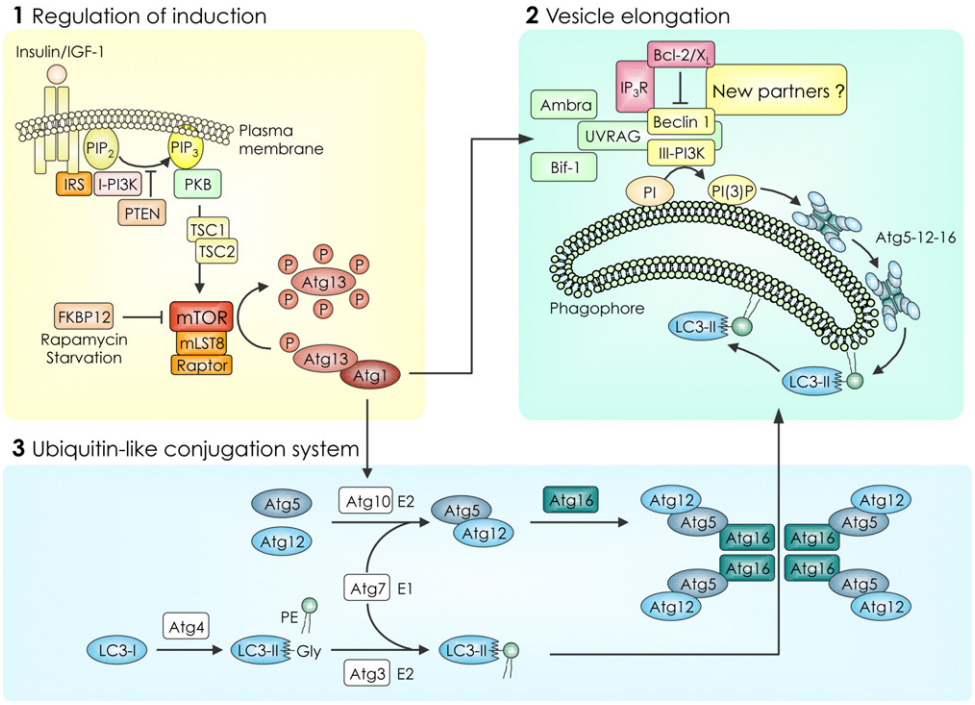

Fig. 2. Molecular regulation of macroautophagy.
The regulation of induction in the process of macroautophagy depends on the kinase activity of mTOR, which phosphorylates Atg13 (Section 1). Inhibition of mTOR by the absence of growth factors or rapamycin treatment, leads to Atg13 dephosphorylation and activation of the kinase Atg1 that unleashes the Atg cascade in two directions. One involves the activation of a class-III PI3K complex that stimulates vesicle elongation (Section 2). This complex is composed of Vps34, Beclin, a series of co-activator molecules (UVRAG, Ambra, Bif-1, in green), Beclin 1 repressors Bcl-2/X_L, IP_3R (in red) and perhaps new binding partners. Elongation depends also on the activation of an ubiquitin-like conjugation system that operates in two ways (Section 3); by stepwise processing of Atg5-12-16 phagophore-stabilizing complexes, and by cleavage/conjugation of LC3 to phosphatidylethanolamine present in the phagophore membrane.

tor (IP_3R) [69] are Beclin 1 repressors, hence inhibiting autophagy (Fig. 2).

2.2. Cellular protein degradation: the ubiquitin-proteasome system and the autophagy-lysosomal system

Two main pathways have been implicated in the catabolism of cellular proteins, the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal system (ALS). It has been generally assumed that short-lived and misfolded proteins are substrates for the UPS pathway, while long-lived proteins, aggregates, and a particular subset of proteins containing a lysosomal-targeting consensus motif are rather substrates for the ALS [52]. Currently however, a large body of evidence supports the notion that these pathways interact at several levels [70] (Fig. 3).

Here for the sake of brevity we give just a brief description of the UPS pathway (for reviews see [71,72]). The UPS provides specific and timely recycling of essential proteins. It is also an adaptative response to maintain a vital amino acid pool during acute starvation and contributes significantly to the degradation of recently synthesized defective proteins [73,74]. In eukaryotic cells, the UPS comprises the 26S proteasome and the ubiquitin system. The 26S proteasome is a highly conserved protease, composed of the catalytic 20S and the regulatory 19S subunits. It works concertedly with the ubiquitin system, which tags substrates with polyubiquitin chains as a marker for proteasomal degradation. Ubiquitin (Ub) is conjugated to substrate proteins by the action of three enzymes: E1 (Ub-activation enzyme), E2 (Ub-conjugation enzyme) and E3 (Ub ligase). Once Ub is activated by E1, it is transferred to E2, from which E3 attaches Ub covalently to Lys residues of a substrate protein [75]. Poly-Ub substrates are recognized by the 26S proteasome and subsequently degraded by the 20S catalytic subunit in an ATP-dependent manner, allowing for the resulting amino acids to serve in new protein synthesis. The regulatory 19S subunit and several de-ubiquitylating enzymes, remove poly-Ub chains from the substrates before they enter into the catalytic 20S subunit, therefore generating free Ub that serves to tag new substrates for proteasome-mediated degradation [71].

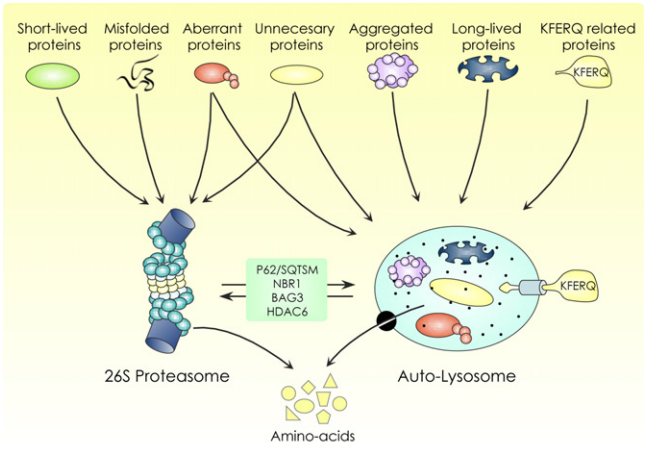

Fig. 3. Catabolic pathways for proteins.
The ubiquitin-proteasome system (UPS) substrates include short-lived, misfolded, aberrant and superfluous or unnecessary proteins; whereas substrates for the autophagy-lysosomal system (ALS) include superfluous, aberrant, aggregated and long-lived proteins, as well as a subset of proteins containing a lysosomal-targeting KFERQ motif. Although the UPS and autophagy have long been considered as independent systems, increasing evidence suggests that they interact at several points, for instance at the level of the proteins p62/SQTSM, NBR1, BAG3 and HDAC6 (see text for details).

The ALS constitutes a second catabolic pathway for the breakdown of proteins. It is responsible for maintaining amino acid pools during chronic starvation and represents the main degradation pathway for long-lived proteins, although its contribution to eliminate defective proteins may equal to that of the UPS [76]. The crosstalk between the two systems is well illustrated by the degradation of α-synuclein, which is substrate for the UPS, macroautophagy and chaperone-mediated autophagy [41,77]. Under conditions in which one degradation system is compromised, enhanced degradation by the alternate pathway may protect against the accumulation of toxic species [52]. For instance, impaired proteosomal degradation of polyglutamine aggregates in a cellular Huntington's model, requires HDAC6-dependent microtubule transport in order to increase the efficiency and selectivity of autophagic degradation [78]. In addition, in a *Drosophila* model of spinobulbar muscular atrophy, triggered by UPS inhibition, overexpression of HDAC6 *in vivo* rescued degeneration in an autophagy-dependent manner [79]. Similarly, rapamycin-induced autophagy was sufficient to rescue the loss of nigral dopaminergic neurons caused by UPS inhibition in a mouse model of Parkinson's disease [80]. Further molecular connections between the UPS and autophagy are represented by p62/SQTSM and NBR1, which both bind ubiquitin and the autophagosomal component LC3, serving as an autophagic receptor for poly-Ub substrates [36–38,81]. In this context, inhibition of autophagy may lead to accumulation of p62, which by scavenging poly-Ub substrates impairs the optimal proteasomal degradation of short-lived proteins [82]. Conversely, loss of p62 can represent a pathomechanisms leading to neurodegeneration [83]. The list of newly discovered components of these systems is continuously growing, and we can certainly predict that further links with neurodegeneration will be exploited in the near future.

3. 'Ca^{2+}-induced autophagy' or 'repair of Ca^{2+}-induced damage by autophagy'? A tour through the ER to mitochondria

The first evidence linking autophagic degradation and cellular Ca^{2+} homeostasis was reported in 1991 by the group of Per O. Seglen, demonstrating that the presence of Ca^{2+}, in an unidentified intracellular compartment, is necessary for the efficient autophagy-lysosomal degradation of proteins, independently of Ca^{2+}-activated effector kinases [84]. During the following years, various studies have identified a series of Ca^{2+}-signaling related proteins in the molecular toolkit of autophagy, unveiling a vastly complex interaction between the two processes. Indeed, as discussed below, the currently available data do not support a simple and straightforward correlation between Ca^{2+} signals and autophagic activity.

3.1. Ca^{2+}-mediated induction of autophagy during nutrient deprivation

Adenosine monophosphate-activated protein kinase (AMPK) is a major nutrient-sensing protein that leads to autophagy induction during starvation, thus guaranteeing the classic adaptive outcome of autophagy [85–87]. The group of Marja Jäättelä reported in 2006 that in MCF-7 breast cancer cells, agents mediating Ca^{2+} release from the ER such as extracellular ATP and 1,25 dihydroxyvitamin D_3 (or its analogue EB1089), induce autophagy by the sequential activation of Ca^{2+}-calmodulin-dependent kinase kinase-β (CaMKK-β) and AMPK, mediating the inhibition of mTOR [88]. This led to the proposal that increased cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) might be a common trigger of autophagy, supported also by the findings that BAPTA-AM suppressed and ionomycin mimicked the effect of effect ATP and EB1089 [88]. In accordance with this concept, depletion of ER Ca^{2+} stores was shown to block nutrient starvation mediated autophagy in the cardiomyocyte HL-1 cell line, although in a Beclin-1 independent manner [89]. However, none of these studies could demonstrate an unambiguous correlation between cytosolic Ca^{2+} levels and autophagic activity, while it appeared dependent rather on the state of filling of the ER Ca^{2+} store. Indeed, ER targeted Bcl-2 appeared to be the most potent inhibitor of autophagy in these studies by lowering ER luminal Ca^{2+} levels ($[Ca^{2+}]_{er}$). Along these lines, several further works demonstrated that ER Ca^{2+} depletion, a condition frequently observed in cellular stress conditions, is an effective inducer of autophagy. In the next sections, we will discuss the possible mechanism and Ca^{2+} dependency of autophagy induction in these cases.

3.2. Autophagy and ER stress: friends and foes

The ER lumen represents both the major site of intracellular Ca^{2+} storage and the site where transmembrane and secreted proteins are folded and post-translationally modified [3]. ER stress refers to conditions that disrupt the luminal environment, including redox balance and luminal Ca^{2+} homeostasis, resulting in the accumulation of unfolded/misfolded proteins. ER stress triggers 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). This signaling pathway aims to restore the homeostatic conditions in the ER lumen by limiting protein translation and import, increasing its folding capacity and promoting the degradation of misfolded proteins [90]. Importantly, autophagy is strongly induced by protein aggregation and oxidative stress. In this manner, ER stress and autophagy often become activated in parallel and partly share their signaling pathways (particularly the ones driven by PERK, ATF6 and ATF4) and team up to remove toxic byproducts of protein misfolding [53,91,92]. However, the interaction between these pathways is not as simple in all cases (see e.g. a very recent work on the role of IRE-1 by the group of Laurie Glimcher [93]) partly because beyond a certain ER stress threshold, the pro-survival effects of the UPR become insufficient and transducers of this pathway ultimately induce cell death, hence preventing the progression of damaged and potentially dangerous cells for the organism [94–96].

ER stress-induced cell death can proceed in both Ca^{2+}-independent and Ca^{2+}-dependent ways. The proapoptotic Bcl-2 family members Bax and Bak are able to directly interact with IRE1, which mediates apoptotic cell death through Jun-N-terminal kinase (JNK)-dependent phosphorylation and inactivation of Bcl-2 [97], parallel with activation of another proapoptotic signal, Bim [98]. In a different model, ER stress-based photodynamic therapy has been documented to mediate degradation of SERCA2 and consequent depletion of the ER Ca^{2+} pool, leading to Bax/Bak-dependent apoptosis [99]. Likewise, ER stress induces the expression of a truncated SERCA1 isoform, causing ER Ca^{2+} leak, mitochondrial Ca^{2+} overload and Bax mediated cytochrome *c* release [100]. Although the role of autophagy has not been directly assessed in these instances, it is sensible to propose that activation of cell death pathways during fervent or prolonged ER stress, sets off a protective response mediated at least partly by autophagy, as already shown in several apoptotic settings [101]. Apart from this teleological argument, two sets of data have provided experimental evidence to this concept, highlighting an indirect role of Ca^{2+} in the activation of adaptive autophagy.

First, the anti-apoptotic proteins Bcl-2 and Bcl-X_L have been repeatedly reported to mediate a decrease of $[Ca^{2+}]_{er}$ and conse-

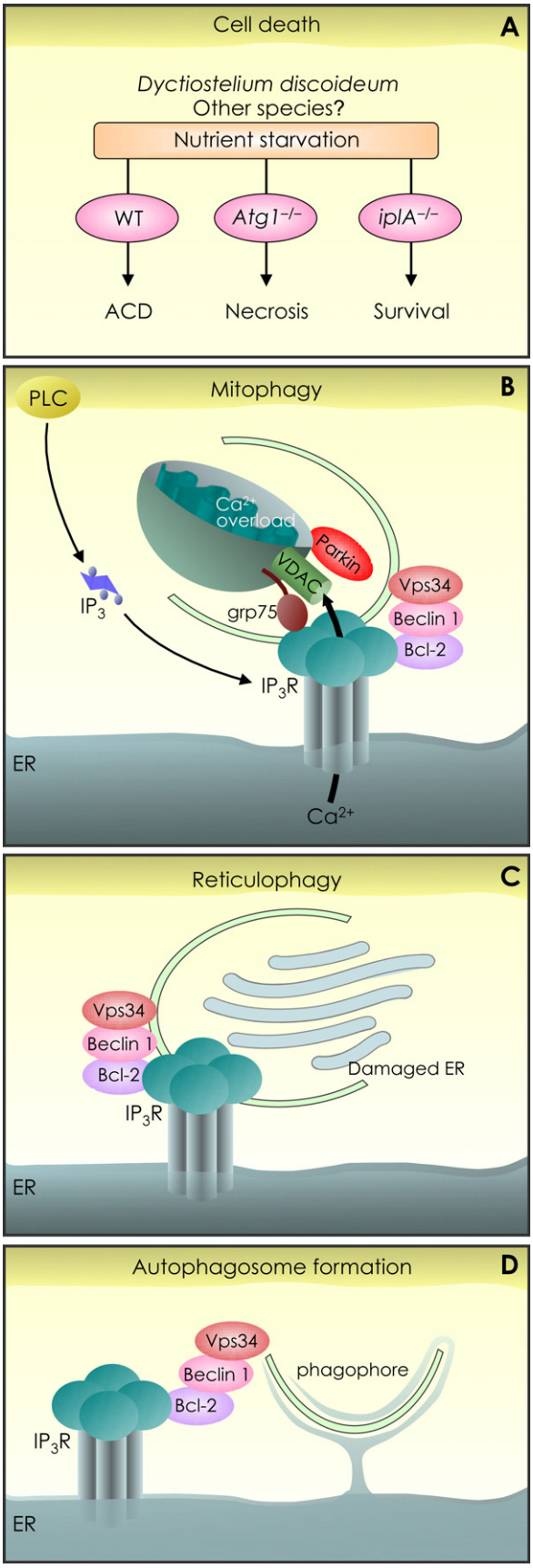

Fig. 4. Proposed implications of IP_3R-dependent autophagy.
(A) Nutrient starvation represents a strong stimulus for autophagic cell death (ACD) in the protist *Dyctiostelium discoideum*. In this organism, *Atg1* null cells do not die by ACD, but die by necrosis. However, mutations in the gene *iplA* (which encodes for the unique form of IP_3R in this species) rescue both, autophagic and necrotic cell death in this and perhaps other organisms. (B) Agents that increase cytosolic calcium (such as IP_3) lead to calcium uptake by neighboring mitochondria. If an overload of mitochondrial calcium occurs, a mechanism mediated by the ubiquitin ligase Parkin (which is mutated in Parkinson's disease) targets damaged mitochondria to autophagic degradation. By bridging ER and mitochondrial networks, as well as being a scaffolding site for autophagic proteins, the IP_3R is proposed to play an important role in selective mitophagy. (C) One of the cellular responses induced by the unfolded protein response, is dependent on the selective degradation of ER by autophagy. The IP_3R might provide the molecular toolkit for the efficient degradation of specific zones of stressed ER. (D) It has recently been proposed that phagophores originate from ER membranes via a cup-shaped cradle that surrounds the nascent autophagosome. The IP_3R could play an important role by recruiting the Beclin 1/class-III PI3K complex that catalyzes autophagosome formation.

quently Ca^{2+} release [3,102–106], and these effects are protective against mitochondrial apoptosis [107,108]. In an autophagic context, Bcl-2/X_L-mediated inhibition of autophagy has primarily been attributed to its interaction with the essential autophagic protein Beclin 1 [109–111], but increasing evidence suggests that Bcl-2 also inhibits autophagy by lowering ER calcium levels [88,89,112,113]. In this fashion, Bcl-2 acts as a suppressor of both Ca^{2+}-dependent apoptotic and autophagic pathways [114–116]. An apparent explanation for these observations is that by reducing the releasable amount of Ca^{2+} from the ER, mitochondrial Ca^{2+} overload will not occur, but a direct effect of Bcl-2/X_L or Ca^{2+} on ER membrane-processes cannot be excluded. In fact, it has been reported that autophagy is strongly activated by IP_3R inhibition [117]. In this model, the IP_3R interacts with the essential autophagic protein Beclin 1, inhibiting its autophagic functions [69]. Importantly, the knockdown or overexpression of Beclin 1, does not affect calcium homeostasis in the ER, neither modifies cytosolic or mitochondrial calcium levels (unpublished data), indicating that the IP_3R acts beyond its calcium channel functions, as an ER-resident transducer of autophagy. The relationship between the IP_3R and Beclin is indirect, mediated by Bcl-2. These findings suggest that the autophagic machinery composed of the class-III PI3K multiprotein complex is anchored to the ER membranes through the IP_3R, and could respond to different needs of autophagy induction at the level of the ER. One important connection between autophagy and its regulation by the IP_3R has been documented in the protist *Dyctiostelium discoideum* [118]. This organism adopts a unicellular life form under nutrient-rich conditions, but upon dwindling of nutrient resources, they restrict their number via autophagic cell death (ACD, a form of cell death with vacuolated morphology [119,120]) and group in a multicellular form, able to migrate, proliferate and respond to light or temperature [121,122]. During this adaptative phase, silencing of *Atg1* impairs ACD and shifts cell death to necrosis [123]. Mutagenesis studies revealed that inactivation of the gene *iplA* (the only gene encoding IP_3R in this organism) protects *D. discoideum* cells from ACD and necrosis [124], further supporting the evolutionarily preserved importance of the IP_3R in mediating autophagy. In this manner, the IP_3R might provide important autophagic functions in four different scenarios: by leading to ACD [118,124]; by promoting the specific removal of damaged ER zones by autophagy [91,92,125–127]; by promoting the specific removal of calcium-overloaded mitochondria in regions of close proximity between the ER and mitochondrial networks, in which the IP_3R is the linking column [128–130]; and by serving as a scaffolding site in the ER membranes, to the assembly of the autophagic machinery that catalyzes the nucleation, elongation and closing of autophagosomes [131] (Fig. 4). In a different model, IP_3R opening leads to its ubiquitination and subsequent elimination via the ER-associated degradation (ERAD) pathway, which depends on the UPS system to clear the ER of aberrant proteins [132]. By degrading IP_3Rs, the ERAD pathway reduces the sensitivity of Ca^{2+} stores to IP_3, and may

protect cells from the deleterious effects of over-activation of Ca^{2+} signaling.

Secondly, as pointed out above, ER stress-induced cell death can proceed through direct Ca^{2+} mediated interaction with mitochondria, which provide a further organelle specific platform for autophagy induction. This latter option is discussed in the next paragraph.

3.3. Autophagy and repair of Ca^{2+}-induced cellular damage: the mitochondrial connection

By activating Krebs cycle dehydrogenases, an increase in mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$) promotes the supply of NAD(P)H, stimulating respiratory chain activity and ATP production [6,133]. Hence, mitochondrial function largely depends on its Ca^{2+} load [134]. It appears that low/moderate increases in $[Ca^{2+}]_m$ are necessary and sufficient for adapting ATP production to cellular demands [135], but overload of mitochondria with Ca^{2+} unequivocally leads to disruption of the mitochondrial membrane integrity, permeability transition, irreversible oxidative damage to mitochondrial membranes and loss of mitochondrial ATP production [136]. This phenomenon has been linked to both necrosis and apoptosis and consequently, to a variety of pathological conditions, including neurodegeneration [15,137,138]. Recently, selective mitochondrial autophagy stood up as a principal mechanism that counterbalances mitochondrial damage. By removing damaged mitochondria from the cell, autophagy inhibits the cytotoxic release of proapoptotic molecules, and contributes to maintain a healthy mitochondrial pool [139,140]. In this context, the identification of the mechanisms responsible for triggering these events is of outstanding importance. Endothelin B/Bif-1 has been recently described to control both mitochondrial integrity and an essential component of the Beclin-1 hosted complex initiating autophagosome formation [141]. However, its exact role in selective mitophagy induction has not yet been systematically studied. More relevant to neurodegeneration, a recent study proposed that the initiation of mitophagy depends on the ubiquitin ligase parkin [142]. Recessive mutations in the gene *Park2* constitute a major cause for early-onset recessive Parkinson's disease. By impairing the turnover of mitochondria, mutations in the *Park2* gene thus might lead to the loss of dopaminergic neurons and to the development of Parkinson's disease. Moreover, parkin overexpression also rescued neuronal cells from mitochondrial oxidative stress and mitochondrial fragmentation induced by loss of the Parkinson-related PTEN-induced kinase 1 (Pink-1) [143], an effect dependent on selective mitophagy [144]. Importantly, the GTPase dynamin-related protein-1 (Drp-1) triggers the mitochondrial fission events prior to autophagic engulfment [145], and the activity of Drp-1 itself has been reported to contribute to the effects of Ca^{2+} signals upon mitochondrial motility and fusion/fission dynamics [128]. In this manner, Ca^{2+} dependent mitochondrial function and dysfunction, as well as its effects upon neuronal survival are intimately linked to autophagy, warranting for further studies to explore the key players in these liaisons, holding an enormous potential as pharmacological targets for the treatment of neurodegenerative diseases.

4. Direct effect of cytosolic Ca^{2+} on protein degradation by the ALS and UPS: a further mechanism of Ca^{2+}-mediated neurotoxicity?

As discussed above, removal of aggregate-prone proteins from the cytoplasm by the ALS and UPR represents a promising strategy for the treatment of neurodegenerative diseases. By inhibiting mTOR, rapamycin induces autophagy and contributes to this effect, but with considerable immunosuppressant effects [56]. To overcome this limitation, the group of David Rubinsztein performed a screening of a small molecule library to identify compounds that activate the clearance of α-synuclein aggregates by inducing autophagy. Surprisingly, they identified VDCC inhibitors as potent ALS inducers in this screen, suggesting that increased $[Ca^{2+}]_c$ might act as an inhibitor of ALS or UPR. Although the mechanism by which these compounds work is not entirely clear, since no data were presented on how an increase of $[Ca^{2+}]_c$ in their screening model is generated, they also provided compelling evidence that calpain inhibition enhances the clearance of both mutant huntingtin and of α-synuclein aggregates [146]. Moreover, the same group has also shown that lithium, by depleting the intracellular inositol phosphate pool and thus inhibiting the generation of Ca^{2+} signals, exerts a similar effect [147]. Indeed, beneficial effects of this cation have been shown both in motoneuron and Alzheimer's diseases [148,149]. These findings are in agreement with other reports showing that calpain activation is responsible for blocking autophagy by cleaving Atg5 [150] and for inhibiting autophagy mediated cell death in the presence of caspase inhibitors [151]. On another hand, the presence of the calpain 4 regulatory subunit appears to be necessary for basal autophagy in a mouse model [152] and, as discussed above, an increase of $[Ca^{2+}]_c$ could also be associated with increased autophagic activity [84,88,89,153].

Further studies on comparable cellular and animal models will be necessary to resolve this issue, but another set of data on the role of Ca^{2+} mediated enzymes in UPR might indicate a more general role of pathological Ca^{2+} signals to impair protein degradation in neurons. Indeed, while Ca^{2+} influx in the post-synaptic density appears to induce protein degradation through CaMKII activation as part of physiological synaptic remodeling [154], high $[Ca^{2+}]$ can inhibit ubiquitination and the activation of the proteasome [155,156]. Thus, it would be interesting to see whether the same phenomenon occurs during excitotoxicity (Ca^{2+} deregulation) in animal models of different neurodegenerative diseases. In addition, such an inhibition might also promote compensatory activation of the ALS, which might explain contradictory findings on the role of cytoplasmic $[Ca^{2+}]$ in the regulation of autophagy.

5. Coda

Recent advances in exploring the pathological basis of neurodegenerative disease have contributed to reconnect previously divergent fields of research, such as proteasomal protein degradation, autophagy, ER stress and Ca^{2+} signaling. The results from these combined approaches revealed an extremely complex picture of the disease, which continues to develop with astonishing pace. We attempted to provide a snapshot of these advances based on available data, which is summarized in Fig. 5. Impaired degradation of damaged proteins leads to their gradual accumulation in a subset of neurons, and this event appears now to be central in initiating neurodegeneration (probably accompanying also unhealthy aging). As a consequence, deregulation of Ca^{2+} homeostasis, ER stress and direct toxic effects impairs mitochondrial function, leading to a general energy deficit of the neuron, driving a vicious cycle that ultimately culminates in neuronal cell death. However, several components of the activated signaling pathways (IP_3 levels, IP_3R, Ca^{2+}/CaM dependent kinases, calpains, energy sensors [AMPK], ER stress-induced transcription factors) embark an homeostatic response through autophagy and UPS, in order to rescue cell from this cycle. The multiplicity of these pathways also explains the lack of simple generalization of the role of individual components, such as Ca^{2+} signaling. Evi-

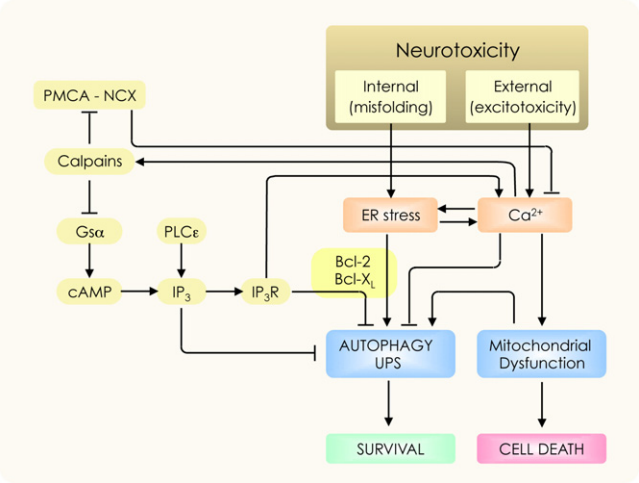

Fig. 5. The roles of Ca^{2+} in autophagy and UPS regulation during neurodegeneration. Ca^{2+} signals exert both, direct and indirect effects on autophagic flow. Ca^{2+}-induced damage on ER protein folding (ER stress) and mitochondria (depolarization), triggers by its own general and organelle specific autophagy, in order to reestablish cellular homeostasis (adaptive autophagy induction). However, several results points to a direct inhibition of autophagy (and the UPS) by direct targets of Ca^{2+}, such as calpain and CaM-dependent kinases. Interestingly, the pathway appears to be potentiated by cAMP signals. Inhibition of cytoplasmic Ca^{2+} signals thus leads to activation of basal autophagy, counterbalancing the deleterious effects of excitotoxicity.

dently, the balance of toxic and adaptive responses will define the fate of these neurons, thus future interventions augmenting the selective proteasomal and autophagic degradation of damaged organelles, or toxic protein species, will certainly increase the likelihood of neuronal survival and reduce the degenerative process.

Conflicts of interest

None declared.

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References

[1] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. 4 (2003) 517–529.
[2] J Hartmann, A. Konnerth, Determinants of postsynaptic Ca^{2+} signaling in Purkinje neurons, Cell Calcium 37 (2005) 459–466.
[3] M.J. Berridge, The endoplasmic reticulum: a multifunctional signaling organelle, Cell Calcium 32 (2002) 235–249.
[4] R. Gomez-Villafuertes, B. Mellstrom, J.R. Naranjo, Searching for a role of NCX/NCKX exchangers in neurodegeneration, Mol. Neurobiol. 35 (2007) 195–202.
[5] O.H. Petersen, M. Michalak, A. Verkhratsky, Calcium signalling: past, present and future, Cell Calcium 38 (2005) 161–169.
[6] G Szabadkai, M.R. Duchen, Mitochondria: the hub of cellular Ca^{2+} signaling, Physiology (Bethesda) 23 (2008) 84–94.
[7] D.G. Nicholls, Mitochondrial calcium function and dysfunction in the central nervous system, Biochim. Biophys. Acta 1787 (2009) 1416–1424.
[8] D. Choi, Ionic dependence of glutamate neurotoxicity, J. Neurosci. 7 (1987) 369–379.
[9] A. Verkhratsky, Calcium and cell death, Subcell. Biochem. 45 (2007) 465–480.
[10] S. Orrenius, B. Zhivotovsky, P. Nicotera, Regulation of cell death: the calcium-apoptosis link, Nat. Rev. Mol. Cell Biol. 4 (2003) 552–565.
[11] M.R. Duchen, Roles of mitochondria in health and disease, Diabetes 53 (Suppl. 1) (2004) S96–S102.
[12] D. Guerini, L. Coletto, E. Carafoli, Exporting calcium from cells, Cell Calcium 38 (2005) 281–289.
[13] D. Bano, K.W. Young, C.J. Guerin, R. Lefeuvre, N.J. Rothwell, L. Naldini, R. Rizzuto, E. Carafoli, P. Nicotera, Cleavage of the plasma membrane Na^+/Ca^{2+} exchanger in excitotoxicity, Cell 120 (2005) 275–285.
[14] A.A. Gerencser, K.A. Mark, A.E. Hubbard, A.S. Divakaruni, Z. Mehrabian, D.G. Nicholls, B.M. Polster, Real-time visualization of cytoplasmic calpain activation and calcium deregulation in acute glutamate excitotoxicity, J. Neurochem. 110 (2009) 990–1004.
[15] D.R. Green, G. Kroemer, The pathophysiology of mitochondrial cell death, Science 305 (2004) 626–629.
[16] A. Ruiz, C. Matute, E. Alberdi, Endoplasmic reticulum Ca(2+) release through ryanodine and IP(3) receptors contributes to neuronal excitotoxicity, Cell Calcium 46 (2009) 273–281.
[17] A. Verkhratsky, E.C. Toescu, Endoplasmic reticulum Ca(2+) homeostasis and neuronal death, J. Cell. Mol. Med. 7 (2003) 351–361.
[18] V. Prahlad, R.I. Morimoto, Integrating the stress response: lessons for neurodegenerative diseases from C. elegans, Trends Cell Biol. 19 (2009) 52–61.
[19] I. Kim, W. Xu, J.C. Reed, Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities, Nat. Rev. Drug Discov. 7 (2008) 1013–1030.
[20] I. Bezprozvanny, M.P. Mattson, Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease, Trends Neurosci. 31 (2008) 454–463.
[21] M.P. Mattson, Calcium and neurodegeneration, Aging Cell 6 (2007) 337–350.
[22] P.S. Vosler, C.S. Brennan, J. Chen, Calpain-mediated signaling mechanisms in neuronal injury and neurodegeneration, Mol. Neurobiol. 38 (2008) 78–7100.
[23] C.S. Chan, T.S. Gertler, D.J. Surmeier, Calcium homeostasis, selective vulnerability and Parkinson's disease, Trends Neurosci. 32 (2009) 249–256.
[24] A.J Meijer, P. Codogno, Autophagy: regulation and role in disease, Crit. Rev. Clin. Lab. Sci. 46 (2009) 210–240.
[25] S.A. Tooze, G. Schiavo, Liaisons dangereuses: autophagy, neuronal survival and neurodegeneration, Curr. Opin. Neurobiol. 18 (2008) 504–515.
[26] A.R Winslow, D.C. Rubinsztein, Autophagy in neurodegeneration and development, Biochim. Biophys. Acta 1782 (2008) 723–729.
[27] L. Bedford, S. Paine, N. Rezvani, M. Mee, J. Lowe, R.J. Mayer, The UPS and autophagy in chronic neurodegenerative disease: six of one and half a dozen of the other—or not? Autophagy 5 (2009) 224–227.
[28] C. De Duve, B.C. Pressman, R. Gianetto, R. Wattiaux, F. Appelmans, Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue, Biochem. J. 60 (1955) 604–617.
[29] C. De Duve, The lysosome turns fifty, Nat. Cell Biol. 7 (2005) 847–849.
[30] S.L. Clark Jr., Cellular differentiation in the kidneys of newborn mice studies with the electron microscope, J. Biophys. Biochem. Cytol. 3 (1957) 349–362.
[31] R.L. Deter, P. Baudhuin, C. De Duve, Participation of lysosomes in cellular autophagy induced in rat liver by glucagon, J. Cell Biol. 35 (1967) C11–C16.
[32] R.L. Deter, C. De Duve, Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes, J. Cell Biol. 33 (1967) 437–449.
[33] H Hoyvik, P.B. Gordon, P.O. Seglen, Use of a hydrolysable probe, [14C]lactose, to distinguish between pre-lysosomal and lysosomal steps in the autophagic pathway, Exp. Cell Res. 166 (1986) 1–14.
[34] D.J. Klionsky, Autophagy: from phenomenology to molecular understanding in less than a decade, Nat. Rev. Mol. Cell Biol. 8 (2007) 931–937.
[35] D.J. Klionsky, A.M. Cuervo, W.A. Dunn Jr., B. Levine, I. van der Klei, P.O. Seglen, How shall I eat thee? Autophagy 3 (2007) 413–416.
[36] V. Kirkin, T. Lamark, Y.S. Sou, G. Bjorkoy, J.L. Nunn, J.A. Bruun, E. Shvets, D.G. McEwan, T.H. Clausen, P. Wild, I. Bilusic, J.P. Theurillat, A. Overvatn, T. Ishii, Z. Elazar, M. Komatsu, I. Dikic, T. Johansen, A role for NBR1 in autophagosomal degradation of ubiquitinated substrates, Mol. Cell 33 (2009) 505–516.
[37] V. Kirkin, D.G. McEwan, I. Novak, I. Dikic, A role for ubiquitin in selective autophagy, Mol. Cell 34 (2009) 259–269.
[38] S. Pankiv, T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, A. Overvatn, G. Bjorkoy, T. Johansen, p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy, J. Biol. Chem. 282 (2007) 24131–24145.
[39] A.M. Cuervo, Autophagy: many paths to the same end, Mol. Cell. Biochem. 263 (2004) 55–72.
[40] J.C. Farre, S. Subramani, Peroxisome turnover by micropexophagy: an autophagy-related process, Trends Cell Biol. 14 (2004) 515–523.
[41] A.M. Cuervo, L. Stefanis, R. Fredenburg, P.T. Lansbury, D. Sulzer, Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy, Science 305 (2004) 1292–1295.
[42] J.F. Dice, S.R. Terlecky, Targeting of cytosolic proteins to lysosomes for degradation, Crit. Rev. Ther. Drug Carrier Syst. 7 (1990) 211–233.
[43] Z. Kouchi, H. Sorimachi, K. Suzuki, S. Ishiura, Proteasome inhibitors induce the association of Alzheimer's amyloid precursor protein with Hsc73, Biochem. Biophys. Res. Commun. 254 (1999) 804–810.
[44] D.J. Klionsky, J.M. Cregg, W.A. Dunn Jr., S.D. Emr, Y. Sakai, I.V. Sandoval, A. Sibirny, S. Subramani, M. Thumm, M. Veenhuis, Y. Ohsumi, A unified nomenclature for yeast autophagy-related genes, Dev. Cell 5 (2003) 539–545.
[45] V.M. Aita, X.H. Liang, V.V. Murty, D.L. Pincus, W. Yu, E. Cayanis, S. Kalachikov, T.C. Gilliam, B. Levine, Cloning and genomic organization of beclin 1, a can-

didate tumor suppressor gene on chromosome 17q21, Genomics 59 (1999) 59–65.
[46] X.H Liang, S. Jackson, M. Seaman, K. Brown, B. Kempkes, H. Hibshoosh, B. Levine, Induction of autophagy and inhibition of tumorigenesis by beclin 1, Nature 402 (1999) 672–676.
[47] Z. Yue, S. Jin, C. Yang, A.J. Levine, N. Heintz, Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 15077–15082.
[48] B Levine, G. Kroemer, Autophagy in the pathogenesis of disease, Cell 132 (2008) 27–42.
[49] U. Pfeifer, J. Bertling, A morphometric study of the inhibition of autophagic degradation during restorative growth of liver cells in rats re-fed after starvation, Virchows Arch. B: Cell Pathol. 24 (1977) 109–120.
[50] G.E. Mortimore, C.M. Schworer, Induction of autophagy by amino-acid deprivation in perfused rat liver, Nature 270 (1977) 174–176.
[51] D.C. Rubinsztein, The roles of intracellular protein-degradation pathways in neurodegeneration, Nature 443 (2006) 780–786.
[52] N.B. Nedelsky, P.K. Todd, J.P. Taylor, Autophagy and the ubiquitin-proteasome system: collaborators in neuroprotection, Biochim. Biophys. Acta 1782 (2008) 691–699.
[53] F. Madeo, T. Eisenberg, G. Kroemer, Autophagy for the avoidance of neurodegeneration, Genes Dev. 23 (2009) 2253–2259.
[54] M.N. Hall, mTOR—what does it do? Transplant. Proc. 40 (2008) S5–S8.
[55] P.B. Dennis, A. Jaeschke, M. Saitoh, B. Fowler, S.C. Kozma, G. Thomas, Mammalian TOR: a homeostatic ATP sensor, Science 294 (2001) 1102–1105.
[56] D.C. Rubinsztein, J.E. Gestwicki, L.O. Murphy, D.J. Klionsky, Potential therapeutic applications of autophagy, Nat. Rev. Drug Discov. 6 (2007) 304–312.
[57] S. Diaz-Troya, M.E. Perez-Perez, F.J. Florencio, J.L. Crespo, The role of TOR in autophagy regulation from yeast to plants and mammals, Autophagy 4 (2008) 851–865.
[58] A. Petiot, E. Ogier-Denis, E.F. Blommaart, A.J. Meijer, P. Codogno, Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells, J. Biol. Chem. 275 (2000) 992–998.
[59] J. Poels, M.R. Spasic, P. Callaerts, K.K. Norga, Expanding roles for AMP-activated protein kinase in neuronal survival and autophagy, Bioessays 31 (2009) 944–952.
[60] T. Yorimitsu, D.J. Klionsky, Autophagy: molecular machinery for self-eating, Cell Death Differ. 12 (Suppl. 2) (2005) 1542–1552.
[61] T. Noda, K. Suzuki, Y. Ohsumi, Yeast autophagosomes: de novo formation of a membrane structure, Trends Cell Biol. 12 (2002) 231–235.
[62] Z. Xie, D.J. Klionsky, Autophagosome formation: core machinery and adaptations, Nat. Cell Biol. 9 (2007) 1102–1109.
[63] S. Pattingre, L. Espert, M. Biard-Piechaczyk, P. Codogno, Regulation of macroautophagy by mTOR and Beclin 1 complexes, Biochimie 90 (2008) 313–323.
[64] A. Kihara, T. Noda, N. Ishihara, Y. Ohsumi, Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in Saccharomyces cerevisiae, J. Cell Biol. 152 (2001) 519–530.
[65] C. Liang, P. Feng, B. Ku, I. Dotan, D. Canaani, B.H. Oh, J.U. Jung, Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG, Nat. Cell Biol. 8 (2006) 688–699.
[66] Y. Takahashi, D. Coppola, N. Matsushita, H.D. Cualing, M. Sun, Y. Sato, C. Liang, J.U. Jung, J.Q. Cheng, J.J. Mule, W.J. Pledger, H.G. Wang, Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis, Nat. Cell Biol. 9 (2007) 1142–1151.
[67] G.M. Fimia, A. Stoykova, A. Romagnoli, L. Giunta, S. Di Bartolomeo, R. Nardacci, M. Corazzari, C. Fuoco, A. Ucar, P. Schwartz, P. Gruss, M. Piacentini, K. Chowdhury, F. Cecconi, Ambra1 regulates autophagy and development of the nervous system, Nature 447 (2007) 1121–1125.
[68] C.G. Noble, J.M. Dong, E. Manser, H. Song, Bcl-xL and UVRAG cause a monomer-dimer switch in Beclin1, J. Biol. Chem. 283 (2008) 26274–26282.
[69] J.M. Vicencio, C. Ortiz, A. Criollo, A.W. Jones, O. Kepp, L. Galluzzi, N. Joza, I. Vitale, E. Morselli, M. Tailler, M. Castedo, M.C. Maiuri, J. Molgo, G. Szabadkai, S. Lavandero, G. Kroemer, The inositol 1,4,5-trisphosphate receptor regulates autophagy through its interaction with Beclin 1, Cell Death Differ. 16 (2009) 1006–1017.
[70] N. Matsuda, K. Tanaka, Does impairment of the ubiquitin-proteasome system or the autophagy-lysosome pathway predispose individuals to neurodegenerative disorders such as Parkinson's disease? J. Alzheimers Dis. (2009), in press.
[71] S. Murata, H. Yashiroda, K. Tanaka, Molecular mechanisms of proteasome assembly, Nat. Rev. Mol. Cell Biol. 10 (2009) 104–115.
[72] H.C. Tai, E.M. Schuman, Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction, Nat. Rev. Neurosci. 9 (2008) 826–838.
[73] U Schubert, L.C. Anton, J. Gibbs, C.C. Norbury, J.W. Yewdell, J.R. Bennink, Rapid degradation of a large fraction of newly synthesized proteins by proteasomes, Nature 404 (2000) 770–774.
[74] R.M. Vabulas, F.U. Hartl, Protein synthesis upon acute nutrient restriction relies on proteasome function, Science 310 (2005) 1960–1963.
[75] Y.C. Liu, Ubiquitin ligases and the immune response, Annu. Rev. Immunol. 22 (2004) 81–127.
[76] B.A. McCray, J.P. Taylor, The role of autophagy in age-related neurodegeneration, Neurosignals 16 (2008) 75–84.
[77] J.L. Webb, B. Ravikumar, J. Atkins, J.N. Skepper, D.C. Rubinsztein, Alpha-synuclein is degraded by both autophagy and the proteasome, J. Biol. Chem. 278 (2003) 25009–25013.
[78] A. Iwata, B.E. Riley, J.A. Johnston, R.R. Kopito, HDAC6 and microtubules are required for autophagic degradation of aggregated huntingtin, J. Biol. Chem. 280 (2005) 40282–40292.
[79] U.B. Pandey, Z. Nie, Y. Batlevi, B.A. McCray, G.P. Ritson, N.B. Nedelsky, S.L. Schwartz, N.A. DiProspero, M.A. Knight, O. Schuldiner, R. Padmanabhan, M. Hild, D.L. Berry, D. Garza, C.C. Hubbert, T.P. Yao, E.H. Baehrecke, J.P. Taylor, HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS, Nature 447 (2007) 859–863.
[80] T. Pan, S. Kondo, W. Zhu, W. Xie, J. Jankovic, W. Le, Neuroprotection of rapamycin in lactacystin-induced neurodegeneration via autophagy enhancement, Neurobiol. Dis. 32 (2008) 16–25.
[81] G. Bjorkoy, T. Lamark, A. Brech, H. Outzen, M. Perander, A. Overvatn, H. Stenmark, T. Johansen, p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death, J. Cell Biol. 171 (2005) 603–614.
[82] V.I. Korolchuk, A. Mansilla, F.M. Menzies, D.C. Rubinsztein, Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates, Mol. Cell 33 (2009) 517–527.
[83] Y. Du, M.C. Wooten, M.W. Wooten, Oxidative damage to the promoter region of SQSTM1/p62 is common to neurodegenerative disease, Neurobiol. Dis. 35 (2009) 302–310.
[84] P.B. Gordon, I. Holen, M. Fosse, J.S. Rotnes, P.O. Seglen, Dependence of hepatocytic autophagy on intracellularly sequestered calcium, J. Biol. Chem. 268 (1993) 26107–26112.
[85] D. Meley, C. Bauvy, J.H. Houben-Weerts, P.F. Dubbelhuis, M.T. Helmond, P. Codogno, A.J. Meijer, AMP-activated protein kinase and the regulation of autophagic proteolysis, J. Biol. Chem. 281 (2006) 34870–34879.
[86] A.J. Meijer, P. Codogno, AMP-activated protein kinase and autophagy, Autophagy 3 (2007) 238–240.
[87] M. Hoyer-Hansen, M. Jaattela, AMP-activated protein kinase: a universal regulator of autophagy? Autophagy 3 (2007) 381–383.
[88] M. Hoyer-Hansen, L. Bastholm, P. Szyniarowski, M. Campanella, G. Szabadkai, T. Farkas, K. Bianchi, N. Fehrenbacher, F. Elling, R. Rizzuto, I.S. Mathiasen, M. Jaattela, Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2, Mol. Cell 25 (2007) 193–205.
[89] N.R Brady, A. Hamacher-Brady, H. Yuan, R.A. Gottlieb, The autophagic response to nutrient deprivation in the hl-1 cardiac myocyte is modulated by Bcl-2 and sarco/endoplasmic reticulum calcium stores, FEBS J. 274 (2007) 3184–3197.
[90] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell Biol. 8 (2007) 519–529.
[91] M. Ogata, S. Hino, A. Saito, K. Morikawa, S. Kondo, S. Kanemoto, T. Murakami, M. Taniguchi, I. Tanii, K. Yoshinaga, S. Shiosaka, J.A. Hammarback, F. Urano, K. Imaizumi, Autophagy is activated for cell survival after endoplasmic reticulum stress, Mol. Cell. Biol. 26 (2006) 9220–9231.
[92] Y. Kouroku, E. Fujita, I. Tanida, T. Ueno, A. Isoai, H. Kumagai, S. Ogawa, R.J. Kaufman, E. Kominami, T. Momoi, ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation, Cell Death Differ. 14 (2007) 230–239.
[93] C Hetz, P. Thielen, S. Matus, M. Nassif, F. Court, R. Kiffin, G. Martinez, A.M. Cuervo, R.H. Brown, L.H. Glimcher, XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy, Genes Dev. 23 (2009) 2294–2306.
[94] H.M. Heath-Engel, N.C. Chang, G.C. Shore, The endoplasmic reticulum in apoptosis and autophagy: role of the BCL-2 protein family, Oncogene 27 (2008) 6419–6433.
[95] T. Nakagawa, H. Zhu, N. Morishima, E. Li, J. Xu, B.A. Yankner, J. Yuan, Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta, Nature 403 (2000) 98–103.
[96] E. Szegezdi, S.E. Logue, A.M. Gorman, A. Samali, Mediators of endoplasmic reticulum stress-induced apoptosis, EMBO Rep. 7 (2006) 880–885.
[97] C. Hetz, P. Bernasconi, J. Fisher, A.H. Lee, M.C. Bassik, B. Antonsson, G.S. Brandt, N.N. Iwakoshi, A. Schinzel, L.H. Glimcher, S.J. Korsmeyer, Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha, Science 312 (2006) 572–576.
[98] H. Puthalakath, L.A. O'Reilly, P. Gunn, L. Lee, P.N. Kelly, N.D. Huntington, P.D. Hughes, E.M. Michalak, J. McKimm-Breschkin, N. Motoyama, T. Gotoh, S. Akira, P. Bouillet, A. Strasser, ER stress triggers apoptosis by activating BH3-only protein Bim, Cell 129 (2007) 1337–1349.
[99] E. Buytaert, G. Callewaert, N. Hendrickx, L. Scorrano, D. Hartmann, L. Missiaen, J.R. Vandenheede, I. Heirman, J. Grooten, P. Agostinis, Role of endoplasmic reticulum depletion and multidomain proapoptotic BAX and BAK proteins in shaping cell death after hypericin-mediated photodynamic therapy, FASEB J. 20 (2006) 756–758.
[100] M. Chami, B. Oules, G. Szabadkai, R. Tacine, R. Rizzuto, P. Paterlini-Brechot, Role of SERCA1 truncated isoform in the proapoptotic calcium transfer from ER to mitochondria during ER stress, Mol. Cell 32 (2008) 641–651.
[101] B. Levine, S. Sinha, G. Kroemer, Bcl-2 family members: dual regulators of apoptosis and autophagy, Autophagy 4 (2008) 600–606.
[102] R. Chen, I. Valencia, F. Zhong, K.S. McColl, H.L. Roderick, M.D. Bootman, M.J. Berridge, S.J. Conway, A.B. Holmes, G.A. Mignery, P. Velez, C.W. Distelhorst, Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to reg-

ulate calcium release from the ER in response to inositol 1,4,5-trisphosphate, J. Cell Biol. 166 (2004) 193–203.
[103] C.J. Hanson, M.D. Bootman, C.W. Distelhorst, R.J. Wojcikiewicz, H.L. Roderick, Bcl-2 suppresses Ca(2+) release through inositol 1,4,5-trisphosphate receptors and inhibits Ca(2+) uptake by mitochondria without affecting ER calcium store content, Cell Calcium 44 (2008) 324–338.
[104] A.E. Palmer, C. Jin, J.C. Reed, R.Y. Tsien, Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 17404–17409.
[105] P. Pinton, R. Rizzuto, Bcl-2 and Ca^{2+} homeostasis in the endoplasmic reticulum, Cell Death Differ. 13 (2006) 1409–1418.
[106] Y.P. Rong, A.S. Aromolaran, G. Bultynck, F. Zhong, X. Li, K. McColl, S. Matsuyama, S. Herlitze, H.L. Roderick, M.D. Bootman, G.A. Mignery, J.B. Parys, H. De Smedt, C.W. Distelhorst, Targeting Bcl-2-IP3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals, Mol. Cell 31 (2008) 255–265.
[107] M.C. Bassik, L. Scorrano, S.A. Oakes, T. Pozzan, S.J. Korsmeyer, Phosphorylation of BCL-2 regulates ER Ca^{2+} homeostasis and apoptosis, EMBO J. 23 (2004) 1207–1216.
[108] Y.P. Rong, P. Barr, V.C. Yee, C.W. Distelhorst, Targeting Bcl-2 based on the interaction of its BH4 domain with the inositol 1,4,5-trisphosphate receptor, Biochim. Biophys. Acta 1793 (2008) 971–978.
[109] A. Oberstein, P.D. Jeffrey, Y. Shi, Crystal structure of the Bcl-XL-Beclin 1 peptide complex: Beclin 1 is a novel BH3-only protein, J. Biol. Chem. 282 (2007) 13123–13132.
[110] W. Feng, S. Huang, H. Wu, M. Zhang, Molecular basis of Bcl-xL's target recognition versatility revealed by the structure of Bcl-xL in complex with the BH3 domain of Beclin-1, J. Mol. Biol. 372 (2007) 223–235.
[111] M.C. Maiuri, G. Le Toumelin, A. Criollo, J.C. Rain, F. Gautier, P. Juin, E. Tasdemir, G. Pierron, K. Troulinaki, N. Tavernarakis, J.A. Hickman, O. Geneste, G. Kroemer, Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1, EMBO J. 26 (2007) 2527–2539.
[112] S. Swerdlow, C.W. Distelhorst, Bcl-2-regulated calcium signals as common mediators of both apoptosis and autophagy, Dev. Cell 12 (2007) 178–179.
[113] A. Uttenweiler, H. Schwarz, A. Mayer, Microautophagic vacuole invagination requires calmodulin in a Ca^{2+}-independent function, J. Biol. Chem. 280 (2005) 33289–33297.
[114] P. Pinton, D. Ferrari, E. Rapizzi, F.D. Di Virgilio, T. Pozzan, R. Rizzuto, The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action, EMBO J. 20 (2001) 2690–2701.
[115] G. Szabadkai, A.M. Simoni, M. Chami, M.R. Wieckowski, R.J. Youle, R. Rizzuto, Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca^{2+} waves and protects against Ca^{2+}-mediated apoptosis, Mol. Cell 16 (2004) 59–68.
[116] C.W. Distelhorst, G.C. Shore, Bcl-2 and calcium: controversy beneath the surface, Oncogene 23 (2004) 2875–2880.
[117] A. Criollo, M.C. Maiuri, E. Tasdemir, I. Vitale, A.A. Fiebig, D. Andrews, J. Molgo, J. Diaz, S. Lavandero, F. Harper, G. Pierron, D. di Stefano, R. Rizzuto, G. Szabadkai, G. Kroemer, Regulation of autophagy by the inositol trisphosphate receptor, Cell Death Differ. 14 (2007) 1029–1039.
[118] D. Lam, A. Kosta, M.F. Luciani, P. Golstein, The inositol 14,5-trisphosphate receptor is required to signal autophagic cell death, Mol. Biol. Cell 19 (2008) 691–700.
[119] B. Levine, G. Kroemer, Autophagy in aging, disease and death: the true identity of a cell death impostor, Cell Death Differ. 16 (2009) 1–2.
[120] G Kroemer, L. Galluzzi, P. Vandenabeele, J. Abrams, E.S. Alnemri, E.H. Baehrecke, M.V. Blagosklonny, W.S. El-Deiry, P. Golstein, D.R. Green, M. Hengartner, R.A. Knight, S. Kumar, S.A. Lipton, W. Malorni, G. Nunez, M.E. Peter, J. Tschopp, J. Yuan, M. Piacentini, B. Zhivotovsky, G. Melino, Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009, Cell Death Differ. 16 (2009) 3–11.
[121] Y. Maeda, Regulation of growth and differentiation in Dictyostelium, Int. Rev. Cytol. 244 (2005) 287–332.
[122] P. Devreotes, *Dictyostelium discoideum*: a model system for cell-cell interactions in development, Science 245 (1989) 1054–1058.
[123] A. Kosta, C. Roisin-Bouffay, M.F. Luciani, G.P. Otto, R.H. Kessin, P. Golstein, Autophagy gene disruption reveals a non-vacuolar cell death pathway in Dictyostelium, J. Biol. Chem. 279 (2004) 48404–48409.
[124] D. Lam, P. Golstein, A specific pathway inducing autophagic cell death is marked by an IP3R mutation, Autophagy 4 (2008) 349–350.
[125] S. Bernales, K.L. McDonald, P. Walter, Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response, PLoS Biol. 4 (2006) e423.
[126] S.H. Oh, S.C. Lim, Endoplasmic reticulum stress-mediated autophagy/apoptosis induced by capsaicin (8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin is regulated by the extent of c-Jun NH2-terminal kinase/extracellular signal-regulated kinase activation in WI38 lung epithelial fibroblast cells, J. Pharmacol. Exp. Ther. 329 (2009) 112–122.
[127] T. Yorimitsu, U. Nair, Z. Yang, D.J. Klionsky, Endoplasmic reticulum stress triggers autophagy, J. Biol. Chem. 281 (2006) 30299–30304.
[128] M. Saotome, D. Safiulina, G. Szabadkai, S. Das, A. Fransson, P. Aspenstrom, R. Rizzuto, G. Hajnoczky, Bidirectional Ca^{2+}-dependent control of mitochondrial dynamics by the Miro GTPase, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 20728–20733.
[129] G. Szabadkai, K. Bianchi, P. Varnai, D. De Stefani, M.R. Wieckowski, D. Cavagna, A.I. Nagy, T. Balla, R. Rizzuto, Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels, J. Cell Biol. 175 (2006) 901–911.
[130] G. Szabadkai, R. Rizzuto, Participation of endoplasmic reticulum and mitochondrial calcium handling in apoptosis: more than just neighborhood? FEBS Lett. 567 (2004) 111–115.
[131] M. Hayashi-Nishino, N. Fujita, T. Noda, A. Yamaguchi, T. Yoshimori, A. Yamamoto, A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation, Nat. Cell Biol. 11 (2009) 1433–1437.
[132] R.J. Wojcikiewicz, M.M. Pearce, D.A. Sliter, Y. Wang, When worlds collide: IP(3) receptors and the ERAD pathway, Cell Calcium 46 (2009) 147–153.
[133] M.R. Duchen, Mitochondria in health and disease: perspectives on a new mitochondrial biology, Mol. Aspects Med. 25 (2004) 365–451.
[134] A.L. Lehninger, Mitochondria and calcium ion transport, Biochem. J. 119 (1970) 129–138.
[135] A. Spat, G. Szanda, G. Csordas, G. Hajnoczky, High- and low-calcium-dependent mechanisms of mitochondrial calcium signalling, Cell Calcium 44 (2008) 51–63.
[136] A. Rasola, P. Bernardi, The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis, Apoptosis 12 (2007) 815–833.
[137] G. Kroemer, Mitochondrial control of apoptosis: an introduction, Biochem. Biophys. Res. Commun. 304 (2003) 433–435.
[138] M. Mancuso, F. Coppede, L. Migliore, G. Siciliano, L. Murri, Mitochondrial dysfunction, oxidative stress and neurodegeneration, J. Alzheimers Dis. 10 (2006) 59–73.
[139] S.P Elmore, T. Qian, S.F. Grissom, J.J. Lemasters, The mitochondrial permeability transition initiates autophagy in rat hepatocytes, FASEB J. 15 (2001) 2286–2287.
[140] G. Twig, A. Elorza, A.J. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, L. Stiles, S.E. Haigh, S. Katz, G. Las, J. Alroy, M. Wu, B.F. Py, J. Yuan, J.T. Deeney, B.E. Corkey, O.S. Shirihai, Fission and selective fusion govern mitochondrial segregation and elimination by autophagy, EMBO J. 27 (2008) 433–446.
[141] T.K. Rostovtseva, H. Boukari, A. Antignani, B. Shiu, S. Banerjee, A. Neutzner, R.J. Youle, Bax activates endophilin B1 oligomerization and lipid membrane vesiculation, J. Biol. Chem. 284 (2009) 34390–34399.
[142] D. Narendra, A. Tanaka, D.F. Suen, R.J. Youle, Parkin is recruited selectively to impaired mitochondria and promotes their autophagy, J. Cell Biol. 183 (2008) 795–803.
[143] R.K. Dagda, S.J. Cherra III, S.M. Kulich, A. Tandon, D. Park, C.T. Chu, Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission, J. Biol. Chem. 284 (2009) 13843–13855.
[144] H.M. McBride, Parkin mitochondria in the autophagosome, J. Cell Biol. 183 (2008) 757–759.
[145] D. Narendra, A. Tanaka, D.F. Suen, R.J. Youle, Parkin-induced mitophagy in the pathogenesis of Parkinson disease, Autophagy 5 (2009) 706–708.
[146] A. Williams, S. Sarkar, P. Cuddon, E.K. Ttofi, S. Saiki, F.H. Siddiqi, L. Jahreiss, A. Fleming, D. Pask, P. Goldsmith, C.J. O'Kane, R.A. Floto, D.C. Rubinsztein, Novel targets for Huntington's disease in an mTOR-independent autophagy pathway, Nat. Chem. Biol. 4 (2008) 295–305.
[147] S. Sarkar, R.A. Floto, Z. Berger, S. Imarisio, A. Cordenier, M. Pasco, L.J. Cook, D.C. Rubinsztein, Lithium induces autophagy by inhibiting inositol monophosphatase, J. Cell Biol. 170 (2005) 1101–1111.
[148] F. Fornai, P. Longone, L. Cafaro, O. Kastsiuchenka, M. Ferrucci, M.L. Manca, G. Lazzeri, A. Spalloni, N. Bellio, P. Lenzi, N. Modugno, G. Siciliano, C. Isidoro, L. Murri, S. Ruggieri, A. Paparelli, Lithium delays progression of amyotrophic lateral sclerosis, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 2052–2057.
[149] H.L. Yeh, S.J. Tsai, Lithium may be useful in the prevention of Alzheimer's disease in individuals at risk of presenile familial Alzheimer's disease, Med. Hypotheses 71 (2008) 948–951.
[150] S. Yousefi, R. Perozzo, I. Schmid, A. Ziemiecki, T. Schaffner, L. Scapozza, T. Brunner, H.U. Simon, Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis, Nat. Cell Biol. 8 (2006) 1124–1132.
[151] D.T. Madden, L. Egger, D.E. Bredesen, A calpain-like protease inhibits autophagic cell death, Autophagy 3 (2007) 519–522.
[152] F. Demarchi, C. Bertoli, T. Copetti, I. Tanida, C. Brancolini, E.L. Eskelinen, C. Schneider, Calpain is required for macroautophagy in mammalian cells, J. Cell Biol. 175 (2006) 595–605.
[153] W. Gao, W.X. Ding, D.B. Stolz, X.M. Yin, Induction of macroautophagy by exogenously introduced calcium, Autophagy 4 (2008) 754–761.
[154] S.N. Djakovic, L.A. Schwarz, B. Barylko, G.N. DeMartino, G.N. Patrick, Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II, J. Biol. Chem. 284 (2009) 26655–26665.
[155] H. Chen, S. Polo, P.P. Di Fiore, P.V. De Camilli, Rapid Ca^{2+}-dependent decrease of protein ubiquitination at synapses, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 14908–14913.
[156] C. Realini, M. Rechsteiner, A proteasome activator subunit binds calcium, J. Biol. Chem. 270 (1995) 29664–29667.