The Stress Rheostat: An Interplay Between the Unfolded Protein Response (UPR) and Autophagy in Neurodegeneration

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Abstract: The unfolded protein response (UPR) is a conserved adaptive reaction that increases cell survival under conditions of endoplasmic reticulum (ER) stress. The UPR controls diverse processes such as protein folding, secretion, ER biogenesis, protein quality control and macroautophagy. Occurrence of chronic ER stress has been extensively described in neurodegenerative conditions linked to protein misfolding and aggregation, including Amyotrophic lateral sclerosis, Prion-related disorders, and conditions such as Parkinson's, Huntington's, and Alzheimer's disease. Strong correlations are observed between disease progression, accumulation of protein aggregates, and induction of the UPR in animal and *in vitro* models of neurodegeneration. In addition, the first reports are available describing the engagement of ER stress responses in brain postmortem samples from human patients. Despite such findings, the role of the UPR in the central nervous system has not been addressed directly and its contribution to neurodegeneration remains speculative. Recently, however, pharmacological manipulation of ER stress and autophagy – a stress pathway modulated by the UPR – using chemical chaperones and autophagy activators has shown therapeutic benefits by attenuating protein misfolding in models of neurodegenerative disease. The most recent evidence addressing the role of the UPR and ER stress in neurodegenerative disorders is reviewed here, along with therapeutic strategies to alleviate ER stress in a disease context.

INTRODUCTION

Neurodegenerative disorders such as Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), Prion-related disorders (PrD) and Huntington's disease (HD) share a common neuropathology, primarily featuring the presence of abnormal protein inclusions containing specific misfolded proteins. These groups of diseases are now classified as Protein Misfolding Disorders [1-4]. Increasing evidence indicates that organelle stress is a key event in neurodegeneration. For example, several disease-related mutant proteins activate stress signaling responses through a pathway known as the Unfolded Protein Response (UPR), which originates from the endoplasmic reticulum (ER) (Table **1**). The ER is a specialized subcellular compartment essential for the folding of proteins destined for the secretory pathway. The UPR is an adaptive reaction that aims to reestablish homeostasis under stress conditions by restoring the cells capacity to produce properly folded proteins. In doing so, activation of the UPR affects expression of proteins involved in nearly every aspect of the secretory pathway, including folding, ER-associated degradation, protein entry to the ER, as well as other adaptive responses such as macroautophagy. Under chronic or irreversible ER damage the UPR ultimately initiates apoptosis to eliminate damaged cells. It has been proposed that the accumulation of protein aggregates and/or misfolded proteins in neurodegenerative conditions may exert their deleterious effects through direct engagement of ER stress. However, the actual contribution of the pathway to the disease process remains speculative and most of the available data is either correlative or based on *in vitro* evidence. This review focuses on recent findings implicating the activation of the UPR in pathological conditions affecting the nervous system. We also discuss the role of autophagy in protein misfolding disorders as a novel mechanism that may participate in the clearance of abnormal protein aggregates.

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I. MECHANISM OF ADAPTATION TO CELLULAR STRESS

ER Stress and the UPR Pathway

The primary function of the ER is to facilitate protein folding and secretion. An efficient and complex system of protein chaperones is employed to promote folding and prevent abnormal aggregation or misfolding of proteins. This organelle is responsible for regulating and executing many posttranslational modifications, ensuring proper protein function and facilitating the formation of protein complexes. The ER also serves as the major calcium store, and biosynthesis of steroids, cholesterol, and other lipids occurs within, playing a crucial role in organelle biogenesis and signaling. A number of stress conditions can interfere with its function and therefore lead to abnormal protein folding in the ER lumen. Accumulation of unfolded and/or misfolded proteins

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Table 1. ER Stress and Neurodegeneration. The table summarizes information obtained from animal and cellular models of neurodegeneration. Experimental validation for activation of the UPR in human patients affected with neurological diseases is also indicated from analysis of post-mortem samples

Protein Conformational Disorder	ER stress -mediated Apoptosis	UPR markers	Human disorder
Alzheimer	*In vitro* and *in vivo*	✓	✓
Parkinson	*In vitro* and *in vivo*	✓	✓
Amyotrophic Lateral Sclerosis	*In vitro* and *in vivo*	✓	✓
Prion disorders (TSEs)	*In vitro* and *in vivo*	✓	✓
Retroviral Spongiform degeneration	*In vitro* and *in vivo*	✓	
Huntington	*In vitro*	✓	
Kennedy disease	*In vitro*	✓	
Spinicerebellar Ataxias	*In vitro*	✓	
Vanishing White Matter Disease	ND	✓	✓
Brain Ischemia/Brain trauma	*In vitro* and *in vivo*	✓	

causes an imbalance between the synthesis of new proteins and the ER's ability to process newly synthesized proteins, resulting in a condition termed 'ER stress' [5]. As a result, cells activate an integrated intracellular signaling cascade, the UPR, to avert ER stress. Activation of the UPR results in an attenuation of the rate of protein synthesis, upregulation of genes encoding chaperones, foldases, and proteins involved in the retrotranslocation and degradation of ER-localized proteins by the proteasome. These responses are initiated to minimize accumulation and aggregation of misfolded proteins by increasing the functional capacity of the ER to facilitate folding and ER-associated degradation (ERAD) [6,7].

There are three main ER resident transmembrane proteins that act as stress sensors to initiate different UPR signaling cascades: double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6) alpha and beta, and inositol requiring kinase 1 (IRE1) alpha and beta (Fig. **1A**). Each of these stress sensors transduces information regarding protein folding status from the ER to the nucleus by controlling expression of specific transcription factors. Thus, PERK, ATF6, and IRE1α operate in concert to ensure adaptation to protein folding stress (reviewed in [8]).

Activation of PERK is mediated by its oligomerization and autophosphorylation, leading to the phosphorylation and inhibition of eukaryotic translation initiation factor 2α (eIF2α) [9]. Alternatively, eIF2α phosphorylation augments the specific translation of ATF4, a UPR transcription factor essential for the upregulation of many essential UPR genes such as CHOP and Grp78/BiP. Activated ATF6 translocates from the ER to the Golgi apparatus where it is proteolyzed, releasing its cytosolic domain which is then translocated to the nucleus to function as an active transcription factor. This cleaved form of ATF6 leads to the upregulation of several ER chaperones [10-12].

The third adaptive response is regulated by IRE1α and its downstream target X-Box-binding protein 1 (XBP-1). IRE1α is a Serine/Threonine protein kinase and endoribonuclease that, upon activation, initiates the unconventional splicing of the mRNA encoding the transcription factor XBP- 1 [13-15] (Fig. **1A**). A 26 nucleotide intron of XBP-1 mRNA is removed by activated IRE1α, resulting in an alternative splice form which shifts the mRNA reading frame. This splicing event promotes the expression of a more stable and potent transcriptional activator, XBP-1s, that controls the upregulation of a subset of UPR-related genes (Fig. **1A**). Through this process IRE1α transduces survival signals to the nucleus to increase the folding capacity of the ER, therefore alleviating stress. In addition, activated IRE1α binds the adaptor protein TRAF2, leading to the activation of several signaling pathways including ERK, JNK and NF-κB [6]. The signaling activity of IRE1α is modulated by the formation of a protein complex termed the *UPRosome* (reviewed in [8,16]), which is controlled by the binding of different accessory proteins including some members of the BCL-2 protein family and other components [16,17].

Autophagy

Autophagy is a "large-scale" cellular degradation process for proteins and damaged organelles [18]. Autophagy is divided in three subtypes, including macroautophagy, microautophagy, and chaperone-mediated autophagy. This review will focus on macroautophagy, hereafter referred to as autophagy. During autophagy, double membrane vesicles termed autophagosomes are formed, sequestering cytosolic proteins and/or organelles as cargoes. Autophagosomes then fuse with lysosomes, and their intracellular components are degraded to ultimately result in macromolecule recycling [18] (Fig. **1B**). Autophagy occurs at basal levels in most tissues [19], yet

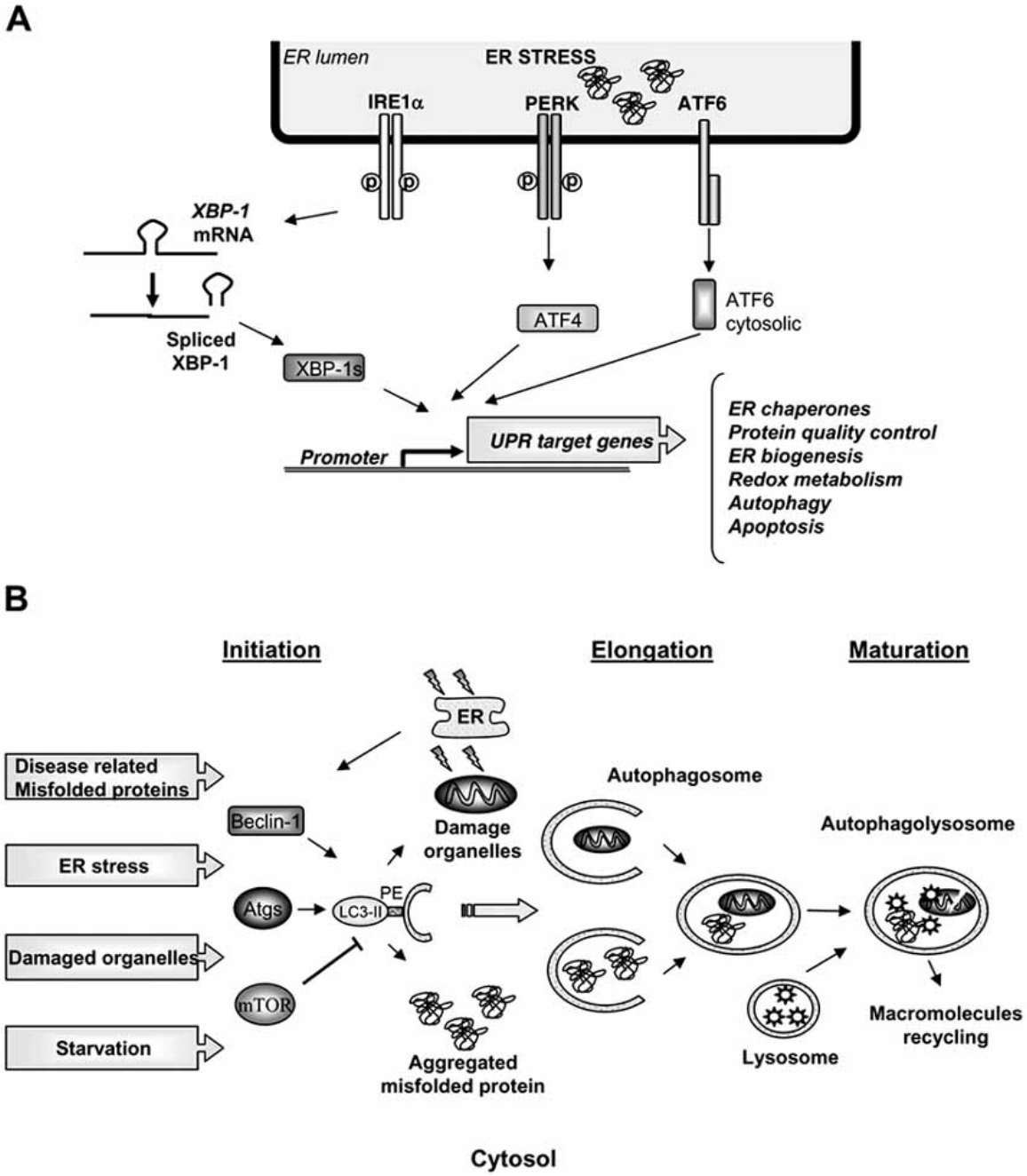

Fig. (1). The unfolded protein response and autophagy. A) Accumulation of misfolded protein inside the endoplasmic reticulum (ER) lumen triggers a stress response known as the unfolded protein response (UPR). In cells undergoing ER stress, IRE1α auto-phosphorylates, leading to the activation of its endoribonuclease activity. This activity mediates the processing of the mRNA encoding *XBP-1*, which is a transcription factor that upregulates many essential UPR genes involved in folding and protein quality control. Alternatively, activation of PERK increases the translation of *ATF4*, a transcription factor that induces the expression of genes such as CHOP that function in amino acid metabolism, antioxidant response, and apoptosis. A third UPR pathway is initiated by ATF6, a type II ER transmembrane protein encoding a bZIP transcriptional factor on its cytosolic domain and localized in the ER in unstressed cells. Upon ER stress induction, ATF6 is processed, increasing the expression of some ER chaperones. **B**) Autophagy can be induced for several stress stimuli including ER stress, nutrient starvation and accumulation of protein aggregates. Different proteins regulate the initiation of autophagy, and the formation and activation of kinase complexes with Atg proteins triggers nucleation and elongation leading to formation of the double-membrane autophagosome. Beclin-1 also mediates the initiation of autophagy and is negatively regulated through an interaction with BCL-2 at the ER membrane. Inhibition of the mammalian target of rapamycin (mTOR) activates autophagy. Finally, the autophagosome matures by fusing with endosomes and lysosomes forming the autophagolysosome where the internal material is degraded, resulting in macromolecule recycling. Phosphatidylethanolamine (PE)-conjugated LC3-II is the most used marker that specifically localizes to autophagosomes.

activation preferentially occurs mainly under two different stress conditions: during starvation it acts to promote survival, and in times when misfolded proteins or damaged organelles are present it acts to rid the cell of altered intracellular components [20,21]. These complex, highly regulated processes are controlled by a family of autophagy-related genes of the *atg* family. Atg proteins have different functions in the process, including the formation of a protein kinase-autophagy regulatory complex that responds to upstream signals such as nutrient limitation, a lipid kinase signaling complex which is involved in vesicle nucleation, ubiquitin-like protein conjugation pathways required for vesicle expansion and completion, and a retrieval pathway for catalyzing the disassembly of Atg protein complexes from matured autophagosomes. LC3, also known as Atg8, is the most commonly used intracellular autophagy marker, as it specifically localizes to autophagosomes (Fig. **1B**). Beclin-1, also known as Atg6, was the first mammalian autophagy gene product identified as being essential for initiation of autophagy. Beclin-1 is regulated at the ER membrane by members of the BCL-2 protein family, suggesting that signaling events originating from the ER are crucial for autophagy.

Recent reports indicate that autophagy is critical for the maintenance of neuronal homoeostasis and has a role in the basal elimination of misfolded, ubiquitinated proteins. Brain specific knockout animals for essential autophagic-related genes develop spontaneous neurodegeneration with pathological features similar to Alzheimer's and Parkinson's disease [22,23]. These data suggest that the continuous clearance of cellular proteins in the brain through basal autophagy is required to prevent abnormal accumulation, therefore acting as a neuroprotective mechanism.

A connection between the ER and autophagy was recently proposed to occur through the IP3 receptor and BCL-2 [24-26] (reviewed in [25]). IP3 receptor-dependent autophagy was attributed to the activation of Beclin-1 and other autophagic related genes, but, unexpectedly, this regulation was independent of calcium release. Many laboratories have shown that ER stress triggers autophagy, and this effect is also regulated by UPR stress sensors such as IRE1α and PERK [24,27-32]. Autophagy may serve as a mechanism to eliminate portions of damaged ER under stress conditions or to control the rate of ER expansion [30]. Unexpectedly, the activation of autophagy by ER stress requires IRE1α and is not inhibited by BCL-2 overexpression (a known inhibitor of Beclin-1), suggesting the occurrence of different autophagy pathways emerging from the ER [18].

Apoptosis, Chronic ER Stress, and the BCL-2 Protein Family

Complex signaling responses mediate adaptation to organelle stress or initiation of cell death processes when a critical threshold of damage has been reached. Execution of apoptosis depends on the activation of caspases, a process tightly regulated by the BCL-2 family of proteins. The BCL-2 family is comprised of pro- and anti-apoptotic members that are defined by the presence of up to four conserved domains (Fig. **2A**). Anti-apoptotic BCL-2 family members display sequence homology in four α-helical domains called BCL-2 homology (BH) 1 to BH4. Pro-apoptotic BCL-2 members can be further subdivided into more highly conserved, "multidomain" members displaying homology in the BH1, BH2 and BH3 domains (i.e. BAX and BAK), or the "BH3-only" members (i.e. BIM, PUMA and NOXA) which contain a single domain critical for activation of apoptosis. BH3-only proteins are thought to operate as sentinels of cellular damage [33], as they are activated in response to various death stimuli such as oxidative stress, DNA damage, or death receptor engagement by either transcriptional upregulation or post-translational modification. BH3-only proteins then promote the activation of the core pro-apoptotic components BAX and/or BAK [34], resulting in mitochondrial membrane permeabilization. Released mitochondrial proteins such as cytochrome *c* then trigger caspase-mediated cell death. BH3-only proteins can be functionally separated into two subtypes (Fig. **2B**): (i) *activators* (i.e. BID, BIM, and PUMA) that directly activate BAX and BAK to trigger cytochrome c release, but are sequestered by anti-apoptotic BCL-2 molecules, and (ii) *sensitizers* (i.e. BAD and NOXA) that only bind to and antagonize anti-apoptotic BCL-2 members to release activator BH3-only proteins [35-37] (Fig. **2B**). Alternatively, differential binding to anti-apoptotic proteins may explain the separation between activator and sensitizer BH3-only proteins [38].

Chronic or irreversible ER stress results in apoptosis. The initial signal or sensing mechanism that activates apoptosis in ER damaged cells has yet to be defined, but many different players have been identified which mediate caspase-dependent cell death downstream of ER stress [39]. Two BH3-only proteins, PUMA and NOXA, are strongly induced at the transcriptional level in cells undergoing prolonged ER stress. *Puma* or *noxa* deficient cells are partially resistant to apoptosis induced by ER injuries. Another BH3-only family member, BIM, is highly induced by ER stress. Under normal conditions BIM is found in the dynein motor complex of the microtubule cytoskeleton, whereas induction of ER stress causes BIM to translocate to the ER, where it may promote caspase activation through an unknown mechanism. In addition, dephosphorylation of BIM by the phosphatase 2A under ER stress increases BIM levels by preventing its ubiquitination and subsequent proteasomal degradation in different cell types. Further, expression of the pro-apoptotic UPR transcription factor CHOP triggers the upregulation of BIM mRNA. These results provide a direct connection between activation of the UPR and the core pro-apoptotic program, a phenomenon that has remained largely unclear. Moreover, *in vivo* studies have shown that BIM deficient mice are resistant to ER stress-induced apoptosis, similar to the phenotype described for *chop* deficient mice [40,41]. In summary,

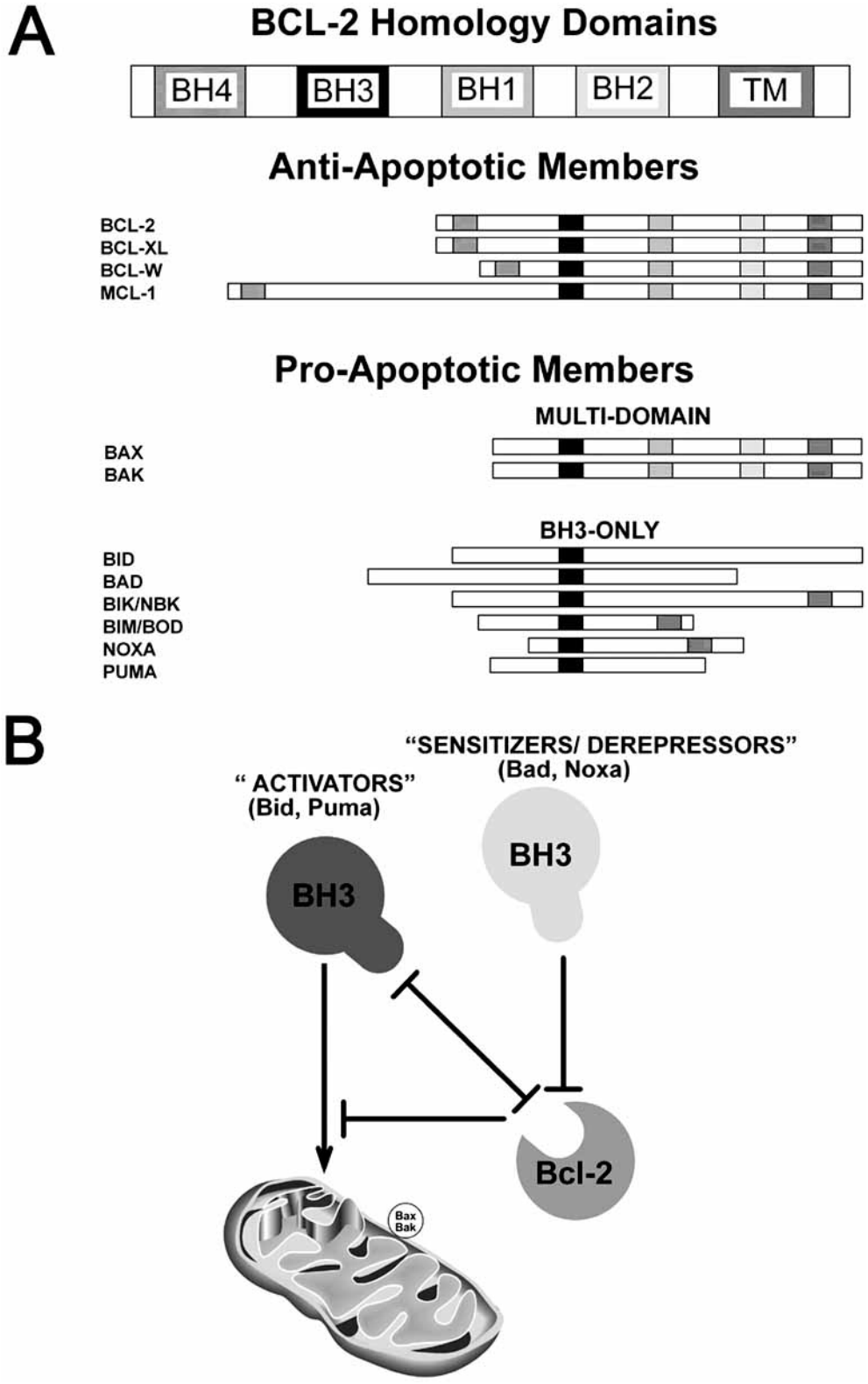

Fig. (2). The BCL-2 family members. A) The BCL-2 family of proteins is defined by the presence of up to four domains homologous to BCL-2. This group of proteins is functionally subdivided into pro- and anti-apoptotic proteins. Pro-apoptotic members can be further subdivided into more fully conserved "multidomain" members possessing BCL-2 homology domains 1, 2, and 3, or "BH3-only" members which display only about nine amino acids of sequence homology within the single death-promoting domain. **B**) Model of activation of BAX/BAK by BH3-only protein. Some BH3-only proteins have been proposed to activate BAX/BAK directly (BID, PUMA), whereas others work by neutralizing prosurvival BCL-2 proteins (BAD, NOXA), lowering the threshold for activation of apoptosis.

irreversible ER damage triggers the upregulation of pro-apoptotic BH3-only proteins, which may then converge to initiate activation of BAX and BAK at the mitochondria to cause cell death.

In murine cells the processing of ER-resident caspase-12 depends on the expression of BAX and BAK at the ER. Caspase-12 has been suggested to be linked to the UPR pathway through an interaction with TNF-receptor associated factor-2 (TRAF2) and possibly with active IRE1α, but a complex between procaspase-12/TRAF2/IRE1α has not been described. Although caspase-12 processing is a well established ER stress marker, its contribution to apoptosis is actively debated. Other components have been shown to control ER stress-induced apoptosis, and for extensive reviews see [42,43].

II. PROTEIN MISFOLDING AND NEURODEGENERATION

As previously mentioned, a common feature of many neurodegenerative diseases is the accumulation and deposition of misfolded proteins, thus affecting neuronal function and viability [1-4]. Experimental evidence using mouse models of neurodegeration indicate that several mutations associated with hereditary forms of disease activate various components of the UPR (Table **1**), implying impaired ER function (Fig. **3**). More importantly, upregulation of ER stress markers has been observed in post-mortem brain tissues and cell culture models of many protein conformational disorders, including Parkinson's, Amyotrophic lateral sclerosis**,** Alzheimer's and Creutzfeldt-Jacob disease [44]. The question remains, however, as to how much of the neurodegeneration is due to ER stress as opposed to other pathways. Little is known about crosstalk and signalling between organelles and how such interactions result in irreversible neuronal damage. The expected functional significance of ER stress to the disease process is proposed to be both a protective component during early UPR responses and more deleterious during prolonged response due to disturbance of ER homeostasis [44]. In the following sections we discuss specific evidence linking ER stress to neurodegeneration and the possible involvement of other stress pathways, such as autophagy, in the process.

Amyotrophic Lateral Sclerosis (ALS)

ALS is a progressive adult-onset motoneuron disease characterized by muscle weakness, atrophy, pa-

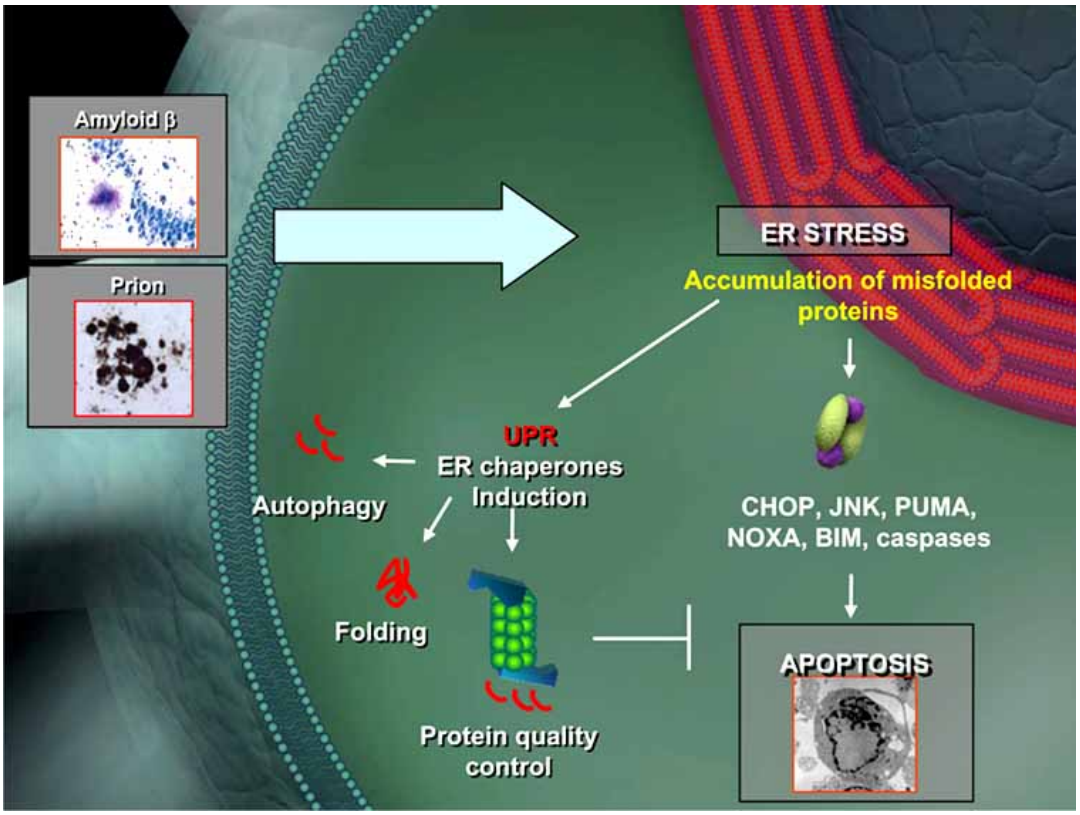

Fig. (3). Protein misfolding and neurodegeneration. The accumulation and deposition of misfolded protein is a common feature of many neurodegenerative diseases. Activation of various components of the UPR is observed in several Protein misfolding disorders affecting the nervous system. The early UPR response activates survival pathways to reestablish cellular homeostasis and inhibit apoptosis. These responses include increased protein folding through the upregulation of chaperones, increased protein quality control, and enhancement of autophagy. Under chronic or irreversible ER damage the UPR ultimately initiates apoptosis to eliminate damaged cells contributing to neurodegenerative processes of disease.

ralysis and premature death. The pathological hallmark of ALS is the selective degeneration of motoneurons in the spinal ventral horn, most brainstem nuclei and the cerebral cortex [45]. The majority of ALS patients lack a defined hereditary genetic component and are considered sporadic, whereas approximately 10% of cases are familial (fALS). Over 100 mutations affect the gene encoding superoxide dismutase-1 (SOD1), a causative mutation of some forms of fALS. Mutations in SOD1 trigger its misfolding and abnormal aggregation, leading to a gain of neurotoxic activity. Overexpression of human fALS-linked SOD1 mutations in transgenic mice recapitulates some essential features of the human pathology, provoking age-dependent protein aggregation, paralysis and motoneuron degeneration [45,46]. The primary mechanism by which mutations in SOD1 contribute to progressive motoneuron loss in fALS remains unknown, but it has been proposed that motoneuron apoptosis is mediated by different mechanisms including mitochondrial dysfunction, altered axonal transport, endoplasmic reticulum stress and other non-neuronal components [45].

Recently, the activation of UPR responses in human post-mortem samples was described in both fALS and sporadic ALS patients, indicating that ER stress may be a general phenomena in different forms of ALS [45]. There are several lines of evidence for the occurrence of ER stress in transgenic rodents expressing different fALS-related SOD1 mutations. Pre-symptomatic upregulation of the ER chaperone Grp78/BiP was initially reported in spinal motor neurons of transgenic $SOD1^{H46R}$ and $SOD1^{L84V}$ mice [47]. However, this finding was not clear in transgenic $SOD1^{G93A}$ mice even at paralysis. Two studies recently evaluated UPR signaling in transgenic $SOD1^{G93A}$ rodents and found activation of numerous UPR elements in the spinal cord, including IRE1α, PERK, and processed ATF6. Upregulation of their downstream targets XPP-1s, ATF4 and phospho-eIF2α was also observed [48]. An age-dependent activation of UPR sensors specific to degenerating areas was found in mutant SOD1 mice and associated with a direct accumulation of SOD1 aggregates at the ER [49]. Moreover, a physical interaction between the ER chaperone Grp78/BiP and mutant SOD1 was observed in microsomal fractions of spinal cord extracts. Additionally, a proteomic analysis of spinal cord extracts from $SOD1^{G93A}$ transgenic mice revealed that two UPR targets, protein disulfide isomerase (PDI) and endoplasmic reticulum protein 57 (Grp58/ERp57), are the most highly induced proteins in this ALS model, highlighting the relevance of ER stress in the disease process. Similar to Grp78/BiP, an interaction between PDI and mutant SOD1 was described at the ER which may have a neuroprotective function against SOD1 aggregation [50]. PDI and Grp58 are both ER chaperones of the PDI family that catalyze the formation and rearrangement of intra- and intermolecular disulfide bonds. The importance of the Cys57–Cys146 disulfide bond in stabilizing dimeric SOD1 to prevent aggregation has been increasingly recognized. In this line, mutant SOD1 aggregates are thought to be formed by abnormally disulfide-bonded

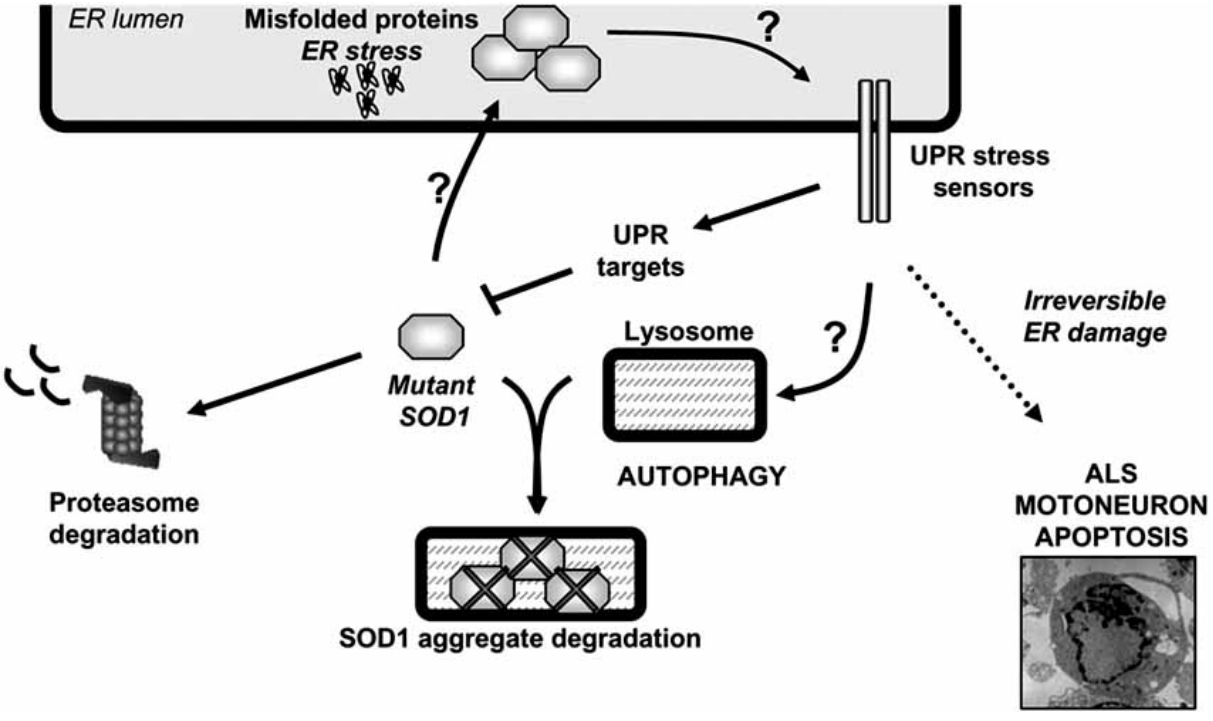

Fig. (4). Signaling UPR and Autophagy in ALS. In animal models of ALS, mutant SOD1 accumulates at the ER, inducing the UPR by an unknown mechanism. Extensive ER stress may contribute to neuronal apoptosis in the disease process. In addition, there is evidence suggesting that the UPR may control autophagy, a degradation pathway known to be involved in the clearance of mutant SOD1 aggregates.

multimers in the spinal cord of affected SOD1 mutant mice [51]. It is possible that PDIs may play an important role in forming the correct disulfide bonds in SOD1, suggesting a direct involvement of ER-related components in the pathogenesis of SOD1 mutants. Finally, two reports have shown that, in addition to mislocating to the ER, SOD1 mutants translocate to the Golgi to be secreted into the extracellular space [52,53]. In summary, increasing reports indicate that adaptive responses against ER stress are activated in fALS, possibly due to a direct accumulation of mutant SOD1 aggregates at the ER (Fig. **4**).

Different pieces of evidence suggest that chronic or irreversible ER stress may play an important role in motoneuron loss in ALS. Many groups have described the upregulation of ER stress related pro-apoptotic factors in the spinal cord of late stage disease animals, including CHOP and caspase-12 processing [50,54-57]. In addition, ER stress inducible BCL-2 pro-apoptotic genes such as BIM and PUMA are upregulated in symptomatic mutant SOD1 transgenic mice [55,58]. More importantly, genetic deletion of BIM or PUMA delays ALS disease onset, possibly due to a significant decrease of apoptosis in motoneurons. This data is in agreement with the fact that BAX deficient or BCL-2 transgenic mice exhibit an increased life span due to augmented motoneuron survival [59,60].

The question remains as to how cells manage to accumulate mutant SOD1. Kabuta and colleagues reported that activation of autophagy reduces $SOD1^{G93A}$ mediated toxicity and overall protein levels a neuroblastoma cell line. The authors proposed that the contribution of autophagy to mutant SOD1 degradation was comparable to that of proteasome pathway [61] (Fig. **4**). Increased levels of LC3 processing and inhibition of mTOR were observed in $SOD1^{G93A}$ transgenic mice [62], possibly inferring that autophagy has a relevant role as a mechanism of elimination of SOD1 protein aggregates. The therapeutic benefits of targeting the pathway are yet to be established.

Prion-Related Disorders

PrDs, also known as transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders characterized by spongiform degeneration of the brain accompanied by accumulations of a misfolded and partially protease-resistant form of the prion protein (PrP^{Sc} or PrP^{RES}) [63]. Etiologically, TSEs can be classified as sporadic, infectious, or autosomal dominant inherited forms, affecting both humans and other mammals. In humans, Prion disorders include classic Creutzfeldt-Jakob disease (CJD), new variant CJD, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia and kuru. Prion diseases in other mammals include bovine spongiform encephalopathy, commonly known as mad cow disease, chronic wasting disease in elk and deer, and scrapie in sheep and goats [64]. This group of diseases generally presents a long incubation period followed by rapid progression to death shortly after the first clinical signs appear. Although clinical symptoms and progression vary among TSEs, they commonly include progressive dementia and ataxia as a result of extensive neuronal death due to reactive astrocytosis and activated microglia during neuro-inflammation [65].

The most widely accepted “protein-only” hypothesis postulates that infectious prion pathogenicity results from a conformational change of normal cellular prion protein (PrP^{C}) from its primarily α-helical structure to a insoluble β sheet conformation (PrP^{Sc}) initiated by an direct interaction between the two PrP forms. No amino acid sequence or post-translational modification differences have been detected between the normal host PrP^{C} and its pathological form, PrP^{Sc} [66]. Like other membrane proteins, PrP^{C} undergoes post translational processing in the ER and Golgi. It then localizes to cholesterol-rich lipid rafts, where it is anchored to the outer surface of the plasma membrane with a glycosyl phosphatidyl inositol (GPI) anchor [67]. Despite the vast body of research, the physiological role of PrP^{C} is still under debate [68].

Apoptosis has been described in the brains of patients affected by CJD, Fatal Familial Insomnia, scrapie-infected hamsters, mice and sheep (reviewed in [69]). Several *in vitro* and *in vivo* models for Prion-related disorders have been developed to understand the molecular basis of neuronal dysfunction in infectious and inherited forms of Prion disease [70-73]. However, the exact mechanism by which Prion misfolding affects neuronal function is not well understood. Our group, as well as many others, has shown an engagement of ER stress responses in PrD models, where XBP-1 splicing [74] and the activation of stress signaling pathways linked to ER stress, such as JNK and ERK, are observed in scrapie infected mice [74,75]. In both human CJD patients and mouse models, the upregulation of UPR-responsive chaperones such as Grp78/BiP, Grp94, and Grp58 is observed in the brain [76,77]. In addition, a proteomic analysis of post-mortem CJD brain samples demonstrated that the disulfide isomerase Grp58 was highly expressed in the cerebellum of humans patients affected with sporadic CJD [78]. *In vitro* studies using Neuro2a neuroblastoma cells demonstrated that inhibition of Grp58 expression using RNA interference leads to a significant enhancement of PrP^{Sc} toxicity. Conversely, overexpression of Grp58 protected cells against PrP^{Sc} toxicity and decreased the rate of caspase-12 activation, suggesting that ER stress is an early protective cellular response to prion replication, acting as a neuroprotective mechanism against prion neurotoxicity [79]. Recent evidence indicates that the activation of UPR components such as IRE1α, XBP-1 and others determines the rate of PrP^{C} aggregation *in vitro* under stress conditions [80,81] and in yeast models [82], suggesting that the UPR has an active role in preventing neurodegeneration. We recently described conditions in which ER stress facilitates *in vitro* conversion of PrP^{C} into PrP^{Sc} due to partial misfolding of PrP^{C} [80], which was reverted by activation of UPR components such as XBP-

1, ATF4 and ATF6. In addition, scrapie infected neuroblastoma cells are more sensitive to ER stress mediated apoptosis [76]. Because PrP^{Sc} induces ER stress, this data present a vicious cycle where alterations of ER physiology prompt PrP^{C} more susceptible to conversion into PrP^{res}, most likely explaining the rapid progression of the disease.

Despite the fact that chronic ER stress has been extensively described in neurodegenerative conditions linked to protein misfolding and aggregation, the role of the UPR in the central nervous system had not been studied directly. To address this question, we recently described the generation of a brain specific XBP-1 conditional knockout strain ($XBP\text{-}1^{Nes\text{-}/\text{-}}$) and assessed the function of XBP-1 prion pathogenesis [74]. To our surprise, the activation of stress responses triggered by prion replication, such as Grp58 and PDI induction, caspase-12 processing, JNK and ERK phosphorylation, were not influenced by XBP-1 deficiency. Neither prion aggregation, neuronal loss or animal survival were affected. These findings imply that this highly conserved arm of the UPR may not contribute to the occurrence or pathology of neurodegenerative conditions associated with Prion misfolding, despite predictions that such diseases are related to ER stress. Alternatively, one may speculate that the upregulation of ER chaperones is due to specific regulation of their promoters and not UPR activation. Since the UPR is not restricted to the IRE1α/XBP-1 pathway in mammals, an activation of other UPR pathways may possibly compensate for XBP-1 deficiency in our prion model. The contribution of ATF6 and PERK to prion pathogenesis remains to be determined using genetic manipulation *in vivo*.

Similarly, Steele *et al.* recently described that caspase-12 knockout mice are normally susceptible to prion infection and pathogenesis despite a clear activation of the UPR in their model [in press]. Pro-apoptotic BH3-only proteins such as BIM and PUMA are induced in two different models of scrapie, suggesting that other ER stress related pro-apoptotic pathways may modulate neuronal loss in infectious Prion-related disorders [74, in press]. However, neither BAX deletion or BCL-2 overexpression affect infectious prion pathogenesis, suggesting that apoptosis is not essential for disease progression, and that it may be a downstream effect of neuronal dysfunction (in press).

In addition to cell death with apoptotic features, autophagic vesicles are also observed in the brain of patients affected with TSE [84]. As mentioned, the autophagic pathway normally functions as a cellular defense process to degrade cellular contents, yet overproduction of autophagic vesicles may cause autophagic cell death with the degradation of cellular organelles [85]. In another report using prion-infected neuronal cells, mild proteasome impairment resulted in the formation of large cytosolic perinuclear aggresomes that contained PrP^{Sc}, heat shock chaperone 70, ubiquitin, proteasome subunits and vimentin [86]. The role of autophagy in the progression of prion diseases is unknown, but the potential to promote clearance of misfolded protein aggregates may be a promising therapeutic approach [87].

Parkinson's Disease

PD is the second most common chronic and progressive neurodegenerative disease, affecting at least one in one hundred of the population over 55 years old. The major clinical symptom of PD is the impairment of motor control as a result of extensive dopaminergic neuron death in the *substantia nigra pars compacta* (SNpc) [88,89]. The pathological hallmark of the disorder is the presence of intracellular inclusion bodies, called Lewy bodies (LBs), which contain aggregated and misfolded α-synuclein protein [90]. Although the cause of selective dopaminergic neuron loss and accumulation of α-synuclein in patients with Parkinson´s disease is unknown, oxidative stress as a result of mitochondrial dysfunction and impairment in the major cellular proteolytic systems have been proposed to contribute to the accumulation of intracellular protein aggregates [91].

Genetic mutations have provided important insight into cellular pathways and molecular mechanisms involved in PD. Some familial cases of PD are associated with mutations in *α-synuclein* [92], but LBs contain α-synuclein even in sporadic PD cases lacking mutations, suggesting a more central role for this protein in PD's pathogenesis. A recent yeast screening identified the earliest defect following α-synuclein expression in both wild type or A53T mutants is a block in ER to Golgi vesicular trafficking with concomitant UPR activation [93]. The largest class of α-synuclein toxicity modifiers described from this screen are proteins functioning in ER/Golgi trafficking, including Rab1, which physically associates with cytoplasmic α-synuclein inclusions. Remarkably, Rab1 protected against α-synuclein-induced dopaminergic neuron loss in animal models of PD [93]. A recent study reported the first evidence of UPR activation in post-mortem tissue from sporadic PD human cases, observing activation of the PERK/eIF2α pathway in dopaminergic neurons of the SNpc. A strong co-localization between α-synuclein inclusions and PERK phosphorylation was observed [94]. In addition, the pancreatic PDI isoform PDIp is highly induced in PD post-mortem human brain samples [95], and brains manifesting sporadic Parkinson's or Alzheimer's disease were observed to have inactivated PDI due to S-nitrosylation [96]. Inhibition of PDI activity was proposed to result in abnormal folding, triggering the accumulation of polyubiquitinated proteins and consequent ER stress and cell death. Finally, *in vitro* studies indicate that overexpression of $\alpha\text{-synuclein}^{A53T}$ decreases proteasome activity and leads to ER stress [97]. Knocking-down caspase-12 or treating of cells with salubrinal, an inhibitor of eIF2a dephosphorylation, partially protects against $\alpha\text{-synuclein}^{A53T}$ toxicity.

The most frequent type of familial PD is autosomal recessive, juvenile Parkinsonism (AR-JP) [98] caused by mutations in the *parkin* gene [98,99]. Parkin is ex-

pressed diffusely in neurons throughout the brain [100] and is suggested to be a component of the ubiquitin-proteasome system (UPS) [101,102]. Parkin may be involved in ERAD, which acts to eliminate misfolded or unassembled proteins from the secretory pathway. The absence of Parkin function presumably allows its protein substrates to accumulate and result in ER stress, but data regarding the involvement of Parkin in the proteasome is controversial [103]. Experimental ER stress results in significant upregulation of Parkin at both the mRNA and protein level [101]. In the same study, Parkin transfected cells were significantly more resistant to cell death induced by ER stress, indicating that it may have a protective role [101]. A recent study in mice found that viral overexpression of Parkin-associated endothelin-receptor (Pael-R) in the SNpc results in increased expression of ER chaperones Grp78 and ORP150 [104]. Conversely, overexpression of Pael-R in Parkin null mice results in apoptotic cell death in the SNpc.

A newly described autosomal recessive loss of function mutation linked to PD was recently characterized in the Chilean population. The protein, identified as a neuronal P-type ATPase gene, ATP13A2, results in early-onset parkinsonism with pyramidal degeneration and dementia (PARK9, Kufor-Rakeb syndrome) [105]. ATP13A2 is normally located in the lysosome, but mutants were retained in the ER and degraded by the proteasome. It remains to be determined if ER stress or organelle dysfunction is altered by ATP13A2 mutants.

Toxicological models that resemble sporadic PD also suggest an involvement of ER stress in the disease process. 6-hydroxy-dopamine (6-OHDA) and MPP^+ are the most commonly used compounds to study PD, as they specifically induce death of dopaminergic neurons. Unlike MPP^+, 6-OHDA is produced endogenously by dopamine metabolism and oxidation in the SNpc, leading to mitochondrial dysfunction and oxidative stress [106]. This neurotoxin is commonly found in post-mortem brains of PD patients [107]. Several pieces of evidence indicate that 6-OHDA, MPP^+, and rotenone are capable of specifically inducing ER stress and the UPR. Remarkably, a series of gene expression profile analyses indicate an active UPR-transcriptional response to these neurotoxic agents. 6-OHDA induces a clear phosphorylation of IRE1α, PERK and eIF2α followed by induction of ATF4, CHOP/GADD153 and Grp78/BiP *in vitro* [108], and post-mortem studies on sporadic PD cases exhibit activation of IRE1α and PERK as well as induction of their downstream targets [94]. Sympathetic neurons from PERK null mice were more sensitive to 6-OHDA, suggesting a functional role of the UPR in this PD model [108]. At the mechanistic level, generation of radical oxygen species by PD-triggering neurotoxins may lead to a rapid accumulation of oxidized proteins which can activate the UPR [109]. The expression of CHOP/GADD153 in neurotoxin models of PD is observed, displaying nuclear expression in models of dopaminergic neuron death induced by intrastriatal injection of 6-OHDA and MPTP, a precursor to MPP+ [110]. In chronic MPTP models, however, while CHOP/GADD153 is robustly expressed, *chop* deficient mice are not protected from the loss of neurons, but they are protected against 6-OHDA [110].

There is an increasing interest in studying the role of autophagy and lysosomal pathways in PD. For example, studies in SH-SY5Y human neuroblastoma cells show that dopamine induces features of autophagic cell death and an increase in α-synuclein expression [111]. Depending on its conformational state and cellular conditions, α-synuclein can be degraded by both the ubiquitin-proteasome system and autophagy [112]. However, only soluble α-synuclein forms can be degraded by the proteasome. In fact, fibrillar forms of α-synuclein usually block proteasomal activity [113]. These soluble forms can also reach the lysosomal compartment for degradation *via* chaperone-mediated autophagy (CMA) [114]. Pathogenic α-synuclein mutants, however, are poorly degraded by CMA because, while they bind to the lysosomal membrane with high affinity, they are not translocated into the lysosomal lumen. Furthermore, because of their high-affinity binding to the CMA receptor, mutant α-synuclein inhibits the uptake and degradation of other CMA substrates, leading to a general CMA blockage and cellular dysfunction [114].

A recent work in SH-SY5Y cells showed that, although there are several modified forms of α-synuclein, only the aggregated and oligomeric forms of dopamine-α-synuclein (DA-α-synuclein) cannot be taken-up by lysosomes *via* CMA, therefore interfering with the pathways degredation ability [20]. This idea comes from both *in vivo* mouse and human post-mortem studies of PD, where modified forms of α-synuclein have been observed in the SNpc [115-118]. Multiple post-translational modifications have been shown to impair the degradation of α-synuclein by CMA, favoring its oligomerization and aggregation [114,119]. A crosstalk between both α-synuclein and clearance pathways may exist [120,121], and inhibition of the ubiquitin-proteasome system and CMA activate macroautophagy, possibly to maintain normal levels of protein degradation and removal of cytosolic toxic aggregates [122,123]. While many studies hint at a very important role for autophagy in the control of PD, much remains to clarify our understanding of its involvement in the disease process *in vivo*.

Huntington's Disease and Therapeutic Strategies to Target the UPR and Autophagy

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive cognitive impairment, neuropsychiatric symptoms, and chorea. Onset depends on the severity of the mutation, usually resulting in symptoms beginning in the fourth or fifth decade of life. Patients typically decline over a 15-20 year period until complications lead to death, as there is no effective treatment for the

selective neuronal loss affecting the frontal lobe, striatum, and basal ganglia [124].

HD is caused by a mutation encoding an abnormal expansion of CAG-encoded polyglutamine repeats within the Huntingtin protein. The general population exhibits an average of 18 poly(Q) repeats, yet expansions exceeding 35 repeats will almost always result in disease. Increasing numbers of repeats cause the age of onset to decrease, and individuals with more than 60 repeats will develop disease before the age of twenty [125].

The primary histopathological feature observed in HD is the co-localization of Huntingtin positive-aggregates with ubiquitin [126]. In many cases, the formation of intracellular Huntingtin inclusions precede cell death [127-129], and neurotoxicity is also plausible when considering the array of other proteins found within such aggregates [130,131]. Changes in subcellular localization of Huntingtin-interacting proteins may also contribute to the pathology [131].

Although the mechanism of expanded poly(Q) pathogenesis is still highly controversial, it is well established that neurotoxicity is provoked by a dominant gain of function. Neuronal apoptosis in HD is proposed to be mediated by several different mechanisms, including mitochondrial dysfunction, altered axonal transport, and proteasome dysfunction, to name a few (reviewed in [132]). Recent data also suggests that pro-apoptotic pathways originating from the ER may contribute to HD neurodegeneration. Mutant Huntingtin aggregates impair the function of the ubiquitin-proteasome system [132], resulting in ER stress through general accumulation of abnormally folded proteins, poisoning the cell with abnormal folding intermediates or immature proteins [133].

Recent attempts to understand the function of wild type Huntingtin demonstrated that inhibition of its expression significantly alters ER morphogenesis [134]. In addition, mutant Huntingtin perturbs ER calcium homeostasis [135], and the experimental targeting of poly(Q) peptides to the ER decreases aggregation [136]. Thus, increasing evidence suggests that mutant Huntingtin may exert its neurotoxic effect by directly causing ER stress. However, the actual involvement of ER stress-related pathways in the disease remains speculative, as no *in vivo* experiments have validated these findings.

The accumulation of mutant poly(Q) inclusions triggers activation of UPR stress sensors IRE1α and PERK *in vitro*, resulting in upregulation of downstream targets [27]. Many groups have shown that ER stress partially mediates toxicity caused by mutant HD associated with activation of JNK, ASK1 and caspase-12 processing. ASK1 deficient neurons have been shown to protect from poly$(Q)_{79}$ toxicity [136,137,141]. One of primary roles of the UPR is to induce expression of a diverse set of chaperones, and many reports have shown that mutant Huntingtin aggregation can be diminished by the expression of chaperones such as Hsp70 and Hsp40 [129,138].

Evidence is beginning to emerge indicating that clearance pathways responsible for elimination of poly(Q) aggregates are closely regulated by the ER through UPR stress sensors such as PERK/eIF2α, which control expression of the autophagy-related gene ATG12 [27]. Thus, the UPR and ER stress pathways may affect the accumulation of mutant Huntingtin in the cell. The therapeutic effects of targeting the UPR were recently demonstrated in models of diabetes where ER stress mediates insulin resistance [139]. The authors employed chemical chaperones, including 4-phenyl butyric acid (4-PBA) and trimethylamine N-oxide dihydrate (TMAO), which are a group of low molecular weight compounds known to stabilize protein conformation and improve ER folding capacity (reviewed in [140]). Likewise, endogenous bile acid derivates, such as TUDCA, also modulate ER function [141]. Remarkably, a positive effect of TUDCA administration was demonstrated on different models of Huntington's disease [142], reducing striatal-neuron apoptosis and the size of intranuclear Huntingtin inclusions. Locomotor and sensorimotor deficits were significantly improved in HD animal models upon treatment [142]. The chemical chaperone 4-PBA also significantly extended survival, improved motor performance, and delayed the neuropathological features in the R6/2 transgenic mouse model of HD [143]. The impact these drugs have on ER stress in HD transgenic mouse models remains to be determined.

The ability of chemical chaperones to alleviate ER stress *in vitro* demonstrates the feasibility of targeting organelle function for therapeutic gain. In this line, activation of autophagy with rapamycin, which targets the mTor pathway, has the ability to increase the survival of HD animal models and recover motor performance. These therapeutic effects were associated with a decrease in Huntingin aggregate size, indicating that pharmacological strategies aiming increasing autophagy-mediated clearance of protein aggregates may have real therapeutic benefits [144]. A relatively new drug, trehalose, was recently shown to act both as a chemical chaperone and an autophagy activator, decreasing the aggregation of mutant Huntingtin and α–synuclein *in vitro* [145]. A recent small-molecule screen performed in yeast by the same group yields novel small-molecule modulators of mammalian autophagy [146]. They identified new small-molecule enhancers and inhibitors of the cytostatic effects of rapamycin in yeast. Three enhancers induced autophagy independently of rapamycin in mammalian cells, facilitating the clearance of mutant Huntingtin and α-synuclein^{A53T}. These autophagy enhancers, which seem to act either independently or downstream of rapamycin, attenuated mutant Huntingtin-fragment toxicity in Huntington's disease *in vitro* models as well as in *D. melanogaster* [146], reinforcing the therapeutic potential to clear mutant proteins responsible for many neurodegenerative conditions.

Alzheimer's Disease

Alzheimer disease (AD) is the most common neurodegenerative disease in elderly people, characterized by the progressive decline of cognitive functions [147]. The pathological hallmarks are intracellular deposits containing neurofibrillary tangles of Tau protein and extracellular plaques of aggregated amyloid β-peptide (Aβ), as well as inflammation, oxidative stress and axonal transport inhibition [147]. Genetic forms of AD are associated with mutations in β-amyloid precursor protein (β-APP) and presenilin-1 (PS1) and 2 (PS2) genes. These mutations cause increased processing of APP and Aβ production, leading to early onset familial AD [148]. A recent study demonstrated that the distribution of phosphorylated PERK correlates with abnormally phosphorylated Tau in post mortem AD brains [149]. Similarly, BiP/Grp78 levels are augmented in AD brains, suggesting that UPR response is relevant to the disease [150].

PS1 and PS2 are components of the γ-secretase complex, located primarily in the ER [151], and their mutations increase the production of Aβ peptide in subjects with familial AD [152]. Cellular studies have demonstrated that PS mutations increase calcium release from the ER [153-156] and enhance sensitivity to ER stress mediated apoptosis [157] [158,159]. However, evidence linking PS1/2 to the UPR is controversial. Some *in vitro* studies report a reduced induction of BiP in PS1 mutant cells [160,161], while others show that the UPR is not affected under ER stress conditions [162]. Interestingly, one study showed that PS1 physically interacts with IRE1α on the ER membrane [160]. In other cases, PS1 mutant cells showed decreased BiP expression [163], PERK and eIF2α phosphorylation after ER stress induction. Thus, familial AD-linked PS1 mutations may down-regulate the UPR, leading cells more vulnerable to ER stress.

Diverse studies have demonstrated that Aβ-42 signals to the ER and induces the UPR [164,165]. It has been shown that caspase-12 deficient neurons have reduced sensitivity to Aβ peptide [158]. Another example associating AD with ER-stress mediated neuronal dysfunction comes from *in vivo* studies injecting amyloid-β in the hippocampus of rabbits. These experiments showed activation of a clear ER stress response, supporting the idea that AD-related events can activate the UPR *in vivo* [166,167]. Despite AD being the most prevalent neurological disorder in humans, the role of ER stress in the disease is still poorly understood, and more *in vivo* studies are required to address the pathways involvement in disease pathology.

III. CONCLUDING REMARKS

The UPR is an essential pathway that controls adaptive processes to stress, which result in global changes of ER function including ERAD, ER/Golgi biogenesis, protein folding, translocation into the ER, and autophagy [168]. UPR genes such as XBP-1 enforce changes in cellular structure and function consistent with the requirements of the UPR to maintain proper function of professional secretory cells, but the exact role of the UPR in the CNS is mostly unknown. A genetic link is observed between an XBP-1 promoter polymorphism and the occurrence of bipolar disorders [169], schizophrenia [170] and certain personality types in the Japanese population [171]. As discussed here, extensive studies indicate a strong association between accumulation of misfolded proteins and ER stress induction in several important neurodegenerative conditions such as AD, PD, HD, ALS, PrD, and many others [44,172]. Direct evidence indicating that perturbation of ER function could result in neurodegeneration came from the characterization of the Woozy mutant mice, where disruption of a BiP co-chaperone triggers neuronal dysfunction associated with spontaneous protein aggregation in the brain [173]. However, most of the evidence supporting the involvement of ER stress in neurodegeneration is correlative, and manipulation of the UPR *in vivo* was required to define the actual contribution of the pathway to the disease process.

Strong correlations have been described between the misfolding and aggregation of an underlying protein and the occurrence of ER stress in neurodegenerative conditions. In addition, increasing reports describe the activation of the UPR in human post-mortem samples from patients affected with diverse types of protein misfolding disorders, suggesting a role as a general cellular response to neurodegeneration (Table **1**). Despite this evidence, little causal relationship is available to link the UPR and ER stress to neurological disorders. Predictions for the role of ER stress in disease processes are not obvious because activation of the UPR may decrease neurodegeneration by increasing folding, protein quality control and autophagy. However, extensive or chronic ER stress may result in irreversible neuronal damage and apoptosis (Fig. **3**).

Small molecules such as chemical chaperones, known to decrease ER stress, or autophagy activators such as rapamycin have been shown to have protective effects against neurodegeneration in certain disease models *in vivo*. However, genetic manipulation of the UPR has been needed to directly address the role of the pathway in the disease process. To address this question, we have recently described the generation of a brain specific XBP-1 deficient mouse model. XBP-1 deficiency did not result in spontaneous neurological dysfunction. Based on previous reports linking ER stress with neurodegeneration, we hypothesized that the IRE1α/XBP-1 pathway might contribute to conditions of chronic stress rather than basal neuronal function. Thus, we challenged XBP-1$^{Nes-/-}$ mice with a murine prion model which evokes several central features of diverse neurodegenerative diseases, such as the accumulation of misfolded protein aggregates, neuronal loss, and progressive appearance of neurological-disease signs leading to death of the animal. Unexpectedly, infection of XBP-1$^{Nes-/-}$ with prions did not affect the appearance of any of these ER stress markers, nor did it affect prion misfolding, replication or animal survival. The mammalian UPR is not restricted to the

IRE1α/XBP-1 pathway, however, and activation of other UPR pathways may compensate for XBP-1 deficiency in Prion-disease models. The possible effects of ATF6 and PERK remain to be studied in regard to prion pathogenesis using *in vivo* genetic manipulation. In addition, it may be possible that involvement of the UPR in neurodegeneration may depend on the specific disease condition, type, and/or localization of the disease-related misfolded protein.

In summary, the signaling pathways linking protein misfolding and neuronal dysfunction are not well understood. Activation of the UPR may be a general phenomenon observed in these diseases, possibly reflecting a general failure of organelle function or direct engagement of stress sensors by the disease-related misfolded proteins. The latter is an interesting possibility based on recent structural data of the ER stress sensing domain of IRE1 (reviewed in [8]). A direct binding of misfolded proteins to the stress sensor has been proposed to mediate its activation and signaling. Mislocation of mutant proteins at the ER lumen may be a signal related to ER stress engagement, as described for ALS and Prion disease models. While the association between ER stress and neurodegeneration is very strong, it remains primarily correlative. However, promising preliminary studies employing drugs known to attenuate ER stress have proven effective in many disease models, suggesting a causal role of ER stress in their pathology.

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