Published in final edited form as:

Cell Calcium. 2016 September ; 60(3): 218–234. doi:10.1016/j.ceca.2016.04.010.

The Entangled ER-Mitochondrial axis as a potential therapeutic strategy in Neurodegeneration: A Tangled Duo Unchained

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Abstract

Endoplasmic reticulum (ER) and mitochondrial function have both been shown to be critical events in neurodegenerative diseases. The ER mediates protein folding, maturation, sorting as well acts as calcium storage. The unfolded protein response (UPR) is a stress response of the ER that is activated by the accumulation of misfolded proteins within the ER lumen. Although the molecular mechanisms underlying ER stress-induced apoptosis are not completely understood, increasing evidence suggests that ER and mitochondria cooperate to signal cell death. Similarly, calcium-mediated mitochondrial function and dynamics not only contribute to ATP generation and calcium buffering but are also a linchpin in mediating cell fate. Mitochondria and ER form structural and functional networks (mitochondria-associated ER membranes [MAMs]) essential to maintaining cellular homeostasis and determining cell fate under various pathophysiological conditions. Regulated Ca^{2+} transfer from the ER to the mitochondria is important in maintaining control of pro-survival/pro-death pathways. In this review, we summarize the latest therapeutic strategies that target these essential organelles in the context of neurodegenerative diseases.

Keywords

Mitochondria; ER; Calcium; Neurodegeneration; Mitochondrial Dynamics; Mitochondrial Transport; UPR

A. The Unfolded protein response

The unfolded protein response (UPR) plays a vital role in maintaining cell homeostasis as a consequence of endoplasmic reticulum (ER) stress (1, 2). Failure in assembly of protein complexes, protein degradation, formation of disulfide bonds, and protein glycosylation in the ER are common triggers of the UPR. Under moderate misfolded protein accumulation, the UPR transduces information from the ER to the nucleus and cytosol and thereby inhibits

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Disclosures

All authors have read and approved the final article. Authors reports no conflicts

protein translation, expands the ER membrane, recruits ER chaperones to aid in the correct folding of misfolded proteins, and promotes protein degradation in order to reduce the load of unfolded or misfolded proteins (3, 4).

Three ER stress sensors mediate the UPR; PKR-like endoplasmic reticulum kinase (PERK), inositol-requiring kinase 1 (IRE1), and activating transcription factor 6 (ATF6) (4) (Figure 1). These sensors transmit signals from the ER to the cytoplasm or nucleus, inducing the suppression of protein translation, expression of ER molecular chaperones or activate of ER-associated degradation (ERAD) reducing the accumulation of unfolded proteins in the ER. In the first pathway, PERK directly phosphorylates and inhibits the ubiquitous eukaryotic translation initiation factor 2 α (eIF2 α) leading to attenuation global protein synthesis to decrease protein influx into the ER lumen (5). Phosphorylation of eIF2 α also favors the selective translation of the mRNA encoding the transcription factor ATF4. Upon translocation to the nucleus, ATF4 induces the expression of ER chaperones thereby increasing the refolding of misfolded proteins. The second pathway of UPR involves the membrane protein ATF6. During ER stress, ATF6 is transported to the Golgi apparatus, where it is cleaved sequentially by the Golgi resident serine proteases S1P and S2P (site 1 and site 2 proteases, respectively) to release its cytosolic domain (6). The resulting cytosolic ATF6 fragment (ATF6f) translocates to the nucleus to induce gene expression of ER chaperones, ERAD components and XBP-1 (7). The third pathway of UPR is initiated by IRE1 that is activated upon its dimerization and auto-phosphorylation (8). IRE1 contains an N-terminal ER stress-sensing domain facing ER lumen, an ER transmembrane domain, and a serine/threonine kinase domain and a C-terminal endoribonuclease domain that faces cytosol. IRE1 binds the RING finger protein, TRAF2, and activates ASK1 (apoptosis signal-regulating kinase 1), leading to activation of JNK and p38. IRE1 degrades a subset of mRNAs, which encode for ER-localized proteins by means of regulated IRE1-dependent decay (RIDD), thereby reducing protein synthesis in the ER. Further, IRE1 activates caspase 12, which activates a cascade of caspases leading to cell death. Also, through decay of anti-caspase 2 pre-miRNAs, IRE1 α activates apoptosis through up regulation of caspase 2 (1, 2, 7).

B. ER stress and disease

Protein misfolding and abnormal aggregation is a common theme observed in most of the neurodegenerative diseases; including Huntington's disease, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis as well as other neurodegenerative diseases involving protein defects (9–11). The abnormal protein aggregation observed in these diseases involves the accumulation of small oligomers, fibrils and large amyloid aggregates.

1. Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease causing progressive motor abnormalities and cognitive defects (12). HD is characterized by a widespread neuronal dysfunction and selective neurodegeneration, particularly in the striatum. It arises from an expanded CAG repeat, coding for a polyglutamine (polyQ) tract

in the huntingtin (Htt) protein. The average polyQ length is ~18 glutamines on Htt gene, which does not confer neurotoxic activity, but expansions exceeding 35 glutamines result in disease development (13). Expression of mHtt (mutant huntingtin) in yeast and mammalian cells induces UPR (14). Furthermore, neurons of animal expressing mHtt up-regulate Rrs1, a regulator of ribosome synthesis, through ER stress, also suggests a link between ER stress and HD (15). UPR markers such as Chop, BiP, XBP1 and Herp are up regulated in postmortem samples of HD patients (16). mHtt protein associates with the ER and may play a direct role in induction of ER stress. Expanded polyQ on mHtt may compromise the proteasomal degradation of misfolded proteins in the ER, leading to ER stress (17). Furthermore, XBP1-deficient mice are more resistant to developing disease symptoms, showed improved neuronal survival and motor performance, as well as a drastic decrease in mHtt levels (18, 19). By contrast, ATF4 deficiency showed no effect, highlighting the involvement of XBP-1 in HD pathogenesis (18, 19). Treatment with ER stressors, tunicamycin or dithiothreitol (DTT), rapidly decreased BiP-GFP mobility in mHtt containing striatal cells and accelerated UPR activation compared with wild-type cells (20). Further, overexpressing GRP78 protected N2a cells, reduced formation of mHtt aggregates, inhibited caspase-12 activation and blocked cell death (21). SCAMP5 was recently identified as a novel regulator of the accumulation of mHtt in the striatum of HD patients and in cultured striatal neurons (22). In the study, the increase of SCAMP5 impaired endocytosis, which in turn enhanced mHtt aggregation (22). Thus, modulating ER stress could reduce mHtt-induced striatal neuronal death and improve ER Ca^{2+} loss and reduce UPR activation thus providing novel therapeutic opportunities.

2. Alzheimer's disease

Alzheimer's disease (AD) manifests a gradual decline in many cognitive processes ultimately leading to dementia (23). These traits are accompanied by a selective neuronal and synaptic loss in cortical and subcortical regions, deposition of extracellular senile plaques, mainly composed of amyloid β ($\text{A}\beta$) peptide, presence of intracellular neurofibrillary tangles (NFT) containing hyperphosphorylated tau protein, and cerebral amyloid angiopathy in the brains of AD patients (24, 25). Accumulation of unfolded or aggregated proteins, increased oxidative stress, and metabolic disturbances are characteristic features of AD. Exogenous $\text{A}\beta$ induces ER stress in primary neuronal cultures and activates mitochondria- and ER-mediated cell death pathways (23). Brains of AD patients have been found to have increased expression of ER chaperone, BiP, and the activated, phosphorylated form of UPR sensor PERK, as well as IRE1 and eIF2 α in the temporal cortex and hippocampus (26). In AD brains, the ER stress marker GRP78/BiP positively correlated with Braak clinical staging (26). Other markers of ER stress, including cleaved caspase-12, cleaved caspase-3, cleaved caspase-4, and CHOP, were elevated in the temporal cortex of AD patients (27, 28). PERK knockdown in neuronal cells enhanced $\text{A}\beta$ toxicity through reduced activation of eIF2 α (28). Furthermore, pharmacological activation of the eIF2 α pathway significantly reduced caspase-4-dependent apoptosis in $\text{A}\beta$ treated neurons (28). These results suggest that the PERK-eIF2 α pathway may play a role in cell survival, rather than apoptosis during ER stress. Interestingly, pathological events described in AD, such as neurofibrillary tangles, neuroinflammation, altered calcium signaling, and excitotoxicity, were also recently linked to the occurrence of pathological ER stress. Similarly, iPSC-

derived neurons and astrocytes from APP-linked familial and sporadic AD patients accumulated A β oligomers, leading to ER and oxidative stress (29). ER stress may interfere with the normal trafficking of APP through the normal secretory pathway, leading to the production of A β , and subsequent toxicity (30). Taken together, there is mounting evidence to support that ER stress is a trigger for A β formation that leads to prolonged UPR activation and ER Ca²⁺ release. Hence, it is an attractive target for therapeutic intervention to halt or slow down the disease progression.

3. Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra in the midbrain. It is characterized by motor symptoms including bradykinesia, rigidity, resting tremor, and postural instability (31). While the etiology of PD is unknown in most cases, degenerating neuronal populations in PD exhibit α -synuclein abnormalities and mutations in that gene cause familial PD, indicating that the α -synuclein abnormalities are mechanistically linked to pathogenesis of PD (31). The genes involved in autosomal recessive PD include Parkin (PARK2), PTEN-induced putative kinase 1 (PINK1; PARK6), DJ-1 (PARK7), and ATP13A2 (PARK9), whereas the genes involved in autosomal dominant PD include α -synuclein (PARK1/4), leucine-rich repeat kinase 2 (LRRK2; PARK8), and ubiquitin carboxy-terminal hydrolase L1 (UCHL-1; PARK5) (32, 33). Many reports suggest the involvement of ER stress in the pathology of Parkinson's disease (34, 35). In yeast, the major physical target of α -synuclein is Rab1, an essential component of the ER-to-Golgi trafficking machinery. Overexpression of Rab1 and some of its homologs rescues dopaminergic neuron loss induced by α -synuclein overexpression (36). UPR activation is noted in PD post-mortem tissues (31) and ER stress markers are up-regulated in the brain of α -synuclein transgenic mice (37, 38). The two hallmarks of ER stress – PDI and Bip levels were substantially increased also in cortical neurons derived from iPSCs of PD patients (31). Mutations in the serine/threonine kinase and GTPase LRRK2 are the most frequent genetic defect identified in PD patients, and LRRK2 partially localizes to the ER in dopaminergic neurons of PD patients (39). *C. elegans*, lacking the LRRK2 homolog develop spontaneous neurodegeneration and hyper-susceptibility to experimental ER stress and LRRK2-mediated neuroprotection involves the up regulation of Bip through p38 signaling (40). The diverse functional roles of LRRK2 have been under recent and extensive investigation. LRRK participates in the regulation of mitochondrial function, autophagy, and cytoskeletal dynamics (41). Most relevant to the topic of this review, LRRK2 has been shown to regulate calcium release from lysosomes through NAADP-receptors (42). It is therefore not surprising that mutations in LRRK2 result in mitochondrial depolarization and disruption in calcium homeostasis in a cultured cortical neuron model (43). Taken together, perturbation of ER homeostasis is emerging as a common pathological event triggered by genes linked to PD. The mechanisms of action for individual mutant PD genes may involve diverse pathways, but they may culminate in a final related outcome that includes pathogenic ER stress. Thus, targeting ER stress might be a potential therapeutic target for PD.

4. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset motor neuron disease and is characterized by selective loss of motor neurons. Approximately 90% of ALS patients have sporadic ALS (sALS) and about 10% have the inherited or familial form of ALS (fALS). Changes in ER morphology have been observed in ALS patients and the G93A-SOD1 ALS model in mice (44–46). Enhanced phosphorylation of eIF2 α and increased levels of the ER foldase PDIA1 along with elevated levels of oxidized proteins and PERK, IRE1, and ATF6 were observed in spinal cords of ALS patients (44, 47, 48). Upregulation of the ER foldase ERp57, XBP-1 and ATF4 have been reported in sALS and fALS (49). Neuronal cells expressing mutants of FUS, TDP-43, C9ORF72 and in TDP-43 animal models exhibit ER stress (46, 50, 51). Impairment of protein transport between the ER and Golgi has been thought to be a major factor in driving the ER stress in ALS. Overexpression of Rab1 prevented ER stress in cells expressing mutant SOD1, TDP-43 and FUS (52). Furthermore, the presence of Rab1-positive inclusions in motor neurons of human spinal cord tissues from sALS patients implies that Rab1 is misfolded and loses its normal vesicular distribution in sALS. SOD1G85R/Perk^{+/-} transgenic mice have an earlier activation of the UPR and apoptosis pathways than G85R transgenic mice; an enhanced and accelerated accumulation of misfolded SOD1 in SOD1G85R/Perk^{+/-} transgenic mice was also observed (47, 53). In another study, increased expression of activated caspase-12 was observed in both the pre-symptomatic and the late symptomatic stages (54). Thus, the evidence linking ER stress and Ca²⁺ deregulation with misfolding and aggregation of several ALS-associated proteins suggests that prevention of dysfunctional ER would limit motor loss and improve the outcome in ALS patients.

C. Recent drug development targeting ER stress pathways

Due to the mounting evidence for UPR activation and ER stress in neurodegenerative diseases, various drug strategies are being pursued. Salubrinal was first identified as a selective inhibitor of cellular complexes that dephosphorylate eIF2 α (5); Salubrinal treatment showed positive outcomes through the reduction of protein misfolding overload and thus overall ER stress in multiple disease models (55, 56). Salubrinal also inhibits the expression of proteoglycans and favors neurite outgrowth from cortical neurons and significantly reduced kainic acid-induced ER stress and neuronal death in vivo and in vitro (57, 58). It significantly increased the Grp78/Bip ER chaperone resulting in attenuation of caspase-4 dependent apoptosis in A β treated neurons (28). In SOD1G93A mice, Salubrinal decreased muscle strength loss and extended survival (59). GSK2606414, a inhibitor of PERK, is neuroprotective in mice against damage caused by prions, and prevents the development of cognitive deficits and other clinical manifestations of prion disease (60, 61). Even when dosed in the symptomatic phase of the prion disease, GSK2606414 significantly reduced neuronal cell death (61). Recently, an optimized version of GSK2606414, GSK2656157, was developed, has favorable pharmacokinetics and crossed the blood brain barrier when dosed orally in mice (62, 63). Using a cell-based screen for inhibitors of PERK signaling, a small molecule, ISRIB (*trans*-N,N'-(cyclohexane-1,4-diyl)bis(2-(4-chlorophenoxy) acetamide) that potently (IC₅₀ = 5 nM) reverses the effects of eIF2 α phosphorylation was identified (64). ISRIB was shown to reduce the viability of cells

subjected to PERK-activation by chronic endoplasmic reticulum stress as well as significant enhancement in spatial and fear-associated learning (64). ISRIB reverses the effect of eIF2 α phosphorylation on translation and stress granule assembly (65). So far there have been no studies of ISRIB in neurodegenerative disease models. AMG PERK44 was recently developed as a potent and selective PERK inhibitor ($IC_{50} = 6$ nM) exhibiting >160-fold selectivity for PERK over B-Raf and GCN2, as well as a panel of 387 other kinases (66). Guanabenz, an α_2 -adrenergic receptor agonist has been used in the treatment of hypertension and was observed to have anti-prion activity (67, 68). Further, it was shown to be beneficial in a SOD1 as well as a TDP-43 transgenic mouse model (69, 70). PPP1R15A is a regulator subunit of protein phosphatase 1 and regulates stress-induced eIF2 α . Recently, Sephin1 was identified as a selective inhibitor of stress-induced PPP1R15A and targeted disease associated with accumulation of misfolded protein (71). Oral treatment of 5 mg/kg of Sephin1 per day was shown to reduce motor deficits, motor neuron loss and the molecular defects in SOD1G93A mice (71).

IRE1, the ER transmembrane sensor that activates UPR to maintain ER and cellular function promotes cell survival, but can also initiate apoptosis via decay of anti-apoptotic microRNAs (8). Recently, multiple small molecule inhibitors that selectively block IRE1–XBP1 activation have been identified. 4 μ 8C, an IRE1 inhibitor, that blocks substrate access to the active site of IRE1, selectively inactivates both Xbp1 splicing and IRE1-mediated mRNA degradation (72). STF-083010, a small molecule inhibitor of IRE-1, inhibited Ire1 endonuclease activity, without affecting its kinase activity, after endoplasmic reticulum stress both in vitro and in vivo in tumor models (73). KIRA6, another IRE-1 inhibitor, preserved photoreceptor numbers in a rodent model of retinal degeneration (74). Apigenin (4',5,7-trihydroxyflavone, Baicalein and Kaempferol, pharmacologically active agents that target the expression of ATF-6, exerted neuroprotective effects AD and PD models (75–77). However, some of these molecules need to be tested in the context of AD, PD, HD and ALS.

4-Phenylbutyric acid (4-PBA) is a chemical chaperone that eliminates the accumulation of unfolded proteins in the ER. Recently, multiple analogues of 4-PBA have been developed and are in various phases on animal studies. These analogues strongly inhibit the induction of the IRE1 and ATF6 pathways and downstream pathogenic events including activation of NF- κ B and phosphorylation of Akt in ER stress-exposed cells (78, 79).

Tauroursodeoxycholic acid (TUDCA), a taurine conjugate of ursodeoxycholic acid (UDCA), reduces endoplasmic reticulum stress, showed neuroprotective effects in AD, PD, HD and ALS models (80). Further, in a recently published pilot study, TUDCA was well tolerated and may be effective in ALS (81).

The Sigma-1 receptor, is a chaperone protein at the ER that modulates calcium signaling through the IP3R, which has been reported to be down regulated in the brains of PD and AD patients as well as in lumbar spinal cord of ALS patients. The Sigma-1 receptor agonists PRE-084 and SA4503 prevented neuronal loss extended survival of SOD1G93A mice and spatial learning deficits in other ND models (82). Further studies using them would help developing new potential therapeutics.

Encouraging natural protein folding machinery through the activation of heat shock transcription factor 1 (Hsf1), which is a critical regulator of chaperone protein expression, is yet another approach to target ER stress. Treatment with Arimoclomol, a co-inducer of the heat shock response, delayed disease progression and extended the lifespan of SODG93A mice, and is currently being investigated in phase II/III clinical trial for ALS patients with SOD1 mutations (83). Celastrol and Tanespimycin, which target the heat shock protein response, are other potential drugs that have shown promise in transgenic mouse model of Alzheimer's disease as well as ALS (84–86) (Table 1). Thus, multiple studies reviewed above are promising and suggest that targeting the cellular protein quality control system of the ER is an attractive strategy for the treatment of neurodegenerative conditions.

D. Dysfunctional Endoplasmic Reticulum Ca^{2+} in neurodegeneration

The ER is an essential organelle in the cell and is involved in functions such as folding, assembly, quality control of secretory and membrane proteins, lipid biosynthesis as well as Ca^{2+} storage and signaling (87–89). Ca^{2+} homeostasis is fundamental for cell metabolism, proliferation, differentiation, and cell death. A range of Ca^{2+} channels are involved in Ca^{2+} signaling that have been discussed elsewhere (88). In the plasma membrane, ionotropic receptor-operated (ligand-gated) channels (ROCs), and voltage-gated Ca^{2+} channels (VGCCs) control the entry of Ca^{2+} into the cell. These are critical in maintaining in Ca^{2+} homeostasis that are reported to be altered in neurodegenerative diseases and have been reviewed elsewhere (90, 91). VGCCs comprise two groups of high voltage-activated channels, dihydropyridine-sensitive Cav1 (L-type) channels and Cav2 (P/Q-, N- and R-type) channels, as well as low voltage-activated Cav3 (T-type) channels (92). Patients with amyotrophic lateral sclerosis possess antibodies (ALS IgGs) that bind to L-type skeletal muscle VGCCs and inhibit L-type calcium current (93). Subcutaneous delivery of isradipine, inhibitor of $\text{Ca}_v1.3$ L-type Ca^{2+} channels, significantly protected SNc neurons in animal models of PD. Inositol-1,4,5- trisphosphate receptors (IP3Rs) and ryanodine receptors (RyRs) are the two ubiquitously expressed major intracellular Ca^{2+} channels (ICCs) that release ER Ca^{2+} stores. IP3Rs constitute a family of three paralogs (IP3R1, IP3R2, IP3R3) and are critical for Ca^{2+} mediated processes such as gene expression, neurotransmission, memory formation, cell growth and development. Mice either lacking IP3R1 or carrying a spontaneous mutation in IP3R1 gene display severe ataxic behavior. Similar to IP3Rs, RyRs are also found in three paralogs (RyR1, RyR2, and RyR3) and are extremely important especially in neurons. Aberrant intracellular Ca^{2+} signaling has been observed in a variety of neurodegenerative diseases including AD, ALS, HD as well as spinocerebellar ataxia. Using single channel recordings of IP3Rs it was observed that expression of mutant PS1 and PS2 is associated with an apparent sensitization of the IP3R channel to IP3, resulting in enhanced channel gating (94). IP3R levels are reduced in the brains of patients with AD and correlates with the increased occurrence of amyloid plaques and neurofibrillary tangles (95, 96). Further, IP3R activity is altered in neurons cultured from AD mouse models (97–99). Recent studies have also shown altered RyR mediated Ca^{2+} release in AD models (100, 101). In patients, alterations in the ryanodine receptor calcium release channel correlated with Alzheimer's disease neurofibrillary and beta-amyloid pathologies (102). In HD, Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by IP3R1

(103). Disruption of IP3R binding to Htt reduced glutamate induced apoptosis by stabilizing intracellular Ca^{2+} homeostasis in a YAC128 HD model (104). Inhibition of RyR and IP3R through Dantrolene and xestospongin C respectively, prevented neuronal death in multiple models (89, 105–107). ER Ca^{2+} depletion is coupled to an increase of Ca^{2+} entry into the cell. STIM1 and STIM2 are plasma-membrane proteins with a luminal Ca^{2+} sensor and interact with Orai1 proteins (108). These tetrameric Ca^{2+} channels in the plasma membrane are then responsible for an increased Ca^{2+} entry (108). Ca^{2+} signaling is often abnormal in neurodegenerative diseases. Studies in AD postmortem brain tissue samples and AD animal models support a role for disruption of synaptic Ca^{2+} regulation in the neurotoxic action of $\text{A}\beta$ (109). Further, similar observations have been made in HD patient's brains and animal models as well as in PD and ALS and have been reviewed (109). The depletion of ER Ca^{2+} stores results in activation of store-operated channels (SOCs) at the plasma membrane, mediating store-operated calcium entry (SOCE) from the extracellular space, followed by the removal of cytosolic Ca^{2+} and replenishment of luminal Ca^{2+} through sarcoplasmic/ER Ca^{2+} -adenosine triphosphatases Presenilins (PS1 and PS2) also govern store-operated calcium entry (SOCE), a refilling mechanism for depleted intracellular calcium stores (110). Modulation of functional PS1, by either knocking out PS1 or expressing inactive PS1, markedly potentiated SOCE, while familial Alzheimer's disease (FAD)-linked mutant PS1 or PS2 significantly attenuated SOCE and store depletion-activated currents (111). Further, purinergic stimulation induced excess calcium release from the ER stores in SOD1G93A astrocytes, resulting from the abnormal ER calcium accumulation and independent of clearance mechanisms. Pharmacological studies suggested that store-operated calcium entry was enhanced in the SOD1G93A astrocytes (112). In transgenic *Drosophila* HD flies and in YAC128 mice, activity of SOCE was enhanced in neuronal cells expressing mutant Htt (113, 114). Thus, dysfunctional intracellular calcium homeostasis is a central cause of neurodegeneration.

E. Mitochondria-associated ER membranes in neurodegenerative diseases

ER and mitochondria establish a tight interplay, which is structurally and functionally modulated through a proteinaceous tether formed at specific subdomains of the ER membrane, designated mitochondria-associated membranes or MAMs. Dysfunctional ER affects mitochondrial dynamics due to the functional and morphological connectivity between the two highly dynamic organelles. The main functions that have been characterized for ER/mitochondria contacts include control of lipid biosynthesis, mitochondrial division, Ca^{2+} signaling and coordinated dynamics of the two organelles (115). Many proteins are enriched at the MAMs (116, 117). VDAC1, a mitochondrial protein, is physically linked to the ER Ca^{2+} release channel IP3R through the molecular chaperone glucose-regulated protein 75 (grp75), optimizing calcium transfer from IP3Rs to mitochondria (Figure 2) (118). Sigma-1 receptor chaperones at the ER–mitochondrion interface regulating Ca^{2+} signaling and cell survival (119). Mfn2, the mitochondrial fusion protein, controls ER–mitochondria juxtaposition and calcium signaling on the MAM as well as modulates the UPR and mitochondrial function via repression of PERK (120). Bap31 is an ER protein sorting factor proposed to escort integral membrane client proteins from their biogenesis site at the Sec61 translocon to other ER complexes that determine their fate in the

ER. Further, outer membrane fission protein of Fis1, makes physical contact with ER-localized Bap31. Prolonged ER stress leads to release of Ca^{2+} from the ER lumen at the MAM and an increased Ca^{2+} uptake into the mitochondrial matrix (115). Sustained Ca^{2+} accumulation in the mitochondrial matrix induced by ER stress triggers proapoptotic mitochondrial alteration, including permeability transition, dissipation of the electrochemical potential, matrix swelling, relocalization of Bax to mitochondria and the release of cytochrome c and apoptosis-inducing factor from mitochondria (121, 122). The spacing between the ER and mitochondria changes with different cell physiological and pathological conditions and artificial modification of this contact can lead to ER stress (123). MAM function and ER/mitochondrial communication was significantly increased in presenilin-mutant cells and in fibroblasts from patients with both the familial and sporadic forms of AD (124). Similar observations were made in human AD brain and related AD mouse and neuronal cell models (125). Furthermore, MAM is a site of production of $\text{A}\beta$ (126). Overexpression of both wild-type and familial ALS/FTD mutant TDP-43 reduced ER–mitochondria associations (127). DJ-1, a PD associated gene, counteracts mitochondrial impairment induced by the tumor suppressor protein p53 by enhancing ER/mitochondria tethering (128). Together, the above findings highlight damage to ER/mitochondria associations as a new pathogenic mechanism in AD, PD, and ALS/FTD. Thus, correcting damaged ER/mitochondria associations may correct damage to other neurodegenerative disease-linked features.

F. Calcium is a physiological activator of mitochondrial functions

After reaching what was considered saturation over two decades ago, advanced microscopy techniques, better biological probes, and more powerful pharmaceutical perturbations resulted in renewed interest in mitochondria biology. These tools demonstrated that mitochondria are not static organelles, but form networks that continuously undergo morphological change. In addition, mitochondria spatiotemporally modulate cell calcium levels and produce reactive oxygen species (ROS) that play essential roles in both pathological (e.g. apoptosis) and physiological (e.g. bioenergetics) signaling (129, 130). Here we focus on the role of mitochondrial calcium in neurodegeneration. Whereas calcium is already well recognized as an activator of mitochondria's canonical metabolic function, calcium regulates mitochondria's other functions, such as mitochondrial dynamics and ROS production that are intimately associated with neuronal function.

G. Calcium is an activator of mitochondrial metabolic function

Calcium is positive effector of mitochondrial energetics. This feature is particularly relevant in neurons as the brain utilizes approximately 20% of the body's oxygen consumption while only comprising 2% of body mass (131, 132). Neurons specifically rely on mitochondria's oxidative phosphorylation (OXPHOS) function due to their lower glycolytic capacity (133). This is because neurons do not generate fructose-2,6-bisphosphate, an activator of 6-phosphofructo-1-kinase, which regulates the rate-limiting step of glycolysis (134). Calcium is a potent activator of OXPPHOS, resulting in approximately a 3-fold reversible increase in respiration (135, 136). Treatment with either a competitive (La^{2+}) or a non-competitive (ruthenium red) inhibitor of calcium transport results in a decrease of oxygen consumption

by isolated mitochondria (137). The mechanisms by which calcium stimulates mitochondrial respiration through the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) in neurons have been previously reviewed (132). In the TCA cycle, calcium directly binds and activates isocitrate dehydrogenase, α -ketoglutarate dehydrogenase, and pyruvate dehydrogenase phosphatase, an activator of pyruvate dehydrogenase (as shown in Figure 2). In parallel, calcium also activates each complex in the ETC in isolated mitochondria by approximately two-fold (138). It is unlikely that calcium directly binds complexes of the ETC (139, 140), but it may regulate post-translational modifiers. Importantly, physiological levels of calcium are necessary to respond to the energetic demands of neurons by stimulating components of both the TCA and ETC (132).

H. Calcium, ROS, and Neurodegeneration

1. Calcium is a mediator of mitochondrial ROS production

Mitochondria are a major source of cellular ROS (129); approximately 0.1% to 0.2% of oxygen consumed is associated with leakage of electrons and results in the production of ROS (141). At least 10 sites of mitochondrial ROS production have been identified including the ETC and pyruvate dehydrogenase (Figure 2). Because calcium stimulates mitochondrial metabolism through both the TCA and ETC, calcium can induce excessive ROS through stimulating mitochondrial metabolism. Not surprisingly, a higher metabolic rate increases mitochondrial ROS production (142). Other mechanisms of calcium-dependent mitochondrial ROS generation have been previously reviewed (130, 143). Calcium does not always increase mitochondrial ROS production. For example, calcium stimulation of mitochondria from brains did not result ROS production in the presence of antimycin A, a complex III inhibitor, but did in the presence of rotenone, a complex I inhibitor (144). Therefore, whether calcium induces an increase or decrease in mitochondrial ROS production depends on the source of the mitochondria, their metabolic state (brain mitochondria produce more ROS in state 4 rather than state 3), and the presence of electron transport chain inhibitors (143).

2. Excessive ROS as a therapeutic target for neurodegeneration

The Harman free radical theory from 1956 suggested that ROS accumulate spontaneously and in response to the environment (145). Neurodegenerative pathogenesis including AD, PD, ALS, and HD are associated with increasing accumulated ROS (146–148). A transcriptional profiling study of postmortem frontal cortex samples showed a decrease in expression of genes involving mitochondrial function and an increase in genes responsible for oxidative stress response after the age of 40 (149). In addition, manganese superoxide dismutase (SOD2) knockout mice exhibit neurodegeneration in the basal ganglia and the brainstem and motor deficiencies (150). SOD2 serves as the primary defense enzyme against increasing levels of mitochondrial ROS. It is therefore not surprising that lowering levels of ROS posed as an attractive therapeutic target for neurodegeneration.

Unfortunately, targeting ROS using generalized antioxidants has been ineffective in multiple neurodegeneration models including AD, ALS, and PD (Table 2). For example, a comprehensive randomized clinical trial for the treatment of AD using either a combination

of vitamin E, vitamin C, and α -lipoic acid or coenzyme Q showed that neither treatment led to changes in biomarkers obtained from cerebrospinal fluid samples including A β 42, tau, and p-tau. Treatment with the combination of antioxidants led to a decrease in F2-isoprostane, a marker of oxidative stress. However, this was accompanied by an accelerated decline in Mini-Mental State Examination scores (151). Furthermore, a high dose of coenzyme Q in another large randomized clinical trial showed no effect in patients with PD (152). Finally, edaravone, a potent ROS scavenger, showed no efficacy in a phase III clinical trials for ALS (153). Sadly, the lack of efficacy of antioxidants as therapeutics is not limited to neurodegenerative disorders and has been also observed in cardiovascular disorders as well (129).

General antioxidants have multiple theoretical limitations that hinder them from use in the clinic. First, antioxidants that act as ROS scavengers, such as vitamin E or C, must be provided in stoichiometric ratios relative to excessive ROS. This translates to larger doses and for prolonged periods of time for chronic disorders. Second, ROS scavengers such as N-acetylcysteine are unstable due to auto-oxidation even prior to treatment. Auto-oxidized ROS scavengers become oxidants and therefore increase oxidative stress (154). Finally, ROS have important physiological functions including signaling of stress response, differentiation, and immunity as reviewed elsewhere (129, 155). Therefore, eliminating ROS below physiological levels may be detrimental to physiological processes and may exemplify potential harmful effects of some antioxidants (151). Calcium, like ROS, has physiological roles, as discussed above. Therefore, it is not surprising that drugs such as dimebon (latrepiridine), an L-type calcium channel blocker were inefficient in clinical trials for both AD and HD (156–158).

An alternative therapeutic strategy to generalized antioxidants is mitochondria-targeted antioxidants. By targeting the sites of ROS production directly, these antioxidants may eliminate some of the limitations of generalized antioxidants. For example, the aromatic-cationic peptides SS31, or Bendavia, and SS20. These synthetic peptides are localized to the inner mitochondrial membrane rather than the mitochondrial matrix. While the mechanism of action of these peptides is not full understood, the SS31 peptide scavenges ROS through a dimethyltyrosine group, but also reduces mitochondrial ROS production and prevents opening of the mitochondrial permeability transition pore (mPTP) (159). Furthermore, SS31 inhibits cytochrome c interactions with cardiolipin, enabling cytochrome c to act as an effective electron carrier (160). In a SOD1 mutant ALS mouse model, daily injections of SS31 led to improvement in survival and motor performance associated with a decrease in neuronal cell death (161). In a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity PD mouse model, SS31 increased viability of dopaminergic neurons and a dose-dependent rescue of dopamine loss (162). Therefore, SS31 may provide effective treatment for neurodegenerative diseases. In addition to the aromatic-cationic peptides, attachment of the lipophilic triphenylphosphonium cation (TPP⁺) to a general antioxidant enabled its delivery to the mitochondrial matrix. One limitation of this mechanism is that compound delivery requires an active mitochondrial membrane potential (159). Mitochondria produce excessive ROS when their membrane potential is compromised and ATP production is inefficient. Therefore, antioxidants must to target the mitochondria regardless of their membrane potential to effectively eliminate the production of excessive ROS. Nevertheless TPP⁺-

conjugated ROS scavengers have been developed including MitoVitE (mitocopherol), MitoTEMPOL (TPP⁺ conjugated to 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl), SkQ1 (TPP⁺ conjugated to plastoquinone), MitoPBN (TPP⁺ conjugated to phenoxy-butyl-nitrene), and MitoQ (TPP⁺ conjugated to coenzyme Q) (163). The most studied, MitoQ, showed cardioprotection in several animal models and was shown to be well tolerated in a Phase I clinical study (164). However, MitoQ failed to show efficacy in PD patients (164). While potentially more efficacious than generalized antioxidants, the relevance of mitochondria-targeted antioxidants in humans is yet to be shown.

I. Calcium regulates mitochondrial morphology and intracellular transport

Rather than pharmacologically targeting ROS as an output of dysfunctional mitochondria, another therapeutic strategy involves directly targeting dysfunctional mitochondria and improving overall mitochondrial health. Neurons' complex shape and energetic demands pose a challenge for mitochondria. Neurons have three main structural domains: the cell body (soma), a long axon, and branch-like dendrites. Areas such as postsynaptic and presynaptic terminals are remote from the cell body yet require a higher ATP supply and a more responsive calcium buffering system (165). Release of neurotransmitters at presynaptic termini is mediated by calcium flow through voltage-gated channels and an increase of cytoplasmic calcium (Ca²⁺_{cyto}). To restore presynaptic termini, mitochondria must sequester Ca²⁺_{cyto} to prepare for an additional neuronal firing (166). Mitochondrial biogenesis and degradation primarily occurs in the cell body. Therefore, mitochondria must have dynamic mechanisms to regulate mitochondrial number, shape, and subcellular localization, to meet the energetic and calcium buffering demands of specialized cells like neurons (165). The previous view of mitochondria as static, uniformly shaped organelles fails to explain this biology.

1. Calcium regulates mitochondrial morphology

One of the observations that reignited mitochondrial biology research is that within a cell, thousands of mitochondria form a dynamic network (167). The functionality of this network is dependent both on the shape and localization of mitochondria. The number and morphology of mitochondria are both mediated by two opposing processes that are at the core of mitochondrial dynamics: fission and fusion (Figure 3A). Fission, the process by which a single mitochondrion divides into two or more mitochondria increases mitochondrial number and regulated mitochondrial quality control. Damage segregates asymmetrically and fission separates damaged from healthy mitochondria (168). Mitochondrial fission is primarily mediated by dynamin-related protein 1 (Drp1), a large GTPase that constricts the mitochondrial membrane to lead to scission (169, 170). Conversely, mitochondrial fusion is the stitching of two or more mitochondria to form a single mitochondrion. Fusion facilitates complementation of mitochondrial DNA and proteins (168). Three large GTPases drive mitochondrial fusion: mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) that stitch the outer mitochondrial membrane, and optic atrophy 1 (Opa1), responsible for joining the inner mitochondrial membrane (171, 172). Maintaining a balance between these two processes is essential for maintaining mitochondrial network homeostasis and cell viability (168).

Calcium mediates mitochondrial network dynamics by regulating mitochondrial morphology through modulating the expression and localization of several factors, including Drp1, the canonical driver of mitochondrial fission. Drp1 is post-translationally regulated by multiple modifiers including cyclin-dependent kinase I (cdk1)/cyclin B phosphorylation at Ser585 and Ser616, cyclic AMP-dependent protein kinase (PKA) phosphorylation at Ser637, calcineurin dephosphorylation at Ser637, delta protein kinase C (PKC δ) phosphorylation at Ser579, SUMOylation by SUMO-conjugating enzyme Ubc9, SUMO-1, and mitochondrial anchored RING-finger containing protein (MAPL), deSUMOylation by SUMO1 specific protease 3 (SEN3), and mitochondrial E3 ubiquitin ligase MARCH5 (173–177). These post-translational modifications are summarized in Figure 3B.

The role of calcium in modulating localization of Drp1 is best shown in the context of the phosphorylation state of Drp1 at Ser637 (178–180). Drp1 is primarily localized in the cytoplasm when Ser637 is phosphorylated. Upon activation by calcium, calcineurin dephosphorylates Drp1 at Ser637, which in turn signals Drp1 localization to the mitochondrial membrane, where it induces mitochondrial fission. In an *in vivo* mouse heart model, activation of PKA with forskolin induces phosphorylation of Drp1 at Ser637. Addition of BayK8644, an L-type Ca²⁺ channel activator, together with forskolin reduces phosphorylated Drp1 at Ser637 (178). In a HeLa cell culture model, depolarizing mitochondria with arachidonic acid or carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone increases cytosolic calcium and results in fragmented mitochondria. However, addition of cyclosporine A, an inhibitor of calcineurin, both reduces calcineurin catalytic activity and decreases mitochondrial fission (180). It is important to note that in these two examples, calcium-induced mitochondrial fission is induced by stress. However, mitochondrial fission is an essential physiological process. Drp1 homozygote knockout mice are embryonic lethal with developmental abnormalities in the forebrain (181, 182) and neural-specific Drp1 homozygote knockout mice have brain hypoplasia and die shortly following birth. Primary neuronal culture from the forebrain of these mice have defective synapse formation due to inability of mitochondria to distribute properly (181). These findings suggest that calcium, through the activation of calcineurin, may have a role in the activation of Drp1 and mitochondrial fission under physiological conditions as well. It is possible that levels of dephosphorylated Drp1 at Ser637 and therefore frequency of mitochondrial fission may dependent on localized calcium concentrations.

Calcium regulation of other post-translational modifiers of Drp1 remains an unexplored area of research. For example, calcium may regulate SUMOylation of Drp1 (183, 184). However, in these reports, calcium did not directly regulate SUMOylation, but instead, mediated a switch from SUMOylation to acetylation through calcineurin-dependent dephosphorylation (184). A calcium-dependent PKC signaling pathway was recently found to regulate trapping of Ubc9, a SUMOylating enzyme, to dendritic spines in cultured hippocampal neurons (185). Ubc9-dependent SUMOylation of Drp1 is associated in greater accumulation of Drp1 at the mitochondrial membrane (186), yet increased mitochondrial fission at dendritic spines due to calcium-dependent Ubc9 localization has not been reported. Therefore, calcium regulation of mitochondrial fission through phosphorylation-independent post-translational modification of Drp1 still remains to be explored.

More recent reports have attributed calcium-dependent changes in mitochondrial morphological shape to processes may be independent of Drp1-mediated mitochondrial fission (187, 188). In cortical astrocytes, treatment with a calcium ionophore, 4Br-A23187, increases intracellular calcium levels and leads to a decrease in mitochondrial length (187). Yet, calcineurin inhibitors, CsA and FK506, did not inhibit 4Br-32187-mediated reduction in mitochondrial length (187), indicating a calcineurin-independent mechanism. However, calcineurin inhibitors attenuate the 4Br-A23187-induced increase in mitochondrial number, suggesting that calcium-mediated changes in mitochondrial morphology can occur independently of a complete fission event. The fission-independent remodeling process is mediated through reactive oxygen species (ROS) as treatment with the antioxidant N-acetylcysteine (NAC) does not increase mitochondrial in combination with 4Br-A23187 treatment but attenuates mitochondrial length shortening (188). Therefore, it is not clear from these findings whether mitochondrial remodeling occurs independently of the canonical fission protein Drp1. For example, it is possible that calcium- and ROS-mediated remodeling occurs through another post-translational modification of Drp1.

2. Mitochondrial dynamics as a therapeutic target

As mentioned earlier, mitochondrial dynamics, or the continuous cycle of mitochondrial division and amalgamation, is essential for maintaining a balanced mitochondrial network. Uncontrolled fission, through which mitochondria become fragmented and therefore too small to function properly, leads to cell death (168), and is a hallmark of numerous neurodegenerative disorders including HD, AD, PD, dementia, ataxia, and hypertension-induced encephalopathy (189–195). Excessive mitochondrial fission is therefore an attractive therapeutic target for neurodegenerative disorders. Efforts using small molecules to inhibit fission by targeting its main mediator Drp1 have led to the development of a specific inhibitor: mdivi-1 (Figure 3C) (196). Treatment with mdivi-1 in a cell culture model rescued propofol-induced toxicity in stem cell-derived neurons as well as functional and structural mitochondrial defects in a PINK1 mutant Parkinson's cell culture model (197, 198). Unfortunately, general toxicity and developmental defects are associated with mdivi-1 in animal models (199–203). The inherent limitation of these general inhibitors is their attenuation of the less-understood physiological fission. While excessive fission is pathological, tightly-regulated physiological fission is necessary for cell survival (168). However, the mechanism distinguishing between physiological and pathological fission are only now being uncovered (129). A peptide inhibitor of Drp1 activation specifically under stress conditions has been recently developed: P110 (Figure 3C) (204). The cytosolic Drp1 anchors onto to the mitochondrial outer membrane by four separate adaptors: mitochondrial fission protein 1 (Fis1), mitochondrial fission factor (Mff), and mitochondrial dynamics proteins of 49 and 51 kDa (Mid49 and Mid51) (129). P110 specifically inhibits the interaction between Drp1 and Fis1 and thus rescues mitochondrial function and morphology in a 1-methyl-4-phenylpyridium neuronal cell culture model of Parkinson's disease (204). In addition, P110 showed efficacy in both cell culture and animal models of HD (205). In patient-derived iPSCs and neurons, P110 inhibited excessive fission and restored mitochondrial function. In an R6/2 mouse model of HD, P110 improved mouse behavior and increased survival (205). Importantly, P110 had no effect on mitochondria under basal conditions (205). Small peptide inhibitors are increasingly used as pharmacological tools

both in basic science research and in the clinic due to their increased conformational flexibility, selectivity, cell permeability, bioavailability, and their ability to cross the blood-brain barrier opening a new therapeutic avenue for pathological protein-protein interactions, such as the one between Fis1 and Drp1 (206–208).

3. Calcium as a regulator of mitochondrial motility

To meet the energetic and buffering challenge posed by the complexity of neuronal morphology, mitochondria must also migrate to various areas of the cell in addition to changing shape. Mitochondria rely on microtubules for both anterograde (away from the cell body) and retrograde (towards the cell body). Anterograde transport of mitochondria is primarily mediated through kinesin-1 (KIF5). KIF5 forms a complex with mitochondrial Rho GTPase (Miro 1 and Miro 2) and Milton (TRAK1 and TRAK2) (Figure 4D) (209). A recent report showed that mitochondrial bidirectional mitochondrial migration to either dendrites or axons is mediated through interactions with either TRAK1 or TRAK2 (210). TRAK1 interactions with both KIF5 and dynein/dynactin mediate mitochondrial transport to axons whereas TRAK2 interactions with dynein/dynactin promote mitochondrial migration to dendrites (210). In the context of microtubule-driven mitochondrial transport, calcium acts as a “stop sign” (211–213). In both H9C2 cardiac cells and primary neurons, overexpressing Miro increases mitochondrial movement at basal cytoplasmic calcium levels. In contrast, at high cytoplasmic calcium levels, increasing Miro overexpression results in more frequent arrest of mitochondrial motility. This arrest is mediated by two EF-hands that act as calcium binding domains (213). Two models were suggested for calcium-mediated attenuation of mitochondrial movement along microtubules: 1) upon calcium binding to Miro through the EF-hands, KIF5 detaches from microtubules and binds Miro (212), and 2) upon calcium binding to Miro, TRAK2 detaches from KIF5 (Figure 4D) (211). Therefore, calcium plays an important role in regulating mitochondrial localization and proteins associated with mitochondrial transport pose as attractive therapeutic targets.

J. Calcium as a regulator of mitochondrial permeability transition pore opening

Calcium is a potent activator of the mitochondrial permeability transition pore (mPTP) (214). At the onset of cellular pathology and associated calcium overload, mPTP opening leads to matrix expansion and membrane rupturing. Following these events, mitochondria discharge a multiple proapoptotic factors, excessive ROS, and stored calcium, abrogating the mitochondria’s calcium buffering capability (214). While the structure of the mPTP has been under great controversy, a recently suggested and more consistent model includes a dimer of F₀F₁ ATP synthase under the regulation of cyclophilin D and calcium (215). Pathological opening of the mPTP is associated with a variety of neurodegenerative disorders, including AD, PD, and HD (216). Cyclophilin D’s most well known inhibitor, Cyclosporin A (CsA), inhibits mPTP opening (217). Unfortunately as discussed above, CsA is also an inhibitor of calcineurin. CsA’s inhibitory interactions with calcineurin elicits immunosuppressive effects, making it a less ideal drug candidate (218). Novel candidates for CsA have been proposed, including alisporivir, SCY-635, and NIM811 as reviewed elsewhere (219). A highly specific cyclophilin D inhibitor, Antmanide, had no effect in the absence of

cyclophilin D on cells in culture and isolated mitochondria. Combined with its functional efficacy, Antmanide serves as a promising drug candidate for the treatment of mPTP-mediated neurodegenerative pathologies (220). Since calcium-mediated pathological mPTP opening is associated with a range of neurodegenerative disorders, compounds that target the regulation of mPTP activity pose as strong therapeutic candidates.

Concluding Remarks

Endoplasmic reticulum (ER) stress and mitochondrial dysfunction play an important role in a range of neurodegenerative disorders, including AD, PD, HD and ALS. These diseases are characterized by accumulation of misfolded proteins and aggregates as well as by mitochondrial dysfunction. Although the contribution of these key organelles in these disorders is now well recognized, the mechanisms by which they contribute to neurodegeneration remains unclear. However, increasing evidence obtained using disease models supports that alterations in ER homeostasis cause accumulation of disease-associated misfolded proteins, which in turn may initiate the ER stress response and contribute to synaptic and neuronal dysfunction in neurodegenerative diseases. Furthermore, calcium directly modulates mitochondrial function, dynamics and mobility. Therefore, signaling components of the UPR, ER Ca^{2+} signaling as well as direct regulators of mitochondrial functions are emerging as potential targets for intervention, and so the treatment of a range of neurodegenerative disorders might become possible.

Acknowledgments

Sources of Funding

This work was supported by National Institutes of Health grants HL52141 and NIAAA11147 to Dr Mochly-Rosen. OSK is supported by a Smith Stanford Graduate Fellowship.

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Highlights

- The unfolded protein response plays a vital role in maintaining cell homeostasis as a consequence of endoplasmic reticulum stress.
- ER and mitochondrial dysfunction are common themes in neurodegenerative diseases.
- Targeting these pathways provides an opportunity to develop new therapeutics for neurodegenerative disease.

Figure 1. The Unfolded Protein Response

The unfolded protein response consists of three independent signaling pathways that work in parallel and are activated upon accumulation of misfolded proteins inside the ER. ER-stress sensors: RNA-activated protein kinase R (PKR)-like ER kinase (PERK), inositol requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) are membrane resident proteins. The intracellular systems involved in regulating Ca²⁺ include IP3R: inositol-1,4,5-trisphosphate receptor, RyR: ryanodine receptor, SERCA: ER Ca²⁺-ATPase at the ER and mPTP: mitochondrial permeability transition pore Bax/Bak and mitochondrial uniporter at the mitochondria. ER stress increases Ca²⁺ release thus overloading mitochondria, leading to mPTP opening and cytochrome c release. Activation of the UPR leads to an overall translational block and specific activation of ER stress responsive genes, which will increase the protein folding capacity and decrease the protein-folding load in the ER.

Figure 2. ER-mitochondria contacts

VDAC is an important regulator of Ca²⁺ transport in and out of the mitochondria. Accumulation of Ca²⁺ into the mitochondrial matrix occurs via the mitochondrial Ca²⁺ uniporter (MCU), which rapidly accumulates Ca²⁺ across the steep electrochemical gradient. Mitofusin 2 (MFN2) along with its role in mitochondrial fusion regulates mitochondrial and endoplasmic reticulum morphology and tethering. Sigma 1 receptor stabilizes IP3Rs when ER Ca²⁺ stores are depleted ensuring proper Ca²⁺ fluxes from the ER to the mitochondria. GRP75 affects the activity of ion channels by mediating the interaction of VDAC1 with IP3Rs, and thus facilitates mitochondrial Ca²⁺ uptake. Also, Fis1, a mitochondrial fission protein and Bap31 at ER, bridge the mitochondria–ER interface to establish a platform for apoptosis induction. Calcium directly activates pyruvate dehydrogenase phosphatase (PDHP), isocitrate dehydrogenase (IDH), α-ketoglutarate dehydrogenase (α-KGDH), complexes I, III, and IV, as well as ATP synthase. Pyruvate dehydrogenase (PDH) complexes I, II, and III are also sites of ROS production. Abbreviations used in the illustration not mentioned above: pyruvate dehydrogenase kinase (PDK), phosphate group (P), and cytochrome c (CytC).

Figure 3. Calcium regulates mitochondrial dynamics and transport

A) Mitochondrial fission/fusion cycle. Mitochondrial fusion is mediated by the outer mitochondrial membrane GTPases Mfn1 and Mfn2, and the inner mitochondrial membrane GTPase Opa1. Mitochondrial fission is regulated by the cytosolic GTPase Drp1 that anchors on the outer mitochondrial membrane. B) Post-translational modifications of Drp1 determine its localization in the cytosol or the mitochondrial membrane. PKA, cdk1, and PKC δ phosphorylate Drp1. Calcineurin (Cn) dephosphorylates Drp1. SUMO-1, Ubc9, and MAPL SUMOylate Drp1. SENP3 deSUMOylates Drp1. MARCH 5 ubiquitinates Drp1. Calcium is an activator of calcineurin and potentially Ubc9, thus promoting Drp1 translocation to the mitochondria. Drp1 is anchored to the mitochondrial membrane through four adaptors: Fis1, Mff, Mid49, and Mid51. C) Drp1 drives both physiological fission and Fis1-dependent pathological fission. While mdivi-1 is a general inhibitor of Drp1, P110 selectively inhibits pathological fission. D) Mitochondria traverse microtubules through motor adaptor complexes including Miro, Milton, and KIF5. Calcium detaches mitochondria from microtubules through two potential mechanisms: 1) KIF1 detachment from microtubules and attachment to calcium-bound Miro, and 2) Milton detachment from KIF5 and attachment to calcium-bound Miro.

Table 1**Drugs targeting the Unfolded Protein Response**

Therapeutic	Mechanism	Indication	Ref
PERK arm			
Salubrinal	eIF2 α -phosphorylation inhibitor	AD, ALS, HD, PD	(28, 56)
Guanabenz	Inhibitor of eIF2 α phosphatase	AD, ALS, HD	(69, 70)
GSK265157/GSK2606414	PERK kinase inhibitor	AD, ALS, HD	(60, 62)
ISRIB	Inhibitor of eIF2 α phosphatase	Prion disease	(64, 65)
Sephin1	Inhibitor of eIF2 α phosphatase	ALS	(71)
AMG PERK 44	PERK kinase inhibitor	Not tested	(66)
IRE1α arm			
STF-083010	IRE1 α RNase active site inhibitor	AD, ALS, HD models	(73)
MKC-3946	IRE1 α RNase active site inhibitor	AD, ALS, HD models	(72)
4u8C	IRE1 α RNase active site inhibitor	AD, ALS, HD models	(72)
KIRA6	IRE1 α type II kinase inhibitor	Other models	(74)
OICR464	Binds to the RNase domain of murine IRE1	Not tested	(74)
OICR573	Binds to the RNase domain of murine IRE1	Not tested	(74)
ATF6 arm			
Apigenin	Up regulation of ATF 6 expression	PD, AD	(75, 77)
Baicalein	Up regulation of ATF 6 expression	PD	(76)
Kaempferol	Down regulation of ATF 6 expression	AD	(221)
Chemical chaperones			
4-phenylbutyrate (4-PBA)	Stabilizes misfolded/unfolded proteins	AD, ALS, HD	(78, 79)
Tauroursodeoxycholic acid (TUDCA)	Stabilizes misfolded/unfolded proteins	AD, ALS, HD	(80, 81)
Miscellaneous			
Arimoclomol	Co-inducer of heat shock response	ALS	(83)
Celastrol	Co-inducer of heat shock response	AD, ALS	(84, 85)
Tanespimycin	Co-inducer of heat shock response	AD, ALS	(86)
PRE-084	Sigma-1 receptor agonist	AD, HD, ALS	(82)
SA4503	Sigma-1 receptor agonist	ALS	(82)

Table 2

Drug targeting mitochondrial dysfunction

Therapeutic	Mechanism	Indication	Ref
General antioxidants			
Vitamin E	General antioxidant	Alzheimer's Disease (potentially harmful, delay of progression) Mild Cognitive Impairment (no effect)	(151, 222, 223)
Vitamin C	General antioxidant	Alzheimer's Disease Clinical Trial (potentially harmful)	(151)
α -lipoic acid	General antioxidant	Alzheimer's Disease Clinical Trial (potentially harmful)	(151)
Coenzyme Q	General antioxidant	Alzheimer's Disease Clinical Trial (no effect) Parkinson's Disease Clinical Trial (no effect) ALS Clinical Trial (no effect)	(151, 152, 224–226)
Curcumin	General antioxidant	Alzheimer's Disease Clinical Trial (increased plasma A β)	(227)
Melatonin	General antioxidant	Alzheimer's Disease Clinical Trial (no effect)	(228)
Pramipexole	General antioxidant	Parkinson's Disease Clinical Trial (no effect)	(229)
N-Acetylcysteine	General antioxidant	Parkinson's Disease Clinical Trial (increase brain and blood glutathione)	(230)
Edaravone	General antioxidant	ALS Clinical Trial (no effect)	(153)
Mitochondria-targeted therapies			
TPP ⁺ -conjugated ROS Scavengers (MitoQ)	Mitochondria-targeted antioxidant	Parkinson's Disease Clinical Trial (no effect)	(164)
Dimebon (latrepiridine)	L-type calcium channel blocker, MPTP inhibitor	Huntington's Disease Clinical Trial (no effect) Alzheimer's Disease Clinical Trial (beneficial phase II, no effect phase III)	(156–158)
Dexpramipexole	Mitochondria-targeted antioxidant	ALS Clinical Trial (no effect)	(231)
Aromatic-cationic peptides (SS31 or Bendavia, SS20)	Mitochondria-targeted antioxidant	ALS SOD1 mouse model (delayed onset) MPTP neurotoxicity in mice (neuroprotection)	(161, 162)
Mdivi-1	General inhibitor of Drp1	Cell culture model of propofol toxicity (decreased cell death) Cell culture model of Parkinson's Disease (rescued mitochondrial structural and functional defects)	(197, 198)
P110	Inhibition of mitochondrial Drp1 recruitment through Fis1	Cell culture model of Parkinson's Disease (increased mitochondrial function) Cell culture model and mouse model of Huntington's Disease (increased mitochondrial function, mouse survival, and mouse behavior).	(204) (205)
CyclosporinA	Calcineurin inhibitor, cyclophilin D inhibitor	No indication (off-target effects leading to immunosuppression).	(218)
Antmanide	cyclophilin D inhibitor		(220)