**OXIDATIVE STRESS AND ALTERED MITOCHONDRIAL FUNCTION IN
NEURODEGENERATIVE DISEASES: LESSONS FROM MOUSE MODELS.**

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Abstract

Oxidative stress has been consistently linked to ageing-related neurodegenerative diseases leading to the generation of lipid peroxides, carbonyl proteins and oxidative DNA damage in tissue samples from affected brains. Studies from mouse models that express disease-specific mutant proteins associated to the major neurodegenerative processes have underscored a critical role of mitochondria in the pathogenesis of these diseases. There is strong evidence that mitochondrial dysfunction is an early event on neurodegeneration. Mitochondria are the main cellular source of ROS and key regulators of cell death. Moreover, mitochondria are highly dynamic organelles that divide, fuse and move along axons and dendrites to supply cellular energetic demands, and therefore, impairment of any of these processes would directly impact on neuronal viability. Most of the disease-specific pathogenic mutant proteins have been shown to target mitochondria promoting oxidative stress and the mitochondrial apoptotic pathway. In addition, disease-specific mutant proteins may also impair mitochondrial dynamics and recycling of damaged mitochondria via autophagy. Thus, all these data suggest that ROS-mediated defective mitochondria may accumulate during and contribute to disease progression, indicating that strategies aimed to improve mitochondrial function or ROS scavenging may be of potential clinical relevance.

Key words: Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, oxidative stress, mitochondria, autophagy, mitochondrial dynamics.

1. INTRODUCTION

Neurodegenerative diseases are hereditary or sporadic conditions characterized by progressive atrophy and dysfunction of anatomically or physiologically related neurologic systems. Huge efforts are being made to develop therapies that counteract these devastating disorders, which encompass Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). However, until now none of the strategies tested have been proved successful, mainly due to our incomplete understanding of the pathogenesis of these ageing-related disorders. Despite their heterogeneity, these diseases share common features such as formation of insoluble disease-specific protein aggregates and oxidative stress that ultimately result in neuronal death. Therefore, it is conceivable to speculate the existence of a unifying etiological factor. Among all the initial cellular derangements, mitochondrial dysfunction seems to play a pivotal role [1, 2]. Mitochondria are the main cellular source of reactive oxygen species (ROS) and have been described as key regulators of cell survival and death [3-5]. Mitochondrial metabolism decline and mitochondrial oxidative stress have been directly implicated in ageing [6, 7], arising as a risk factor for the sporadic forms of the neurodegenerative diseases. Moreover, different studies have shown that most of the disease-specific proteins are able to interact with the organelle resulting in mitochondrial failure and ROS production [8-10]. However, despite evidence of abnormalities in mitochondrial localization, structure and function in samples from symptomatic patients, it is still largely debated whether mitochondrial impairment and enhanced oxidative stress are causal or merely a consequence of the neurodegenerative processes. Recently, studies from genetically-modified mice have proved useful to unravel the contribution of mitochondria as a major pathogenic factor leading to brain neurodegeneration. This review will focus on

some of these relevant new insights, lending further support for the involvement of oxidative stress and altered mitochondrial function in neurodegenerative diseases. Clearly, uncontrolled generation of ROS would be expected to affect different cells and hence the course of progression of multiple pathologies linked to neurodegeneration, such as obesity, insulin sensitivity, cardiovascular and liver diseases, which have been covered in recent reviews [11-13].

2. MITOCHONDRIAL CONTROL OF OXIDATIVE STRESS.

Mitochondria provide more than 90 percent of the cellular ATP requirement, and hence can be considered the powerhouse of cells. Maintenance of mitochondrial function is particularly important in neurons given that they rely on glucose oxidation for energy production [14]. Moreover, neurons are highly specialized cells whose major function is intercommunication. This interactive activity requires tightly regulated signalling systems, mainly in the synaptic compartments, that rely on the correct function of ATP-dependent processes such as ion channels, receptors, pumps, vesicle release, and neurotransmitters recycling [14]. The ability of mitochondria to divide, fuse, and migrate will facilitate the transmission of energy across long distances and will help neurons to meet the high-energy demands, especially on synapses. In addition to its well-known role on meeting bioenergetic demands, mitochondria display other important cellular functions including regulation of Ca^{2+} homeostasis [2], ROS signalling [4] and apoptosis [3, 5]. Derangements in any of these intimately interrelated mitochondrial functions result in oxidative stress, perturbed Ca^{2+} signaling, and ultimately cell death, underscoring the crucial role of mitochondria in cell fate decisions. Therefore, in this section we will briefly describe the role of mitochondria in ROS generation, Ca^{2+} homeostasis and cell death regulation.

2.1. Mitochondria and ROS generation

Mitochondria are the major endogenous source of ROS, which are generated mainly as by-products of the oxidative phosphorylation [15] (Fig. 1). When produced in an uncontrolled fashion, mitochondrial-derived ROS may compromise mitochondrial function and cell survival [5]. Indeed, in the course of normal physiological respiration it is estimated that up to 2-4 % of the O₂ consumed by mitochondria is converted into superoxide radicals (O₂^{•-}), although recent determinations have reduced this figure to about 0.1-0.5% [16]. O₂^{•-} generation is stimulated by high mitochondrial membrane potential ($\Delta\Psi_m$) [17], whereas a mild uncoupling activity mediated by uncoupling proteins (UCPs) results in up to 70% drop in ROS generation. [18]. Moreover, UCP2 overexpression in brain mitochondria has been shown to be cytoprotective in several models of ischemic injury [19].

In addition to the respiratory chain, several mitochondrial flavoenzymes have been shown to produce ROS with appreciable rates as shown with either isolated enzymes or mitochondria, although their specific contribution under physiological conditions to ROS generation and cell regulation is unknown. Especially relevant in brain are monoamine oxidases (MAOs) located in the outer membrane that catalyze oxidation of biogenic amines accompanied by release of hydrogen peroxide (H₂O₂) [20] (Fig. 1). Some authors suggested that an upregulation of MAO-B and the resulting ROS production might be responsible for the mitochondrial damage in PD [21, 22]. Elevated MAO-B activity in brain tissue as well as in platelets has been also found in other neurodegenerative diseases, including AD [23, 24]. Indeed, MAO-B inhibitors (L-deprenyl and rasagiline) have been shown to be effective in treating PD and possibly AD, with concomitant extension of life span, although its neuroprotective effect may involve actions other than the inhibition of the enzyme, including anti-apoptotic activities and induction of antioxidant enzyme expression [25]. Intriguingly, recent data from a double blind trial in PD patients, showed that low (1 mg per

day) but not high (2 mg per day) rasagiline displayed disease-modifying effects [26]. Other well-established source of ROS is the flavin-containing enzyme dihydrolipoyl dehydrogenase within the α -ketoglutarate dehydrogenase complex (KGDHC) [27-29] (Fig. 1). KGDHC is tightly associated with the matrix side of the inner membrane and catalyzes oxidation of α -ketoglutarate to succinyl-CoA using NAD^+ as electron acceptor. Low availability of NAD^+ stimulates ROS generation, whereas the physiological catalytic activity of the enzyme is inhibited [28]. Therefore, any condition that restricts the re-oxidation of NADH in the respiratory chain increases the intramitochondrial NADH/NAD^+ ratio, hence promoting KGDHC-mediated ROS production. KGDHC is also sensitive to oxidative stress [29] and the enzyme activity is substantially reduced in postmortem brain samples from AD and PD patients [30, 31]. Moreover, recent studies in AD transgenic mouse models show that KGDHC impairment accelerates amyloid pathology and memory deficits through increased mitochondrial oxidative stress [32].

Specific ROS can be stimulated by nitrogen species [33, 34] (Fig. 1). Nitric oxide (NO) is produced by nitric oxide synthases (NOSs), a family of enzymes that catalyze the NADPH-dependent oxidation of L-arginine to yield L-citrulline and NO. Moreover, the generation of $\text{O}_2^{\cdot -}$ and NO in close spatial proximity can give rise to the potent oxidant peroxynitrite. The first reports of a mitochondrial NOS isoform (mtNOS) were from Bates *et al.*, who demonstrated the presence of NOS in nonsynaptosomal rat brain and liver mitochondria [35]. However, although the existence of mtNOS has been described in the last decade in mitochondrial fractions isolated from different sources, recent evidence in ultrapurified rat liver mitochondria has questioned its existence (at least in rat liver) and hence the *in situ* generation of NO and the consequent generation of peroxynitrite within mitochondria [36]. NO might affect respiratory chain activity in different ways. Reversible inhibition of

mitochondrial respiration can result from the interaction of NO with cytochrome c oxidase, i.e., by S-nitrosation of key cysteine residues of complex IV [33]. In fact, NO has been identified as a physiological regulator of electron transfer and ATP synthesis. The inhibition of cytochrome c oxidase not only affects O₂ consumption, but also impacts on the redox status of the respiratory chain and thus on O₂^{•-} generation. Further, NO and peroxynitrite can also cause S-nitrosation of complex I resulting in reversibly increased ROS production [34].

Although low levels of ROS are important for many cellular metabolic processes through activation of different enzymatic cascades and several transcriptional factors [4], an imbalance that favors the production of ROS over its removal will irretrievably result in oxidative stress.

2.2. Mitochondria as a target of ROS.

Mitochondria are not only an important source of ROS generation in aerobic cells, but they are also sensitive targets for the damaging effects of oxygen radicals [5]. The accumulation of specific oxidant species within mitochondria can oxidize a broad range of bio-molecules, including lipids, proteins and nucleic acids, which can extensively impair mitochondrial function resulting in further enhancement of ROS production and cell death. For instance, damage of mitochondrial DNA (mtDNA), which is especially susceptible to attack by ROS owing to its close proximity to the electron transport chain and the lack of protective histones, not only can compromise the provision of energy to satisfy the cellular demands but also can result in enhanced ROS generation through the loss of functional respiratory complexes [7].

Membrane lipids and proteins are also critical targets for oxidative damage. The inactivation of mitochondrial iron-sulfur (Fe-S) proteins, in particular aconitase, may have

major consequences. First, the formation of an inactive $[3\text{Fe-4S}]^+$ cluster results in the simultaneous release of Fe_2^+ and H_2O_2 that stimulates hydroxyl radical generation via Haber-Weiss and Fenton reactions [37]. Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a model of PD in mice, has been shown to mobilize an early mitochondrial pool of iron concomitant to aconitase inactivation, an effect that is significantly attenuated in transgenic mice overexpressing MnSOD [38]. Inhibition of mitochondrial aconitase could also result in Krebs cycle impairment and hence energy production. Moreover, aconitase is associated with protein-mtDNA complexes called nucleoids. In this context, aconitase stabilizes mtDNA and therefore, the reversible modulation of the nucleoids by aconitase inactivation may directly influence mitochondrial gene expression [39].

Lipid peroxidation has been reported to impair the barrier function of cellular membranes resulting in cell death [40]; an increase in mitochondrial lipid peroxides may contribute to reduce mitochondrial membrane potential and Ca^{2+} buffering capacity. Cardiolipin molecules are particularly susceptible to oxidation due to their high content of unsaturated fatty acids [41, 42]. Interestingly, compared to other tissues, brain cardiolipin displays differences in fatty acid composition that could render this phospholipid more susceptible to peroxidation [43]. Cardiolipin molecules are required for functional activity of a number of inner mitochondrial membrane proteins, including respiratory chain complexes and mitochondrial anion carriers such as the adenine nucleotide translocase (ANT). Thus, oxidative damage to cardiolipin may have deleterious effects in mitochondrial function. Additionally, recent studies have shown that peroxidation of cardiolipin may promote cell death induced by an apoptotic stimulus, by facilitating the detachment of cytochrome c

from the inner membrane [44, 45], restructuring of the biophysical properties of the bilayer [45] and promoting Ca^{2+} -induced mitochondrial permeability transition (MPT) [46].

2.3. Mitochondrial defenses against oxidative stress.

Mitochondria possess a wide network of antioxidant systems to eliminate ROS and repair oxidative damage generated during normal aerobic metabolism that include several enzymatic systems as well as water- and lipid-soluble antioxidants (e.g., coenzyme Q, vitamins C and E) (Fig. 2). Most important for mitochondrial antioxidant protection is the tripeptide glutathione (L- γ -glutamyl-L-cysteinylglycine, GSH) and the different GSH-linked enzymatic defense systems [47]. Mitochondrial GSH arises from the transport of cytosol GSH into the mitochondrial matrix [48]. One of the highlights of this transport mechanism is its dependence on appropriate membrane dynamics; the loss of mitochondrial fluidity impairs the function of the mitochondrial carrier of GSH, resulting in its depletion [49, 50]. Cumulating evidence shows that oxidative cell death is caused by the specific depletion of this particular pool of GSH [45, 51-53]. GSH can act non-enzymatically by direct interaction with electrophiles, although this reaction is greatly enhanced in the presence of GSH-S-transferases. Among GSH-linked enzymes involved in maintaining mitochondrial redox homeostasis are selenium (Se)-dependent glutathione peroxidases (Gpx) 1 and 4 (Fig. 2). Gpx4 reduces hydroperoxide groups on phospholipids, lipoproteins, and cholesteryl esters and is considered to be the primary enzymatic defense against oxidative damage to cellular membranes [54]. The critical role of Gpx4 is underscored by the fact that whereas Gpx1-knockout mice are fully viable, disruption of the *Gpx4* gene in the mouse results in embryonic lethality [55]. Other members of the family of mitochondrial GSH-linked redox enzymes are glutaredoxin 2 (Grx2), which catalyzes

glutathione-dependent dithiol reaction mechanisms, reducing protein disulfides, monothiol reactions, and mixed disulfides between proteins and GSH [56, 57].

The mitochondrial thioredoxin system, which includes thioredoxin 2 (Trx2) and thioredoxin reductase 2 (TrxR2), is another potential source of disulfide reductase activity (Fig. 2). Trx2 is a multifunctional selenoprotein containing two redox-active cysteines in its conserved active site that form a dithiol group which catalyzes the reduction of disulfides [58]. Thioredoxins reduce protein disulfides at much higher rates than Grx [59]. The physiological significance of this system is emphasized by the finding that Trx2 deficiency is embryonic lethal [60]. The thioredoxin system can also interact with peroxiredoxins (Prx), which constitute a novel family of thiol-specific peroxidases that rely on Trx as the hydrogen donor for the reduction of H₂O₂ and lipid hydroperoxides [61].

A key mechanism to control the production of ROS is through the transcriptional regulation of these enzymatic strategies. For instance, a number of transcription factors including nuclear respiratory factor 1/2 (Nrf1/2), along with PPARgamma coactivator 1 α (PGC-1 α) and forkhead box class O (FOXO) have been described to modulate the expression of antioxidant enzymes such as MnSOD, Prx3, and Trx2, protecting against oxidative stress in a number of conditions and cell types [62, 63]. PGC-1 α null mice are much more sensitive to the neurodegenerative effects of MPTP and kainic acid, which affect the substantia nigra and hippocampus, respectively, in an oxidative stress-dependent fashion [62]; similarly, increased sensitivity to oxidant-mediated cell death has been seen in models of PD, HD and ALS due to the deletion of the *Nrf2* gene [64-66]. Overall, these findings unravel novel neuroprotective pathways that confer resistance to a variety of stress-related neurodegenerative insults.

Intermediates of the mitochondrial respiratory chain can also contribute to the mitochondrial antioxidant defense. Ubiquinol (QH₂) has been shown to act as a reducing agent in the elimination of various peroxides including lipid radicals and the regeneration of vitamin E from the α -tocopheroxyl radical [67]. Thus, coenzyme Q (CoQ10) acts a source of O₂^{•-} when partially reduced (semiquinone form) and as an antioxidant when fully reduced (ubiquinol). Besides its free radical scavenging activity, CoQ10 has been described to prevent apoptotic cell death by blocking Bax binding to mitochondria and by inhibiting activation of the MPT [68, 69]. Moreover, CoQ10 is a cofactor of mitochondrial UCPs and therefore, it may also be of benefit through reducing ROS generation via activation of these proteins. In several *in vivo* and *in vitro* models of neurodegenerative diseases administration of CoQ10 has been shown to exert neuroprotection [70-72], and based on these experimental data several clinical trials have been recently initiated to test its efficacy as a therapy for neurodegenerative disorders [73-75].

2.4. Ca²⁺ homeostasis, MPT and cell death.

Mitochondria actively regulate cellular Ca²⁺ homeostasis. It is well known that the removal of Ca²⁺ from the cytoplasm results in mitochondrial accumulation in the matrix. Indeed, mitochondria are often located in close contact with the sources that contribute to the rise of cytosolic Ca²⁺, such as the endoplasmic reticulum (ER) and the plasma membrane. For instance, in excitotoxic neuronal cell death, overactivation of N-methyl-D-aspartate (NMDA) receptors induces cytosolic and mitochondrial rises in Ca²⁺ concentrations, underlying the relevance between cytosolic and mitochondria Ca²⁺ regulation in cell death [76]. The major metabolic effect of elevated mitochondrial Ca²⁺ is the upregulation of ATP production through activation of dehydrogenases of the TCA cycle as well as stimulation of the ATP synthase (complex V) and the ANT [77] (Fig. 1). Mitochondria also participate in

shaping the space and temporal patterning of cytosolic Ca^{2+} , contributing to many Ca^{2+} -mediated signalling processes [78]. Conversely, excessive Ca^{2+} uptake has been shown to induce MPT, which results in secondary burst of ROS [79] and the subsequent release of proapoptotic peptides such as cytochrome c that trigger apoptosis [80]; thus, illustrating the critical role of mitochondria in the excitotoxicity observed in different neurodegenerative diseases [2].

Several lines of evidence suggest the existence of an intricate crosstalk between Ca^{2+} handling and mitochondrial ROS generation [81] (Fig. 1). Mitochondrial Ca^{2+} influx causes mild uncoupling of $\Delta\Psi_m$, which should decrease ROS production; however, it has been shown to enhance its production when respiratory complexes are inhibited [82]. Stimulation of the TCA cycle and oxidative phosphorylation by Ca^{2+} may also enhance ROS output by stimulating electron flow and mitochondrial load work. Indeed, mitochondrial ROS generation correlates well with metabolic rate [83], suggesting that a faster metabolism results in more electron leakage. In addition, Ca^{2+} -induced NO synthesis may increase ROS production by inhibiting complex IV, as previously mentioned. Furthermore, Ca^{2+} can promote cytochrome c dissociation from the mitochondrial inner membrane, either by competing for cardiolipin binding sites or by inducing the MPT [84, 85]. These events can result in blockage of respiratory chain at complex III, which in turn would stimulate O_2^- generation. Recently, to illustrate the relevance of the ER-mitochondria cross talk in Ca^{2+} -mediated neurodegeneration, Sano *et al.* reported in monosialo gangliosides-1 (GM1) gangliosidosis, a neurological disorder characterized by β -galactosidase deficiency, the accumulation of GM1 at the mitochondrial associated ER membranes [86].

MPT has been shown to be promoted by thiol oxidation and inhibited by antioxidants, lending support for a role of ROS in pore opening [87]. The nature and composition of

MPT is still unclear, although several components have been described including the VDAC, the ANT and the soluble matrix protein cyclophilin D (CypD). In addition, the phosphate carrier has also been implicated in MPT because its depletion results in delayed cytochrome c mobilization and apoptosis [88]. The association of ANT and VDAC in contact sites between the inner and outer mitochondrial membrane forms a large conductance channel that, when opened, dissipates $\Delta\Psi_m$ and allows matrix solutes < 1.5 kDa and Ca^{2+} to be released from the mitochondrial matrix. Pro-oxidant agents that target the MPT pore components have been shown to cause VDAC oxidation, protein thiol modifications in the ANT, and CypD recruitment to the inner membrane and binding to ANT, conditions that promote Ca^{2+} -induced pore opening [89-91].

The role of MPT in apoptosis is still under debate. For instance, recent experiments have shown a major role for MPT in ischemia-reperfusion injury but not in other forms of cell death such as developmental apoptosis. Mitochondria from CypD-deficient mice showed a general defect in the Ca^{2+} -induced permeability transition, but had no developmental defects associated with a lack of apoptosis [92]. Mitochondrial outer membrane permeabilization induced by the pro-apoptotic protein Bid, Bax or by apoptotic stimuli was intact in CypD null mice. In contrast, cell death induced by ischemia-reperfusion injury in the heart or brain was defective in CypD-deficient mice. Moreover, although VDAC has been proposed as an integral component of MPT and in mitochondrial outer membrane permeabilization, recent data have discarded a role for VDAC in apoptosis. Murine cells deficient in all three VDAC isoforms successfully undergo intrinsic apoptosis [93]. Furthermore, the isoform VDAC2 has been described to be antiapoptotic [94]. Similarly, mitochondria from murine cells lacking ANT1 and ANT2 can still undergo Ca^{2+} -induced swelling and MPT, although at a higher threshold, which has been considered as a sign

against a role for ANT in MPT and hence in mitochondrial outer membrane permeabilization [95]. However, the ability of ANT1/ANT2-deficient cells to undergo MPT might be due to the functional compensation by a novel ANT isoform identified recently [96], or by other mitochondrial carriers able to form pores in the inner membrane such as the ornithine/citrulline transporters or the phosphate carrier [88]. Thus, regardless of the ultimate phenotype of cell death, MPT plays a critical role in mitochondrial pathophysiology and hence its targeting can be of relevance in neurodegenerative diseases.

3. PATHOGENESIS OF NEURODEGENERATIVE DISEASES: CONTRIBUTION OF OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION.

The brain is especially susceptible to free radical damage. This vulnerability can be explained in part by its elevated metabolic rate, the high content of membrane polyunsaturated fatty acids susceptible to ROS-mediated peroxidation [97] and its relative low abundance of antioxidant defense compared with other organs.

Oxidative stress has been consistently associated with neurodegenerative disorders. This tenet is mainly based on analyses of postmortem brain tissues from affected patients showing lipid peroxidation, elevated protein carbonyls content and presence of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative DNA damage [98-101]. In addition, reduced levels of antioxidants and higher content of lipid peroxides have been observed in aged compared with young brain tissues [102]. Similarly, many studies have shown that old mitochondria are morphologically altered and functionally less efficient relative to young ones contributing to more oxidants and less ATP generation [6, 7], indicating that the accumulation of oxidative damage during life span might influence the neuropathology of late-onset neurodegenerative diseases [102]. However, based on the correlative nature of these observations, it is difficult to distinguish whether oxidative stress is only a late sign of

brain injury and hence a consequence rather than a cause of the disease, questioning the contribution of oxidative stress as an etiological factor in neurodegenerative diseases. Recently, although the onset of most neurodegenerative diseases is sporadic (not inherited) and the genetic mutations discovered until now account for only a small fraction of early-onset cases, the generation of genetically-modified mice that express these disease-causing mutations has emerged as an useful tool to underline the contribution of oxidative stress and the mitochondrial dysfunction in the pathogenesis of the main ageing-related neurodegenerative disorders. Some of these major breakthroughs will be briefly summarized in the following subsections.

3.1. Alzheimer's disease (AD)

AD is a progressive neurodegenerative dementia associated with the extracellular presence of senile plaques composed of fibrillar amyloid- β peptide ($A\beta$), intracellular neurofibrillary tangles (NFT) of hyperphosphorylated tau, and selective synaptic and neuronal loss, mainly in brain regions related to memory and cognition such as entorhinal and frontal cortices and hippocampus [103]. Cumulating evidence suggests that increased $A\beta$ levels, resulting from altered proteolytic processing of the amyloid precursor protein (APP) and reduced clearance of these neurotoxic forms, play a pivotal role in the pathogenesis of the disease [103, 104]. Among the mechanisms through which $A\beta$ exerts its toxicity there is extensive literature supporting a role for oxidative stress [105-107]. Moreover, oxidative stress can further stimulate amyloidogenic processing and tau phosphorylation [108-110], initiating an auto-amplification loop that culminates in a toxic vicious cycle. Studies from AD transgenic mice show that oxidative damage precedes amyloid plaque deposition [106, 111] with alterations in mitochondrial proteome and function occurring even earlier [112-114].

Intracellular A β accumulates in brain mitochondria from AD transgenic mice and patients. It has been widely reported that A β interaction with mitochondria, results in ROS generation and mitochondrial dysfunction [115-117] (Fig. 3). Specifically, its interaction with A β -binding alcohol dehydrogenase (ABAD) results in ROS generation and mitochondrial dysfunction, and overexpression of ABAD in mutant APP transgenic mice has shown to exacerbate neuronal oxidative stress and spatial and temporal memory impairment [117, 118] (Fig. 3). Further support for the involvement of mitochondrial oxidative stress comes from a recent study of Dumont *et al.* where the partial genetic deletion of dihydrolipoyl succinyltransferase enzyme, a key subunit specific to the mitochondrial KGDHC, increases A β plaque burden, nitrotyrosine levels and accelerates the occurrence of deficits in spatial learning and memory in a transgenic mouse model that carry the human APP with two mutations (Tg19959 mice) [32]. A β may also promote MPT by binding to CypD [119] (Fig. 3). CypD-deficient cortical mitochondria are resistant to A β - and Ca²⁺-induced mitochondrial swelling. Moreover, CypD deficiency substantially improves mitochondrial stress, alleviates A β -mediated reduction of long-term potentiation, and ameliorates behavioral and synaptic function in mutant APP transgenic mice [119]. Overall these studies underscore the relevance of mitochondria in A β neurotoxicity and suggest that the control over the mitochondrial oxidative state could determine AD pathogenesis. Indeed, hemizygous deficiency of the mitochondrial antioxidant enzyme MnSOD significantly increases brain A β levels and accelerates the onset of behavioral changes in AD transgenic mice [120, 121]. Conversely, MnSOD overexpression reduces oxidative stress and markedly attenuates the phenotype of Tg19959 mice [122]. In agreement with these studies, we have recently demonstrated that the mitochondrial pool of GSH rather than cytosol GSH determines A β susceptibility [51]. Reduced mitochondrial

GSH content can result from cholesterol-mediated perturbation of mitochondrial membrane dynamics [49, 50]. Using genetic mouse models of cholesterol loading such as sterol-regulatory element-binding protein 2 (SREBP-2) transgenic mice and Niemann-Pick disease type C1 (NPC-1) knockout mice, we have shown that increased brain cholesterol selectively depletes mitochondrial GSH levels and exacerbates A β -induced neuroinflammation and neurotoxicity. *In vivo*, following a continuous intracerebroventricular delivery of human A β , we found that the SREBP-2 transgenic mice display increased oxidative damage, neuroinflammation, synaptic loss, and neuronal death with markers of apoptosis and oxidant-dependent cell death [51]. All these endpoints were reversed by treatment with GSH ethyl ester, a cell-permeable form of GSH, which significantly increased the pool of mitochondrial GSH in Tg-SREBP-2 mice. Thus, these findings define a novel role of cholesterol in AD pathogenesis, which has been previously identified as a major risk factor for the disease [123]. In addition to fostering A β generation by facilitating β - and γ -secretases cleavage of APP in lipid rafts [124-127], the trafficking of cholesterol to mitochondria specifically sensitizes neurons to A β -mediated cell death by depleting the mitochondrial pool of GSH. Interestingly, we observed enhanced mitochondrial cholesterol accumulation and mitochondrial GSH depletion in old APP/PS1 transgenic mice after A β accumulation suggesting that A β might itself regulate cholesterol levels [51]. However, whether the alterations of mitochondrial GSH levels can modulate A β -induced memory impairment in these mice is unknown and will require further analysis. The regulatory role of A β on cholesterol homeostasis has been also reported in previous studies where both pathological presenilin mutations [128] and A β -induced membrane oxidative stress [129] results in increased amounts of cholesterol accompanied

with an altered sphingolipid metabolism. Elevated levels of ceramides and membrane cholesterol are found in vulnerable brain regions of AD patients with mild to moderate symptoms [129], suggesting that these abnormalities occur relatively early in the course of the disease. *In vitro*, A β has been shown to induce apoptosis via the sphingomyelin/ceramide pathway in various brain cells [130, 131]. Studies in cortical neurons report early increases in intracellular levels of the ceramide glycosylated derivative disialolactosylceramide (GD3) after A β challenge, describing a causal relationship among GD3 formation, cell-cycle activation, and neuronal death [132]. Recent observations further support a role for GD3 in promoting A β neurotoxicity, as the genetic downregulation of GD3 synthase, the enzyme responsible for the synthesis of GD3 from monosialolactosylceramide (GM3), reduces A β plaque load and improves memory in APP/PS1 transgenic mice [133]. Both ceramide and ganglioside GD3 has been shown to act directly on mitochondria and induce MPT with the release of cytochrome c [134, 135], however, the possibility that these sphingolipids contribute to A β -induced cell death by targeting the mitochondrial pathway has not been established yet and will required future work.

Recent observations have described that memantine, an uncompetitive, moderate-affinity antagonist of NMDA receptors, exhibit disease-modifying effects in triple transgenic (3xTg-AD) mice, which harbor PS1_{M146V} knockin alleles and APP_{Swe} and tau_{P301L} transgenes. Memantine restored cognition, reduced the levels of toxic A β species as well as the content of tau and hyperphosphorylated tau, and strikingly, prevented A β -induced inhibition of long-term potentiation (LTP) in the hippocampus of cognitively normal mice [136]. Whether these therapeutics effects of memantine were accompanied by the reversal

of mitochondrial Ca^{2+} rise, expected from the overactivation of NMDA, was not analyzed and deserves further investigation.

3.2. Parkinson's disease (PD)

PD is characterized by the loss of dopaminergic (DA) neurons in the substantia nigra and the accumulation of intraneuronal proteinaceous inclusions, known as Lewy bodies. Pioneering evidence pointing to mitochondrial oxidative stress in PD came from studies where agents that cause Parkinsonism (MPTP, rotenone, 6-hydroxydopamine) inhibited complex I of the mitochondrial respiratory chain resulting in ROS production [137, 138]. Different reports showed that mice overexpressing antioxidant enzymes are protected against these parkinsonian agents [139-141] whereas mice deficient in Gpx, MnSOD or dihydrolipoyl dehydrogenase display increased vulnerability [142-144], emphasizing the concept that mitochondrial oxidative stress may play a major role in PD. However, the relevance of these findings using neurotoxins is uncertain by the fact that they may have pleiotropic pharmacological effects in DA neurons, effects on non-DA neurons, and most importantly, the vast majority of patients are never exposed to these poisons.

Another interesting approach was the generation of a conditional knockout mouse with disruption of the gene for mitochondrial transcription factor A (Tfam) in DA neurons. The knockout mice have reduced mtDNA expression and respiratory chain deficiency in midbrain DA neurons, which, in turn, leads to a parkinsonism phenotype with adult onset of slowly progressive impairment of motor function, thus providing experimental support for the hypothesis that acquired respiratory chain dysfunction may be of pathophysiological importance in PD [145].

However, although the concept that mitochondrial dysfunction as a potential contributory cause to PD pathogenesis has been launched about two decades ago, the most convincing

evidence came from the characterization of genes mutated in familial PD with putative functional roles within mitochondria. Several studies have suggested an association of many of the proteins linked to PD with the mitochondrion, including, α -synuclein, DJ-1 (PARK7, Parkinson disease protein 7), parkin, PTEN induced putative kinase 1 (PINK1), and HtrA serine peptidase 2 (HtrA2/OMI) (Fig. 3). How these proteins regulate mitochondrial function is not fully clear, even though recent data indicate an intricate relationship between them [146-148] and their loss of function significantly affects mitochondria. For instance, PINK1 and parkin knockout mice both display impaired mitochondria and decreased antioxidant capacity specifically within nigrostriatal dopaminergic circuit [149-151]. Studies in PINK1 knockout mouse brain describe elevated susceptibility to H₂O₂ or heat-shock with decreased activities of the respiratory complexes as well as aconitase [149]. Parkin, but not its pathogenic mutants, stabilizes PINK1 by interfering with its degradation via the ubiquitin-mediated proteasomal pathway [152]. In addition, recent reports showed that PINK1 can regulate parkin function through direct phosphorylation [153]. The phosphorylation of parkin by PINK1 activates parkin E3 ligase function and enhances parkin-mediated ubiquitin signaling through the NF- κ B pathway [153]. Parkin-mediated ubiquitin signaling is impaired by PD-linked pathogenic PINK1 mutations [153]. PINK1 may also regulate calcium efflux from mitochondria via the mitochondrial Na⁺/Ca²⁺ exchanger and therefore, its deficiency causes mitochondrial calcium overload, resulting in ROS generation and MPT [154]. Moreover, disease-causing mutations in PINK1 have been reported to decrease HtrA2 phosphorylation affecting its protease activity [148]. Recent studies have shown that loss of HtrA2 results in mitochondrial accumulation of unfolded proteins and oxidative stress that activates a mitochondrial stress response resulting in the induction of the transcription factor C/EBP-

homologous protein (CHOP) and neuronal death, likely involving a mitochondria-ER crosstalk [155]. However, the role of HtrA2 within the mitochondria remains unclear and some studies have questioned its contribution in PD pathogenesis at least as a component of the Parkin/PINK1 pathway [156]. Transgenic mice expressing pathogenic mutants of α -synuclein (A53T mice) also show mitochondrial abnormalities linked to neural degeneration [157]. Although these mice develop profound adult-onset motor abnormalities that progress to paralysis and death, a definitive role of mitochondrial dysfunction as a contributing factor in this mouse model needs to be established. The possible relationship between increased alpha synuclein levels, mitochondrial defects and PD pathology is reinforced by a recent study where accumulation of α -synuclein in the mitochondria of human dopaminergic neurons results in reduced complex I activity and increased production of ROS, whereas α -synuclein lacking the mitochondrial targeting signal fails to associate with mitochondria and induce mitochondrial dysfunction [158]. The physiological task of α -synuclein is poorly defined, however, consistent with its ubiquitous distribution in brain. However, different studies have described a role for α -synuclein in brain lipid metabolism, where its deletion results in an elevation of cholesterol and cholesteryl esters [159]. Interestingly, in α -synuclein knockout mice the elevated cholesterol levels correlate with increased mitochondrial membrane order and electron transport chain impairment [160]. Although mitochondrial GSH levels have not been specifically analyzed in this mouse model, it is conceivable that lipid abnormalities, and in particular the cholesterol upregulation may result in decreased mitochondrial GSH stores, which in turn, may play a role in PD progression as described in AD. To date whether cholesterol homeostasis is altered in PD is unknown and will need future studies. Altogether, although it seems well established that PD has a mitochondrial component,

however, whether dysfunctional mitochondria are the common denominator that leads to the different aspects of PD pathogenesis is still an open question.

3.3. Huntington's disease (HD)

HD neuropathology is characterized by the preferential degeneration of striatal medium spiny neurons secreting γ -aminobutyric acid (GABAergic neurons), which extends to other brain regions as the disease progresses. The abnormal polyglutamine tract expansion within a cytosolic protein called huntingtin (htt) has been described as the causative factor for HD [161]. Although the pathogenic processes triggered by mutant htt have not yet been fully identified, a fundamental facet of htt is the blockade of transcription by interfering the activity of transcription factors [162]. In addition to this crucial role, it is becoming increasingly clear that alterations in mitochondrial function by htt contribute to the HD pathogenesis. Studies with immortalized striatal cells from knock-in HD-mouse models and brain mitochondria from transgenic mice expressing full-length mutant htt have demonstrated that mutant htt interact directly with mitochondria resulting in impaired respiration, loss of membrane potential and Ca^{2+} -handling defects [10, 163] (Fig. 3). Mutant htt can also initiate the mitochondrial apoptotic pathway by increasing the levels and transcriptional activity of p53 [164] (Fig. 3). Moreover, genetic deletion of p53 suppresses mitochondrial membrane depolarization and cytotoxicity in HD cells, and prevents the neurobehavioral abnormalities of mutant htt transgenic mice. In addition, studies in knockout mice have linked deficiencies on PGC-1 α , a potent stimulator of mitochondrial biogenesis, with HD-like phenotype [165, 166]. Mutant htt has been shown to impair PGC-1 α activity by blocking the transcription of its gene [167] (Fig. 3). As previously mentioned PGC-1 α is also a powerful regulator of ROS metabolism, required for the induction of many ROS-detoxifying enzymes [62]. Thus, the ability of PGC-1 α to

increase mitochondrial electron transport activity while stimulating a broad anti-ROS program makes this protein a powerful tool for controlling the damage associated with defective mitochondrial function seen not only in HD but also in other neurodegenerative diseases. PGC-1 α is directly deacetylated and activated by sirtuin type1 (SIRT1), a member of the sirtuin family of protein deacetylases [168]. SIRT1 is involved in the regulation of transcriptional silencing as well as in the deacetylation of both histone and a growing number of non-histone substrates, modulating not only the mitochondrial function, via PGC-1 α activation, but also other critical mechanisms in ageing-related neurodegeneration, including removal of protein aggregates (decay-accelerating factor -16, DAF-16), regulation of stress responses (FOXO; p53) and inflammatory processes (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, NF-kB; liver X-activated receptor, LXR) [169]. Therefore, the manipulation of sirtuin activities, either by SIRT-activating compounds such as resveratrol or by metabolic conditioning associated with caloric restriction has neuroprotective effects that hold great promise as a potential therapeutic strategy for neurodegenerative diseases.

Finally, in further supporting a role for mitochondria dysfunction in HD, we have observed that mitochondrial GSH depletion in caveolin-1 knockout mice due to cholesterol accumulation sensitized to 3-nitropropionic acid (3-NP)-induced neuronal death and HD pathology (Bosch *et al.*, unpublished data). In line with these observations involving mitochondrial dysfunction, it has been recently reported that the targeting of hypoxia inducible factor (HIF-1 α) and prolyl hydroxylases by chemical inhibitors protect against 3-NP and MPTP-mediated neuronal death, establishing a link between mitochondrial dysfunction and HIF-1 α signaling pathway in neurodegeneration [170, 171]. Parallel to the

caveat outlined above for PD disease, the relevance of these studies using neurotoxic agents as an experimental approach for HD has to be taken cautiously.

3.4. Amyotrophic lateral sclerosis (ALS)

ALS is characterized by selective premature degeneration and death of motor neurons, which control voluntary actions. Familial ALS-linked mutations in the Cu,Zn-superoxide dismutase (SOD1) gene cause motor neuron death in about 3% of ALS cases. Intriguingly, mutations in SOD1 associated with ALS phenotype results in a gain-of-function with an enhanced free radical-generating capacity compared to wild type enzyme [172, 173]. In affected neurons mutated SOD1 (mutSOD) targets mitochondria and forms aggregates [174, 175] that lead to structural alterations, such as mitochondrial swelling and vacuolization [176, 177]. The presence of cross-linked oligomers of mutSOD1 causes a shift in the redox state of these organelles and inhibits complex II and IV of the mitochondrial electron transport chain resulting in loss of membrane potential [178, 179] (Fig. 3).

Mice expressing mutations in SOD1 recapitulate the main clinical and pathological features of the disease. Mitochondria from these mouse models of human ALS show increased vulnerability and impaired Ca^{2+} homeostasis [180] that precedes the onset of motor symptoms [181]. In fact, while the wild-type protein is anti-apoptotic, mutSOD1 seems to promote the mitochondrial apoptotic pathway. First, it has been suggested that mutSOD1 by increasing the levels of oxidized cardiolipin in the brain of mutSOD1 transgenic mice may disrupt the association of cytochrome *c* with the inner mitochondrial membrane, thereby priming the apoptotic program [182]. In addition, Bcl-2 can bind to mutSOD1-containing aggregates in mitochondria contributing to the depletion of this anti-apoptotic protein in motor neurons [183]. Moreover, recent data show that the genetic deletion of

CypD has robust effects in ALS mice by delaying disease onset and extending survival, supporting the participation of MPT in the causal mechanisms of motor neuron degeneration [184] (Fig. 3). The mechanisms whereby mutSOD1 contribute to mitochondrial dysfunction are still unknown. Since mutSOD1 exhibits a gain-of-function it may be conceivable that accelerated superoxide anion scavenging to hydrogen peroxide in mitochondria could overwhelm the GSH redox system, particularly if GSH availability in mitochondria becomes limited, resulting in potential generation of hydroxyl radical, although this remains to be critically established.

Interestingly, before the activation of apoptotic proteins, motor neurons exhibit mitochondrial abnormalities at the innervated neuromuscular junction with high levels of mutSOD1 in the presynaptic terminals. Indeed, neuromuscular denervation and mitochondrial vacuolization is present in the absence of apoptotic death in mutSOD1 transgenic mice crossed with Bax knockout mice [185], indicating that mitochondrial changes in distal axons may trigger the mechanisms responsible for axonal degeneration and denervation. Furthermore, overexpression of mutSOD1 in cell cultures has shown to significantly disrupt both anterograde and retrograde mitochondrial transport [186, 187]. These trafficking abnormalities along with the observations of mitochondrial fragmentation and axonal clustering [188, 189] are highly suggestive that mitochondrial dynamics modulated by mutSOD1 may play a critical role in ALS pathogenesis. However, as mutSOD1 only account for 3% of ALS cases the mitochondrial role in the pathogenesis of most common sporadic form of ALS has to be yet proved.

4. AUTOPHAGY, MITOCHONDRIAL DYNAMICS AND MITOPHAGY.

Autophagy is the generic term for pathways that transport cytosolic contents to lysosomes for its degradation, thought to play a critical housekeeping role to nourish cells and prevent

accumulation damaged organelles, including mitochondria. First, we will briefly describe this process to focus then on mitophagy and relation to neurodegeneration.

Two main types of autophagy has been described (macroautophagy and chaperone-mediated autophagy) that differ with respect to their physiological functions and the mode of cargo delivery to lysosomes. In brief, macroautophagy is a bulk degradation pathway capable of getting ride of large protein complexes or organelles and involves the formation of double-membrane vesicles (autophagosomes or autophagic vacuoles) which fuse with lysosomes (autolysosomes or autophagolysosomes) [190]. This is a highly regulated process orchestrated by a family of autophagy-related (Atg) proteins and controlled by the mammalian target of rapamycin (mTOR) kinase pathway [190]. By contrast, chaperone-mediated autophagy (CMA) is selective for specific cytosolic proteins that contain a pentapeptide Lys-Phe-Glu-Arg-Gln (KFERQ)-like motif, which is recognized by the 70 kDa heat shock cognate protein (Hsc70) transferring the protein to the lysosomal membrane where, through binding to the receptor lysosome-associated membrane protein type 2a (lamp2a), is then translocated into the lysosomal lumen [191].

Autophagy has a fundamental constitutive function in maintaining cellular homeostasis by performing a constant turnover of cellular components. This basal clearing mechanism is exceptionally efficient in postmitotic cells like neurons with extended life span. Interestingly, neuron-specific ablation of autophagy-related genes in mice causes ubiquitinated protein aggregates, inclusion bodies and progressive neurodegeneration [192, 193]. The requirement for autophagy is even more evident under cellular stress conditions. Autophagy is upregulated as a catabolic mechanism to provide metabolites for ATP generation and biosynthesis in situations of nutrient and growth factor deprivation but also as a clearing system that protects cells from cumulative proteins aggregates and oxidatively

damaged organelles, such as mitochondria. Therefore, an inadequate or defective autophagy may determine cell fate in response to stress conditions and have major consequences in several diseases including neurodegenerative disorders. In this regard, accumulation of autophagic vacuoles (AV) and mitochondrial autophagy (mitophagy) has been reported in several neurodegenerative diseases including AD [194, 195], PD [196, 197] and HD [198, 199]. Although the role of autophagy in brain neurodegeneration is not fully deciphered, the emerging view is that the process is initially induced as a neuroprotective response in stressed neurons but is subsequently impaired by several disease-related factors. For example, it has been described that autophagosome-like structures accumulate in dystrophic neurites of AD patients and model mice due to defective maturation of autophagosomes to lysosomes [200]. The immature autophagosomes are enriched in APP and become active sites of A β synthesis [201]. Further support for the correlation between autophagy and A β metabolism is shown in APP transgenic mice with a genetic reduction in the expression of the autophagic key regulator Beclin-1 that display increased A β accumulation and profound neuronal abnormalities [202]. Autophagy-lysosomal dysfunction has been also described in NPC-1 knockout mice indicating that disturbances in cholesterol metabolism might play a regulatory role [203, 204]. Recent studies show that mutant α -synuclein can block CMA, which in turn, leads to lysosomal dysfunction [205, 206]. Dysregulation of this pathway impairs the activity of myocyte enhancer factor 2D (MEF2D), a transcription factor required for neuronal survival [207]. Additionally, aggregates of mutant huntingtin have been reported to recruit Beclin-1, and therefore, inhibiting the induction of autophagy [208]. Similarly, dynein mutations cause aggregates clearance defects in HD animal models indicating that autophagy dysfunction may also arise from impaired vesicle transport [209].

Dysfunctional mitochondria have been previously proposed to activate mitophagy [210, 211]. Interestingly, recent data from Narendra *et al.* have linked Parkin to the regulation of mitochondrial elimination; Parkin is selectively recruited to mitochondria with low membrane potential and subsequently promotes their autophagy [197, 212]. Therefore, the loss-of-function of Parkin, implicates a failure to eliminate dysfunctional mitochondria in the pathogenesis of Parkinson's disease. Mitophagic insufficiency may culminate in a progressive accumulation of ROS-generating mitochondria exacerbating the cellular stress [213], however, whether this is the case in neurodegenerative diseases remains unknown. A detailed understanding of the regulatory relationship between mitochondria and autophagy would help to decipher their beneficial or detrimental effects on neurodegeneration.

Mitochondria are dynamic organelles that actively divide, fuse with one another and interact with other cellular organelles. Fission and fusion processes are important in maintaining the integrity of mitochondria, in mitochondrial redistribution and turnover, as well as in segregation, stabilization, and protection of mtDNA [214, 215]. In neurons, the mitochondrial fission/fusion machinery is intimately involved in the formation of synapses and dendritic spines. Prevention of mitochondrial fission leads to a loss of mitochondrial network from dendritic spines and a reduction of synapse formation [216]. A group of conserved large dynamin-related GTPases is responsible for the balance between mitochondrial fission and fusion. Dynamin-related protein (Drp1) is a key mediator of mitochondrial fission [217]. Mitochondrial fission (Fis1) in yeast and its mammalian homologue hFis1 are also involved in mitochondrial fission, likely playing a role in Drp1 recruitment to the mitochondria. GTP hydrolysis is thought to cause a conformational change in Drp1 that drives the mitochondrial outer membrane fission event [218, 219]. In contrast to fission, mitochondrial fusion requires both outer and inner membrane

components. Mitofusins 1, 2 (Mfn 1, 2) facilitate outer membrane fusion in mammals, likely through trans interactions that promote membrane curvature and fusion, whereas, the GTPase optic atrophy 1 (OPA1) is the main mediator of inner membrane fusion [220, 221]. Recent data suggest that mitochondrial dynamics may have a role in the management of oxidative damaged organelles. Mitochondrial fusion could complement an injured unit and possibly recover its activity, thereby maintaining the metabolic efficiency [222]. Mitochondrial fission could also have a protective role by the segregation of damaged and inactive mitochondria that facilitates autophagic clearance [214, 223]. In particular, studies from Twing *et al.* reveal that the fission events often generate a subpopulation of non-fusing and autophagocytosed mitochondria with reduced $\Delta\Psi_m$ and decreased levels of the fusion protein OPA1 [223]. Conversely, either inhibition of the fission machinery or overexpression of OPA1 decrease mitochondrial autophagy and result in accumulation of oxidized mitochondrial proteins and impaired respiration [223].

The clinical relevance of a finely tuned balance between fission and fusion processes is underscored by the fact that the pathogenesis of certain hereditary neurodegenerative disorders such as autosomal dominant optic atrophy (ADOA) and Charcot-Marie-Tooth neuropathy type 2A (CMT2A) can be linked to mutations in genes encoding mediators of mitochondrial fusion [224, 225]. The association of neurodegeneration with impaired mitochondrial dynamics is further supported by growing evidence suggesting that mutant proteins which cause more common chronic neurodegenerative diseases, may interact with fission/ fusion GTPases resulting in increased mitochondrial fragmentation. Thus, A β overproduction has been shown to cause abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins [226]. Nitric oxide produced in response to A β , has been proposed to trigger mitochondrial fission, synaptic loss, and

neuronal damage, in part via S-nitrosylation of Drp1 [227]. In addition, studies from several groups either in *Drosophila melanogaster* and mammalian cells suggest an emerging consensual view that PD-related PINK1/Parkin pathway regulates mitochondrial fission and fusion machinery [228, 229]. PINK1 deficiency leads to fragmented mitochondrial cristae, hypersensitivity to oxidative stress, and neuronal degeneration and is rescued by overexpression of parkin and fission factors Drp1 or Fis1 [146, 230-232]. Interestingly, a recent study indicates that a coordinated regulation of mitochondrial dynamics and mitophagy triggered by mitochondrial oxidant generation limits cell death associated with loss of PINK1 function [233]. In this work Parkin overexpression enhances the protective mitophagic response, whereas RNA-interference knockdown of autophagy proteins decreases mitochondrial fragmentation, underscoring the inter-relationship between both processes.

5. CONCLUDING REMARKS

Evidence strongly supports the role of mitochondrial oxidative stress as a common denominator in the pathogenesis of ageing-related neurodegenerative diseases. Data from genetically-modified mice that express the disease-causing mutations have provided a valuable tool to demonstrate that oxidative damage, mitochondrial dysfunction and defective degradation of protein aggregates and impaired organelles are three interrelated molecular processes responsible for cell death in neurodegeneration. Moreover, the discovery that most of the disease-specific proteins interact with mitochondria has unraveled new potential therapeutic targets; still, an important point to take into consideration is that genetic forms of the neurodegenerative diseases are only a small percentage of early-onset cases, therefore, the relevant role of mitochondria observed in mouse models must be also proved for sporadic late-onset cases. Beside that the onset of

oxidative stress and functionally affected mitochondria are features typically associated with brain ageing, further research is required first to elucidate whether mitochondrial dysfunction plays only a contributory role or is an early seminal event in non-inherited forms of neurodegenerative diseases and second and, most important, to design effective strategies to cope with mitochondrial oxidative stress. To date antioxidant therapies have been modestly successful in clinical trials, with best outcomes reflected in just delaying the progression of neurodegeneration. Indeed, increasing the antioxidant cellular content may not necessarily result in mitochondrial protection. We have recently shown that elevated cholesterol in AD may specifically deplete the mitochondrial pool of GSH by impairing its transport into mitochondria, suggesting that efforts should be directed to bypass the transport blockade imposed by cholesterol deposition. These observations on the role of mitochondrial cholesterol in the susceptibility to AD imply that not all GSH precursors would be expected to be of benefit in exerting disease-modifying effects in AD progression, although they may be able to boost the cytosolic GSH pool. Further implications from these findings indicate that only permeable GSH prodrugs able to penetrate mitochondria would result in effective replenishment of this critical pool of GSH, which may protect mitochondria from A β -mediated neurotoxicity.

The generation and use of genetically modified mice as experimental models of neurodegeneration have been extremely valuable in increasing our understanding of the basics of neurodegenerative diseases and in the identification of potential therapeutic targets for the future. However, they may also highlight the disparity between these models and human neurodegeneration, as recently illustrated for the case of memantine showing a significant effectiveness in transgenic AD mice but very modestly so in humans. In addition to our expectation to discover novel therapies for these devastating diseases, the

available mouse models may be very helpful to test new or current therapy as disease or hypothesis-modifying drugs. With respect to mitochondrial function and oxidative stress, it is to be expected that therapeutic agents having the ability to target mitochondria may exhibit hypothesis or disease-modifying effects. Moreover, although the expectation of the translational application of the experimental therapeutics identified in mouse models to human disease has not been fulfilled, this could well reflect our limitation in the understanding the complex interactions/mechanisms of oxidant and antioxidant actions in mitochondria to design effective therapies. For instance, a broad approach might include the activation of transcription factors like PGC-1 α , a potent stimulator of mitochondrial biogenesis but also required for the induction of many ROS-detoxifying enzymes, in combination with the activation of mitophagy processes that would eliminate damaged mitochondria and prevent the accumulation of ROS-generating organelles. Clearly, a greater understanding of how mitochondrial dynamics are involved with the pathogenesis of neurodegenerative diseases and the significant neuroprotective properties of mitophagy activation could be pivotal to develop new therapeutic interventions.

ACKNOWLEDGEMENTS

The work was supported by grants: SAF2006-06780, SAF2008-02199, SAF2008-04974 and SAF2009-11417 (Plan Nacional de I+D), PI070193 and PI0900056 (Instituto de Salud Carlos III), P50-AA-11999 (Research Center for Liver and Pancreatic Diseases, US National Institute on Alcohol Abuse and Alcoholism) and by CIBEREHD (Intituto de Salud Carlos III). **Footnotes:** 1. Bosch, M.; Marí, M.; Herms, A.; Fernández, A.; Kassan, A.; Giralt, A.; Colell, A.; González-Moreno, E.; Tebar, F.; Camps, M.; García-Ruiz, C.; Pérez-

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ABBREVIATIONS: A β , amyloid- β peptide; APP, amyloid precursor protein; ANT, adenine nucleotide translocase; CoQ10, coenzyme Q; CypD, cyclophilin D; GSH, glutathione; Gpx, GSH peroxidase; KGDHC, α -ketoglutarate dehydrogenase complex; MAO, monoamine oxidase; MPT, mitochondrial permeability transition; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; PGC-1 α , PPARgamma coactivator 1; PINK1, PTEN induced putative kinase 1; Prx, peroxiredoxin; PS1, preseniline; ROS, reactive oxygen species; SOD, superoxide dismutase; TCA, tricarboxylic acid; Trx, thioredoxin; TrxR, Trx reductase; UCP, uncoupling protein; VDAC, voltage-dependent anion channel.

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FIGURE LEGENDS

Fig. (1). Mitochondrial ROS generation. The main superoxide ($O_2^{\cdot-}$)-producing sites in mitochondria are the redox centers in complex I and complex III of the electron transport chain (ETC). $O_2^{\cdot-}$ formation at complex I occurs primarily on the matrix side of the inner membrane (IM) through both forward electron transfer, involving electrons originating from NADH, and reverse electron transfer, with electrons derived from succinate, the substrate of complex II. The formation of ROS by complex III appears at both sides of the IM and is attributed to the access of O_2 to semiquinone radical intermediate ($QH^{\cdot}$) formed at the Q_o site during redox cycling of coenzyme Q (Q-cycle). Mitochondrial flavoenzymes like monoamine oxidase (MAO) in the mitochondrial outer membrane (OM), and the dihydrolipoyl dehydrogenase within the α -ketoglutarate dehydrogenase complex (KGDHC), localized in the matrix side of the IM, are also sources of ROS. Additionally, Ca^{2+} overload may favor ROS generation by several mechanisms: 1. Stimulation of the TCA cycle that will enhance electron flow through the ETC, 2. induction of nitric oxide synthase (NOS) and subsequent nitric oxide (NO) generation that would inhibit complex I and IV, and 3. dissociation of cytochrome c (cytC) and its release via the mitochondrial permeability transition (MPT) pore.

Fig. (2). Mitochondrial control of oxidative stress. Anion superoxide ($O_2^{\cdot-}$) has a very short half-life being rapidly dismutated into H_2O_2 and O_2 by manganese superoxide dismutase (Mn-SOD) in the mitochondrial matrix. Excessive $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2) levels can result in generation of the highly reactive hydroxyl radicals ($\cdot OH$) via Haber-Weiss and Fenton reactions. A network of antioxidant enzymatic reactions takes place in mitochondria to eliminate ROS and repair oxidative modification in proteins, membrane lipids and DNA. Glutathione peroxidases (Gpx) catalyze the reduction of H_2O_2

and various hydroperoxides, with glutathione (GSH) as the electron donor and the subsequent conversion of GSH disulfide (GSSG) back into GSH by the NADPH-dependent glutathione reductase (GR). Protein disulfides can be also reduced by the action of mitochondrial thioredoxins (Trx2) and glutaredoxins (Grx2). In addition, mitochondrial Peroxiredoxins (PrxIII) use the peroxidatic cysteine (reactive center) to reduce hydroperoxides in two-steps; first, the Cys residue of each subunit of the Prx homodimer is oxidized to Cys-SOH, which then reacts with neighboring Cys-SH of the other subunit to form an intermolecular disulfide. The disulfide is reduced specifically by Trx2, which is subsequently regenerated by thioredoxin reductase 2 (TrxR2) at the expense of NADPH.

Fig. (3). Mitochondria are a critical target in ageing-related neurodegenerative diseases. Scheme depicting different interactions of disease-specific proteins with mitochondria that lead to ROS, mitochondrial failure and cell death. Most of mutated proteins target different complexes of the electron transport chain (ETC) resulting in ROS generation. Specifically, amyloid- β (A β) also induces oxidative stress by interacting with the amyloid-binding alcohol dehydrogenase (ABAD) and promotes mitochondrial permeability transition (MPT) by binding to cyclophilin D (CypD). In Parkinson's disease, the alteration of the parkin/PINK1 inter-regulatory pathway impairs mitochondrial dynamics and function. Mutated PINK1 might also affect the protease activity of HtrA2 that, in turn, would activate a mitochondrial stress response with the induction of the transcription factor CHOP. In addition, PINK1 can increase the mitochondrial Ca^{2+} levels and promote MPT, similarly as described for the pathogenic mutant huntingtin (htt) and Cu,Zn-superoxide dismutase (SOD1). Both, htt and SOD1 enhance the mitochondrial apoptotic pathway by increasing p53 levels and reducing the anti-apoptotic Bcl-2 levels, respectively. Decreased mitochondrial antioxidant capacity, i.e. as a result of an increase in

mitochondrial membrane cholesterol (CHOL) that affects the mitochondrial GSH transport or an impairment of the transcriptional activity of PGC-1 α , would exacerbate the oxidative stress mediated by pathogenic mutant proteins. Glutathione (GSH), GSH peroxidase (Gpx), α -ketoglutarate dehydrogenase complex (KGDHC), Mn-dependent superoxide dismutase (MnSOD).