Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases

Michael T. Lin[1] & M. Flint Beal[1]

Many lines of evidence suggest that mitochondria have a central role in ageing-related neurodegenerative diseases. Mitochondria are critical regulators of cell death, a key feature of neurodegeneration. Mutations in mitochondrial DNA and oxidative stress both contribute to ageing, which is the greatest risk factor for neurodegenerative diseases. In all major examples of these diseases there is strong evidence that mitochondrial dysfunction occurs early and acts causally in disease pathogenesis. Moreover, an impressive number of disease-specific proteins interact with mitochondria. Thus, therapies targeting basic mitochondrial processes, such as energy metabolism or free-radical generation, or specific interactions of disease-related proteins with mitochondria, hold great promise.

Neurodegenerative diseases are a heterogeneous group of disorders characterized by gradually progressive, selective loss of anatomically or physiologically related neuronal systems. Prototypical examples include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). Despite this heterogeneity, we argue that mitochondrial involvement is likely to be an important common theme in these diseases. Mitochondria are key regulators of cell survival and death (Fig. 1; for a review see ref. 1), have a central role in ageing, and have recently been found to interact with many of the specific proteins implicated in genetic forms of neurodegenerative diseases (Table 1).

Mitochondria and ageing

By far the greatest risk factor for neurodegenerative diseases such as AD, PD and ALS is ageing, and mitochondria have been thought to contribute to ageing through the accumulation of mitochondrial DNA (mtDNA) mutations and net production of reactive oxygen species (ROS).

Although most mitochondrial proteins are encoded by the nuclear genome, mitochondria contain many copies of their own DNA. Human mtDNA is a circular molecule of 16,569 base pairs that encodes 13 polypeptide components of the respiratory chain, as well as the rRNAs and tRNAs necessary to support intramitochondrial protein synthesis using its own genetic code. Inherited mutations in mtDNA are known to cause a variety of diseases, most of which affect the brain and muscles — tissues with high energy requirements. One hypothesis has been that somatic mtDNA mutations acquired during ageing contribute to the physiological decline that occurs with ageing and ageing-related neurodegeneration.

It is well established that mtDNA accumulates mutations with ageing, especially large-scale deletions[2] and point mutations. In the mtDNA control region, point mutations at specific sites can accumulate to high levels in certain tissues: T414G in cultured fibroblasts, A189G and T408A in muscle, and C150T in white blood cells[3]. However, these control-region 'hot spots' have not been observed in the brain[4]. Point mutations at individual nucleotides seem to occur at low levels in the brain[5], although the overall level may be high. Using a polymerase chain reaction (PCR)-cloning-sequencing strategy, we found that the average level of point mutations in two protein-coding regions of brain mtDNA from elderly subjects was ~2 mutations per 10 kb[6]. Noncoding regions, which may be under less selection pressure, potentially accumulate between twice and four times as many[7].

The accumulation of these deletions and point mutations with ageing correlates with decline in mitochondrial function. For example, a negative correlation has been found between brain cytochrome oxidase

Table 1 | Proteins that have a function in major neurodegenerative diseases with mitochondrial involvement

Disease	Genetic causes	Function
Alzheimer's disease	APP	Gives rise to Aβ, the primary component of senile plaques
	PS1 and PS2	A component of γ-secretase, which cleaves APP to yield Aβ
Parkinson's disease	α-Synuclein	The primary component of Lewy bodies
	Parkin	A ubiquitin E3 ligase
	DJ-1	Protects the cell against oxidant-induced cell death
	PINK1	A kinase localized to mitochondria. Function unknown. Seems to protect against cell death
	LRRK2	A kinase. Function unknown
	HTRA2	A serine protease in the mitochondrial intermembrane space. Degrades denatured proteins within mitochondria. Degrades inhibitor of apoptosis proteins and promotes apoptosis if released into the cytosol
Amyotrophic lateral sclerosis	SOD1	Converts superoxide to hydrogen peroxide. Disease-causing mutations seem to confer a toxic gain of function
Huntington's disease	Huntingtin	Function unknown. Disease-associated mutations produce expanded polyglutamine repeats

[1]Department of Neurology and Neuroscience, Weill Medical College of Cornell University, Room F-610, 525 East 68th Street, New York 10021, USA.

©2006 Nature Publishing Group

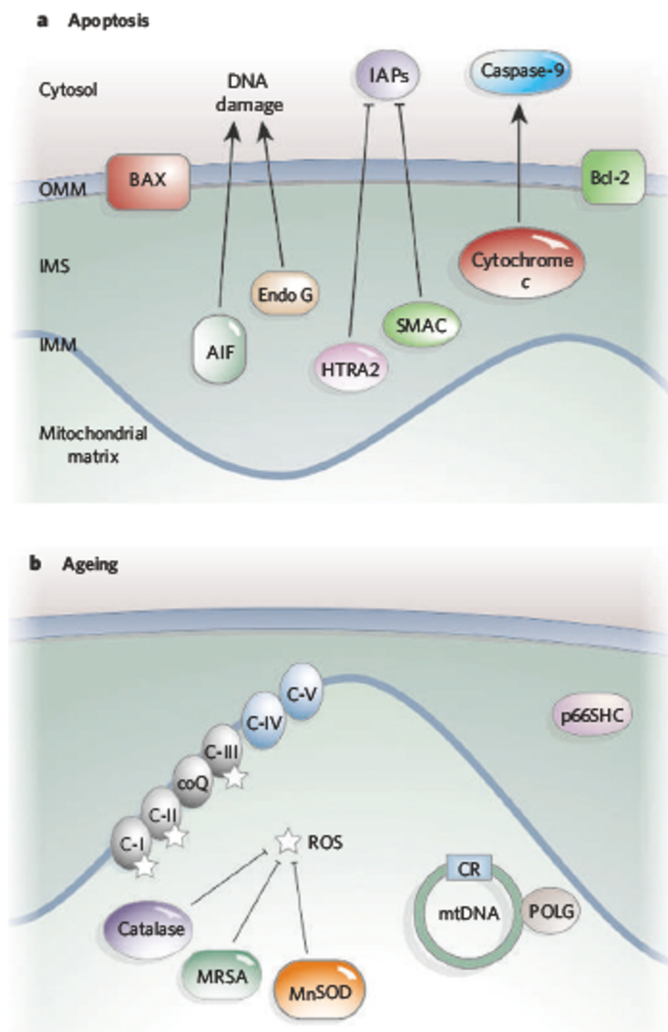

Figure 1 | Role of mitochondria in apoptosis and ageing. **a**, Several intermembrane space (IMS) proteins are pro-apoptotic if released into the cytosol. Cytochrome *c* (C) activates caspase-9. SMAC (second mitochondrial activator of caspases) and HTRA2 inhibit cytosolic inhibitor of apoptosis proteins (IAPs). HTRA2 is a serine protease that might function to remove denatured proteins within mitochondria, but degrades IAPs when released from mitochondria. Inhibiting HTRA2's normal quality control function or enhancing its IAP-degrading activity could both promote cell death. Apoptosis inhibitor factor (AIF) and endonuclease G (endo G) translocate to the nucleus and induce chromatin condensation and DNA fragmentation. Release of these proteins into the cell is modulated by recruitment of BAX (which is proapoptotic) or Bcl-2 (anti-apoptotic) to the outer mitochondrial membrane (OMM). Numerous extracellular and intracellular signals converge to regulate mitochondrial apoptosis (reviewed in ref. 1; see also page 796). **b**, Mitochondrial DNA (mtDNA) mutations and oxidative stress may contribute to ageing. Somatic mutations accumulate in mtDNA with age. Inducing mtDNA mutations by disabling the proofreading activity of mtDNA POLG accelerates ageing-related pathology in transgenic mice. Certain mtDNA polymorphisms are associated with increased longevity, possibly by reducing membrane potential and decreasing the generation of reactive oxygen species (ROS, white stars). Overexpression of ROS-scavenging enzymes manganese superoxide dismutase (MnSOD), methionine sulphoxide reductase A (MSRA) or catalase within mitochondria prolongs lifespan. Knockout of p66SHC, a protein that promotes ROS generation and mitochondrial apoptosis, also prolongs lifespan. Complex IV (C-IV) and complex V activities decline with ageing, and knockdown of complex V activity causes oxidative damage to nuclear DNA, which probably results in decreased gene expression with ageing. IMM, inner mitochondrial membrane.

activity and increased point-mutation levels in a cytochrome oxidase gene (*CO1*)[6]. Moreover, somatic deletions can be clonally expanded in individual neurons, and high levels of such deletions correlate with cytochrome oxidase deficiency on a cell-by-cell basis in the substantia nigra, perhaps contributing to the age dependence of PD[8,9]. However, although the cell-by-cell correlation provides strong circumstantial evidence, correlations do not prove that somatic mtDNA mutations cause age-related pathology.

Recently, several groups have addressed the issue of causation using a clever approach to generate mtDNA mutations experimentally (for a review see ref. 10). MtDNA replication is carried out by mtDNA polymerase-γ (POLG), which has 3′-to-5′ exonuclease (proofreading) activity in addition to its 5′-to-3′ polymerase activity. If the proofreading activity of POLG is eliminated and the polymerase activity preserved, mtDNA mutations accumulate because of uncorrected errors during replication. In mice with such proofreading-deficient POLG (mtDNA-mutator mice), mtDNA mutations accumulate to high levels in all tissues. By 8 weeks of age, homozygous *Polg*$^{-/-}$ animals had 9 point mutations per 10 kb in cytochrome *b*. By contrast, normal mice had less than 1 mutation per 10 kb. This marked increase in mtDNA mutations resulted in decreased respiratory enzyme activity and ATP production. To begin with, the mice appeared normal, but by 25 weeks of age began to exhibit pathology frequently seen in human (although not necessarily murine) ageing, including weight loss, alopecia, osteoporosis, kyphosis, cardiomyopathy, anaemia, gonadal atrophy and sarcopaenia. The median lifespan of such mice was 48 weeks (none lived beyond 61 weeks of age) — much shorter than the typical murine lifespan of 2 years.

A second, independent mtDNA-mutator mouse showed a similar marked increase in mtDNA mutations, progeric features and early mortality. Notably, neuropathology was not reported in either mouse model, although detailed examination was not carried out. Humans with *POLG* mutations exhibit parkinsonism, ophthalmoplegia and myopathy (see below), and the reasons for the differences between mice and humans with such mutations are not yet known.

In both mtDNA-mutator mouse models, markers of apoptosis such as activated caspase 3 were increased at times coinciding with tissue degeneration, suggesting that apoptosis mediates deleterious effects of somatic mtDNA mutations. Interestingly, tissues from the mtDNA-mutator mice did not show increased levels of lipid, protein or DNA oxidation, hydrogen peroxide production, or sensitivity to oxidative stress. Thus, the effects of mtDNA mutations in these mice do not seem to be mediated through ROS production.

Net production of ROS is another important mechanism by which mitochondria are thought to contribute to ageing. Mitochondria contain multiple electron carriers capable of producing ROS, as well as an extensive network of antioxidant defences (Fig. 2). Mitochondrial insults, including oxidative damage itself, can cause an imbalance between ROS production and removal, resulting in net ROS production[11]. The importance to ageing of net mitochondrial ROS production is supported by observations that enhancing mitochondrial antioxidant defences can increase longevity. In *Drosophila*, overexpression of the mitochondrial antioxidant enzymes manganese superoxide dismutase (MnSOD)[12] and methionine sulphoxide reductase[13] prolongs lifespan. This strategy is most successful in short-lived strains of *Drosophila*, and has no effect in already long-lived strains. However, it has recently been shown that overexpression of catalase experimentally targeted to mitochondria increased lifespan in an already long-lived mouse strain[14]. The authors of this work generated transgenic mice overexpressing catalase targeted to peroxisomes, nuclei or mitochondria. The mitochondrially targeted construct provided the maximal benefit, increasing median and maximal lifespan by 20%. Hydrogen peroxide production and oxidative inactivation of aconitase were reduced in isolated cardiac mitochondria, DNA oxidation and levels of mitochondrial deletions were reduced in skeletal muscle, and cardiac pathology, arteriosclerosis and cataract development were delayed.

In humans, a recent study of gene expression in the brain suggests that oxidative damage has a major role in the cognitive decline that

©2006 Nature Publishing Group

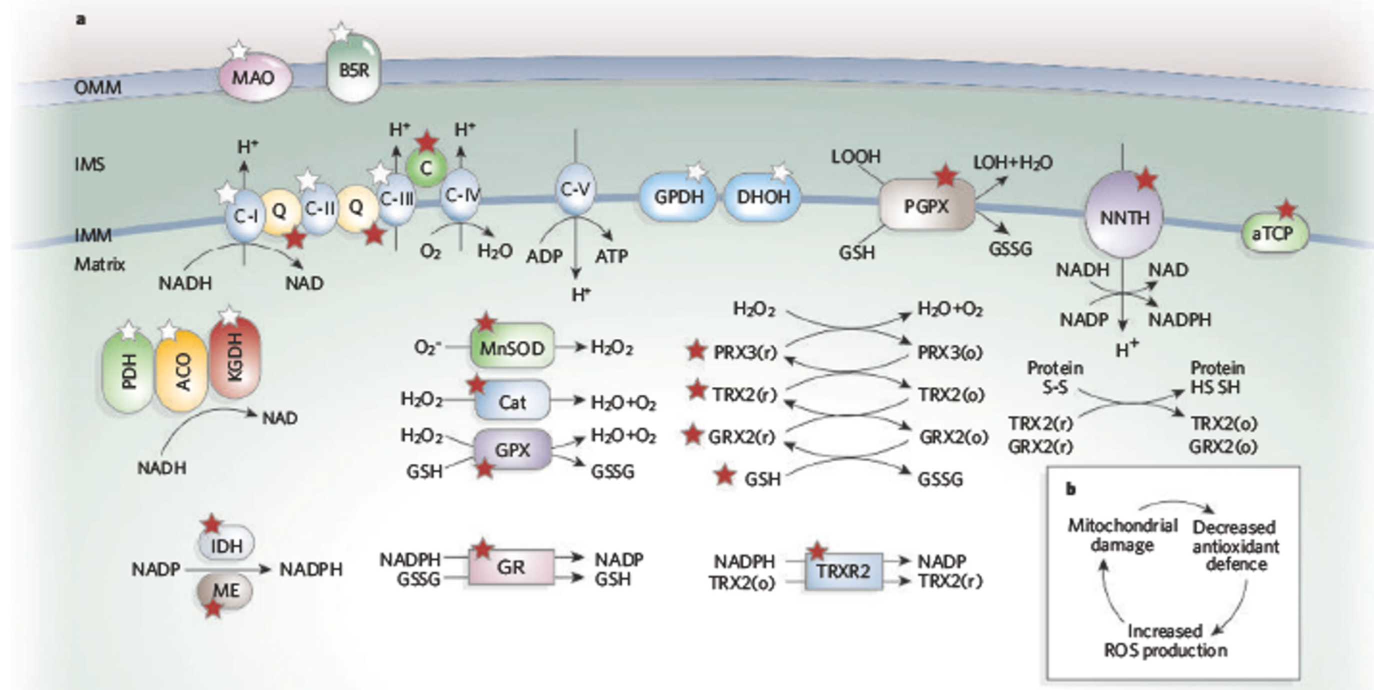

Figure 2 | Role of mitochondria in reactive oxygen species metabolism. The processes and components involved in ROS generation (white stars) and antioxidant defence (red stars). Mitochondria are the primary cellular consumers of oxygen and contain numerous redox enzymes capable of transferring single electrons to oxygen, generating the ROS superoxide ($O_2^{•-}$). Mitochondrial enzymes so far known to generate ROS include the tricarboxylic acid (TCA) cycle enzymes aconitase (ACO) and α-ketoglutarate dehydrogenase (KGDH); the electron-transport chain (ETC) complexes I, II and III; pyruvate dehydrogenase (PDH) and glycerol-3-phosphate dehydrogenase (GPDH); dihydroorotate dehydrogenase (DHOH); the monoamine oxidases (MAO) A and B; and cytochrome b_5 reductase (B5R). The transfer of electrons to oxygen, generating superoxide, is more likely when these redox carriers are abundantly charged with electrons and the potential energy for transfer is high, as reflected by a high mitochondrial membrane potential. ROS generation is decreased when available electrons are few and potential energy for the transfer is low. Mitochondria also contain an extensive antioxidant defence system to detoxify the ROS generated by the reactions described above. Both the membrane-enclosed and soluble compartments are protected. Nonenzymatic components of the system include α-tocopherol (aTCP), coenzyme Q10 (Q), cytochrome *c* (C) and glutathione (GSH). Enzymatic components include manganese superoxide dismutase (MnSOD), catalase (Cat), glutathione peroxidase (GPX), phospholipid hydroperoxide glutathione peroxidase (PGPX), glutathione reductase (GR); peroxiredoxins (PRX3/5), glutaredoxin (GRX2), thioredoxin (TRX2) and thioredoxin reductase (TRXR2). The regeneration of GSH (through GR) and reduced TRX2 (through TRXR2) depends on NADPH, which is derived from substrates (through isocitrate dehydrogenase, IDH, or malic enzyme, ME) or the membrane potential (through nicotinamide nucleotide transhydrogenase, NNTH). So, like ROS generation, antioxidant defences are also tied to the redox and energetic state of mitochondria. GSSG, glutathione disulphide; LOH, lipid hydroxide; LOOH, lipid hydroperoxide; o, oxidized state; r, reduced state. In structurally and functionally intact mitochondria, a large antioxidant defence capacity balances ROS generation, and there is little net ROS production. Mitochondrial damage with decrease of antioxidant defence capacity is a prerequisite for net ROS production. Once this occurs, a vicious cycle (inset) can ensue whereby ROS can further damage mitochondria, causing more free-radical generation and loss or consumption of antioxidant capacity. For example, the Fe–S cluster in aconitase is easily inactivated by superoxide, the iron is released, and this induces hydroxyl radical production. (For a review of the role of mitochondria in ROS metabolism, see ref. 11.)

accompanies ageing[15]. Transcriptional profiling of postmortem frontal cortex samples from individuals aged from 26 to 106 revealed that after the age of 40 there was a decrease in the expression of genes involved in synaptic plasticity, vesicular transport and mitochondrial function, followed by increased expression of stress-response, antioxidant and DNA-repair genes. In the brain, the age-downregulated genes suffered markedly increased oxidative DNA damage compared with the age-stable or age-upregulated genes. Promoter regions were particularly affected, perhaps because they contain G/C-rich sequences that are sensitive to oxidation, or do not undergo transcription-coupled repair. In SH-SY5Y cells, promoters of the same age-downregulated genes were both more sensitive to hydrogen-peroxide-induced damage and less able to undergo base excision repair of such damage than promoters of age-stable or age-upregulated genes.

To investigate whether impaired mitochondrial function could predispose these age-downregulated genes to DNA damage, small interfering RNA (siRNA) was used to reduce the expression of mitochondrial F_1-ATPase 2.5-fold in SH-SY5Y cells, approximating the reduction seen in the aged human cortex. This resulted in significantly increased promoter DNA damage in age-downregulated genes, which was partly reversed by the antioxidant vitamin E. These findings support the idea that mitochondrial dysfunction contributes to the damage of vulnerable genes in the ageing brain. The vulnerable-gene promoters are both more sensitive to oxidative stress and deficient in repair, and mitochondrial dysfunction could potentially exacerbate both by increasing ROS or decreasing the availability of ATP, which is necessary for repair.

Mitochondria and Alzheimer's disease

AD is characterized clinically by progressive cognitive decline, and pathologically by the presence of senile plaques composed primarily of amyloid-β peptide (Aβ) and neurofibrillary tangles made up mainly of hyperphosphorylated tau. About 5–10% of cases are familial, occurring in an early-onset, autosomal-dominant manner. Three proteins are known to be associated with such familial cases: amyloid precursor protein (APP) — which is cleaved sequentially by β- and γ-secretases to produce Aβ — and presenilins 1 and 2 (PS1 and PS2), one or other of which is a component of each γ-secretase complex.

There is extensive literature supporting a role for mitochondrial dysfunction and oxidative damage in the pathogenesis of AD. Oxidative damage occurs early in the AD brain, before the onset of significant

©2006 Nature Publishing Group

plaque pathology[16]. Oxidative damage also precedes Aβ deposition in transgenic APP mice[17], with upregulation of genes relating to mitochondrial metabolism and apoptosis occurring even earlier and co-localizing with the neurons undergoing oxidative damage[18].

Moreover, such oxidative damage and mitochondrial dysfunction probably contribute causally to AD-related pathology. In fetal guinea pig neurons, hydrogen peroxide treatment increased intracellular Aβ levels[19]. Treatment with the mitochondrial uncoupler CCCP (carbonyl cyanide m-chlorophenylhydrazone) caused cultured astrocytes to mimic the amyloidogenic APP processing and intracellular Aβ accumulation that is seen in Down syndrome astrocytes[20]. In a transgenic APP-mutant mouse, hemizygous deficiency of the mitochondrial antioxidant enzyme MnSOD markedly increased brain Aβ levels and plaque deposition[21]. In another transgenic APP-mutant mouse, energy metabolism inhibitors (insulin, 2-deoxyglucose, 3-nitropropionic acid and kainic acid) elevated β-secretase levels and activity and Aβ levels[22].

Several pathways connecting oxidative stress and AD pathology have recently been uncovered. Oxidative stress may activate signalling pathways that alter APP or tau processing. For example, oxidative stress increases the expression of β-secretase through activation of c-Jun amino-terminal kinase and p38 mitogen-activated protein kinase (MAPK)[23], and increases aberrant tau phosphorylation by activation of glycogen synthase kinase 3 (ref. 24). Oxidant-induced inactivation of critical molecules may also be important. In a proteomic study, the prolyl isomerase PIN1 was found to be particularly sensitive to oxidative damage[25]. PIN1 catalyses protein conformational changes that affect both APP and tau processing. Knockout of *Pin1* increases amyloidogenic APP processing and intracellular Aβ levels in mice[26]. *Pin1*-knockout mice also exhibit tau hyperphosphorylation, motor and behavioural deficits, and neuronal degeneration[27].

There is some evidence that mtDNA may be involved in the mitochondrial dysfunction seen in AD. When patient mtDNA is transferred into mtDNA-deficient cell lines, the resulting 'cybrids' reproduce the respiratory enzyme deficiency seen in the brain and other tissues in AD, suggesting that the defect is carried at least in part by mtDNA abnormalities[28]. However, identifying AD-specific mtDNA mutations has been a challenge. Complete sequencing of mtDNA from 145 AD patients and 128 controls did not reveal any significant association with mitochondrial haplogroup or with inherited mtDNA mutations[29]. There was also no association with acquired mtDNA mutations when a coding region (for CO1) was examined[6]. However, in the same way that promoters appeared more sensitive to damage than coding regions in nuclear genes[15], the mtDNA control region showed an increase in acquired mutations in AD[30]. AD brains had on average a 63% increase in heteroplasmic mtDNA control-region mutations, and in individuals older than 80 years there was a 130% increase in mutations. These mutations preferentially altered known mtDNA regulatory elements and suppressed mitochondrial transcription and replication.

Finally, several recent reports suggest that many of the proteins implicated in AD pathogenesis have direct physical involvement with mitochondria or mitochondrial proteins (Fig. 3). APP has been found to have a dual endoplasmic reticulum/mitochondrial-targeting sequence, and in transfected cells and transgenic mice overexpressing APP it clogged the mitochondrial protein importation machinery, causing mitochondrial dysfunction and impaired energy metabolism[31]. Aβ binds to a mitochondrial-matrix protein termed Aβ-binding alcohol dehydrogenase (ABAD)[32]. Blocking the interaction of Aβ and ABAD with a 'decoy peptide' suppressed Aβ-induced apoptosis and free-radical generation in neurons. Conversely, overexpression of ABAD in transgenic APP-mutant mice exaggerated neuronal oxidative stress and impaired memory. Two other groups have also reported that Aβ interacts with mitochondria, inhibiting cytochrome oxidase activity and increasing free-radical generation[33,34]. Aβ also inhibits α-ketoglutarate dehydrogenase activity in isolated mitochondria[35], and deficiency of α-ketoglutarate dehydrogenase[36] and cytochrome oxidase activities[37] has previously been observed in the brain and other tissues in AD. Aβ also interacts with the serine protease HTRA2 (also known as OMI)[38]. Presenilin and all the other components of the γ-secretase complex have also been localized to mitochondria, where they form an active γ-secretase complex[39].

Mitochondria and Parkinson's disease

PD is characterized clinically by progressive rigidity, bradykinesia and tremor, and pathologically by loss of pigmented neurons in the substantia nigra and the presence of Lewy bodies — distinctive cytoplasmic inclusions that immunostain for α-synuclein and ubiquitin.

Mitochondria were first implicated in PD because MPTP (1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine), whose metabolite MPP+ inhibits complex I of the mitochondrial electron-transport chain, caused parkinsonism in designer-drug abusers. This model has since been refined in laboratory animals, in which chronic infusion of rotenone[40] — another complex-I inhibitor — or MPTP[41] results clinically in a parkinsonian phenotype and pathologically in nigral degeneration with cytoplasmic inclusions immunoreactive for α-synuclein and ubiquitin. The mechanism of toxicity in these complex-I inhibition models probably involves oxidative stress[42]. Complex-I inhibition and oxidative stress were shown to be relevant to naturally occurring PD when complex-I deficiency and glutathione depletion were found in the substantia nigra of patients with idiopathic PD and in patients with pre-symptomatic PD[43].

Many of the genes associated with PD also implicate mitochondria in disease pathogenesis. So far, mutations or polymorphisms in mtDNA and at least nine named nuclear genes have been identified as causing PD or affecting PD risk: α-synuclein, parkin, ubiquitin carboxy-terminal hydrolase L1, *DJ-1*, phosphatase and tensin homologue (PTEN)-induced kinase 1 (*PINK1*), leucine-rich-repeat kinase 2 (*LRRK2*), the nuclear receptor *NURR1*, *HTRA2* and tau. Of the nuclear genes, α-synuclein, parkin, *DJ-1*, *PINK1*, *LRRK2* and *HTRA2* directly or indirectly involve mitochondria.

In a small number of cases, inherited mtDNA mutations result in parkinsonism, typically as one feature of a larger syndrome. In one family, we found that the Leber's optic atrophy G11778A mutation was associated with L-DOPA-responsive parkinsonism, variably co-occurring with dementia, dystonia, ophthalmoplegia and ataxia[44]. Notably, this mutation is in a subunit of complex I. Mutations in the nuclear-encoded mtDNA polymerase-γ (*POLG*) gene impair mtDNA replication and result in multiple mtDNA deletions, typically causing chronic progressive external ophthalmoplegia and myopathy. In such families, *POLG* mutations also cosegregate with parkinsonism[45].

There is less evidence for mtDNA involvement in non-syndromic PD. Nigral neurons from PD patients contain increased levels of clonally expanded somatic mtDNA deletions compared with those from age-matched controls, although high levels are also seen in normal ageing[8,9]. We found no difference between PD and control subjects in inherited or acquired complex-I or tRNA point mutations[46,47]. Interestingly, however, several groups have found that certain continent-specific clusters of polymorphisms, termed mtDNA haplogroups, may decrease the risk of developing PD. Among Europeans, the haplogroup cluster UJKT is associated with a decreased risk for PD compared with haplogroup H (ref. 48). It is of note that haplogroups underrepresented in PD patients are overrepresented in healthy centenarians[49]. Protective mtDNA lineages seem to have arisen from areas requiring cold-adaptation, including relative uncoupling of mitochondria to increase heat generation at the expense of ATP production. It has been proposed that this partial uncoupling increases longevity and decreases risk of neurodegeneration by decreasing free-radical generation[50].

Mutations in α-synuclein are associated with autosomal dominant familial PD. α-Synuclein is a major component of Lewy bodies, and the primary effect of α-synuclein mutations is likely to be an increased formation of oligomeric or fibrillar aggregates. However, there seem to be close interrelationships between abnormal protein accumulation or degradation, oxidative stress and mitochondrial dysfunction. In transgenic mice, overexpression of α-synuclein impairs mitochondrial function, increases oxidative stress and enhances nigral pathology induced by MPTP[51]. Moreover, in a recent study of mice overexpressing

©2006 Nature Publishing Group

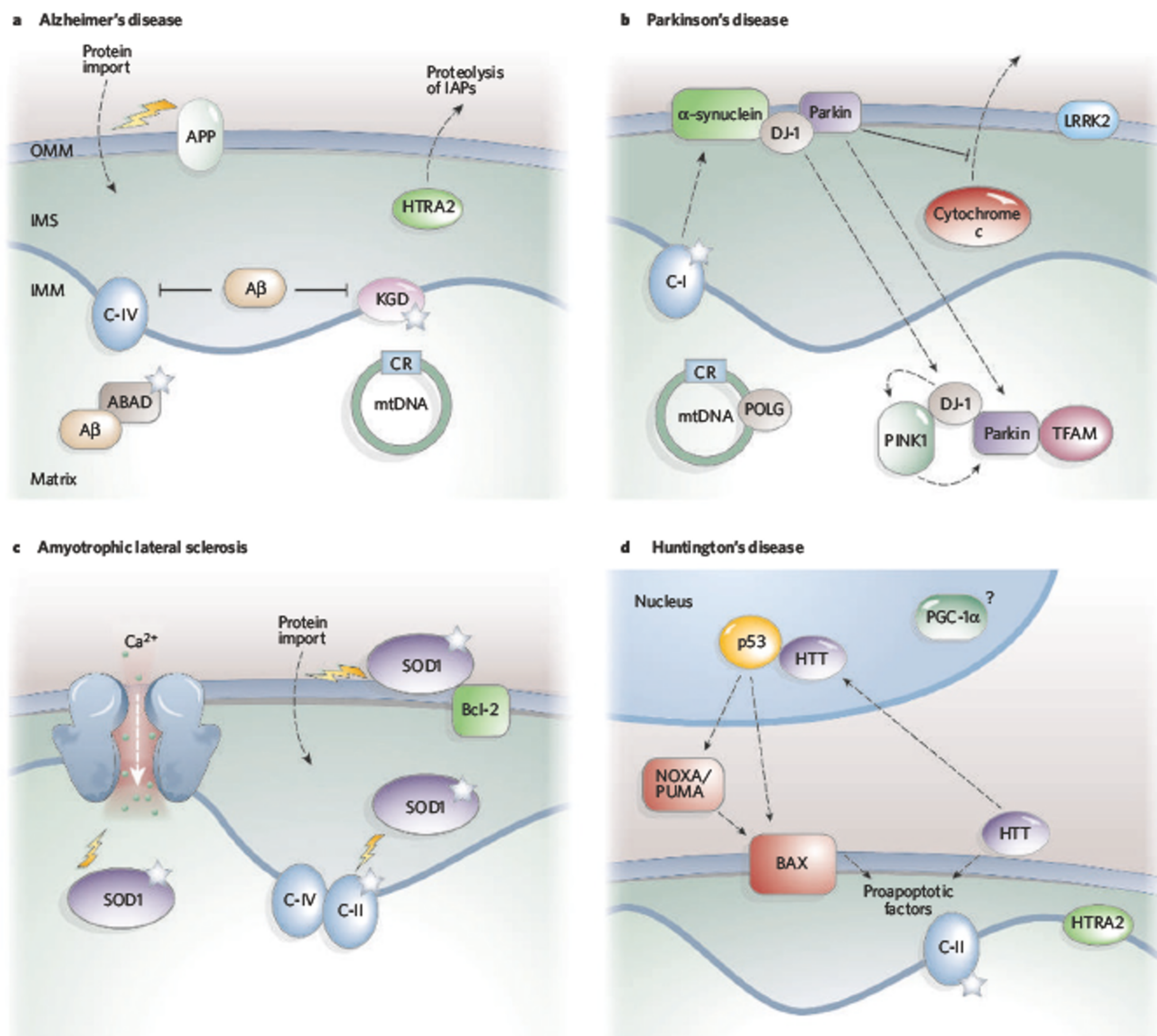

Figure 3 | The role of mitochondria in ageing-related neurodegenerative diseases. **a**, In AD, mitochondrial ROS generation and inhibition of energy metabolism increase Aβ levels in cells and transgenic mice, and Aβ can interact with mitochondria and cause mitochondrial dysfunction. Aβ inhibits complex IV and α-ketoglutarate dehydrogenase (KGD), and binds Aβ-binding alcohol dehydrogenase (ABAD). Both KGD and ABAD produce ROS (white stars). Amyloid precursor protein (APP) may be targeted to the OMM and interfere with protein import. Mitochondria have also been reported to contain active γ-secretase complexes, which are involved in cleaving APP to form Aβ and contain presenilin 1, which increases the proteolytic activity of HTRA2 towards IAPs. AD patients have on average more somatic mutations in the mtDNA control region than control subjects. **b**, Complex I activity is decreased in PD, and inhibition of complex I by MPTP or rotenone causes parkinsonism. Mutations in mtDNA-encoded complex I subunits, 12SrRNA, and POLG also cause parkinsonism. Many genes associated with PD also implicate mitochondria in disease pathogenesis. α-Synuclein immunostaining is seen in degenerating mitochondria from mice overexpressing A53T α-synuclein. α-Synuclein overexpression impairs mitochondrial function and enhances the toxicity of MPTP. Parkin associates with the OMM and protects against cytochrome *c* release. It may also associate with mitochondrial-transcription-factor A (TFAM) and enhance mitochondrial biogenesis. When oxidized, DJ-1 translocates to mitochondria (IMS and matrix), downregulates the PTEN-tumour suppressor (not shown), and protects the cell from oxidative-stress-induced cell death. The mitochondrial kinase PINK1 protects against apoptosis, an effect that is reduced by PD-related mutations or kinase inactivation. Physical associations have been reported between DJ-1 and α-synuclein, DJ-1 and parkin, and DJ-1 and PINK1, and there is genetic evidence that DJ-1, PINK1 and parkin function sequentially in the same pathway. About 10% of the kinase LRRK2 is localized to mitochondria, and PD-related mutations augment its kinase activity. A mutation in HTRA2 was found in ~1% of sporadic PD patients. Overexpression of the mutant impaired normal HTRA2 protease activity, and HTRA2 knockout results in striatal degeneration and parkinsonism. **c**, Overexpression of mutant SOD1 in ALS impairs electron-transport-chain activities and decreases mitochondrial calcium-loading capacity. SOD1 has been localized to the OMM, IMS and matrix, and targeting of mutant SOD1 to mitochondria causes cytochrome *c* release and apoptosis. Mutant SOD1 promotes aberrant mitochondrial ROS production and forms aggregates that may clog the OMM protein importation machinery or bind and sequester the antiapoptotic protein Bcl-2. **d**, Complex II activity is decreased in the HD brain, and the complex-II inhibitor 3-nitropropionic acid induces striatal degeneration and movement disorder in rodents and primates. Overexpression of complex-II subunits reduces cell death in striatal neurons expressing mutant HTT. Mutant HTT associates with the OMM and increases sensitivity to calcium-induced cytochrome *c* release. Mutant HTT also translocates to the nucleus, where it binds and increases the level and transcriptional activity of p53. p53 activates the pro-apoptotic protein BAX, either directly or by increasing expression of BH3-only Bcl-2 family members NOXA and PUMA. In mice, knockout of *Pgc-1α* or a missense mutation in *Htra2* causes involuntary movements and striatal degeneration.

©2006 Nature Publishing Group

A53T mutant α-synuclein, degenerating mitochondria were immunostained for α-synuclein, raising the possibility that mutant α-synuclein might damage mitochondria directly[52]. Whereas overexpression of α-synuclein increases sensitivity to MPTP, α-synuclein-null mice are resistant to MPTP[41] and other mitochondrial toxins such as malonate and 3-nitropropionic acid[53]. Thus, α-synuclein seems to mediate the toxic effects of MPTP.

Mutations in parkin are associated with autosomal recessive juvenile PD. Parkin encodes a ubiquitin E3 ligase, and the primary abnormality, therefore, is in the ubiquitin–proteasome system. However, as above, there seem to be close interrelationships between the ubiquitin–proteasome system, oxidative stress and mitochondrial dysfunction. On one hand, parkin deficiency or mutations lead to oxidative stress and mitochondrial dysfunction. *Parkin*-null *Drosophila*[54] and mouse[55] strains exhibit mitochondrial impairment and increased oxidative stress, and leukocytes from individuals with parkin mutations have a selective impairment in complex-I activity[56]. Parkin can associate with the outer mitochondrial membrane and prevent mitochondrial swelling, cytochrome *c* release and caspase activation, and this protective effect is abrogated by proteasome inhibitors and parkin mutations[57]. Parkin has also been localized to mitochondria in proliferating cells, where it has been shown to associate with mitochondrial transcription factor A and to enhance mitochondrial biogenesis[58]. On the other hand, mitochondrial dysfunction and oxidative stress can affect parkin function and exacerbate parkin mutations. S-nitrosylation of parkin, an oxidative modification, impairs its ubiquitin-ligase activity and compromises its protective function[59]. Conversely, overexpression of glutathione *S*-transferase, which has a role in detoxifying products of oxidative damage, suppresses neurodegeneration in *Drosophila parkin* mutants[60].

Mutations in *DJ-1* are also associated with autosomal recessive juvenile PD[61]. DJ-1 has been reported to interact with α-synuclein[62], parkin[63] and PINK1 (ref. 64). The overall function of DJ-1 seems to be to protect against cell death, especially that induced by oxidative stress. It can act as a redox sensor: oxidative stress causes a critical cysteine residue (C106) to be acidified, which leads to its relocalization to mitochondria. C106 mutations prevent this mitochondrial relocalization and impair the cell's response to oxidative stress and mitochondrial damage[65]. DJ-1 is a negative regulator of the PTEN tumour-suppressor protein, which promotes apoptosis by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate, which is necessary for phosphatidylinositol 3OH-kinase-mediated activation of the cell-survival kinase Akt. *Dj-1* knockdown results in decreased phosphorylation of Akt[66], whereas *Dj-1* overexpression leads to Akt hyperphosphorylation and increased cell survival[67]. DJ-1-deficient mice are hypersensitive to MPTP and oxidative stress[68]. Moreover, in flies, DJ-1 undergoes progressive oxidative inactivation with ageing, which, in turn, increases sensitivity to oxidative stress, and could provide one potential explanation for the age dependence of sporadic PD[69].

Mutations in *PINK1* represent a third form of autosomal recessive juvenile PD[70]. PINK1 is a kinase localized to mitochondria[71], and, like DJ-1, seems to protect against cell death. Overexpression of wild-type PINK1 prevents apoptosis under basal and staurosporine-induced conditions by decreasing cytochrome *c* release and caspase activation, and this effect is abrogated by PD-related mutations and a kinase-inactive mutation[72]. In *Drosophila*, PINK1 deficiency causes mitochondrial pathology, increased sensitivity to paraquat and rotenone, and degeneration of flight muscles and dopaminergic neurons. This pathology resembles that of *parkin*-mutant flies, and can be rescued by overexpression of parkin, but not DJ-1 (ref. 73). Thus, PINK1 probably functions in the same pathway as parkin, with parkin downstream.

Mutations in *LRRK2* are the most common known cause of familial late-onset PD, and also account for 1–2% of sporadic late-onset PD cases. On the basis of its sequence, LRRK2 is predicted to have a ROC–COR GTPase domain, a MAPK kinase kinase domain and WD40 domains. Recently, West and colleagues showed that LRRK2 is a kinase, that two disease-associated mutations, including the most common G2019S mutation, augment the kinase activity, and that ~10% of LRRK2 is associated with mitochondria[74].

The gene most recently associated with PD is *HTRA2* (also known as *OMI*)[75]. A G399S mutation was found in 4 of 414 individuals with sporadic PD and none of 313 controls. An additional polymorphism, A141S, was found in a heterozygous state in 3% of controls and 6.2% of PD subjects ($P=0.039$, odds ratio=2.15). As noted above (Fig. 1), HTRA2 may be a protein quality control agent within mitochondria, and a pro-apoptotic factor when released into the cytosol from the mitochondrial intermembrane space through a mechanism mediated by the pro-apoptotic proteins BAX and BAK. Consistent with a role for HTRA2 in maintaining mitochondrial function, homozygous *Htra2*-knockout mice develop striatal degeneration and parkinsonism[76]. Expression in cultured cells of the G399S and A141S mutations found in people with PD impairs normal HTRA2 protease activity, causes mitochondrial swelling, decreases mitochondrial membrane potential and increases staurosporine-induced cell death[75].

Mitochondria and amyotrophic lateral sclerosis

ALS is characterized clinically by progressive weakness, atrophy and spasticity of muscle tissue, reflecting the degeneration of upper and lower motor neurons in the cortex, brainstem and spinal cord. Approximately 90% of cases are sporadic (SALS) and 10% are familial (FALS). About 20% of familial cases are caused by mutations in Cu/Zn-superoxide dismutase (*SOD1*).

In both SALS and FALS, postmortem and biopsy samples from the spinal cord, nerves and muscles show abnormalities in mitochondrial structure, number and localization. Defects in activities of respiratory-chain complexes have also been detected in muscle and spinal cord. However, it is difficult to know from single snapshots of already symptomatic individuals whether mitochondria contribute to pathogenesis or are innocent bystanders. Thus, research on mitochondrial involvement in ALS has focused on expression of mutant SOD1 in animal and cellular models of the disease.

Overexpressing the G93A *Sod1* mutation in transgenic mice causes impaired mitochondrial energy metabolism in the brain and spinal cord at disease onset[77]. However, long before disease onset there is a decrease in the calcium-loading capacity in mitochondria from the brain and spinal cord, but not the liver, of mice overexpressing G93A or G85R mutant *Sod1* (ref. 78). In G93A *Sod1* mice there is a transient explosive increase in vacuolar mitochondrial degeneration just preceding motor-neuron death[79], suggesting that mitochondrial abnormalities trigger the onset of ALS.

Interestingly, SOD1 immunoreactivity is concentrated inside vacuolated mitochondria[80]. SOD1 has traditionally been thought to be a cytoplasmic protein, but localization of a fraction of cellular SOD1 to the mitochondrial outer membrane, intermembrane space and matrix has now been demonstrated[81,82]. The localization of SOD1 to mitochondria has been reported to occur only in affected tissues and to occur preferentially for mutant SOD1 (ref. 83).

Interaction between SOD1 and mitochondria suggests a number of mechanisms by which mitochondrial function and cell survival may be adversely affected. Mitochondrial targeting of mutant SOD1 caused cytochrome *c* release and apoptosis, whereas targeting to the endoplasmic reticulum or nucleus did not cause cell death[84]. Cleveland and colleagues suggest that mutant SOD1 accumulates and aggregates in the outer mitochondrial membrane and clogs the protein importation machinery, eventually resulting in mitochondrial dysfunction[83]. Mutant SOD1 has been proposed to promote aberrant ROS production, and we found oxidative damage to mitochondrial lipids and proteins, accompanied by impaired respiration and ATP synthesis, in mice expressing mutant human SOD1 (ref. 77). Mutant, but not wild-type, SOD1 species bind to and aggregate with cytosolic heat-shock proteins[85] and mitochondrial Bcl-2 (ref. 86), rendering them unavailable for anti-apoptotic functions.

Mitochondria and Huntington's disease

HD is characterized clinically by chorea, psychiatric disturbances and dementia, and pathologically by loss of long projection neurons in the cortex and striatum. HD is inherited in an autosomal dominant

©2006 Nature Publishing Group

Table 2 | Mitochondrial involvement in less common neurodegenerative diseases

Disease	Clinical features	Protein	Function
Friedreich's ataxia	Ataxia and neuropathy due to degeneration of spinocerebellar tracts and dorsal-root ganglia; diabetes; cardiomyopathy Autosomal recessive	Frataxin	A mitochondrial iron chaperone that promotes the biogenesis of enzymes with Fe–S clusters and detoxifies excess iron. Frataxin deficiency causes iron accumulation and impairs the activity of Fe–S cluster-containing enzymes (complexes I and II, and aconitase). It may also perturb manganese balance and impair MnSOD activity
Hereditary spastic paraplegia (HSP)	Slowly progressive weakness and spasticity of the legs Autosomal recessive	SPG7*	An inner mitochondrial membrane m-AAA metalloprotease. It functions as a chaperone and is involved in the assembly of respiratory-chain complexes. SPG7 deficiency causes recessive HSP with mitochondrial myopathy
	Autosomal dominant	SPG13†	A mitochondrial protein chaperone
Neurodegeneration with brain iron accumulation (NBIA)	Progressive dementia, rigidity, involuntary movements, spasticity and retinal degeneration accompanied by iron deposition in globus pallidus and substantia nigra Autosomal recessive, paediatric onset	PANK2	PANK2 is localized to mitochondria and catalyses the first step in coenzyme A synthesis. PANK2 deficiency is the most common cause of NBIA, accounting for ~50% of cases
Optic atrophy type 1	Autosomal dominant optic neuropathy causing progressive visual loss	OPA1	OPA1 is a dynamin-related GTPase localized to mitochondria. It organizes the mitochondrial inner membrane and is necessary for maintaining cristae integrity. SiRNA knockdown of OPA1 causes fragmentation of the mitochondrial network, loss of mitochondrial membrane potential, disorganization of cristae, release of cytochrome *c* and activation of caspases

*Also known as paraplegin. †Also known as heat-shock protein 60. m-AAA, mitochondrial ATPases associated with diverse cellular activities; PANK2, pantothenate kinase 2; SPG, spastic paraplegia gene.

manner, and is due to expansion of a CAG trinucleotide repeat in the huntingtin (*HTT*) gene, which gives rise to an expanded polyglutamine stretch in the corresponding protein. The normal number of CAG (Q) repeats is less than 36; repeat numbers greater than 40 are associated with human disease.

Various lines of evidence demonstrate the involvement of mitochondrial dysfunction in HD. Nuclear magnetic resonance spectroscopy reveals increased lactate in the cortex and basal ganglia[87]. Biochemical studies show decreased activities of complexes II and III of the electron-transport chain in the human HD brain[88]. In striatal cells from mutant *Htt*-knock-in mouse embryos, mitochondrial respiration and ATP production are significantly impaired[89].

The mitochondrial dysfunction observed above is likely to be pathogenically important, because 3-nitropropionic acid and malonate — mitochondrial toxins that selectively inhibit succinate dehydrogenase and complex II — induce a clinical and pathological phenotype that closely resembles HD[90]. Moreover, in striatal neurons expressing the first 171 amino acids of HTT with an insertion of 82 glutamines, overexpression of complex-II subunits restored complex-II activity and blocked mitochondrial dysfunction and cell death[91].

There are several mechanisms by which the mutation could result in mitochondrial dysfunction. First, HTT may interact directly with mitochondria. In one study[92], lymphoblast mitochondria from HD patients and brain mitochondria from YAC transgenic mice expressing HTT with 72 repeats were found to have lower membrane potentials and to depolarize at lower calcium loads than control mitochondria. Amino-terminal mutant HTT was identified on neuronal mitochondrial membranes with immunoelectron microscopy, and the incubation of normal mitochondria with mutant HTT reproduced the calcium-handling defect seen in HD patients and transgenic mice. In another study[93], subfractionation of mitochondria from a knock-in HD-mouse model showed HTT in association with the outer mitochondrial membrane. Mitochondria from the knock-in HD mouse were more sensitive to calcium-induced mitochondrial permeabilization and cytochrome *c* release, an effect that was mimicked by incubating normal mitochondria with mutant, but not wild-type, HTT.

Another mechanism through which mutant HTT could affect mitochondrial function is by altering transcription[94]. HTT interacts with a number of transcription factors, including p53, CREB-binding protein and SP1 (for a review see ref. 95). p53 is a tumour suppressor known to regulate genes involved in mitochondrial function and oxidative stress. In response to genotoxic injury, p53 activates mitochondrial pathway apoptosis by increasing transcription of the pro-apoptotic BH3-only Bcl-2 family members such as PUMA[96]. p53 can also translocate to mitochondria and directly activate BAX[97]. In a recent study[98], mutant HTT bound p53 and increased p53 levels and transcriptional activity, leading to upregulation of downstream targets BAX and PUMA, and mitochondrial membrane depolarization. Pharmacological suppression or genetic deletion of *p53* prevented HTT-induced mitochondrial depolarization, cytochrome oxidase deficiency and cytotoxicity. Moreover, expression of mutant HTT in a p53-null background diminished retinal degeneration in *Drosophila* and reversed behavioural abnormalities in mice.

Although they have not yet been directly related to human HD, two mitochondria-related proteins are associated with HD-like phenotypes in transgenic mice. PGC-1α is a transcriptional coactivator that regulates mitochondrial biogenesis and metabolic pathways. *Pgc-1α*-knockout mice exhibit impaired mitochondrial function, a hyperkinetic movement disorder and striatal degeneration — features that are all also observed in HD[99]. HTRA2 is a serine protease that resides in the mitochondrial intermembrane space. In mice, a missense mutation in HTRA2 that reduces protease activity results in the Mnd2 (motor-neuron degeneration 2) phenotype, which shares many features of HD, including involuntary movements, abnormal postures and massive loss of striatal neurons[100].

Mitochondrial involvement in other, less common, neurodegenerative diseases is reviewed in Table 2.

Summary and future directions

Recent findings have greatly expanded our understanding of the role of mitochondria in the pathogenesis of neurodegenerative diseases. Mitochondrial-DNA mutations and oxidative stress contribute to ageing, the greatest risk factor for neurodegenerative diseases. Mitochondrial dysfunction and oxidative stress occur early in all major neurodegenerative diseases, and there is strong evidence that this dysfunction has a causal role in disease pathogenesis. Most impressively, specific interactions of disease-related proteins with mitochondria have recently been uncovered: APP, Aβ, presenilin, α-synuclein, parkin, DJ-1, PINK1, LRRK2, HTRA2, SOD1 and huntingtin have all been found within mitochondria, as discussed above. This explosion of findings raises further questions.

First, what is the basis for cell-type specificity in neurodegenerative disorders? For example, why does systemic overexpression of mutant SOD1 affect calcium handling in brain but not liver mitochondria[78]? The propensity of mitochondrial disorders to affect the brain and muscles has thus far been explained by the different tissue requirements for mitochondrial function — brain and muscle tissues have high energy requirements. However, we speculate that mitochondrial differences between different cell types might be at least as important in this selectivity,

©2006 Nature Publishing Group

if not more so. Most mitochondrial proteins are encoded by nuclear genes, and it is to be expected that the distinct nuclear programmes required to produce different cell types should also produce different mitochondria. However, knowledge of mitochondrial biology in different cell types is extremely rudimentary. Further investigation will probably provide a more fundamental understanding of neurodegenerative diseases, because cell selectivity is a fundamental characteristic of these disorders.

Second, the complexity of mitochondrial ROS metabolism suggests that interventions such as the administration of one or a few antioxidants may be too simplistic. Indeed, such interventions have generally been, at best, modestly successful in clinical trials, despite abundant evidence for oxidative stress in disease pathogenesis. A more complete approach to antioxidant therapy would be to decrease ROS generation (for example, by expressing uncoupling proteins) and to upregulate the multilayered endogenous mitochondrial and intracellular antioxidant defence network. However, this will require a considerably better understanding of ROS biology than we have at present. It will also require the global 'ROS bad, antioxidants good' impression to be replaced with knowledge of the specific targets of ROS (such as PIN1 in AD).

Finally, the interaction of mitochondria with specific disease-related proteins opens exciting new possibilities for therapeutic targets. For example, in HD, reducing p53 protects against mutant huntingtin. However, in the case of AD, PD and ALS, genetic forms of disease account for only a small percentage of cases, and the relevance of mitochondrial interactions with these proteins must be determined for sporadic cases.

Knowledge of neurodegenerative diseases has advanced rapidly in the last few years, and the field holds great promise for furthering our understanding and the eventual treatment of these devastating illnesses. ■

1. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116**, 205–219 (2004).
2. Corral-Debrinski, M. *et al.* Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nature Genet.* **2**, 324–329 (1992).
3. Zhang, J. *et al.* Strikingly higher frequency in centenarians and twins of mtDNA mutation causing remodeling of replication origin in leukocytes. *Proc. Natl Acad. Sci. USA* **100**, 1116–1121 (2003).
4. Chinnery, P. F. *et al.* Point mutations of the mtDNA control region in normal and neurodegenerative human brains. *Am. J. Hum. Genet.* **68**, 529–532 (2001).
5. Simon, D. K. *et al.* Low mutational burden of individual acquired mitochondrial DNA mutations in brain. *Genomics* **73**, 113–116 (2001).
6. Lin, M. T., Simon, D. K., Ahn, C. H., Kim, L. M. & Beal, M. F. High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. *Hum. Mol. Genet.* **11**, 133–145 (2002).
7. Jazin, E. E., Cavelier, L., Eriksson, I., Oreland, L. & Gyllensten, U. Human brain contains high levels of heteroplasmy in the noncoding regions of mitochondrial DNA. *Proc. Natl Acad. Sci. USA* **93**, 12382–12387 (1996).
8. Bender, A. *et al.* High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nature Genet.* **38**, 515–517 (2006).
9. Kraytsberg, Y. *et al.* Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nature Genet.* **38**, 518–520 (2006).
10. Trifunovic, A. Mitochondrial DNA and ageing. *Biochim. Biophys. Acta* **1757**, 611–617 (2006).
11. Andreyev, A. Y., Kushnareva, Y. E. & Starkov, A. A. Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Mosc.)* **70**, 200–214 (2005).
12. Sun, J., Folk, D., Bradley, T. J. & Tower, J. Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult *Drosophila melanogaster*. *Genetics* **161**, 661–672 (2002).
13. Ruan, H. *et al.* High-quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proc. Natl Acad. Sci. USA* **99**, 2748–2753 (2002).
14. Schriner, S. E. *et al.* Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* **308**, 1909–1911 (2005).
15. Lu, T. *et al.* Gene regulation and DNA damage in the ageing human brain. *Nature* **429**, 883–891 (2004).
16. Nunomura, A. *et al.* Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **60**, 759–767 (2001).
17. Pratico, D., Uryu, K., Leight, S., Trojanoswki, J. Q. & Lee, V. M. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J. Neurosci.* **21**, 4183–4187 (2001).
18. Reddy, P. H. *et al.* Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. *Hum. Mol. Genet.* **13**, 1225–1240 (2004).
19. Ohyagi, Y. *et al.* Selective increase in cellular A β 42 is related to apoptosis but not necrosis. *Neuroreport* **11**, 167–171 (2000).
20. Busciglio, J. *et al.* Altered metabolism of the amyloid β precursor protein is associated with mitochondrial dysfunction in Down's syndrome. *Neuron* **33**, 677–688 (2002).
21. Li, F. *et al.* Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J. Neurochem.* **89**, 1308–1312 (2004).
22. Velliquette, R. A., O'Connor, T. & Vassar, R. Energy inhibition elevates β-secretase levels and activity and is potentially amyloidogenic in APP transgenic mice: possible early events in Alzheimer's disease pathogenesis. *J. Neurosci.* **25**, 10874–10883 (2005).
23. Tamagno, E. *et al.* β-Site APP cleaving enzyme up-regulation induced by 4-hydroxynonenal is mediated by stress-activated protein kinases pathways. *J. Neurochem.* **92**, 628–636 (2005).
24. Lovell, M. A., Xiong, S., Xie, C., Davies, P. & Markesbery, W. R. Induction of hyperphosphorylated tau in primary rat cortical neuron cultures mediated by oxidative stress and glycogen synthase kinase-3. *J. Alzheimers Dis.* **6**, 659–671 (2004).
25. Sultana, R. *et al.* Oxidative modification and down-regulation of Pin1 in Alzheimer's disease hippocampus: a redox proteomics analysis. *Neurobiol. Aging* **27**, 918–925 (2006).
26. Pastorino, L. *et al.* The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-β production. *Nature* **440**, 528–534 (2006).
27. Liou, Y. C. *et al.* Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. *Nature* **424**, 556–561 (2003).
28. Swerdlow, R. H. *et al.* Cybrids in Alzheimer's disease: a cellular model of the disease? *Neurology* **49**, 918–925 (1997).
29. Elson, J. L. *et al.* Does the mitochondrial genome play a role in the etiology of Alzheimer's disease? *Hum. Genet.* **119**, 241–254 (2006).
30. Coskun, P. E., Beal, M. F. & Wallace, D. C. Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc. Natl Acad. Sci. USA* **101**, 10726–10731 (2004).
31. Anandatheerthavarada, H. K., Biswas, G., Robin, M. A. & Avadhani, N. G. Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. *J. Cell Biol.* **161**, 41–54 (2003).
32. Lustbader, J. W. *et al.* ABAD directly links A β to mitochondrial toxicity in Alzheimer's disease. *Science* **304**, 448–452 (2004).
33. Crouch, P. J. *et al.* Copper-dependent inhibition of human cytochrome *c* oxidase by a dimeric conformer of amyloid-β_{1-42}. *J. Neurosci.* **25**, 672–679 (2005).
34. Manczak, M. *et al.* Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum. Mol. Genet.* **15**, 1437–1449 (2006).
35. Casley, C. S., Canevari, L., Land, J. M., Clark, J. B. & Sharpe, M. A. β-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J. Neurochem.* **80**, 91–100 (2002).
36. Gibson, G. E. *et al.* Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease. *Arch. Neurol.* **45**, 836–840 (1988).
37. Parker, W. D., Filley, C. M. & Parks, J. K. Cytochrome oxidase deficiency in Alzheimer's disease. *Neurology* **40**, 1302–1303 (1990).
38. Park, H. J., Seong, Y. M., Choi, J. Y., Kang, S. & Rhim, H. Alzheimer's disease-associated amyloid β interacts with the human serine protease HtrA2/Omi. *Neurosci. Lett.* **357**, 63–67 (2004).
39. Hansson, C. A. *et al.* Nicastrin, presenilin, APH-1, and PEN-2 form active γ-secretase complexes in mitochondria. *J. Biol. Chem.* **279**, 51654–51660 (2004).
40. Betarbet, R. *et al.* Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neurosci.* **3**, 1301–1306 (2000).
41. Fornai, F. *et al.* Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin–proteasome system and α-synuclein. *Proc. Natl Acad. Sci. USA* **102**, 3413–3418 (2005).
42. Sherer, T. B. *et al.* Mechanism of toxicity in rotenone models of Parkinson's disease. *J. Neurosci.* **23**, 10756–10764 (2003).
43. Schapira, A. H. *et al.* Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* **i**, 1269 (1989).
44. Simon, D. K. *et al.* Familial multisystem degeneration with parkinsonism associated with the 11778 mitochondrial DNA mutation. *Neurology* **53**, 1787–1793 (1999).
45. Luoma, P. *et al.* Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. *Lancet* **364**, 875–882 (2004).
46. Vives-Bauza, C. *et al.* Sequence analysis of the entire mitochondrial genome in Parkinson's disease. *Biochem. Biophys. Res. Commun.* **290**, 1593–1601 (2002).
47. Simon, D. K. *et al.* Somatic mitochondrial DNA mutations in cortex and substantia nigra in aging and Parkinson's disease. *Neurobiol. Aging* **25**, 71–81 (2004).
48. Pyle, A. *et al.* Mitochondrial DNA haplogroup cluster UKJT reduces the risk of PD. *Ann. Neurol.* **57**, 564–567 (2005).
49. Tanaka, M. Mitochondrial genotypes and cytochrome b variants associated with longevity or Parkinson's disease. *J. Neurol.* **249** (Suppl. 2), II11–II18 (2002).
50. Wallace, D. C. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* **39**, 359–407 (2005).
51. Song, D. D., Shults, C. W., Sisk, A., Rockenstein, E. & Masliah, E. Enhanced substantia nigra mitochondrial pathology in human α-synuclein transgenic mice after treatment with MPTP. *Exp. Neurol.* **186**, 158–172 (2004).
52. Martin, L. J. *et al.* Parkinson's disease α-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *J. Neurosci.* **26**, 41–50 (2006).
53. Klivenyi, P. *et al.* Mice lacking α-synuclein are resistant to mitochondrial toxins. *Neurobiol. Dis.* **21**, 541–548 (2006).
54. Pesah, Y. *et al.* *Drosophila* parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development* **131**, 2183–2194 (2004).
55. Palacino, J. J. *et al.* Mitochondrial dysfunction and oxidative damage in *parkin*-deficient mice. *J. Biol. Chem.* **279**, 18614–18622 (2004).
56. Muftuoglu, M. *et al.* Mitochondrial complex I and IV activities in leukocytes from patients with parkin mutations. *Mov. Disord.* **19**, 544–548 (2004).
57. Darios, F. *et al.* Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. *Hum. Mol. Genet.* **12**, 517–526 (2003).
58. Kuroda, Y. *et al.* Parkin enhances mitochondrial biogenesis in proliferating cells. *Hum. Mol. Genet.* **15**, 883–895 (2006).
59. Chung, K. K. *et al.* S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science* **304**, 1328–1331 (2004).

©2006 Nature Publishing Group

60. Whitworth, A. J. *et al.* Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease. *Proc. Natl Acad. Sci. USA* **102,** 8024–8029 (2005).
61. Bonifati, V. *et al.* Mutations in the *DJ-1* gene associated with autosomal recessive early-onset parkinsonism. *Science* **299,** 256–259 (2003).
62. Meulener, M. C. *et al.* DJ-1 is present in a large molecular complex in human brain tissue and interacts with α-synuclein. *J. Neurochem.* **93,** 1524–1532 (2005).
63. Moore, D. J. *et al.* Association of DJ-1 and parkin mediated by pathogenic DJ-1 mutations and oxidative stress. *Hum. Mol. Genet.* **14,** 71–84 (2005).
64. Tang, B. *et al.* Association of PINK1 and DJ-1 confers digenic inheritance of early-onset Parkinson's disease. *Hum. Mol. Genet.* **15,** 1816–1825 (2006).
65. Canet-Aviles, R. M. *et al.* The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc. Natl Acad. Sci. USA* **101,** 9103–9108 (2004).
66. Yang, Y. *et al.* Inactivation of *Drosophila* DJ-1 leads to impairments of oxidative stress response and phosphatidylinositol 3-kinase/Akt signaling. *Proc. Natl Acad. Sci. USA* **102,** 13670–13675 (2005).
67. Kim, R. H. *et al.* DJ-1, a novel regulator of the tumor suppressor PTEN. *Cancer Cell* **7,** 263–273 (2005).
68. Kim, R. H. *et al.* Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. *Proc. Natl Acad. Sci. USA* **102,** 5215–5220 (2005).
69. Meulener, M. C., Xu, K., Thompson, L., Ischiropoulos, H. & Bonini, N. M. Mutational analysis of DJ-1 in *Drosophila* implicates functional inactivation by oxidative damage and aging. *Proc. Natl Acad. Sci. USA* **103,** 12517–12522 (2006).
70. Valente, E. M. *et al.* Hereditary early-onset Parkinson's disease caused by mutations in *PINK1*. *Science* **304,** 1158–1160 (2004).
71. Silvestri, L. *et al.* Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum. Mol. Genet.* **14,** 3477–3492 (2005).
72. Petit, A. *et al.* Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J. Biol. Chem.* **280,** 34025–34032 (2005).
73. Yang, Y. *et al.* Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. *Proc. Natl Acad. Sci. USA* **103,** 10793–10798 (2006).
74. West, A. B. *et al.* Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc. Natl Acad. Sci. USA* **102,** 16842–16847 (2005).
75. Strauss, K. M. *et al.* Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum. Mol. Genet.* **14,** 2099–2111 (2005).
76. Martins, L. M. *et al.* Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol. Cell. Biol.* **24,** 9848–9862 (2004).
77. Mattiazzi, M. *et al.* Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. *J. Biol. Chem.* **277,** 29626–29633 (2002).
78. Damiano, M. *et al.* Neural mitochondrial Ca^{2+} capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. *J. Neurochem.* **96,** 1349–1361 (2006).
79. Kong, J. & Xu, Z. Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J. Neurosci.* **18,** 3241–3250 (1998).
80. Jaarsma, D. *et al.* CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. *Acta Neuropathol. (Berl.)* **102,** 293–305 (2001).
81. Higgins, C. M., Jung, C. & Xu, Z. ALS-associated mutant SOD1G93A causes mitochondrial vacuolation by expansion of the intermembrane space and by involvement of SOD1 aggregation and peroxisomes. *BMC Neurosci.* **4,** 16 (2003).
82. Vijayvergiya, C., Beal, M. F., Buck, J. & Manfredi, G. Mutant superoxide dismutase 1 forms aggregates in the brain mitochondrial matrix of amyotrophic lateral sclerosis mice. *J. Neurosci.* **25,** 2463–2470 (2005).
83. Liu, J. *et al.* Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. *Neuron* **43,** 5–17 (2004).
84. Takeuchi, H., Kobayashi, Y., Ishigaki, S., Doyu, M. & Sobue, G. Mitochondrial localization of mutant superoxide dismutase 1 triggers caspase-dependent cell death in a cellular model of familial amyotrophic lateral sclerosis. *J. Biol. Chem.* **277,** 50966–50972 (2002).
85. Okado-Matsumoto, A. & Fridovich, I. Amyotrophic lateral sclerosis: a proposed mechanism. *Proc. Natl Acad. Sci. USA* **99,** 9010–9014 (2002).
86. Pasinelli, P. *et al.* Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. *Neuron* **43,** 19–30 (2004).
87. Jenkins, B. G., Koroshetz, W. J., Beal, M. F. & Rosen, B. R. Evidence for impairment of energy metabolism *in vivo* in Huntington's disease using localized 1H NMR spectroscopy. *Neurology* **43,** 2689–2695 (1993).
88. Gu, M. *et al.* Mitochondrial defect in Huntington's disease caudate nucleus. *Ann. Neurol.* **39,** 385–389 (1996).
89. Milakovic, T. & Johnson, G. V. Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin. *J. Biol. Chem.* **280,** 30773–30782 (2005).
90. Brouillet, E. *et al.* Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proc. Natl Acad. Sci. USA* **92,** 7105–7109 (1995).
91. Benchoua, A. *et al.* Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin. *Mol. Biol. Cell* **17,** 1652–1663 (2006).
92. Panov, A. V. *et al.* Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nature Neurosci.* **5,** 731–736 (2002).
93. Choo, Y. S., Johnson, G. V., MacDonald, M., Detloff, P. J. & Lesort, M. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome *c* release. *Hum. Mol. Genet.* **13,** 1407–1420 (2004).
94. Luthi-Carter, R. & Cha, J.-H. J. Mechanisms of transcription dysregulation in Huntington's disease. *Clin. Neurosci. Res.* **3,** 165–177 (2003).
95. Sugars, K. L. & Rubinsztein, D. C. Transcriptional abnormalities in Huntington disease. *Trends Genet.* **19,** 233–238 (2003).
96. Yu, J., Zhang, L., Hwang, P. M., Kinzler, K. W. & Vogelstein, B. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol. Cell* **7,** 673–682 (2001).
97. Chipuk, J. E. *et al.* Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* **303,** 1010–1014 (2004).
98. Bae, B. I. *et al.* p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. *Neuron* **47,** 29–41 (2005).
99. Lin, J. *et al.* Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice. *Cell* **119,** 121–135 (2004).
100. Rathke-Hartlieb, S. *et al.* Progressive loss of striatal neurons causes motor dysfunction in MND2 mutant mice and is not prevented by Bcl-2. *Exp. Neurol.* **175,** 87–97 (2002).

Acknowledgments This work was supported by grants from the National Institutes of Health and the American Federation for Aging Research/Beeson Program. We apologize to our many colleagues whose work we were unable to cite due to space limitations.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence should be addressed to M.F.B. (fbeal@med.cornell.edu).

©2006 Nature Publishing Group