Mitochondriopathy in Parkinson Disease and Amyotrophic Lateral Sclerosis

Lee J. Martin, PhD

Abstract

The causes of the selective neurodegeneration in Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS) are unknown and commonalities among these and other age-related neurodegenerative diseases continue to be sought. Morphologic, biochemical, molecular genetic, and cell/animal model studies suggest that mitochondria might be a convergence point for neurodegeneration. The functions and properties of mitochondria might render subsets of selectively vulnerable neurons intrinsically susceptible to cellular aging and stress. In PD, mutations in putative mitochondrial proteins have been identified recently and mitochondrial DNA mutations have been found in nigral neurons. In ALS, changes occur in mitochondrial respiratory chain enzymes and in mitochondrial cell death proteins, indicative of an activation of programmed cell death impulses. The mitochondrial potential, \( \Delta \psi_m \), provides the driving force for the accumulation of Ca\(^{2+} \) into the matrix. Cytosolic Ca\(^{2+} \) concentrations above set point levels are believed to be achieved during tetanic stimulation and glutamate receptor activation (1). In settings of excitotoxicity (4), resulting from excessive overstimulation of glutamate receptors, Ca\(^{2+} \) overload in neurons is significant. When mitochondria become overloaded with Ca\(^{2+} \), they undergo mitochondrial permeability transition (see subsequently) resulting in osmotic swelling and rupture of the outer mitochondrial membrane. Interestingly, mitochondria within synapses appear to be more susceptible than non-synaptic mitochondria to Ca\(^{2+} \) overload (5).

MITOCHONDRIUM AND REACTIVE OXYGEN SPECIES

Mitochondria generate most of the endogenous ROS as a byproduct of oxidative phosphorylation (6). Electrons in the electron carriers, such as the unpaired electron of ubisemiquinone bound to coenzyme Q-binding sites of complexes I, II, and III, can be donated directly to O\(_2\) to generate O\(_2^-\) (Fig. 1). The mitochondrial matrix enzyme manganese superoxide dismutase (MnSOD or SOD2; Fig. 1) or copper/zinc SOD (Cu/ZnSOD or SOD1) in the mitochondrial...
intermembrane space and matrix as well as in the cytosol convert $O_2^-$ to hydrogen peroxide ($H_2O_2$) in the reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. $H_2O_2$ is more stable than $O_2^-$ and can diffuse from mitochondria and into the cytosol and nucleus. $H_2O_2$ is detoxified by glutathione peroxidase in mitochondria and the cytosol and by catalase in peroxisomes. When $H_2O_2$ is in the presence of reduced transitional metals ($Fe^{2+}$), it is catalyzed to "OH (Fig. 1). $O_2^-$ can also react with nitric oxide (NO) to form peroxynitrite (ONOO$^-\$). ONOO$^-\$ or products of ONOO$^-\$ can damage proteins by nitration. Bcl-2 family members regulate apoptosis by modulating the release of cytochrome c from mitochondria into the cytosol. In the Bax channel model (left), Bax is a proapoptotic protein found in the cytosol that translocates to the outer mitochondrial membrane (OMM). Bax monomers physically interact and form tetrameric channels that are permeable to cytochrome c. The formation of these channels is blocked by Bcl-2 and Bcl-xL at multiple sites. BH3-only members (Bad, Bid, Noxa, Puma) are proapoptotic and can modulate the conformation of Bax to sensitize this channel, possibly by exposing its membrane insertion domain or by inactivating Bcl-2 and Bcl-xL. The mitochondria apoptosis-induced channel (MAC) may be a channel similar to the Bax channel but possibly has additional components. Release cytochrome c participates in the formation of the apoptosomes in the cytosol that drives the activation of caspase-3 leading to apoptosis. Smac/DIABLO are released to inactivate the antiapoptotic actions of inhibitor of apoptosis proteins that inhibit caspases. AIF and EndoG are released and translocate to the nucleus to stimulate DNA fragmentation. Another model involves the permeability transition pore (PTP). The PTP is a transmembrane channel formed by the interaction of the adenine nucleotide translocator (ANT) and the voltage-dependent anion channel (VDAC) at contact sites between the inner mitochondrial membrane (IMM) and the OMM and is modulated by cyclophilin D (cy-D). Opening of the PTP induces matrix swelling and OMM rupture leading to release of apoptogenic proteins (cytochrome c, AIF, EndoG) or to cellular necrosis.

Mitochondrial dysfunction and regulation of apoptosis. Mitochondria generate reactive oxygen species (ROS) in the respiratory chain (lower left). Complexes I, II, and III can generate $O_2^-$. MnSOD or CuZnSOD convert $O_2^-$ to $H_2O_2$ that can form "OH. $O_2^-$ can react with NO (generated by a form of mtNOS or iNOS) to form peroxynitrite (ONOO$^-\$). ONOO$^-\$ or products of ONOO$^-\$ can damage proteins by nitration. Bcl-2 family members regulate apoptosis by modulating the release of cytochrome c from mitochondria into the cytosol. In the Bax channel model (left), Bax is a proapoptotic protein found in the cytosol that translocates to the outer mitochondrial membrane (OMM). Bax monomers physically interact and form tetrameric channels that are permeable to cytochrome c. The formation of these channels is blocked by Bcl-2 and Bcl-xL at multiple sites. BH3-only members (Bad, Bid, Noxa, Puma) are proapoptotic and can modulate the conformation of Bax to sensitize this channel, possibly by exposing its membrane insertion domain or by inactivating Bcl-2 and Bcl-xL. The mitochondria apoptosis-induced channel (MAC) may be a channel similar to the Bax channel but possibly has additional components. Release cytochrome c participates in the formation of the apoptosomes in the cytosol that drives the activation of caspase-3 leading to apoptosis. Smac/DIABLO are released to inactivate the antiapoptotic actions of inhibitor of apoptosis proteins that inhibit caspases. AIF and EndoG are released and translocate to the nucleus to stimulate DNA fragmentation. Another model involves the permeability transition pore (PTP). The PTP is a transmembrane channel formed by the interaction of the adenine nucleotide translocator (ANT) and the voltage-dependent anion channel (VDAC) at contact sites between the inner mitochondrial membrane (IMM) and the OMM and is modulated by cyclophilin D (cy-D). Opening of the PTP induces matrix swelling and OMM rupture leading to release of apoptogenic proteins (cytochrome c, AIF, EndoG) or to cellular necrosis.

Mitochondrial Regulation of Apoptosis

Apoptosis is a structurally and biochemically organized form of cell death. The basic machinery of apoptosis is conserved in yeast, hydra, nematode, fruit fly, zebrafish, mouse, and human (9). Mitochondria are critical regulators of the apoptotic process (Fig. 1). A variety of mitochondrial proteins function in apoptosis (Table 1; Fig. 1), including Bcl-2 family members, cytochrome c, apoptosis-inducing factor (AIF), endonuclease G, second mitochondrial activator of

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TABLE 1. Mitochondrial-Associated Proteins That Function in Cell Death

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2*</td>
<td>Antiapoptotic, blocks Bax/Bak channel formation</td>
</tr>
<tr>
<td>Bcl-X{sub}L</td>
<td>Antiapoptotic, blocks Bax/Bak channel formation</td>
</tr>
<tr>
<td>Bax*</td>
<td>Proapoptotic, forms pores for cytochrome release</td>
</tr>
<tr>
<td>Bak*</td>
<td>Proapoptotic, forms pores for cytochrome release</td>
</tr>
<tr>
<td>Bad</td>
<td>Proapoptotic, decoy for Bcl-2/Bcl-X{sub}L promoting Bax/Bak pore formation</td>
</tr>
<tr>
<td>Bid</td>
<td>Proapoptotic, decoy for Bcl-2/Bcl-X{sub}L promoting Bax/Bak pore formation</td>
</tr>
<tr>
<td>Noxa</td>
<td>Proapoptotic, decoy for Bcl-2/Bcl-X{sub}L promoting Bax/Bak pore formation</td>
</tr>
<tr>
<td>Puma</td>
<td>Proapoptotic, decoy for Bcl-2/Bcl-X{sub}L promoting Bax/Bak pore formation</td>
</tr>
<tr>
<td>p53*</td>
<td>Antagonizes activity of Bcl-2/Bcl-X{sub}L, promotes Bax/Bak oligomerization</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>Activator of apoptosis</td>
</tr>
<tr>
<td>Smac/DIABLO</td>
<td>IAP inhibitor</td>
</tr>
<tr>
<td>AIF</td>
<td>Antioxidant flavoprotein/released from mitochondria to promote nuclear DNA fragmentation</td>
</tr>
<tr>
<td>Endonuclease G</td>
<td>Released from mitochondria to promote nuclear DNA fragmentation</td>
</tr>
<tr>
<td>HtrA2/Omi</td>
<td>IAP inhibitor</td>
</tr>
<tr>
<td>VDAC</td>
<td>PTP component in outer mitochondrial membrane</td>
</tr>
<tr>
<td>ANT</td>
<td>PTP component in inner mitochondrial membrane</td>
</tr>
<tr>
<td>Cyclophilin D</td>
<td>PTP component in mitochondrial matrix</td>
</tr>
<tr>
<td>Peripheral benzodiazepine receptor</td>
<td>PTP component in outer mitochondrial membrane</td>
</tr>
</tbody>
</table>

* Known to be changed in human ALS (69, 72).
AIF, apoptosis-inducing factor; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocator.

caspases (Smac/DIABLO), and Omi/high-temperature requirement protein A2 (HtrA2) inhibitor of the inhibitors of apoptosis proteins (10–12). Other proteins (e.g. humanin, Ku70, 14-3-3 proteins) that are not mitochondrial can modulate mitochondrial death proteins.

Bcl-2 Family

The bcl-2 protooncogene family is a group of apoptosis regulatory genes encoding for proteins defined by at least one conserved Bcl homology domain (BH1–BH4 can be present) that function in the protein–protein interactions (10, 11). This family of genes encodes for more than 20 proteins. Some of the products of these genes are anti-apoptotic, whereas other gene products are proapoptotic (Table 1; Fig. 1). Because Bcl-x{sub}L possesses structural similarities to the pore-forming subunit of diphtheria toxin, some Bcl-2 family members appear to function by conformation-induced insertion into the outer mitochondrial membrane to form channels or pores that release apoptogenic factors (Fig. 1).

Bax, Bad, and Bcl-2 reside primarily in the cytosol, whereas Bak resides primarily in mitochondria. Bcl-2 family members can form homodimers or heterodimers and higher-order multimers with other family members. Bak forms homodimers or heterodimers with Bax, Bcl-2, or Bcl-x{sub}L. When Bax and Bak are present in excess, the antiapoptotic activity of Bcl-2 is antagonized. The formation of Bax homooligomers promotes apoptosis, whereas Bax heterodimerization with either Bcl-2 or Bcl-x{sub}L prevents apoptosis by blocking the release of cytochrome c residing in the mitochondrial intermembrane space (Fig. 1). Release of apoptogenic proteins from mitochondria may occur through mechanisms that involve the formation of membrane channels comprised of Bax (13), Bax and the adenine nucleotide translocator (14), and the voltage-dependent anion channel (15). Bcl-2 and Bcl-x{sub}L block the release of cytochrome c and AIF (16–18) from mitochondria and thus the activation of caspase-3 leading to internucleosomal cleavage of DNA. The blockade of cytochrome c release from mitochondria by Bcl-2 and Bcl-x{sub}L (19) is caused by inhibition of Bax channel-forming activity in the outer mitochondrial membrane (13) or by modulation of mitochondrial membrane potential and volume homeostasis (19). Bax and Bak double knockout cells are completely resistant to mitochondrial cytochrome c release during apoptosis (20). BH3-only proteins such as Bid, Puma, and Noxa appear to induce a conformaional change in Bax that allows it to form pores in the outer mitochondrial membrane or they serve as decoys for Bcl-x{sub}L (21). Mitochondria and the endoplasmic reticulum appear to participate in a loop involving intracellular Ca{sup 2+} to modulate mitochondrial permeability transition and cytochrome c release (22).

Inhibitors of the Inhibitors of Apoptosis

Mitochondrial proteins exist that inhibit mammalian inhibitors of apoptosis (IAPs) that reside in the cytoplasm. A protein called Smac (second mitochondrial activator of caspases) and its human ortholog DIABLO (direct IAP-binding protein with low pl) inactivate the antiapoptotic actions of IAPs and thus exert proapoptotic actions (12). These IAP inhibitors are 23-kDa mitochondrial proteins (derived from 29-kDa precursor proteins processed in the mitochondria) that are released from the intermembrane space (Fig. 1) and sequester IAPs. Omi/Htr2A serine peptidase is another mitochondrial protein that functions in apoptosis, stress response, and neurodegeneration. Omi/HtrA2 localizes to the mitochondrial intermembrane space and is released into the cytosol during apoptosis to inhibit IAPs. Mutations in Omi/HtrA2 cause the neuromuscular disorder in mnd2 mutant mice (23) and might cause some forms of familial Parkinson disease (PD) (24).

Apoptosis-Inducing Factor

Apoptosis-inducing factor is a mammalian cell mitochondrial protein identified as a flavoprotein (17). AIF is synthesized as a 67-kDa protein that has an N-terminal mitochondrial localization signal, and after import into the inner mitochondrial space, the mitochondrial localization signal is cleaved off to generate a mature protein of 57 kDa. AIF appears to have dual functions. Under normal physiological conditions, AIF might function as an ROS scavenger.
targeting \( \text{H}_2\text{O}_2 \) (25) or in redox cycling with NAD(P)H (26). In cells after exposure to an apoptotic stimulus, AIF functions proapoptotically and translocates to the nucleus where it has DNA-binding activity (17). Overexpression of AIF induces cardinal features of apoptosis, including chromatin condensation, high-molecular-weight DNA fragmentation, and loss of mitochondrial transmembrane potential (17).

MITOCHONDRIAL PERMEABILITY TRANSITION

Conditions of mitochondrial \( \text{Ca}^{2+} \) overload, excessive oxidative stress, and decreased electrochemical gradient (\( \Delta P \)), ADP, and ATP can favor mitochondrial permeability transition. Mitochondrial permeability transition is a mitochondrial state in which the proton-motive force is disrupted (27–30). This disruption involves mitochondrial permeability transition pore (PTP), which functions as a voltage, thiol, and \( \text{Ca}^{2+} \) sensor. The PTP has been studied mostly in vitro systems and its role in vivo remains debatable. The PTP is believed to be a polyprotein transmembrane channel formed at the contact sites between the inner mitochondrial membrane and the outer mitochondrial membrane. The components of the PTP (Table 1; Fig. 1) are still controversial, but they appear to include the voltage-dependent anion channel (VDAC or porin) and the peripheral benzodiazepine receptor in the outer mitochondrial membrane, the adenine nucleotide translocator (ANT) in the inner mitochondrial membrane, and matrix cyclophilin D (27). It might also contain hexokinase from the cytosol and creatine kinase in the intermembrane space. The VDAC makes the inner mitochondrial membrane permeable to most small molecules up to 1.3 kDa for free exchange of respiratory chain substrates, but the functions of the VDAC and its role in cell death is still uncertain (28). The ANT mediates the exchange of ADP for ATP. During normal mitochondrial function, the intermembrane space separates the outer and inner mitochondrial membranes, and the VDAC and the ANT do not interact and the VDAC might be open (28). When mitochondrial permeability transition is activated by the formation of the PTP, the inner mitochondrial membrane loses its integrity and the ANT deforms from its native state into a nonselective pore and oxidative phosphorylation is uncoupled. When this occurs, small ions and metabolites permeate freely across the inner membrane and oxidation of metabolites by \( \text{O}_2 \) proceeds with electron flux not coupled to proton pumping, resulting in collapse of \( \Delta P \), dissipation of ATP production, production of ROS, equilibration of ions between the matrix and cytosol, and mitochondrial swelling (29, 30). Bcl-2 family members (Bax, Bcl-2, Bid, and Bcl-xL) appear to modulate mitochondrial permeability transition.

p53

Apoptosis can be induced by the tumor suppressor protein p53 and related DNA binding proteins (31). In the canonic pathway, p53 commits to death cells that have sustained DNA damage from free radicals, irradiation, and other genotoxic stresses through transcriptionally dependent mechanisms (31). Activated p53 binds the promoters of several genes encoding proteins associated with growth control and cell cycle checkpoints (p21, Gadd45, Mdm2) and apoptosis (Bax, Bcl-2, Bcl-xL, Fas). The BH3-only proteins Puma and Noxa are critical mediators of p53-mediated apoptosis (32). Recent studies have shown that p53 functions at mitochondria to drive transcription-independent apoptosis (33). At mitochondria, p53 localizes primarily to the surface where it translocates before cytochrome c release by mechanisms that might be independent of significant posttranslational modification. p53 interacts directly with Bcl-2 and Bcl-xL, thereby neutralizing their antiapoptotic activity. p53 can mediate mitochondrial membrane permeabilization and apoptosis by inducing Bak and Bax oligomerization (34, 35).

MITOCHONDRIAL DNA

Each human cell contains hundreds of mitochondria and thousands of maternally inherited mitochondrial DNA (mtDNA) copies residing in the matrix as a double-stranded circular molecule of approximately 16.5 kb with 37 genes that are all transcribed (6). mtDNA has the genes for the 12S and 16S rRNAs and the 22 tRNAs required for mitochondrial protein synthesis occurring at mitochondrial ribosomes. mtDNA also has the genes encoding 13 proteins that are structural subunits of oxidative phosphorylation enzyme complexes, including 7 of the 46 proteins of complex I (NADH dehydrogenase), one of the 11 proteins of complex III (bc1 complex), 3 of the 13 proteins of complex IV (cytochrome c oxidase), and 2 of the 16 proteins of complex V (ATP synthetase) (6). Mitochondrial ROS can damage mtDNA, fostering the belief that mtDNA has a very high mutation rate (6). Cells containing a mixed population of normal and mutant mtDNA are known as heteroplasmic. In postmitotic tissues, mutant mtDNA can be preferentially, clonally amplified. One type of mtDNA mutation, called the common mtDNA deletion (mtDNA4977), is found nonuniformly within different human brain areas with the highest levels detected in the striatum and substantia nigra (36, 37).

MITOCHONDRIAL ROLE IN PARKINSON DISEASE

Parkinson disease is a chronically progressive, age-related, fatal neurologic disease in humans that affects approximately 2% of the population at some time in life (38, 39). PD is the second most common neurodegenerative disease with an adult onset (after Alzheimer disease) with the greatest prevalence occurring in the United States (between 100 and 250 cases per 100,000). The disease is characterized clinically by complex symptomatology such as progressive resting tremor (4–7 Hz), rigidity, bradykinesia/akinesia, gait disturbance, postural instability, mood disturbances, dementia, sleep disturbances, and autonomic dysfunction. A major neuropathologic feature of PD is the degeneration and elimination of dopamine neurons in...
Approximately 5% to 10% of patients with PD have familial patterns of inheritance (42). Over the past decade, several genes have been implicated in Mendelian forms of PD. Gene mutations with autosomal-dominant or autosomal-recessive inheritance patterns have been identified in familial forms of PD (Table 2). PD-linked mutations occur in the genes encoding α-Syn, parkin, ubiquitin carboxyterminal hydrolase-L1 (UCH-L1), PTEN-induced putative kinase 1 (PINK1), DJ-1, and leucine-rich repeat kinase-2 (LRRK2) (43).

α-Syn is a relatively small (140 amino acids) very abundant protein (approximately 1% of total protein) found in cells throughout the nervous system and is particularly enriched in neuronal axon terminals (44, 45). In vitro, overexpression of human wild type (wt) or mutant α-Syn elevates the generation of intracellular ROS (46) and causes mitochondrial deficits (46).

The PINK1 gene is at the PARK6 locus. PINK1 is a 581 amino acid protein (approximately 63 kDa) and contains a domain highly homologous to the serine/threonine protein kinases of the calcium/calmodulin family and a mitochondrial targeting motif, suggesting that PINK1 is a mitochondrial kinase. It is processed at the N-terminus in a manner consistent with mitochondrial import. Both human wt and mutant PINK1 localize to mitochondria (47). Interestingly, most of the reported mutations are in the putative kinase domain. Speculation has PINK1 protecting dopaminergic neurons against mitochondrial malfunction under conditions of cell stress.

The PARK7 locus contains the DJ-1 gene. PARK7 kindreds can have homozygous deletion of a large region within the DJ-1 gene causing complete loss of DJ-1 expression or homozygous missense mutations in the DJ-1 gene resulting in single amino acid substitutions in the DJ-1 molecule. DJ-1 is a small (189-amino acid, approximately 20–25 kDa) protein with multiple apparent functions, including oxidative stress response. DJ-1 is expressed throughout the nervous system and it might act as a neuroprotective intracellular redox sensor that can localize to the cytoplasmic side of mitochondria (48). The localization of DJ-1 to mitochondria is associated with protective actions against some mitochondrial poisons (48).

Mutations in the nuclear-encoded mitochondrial gene DNA polymerase γ (POLG), which functions specifically in mitochondrial DNA replication, were found in families with PD associated with progressive external ophthalmoplegia (49). These mutations cause multiple mtDNA deletions and neuronal loss in the SNc, possibly as a result of clonal expansion of mtDNA deletions and respiratory chain deficits. Recently, high levels of mtDNA deletions have been detected in SNc neurons of PD cases (50, 51) showing that accumulation of somatic mtDNA mutations associates with neurodegeneration.

It has been believed for over a decade that mitochondrial dysfunction is related to the pathogenesis of PD. Complex I activity was found to be reduced in the substantia nigra and platelets of PD cases with changes in skeletal muscle being contentious (42). Strong evidence emerged when drug abusers exposed to 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) presented with PD (52), and it was discovered that the active metabolite of MPTP, the 1-methyl-4-phenylpyridinium ion (MPP+), is a complex I inhibitor (53). Subsequently, it was found that complex I inhibitors cause damage to dopaminergic neurons in animal models (54). Recently, it has been shown that mitochondrial KATP channel activity has a role in SNc neuron degeneration in PD models (55).

### Mutant α-Syn Mice Develop Neuronal Mitochondrial Degeneration and Cell Death

Several tg mouse lines have been generated using a variety of different promoters to drive overexpression of human wt or mutant α-Syn (43, 56). Currently, there have been no reports of nigral neuron death in α-Syn tg mice or any other tg or null mouse models of PD-linked

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**TABLE 2. Mutant Genes Linked to Familial Parkinson Disease**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Protein Name/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1/4q21</td>
<td>Autosomal dominant</td>
<td>α-Syn</td>
<td>α-Syn/presynaptic maintenance?</td>
</tr>
<tr>
<td>PARK2/6q25.2–27</td>
<td>Autosomal recessive</td>
<td>Parkin</td>
<td>Parkin/ubiquitin E3 ligase</td>
</tr>
<tr>
<td>PARK3/2p13</td>
<td>Autosomal dominant</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>PARK4/4p15</td>
<td>Autosomal dominant</td>
<td>α-Syn</td>
<td>α-Syn/presynaptic maintenance?</td>
</tr>
<tr>
<td>PARK5/4p14</td>
<td>Autosomal dominant</td>
<td>UCHL1</td>
<td>UCHL1/polyubiquitin hydrolase</td>
</tr>
<tr>
<td>PARK6/1p36</td>
<td>Autosomal recessive</td>
<td>PINK1</td>
<td>PTEN-induced putative kinase 1/mitochondrial protein kinase</td>
</tr>
<tr>
<td>PARK7/1p36.33–36–12</td>
<td>Autosomal recessive</td>
<td>DJ-1</td>
<td>DJ-1/mitochondrial antioxidant, chaperone</td>
</tr>
<tr>
<td>PARK8/12q12</td>
<td>Autosomal dominant</td>
<td>UCHL1</td>
<td>UCHL1/polyubiquitin hydrolase</td>
</tr>
<tr>
<td>Unmapped</td>
<td>Autosomal dominant or recessive</td>
<td>POLG</td>
<td>POLG/mitochondrial DNA polymerase γ</td>
</tr>
<tr>
<td>Unmapped</td>
<td>Autosomal dominant?</td>
<td>Omi/HtrA2</td>
<td>Omi/HtrA2, mitochondrial serine peptidase, inhibitor of IAPs</td>
</tr>
</tbody>
</table>

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substantia nigra pars compacta (SNc) and in other brainstem regions. The movement disorder in PD is thought to arise from reduced dopaminergic innervation of the striatum resulting from the loss of SNc neurons and can be explained functionally by overactivity of the subthalamic nucleus and GPi (40). Another neuropathologic feature of PD is the formation of eosinophilic proteinaceous intraneuronal or intraglial inclusions known as Lewy bodies. Lewy bodies are comprised of a dense core of filamentous material enshrouded by filaments 10 to 20 nm in diameter (41) and are usually positive for ubiquitin and α-synuclein (α-Syn).

It has been believed for over a decade that mitochondrial dysfunction is related to the pathogenesis of PD. Complex I activity was found to be reduced in the substantia nigra and platelets of PD cases with changes in skeletal muscle being contentious (42). Strong evidence emerged when drug abusers exposed to 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) presented with PD (52), and it was discovered that the active metabolite of MPTP, the 1-methyl-4-phenylpyridinium ion (MPP+), is a complex I inhibitor (53). Subsequently, it was found that complex I inhibitors cause damage to dopaminergic neurons in animal models (54). Recently, it has been shown that mitochondrial KATP channel activity has a role in SNc neuron degeneration in PD models (55).
Gene mutations. Despite no prominent changes in the SNc, tg α-Syn mice have a shortened lifespan and develop a severe fatal movement disorder, robust synucleinopathy, and neurodegeneration (57). Mice expressing A53T α-Syn, but not mice expressing wt or A30P α-Syn, develop adult-onset progressive motor deficits, including reduced spontaneous activity with bradykinesia, mild ataxia, and dystonia at approximately 10 to 15 months of age followed by rapidly progressive paralysis and death (43). A53T mice develop intraneuronal Lewy body-like inclusions, mitochondrial degeneration, and cell death in the neocortex, brainstem, and spinal cord (57). A53T mice have progressive, profound loss (approximately 75%) of motor neurons (MNs) (57).

Mitochondria in brainstem and spinal cord cells in A53T mice appear dysmorphic, becoming shrunken, swollen, or vacuolated, and some mitochondria contain human α-Syn (57). Some abnormal intracellular inclusions in these cells are degenerating mitochondria. A mitochondrial defect is further indicated by biochemical evidence revealing loss of complex IV activity (57). Mitochondrial pathology in A53T mice involving mitochondrial DNA damage is seen frequently in the absence of nuclear DNA damage in large brainstem neurons and spinal MNs in A53T mice (57). The mechanisms for this mitochondrial DNA damage are possibly related to ONOO− mediated oxidative/nitrosative stress in A53T mouse MNs as indicated by the presence of nitrated synuclein and inclusions (57). Mitochondrial DNA damage is consistent with the presence of ONOO− or its derivatives near mitochondria, because ONOO− or products of ONOO− are directly genotoxic by causing single- and double-strand breaks in DNA (8). Overall, ONOO− mediated damage in mitochondria may be a key pathologic mechanism leading to MN degeneration in A53T mice.

**MITOCHONDRIAL ROLE IN AMYOTROPHIC LATERAL SCLEROSIS**

Amyotrophic lateral sclerosis (ALS) is a progressive and severely disabling fatal neurologic disease in humans characterized by weakness, muscle atrophy, spasticity, and eventual paralysis and death within 3 to 5 years after symptoms begin (58). The cause of the spasticity, paralysis, and death is progressive degeneration and elimination of upper MNs in the cerebral cortex and lower MNs in the brainstem and spinal cord (58, 59). More than 5,000 people in the United States are diagnosed with ALS each year, and, in parts of the United Kingdom, one in approximately 500 deaths is caused by some form of ALS. It is still not understood why MNs are selectively vulnerable in ALS. The molecular pathogenesis of most forms of ALS is understood poorly, contributing to the lack of effective mechanism-based therapies to treat this disease. Two forms of ALS exist: idiopathic (sporadic) and heritable (familial). The majority of ALS cases are sporadic with no known genetic component. Aging is a strong risk factor for ALS. Familial forms of ALS (FALS) have autosomal-dominant or autosomal-recessive inheritance patterns and make up approximately 10% or less of all ALS cases. ALS-linked mutations occur in the genes encoding SOD1 (ALS1), Alsin (ALS2), senataxin (ALS4), vesicle-associated membrane protein (VAMP/synaptobrevin)-associated protein B (ALS8), and dynactin (43).

Mitochondrial dysfunction might play a role in the pathogenesis of ALS. Electron microscopic studies have shown mitochondrial morphology abnormalities in skeletal muscle, liver, and spinal MNs of some patients with ALS (60). Skeletal muscle biopsies of patients with sporadic ALS have changes in mitochondrial Ca2+ levels (61). Markers of oxidative stress and ROS damage are elevated in ALS tissues (62). Studies of respiratory chain enzyme activities are discrepant. Increases have been reported in complex I, II, and III activities in vulnerable and nonvulnerable brain regions in patients with mutant SOD1-FALS (63). Other studies have found decreased complex IV activity in the spinal cord ventral horn (64) and skeletal muscle (65) of sporadic ALS cases. A mutation in cytochrome c oxidase subunit I was found in a patient with a MN disease phenotype (66). Another patient with MN disease had a mutation in a mitochondrial tRNA gene (67). Single-cell analysis of MNs from sporadic ALS cases has shown no significant accumulation of the 5 kb common deletion in mtDNA (68).

**Motor Neuron Degeneration in Amyotrophic Lateral Sclerosis Has Features of Programmed Cell Death**

Programmed cell death appears to contribute to the selective degeneration of MNs in human sporadic ALS and FALS, albeit perhaps as a nonclassic form of apoptosis (69). MNs appear to pass through sequential stages of chromatolysis (suggestive of initial axonal injury), somatodendritic attrition without extensive cytoplasmic vacuolation, and then cell death. MNs in sporadic ALS and FALS cases generally show the same patterns of degeneration (69). This death is defined by DNA fragmentation and cell loss and is associated with accumulation of cytochrome c and cleaved caspase-3 (70). However, the morphology of this cell death is distinct from classic apoptosis (2). Nevertheless, Bax and Bak protein levels are increased and Bcl-2 protein level is decreased in mitochondria-enriched fractions of selectively vulnerable motor regions (spinal cord anterior horn and motor cortex gray matter), but not in regions unaffected by the disease (somatosensory cortex gray matter), although these biochemical observations lack direct specificity for MN events (69). Subsequently, immunohistochemistry (70) and laser capture microdissection of MNs combined with protein profiling (71) have confirmed the presence of intact active caspase-3 in human ALS MNs. MNs in human ALS also upregulate p53 with competent DNA binding function (72). These data support the concept of an inappropriate reemergence of a programmed cell death mechanism involving p53 activation and cytosol-to-mitochondria redistributions of cell death proteins participating in the pathogenesis of MN degeneration in human ALS (69, 72). The changes seen in human ALS are modeled robustly and reproducibly at structural and...
molecular levels in axotomy models of MN degeneration (70, 73). In contrast, the morphology of MN degeneration in human ALS is very different from that seen in the commonly used G93A-mSOD1 mouse model of ALS (70, 74).

**Mitochondria in Motor Neurons of Mutant SOD1 Mice: Trojan Horse Effectors of Motor Neuron Death**

Motor neurons in G93A-mSOD1 tg mice, a widely used mouse model of ALS, undergo slow degeneration lacking similarity to apoptosis structurally and biochemically (74). It is characterized by somal and mitochondrial swelling and formation of DNA single-strand breaks before double-strand breaks occurring in nuclear and mitochondrial DNA (74). The MN death is independent of activation of caspases-1, 3, and 8 or apoptosis-inducing factor within MNs with a blockade of apoptosis possibly mediated by IAP upregulation. mSOD1 mouse MNs accumulate mitochondria from the axon terminals and generate higher levels of O$_2^-$, NO, and ONOO$^-$ than MNs in mice expressing wt human SOD1 (74). Nitrated and aggregated cytochrome c oxidase subunit-I and α-Syn as well as nitrated SOD2 accumulate in mSOD1 mouse spinal cord (74). Mitochondria in mSOD1 mouse MNs accumulate NADPH diaphorase and inducible NO synthase (iNOS)-like immunoreactivity (Fig. 1), and iNOS gene deletion extends significantly the lifespan of G93A-mSOD1 mice (74). This recent work identifies novel mechanisms for mitochondriopathy and MN degeneration in ALS mice involving blockade of apoptosis, accumulation of MN mitochondria with enhanced ROS toxic potential from distal terminals, NOS localization in MN mitochondria, and ONOO$^-$ damage. Thus, mitochondrial oxidative stress, protein nitration, and aggregation are likely participants in the process of MN degeneration caused by mSOD1. The mechanistic basis for the difference between human ALS and mSOD1 mice is not yet clear but could be related to the supranormal expression of toxic mSOD1 or to the robust upregulation of iNOS that drives MNs to necrotic-like death along the apoptosis-necrosis cell death continuum (2).

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**REFERENCES**


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