From the Department of Laboratory Medicine
Karolinska Institutet, Stockholm, Sweden

MITOCHONDRIAL DYSFUNCTION IN DOPAMINE NEURONS - IMPLICATIONS FOR PARKINSON'S DISEASE

Fredrik H. Sterky

Karolinska Institutet

Stockholm 2012

Success is the ability to go from one failure to another with no loss of enthusiasm.

–Churchill

Cover: Mitochondria (gray) in dopaminergic dendrites projecting from the pars compacta area to the pars reticulata area of the substantia nigra.

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by Larserics Digital Print AB.

ISBN 978-91-7457-678-8

ABSTRACT

Mitochondria are essential for cellular homeostasis and contain the respiratory chain (RC). Decreased mitochondrial function is associated with ageing, as exemplified by the finding of a mosaic pattern of RC-deficient cells in aged tissues. Parkinson's disease (PD) is a common age-related disorder characterized by loss of midbrain dopamine (DA) neurons and formation of intracellular inclusions. A number of observations suggest a role for mitochondrial dysfunction in the pathophysiology of PD: (1) toxins linked to PD have been shown to impair RC function, (2) reduced RC enzyme activities are found in patient tissues, (3) the proportion of DA neurons that are RC-deficient, due to accumulation of mtDNA deletions, is higher in PD patients than in controls, and (4) an inherited form of PD is caused by loss-of-function mutations in the gene for Parkin, an E3 ubiquitin ligase reported to facilitate clearance of defective mitochondria.

In this thesis, experimental genetics in mice have been used to study consequences of mitochondrial dysfunction in the brain, with particular focus on DA neurons. First, we addressed the role of mosaic RC deficiency in the brain by creating chimeric mice with a mixture of normal and RC-deficient forebrain neurons. A low proportion (>20%) of respiratory chain-deficient forebrain neurons was sufficient to cause symptoms. On the one hand, surrounding normal neurons could prevent mortality and delay the onset of symptoms. On the other hand, RC-deficient neurons could induce death of surrounding normal neurons by a trans-neuronal degeneration mechanism.

We also developed so-called 'MitoPark' mice, which have DA-specific disruption of *Tfam*, a gene critical for maintenance and expression of mtDNA. MitoPark mice have severe RC deficiency in DA neurons and develop slow, progressive loss of DA neurons accompanied by motor symptoms resembling those seen in PD. To study changes in mitochondrial morphology and distribution, we developed a novel reporter mouse for cell type-specific labeling of mitochondria. We found that mitochondria in RC-deficient neurons fragmented and concomitantly formed severely enlarged mitochondrial bodies in the soma and proximal dendrites. Mitochondria in distal axon segments had normal morphology, but the RC-deficient neurons had an impaired anterograde supply of new mitochondria to their axon terminals. We did not find support for a role of Parkin in MitoPark DA neurons, as overexpressed Parkin was not recruited to the aberrant mitochondria and the absence of Parkin did not affect their clearance. Finally, we studied the consequences of a complex I defect in DA neurons. Disruption of the complex I subunit gene *Ndufs4* resulted in a mild complex I deficiency, which did not cause degeneration of DA neurons or PD-like symptoms. Nevertheless, we found increased levels of DA metabolites and impaired DA release at the level of the axon terminals, compatible with early changes seen in PD. *Ndufs4* knockout DA neurons were in addition more vulnerable to toxic insult. In summary, these results support a role for mitochondrial dysfunction in PD and show that RC function is important for axonal mitochondrial transport and synaptic DA release.

LIST OF PUBLICATIONS

I Age-associated mosaic respiratory chain deficiency causes trans-neuronal degeneration.
E Dufour, M Terzioglu, **F H Sterky**, L Sörensen, D Galter, L Olson, J Wilbertz, and NG Larsson.
Hum Mol Genet 2008, 17(10): 1418-1426

II Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons.
M I Ekstrand, M Terzioglu, D Galter, S Zhu, C Hofstetter, E Lindqvist, S Thams, A Bergstrand, **F Sterky Hansson**, A Trifunovic, B Hoffer, S Cullheim, A H Mohammed, L Olson, and NG Larsson.
Proc Natl Acad Sci USA 2007, 104(4): 1325-1330

III Impaired mitochondrial transport and Parkin-independent degeneration of respiratory chain-deficient dopamine neurons in vivo.
F H Sterky, S Lee, R Wibom, L Olson, and NG Larsson.
Proc Natl Acad Sci USA 2011, 108(31): 12937-12942

IV Altered dopamine metabolism and increased vulnerability to MPTP in mice with partial deficiency of mitochondrial complex I in dopamine neurons.
F H Sterky, A F Hoffman, D Milenkovic, B Bao, A Paganelli, D Edgar, R Wibom, C R Lupica, L Olson, and NG Larsson.
Hum Mol Genet 2011, *Epub ahead of print*

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
ADP	Adenosine di-phosphate
ANT	Adenine nucleotide translocase
AR-JP	Autosomal recessive juvenile parkinsonism
ATP	Adenosine tri-phosphate
bp	Base pair
CCCP	Carbonyl cyanide m-chlorophenyl hydrazone
COMT	Catechol-O-methyltransferase
COX	Cytochrome c oxidase
DA	Dopamine
Da	Dalton (g/mol)
DAT	Dopamine transporter (Slc6A3)
DDC	Dopa decarboxylase
DOPAC	3,4-Dihydroxyphenylacetic acid
Drp1	Dynamin-related protein 1
FAD	flavine-adenine dinucleotide
HPLC	High-performance liquid chromatography
HSP	Heavy strand promoter
HVA	Homovanillic acid
KSS	Kearns-Sayre syndrome
L-DOPA	L-3,4-dihydroxyphenylalanine
LacZ	β-galactosidase
LB	Lewy body
LC3	Microtubule-associated protein 1 light chain 3
LSP	Light strand promoter
MAO	Monoamine oxidase
Mfn1/2	Mitofusin1/2
MILON	Mitochondrial late-onset neurodegeneration
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtDNA	Mitochondrial DNA
NAD	Nicotinamide adenine dinucleotide
ND	NADH dehydrogenase
Ndufs4	ND iron-sulfur protein 4
Ndufs4-DA	DA neuron-specific Ndufs4 gene knockout
O_H	origin of heavy strand replication
O_L	origin of light strand replication
Opa1	Optic atrophy protein 1
OxPhos	Oxidative phosphorylation
PD	Parkinson's disease
PEO	Progressive external ophthalmoplegia
P_i	Inorganic phosphate
PINK1	Pten-induced kinase-1
POLRMT	Mitochondrial RNA polymerase
POLγ	Mitochondrial DNA polymerase γ

Q	Ubiquinone (coenzyme Q)
QH_2	Ubiquinol (reduced coenzyme Q)
RC	Respiratory chain
ROS	Reactive oxygen species
SN	Substantia nigra
SNc	SN, pars compacta
TCA	Tricarboxylic acid
TFAM	Mitochondrial transcription factor A
TFB2M	Mitochondrial transcription factor B2
VMAT	Vesicular monoamine transporter 2
VTA	Ventral tegmental area
w	Week(s)
YFP	Yellow fluorescent protein

INTRODUCTION

MITOCHONDRIA

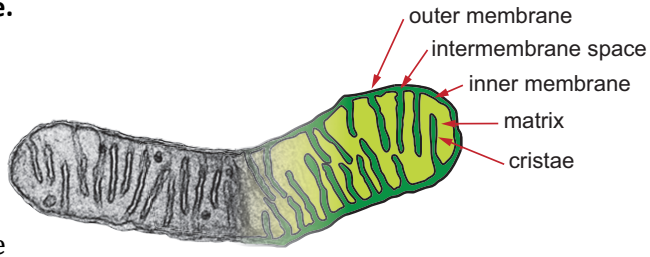
Two fundamental activities of everyday life, to eat and to breathe, converge at the level of an organelle present in nearly all cells. Mitochondria use oxygen that we inhale to convert energy in the carbohydrates and lipids in the food into adenosine triphosphate (ATP), the energy carrier used to drive almost all energy-dependent processes in the cell.

Mitochondria have prokaryotic ancestry and originate from the entry of a Proteobacteria species into the cytoplasm of a primitive host cell about 2 billion years ago (Gray, 1999; Andersson et al., 2003); a symbiosis that gave the host cell the evolutionary advantageous capacity for aerobic metabolism. Deepened integration of the proteobacterium in cellular physiology shaped a ubiquitous organelle with several essential functions. In addition to intermediary metabolism and ATP synthesis, mitochondria have important roles in calcium buffering, regulation of apoptosis, reactive oxygen species (ROS) production and the assembly of iron-sulfur cluster proteins (Lill and Mühlenhoff, 2008). As a consequence of their evolutionary origin, mitochondria share several features with prokaryotes such as containing their own bacteria-like genome and translation machinery. Lateral gene transfer from the mitochondrial DNA (mtDNA) to the nuclear genome has reduced gene content, and mammalian mtDNA only contain 13 protein-coding genes. The remaining ~1000 mitochondrial proteins (Pagliarini et al., 2008) are encoded by nuclear DNA. Nuclear-encoded mitochondrial proteins are imported into mitochondria by an import machinery following recognition of an N-terminal targeting peptide (Neupert and Herrmann, 2007).

Mitochondrial ultrastructure

Mitochondria consist of two lipid bi-layers that create four separate mitochondrial compartments (Fig. 1): the mitochondrial outer membrane, the intermembrane space, the inner membrane and the matrix. The outer membrane is semipermeable due to high amounts of channel proteins of the porin family that allows diffusion of molecules with a molecular weight of less than ~5 kDa across the membrane. Concentrations of small molecules within the intermembrane space are therefore in equilibrium with the cytosol.

Figure 1. Mitochondrial ultrastructure. An electron micrograph shows the mitochondrial outer and inner membranes that separate the intermembrane space and the mitochondrial matrix. Folding of the inner membrane into the matrix give rise to cristae.



The inner membrane is very protein-dense and rich in cardiolipin, a specialized diphosphatidylglycerol lipid. Unlike the outer membrane, the inner membrane is impermeable to ions and solutes. Transfer across the inner membrane depends on specialized transporters, for example the adenine nucleotide translocator (ANT) that exchanges ADP and ATP. This impermeability is important for the respiratory chain (RC) complexes, which are embedded in the inner membrane. Invaginations of the inner membrane create subcompartments of the intermembrane space called *cristae*. Formation of cristae may facilitate metabolism by greatly expanding the surface area between the RC in the inner membrane and the matrix compartment. The morphology of cristae can vary widely between different organisms, tissues and conditions (Zick et al., 2009). The mechanism that shape cristae are incompletely understood but may include dimerization of the ATP synthase (Davies et al., 2011). The matrix harbors the mtDNA and enzymes of the tricarboxylic acid (TCA) cycle, β-oxidation and some of the enzymes involved in steroid biosynthesis, gluconeogenesis and the urea cycle.

Mitochondrial dynamics

The size and shape of mitochondria varies between cell types and tissues, from being sphere-like to forming long interconnected tubules (Kuznetsov et al., 2009). The morphology is dynamic and mitochondria can move and remodel through fusion of separate mitochondria and division into daughter units (Fig. 2A). Conserved protein machineries regulate mitochondrial fusion and division (fission) (Detmer and Chan, 2007; Westermann, 2010). The relative activity of these opposing processes dictates the overall shape, which can rapidly change in response to altered conditions.

Mitochondrial fusion

Fusion of the outer membrane of mammalian mitochondria is mediated by two closely related proteins, Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) (Chen et al., 2003). Mitofusins are large dynamin-related GTPases that are anchored to the outer membrane with both the N- and C-terminal domains facing the cytoplasm. C-terminal heptad repeat domains of mitofusins on adjacent mitochondria can

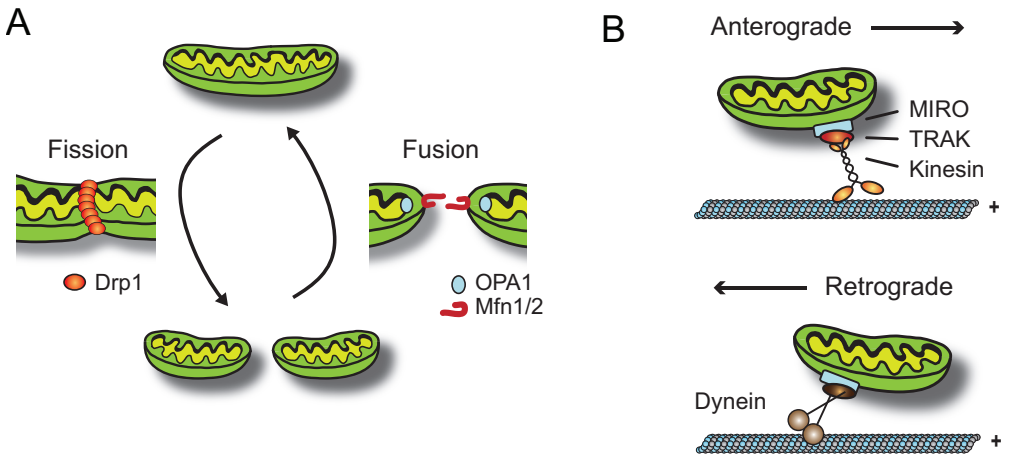


Figure 2. Mitochondrial morphology and motility is regulated by conserved protein machineries. (A) Remodeling of the mitochondrial network by fission and fusion is governed by four large GTPases. Oligomerization of Drp1 mediates mitochondrial fission by scission of the outer membrane. During fusion, mitofusins of juxtaposed mitochondria interact in *trans* to mediate fusion of the outer membrane. Opa1 is required for inner membrane fusion.
(B) Fast axonal transport of mitochondria occurs along microtubules. Kinesin motors attached to mitochnodria via the adaptor molecules TRAK and MIRO drive anterograde transport. Dynein mediates retrograde transport.

interact in *trans* to form either homotypic (Mfn1-Mfn1 or Mfn2-Mfn2) or heterotypic (Mfn1-Mfn2) complexes that tether the membranes in close proximity (Koshiba et al., 2004). It is believed that subsequent GTPase activity induce a conformational change that fuse the juxtaposed membranes. Loss of the gene for either *Mfn1* or *Mfn2* decreases mitochondrial fusion and leads to fragmentation of the mitochondrial network (Chen et al., 2003). Loss of both genes completely abolishes fusion (Koshiba et al., 2004). Only a single Mfn homologue is found in yeast, and the role for the two separate mitofusins in mammals is not fully understood. Additional functions have been ascribed to Mfn2, including a role in mitochondrial axonal transport (Misko et al., 2010) and the tethering of mitochondria to the endoplasmatic reticulum (de Brito and Scorrano, 2008).

The mechanisms that shape inner membrane morphology are less well understood. The inner membrane large GTPase OPA1 is required for fusion, and loss of *OPA1* results in mitochondrial fragmentation and aberrant cristae formation (Song et al., 2009). Alternative splicing generates multiple OPA1 isoforms. In addition, OPA1 is subject to proteolytic processing by inner membrane proteases to generate shorter isoforms of the protein (Westermann, 2010). Fusion requires both the long and short isoforms (Song et al., 2007).

Reduced mitochondrial membrane potential promotes processing and inhibits inner membrane fusion through loss of long OPA1 isoforms (Duvezin-Caubet et al., 2006).

Fusion allows inter-mitochondrial content mixing and is essential to maintain a functional mitochondrial network. Knockout of either *Mfn1* or *Mfn2* in mice results in embryonic lethality (Chen et al., 2003), and tissue-specific knockout of *Mfn2* in cerebellar neurons leads to neurodegeneration and mitochondrial dysfunction in affected neurons (Chen et al., 2007). In humans, mutations in *MFN2* cause Charcot-Marie-Tooth type 2A, a peripheral neuropathy that affects long axons and cause distal weakness and sensory loss in the limbs. Mutations in *OPA1* is a major cause of dominant optic atrophy, a disease that leads to blindness due to degeneration of retinal ganglion cells (Delettre et al., 2002).

Mitochondrial fission

Fission of mitochondria depends on the large GTPase dynamin-related protein 1 (Drp1). Drp1 is predominantly located in the cytoplasm with a minor fraction found on punctate spots on the mitochondrial surface. During fission, these sites recruit cytosolic Drp1, which oligomerize to encircle and constrict the mitochondrial tubule to promote scission of the membranes (Ingerman et al., 2005). The mechanism that dictates future fission sites is not well understood. In yeast, a small outer membrane protein called Fis1 is required for the recruitment of cytosolic Drp1, but the mammalian homologue is dispensable for the process (Lee et al., 2004). Instead, recruitment in mammals appears to depend on mitochondrial fission factor (Mff) (Otera et al., 2010).

Fission is important for proper segregation and distribution of mitochondria, and Drp1-mediated fission is also an early event during apoptosis. *Drp1* knockout mice are embryonic lethal and have highly connected mitochondria with aberrant morphology (Ishihara et al., 2009; Wakabayashi et al., 2009). *Drp1* knockout neurons fail to distribute mitochondria in neural processes and have impaired synapse formation (Ishihara et al., 2009). The importance of mitochondrial fission in humans has been illustrated by a case with fatal brain malformation due to a spontaneous dominant-negative *DRP1* mutation (Waterham et al., 2007).

Mitochondrial motility

Distribution of mitochondria within the cell is essential during cell division and to position mitochondria at sites with high energy demands (Hollenbeck and Saxton, 2005). Neurons are extreme in their need to transport mitochondria over

vast distances. In axons of lower motor neuron explants, mitochondria travel at peak velocities of 1.0 μm/s in the anterior direction and 1.4 μm/s in the retrograde direction (Misgeld et al., 2007). Although referred to as fast axonal transport, this corresponds to a minimal transit time of ~10 days from cell body to axon terminal in the longest of human axons. However, mitochondrial transport includes frequent pauses (saltatory movement), and net velocity over a distance is therefore likely slower (Hollenbeck and Saxton, 2005).

Fast axonal transport of mitochondria depends on microtubules (Fig. 2B). The kinesin-1 family of molecular motors drives anterograde transport, i.e. movement from the cell body towards the axon terminal and the microtubule (+)-end. Retrograde transport towards the microtubule (-)-end is driven by dynein (Pilling et al., 2006). Mutational screens in *Drosophila* have revealed two adaptor proteins for mitochondria-specific transport, Milton (Stowers et al., 2002) and Miro (Guo et al., 2005). Both genes have two mammalian orthologues named *TRAK*(1/2) and *MIRO*(1/2), respectively. The RHO family GTPase MIRO is attached to the outer membrane and binds the adaptor protein Milton/TRAK, which in turn interacts with kinesin (Fransson et al., 2006; Wang and Schwarz, 2009). MIRO contains two Ca^{2+}-binding EF-hand motifs that arrest mitochondrial motility upon Ca^{2+}-binding (Wang and Schwarz, 2009), suggesting a mechanism to direct mitochondria to subcellular regions with high Ca^{2+} levels. Phosphorylation of MIRO by the kinase PINK1 (Wang et al., 2011) may arrest transport in a membrane potential-dependent manner. Several other proteins have been implied in the regulation of mitochondrial transport (Sheng and Cai, 2012).

Oxidative phosphorylation and the respiratory chain

Catabolism of carbohydrates and lipids by glycolysis, the TCA cycle and β-oxidation generates reduced forms of the electron carriers NADH and $FADH_2$. The mitochondrial RC consists of four enzyme complexes (complexes I-IV) situated in the mitochondrial inner membrane (Fig. 3). The RC harvests electrons from NADH and $FADH_2$ and transfer them via a series of redox reactions to finally reduce molecular oxygen to water. This series of redox reactions is coupled to the translocation of protons across the inner membrane at complexes I, III and IV to generate an electrochemical gradient of protons across the inner membrane. This so-called proton motive force drives the ATP synthase and couples cellular respiration to ATP production by oxidative phosphorylation (OxPhos) (Mitchell, 1961).

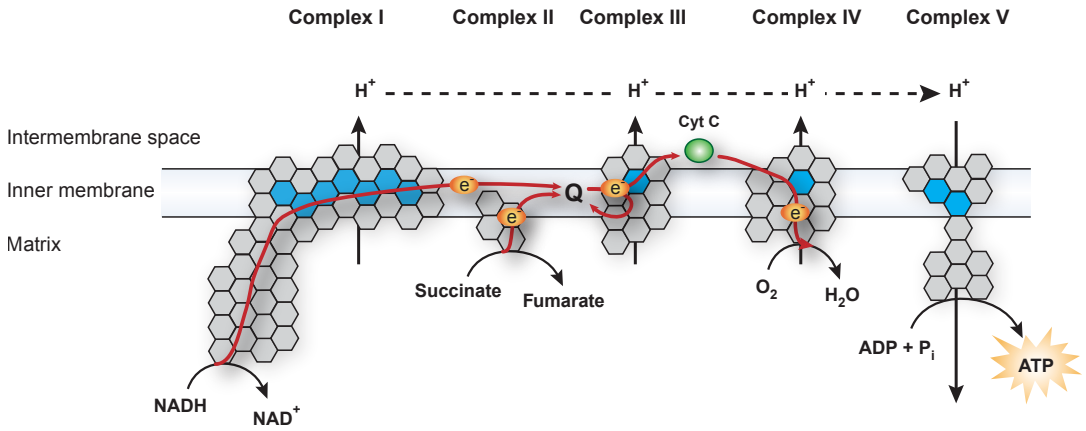


Figure 3. The five enzyme complexes of the OxPhos machinery. Complex I (NADH:ubiquinone oxidoreductase) and complex II (succinate dehydrogenase) transfer electrons (e^-) from intermediary metabolites to the inner membrane electron carrier coenzyme Q (Q). Complex III (ubiquinol-cytochrome c reductase) subsequently transfers electrons from reduced coenzyme Q to the electron shuttling protein cytochrome c (Cyt C). The electron carried by reduced cytochrome c is used in the final step to reduce oxygen (O_2) to water (H_2O) by complex IV (cytochrome c oxidase). The transfer of electrons (red arrows) is coupled to the translocation of protons (H^+) across the inner mitochondrial membrane by complexes I, III, and IV. This generates the proton gradient that drives the formation of ATP from ADP and inorganic phosphate (P_i) by complex V (ATP synthase). Each hexagon represents a unique subunit, with those encoded by mtDNA shown in blue; adapted from (Schapira 2006). Oligomerization of complex V subunits and higher order structure of complexes are not illustrated.

The OxPhos machinery is unique in its dependence on both nuclear and mitochondrial genes. The mtDNA encodes 13 essential subunits of complexes I, III, IV and V, whereas the remaining ~80 subunits of complexes I-V are encoded by nuclear DNA. In addition, correct complex assembly is at least partly dependent on a number of nuclear-encoded assembly chaperones. Higher order assemblies of RC complexes, called supercomplexes, can be isolated by native gel electrophoresis (Acín-Pérez et al., 2008). Electron microscopy studies have shown that complexes I, III and IV form a supercomplex (in 1:2:1 stoichiometry) that brings the sites for electron transfer between the complexes in close proximity (Vonck and Schäfer, 2009; Althoff et al., 2011).

Complex I (NADH:ubiquinone oxidoreductase) couples the oxidation of NADH to the reduction of the membrane-soluble electron carrier coenzyme Q (ubiquinone). Complex I is the largest of the electron transport chain complexes and consists of 45 subunits that assemble into a ~1 MDa structure. The overall architecture is L-shaped with a hydrophobic membrane-embedded arm and a hydrophilic peripheral arm that protrude into the matrix. The peripheral arm

contains the redox centers necessary for electron transport, whereas the membrane arm, which contains all of the mtDNA-encoded subunits (ND1-6, ND4L), makes up the proton translocation machinery. Oxidation of NADH takes place in the peripheral arm by the transfer of two electrons to an acceptor flavin mononucleotide (FMN). These electrons are then sequentially transferred via a series of iron-sulfur clusters (Sazanov and Hinchliffe, 2006) to the proximal part of the peripheral arm, where ubiquinone (Q) accepts the electrons to form first semiquinone ($QH^{\bullet-}$), then ubiquinol (QH_2). The energy released by the electron transfer is coupled to the translocation of protons across the inner membrane by the membrane arm. Recent crystal structures (Hunte et al., 2010; Efremov and Sazanov, 2011) suggest that a rod-like helix parallel to the membrane arm acts like a piston to transfer conformational energy from the Q reduction site to two separate proton channels in the membrane arm (Brandt, 2011). Each electron transferred drives translocation of one proton at each channel, so that each NADH (2 electrons) in total drives translocation of 4 protons.

Complex II (succinate:ubiquinone reductase or succinate dehydrogenase) is another source of QH_2, but does not directly contribute to the proton motive force. Complex II consists of four subunits and is integrated in the TCA cycle by the SDH-A subunit that catalyzes the succinate to fumarate reaction. In doing so it acquires electrons to the covalently bound cofactor FAD, from which the electrons are further transferred via three iron sulfur clusters in the SDH-B subunit to the final electron acceptor coenzyme Q. Two hydrophobic subunits provide the Q binding site and anchor the complex to the inner membrane. All complex II subunits are nuclear-encoded, and the complex forms a homotrimer.

A third source of QH_2 comes from the catabolism of fatty acids. Electrons from $FADH_2$ generated by fatty acyl-CoA dehydrogenases are collected by the electron transfer flavoprotein (ETF) and transferred to the pool of coenzyme Q in the inner membrane by the enzyme ETF:ubiquinone reductase.

Complex III (ubiquinol-cytochrome c reductase) transfers electrons from QH_2 to cytochrome c, a soluble protein in the intermembrane space, and couples this reaction to the translocation of protons across the inner membrane. Complex III consists of eleven subunits and functions as a homodimer. Only three of the subunits, including the only mtDNA-encoded subunit, cytochrome b, contain redox centers and participate in electron transport (Saraste, 1999). The first electron from QH_2 is transferred directly to cytochrome c, which is only a single-electron acceptor. Thus, the second electron cannot be transferred directly to cytochrome c and is instead recycled by a mechanism called the Q cycle. This is achieved by transferring the second electron via the cytochrome b subunit to

another Q molecule at a second binding site to generate semi-ubiquinone. During the next round of cytochrome c reduction, this semi-ubiquinone is further reduced to QH_2. The net effect of the Q cycle is that every QH_2 results in the reduction of two cytochrome c molecules while translocating four protons (Saraste, 1999).

Complex IV (cytochrome c oxidase) harvests electrons from reduced cytochrome c and delivers them to the final electron acceptor, molecular oxygen. Three of the 13 subunits are mtDNA-encoded (COXI-III), and together make up the catalytic core. Four molecules of reduced cytochrome c sequentially deliver electrons to heme and copper centers in the oxidase, which in turn passes them on to O_2 to generate two molecules of H_2O (Saraste, 1999). This reaction is coupled to the translocation of four protons.

Complex V (ATP synthase) uses the proton gradient generated by complexes I, III and IV to synthesize ATP. The complex consists of 14 different subunits, two of which are mtDNA-encoded, and hetero-oligomerize to form a 600 kDa complex with a membrane-bound part (F_0) and a catalytic part (F_1) that protrudes into the matrix. Movement of protons through a channel in the F_0 part causes rotation of a central "rotor" that connects the F_0 and F_1 parts. The F_1 part is prevented from rotating by a peripheral stalk that anchors it to the static membrane part (Rubinstein et al., 2003). Instead, the rotational energy induces conformational changes that catalyze the formation of ATP from ADP and inorganic phosphate. Under some conditions, activity of the ATP synthase can be reversed so that ATP hydrolysis instead drives proton pumping from the matrix to the intermembrane space. Recent electron microscopy studies has shown that complex V forms dimers situated along cristae ridges and may be responsible for shaping inner membrane curvature (Davies et al., 2011).

Mitochondrial ROS production

Reactive oxygen species (ROS) are important byproducts of the RC and form when an electron escapes from the preferred path. Such electrons often react with oxygen to generate a superoxide anion ($O_2^{\bullet-}$). Complexes I and III are the main sites for mitochondrial ROS production and it has been estimated that 0.2% of molecular oxygen forms superoxide (Balaban et al., 2005). Mitochondrial superoxide dismutase (SOD2) converts $O_2^{\bullet-}$ into another oxidant, hydrogen peroxide (H_2O_2). H_2O_2 can be enzymatically reduced to water by catalase or glutathione peroxidase, but can also react with transition metal ions to generate a highly reactive hydroxyl radical ($OH^{\bullet}$) by the Fenton reaction. ROS in the form of $O_2^{\bullet-}$, H_2O_2 or $OH^{\bullet}$ can cause oxidative damage to DNA, proteins and lipids. ROS

may also play a physiological role in redox signaling (Finkel, 2011; Murphy et al., 2011).

Mitochondrial genetics

The mammalian mitochondrial genome is a circular DNA molecule (Fig. 4) of ~16.5 kb (16.6 kb in humans, 16.3 kb in mice). Each cell contains thousands of copies of mtDNA but it only makes up ~1% of the total cellular DNA content. The mtDNA encodes 13 proteins that are all subunits of the oxidative phosphorylation system as well as 22 tRNAs and two ribosomal RNAs (12S and 16S rRNAs) (Anderson et al., 1981). All proteins necessary for transcription, replication and maintenance of mtDNA are nuclear-encoded. The two mtDNA strands are called the heavy strand (H-strand) and the light strand (L-strand), based on the difference in molecular weight that results from an imbalance in the content of purines. The sequence is extremely compact with no introns and consists almost exclusively of coding sequence. The major exception is the ~1 kb region of regulatory sequence known as the displacement loop (D-loop).

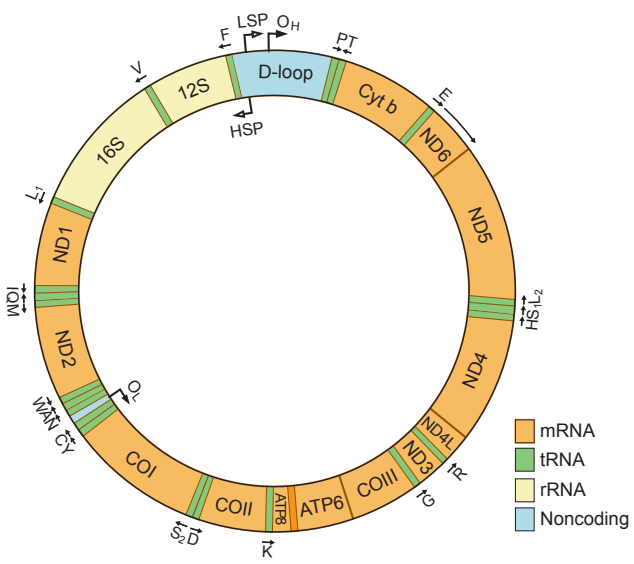


Figure 4. Gene organization of mammalian mtDNA. Each gene is represented by a box with tRNA genes indicated by one-letter amino acid codes and direction of transcription indicated by arrows. The D-loop is the only longer non-coding region and contains promoters for transcription of both strands (LSP and HSP) and the origin of leading strand replication (O_H). Lagging strand replication is initiated from a site (O_L) within a cluster of tRNA genes. The H-strand is transcribed from HSP and encodes 2 rRNAs (12S and 16S rRNA), 12 mRNAs (ND1–5, ND4L, Cyt b, COI–III, ATP6, and ATP8), and 14 tRNAs (F, V, L_1, I, M, W, D, K, G, R, H, S_1, L_2, T). The L-strand is transcribed from LSP and encodes the mRNA for ND6 and 8 tRNAs (P, E, S_2, Y, C, N, A, Q). Adapted from (Larsson, 2010).

Organization of the mitochondrial genome

The mitochondrial genome is organized by associated protein components to form structures called nucleoids (Bogenhagen, 2011). Each nucleoid has a size of ~100 nm (Kukat et al., 2011) and may be associated with the inner membrane (Wang and Bogenhagen, 2006). The number of mitochondrial genomes

contained in each nucleoid is debated (Bogenhagen, 2011), but a recent study combining quantitative PCR with super-resolution light microscopy estimated that each nucleoid on average contains 1.4 mtDNA molecules (Kukat et al., 2011), suggesting that most nucleoids contain only a single mitochondrial genome. The mitochondrial transcription factor A (TFAM) is an abundant mitochondrial protein, present in about 1,000 molecules per mtDNA copy, and it is a major component of the nucleoid structure (Kaufman et al., 2007; Bogenhagen, 2011). TFAM contains two high mobility group (HMG)-box domains that can bind double stranded DNA in both sequence-specific and sequence-unspecific manner. Binding of TFAM bends the DNA helix almost 180° (Ngo et al., 2011; Rubio-Cosials et al., 2011), and binding of increasing amounts of TFAM compacts the DNA (Kaufman et al., 2007). Consistent with a role in DNA packaging, mtDNA copy number co-varies with the levels of Tfam (Larsson et al., 1994; 1998; Ekstrand et al., 2004).

Transcription of mtDNA

Both the H-strand and the L-strand are transcribed in a bi-directional fashion from two promoters in the D-loop to create long polycistronic transcripts (Fig. 4). These primary transcripts are post-transcriptionally cleaved to generate individual mRNA, tRNA and rRNA molecules (Ojala et al., 1981). Mitochondrial mRNAs are polyadenylated but are not capped in the 5´-end. Transcription of the H-strand is initiated upstream of $tRNA^{Phe}$ by the H-strand promoter (HSP) and generates a transcript containing the 12S and 16S rRNAs, 14 tRNAs, and all protein coding genes except ND6. The L-strand promoter (LSP) initiates transcription of the L-strand that contains 8 tRNAs and the gene encoding ND6. A second H-strand promoter (HSP2) just upstream of the 12S rRNA gene has been reported but its existence is debated as it has not been possible to reconstitute *in vitro* (Litonin et al., 2010).

The organelle-specific mitochondrial RNA polymerase (POLRMT) mediates mtDNA transcription. POLRMT consists of a single subunit and shares homology with bacteriophage RNA polymerases. TFAM and the mitochondrial transcription factor B2 (TFB2M) are additionally required for transcription initiation and together sufficient to initiate transcription from HSP and LSP templates *in vitro* (Falkenberg et al., 2002). Both promoters contain an upstream recognition sequence to which TFAM can bind with sequence-specificity (Fisher et al., 1989). TFB2M and POLRMT are recruited by the C-terminal domain of TFAM and cooperatively bind the transcription start site (Sologub et al., 2009).

Replication of mtDNA

Replication of mtDNA is under relaxed control, and each mtDNA molecule may replicate several times during the cell cycle or not at all (Bogenhagen and Clayton, 1977). The mitochondrial DNA polymerase-γ (POLγ) consists of a catalytic A subunit and two accessory B subunits. The minimal replication machinery also includes mitochondrial single-stranded DNA-binding protein (mtSSB) and the hexameric mitochondrial DNA helicase TWINKLE (Korhonen et al., 2004). In addition, POLRMT is required for replication initiation. RNA synthesis initiated at LSP is frequently terminated prematurely at a conserved sequence block within the D-loop and thereby creates the primer for H-strand replication (Falkenberg et al., 2007; Wanrooij et al., 2010). DNA synthesis from origin of H-strand replication (O_H) is often aborted after ~700 bp. The resulting nascent strand (called 7S DNA) displaces the parental H-strand and makes the D-loop a triple-stranded structure.

The model for leading and lagging strand replication is debated. In the *strand-displacement* model (Clayton, 1982), replication of the leading strand alone proceeds around two thirds of the genome until it reaches the origin of L-strand replication (O_L). When the O_L sequence becomes single-stranded, it adopts a stem-loop structure that facilitates the generation of a short primer by POLRMT to initiate replication of the lagging strand (Fusté et al., 2010). The *strand-synchronous* model instead propose replication of both strands is coupled and resembles that of nuclear DNA replication in that lagging strand replication involves the formation of Okazaki fragments (Yang et al., 2002).

Segregation and transmission of mtDNA

MtDNA may acquire both point mutations and rearrangements such as deletions. The mutation rate of mtDNA is many times higher than that of nuclear DNA (Khrapko et al., 1997). Both mutant and wildtype alleles may coexist within a single cell, a state known as heteroplasmy. Homoplasmy refers to the situation when all mtDNA molecules carry the same allele. The pool of mtDNA molecules distribute stochastically to daughter cells during cell division, and such random segregation may lead to differences in the levels of heteroplasmy between different cells and tissues. Even in postmitotic cells, selective replication of a subset of mtDNA molecules may cause random drift and accumulation of certain mtDNA species over time, so called clonal expansion.

The mitochondrial genome is maternally inherited by contribution of oocyte mitochondria. The level of a heteroplasmic mtDNA variant transmitted from mother to offspring can vary significantly. This phenomenon is attributed to a

significant reduction in the number of transmitted genomes and referred to as the 'genetic bottleneck' (Shoubridge and Wai, 2007; Carling et al., 2011). Paternal mitochondria in the spermatozoon midpiece, that enter the oocyte during fertilization, are rapidly degraded by autophagy (Rawi et al., 2011). Paternal inheritance of mtDNA has been reported in a patient (Schwartz and Vissing, 2002), but this appears to be a rare exception (Taylor et al., 2003). Escape from degradation is also seen in interspecific crosses between distantly related mouse species (Gyllensten et al., 1991; Kaneda et al., 1995), suggesting the involvement of a specific recognition receptor.

Mitochondrial disease

Mitochondrial disease constitutes a heterogeneous group of inborn errors of metabolism that result from impaired OxPhos capacity. Rolf Luft was the first to establish mitochondrial dysfunction as a cause of disease. Already 50 years ago he and his colleagues identified abnormal uncoupling of cellular respiration as the underlying cause of hypermetabolism (increased perspiration, weakness and cachexia despite high food intake) in a young adult female (Luft et al., 1962). Today, a large number of mitochondrial disorders have been described that collectively affect about 1 per 8,500 individuals (Chinnery and Turnbull, 2001). Mitochondrial disorders may present from infancy to adulthood with symptoms of either single- or multi-organ failure. The central nervous system, heart and skeletal muscle are commonly affected, presumably due to high energy demands. Diagnosis normally involves biopsy for histology and biochemical measurements. Molecular diagnosis became possible following the identification of pathogenic mtDNA deletions (Holt et al., 1988) and point mutations (Wallace et al., 1988). Mutations in a growing list of nuclear-encoded genes can also cause mitochondrial disease. Pathogenic mutations either directly interfere with OxPhos enzyme kinetics or assembly, or interfere with the production of mtDNA-encoded subunits (Debray et al., 2008). Mutations in either genome may underlie a common clinical presentation. Leigh syndrome, for example, a condition with degenerative lesions in the brainstem and basal ganglia that develop during infancy or early childhood, can result from both nuclear DNA and mtDNA mutations (Naess et al., 2009), and the inheritance can thus be either maternal, autosomal recessive or X-linked.

Pathogenic mtDNA variants are often heteroplasmic, and the proportion of mutated mtDNA referred to as the mutation load. Wildtype mtDNA can normally complement the mutated mtDNA if present above a certain threshold level (Rossignol et al., 2003). The mutation load required to develop disease depends

on the type of mutation and can vary from 60% for deletions (Hayashi et al., 1991) to >90% for some point mutations (Chomyn et al., 1992). An uneven distribution of the mutation load between different tissues contributes to, but cannot fully explain, clinical heterogeneity. An example of this is Leber's hereditary optic neuropathy (LHON), a cause of subacute blindness and sometimes additional symptoms. A majority of LHON patients carry a homoplasmic mutation in ND1, ND4 or ND6, but the penetrance is incomplete and may depend on nuclear gene variants (McFarland and Turnbull, 2009). Some "classical" syndromes are associated with specific mutations (McFarland and Turnbull, 2009). For example the 3243A>G mutation in the $tRNA^{Leu(UUR)}$ gene causes MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) (Goto et al., 1990) and the 8344A>G $tRNA^{Lys}$ mutation causes MERFF (myoclonic epilepsy and ragged red fibers) (Shoffner et al., 1990). However, mutations in other genes may present with a similar phenotype. Conversely, a heteroplasmic mutation in the protein-coding ATP6 gene is associated with NARP (neurogenic weakness, ataxia and retinitis pigmentosa) at high mutation loads and infantile Leigh syndrome at very high mutation loads (Santorelli et al., 1993).

Large mtDNA deletions arise spontaneously and are rarely inherited. Disease severity correlates with tissue distribution and mutation load (McFarland and Turnbull, 2009). MtDNA deletion is a common cause of chronic progressive external ophthalmopleiga (PEO), which may present in adults or during childhood in combination with myopathy, pigmentary retinopathy and cardiac conduction defects (Kearns-Sayre syndrome; KSS). High mutation loads may lead to severe bone marrow failure during infancy (Pearson syndrome). Survivors of Pearson syndrome go on to develop KSS (Larsson et al., 1990). Mutations in nuclear genes necessary for mtDNA replication, including ANT1 (Kaukonen et al., 2000), TWINKLE (Spelbrink et al., 2001) and the catalytic subunit of the mitochondrial DNA polymerase (Van Goethem et al., 2001) can also cause PEO and are associated with accumulation of multiple mtDNA deletions.

Mitochondria in ageing and degenerative disease

Mitochondrial dysfunction is implicated in the process of normal ageing (Trifunovic and Larsson, 2008; Larsson, 2010). RC function declines with age (Trounce et al., 1989) and ageing is associated with increasing amounts of somatic mtDNA mutations (Corral-Debrinski et al., 1992; Cortopassi et al., 1992; Soong et al., 1992). Although the total level of mutated mtDNA in a tissue is low, clonal expansion and random segregation can raise the mutation level in some

individual cells to exceed the threshold for RC deficiency (Larsson, 2010). A mosaic pattern of RC-deficient cells is indeed found in several aged tissues including heart (Müller-Höcker, 1989), skeletal muscle (Müller-Höcker, 1990) and brain (Cottrell et al., 2001; Bender et al., 2008). Affected cells have accumulation of mtDNA molecules carrying point mutations or large deletions (Fayet et al., 2002; Bender et al., 2006; Kraytsberg et al., 2006). A causative role for such mutations in ageing is strengthened by the finding that mice that accumulate high levels of somatic mtDNA point mutations due to expression of a proofreading-deficient POLγ develop a phenotype resembling premature ageing (Trifunovic et al., 2004). Mitochondrial dysfunction is also implicated in the pathophysiology of several neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease (Schapira, 2006).

PARKINSON'S DISEASE

Parkinson's disease (PD) is named after the English physician James Parkinson, who in 1817 described six subjects, some of which were patients at his clinic and others only seen in the streets, afflicted with a syndrome of tremor, weakness and disturbed gait (Parkinson, 1817). The pioneering French neurologist Jean-Martin Charcot later renamed the disease in honor of Parkinson's observations, although symptoms of the "shaking palsy" are described already in ancient literature. The term parkinsonism is used to describe the syndrome of motor manifestations seen in PD, but that may also result from certain medications or brain damage acquired by cerebrovascular insults or CNS infections.

In the early 1900s, studies of post-mortem brains identified two major pathological hallmarks of the disease: Frederic Lewy described the intraneuronal cytoplasmic inclusion bodies that where later called Lewy bodies (Lewy, 1912). Lewy bodies are, however, not pathognomonic for PD as they are also present in some other neurodegenerative conditions. Some years later, the Russian pathologist Tretiakoff described the loss of the endogenously pigmented neurons in the pars compacta area of substantia nigra (SNc) in PD patients brains (Tretiakoff, 1919). The relevance of this finding was however not appreciated until later. In 1957 Arvid Carlsson and colleagues discovered that dopamine (DA) acts as a signaling substance in the brain (Carlsson et al., 1958) and, based on the phenotype of animals with drug-induced DA depletion, proposed that impaired DA signaling plays a role in PD (Carlsson, 1959). Reduced DA content in brains of PD patients was soon confirmed (Ehringer and Hornykiewicz, 1960).

The midbrain dopamine system

The DA neurons in SNc project to areas of the striatum and form a part of the neural circuitry known as the basal ganglia. The basal ganglia are part of cortico-thalamic loops that process cortical input to facilitate a variety of functions including voluntary motor control. Two main loops exist, the excitatory *direct* pathway, and the inhibitory *indirect* pathway (Fig. 5A). DA in striatum modulates the signaling of both pathways. In PD, striatal DA deficiency resulting from loss of SNc DA neurons disrupts basal ganglia signaling and leads to classical motor symptoms. However, PD pathology is not restricted to DA neurons, and several other populations of neurons are also variably affected, in areas of the gastrointestinal tract to cortex cerebri, possibly contributing to non-motor symptoms (Braak et al., 2004).

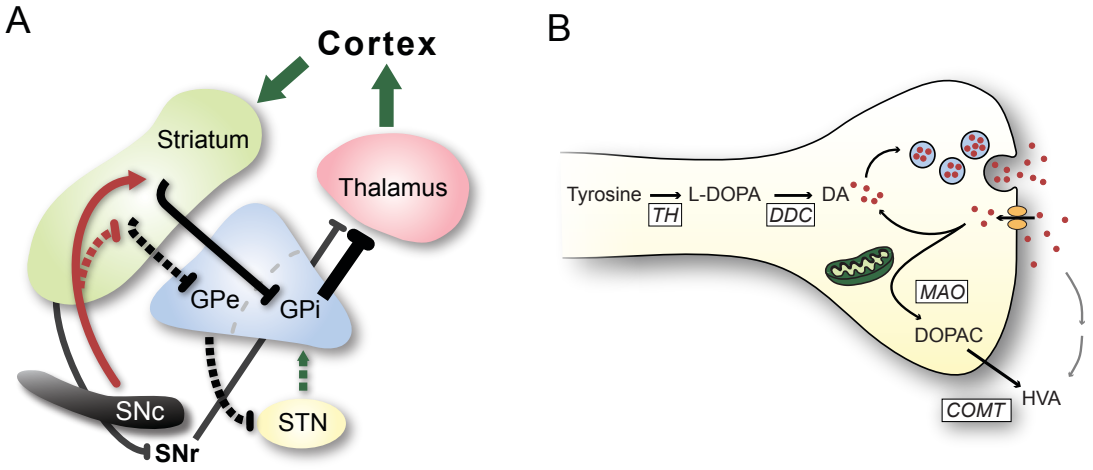


Figure 5. Dopamine metabolism and circuitry affected in PD. (A) Simplified schematic of basal ganglia circuitry affected in PD. The basal ganglia process excitatory input from cortex via the direct and indirect pathways to enhance or reduce thalamic stimulation of the motor cortex, respectively. DA neurons (red) projecting from substantia nigra pars compacta (SNc) modulate the strength of the cortical input to striatum. Striatal neurons of the direct pathway (solid line) have excitatory D1 dopamine receptors and project to the internal segment of Globus Pallidus (GPi). Striatal neurons of the indirect pathway (dashed line) are inhibited by DA acting on D2 inhibitory receptors, and project to the external part of Globus Pallidus (GPe). The GPe signals to GPi via the subthalamic nucleus (STN). Striato-thalamic signaling also involves the substantia nigra pars reticulata (SNr). Red denotes DA, green glutamate (excitatory) and black GABA (inhibitory) signaling.
(B) DA synthesis and degradation. The rate-limiting step in DA synthesis is the hydroxylation of the amino acid L-tyrosine into L-DOPA by the enzyme tyrosine hydroxylase (TH). The carboxylase group of L-DOPA is removed by dopa decarboxylase (DDC) to form DA (depicted as red dots). DA is concentrated into synaptic vesicles by the transporter VMAT2. A majority of released DA is recycled via reuptake by the DA transporter (DAT) (yellow). DA is primarily degraded by monoamine oxidase (MAO), situated on the outer mitochondrial membrane, to form 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC is further metabolized into homovanillic acid (HVA) by catechol-o-methyl-transferase (COMT). DA can also be degraded directly by COMT to 3-methoxytyramine, which is further degraded to HVA by MAO. Also other cell types participate in DA degradation, e.g. astrocytes that contain MAO.

Clinical aspects of PD

Parkinson's disease affects over 1% of the population above the age of 60 (de Lau and Breteler, 2006; Wirdefeldt et al., 2011), making it the second most common neurodegenerative disease after Alzheimer's disease. The incidence is heavily age-dependent and 60 years is the mean age at time of diagnosis. Diagnosis is primarily clinical and based on characteristic motor symptoms. Unilateral onset of tremor in a hand or a foot are typical presenting symptoms,

but these may have been preceded by more subtle non-motor symptoms such as constipation, loss of olfaction, and sleep disturbances. Tremor, rigidity, difficulties to initiate movements and impaired balance characterize disease progression and may become severely disabling. At late stages autonomic dysfunction, dementia and depression may develop. Treatment primarily includes medications that increase striatal DA signaling to restore balance to affected basal circuitries, but surgical interventions are also used. An initially good response to symptomatic pharmacological treatment declines with time, and severe side effects may develop. There is currently no effective treatment to slow down disease progression.

PD genetics

PD has traditionally been regarded as a 'sporadic' disease, and lack of family history has even been considered diagnostic. During the last 15 years, linkage analysis and genome wide association screens have revealed over ten genes in which mutations cause or seriously influence the risk for PD. For example, mutations in the gene LRRK2 are found in 10% of PD cases previously considered 'sporadic' (Hardy, 2010).

Alpha-synuclein and Lewy bodies

α-Synuclein was the first gene identified to cause PD with a Mendelian inheritance, when the 209G>A (Ala53Thr) pathogenic mutation was identified in Italian and Greek families with autosomal dominant PD (Polymeropoulos et al., 1997). Soon thereafter, misfolded α-synuclein protein was identified as a major component of Lewy bodies in idiopathic PD (Spillantini et al., 1997; 1998), suggesting that mishandling of wildtype α-synuclein protein plays a role in PD pathology. This was further supported by discoveries that duplication (Chartier-Harlin et al., 2004) or triplication (Singleton et al., 2003) of the wildtype allele cause PD with gene-dose dependent severity.

α-Synuclein is a 140 amino-acid protein that is highly expressed in the brain and enriched in presynaptic nerve endings. It belongs to a family of synucleins (α–/β–/γ–) that play a role in the recycling of synaptic vesicles by facilitating SNARE-complex assembly (Burre et al., 2010). α-Synuclein folds natively into a tetramer (Bartels et al., 2011), but can also adopt a β–sheet conformation that favors polymerization into fibrils. The so called 'permissive templating' hypothesis (Hardy, 2005) puts forward the prion-like behavior of α-synuclein. This idea is supported by findings that Lewy bodies can form in grafted, embryonic dopamine neuron about a decade after transplantation into the

brains of PD patients (Kordower et al., 2008; Li et al., 2008). *In vitro* experiments show that preformed α-synuclein fibrils can enter cells and induce Lewy pathology (Volpicelli-Daley et al., 2011). Compatible with this idea is the hypothesis put forward by Braak and colleagues (Braak et al., 2004) that Lewy body pathology starts in the enteric nervous system and the vagus nerve and with times spreads cranially to the basal ganglia and cortex. What precipitates α-synuclein misfolding and Lewy body formation is not known.

Mitochondrial dysfunction in PD

Several different experiments support a role for mitochondrial dysfunction in the pathophysiology of PD:

1. Several toxins linked to PD, most notably MPTP, can act as complex I inhibitors. MPTP was identified following a small outbreak of parkinsonism among drug abusers in California who had injected contaminated drugs (Langston et al., 1983). MPTP induces dopamine neuron degeneration in humans and laboratory animals (Burns et al., 1983; Kopin and Markey, 1988), and is widely used in PD research. Within the brain, monoamine oxidase converts MPTP to its active metabolite MPP+, which is concentrated in dopamine neurons by the dopamine transporter (Smeyne and Jackson-Lewis, 2005). MPP+ accumulates in mitochondria (Ramsay et al., 1986) where it can inhibit complex I (Heikkila et al., 1985; Nicklas et al., 1985; Mizuno et al., 1987).
2. Decreased mitochondrial complex I activity has been measured in postmortem SN tissue from PD patients (Schapira et al., 1989). Other groups reported reduced RC activities measured in skeletal muscle (Bindoff et al., 1989) and platelets (Parker et al., 1989).
3. Expression levels of PGC-1α, a key regulator of mitochondrial biogenesis, are decreased in the brains of patients with PD-like pathology (Zheng et al., 2010). Consistent with this finding, SNc homogenates from PD patients contain increased levels of PARIS, a zinc finger protein shown to act as a transcriptional repressor of PGC-1α (Shin et al., 2011).
4. Higher proportions of mtDNA molecules carrying deletions are found in homogenates of SN and dorsal striatum than in other brain regions (Corral-Debrinski et al., 1992; Soong et al., 1992). DA neurons with high levels of deleted mtDNA become RC-deficient (Bender et al., 2006; Kraytsberg et al., 2006). In brains of PD patients ~3% of the remaining DA neurons are RC-deficient, as compared to ~1% of all DA neurons in age-matched controls (Bender et al., 2006). Although these numbers are low, they may represent a

temporal snapshot of slowly progressive cell loss. High levels of mtDNA deletions can cause degeneration of DA neurons; this is illustrated in patients carrying mutations in the polymerase domain of *POLγ,* who accumulate high levels mtDNA deletions and may develop loss of DA neuron and parkinsonism (Luoma et al., 2004).

Recessive parkinsonism and the PINK1/Parkin pathway

Loss-of-function mutations in the genes *Parkin* (Kitada et al., 1998), *PINK1* (Valente et al., 2004) and *DJ-1* (Bonifati et al., 2003) cause a form of autosomal recessive juvenile parkinsonism (AR-JP). Symptoms are similar to idiopathic PD, but onset is earlier, around an age of 30 years, and disease progression slower. Neuropathological examination shows dopamine neuron degeneration, but Lewy bodies are typically absent, at least in carriers of Parkin mutations. AR-JP thus represents a partially distinct clinical entity, and pathophysiology may differ from that of PD (Ahlskog, 2009).

Parkin is a cytosolic E3 ubiquitin ligase protein that is upregulated in response to cell stress and is protective against various stresses when overexpressed (Pilsl and Winklhofer, 2011). PINK1 is a serine/threonine kinase that carries an N-terminal targeting sequence that inserts PINK1 in the outer mitochondrial membrane with the kinase domain facing the cytoplasm (Zhou et al., 2008). Studies in flies first established that PINK1 and Parkin act in the same pathway, with PINK1 acting upstream of Parkin (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Results from mammalian cells have proposed a model for how this pathway governs mitochondrial quality control.

PINK1 is continuously expressed and its steady state levels regulated by proteolysis. Under basal conditions, PINK1 is cleaved by the inner membrane protease PARL and possibly additional proteases to generate a short isoform that is rapidly degraded (Jin et al., 2010; Matsuda et al., 2010; Narendra et al., 2010). Processing of PINK1 depends on the mitochondrial membrane potential, and depolarization by uncoupling agents such as CCCP impairs processing and cause a longer, stable isoform to accumulate. Stabilization of PINK1 on the outer membrane recruits cytosolic Parkin to the mitochondrial outer surface and activates its ubiquitin ligase domain (Matsuda et al., 2010). In cultured cells, this pathway mediates selective removal of the depolarized mitochondria by an organelle-specific form of macro-autophagy, also referred to as mitophagy (Narendra et al., 2008; Geisler et al., 2010; Narendra et al., 2010; Vives-Bauza et al., 2010). These results suggest that PINK1 and Parkin act in a conserved pathway that protects against parkinsonism by degradation of impaired

mitochondria to prevent accumulation of mitochondrial damage. However, *in vivo* data to support this hypothesis is currently lacking and other roles for Parkin have been proposed (see Discussion).

THESIS AIM

The general aim of this thesis was to investigate consequences of mitochondrial dysfunction in the central nervous system with particular focus on midbrain dopamine neurons. To achieve this, several new mouse models were generated. The specific aim of each paper was to:

Paper I: study the consequences of mosaic RC-deficiency in forebrain neurons.

Paper II: study if disruption of mtDNA in DA neurons can cause Parkinsonism in mice.

Paper III: develop a reporter mouse for mitochondrial morphology to study distribution and turnover of mitochondria in healthy and RC-deficient DA neurons.

Paper IV: address the role of an isolated complex I deficiency in DA neurons in mice.

RESULTS AND DISCUSSION

Consequences of mosaic respiratory chain deficiency in the brain (paper I).

A mosaic pattern of RC-deficient neurons is found in the SNc and other brain regions in aged humans. It is also a characteristic feature of encephalopathies caused by pathogenic mtDNA mutations. In paper I, we addressed the role of mosaic RC deficiency by developing a chimeric mouse model. We based the model on a previously characterized mouse model with RC deficiency in forebrain neurons, so called mitochondrial late-onset neurodegeneration (MILON) mice (Sörensen et al., 2001). In MILON mice, *Tfam* is loxP-flanked and disrupted postnatally in neurons of neocortex and hippocampus by expression of *cre* recombinase under control of the calmodulin kinase II (CamKII) promoter (Xu et al., 2000). MILON mice appear healthy up to about 5 months of age, when widespread degeneration of affected brain regions is accompanied by rapid deterioration and premature death. We generated mice that contained mixed proportions of MILON cells and cells carrying a *LacZ* reporter gene. Pre-implantation embryos were harvested and fused *in vitro*, and subsequently implanted in pseudopregnant females to obtain chimeric mice. From a total of 216 pups, 61% were chimeric and carried a combination of either MILON and LacZ genotypes (MILON chimeras) or control and LacZ genotypes (control chimeras). The relative contribution of each genotype was assessed by Southern blotting and results correlated with the proportion of LacZ-positive neurons in hippocampus.

Chimeric mice model mosaic RC deficiency and reveal phenotypic thresholds

While control chimeras were healthy, some of the MILON chimeras developed symptoms and premature death. The risk for developing this phenotype correlated with the proportion of RC-deficient (MILON) neurons. Low proportions (>20%) were sufficient to cause symptoms in a subset of mice, and those with very high proportions (>80%) invariably developed symptoms. Premature death only occurred in mice with high proportions (>60%). Chimeric mice had a slight but significant delay the onset of symptoms compared with non-chimeric MILON mice. This suggests that surrounding healthy neurons can partially rescue the phenotype.

Trans-*neuronal degeneration of neurons with intact RC function in chimeric mice*

Neurodegeneration was primarily seen in the neocortex and medial hippocampus, as expected from the previous study (Sörensen et al., 2001). However, a subset of degenerating neurons in the chimeric mice was LacZ-positive, indicating that they degenerate despite having intact RC function. In chimeras with high proportions, such cells represented ~1% of all degenerating neurons. This represents a substantial proportion of neurons with normal RC function, as these are relatively few in mice with high proportions of MILON neurons. The mechanism for this *trans*-neuronal degeneration is not known. Neurons depend on trophic support and it has been suggested that interconnected neurons promote mutual survival by forming trophic units (Agnati et al., 1995). We were unable to address the anatomical relationships between the different degenerating neurons, as many may already have been lost. Another possibility is that the ongoing cell death creates a milieu that can harm cells in proximity even without being in direct contact. The relevance for DA neurons is not known. The level of RC-deficient DA neurons in a PD brains is low (~3%) at a given time point. Nevertheless, also surrounding neurons likely carry some degree of impairment, and may become affected by the gradual loss of surrounding neurons.

Consequences of combined respiratory chain defect in DA neurons (papers II and III)

PD is associated with declined mitochondrial function and accumulation of mtDNA molecules carrying deletion in SNc DA neurons. In paper II, we experimentally tested the consequences of mtDNA depletion by disrupting *Tfam* specifically in DA neurons. Restricted expression of the *cre*-recombinase in DA neurons was achieved by targeted insertion of the coding sequence into the DA transporter (DAT) locus by homologous recombination in embryonic stem cells. In situ hybridizations and breeding with a reporter mouse confirmed that DAT^{cre} mice express *cre* exclusively in DA neurons of the SNc and the neighboring ventral tegmental area (VTA). We crossed DAT^{cre} mice to mice with $Tfam^{loxP}$ mice for two consecutive generations to obtain DA-specific Tfam knockout mice (genotype $DAT^{cre/+}$; $Tfam^{loxP/loxP}$) and named these MitoPark mice.

MitoPark mice have RC deficiency in DA neurons and develop progressive Parkinsonism

MitoPark mice were born in Mendelian proportions and appeared healthy at birth. COXI transcripts were lost in MitoPark DA neurons at 6 w of age, and

reduced COX activity was found by enzymatic staining at later time points. At an age of ~15-20 w, symptoms of parkinsonism (reduced activity, twitching of the limbs and abnormal gait) appeared. These symptoms progressed with age and were accompanied by tremor, rigidity, weight loss and a deteriorated general condition that demanded euthanasia at an age of ~45 w. We quantified motor activity using locomotor boxes, in which light beams record mouse movements. MitoPark mice did not differ from controls at 10 w of age, but from 14 w displayed significantly reduced activity that progressively declined with increasing age. We measured monoamine levels in different brain regions by high performance liquid chromatography (HPLC) and found that symptomatic MitoPark mice had drastically reduced DA levels in striatum. Consistent with this, administration of standard PD medication L-DOPA plus benserazide drastically improved motor performance.

Immunolabeling of midbrain DA neurons with antibodies against tyrosine hydroxylase (TH) revealed progressive degeneration of DA terminals in striatum and cell bodies in SNc and VTA. SNc neurons degenerated somewhat prior to those of the VTA, suggesting that SNc neurons may be relatively more susceptible than VTA neurons to mitochondrial dysfunction. Mice with *Tfam* knockout hearts have increased apoptosis but little evidence of increased ROS levels (Wang et al., 2001). We failed to detect TUNEL- or FluoroJade-positive DA neurons in MitoPark mice, possibly reflecting a relatively slow rate of cell loss.

α-Synuclein is dispensable for neuropathology in MitoPark mice

MitoPark DA neurons contained spherical TH-pale regions in the cytoplasm of the soma and proximal dendrites, suggesting the presence of intracellular inclusions. These space-occupying formations were immunoreactive for several mitochondrial proteins as well as a polyclonal antiserum raised against residues 116-131 of human α-synuclein. The latter allowed quantifications showing that these pathological bodies increased in both number and size with age. However, α-synuclein immunoreactivity was unspecific, as it was also seen in MitoPark mice bred onto an α-synuclein knockout background. The epitope responsible for this cross-reactivity is unknown but likely consists of a possibly misfolded, mitochondrial protein. MitoPark mice with α-Synuclein knockout developed the same phenotype as normal MitoPark mice, demonstrating that α-synuclein is dispensable for inclusion formation and neurodegeneration in MitoPark mice. Although loss of α-synuclein can be compensated by redundancy within the (α–/β–/γ–) synuclein family (Burre et al., 2010), we did not find evidence that mitochondrial dysfunction leads to α-synuclein aggregation in mouse DA neurons.

Developing a reporter mouse for in vivo labeling of mitochondria

We visualized the large intracellular bodies by electron microscopy and found several of them to consist in part of tubular double layer membrane formations resembling disorganized cristae and in part fairly electron dense regions. To further study mitochondrial morphology and distribution in MitoPark DA neurons, we developed a reporter mouse for tissue-specific labeling of mitochondria (paper III). We used the yellow fluorescent protein (YFP) and directed this to the mitochondrial matrix by N-terminal fusion with a mitochondrial targeting sequence. A stop cassette flanked by non-standard sequence loxP-sites was inserted upstream of the coding sequence to restrict mito-YFP expression to cells that express *cre*. The construct was targeted to the ROSA26 locus by homologous recombination in embryonic stem cells. The endogenous ROSA26 promoter produces a near-ubiquitously expressed non-coding transcript that can be replaced by any transgene of choice (Zambrowicz et al., 1997; Soriano, 1999). This strategy ensured wider versatility than would have been achieved using a tissue-specific promoter. When these knock-in mice were crossed to mice with ubiquitous *cre* expression via the β-actin promoter, the resulting offspring displayed YFP-labeled mitochondria in a range of tissues, including neurons, heart and skeletal muscle, kidney, liver and hematopoietic cells. Expression of mitochondria-targeted YFP (mito-YFP) did not affect mouse viability or compromise OxPhos capacity. Expression of mito-YFP could also be restricted to DA neurons by crossing to DAT^{cre} mice (Fig. 6A). Mitochondria in DA neurons formed a dense network of mitochondrial tubules in the soma that extended to proximal parts of the dendrites (Fig. 6B). In distal parts of axons and dendrites, mitochondria were gradually shorter and arranged in rows.

Large mitochondrial aggregates are formed in MitoPark DA neurons

In MitoPark DA neurons, mitochondria in the soma and proximal parts of dendrites were condensed. Large spherical YFP-labeled structures were also found that corresponded in size and distribution to the previously studied intracellular TH-negative bodies (Fig. 6C). This led us to conclude that the structures found by electron microscopy consisted of abnormal and enlarged mitochondria. Immunohistochemistry confirmed that the aggregates were surrounded by an outer mitochondrial membrane and contained mitochondrial matrix proteins. Small aggregates were found already at 4 weeks of age, suggesting that their formation is an early consequence of mitochondrial dysfunction. Associated with their appearance was a fragmentation of surrounding mitochondria and shortening of mitochondria in axons and dendrites.

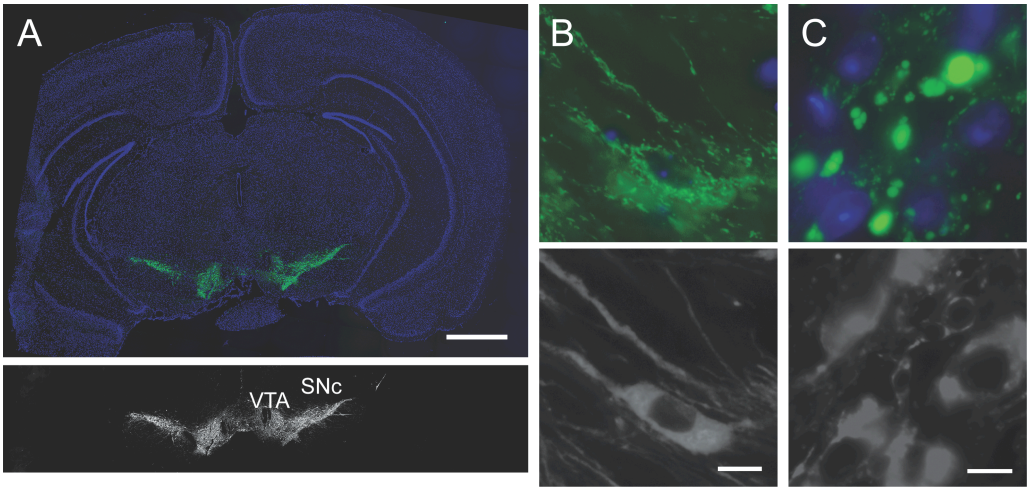


Figure 6. A mitochondrial reporter mouse allows visualization of mitochondria specifically in DA neurons. (A) Coronary section of the midbrain. Transgene expression is restricted to DA neurons in SNc and VTA regions. (B) Mitochondrial morphology in healthy SNc DA neurons. (C) Mitochondrial morphology in MitoPark DA neurons. Mitochondrial YFP shown in green and nuclei counterstained blue. DA neurons are outlined by TH immunoreactivity (gray; lower panels).
Scale bars 1 mm (A), 10 μm (B-C).

Supply of distal mitochondria is impaired

Cell type-specific labeling allowed us to study the distal pool of mitochondria in striatal DA nerve terminals. The number of striatal DA mitochondria declined in parallel with loss of TH-positive fibers, but remaining mitochondria looked normal. We addressed the turnover of striatal mitochondria by employing a pulse-labeling strategy. By stereotaxic injections into the SNc area of adult mice, we delivered an adeno-associated virus (AAV) encoding a mitochondrial matrix-targeted red fluorescent protein (mito-dsRed). Mitochondrial biogenesis occurs primarily in the perinuclear region (Davis and Clayton, 1996) and mito-dsRed accumulated in proximal mitochondria of transduced cells. In control mice, a significant proportion of striatal mitochondria became labeled with mito-dsRed when studied 1 w after viral delivery. However, only few such mitochondria could be observed in the striatum of MitoPark mice. In relation to the number of dsRed-expressing cells in SNc, the reduction was ~10-fold. Thus MitoPark mice have a drastically impaired supply of mitochondria to distal axon segments.

Axonal degeneration in PD precedes loss of cell bodies in SNc (Cheng et al., 2010), and a similar 'dying back' pattern is also seen in MitoPark mice. Brain mitochondria have an estimated half-life of ~24-30 days (Gross et al., 1969; Menzies and Gold, 1971) and it is believed that dysfunctional mitochondria in axon terminals are continuously replaced and transported back to the soma for

degradation (Sheng and Cai, 2012). Impaired anterograde supply may cause depletion of axonal mitochondria and suggests a mechanism for the early axonal degeneration.

Neurodegeneration in MitoPark mice occurs independently of Parkin

Given the proposed role for Parkin to target dysfunctional mitochondria for degradation, we tested the role of Parkin in MitoPark mice. To this end, we crossed MitoPark mice onto a Parkin knockout background. We found that MitoPark mice lacking Parkin developed a similar phenotype to that of MitoPark mice wildtype for Parkin, with similar mitochondrial morphology, formation of large mitochondrial aggregates and rate of cell loss. We also tested whether Parkin translocated to the impaired mitochondria in MitoPark DA neurons *in vivo*. As endogenous levels of Parkin could not reliably be detected by immunohistochemistry, possibly due to low expression levels, we overexpressed Parkin fused to a fluorescent protein. Viral delivery of mCherry-fused mouse Parkin resulted in a cytoplasmic expression pattern, sometimes with punctate accumulation, but no co-localization with mitochondria of any size or shape was found in either control of MitoPark neurons. Furthermore, we found no co-localization with a marker for autophagy, cyan fluorescent protein-tagged LC3 (data not shown). Taken together, this suggested that the aberrant mitochondria in MitoPark DA neurons were not subject to Parkin-mediated autophagy.

Parkin-recruitment requires depolarization of the inner membrane, while mitochondrial dysfunction *per se* appears insufficient (Narendra et al., 2009). A possible explanation for the above findings is thus a partially maintained membrane potential, despite loss of mtDNA. The accumulation of high levels of mito-dsRed in the mitochondrial aggregates soon after expression suggested the possibility of retained mitochondrial protein import and residual membrane potential. Import of polypeptides via the TOM/TIM complex depends on a membrane potential across the inner membrane and is blocked by CCCP. In analogy, cultured cells depleted of mtDNA (so-called rho0 cells) maintain a mitochondrial membrane potential sufficient for protein import by influx of ATP^{4-} to the matrix by ANT and F_1-ATPase activity (Buchet, 1998). Rho0 cells can partly compensate the resulting ATP consumption by anaerobic metabolism in buffered media. Likewise, cells surrounding MitoPark DA neurons can possibly provide support for prolonged anaerobic ATP production.

On the physiological role of Parkin

Despite recent advances, the physiological role of Parkin *in vivo* remains unclear. Numerous experiments lend support to a model in which cytosolic Parkin is regulated by PINK1 on the outer mitochondrial surface in response to the membrane potential. However, Parkin-dependent mitophagy has only been demonstrated *in vitro* and data are almost exclusively derived from cells with forced Parkin expression and high levels of toxins. Mitophagy in Parkin-overexpressing cells exposed to CCCP is preceded by widespread ubiquitination and proteasome-mediated degradation of a large number of mitochondrial outer membrane proteins (Chan et al., 2011) and even rupture of the outer membrane (Yoshii et al., 2011), although the relevance of this in the process of mitophagy is unclear. Under physiological conditions, Parkin may instead have a limited number of substrates that regulate mitochondrial functions other than mitophagy. For example, Parkin may regulate mitochondrial morphology by targeting Mfn1 and Mfn2 for degradation (Gegg et al., 2010; Tanaka et al., 2010; Ziviani et al., 2010; Rakovic et al., 2011), consistent with results from flies showing that increased mitochondrial fission can suppress loss of PINK1 or Parkin (Deng et al., 2008; Poole et al., 2008; Yang et al., 2008). Other studies have shown that the PINK1/Parkin pathway may regulate mitochondrial motility by direct interaction with Miro1 and Miro2 (Weihofen et al., 2009; Wang et al., 2011). Another possibility is that Parkin, even if activated on the mitochondrial surface, regulates cytosolic proteins. One such example is PARIS, a cytoplasmic protein that may regulate mitochondrial biogenesis by repression of PGC-1α expression (Shin et al., 2011).

Studies of the *in vivo* role of Parkin have been complicated by the lack of good mammalian model systems. Flies with disruption of either PINK1 or Parkin develop a degenerative phenotype accompanied by mitochondrial abnormalities. However, PINK1 (Kitada et al., 2007; Gautier et al., 2008; Gispert et al., 2009) or Parkin (Goldberg et al., 2003; Itier et al., 2003; Coelln et al., 2004) knockout mice only show subtle phenotypes and no loss of DA neurons (Perez and Palmiter, 2005; Kitada et al., 2009). This is often attributed to functional redundancy, but because a single conserved gene encodes Parkin, such an explanation suggests presence of unrelated pathways able to perform the same task. Other mechanisms to ensure mitochondrial quality certainly exist, but may not depend on selective removal of entire organelles (Tatsuta and Langer, 2008). It is however important to point out that our data are negative and do not exclude a role for Parkin in mitophagy. Translocation of Parkin may be difficult to visualize *in vivo*, as it is a transient and possibly rapidly occurring event.

Consequences of a mild complex I deficiency in DA neurons (paper IV)

Biochemical defects found in patient material as well as studies of several PD-linked toxins have pointed towards a specific involvement of complex I in the pathophysiology of PD. In paper IV, we addressed the consequences of complex I in DA neurons by generating mice with DA-specific disruption of the complex I subunit Ndufs4.

Loss of Ndufs4 cause a mild complex I deficiency in vivo

Ndufs4 is a nuclear-encoded subunit situated in the peripheral arm of complex I. We first characterized the biochemical consequences of *Ndufs4* disruption in heart. Heart knockouts were generated by crossing mice with loxP-flanked *Ndufs4* alleles to mice that express *cre* in heart and skeletal muscle. Biochemical measurements and blue-native gel electrophoresis demonstrated that loss of Ndufs4 impairs stability and/or assembly of complex I. Isolated enzyme activity was completely absent in disrupted mitochondrial particles, but, in contrast, intact mitochondria displayed near-normal ATP production rates also with substrates that enter the TCA cycle at the level of complex I. We attributed this discrepancy to impaired complex stability and concluded that *Ndufs4* ablation only leads to a mild complex I deficiency *in vivo*.

Ndufs4 disruption in DA neurons does not lead to neurodegeneration or Parkinsonism

We used *DAT*^{cre} mice to generate DA-specific *Ndufs4* knockout mice, hereafter referred to as Ndufs4-DA mice. These mice appeared healthy and did not develop symptoms of Parkinsonism even when aged up to 2 years. Midbrain DA neurons looked normal and large mitochondrial aggregates were not formed. In very old mice, a significant (20%) reduction in the number of SNc DA neurons was found using one of two different approaches to count cells, but accompanying striatal denervation was not seen.

Of note, full-body *Ndufs4* knockouts develop a fatal neurodegenerative phenotype within 2 months of age (Kruse et al., 2008; Quintana et al., 2010). This suggests that DA neurons are not particularly vulnerable to a systemic complex I deficiency, although complex I deficiencies following other types of primary defects may have other outcomes (Sterky and Larsson, 2008).

Ndufs4-DA mice have altered striatal DA homeostasis

Measurements of the levels of brain monoamine levels in Ndufs4-DA mice at different ages consistently showed slightly (~15%) reduced striatal DA levels,

which was accompanied by increased amounts of major DA metabolites DOPAC and HVA. An increased metabolite:DA ratio typically reflects increased DA turnover and suggest an altered striatal DA homeostasis. We therefore measured DA release and re-uptake by fast scan cyclic voltammetry in acute striatal brain slices, and found that DA release was reduced following either a single pulse or repeated phasic stimulation. The decay constant, an indirect measure of DA reuptake, was unaffected and suggested intact DAT activity. These results are consistent with findings in young MitoPark mice (Good et al., 2011) and reduced striatal DA release thus appears to be an early consequence of mitochondrial dysfunction. Interestingly, impaired DA release has also been found in *PINK1* knockout mice (Kitada et al., 2007), possibly as a consequence of impaired complex I activity (Morais et al., 2009). Decreased DA release may result from impaired DA exocytosis, but also from reduced vesicular DA uptake by VMAT-2. The latter idea gains support from analogy with DA release curves obtained using VMAT-2 inhibitors (Good et al., 2011) and the increase in DA metabolites seen in mice with severely reduced VMAT-2 levels (Caudle et al., 2007).

Ndufs4-DA mice are more vulnerable to MPTP exposure

Previous studies suggest that the PD-linked toxin MPTP may act by inhibition of complex I. We hence tested the effects of MPTP in our Ndufs4-DA mice. Both control and Ndufs4-DA mice showed significant reductions in striatal DA levels one week after exposure to MPTP. Striatal DA reduction was more pronounced in Ndufs4-DA mice, which was also reflected by increased metabolite:DA ratios. These results show that also a mild complex I deficiency in DA neurons can increase the vulnerability to stress by toxins such as MPTP.

In summary, we conclude that complex I deficiency in DA neurons can contribute to PD pathophysiology.

CONCLUDING REMARKS

Is mitochondrial dysfunction a driving force in PD pathophysiology? Data from patient studies is correlative and cannot conclude causality. The results presented herein demonstrate that RC dysfunction in DA neurons *can* cause parkinsonism. On the other hand, we find no support for the hypothesis that DA neurons are more sensitive than other cells to systemic RC deficiency. However, studies in patients suggest that DA neurons may be particularly vulnerable to age-related accumulation of mtDNA mutations, which would result in a focal RC deficiency.

In this thesis, I have investigated the consequences of RC deficiency in DA neurons and identified several mechanisms that may contribute to PD pathology. Already a mild complex I deficiency alters DA release at the synapse, and the axon terminals may also be more vulnerable to severe RC dysfunction because of impaired axonal transport. We also identified *trans*-neuronal degeneration as a mechanism that may add stress to cells surrounding degenerating neurons. However, we found no support for a role of Parkin in our model. As part of this work I also developed a reporter mouse for cell type-specific labeling of mitochondria, which can be a valuable tool for future studies of mitochondria in the brain and other tissues.

Even if mitochondrial dysfunction is heavily associated with sporadic PD, it may not be a driving factor for disease onset or progression. It is clear that increased levels of α-synuclein can cause PD pathology, but the link between α-synuclein accumulation and RC dysfunction is unclear. As shown herein, even mild RC dysfunction can impair DA release at the synapse, and given the role of α-synuclein in synaptic vesicle recycling, such stress could possibly alter α-synuclein levels and with time promote aggregation. Future studies will be needed to clarify potential relationships between α-synuclein pathology, mitochondrial quality and age-related changes.

ACKNOWLEDGEMENTS

This thesis summarizes work done in both the Larsson and Olson labs over a number of years during which I have learnt plenty about labwork, science and life. Warm THANKS to:

Nils-Göran Larsson for giving me the opportunity to join the lab. It has been exciting and incredibly instructive to be part of your group! Things usually take longer than I anticipate, and this was no exception, but you have been patient and allowed me to develop (just enough?) independence. I am thankful that you let some resources remain in Stockholm to continue some interesting work and help me finish my PhD.

Lars Olson for your inspiring never-ending curiosity and for always being helpful; your door has always been open and no matter how busy, you always seemed to have time for me.

Barry Hoffer, my mentor at NIDA, for fantastic hospitality and for showing me NIH and Annapolis.

Claes Gustafsson for supportive chats, laughs and for sharing some your wisdom.

Past and present members in the NGL group, in Stockholm, Cologne and elsewhere:
Mügen Terzioglu for Turkish delights and some early private journal clubs.
Eric Dufour for intricate discussions and for introducing me to some cloning techniques (despite I had been warned!).
Chris Kukat for lessons in microscopy.
Chan Bae Park for demonstrating how the work should be done!
Benedetta Ruzzenente, **Metodi Metodiev** and warmhearted **Yolanda Camara** for patiently answering questions in the lab (and Betty for being Betty!).
Jim Stewart for interesting and often geeky facts and discussions and for successful apartment swapping.
Chris Freyer –I miss your company in the writing room (but hope to see you soon)!
Camilla Koolmeister for management with a smile!
Zekiye Cansu for heartful attempts to babysit during meetings.
Erik Hagström for always being around for a discussion (about politics, fish and more)**.**
Seungmin Lee for keeping up good work in Stockholm.
Anna Wredenberg, Mats Ekstrand, Anna Hansson, Nicole Hance, Emmanouella Chatzidaki, **Ana Bratic, Marie Lagouge, Arnaud Mourier**, **Dusanka Milenkovic** and **Arianna Paganelli** for being helpful and for contributing to a nice environment.

People working in the groups of **Alexandra Triunovich**, **Mia Falkenberg**, **Claes Gustavsson** and **Anna Karlsson** at our once-great research unit in NOVUM, in particular **Daniel Edgar** for friendship, **Thomas Linder** for pubs and more, and **Mia Bjerke** (espresso mousse!).

Lene Sörensen, **Venus Azhary**, **Stina Nordman** for help with arranging trips, paperwork etc. For this I must also thank **Corry Schwierzy** at MPI.

People at the Department of Neuroscience, especially past and present members of the extended LO group: **Andrea Carmine Belin, Anna Anvret, Anna Josephson, Anna Mattsson, Caroline Ran, Matt Abrams, Mimi Westerlund, Jacob Kjell, Jaime Ross, Linus Olson, Lisette Graae, Sandra Gellhaar, Simone Codeluppi, Sophia Savage**, **Tobias Karlsson** and **Dagmar Galter** (for MitoPark and neuroanatomy expertise!). For fantastic technical assistance: **Eva Lindqvist** for patiently struggling with the HPLC system, **Karin Lundströmer** for flawless sectioning and **Karin Pernold** who finally deserves a special thanks for helping me with all kinds of things, especially during periods when I was away from the lab; you even got to adopt one of my projects! I hope you will enjoy your new position, but I already worry about how to manage without you!

Staff at the animal departments, in particular Niklas Lilja and Mikael Zmarzlak for awesome help with animal transfers.

Collaborators: Rolf Wibom at CMMS, Staffan Cullheim and Anita Bergstrand at the Department of Neuroscience and Carl Lupica, Alex Hoffman and Brandon Harvey at NIDA.

Friends and family:

A collective thanks to all friends from times in Göteborg, Uppsala and Stockholm – you know who you are! A special thanks to Erik for almost being family and always just a phone call away.

My supportive parents Tusse and Gunnar, my brother Patrik and sister Lisa, as well as all fantastic in-laws!

My wonderful **KARIN** for love, support and a great deal of patience! In the journey that is our lives, you are the perfect travel partner; I love you!

Hedda and **Henning** for being wonderful and ensuring that life never becomes boring (and for failing sincere attempts to prevent this from ever being finished)!

REFERENCES

Acín-Pérez R, Fernández-Silva P, Peleato ML, Pérez-Martos A, Enriquez JA (2008) Respiratory active mitochondrial supercomplexes. Mol Cell 32:529–539.

Agnati LF, Cortelli P, Pettersson R, Fuxe K (1995) The concept of trophic units in the central nervous system. Prog Neurobiol 46:561–574.

Ahlskog JE (2009) Parkin and PINK1 parkinsonism may represent nigral mitochondrial cytopathies distinct from Lewy body Parkinson's disease. Parkinsonism Relat Disord 15:721–727.

Althoff T, Mills DJ, Popot J-L, hlbrandt WKU (2011) Arrangement of electron transport chain components in bovine mitochondrial supercomplex I1III2IV1. EMBO J 30:4652–4664.

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465.

Andersson GE, Karlberg O, Canback B, Kurland CG (2003) On the origin of mitochondria: a genomics perspective. Philosophical Transactions of the Royal Society B: Biological Sciences 358:165–179.

Balaban RS, Nemoto S, Finkel T (2005) Mitochondria, oxidants, and aging. Cell 120:483–495.

Bartels T, Choi JG, Selkoe DJ (2011) α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature 477:107–110.

Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betts J, Klopstock T, Taylor RW, Turnbull DM (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet 38:515–517.

Bender A, Schwarzkopf R-M, McMillan A, Krishnan KJ, Rieder G, Neumann M, Elstner M, Turnbull DM, Klopstock T (2008) Dopaminergic midbrain neurons are the prime target for mitochondrial DNA deletions. J Neurol 255:1231–1235.

Bindoff LA, Birch-Machin M, Cartlidge NE, Parker WD, Turnbull DM (1989) Mitochondrial function in Parkinson's disease. Lancet 2:49.

Bogenhagen D, Clayton DA (1977) Mouse L cell mitochondrial DNA molecules are selected randomly for replication throughout the cell cycle. Cell 11:719–727.

Bogenhagen DF (2011) Mitochondrial DNA nucleoid structure. Biochim Biophys Acta.

Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MCJ, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC,

Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299:256–259.

Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K (2004) Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 318:121–134.

Brandt U (2011) A two-state stabilization-change mechanism for proton-pumping complex I. Biochim Biophys Acta 1807:1364–1369.

Buchet K (1998) Functional F1-ATPase Essential in Maintaining Growth and Membrane Potential of Human Mitochondrial DNA-depleted rho Cells. Journal of Biological Chemistry 273:22983–22989.

Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci USA 80:4546–4550.

Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC (2010) alpha-Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro. Science 329:1663–1667.

Carling PJ, Cree LM, Chinnery PF (2011) The implications of mitochondrial DNA copy number regulation during embryogenesis. Mitochondrion 11:686–692.

Carlsson A (1959) The occurrence, distribution and physiological role of catecholamines in the nervous system. Pharmacol Rev 11:490–493.

Carlsson A, Lindqvist M, Magnusson T, Waldeck B (1958) On the presence of 3-hydroxytyramine in brain. Science 127:471.

Caudle WM, Richardson JR, Wang MZ, Taylor TN, Guillot TS, McCormack AL, Colebrooke RE, Di Monte DA, Emson PC, Miller GW (2007) Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. J Neurosci 27:8138–8148.

Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RLJ, Hess S, Chan DC (2011) Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. Hum Mol Genet 20:1726–1737.

Chartier-Harlin M-C, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, Levecque C, Larvor L, Andrieux J, Hulihan M, Waucquier N, Defebvre L, Amouyel P, Farrer M, Destée A (2004) Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 364:1167–1169.

Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J Cell Biol 160:189–200.

Chen H, McCaffery JM, Chan DC (2007) Mitochondrial fusion protects against neurodegeneration in the cerebellum. Cell 130:548–562.

Cheng H-C, Ulane CM, Burke RE (2010) Clinical progression in Parkinson disease and the neurobiology of axons. Ann Neurol 67:715–725.

Chinnery PF, Turnbull DM (2001) Epidemiology and treatment of mitochondrial disorders. Am J Med Genet 106:94–101.

Chomyn A, Martinuzzi A, Yoneda M, Daga A, Hurko O, Johns D, Lai ST, Nonaka I, Angelini C, Attardi G (1992) MELAS mutation in mtDNA binding site for transcription termination factor causes defects in protein synthesis and in respiration but no change in levels of upstream and downstream mature transcripts. Proc Natl Acad Sci USA 89:4221–4225.

Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA, Guo M (2006) Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature 441:1162–1166.

Clayton DA (1982) Replication of animal mitochondrial DNA. Cell 28:693–705.

Coelln Von R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, Dawson VL, Dawson TM (2004) Loss of locus coeruleus neurons and reduced startle in parkin null mice. Proc Natl Acad Sci USA 101:10744–10749.

Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC (1992) Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. Nat Genet 2:324–329.

Cortopassi GA, Shibata D, Soong NW, Arnheim N (1992) A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues. Proc Natl Acad Sci USA 89:7370–7374.

Cottrell DA, Blakely EL, Johnson MA, Ince PG, Borthwick GM, Turnbull DM (2001) Cytochrome c oxidase deficient cells accumulate in the hippocampus and choroid plexus with age. Neurobiol Aging 22:265–272.

Davies KM, Strauss M, Daum B, Kief JH, Osiewacz HD, Rycovska A, Zickermann V, Kühlbrandt W (2011) Macromolecular organization of ATP synthase and complex I in whole mitochondria. Proceedings of the National Academy of Sciences 108:14121–14126.

Davis AF, Clayton DA (1996) In situ localization of mitochondrial DNA replication in intact mammalian cells. J Cell Biol 135:883–893.

de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456:605–610.

de Lau LML, Breteler MMB (2006) Epidemiology of Parkinson's disease. Lancet neurology 5:525–535.

Debray F-G, Lambert M, Mitchell GA (2008) Disorders of mitochondrial function. Curr Opin Pediatr 20:471–482.

Delettre C, Lenaers G, Pelloquin L, Belenguer P, Hamel CP (2002) OPA1 (Kjer Type) Dominant Optic Atrophy: A Novel Mitochondrial Disease. Molecular Genetics and Metabolism 75:97–107.

Deng H, Dodson MW, Huang H, Guo M (2008) The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila. Proc Natl Acad Sci USA 105:14503–14508.

Detmer SA, Chan DC (2007) Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol 8:870–879.

Duvezin-Caubet S, Jagasia R, Wagener J, Hofmann S, Trifunovic A, Hansson A, Chomyn A, Bauer MF, Attardi G, Larsson N-G, Neupert W, Reichert AS (2006) Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology. J Biol Chem 281:37972–37979.

Efremov RG, Sazanov LA (2011) Structure of the membrane domain of respiratory complex I. Nature 476:414–420.

Ehringer H, Hornykiewicz O (1960) [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. Klin Wochenschr 38:1236–1239.

Ekstrand MI, Falkenberg M, Rantanen A, Park CB, Gaspari M, Hultenby K, Rustin P, Gustafsson CM, Larsson N-G (2004) Mitochondrial transcription factor A regulates mtDNA copy number in mammals. Hum Mol Genet 13:935–944.

Falkenberg M, Gaspari M, Rantanen A, Trifunovic A, Larsson N-G, Gustafsson CM (2002) Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. Nat Genet 31:289–294.

Falkenberg M, Larsson N-G, Gustafsson CM (2007) DNA replication and transcription in mammalian mitochondria. Annu Rev Biochem 76:679–699.

Fayet G, Jansson M, Sternberg D, Moslemi AR, Blondy P, Lombès A, Fardeau M, Oldfors A (2002) Ageing muscle: clonal expansions of mitochondrial DNA point mutations and deletions cause focal impairment of mitochondrial function. Neuromuscul Disord 12:484–493.

Finkel T (2011) Signal transduction by reactive oxygen species. J Cell Biol 194:7–15.

Fisher RP, Parisi MA, Clayton DA (1989) Flexible recognition of rapidly evolving promoter sequences by mitochondrial transcription factor 1. Genes Dev 3:2202–2217.

Fransson S, Ruusala A, Aspenström P (2006) The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. Biochem Biophys Res Commun 344:500–510.

Fusté JM, Wanrooij S, Jemt E, Granycome CE, Cluett TJ, Shi Y, Atanassova N, Holt IJ, Gustafsson CM, Falkenberg M (2010) Mitochondrial RNA polymerase is needed for activation of the origin of light-strand DNA replication. Mol Cell 37:67–78.

Gautier CA, Kitada T, Shen J (2008) Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. Proc Natl

Acad Sci USA 105:11364–11369.

Gegg ME, Cooper JM, Chau K-Y, Rojo M, Schapira AHV, Taanman J-W (2010) Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. Hum Mol Genet 19:4861–4870.

Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 12:119–131.

Gispert S et al. (2009) Parkinson phenotype in aged PINK1-deficient mice is accompanied by progressive mitochondrial dysfunction in absence of neurodegeneration. PLoS ONE 4:e5777.

Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, Meloni EG, Wu N, Ackerson LC, Klapstein GJ, Gajendiran M, Roth BL, Chesselet M-F, Maidment NT, Levine MS, Shen J (2003) Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J Biol Chem 278:43628–43635.

Good CH, Hoffman AF, Hoffer BJ, Chefer VI, Shippenberg TS, Bäckman CM, Larsson N-G, Olson L, Gellhaar S, Galter D, Lupica CR (2011) Impaired nigrostriatal function precedes behavioral deficits in a genetic mitochondrial model of Parkinson's disease. FASEB J 25:1333–1344.

Goto Y, Nonaka I, Horai S (1990) A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 348:651–653.

Gray MW (1999) Mitochondrial Evolution. Science 283:1476–1481.

Gross NJ, Getz GS, Rabinowitz M (1969) Apparent turnover of mitochondrial deoxyribonucleic acid and mitochondrial phospholipids in the tissues of the rat. J Biol Chem 244:1552–1562.

Guo X, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfield M, Marin L, Charlton MP, Atwood HL, Zinsmaier KE (2005) The GTPase dMiro is required for axonal transport of mitochondria to Drosophila synapses. Neuron 47:379–393.

Gyllensten U, Wharton D, Josefsson A, Wilson AC (1991) Paternal inheritance of mitochondrial DNA in mice. Nature 352:255–257.

Hardy J (2005) Expression of normal sequence pathogenic proteins for neurodegenerative disease contributes to disease risk: “permissive templating” as a general mechanism underlying neurodegeneration. Biochem Soc Trans 33:578–581.

Hardy J (2010) Genetic analysis of pathways to Parkinson disease. Neuron 68:201–206.

Hayashi J, Ohta S, Kikuchi A, Takemitsu M, Goto Y, Nonaka I (1991) Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking

mitochondrial DNA results in mitochondrial dysfunction. Proc Natl Acad Sci USA 88:10614–10618.

Heikkila RE, Nicklas WJ, Vyas I, Duvoisin RC (1985) Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: implication for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. Neurosci Lett 62:389–394.

Hollenbeck PJ, Saxton WM (2005) The axonal transport of mitochondria. J Cell Sci 118:5411–5419.

Holt IJ, Harding AE, Morgan-Hughes JA (1988) Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature 331:717–719.

Hunte C, Zickermann V, Brandt U (2010) Functional modules and structural basis of conformational coupling in mitochondrial complex I. Science 329:448–451.

Ingerman E, Perkins EM, Marino M, Mears JA, McCaffery JM, Hinshaw JE, Nunnari J (2005) Dnm1 forms spirals that are structurally tailored to fit mitochondria. J Cell Biol 170:1021–1027.

Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, Otera H, Nakanishi Y, Nonaka I, Goto Y-I, Taguchi N, Morinaga H, Maeda M, Takayanagi R, Yokota S, Mihara K (2009) Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. Nat Cell Biol 11:958–966.

Itier J-M et al. (2003) Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. Hum Mol Genet 12:2277–2291.

Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ (2010) Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J Cell Biol 191:933–942.

Kaneda H, Hayashi J, Takahama S, Taya C, Lindahl KF, Yonekawa H (1995) Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. Proc Natl Acad Sci USA 92:4542–4546.

Kaufman BA, Durisic N, Mativetsky JM, Costantino S, Hancock MA, Grutter P, Shoubridge EA (2007) The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. Mol Biol Cell 18:3225–3236.

Kaukonen J, Juselius JK, Tiranti V, Kyttälä A, Zeviani M, Comi GP, Keränen S, Peltonen L, Suomalainen A (2000) Role of adenine nucleotide translocator 1 in mtDNA maintenance. Science 289:782–785.

Khrapko K, Coller HA, André PC, Li XC, Hanekamp JS, Thilly WG (1997) Mitochondrial mutational spectra in human cells and tissues. Proc Natl Acad Sci USA 94:13798–13803.

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause

autosomal recessive juvenile parkinsonism. Nature 392:605–608.

Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, Bonsi P, Zhang C, Pothos EN, Shen J (2007) Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proc Natl Acad Sci USA 104:11441–11446.

Kitada T, Tong Y, Gautier CA, Shen J (2009) Absence of nigral degeneration in aged parkin/DJ-1/PINK1 triple knockout mice. J Neurochem 111:696–702.

Kopin IJ, Markey SP (1988) MPTP toxicity: implications for research in Parkinson's disease. Annu Rev Neurosci 11:81–96.

Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med 14:504–506.

Korhonen JA, Pham XH, Pellegrini M, Falkenberg M (2004) Reconstitution of a minimal mtDNA replisome in vitro. EMBO J 23:2423–2429.

Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC (2004) Structural basis of mitochondrial tethering by mitofusin complexes. Science 305:858–862.

Kraytsberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW, Khrapko K (2006) Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. Nat Genet 38:518–520.

Kruse SE, Watt WC, Marcinek DJ, Kapur RP, Schenkman KA, Palmiter RD (2008) Mice with mitochondrial complex I deficiency develop a fatal encephalomyopathy. Cell Metab 7:312–320.

Kukat C, Wurm CA, Spåhr H, Falkenberg M, Larsson N-G, Jakobs S (2011) Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. Proceedings of the National Academy of Sciences 108:13534–13539.

Kuznetsov AV, Hermann M, Saks V, Hengster P, Margreiter R (2009) The cell-type specificity of mitochondrial dynamics. Int J Biochem Cell Biol 41:1928–1939.

Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219:979–980.

Larsson N-G (2010) Somatic mitochondrial DNA mutations in mammalian aging. Annu Rev Biochem 79:683–706.

Larsson NG, Holme E, Kristiansson B, Oldfors A, Tulinius M (1990) Progressive increase of the mutated mitochondrial DNA fraction in Kearns-Sayre syndrome. Pediatr Res 28:131–136.

Larsson NG, Oldfors A, Holme E, Clayton DA (1994) Low levels of mitochondrial transcription factor A in mitochondrial DNA depletion. Biochem Biophys Res Commun 200:1374–1381.

Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS, Clayton DA (1998) Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat Genet 18:231–236.

Lee Y-J, Jeong S-Y, Karbowski M, Smith CL, Youle RJ (2004) Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol Biol Cell 15:5001–5011.

Lewy F (1912) Paralysis agitans. In: Pathologische Anatomie. Handbuchder Neurologie. (Lewandowsky M, ed), pp 920–933. Berlin: Springer Verlag.

Li J-Y, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehncrona S, Björklund A, Widner H, Revesz T, Lindvall O, Brundin P (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med 14:501–503.

Lill R, Mühlenhoff U (2008) Maturation of Iron-Sulfur Proteins in Eukaryotes: Mechanisms, Connected Processes, and Diseases. Annu Rev Biochem 77:669–700.

Litonin D, Sologub M, Shi Y, Savkina M, Anikin M, Falkenberg M, Gustafsson CM, Temiakov D (2010) Human mitochondrial transcription revisited: only TFAM and TFB2M are required for transcription of the mitochondrial genes in vitro. Journal of Biological Chemistry 285:18129–18133.

Luft R, Ikkos D, Palmieri G, Ernster L, Afzelius B (1962) A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study. J Clin Invest 41:1776–1804.

Luoma P, Melberg A, Rinne JO, Kaukonen JA, Nupponen NN, Chalmers RM, Oldfors A, Rautakorpi I, Peltonen L, Majamaa K, Somer H, Suomalainen A (2004) Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. Lancet 364:875–882.

Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, Tanaka K (2010) PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol 189:211–221.

McFarland R, Turnbull DM (2009) Batteries not included: diagnosis and management of mitochondrial disease. J Intern Med 265:210–228.

Menzies RA, Gold PH (1971) The turnover of mitochondria in a variety of tissues of young adult and aged rats. J Biol Chem 246:2425–2429.

Misgeld T, Kerschensteiner M, Bareyre FM, Burgess RW, Lichtman JW (2007) Imaging axonal transport of mitochondria in vivo. Nat Methods 4:559–561.

Misko A, Jiang S, Wegorzewska I, Milbrandt J, Baloh RH (2010) Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the

Miro/Milton complex. J Neurosci 30:4232–4240.

Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:144–148.

Mizuno Y, Suzuki K, Sone N, Saitoh T (1987) Inhibition of ATP synthesis by 1-methyl-4-phenylpyridinium ion (MPP+) in isolated mitochondria from mouse brains. Neurosci Lett 81:204–208.

Morais VA, Verstreken P, Roethig A, Smet J, Snellinx A, Vanbrabant M, Haddad D, Frezza C, Mandemakers W, Vogt-Weisenhorn D, Van Coster R, Wurst W, Scorrano L, De Strooper B (2009) Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function. EMBO Mol Med 1:99–111.

Murphy MP, Holmgren A, Larsson N-G, Halliwell B, Chang CJ, Kalyanaraman B, Rhee SG, Thornalley PJ, Partridge L, Gems D, Nyström T, Belousov V, Schumacker PT, Winterbourn CC (2011) Unraveling the Biological Roles of Reactive Oxygen Species. Cell Metab 13:361–366.

Müller-Höcker J (1989) Cytochrome-c-oxidase deficient cardiomyocytes in the human heart--an age-related phenomenon. A histochemical ultracytochemical study. Am J Pathol 134:1167–1173.

Müller-Höcker J (1990) Cytochrome c oxidase deficient fibres in the limb muscle and diaphragm of man without muscular disease: an age-related alteration. Journal of the Neurological Sciences 100:14–21.

Naess K, Freyer C, Bruhn H, Wibom R, Malm G, Nennesmo I, Döbeln von U, Larsson N-G (2009) MtDNA mutations are a common cause of severe disease phenotypes in children with Leigh syndrome. BBA - Bioenergetics 1787:484–490.

Narendra D, Tanaka A, Suen D-F, Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183:795–803.

Narendra D, Tanaka A, Suen D-F, Youle RJ (2009) Parkin-induced mitophagy in the pathogenesis of Parkinson disease. Autophagy 5:706–708.

Narendra DP, Jin SM, Tanaka A, Suen D-F, Gautier CA, Shen J, Cookson MR, Youle RJ (2010) PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol 8:e1000298.

Neupert W, Herrmann JM (2007) Translocation of proteins into mitochondria. Annu Rev Biochem 76:723–749.

Ngo HB, Kaiser JT, Chan DC (2011) The mitochondrial transcription and packaging factor Tfam imposes a U-turn on mitochondrial DNA. Nat Struct Mol Biol 18:1290–1296.

Nicklas WJ, Vyas I, Heikkila RE (1985) Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the

neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. Life Sci 36:2503–2508.

Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290:470–474.

Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, Youle RJ, Mihara K (2010) Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. J Cell Biol 191:1141–1158.

Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong S-E, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA, Mootha VK (2008) A mitochondrial protein compendium elucidates complex I disease biology. Cell 134:112–123.

Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim J-M, Chung J (2006) Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature 441:1157–1161.

Parker WD, Boyson SJ, Parks JK (1989) Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann Neurol 26:719–723.

Parkinson J (1817) An essay on the shaking palsy. London: Sherwood, Neely and Jones.

Perez FA, Palmiter RD (2005) Parkin-deficient mice are not a robust model of parkinsonism. Proc Natl Acad Sci USA 102:2174–2179.

Pilling AD, Horiuchi D, Lively CM, Saxton WM (2006) Kinesin-1 and Dynein are the primary motors for fast transport of mitochondria in Drosophila motor axons. Mol Biol Cell 17:2057–2068.

Pilsl A, Winklhofer KF (2011) Parkin, PINK1 and mitochondrial integrity: emerging concepts of mitochondrial dysfunction in Parkinson’s disease. Acta Neuropathol 123:173–188.

Polymeropoulos MH et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047.

Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ (2008) The PINK1/Parkin pathway regulates mitochondrial morphology. Proceedings of the National Academy of Sciences 105:1638–1643.

Quintana A, Kruse SE, Kapur RP, Sanz E, Palmiter RD (2010) Complex I deficiency due to loss of Ndufs4 in the brain results in progressive encephalopathy resembling Leigh syndrome. Proceedings of the National Academy of Sciences 107:10996–11001.

Rakovic A, Grünewald A, Kottwitz J, Brüggemann N, Pramstaller PP, Lohmann K, Klein C (2011) Mutations in PINK1 and Parkin impair ubiquitination of Mitofusins in human fibroblasts. PLoS ONE 6:e16746.

Ramsay RR, Salach JI, Singer TP (1986) Uptake of the neurotoxin 1-methyl-4-phenylpyridine (MPP+) by mitochondria and its relation to the inhibition of

the mitochondrial oxidation of NAD+-linked substrates by MPP+. Biochem Biophys Res Commun 134:743–748.

Rawi Al S, Louvet-Vallée S, Djeddi A, Sachse M, Culetto E, Hajjar C, Boyd L, Legouis R, Galy V (2011) Postfertilization autophagy of sperm organelles prevents paternal mitochondrial DNA transmission. Science 334:1144–1147.

Rossignol R, Faustin B, Rocher C, Malgat M, Mazat J-P, Letellier T (2003) Mitochondrial threshold effects. Biochem J 370:751–762.

Rubinstein JL, Walker JE, Henderson R (2003) Structure of the mitochondrial ATP synthase by electron cryomicroscopy. EMBO J 22:6182–6192.

Rubio-Cosials A, Sidow JF, Jiménez-Menéndez N, Fernández-Millán P, Montoya J, Jacobs HT, Coll M, Bernadó P, Solà M (2011) Human mitochondrial transcription factor A induces a U-turn structure in the light strand promoter. Nat Struct Mol Biol 18:1281–1289.

Santorelli FM, Shanske S, Macaya A, DeVivo DC, DiMauro S (1993) The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh's syndrome. Ann Neurol 34:827–834.

Saraste M (1999) Oxidative Phosphorylation at the fin de siècle. Science 283:1488–1493.

Sazanov LA, Hinchliffe P (2006) Structure of the hydrophilic domain of respiratory complex I from Thermus thermophilus. Science 311:1430–1436.

Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD (1989) Mitochondrial complex I deficiency in Parkinson's disease. Lancet 1:1269.

Schapira AHV (2006) Mitochondrial disease. Lancet 368:70–82.

Schwartz M, Vissing J (2002) Paternal inheritance of mitochondrial DNA. N Engl J Med 347:576–580.

Sheng Z-H, Cai Q (2012) Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. Nat Rev Neurosci.

Shin J-H, Ko HS, Kang H, Lee Y, Lee Y-I, Pletinkova O, Troconso JC, Dawson VL, Dawson TM (2011) PARIS (ZNF746) Repression of PGC-1α Contributes to Neurodegeneration in Parkinson's Disease. Cell 144:689–702.

Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC (1990) Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. Cell 61:931–937.

Shoubridge EA, Wai T (2007) Mitochondrial DNA and the mammalian oocyte. Curr Top Dev Biol 77:87–111.

Singleton AB et al. (2003) alpha-Synuclein locus triplication causes Parkinson's disease. Science 302:841.

Smeyne RJ, Jackson-Lewis V (2005) The MPTP model of Parkinson's disease. Brain Res Mol Brain Res 134:57–66.

Sologub M, Litonin D, Anikin M, Mustaev A, Temiakov D (2009) TFB2 is a

transient component of the catalytic site of the human mitochondrial RNA polymerase. Cell 139:934–944.

Song Z, Chen H, Fiket M, Alexander C, Chan DC (2007) OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J Cell Biol 178:749–755.

Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC (2009) Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. Mol Biol Cell 20:3525–3532.

Soong NW, Hinton DR, Cortopassi G, Arnheim N (1992) Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. Nat Genet 2:318–323.

Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet 21:70–71.

Sörensen L, Ekstrand M, Silva JP, Lindqvist E, Xu B, Rustin P, Olson L, Larsson NG (2001) Late-onset corticohippocampal neurodepletion attributable to catastrophic failure of oxidative phosphorylation in MILON mice. Journal of Neuroscience 21:8082–8090.

Spelbrink JN et al. (2001) Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. Nat Genet 28:223–231.

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci USA 95:6469–6473.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388:839–840.

Sterky FH, Larsson N-G (2008) Complex I: a complex gateway to the powerhouse. Cell Metab 7:278–279.

Stowers RS, Megeath LJ, Górska-Andrzejak J, Meinertzhagen IA, Schwarz TL (2002) Axonal transport of mitochondria to synapses depends on milton, a novel Drosophila protein. Neuron 36:1063–1077.

Tanaka A, Cleland MM, Xu S, Narendra DP, Suen D-F, Karbowski M, Youle RJ (2010) Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J Cell Biol 191:1367–1380.

Tatsuta T, Langer T (2008) Quality control of mitochondria: protection against neurodegeneration and ageing. EMBO J 27:306–314.

Taylor RW, McDonnell MT, Blakely EL, Chinnery PF, Taylor GA, Howell N, Zeviani M, Briem E, Carrara F, Turnbull DM (2003) Genotypes from patients indicate no paternal mitochondrial DNA contribution. Ann Neurol 54:521–524.

Tretiakoff C (1919) Contribution a l“Etude de L”Anatomie pathologique du Locus Niger de Soemmering avec quelques déductions relatives à la pathogénie des

troubles du tonus musculaire et De La Maladie de Parkinson. Paris.

Trifunovic A, Larsson NG (2008) Mitochondrial dysfunction as a cause of ageing. J Intern Med 263:167–178.

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly-Y M, Gidlöf S, Oldfors A, Wibom R, Törnell J, Jacobs HT, Larsson N-G (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429:417–423.

Trounce I, Byrne E, Marzuki S (1989) Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. The Lancet 1:637–639.

Valente EM et al. (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304:1158–1160.

Van Goethem G, Dermaut B, Löfgren A, Martin JJ, Van Broeckhoven C (2001) Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. Nat Genet 28:211–212.

Vives-Bauza C, Zhou C, Huang Y, Cui M, De Vries RL, Kim J, May J, Tocilescu MA, Liu W, Ko HS, Magrane J, Moore DJ, Dawson VL, Grailhe R, Dawson TM, Li C, Tieu K, Przedborski S (2010) PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proceedings of the National Academy of Sciences 107:378–383.

Volpicelli-Daley LA, Luk KC, Patel TP, Tanik SA, Riddle DM, Stieber A, Meaney DF, Trojanowski JQ, Lee VM-Y (2011) Exogenous alpha-Synuclein Fibrils Induce Lewy Body Pathology Leading to Synaptic Dysfunction and Neuron Death. Neuron 72:57–71.

Vonck J, Schäfer E (2009) Supramolecular organization of protein complexes in the mitochondrial inner membrane. BBA - Molecular Cell Research 1793:117–124.

Wakabayashi J, Zhang Z, Wakabayashi N, Tamura Y, Fukaya M, Kensler TW, Iijima M, Sesaki H (2009) The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. J Cell Biol 186:805–816.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ, Nikoskelainen EK (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427–1430.

Wang J, Silva JP, Gustafsson CM, Rustin P, Larsson NG (2001) Increased in vivo apoptosis in cells lacking mitochondrial DNA gene expression. Proc Natl Acad Sci USA 98:4038–4043.

Wang X, Schwarz TL (2009) The Mechanism of Ca2+-Dependent Regulation of Kinesin-Mediated Mitochondrial Motility. Cell 136:163–174.

Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL (2011) PINK1 and Parkin Target Miro for Phosphorylation and Degradation to Arrest Mitochondrial Motility. Cell 147:893–906.

Wang Y, Bogenhagen DF (2006) Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. J Biol Chem 281:25791–25802.

Wanrooij PH, Uhler JP, Simonsson T, Falkenberg M, Gustafsson CM (2010) G-quadruplex structures in RNA stimulate mitochondrial transcription termination and primer formation. Proceedings of the National Academy of Sciences 107:16072–16077.

Waterham HR, Koster J, van Roermund CWT, Mooyer PAW, Wanders RJA, Leonard JV (2007) A lethal defect of mitochondrial and peroxisomal fission. N Engl J Med 356:1736–1741.

Weihofen A, Thomas KJ, Ostaszewski BL, Cookson MR, Selkoe DJ (2009) Pink1 forms a multiprotein complex with Miro and Milton, linking Pink1 function to mitochondrial trafficking. Biochemistry 48:2045–2052.

Westermann B (2010) Mitochondrial fusion and fission in cell life and death. Nature Publishing Group 11:872–884.

Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 26 Suppl 1:S1–58.

Xu B, Zang K, Ruff NL, Zhang YA, McConnell SK, Stryker MP, Reichardt LF (2000) Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor TrkB. Neuron 26:233–245.

Yang MY, Bowmaker M, Reyes A, Vergani L, Angeli P, Gringeri E, Jacobs HT, Holt IJ (2002) Biased incorporation of ribonucleotides on the mitochondrial L-strand accounts for apparent strand-asymmetric DNA replication. Cell 111:495–505.

Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang J-W, Yang L, Beal MF, Vogel H, Lu B (2006) 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.

Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel H, Lu B (2008) Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. Proc Natl Acad Sci USA 105:7070–7075.

Yoshii SR, Kishi C, Ishihara N, Mizushima N (2011) Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. Journal of Biological Chemistry 286:19630–19640.

Zambrowicz BP, Imamoto A, Fiering S, Herzenberg LA, Kerr WG, Soriano P (1997) Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. Proc Natl Acad Sci USA 94:3789–3794.

Zheng B et al. (2010) PGC-1α, a potential therapeutic target for early

intervention in Parkinson's disease. Sci Transl Med 2:52ra73.

Zhou C, Huang Y, Shao Y, May J, Prou D, Perier C, Dauer W, Schon EA, Przedborski S (2008) The kinase domain of mitochondrial PINK1 faces the cytoplasm. Proc Natl Acad Sci USA 105:12022–12027.

Zick M, Rabl R, Reichert AS (2009) Cristae formation—linking ultrastructure and function of mitochondria. BBA - Molecular Cell Research 1793:5–19.

Ziviani E, Tao RN, Whitworth AJ (2010) Drosophila Parkin requires PINK1 for mitochondrial translocation and ubiquitinates Mitofusin. Proc Natl Acad Sci USA 107:5018–5023.