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Review

Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism

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ABSTRACT

The pathogenic mechanisms that underlie Parkinson's disease remain unknown. Here, we review evidence from both sporadic and genetic forms of Parkinson's disease that implicate both mitochondria and oxidative stress as central players in disease pathogenesis. A systemic deficiency in complex I of the mitochondrial electron transport chain is evident in many patients with the disease. Oxidative stress caused by reactive metabolites of dopamine and alterations in the levels of iron and glutathione in the substantia nigra accompany this mitochondrial dysfunction. Recent evidence from studies on the genetic forms of parkinsonism with particular stress on DJ-1, parkin, and PINK-1 also suggest the involvement of mitochondria and oxidative stress.

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Introduction

In 1817, Dr. James Parkinson of London published his *Essay on the Shaking Palsy* (Parkinson, 2002). Parkinson defined a disorder that was characterized by an involuntary resting tremor, a stooped posture, weakness of the limbs, and a festinating gait with little to no cognitive deficits. Today, we recognize this disorder as being characterized clinically by four cardinal symptoms; resting tremor, rigidity, bradykinesia (slowness of movement), and postural instability.

A profound depletion of the neurotransmitter dopamine (DA) in the striatum is the primary cause of these motor symptoms, collectively known as parkinsonism. Parkinson's disease (PD) is the main cause of parkinsonism and generally presents as parkinsonism along with varying extrastriatal effects such as gastrointestinal, olfactory, and sleep disorders. Because the symptoms of PD can vary widely

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amongst patients and many neurological insults can cause parkinsonism, a definitive diagnosis of PD can only be done upon post-mortem examination of the neural tissue.

Pathologically, dopamine depletion is a consequence of the loss of pigmented dopaminergic (DAergic) projection neurons in the substantia nigra pars compacta (SNpc). These neurons project onto medium spiny neurons in the striatum where they release DA and facilitate movement. Additionally, proteinaceous inclusions known as Lewy bodies and Lewy neurites can be found localized to the soma and processes of neurons, respectively, in many areas of the PD brain. Lewy bodies and Lewy neurites are composed of several proteins including a-synuclein, as well as lipids (Spillantini et al., 1997). The deposition of Lewy bodies and neurites has been demonstrated to occur years before the degeneration of the SNpc and the appearance of parkinsonism (Braak et al., 2003). Therefore, PD is a disease defined pathologically by the presence of Lewy bodies in the context of nigral cell loss and parkinsonism.

A recent epidemiological study estimated that there were 4.1 to 4.6 million people with PD worldwide in 2005 (Dorsey et al., 2007). This number was projected to double by the year 2030 as populations age, forecasting an impending burden on the healthcare systems of many countries. Current treatments for PD are relatively efficacious in the alleviation of the symptoms of parkinsonism in the early stages of the disease. However, symptomatic treatments become less effective as the disease worsens and there are no therapies currently available that prevent the onset or progression of the disease. Therefore, it is of great importance to understand the molecular basis of PD so that therapeutic advances can be made in the near future.

Mitochondrial dysfunction in PD

Complex I deficiency

A major breakthrough in our understanding of the pathogenic mechanisms underlying PD came from specific cases of induced parkinsonism in California during the 1980s. Several drug users accidently injected themselves with the synthetic heroine analog 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Within days, they developed parkinsonism and *post-mortem* analysis revealed significant lesions of DAergic neurons in the SNpc (Langston et al., 1983).

MPTP crosses the blood–brain barrier easily and is taken up by astrocytes where it is metabolized into 1-methyl-4-phenylpyridinium (MPP+) and released into the extracellular space. MPP+ is a substrate for the dopamine transporter and is taken up selectively into DAergic neurons where it inhibits complex I of the mitochondrial respiratory chain. Once inhibited, complex I produces excess superoxide that overwhelms the antioxidant capacity of the DAergic neurons and leads to their death. Importantly, MPP+ has been demonstrated to be toxic to DAergic neurons in both non-human primates and rodents (Heikkila et al., 1984; Langston et al., 1984).

Shortly following the discovery of parkinsonism caused by MPTP administration, it was reported that complex I activity is decreased in the SNpc of patients with sporadic PD but remains normal in other neuronal regions (Schapira et al., 1989, 1990). Complex I deficiencies have also been reported in the platelets and skeletal muscle of those with PD (Bindoff et al., 1991; Krige et al., 1992; Parker et al., 1989). The somewhat paradoxical findings that complex I deficiency is observed in the peripheral tissue yet confined to the SNpc in brain were later clarified, as it was demonstrated that mitochondria from the frontal cortex of PD patients had significantly decreased complex I activity if the mitochondria were sufficiently purified (Parker et al., 2008). It should be noted that not all groups have reported deficient complex I activity in PD tissue, with particularly conflicting evidence from skeletal muscle biopsies (Taylor et al., 1994). The failure of such studies to find differences between PD and controls may be due to the methodological issues brought up in the study of frontal cortex mitochondria (Parker et al., 2008). Regardless, it is clear that in many cases of PD there is a modest (~20–30%) decrease in complex I activity (For further review (Schapira, 2007)).

The finding that peripheral mitochondrial dysfunction can be associated with a disease primarily affecting the nervous tissue led to the hypothesis that neuronal death observed in PD may result from broader mitochondrial defects. This has been tested by the systemic administration of the complex I inhibitor rotenone to rats. Rotenone is a lipophilic molecule, which allows it to freely enter and inhibit complex I in both peripheral tissues and the central nervous system. It was found that the chronic administration of rotenone in rats caused selective degeneration of the SNpc and the accumulation of proteinaceous inclusions similar to Lewy Bodies within those neurons (Betarbet et al., 2000; Cannon et al., 2009). Further studies have demonstrated that the gastrointestinal pathology of PD is also recapitulated using this model (Drolet et al., 2009). Taken together, the rotenone model suggests that a mild systemic impairment of mitochondrial complex I is sufficient to cause many of the pathological and behavioral hallmarks of PD in mammals (Betarbet et al., 2000; Cannon et al., 2009; Drolet et al., 2009).

Molecular mechanisms underlying complex I deficiency in PD

Although it is accepted by many that there is a complex I defect in the mitochondria from PD patients, there is no conclusive explanation for the deficit. The possibility that exposure to an environmental agent causes the inhibition has been considered because several common pesticides, including rotenone, can directly inhibit complex I. Indeed, environmental exposure to rotenone and paraquat has been linked to PD (Tanner et al., 2011). However, MPTP exposure is the only confirmed pure environmental cause of PD. In this context, there are several other explanations proposed in the literature for a decrement in complex I in PD.

A recent meta-analysis that combined gene expression data from numerous PD studies found that the expression of ten sets of genes differs between PD and control DAergic SNpc cells isolated using laser capture microdissection (Zheng et al., 2010). Although the ten sets of genes were distinct, they each represented pathways dealing with bioenergetics (nuclear-encoded mitochondrial electron transport, mitochondrial biogenesis, glucose utilization, and glucose sensing). Interestingly, these sets of genes were demonstrated to be under the control of the master transcriptional regulator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). Although the authors did not report any differences in the expression of PGC-1α between PD and controls, they did find that overexpression of PGC-1α protected mouse neurons from rotenone toxicity in culture (Zheng et al., 2010). This could indicate that the complex I deficits in PD are the result of a widespread deregulation of cellular bioenergetics.

Another intriguing possibility that could underlie the mitochondrial defects seen in PD is the accumulation of point mutations and deletions in mitochondrial DNA (mtDNA). In eukaryotic cells, mtDNA is organized into protein/nucleic acid structures known as nucleoids. Each nucleoid contains on average 1.4 copies of mtDNA and cells can contain upwards to 2000 nucleoids (Kukat et al., 2011). The mtDNA is circular and codes for 13 proteins along with the mitochondrial tRNA and ribosomal RNA. The proteins coded by mtDNA include subunits from all parts of the electron transport chain, with 6 of the genes coding for complex I subunits. Hence, point mutations or deletions in any of these 6 genes could feasibly alter complex I activity.

The organization of mtDNA into discrete nucleoids can lead to a situation in which individual cells can contain multiple mtDNA sequence variants, termed heteroplasmy. Recent measurements found heteroplasmic mtDNA in approximately 25% of the humans tested (Li et al., 2010). The proportion of heteroplasmic mtDNA correlates with age, with some mutations increasing over time and others decreasing (Sondheimer et al., 2011). Currently, it is known that only a proportion of heteroplasmy

is inherited, that somatic *de novo* mtDNA mutations occur often, and there are active mechanisms controlling the abundance of mtDNA variants in an individual (Sondheimer et al., 2011; Suen et al., 2010; Wai et al., 2008).

Multiple lines of evidence support the hypothesis that the complex I defect observed in PD is the result of point mutations or deletions of mtDNA. First, when PD patient mitochondria are transferred into cells that contain no mtDNA of their own (PD cybrid cells), the complex I deficiency is observed in the resulting cells (Swerdlow et al., 1996). Numerous studies have sought to identify individual mtDNA point mutations associated with PD, but studies using large sample sizes have not demonstrated differences between the mutational load of PD patients and controls (Bandmann et al., 1997). One problem when trying to identify alterations in mtDNA is that they could be clonal and occur at a low frequency in the tissue analyzed. This is problematic because only a small percentage of cells within a given tissue could harbor mutated mtDNA, making it hard to detect these mutant mtDNA molecules using techniques in which the whole tissue is homogenized. This difficulty can be overcome using a mixture of histochemical and molecular genetic techniques. Two studies analyzed SNpc neurons from human brains by staining for the nuclear encoded mitochondrial protein succinate dehydrogenase (SDH) and the mtDNA encoded protein cytochrome c oxidase (COX). This method readily differentiates individual cells with mtDNA defects as they lack COX staining yet retain SDH staining, causing them to stain bright blue. The studies found that COX deficient neurons are abundant in the SNpc and that their numbers increase with age, with PD brains containing more than controls (Bender et al., 2006; Kraytsberg et al., 2006). In both studies, mtDNA from individual COX deficient neurons was sequenced. No known pathogenic mutations were found, but COX deficient neurons had high levels of mtDNA deletions that were of clonal origin. Deletions were prevalent in the SNpc tissue from both control and PD brains, but there was no significant difference in the number of deletions between the two.

mtDNA is synthesized by mitochondrial polymerase gamma (Polg), a nuclear encoded protein with both polymerase and exonuclease activities. Several pathogenic mutations in Polg exist in humans, and manifest themselves as a variety of neurological presentations (Milone and Massie, 2010). Frequently, Polg mutations present as Alper's disease, progressive external ophthalmoplegia (PEO), or ataxia–neuropathy syndrome. Parkinsonism is also seen in some families with Polg mutations. One study reported that L-dopa responsive parkinsonism occurred several years after the onset of PEO in some families (Luoma et al., 2004). Two patients had decreased striatal [^{18}F]β-CFT uptake while two others had significant cell death in the SNpc, albeit without Lewy bodies. Other cases of Polg-associated parkinsonism have been reported including at least two cases without PEO (Davidzon et al., 2006; Synofzik et al., 2010). The Polg gene includes a region that encodes a polyglutamine (polyQ) tract normally 10Q long but that can range between 6 and 14Q. One study found no association between polyQ length and sporadic PD, however two larger studies later identified a significant association between rare polyQ lengths and sporadic PD (Anvret et al., 2010; Eerola et al., 2010; Taanman and Schapira, 2005).

Two groups have separately generated mice that harbor a proofreading deficient Polg knocked in to the endogenous Polg locus ($Polg^{D257A}$, termed Polg mutator mice) (Kujoth et al., 2005; Trifunovic et al., 2004). Each has a premature aging phenotype with reduced lifespan and multiple age-related features such as alopecia, graying of the hair, osteoporosis, weight loss, and enlargement of the heart, each occurring earlier than wild type mice. However, no apparent parkinsonism has been reported in either strain of mice.

COX negative cells, indicating likely deletions of complex I encoding regions of the mtDNA genome, were prevalent in the brain and heart tissue from Polg mutator mice, and a qPCR based strategy revealed that the mice accumulate mtDNA deletions at a much higher rate than the wild type mice (Vermulst et al., 2008). Next generation sequencing has been used to analyze mtDNA from Polg mutator mice and two studies have found between two and tenfold increases in mtDNA mutations in the mutator mice (Ameur et al., 2011; Williams et al., 2010). Interestingly, these approaches did not find abundant mtDNA deletions; rather they reported significant increases in multimers of the mtDNA control region. The conflicting reports about whether or not mtDNA deletions occur in Polg mutator mice merit further study, but it has been suggested that these mice may not accurately model the mtDNA defects in PD where both point mutations and deletions occur (Clark et al., 2011).

Oxidative stress in PD

The electron transport chain in the mitochondria is a major source of reactive oxygen species (ROS) in eukaryotic cells (Chance et al., 1979). As molecular oxygen is sequentially reduced to water by the electron transport chain complexes, a small percentage of superoxide ($O_2^{\bullet-}$) is produced by complexes I and III. Once produced inside the mitochondria, superoxide may be converted to hydrogen peroxide (H_2O_2) by the enzyme manganese superoxide dismutase (MnSOD). Numerous other enzymes (glutathione peroxidase 4, peroxiredoxin 3, peroxiredoxin 5) localized in the mitochondria catalyze the further reduction of hydrogen peroxide to water and molecular oxygen. Under normal conditions, ROS participate in signaling events mediated by select thiol residues in proteins that have the potential to control large scale changes in transcription amongst other things (Fomenko et al., 2011). However, certain situations can cause ROS production to surpass the antioxidant capacity of a cell. This condition, termed oxidative stress, causes irreversible damage to cellular macromolecules and can ultimately lead to cell death. Markers of oxidative stress, including high levels of oxidatively modified lipids, proteins, and DNA have all been found in the SNpc of samples from patients with PD (Alam et al., 1997; Dexter et al., 1989a; Floor and Wetzel, 1998).

Partial inhibition of complex I using drugs such as rotenone or MPP+ has long been known to increase the amount of superoxide produced by complex I (Hasegawa et al., 1990; Takeshige and Minakami, 1979; Votyakova and Reynolds, 2005). Oxidative stress caused by an excess of superoxide, as opposed to a loss of ATP, has been suggested to underlie the toxic effects of rotenone both *in vitro* and *in vivo* (Li et al., 2003; Sherer et al., 2003). Hence, the complex I deficiency observed in sporadic PD may result in increased oxidative stress. This data is supported by the finding that PD cybrid cell lines, which have decreased complex I activity compared to control cybrid lines, exhibit increased oxidative stress (Cassarino et al., 1997; Esteves et al., 2009).

In addition to complex I impairment, alterations in the levels of antioxidant molecules have also been observed in the PD brain. Glutathione (GSH), a tripeptide present at millimolar concentrations in the cytosol of most cells, is a major antioxidant molecule (Smith et al., 1996). Some tissues, such as the liver, have higher concentrations of GSH and secrete it for transportation to other organs. In the brain, both neurons and glia can synthesize GSH, and it has been demonstrated that astrocytes are able to release GSH into the extracellular space where it is subsequently metabolized into components which are taken up by neurons and resynthesized into GSH (Hirrlinger et al., 2002; Rice and Russo-Menna, 1998). It has been consistently observed that GSH levels are reduced in the SNpc of PD brains compared to controls (Perry et al., 1982; Sian et al., 1994; Sofic et al., 1992). Decreased GSH levels are not specific for PD as SNpc tissue from patients with progressive supranuclear palsy, another parkinsonian disorder, has also been reported to be GSH deficient (Fitzmaurice et al., 2003). However, reduced GSH levels in PD may be particularly important as this alteration has been shown to occur early in the disease process (Dexter et al., 1994).

The SNpc of the PD brain has also been found to have higher levels of iron than the control brains (Dexter et al., 1989b; Hirsch et al., 1991; Sofic et al., 1988). Recently, it was demonstrated that the increased iron is located within individual DAergic neurons in the PD SNpc (Oakley et al., 2007). The interaction of ferrous iron (Fe(II)) with H_2O_2 readily generates highly toxic hydroxyl radicals ($HO^{\bullet}$) via Fenton chemistry, making the high iron levels in the SNpc potentially detrimental to dopamine neuron survival. The neuroprotective actions of iron chelators in neurodegenerative models appear to have the dual mechanism of reducing oxidative stress as well as inhibiting iron dependent enzymes such as HIF prolyl-4-hydroxylases (Weinreb et al., 2010).

One possible mechanism underlying the increased iron levels in the SNpc may involve dysfunctional iron transport to the mitochondria in DAergic neurons. The iron transport protein, transferrin, was found to be increased in DAergic SNpc cells of human PD brain and in both rats and monkeys treated with rotenone (Mastroberardino et al., 2009). Both transferrin and the transferrin receptor 2 localized to the mitochondria, possibly representing a novel mechanism for iron transport into the mitochondria. Moreover, transferrin was oxidized at thiol residues in human PD SNpc cells, and the authors provided data that suggested that oxidized transferrin releases Fe(II) making it available for Fenton reactions. Thus, perturbations in transferrin mediated iron transport may be the result of increased oxidative stress in the PD SNpc and may also contribute to the generation of ROS in the SNpc (Mastroberardino et al., 2009).

Oxidative stress caused by dopamine

In addition to complex I inhibition, decreased antioxidant levels, and increased iron levels, DAergic neurons found in the SNpc may also be under greater amounts of oxidative stress than other neurons in the brain because they contain DA. The metabolism of DA by monoamine oxidase yields H_2O_2, which can participate in Fenton type reactions with Fe(II) to generate ROS. DA may also oxidize to the electron-deficient DA quinone (DAQ) either spontaneously in the presence of transition metals or enzymatically (Hastings, 1995). The DAQ contains a partial positive charge predominantly localized at the number five carbon atom of the catechol ring that can readily be attacked by thiolate ($-S^-$) ions contained in the cysteine residues of both GSH and proteins (Graham et al., 1978). 5-Cysteinyl-DA, the major product of the reaction between a cysteine thiolate and DAQ, has been detected in the human SNpc and its levels are elevated in the PD brain (Spencer et al., 1998). Because cysteine residues often play critical roles in protein function, the modification of these residues by the irreversible covalent reaction between DAQ and cysteine can have adverse effects on cellular health. Also in a similar reaction, GSH covalently bound to DA cannot participate in redox reactions, effectively decreasing the pool of reduced GSH, further jeopardizing cellular viability (Rabinovic and Hastings, 1998).

The potential for DA to be toxic *in vivo* has been demonstrated in multiple rodent models. When DA is injected into the rat striatum, DAergic terminals are selectively lesioned and the lesion size positively correlates with levels of protein 5-cysteinyl-DA and 5-cysteinyl-DOPAC formed following injection (Hastings et al., 1996; Rabinovic et al., 2000). The loss of DA terminals following exogenous DA is dependent upon DA uptake into the terminals, independent of MAO metabolism of DA, and at 4 weeks results in the loss of DAergic neurons in the SN (unpublished findings, Hastings laboratory.) These findings showed the potential of exogenous DA to be toxic, but left open the possibility that endogenous DA may not be toxic. Normally, DA is safely sequestered into acidic vesicles where it is less likely to oxidize and cannot react with either proteinaceous thiols or GSH. However, if DA cannot be sequestered correctly it can exert its toxic effects. This concept has been elegantly demonstrated in mice that expressed VMAT2 at only ~5% of normal levels. These mice, which cannot readily sequester DA into vesicles, develop a progressive loss of SNpc neurons along with both motor and non-motor symptoms associated with PD (Caudle et al., 2007; Taylor et al., 2011, 2009). A deficiency in VMAT2 has also recently been shown to enhance the toxicity of α-synuclein overexpression in SNpc neurons further suggesting the potential for endogenous DA participating in the vulnerability of DA neurons (Ulusoy et al., 2012). In a separate *in vivo* model, the importance of intracellular accumulation of unsequestered DA and its ability to oxidize to reactive DAQs was demonstrated (Chen et al., 2008).

Because of the role that mitochondrial impairment may play in the pathogenesis of PD, the effects of DA oxidation products on mitochondria have been examined. When intact, well-coupled mitochondria isolated from rat brain were exposed to DAQ *in vitro*, a dramatic increase in resting state 4 respiration was observed (Berman and Hastings, 1999). This change, indicative of the uncoupling of ATP production from substrate utilization, was accompanied by the opening of the mitochondrial permeability transition pore. Importantly, GSH prevented the effects of DAQ on the mitochondria while antioxidant enzymes could not. These data indicate that the direct effects of DAQ on thiol residues in the mitochondria underlie the observed changes, as opposed to indirect effects from ROS produced by DAQ (Berman and Hastings, 1999).

The protein targets of DAQ have been studied by exposing neuronally differentiated immortalized cells and isolated rat brain mitochondria to DA and DAQ, followed by analysis using proteomic methods (Dukes et al., 2008; Van Laar et al., 2008, 2009). After the addition of DA to their media, differentiated PC12 cells displayed increased levels of several endoplasmic reticulum (ER) chaperone proteins suggesting the activation of ER stress response pathways (Dukes et al., 2008). Several proteins including subunits of complex I, mitofilin, mitochondrial creatine kinase, and isocitrate dehydrogenase were covalently modified by DA when isolated rat brain mitochondria were exposed to radiolabelled DAQ *in vitro* (Van Laar et al., 2009). A parallel study identified that the abundance of numerous proteins decreased rapidly in isolated mitochondria exposed to DAQ (Van Laar et al., 2008). Because several of the proteins that were modified by DA were also shown to be decreased in abundance, it is possible that DA-modified proteins are targeted for degradation by mitochondrial proteases. The mitochondrial Lon protease is known to selectively degrade oxidized proteins in the mitochondria, making it a plausible candidate for the degradation of DA-modified proteins (Bota and Davies, 2002). In addition to mitochondrial proteins, several PD associated proteins including α-synuclein, parkin, DJ-1, and UCH-L1 have all been shown to be modified by DA quinones (Conway et al., 2001; LaVoie et al., 2005; Van Laar et al., 2009).

Monogenic parkinsonism

For a long time, PD was viewed as a sporadic disease with little or no genetic component. However, in 1997 it was discovered that a point mutation in the gene coding for a small protein called alpha-synuclein caused dominantly inherited parkinsonism in a Greek family (Polymeropoulos et al., 1997). Subsequently, it was reported that antibodies against alpha-synuclein stained Lewy bodies found in PD brains (Spillantini et al., 1997). Taken together, these data provided a clear link between sporadic PD and familial parkinsonism. In the years since the discovery of point mutations in alpha-synuclein, there have been at least ten more genes identified that cause monogenic parkinsonism when they are mutated or deleted in humans (Hardy, 2010).

Point mutations and deletions in several genes including *parkin* (*PARK2*), *PINK-1* (*PARK6*), and *DJ-1* (*PARK7*) have been identified as causes of autosomal recessive parkinsonism in humans (Bonifati et al., 2003; Kitada et al., 1998; Valente et al., 2004). The *parkin* gene codes for an E3-ubiquitin-ligase, whereas the *PINK-1* gene encodes a serine–threonine kinase with a mitochondrial targeting sequence. *DJ-1* codes

for a small protein of unknown function that is highly conserved amongst prokaryotes and eukaryotes. Since large deletions in the *parkin*, *PINK-1*, and *DJ-1* genes have been found in patients with autosomal recessive parkinsonism, it is likely that the loss of the function(s) of the protein products of these genes underlies the parkinsonian phenotype observed in these patients. Thus, unraveling what the functions of parkin, PINK-1, and DJ-1 may point to clues about commonalities between sporadic PD and monogenic parkinsonism. Even more so, these proteins and any they interact with may represent targets for therapeutic strategies for PD. The focus of the remainder of this review will be on PINK-1/parkin and DJ-1 because of their roles in the maintenance of mitochondrial integrity.

PINK-1/parkin

In Drosophila, loss of *parkin* leads to mitochondrial defects and muscle degeneration (Greene et al., 2003). Subsequent studies have reported both aberrant morphology and degeneration of dopaminergic neurons in *parkin* mutant flies (Cha et al., 2005; Whitworth et al., 2005). Interestingly, *PINK-1* mutant flies have similar phenotypes to *parkin* mutants and the overexpression of parkin protein can rescue the phenotype of *PINK-1* mutant flies (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). These data indicate that parkin and PINK-1 function in the same pathway with parkin being genetically downstream of PINK-1 in *Drosophila*.

Recently, it has been demonstrated that parkin translocates from the cytoplasm to the mitochondria when the organelles are depolarized for extended periods of time using the protonophore CCCP (Narendra et al., 2008). Once at the mitochondria, parkin ubiquitylates multiple mitochondrial substrates including the voltage-dependent anion channel VDAC1 and mitofusins, outer mitochondrial membrane proteins involved in mitochondrial fusion (Geisler et al., 2010; Tanaka et al., 2010; Ziviani et al., 2010). Ubiquitylation of outer mitochondrial membrane proteins by parkin can lead to their rapid degradation through the proteasome and/or the binding of p62/SQSTRM and the elimination of mitochondria by selective autophagy (mitophagy) (Chan et al., 2011; Narendra et al., 2010a). PINK-1 localizes to the mitochondrial outer membrane where it is rapidly degraded until the mitochondria become depolarized. Upon depolarization, PINK-1 degradation is slowed, allowing the protein to build up on the outer membrane (Narendra et al., 2010b). The kinase domain of PINK-1 faces the cytoplasm and is required for parkin translocation to the mitochondria (Narendra et al., 2010b; Zhou et al., 2008). Bona fide protein targets of PINK-1 kinase activity have yet to be identified, but it has been hypothesized that the accumulation of PINK-1 on depolarized mitochondria allows for the sufficient phosphorylation of substrates which signal parkin to translocate to those mitochondria and target them for destruction.

Although the PINK-1/parkin pathway involved in mitophagy has been clearly demonstrated in non-neuronal mammalian cells, the physiological relevance of this pathway in neurons remains unclear (Van Laar et al., 2011). The cultured cells used in the majority of experiments to elucidate the functions of PINK-1 and parkin rely heavily on glycolysis for ATP production, whereas neurons that favor mitochondrial oxidative phosphorylation, are unable to switch to glycolysis under stress conditions as immortalized cells will (Herrero-Mendez et al., 2009; Reitzer et al., 1979). Neurons are thought to rely more on astrocyte derived lactate as substrates for oxidative phosphorylation, but they continue to be dependent on mitochondrial function for survival (Pellerin and Magistretti, 1994; Wyss et al., 2011). In turn, neurons may be much more reluctant to dispose of their mitochondria *in vivo*, even if the mitochondria are damaged. This notion is supported by a recent study that demonstrated a lack of parkin translocation to the mitochondria following CCCP treatment in primary neuronal cultures (Van Laar et al., 2011). It must be noted that the authors were able to observe a modest accumulation of parkin at the mitochondria in primary neurons when they depolarized mitochondria and inhibited the reversal of ATP synthase thus preventing the concurrent loss of ATP levels. Interestingly, the authors also demonstrated that forcing cultured immortalized cells (HeLa) to rely on oxidative phosphorylation instead of glycolysis prevented the translocation of parkin to depolarized mitochondria. These data suggest, at the very least, that neurons are less inclined to the disposal of dysfunctional mitochondria than immortalized cell lines due to their unique bioenergetic requirements.

DJ-1

Human DJ-1 is a member of the DJ-1/PfpI superfamily of proteins containing members from both eukaryotic and prokaryotic species. Members of this family include proteins of known function such as the *Escherichia coli* chaperone HSP31 and the bacterial proteases PfPI and PH1704 (Bandyopadhyay and Cookson, 2004). Both sequence and structural similarity analyses of the DJ-1/PfpI superfamily suggest that human DJ-1 is part of a unique clade of proteins with a function unlike other members of the superfamily (Bandyopadhyay and Cookson, 2004; Wei et al., 2007).

The crystal structures of both human DJ-1 and its *E. coli* homologue YajL have been solved (Wilson et al., 2003, 2005). Despite having roughly 40% sequence homology, the backbone structures of the two proteins are remarkably similar. Each protein is a dimer in both the crystal and solution, and each contains a cysteine residue positioned at the nucleophile elbow that is conserved across the DJ-1/PfPI superfamily. This cysteine (C106 in human DJ-1) is oxidized to sulfinic acid in both human DJ-1 and YajL crystals (Canet-Aviles et al., 2004; Wilson et al., 2005). Numerous studies have demonstrated that DJ-1 is oxidized in cultured cells exposed to oxidative stress, and that C106 is the residue most sensitive to oxidation (Canet-Aviles et al., 2004; Kinumi et al., 2004; Mitsumoto et al., 2001). The accumulation of oxidized forms of DJ-1 has also been reported in the lungs of mice injected intraperitoneally with lipopolysaccharide, the brains of rats treated systemically with rotenone, and in post-mortem brain tissue from those with sporadic PD (Bandopadhyay et al., 2004; Betarbet et al., 2006; Mitsumoto and Nakagawa, 2001).

Site-directed mutagenesis of C106 has established a clear role for the oxidation of C106 in DJ-1 function. Exposing cells to oxidative stress resulted in the accumulation of DJ-1 at the mitochondria (Canet-Aviles et al., 2004). The replacement of C106 with a non-oxidizable alanine residue completely abolished mitochondrial accumulation in response to oxidative stress, whereas the mutation of the two other cysteine residues to alanine had a little effect. Overexpressed DJ-1 was able to protect cells from rotenone toxicity while the C106A mutant conferred no protection. Further studies demonstrated that the formation of sulfinic acid at C106 was necessary to drive DJ-1 to accumulate at the mitochondria and protect cells from oxidative stress (Blackinton et al., 2009).

Several groups have independently generated DJ-1$^{-/-}$ mice (Chandran et al., 2008; Chen et al., 2005; Goldberg et al., 2005; Kim et al., 2005; Manning-Bog et al., 2007). No overt changes in the nigrostriatal pathway have been observed in any of these mice regardless of age. However, more subtle phenotypes such as hypoactivity and increased DA uptake in isolated striatal terminals have been reported. Despite the lack of overt SNpc pathology, further studies have demonstrated that the loss of DJ-1 sensitizes these animals to oxidative stress. One group has found that DJ-1$^{-/-}$ mice are more sensitive to MPTP toxicity than wild type mice (Kim et al., 2005). Furthermore, two-photon imaging of living midbrain slices from DJ-1$^{-/-}$ mice demonstrated that their SNpc neurons had increased levels of mitochondrial oxidative stress (Guzman et al., 2010).

DJ-1 deficiency has also been modeled in both *Drosophila melanogaster* and *Caenorhabditis elegans* (Hao et al., 2010; Meulener et al., 2005, 2006; van der Brug et al., 2008; Ved et al., 2005). In flies, there are two DJ-1 homologues named DJ-1alpha and DJ-1beta. Flies with both homologues deleted do not display any DAergic neuronal degeneration, but are more sensitive to toxins that cause oxidative stress

(Hao et al., 2010; Meulener et al., 2005; van der Brug et al., 2008). Accordingly, overexpression of either wild type human DJ-1 or fly DJ-1beta is able to protect DJ-1alpha/beta knock out flies from oxidative stress, whereas the overexpression of C104A DJ-1beta (homologous to human C106A) cannot confer protection (Meulener et al., 2006). In nematodes, the knockdown of DJ-1 rendered them more sensitive to death caused by rotenone toxicity (Ved et al., 2005).

In cell culture models, loss of DJ-1 causes several phenotypes associated with mitochondrial dysfunction and autophagy. Mouse embryonic fibroblasts from DJ-1$^{-/-}$ mice contain fragmented mitochondria with defects in the mitochondrial oxygen consumption along with a marked inability to degrade these dysfunctional organelles through autophagic pathways (Krebiehl et al., 2010). Similarly, human neuroblastoma cells expressing stable shRNA for DJ-1 displayed fragmented mitochondria, increased ROS production, and deficiencies in mitophagy (Thomas et al., 2011).

Taken together, these data suggest a clear role for DJ-1 as a sensor of oxidative stress that interacts with mitochondria. Although the means by which DJ-1 senses oxidative stress have been determined, it remains unclear what effect oxidation at C106 has on DJ-1 and what the physiological function of the protein is.

Closing remarks

It has been nearly thirty years since it was discovered that MPTP caused parkinsonism in humans and mitochondria were first implicated in the pathogenesis of PD. Since then, it has become increasingly clear that both mitochondrial dysfunction and oxidative stress underlie the death of SNpc neurons in the disease. Our understanding of genetic forms of parkinsonism, with particular emphasis on the protein products of the *parkin*, *PINK-1*, and *DJ-1* genes, has also pointed towards mitochondrial dysfunction and oxidative stress.

Despite the breadth of our understanding of PD, many difficult questions remain unanswered. What is the cause of the complex I deficiency observed in many patients with PD? Why do SNpc neurons preferentially degenerate in the disease despite seemingly systemic mitochondrial dysfunction? Are the changes in the levels of GSH and iron in the SNpc the cause or the result of oxidative stress? What role do the reactive metabolites of DA have in the degeneration of SNpc neurons? Does mitochondrial dysfunction and oxidative stress also underlie the extranigral pathology of PD? These questions, and many others will be the subject of future research and may lead to therapeutic advances for PD and parkinsonism.

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