

# Mitochondria and Dopamine: New Insights into Recessive Parkinsonism

## Minireview

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**Recessively inherited mutations in *parkin*, *DJ-1*, and *PINK1* have recently been linked to familial forms of parkinsonism. These syndromes are often clinically indistinguishable from Parkinson's disease, as similar neuronal groups, notably dopaminergic neurons, are selectively affected. Studies of the functions of these gene products may provide insights into the pathogenic mechanisms underlying the selective degeneration of dopaminergic neurons. Emerging evidence that one or several of these genes play important roles in mitochondrial function and the dopaminergic system suggests that these events may be early steps of the pathophysiological changes of the disease. This review will summarize recent advances in our understanding of these gene products, with emphasis on the surprising convergence of their functions.**

The primary neuropathological hallmarks of Parkinson's disease (PD) are progressive degeneration of various neuronal groups and the presence of intraneuronal cytoplasmic inclusions known as Lewy bodies (LBs). Many of the clinical manifestations of PD are a consequence of the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). These neurons give rise to the nigrostriatal pathway, and as a consequence of their loss, there is depletion of dopamine (DA) in the striatum, where DA is required for normal motor function. This depletion therefore causes a spectrum of movement disorders, such as the clinical triad of resting tremor, rigidity, and bradykinesia. Neurodegeneration in PD, however, is not limited to the SNpc, as it also occurs in other brain subregions, including noradrenergic neurons in the locus ceruleus and serotonergic neurons in the dorsal raphe nucleus. The occurrence of PD is largely sporadic, but clinical syndromes mimicking sporadic PD occur with an autosomal-recessive pattern of inheritance in a number of families, which represent a small percentage of all PD cases.

Although degeneration of the nigrostriatal pathway accounts for many of the clinical manifestations of PD, the underlying mechanisms that provoke neuronal cell loss remain unclear. Much attention has been devoted to the apparent selective vulnerability of nigral neurons. For example, it has been known for several years that

parkinsonian syndromes can be induced by exposure to rotenone, which causes highly selective nigrostriatal dopaminergic degeneration, even though it inhibits mitochondrial complex I activity across all brain subregions (Betarbet et al., 2000). The discovery of autosomal-recessively inherited mutations in the *parkin* (Kitada et al., 1998), *DJ-1* (Bonifati et al., 2003), and *PINK1* (Valente et al., 2004) genes associated with familial parkinsonism demonstrated that the loss of function of a single gene product can lead to nigral degeneration and the clinical manifestations of parkinsonism (Table 1). This infers that these gene products are essential for the survival of human nigral neurons (although autopsy studies for DJ-1 and PINK1 cases are not yet available). Identification of the cellular and physiological functions of these gene products, therefore, would be invaluable for the elucidation of the pathogenic mechanisms underlying the selective degeneration of dopaminergic neurons. In this review, we will outline emerging experimental evidence and our developing concepts about the function and dysfunction of parkin, DJ-1, and PINK1. One surprising outcome of these analyses is the possible convergence of these genes in the following essential cellular functions.

### **Ubiquitin Proteasome System**

Following the identification of *parkin* mutations, several groups reported that parkin is an E3 ubiquitin-protein ligase. The addition of chains of ubiquitin to target proteins by E3 ligases regulates the rate of entry of proteins to the proteasome. Hence, E3 ligases act as gatekeepers for degradation of proteasome substrates. The domain structure of parkin is similar to other E3 ligases, with two C-terminal really interesting new gene (RING) domains separated by an in-between-ring (IBR) domain. The E2 enzymes UbCH7 or UbCH8, which carry activated ubiquitin, bind to this RING-IBR-RING motif. Most substrates (see below) also bind to the RING-IBR-RING region, often to RING1. By analogy to other E3 ligases with similar domain structures, it is likely that substrates are ubiquitylated by binding to RING1 in close proximity to the E2-ubiquitin and the ubiquitin is then transferred to a substrate lysine residue. The structural basis of substrate recognition by parkin has not yet been elucidated and is a major unanswered question in the field. At the N-terminal region of parkin is a ubiquitin-like domain that interacts directly with the proteasome cap protein Rpn10 (Sakata et al., 2003). Hence, the most likely function of parkin is to promote polyubiquitylation and transfer to the proteasome. It should be acknowledged, however, that many cellular processes employ ubiquitylation and related modifications to control protein activity rather than degradation. For example, there is increasing evidence that ubiquitylation (especially monoubiquitylation) affects intracellular targeting of proteins rather than degradation. This leads to effects of the ubiquitin proteasome system (UPS) on synaptic function, receptor internalization, and other cellular processes, such as transcriptional regulation (Marx, 2002). Whether parkin has effects on some of these other processes has not been addressed.

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Table 1. Recessively Inherited Mutations Linked to Familial Parkinsonism

Gene	Locus	Inheritance	# of Mutations	Type of Mutations	Putative Function
<i>parkin</i> ( <i>PARK2</i> )	6q25-27	AR	>40	exonic deletion and duplication; nonsense; frame-shift; missense	E3 ubiquitin ligase
<i>DJ-1</i> ( <i>PARK7</i> )	1p36	AR	>2	exonic deletion (exons 1-5); missense (L166P)	oncogene??? antioxidant?
<i>PINK1</i> ( <i>PARK6</i> )	1p35-36	AR	2	nonsense (W437Stop); missense (G309D)	kinase?

Parkin appears to conjugate ubiquitin to multiple substrate proteins, at least in vitro, some of which may be toxic if allowed to accumulate. For example, the septin CDC-rel1 has been shown to be toxic in rats when overexpressed using viral vectors in vivo. Parkin suppresses the toxicity of another substrate, Pael receptor 1 (Yang et al., 2003), and similar relationships may hold for other substrates. Whether parkin regulates the degradation of these proteins in vivo is difficult to address, although it is important to note that analysis of *parkin* null mice did not identify altered steady-state levels of CDC-rel1 and other substrates (Goldberg et al., 2003). Parkin also protects against the deleterious effects of overexpression of mutant  $\alpha$ -synuclein in some models (Petrucci et al., 2002; Yang et al., 2003). Overall, it appears that parkin's E3 ligase activity is required for protection of cell survival, as pathogenic inactivating mutations do not protect cells.

These observations support two concepts. First, most evidence to date suggests that parkin can function as E3 ubiquitin ligase, although these studies tend to be done in vitro or using overexpressing cell lines. Thus, it is logical to predict that parkin regulates the steady-state levels of substrate proteins, thereby limiting the abundance of damaged and/or damaging proteins. The physiological substrates of parkin, however, remain unclear. Second, parkin is neuroprotective against a number of stresses, including but not limited to overexpression of mutant  $\alpha$ -synuclein. This is consistent with parkin acting downstream of cellular stress triggers. The key questions remain: what are the downstream targets of parkin, through which parkin mediates its protection of nigral neurons; and is there any evidence linking parkin to other recessive parkinsonian genes, or does each impinge on distinct pathways?

#### **Mitochondrial Dysfunction and Oxidative Stress**

The idea that mitochondrial dysfunction may play a key role in PD pathogenesis has been around for a long time, since postmortem studies have shown mitochondrial impairment and oxidative damage in PD brains (Beal, 2003; Jenner and Olanow, 1998). This is further supported by observations that mitochondrial complex I inhibitors, such as MPTP, rotenone, and paraquat, can be used to produce a parkinsonian syndrome in experimental models and humans (Dauer and Przedborski, 2003). The coexistence of mitochondrial dysfunction and nigral cell loss in these systems makes it difficult to establish whether mitochondrial dysfunction is causal or a secondary consequence of nigral degeneration. Interestingly, recent studies have surprisingly indicated a role for parkin in mitochondrial function. First, using an unbiased proteomics approach, alterations in the apparent abundance of several mitochondrial proteins in the electron transport chain and several proteins in-

involved in the protection of oxidative stress were found in the ventral midbrain of *parkin* knockout mice (Palacino et al., 2004). These alterations were accompanied by decreases in mitochondrial respiratory capacity and age-dependent increases of oxidative damage. Second, mitochondrial pathology is a prominent feature in the flight muscle of *Drosophila parkin* null mutants (Greene et al., 2003; Pesah et al., 2004). This mitochondrial damage may be responsible for the observed apoptosis in the flight muscle, which is a heavily energy-dependent tissue in insects. Third, overexpression of parkin in vitro decreases cellular sensitivity to agents (e.g., ceramide) that trigger mitochondria-dependent apoptosis (Darios et al., 2003). The fact that loss of parkin function in mice causes mitochondrial dysfunction and oxidative damage in the absence of neuronal degeneration seems to lend support for a causal role of mitochondrial dysfunction in nigral degeneration. Of course, this notion will have to be confirmed in a model system that exhibits mitochondrial dysfunction and eventually develops substantial degeneration of nigral neurons.

How and whether parkin regulates mitochondrial function through its E3 ligase activity is not yet clear. None of the mitochondrial proteins were found to accumulate in the absence of parkin, suggesting that parkin does not regulate their abundance directly through its E3 ligase activity (Palacino et al., 2004). In addition, none of the substrates identified for parkin's E3 ligase activity have been implicated in mitochondrial function. However, it remains possible that physiological substrates of parkin's E3 ligase activity, which are yet to be identified, may be involved in the biogenesis of these mitochondrial proteins. Interestingly, a small proportion of parkin is associated with the outer mitochondrial membrane, at least in cultured cells (Darios et al., 2003), though parkin lacks an obvious mitochondrial targeting sequence. It has been reported that inhibition of proteasome function decreases mitochondrial protein synthesis and activity and increases the production of reactive oxygen species (Sullivan et al., 2004). Therefore, it is also possible that the observed changes in mitochondrial protein expression are a downstream effect of parkin on net proteasome function.

Given the evidence mentioned above, several laboratories have evaluated whether the more recently identified DJ-1 and PINK1 affect mitochondrial function and/or oxidative stress. Although initial evidence suggested that wild-type and mutant DJ-1 localized differentially to mitochondria in transfected cells (Bonifati et al., 2003), a more recent report suggests that oxidizing conditions favor DJ-1 localization to the outer mitochondrial membrane (Canet-Aviles et al., 2004). It is important to note that this observation has not yet been confirmed in vivo at physiological expression levels and the mechanistic

Table 2. Parkin-Deficient Models

	References	Phenotypes
Mouse	1	nigrostriatal dysfunction (e.g., increased striatal extracellular dopamine, reduced synaptic excitability of striatal spiny neurons, behavioral impairments) but absence of dopaminergic neuron loss
	2	behavioral deficits, inhibition of glutamate neurotransmission, increased dopamine, increased GSH levels, absence of dopaminergic neuron loss
	3	impaired mitochondrial function, alterations in mitochondrial proteins and proteins involved in protection of oxidative stress, age-dependent increases in oxidative damage
<i>Drosophila</i>	4	reduced lifespan, apoptotic muscle degeneration and male sterility associated with mitochondrial pathology, absence of dopaminergic neuron loss
	5	male and female sterility, shorter lifespan by paraquat treatment, muscle degeneration, absence of dopaminergic neuron loss

The quaking mouse, which contains a large genomic deletion encompassing both the *parkin* gene and the adjacent *parkin co-regulated* gene, also exhibits intact dopaminergic neurons in the substantia nigra (Lorenzetti et al. 2004). 1, Goldberg et al., 2003; 2, Itier et al., 2003; 3, Palacino et al., 2004; 4, Greene et al., 2003; 5, Pesah et al., 2004.

basis for DJ-1 localization to mitochondria has not been elucidated. Importantly, DJ-1 overexpression protects against mitochondrial damage (Canet-Aviles et al., 2004), and knockdown models show heightened sensitivity to oxidative stress (Yokota et al., 2003). Very recently, the first report on the linkage of *PINK1* mutations to familial parkinsonism shows that *PINK1* contains a mitochondrial localization sequence and is indeed localized to mitochondria (Valente et al., 2004). *PINK1* also protects cells against loss of mitochondrial membrane potential triggered by application of proteasome inhibitors. Overall, it is still very early for the studies of DJ-1 and *PINK1*. For example, how and whether these proteins regulate mitochondrial function is unknown, and the substrates for the presumed *PINK1* kinase activity are not yet identified. Given that *PINK1* has a mitochondrial targeting sequence, it is reasonable to expect that its physiological substrates may include mitochondrial proteins. It should be emphasized that knockout models for DJ-1 or *PINK1* have not yet been reported. In one knockdown model for DJ-1, increased sensitivity of cells to a number of stresses was noted, but mitochondrial function was not examined (Yokota et al., 2003). Results from such experiments are eagerly awaited, especially using relevant *in vivo* systems.

Thus, it seems that the triad of genes responsible for recessive parkinsonism are all variably associated with mitochondria and protect against some combination of mitochondrial dysfunction and/or oxidative stress. More broadly, selective vulnerability of nigral neurons in parkinsonism is likely to be multifaceted and may involve either mitochondrial or proteasomal pathways or both. Although beyond the scope of the present minireview, it is also of interest that the known dominant mutation associated with LB-positive PD,  $\alpha$ -synuclein, has been linked to both proteasomal dysfunction (Petruccioli et al., 2002, and references therein) and mitochondrial damage (Dauer and Przedborski, 2003; Dawson and Dawson, 2003). DJ-1 (Yokota et al., 2003) and *PINK1* (Valente et al., 2004) have both been suggested to protect cells against proteasome dysfunction, although, as neither gene has an apparent direct link to the UPS, it is unclear how either DJ-1 or *PINK1* is protective against these stresses. There is evidence that inhibitors of the proteasome and mitochondrial complex I have additive effects

on dopaminergic neurons (Hoglinger et al., 2003). It is possible that both pathways are affected to induce neuronal death in recessive parkinsonism, and selective vulnerability results from a complex interplay between the two stresses. In this context, it is worth noting that several recent studies implicate another old suspect for dopaminergic cell loss in parkinsonism: dopamine itself.

#### **Dopaminergic System**

As stated above, a key feature of parkinsonian disorders is the selective loss of dopaminergic neurons in the SNpc, resulting in reduced dopamine input to the striatum. Postmortem analysis of parkin mutation-bearing patients confirmed the selective loss of DA neurons in the SNpc, though neuropathological studies of patients carrying mutations in DJ-1 and *PINK1* are still lacking. Inactivation of parkin function in mice and flies, however, fails to reproduce the selective loss of dopaminergic neurons seen in human patients (Goldberg et al., 2003; Greene et al., 2003; Itier et al., 2003) (Table 2). The same preservation of nigral cells is seen in the quaking mouse strain, which harbors deletions of both the *parkin* and *parkin co-regulated* genes (Lorenzetti et al., 2004). These observations suggest that frank dopaminergic cell death may occur over a more protracted time scale than that available in experimental animals or that an additional pathogenic event(s) may be necessary to produce such cell death. These models are nevertheless valuable for the identification of the early changes in dopaminergic neuronal physiology that may promote or eventually precipitate selective nigral degeneration. At the same time, such analyses will shed light on the normal function of parkin and its role in dopaminergic neuronal survival.

Recent analysis of *parkin* null mice has indeed revealed disturbances in dopaminergic physiology. Using *in vivo* microdialysis, extracellular concentrations of dopamine were found to be increased in the striatum, though striatal levels of DA and its metabolites DOPAC and HVA were unaffected (Goldberg et al., 2003). This effect appears to be specific for dopamine, as levels of other neurotransmitters, such as serotonin and noradrenaline, are not altered in the absence of parkin (Itier et al., 2003). However, the precise mechanism by which loss of parkin leads to the increase in extracellular DA is less clear. Additional deficits in the dopaminergic sys-

tem in *parkin* null mice include reduced synaptic responses in the striatal spiny neurons and behavioral impairment in paradigms that are sensitive to the nigrostriatal pathway (Goldberg et al., 2003). Interestingly, a proportion of parkin protein localizes to the postsynaptic density via an interaction with CASK/lin-1, and at least one of its putative substrates (CDC-rel1) is involved in synaptic function. In view of these findings, it is possible that a synaptically localized parkin substrate(s) may be involved in the regulation of dopamine uptake or release.

It is interesting that patients with *parkin* mutations often have dystonia as an early symptom. Mutations in dopamine synthesis genes such as GTP cyclohydrolase I or tyrosine hydroxylase also produce dopa-responsive dystonia. Therefore, the alterations in dopamine transmission in these model systems may reflect some of these early symptomatic changes in human patients. Nevertheless, it is unclear whether these parkin-dependent alterations in the mouse nigrostriatal system truly represent a precursor to subsequent nigral degeneration, as *parkin* null mice do not progress to develop dopaminergic cell loss. It is therefore essential to develop an animal model that recapitulates the selective loss of dopaminergic neurons, which will enable the dissection of the underlying mechanisms and molecular events promoting the selective vulnerability of DA neurons. Although knockout models of DJ-1 and PINK1 have not yet been reported, it will be interesting to find out whether either of these genes plays physiological roles in the dopaminergic system or in neurotransmission more generally and whether they will show dopaminergic cell loss. As each of these recessive parkinsonian genes appears to be required for the survival of dopaminergic neurons in humans, inactivation of all three of these genes may be necessary to reproduce the substantial nigral degeneration in mice. Finally, it is worth noting that there are several lines of evidence supporting an involvement of dopamine in the toxic effects of  $\alpha$ -synuclein (Dauer and Przedborski, 2003; Lotharius and Brundin, 2002), although the relationships between dominant  $\alpha$ -synuclein mutations and recessive parkinsonism are not clear and are beyond the scope of the present review.

### Conclusion

Studies of the *parkin* gene in a variety of experimental systems are now reaching a new level of maturity. A series of observations has implicated a role for parkin in dopaminergic neurotransmission and/or mitochondrial function. However, the mechanistic links between E3 ligase activity and these two measures are not yet clear. There are already some hints from the study of DJ-1 and PINK1 implicating possible roles in mitochondria, but here we have to be careful, as studies of these two proteins are still at very early stages. It is clear that examining mitochondrial function and the dopaminergic system in DJ-1 and PINK1 knockout models will be important, when they become available.

In this minireview, we have suggested that there is surprising convergence at the cellular level between these different genes that are associated with familial parkinsonism. A more simplified and clearer model would require further studies of these gene products in a variety of experimental model systems. By refining our knowledge of the cellular and physiological roles

of these genes, we hope to understand the complex relationships between idiopathic PD and the recessive parkinsonian syndromes and eventually to develop novel ways in which to protect nigral neurons from the disease process.

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