Pink1, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson’s disease
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Mutations in PARKIN, PTEN-induced kinase 1 (PINK1) and DJ-1 are found in autosomal recessive forms and some sporadic cases of Parkinson’s disease. Recent work on these genes underscores the central importance of mitochondrial dysfunction and oxidative stress in Parkinson’s disease. In particular, pink1 and parkin loss-of-function mutants in Drosophila show similar phenotypes, and pink1 acts upstream of parkin in a common genetic pathway to regulate mitochondrial function. DJ-1 has a role in oxidative stress protection, but a direct role of DJ-1 in mitochondrial function has not been fully established. Importantly, defects in mitochondrial function have also been identified in patients who carry both PINK1 and PARKIN mutations, and in those who have sporadic Parkinson’s disease. Future studies of the biochemical interactions between Pink1 and Parkin, and identification of other components in this pathway, are likely to provide insight into Parkinson’s disease pathogenesis, and might identify new therapeutic targets.

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Introduction
Parkinson’s disease pathiology is characterized most prominently by loss of dopaminergic neurons in the substantia nigra and formation of intraneuronal protein aggregates called Lewy bodies [1]. Over the past decade, mutations that mediate familial forms of Parkinson’s disease have been definitively identified in five genes: those that encode α-Synuclein (SNCA, also known as PARK1) and Leucine-rich repeat kinase 2 (LRRK2, also known as PARK8), which mediate autosomal dominant forms of Parkinson’s disease, and those that encode Parkin (PARKIN, also known as PARK2), DJ-1 (also known as PARK7) and PTEN-induced kinase 1 (PINK1, also known as PARK6), which mediate autosomal recessive forms [1]. Although familial forms of Parkinson’s disease account for only 5–10% of all cases, mutations in several of these genes have also been identified in sporadic Parkinson’s disease [1], suggesting that studies of these genes might provide insight into both familial and sporadic disease.

Mitochondrial dysfunction and oxidative stress were originally implicated in Parkinson’s disease pathogenesis because exposure to environmental toxins that inhibit mitochondrial respiration and promote production of reactive oxygen species (ROS) cause loss of dopaminergic neurons in humans and animal models [2]. Recent demonstrations that pink1, parkin and DJ-1 have crucial roles in mitochondrial function and resistance to oxidative stress reinforce the central importance of these themes in Parkinson’s disease pathogenesis and have begun to enable understanding of these processes at the mechanistic level. This review highlights work from the past two years on the roles of parkin, pink1 and DJ-1 in mitochondrial function.

Parkin: an E3 ubiquitin ligase essential for mitochondrial function
The Parkin protein bears two RING-finger motifs and has E3 ubiquitin ligase activity in vitro [3]. It is not yet known whether the ligase activity of Parkin is required for its protective role with respect to Parkinson’s disease pathogenesis. Nonetheless, because protein aggregation is a pathological feature of Parkinson’s disease, a major hypothesis is that mutations in parkin result in the aberrant accumulation of toxic substrates via dysfunction of the ubiquitin–proteasome system [4]. Several putative Parkin substrates have been identified in vitro [4]. However, only two of these, the aminoacyl-tRNA synthetase cofactor p38 [5] and far upstream element-binding protein 1 [6] have been found to accumulate in parkin null mice, and their roles in Parkinson’s disease pathogenesis remain unclear. Interestingly, recent studies suggest that the ubiquitin ligase activity of Parkin can have proteasome-independent functions, offering a potential explanation for the lack of accumulation of some putative substrates in parkin null mice. In addition to lysine48 (K48)-linked polyubiquitination, which generally targets substrates for proteasomal degradation, Parkin is able to catalyze both monoubiquitination [7,8] and K63-linked polyubiquitination [9,10]. These modifications can influence cellular processes such as signal transduction, transcriptional
regulation, and protein and membrane trafficking, without promoting substrate degradation [11]. For example, it was recently reported that ubiquitination of the endocytic protein Ep15 by Parkin modulates the kinetics of endocytosis of epidermal growth factor receptor (EGFR) and, downstream of EGFR, signaling by phosphoinositide 3-kinase and Akt [12*].

Important clues to the cellular processes that rely on parkin function have come from studies of parkin loss-of-function mutants in Drosophila. parkin mutant flies exhibit dramatic mitochondrial defects — swollen mitochondria that have severely fragmented cristae — in several energy-intensive tissues, including the male germline and adult flight muscle [13,14]. The flight muscles ultimately die, and their death shows features of apoptosis [14]. parkin mutant flies also display a small but significant degeneration of a subset of dopaminergic neurons [15]. Although severe defects in mitochondrial morphology are not observed in parkin knockout mice [16], these animals display reduced mitochondrial respiratory activity, which is associated with signs of oxidative tissue damage in the brain [16].Interestingly, defects of the mitochondrial respiratory chain have also been detected in peripheral tissues taken from human Parkinson’s disease patients who have parkin mutations [17]. Together, these observations suggest that parkin has a crucial and evolutionarily conserved role in mitochondrial function.

pink1 and parkin function in a common genetic pathway to regulate mitochondrial function

Recent studies of pink1 and its interaction with parkin strengthen the idea that parkin regulates mitochondrial function. We and others reported that pink1 loss-of-function mutants in Drosophila, parkin mutant flies exhibit dramatic mitochondrial defects — swollen mitochondria that have severely fragmented cristae — in several energy-intensive tissues, including the male germline and adult flight muscle [18,19,20]. pink1 mutants also show reduced ATP levels and mitochondrial DNA (mtDNA) content [18,19,20]. As in parkin mutant flies, mitochondria in pink1 mutant flight muscle are swollen with fragmented cristae, and these cells ultimately undergo apoptotic death [18,19,20]. Muscle degeneration and reduced ATP and mtDNA content can be rescued by overexpression of buffy, a fly Bel-2 homolog [19*], but it is unclear if buffy overexpression exerts protective effects through direct inhibition of apoptosis or through other effects on mitochondrial physiology. Mitochondria within dopaminergic neurons in pink1 mutants also display aberrant morphology [19*], and a small but statistically significant loss of a subset of these neurons occurs with age [19,20,21]. The striking similarity between the phenotypes of pink1 and parkin mutant flies led us and others to search for genetic interactions between the two genes. parkin overexpression in flies suppresses all pink1 mutant phenotypes tested [18,19,20] and pink1 overexpression does not compensate for loss of parkin function [18,19]. Furthermore, double mutants in which both pink1 and parkin are absent have phenotypes identical to, rather than stronger than, either single mutant [18,19]. Together, these data provide compelling in vivo evidence that pink1 and parkin act in a linear pathway that affects mitochondrial function, with parkin downstream of pink1.

Several lines of evidence suggest that these observations on pink1 and parkin function are relevant to humans. First, expression of human PINK1 [18,20] or PARKIN (LJ Pallanck, personal communication) in Drosophila suppresses phenotypes caused by loss-of-function of pink1 or parkin, respectively, suggesting that the human and fly proteins are functionally conserved. Second, in addition to being expressed in the brain, human PINK1 and PARKIN are highly expressed in testes and muscle [22,23], the tissues in which mitochondrial defects are most prominently seen in pink1 and parkin mutant flies. Third, pathological changes and defects in mitochondrial respiration have been detected in peripheral tissues from patients with PINK1 [24] or PARKIN [17] mutations, as well as those with sporadic Parkinson’s disease [25–27], suggesting that such defects accompany loss of dopaminergic neurons as part of a more global pathology in Parkinson’s disease. Finally, Parkinson’s disease patients who harbor mutations in PINK1 or PARKIN are clinically indistinguishable [28], a finding consistent with the hypothesis that these genes function in a common genetic pathway.

What is the biochemical relationship between Pink1 and Parkin?

An important unanswered question is whether Pink1 and Parkin directly bind to each other, and whether Parkin is a substrate for Pink1 kinase activity. We have found that Pink1 and Parkin can physically interact in cultured Drosophila cells (R Feldman and M Guo, unpublished data), although the functional consequences of this interaction remain unclear. No substrates for Pink1 kinase activity have been identified to date but the ubiquitin ligase activity of Parkin can be modulated by phosphorylation [29]. Thus, Pink1 might regulate Parkin activity post-translationally, either by direct phosphorylation or by activation of intermediate signaling proteins (Figure 1). Alternatively, it has been reported that knockdown of pink1 by RNA interference (RNAi) in Drosophila results in reduced abundance of the Parkin protein [20*], suggesting that Pink1 functions directly (perhaps through modulation of Parkin autoubiquitination and degradation) or indirectly (through actions on other proteins) to maintain Parkin levels. The aforementioned genetic data are also consistent with a model in which Pink1 and Parkin act in series on shared targets. For example, Pink1-dependent phosphorylation of a substrate might facilitate the interaction of this protein with Parkin (Figure 1).
Further genetic and biochemical characterization of the Pink1–Parkin interaction is required to clarify the mechanisms by which this pathway functions.

**Where do Pink1 and Parkin function?**

Several lines of evidence suggest that Pink1 functions within mitochondria. Pink1 bears an N-terminal mitochondrial targeting sequence (MTS) and colocalizes predominantly with mitochondrial markers in cultured mammalian cells [19**,30] and *in vivo* [18**]. In human and rat brain, endogenous Pink1 fractionates with markers of the outer and inner mitochondrial membranes but not with those of the matrix or intermembrane space [31]. Immunoelectron microscopy studies show Pink1 to be closely associated with the inner mitochondrial membrane [32]. Removal of the outer membrane exposes Pink1 to protease digestion [32], suggesting that at least a portion of the protein is exposed to the intermembrane space. Pink1 contains a predicted transmembrane domain after its N-terminal MTS, suggesting that it might be an integral membrane protein [32], although this hypothesis has yet to be tested directly. Interestingly, an N-terminally truncated form of the protein can be detected in the cytosolic fraction when *pink1* is overexpressed in cultured mammalian cells [33], raising the possibility that mature Pink1 is released from the mitochondrion under certain conditions [33].

Unlike Pink1, Parkin does not contain a MTS and localizes predominantly to the cytosol and endoplasmic reticulum [34,35]. However, part of the cellular Parkin pool associates with the cytoplasmic surface of the outer mitochondrial membrane, as determined by limited protease digestion of mitochondria-enriched fractions from cultured mammalian cells [35]. Furthermore, although Parkin had not previously been identified within mitochondria, one recent study using immunoelectron microscopy has detected Parkin in association with mitochondrial cristae under certain conditions [36**]. Thus, it is likely that both Pink1 and Parkin are present within or at the surface of the mitochondrion in at least some contexts. Given that Pink1 localizes predominantly within mitochondria and is required for mitochondrial function, the most parsimonious hypothesis is that...
Pink1 functions directly at the mitochondrion, perhaps through direct phosphorylation of Parkin or of other signaling molecules that regulate the activity or abundance of Parkin. Alternatively, because Pink1 might be released from the mitochondrion under certain conditions, it remains possible that Pink1 has extra-mitochondrial functions, perhaps involving interaction with cytosolic Parkin, that regulate mitochondrial function (Figure 1). By the same logic, the partial mitochondrial localization of Parkin might reflect a direct function on mitochondria, but it might also be that actions carried out by Parkin in other cellular compartments have a downstream effect on mitochondrial function.

**Which aspects of mitochondrial function rely on Pink1 and Parkin?**

Mitochondria have crucial roles in multiple cellular processes, including ATP production, regulation of cell death, Ca$^{2+}$ homeostasis and cellular signaling [37]. *pink1* mutant *Drosophila* exhibit decreased ATP content [18**,19**,20*], and deficiencies in mitochondrial respiratory chain activity have been detected in *parkin* knockout mice [16] and in human Parkinson’s disease patients who have mutations in *PARKIN* [17] or *PINK1* [24]. Furthermore, *parkin* loss-of-function sensitizes cultured mammalian cells [38] and *Caenorhabditis elegans* [39] to inhibition of mitochondrial complex I, whereas *parkin* overexpression increases complex I activity [40]. It is unclear whether these defects in respiration and energy production reflect direct or indirect roles of *pink1* and *parkin* in these processes. For example, *fly pink1* mutants display additional mitochondrial defects, such as loss of cristae [18**,19**,20*] and mtDNA [19**], which could account for the reduced ATP production. In the *Drosophila* flight muscle, *pink1* [18**,19**] and *parkin* [14] are required for proper mitochondrial maintenance, because mitochondria initially appear normal during pupal development but progressively deteriorate as the fly ages. Given this, it will be interesting to determine whether biochemical defects in mitochondrial function precede mitochondrial morphological defects, or vice versa. One possible mechanism by which *pink1* and *parkin* could influence mitochondrial physiology is suggested by the intriguing observation that Parkin can associate with mitochondrial transcription factor A (TFAM) and positively regulate the transcription of mtDNA-encoded respiratory chain complexes [36**]. Thus, it is possible that *parkin* and *pink1* have a direct role in the maintenance of proper mitochondrial respiratory activity. Given that pharmacological inhibition of complex I recapitulates key features of Parkinson’s disease [2], it is conceivable that defects in mitochondrial respiration caused by mutations in *PARKIN* or *PINK1* could contribute to Parkinson’s disease pathogenesis by a similar mechanism. In addition, mitochondria undergo dynamic fusion and fission events, which are linked to the maintenance of proper mitochondrial function and the regulation of cell death [41]. Therefore, it is not unreasonable to speculate that *parkin* and *pink1* also contribute to the regulation of mitochondrial dynamics.

**DJ-1 functions in resistance to oxidative stress**

Unlike *pink1* and *parkin*, there is little evidence that DJ-1 has a direct role in mitochondrial function. However, studies in both cell culture and animal models have demonstrated that DJ-1 deficiency increases sensitivity to cell death induced by oxidative stress, whereas overexpression is protective [42]. Interestingly, these effects seem to be specific to oxidative stress because DJ-1 deficiency sensitizes flies to H2O2 and the ROS-generating compound paraquat, but not to various non-oxidative stresses [43]. Cysteine residues in both human [44,45] and *Drosophila* [46*] DJ-1 are modified to cysteine-sulfenic and cysteine-sulfonic acids under oxidative conditions; this modification is required for the protective function of DJ-1 [45,46*]. These data suggest that, through cysteine modification, DJ-1 senses the redox state of the cell and under oxidative conditions is activated to exert protective effects. DJ-1 has been suggested to function through various mechanisms, including as a transcriptional coactivator, a protease or a molecular chaperone [42], but exactly how DJ-1 exerts its protective effects remains unclear.

Endogenous DJ-1 can be found in the mitochondrial matrix and intermembrane space in mouse brain, although its localization is predominantly cytosolic [47]. This partial mitochondrial localization might reflect a role for DJ-1 in mitochondrial function, but mitochondrial morphological defects due to DJ-1 loss-of-function have not been reported in any model organism. Interestingly, *in vitro* studies have reported physical interactions of DJ-1 with Pink1 [48] and, under oxidative conditions, with Parkin [49]; however, it remains unclear whether these associations occur *in vivo*. Overexpression of DJ-1 does not rescue muscle phenotypes [20*] or male sterility (M. Guo, unpublished data) that is due to lack of *pink1*, or muscle degeneration that is caused by lack of *parkin* (L. Pallanck, personal communication), suggesting that DJ-1 might function in a distinct pathway from that of *pink1* and *parkin*. Interestingly, the mild neuronal death caused by loss of *pink1* function [21] or *parkin* function [15] in *Drosophila* can be suppressed by overexpression of genes that encode antioxidant proteins, suggesting that oxidative stress is also a component of pathology due to *pink1* and *parkin* loss-of-function. Because defective mitochondrial respiration can increase ROS production, a reasonable hypothesis is that DJ-1 functions to detect and/or defend against oxidative stress associated with mitochondrial respiration.

**A convergence between mitochondrial dysfunction and protein aggregation?**

Two major processes, mitochondrial dysfunction and protein aggregation, have been implicated in Parkinson’s
Inhibition of complex I of the mitochondrial respiratory chain complex [50] (see also the Update section of this review). Mutant SNCA expression also increases sensitivity to inhibitors of mitochondrial complex I, whereas loss-of-function of α-Synuclein promotes resistance to these compounds [51]. Intriguingly, pharmacological inhibition of complex I of the mitochondrial respiratory chain in rodents causes aggregation of α-Synuclein [52,53]. Together, these data suggest a possible convergence between mitochondrial function and protein aggregation. One may envisage a positive feedback model in which mitochondrial dysfunction promotes α-Synuclein aggregation, which promotes further mitochondrial dysfunction. In such a model, either stimulus — mitochondrial dysfunction or α-Synuclein aggregation — could initiate the cycle but the end result would be similar.

Conclusions
Recent work has underscored the central importance of mitochondrial dysfunction in Parkinson’s disease. In particular, studies from Drosophila have demonstrated that pink1 and parkin act in a linear genetic pathway required for the maintenance of mitochondrial function. Meanwhile, cumulative data suggest that DJ-1 functions in protection from oxidative stress but does not directly affect mitochondrial morphology. Whereas a large body of evidence suggests that Pink1 is present within the mitochondrion, Parkin localizes predominantly to the cytosol, although recent studies have demonstrated mitochondrial localization for a portion of the cellular Parkin pool. Thus, it remains unclear how Pink1 and Parkin act together to regulate mitochondrial function. It is crucial that future studies address the mechanisms through which Pink1 and Parkin interact with each other to affect mitochondrial physiology. Towards this end, genetic approaches in Drosophila have tremendous potential to identify further components of the pink1–parkin pathway as enhancers or suppressors of the robust phenotypes associated with mutations in these genes. Future studies on the pink1–parkin pathway will provide further insight into Parkinson’s disease pathogenesis, and have the potential to identify new therapeutic targets.

Update
Recently, Stichel et al. have identified defects in mitochondrial morphology in dopaminergic neurons in parkin knockout mice expressing mutant human SNCA [54]. These mice display degenerative mitochondrial changes, such as dilated cristae, which are absent in young mice but frequent in 12–14 month old mice. The same types of age-dependent mitochondrial structural defects were also seen in mice either mutant for parkin or expressing mutant SNCA more frequently than in wild type controls, but the number of damaged mitochondria only reached statistical significance in mice with both genetic modifications. These results suggest that the effects of parkin loss-of-function and SNCA expression on mitochondrial morphology are additive. These data also extend the findings of Martin et al. [50] by demonstrating that mutant human SNCA expression in mice causes mitochondrial degeneration in dopaminergic neurons in the substantia nigra.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


Although several Parkin substrates were identified in vitro, this study demonstrates the accumulation of only one, the aminoacyl-tRNA synthetase cofactor p38 protein, in parkin knockout mice.


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18. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA, Guo M: *Drosophila* pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* 2006, 441:1162-1166. This study and [19**]** were the first to report the consequences of pink1 loss-of-function in vivo and the genetic interaction between pink1 and parkin. Deletion mutants of pink1 in *Drosophila* showed mitochondrial morphological defects in the male germline and muscle, stress sensitivity and reduced ATP content. The authors also showed unequivocally that Pink1 localizes predominantly to mitochondria in vivo. Pink1 mutant phenotypes were rescued by overexpression of parkin, but overexpression of pink1 did not rescue parkin mutant phenotypes. In addition, human PARKIN rescued fly pink1 mutant phenotypes, establishing the functional conservation between human and fly Pink1.

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45. Stichel CC, Zhu XR, Bader V, Linnartz B, Schmidt S, Lubbert H: Mono- and double-mutant mouse models of Parkinson’s disease display severe mitochondrial damage. Hum Mol Genet 2007 doi: 10.1093/hmg/ddm083 http://hmg.oxfordjournals.org/. The authors demonstrate that mitochondria from parkin knockout mice and mice expressing mutant human SNCA exhibit defects in mitochondrial respiration. parkin mutant mice expressing SNCA also displayed age-dependent defects in mitochondrial morphology within dopaminergic neurons. These defects were also seen in mice either mutant for parkin or expressing SNCA more frequently than in wild type mice, but the number of damaged mitochondria only reached statistical significance in mice with both genetic modifications.


This study reports that expressing pathogenic mutant forms of SNCA alone in mice results in the presence of aberrant mitochondria in axons and dendrites of affected neurons, reduced activity of the mitochondrial respiratory chain, and mtDNA damage.


The authors demonstrate that mitochondria from parkin knockout mice and mice expressing mutant human SNCA exhibit defects in mitochondrial respiration. parkin mutant mice expressing SNCA also displayed age-dependent defects in mitochondrial morphology within dopaminergic neurons. These defects were also seen in mice either mutant for parkin or expressing SNCA more frequently than in wild type mice, but the number of damaged mitochondria only reached statistical significance in mice with both genetic modifications.