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Mitochondrial Dynamics and Mitophagy in Parkinson's Disease: Disordered cellular power plant becomes a big deal in a major movement disorder

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Abstract

Parkinson's disease (PD), the most common movement disorder, is characterized by age-dependent degeneration of dopaminergic neurons in the substantia nigra of the mid-brain. Non-motor symptoms of PD, however, precede the motor features caused by dysfunction of the dopaminergic system, suggesting that PD is a systemic disorder. Mitochondrial dysfunction has long been observed in PD patients and animal models, but the mechanistic link between mitochondrial dysfunction and PD pathogenesis is not well understood. Recent studies have revealed that genes associated with autosomal recessive forms of PD such as *PINK1* and *Parkin* are directly involved in regulating mitochondrial morphology and maintenance, abnormality of which is also observed in the more common, sporadic forms of PD, although the autosomal recessive PDs lack Lewy-body pathology that is characteristic of sporadic PD. These latest findings suggest that at least some forms of PD can be characterized as a mitochondrial disorder. Whether mitochondrial dysfunction represents a unifying pathogenic mechanism of all PD cases remains a major unresolved question.

Introduction

Mitochondrial dysfunction has long been implicated in the etiology of PD. The discovery of the Parkinsonism-inducing neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), which is a selective inhibitor of mitochondrial complex I, directed researchers' attention to pathological roles of mitochondria in PD and raised the possibility that environmental toxins affecting mitochondria might cause PD. Other mitochondrial toxins characterized as parkinsonism-inducing reagents include 6-Hydroxy-Dopamine (6-OHDA), rotenone and paraquat. Studies of animal models of PD induced with these toxins suggest that mitochondrial dysfunction and oxidative stress are important pathogenic mechanisms [1]. In humans, reduced complex I activity has been reported in both post-mortem brain samples and platelets of sporadic PD cases [2-4], and mutations or polymorphisms in mitochondrial DNA can confer genetic risk for PD [5]. Genetic evidence has also come from studies of familial forms of PD (FPD). The identification and characterization of FPD genes

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have provided an unprecedented opportunity to understand pathogenic mechanisms underlying dopaminergic neurodegeneration. Studies of FPD have revealed two distinct but potentially inter-connected disease pathways: the autosomal dominant genes represented by α-Synuclein that lead to Lewy-body pathology, and the autosomal recessive genes *Parkin, PINK1, DJ-1, and HtrA2/Omi* that have been linked to regulation of mitochondria. In this review, we focus on recent findings from molecular genetic and cell biological studies that reveal the roles of the autosomal recessive FPD genes in governing mitochondrial functions and discuss how loss of function of these genes may lead to neurodegeneration. It is anticipated that studies of these autosomal recessive FPD genes will also help understand the pathogenesis of sporadic and the autosomal dominant FPD cases, which also feature mitochondrial pathology.

Regulation of mitochondrial dynamics by PINK1 and Parkin

Mutations of the *Parkin* gene cause an autosomal recessive juvenile form of PD (AR-JP). The gene product contains a ubiquitin-like (Ubl) domain at the N-terminus and two RING fingers flanking a cysteine-rich domain, termed In Between RING fingers (IBR), which confer E3 ubiquitin-ligase activity. To study Parkin function, several *Parkin*-deficient mice have been generated. However, most of them do not fully recapitulate dopaminergic neurodegeneration, which has hindered elucidation of the pathological mechanisms of AR-JP. The discovery of a genetic interaction between *Parkin* and *PINK1* in *Drosophila* has shed light on Parkin function *in vivo* [6-8]. The *PINK1* gene, mutations of which also cause juvenile PD, encodes a serine-threonine kinase with a mitochondria-targeting signal at the N-terminus. Loss of PINK1 or Parkin genes in *Drosophila* results in mitochondrial aggregation and cellular degeneration in dopaminergic neurons muscle and sperm, leading to motor impairment and decreased fertility [6-8]. Overexpression of wild-type *Parkin* can rescue the phenotypes caused by *PINK1* deficiency, but not the other way around [6-8]. These studies suggest that *Parkin* is epistatic to PINK1 and that it affects mitochondrial function. Parkin protein is mainly localized to the cytosol, and the molecular mechanism by which it regulates mitochondrial function is an open question.

In contrast to the textbook view of kidney bean-shaped organelles, mitochondria exhibit dynamic morphological changes *in vivo* associated with changes in distribution and function. These morphological changes are regulated by a delicate balance between the opposing processes of mitochondrial fusion and fission. Increased fission leads to mitochondrial fragmentation, while increased fusion leads to mitochondrial elongation or aggregation. One remarkable feature of the *PINK1*-deficient fly is the presence of highly aggregated mitochondria in dopaminergic neurons [8,9]. A similar mitochondrial morphological abnormality is observed in the flight muscle of *PINK1*- and *Parkin*-deficient flies, in which swollen mitochondria, often with disintegrated cristae, are observed [10,11]. Interestingly, *PINK1* and *Parkin* mutant phenotypes are partly rescued by increased activity of Drp1, which is a major component of the mitochondrial fission machinery, or by reduced activity of Mitofusin (Mfn) or OPA1, which together control mitochondrial fusion [9,11,12]. Abnormal mitochondrial morphology and dynamics are also observed in mammalian cultured cells and hippocampal and dopaminergic neurons [9,13]. These findings suggest that PINK1 and Parkin may have conserved roles in the regulation of neuronal mitochondrial morphology and function. This represents a breakthrough in PD research.

Regulation of mitophagy by PINK1 and Parkin

Another breakthrough in our understanding of PINK1/Parkin function came from a series of elegant cell biological studies. When the mitochondrial membrane potential is disrupted by mitochondria-damaging reagents such as carbonyl cyanide m-chlorophenylhydrazone

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(CCCP) in mammalian [14-18] or *Drosophila* cultured cells [19], Parkin translocates to mitochondria with low membrane potential, where it promotes LC3-mediated autophagic elimination of the damaged mitochondria in a process called mitophagy (Figure 1) [20]. After Parkin translocation, mitochondrial accumulation of poly-ubiquitinated proteins, consisting mainly of Lys63-linked poly-ubiquitin and a small portion of Lys48-linkages [21,22], recruits the ubiquitin- and LC3-binding adaptor protein p62/SQSTM1 [16,23,24] and the ubiquitin-binding deacetylase HDAC6 [21]. Although important details are still unresolved, Lys63-linked poly-ubiquitination may contribute to proteasomal degradation of mitochondrial proteins [25] and HDAC6- and/or p62-mediated sequestration of mitochondria [21,22]. Mitochondria depolarized by CCCP or paraquat accumulate in the perinuclear compartment in a p62/SQSTM1-dependent manner [16,23,24]. This is followed by engulfment of the damaged mitochondria by autophagosomes and subsequent lysosomal degradation [20]. The clustering of ubiquitinated mitochondria by p62 and HDAC6 is reminiscent of their sequestration of ubiquitinated proteins into aggresomes [26,27].

The translocation of Parkin from the cytosol to the mitochondria, which requires intact PINK1 with kinase activity, is an essential step for mitophagy [17,18]. Through the ubiquitin-proteasome pathway, Parkin ubiquitinates and degrades several proteins localized at the mitochondrial outer membrane, including Mfn [28-30], Drp1 [31], voltage-dependent anion channel 1 (VDAC1) [16,30] and Bcl-2 [32]. The degradation of the mitochondrial fusion factor Mfn by Parkin was also observed in *Drosophila* cultured cells [19,33]. This may contribute to the fragmentation of mitochondria and facilitate mitophagy. This finding is consistent with the *in vivo* observations that loss of *PINK1* or *Parkin* leads to mitochondrial elongation, which is rescued by a reduction of Mfn activity. However, the elimination of Mfn by Parkin and the perinuclear aggregation of mitochondria by p62/SQSTM1 appear to be dispensable for mitophagy in mammalian cells [23,24,30], although the requirement of p62 is controversial [16]. Mfn degradation and mitochondrial perinuclear clustering may prevent the re-fusion of depolarized mitochondria with healthy ones, or compromise the axonal transport of damaged mitochondria [23,28]. In addition, these events may facilitate the isolation of mitochondria by the autophagosomes [28].

How is the autophagy machinery targeted to mitochondria? In yeast, an outer mitochondrial protein ATG32 is reported to recruit the autophagy machinery [34,35]. Although there is no homologue of ATG32 in higher animals, mammalian BNIP3 (BCL2 and adenovirus E1B 19 kDa-interacting protein 3) and NIX/BNIP3-like (BNIP3L), which belong to the BH3-only mitochondrial protein family, induce both cell death and mitophagy. NIX is involved in the programmed mitochondrial clearance by mitophagy during reticulocyte maturation [36,37], and is reported to be required for Parkin translocation to depolarized mitochondria treated with CCCP [38]. But it is unclear whether NIX functions to prime the recruitment of the autophagy machinery in this context as ATG32 does in yeast, or whether it acts as a regulator of PINK1.

Regulation of PINK1 and Parkin

Although endogenous PINK1 is difficult to detect under normal conditions, PINK1 rapidly accumulates in depolarized mitochondria [14,17,18]. This suggests that PINK1 protein is regulated by a post-translational degradation mechanism (Figure 2). Several studies indicate that the rhomboid family protease presenilin-associated rhomboid-like protein (PARL), which is localized to the mitochondrial inner membrane, processes PINK1 in a mitochondrial membrane potential-dependent manner [39-43]. Newly synthesized PINK1 in the cytosol is imported and inserted into the mitochondrial inner membrane (IM), and is cleaved in its putative transmembrane domain by PARL to generate the 52-kD form of PINK1, which is rapidly removed by a proteasome-dependent pathway, likely after its

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release into the cytosol from the mitochondrial intermembrane space (IMS) [40-42]. Upon depolarization of the mitochondrial membrane potential, the IM insertion and the subsequent processing of PINK1 by PARL may be inhibited, leading to full-length PINK1 accumulating in the mitochondrial outer membrane (OM), probably facing the cytosol [41,42,44]. However, there is discrepancy as to whether the processing of PINK1 by PARL is required for Parkin recruitment upon mitochondria depolarization, and further studies are necessary to completely resolve the changes in topology of the processed forms of PINK1 [41,43]. The accumulation of PINK1 with kinase activity is sufficient for Parkin recruitment to the mitochondrial surface, where Parkin's E3 activity appears to be stimulated, although the phosphorylation target(s) of PINK1 remains unknown [17]. Conformational change of Parkin on the mitochondria may serve to activate its E3 activity, as the Ubl domain of Parkin normally inhibits its E3 activity intramolecularly [45]. Parkin is also upregulated by ATF4, a transcription factor of the unfolded protein response (UPR) [46]. Mitochondrial damage may induce the activation of the UPR, leading to the upregulation of Parkin expression [46].

Loss of mitochondrial control and possible disease relevance

Although the relevance of mitophagy observed with mitochondria-damaging reagents to PD etiology remains debatable, accumulating evidence of mitochondrial abnormality in animal models and PD patients has increased our understanding of disease pathogenesis. The ubiquitination and elimination of Mfn following oxidative stress induction as well as mitochondrial depolarization are reproduced in human fibroblasts derived from *PINK1*- or *Parkin* patients [47]. Recent studies of *Parkin*- or *PINK1*-deficient mice have reported morphological and functional alterations of mitochondria in both neurons [48,49] and astrocytes [50]. A missense mutation in PARL found in PD cases abolishes its PINK1-processing activity and the ensuing Parkin-mediated mitophagy [43]. Like the muscle degeneration in *Drosophila*, the function of cardiac muscle, in which mitochondria are abundant, is also impaired by increased oxidative stress in *PINK1*-null mice [51]. Ischemic preconditioning has cardioprotective effects in heart failure models, where the mitochondrial translocation of Parkin is induced. *Parkin* deletion abolishes this effect [52]. Although the roles of PINK1 and Parkin in human cardiac function are unknown, it is worth noting that the prevalence of heart failure in elderly PD patients is double that of non-PD controls [53].

Contribution of DJ-1 and HtrA2 to mitochondrial regulation

DJ-1, which can exert neuroprotective effect by scavenging hydrogen peroxide through self-oxidation, has been reported to be involved in mitochondrial maintenance. Recent data suggest that DJ-1 acts in parallel to the PINK1-Parkin pathway to control mitochondrial polarization and morphology in cultured cells [54,55] and mitochondrial coupling and ATP production in *Drosophila* [56] in certain contexts, but surprisingly DJ-1/PINK1/Parkin triple knockout mice do not exhibit degeneration in the nigrostriatal system [57]. Although the linkage of the *HtrA2* gene to PD pathogenesis is under debate [58], loss of the *HtrA2* gene, which encodes a mitochondrial serine protease, leads to selective loss of striatal neurons in mice [59]. Genetic studies in *Drosophila* showed that HtrA2 mutants do not exhibit mitochondrial morphological defects and there is no genetic interaction that supports HtrA2 functioning in the same genetic pathway as Pink1 in terms of regulation of mitochondrial integrity and dynamics [60]. Furthermore, HtrA2-associated neurodegeneration was not rescued by a Parkin transgene in mice [61]. Together, these results suggest that HtrA2 may not be functioning in the PINK1-Parkin pathway.

Concluding remarks

Prominent pathological features of PD include mitochondrial dysfunction and the accumulation of protein inclusions into Lewy-bodies. These disease phenotypes could arise

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from impairments in the cellular quality control systems for mitochondria and cytoplasmic proteins involving mitochondrial fission/fusion dynamics, the ubiquitin-proteasome system, and the autophagy pathway. These cellular quality control systems do not work in isolation but rather are inter-connected. This could explain why mutations in the autosomal recessive and autosomal dominant FPD genes, which impair the mitochondrial quality control and cytoplasmic protein quality control, respectively, lead to distinct pathological hallmarks but similar clinical outcomes. Impairment of the ubiquitin-proteasome pathway can induce the accumulation of reactive oxygen species in mitochondria [62], with the affected mitochondria later removed by the autophagy pathway [62]. In addition to impaired mitophagy, decreased mitochondrial biogenesis, which may be closely linked to the TOR-mediated protein translation pathway [64], is also implicated in PD pathogenesis [63,65,66]. Thus, pathways for protein synthesis, quality control, mitochondrial maintenance, and mitochondrial dynamics are mechanistically inter-connected in the pathogenesis of PD, and represent novel targets for disease prevention and treatment.

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Box 1

Physiological meaning of mitophagy

The elimination of damaged mitochondria by the PINK1-Parkin pathway appears to be divided into two phases. The first step may be characterized as Parkin- and proteasome-dependent protein degradation of a broad range of the mitochondrial OM proteins, including Mfn1, Mfn2, Tom70 and Tom20 [30,67]. The second step involves Parkin-dependent mitophagy, with which the proteasomal activity may be functionally coupled [30]. Alternatively, proteasomal activity in the second step may be required only for destruction of the mitochondrial OM [67]. The degradation of a wide range of OM proteins by a proteasome- and a AAA+ family ATPase p97-dependent pathway raises the possibility that Parkin performs quality control of the OM proteins even under steady state, which bears some resemblance to ER-associated degradation (ERAD). ERAD is an important cellular event needed to eliminate aberrant membrane and secretory proteins at the ER, which also involves the proteasome and p97 activities.

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Highlights

- *PINK1* and *Parkin* are involved in regulating mitochondrial fission and fusion dynamics.
- *PINK1* and *Parkin* are implicated in a process of autophagic removal of dysfunctional mitochondria called mitophagy.
- Mitochondrial dynamics and Mitophagy are thought to be required for the maintenance of a healthy mitochondrial network.
- The pathological relevance of mitophagy in PD etiology awaits further investigation.

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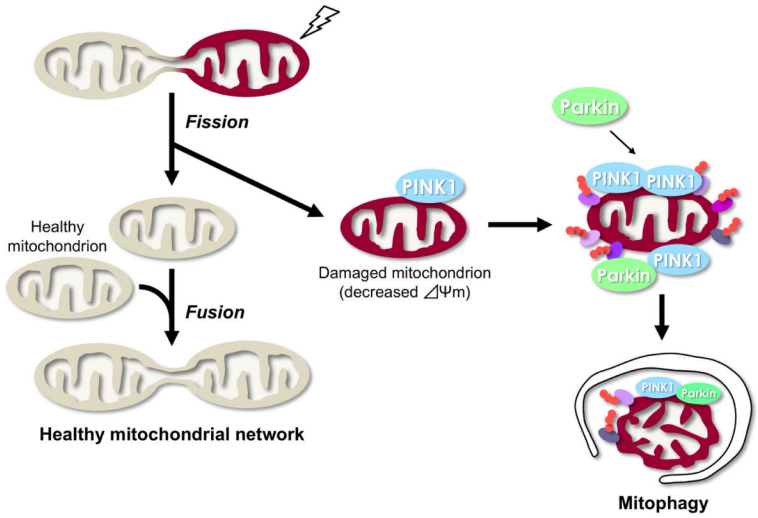

Figure 1.

Mitochondrial fusion and fission events are required for the maintenance of a healthy mitochondrial population (beige). Mitochondrial fusion is thought to facilitate the interchange of internal components such as copies of the mitochondrial genome, respiratory proteins and metabolic products. Mitochondrial fission may play a role in the removal of dysfunctional mitochondria (dark red) with reduced mitochondrial membrane potential (Δψm), through an autophagy-lysosomal pathway named “mitophagy”. PINK1 and Parkin are likely to be involved in this process. PINK1 normally has a short half-life in healthy mitochondria. Upon reduction of the Δψm, PINK1 is stabilized on the OM. Accumulation of PINK1 induces the translocation of Parkin from the cytosol to the mitochondria, leading to Parkin-dependent ubiquitination and degradation of the mitochondrial proteins, and subsequent activation of the autophagy machinery. Ubiquitnated proteins of the mitochondria are shown as ovals with small orange circles.

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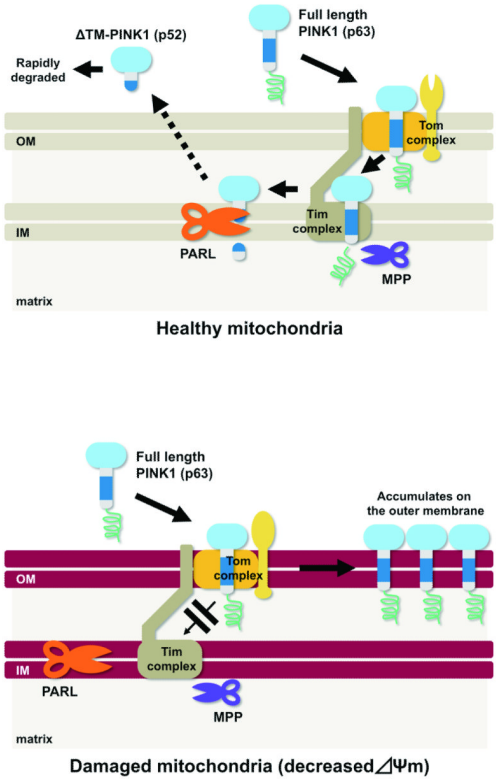

Figure 2.

Proposed model of post-translational processing of PINK1. (Upper) Newly synthesized PINK1 (p63) is targeted to the IM via the Tom and Tim complexes. PINK1 p63 may be processed by mitochondrial processing protease (MPP), which cleaves the mitochondrial targeting sequence to generate a 60-kD PINK1. PINK1 is then cleaved to a 52-kD form within the IM by PARL. The 52-kD PINK1 is released into the cytosol and is degraded by proteasome activity. (Lower) Upon reduction of the Δψm, PINK1 is accumulated at the OM, probably due to inhibition of the translocation through the Tim complex.