

Mitochondrial quality control turns out to be the principal suspect in *parkin* and *PINK1*-related autosomal recessive Parkinson's disease

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Abstract

Mitochondrial dysfunction has long been suspected to play a key role in neurodegeneration in Parkinson's disease. PINK1 and Parkin, the products of two genes responsible for autosomal recessive parkinsonian syndromes with early onset, act as a quality control system on the outer mitochondrial membrane to preserve mitochondrial integrity. While doing so, they interact with multiple molecular actors in processes regulating mitochondrial biology and cell survival. The physiological conditions that mobilize these processes in neurons, and the mechanisms underlying their integration and spatiotemporal coordination, remain to be elucidated. Understanding how dysfunction of these house-keeping pathways leads to the preferential degeneration of a specific neuronal population in Parkinson's disease is a major challenge for future research.

Introduction

Parkinson's disease (PD) is the most frequent movement disorder in modern societies, with a prevalence of 1-2% over age 60. Its cardinal motor symptoms are due to the progressive degeneration of the dopaminergic (DA) neurons in the *substantia nigra pars compacta* accompanied by characteristic intraneuronal protein inclusions, termed Lewy bodies. Environmental factors were long believed to play a predominant role in PD pathogenesis, but this view has evolved in the last 15 years through the study of rare Mendelian forms representing approximately 10% of all cases. At least seven causative genes have been identified, some of which predispose to the more common sporadic disorder [1]. Autosomal recessive early-onset PD is, in most cases, due to homozygous or compound heterozygous mutations in the *parkin* and *PTEN-induced putative kinase 1 gene (PINK1)* genes, encoding a cytosolic E3 ubiquitin ligase and a mitochondrial serine/threonine kinase.

Mitochondrial dysfunction has been suspected to be central to neurodegeneration in PD since the early 1980s marked by the discovery of the mechanism of action of the mitochondrial neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes selective DA neuron death and a parkinsonian syndrome in humans. This idea has been nurtured by repeated reports of moderate deficits in mitochondrial complex I in PD patients, descriptions of transmission of such deficits from patient-derived mitochondrial DNA to healthy recipient cells, and demonstrations of particularly high burdens of somatic mitochondrial DNA mutations associated with a respiratory chain deficiency in DA neurons in the aged human *substantia nigra* [1,2]. Studies in animal and cell models mimicking the genetic conditions responsible for familial forms of PD in humans are revealing an ever-increasing number of putative connections between dysfunction in the corresponding genes and alterations in various aspects of mitochondrial biology [1,2]. However, causal links to mitochondrial dysfunction appear to be particularly straightforward in the case of mutations in the *parkin* and *PINK1* genes. Genetic approaches in *Drosophila* have provided compelling evidence that these genes act in a common pathway regulating mitochondrial morphology, dynamics and function [1,3]. Loss of function of these genes in zebrafish and mammalian models cause defects in mitochondrial respiration, signs of oxidative stress, a decline in mitochondrial membrane potential ($\Delta\Psi$), calcium dyshomeostasis as well as abnormal morphology and dynamics. These studies raise the question as to how the products of these genes cooperate to preserve, together, a plethora of functions related to mitochondrial biology. A ground-breaking discovery in 2008, rapidly extended by several groups world-wide, provided insight: PINK1 and Parkin act jointly in mitochondrial quality control, by sensing mitochondrial dysfunction on the outer mitochondrial membrane (OMM) and coupling it with a program of organelle degradation [4,5]. This review summarizes major advances

made in the past two years and unresolved questions about the molecular mechanisms involved in this program and its relation to other processes affecting mitochondrial quality.

The complex life-cycle of PINK1: a matter of life and death for mitochondria

Initiation of the cascade of events leading to the degradation of dysfunctional mitochondria depends on the peculiar life cycle of PINK1, predicted to carry an N-terminal mitochondrial targeting signal (MTS). Classically, mitochondrial proteins with MTS are imported through the translocases TOM and TIM in the outer and inner mitochondrial membranes in a $\Delta\Psi$ -dependent manner [6]. Their MTS is cleaved in the matrix by the mitochondrial processing peptidase, MPP, in a step thought to be tightly coupled with protein import. Alternatively, atypical mitochondrial targeting modes have been described for PINK1. In functional mitochondria, after processing by MPP, the N-terminal membrane-spanning domain of PINK1 is cleaved by the redundant action of at least two proteases in the inner mitochondrial membrane [7-10]; the resulting smaller fragment is then rapidly degraded by a protease sensitive to proteasome inhibitors [7,11]. Following $\Delta\Psi$ collapse, newly translated PINK1 accumulates as a full-length protein in the OMM with its C-terminal kinase domain facing the cytosol [12-14] and recruits Parkin, in a kinase-dependent manner, to initiate mitochondrial clearance [12,13,15,16]. This targeting mode is also activated by the downregulation of MPP, which artificially blocks import of PINK1 [9]. In this case, Parkin recruitment and mitochondrial clearance are independent of a decline in $\Delta\Psi$ believed to be sensed by PINK1, suggesting functional coupling between loss of import efficiency and mitochondrial degradation [9]. Not surprisingly, full-length PINK1 associates stably with the TOM complex specifically after $\Delta\Psi$ collapse [17]. Does it recruit Parkin from this location (Figure 1)? Does its proximity with the TOM complex constitute an “eat-me signal” for the removal of damaged portions of the mitochondrial network? Or does it merely ensure prompt resumption of its import when $\Delta\Psi$ is restored [17]?

The fate of the unstable smaller fragment of PINK1 is also unclear. It is produced in functional mitochondria but has been found either in the mitochondrion [7] or in the cytosol [9,11]. The cytosolic location is more compatible with the reported sensitivity of PINK1 to specific proteasome inhibitors, but less with putative intramitochondrial protein interactions [18,19]. The presence of a C-terminal OMM retention signal is suggested to preclude full translocation of PINK1 across the OMM during import [20]. How then is the fragment released into the cytosol to be degraded? Regardless of its submitochondrial localization, is the fast turnover of PINK1 compatible with any hypothesized protective functions [18,21]?

PINK1 meets the E3 ubiquitin ligase Parkin on the OMM to trigger mitochondrial clearance: an intricate affair.

Many aspects of the molecular mechanisms regulated by the PINK1/Parkin pathway underlying mitochondrial clearance remain obscure. Initially thought to be an autophagy-dependent event, “mitophagy” also mobilizes the ubiquitin-proteasome pathway [22-24]. The enzymatic activity of Parkin is unmasked on damaged mitochondria by mechanisms that may involve interaction with a privileged binding partner [25]. Ubiquitin, Lys48-linked ubiquitin chains and proteasome subunits associate with mitochondria shortly after $\Delta\Psi$ collapse [23]. The degradation of OMM proteins precedes that of proteins targeted to other submitochondrial compartments. It is independent of autophagy, prevented by proteasome inhibitors and associated with OMM rupture in the absence of apoptotic features [23,24]. It may require intervention of the chaperone p97/VCP to facilitate extraction of the proteins from the OMM [26]. Proteasome and lysosome inhibitors prevent elimination of whole mitochondria, suggesting that the ubiquitin-proteasome and autophagy pathways cooperate in this process [22]. Mitochondria with ultrastructurally preserved OMM are found within autophagosomes, indicating that digestion of the entire OMM is not required for initiation of mitophagy [24]. Rather, the proteasome-dependent proteolysis of one or a few critical targets may suffice. There is no evidence for an involvement of any mitochondrial factor besides PINK1 in this process. Although VDAC1 and Mitofusin 1/2 are early targets of Parkin after $\Delta\Psi$ collapse [22,27,28], these proteins are not needed for mitochondrial degradation [22,29]. TOM subunits are appealing candidates, as they are among the first OMM proteins to be degraded following mitochondrial recruitment of Parkin [23,24]. However, PINK1 chimeras targeted artificially to the OMM did not associate with the TOM complex but still triggered organelle elimination, suggesting that TOM is not required for this process [17].

The role of PINK1 and Parkin in the recruitment of autophagy-related proteins, and the mechanisms by which autophagosomal membranes are generated or translocated in proximity to damaged mitochondria are also unclear. Ubiquitylated OMM proteins may be recognized by the ubiquitin-binding adaptor protein p62 which binds the LC3 family of autophagosomal proteins, but neither massive ubiquitylation of mitochondria nor p62 are essential for mitophagy [29,30]. In the presence of Parkin, “upstream” autophagy (Atg)-related proteins essential for autophagy induction, including components of the class III phosphatidylinositol 3-kinase (class III PI3K) complex, are found in proximity of depolarized mitochondria [31]. Autophagosomes may therefore emerge *de novo*, possibly from the adjacent rough endoplasmic reticulum [31]. Parkin and PINK1 might stimulate the nucleation of preautophagosomal isolation membranes (Figure 1), as they have been independently

implicated in the recruitment of Ambra1, and activator of the class III PI3K complex [32], and in the interaction with its component Beclin 1 [33].

The specific roles of the enzymatic activities of PINK1 and Parkin in the different steps of the mitochondrial clearance program remain mysterious. Autophosphorylation of PINK1 on specific serine residues is essential for recruitment of Parkin to mitochondria [34] and phosphorylation of Parkin by PINK1 may activate Parkin on mitochondria [35]. Whether other critical Atg or OMM proteins are phosphorylated in a PINK1-dependent manner and the relative contribution of PINK1-dependent phosphorylation to mitochondrial degradation remain unclear. The significance of the Lys63- and Lys27-linked ubiquitin-chains formed in the presence of Parkin on depolarized mitochondria also requires investigation [15,23,29].

Is PINK1/Parkin-regulated mitochondrial clearance relevant to neurons?

In mammalian cells, specific mitophagy programs are activated to remove mitochondria during erythrocyte maturation, destroy sperm-derived mitochondria after oocyte fertilization and eliminate damaged mitochondria [36]. The role of the PINK1/Parkin pathway in the degradation of dysfunctional mitochondria has mostly been investigated in non-neuronal cells that, in contrast to neurons, do not rely on mitochondrial respiration for energy production. Van Laar *et al.* [37] questioned the relevance of this pathway in neurons, as they did not observe a redistribution of Parkin to mitochondria or signs of mitophagy at early time points following $\Delta\Psi$ collapse in these cells. These responses were also blocked in non-neuronal cells forced to depend on mitochondrial oxidative phosphorylation for ATP production, suggesting that the activity of the PINK1/Parkin pathway depends on the bioenergetic properties of the cells [37]. Accordingly, Parkin did not associate with respiratory chain-deficient DA neurons in a mouse model in which the gene encoding the mitochondrial transcription factor TFAM was selectively disrupted in DA neurons, and Parkin-deficiency did not modify the mitochondrial phenotype of these mice [38]. Others, however, have reported accumulation of Parkin on mitochondria and Parkin-dependent mitochondrial degradation in primary rat neurons and in human DA neurons derived from induced pluripotent stem cells (iPSc) after prolonged mitochondrial depolarization [39-41]. Therefore, the PINK1/Parkin pathway can, in principle, promote mitochondrial clearance in neurons, possibly with slower kinetics than in other types of cells. Whether it plays a special role in DA neurons remains to be determined. In a Parkin-deficient *Drosophila* model, morphological and functional alterations of mitochondria were exacerbated in these neurons compared to other neuronal subtypes [42]. DA neurons of the *substantia nigra*, are at particular risk because of intrinsic properties, which may explain their preferential vulnerability to PD [1,2]: the cytoplasmic nonenzymatic and enzymatic metabolism of

dopamine leads to the production of reactive oxygen species (ROS); byproducts of dopamine metabolism may cause damage to mitochondrial respiratory chain components and in turn enhance ROS production; the vulnerable neurons rely on L-type $\text{Ca}_v1.3$ channels rather than sodium channels for their pacemaker activity, which exposes them to continuous calcium influxes that may, in the long term, compromise the capacity of mitochondria to buffer calcium. These neurons are also vulnerable to excitotoxicity, as they receive rich excitatory glutamatergic input from the subthalamic nucleus, which can again overburden mitochondrial calcium stores through excessive calcium inflow and fuel a vicious cycle of mitochondrial ROS production. Under these delicate circumstances, rejuvenating mitochondria by appropriate quality control mechanisms may be vital.

Future work must focus on the physiological conditions and signaling pathways that regulate mitochondrial degradation in neuronal and non neuronal cells. Most of the studies have used mitochondrial uncouplers to trigger and investigate this process. These drugs depolarize the entire mitochondrial network and lead to the elimination of all mitochondria from cells. Under physiological conditions, changes in $\Delta\Psi$ would probably be less dramatic and restricted to portions of the network. Exploring mitochondrial degradation under physiological conditions will require the development of more sensitive and precise methods to investigate the loss of mitochondrial material. Such methods will have to distinguish between different, possibly coexisting types of mitochondrial quality control mechanisms in which PINK1 and Parkin could participate, including ubiquitin-proteasome pathway-dependent degradation of mitochondrial proteins [26] and direct lysosomal delivery of selected cargo by mitochondria-derived vesicles [43]. They would also have to take into account and circumvent biases related to the possible functional coupling between mitochondrial degradation and biogenesis, which may also be coordinated by the PINK1/Parkin pathway [44].

Can other functions of PINK1 and Parkin be reconciled with a role in mitochondrial quality control?

The quality of the mitochondrial network is maintained by a number of intertwined processes that, in addition to mitochondrial degradation, include fission and fusion, transport and biogenesis. PINK1 and Parkin appear to regulate each of these processes. However, whether they always do so concertedly remains to be determined. *Drosophila* models brought to light a role of the PINK1/Parkin pathway in promoting mitochondrial fission [1]: increasing the expression of the mitochondrial fission-promoting GTPase Drp1, or decreasing that of the mitochondrial fusion-promoting GTPases OPA1 and Mfn, suppressed the mitochondrial phenotypes caused by loss-of-function mutations in *PINK1* and *parkin*. A role of the PINK1/Parkin pathway in the control of the balance between mitochondrial fusion and fission was confirmed in mammalian cells, although the outcome varied

according to the cell type and experimental context [1,2,45-52] (Figure 1). Parkin was reported to promote the ubiquitin-dependent degradation of the pro-fission proteins, Drp1 and Fis1, and of the OMM profusion GTPases, Mfn1 and Mfn2 [22,27,28,51,53] (Figure 2). These antagonistic, apparently paradoxical, interactions may support maintenance of mitochondrial quality in circumstances requiring different adaptations by the mitochondrial network [54]. For instance, mitochondrial hyperfusion occurs in response to cell stress, whereas during degradation mitochondria must be separated from the network in order to be engulfed by the autophagosome. The Parkin-dependent degradation of Mfn1/2 participates in this process by preventing refusion of damaged mitochondria with the healthy network [22]. Isolation of dysfunctional mitochondria also requires their detachment from cytoskeletal elements. PINK1 and Parkin may affect both kinesin-dependent anterograde and dynein-dependent retrograde movements of mitochondria along microtubules [39,40]. Following $\Delta\Psi$ collapse, they associate with the OMM Rho-like GTPase Miro and its adaptor Milton, which connect mitochondria with microtubules through the kinesin heavy chain; Miro is ubiquitinated and degraded in a PINK1-, Parkin- and proteasome-dependent manner, leading to the detachment of mitochondria from microtubules, mitochondrial arrest [39] (Figure 2) and facilitation of mitochondrial degradation [55]. Whether Miro is a direct substrate of PINK1 or Parkin needs further investigation [39,55].

Parkin is a powerful neuroprotective agent in multiple contexts. Because of its widespread distribution within cells and the variety of proteins with which it interacts, its functions probably extend beyond cooperative interactions with PINK1 on the OMM [56]. Parkin may promote mitochondrial biogenesis by facilitating the degradation of the transcriptional repressor PARIS, which inhibits the expression of the peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α), involved in the upstream control of mitochondrial biogenesis [44]. Whether this function of Parkin is coupled with its role in mitochondrial degradation is not known (Figure 1). Not surprisingly, Parkin also regulates evolutionary conserved cell death pathways by interacting functionally with Bcl-2 family members in the cytosol [57,58]. Following induction of apoptosis, it controls the cytoplasmic levels of proapoptotic Bax, limiting its translocation to mitochondria [58] and increasing the threshold for mitochondrial release of cytochrome c [59,60]. Mitophagy may be a critical checkpoint determining the fate of the cell following the mitochondrial permeability transition and the release of pro-apoptotic proteins [61,62]. The desensitization of mitochondria to Bax-dependent OMM permeabilization by Parkin might therefore shift the balance towards protective mitophagy in critical conditions (Figure 2).

Conclusions

Contributions from many independent laboratories, in the past four years, have provided evidence that PINK1 and Parkin act as a sensor/effector pair, coupling mitochondrial dysfunction with mechanisms of quality control. This function may well be the central route by which the PINK1/Parkin pathway preserves the integrity of mitochondria. Future work will have to focus on the physiological conditions capable of mobilizing this pathway in neurons. What are the physiological signals leading to the accumulation of PINK1 on the OMM and the mitochondrial recruitment of Parkin? The sensor function of PINK1 is intimately linked to its sensitivity to loss of mitochondrial import efficiency, suggesting that it becomes essential when protein flux through the TOM machinery is impaired. Recent studies in *Caenorhabditis elegans* provide a remarkable example of how an analogous mechanism coordinates mitochondrial dysfunction with a protective mitochondrial unfolded-protein response via the mitochondrial import-sensitive transcriptional activator ATFS-1 [63]. Changes to protein import efficiency and reductions in protein-folding capacity in mitochondria are interdependent and have both been reported to occur in PINK1-deficient models and in patients with *PINK1* mutations [64,65]. They may be associated with normal aging and could in turn be exacerbated by defective house-keeping mitochondrial quality control mechanisms.

While assuring their role in these mechanisms, PINK1 and Parkin interact concertedly or separately with a number of molecular actors in processes relevant to mitochondrial biology, which supposedly requires finely tuned spatiotemporal coordination. What are the underlying mechanisms? Intriguingly, many if not all of the processes regulated by the PINK1/Parkin pathway are integrated by a physical platform that recruits several of the key molecular actors involved: the endoplasmic reticulum-mitochondria interface [66]. The possibility that the various functions of PINK1 and Parkin are co-regulated at this interface suspected to play a role in neurodegeneration is therefore an attractive hypothesis to explore.

Finally, is there any contribution of mitochondrial quality control mechanisms and their dysfunction to the degeneration of the DA neurons in the *substantia nigra pars compacta* in sporadic PD or other genetic forms of the disease? These pathways are probably redundant, as indicated by the activation of compensatory mitochondrial autophagy following downregulation of PINK1 in cell models [48]. A comparative analysis of the effects of chemical stressors in iPSc-derived neurons from PD patients with *PINK1* and *LRRK2* mutations has recently confirmed that cellular vulnerability to mitochondrial stress could be a convergent physiopathological mechanism [67]. iPSc-derived neurons from patients with different genetic or idiopathic forms of PD are, therefore, an appealing model for large-scale

exploration of the efficiency of mitochondrial quality pathways in different neuronal contexts, thereby paving the way for the development of targeted neuroprotective treatments.

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Figure 1: The PINK1/Parkin pathway regulates the balance between mitochondrial fusion and fission.

Overview of the key studies describing the effects of overproduction (_{over}) of PINK1 and Parkin or downregulation (_{down}) of the endogenous genes by RNA interference on the appearance of the mitochondrial network in mammalian cells. Variable phenotypes have been reported depending on the cell context and experimental conditions, compatible with excessive mitochondrial fragmentation or mitochondrial hyperfusion. Most of these studies have demonstrated that manipulation of the expression levels of pro-fission (Drp1, hFis) or pro-fusion proteins (OPA1, Mfn2) suppresses the abnormal phenotypes observed, consistent with a role of the PINK1/Parkin pathway in the regulation of mitochondrial dynamics. Drp1K38A: dominant negative variant of Drp1. Mdivi-1: small molecule inhibitor of the GTPase activity of Drp1. Rotenone: mitochondrial complex I inhibitor. Nd: not determined.

Figure 2: Mitochondrial quality control and survival mechanisms regulated by the PINK1/Parkin pathway.

PINK1 and Parkin have cooperative and independent effects on several processes implicated in mitochondrial physiology and cell survival. Their cooperative functions may be exerted through a common platform on the OMM, in proximity to the TOM complex and possibly at the endoplasmic reticulum-mitochondria interface. Parkin has additional regulatory roles in the cytoplasm. Under stress, the PINK1/Parkin pathway may act directly or indirectly on the machinery controlling mitochondrial dynamics, in order to induce either protective mitochondrial hyperfusion or the mitochondrial fission that precedes mitochondrial degradation. Parkin could contribute by ubiquitylating the pro-fission factor Drp1 or the pro-fusion GTPases Mfn1/2. PINK1 and Parkin co-regulate the Rho GTPase of the OMM Miro, which connects mitochondria to the microtubule network through its adaptor Milton and the kinesin heavy chain, to promote isolation and arrest of dysfunctional mitochondria in view of their degradation. Parkin and PINK1 also intervene in the recruitment of the class III PI3K complex activator Ambra 1 and its component Beclin 1 to promote mitophagy. In addition, Parkin activates mitochondrial biogenesis by promoting the degradation of PARIS, a repressor of the transcriptional co-activator PGC-1 α . By regulating Bcl-2 family members in the cytosol, particularly the pro-apoptotic protein Bax, Parkin exerts anti-apoptotic effects by increasing the threshold for the release of cytochrome *c*. Parkin and PINK1 may also contribute to other quality control mechanisms, including the degradation of OMM proteins by the ubiquitin-proteasome pathway or the direct delivery of mitochondrial cargo to the lysosome through mitochondria-derived vesicles (MDV). Question marks indicate open questions in the field.

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