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Current Opinion in Neurobiology

Novel insights into Parkin-mediated mitochondrial dysfunction and neuroinflammation in Parkinson's disease

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Abstract

Mutations in *PRKN* cause the second most common genetic form of Parkinson's disease (PD)—a debilitating movement disorder that is on the rise due to population aging in the industrial world. *PRKN* codes for an E3 ubiquitin ligase that has been well established as a key regulator of mitophagy. Together with PTEN-induced kinase 1 (PINK1), Parkin controls the lysosomal degradation of depolarized mitochondria. But Parkin's functions go well beyond mitochondrial clearance: the versatile protein is involved in mitochondria-derived vesicle formation, cellular metabolism, calcium homeostasis, mitochondrial DNA maintenance, mitochondrial biogenesis, and apoptosis induction. Moreover, Parkin can act as a modulator of different inflammatory pathways. In the current review, we summarize the latest literature concerning the diverse roles of Parkin in maintaining a healthy mitochondrial pool. Moreover, we discuss how these recent discoveries may translate into personalized therapeutic approaches not only for *PRKN*-PD patients but also for a subset of idiopathic cases.

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Current Opinion in Neurobiology 2023, **80**:102720

This review comes from a themed issue on **Metabolic underpinnings of normal and diseased neural function**

Edited by **Russell H. Swerdlow** and **Inna Slutsky**

For a complete overview see the Issue and the Editorial

Available online 4 April 2023

https://doi.org/10.1016/j.conb.2023.102720

Keywords

Parkin, Parkinson's disease, Mitochondria, mtDNA, Metabolism, Inflammation, Mitophagy, Calcium, PINK1, Biogenesis.

Abbreviations

BAK, BCL2-antagonist/killer 1; BAX, Bcl-2-associated X protein; ccf, circulating cell-free; cGAS, cyclic GMP–AMP synthase; mtDNA, mitochondrial DNA; MERCS, mitochondria-ER contact sites; mitoDAMPs, mitochondrial damage-associated molecular patterns; MCU, mitochondrial calcium uptake; MDVs, mitochondrial-derived vesicles; MFN, Mitofusin; NLRP3, NLR family pyrin domain containing 3; OPTN, Optineurin; PARIS, Parkin-interacting substrate; PRKN, Parkin; PINK1, PTEN induced kinase 1; PGC-1a, peroxisome proliferator-activated receptor-gamma coactivator 1 alpha; POLG, mitochondrial polymerase gamma; PD, Parkinson's disease; SIRT1, Sirtuin 1; SN, substantia nigra; STING, stimulator of interferon genes; TOM, translocase of the outer membrane; TBK1, TANK-binding kinase 1; ULK1, unc-51-like autophagy activating kinase 1; VDAC1, voltage-dependent anion-selective channel 1.

Introduction

Parkinson's disease (PD) caused by mutations in the *PRKN* gene was initially reported by Kitada and colleagues in 1998 [1]. The first described index patient of Japanese origin suffered from autosomal recessive early onset parkinsonism with mild foot dystonia, hyperreflexia, diurnal fluctuation, and dopa-induced dyskinesia in addition to the cardinal signs [1]. These symptoms are representative for the majority of *PRKN*-PD patients, who according to the MDSGene database (www.mdsgene.org), have a median age at the onset of 31 years with a classical dopa-responsive PD. While dystonia and dyskinesia are relatively frequently occurring in about 17% of mutation carriers, respectively, cognitive decline does not [2]. Among index patients, exon rearrangements are the most common mutation type with exon 3 deletions being the most abundant [3]. Interestingly, even in monoallelic state, *PRKN* mutations may increase the risk to develop PD. Our previously published meta-analysis of *PRKN* mutation carriers revealed that manifesting heterozygotes have an on average 10-year earlier disease onset than idiopathic PD patients [4].

PRKN encodes an E3 ubiquitin ligase that belongs to the RING-between-RING (RBR) family and which facilitates the transfer of ubiquitin onto target

proteins, thereby regulating their function or labeling them for degradation by the proteasome [5]. By ubiquitination of various targets, Parkin interferes with different levels of mitochondrial quality control. This includes (i) the restoration of dysfunctional mitochondria by fission and fusion or (ii) via mitochondrial-derived vesicles (MDVs), as well as (iii) the removal of mitochondria by mitophagy, which is (iv) paralleled by activation of mitochondrial biogenesis and mtDNA maintenance processes [3].

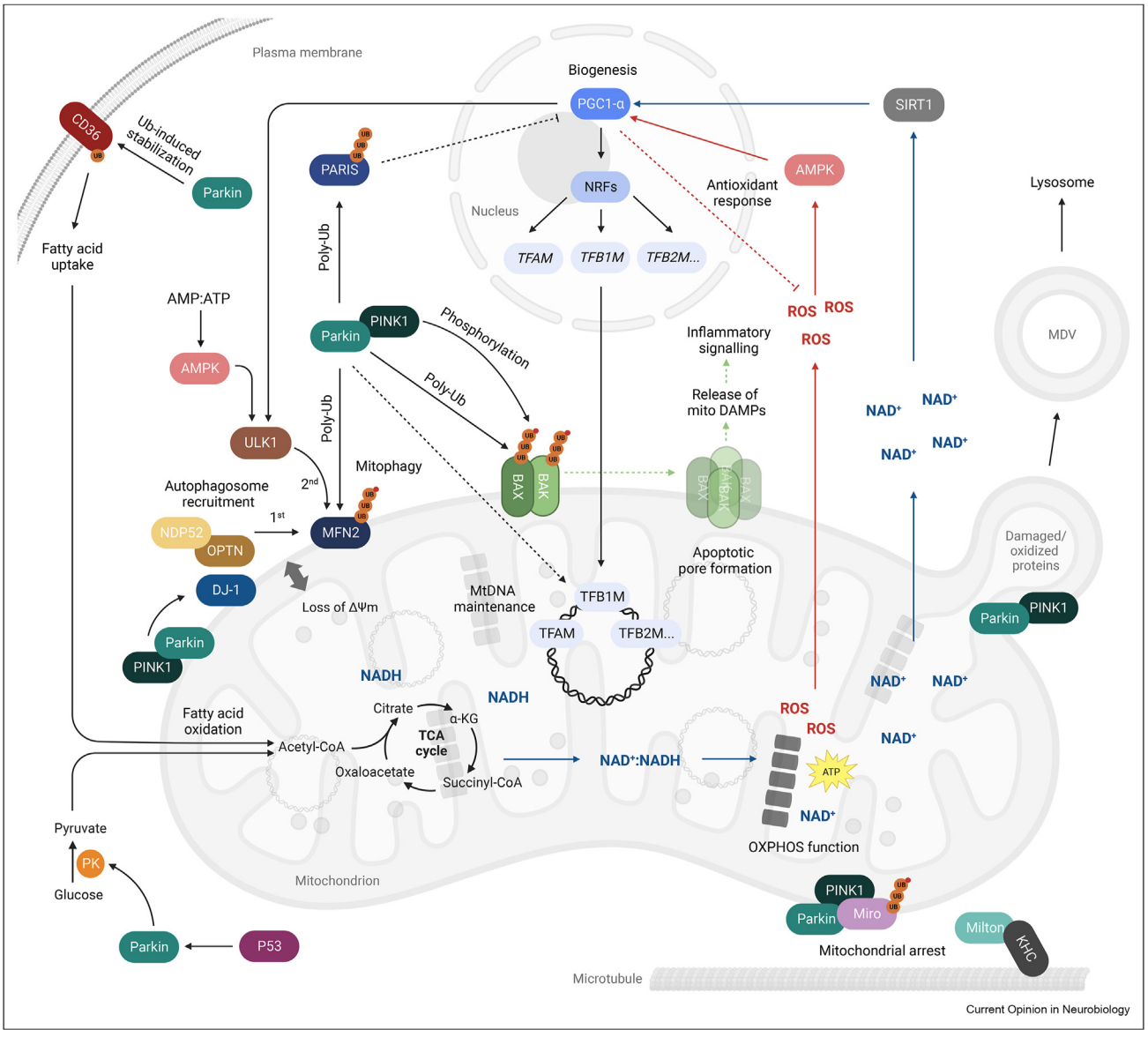
Parkin as regulator of mitochondrial clearance

After the first evidence emerged that Parkin can translocate from the cytosol to mitochondria under the impact of various stressors [6], Narendra and colleagues performed their seminal work, which revealed that Parkin is selectively recruited to the depolarized organelles [7]. This recruitment is mediated by the PD-linked PTEN-induced kinase 1 (PINK1), which accumulates at mitochondria in response to a decline in membrane potential [3]. Mitochondrially localized Parkin then (poly-)ubiquitinates various targets situated at the outer membrane, including Mitofusin 1 and 2 (MFN1 and 2), voltage-dependent anion-selective channel 1 (VDAC1) [8], translocase of the outer membrane (TOM) 20, 40, and 70 [9]. The mitochondrial elimination signal is further amplified by the PINK1-mediated phosphorylation of the resulting poly-ubiquitin chains (at serine 65), which serve as linkers for the autophagy machinery [10]. More specifically, phospho-ubiquitin triggers the recruitment of adapter proteins, such as calcium-binding and coiled-coil domain-containing protein 2 (NDP52/CALCOCO2) and Optineurin (OPTN), which in turn attract the unc-51-like autophagy activating kinase 1 (ULK1) complex to induce the engulfment of damaged mitochondria by autophagosomes [11,12]. This process is AMP-activated protein kinase (AMPK)-dependent [13] and enhanced by TANK-binding kinase 1 (TBK1), which phosphorylates OPTN to increase LC3 binding affinity and to strengthen the link between OPTN and the ubiquitin chains [11]. The AMPK-ULK1 axis is induced by an imbalance in the AMP/ATP ratio and elevated mitochondrial reactive oxygen species (ROS) levels in response to mitochondrial depolarization [14]. Moreover, recent work suggests that the ROS scavenger and PD-associated protein DJ-1 acts as a downstream mediator of the PINK1/Parkin pathway. Imberechts and colleagues showed that the protein encoded by the *PARK7* gene is localized in close proximity to OPTN at the outer mitochondrial membrane and facilitates the recruitment of the autophagy receptor to depolarized mitochondria [15] (Figure 1). By contrast, there are also older reports that contradict these findings [16]. For instance, experiments in M17 cells—a human DA neuroblastoma line—implicate DJ-1 as a modulator of mitochondrial homeostasis that acts in parallel to the PINK1/Parkin pathway [17]. Beyond its function in labeling dysfunctional mitochondria for lysosomal degradation, recent work also showed that Parkin is involved in the regulation of phosphatidic acid (PA) formation and its conversion to diacylglycerol (DAG), thereby enabling the local assembly of autophagosomes [18].

However, for damaged mitochondria to be efficiently eliminated from the general pool, they need to be separated from the network, which is mediated by an interplay of dynamin-related protein 1 (DRP1) and mitofusins [3]. Given that MFN 1 and 2 are targets of PINK1 and Parkin, patient-derived cellular models harboring mutations in these proteins present a wide range of morphological alterations [19]. At the same time, mitophagy induction is closely intertwined with mitochondrial biogenesis. Treatment with the mitochondrial ionophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) (which causes a loss in membrane potential) suffices to trigger the translocation of the transcription factors nuclear respiratory factor 2 (NRF2) and transcription factor EB (TFEB) to the nucleus, where they regulate the expression of mitochondrial proteins, such as the master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) [20] (more details on Parkin's biogenesis-modulating function are provided in the next chapter).

An additional route of mitochondrial quality control is mediated via mitochondrial-derived vesicles (MDVs) that can transport cargo such as lipids or proteins to other organelles [21]. Depending on the cargo composition, MDVs can emerge from the inner or outer mitochondrial membrane and thus be single or double membrane-bound [21]. Parkin and PINK1 are involved in the formation of MDVs that are directed toward lysosomes [21,22]. Under the impact of oxidative stress, these vesicles are budding off from mitochondria potentially with the aim to selectively degrade oxidized and damaged proteins [22] (Figure 1). Of note, recent research revealed that at least two distinct MDV pathways exist. While Parkin/PINK1-dependent MDVs transport mitochondrial content to the lysosome, optic atrophy 1 (OPA1) and sorting nexin 9 (SNX9) mediate the formation of MDVs that target mitochondrial proteins to extracellular vesicles [23]. At the lysosome or late endosome, the fusion of Parkin/PINK1-dependent MDVs is mediated by syntaxin-17, which interacts with synaptosome-associated protein 29 (SNAP29) and vesicle-associated membrane protein 7 (VAMP7) to form a ternary SNARE complex [24]. In addition, Matheoud and colleagues uncovered that Parkin works together with PINK1 to suppress the formation of a subtype of MDVs that present mitochondrial proteins on major histocompatibility (MHC) class I molecules at

Figure 1



Mitochondrial functions of Parkin. By poly-ubiquitinating of outer mitochondrial proteins, such as MFN2, Parkin, together with PINK1, induces the lysosomal degradation of depolarized mitochondria. By facilitating the recruitment of the autophagy receptor OPTN to depolarized mitochondria, DJ-1 has recently been identified as a downstream mediator of this pathway. PINK1/Parkin also coordinate the formation of mitochondria-derived vesicles (MDVs), which allow for the selective removal of damaged mitochondrial components. Beyond the regulation of mitochondrial quality control, Parkin is involved in a multitude of mitochondrial signaling pathways. For instance, Parkin targets PARIS, which inhibits the mitochondrial biogenesis master regulator PGC-1α. Through PGC-1α, Parkin also controls mtDNA maintenance processes. In addition, the generation of novel mitochondria is influenced by cellular metabolism via the SIRT1-PGC-1α route. Moreover, the p53 target Parkin interferes with TCA cycle turnover by acting on pyruvate kinase (PK). A shift in the NAD+:NADH ratio impacts on respiratory chain function and ROS production. ROS can be sensed by AMPK, which—just like SIRT1—affects PGC-1α activity. Also, autophagosome recruitment via ULK1 depends on AMPK. In addition, Parkin-mediated mono-ubiquitination stabilizes CD36, which mediates fatty acid uptake. Moreover, Parkin has been shown to poly-ubiquitinate BAX and BAK (apoptotic proteins that participate in mitochondrial pore formation), thereby controlling the release of mitochondrial damage-associated molecular patterns (mitoDAMPs). Finally, PINK1/Parkin regulate the degradation of Miro, which is required for mitochondrial movement along microtubules. Abbreviations: AMPK, AMP-activated protein kinase; BAK, BCL2-antagonist/killer 1; BAX, Bcl-2-associated X protein; KHC, kinesin heavy chain; Mono-Ub, mono-ubiquitination; NDP52/CALCOCO2, calcium binding and coiled-coil domain 2; Nrf, nuclear respiratory factor; OPTN, Optineurin; p53, cellular tumor antigen p53; PARIS, Parkin-interacting substrate; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1 alpha; Poly-Ub, poly-ubiquitination; SIRT1, Sirtuin 1; TCA, tricarboxylic acid; TFAM, mitochondrial transcription factor A; TFB2M, mitochondrial transcription factor 2B; ULK1, Unc-51 like autophagy activating kinase 1; Ub, ubiquitin. This figure was created with BioRender.com.

the surface, creating a first link between mitochondrial quality control and the immune system [25]—a topic that will be further explored in a separate chapter below.

Parkin's involvement in mitochondrial biogenesis and mtDNA maintenance

Mitochondrial biogenesis is controlled by the master regulator PGC-1α, which acts as a transcription factor that promotes the expression of the nuclear respiration factors NRF1 and 2. In turn, the NRFs regulate the expression of several proteins involved in mtDNA transcription (such as the mitochondrial transcription factors TFAM, TFB1M, and TFB2M), replication, and translation [26].

Initial experiments to unravel the role of Parkin in mitochondrial biogenesis regulation suggested that the protein directly interacts with the mitochondrial transcription factor TFAM. The authors of the study used a combination of chromatin immunoprecipitation (ChIP) and Co-immunoprecipitation (Co-IP) analysis to reveal that Parkin can be imported into mitochondria where it can be associated with the mitochondrial genome via TFAM [27]. Strengthening this finding, Rothfuss and colleagues confirmed an association between Parkin and mitochondrial DNA (mtDNA) in mouse and human brain tissue as well as in SH-SY5Y cells [28].

However, the ability of Parkin to enter mitochondria is still being debated. An indirect way of Parkin affecting mtDNA maintenance mechanisms emerged when the Parkin-PARIS-PGC-1α axis was uncovered. PARIS is a repressor of *PPARG Coactivator 1 Alpha* (*PPARGC1A*) expression (the gene coding for PGC-1α) and its proteasomal degradation is regulated by Parkin-mediated ubiquitination [29]. More recent work in *drosophila* showed that PARIS specifically accumulates in dopaminergic (DA) neurons highlighting the pathway as a possible cause for the selective vulnerability of these cells [30]. Moreover, in human iPSC-derived Parkin KO DA neurons, an upregulation of PARIS was observed, which coincided with a reduction in mitochondrial mass and mtDNA copy number [31]. By contrast, our own work in control and *PRKN*-PD patient-derived midbrain neuronal cultures did not show differences in terms of PARIS protein levels under basal conditions [32]. Nevertheless, in the patient cultures, we observed a disruption of the mitochondrial biogenesis pathway that resulted in mtDNA dyshomeostasis [32]. Interestingly, the NAD^+-dependent energy sensor sirtuin 1 (SIRT1), which acts on mitochondrial biogenesis by modulating *PPARGC1A* expression and PGC-1α protein deacetylation, was downregulated in untreated Parkin-deficient neurons and SH-SY5Y cells [32]. Suggesting that a metabolic shift is the underlying cause of the observed mtDNA phenotypes in the *PRKN*-PD patient neurons, we succeeded to recapitulate the alterations in the mitochondrial biogenesis pathway by increasing the NAD^+:NADH ratios under hypoxic conditions [32]. We observed a lowered mitochondrial complex I activity in our *PRKN*-mutant models, which plausibly contributes to the NAD^+ deficit in our cells. Given SIRT1's central position at the interface between mitochondrial biogenesis and clearance [33,34], it is possible that impaired mitophagy in the absence of Parkin is also triggering mtDNA dyshomeostasis (Figure 1). In fact, transient Parkin overexpression in heteroplasmic cybrid cells sufficed to reduce the abundance of deleterious *COXI* mutations and to permanently augment the amount of wildtype mtDNA molecules [35]. In accordance with this finding, when assessing the somatic mutational load in our midbrain cultures, we found an increase in the number of heteroplasmic variants in mtDNA derived from *PRKN*-PD neurons or blood [36]. Moreover, Nanopore sequencing of mtDNA extracted from nigral and non-nigral postmortem midbrain tissue of a *PRKN*-PD patient and a control indicated that this maintenance impairment is worsened in the oxidative environment of DA neurons [36].

Further strengthening the relevance of Parkin deficiency-induced mtDNA maintenance impairments, the Parkin KO-mtDNA mutator mouse is one of the rare PD rodent models that was reported to present nigrostriatal neurodegeneration [3,37]. In these animals, Parkin depletion was combined with a proofreading-deficient version of mitochondrial polymerase gamma ($polg^{D257A/D257A}$), which leads to an accumulation of mtDNA replication errors [37]. By contrast, more recent experiments in a transgenic model that was equally created by crossing $Parkin^{-/-}$ mice with $polg^{D257A/D257A}$ mice resulted in conflicting findings. Here, the authors neither detected DA neuron loss nor nigrostriatal neurobehavioral deficits. Moreover, they did not observe synergistic effects of the two genotypes with respect to mitochondrial dysfunction, potentially questioning the value of these mice as a reliable PD model [38]. The discrepancy between the two studies may potentially be explained by the different Parkin KO strains (deletion of first RING finger domain versus replacement of exon 3 by EGFP) used to create $Parkin^{-/-}$ $polg^{D257A/D257A}$ mice. Moreover, Scott and colleagues suggest that differences in data analysis approaches as well as the handling of the animals may have contributed to the diverging results [38].

Parkin intervention in cellular bioenergetics

Bioenergetic changes in the PD brain have been well documented with shifts in glucose utilization rates, which reflect neuronal activity and integrity occurring divergently in different regions of the brain [39]. Such changes reflect the ongoing pathological mechanisms of the disease and can be sustained by the inefficient provision of O_2 and energy substrates secondary to cerebrovascular degeneration and hypoperfusion [40,41] and by mitochondrial dysfunction [19]. At the cellular level,

these changes incur in decreased ATP and pronounced oxidative stress compromising cell viability. Recently, this bioenergetic imbalance was further supported by the assessment of activated AMPK levels in distinct regions of the adult mouse brain. pAMPK/AMPK ratios serve as a proxy for cellular energy requirements, and they were found diminished as a consequence of aging and PD (*prkn* or *pink1* null mice) particularly in the ventral midbrain region [42] (Figure 1).

Mitochondrial dysfunction and altered glucose metabolism are likely to be early effectors in the pathomechanism of PD. Both these phenotypes were elevated in peripheral blood mononuclear cells from early idiopathic PD and prodromal patients with rapid eye movement −sleep behavior disorder [43]. Recently, *in vivo* glucose metabolism has been studied in a cohort of genetically stratified PD patients. The contribution of gluconeogenesis to total glucose production was greater in idiopathic PD, but unchanged in biallelic *PRKN* mutation carriers when compared to healthy controls [44]. As discussed by the authors, effects in glucose metabolism may only be detected in *PRKN*-PD patients under stress conditions, such as physical activity, similarly to what was previously reported for *prkn* null mice in an inflammatory context [45].

Insights from tumor biology studies show that Parkin is a target of p53 [46] and can directly regulate the activity of glycolytic enzymes, such as pyruvate kinase [47], favoring oxidative phosphorylation (OXPHOS) and downregulating glycolysis and thus disabling the Warburg effect. Interestingly, proteomic analyses of human neurons derived from a healthy control iPSC line and an isogenic Parkin KO-engineered line revealed the downregulation of distinct enzymes impacting glycolysis and OXPHOS in the mutant cells [48]. The same group later found that increased lactate production, and an accumulation of tricarboxylic acid (TCA) cycle intermediates was present in Parkin KO and patient neurons [49]. These findings correlated with a decreased NAD^+:NADH ratio, which was also detected in our own study [32]. Lowered NAD^+:NADH ratios can be caused by OXPHOS impairments, particularly through the dysfunction of respiratory complex I (NADH:ubiquinone oxidoreductase), which oxidizes NADH to NAD^+ [50], and the activity of which we found to be reduced in Parkin-deficient models [32,51]. The perturbation of NAD^+:NADH levels can lead to redox imbalances, which besides directly affecting enzymes that use these molecules as cofactors, can result in overt oxidative stress [52] (Figure 1).

Lipid metabolism is also impacted by Parkin deficiency. Parkin stabilizes CD36, a fatty acid (FA) transport protein through ubiquitination, and therefore regulates fat uptake and lipid homeostasis [53]. Accordingly, *PRKN*-PD patients present elevated FA metabolites and oxidized lipids in serum [54], plausibly caused by inefficient FA uptake. Noteworthy, impaired mitochondrial FA import with effects on lipid β-oxidation was also observed in Parkin KO neurons [49] (Figure 1).

Parkin's involvement in the regulation of calcium homeostasis

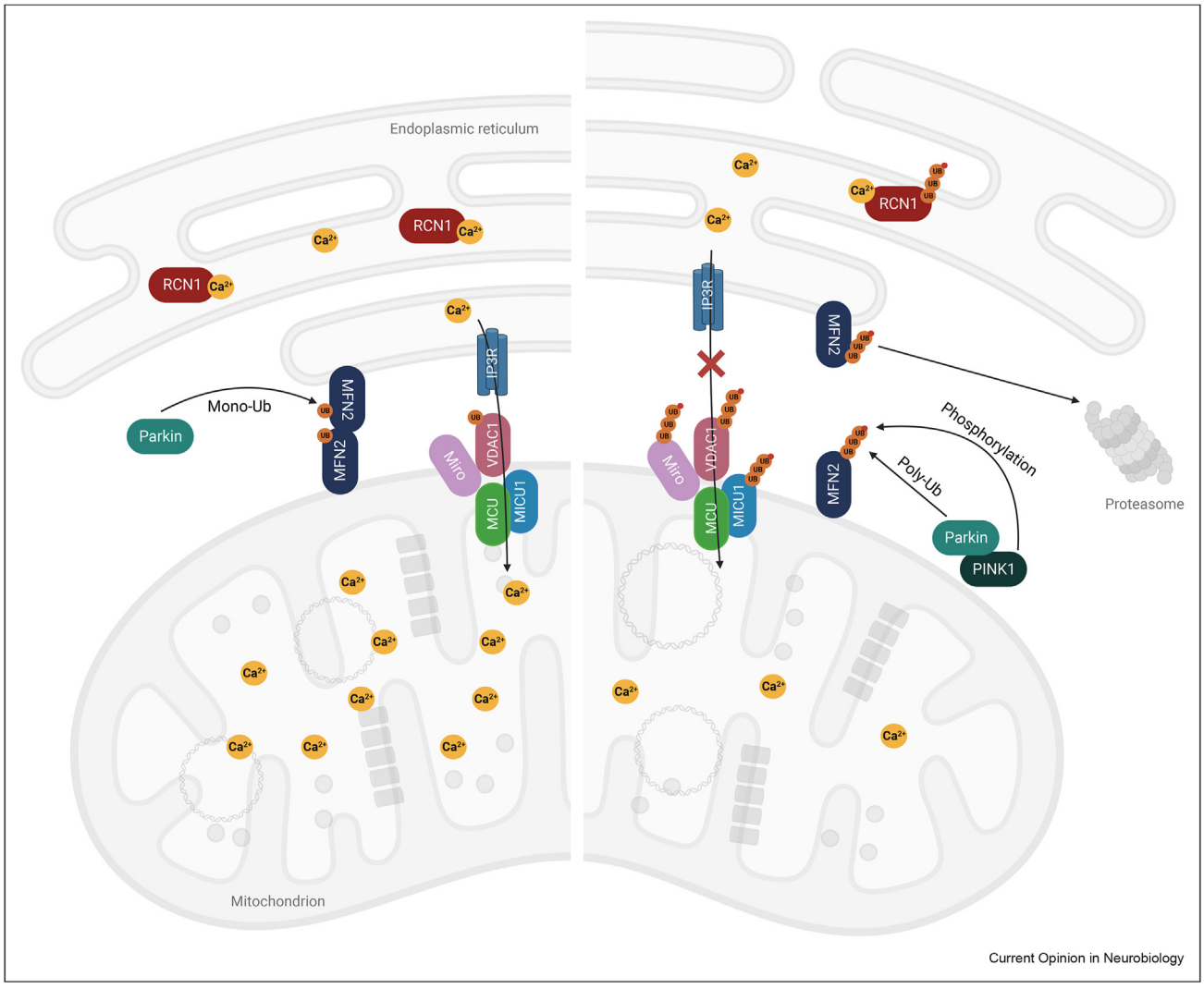
Cellular calcium homeostasis is ensured by complex signaling pathways of various proteins and the interaction of mitochondria with the endoplasmic reticulum (ER) at so-called mitochondria-ER contact sites (MERCS), which provide a hub for cellular calcium handling via the ER and mitochondria. Parkin is engaged in this homeostasis through the regulation of a number of proteins, such as, for instance, Mitofusin 2 (MFN2), which is an important tether for MERCS. Studies in *PRKN*-mutant patient-derived fibroblasts suggest that the ubiquitination of Lysine 416 of MFN2 by Parkin is important for MERCS stability to sustain mitochondrial calcium handling [55,56]. In line with this observation, overexpression of *PRKN* caused an increase of mitochondria-ER tethering, consequently elevating mitochondrial calcium levels, while siRNA-mediated knockdown of *PRKN* reduced MERCS and diminished mitochondrial calcium levels in HeLa or SH-SY5Y cells [57] (Figure 2).

However, in other studies, Parkin deficiency was shown to cause an increase in MERCS, e.g. in Parkin-mutant flies or fibroblasts from PD patients with homozygous mutations in *PRKN* [58,59], as Parkin/PINK1 regulates the phospho-ubiquitination of MFN2, thereby initiating the removal of MFN2 and detethering MERCS to enable mitophagy of liberated depolarized mitochondria [60] (Figure 2).

Contradicting results from different studies implicate that Parkin is critically involved in the stabilization and dissociation of MERCS through the ubiquitination of key proteins. A recent study suggests that the type of Parkin-mediated ubiquitination determines whether MERCS are dissolved in favor of mitophagy: poly-ubiquitination of VDAC1 by Parkin is required for mitophagy, while mono-ubiquitination suppresses apoptosis and fine-tunes mitochondrial calcium uptake (MCU) in a drosophila model [8].

Parkin is also directly responsible for the turnover of various proteins that regulate calcium homeostasis. One of these proteins is phospholipase Cγ1 (PLCγ1). Parkin mutants and knockdown of *PRKN* via siRNA lead to a PLCγ1-dependent increase in cellular calcium levels [61]. Further, Parkin facilitates the degradation of the calcium-binding protein reticulocalbin 1 (RCN1). In astrocytes in the substantia nigra (SN) of *prkn*-deficient mice, RCN1 is upregulated, leading to impaired ER calcium homeostasis [62]. Additionally, Parkin facilitates the turnover of Miro1 and mitochondrial calcium

Figure 2



Parkin at the ER-mitochondria contact sites. Parkin is a crucial regulator of MERCS and proteins involved in calcium homeostasis. Mono-ubiquitination of MFN2 by Parkin is required for the stabilization of MERCS. Likewise, Parkin mediates mono-ubiquitination of VDAC1, thereby favoring calcium influx into mitochondria. In contrast, poly-ubiquitination by Parkin and subsequent phosphorylation via PINK1 primes proteins, such as Miro, VDAC1, MFN2, MICU1, and RCN1, for proteasomal degradation, thus dissolving MERCS and hampering calcium handling. Abbreviations: IP3R, inositol 1,4,5-trisphosphate receptor; MCU - mitochondrial calcium uniporter; MICU1, mitochondrial calcium uptake 1; MFN2, Mitofusin 2; Mono-Ub, mono-ubiquitination; Poly-Ub, poly-ubiquitination; RCN1, reticulocalbin 1; Ub, ubiquitin; VDAC1, voltage-dependent anion-selective channel 1. This figure was created with BioRender.com.

uptake 1 (MICU1) [63,64]. While Miro1 is a calcium-binding GTPase located at the outer mitochondrial membrane that participates in the regulation and stabilization of MERCS [65,66] and directly interacts with the mitochondrial calcium uniporter for the regulation of MCU [67], MICU1 is a regulatory subunit of the MCU complex [68] (Figure 2).

Taken together, Parkin is involved in the regulation of calcium homeostasis by (i) facilitating MERCS stability and thus calcium flux between ER and mitochondria via mono-ubiquitination of proteins, such as MFN2 or VDAC1, (ii) dissociating ER and mitochondria through poly-ubiquitination of MERCS-residing proteins for subsequent proteasomal degradation, thereby initiating mitophagy and hindering ER-mitochondria calcium exchange, and (iii) by direct poly-ubiquitin-mediated regulation of proteins involved in calcium homeostasis, e.g. PLCγ1, RCN1, Miro1, and MICU1.

Parkin and mitochondrial-induced inflammation

Recent years witnessed intense research on the topic of neuroinflammation in aging and neurodegeneration. *PRKN*-associated PD has been in the center of this interest with the publication of landmark studies, such as the one by Sliter and colleagues [45]. This paper establishes a link between mitochondrial stress involving ineffective mitophagy and inflammation in Parkin and

PINK1-deficient models. *In vivo* mitochondrial stress, either applied acutely via exhaustive physical exercise or chronically through the expression of a proofreading-defective mtDNA polymerase (polg), was shown to facilitate the accumulation of mtDNA mutations [37] and increase serum levels of pro-inflammatory cytokines in Parkin-deficient mice. Such increase was accompanied by elevated serum levels of circulating cell-free (ccf)-mtDNA (see Box for more details on the quantification of ccf-mtDNA in biofluids) and was strictly dependent on the cGAS-STING-mediated type I interferon response. Under stress conditions, mtDNA can be released from the mitochondrial compartment through mitochondrial pores [5,69,70], which are formed by the Parkin (poly)-ubiquitination targets BAX [71], BAK [72], and VDAC1 [8]. When mtDNA reaches the cytosol, it can elicit immunological cascades, by triggering DNA sensors, such as cGAS [73]. Moreover, loss of STING is sufficient to rescue motor impairments and neurodegeneration in the Parkin-deficient/polg-mutant mice, emphasizing that inflammation facilitates neurodegeneration [45]. Conversely, constitutively active STING caused neuro-inflammation and degeneration of DA neurons in mice [74]. Interestingly, elevated levels of cytokines and circulating mtDNA were also detected in the serum of *PRKN*-PD patients [45] and positively correlated with disease duration [75]. Recently, our group has strengthened the connection between Parkin, PINK1, and mtDNA homeostasis. Mutations in *PRKN*/*PINK1* increased the heteroplasmic mtDNA variant burden in a dose and PD manifestation-dependent manner. Interestingly, the mtDNA variant load was correlated with serum IL6 levels [36], suggesting a mechanistic link between the mtDNA mutational load and release. Furthermore, NAD^+ reductive stress induced by Parkin deficiency led to impaired biogenesis and mtDNA dyshomeostasis, which resulted in its release from the mitochondrial compartment [32].

Box 1. Technical aspects of circulating cell-free mtDNA analysis

Circulating cell-free mitochondrial DNA copy number (ccf-mtDNAcn) has emerged as a potential biomarker for several disorders, such as cancer or diabetes, and it is now clear that it does not reflect mitochondrial bioenergetics [76]. It is present in the blood as naked DNA, encapsulated in vesicles, or in whole mitochondria [77].

However, ccf-mtDNAcn can easily be measured by PCR. The main determinats for the quality and relevance of the data are the sample (1) collection, (2) preparation, and (3) extraction.

(1) The type of tube used for blood collection can influence the results: EDTA tubes lead to lower amounts of plasma ccf-mtDNA [78]. The processing of the tubes, including centrifugation conditions, also impacts the ccf-mtDNAcn [77].
(2) Ccf-mtDNAcn is commonly measured in plasma or serum. However, it has emerged that during the clotting process of serum, platelets release mtDNA leading to an increased copy number in serum compared to plasma [77].
(3) The method to extract the DNA from the sample also influences the outcome of the assay. It has recently been demonstrated that direct lysis buffers are more efficient than column-based extraction methods. Indeed, lower sample volumes are needed, and it is cheaper and allows for high-throughput quantification using 96-well plates. Moreover, direct lysis presents less bias in the sense where column-based extraction might not retain DNA of all sizes. Nevertheless, direct lysis does have some limitations. Since the initial amount of sample is quite low, the PCR signal is sometimes at the limit of detection of the instrument, which leads to increased variability. Moreover, since this extraction method lacks a purification step, the product might not be compatible with downstream analyses such as sequencing [78].

Despite accumulating evidence suggesting mtDNA as a vector of neuroinflammation via the cGAS-STING axis, other pattern-recognition receptors also participate in Parkin loss-mediated pathology [79]. Panicker and co-authors recently proposed the NLRP3 inflammasome as a Parkin substrate in DA neurons. Following their model, Parkin signals NLRP3 for proteasomal degradation through ubiquitination, impeding its accumulation and priming. Concurrently, under conditions of Parkin deficiency, PARIS accumulates and drives mitochondrial dysfunction and ROS generation, which activates the primed NLRP3 complex contributing to DA neuron demise in a cell-autonomous manner [80]. Noteworthy, previous reports suggested the engagement of NLRP3 inflammatory signaling in PD [81], which does, however, not exclude a role for mtDNA as the immunostimulatory molecule [79,82].

Conclusion and outlook

While significant progress has been made in understanding Parkin's involvement in individual aspects of mitochondrial signaling, the interconnection of these processes [83] remains to be explored in more detail. For instance, it is currently unclear whether certain functions of Parkin are more relevant in specific cell types—e.g. Parkin's immune modulatory role may be most crucial in microglia, whereas its metabolic influence could be critical for the neuronal support by astrocytes.

However, first attempts at tackling these overarching research questions suggest that the various mitochondrial phenotypes caused by mutations in Parkin may be the consequence of (aberrant mitophagy-induced) dysmetabolism and inflammation—two processes, that themselves are tightly linked [84]. Longitudinal studies in multi-cellular systems are now needed to elucidate the cascade of events that accounts for bioenergetic impairments and immune responses in *PRKN*-PD. Only experiments in co-cultures (or even triple-cultures) will ultimately help to determine the relative contribution of mitochondrial deficits in neurons and glia to neuroinflammation.

However, even considering our current understanding of mitochondrial signaling in *PRKN*-PD, the newly uncovered link between metabolic stress and neuroinflammation opens novel routes for therapeutic intervention: First, targeting cerebral bioenergetics in patients with *PRKN* mutations could be a promising approach. NAD^+ replenishment therapy, which acts on mitochondrial biogenesis via SIRT1, was suggested to be neuroprotective in aging and neurodegeneration, and has recently been shown to induce mild clinical improvements in PD patients in a process that involved immune modulation [85]. Second, the application of anti-inflammatory drugs, which was associated with reduced PD risk in population studies [86], may be explored as a treatment strategy for *PRKN*-PD. Third, one may speculate that tightly monitored (e.g. by measuring cytokine and ccf-mtDNAcn concentrations in blood) physical exercise may improve the clinical outcome of *PRKN*-PD patients, if a level of activity were reached that allows to improve mitochondrial fitness [87] without triggering detrimental inflammatory effects. Given that Parkin deficiency was also documented in idiopathic PD [88], the proposed interventions may not only be of relevance for *PRKN*-PD patients but could ultimately benefit a larger number of individuals suffering from the movement disorder.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data were used for the research described in the article.

Acknowledgements

S.L.P. and A.G. were supported by the Luxembourg National Research Fund (FNR) within the ATTRACT Program (Model-IPD, FNR9631103). A.G. and S.L.P. were generously supported by a donation from Le Foyer Assurances Luxembourg. S.L.P. was further supported by the FNR INTER Mobility Scheme (INTER/MOBILITY/19/13512561). A.G. and S.D. received funding through an FNR INTER grant (ProtectMove, FNR11250962 and INTER/DFG/19/14429377) and via a grant from The Michael J. Fox Foundation within the framework of the Mitochondrial Biomarkers Spring 2018 Program. Moreover, A.G. received funding from the FNR as part of the National Centre of Excellence in Research on Parkinson's disease initiative. In addition, this work was supported by the Nachwuchsförderung der Deutschen Gesellschaft für Parkinson und Bewegungsstörungen e.V. to DG. Finally, AH received funding from the "Hermann und Lilly Schilling-Stiftung für medizinische Forschung im Stifterverband".

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In this study, Ham and colleagues assessed how mono- versus poly-ubiquitination of VDAC1/Porin by Parkin affects the function of the mitochondrial core component. While both processes, VDAC1 mono- and poly-ubiquitination, depend on PINK1/Parkin, they take place independently from each other. The authors reveal that by controlling the number of ubiquitin molecules linked to VDAC1, Parkin determines whether mitophagy (poly-ubiquitination) will be induced or apoptosis (mono-ubiquitination) inhibited in the cells. Moreover, studies in porin-mutant flies suggested that porin mono-ubiquitination is critical for the regulation of mitochondrial calcium uptake, which in turn affects the initiation of apoptosis. In conclusion, this work uncovered a dual role for Parkin-mediated ubiquitination of VDAC1.

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Using PD patient fibroblasts and iPSC-derived neuronal models, Imberechts and colleagues explored whether the *PARK7* encoded protein DJ-1 is involved in PINK1/Parkin-mediated mitophagy. While

loss of DJ-1 did not prevent the recruitment of PINK1 and Parkin to depolarized mitochondria, the recruitment of the autophagy receptor OPTN was impaired under these conditions. Moreover, after inducing mitophagy in wildtype fibroblasts or neurons, DJ-1 was localized in close proximity to OPTN at the outer mitochondrial membrane. Finally, the authors performed a set of experiments in *PINK1*- and *PRKN*-mutant fibroblasts and neurons to demonstrate that the translocation of DJ-1 to depolarized mitochondria was disrupted in the absence of these proteins. Together, these results suggest that DJ-1 acts downstream of PINK1 and Parkin in the mitophagy pathway.

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Here, we showed that mitochondrial biogenesis is affected in neuronal cultures derived from *PRKN*-PD patient iPSCs and in Parkin KO SH-SY5Y cells, being accompanied by mtDNA dyshomeostasis. We have detected altered bioenergetics in the Parkin-deficient models resulting in decreased NAD+/NADH ratio, which impacted on SIRT1 activity, a major regulator of mtDNA biogenesis and mitophagy. *PRKN*-mutant models presented increased mtDNA extrusion from the mitochondrial compartment, which can elicit inflammation. mtDNA dyshomeostasis was confirmed in a postmortem brain from a *PRKN*-PD patient, which concurrently presented increased microgliosis. Finally mtDNA/LPS treatments elicited a greater immune response in microglia-neuronal co-cultures derived from *PRKN*-PD patients. Together, these findings reinforce the existence of a crosstalk between mitochondrial homeostasis, cell bioenergetics and neuroinflammation, which is regulated by Parkin.

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This study performs metabolic profiling of iPSC-derived neurons to explore bioenergetic impairments induced by Parkin deficiency. The authors found overall changes in glycolysis, the TCA cycle and carnitine metabolism, which are associated with diminished ATP levels and unbalanced NAD^+/NADH ratios. Mitochondrial ultrastructural and functional alterations were also identified. Furthermore Parkin-deficient models exhibited increased oxidative stress and lowered antioxidant response. Overall, this paper adds more detail to a prototypical bioenergetic profile of Parkin-deficient neurons, which can sustain some of the pathological mechanisms of PD.

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Interleukin 6 (IL6) and circulating cell-free mitochondrial DNA (ccf-mtDNA) were assessed in the serum of 245 participants. The authors showed that Parkinson's disease (PD) patients with biallelic *PRKN/PINK1* mutations had increased levels of IL6 in the serum and that the levels of IL6 correlated with disease duration in those patients, while this was not the case for idiopathic PD (iPD) patients. Moreover, in PD patients with biallelic and heterozygous *PRKN/PINK1* mutations, the levels of ccf-mtDNA were increased compared to iPD patients, while the levels in controls and unaffected heterozygotes were similar. Finally, they showed that ccf-mtDNA could potentially be used to discriminate between iPD cases and manifesting carriers of heterozygous *PRKN/PINK1* mutations.

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Trumpff and colleagues highlight the variation in sample collection, processing and analysis across studies focusing on circulating cell-free mitochondrial DNA (ccf-mtDNA). cf-mtDNA levels are highly dynamic and vary quickly upon stress or exercise. Therefore, even slight differences in the experimental design lead to conflicting results across studies. The authors propose guidelines for sample collection, ccf-mtDNA isolation and quantification together with reporting standards to facilitate reproducible research in this field.

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By depleting parkin specifically in mouse SN pars compacta DA neurons, the authors showed a cell-autonomous activation of the NLRP3 inflammasome, which was sufficient to induce DA neuron degeneration. The authors go further to show that Parkin regulates NLRP3 activation in two ways: i) by directly poly-ubiquitinating NLRP3 and targeting it for proteasomal degradation, and ii) by suppressing PARIS, which would inhibit mitochondrial biogenesis, leading to mitochondrial stress and ROS, which can serve as a second stimulus to drive NLRP3 activation.

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Brakedal and colleagues performed a double-blinded phase I clinical trial to test the safety and effectiveness of nicotinamide riboside (NR) in replenishing cerebral nicotinamide adenine (NAD) levels. Thousand mg NR or a placebo was taken orally by 30 newly diagnosed treatment-naive Parkinson's disease (PD) patients for 30 days. Individuals who received NR and who showed elevated brain NAD levels also presented with metabolic alterations and mild clinical improvements. Of note, NR treatment led to increased expression of genes involved in the regulation of mitochondrial, lysosomal and proteasomal processes. Finally, NR intake reduced cytokine levels in serum and cerebrospinal fluid strengthening the link between mitochondrial metabolism and inflammation in PD.

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