Exploring the contribution of the mitochondrial disulfide relay system to Parkinson's disease: the PINK1/CHCHD4 interplay

Giuseppe Arena*, Nazanine Modjtahedi, Rejko Kruger

Parkinson's disease (PD) is a common movement disorder of the elderly caused by the degeneration of dopaminergic neurons in the substantia nigra pars compacta of the brain.

Both environmental and genetic factors pointed out mitochondrial dysfunction as a major cause of neurodegeneration in PD. Pioneering studies using mitochondrial toxins revealed their ability to trigger dopaminergic cell death and irreversible parkinsonism in different animal models (Poewe et al., 2017). Typical features of mitochondrial dysfunction have been also observed in the human brain of idiopathic PD cases, showing alterations of respiratory chain complex I and IV activity, accumulation of mtDNA deletions and increased oxidative stress (Bender et al., 2006). Moreover, a number of genes found mutated in familial PD forms encode for proteins involved in the maintenance of mitochondrial homeostasis and quality control. Among these, the PINK1 gene encodes a mitochondrial serine/threonine kinase implicated in key neuroprotective functions, including mitophagy, regulation of mitochondrial transport, control of the mitochondria/endoplasmic reticulum crosstalk and calcium homeostasis (Brunelli et al., 2020).

Like the majority of nuclear-encoded mitochondrial proteins containing an N-terminal mitochondrial localization signal (MLS), PINK1 is actively transported into mitochondria through the translocases of the outer and inner mitochondrial membranes (TOM/TIM) complex, driven by the electrical potential (Δψm) across the inner mitochondrial membrane (IMM). Once the N-terminal domain of PINK1 reaches the mitochondrial matrix, the full-length 63kDa protein is first cleaved by the MPPα/β protease and then by the IMM-resident PGAM5-associated rhomboid-like (PARL) protease, generating an unstable 52 kDa product that is retro-translocated to the cytosol and finally degraded by the proteasome via the N-end rule pathway (**Figure 1**, left panel). Other proteases embedded in the IMM, such as YME1L and AFG3L2, have been reported to cooperate with PARL in order to fulfill the processing of PINK1 in healthy mitochondria. Conversely, PINK1 cleavage is impaired upon Δψm dissipation induced by oxidative phosphorylation (OXPHOS) uncoupling agents, which leads to the accumulation of full-length PINK1 on the outer mitochondrial membrane (OMM). This induces the translocation of the ubiquitin-ligase Parkin from the cytosol to the surface of depolarized organelles, thus activating a complex signaling cascade that (i) excludes damaged mitochondria from the network by preventing their movement and (ii) ensures their selective elimination through the autophagy pathway (Sekine and Youle, 2018; **Figure 1**, right panel).

It is worth noting that not only the collapse of Δψm, but also generation of mitochondrial reactive oxygen species or even the accumulation of misfolded proteins in the mitochondrial matrix have been previously described as potential inducers of PINK1-dependent mitophagy. Further expanding the plethora of stress conditions and signals triggering this pathway, Gao et al. (2020) recently found that the redox-regulated CHCHD4/GFER mitochondrial import pathway was required for PINK1 stabilization and mitophagy execution. This evolutionary conserved disulfide relay system, mammalian homologue of the yeast Mia40/Erv1 pathway, operates in the mitochondrial intermembrane space (IMS) to regulate the import and/or proper folding of a set of substrates carrying specific cysteine motifs; the CHCHD4 protein is the core component of this machinery that catalyzes the oxidation of the cysteine residues to disulfide bridges (**Figure 1**). Notably, many of these substrates participate in potentially relevant pro-survival and neuroprotective activities, including biogenesis and assembly of the respiratory chain complexes, regulation of mitochondrial ultrastructure and dynamics, general mitochondrial import control, calcium storage, lipid homeostasis and mitochondrial protein translation (Reinhardt et al., 2020). In this light, impairment of the CHCHD4-dependent pathway would have profound effects on mitochondrial function and neuronal metabolism, thus representing a candidate mechanism contributing to neurodegeneration in PD. In accordance with this hypothesis, heterozygous mutations in the CHCHD4/Mia40 substrates CHCHD2/MIX17A and CHCHD10/MIX17B have been detected in distinct families affected by late-onset autosomal dominant PD as well as in sporadic PD patients (Reinhardt et al., 2020). Both CHCHD2 and CHCHD10 mutations are suggested to disrupt the integrity of respiratory complexes by impinging on the mitochondrial contact site and cristae organization system (MICOS). Of note, CHCHD2 and CHCHD10 are binding partners and recent studies suggest that mitochondrial dysfunction caused by PD-associated mutations of CHCHD2 could implicate the perturbation of the physical and functional interaction with CHCHD10 (Reinhardt et al., 2020). Deficiency in the unique Drosophila orthologue of CHCHD2/CHCHD10 results in loss of dopaminergic neurons and motor dysfunction, a phenotype likely due to alteration of cristae ultrastructure, which in turn leads to impaired mitochondrial respiration and increased oxidative stress. In line with this, mitochondrial phenotypes observed in mutant flies are rescued by overexpressing the wild-type human CHCHD2, but not its PD-associated mutant variants (Imai et al., 2019).

CHCHD2 and CHCHD10 are prototypes of proteins that carry simultaneously an N-terminal MLS and a coiled-coil-helix-coiled-coil-helix (CHCH) domain, characterized by four cysteine residues that form two disulfide bonds. Interestingly, a recent study indicated that the CHCH domain of CHCHD10 was required for its CHCHD4-mediated import to the IMS, rather than its N-terminal positively charged MLS (Imai et al., 2019). A similar mechanism could be also responsible for PINK1 stabilization on the OMM of depolarized mitochondria during mitophagy, as demonstrated by inhibition of PINK1 accumulation upon CHCHD4 or GFER knockdown, or after treatment with chemical inhibitors of the CHCHD4/GFER disulfide relay system (Gao et al., 2020).

Structural analysis of the PINK1 protein as well as sub-mitochondrial localization studies of PINK1 deletion mutants allowed to identify a putative OMM localization signal (OMS) between the amino acids 74 and 93 of PINK1, which deletion impairs PINK1 retention on the surface of damaged mitochondria and the subsequent mitophagy cascade (Sekine and Youle, 2018). It is worth noting that a cysteine (amino acid 92) is present in this region, whereas two other cysteine residues (amino acids 96 and 125) are located in the just adjacent transmembrane domain of PINK1. In this light, one could speculate that, upon mitochondrial damage, activation of the CHCHD4/GFER disulfide relay system may induce the formation of disulfide bonds

in the region encompassing the OMS and the transmembrane domain of PINK1. This could in turn inhibit the mitochondrial import and full proteolytic processing of PINK1 and thus promote its accumulation on the OMM required for mitophagy induction. According to this model, two PD-associated mutations involving cysteine residues located within this region have been described, namely the C92F and C125G substitutions. Importantly, both display reduced stabilization of PINK1 and, limited to the C125G mutant, impaired Parkin recruitment to mitochondria in response to carbonyl cyanide m-chlorophenyl hydrazone (CCCP) treatment (Sekine et al., 2019).

Another PINK1 domain that could interact with CHCHD4 is the amphipathic helix (amino acids 166-172) containing hydrophobic and aromatic residues in the vicinity of a potential docking cysteine 166. This segment of PINK1 could represent a putative internal IMS-targeting sequence, which is proposed to allow the incoming protein substrate to recognize and interact with the CHCHD4 oxidase (Reinhardt et al., 2020). Indeed, deletion of this region (PINK1 Δ166-171) strongly decreased the PINK1 binding to CHCHD4 and, most importantly, two PD-related mutations in PINK1 (i.e., A168P and V170G) also display a reduced interaction with CHCHD4 and impaired accumulation upon CCCP treatment (Gao et al., 2020).

If, on the one hand, the CHCHD4/GFER mitochondrial import machinery seems to be important to assist PINK1 accumulation on the OMM in the early phase of mitophagy, on the other hand PINK1 could also regulate the activity of CHCHD4/GFER, thus providing a positive feedback loop that sustains the import of other CHCHD4 substrates. In fact, the interaction between endogenous PINK1 and CHCHD4 was detected not only in cells treated with CCCP but also under basal conditions, suggesting a potential interplay between these two proteins that extends beyond the mere regulation of PINK1 (Gao et al., 2020). By controlling the import and/or proper folding of specific subsets of proteins, impairment of CHCHD4/Mia40 activity is known to (i) alter mitochondrial morphology and dynamics, (ii) decrease the formation of electron transport chain complexes, (iii) induce mitochondrial calcium dyshomeostasis and (iv) disrupt phospholipid metabolism, thus impinging on the integrity of the IMM and cristae ultrastructure (Reinhardt et al., 2020). Notably, many of these alterations are often observed in PINK1 deficient cells as well. At least six CHCHD4 substrates (NDUFA8, NDUFAF8, NDUFB7, NDUFB10, NDUFS5 and NDUFS8) are directly involved in the biogenesis and proper assembly of

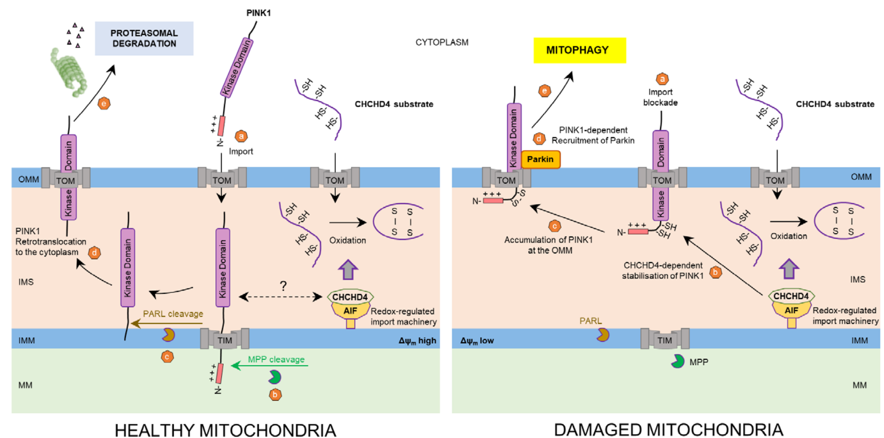

Figure 1 | The cross-talk between PINK1 and the CHCHD4-dependent import machinery.
Left panel: healthy mitochondria. In polarized mitochondria (Δψm high), PINK1 is actively imported into the organelle through the TOM/TIM translocases (a). The positively charged N-terminal (N-ter) region of the full-length PINK1 is first cleaved by the mitochondrial matrix (MM) protease MPP (b), followed by a further processing operated by the inner mitochondrial membrane (IMM)-resident protease PARL (c). This generates an unstable shorter form of PINK1 (52 kDa) that is retrotranslocated to the cytosol (d) and finally degraded by the proteasome (e). During its transit in the intermembrane space (IMS) of healthy mitochondria, PINK1 was found to interact with the CHCHD4 oxidase, the core component of a redox-regulated import machinery that regulates the import and proper folding of cysteine motif-carrying protein substrates (Imai et al., 2019). The interplay between PINK1 and the AIF/CHCHD4 complex could be determinant in the physiological fine-tuning of mitochondrial homeostasis and function. Right panel: damaged mitochondria. Upon dissipation of the mitochondrial membrane potential (Δψm low), PINK1 import is impaired (a) and the full-length protein accumulates on the outer mitochondrial membrane (OMM, c) thus triggering the signal for Parkin recruitment (d) and mitophagy activation (e). The CHCHD4-dependent pathway was found to be required for PINK1 stabilization (b) and mitophagy execution, an effect likely mediated by the formation of disulfide bonds (S-S) that inhibit the proteolytic cleavage of PINK1 (Gao et al., 2020). PARL: PGAM5-associated rhomboid-like protease.

mitochondrial respiratory complex I, which activity is impaired in PD patients carrying distinct PINK1 loss-of-function mutations and, more in general, in the brain of sporadic PD patients (Brunelli et al., 2020; Reinhardt et al., 2020). Decreased complex IV activity is also observed in PINK1-KO flies and in PINK1 null dopaminergic neurons, an effect previously linked to down-regulation of specific mitochondrial chaperons involved in the assembly of complex IV subunits (Kim et al., 2012). Interestingly, the CHCHD4/Mia40 substrates CMC1, CMC2, COX6B1 (otherwise called COXG), COA4, COA5, COA6, COX17 and the copper chaperone CHCHD7 (else known as COX23) also participate in the biogenesis and assembly of mitochondrial complex IV (Reinhardt et al., 2020). Another CHCHD4/Mia40 substrate, namely TRIAP1/Mdm35, could indirectly influence the activity of electron transport chain by regulating the phospholipid composition (i.e., cardiolipin) of the IMM, which is essential for the accurate assembly of respiratory complexes and supercomplexes (Maguire et al., 2017; Reinhardt et al., 2020). To this regard, cardiolipin levels are significantly reduced in mitochondrial fractions from PINK1-KO mouse embryonic fibroblasts (MEFs) compared to control MEFs, whereas cardiolipin supplementation rescues the impairment of complex I activity observed in PINK1 deficient flies (Brunelli et al., 2020). As a structural component of the IMM, cardiolipin also plays a role in other key functions that were found altered in PINK1 mutant cells, including mitochondrial dynamics and apoptotic cell death.

Even if it appears quite clear that PINK1 and CHCHD4 regulate common downstream processes maintaining mitochondrial homeostasis and function, further efforts are needed to provide a more comprehensive characterization of the molecular mechanisms regulating the PINK1/CHCHD4 interplay as well as its physiological relevance in the neuronal context. To this regard, useful hints may come from the discovery of apoptosis-inducing factor (AIF) as a major CHCHD4 binding partner in the IMS (Reinhardt et al., 2020). Aifm1 depletion in the mouse forebrain results in a neurodegenerative phenotype characterized by altered cortical development and decreased neurons survival, an effect likely due to the accumulation of dysfunctional mitochondria, which appeared fragmented, with aberrant cristae ultrastructure and reduced OXPHOS capacity. A viral insertion in the Aifm1 gene, which causes an 80% reduction in the expression of AIF, underlies the progressive neuronal degeneration observed in the *Harlequin* (*Hq*) mutant mice, one of the most reliable models of mitochondrial complex I deficiency. Moreover, *Hq* mice are highly susceptible to the MPTP toxin previously associated to PD,

as demonstrated by the strong nigrostriatal dopaminergic loss even in presence of subtoxic doses (Reinhardt et al., 2020). Based on the current knowledge, AIF acts upstream of CHCHD4 to regulate the co-translational import of CHCHD4 itself and, in turn, the activity of the whole CHCHD4/GFER disulfide relay system. Thus, mitochondrial dysfunctions observed in AIF deficient models, including impaired complex I activity, are significantly restored upon CHCHD4 over-expression, whereas the beneficial effects of ectopically expressed AIF are abolished after CHCHD4 downregulation (Reinhardt et al., 2020).

The exact molecular mechanism through which AIF may control the CHCHD4-dependent mitochondrial import machinery has not been fully elucidated yet. Previous findings revealed that, upon binding to the reduced pyridine nucleotide cofactors nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), AIF undergoes a conformational modification, which appears to enhance its interaction with CHCHD4 (Reinhardt et al., 2020). Interestingly, PD-related mutations in PINK1 and GBA genes decreased nicotinamide adenine dinucleotide (NAD^+) levels, leading to mitochondrial dysfunction and neurodegeneration. In contrast, boosting the intracellular pool of pyridine nucleotide cofactors upon supplementation with the NAD^+ precursors Nicotinamide or Nicotinamide Riboside rescued mitochondrial defects and neuronal loss in Drosophila and human induced pluripotent stem cells-derived models of PD (Schöndorf et al., 2018). If this translates in increased AIF/CHCHD4 binding and enhanced CHCHD4 activity remains to be demonstrated, as well as the potential contribution of this pathway in the neuroprotective effects of NAD^+ precursors.

Another mechanism underlying regulation of the CHCHD4/GFER disulfide relay system could involve potential post-translational modifications of either AIF or CHCHD4, which may control their protein-protein interaction and modulate the activity of AIF/CHCHD4 complex. If PINK1 could play a role in that sense, for instance by means of its kinase activity, still needs further investigation, but distinct observations make this possibility worthy of being explored. First, PINK1 and CHCHD4 bind to each other not only in dysfunctional mitochondria, destined to mitophagy, but also in healthy polarized organelles, thus extending the functional consequences of their interaction behind the simple control of PINK1 accumulation on the OMM by CHCHD4 (Gao et al., 2020). This also highlights the importance, often underestimated, of the mitochondrial IMS, where PINK1 localizes during its mitochondrial import, just before its processing and retro-translocation to the cytosol (**Figure 1**). Moreover, even if some *in vitro* experiments suggested that the C-terminal domain of PINK1 could remain in the cytosol during the import process, previous findings reported that PINK1 interacts with and phosphorylates a number of IMS proteins, such as HtrA2/Omi and TRAP1 (Arena G and Valente, 2017).

Future research aiming to better characterize the PINK1 activity in the IMS of healthy mitochondria and its relationship with the AIF/CHCHD4 machinery is warranted, and will answer the question whether stimulating the disulfide relay system could represent an effective strategy to rescue mitochondrial dysfunction in PD.

This work was supported by grants from the Luxembourg National Research Fund (FNR) for the National Centre for Excellence in Research on Parkinson's disease (NCER-PD to RK), the PEARL program (FNR/P13/6682797 to RK) and MiRisk-PD (C17/BM/11676395 to RK and GA). RK has also received research grants from the Michael J Fox Foundation, the European Union's Joint Program-Neurodegenerative Diseases (JPND; COURAGE-PD), the European Union's Horizon2020 research and innovation program (No. 692320), and the Federal Ministry for Education and Research (BMBF; Mito-PD 031 A 430 A). NM was supported by the French National Cancer Institute (INCA2017-1-PLBIO-08) and by Fondation pour la recherche contre le cancer (ARC).

Giuseppe Arena*, Nazanine Modjtahedi, Rejko Kruger

Translational Neuroscience group, Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-sur-Alzette, Luxembourg (Arena G, Kruger R)

Université Paris-Saclay, Gustave Roussy Institute, CNRS, Metabolic and systemic aspects of oncogenesis for new therapeutic approaches, Villejuif, France (Modjtahedi N)

Parkinson Research Clinic, Centre Hospitalier du Luxembourg (CHL), Esch-sur-Alzette, Luxembourg (Kruger R)

Transversal Translational Medicine, Luxembourg Institute of Health (LIH), Esch-sur-Alzette, Luxembourg (Kruger R)

***Correspondence to:** Giuseppe Arena, PhD, giuseppe.arena@uni.lu.

https://orcid.org/0000-0003-2398-5503 (Giuseppe Arena)

Date of submission: October 5, 2020
Date of decision: November 13, 2020
Date of acceptance: January 18, 2021
Date of web publication: March 25, 2021

https://doi.org/10.4103/1673-5374.310679

How to cite this article: *Arena G, Modjtahedi N, Kruger R (2021) Exploring the contribution of the mitochondrial disulfide relay system to Parkinson's disease: the PINK1/CHCHD4 interplay. Neural Regen Res 16(11):2222-2224.*

Copyright license agreement: *The Copyright License Agreement has been signed by all authors before publication.*

Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open access statement: *This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.*

References

Arena G, Valente EM (2017) PINK1 in the limelight: multiple functions of an eclectic protein in human health and disease. J Pathol 241:251-263.

Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betts J, Klopstock T, Taylor RW, Turnbull DM (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet 38:515-517.

Brunelli F, Valente EM, Arena G (2020) Mechanisms of neurodegeneration in Parkinson's disease: keep neurons in the PINK1. Mech Ageing Dev 189:111277.

Gao F, Zhang Y, Hou X, Tao Z, Ren H, Wang G (2020) Dependence of PINK1 accumulation on mitochondrial redox system. Aging Cell 19:e13211.

Imai Y, Meng H, Shiba-Fukushima K, Hattori N (2019) Twin CHCH proteins, CHCHD2, and CHCHD10: key molecules of Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal dementia. Int J Mol Sci 20:908.

Kim KH, Song K, Yoon SH, Shehzad O, Kim YS, Son JH (2012) Rescue of PINK1 protein null-specific mitochondrial complex IV deficits by ginsenoside Re activation of nitric oxide signaling. J Biol Chem 287:44109-44120.

Maguire JJ, Tyurina YY, Mohammadyani D, Kapralov AA, Anthonymuthu TS, Qu F, Amoscato AA, Sparvero LJ, Tyurin VA, Planas-Iglesias J, He RR, Klein-Seetharaman J, Bayır H, Kagan VE (2017) Known unknowns of cardiolipin signaling: The best is yet to come. Biochim Biophys Acta Mol Cell Biol Lipids 1862:8-24.

Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag AE, Lang AE (2017) Parkinson disease. Nat Rev Dis Primers 3:17013.

Reinhardt C, Arena G, Nedara K, Edwards R, Brenner C, Tokatlidis K, Modjtahedi N (2020) AIF meets the CHCHD4/Mia40-dependent mitochondrial import pathway. Biochim Biophys Acta Mol Basis Dis 1866:165746.

Schöndorf DC, Ivanyuk D, Baden P, Sanchez-Martinez A, De Cicco S, Yu C, Giunta I, Schwarz LK, Di Napoli G, Panagiotakopoulou V, Nestel S, Keatinge M, Pruszak J, Bandmann O, Heimrich B, Gasser T, Whitworth AJ, Deleidi M (2018) The NAD+ precursor nicotinamide riboside rescues mitochondrial defects and neuronal loss in iPSC and fly models of Parkinson's disease. Cell Rep 23:2976-2988.

Sekine S, Wang C, Sideris DP, Bunker E, Zhang Z, Youle RJ (2019) Reciprocal roles of Tom7 and OMA1 during mitochondrial import and activation of PINK1. Mol Cell 73:1028-1043.e5.

Sekine S, Youle RJ (2018) PINK1 import regulation; a fine system to convey mitochondrial stress to the cytosol. BMC Biol 16:2.

C-Editors: Zhao M, Wang L; T-Editor: Jia Y