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

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 (1). 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 (2). 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 (3).

Like the majority of nuclear-encoded mitochondrial proteins containing an N-terminal mitochondrial localization signal (MLS), PINK1 is actively transported into mitochondria through the TOM/TIM complex (translocases of the outer and inner mitochondrial membranes, respectively), driven by the electrical potential ($\Delta\psi$) 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 52kDa product that is retro-translocated to the cytosol and finally degraded by the proteasome via the N-end rule pathway. 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 dissipation of the mitochondrial membrane potential ($\Delta\psi_m$) by oxidative phosphorylation uncoupling agents, which lead 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 (4).

It is worth noting that not only the collapse of $\Delta\psi_m$, but also generation of mitochondrial reactive oxygen species (ROS) 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 and colleagues recently found that the redox-regulated CHCHD4/GFER mitochondrial import pathway was required for PINK1 stabilization and mitophagy execution (5). 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. 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 (6). 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 (6). 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 (6). 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 (7).

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 of CHCHD10 indicated that the CHCH domain of the protein was required for its CHCHD4-mediated import to the IMS, rather than its N-terminal positively charged MLS (7). 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 (5).

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 (4). 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 (TMD) 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 TMD 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 CCCP treatment (8).

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 (6). Indeed, deletion of this region (PINK1 Δ166-171) strongly decrease 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 (5).

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 (5). 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 (6). 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 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 (3,6). 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 (9). 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 (6). 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 (6,10). To this regard, cardiolipin (CL) levels are significantly reduced in mitochondrial fractions from PINK1-KO mouse embryonic fibroblasts (MEFs) compared to control MEFs, whereas CL supplementation rescues the impairment of complex I activity observed in PINK1 deficient flies (3). As a structural component of the IMM, CL also play 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 (6). *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 (6). 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 (6).

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 NADH and NADPH, AIF undergoes a conformational modification, which appears to enhance its interaction with CHCHD4 (6). Interestingly, PD-related mutations in PINK1 and GBA genes decreased 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 (NAM) or Nicotinamide Riboside (NR) rescued mitochondrial defects and neuronal loss in Drosophila and human induced pluripotent stem cells (iPSC)-derived models of PD (11). 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 (5). This also highlights the importance, often underestimated, of the mitochondrial intermembrane space (IMS), where PINK1 localizes during its mitochondrial import, just before its processing and retro-translocation to the cytosol. 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 (12).

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.

REFERENCES

1) Poewe W, Seppi K, Tanner CM, et al. Parkinson disease. *Nat Rev Dis Prim* (2017) **3**:1-21.
2) Bender A, Krishnan KJ, Morris CM, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet* (2006) **38**:515-7.
3) Brunelli F, Valente EM, and Arena G. Mechanisms of neurodegeneration in Parkinson's disease: keep neurons in the PINK1. *Mech Ageing Dev* (2020), **189**:111277.
4) Sekine S and Youle RJ. PINK1 import regulation; a fine system to convey mitochondrial stress to the cytosol. *BMC Biology* (2018) **16**:2.
5) Gao F, Zhang Y, Hou X, et al. Dependence of PINK1 accumulation on mitochondrial redox system. *Aging Cell* (2020) **19**:e13211.
6) Reinhardt C, Arena G, Nedara K, et al. AIF meets the CHCHD4/Mia40-dependent mitochondrial import pathway. *Biochim Biophys Acta Mol Basis Dis* (2020) **1866**:165746.
7) Imai Y, Meng H, Shiba-Fukushima K, et al. Twin CHCH Proteins, CHCHD2, and CHCHD10: Key Molecules of Parkinson's Disease, Amyotrophic Lateral Sclerosis, and Frontotemporal Dementia, *Int J Mol Sci* (2019) **20**: 908.

8) Sekine S, Wang C, Sideris DP, et al. Reciprocal Roles of Tom7 and OMA1 during Mitochondrial Import and Activation of PINK1. *Mol Cell* (2019) **73**:1028–1043.
9) Kim KH, Song S, Yoon SH, et al. Rescue of PINK1 Protein Null-specific Mitochondrial Complex IV Deficits by Ginsenoside Re Activation of Nitric Oxide Signaling. *J Biol Chem* (2012) **287**:44109-44120.
10) Maguire JJ, Tyurina YY, Mohammadyani D, et al. Known unknowns of cardiolipin signaling: The best is yet to come. *Biochim Biophys Acta Mol Cell Biol Lipids* (2017) **1862**:8-24.
11) Schondorf DC, Ivanyuk D, Baden P, et al. The NAD+ Precursor Nicotinamide Riboside Rescues Mitochondrial Defects and Neuronal Loss in iPSC and Fly Models of Parkinson's Disease. *Cell Reports* (2018) **23**:2976–2988.
12) Arena G & Valente EM. PINK1 in the limelight: multiple functions of an eclectic protein in human health and disease. *J Pathol (*2017) **241:** 251–263.