

# Characterization of a novel mitochondrial function of PINK1 in Parkinson disease pathogenesis

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## Background:

Parkinson's disease (PD) is a frequent neurodegenerative disorder resulting from massive degeneration of the dopaminergic neurons in the substantia nigra. Although most cases are sporadic, several genes are known to cause early onset familial PD. Among these, 2% of positive case are explained with the presence of recessive variants in phosphatase and tensin homologue-induced kinase 1 (*PINK1*), encoding a serine-threonine kinase first characterized for the neuroprotective role. *PINK1* is a mitochondrial protein known to enter the mitochondrion and to interact with some mitochondrial proteins such as the chaperon protein TRAP1, with antioxidant activity, or the complex I subunit NdufA10. However, most of research on this protein has focused so far on its accumulation on the outer surface of damaged mitochondria to trigger mitophagy, while *PINK1* functions within mitochondria are still poorly elucidated.

Recently, in our lab, we disclosed a possible interaction between *PINK1* and UQCRC1 (Ubiquinol-Cytochrome C Reductase Core Protein 1), a core component of complex III in the respiratory chain whose function is not completely known. Of note, mutations in *UQCRC1* have recently been reported in patients with Parkinson disease.

Aim of this PhD project is to first confirm this interaction and, starting from this, determine its physiological significance and potential implications for PD pathogenesis. This will be pursued through the following tasks: (a) confirm the interaction using different techniques such as co-GST-pulldown assay, co-Immunoprecipitation and FLIM-FRET analysis; (b) develop and employ *PINK1* and *UQCRC1* mutated / truncated constructs to determine the proteins' domains required for the interaction and whether this is impaired by PD-associated mutations; (c) establish whether *UQCRC1* could represent a potential substrate for the phosphorylation activity of *PINK1*; d) determine if *UQCRC1* could be a

potential mitochondrial processing peptidase that regulates the processing and importation of *PINK1*; e) evaluate the significance of this interaction on key mitochondrial-related pathways, such as respiratory chain activity, energy production, oxidative stress and apoptosis.

The project will be carried out in the unit of Biology and Genetics, Department of Molecular Medicine. A variety of cell models will be employed, including neuronal cell lines engineered to stably express wild type or mutant proteins, or silenced for *PINK1* expression, as well as patient-derived iPSCs differentiated towards dopaminergic neurons.

## Main techniques:

Cell cultures (HeLa, SH-SY5Y, iPSCs), cell transfection, electroporation, cloning, recombinant protein expression and purification, immunofluorescence, confocal imaging, FLIM-FRET analysis, co-immunoprecipitation, co-GST-pulldown, western blotting.

