Unravelling the function of the PD-linked kinase PINK1 using proteomics and interaction partner validation

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Parkinson's disease is the most common neurodegenerative motor disorder and is pathologically characterized by the selective loss of dopaminergic neurons in the substantia nigra and by the presence of cytoplasmic protein inclusions known as Lewy bodies. The major symptoms are tremor at rest, rigidity, slowness or absence of voluntary movement, postural instability and freezing. Although the etiology of the disease is incompletely understood, genetic studies have identified mutations in several genes that are associated with rare familial forms of the disease. Mutations in PTEN-induced putative kinase 1 (PINK1), a mitochondrial serine/threonine kinase, cause an autosomal recessive form of PD. Despite the fact that several studies show that PINK1 protects against stress-induced mitochondrial dysfunction and cell death, the exact biological function of this protein as well as its implication in PD remains to be further elucidated.

In this study we aim to identify cellular protein partners of PINK1 via 2D-DIGE proteomics and interactomics in the rodent brain in order to unravel PINK1 related cellular pathways. These pathways are predicted to play a crucial role in the pathogenesis of PD and may therefore allow to identify potential new drug targets for PD therapy.

First, to gain insight in cellular processes that involve PINK1, we performed a 2D-DIGE proteomics study on a total brain extract from PINK1 KO mice compared to control mice. Because of the mitochondrial localization of PINK1, we will also perform the 2D-DIGE analysis on a mitochondrial brain extract. Complementary to the proteomic analysis, we are also performing *in vivo* co-immunoprecipitations with hPINK1-3flag to identify direct PINK1 interactors. To this end, recombinant adeno-associated viral vectors (rAAV) coding for CMV-hPINK1-3flag were generated and stereotactically injected in the right striatum of C57BL6 mice. After immunoprecipitation of hPINK1-3flag, the co-purifying proteins are identified by nanoLC/MS. Interesting hits will be further validated in vitro in secondary binding assays and in vivo by overexpression and RNAi-mediated knock-down of the particular protein using viral vector technology.