Parkinson’s disease is a common neurodegenerative disease marked by the progressive loss of dopaminergic neurons in the substantia nigra in the brain, but the exact etiology of the disease is unknown (1). Mitochondrial dysfunction is strongly linked to early-onset autosomal recessive Parkinson’s disease (2). Dysfunctional mitochondria in normal cells are selectively degraded through mitophagy. The gene product PINK1 is upstream of the Parkin protein in the signaling of mitophagy. Mutations in PINK1 can disrupt normal mitophagy in cells, which leads to the accumulation of dysfunctional mitochondria, causing cell death. This is believed to be one of the causes for dopaminergic neuronal cell death in Parkinson’s disease. It is known that phosphorylation of Parkin by PARK1 is an important step in mitophagy, but the mechanism by which PINK1 kinase activity is regulated is largely **unknown** (3). There is some evidence that PINK1 activation is affected by changes in the mitochondrial membrane potential.

My **primary goal** is to characterize the mechanism by which PINK1 kinase activity is regulated.

My **hypothesis** is that depolarization of the mitochondrial membrane potential will result in significant increases in PINK1 phosphorylation of Parkin, which will result in higher mitophagy and lower dopaminergic cell death. My **long-term** goal is identify therapeutic targets within the PINK1/Parkin signaling pathway that could lead to prevention and treatment of early-onset Parkinson’s disease.

**Aim 1: Determine the effects of mutations surrounding and including the kinase domain of PINK1 on zebrafish motor function and dopaminergic neuron phenotypes.**

**Approach:** CRISPR/Cas9 will be used to create mutations in and around the protein kinase domain of PINK1 in zebrafish. Next-generation sequencing will be used to confirm the genotypes of mutants. Motor function will be observed after three months. At the end of their natural lifespan, the number of dopaminergic neurons present at death will be measured using TH immunohistochemistry.

**Hypothesis:** I hypothesize that mutations that cause distinct changes in PINK1 folding will result in uncoordinated movement and loss of dopaminergic neurons.

**Rationale:** Performing this CRISPR/Cas9 screen will help identify the specific regions of PINK1 that are necessary for normal kinase function and phosphorylation of Parkin so that the normal mitophagy pathway can proceed.

Aim 2:

Aim 3:

References:

[1] de Rijk MC, Breteler MM, Graveland GA, Ott A, Grobbee DE, van der Meche FG, Hofman A. Prevalence of Parkinson's disease in the elderly: the Rotterdam Study. Neurology. 1995;45:2143–2146.

[2] Bose, A. and Beal, M. F. (2016), Mitochondrial dysfunction in Parkinson's disease. J. Neurochem., 139: 216-231. doi:[10.1111/jnc.13731](https://doi.org/10.1111/jnc.13731)

[3] Kondapalli, C., Kazlauskaite, A., Zhang, N., Woodroof, H. I., Campbell, D. G., Gourlay, R., Burchell, L., Walden, H., Macartney, T. J., Deak, M., Knebel, A., Alessi, D. R., … Muqit, M. M. (2012). PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open biology*, *2*(5), 120080.