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 when PINK1 is activated, it phosphorylates Parkin, which is an important step in mitophagy, but the mechanism by which PINK1 kinase activity is regulated is largely **unknown** (3).

My **primary goal** is to better understand how regulation of PINK1 kinase activity contributes to mitochondrial regulation and neurodegeneration. My **long-term goal**of this research is to understand how regulation of PINK1 contributes to regulation of mitophagy so that effective treatments can be made for Parkinson’s disease patients. I will test the **hypothesis** that PINK1 kinase activity can be regulated by altering autophosphorylation sites in PINK1. I will use zebrafish as a model organism because the region of the zebrafish brain that contains dopaminergic neurons is strikingly similar to the human ascending midbrain dopaminergic neurons in humans, and dysfunctional mitochondria phenotypes are easy to see as they are increased in size (4).

**Aim 1: Identify conserved autophosphorylation sites of PINK1 crucial for normal mitophagy pathway.**

**Approach:** First, I will use BLAST to find homologs of PINK1. I will then align protein sequences using Clustal Omega to identify conserved autophosphorylation sites that have been found in insect PINK1 among the homologs. I will then mutate the well conserved autophosphorylation sites with CRISPR-Cas9 using zebrafish as a model to understand how these mutations affect the mitophagy pathway. I will then screen the mutated zebrafish for the increased mitochondrial size phenotype, as well as dopaminergic death at different stages of life.

**Rationale:** Autophosphorylation sites have been identified in insect PINK1 that are believed to autophosphorylate before PINK1 can phosphorylate Parkin. Screening of zebrafish with the induced gene mutations from those conserved sites should result in zebrafish with dysfunctional mitochondria that are increased in size, which will help explain what mutations in PINK1 do to the mitophagy pathway.

**Hypothesis:** I hypothesize that specific autophosphorylation sites in PINK1 correlate with normal mitophagy pathway and decreased dopaminergic death.

**Aim 2: Identify small molecules that rescue PINK1 mutant phenotypes.**

**Approach:** I will perform a high-throughput chemical genomic screen on wild type and PINK1 mutant zebrafish from Aim 1 at different ages. This will be done using a diversity-oriented library to identify small molecules that rescue the PINK1 mutant dysfunctional mitochondria and dopaminergic death.

**Rationale:** Treating mutant PINK1 zebrafish with the identified small molecules will restore the mitophagy pathway and restore wild type function of PINK1.

**Hypothesis:** I hypothesize that small molecules that can rescue the PINK1 mutant phenotypes will restore the normal mitophagy pathway and decrease dopaminergic death.

**Aim 3: Determine differences in protein interaction complexes required for normal mitophagy pathway between mutant and wildtype zebrafish.**  
**Approach**: Use autophosphorylation site mutants from Aim 1 with dysfunctional mitochondria. For mutant and wildtype groups, PINK1 fragment-containing complexes will be copurified with the affinity-tagged PINK1-fragment proteins and analyzed using mass spectrometry. I will then compare the mutant and wildtype zebrafish to each other and create protein-protein interaction webs. I will find the gene ontology of interaction proteins using Panther.

**Rationale:**​In normal individuals, PINK1 works with other proteins in the mitophagy pathway. I want to elucidate if mutation of the autophosphorylation sites affects the protein-protein interaction network responsible for mitophagy.   
**Hypothesis:** I hypothesize that mutated PINK1 will have reduced protein interactions with mitochondrial regulation and apoptosis regulation proteins.

**References:**

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