Investigating the Role of the PINK1-Parkin Pathway in Mitochondrial Morphology

Avi Adler, Erin Barnhart PhD

Barnhart Lab, Department of Biological Sciences, Columbia University, New York, NY

Introduction

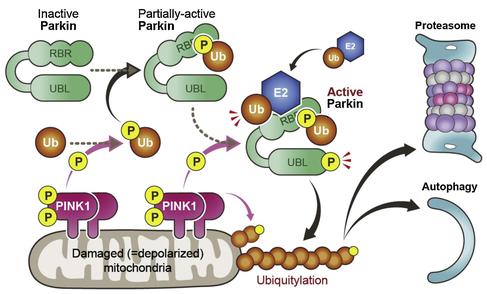

Figure #1: **PINK1/Parkin Degradation Pathway**[1]

A stable and healthy population of mitochondria is crucial to maintain cellular health. This is especially challenging in neurons which contain extensive dendritic arborizations and require large reserves of energy.[2] In normal ATP (energy) production, mitochondria generate reactive oxygen species (ROS) which are damaging to mitochondria themselves. Although equipped to handle the stresses of ROS, mitochondria can get overwhelmed by oxidative stress and become a liability to cellular health.[3] To stave off neurodegeneration, and to maintain cellular health, neurons must regularly turn over their population of mitochondria via varying degradation pathways.[4] Breakdown of the mitochondrial network in neurons has been implicated in numerous neurological diseases, namely Parkinson's Disease.[5] One pathway of mitochondrial degradation, mitophagy, involves the action of two proteins: PTEN-induced kinase 1 (PINK1), and the ubiquitin ligase, Parkin. In healthy mitochondria, PINK1 is imported into the inner mitochondrial membrane, and is subsequently degraded. However, in damaged mitochondria, PINK1 accumulates on the surface of the organelle, and recruits Parkin, which flags the mitochondria for degradation[6](Fig 1). The relative contribution of mitophagy to overall mitochondrial turnover remains unclear.[7] In this study, PINK1 and Parkin proteins were overexpressed in HS neurons of the Drosophila visual system.

Methods

Dissection and Immunostaining

- Mitochondria were labeled using the GAL4/UAS system to drive expression of mitochondria targeted GFP (mitoGFP) and a cytosolic volume marker, tdTomato. these reports were expressed in HS neurons alongside PINK1 and Parkin overexpression.
- I dissected and cleaned multiple sets of brains, fixed them in formaldehyde, permeated in PBST, and blocked in Normal Goat Serum (NGS).
- Samples were then immunostained in anti-GFP, anti-dsRed and anti-NC82 antibodies. For this experiment, I helped to develop a dissection and immunostaining protocol for the Barnhart lab.

Experimental Design of *Drosophila* Brain Imaging

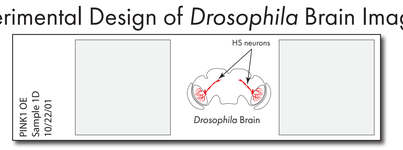

Figure #2: **Microscope Slide** with *Drosophila* Brain expressing HS neurons; bounded by microscope slip covers.

Imaging and image analyses

- I transferred samples to microscope slides and encased them in gold antifade (Fig 2).
- I imaged the brains under an oil emersion, 63x confocal microscope at varying magnifications and laser powers. Brains were imaged in primary regions of the HS neuron: Primary dendrites, distal dendrites, cell bodies and axonal projections (fig 4).
- Microscope data were compiled into MAX projections where the background was then removed. Then, each image was de-speckled for clarity.
- Image analyses and development was done in Fiji.

Results

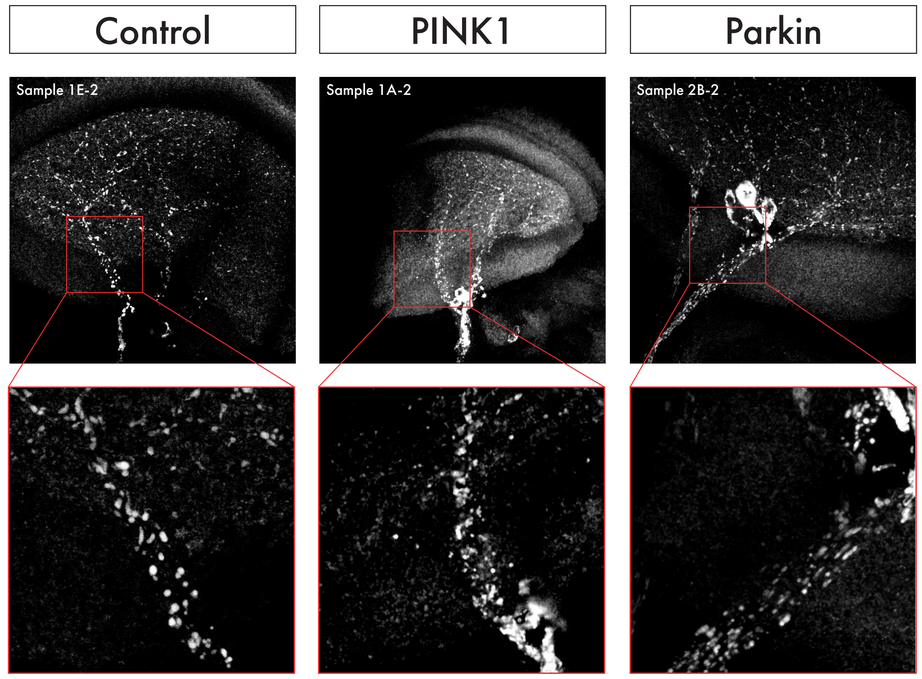

Figure #3: **Mitochondrial Morphology in HS neurons of Drosophila**. Confocal microscopy of mitoGFP reporters in Horizontal system(HS) neurons of 7-day old Drosophila brains. **(A)** Overview of the optic lobe of Wild Type (Control), PINK1-overexpression (PINK1) and Parkin-overexpression (Parkin) flies. **(B)** Focused images of the primary arborization of HS neurons. Fragmentation of mitochondria is evident in PINK1 and Parkin samples. Further quantification is needed to verify this result. (For genotypes analyzed, see below right)

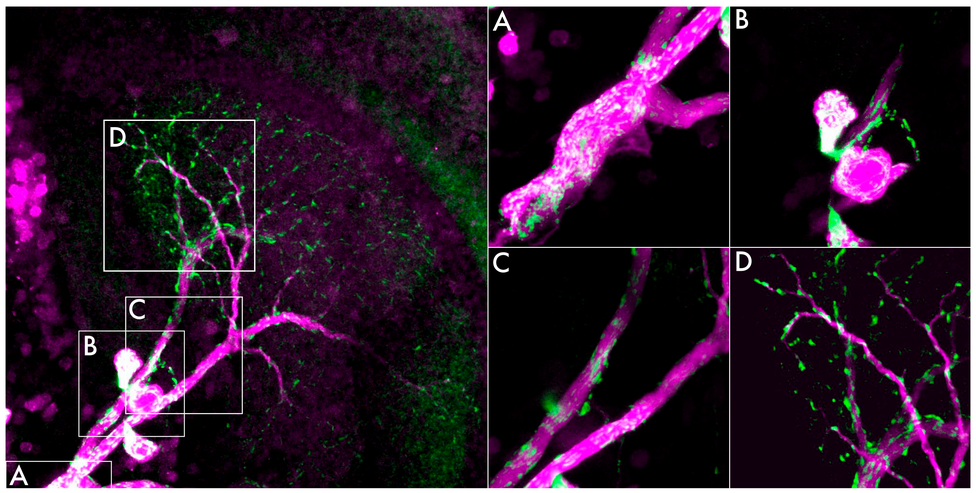

Figure #4: **Horizontal System (HS) neuron overview and structure**. Samples contained transgenic reporters mitochondria targeted GFP (mitoGFP)(green) and tdTomato(magenta). Max projections complied from confocal microscopy data. HS neuron overview with corresponding focused panels (A-D) of **(A)**axonal projections; **(B)**cell bodies; **(C)**primary dendritic arborization; **(D)**distal dendrites.

Conclusions

- Visual inspection indicates that PINK1 and Parkin overexpression leads to greater mitochondria fragmentation (Fig 3). Quantification of mitochondrial morphology as well as greater sample size are needed to confirm this result.
- While the contribution of PINK1 and Parkin to overall mitochondria turnover remains unclear, our results suggest these proteins may affect mitochondrial morphology.
- Overexpression of PINK1 or Parkin may upset a fission/fusion balance typically observed in mitochondria with fission exceeding fusion.8 This result could help explain the increased fragmentation pattern observed here.

Future Studies

- Our future studies will utilize greater sample size, better immunostaining and dissection protocols, and quantitative data analyses.
- We will aim to determine how PINK1 and Parkin mutations effect mitochondrial morphology in varying regions of the neuron (Fig 4).
- While this study focused on HS neurons, future studies will determine how PINK1 and Parkin effect multiple neuronal types. This is important in determining how appreciable mitophagy is across varying cell types as well as overall mitochondrial turnover.

Acknowledgements

- Supported by The Laidlaw Scholarship at Columbia University.
- I would like to thank Adam Lamper for training and guiding my research. I would like to thank Dr. Erin Barnhart for guidance and support. In addition, I would like to thank members of the Barnhart lab for encouragement and critical questioning.

Genotypes of *Drosophila* Samples

$$\text{(A) } W+ ; \frac{\text{UAS-mitoGFP,tdTomato}}{+} ; \frac{\text{HS-Gal4}}{+}$$

$$\text{(B) } W+ ; \frac{\text{UAS-mitoGFP,tdTomato}}{+} ; \frac{\text{HS-Gal4}}{\text{UAS-PINK1}}$$

$$\text{(C) } W+ ; \frac{\text{UAS-mitoGFP,tdTomato}}{+} ; \frac{\text{HS-Gal4}}{\text{UAS-Park}}$$

(A) Control Samples; (B) PINK1 Overexpression; (C) Parkin Overexpression.

References

(1) Tanaka, Keiji. 2020. "The PINK1–Parkin Axis: An Overview." Neuroscience Research 159: 9–15.
(2)Harris, Julia J., Renaud Jolivet, and David Attwell. 2012. "Synaptic Energy Use and Supply." *Neuron* 75(5): 762–77. http://dx.doi.org/10.1016/j.neuron.2012.08.019.
(3)Orrenius, Sten, Vladimir Gogvadze, and Boris Zhivotovsky. 2007. "Mitochondrial Oxidative Stress: Implications for Cell Death." *Annual Review of Pharmacology and Toxicology* 47(1): 143–83. https://doi.org/10.1146/annurev.pharmtox.47.120505.105122.
(4)Rugarli, Elena I., and Thomas Langer. 2012. "Mitochondrial Quality Control: A Matter of Life and Death for Neurons." *EMBO Journal* 31(6): 1336–49. http://dx.doi.org/10.1038/emboj.2012.38.
(5)Vives-Bauza, Cristofol et al. 2010. "PINK1-Dependent Recruitment of Parkin to Mitochondria in Mitophagy." *Proceedings of the National Academy of Sciences of the United States of America* 107(1): 378–83.
(6)Ashrafi, Ghazaleh, Julia S Schlehe, Matthew J Lavoie, and Thomas L Schwarz. 2014. "Mitophagy of Damaged Mitochondria Occurs Locally in Distal Neuronal Axons and Requires PINK1 and Parkin." 206(5): 655–70.
(7)Cummins, Nadia, and Jürgen Götz. 2018. "Shedding Light on Mitophagy in Neurons: What Is the Evidence for PINK1/Parkin Mitophagy *in Vivo*?" *Cellular and Molecular Life Sciences* 75(7): 1151–62
(8) Yu, Wendou, Yaping Sun, Su Guo, and Bingwei Lu. 2011. "The PINK1/Parkin Pathway Regulates Mitochondrial Dynamics and Function in Mammalian Hippocampal and Dopaminergic Neurons." *Human Molecular Genetics* 20(16): 3227–40.