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## Analysis of Mitochondrial Turnover in Neuromuscular Junctions of Parkin Mutants

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### ABSTRACT

The accumulation of dysfunctional or damaged mitochondria in neurons has been linked to the pathogenesis of many neurodegenerative diseases, such as Parkinson's disease. It has been proposed that proteins PINK1 and Parkin regulate mitochondrial quality control by selectively targeting depolarized mitochondria for autophagic degradation, a process known as mitophagy. Though previously analyzed in cell bodies and axons of neurons, the role of the PINK1/Parkin pathway in the synapse is unclear, and it is not known whether mitochondrial turnover occurs in the neuromuscular junctions (NMJs). To study this, intact *Drosophila* nervous systems were analyzed *in vivo* by performing gentle dissections on third instar larvae to expose the ventral ganglia and segmental nerves with their NMJs. Both the control and *parkin* mutants were genetically modified to mark mitochondria via mito-GFP expression and autophagic vacuoles via RFP-atg8 expression in their motor neurons, with *parkin* mutants being additionally modified for the deletion of the Parkin gene. The physiological states of mitochondria were quantified through measurements of mitochondrial membrane potential ( $\Delta\psi_m$ ), and the possible occurrence of mitophagy in nerve terminals was tested through observations of GFP and RFP signal co-localization. Unexpectedly, mitochondria of *parkin* mutants displayed normal  $\Delta\psi_m$  readings in NMJs, indicating that mitochondria from mutant nerve terminals exhibit normal physiological conditions. In addition, co-localization was not observed in thin axons adjacent to NMJs, suggesting that mitophagy is down-regulated *in vivo*. By elucidating the role of Parkin in the synapse of neurons, the manifestation of Parkinson's and other neurodegenerative diseases will be better understood.

### KEYWORDS

Parkin, mitophagy, mitochondrial membrane potential, *Drosophila*, synapse, neuromuscular junction