



The role of mitochondria in the recovery of neurons after injury

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Mitochondria are well characterized by their fundamental functions in regulating cellular homeostasis, including energy and iron metabolism. These functions are essential in neurons with high metabolic demands and elongated neuronal processes. Mitochondria dynamically change morphology, localization, and activity to match neurons' spatial and temporal demands. Mitochondrial dysfunctions have been associated with many neurological disorders. Recent studies highlight that mitochondria also act as central regulators of the neuronal response to injury. Here, we discuss important findings that support the critical regulation of mitochondrial dynamics, energy metabolism, and iron homeostasis in the repair of damaged neurons.

Mitochondrial functions in energy and iron metabolism: The dynamic double-membraned organelles, mitochondria, are responsible for producing adenosine triphosphate (ATP), the energy currency of the cell, via oxidative phosphorylation. This process converts energy from glucose through a series of redox reactions at the electron transport chain of complexes located at the mitochondrial inner membrane. The nervous system is dependent on mitochondria for its high energy demands; however, the mismatch between mitochondrial biogenesis locations (cell body) and high energy consumption sites (axon and synapse) leads to a unique challenge for neurons to maintain energy homeostasis within the peripheral area of cells.

The proper function of neurons also requires appropriate levels of iron, which is an essential metal for almost all organisms and involved in many oxidation/reduction reactions. In fact, depending on the cell type, mitochondria may contain up to 20–50% of total cellular iron. Since mitochondria are responsible for the biosynthesis of iron-sulfur clusters and heme, mitochondria play interrelated roles in iron homeostasis by being a critical regulator of cellular iron homeostasis and primary targets for damage in response to impaired iron homeostasis (Zeidan et al., 2021). In addition, iron is important in central nervous system (CNS) myelination, particularly the oligodendrocytes where iron is indispensable for their myelination and maturation (Levi and Taveggia, 2014).

Mitochondrial transport in axon regeneration after nerve injury: Axon regeneration is one of the essential processes to recover damaged neurons with axonal disconnection. Mature neurons in the CNS have minimal axon regeneration capacity due to declines in intrinsic capacity and the presence of extrinsic factors. In contrast, neurons in the peripheral nervous system (PNS) often have a more robust regenerative capacity, although this capacity declines with age. Interestingly, studies in the PNS have highlighted that the normally stationary mitochondria become motile and move bidirectionally in response to axonal injury, raising the possibility that mitochondria could be primary responders of nerve injury.

Accordingly, recent reports across species in both the CNS and PNS demonstrate that this increased proportion of motile mitochondria enhanced the axon regeneration of injured neurons. In GABAergic neurons of the nematode *C. elegans*, successfully regenerating axons over the injured site had significantly increased mitochondrial translocation to injured axons, resulting in high density compared to non-regenerating neurons. In contrast, neurons with failed axon regeneration contain low mitochondrial density in injured axons. Inhibition or activation of mitochondrial transport into the axon by targeting Miro, a conserved mitochondrial trafficking regulator, leads to corresponding changes in axonal regenerative capacity with a positive correlation (Han et al., 2016).

Manipulating mitochondrial motility in vertebrates is also associated with axon regeneration capacity (Cartoni et al., 2016; Zhou et al., 2016; Xu et al., 2017). For example, in the zebrafish CNS, mitochondrial motility is positively correlated with axon regeneration (Xu et al., 2017). Pharmacological manipulation with dibutyl cyclic adenosine monophosphate, a structural analog of endogenous cyclic adenosine monophosphate, was shown to enhance regeneration in zebrafish axons and increase mitochondrial motility (Xu et al., 2017). In adult murine retinal ganglion cells, the Armadillo Repeat Containing X-Linked 1 (*Armcx1*) protein localizes to mitochondria, interacts with Miro, and increases mitochondrial transport. Importantly, overexpression of *Armcx1* protects axotomized neurons from cell death and promotes axon regeneration after optic nerve injury depending on its mitochondrial localization (Cartoni et al., 2016). In contrast, *Armcx1* knockdown undermines both neuronal survival and axon regeneration in the PTEN knockout condition with high regenerative capacity. In addition, syntaphilin (SNPH) is an axonal mitochondria-anchoring protein whose levels increase as neurons mature. Knock-out of SNPH in mice results in ~70% increase of axonal mitochondria and is associated with the clearance of injured mitochondria and replacement with healthy mitochondria after sciatic nerve injury (Zhou et al., 2016). Thus, decreased mitochondrial transport as an intrinsic factor limiting regenerative capacity in mature neurons could be bypassed through genetic or pharmacological means.

It remains incompletely understood how nerve injury affects mitochondrial transport in the injured axons. In the *C. elegans* GABAergic neurons, the dual-leucine zipper kinase 1 (DLK-1) mitogen activated protein kinase pathway, a conserved axon regeneration regulator, promotes mitochondria trafficking within axons (Figure 1A), indicating that specific injury signals could be involved (Han et al., 2016). The ability of DLK-1 signaling to increase mitochondrial trafficking was largely dependent on the CEBP-1 transcription factor suggesting transcriptional regulation of target genes is involved. However, in neurons lacking DLK-1 signaling, the increase

in mitochondrial density was reduced but not eliminated suggesting other unknown injury pathways working in parallel to link injury to mitochondria (Han et al., 2016). In retinal ganglion cells, *Armcx1* was identified as an up-regulated gene in the PTEN knock-out mice, an axon regeneration inhibitor in both the PNS and CNS. However, it remains to be determined if PTEN signaling regulates *Armcx1* (Cartoni et al., 2016). Recent studies indicate that the phosphorylation status of SNPH regulated by protein kinase B (AKT) growth signaling and subsequent P21-activated kinase 5 activation may act as an axonal mitochondrial signaling pathway that remobilizes damaged mitochondria in response to ischemic injury (Huang et al., 2021; Figure 1A). P21-activated kinase 5 is a brain mitochondrial kinase that declines in expression as neurons mature, but upon axonal injury/ischemia is locally synthesized and activated while AKT signaling further potentiates P21-activated kinase 5 activity.

Mitochondrial energy metabolism in axon regeneration after nerve injury: Axon regeneration involves multiple steps, including membrane sealing of the injured axon, activation of signaling pathways to promote transcription and translation of pro-growth genes, formation of functional growth cones, and cytoskeletal remodeling, all of which require copious amounts of energy. Not surprisingly, the cellular energy factories mitochondria play a pivotal role in providing the energy necessary for axon regrowth (Cartoni et al., 2016; Han et al., 2016; Zhou et al., 2016; Xu et al., 2017). In the *C. elegans* GABAergic neurons, mutations in the core components of the mitochondrial electron transport complex decrease axon regeneration capacity (Han et al., 2016). In the sciatic nerve of mammals, nerve injury depolarizes mitochondria membrane potential and results in a local energy deficit (Zhou et al., 2016). Increased mitochondrial trafficking suppressed the energy deficit along with enhanced axon regeneration capacity (Zhou et al., 2016). Supplying ATP to SNPH-deficient neurons was able to partially recover suppression of axon regrowth (Zhou et al., 2016). Since ATP has a limited diffusion capacity throughout the axon, increased mitochondrial trafficking is likely more efficient in providing ATP to the peripheral axon from the cell body. Although providing additional ATP could be one strategy to treat injured axons and bypass the issue of motility, mitochondria might be the more suitable therapeutic option, given that the rapid degradation of ATP limits its efficacy. As a potential alternative to ATP, creatine has been used as an energetic facilitator to promote the regeneration of axons after spinal cord injury in mice (Han et al., 2020). Creatine elevates creatine kinase activity and restores ATP independent of mitochondrial transport. The combination of genetically knocking down SNPH and administering creatine further enhanced corticospinal tract regenerative capacity in the *SnpH*^{-/-} mice (Huang et al., 2021).

Mitochondrial iron homeostasis in axon regeneration after nerve injury: Besides their critical functions in energy homeostasis, mitochondria's regulation of cellular iron homeostasis also plays a crucial role in the neuronal response to injury. Recent studies reported that peripheral nerve injury led to an increase in the expression levels of transferrin (iron importer) and transferrin receptor, and a decrease in the expression levels of ferroportin

(iron exporter). Moreover, heme oxygenase-1 is induced during nerve regeneration in order to catalyze the release of iron from heme and this is accompanied by an increase in transferrin receptor 1 suggesting recycling of iron for use in the regeneration process. All these changes thereby increase intracellular iron levels and eventually, if not properly regulated, can lead to cellular iron overload. This will increase oxidative stress and jeopardize the mitochondrial ability to produce the ATP required for injury repair. On the other hand, interestingly, transferrin in the PNS is thought to be a pro-differentiation factor and has a role in nerve differentiation, regeneration, and myelination in addition to its role in iron transport (Levi and Taveggia, 2014).

Given that iron is a vital co-factor for ATP production in mitochondria, providing an adequate level of iron to mitochondria located in peripheral axons far from the cell body is important for neuronal recovery after injury, whereas iron chelation can impair nerve degeneration. Recent studies indicate that Schwann cells conceivably supply iron to neurons with long axons, such as the sciatic nerve (Mietto et al., 2021). Importantly, the iron supplied by Schwann cells to long axons is vital for proper axon regeneration and mobility recovery after injury in the PNS. Disrupted iron homeostasis diminished mitochondrial positioning to injured axons and broadly decreased the expression of mitochondrial fusion and transport regulators, including Miro and Armcx1 (Mietto et al., 2021). Interestingly, the activation of AKT by iron signaling could lead to a reduction of cellular iron content and protect neurons from injury. This is achieved by decreasing the transferrin receptor-mediated iron uptake and upregulating the ferroportin-mediated iron efflux (Figure 1B). The reduction in the cellular iron concentration and restoration of iron homeostasis eventually lead to restoration of mitochondrial health and function (Hao et al., 2016).

Recently, the iron-dependent programmed cell death ferroptosis has been shown to negatively affect neuronal function, survival, and plasticity during nerve injury (Feng et al., 2021; Figure 1B). Ferroptosis is characterized by an iron-induced increase in lipid reactive oxygen species and, eventually, decreased cellular glutathione reserve and dampened activity of glutathione peroxidase 4, all of which contribute to the distorted mitochondrial morphology and impaired function, and subsequent cellular death (Zeidan et al., 2021). In fact, ferroptosis inhibition has been suggested as a potential therapeutic for nerve injury. Ferrostatin-1, a ferroptosis inhibitor, was able to reverse the increase of the iron content in injured rat spinal cords. Remarkably, ferrostatin-1 increases glutathione peroxidase 4 expression, induces neuron and astrocyte activation, alleviates the associated pain, and promotes injury healing in rats (Wang et al., 2021).

Collectively, understanding how mitochondria relate to declines in intrinsic capacity for axon regrowth is critical to successful axon regeneration. Mitochondria sit as hubs for interconnected pathways between energy and iron homeostasis and present as attractive therapeutic targets to promote neuronal recovery following injury. The authors would like to acknowledge and apologize that not all relevant literature has been cited due to space constraints.

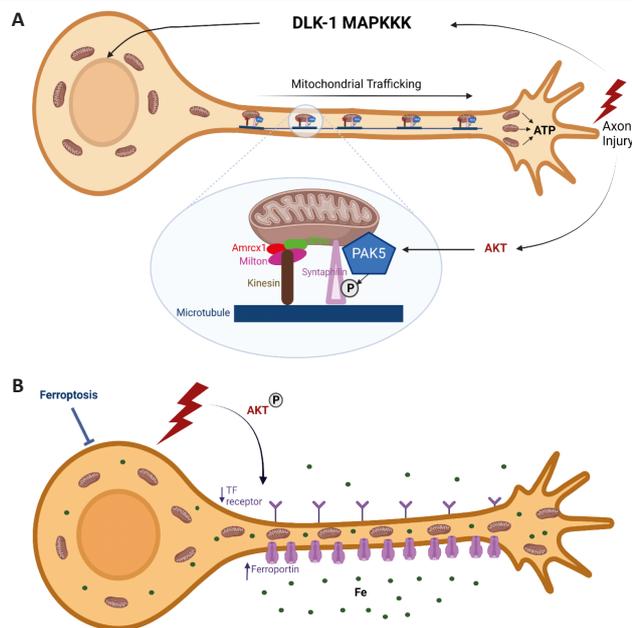


Figure 1 | The multifaceted roles of mitochondria in neuronal recovery after injury.

(A) DLK-1 and AKT signaling promote mitochondrial trafficking. The DLK-1 signaling is required to promote mitochondrial positioning to the injured axon, while AKT phosphorylates syntrophin via PAK5 and releases stalled mitochondria. Additionally, Armcx1 binds to Miro, which along with Milton and kinesin, increases anterograde mitochondrial transport to supply ATP for neuronal repair. (B) AKT activation leads to a reduction in cellular iron concentration by downregulating transferrin levels and upregulating ferroportin levels, which result in a decrease in cellular iron import and an increase in cellular iron export, respectively. Additionally, inhibition of ferroptosis (iron-induced cell death) is another way to promote injury repair. AKT: Protein kinase B; ATP: adenosine triphosphate; DLK-1: dual-leucine zipper kinase 1; MAPKKK: mitogen activated protein (MAP) kinase kinase kinase; PAK5: P21-activated kinase 5; TF: transferrin. This figure was created with BioRender.com.

This work was supported by grants from the National Institute on Aging (AG063766 and AG028740 to RX, AG066654 to SMH, T32AG062728 to TM), the American Cancer Society (RSG-17-171-01-DMC to RX), and the American Federation for Aging Research (AGR DT 07-2502019 and AGR DTD 09-15-2021 to SMH).

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Date of submission: January 6, 2022

Date of decision: February 8, 2022

Date of acceptance: February 19, 2022

Date of web publication: July 1, 2022

<https://doi.org/10.4103/1673-5374.343907>

How to cite this article: McElroy T, Zeidan RS, Rathor L, Han SM, Xiao R (2023) The role of mitochondria in the recovery of neurons after injury. *Neural Regen Res* 18(2):317-318.

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Open peer reviewer: Andrew William James Paterson, Leeds Beckett University, UK.

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P-Reviewer: Paterson AWJ; C-Editors: Zhao M, Liu WJ, Wang Lu; T-Editor: Jia Y