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The interplay of axonal energy homeostasis and mitochondrial trafficking and anchoring

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Abstract

Mitochondria are key cellular power plants essential for neuronal growth, survival, function, and regeneration after injury. Given their unique morphological features, neurons face exceptional challenges in maintaining energy homeostasis at distal synapses and growth cones where energy is in high demand. Efficient regulation of mitochondrial trafficking and anchoring is critical for neurons to meet altered energy requirements. Mitochondrial dysfunction and impaired transport have been implicated in several major neurological disorders. Research into energy-mediated regulation of mitochondrial recruitment and redistribution is thus an important emerging frontier. This review discusses new insight into the mechanisms regulating mitochondrial trafficking and anchoring, and provides an updated overview of how mitochondrial motility maintains energy homeostasis in axons, thus contributing to neuronal growth, regeneration, and synaptic function.

Keywords

Energy deficits; mitochondrial transport; neuronal growth; neurodegeneration; neuronal regeneration; synaptic function

Neurons face unique challenges in maintaining energy homeostasis

Mitochondria are the main cellular energy powerhouses that convert glucose and pyruvate into adenosine triphosphate (ATP) through the electron transport chain and oxidative phosphorylation [1]. Mitochondria provide most of the ATP required in the brain that powers various neuronal functions [2]. A constant ATP supply is thus essential for nerve cell growth, survival, and function [3]. In the brain, synapses are the primary sites of ATP consumption where mitochondria supply ~93% of the ATP, while glycolysis generates only ~7% of the ATP [4]. Due to their high-energy demand and unique polarized structures, neurons require specialized mechanisms to maintain energy homeostasis throughout the cell, particularly at distal synapses and in axons that can extend several centimeters long or even

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up to a meter in some peripheral nerves [5, 6]. Because ATP has a limited diffusion capacity in the long axonal process [7, 8], a fundamental question remains: do energy deficits or bioenergetic failure occur early in distal axons under physiological and pathological stress conditions? This is particularly an important question relevant to a range of neurodegenerative diseases that associate with degeneration of synaptic terminals at early disease stages along with energy deficits [9, 10].

Mitochondria in axons and at synapses maintain energy homeostasis that is essential for synaptic functions [4], including synapse assembly [11]; generation of action and synaptic potentials [12]; and synaptic vesicle (SV) trafficking and recycling [8, 13]. In addition, mitochondria efficiently buffer transient Ca^{2+} by sequestering Ca^{2+} influx [14–17]. While less than 50% of presynaptic terminals in hippocampal regions have mitochondria [18], most synapses may have motile mitochondria passing by. Depleting mitochondria from axon terminals impairs synaptic transmission [8, 13, 19]. Defective mitochondrial transport along with energy deficits is implicated in the failed axonal regeneration after injury and the pathogenesis of several major neurological disorders, including Alzheimer's and Parkinson's diseases [20–22]. Mitochondria also alter their motility under certain stress conditions or when their integrity is impaired [23–26]. Therefore, efficient regulation of mitochondrial motility is critical for neurons to meet altered energy requirements and to remove damaged mitochondria or replenish healthy ones, thus maintaining energy homeostasis in distal axons and at synapses.

Research into the mechanisms regulating mitochondrial motility and distribution in response to changes in energy consumption and homeostasis is thus an important emerging frontier. Recent advances provide exciting lines of evidence as to how mitochondrial trafficking and anchoring are coordinated to sense and respond to altered energy requirements under physiological and pathological stress conditions. This article provides a brief overview of the mechanisms regulating axonal mitochondrial trafficking and anchoring and discusses recent findings on how: (1) mitochondrial transport influences energy homeostasis in distal axons and at synapses, thus regulating axonal growth and regeneration, and synaptic function, and (2) mitochondrial recruitment is regulated in response to changes in bioenergetic status. Additional insights from different perspectives can be found in other outstanding reviews [5, 6, 10, 21, 27–32].

The mechanisms of mitochondrial trafficking and anchoring

Long-distance mitochondrial transport is driven by microtubule (MT)-based and ATP-dependent molecular motors: the plus-end directed kinesin and the minus-end directed dynein [33]. Axonal MTs are uniformly arranged so that their plus-end is directed distally and the minus-end is toward the soma; thus, kinesin motors move in an anterograde direction toward distal axons while dynein motors mediate retrograde transport toward the soma. Kinesin-1 family members, also known as KIF5A, KIF5B, and KIF5C, are the main motors driving mitochondrial transport in neurons [34, 35]. Kinesin-1 motor proteins contain two heavy chains (KHC) and two light chains (KLC). The motor domain of KHC has ATPase activity and binds directly to MTs, whereas its carboxyl-terminal domain associates with a KLC or interacts with cargoes. The monomeric kinesin-3 motor family KIF1B α has also been demonstrated to mediate the anterograde transport of mitochondria in neurons [33]. Motor proteins are recruited to mitochondria through their cargo adaptors, thus ensuring targeted trafficking and regulation of mitochondrial transport [21] (Figure 1) (Box 1).

In axons of the central nervous system (CNS) *in vitro*, the majority of mitochondria remain stationary, while approximately 20–30% are motile [8]. Those motile mitochondria can become stationary and stationary ones can be remobilized in response to changes in bioenergetic status and synaptic activity. Thus, axonal mitochondria deploy an anchoring mechanism in addition to motor-driven transport. This model was recently validated by our study demonstrating that syntaphilin (SNPH) acts as a "static anchor" specific for axonal mitochondria [36, 37]. SNPH selectively targets to the outer-membrane of axonal mitochondria through its carboxyl-terminal mitochondria-targeting domain and axon-sorting sequence. SNPH arrests axonal mitochondrial transport by anchoring them to MTs. Over-expressing SNPH abolishes axonal mitochondrial transport. Conversely, deleting *snph* robustly enhances axonal mitochondrial motility to 78% in two-week-old cultured hippocampal neurons and 71% in *ex vivo* axonal bundles of sciatic nerves from 2-month-old mice [22, 36, 37]. These data indicate that SNPH acts as an anchoring protein that restricts axonal mitochondrial transport. Thus, *snph* knockout (KO) mice serve an ideal genetic model for investigating how enhanced transport of axonal mitochondria influences synaptic function, axonal growth, and regenerative capacity in response to altered energy requirements.

Energy-demanding synaptic activity regulates mitochondrial transport

Synaptic function is driven by ATP [4], which supports synapse assembly [11], powers action potentials [12], and fuels SV trafficking and recycling [8, 13, 38, 39]. Due to the high-energy demand at synapses, the constant and local ATP supply is critical to maintaining ionic gradients and supporting neurotransmission. Dysfunction or loss of synaptic mitochondria leads to synaptic deficits, which are associated with the neuropathologies found in several major neurodegenerative diseases [21]. Elevated intracellular Ca^{2+}, through activation of voltage-dependent calcium channels or NMDA receptors, recruits mitochondria to activated synapses [40–42]. The mechanisms underlying such activity-dependent mitochondrial recruitment were not known until the identification of Miro as a Ca^{2+} sensor [35, 43, 44]. Miro is a mitochondrial outer-membrane protein with Ca^{2+}-binding EF hands [45]. By sensing cytosolic Ca^{2+}, Miro arrests mitochondria at activated synapses through the inactivation of the transport machineries. A Miro-Ca^{2+} sensing model was proposed: when a trafficking mitochondrion passes through an active synapse, elevated Ca^{2+} binds to Miro and induces its conformational changes, thus disrupting the motor-apaotor complexes of KIF5-Trak-Miro (in mammals) or KIF5-Milton-Miro (in *Drosophila*) [35, 44]. By this mechanism, mitochondria are immobilized at activated synapses (Figures 2A, 2B). This model, however, was disputed as to whether KIF5 motor remains associated with arrested mitochondria or is released from the organelle upon immobilization. It is also unclear how this sensing pathway inactivates dynein–mediated retrograde transport. A genetic mouse study using a neuronal *miro1* deletion showed that Miro1 loss does not prevent Ca^{2+}-dependent inhibition of mitochondria motility [46]. A second study consistently showed that mitochondrial trafficking in the *miro1* deletion neurons remians sensitive to neuronal activation [47]. These two mouse genetic studies raise questions as to whether Miro1 is essential for Ca^{2+}-mediated inhibition of mitochondrial transport and whether the remaining Miro2 in these *miro1* mutant neurons or other mechanisms in place can immobilize mitochondria by sensing Ca^{2+}. These studies also suggest the possibility that mitochondrial immobilization may require a static anchoring mechanism.

We recently tested this possibility and surprisingly found that activating the Miro-Ca^{2+} sensing pathway failed to arrest axonal mitochondria in *snph* KO hippocampal neurons [37]. Deleting the *snph* gene abolished the activity-dependent immobilization of mitochondria in axons, but not in dendrites, consistent with SNPH expression that is specific to axonal mitochondria. We further revealed that SNPH competes with Trak2 to bind with KIF5 motors and inhibits motor ATPase activity. Thus synaptic activity favors the KIF5-SNPH anchoring interaction and SNPH coordinates with the Miro-Ca^{2+} sensing mechanism for arresting axonal mitochondrial trafficking. These findings allow us to propose a new "engine-switch and brake" model: when a motile mitochondrion passes by an activated synapse, SNPH responds to elevated Ca^{2+} levels (stop sign) to switch off the engine (motor) and places a brake on the mitochondrion, thereby arresting the mitochondrion on the MT-track. When the Ca^{2+} signal is removed, the cargo-loaded motor-adaptor complexes can be quickly re-activated (Figure 2C). This "engine-switch and brake" model represents an interplay between the KIF5-Trak-Miro complex and the anchoring protein SNPH, thus effectively turning on/off trafficking and anchoring mechanism in response to changes in energy-demanding synaptic activity.

Mitochondrial motility influences energy homeostasis and presynaptic strength

A quantitative analysis of presynaptic ATP levels shows that electrical activity imposes large bioenergetic demands that are met via local ATP synthesis in presynaptic boutons, in which SV recycling consumes most of presynaptic ATP [38]. A brief interruption of local ATP synthesis severely impairs presynaptic function. Consistently, Pathak et al. [39] showed that SV endocytosis requires much higher ATP consumption than SV exocytosis and re-acidification. While these studies suggest that presynaptic ATP supply is required to maintain sustained synaptic transmission, they raise the question of whether defects in mitochondrial anchoring at presynaptic boutons cause local energy deficits, thus impairing the maintenance of SV release and recycling. Previous studies provided evidence that the loss of mitochondria from axonal terminals inhibits synaptic transmission due to insufficient ATP supply. For example, expressing mutant Milton in *Drosophila* photoreceptors impaired synaptic transmission by reducing synaptic mitochondria [29]. Expressing a loss-of-function mutant of syntabulin, a mitochondrial KIF5 motor adaptor [34], reduces mitochondrial trafficking to axonal terminals and accelerates synaptic depression and slows recovery during high-frequency firing [19]. Mutation in the mitochondrial fission protein Drp1 in *Drosophila* reduces synaptic localization of mitochondria and impairs SV mobilization from the reserve pool, thus depleting SVs much faster than in wild-type neurons during prolonged trains of stimulation, a phenotype partially rescued by adding ATP in the synapses [13]. Consistently, a conditional *drp1* KO mouse line with a postnatal deletion in CA1 hippocampal neurons fails to maintain mitochondrial-derived ATP levels at presynaptic terminals during neuronal activity, thus impairing SV recycling and memory in the mutant mice [48].

In cultured mature neurons, approximately 20–30% of axonal mitochondria move bi-directionally, some of which pass through or pause at presynaptic terminals [5, 6]. By imaging both axonal and presynaptic mitochondria in live hippocampal neurons, we recently characterized five patterns of mitochondrial motility and distribution in axons: non-synaptic stationary (54.07 ± 2.53%) and synaptic stationary mitochondria (16.29 ± 1.66%), and motile mitochondria passing through synapses (14.77 ± 1.58%), pausing at synapses briefly (7.01 ± 1.29%) or more than 200 sec (8.30 ± 1.52%) [8]. These patterns are consistent with a previous study in cortical neurons [42], and further supported by a recent *in vivo* 3D electron microscopy analysis of perfusion-fixed hippocampi, where 33% of total synapses contain presynaptic mitochondria, 31% of synapses have the nearest axonal mitochondrion located at a distance less than 3 μm from the synapses, and 36% of synapses have a distance greater than 3 μm to the nearest axonal mitochondrion [49].

The balance between these motile and synaptic pools of mitochondria responds quickly to changes in synaptic activity and likely the status of energy homeostasis. We propose an attractive model: a stationary mitochondrion retained within presynaptic boutons could constantly supply ATP to support ATP-dependent presynaptic functions. Conversely, for a presynaptic terminal lacking an anchored mitochondrion, ATP is mainly supplied through diffusion from mitochondria outside the synapse. When mitochondria move closer, more ATP is supplied to the synapse; when mitochondria move away, less ATP is supplied to the synapse. Therefore, a motile mitochondrion passing through this presynaptic bouton dynamically alters local ATP homeostasis, thus influencing ATP-dependent synaptic functions (Figure 3).

We recently tested this model by combining live neuron imaging and electrophysiological analysis in a *snph* mutant mouse model in which axonal mitochondrial motility was robustly increased (78%) [8]. First, using cultured hippocampal neurons and hippocampal slices from age-matched wild-type and *snph*$^{-/-}$ mice, we revealed that enhanced axonal mitochondrial motility significantly increases the variability of pulse-to-pulse amplitudes of excitatory postsynaptic currents (EPSCs). Over-expressing SNPH reduces the variability found in wild-type neurons by abolishing mitochondria motility. Second, using dual-channel imaging of mitochondrial motility and synapto-pHluorin at single-bouton levels, we further showed that mitochondrial movement either into or out of presynaptic boutons influences SV cycling due to the large fluctuations of synaptic ATP levels. In the absence of an anchored mitochondrion, presynaptic boutons lack a constant on-site ATP supply under intensive synaptic activity. A motile mitochondrion passing through could spatially and temporally supply ATP, thus changing presynaptic energy levels when a mitochondrion moves in or out of synapses and consequently influencing ATP-dependent synaptic activities (Figure 3). Therefore, fluctuations of presynaptic ATP levels contribute to the wide variability of presynaptic strength. Our study thus reveals that axonal mitochondrial motility is one of the primary mechanisms underlying plasticity and reliability of presynaptic strength in the CNS.

In neurons, glycolysis also produces ATP, a process independent of mitochondria. Thus, increased glycolysis could supply ATP in boutons that lack mitochondria. By measuring presynaptic ATP levels, the Ryan group demonstrated that glycolysis supports the maintenance of ATP levels at resting presynaptic boutons [38]. Blockage of the ATP synthesis pathways by application of either glycolysis inhibitor 2-deoxyglucose or mitochondrial ATPase inhibitor oligomycin alone reduces presynaptic ATP levels during synaptic activity. Their study suggests that ATP supply from both glycolysis and mitochondria is required to sustain activity-dependent ATP consumption. It was also reported that mitochondria-derived ATP is dispersed in axons and diffused to non-mitochondria-containing presynaptic bontons. Thus, the capacity for SV recycling is similar in the presynaptic boutons with or without a mitochondrion [39]. These findings raise a question as to whether presynaptic mitochondria could provide more ATP sources to sustain increased synaptic efficacy. By 3D electron microscopy, the Harris group recently demonstrated that sustained synaptic activity is specific to the mitochondria-containing presynaptic boutons during long-term potentiation [49]. Presynaptic boutons with mitochondria have more docked SVs than those without mitochondria in the hippocampal CA1 area. This is largely attributed to the fact that efficient SV mobilization is restricted to presynaptic boutons with or near mitochondria. This study further supports a previous study that ATP production from presynaptic mitochondria is the main local energy source driving SV mobilization from the reserve pool in order to sustain the lasting synaptic efficacy [13].

While MT-based kinesin and dynein motors drive mitochondrial transport along long-range MT-tracks, actin-based myosin motors mediate short-range movement at presynaptic terminals where actin filaments form the major cytoskeletal architecture. It was reported that the actin anchors mitochondria at nerve growth factor stimulation sites although underlying mechanisms remain unclear [50], thus raising an interesting question as to whether motile mitochondria are recruited to and captured at presynaptic terminals through the MTs-actin crosstalk.

Regulation of mitochondrial motility by energy metabolism and growth status

Mitochondrial trafficking and distribution in polarized neurons is the central issue concerning the maintenance of energy homeostasis throughout cells. Proper mitochondrial transport into growth cones and branches in developing neurons ensures an adequate ATP supply in these metabolically active regions. Recent studies have established a correlation between polarized mitochondrial transport and axonal and dendritic morphology. For example, a recent study reported the differential functions of motor adaptors Trak1 and Trak2 in driving polarized mitochondrial transport and thus axonal and dendritic growth were reported [51]. While Trak1 is required for axonal mitochondria transport through binding to both kinesin-1 and dynein motors, Trak2 predominantly mediates mitochondrial transport into dendrites by interacting with the dynein motors. Consistently, depleting Trak1 inhibits axonal outgrowth, while disrupting Trak2-mediated mitochondrial trafficking impairs dendrite morphology. A genetic mouse study provided further evidence showing the differential roles of Miro1 and Miro2 in mitochondrial trafficking and neuronal morphogenesis [47]. Miro1, but not Miro2, is the main regulator of mitochondrial trafficking. Deleting Miro1 *in vivo* during mouse development disrupts neuronal morphogenesis, while Miro1 disruption in mature neurons leads to a loss of distal dendritic complexity.

Emerging evidence suggests that neurons may have a special mechanism activating mitochondrial biogenesis and delivering mitochondria to distal axons by sensing energy requirements. Neuronal growth requires a considerable amount of energy to drive the synthesis of raw building materials and the delivery of these materials to new growing tips. Mitochondrial biogenesis and a local mitochondria-derived ATP supply are required for axonal growth during development [52]. AMPK, an AMP-activated protein kinase, is a master regulator of cellular energy homeostasis and is activated upon stresses that deplete cellular ATP supplies. Earlier activation of mitochondrial biogenesis through the AMPK-PGC-1α–NRF1 axis accelerates generation of new mitochondria and increases mitochondrial density and the ATP/ADP ratio in axonal terminals, thereby ensuring energy-production capability necessary for axonal growth.

It is assumed that stationary mitochondria ideally serve as local energy stations that constantly supply ATP and thus maintain local ATP homeostasis. We recently examined this issue by using cortical neurons and found a solid causal correlation of mitochondrial distribution patterns and the ATP/ADP ratio in axonal terminals [22]. Over-expressing SNPH restricts axonal mitochondria within the proximal axons due to reduced flux to distal axons, which correlates with a reduced ATP/ADP ratio in the most distal axon segment and smaller sized growth cones. Conversely, over-expressing Miro1 increases mitochondrial density in distal axons and the average size of growth cones. These results suggest that proper mitochondrial density in distal axons is required to maintain growth capacity. High ADP levels are thought to suppress mitochondrial motility [53]. Using a motor-assisted transport model combined with probability simulations, Mironov reported that [ADP] gradients in the proximity of active synapses and growth cones slow down mitochondrial motility and thus target mitochondria to “hot spots” with high energy consumption.

Recent studies support the notion that the balance between motile and stationary mitochondria responds to changes in axonal growth status via AMPK. One study highlights a critical role for SNPH in mediating mitochondrial anchoring through the AMPK pathway [54]. Activation of AMPK increases anterograde flux of mitochondria into distal axons and induces axonal branching. As a cellular energy sensor, AMPK activation may replenish ATP supply in distal axons by recruiting mitochondria and anchoring them through signaling pathways that have not been revealed yet. Intriguingly, depleting SNPH reduces the stationary pool size of axonal mitochondria accompanied by decreased axon branching, thus establishing a causal correlation between SNPH-mediated anchoring and AMPK-induced axonal branching. However, an important mechanistic question remains: Do SNPH and/or motor complexes act as a downstream effector of the AMPK pathways in recruiting mitochondria by sensing metabolic signals? KIF5 motor might be a potential target because its light chain is phosphorylated by AMPK [55]. It would be very significant to investigate the mechanisms by which axonal mitochondria are immobilized or remobilized to sense changes in the local ATP/ADP ratio or metabolic signals.

Tao et al. [56] provided further evidence showing that AMPA activation is required for axonal branch formation in an ATP-dependent manner by balancing mitochondrial trafficking and anchoring. Activation of AMPK increases anterograde transport of mitochondria toward axonal terminals and accumulates mitochondrial docking in regions preceding the emergence of new axonal branches. The formation of axonal branches is blocked by the mitochondrial un-coupler FCCP, thus providing a link between mitochondria-derived ATP and formation and/or maintenance of axonal branches. A third study revealed an intriguing mechanism underlying the mitochondrial role in determining axon branching sites [57]. Anchored mitochondria in localized hot spots promote the maturation of axonal filopodia into axon branching through ATP generation that powers local intra-axonal mRNA translation and protein synthesis. Blocking mitochondrial respiration or inhibiting protein synthesis impairs maturation of axonal branches, despite mitochondria having been anchored at these hot spots. These studies support the notion that the balance between motile and stationary mitochondria in axons responds to changes in axonal growth status via AMPK. Thus, regulating mitochondrial trafficking and anchoring is critical to maintaining the local ATP supply necessary for energy-demanding axonal growth and branching.

Glucose is the main carbon source for mitochondria-derived ATP production. In particular, neurons rely heavily on a continuous supply of glucose to maintain mitochondrial energy metabolism. A recent study revealed that glucose levels can also regulate mitochondrial motility in neurons [58]. Extracellular glucose arrests mitochondrial transport through the enzyme OGT, a putative metabolic sensor. Post-translational modification of Milton by OGT-dependent O-GlcNAcylation is required for regulating mitochondrial distribution. Through this mechanism, neurons may accumulate axonal mitochondria in areas where cytosolic glucose is elevated, thus ensuring rapid ATP production by sensing changes in the glucose supply.

Mitochondrial transport facilitates axon regeneration by rescuing energy deficits

While young neurons during early developmental stages have robust axon growth capacity, mature neurons typically fail to regenerate after spinal cord injury or traumatic brain injury, leading to permanent neurological impairments. It was suggested that mature neurons have lost their growth capacity due to an intrinsic decline of permissive conditions for regeneration [59]. Thus, it is critical to understanding which intrinsic mechanisms account for the mature neuron-associated decline of regrowth capacity. Injury in mature neurons usually leads to an inability to reform an active growth cone, where damaged membranes are resealed, cytoskeletal structures are rearranged, and regrowth programs are activated, including synthesis of raw building materials, transport, and assembly of axonal components. All of these regrowth events require high levels of energy consumption [60]. Mitochondria-derived ATP production provides most of the axonal energy. Because of the limited diffusion capacity of intracellular ATP through extremely long axons, axonal mitochondria are the main source of the ATP necessary to assemble a new growth cone and support axon regeneration. Axonal injury is a strong stress condition that induces mitochondrial depolarization [61, 62]. Dysfunctional mitochondria not only supply less ATP, causing local energy deficits, but also release toxic ROS and apoptotic factors which further trigger axonal pathology and degeneration [63]. Therefore, enhancing mitochondrial transport not only removes those damaged mitochondria, but also delivers healthy ones into injured axons to meet increased energy requirements during regeneration. To test the above hypothesis, one would first address two fundamental questions: (1) Do mature neurons maintain an effective capacity to recruit healthy mitochondria to injured axons? (2) If this capacity declines with neuron maturation, does enhancing mitochondrial transport enables mature neurons to regain axon regenerative capacity?

We recently examined these issues by live imaging of mitochondrial transport, mitochondrial integrity, and dynamic ATP levels in injured axons within a microfluidic chamber system combined with an *in vivo* mouse model [22]. First, we examined the relative SNPH expression and axonal mitochondrial transport throughout neuronal developmental stages. To our surprise, the mature neuron-associated decline of regrowth capacity correlated well with progressively increased levels of SNPH expression. SNPH becomes detectable after DIV9, and peaks at DIV22 in culture. Axonal mitochondrial motility at DIV7 is 47%, two times higher than that at DIV18. This *in vitro* SNPH expression pattern is consistent with a robustly increase in SNPH expression in mature neurons of rat brains. These results suggest that mature neuron-associated increase in SHPN expression and declines in mitochondrial transport is one of the intrinsic mechanisms diminishing axonal regenerative capacity. Our study showing a progressive decline of axonal mitochondrial transport over neuronal maturation is also consistent with three recent *in vitro* and *in vivo* studies [64–66]. In ganglion cell dendrites in the intact retina, mitochondria are highly motile (30%) during developmental stages, as dendrites mature, mitochondria reach stable positions such as synapses and branch points [64]. Second, we manipulated axonal mitochondrial transport in mature cortical neurons by over-expressing SNPH or Miro1 transgenes and then evaluated axonal regrowth 6 days after injury. Expressing SNPH arrests all mitochondrial transport and abolishes axons regrowth after injury. In contrast, expressing Miro1 enhances mitochondrial transport and robustly increases axonal regrowth capacity. This study indicates that mature neurons can regain their regrowth capacity by enhancing mitochondrial transport. Third, by monitoring mitochondrial membrane potentials and ATP/ADP ratio, we showed that axonal injury is an acute stress condition that damages local mitochondria and reduces ATP supply, thus triggering energy deficits in the injury sites. Furthermore, by *in vivo* crushing sciatic nerves in adult *snph* KO mice, we found that enhanced mitochondrial transport in *snph* KO sciatic nerves facilitates *in vivo* axonal regeneration [22].

Our findings that mitochondrial transport influence axonal regenerative capacity in mouse models are supported by several previous reports. In *C. elegans* mutant ric-7 with impaired mitochondrial transport to distal axons, injured axons degenerate rapidly; such degeneration can be suppressed by forcing mitochondria into the axons [67]. When mitochondria are eliminated from fly axons by depleting Milton, up-regulation of Nmnat, which is known to suppress axon degeneration [68], fails to suppress axon degeneration [69], suggesting that mitochondrial trafficking to axons is critical for suppressing axonal degeneration. Using both *Drosophila* and mouse models, Avery et al. [70] identified axonal mitochondria as a key target for Wld^S, an effective protein that protects axon from Wallerian degeneration after injury [71]. Wld^S enhances mitochondrial flux into axons, which is essential for maximal axonal protection after injury [70]. A peripheral injury of CNS axons induces a global increase in the axonal transport of organelles including mitochondria, lysosomes, and other axonal building blocks, thus supporting the axon regeneration [72]. Recently, two other studies provided *in vivo* evidence in worms and mice that mitochondrial transport plays a critical role in enhancing neuronal regenerative capacity after injury. In *C. elegans*, axotomy triggers an energy stress in injured axons and recruits and increases axonal mitochondrial density to supply ATP for sustained axonal regeneration [73]. This injury-induced response is via the activation of the dual leucine zipper kinase 1 (DLK-1), a conserved regulator of axon regeneration. Using adult mouse retinal ganglion cells as an *in vivo* injury model, Cartoni et al [74] reported that expression of the mitochondrial protein Armcx1 enhances mitochondrial transport by recruiting stationary mitochondria. Such enhanced transport is critical to protecting axotomized neurons from cell death and promoting axon regeneration. Although the mechanisms enhancing axonal mitochondrial transport by DLK-1 and Armcx1 have yet been elucidated, these studies reveal new molecular players in the regulation of neuronal injury responses.

Energy deficit is defined as insufficient ATP supply when mitochondria are damaged and/or increased energy consumption such as during axonal regeneration. Mitochondrial damage by axonal injury, mature neuron-associated decline of mitochondrial transport, and enhanced energy consumption collectively contribute to energy deficits in injured axons. Enhanced mitochondrial transport rescues energy deficits by replenishing healthy mitochondria to injured axons (Figure 4). Thus, activating an intrinsic “growth program” requires the coordinated recovery of energy supply through enhanced mitochondrial transport. Such coordinated regulation may represent a valid therapeutic strategy to facilitate nerve regeneration and recovery after injury and diseases. Future development of safe and effective small molecule compounds will be an attractive strategy to selectively increase mitochondrial motility and rescue the local energy deficits within injured axons [75].

Conclusion Remarks

Mitochondria are the main cellular power plants that produce energy essential for neuronal growth, survival, and function. Neurons face exceptional challenges in maintaining energy homeostasis especially at distal synapses and growth cones where energy is in high demand. Anchored mitochondria ideally serve as local energy sources. The energy-dependent regulation of mitochondrial trafficking and anchoring ensures that these metabolically active areas are adequately supplied with ATP during the growth of developing neurons, the regeneration of injured mature neurons, and the maintenance of synaptic activity.

It is well documented that mitochondrial dysfunction and impaired mitochondrial transport are involved in the major neurodegenerative diseases and neurological disorders [20, 21, 76]. Damaged mitochondria fail to produce ATP and associate with an altered redox status. Bioenergetic deficits and chronic oxidative stress trigger axonal pathology and synaptic dysfunction, thus contributing to pathogenesis of neurodegenerative diseases [30]. Indeed, energy failure or an impaired bioenergetic metabolism emerges as a common problem in the early stages of aging-associated neurodegenerative diseases, including Alzheimer’s and Parkinson’s diseases [10, 77–79].

SNPH is one intriguing anchoring protein that is specific for axonal mitochondria and thus serves as an attractive target for future investigations into mechanisms that recruit mitochondria into activated synapses and injured axons. Our studies demonstrated that the relative SNPH enrichment on axonal mitochondria controls mitochondrial motility and the mature neuron-associated decline of mitochondrial transport correlates with progressively increased SNPH expression [22, 36]. Declined axonal mitochondrial transport in mature neurons is also supported by recent three *in vitro* and *in vivo* studies [64–66]. Elevated SNPH expression and thus mitochondrial anchoring in mature neurons is necessary to maintain synaptic function. However, when mature neurons are injured and diseased, SNPH-mediated mitochondrial anchoring restricts axonal regrowth and regenerative capacity. These findings allow us to propose a hypothesis: mature neuron-associated SNPH expression is an intrinsic mechanism that restricts the removal of dysfunctional mitochondria from axons, thus leading to energy deficits at distal synapses under pathological conditions. The selective removal of SNPH from those damaged mitochondria would enhance their transport, allowing for efficient repair or elimination in the somatodendritic regions where mature lysosomes are relatively enriched. Thus, spatially and temporally removing SNPH from axonal mitochondria may be one of attractive pathways to replenish healthy mitochondria at distal terminals in order to rescue energy deficits, and thus support neuron regeneration after injury and maintain synaptic transmission in disease. Therefore, the development of new optogenetic tools to test this hypothesis will help advance our understanding as to how mitochondrial trafficking and anchoring in axons and at synapses are regulated through the sensing and integration of changes in local metabolic status under various physiological and pathological stresses. The future studies should be directly relevant to the challenge mature neurons face in maintaining an energy supply in health and similarly recovering energy deficits in neurological disorders and regeneration after injury and diseases (see Outstanding Questions).

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BOX 1

Motors and adaptors in mitochondrial transport

The *Drosophila* protein Milton and its mammal orthologues Trak1 and Trak2 function as adaptors linking the C-terminal domain of KIF5 motors to mitochondria through Miro1 or Miro2, a Rho-GTPase present in the mitochondrial outer membrane [45, 80, 81]. Miro contains EF hand Ca^{2+}-binding motifs and GTPase domains, thus allowing mitochondrial transport to be regulated in response to Ca^{2+} signaling. KIF5, Milton/Trak, and Miro1/2 constitute the motor-adaptor complexes driving anterograde mitochondrial transport. Mutation of the *milton* or *miro* genes in *Drosophila* depletes mitochondria at synaptic terminals [29, 81]. In mouse hippocampal neurons, the Miro1/Trak2 complex is a key regulator of mitochondrial transport [35]. Expressing Miro1 facilitates the recruitment of Trak2 to mitochondria while depleting Trak1 impairs mitochondrial transport in axons [82, 83]. The role of Miro was further confirmed in two recent studies with *miro1* deletion mouse models. The first study showed that neuron-specific loss of Miro1 causes depletion of mitochondria from corticospinal tract axons and progressive neurological deficits [46]. The second study, however, demonstrated that Miro1, but not Miro2, is the primary regulator of mitochondrial transport in both axons and dendrites. The Miro1 deletion causes depletion of mitochondria from distal dendrites accompanied by a marked reduction in dendritic complexity [47]. Syntabulin is an alternative KIF5 adaptor for driving mitochondrial transport. Syntabulin attaches to the outer mitochondrial membrane via its carboxyl-terminal domain and recruits KIF5 motors to mitochondria [34]. Depleting syntabulin or blocking its coupling to KIF5 impairs mitochondrial transport from the soma to distal axons. The presence of several motor adaptors for mitochondria may highlight the complex regulation of their motility in response to various physiological signals.

Dynein motors mediate retrograde mitochondrial movement in axons. Cytoplasmic dynein is composed of two dynein heavy chains (DHCs) and several intermediate (DICs), light intermediate (DLICs), and light chains (DLCs). DHCs function as motors and the association of the dynein motor with cargoes and the regulation of its motility involve other polypeptides. Dynein associates with *Drosophila* mitochondria and mutations in DHC alter the velocities and run lengths of mitochondrial retrograde transport in axons [84]. Compared with Trak-Miro as the KIF5 adaptor complex, adaptors that recruit dynein motors to mitochondria are less known. Recent studies suggest that KIF5 and dynein motors share the same set of mitochondrial adaptor complexes. The loss of *dmiro* impairs both kinesin- and dynein-driven mitochondrial transport while over-expressing dMiro alters bi-directional mitochondrial transport [82, 85]. Trak1 and Trak2 contain separate binding domains for KIF5 and dynein/dynactin, thus allowing their coupling with both KIF5 and dynein [51]. Mitochondria move bi-directionally and frequently change direction, suggesting a model in which the relative activity of opposite-moving motors could be regulated through their interactions with the adaptor complexes.

Trends Box

Mitochondria are the main cellular power plants that supply energy essential for neuronal growth, survival, and function.

Neurons face exceptional challenges in maintaining energy homeostasis especially at distal synapses and growth cones where energy is in high demand.

Anchored mitochondria ideally serve as local energy sources.

The energy-dependent regulation of mitochondrial trafficking to and anchoring at distal axons and synapses is essential to ensure that these metabolically active areas are adequately supplied with ATP during the growth of developing neurons, the regeneration of injured mature neurons, and the maintenance of synaptic activity.

SNPH is one intriguing anchoring protein that is specific for axonal mitochondria and thus serves as an attractive target for future investigations into mechanisms that recruit mitochondria into activated synapses and injured axons.

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Outstanding questions

- How are mitochondrial trafficking and anchoring regulated through the sensing and integration of changes in the local bioenergetics status under various physiological stresses?
- Does anchoring protein SNPH act as downstream effectors of AMPK pathways in recruiting mitochondria by sensing the metabolic signals?
- Are mobile mitochondria captured at active presynaptic terminals through the microtubule-actin track switch and crosstalk?
- Do energy deficits occur in distal axons and at synapses in early stages under certain pathological conditions?
- Is energy deficit associated with a range of neurodegenerative diseases? If this is the case, does recovery of energy supply slow down the pathogenesis?
- How could SNPH be removed from axonal mitochondria in mature neurons in order to spatially and temporally enhance axonal mitochondrial transport and rescue energy deficits, and thus support neuron regeneration after injury and disease?

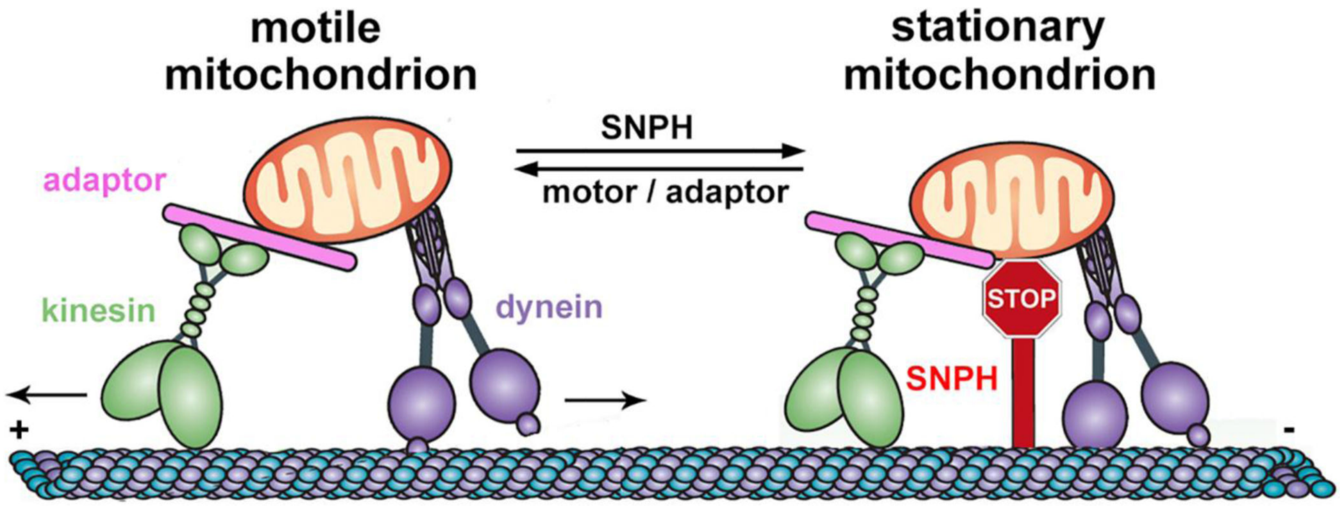

Figure 1. Motors/adaptors and anchoring protein play the opposite roles in regulating axonal mitochondrial motility

Long-distance axonal mitochondrial transport is driven by MT-based molecular motors: the plus-end directed kinesin and the minus-end directed dynein. Axonal MTs are uniformly arranged so that their plus-end is directed distally and the minus-end is toward the soma; thus, most kinesin motors move toward distal axons while dynein motors mediate retrograde transport toward the soma. The kinesin-1 family proteins (KIF5A, KIF5B, and KIF5C) are the main motor driving mitochondrial transport in neurons. Kinesin-1 motors interact with mitochondria through adaptor proteins. Axonal mitochondria also deploy an anchoring mechanism in addition to motor-driven transport. SNPH acts as a "static anchor" specific for axonal mitochondria. SNPH arrests mitochondrial transport by anchoring them to MTs. In CNS axons, the majority of mitochondria remain stationary, while approximately 20–30% are motile. Motile mitochondria can become stationary and stationary ones can be remobilized. The balance of motile versus stationary axonal mitochondria depends on the relative action of the motor/adaptor and SNPH.

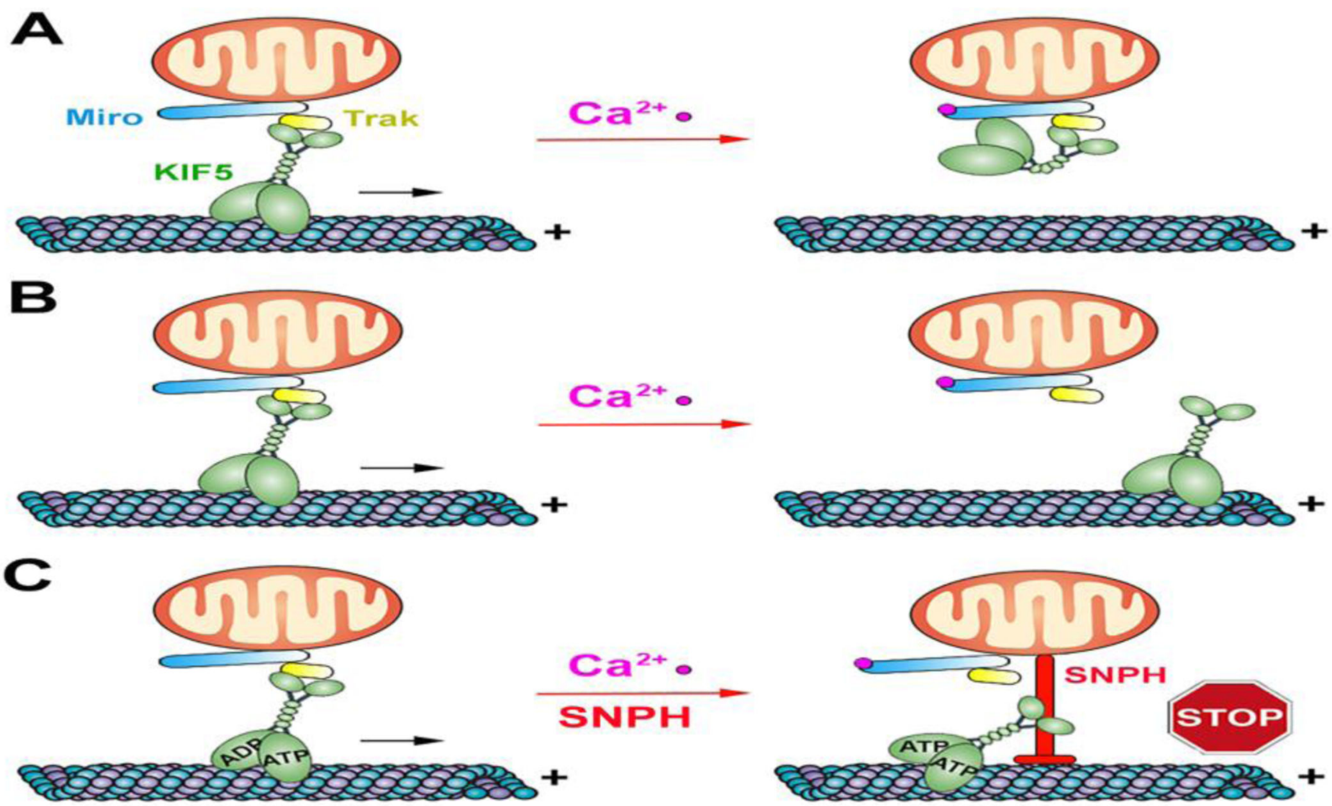

Figure 2. Synaptic activity regulates mitochondrial transport

(**A, B**) Miro-Ca^{2+} sensing models. Miro is a mitochondrial outer membrane protein with two Ca^{2+}-binding EF hands. By sensing cytosolic Ca^{2+} levels, Miro arrests mitochondria at activated synapses by inactivating KIF5 transport machineries. When a trafficking mitochondrion passes through an active synapse, elevated Ca^{2+} binds to Miro and induces its conformational changes, thus disrupting the complexes of KIF5-Trak-Mito complex [35, 44]. Through this mechanism, mitochondria are immobilized at activated synapses. Two alternative models were proposed on whether (**A**) the KIF5 motor remains associated with arrested mitochondria or (**B**) is disconnected with the organelle upon immobilization. It should be noted that two recent genetic studies showed that the loss of Miro1 in neurons does not inhibit the Ca^{2+}-dependent arrest of remaining mitochondria [46, 47], thus raising the possibility that activity-dependent mitochondrial immobilization may require a static anchoring mechanism.

(**C**) Engine-switch and brake model. When a motile mitochondrion passes by an activated synapse, the anchoring protein SNPH responds to elevated Ca^{2+} (stop sign) and switches off the engine (motor) and places a brake on mitochondrion, thereby arresting mitochondria on the MT-track. When the Ca^{2+} signal is removed, the cargo-loaded motor-adaptor complexes can be quickly re-activated to drive the mitochondrion to new active synapses. This engine-switch and brake model suggests an interplay between the motor-adaptor transport complex and the anchoring protein SNPH [37]. Through this mechanism, neurons effectively regulate axonal mitochondrial distribution in response to changes in energy-demanding synaptic activity.

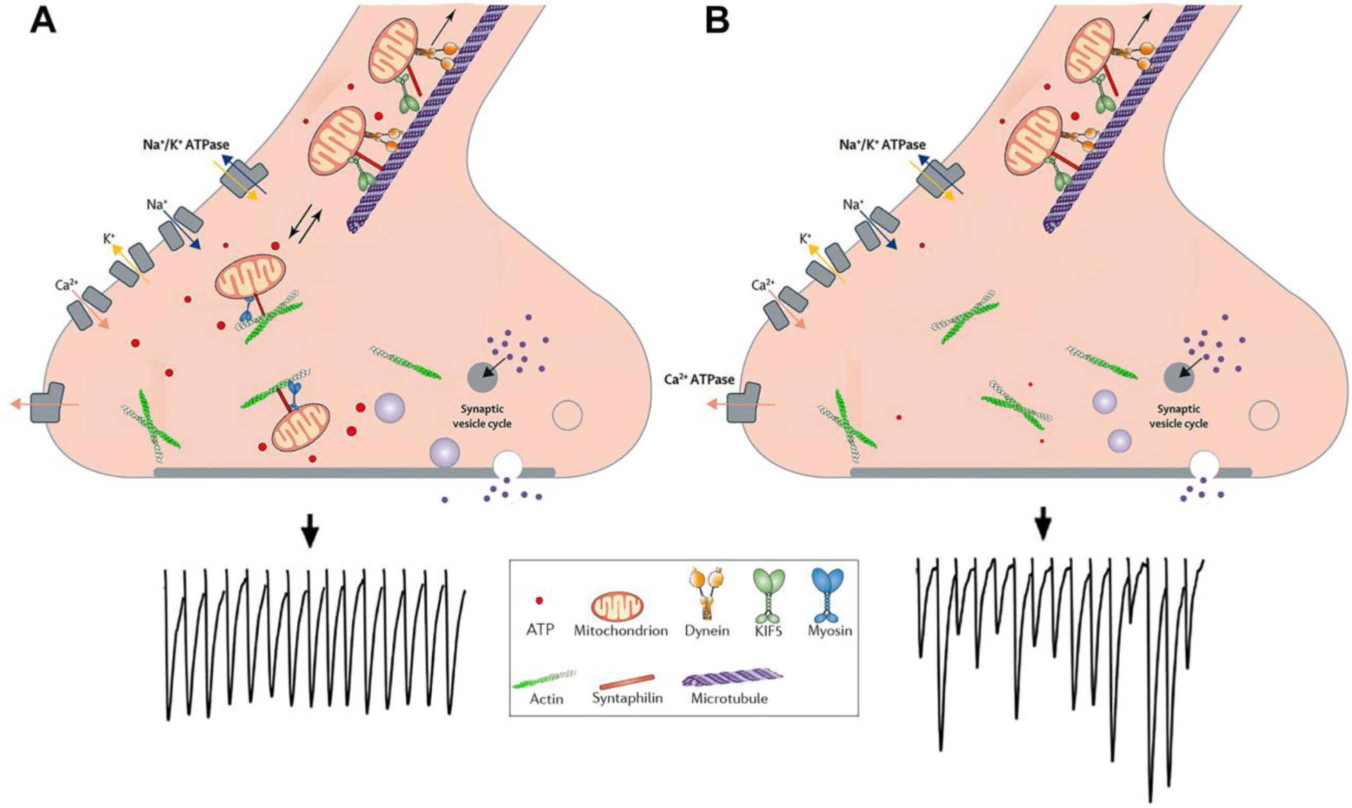

Figure 3. Mitochondrial motility influences energy homeostasis and presynaptic strength

(A) A stationary mitochondrion retained within a presynaptic bouton constantly supplies ATP to support various presynaptic functions such as: establishing the proton gradient necessary for neurotransmitter loading; removing Ca^{2+} from nerve terminals; powering SV transport from reserve pools to release sites; and driving SV exo- and endocytotic recycling, thus maintaining presynaptic strength.

(B) For a presynaptic terminal lacking an anchored mitochondrion, ATP is mainly supplied through diffusion from mitochondria outside synapses. When a mitochondrion moves closer, more ATP supplies the synapse; when a mitochondrion moves away, less ATP supplies the synapse. Thereby, a motile mitochondrion passing through this presynaptic bouton dynamically alters local ATP levels and influences ATP-dependent synaptic functions, thus leading to wide pulse-to-pulse variability of synaptic strength, particularly under increased energy demand during sustained synaptic activity.

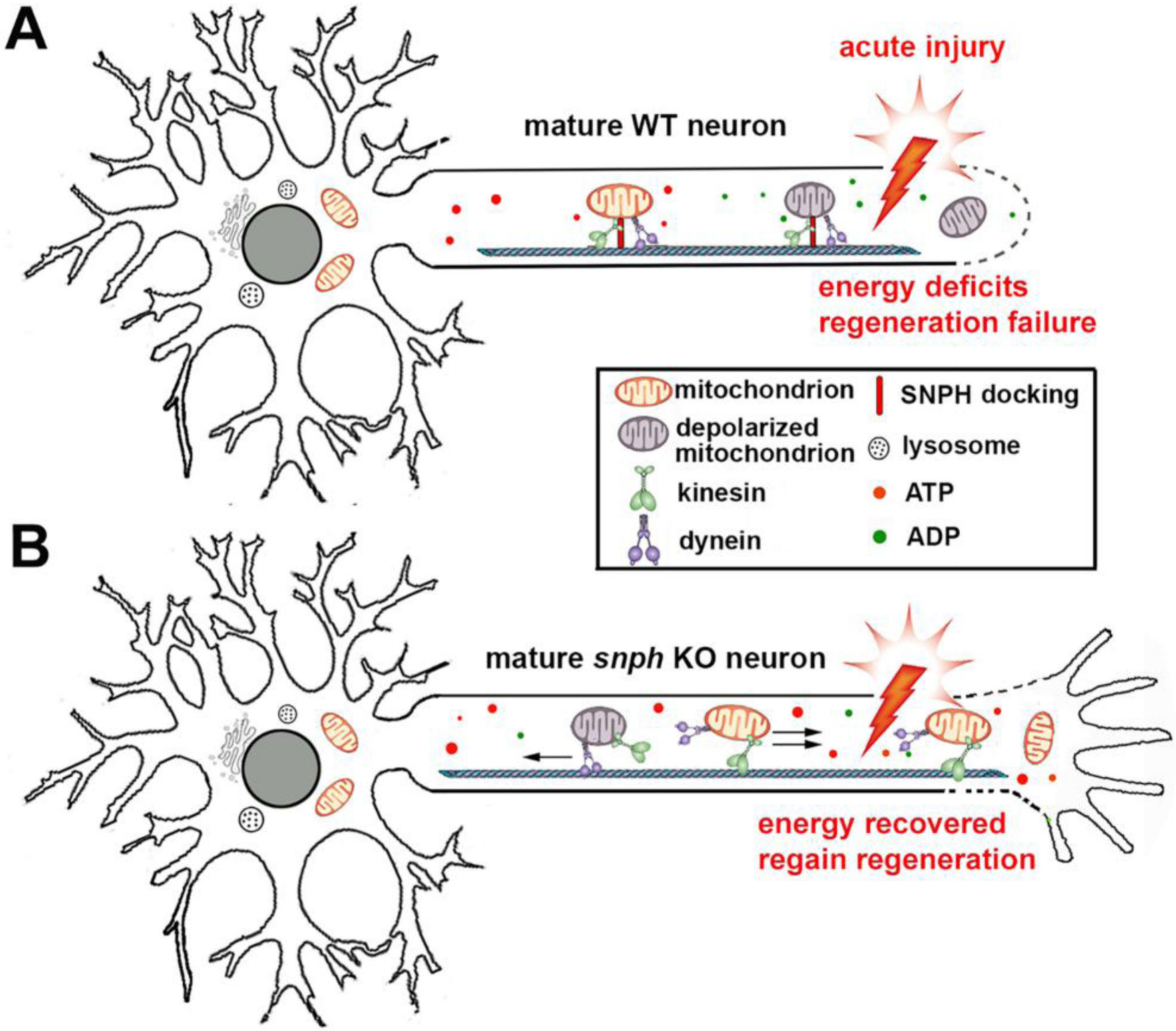

Figure 4. Illustration of enhanced mitochondrial transport critical for mature neurons to regain axonal regenerative capacity

(A) An energy deficit is defined as insufficient ATP supply when mitochondria are damaged and/or there is increased energy consumption during regeneration. Mitochondrial damage by axonal injury and mature neuron-associated decline of mitochondrial transport collectively contribute to local energy deficits in injured axons, thus leading to regeneration failure. Energy deficits may reflect the intrinsic restriction of mature neurons to regenerate following injury.

(B) Enhanced mitochondrial transport by deleting SNPH not only helps remove those dysfunctional mitochondria, but also replenishes healthy ones to the injured axons, thus recovering mitochondrial integrity and rescuing energy deficits. An enhanced local ATP supply is critical to meeting the metabolic requirements of axon regeneration. Thus, activating an intrinsic “growth program” requires the coordinated recovery of energy deficits by enhancing mitochondrial transport. Such coordinated regulation may represent a valid therapeutic strategy to facilitate nerve regeneration and functional recovery after injury and diseases.