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Regulation of Axonal Mitochondrial Transport and Its Impact on Synaptic Transmission

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Abstract

Mitochondria are essential organelles for neuronal survival and play important roles in ATP generation, calcium buffering, and apoptotic signaling. Due to their extreme polarity, neurons utilize specialized mechanisms to regulate mitochondrial transport and retention along axons and near synaptic terminals where energy supply and calcium homeostasis are in high demand. Axonal mitochondria undergo saltatory and bidirectional movement and display complex mobility patterns. In cultured neurons, approximately one-third of axonal mitochondria are mobile, while the rest remain stationary. Stationary mitochondria near synapses serve as local energy stations that produce ATP to support synaptic function. In addition, axonal mitochondria maintain local Ca^{2+} homeostasis at presynaptic boutons. The balance between mobile and stationary mitochondria is dynamic and responds quickly to changes in axonal and synaptic physiology. The coordination of mitochondrial mobility and synaptic activity is crucial for neuronal development and synaptic plasticity. In this update article, we introduce recent advances in our understanding of the motor-adaptor complexes and docking machinery that mediate mitochondrial transport and axonal distribution. We will also discuss the molecular mechanisms underlying the complex mobility patterns of axonal mitochondria and how mitochondrial mobility impacts the physiology and function of synapses.

Keywords

mitochondria; axonal transport; docking; synaptic plasticity; kinesin; motor adaptor; anterograde transport; retrograde transport; stationary mitochondria; mitochondrial mobility

1. Introduction

Proper neuronal function and survival depends upon the supply of appropriate levels of ATP, approximately ninety percent of which is produced by mitochondria via oxidative phosphorylation. Neurons are highly polarized cells whose morphology precludes the efficient diffusion of somally produced ATP to distal processes. While the biogenesis of neuronal mitochondria is not well characterized, it is clear that the majority of mitochondria are produced in the cell body. Thus, distal cellular compartments such as synapses depend upon the efficient delivery of mitochondria through active transport to provide local sources

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of ATP. Additionally, mitochondria have been shown to aid in critical physiological processes, including the establishment of the axonal resting membrane potential required for action potential propagation, the assembly of the actin cytoskeleton within presynaptic boutons (Lee and Peng 2008), and the myosin-driven mobilization of synaptic vesicles from the reserve pool to the readily releasable pool during sustained neuronal activity (Versteken 2005). Furthermore, mitochondria's ability to buffer Ca^{2+} within presynaptic terminals appears to be involved in certain types of short-term synaptic plasticity (Tang and Zucker, 1997, Billups and Forsythe, 2002, Levy et al., 2003, Kang et al., 2008). Thus, removing mitochondria from axon terminals results in aberrant synaptic transmission (Stowers et al., 2002; Guo et al., 2005; Verstreken et al., 2005; Ma et al., 2009). Many neurodegenerative diseases, including Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, involve defects in mitochondrial function and transport (see reviews by Chan, 2006; Stokin and Goldstein, 2006).

Mitochondria are transported from the cell body along axonal microtubules (MTs) by protein motors to reach areas with high ATP and calcium buffering requirements like Nodes of Ranvier, axonal branches, active growth cones, and synapses (Fabricius et al., 1993; Morris and Hollenbeck, 1993; Mutsaers and Carroll, 1998; Ruthel and Hollenbeck, 2003; Kang et al., 2008; Zhang et al., 2010). Generally, kinesin motors drive anterograde mitochondrial transport, while dyneins are responsible for retrograde transport (Fig. 1). Individual mitochondrion, however, rarely move in only one direction. Their transport along MTs typically involves pauses of short and long duration and abrupt changes in direction. At any given time, two-thirds of axonal mitochondria in cultured neurons are stationary (Hollenbeck and Saxton, 2005; Kang et al., 2008). Syntaphilin mediates axonal mitochondrial "docking" and helps establish an appropriate axonal mitochondrial distribution (Kang et al., 2008; Chen et al., 2009). The complex mobility patterns of axonal mitochondria may indicate that individual mitochondria are simultaneously coupled to kinesins, dyneins, and anchoring machinery whose actions compete or oppose one another. Averaging the bidirectional and saltatory components yields a net mitochondrial velocity that falls between fast moving vesicles and slow-moving cytoskeletal proteins: 0.3–2.0 μm $second^{-1}$(Morris and Hollenbeck, 1993; Ligon and Steward, 2000).

During development, mitochondrial mobility is tightly regulated to ensure that metabolically active areas are adequately supplied with ATP and able to respond to changes in intracellular Ca^{2+} levels. Later, the structural and functional plasticity characteristic of synapses and axons can drive changes in mitochondrial mobility. For example, the number of immobile mitochondria adjacent to active synapses increases in response to elevated cytosolic Ca^{2+} levels induced by synaptic activity (Rintoul et al., 2003; Yi et al., 2004). It is probable that neuronal development and synaptic activity are partially regulated by the mechanisms that control mitochondrial position. While these events have not been fully elucidated, two prominent proteins have been identified within the last decade. Milton, a mitochondrial motor-adaptor, provides a link between kinesin motors and mitochondria through an interaction with Miro, a calcium-sensing member of the Rho-GTPase family present in the outer mitochondrial membrane (Glater et al., 2006; Saotome et al., 2008; Macaskill et al., 2009; Wang and Schwarz, 2009; Cai and Sheng, 2009). Continued research will produce more regulatory proteins and increase our understanding of how neurons regulate mitochondrial trafficking to maintain synaptic and axonal homeostasis.

2. Motor proteins driving axonal mitochondrial transport

ATP-dependent kinesin motors mediates anterograde transport along MT-based tracks (Hollenbeck, 1996). Since Kinesin-1 (KIF5) was first reported to drive plus end-directed transport *in vitro*, at least 45 different human and mouse kinesin genes have been identified

(Hirokawa and Takemura, 2004). Most members of the kinesin superfamily are structurally similar to Kinesin-1 and have two heavy chains (KHCs) and two light chains (KLCs) (Fig. 2A). The heavy chains of KIF5 contain coiled-coil domains that facilitate their association into homo- or heterodimers. Mammals have two neuron-specific KIF5s (KIF5A and –C) and another that is expressed in most cell types (KIF5B) (Kanai et al., 2000, also see review by Hirokawa and Takemura, 2005). The amino terminal domain of each KIF5 contains a MT-binding motor domain, while the carboxyl terminal domain binds to KLCs or interacts directly with cargo adaptors. Thus, through their C-terminal cargo-binding domain, kinesins attach to cargoes through either a direct interaction between their cargo-binding domains and cargo adaptor proteins or an indirect interaction via their KLCs. Regarding mitochondrial transport, recent studies demonstrate that the former situation is more likely: KIF5s appear to bind mitochondria via their adaptor proteins independent of their KLCs (Cai et al., 2005; Glater et al., 2006).

Several lines of evidence demonstrate that KIF5s play a major role in the anterograde transport of axonal mitochondria (Hurd and Saxton, 1996; Tanaka et al., 1998; Stowers et al., 2002; Cai et al., 2005; Glater et al., 2006). Both imaging and biochemical analyses have confirmed that KIF5 motors associate with brain mitochondria (Hirokawa et al., 1991; Cai et al., 2005; Pilling et al., 2006; Macaskill et al., 2009; Wang and Schwarz, 2009). Furthermore, motor axons of larval *Drosophila kif5* mutants display impaired mitochondrial transport and a reduced mitochondrial distribution (Pilling et al., 2006). In undifferentiated extra-embryonic cells, the targeted disruption of *kif5b* induces aberrant mitochondrial clustering in the perinuclear region as most mitochondria fail to undergo transport towards the cell periphery (Tanaka et al., 1998). Disrupting KIF5-mitochodria coupling in hippocampal neurons by expressing the KIF5 cargo-binding domain transgene induces impaired axonal mitochondrial transport and reduced mitochondrial density in distal axonal compartments (Cai et al., 2005).

Additionally, a member of the kinesin-3 family, KIF1Bα, is widely expressed in the brain and has been shown to interact directly with mitochondria (Nangaku et al., 1994). *In vitro*, KIF1Bα has been shown to transport mitochondria along MTs at 0.5 μm/sec. While the mutation of murine *kif1B* leads to peripheral neuropathies, its role in anterograde mitochondrial transport remains to be further characterized.

Dynein motors drive the retrograde transport of neuronal organelles, including mitochondria (Pilling et al., 2006). The dynein motor complex is comprised of two heavy chains (DHC) and several intermediate, light intermediate, and light chains (DIC, DLIC and DLC, respectively) thought to regulate the motility of the dynein motor or mediate its association with cargoes (Fig. 2B). Whereas the heterogeneity of KHCs presumably allows for the selective binding of cargoes, relatively few DHCs have been identified. Thus, the cargo selectivity of the dynein complex likely depends more heavily upon accessory proteins (Susalka and Pfister, 2000). Dynactin, though nonessential, has been shown to mediate some dynein-cargo interactions, coordinate bidirectional axonal transport, and enhance the processivity of the dynein motor (Waterman-Storer et al., 1995; Karki and Holzbaur, 1999; King and Schroer, 2000).

One of eleven different dynactin subunits, $p150^{Glued}$, can bind directly to both cytoplasmic dynein and MTs. Additionally, although there is a strong directional bias towards the MT minus end, single dynein-dynactin complexes can move bidirectionally (Mallik et al., 2005). It is possible that this versatility may allow the dynein motor to backtrack in order to maneuver past physical barriers it encounters along MTs. Subsequent experiments have shown that cytoplasmic dynein is necessary for proper mitochondrial retrograde transport in the *Drosophila* nervous system and that dynein and dynactin components interact with

purified mitochondria (Pilling et al., 2006). The mutation of dynactin $p150^{Glued}$ and the dynein heavy chain disrupts bidirectional fast organelle transport. The resultant phenotype is similar to those caused by kinesin mutations and is characterized by axonal swelling as retrograde and anterograde cargoes, including mitochondria, accumulate along the axon (Martin et al., 1999).

Recent evidence suggests that opposing kinesin and dynein motors localized to the same mitochondrion may be responsible for the complex mobility patterns of axonal mitochondria. As axonal mitochondria exhibit bidirectional movement and dynein can co-localize with mitochondria moving in either direction (Hirokawa et al., 1990), it is likely that kinesin and dynein coordinate the transport of individual mitochondria. A *Drosophila* homologue of the mammalian kinesin-interacting scaffold protein JIP-1/JNK, APLIP1, is implicated in retrograde mitochondrial transport (Horiuchi et al., 2005). More recently, it was shown that the *Drosophila* mitochondrial adaptor protein dMiro helps regulate both anterograde and retrograde transport of axonal mitochondria (Russo et al., 2009). Relatively little is know about the roles and mechanisms of these adaptor proteins in regulating bidirectional movement. Future experiments are necessary to determine to what extent regulatory crosstalk between kinesin, dynein, and their respective adaptors coordinate the distribution and mobility state of mitochondria in response to metabolic changes and synaptic activity.

3. Motor adaptors specific for mitochondria

Cargo identity must be preserved to permit the targeted trafficking of cellular components. Generally, this is achieved by specific linkage between cargo vesicles and motors. Regarding neuronal mitochondria, the specificity of anterograde transport is achieved by direct interactions between KIF5s and the mitochondrial membrane or by indirect coupling via an adaptor that binds KIF5s and mitochondria through lipid-binding or transmembrane domains. Several mitochondrial motor-adaptor complexes and receptors have been identified. Further characterization of these and identification of novel protein adaptors is necessary to more fully understand the complex mechanisms regulating neuronal mitochondrial distribution and transport.

The Miro/Milton complex in *Drosophila* illustrates how KIF5s can utilize adaptor proteins to achieve targeted transport. Miro is a mitochondrial outer membrane protein that interacts directly with the mitochondrial transport adaptor Milton (Fig. 3A). Mutation of the *Drosophila dmiro* gene disrupts anterograde mitochondrial transport and inhibits their entry into the axon and synapses, which results in impairment of both neurotransmitter release and Ca^{2+} buffering during prolonged stimulation (Guo et al., 2005). Similarly, mutation of the *Drosophila milton* gene results in reduced levels of synaptic and axonal mitochondria (Stowers et al., 2002). Subsequent experiments established that the association of Milton and KIF5 with mitochondria requires membrane-bound Miro (Glater et al., 2006). Genetic and biochemical evidence indicates that mitochondrial KIF5 recruitment occurs independently of KLCs. Importantly, Miro, a member of the mitochondrial Rho-GTPase family, has two EF-hand Ca^{2+} binding domains that may switch the mitochondrial transport machinery on or off in response to intracellular Ca^{2+} levels (Fransson et al., 2003).

The two mammalian orthologues of dMiro (Miro1 and Miro2) can form complexes with the two mammalian Milton orthologues (TRAK1 and TRAK2, or, OIP106 and OIP98/Grif-1) (Fig. 3A) (Fransson et al., 2003). TRAK1 and TRAK2 share 33.5% and 35.3% homology, respectively, with dMilton. Whereas dMilton serves as a mitochondria-specific adaptor that links Miro to KIF5 (Glater et al., 2006; Wang and Schwarz, 2009), mammalian TRAKs are more generally involved in the intracellular transport of cargoes and organelles, including

mitochondria (Grishin et al., 2006; Kirk et al., 2006; Webber et al., 2008). While TRAKs play an important role in mitochondrial trafficking (Smith et al., 2006), whether they serve as regulators of motor activity or are adaptors that link KIF5 to Miro remains unclear.

Syntabulin was first identified in our laboratory to serves as another KIF5 motor adaptor that links KIF5 to syntaxin-containing vesicles. Syntabulin is required for the anterograde transport of presynaptic active zone components, presynaptic assembly, and activity-dependent plasticity in developing neurons (Su et al., 2004; Cai et al., 2007). Interestingly, we also found that syntabulin contains a carboxyl-terminal transmembrane domain that targets to mitochondria and plays a critical role in the anterograde transport of mitochondria (Cai et al., 2005). In cultured hippocampal neurons, knockdown of syntabulin or inhibition of syntabulin-KIF5 binding via binding-domain transgenes results in prominent mitochondrial clustering in the soma and sparse mitochondrial distribution along processes. Similarly, mobility analyses in live neurons demonstrate that syntabulin loss-of-function reduces anterograde, but not retrograde, transport of mitochondria along axonal processes. This phenotype provides evidence that syntabulin mediates mitochondrial anterograde transport by acting as an adaptor that links KIF5 to mitochondria (Fig. 3B).

Several additional proteins have also been identified as potential mitochondrial transport adaptors. One of these, the mammalian fasciculation and elongation protein zeta-1 (FEZ1) (Kuroda et al., 1999), was shown to be necessary for the anterograde transport of axonal mitochondria in hippocampal neurons (Ikuta et al., 2007). Its *Caenorhabditis elegans* orthologue, UNC-76, was initially identified as a brain-specific coiled-coil protein involved in axonal outgrowth (Kuroda et al., 1999). In *Drosophila*, UNC-76 enables axonal transport of organelles and vesicles through an association with the kinesin motor (Gindhart et al., 2003). Subsequently, the association of FEZ1 with JIP1, the c-Jun N-terminal kinase-interacting protein, was found to activate kinesin motor activity (Blasius et al., 2007). It has been proposed that FEZ1 may enable anterograde mitochondrial transport by regulating motor activity or stabilizing motor-MT binding.

4. Syntaphilin as an axonal mitochondrial docking protein

As the main goal of mitochondrial transport is to deliver organelles to areas with high metabolic and calcium-buffering requirements, it is imperative to understand the mechanisms by which mitochondrial transport terminates. A recent study in our laboratory, using genetic mouse models and time-lapse imaging, identified syntaphilin (SNPH) as a “static anchor” for axonal mitochondria (Kang et al., 2008). SNPH is neuron-specific and targets to axonal mitochondria via its carboxyl-terminal tail (Fig. 4). Mitochondrial anchoring occurs when SNPH binds both mitochondria and MTs, effectively tethering the organelles along the axon. Interestingly, there is a strong correlation between SNPH-enriched axonal mitochondria (65±14%) and the proportion of stationary mitochondria (62±15%). As predicted, mitochondrial mobility is greatly enhanced in *snph* knockout mice relative to wild-type controls. Furthermore, we recently showed that dynein light chain LC8 regulates the SNPH-mediated mitochondrial docking mechanism (Chen et al., 2009). LC8 binds SNPH and enhances the SNPH-microtubule interaction. These findings provide new insight into the mechanisms of mitochondrial mobility. Presumably, the SNPH-mediated anchoring mechanism allows neurons to dynamically regulate axonal mitochondrial distribution in response to changes in axonal and synaptic physiology. Mounting evidence implicates defective mitochondrial trafficking in the pathology of several major neurodegenerative diseases. *Snph* mutant mice provide a unique model with which to study the physiological impact of mitochondrial docking and retention on axonal homeostasis, synaptic transmission, and neurodegeneration. It will be interesting to cross $snph^{-/-}$ mice with neurodegenerative mouse models to test if increased mitochondrial transport may

augment the transport rate of dysfunctional mitochondria to the soma for degradation, thus slowing neurodegeneration.

5. Regulation of mitochondrial transport

In neurons, activity-dependent regulation of mitochondrial transport is largely due to shifts in intracellular calcium levels and ATP availability (Yi et al., 2004). The ratio of mobile and stationary mitochondrial is dynamic and responds to changes in synaptic activity. Depletion of local ATP via glutamate application, for example, reduces local mitochondrial transport velocity. Furthermore, elevated ADP levels due to increased ATP consumption appear to recruit mitochondria to synapses (Mironov, 2007; 2009).

Mitochondria buffer intracellular calcium levels. In neurons, mitochondria provide ATP to actively pump Ca^{2+} across the plasma membrane and sequester excess cytosolic Ca^{2+} during calcium influx into presynaptic terminals and postsynaptic dendritic spines. Mitochondrial mobility decreases after KCl-induced depolarization (Li et al., 2004) and NMDA receptor-mediated Ca^{2+} influx (Rintoul et al., 2003), and the duration of increased intracellular Ca^{2+} levels corresponds to the degree of mitochondrial transport reduction (Mironov, 2006). Conversely, mitochondrial mobility in cultured neurons increases after tetrodotoxin application (Li et al., 2004). These studies provide evidence that specialized activity-dependent mechanisms induce synaptic mitochondrial retention during sustained periods of synaptic activity.

Until recently, the mechanisms by which mobile mitochondria transition to a stationary state were largely unknown. Recent studies of the KIF5-Milton-Miro complex, however, have begun revealing how intracellular Ca^{2+} levels regulate mitochondrial mobility (Saotome et al., 2008; Wang and Schwarz, 2009; Macaskill et al., 2009; also see Cai and Sheng, 2009). Importantly, it was shown that mitochondrial mobility is arrested when the EF-hands of Miro bind Ca^{2+}. In this fashion, Miro acts as an intracellular Ca^{2+} sensor. Synaptic mitochondrial recruitment induced by the activation of glutamate receptors or application of an electric field to stimulate action potentials occurs through a Miro-mediated Ca^{2+}-sensing mechanism. For example, the Ca^{2+}-induced reduction in mitochondrial velocity was effectively blocked in neurons expressing a mutant Miro with EF hands incapable of Ca^{2+} binding. Despite these advances, the precise mechanisms of Miro-mediated Ca^{2+}-induced suppression of mitochondrial mobility are unclear.

Two models have been proposed. The motor-adaptor switch model (Wang and Schwarz 2009) proposes that Miro acts as a calcium dependent on/off switch for mitochondrial transport (Fig. 5A). In the absence of Ca^{2+}, the C-terminal tail of KIF5 binds mitochondria via an interaction with the Milton-Miro complex, while its N-terminal motor domain is free to drive anterograde transport along MTs. A conformational change driven by Ca^{2+} binding to Miro's EF-hands results in a direct interaction between the motor domain of KIF5 and Miro that disrupts the KIF5-MT interaction necessary for transport. A second model (Fig. 5B), however, posits that Miro serves as an adaptor protein that indirectly links mitochondria and KIF5 (Macaskill et al. 2009). In this scenario, Ca^{2+} binding to Miro's EF-hands releases Miro-bound mitochondria from KIF5. While their proposed mechanisms of action differ, both models offer possible explanations of how activity-triggered Ca^{2+} influx induces the recruitment of mitochondria to the synaptic stationary pool.

6. Impact of axonal mitochondrial mobility on synaptic function

Many studies have confirmed that proper axonal transport and synaptic distribution of mitochondria play a crucial role in the maintenance of synaptic homeostasis during neuronal

activity. Presynaptic mitochondria within mammalian central synapses, for example, aid neurotransmission by accelerating recovery from synaptic depression after moderate neuronal activity (Billups and Forsythe, 2002). Additionally, mitochondria sequester excess calcium during tetanic stimulation and release it after stimulus removal. The loss of mitochondria from synapses also affects synaptic transmission (Stowers et al., 2002). For example, reduced synaptic mitochondria in photoreceptors expressing mutant Milton causes aberrant synaptic transmission. Similarly, a *Drosophila* model with defective axonal mitochondrial transport, dMiro, displays reduced mitochondrial levels at neuromuscular junctions, impaired Ca^{2+} buffering capacity, and aberrant neurotransmitter release during prolonged stimulation (Guo et al., 2005). During intense synaptic activity, myosin-driven mobilization of synaptic vesicles from the reserve pool depends upon mitochondrial ATP production; the loss of mitochondria from neuromuscular junctions in *drp1* mutant *Drosophila* results in faster synaptic vesicle depletion during prolonged pulse train stimulation (Verstreken et al., 2005).

While previous studies focus on the depletion of mitochondria from synapses, recent work demonstrates that syntabulin-mediated KIF5-driven mitochondrial transport helps maintain presynaptic function. Genetic manipulation of cultured superior cervical ganglion neurons shows that syntabulin loss-of-function reduces mitochondrial density along neuronal processes. These defects are associated with delayed initiation of synaptic activity in developing neurons and impaired synaptic transmission in mature neurons, including reduced basal neurotransmission, accelerated synaptic depression during high-frequency firing, slowed recovery rates after synaptic vesicle depletion, and impaired presynaptic short-term plasticity. Importantly, these phenotypes are rescued by the introduction of ATP to presynaptic neurons (Ma et al., 2009). Thus, these findings suggest that syntabulin-mediated mitochondrial transport to presynaptic terminals plays a critical role in mobilizing synaptic vesicles to the readily releasable pool for the maintenance of synaptic function and plasticity.

Utilizing *snph*$^{-/-}$ mice, whose axonal mitochondria exhibit high mobility and reduced persistent docking, we recently provided the first direct evidence that the manipulation of mitochondrial anchoring can impact short-term presynaptic plasticity (Kang et al., 2008). Dual whole-cell patch-clamp recordings from paired hippocampal neurons in culture demonstrate that basal synaptic transmission is not significantly impacted by *snph* deletion. However, when short stimulus trains (20Hz, 1s) are applied to presynaptic neurons at 10-second intervals, persistent enhanced facilitation is observed in the *snph*$^{-/-}$ neurons, but not in wild-type controls. Introducing the *snph* transgene into mutant neurons fully rescues the phenotype, indicating that the enhanced short-term facilitation in *snph*$^{-/-}$ neurons is caused by *snph* deletion. We further demonstrated that *snph* deletion changes Ca^{2+} dynamics at presynaptic boutons during prolonged, intense stimulation. This phenotype could be due to an impaired ability to pump excess Ca^{2+} across the plasma membrane due to reduced ATP availability or a reduction in mitochondrial Ca^{2+} buffering capacity. These experiments provide evidence that changes in axonal mitochondrial docking status affect short-term presynaptic plasticity by altering the ability of neurons to respond to influxes of calcium at synapses.

7. Conclusion

The mechanisms that determine the balance between the mobile and stationary phases of axonal mitochondria are likely regulated by intracellular signaling and synaptic activity. How are mobile mitochondria recruited to the stationary pool (or vice versa) in response to changes in neuronal activity and synaptic physiology? While the Miro-Ca^{2+} models explain how Ca^{2+} signal and synaptic activity regulate mitochondrial transport, many questions

remain unaddressed. Several lines of evidence indicate that retrograde mitochondrial transport may be an important component of the mitochondrial recruitment mechanism. For example, the Miro-mediated Ca^{2+}-dependent mechanism suppresses mitochondrial transport in both the anterograde and retrograde directions. Thus, it is critical to assess whether dynein is mechanistically linked to Miro. Additionally, when kinesin-driven anterograde transport is disrupted, dynein-driven retrograde transport does not simply take over. Using a tug-o-war model to explain the direction of mitochondria transport is probably over-simplistic. It seems more likely that the activity of opposing motors bound to a single organelle are coordinated by motor adaptor proteins. The discovery that syntaphilin anchors axonal mitochondria also begs more questions. Is it possible that motor-adaptor complexes and docking machinery may physically displace one another? It will be important to determine if a single pathway regulates the activity of mitochondrial motor-adaptor complexes and the mechanisms of mitochondrial docking. Finally, the neuronal signals that coordinate the molecular interplay between mitochondrial transport machinery and docking receptors must be elucidated. Genetic mouse models will undoubtedly play an important role in future studies seeking to elucidate the complex mechanisms of mitochondrial transport and distribution in axons.

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Abbreviations

SNPH	syntaphilin
MT	microtubule
KIFs	kinesin superfamily proteins
KHC	kinesin heavy chains
KLC	kinesin light chains
DHC	dynein heavy chains
DIC	dynein intermediate chains
DLIC	dynein light intermediate chains
DLC	dynein light chains
dMiro	*drosophila* mitochondrial Rho-GTPase
TRAK1	trafficking protein kinesin-binding 1
FEZ1	fasciculation and elongation protein zeta-1
JIP1	c-Jun N-terminal kinase (JNK)-interacting protein

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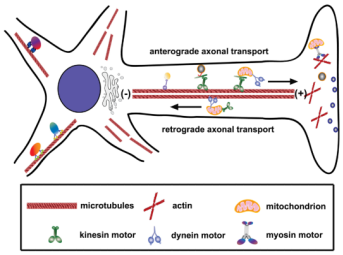

Figure 1. Axonal mitochondrial transport

In axons, MTs are uniformly organized with the plus (+) ends facing toward the axonal terminals and the minus (−) ends toward the cell body. While kinesin motors are mostly plus-end directed, dyneins travel toward the minus ends of MTs. Therefore, kinesin motors generally mediate anterograde axonal transport of mitochondria and dynein drives retrograde axonal transport of mitochondria.

(Adapted with permission from Qian Cai, Zu-Hang Sheng. Mitochondrial transport and docking in axons. Experimental Neurology 218, 257–267, 2009).

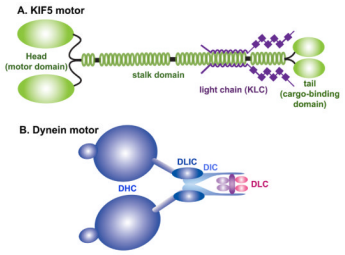

Figure 2. Structure of motor proteins

(A) KIF5 motors form homodimers through the coiled-coil region in the stalk domains. While KIF5 possesses motor function, it also binds to the kinesin-1 light chain (KLC) through its stalk and tail domains. The specific association of KIF5 with cargoes or organelles can be mediated directly through the cargo-binding region in its tail domain or indirectly via the COOH-terminal domains of KLC, indicating the existence of two forms of KIF5 motor-cargo coupling.

(B) Cytoplasmic dyneins consist of heavy chains (DHC), intermediate chains (DIC), light intermediate chains (DLIH), and light chains (DLC). To transport cargoes, cytoplasmic dynein also bind to the dynactin complex (not shown).

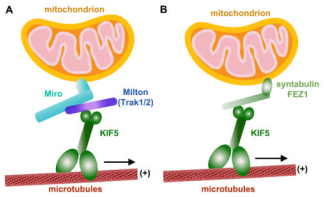

Figure 3. KIF5 motor adaptors for mitochondrial transport

(A) Milton-Miro adaptor complex. Miro is a member of the mitochondrial Rho-GTPase family and a mitochondrial outer membrane protein. Milton attaches indirectly to mitochondria via an interaction with Miro and recruits KIF5 to mitochondria independently of KLC. Two mammalian Milton orthologues, TRAK1 and TRAK2, can form complexes with the two mammalian orthologues of dMiro (Miro1 and Miro2).

(B) Syntabulin is a KIF5 adaptor that targets to mitochondria via its carboxyl-terminal transmembrane domain. It provides a link between KIF5 and mitochondria that mediates mitochondrial anterograde transport. In addition, FEZ1 was reported to serve as a candidate kinesin motor adaptor.

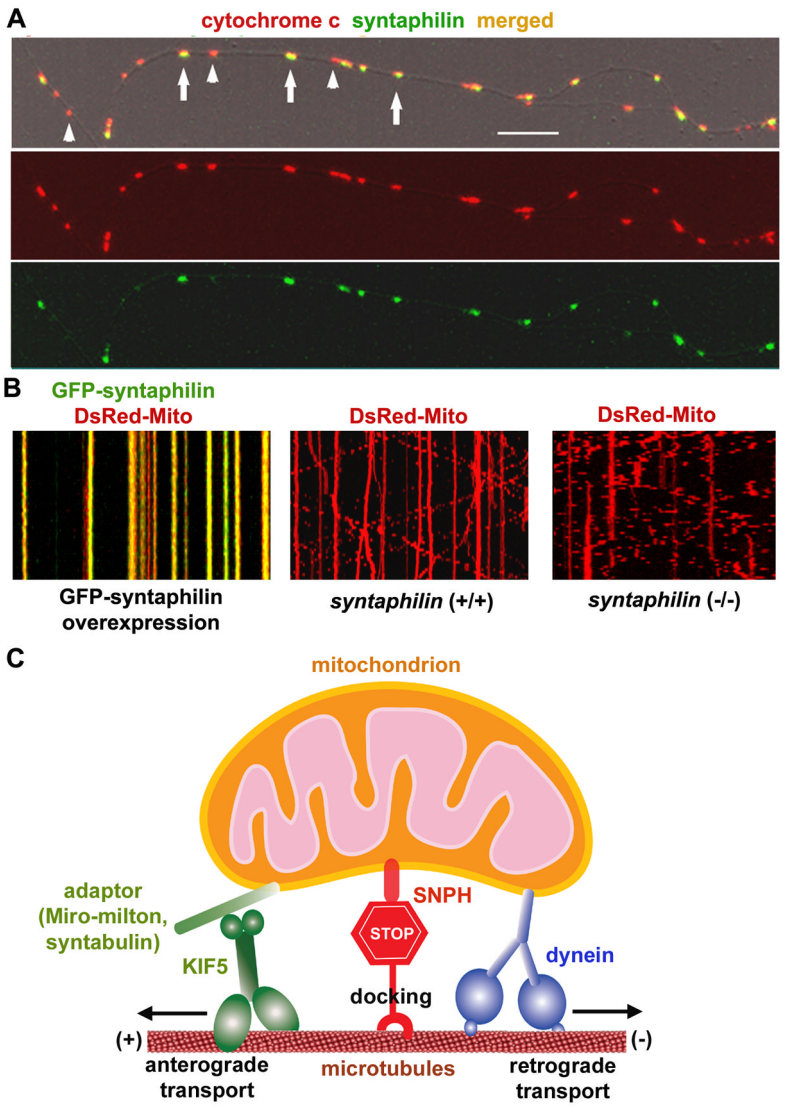

Figure 4. Syntaphilin acts as a receptor for docking/anchoring axonal mitochondria

(A) Syntaphilin (SNPH) targets to axonal mitochondria in cultured hippocampal neurons. Hippocampal neurons at DIV14 were co-immunostained for SNPH and mitochondrial marker cytochrome c. Arrows point to SNPH-enriched mitochondria, and arrowheads indicate mitochondria poorly labeled by SNPH within an axonal process. Scale bars, 10 μm.

(B) SNPH immobilizes axonal mitochondria, while deletion of the *snph* gene in mice robustly increases axonal mitochondrial mobility. Axonal mitochondrial mobility was observed in live neurons one week after transfection. Motion data are presented in kymograph, in which vertical lines represent stationary mitochondria and slant or curved lines indicate mobile ones. Left panel: wild-type neurons co-transfected at DIV6 with DsRed-mito (red) and GFP-SNPH (green); middle panel: wild-type neurons transfected with DsRed-Mito alone; right panel: $snph^{-/-}$ neurons transfected with DsRed-Mito alone.

(C) SNPH acts as a receptor for docking/anchoring mitochondria in axons and is required for maintaining a large stationary axonal mitochondrial pool by interacting with the MT-based cytoskeleton.

(Images in A are adapted with permission from Jian-Sheng Kang, Jin-Hua Tian, Philip Zald, Ping-Yue Pan, Cuiling Li, Chuxia Deng, and Zu-Hang Sheng. Docking of axonal mitochondria by syntaphilin controls their mobility and affects short-term facilitation. Cell 132, 137–148, 2008. Images in B and schematic diagram in C are adapted with permission from Qian Cai, Zu-Hang Sheng. Mitochondrial transport and docking in axons. Experimental Neurology 218, 257–267, 2009).

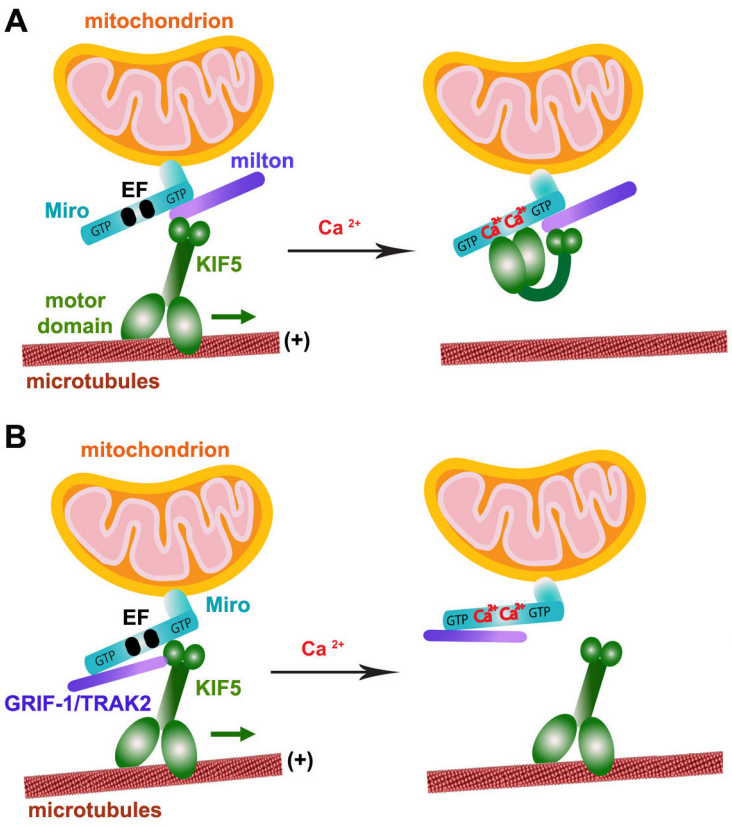

Figure 5. Two proposed models of Miro's role as a Ca^{2+} sensor that regulates mitochondrial mobility

Miro contains two GTPase domains and calcium-binding EF-hand motifs. Thus, Miro regulates axonal mitochondrial mobility by either GTP hydrolysis or calcium binding in response to calcium signals and synaptic activity.

(A) Ca^{2+}-binding turns "off" KIF5 engagement with MTs. The tail of KIF5 links to Miro via Milton in a Ca^{2+}-independent manner, thus leaving its motor domain free to engage with MTs. Ca^{2+}-binding to the EF-hands triggers the direct interaction of the motor domain with Miro, which prevents the motor from engaging MTs (Wang and Schwarz, 2009).

(B) Ca^{2+}-binding detaches KIF5 from mitochondria. Mitochondrial transport is mediated by Miro/KIF5 linkage. Ca^{2+}-binding to the EF-hands dissociates Miro from KIF5 while KIF5-binding protein GRIF-1/TRAK2 (a mammalian homologue of Milton) remains bound to Miro1 (Macaskill et al., 2008; 2009).

(The model diagram is adapted with permission from Qian Cai and Zu-Hang Sheng. Moving or stopping mitochondria: Miro as a traffic cop by sensing calcium. Neuron 61, 493–496. 2009).