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Review

The meaning of mitochondrial movement to a neuron's life ☆

Jonathan R. Lovas, Xinnan Wang *

Stanford Institute for Neuro-innovation and Translational Neurosciences and Department of Neurosurgery, Stanford University School of Medicine, USA

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ABSTRACT

Cells precisely regulate mitochondrial movement in order to balance energy needs and avoid cell death. Neurons are particularly susceptible to disturbance of mitochondrial motility and distribution due to their highly extended structures and specialized function. Regulation of mitochondrial motility plays a vital role in neuronal health and death. Here we review the current understanding of regulatory mechanisms that govern neuronal mitochondrial transport and probe their implication in health and disease. This article is part of a Special Issue entitled: Mitochondrial dynamics and physiology.

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1. Introduction

Mitochondria, best known as the powerhouses of cells, are cytoplasmic organelles of an endosymbiotic origin. Mitochondria have their own distinct haploid DNA which encodes essential enzymes for oxidative phosphorylation and mitochondrial tRNAs, but are assembled from proteins mostly encoded by nuclear DNA. Although ATP conversion by aerobic respiration is their major job, mitochondria also provide a reservoir for cytosolic calcium ions, and synthesize certain heme compounds [1] and steroids [2]. The uniqueness of these features places mitochondria in a significant position for regulating cellular proliferation and death [3].

Mitochondria move and undergo fission and fusion in almost all eukaryotic cells. This dynamic nature holds a particular urgency for neurons because of the problems that can arise during the long-range transport that is necessitated by the remarkable length and complexity of axons and the variable and specialized energetic demands of these highly polarized cells. The far-flung extremities of neurons are especially susceptible to disruption of the proper allocation of mitochondria [4]; both the presynapse and postsynapse have particularly high demands for energy and Ca^{++} buffering. To sustain the periphery, newly assembled mitochondria from the cell body must be transported into neurites, and peripheral mitochondria whose proteins and DNA accumulate defects must be either repaired by fusion with fresh mitochondria or cleared from the cell [5]. Because the cell body is enriched with ribosomes, lysosomes, and other organelles, damaged mitochondria might be transported back to the cell body to be replenished or degraded. Therefore, in order to keep energy homeostasis and maintain essential activities, neurons need not only to precisely set up an adequate distribution of mitochondria, but also to efficiently sustain them in the periphery and clear them away when necessary. An exquisite regulation of mitochondrial movement is required to achieve these goals.

Misregulated mitochondrial motility in neurites is predicted to cause neuronal dysfunction and degeneration. Abnormalities in mitochondrial motility and distribution are implicated in a wide range of

Abbreviations: Akt-GSK3β, Akt-Glycogen Synthase Kinase 3β; ALS, Amyotrophic Lateral Sclerosis; CCCP, Carbonyl cyanide *m*-chlorophenyl hydrazine; CMT, Charcot–Marie–Tooth; DA, Dopamine; DISC1, Disrupted In Schizophrenia 1; FEZ1, Fasciculation and Elongation Protein-Zeta 1; GRIF1, Gamma-Amino Butyric Acid (GABA) Receptor Interacting Factor 1; HAP1, Huntingtin-Associated Protein 1; HBP, Hexamine Biosynthetic Pathway; HIF-1α, Hypoxia-Inducible Factor 1α; HTT, Huntingtin; HUMMR, Hypoxia Up-regulated Mitochondrial Movement Regulator; KBP, KIF1-Binding Protein; KHC, Kinesin Heavy Chain; KIF, Kinesin Family Member; KLC, Kinesin Light Chain; KLP6, Kinesin-like Protein 6; LC8, Dynein Light Chain 8; MFN, Mitofusin; Miro, Mitochondrial Rho GTPase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTS, Mitochondrial Targeting Sequence; OGT, *O*-linked N-acetylglucosamine Transferase; OIP106, *O*-linked N-acetylglucosamine Transferase (OGT) Interacting Protein 106; PINK1, Phosphate Tensin Homologue (PTEN) Induced Kinase 1; RANBP2, Ras-Related Nuclear Protein (RAN) Binding Protein 2; TM, Transmembrane; TRAK, Trafficking Kinesin Binding Protein 1, 2; UDP-GlcNAc, Uridine Diphosphate N-acetylglucosamine; WAVE1, Wiskott–Aldrich Syndrome Protein (WASP) Family Verprolin Homologous Protein 1; 5-HT, 5-hydroxytryptamine

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* Corresponding author at: Building A, Room A152, 1050 Arastradero Rd, Palo Alto, CA 94304, USA. Tel.: +1 650 724 9282; fax: +1 650 725 7813.
E-mail addresses: lovas@stanford.edu (J.R. Lovas), xinnanw@stanford.edu (X. Wang).

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neurodegenerative and psychiatric disease models, such as Charcot–Marie–Tooth (CMT) [6], Amyotrophic Lateral Sclerosis (ALS) [7], Alzheimer's [8–10], Huntington's [11], Parkinson's [12], and schizophrenia [13]. However in these diseases mitochondrial function of oxidative phosphorylation and generation of ATP may be disrupted by primary neurotoxic factors and cellular stresses. Malfunctioning mitochondria can affect mitochondrial motility [12], and cause neuronal cell death by generating reactive oxygen species and triggering apoptosis [14]. Therefore the part the misregulation of mitochondrial motility plays in disease progression merits further investigation. It is certain, however, that both misregulated mitochondrial motility and function can expedite the cell death process in neurons already vulnerable or stressed [15,16]. Here we review the current understanding of the cellular signals that regulate neuronal mitochondrial motility, as well as their significance and implication in neuronal health and disease.

2. Mitochondrial transport machinery

In neurites approximately 30–40% of total mitochondria are engaged in saltatory movement at any given time [17–20]. The densities of mitochondria at different synapses therefore change constantly. This reflects an acute need for mitochondria to buffer Ca^{++} influx and distribute ATP among the microdomains of a neuron [21]. A mitochondrion can either move continually over a long distance at a relatively constant speed, or pause sporadically and start again with a different speed or direction. Fission-and-fusion events can also occur in moving mitochondria, though the relationship between movement and fission-and-fusion is unclear. Study in non-neuronal cells suggests that fusion events happen more frequently while mitochondria are moving [22]. The majority of mitochondrial movement is microtubule-based [17,21,23]. In an axon microtubules are uniformly arrayed, with all (+)-ends pointing to the axonal terminal and the (−)-ends to the cell body, whereas in a dendrite their polarities are typically more mixed [24]. Anterograde movement toward the (+)-ends of microtubules is mediated by kinesin motors, and retrograde toward (−)-ends by dynein motors. These motor proteins are generally shared with other cellular cargoes moving along microtubules. Mitochondria are anchored to motor proteins by idiosyncratic adaptor proteins; in this way their motility is precisely and specifically regulated by cellular signals (Table 1).

Mitochondria are also transported along actin filaments, a process that happens more frequently in dendritic spines, growth cones, and synaptic boutons where the actin cytoskeleton is enriched. This actin-based movement is relatively short-ranged, likely mediated by myosin motors, and important for local and acute translocation and docking of mitochondria in response to action potentials, Ca^{++} influx, or neurotrophic stimulation (Table 1). Actin-based transport can also coordinate, supplement, or even oppose microtubule-based transport [25].

2.1. Anterograde microtubule motors

In mammals, at least 14 families and 45 genes of kinesins have been identified to date, of which the Kinesin-1 family has been shown to be critical for mitochondrial transport [26–29]. Kinesin-1 is also known as the conventional kinesin heavy chain (KHC), or KIF5. Each KIF5 gene contains an N-terminal motor domain that binds to microtubules and hydrolyzes ATP, and a C-terminal domain that can interact with kinesin light chain (KLC) or cargo adaptors. Three KIF5 genes exist in mammals: two are neuronal-specific (KIF5A and KIF5C), while one is ubiquitously expressed (KIF5B). Mutations in mice *KIF5B* lead to perinuclear clustering of mitochondria, which indicates a disruption of kinesin-mediated anterograde transport of mitochondria [29]. In *Drosophila*, when the only orthologue of KIF5 is mutated axonal mitochondrial transport is impaired [30]. In both neuronal and non-neuronal cells KIF5 is recruited exclusively to mitochondria from cytosol by overexpression of mitochondrial adaptor proteins [19,31], and overexpression of the motor domain of KIF5 in hippocampal neurons alters the regulation of mitochondrial motility by interfering with the integrity of the mitochondrial motor/adaptor complex [19].

In addition, two members of the Kinesin-3 family are associated with mitochondria; KIF1Bα and Kinesin-Like Protein 6 (KLP6). KIF1Bα is expressed ubiquitously and is particularly abundant in differentiated neuronal cells. The motor has been shown to colocalize with mitochondria and facilitate their (+)-end directed transport *in vitro* [32]. Knockdown of *KLP6* disrupts anterograde axonal transport of mitochondria in neuronal cells and expression of a dominant negative KLP6 affects mitochondrial morphology in HeLa cells [33].

2.2. Anterograde microtubule adaptors

2.2.1. The KHC/milton/Miro complex

The KHC/milton/Miro complex is the best understood motor/adaptor complex for the regulation of mitochondrial transport. The current model suggests that Miro functions as a receptor with a transmembrane (TM) domain integrated into the outer mitochondrial membrane, and Miro binds to milton, which in turn binds to KHC. This complex allows mitochondria to associate with microtubules and plays key roles in regulating mitochondrial motility (Fig. 1A). Milton came from a genetic screen in *Drosophila* for identification of mutants that disrupt synaptic transmission in photoreceptors, and was named after the 17th-century blind English poet John Milton [34]. Mitochondria are absent from axons deficient in *milton* but are present and functional in cell bodies. Milton is localized to mitochondria, and has a predicted coiled-coil domain interacting directly with KHC. Overexpression of milton in cultured mammalian cells recruits KHC to mitochondria [19,31,34]. In addition, the interaction between milton and KHC is KLC independent: KLC is not recruited to mitochondria by milton nor is it present in the KHC–milton complex [31]. Knockout of *KLC* in flies does not impair mitochondrial transport, suggesting that KLC is dispensable for their movement [31]. Milton has two homologues in mammals, TRAK1 (also known as milton-1, OIP106) and TRAK2 (milton-2, GRIF1), which are about 30% identical to *Drosophila* milton in their amino acid sequence. Both homologues also interact with KHC [35,36]. Knockdown of TRAK1 but not TRAK2 in cultured neurons impairs axonal mitochondrial movement, which can be rescued by expression of either TRAK1 or TRAK2 [37]. These findings reveal an important and conservative role of TRAK as a KHC adaptor in regulating mitochondrial motility. Differences do exist between *Drosophila* and mammalian milton homologues: whereas *Drosophila* mutants appear to be selectively defective in mitochondrial transport, there is evidence that the mammalian homologues may be associated with additional organelles [35,38–40].

KHC and milton need a third protein, Miro, to attach them to mitochondria. There is one *Miro* gene in *Drosophila*, and two (*Miro1* and *Miro2*) in mammals. Each contains two GTPase motifs, a pair of EF-hands involved in Ca^{++} binding, and a C-terminal TM domain that incorporates into the outer mitochondrial membrane [41,42]. Similarly to *milton* mutants, neurons deficient in *Drosophila Miro* lack axonal mitochondria [43]. Miro binds to milton directly, and Miro, milton and KHC

Table 1
Summary of current understanding of transport machineries for mitochondria. See also Section 2.

Motor	Adaptor/associated proteins	Cytoskeleton	Illustration
KHC	Milton, Miro	Microtubules	Fig. 1A
	Syntabulin/FEZ1/RANBP2/more	Microtubules	Fig. 1B
KIF1Bα	KBP, more	Microtubules	Fig. 1C
KLP6	KBP, more	Microtubules	Fig. 1C
Dynein	Dynactin, more	Microtubules	Fig. 1D
Myo19, more	Unknown	Actin	Fig. 1E