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Review

# Calcium regulation of mitochondria motility and morphology

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ABSTRACT

In the Fifties, electron microscopy studies on neuronal cells showed that mitochondria typically cluster at synaptic terminals, thereby introducing the concept that proper mitochondria trafficking and partitioning inside the cell could provide functional support to the execution of key physiological processes. Today, the notion that a central event in the life of every eukaryotic cell is to configure, maintain, and reorganize the mitochondrial network at sites of high energy demand in response to environmental and cellular cues is well established, and the challenge ahead is to define the underlying molecular mechanisms and regulatory pathways. Recent pioneering studies have further contributed to place mitochondria at the center of the cell biology by showing that the machinery governing remodeling of mitochondria shape and structure regulates the functional output of the organelle as the powerhouse of the cell, the gateway to programmed cell death, and the platform for $Ca^{2+}$ signaling. Thus, a raising issue is to identify the cues integrating mitochondria trafficking and dynamics into cell physiology and metabolism. Given the versatile function of calcium as a second messenger and of the role of mitochondria as a major calcium store, evidences are emerging linking $Ca^{2+}$ transients to the modulation of mitochondrial activities. This review focuses on calcium as a switch controlling mitochondria motility and morphology in steady state, stressed, and pathological conditions.


## 1. Introduction

In any given eukaryotic cell, the architecture of the mitochondrial network is determined by the molecular machineries governing the trafficking and shape of the organelle. Its design reflects the physiological state of the cell in steady state, stressed, and pathological conditions because the mechanisms controlling mitochondrial dynamics also regulate the function of the organelle as the powerhouse of the cell, the gateway to programmed cell death, and the platform for $Ca^{2+}$ signaling. In this process, calcium is emerging as a relay preposed to the intracellular distribution of the organelle at sites of high energy demand as well as a molecular switch for a number of mitochondrial membrane remodeling proteins. This review focuses on the aspects of mitochondria motility and dynamics that are directly or indirectly related to calcium, and it is intended to complement recent authoritative reviews on mitochondrial transport [1–5], dynamics [6,7], apoptosis [8–11], control of neuronal activities [12], and link to cell signaling [13]. We apologize in advance for omitting to quote every publication that has contributed to make emerge such exciting fields.

## 2. Distributing, positioning, anchoring, and regulating mitochondria activity at sites of high energy demand through calcium

The first observations describing differential positioning of mitochondria in cultured cells were reported nearly a century ago [14]. Forty years later, in the Fifties, electron microscopy studies showed that mitochondria typically cluster at synaptic terminals [15], eliminating the concept that the architecture of the mitochondrial network is the outcome of a random distribution process of the organelle, and introducing the possibility that proper mitochondria trafficking and partitioning inside the cell could provide functional support to the execution of key physiological processes [16–18]. Today, the notion that a central event in the life of every eukaryotic cell is to

*Abbreviations:* IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; IMS, intermembrane space

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configure, maintain and reorganize the mitochondrial network at sites of high energy demand in response to environmental and physiological cues is well established [1], and the challenge ahead is to define the underlying molecular mechanisms and regulatory pathways.

### 2.1. Mitochondria anterograde and retrograde transport: raison d'être and calcium regulation

*In vivo* studies using time-lapse microscopy of fluorescently labeled mitochondria have shown that a sub-population of the organelle remains stationary, while another commutes, often pausing between movements. In polarized cells like budding yeast and neurons, two widely used models in mitochondria trafficking studies, the organelle movements along the major axis of the cell are prominent, and occur both away from the nucleus (anterograde transport) and toward the center of the cell and nucleus (retrograde transport). In *Drosophila* motor neuron axons, anterograde and retrograde movement velocities averaged 0.26 and 0.45 mm/s, respectively [19]. It should be noted, however, that observations of run length, pausing frequency, average velocity, and persistence of direction can significantly differ, possibly reflecting the diverse physiological states of the cell as well as tissue and experimental approaches used [2,20]. Interestingly, moving mitochondria that switch direction of movement are not observed [19], suggesting that individual organelle reprogramming is required to alternate between anterograde and retrograde trafficking.

Several pioneering laboratories have investigated the raison d'être of mitochondria bidirectional transport and found it to be linked to maintaining bioenergetically competent organelles at sites of high energy demand. In neurons, among the moving mitochondrial sub-population 90% of the organelles with high membrane potential move anterogradely and accumulate in the growth cone, where metabolic activities are intense. Conversely, 81% of mitochondria showing low membrane potential are transported in the opposite direction, toward the cell body [21], suggesting a role of retrograde transport in eliminating energetically compromised organelles. An alternative possibility would be that retrograde transport actively uncouples membrane potential, thereby working as an energy switch; however, this is unlikely because arresting mitochondria motility does not correlate to an overall increase in respiration. Therefore, while anterograde transport generates the network of stationary active organelles required at sites of high metabolic activities, retrograde motility maintains it by removing depolarized and damaged organelles. In line with this notion, elegant imaging and computational studies from the Sheetz's group have shown that, in the axons of neurons isolated from chicken embryos, stationary mitochondria originate from a fast moving, anterogradely transported pool of organelles [21]. In these cells, mitochondria are evenly distributed, a pattern that originates because the organelles are preferentially dispensed in the middle of the gaps existing between stationary mitochondria [21], suggesting that transport at these “drop site” must be inhibited. If so, a gradient of signaling factors such as $Ca^{2+}$, ADP, or a small mitochondrial G-protein like Rab32 [22], could play a role in mitochondria trafficking arrest. In this respect, inhibition of mitochondrial motility has been shown to correlate with altered $Na^{+}$, $K^{+}$ or $Ca^{2+}$ ionic balance, suggesting the possibility that calcium could be part of the mechanism that arrests mitochondrial trafficking [21,23]. Consistent with this concept, in neuronal axons, using a micropipette to locally manipulate ion balance and composition, iso-osmotic replacement of NaCl with mannitol, which does not change overall metabolic activity, increases intracellular $Ca^{2+}$ levels and decreases anterograde and retrograde trafficking [21,24,25]. Further, in hippocampal rat neurons mitochondria motility is suppressed by calcium influx through L-type voltage-gated calcium channels and NMDA receptors. Conversely, reduced calcium-dependent synaptic activities increase overall mitochondrial trafficking [26].

Mitochondrial oxidative phosphorylation uncoupling agents like CCCP also induce calcium transients and inhibit mitochondrial transport. However, this seems to be a non-specific effect, independent of mitochondrial depolarization and calcium release [27] because other uncouplers, such as DNP, FCCP and PCP, and complex III inhibitors like Antimycin A, increase or have no effect on mitochondrial transport [21,27,28]. Since CCCP is one of the most effective uncouplers [29] and can cause mitochondria to become consumers of ATP [25], its effect might occur because it lowers local ATP levels such that transport cannot be sustained [21]. More direct evidence of calcium participation to the organelle trafficking can be inferred from the identification of Miro, a mitochondrial outer membrane protein whose function in anterograde transport depends on intact EF-hand calcium binding domains [30] and, by implication, cytosolic calcium transients (discussed below).

### 2.2. Stationary mitochondria: regulating local power supply through calcium

Anterograde transport distributes functional organelles to sites of intense metabolic activities which, in neurons, include active synapses, nodes of Ranvier, myelination boundaries, axonal branches, and growth cones [1,31]. Mitochondria cluster at these locations, and their density changes in response to physiological cues. In rat hippocampal cultured neurons, the organelle dynamically redistributes into dendritic protrusions in response to synaptic excitation and their number increases during synaptogenesis and spine formation [26]. Conversely, preventing mitochondria to access synapses has profound deleterious effects on neuronal activity and synaptic plasticity [26,32]. In neurons, the pools of stationary mitochondria located at synaptic and dendritic terminals likely support bioenergetic and calcium buffering requirements for dendritic development and synaptic plasticity, two essential processes known to be regulated through $Ca^{2+}$-triggered transcriptional programs that drive the synthesis of the effector molecules required for long-term changes in neuronal function [33]. The mechanisms controlling mitochondrial docking within axons has just started to emerge. A recent study from the laboratory of Zu-Hang Sheng reported a role for axon-targeted syntaphilin (SNPH) in mitochondrial docking through its interaction with microtubules. Axonal mitochondria that contain exogenously or endogenously expressed SNPH lose mobility. Deletion of the mouse *snph* gene results in a substantially higher proportion of axonal mitochondria in the mobile state and reduces the density of mitochondria in axons, a phenotype that is fully rescued by reintroducing the *snph* gene into the mutant neurons. The *snph* mutant neurons exhibit enhanced short-term facilitation during prolonged stimulation, probably by affecting calcium signaling at presynaptic boutons [34].

The cellular strategy of crowding mitochondria to supply chemical energy at sites of intense metabolic activities is intrinsically limited by the typically modest amount of space available at sites like synaptic terminals, spines, and lamellipodia of the growth cone. Consistent with this concept, recent groundbreaking studies from Verburg and Hollenbeck have shown that the cell can also concentrate mitochondrial bioenergetic function by locally increasing respiration of mitochondria located at these sites. Using beads covalently coupled to Nerve Growth Factor (NGF) and Semaphorin to provide localized stimuli, this group showed that these survival and guidance cues could differentially affect mitochondria membrane potential. In areas of intense metabolic activities, organelles immediately adjacent to the site of NGF or Semaphorin stimulation showed a 40–50% increase in membrane potential, whereas mitochondria 10–50 μm away showed a smaller effect that declined to insignificance beyond 50 μm [35]. Inhibitors of PI3 kinase and MAP kinase abolished this effect, implicating these signal-transducing molecules in the upregulation of mitochondrial membrane potential and, by implication, ATP synthesis. Importantly, because simultaneous inhibition of these two