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**Location and Organization of Features of the Mitochondrial Life Cycle in Neurons**

Mitochondria are widely distributed via regulated transport in neurons, but their sites of residence, biogenesis and turnover remain uncertain. We have analyzed mitochondrial positioning and sorting at axonal branch points, mitochondrial membrane potential (MMP) in different regions and under different conditions, and the site(s) of mitochondrial fission, fusion and DNA replication. (1) In hippocampal neurons in culture, anterograde mitochondria sort preferentially into growing branches but retrograde mitochondria do not selectively exit from non-growing branches. Furthermore, specific signaling pathways can halt mitochondria in otherwise undistinguished regions of the axon. (2) Data from some EM studies and vital dye experiments in live neurons have suggested that anterograde mitochondria are intact and metabolically active while retrograde mitochondria are senescent. We studied this using a quantitative measure of MMP, a reasonable stand-in for their metabolic state. We found no difference in MMP among three functionally distinct regions: axonal branch points, distal axons, and the remaining axon shaft. In addition, we found no difference among stationary, retrogradely moving or anterogradely moving mitochondria. However, MMP was significantly higher in the lamellipodia of growth cones, and among a small fraction of mitochondria throughout the axon. We also found that local stimulation of the axonal membrane with survival and guidance cues produced very local, receptor-mediated increases in MMP, and global inhibition of receptor tyrosine kinase activity produced a dramatic decrease in MMP throughout the axon. (3) Most mitochondrial proteins are encoded in the nuclear genome, and evidence has suggested that mitochondrial DNA (mtDNA) replication occurs mainly or entirely in the cell body. However, it has also become clear that nuclear-encoded mitochondrial proteins can be translated in the axon, and that components of the mitochondrial replication machinery reside there as well. We assessed axonal mtDNA replication directly in axotomized arrays of chick peripheral neurons labeled with BrdU, and found that a significant fraction of mtDNA synthesis continued in the absence of connection to the cell bodies. To assess whether other aspects of mitochondrial life cycle also occurred in the axon, we localized the mitochondrial fission protein Drp1 in neurons by immunofluorescence or expression of GFP-Drp1. Drp1 was present on the majority of axonal mitochondria, indicating that a substantial number had undergone recent division in the axon. We also found that inhibition of Drp1 expression overexpression of the fusion protein Mfn1 both resulted in significantly longer mitochondria in axons, including many at a great distance from the cell body. These data indicated that mitochondria can replicate their DNA, divide, and fuse locally within the axon and thus that mitochondrial biogenesis is not limited to the cell body.

**Suggested readings:**

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