Mitochondrial transport, Metabolism and ROS Production in Disease Models

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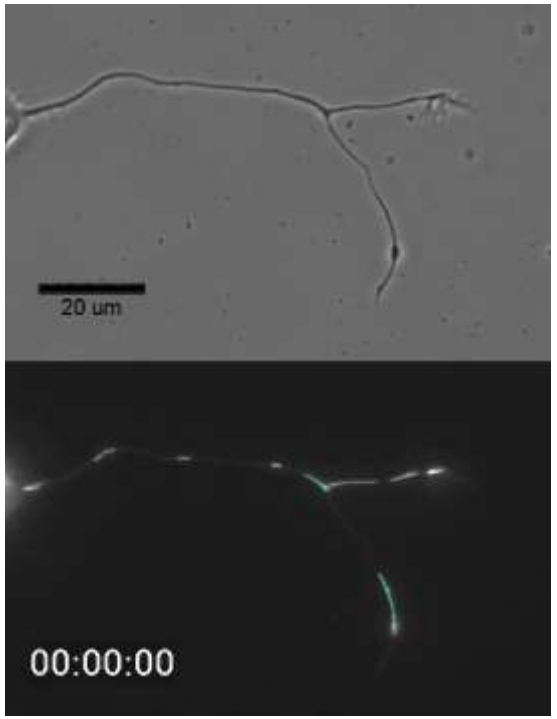


Workshop lecture 7
ICTS-TIFR Advanced School on Axonal Transport & Neurodegenerative Disorders
21 January 2013

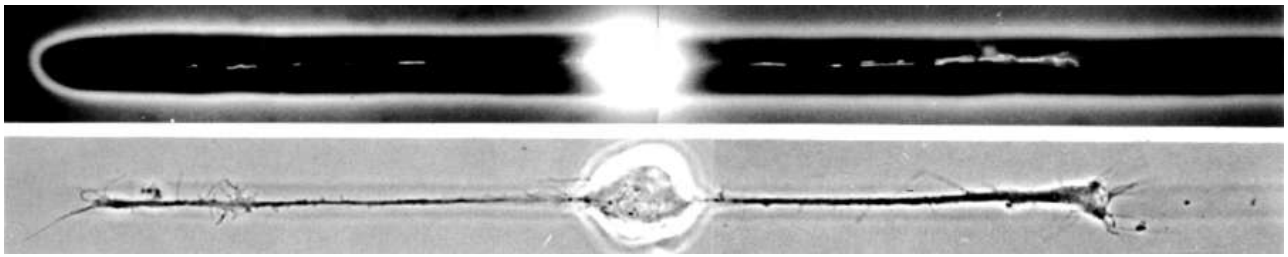
Life cycle of mitochondria in the nervous system: how to supply the whole cell?

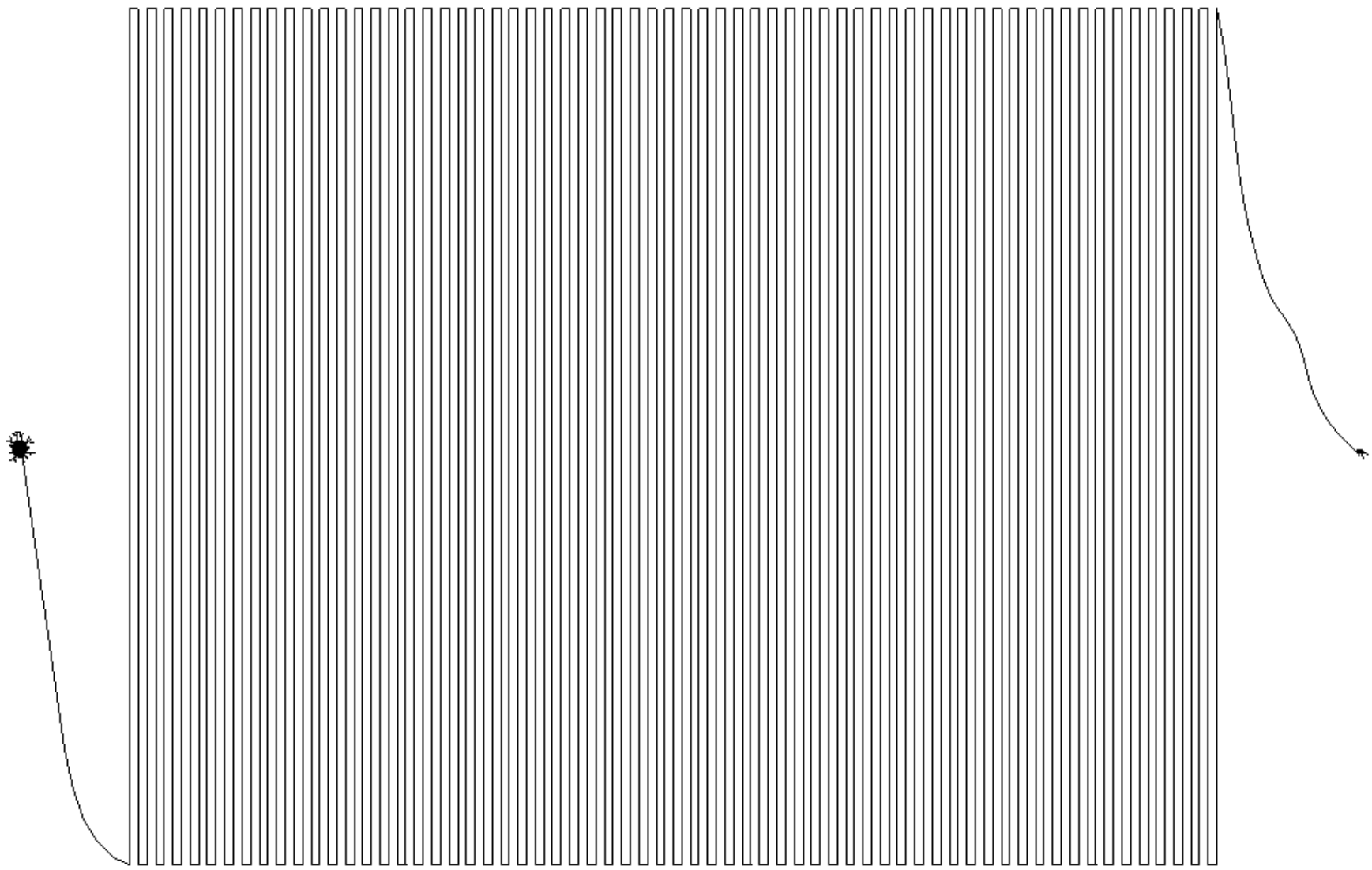
(1) **Movement and distribution**. How do neurons get them to the right place at the right time?

(2) **Metabolic activity.** Is their output modulated to meet the needs of time and place?

(3) **Proliferation/biogenesis.** Can they divide and fuse in the axon, or are they just visitors?

Do any of these go awry in degenerative diseases of the nervous system?



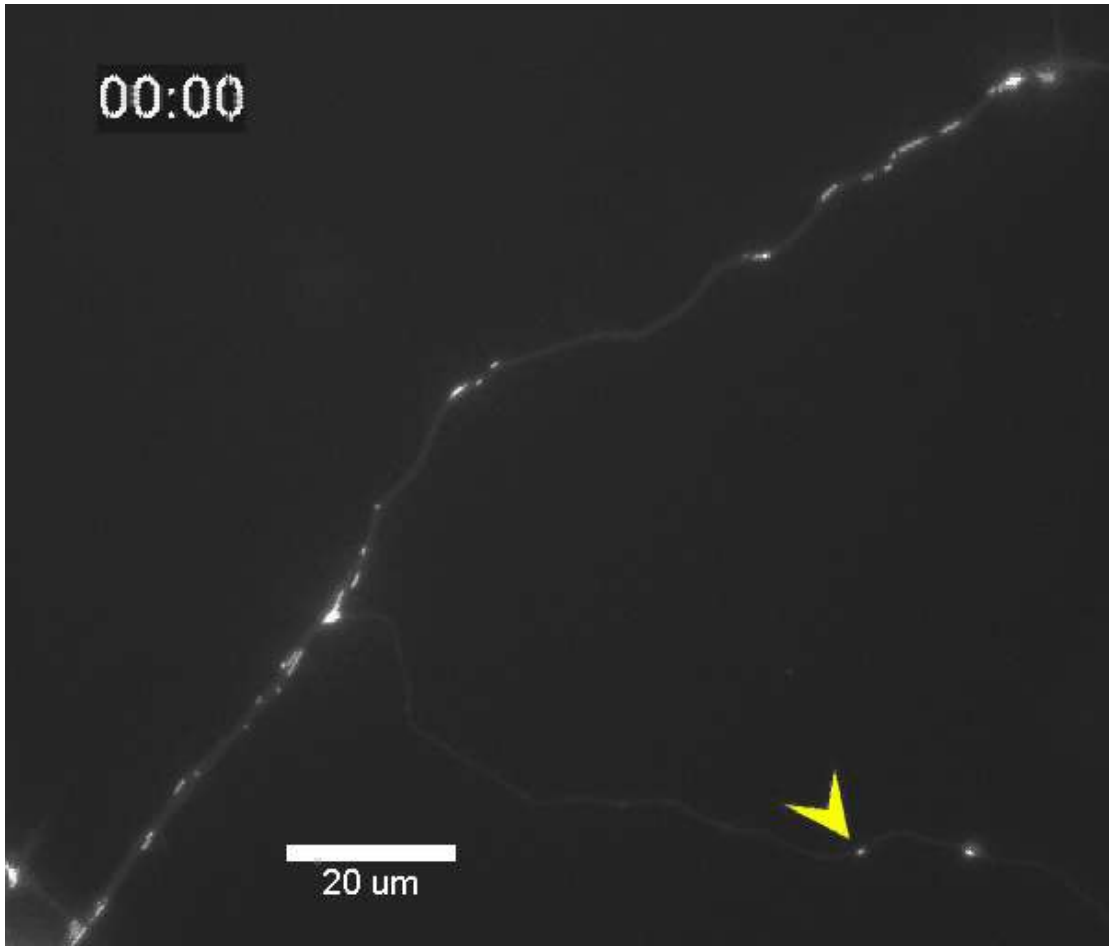


Axonal transport of mitochondria: saltatory, bidirectional

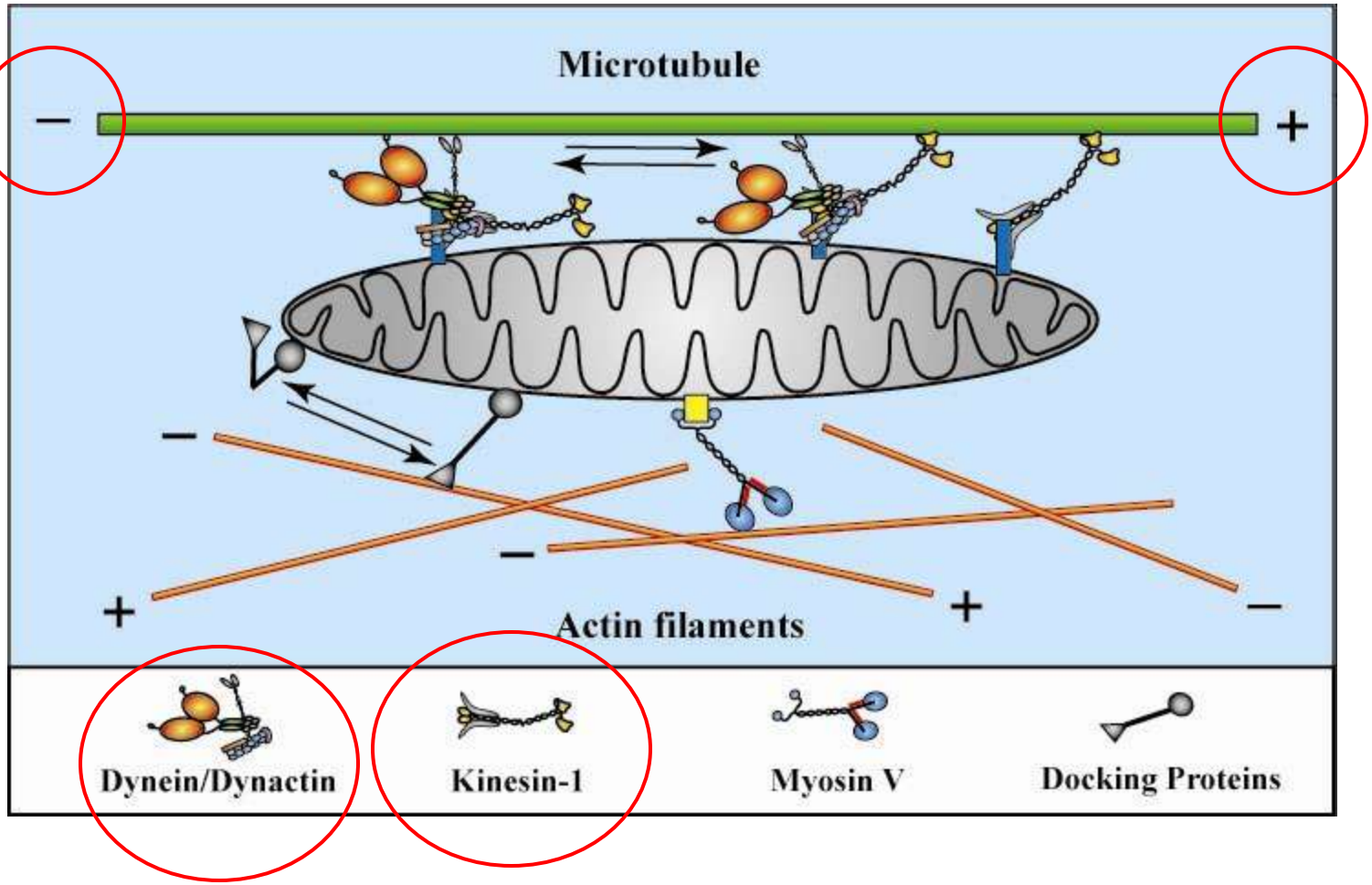
Move both anterograde and retrograde, can stop/start and change directions

Major shifts in mito distribution can be induced by local activity

These result mainly from regulation of anterograde movement & balance between stationary and motile states



Two cytoskeletal tracks for movement: MTs and actin filaments



Model disorder: Friedreich ataxia (FRDA)

Mutated protein:	frataxin, normally supports assembly of Fe-S complexes for enzymes
Known "hits":	unstable GAA repeat in first intron some point mutations
Affected cell types:	sensory & motor neurons cerebellum heart
Cellular phenotype:	decreased activity of cytoplasmic and mitochondrial (Fe-S) enzymes
Gross phenotype:	ataxia, dying-back neuropathy, hypertrophic cardiomyopathy

Summary of current thinking about cellular neuropathology of Friedreich ataxia:

Frataxin deficiency results in abnormal assembly of Fe-S complex proteins, esp those of the mitochondrial electron transport system

Defects in the ETS lead to excessive ROS production and oxidative stress. Other forms of iron-related oxidative stress have also been suggested.

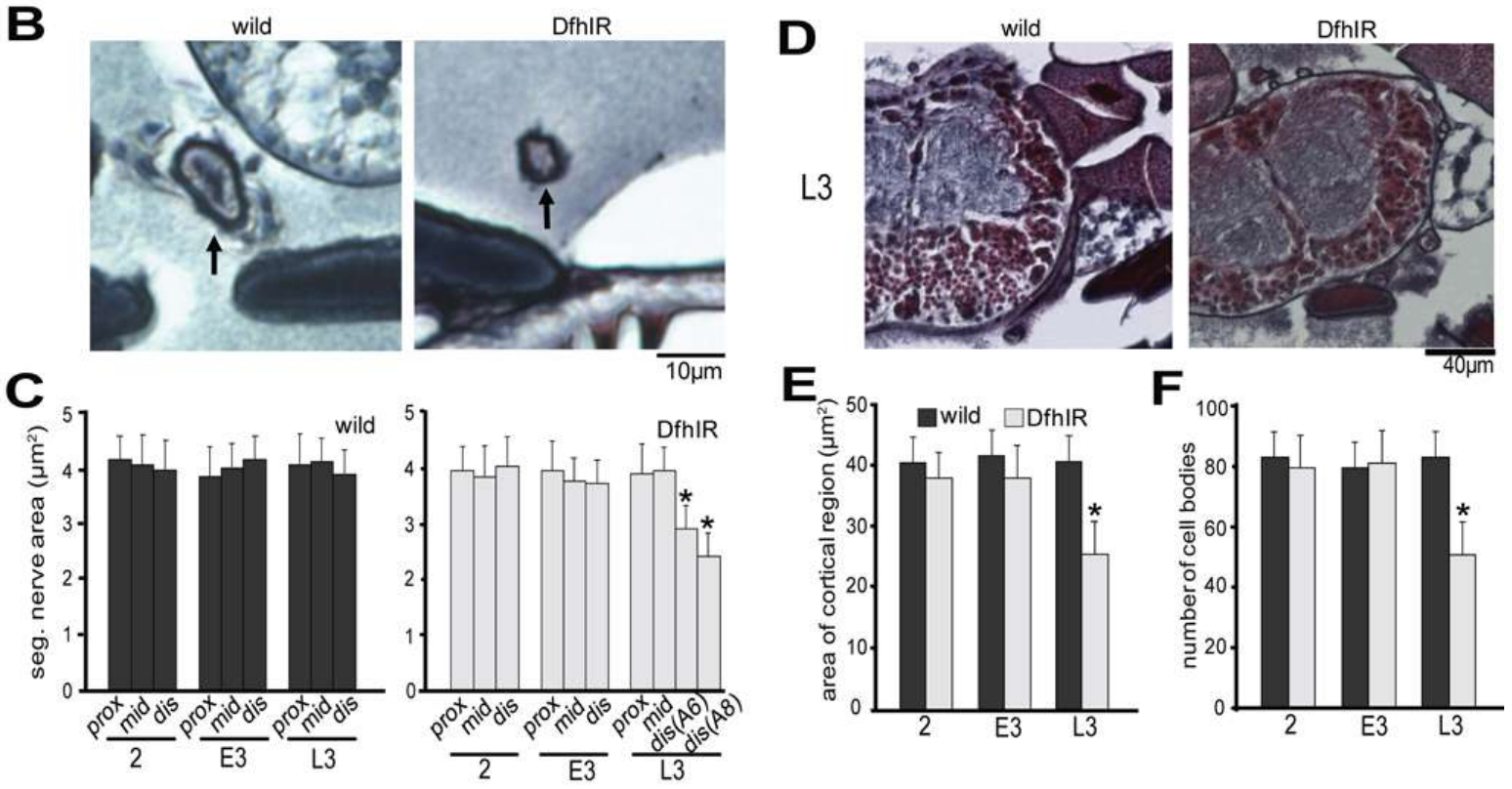
Friedreich ataxia pathology and neuronal death are due in large part to this oxidative stress.

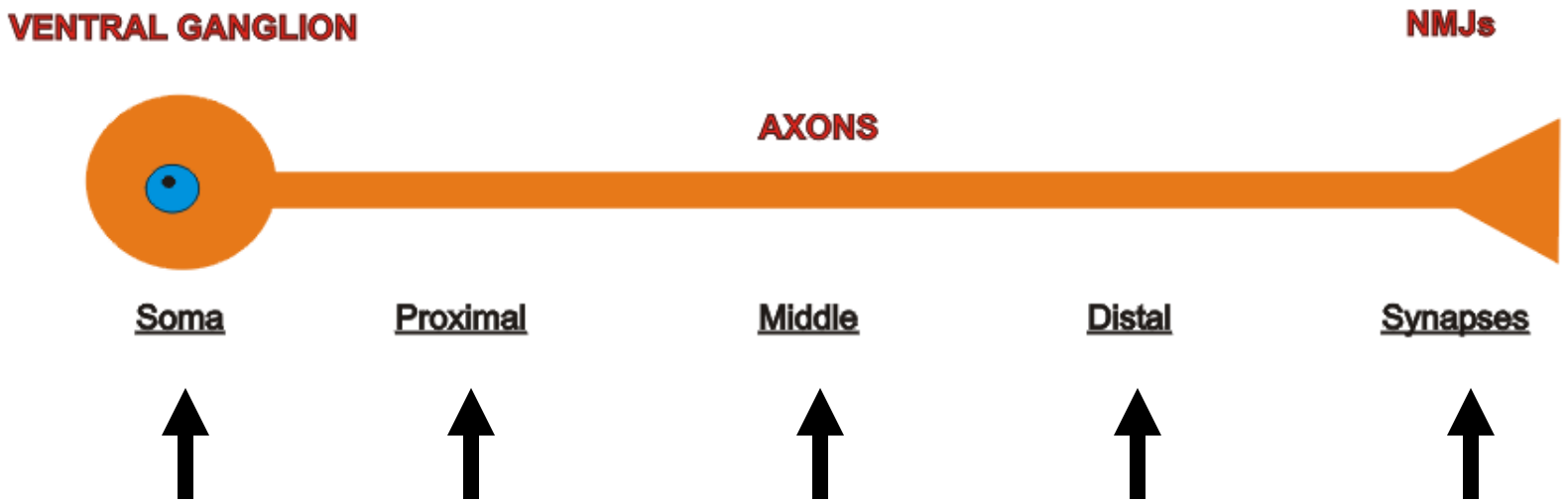
Other data suggest that, instead or in parallel, oxidative stress-independent mechanisms may produce FRDA pathology

Using a *Drosophila* strain with an RNA knock-down of frataxin ("DfhIR," Anderson et al., 2005), we have quantified several parameters of the mitochondrial life cycle in frataxin-deficient neurons in the intact larval nervous system.

Q: What goes wrong in the mitochondrial life cycle when neurons lack frataxin?

Frataxin deficiency causes dying-back neuropathy in late 3rd instar larvae



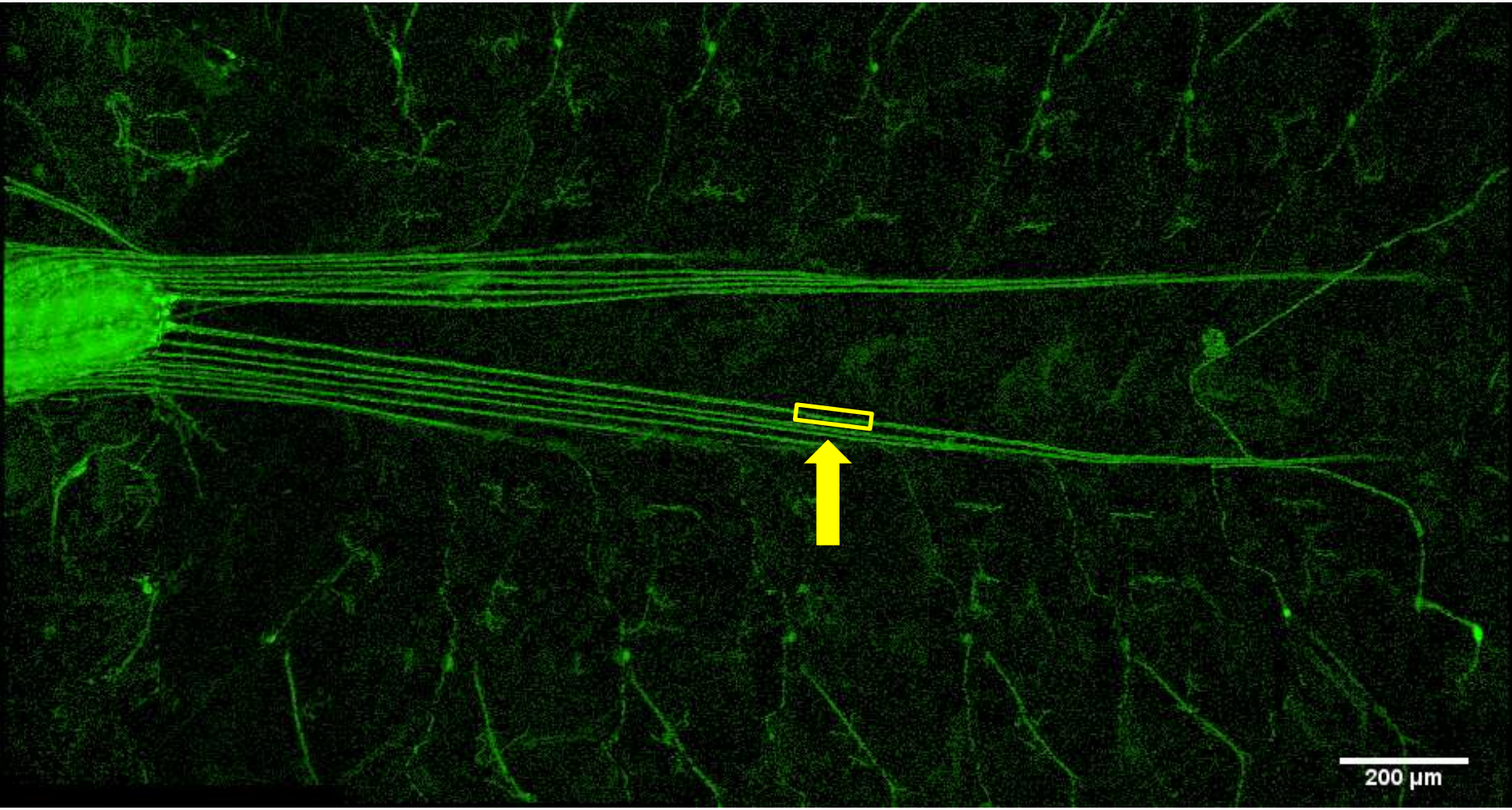


Look at:

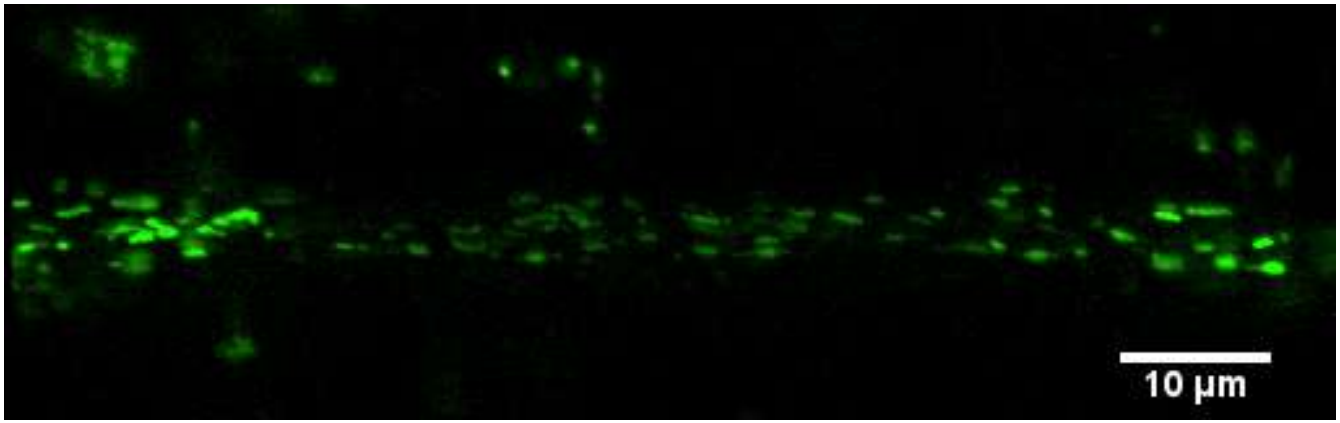
(1) Mitochondrial axonal transport
(2) Mitochondrial distribution and density
(3) Mitochondrial membrane potential
(4) Mitochondrial & cytoplasmic ROS levels

in all regions of neurons, from the cell bodies to the synapses, and throughout larval development, from 2nd to late 3rd instar.

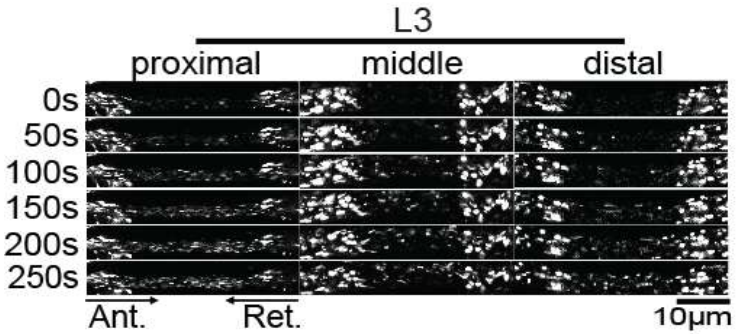
3rd instar larva with D42-GAL4 / UAS-mitoGFP

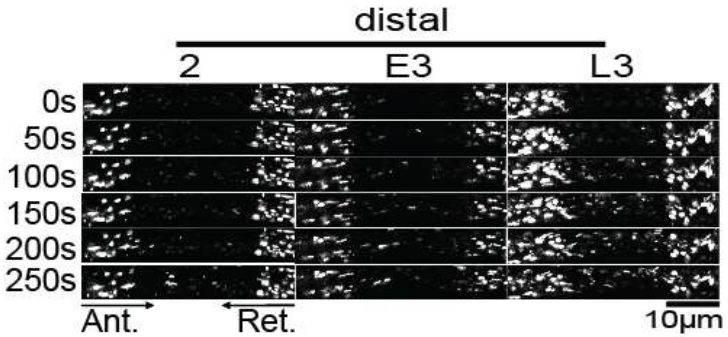


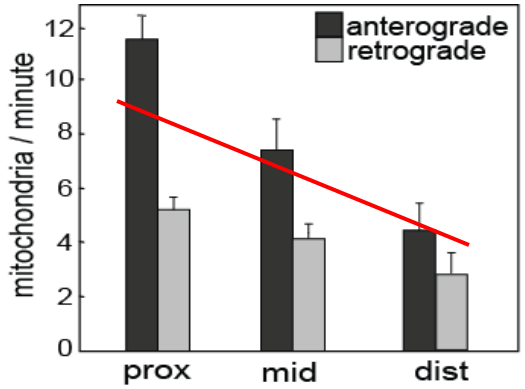
Axonal transport: confocal microscopy of 3rd instar segmental nerve

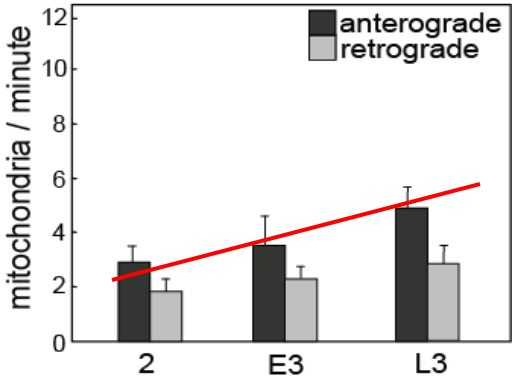


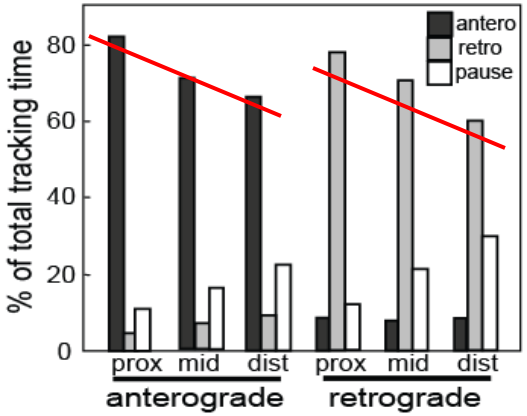
Axonal mitochondrial transport: spatiotemporal variation

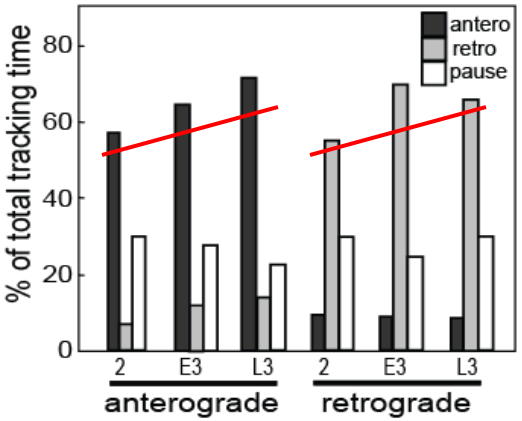




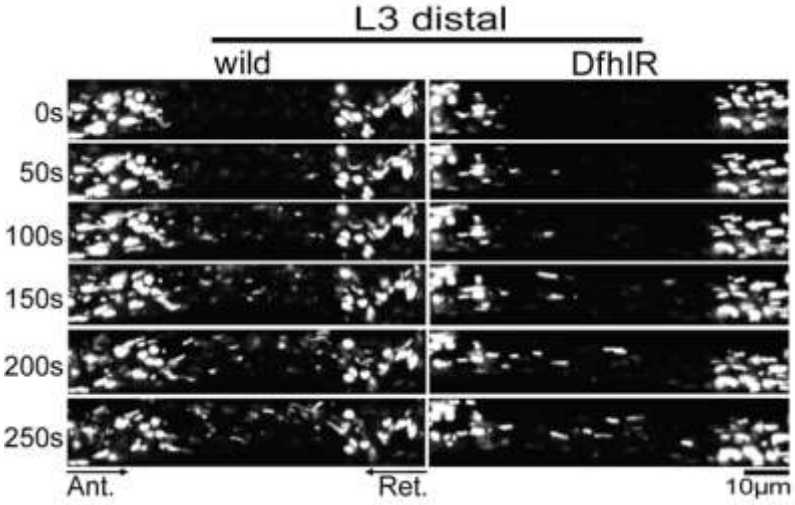


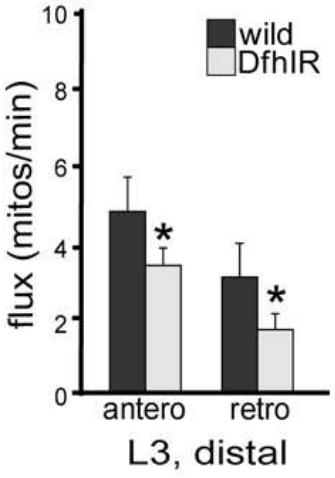


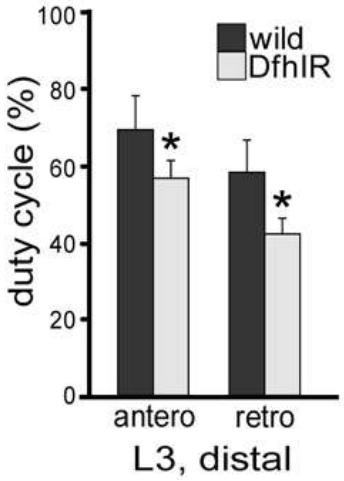


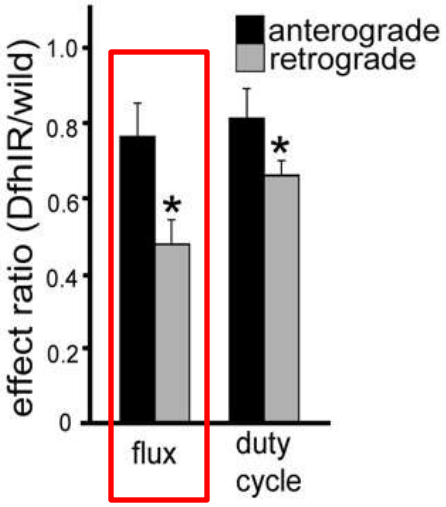


DfhIR axons show transport deficits, but in the distal regions and not until 3rd instar

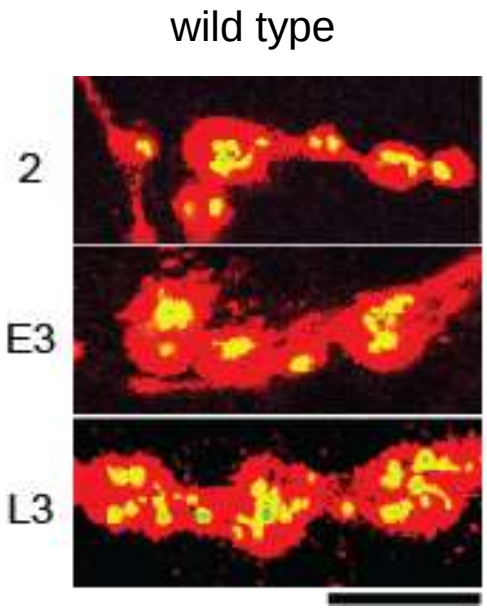


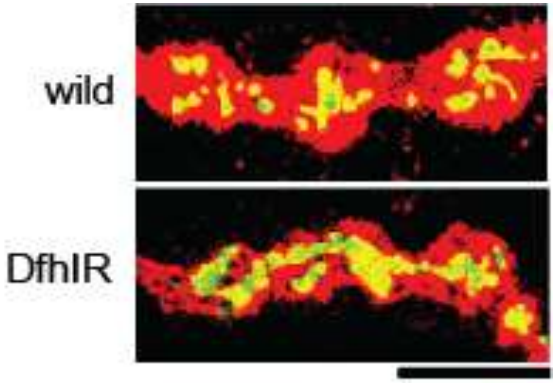


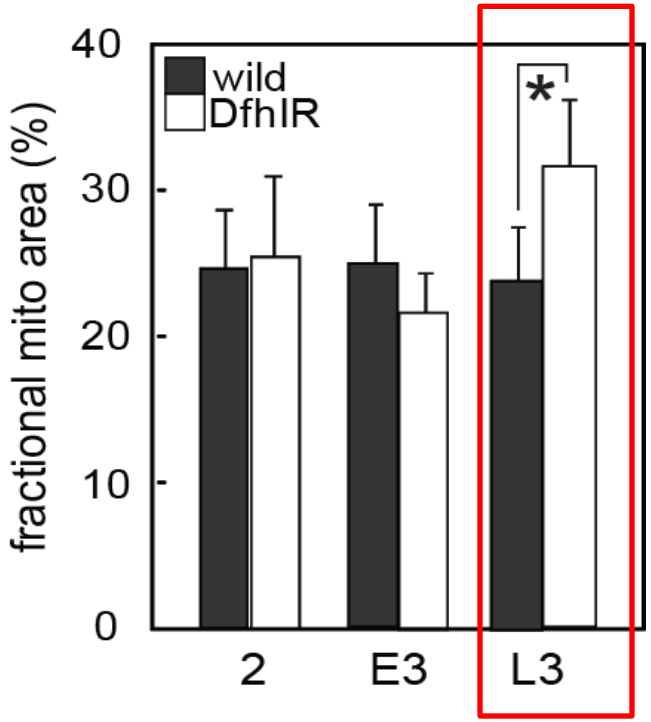




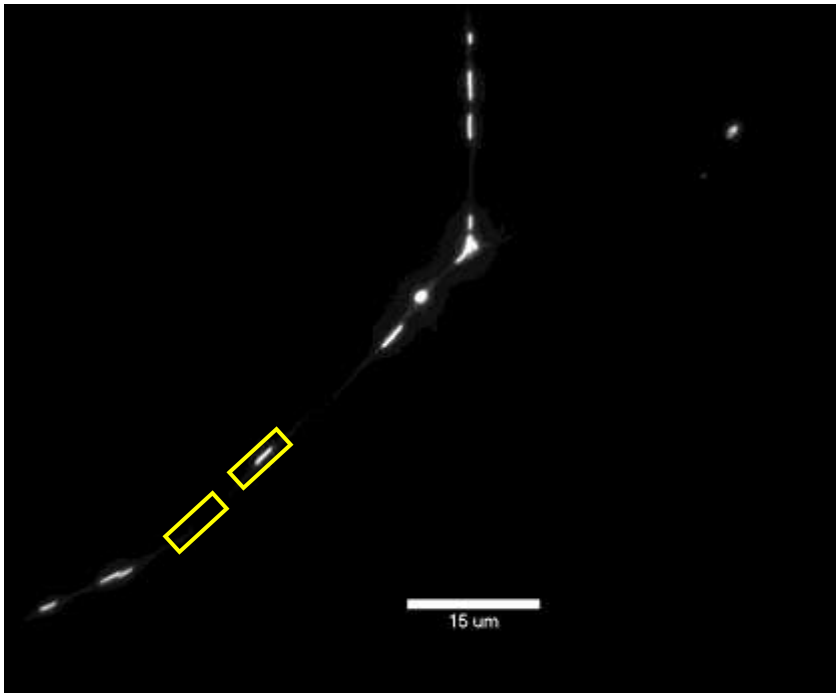
NMJs of DfhIR larvae accumulate excess mitochondria

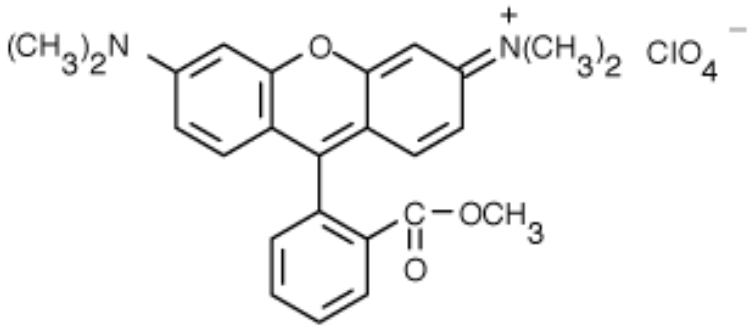






Mitochondrial metabolic state: variation of membrane potential with location, and regulation by signaling

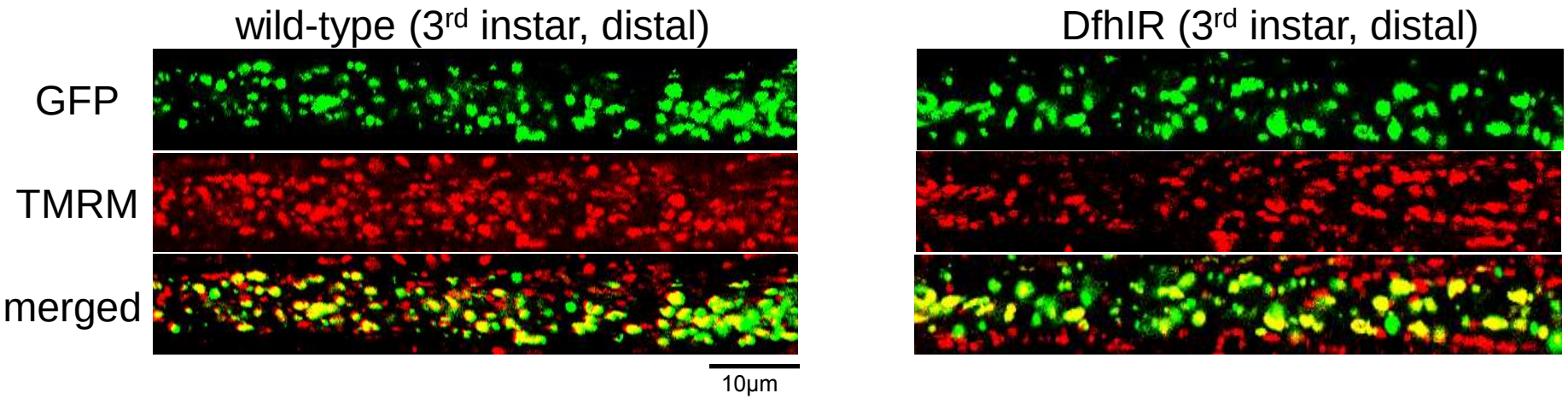


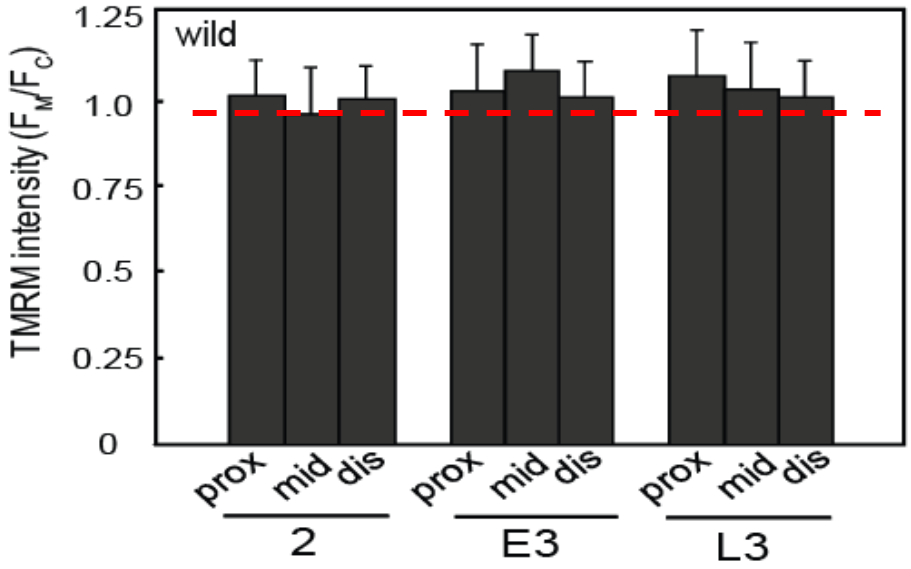


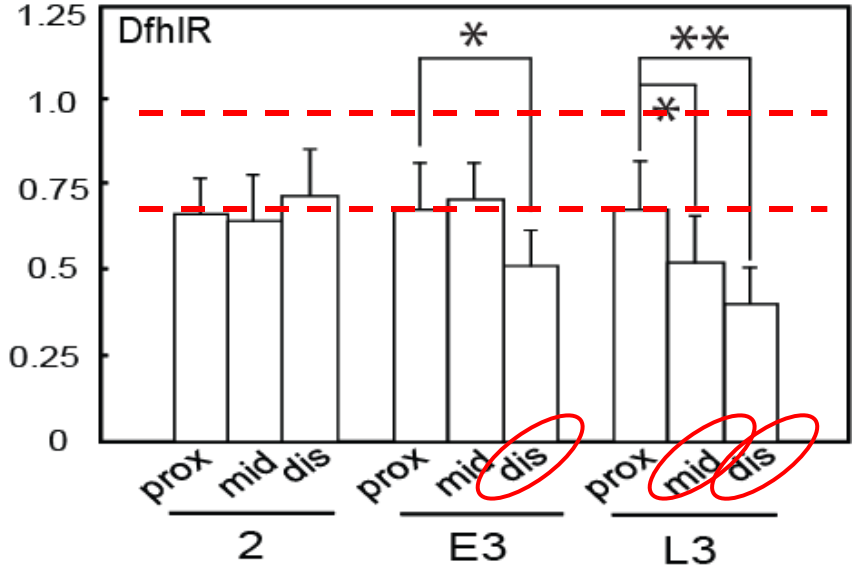
- Tetramethylrhodamine methyl ester (TMRM)
- Low toxicity, minimal interference with respiration
- Equilibrates quickly
- Ratio'd mito:cyto fluorescence intensity is proportional to $\Delta\Psi_m$

Verburg & Hollenbeck, 2008

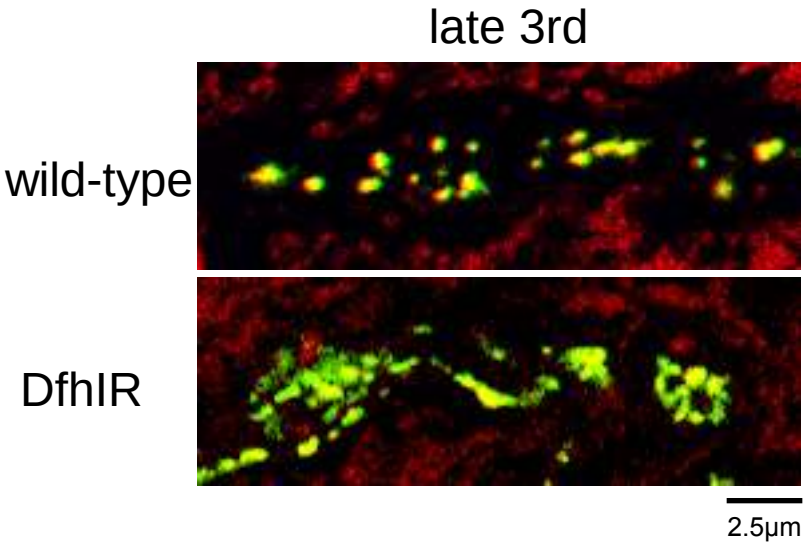
$\Delta\Psi_m$ declines in mitochondria in motor axons



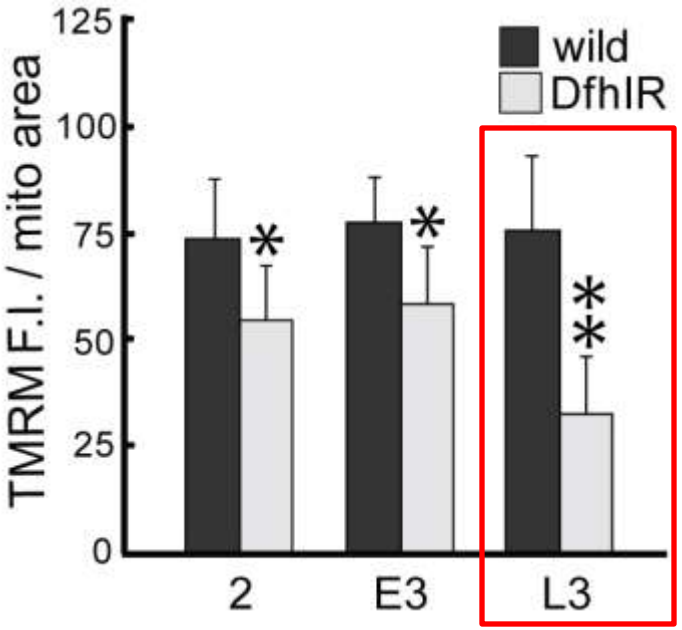




$\Delta\Psi_m$ declines in mitochondria of NMJs



Some mitochondria in DfhIR NMJs are completely depolarized



Are ROS levels higher in frataxin-deficient neurons?

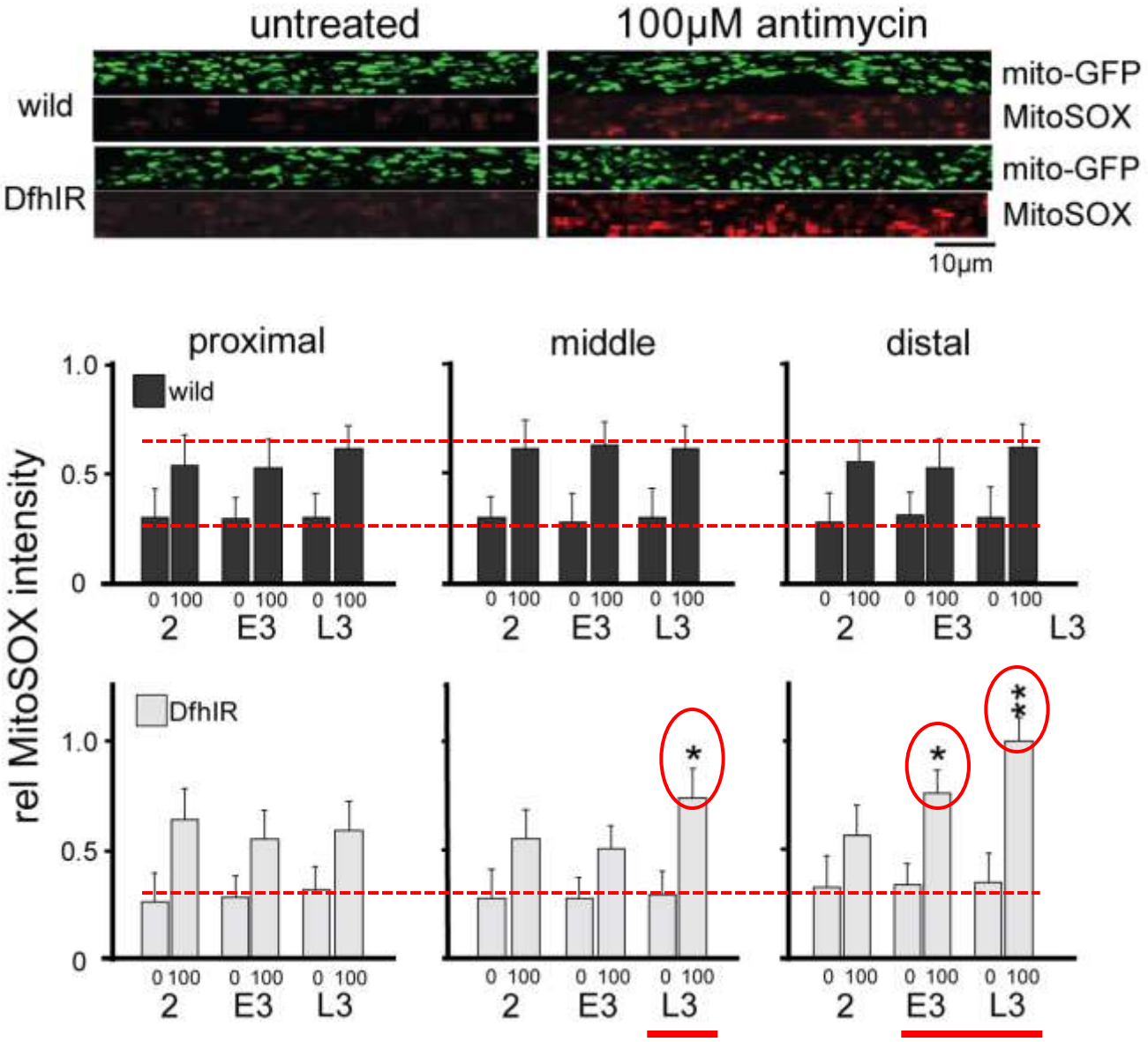
To quantify ROS levels in live neurons of the intact larval nervous system, we used two fluorescent probes:

MitoSOX, to determine mitochondrial [ROS], using the ratio of mitochondrial to adjacent cytoplasmic fluorescence

Dihydroethidium, to determine cytoplasmic [ROS]

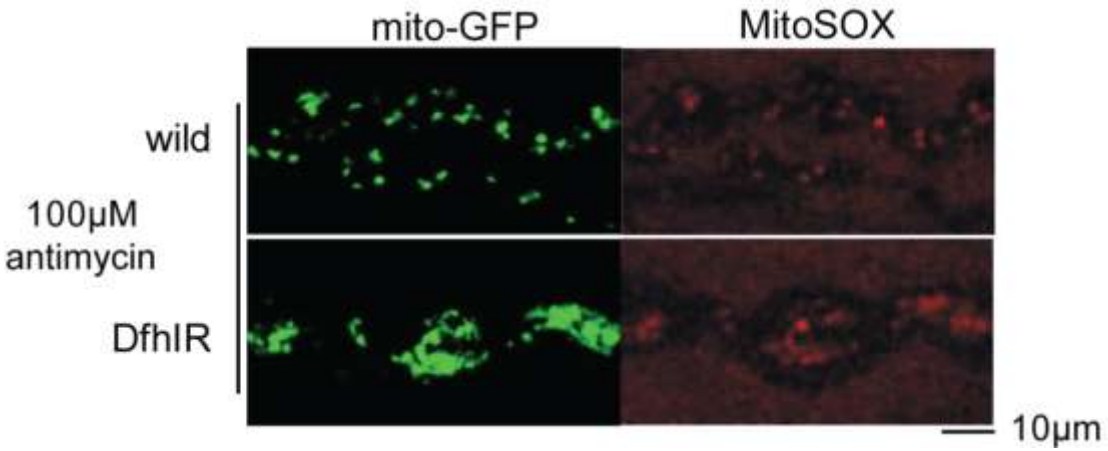
Positive control for ROS detection: complex III inhibitor, antimycin A

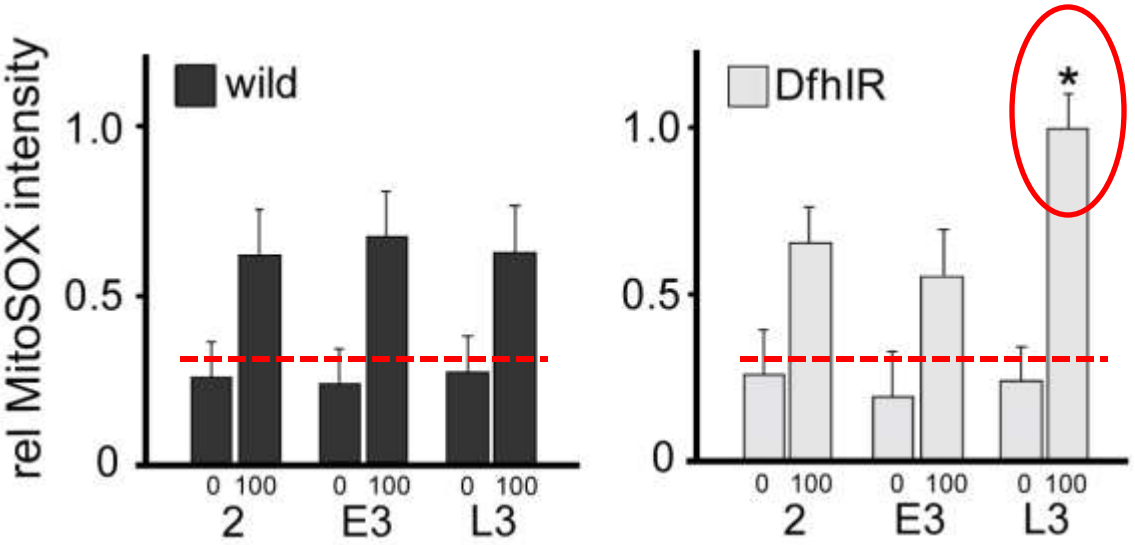
mtROS levels are not elevated in DfhIR axons



No difference in unperturbed ROS levels, but an increased response to antimycin challenge

mtROS levels are not elevated in DfhIR NMJs





No difference in unperturbed ROS levels, but an increased response to antimycin challenge

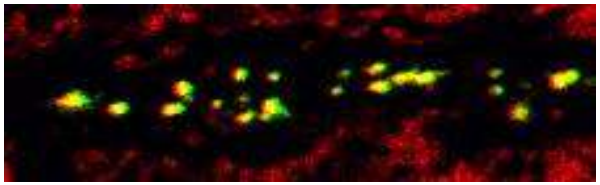
In a frataxin-deficient nervous system:

(1) Mitochondrial membrane potential is diminished in all regions, throughout development. NMJs even have a subset of completely depolarized mitochondria.

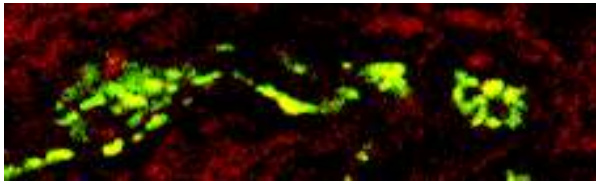
(2) Mitochondrial transport deficits arise during 3rd instar, and retrograde movement declines preferentially.

(3) Due to (1) and (2), NMJs accumulate excess, metabolically-inactive mitochondria

wild-type



DfhIR



In a frataxin-deficient nervous system:

(4) Mitochondrial ROS production is no greater than in a wild-type larva. This is true in all regions and throughout development, if the nervous system is unperturbed.

(5) However, if the nervous system is challenged with a complex III inhibitor, DfhIR neurons react with greater ROS production than wild-type, late in development, in most regions.

(6) These data suggest an oxidative damage-independent mechanism in the cellular neuropathology of Friedreich ataxia, involving defects in motility and membrane potential.

Implications for the cellular neuropathology of Friedreich ataxia:

(1) A metabolic disorder with its origins early in development, rather than a disease of accumulating oxidative damage?

(2) Both ATP deficiency and changes in ATP-dependent signaling might be relevant.

(3) Despite lack of evidence for increased [ROS], both induced ROS increases and ROS signaling might be important.

Parkinsons disease models: fly *PINK1* and *parkin* mutants

PD: loss of DA neurons. Two candidate cellular defects associated with PD are problems are oxidative stress and mitochondrial dysfunction.

Some early-onset PD pedigrees are associated with mutations in known proteins, including PINK1 (ser-thr kinase) and parkin (E3 ubiquitin-protein ligase).

PINK1 and parkin -- associated with mitochondria and implicated in mitochondrial turnover (mitophagy).
One hypothesis: PINK1 associates with the surface of damaged mitochondria, recruiting parkin and targeting those mitochondria for turnover.

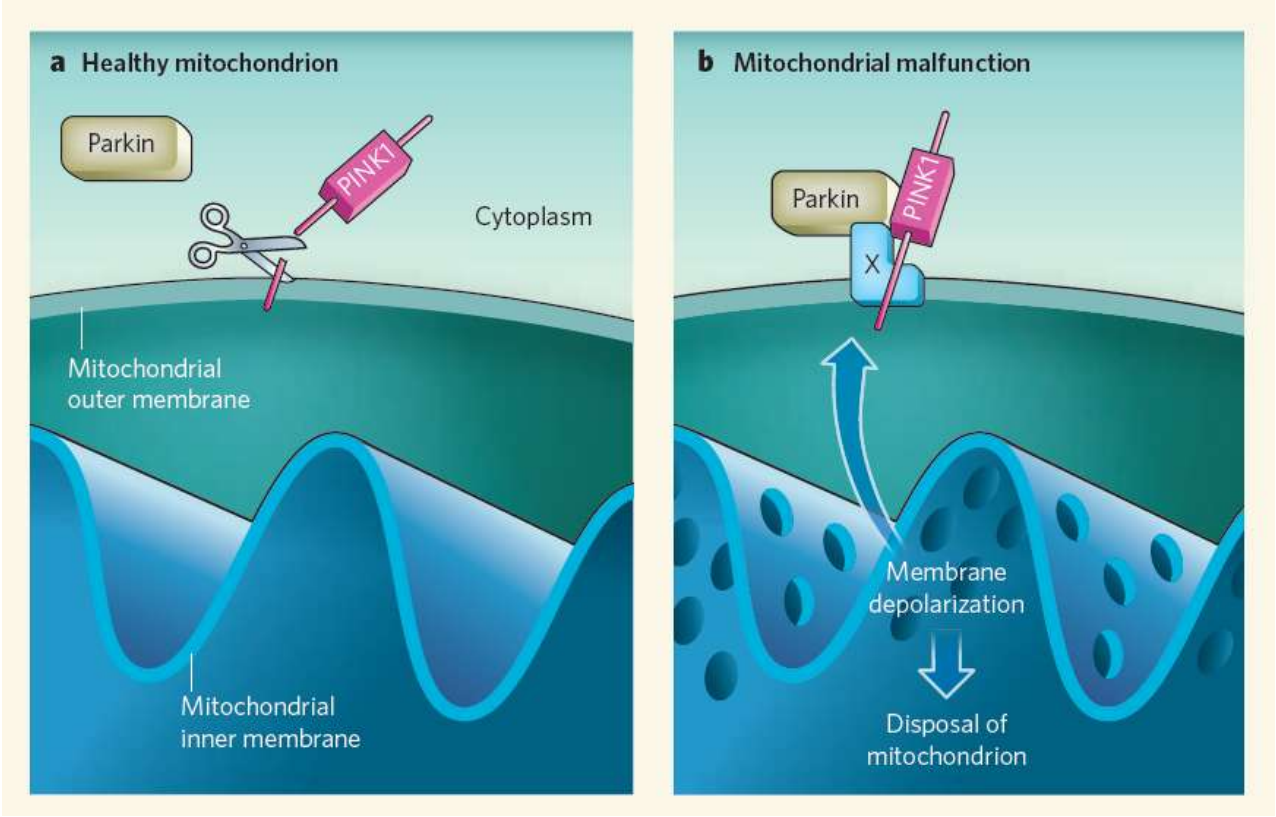
Parkinsons disease models: fly *PINK1* and *parkin* mutants

Pallanck lab used genetic approach to (1) order *PINK1* and *parkin* in a pathway; (2) establish that mutants show a progressive pathology of muscle cells and abnormal morphology of muscle mitochondria.

Question: Does the cellular neuropathology of these mutants support current ideas about PD patho-physiology? What's wrong with the mitochondrial life cycle in *PINK1/parkin* mutants?

Parkinsons disease models: fly *PINK1* and *parkin* mutants

Current hypothesis: PINK1 associates with the surface of damaged mitochondria, recruits parkin, targets those mitochondria for turnover.



Asa Abeliovich
Nature, 2010. Vol 463

Parkinsons disease models: fly *PINK1* and *parkin* mutants

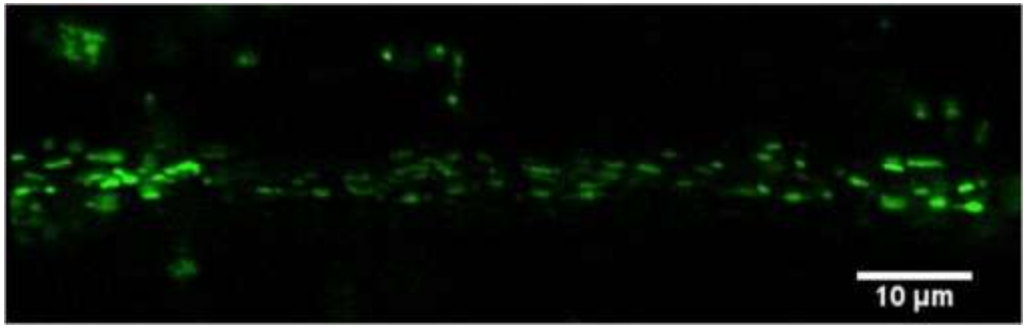
Questions: What's wrong with the mitochondrial life cycle in *PINK1/parkin* mutants? Does the cellular neuropathology of these mutants support current ideas about PD pathophysiology?

Hypotheses: *PINK1/parkin* mutants will show –

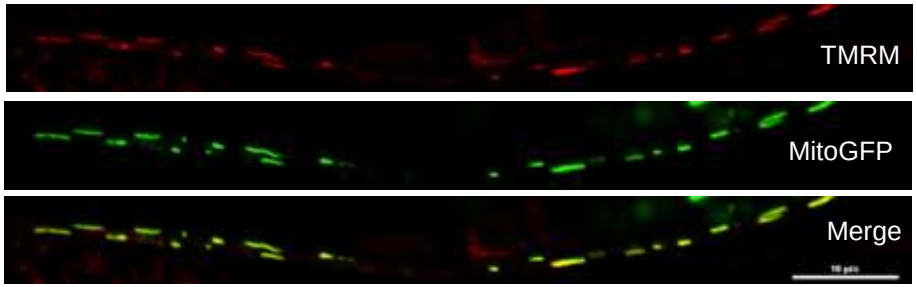
metabolic defects

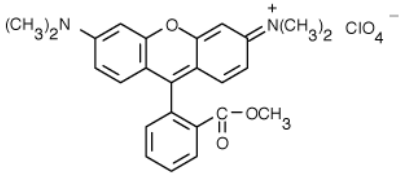
axonal transport deficiencies

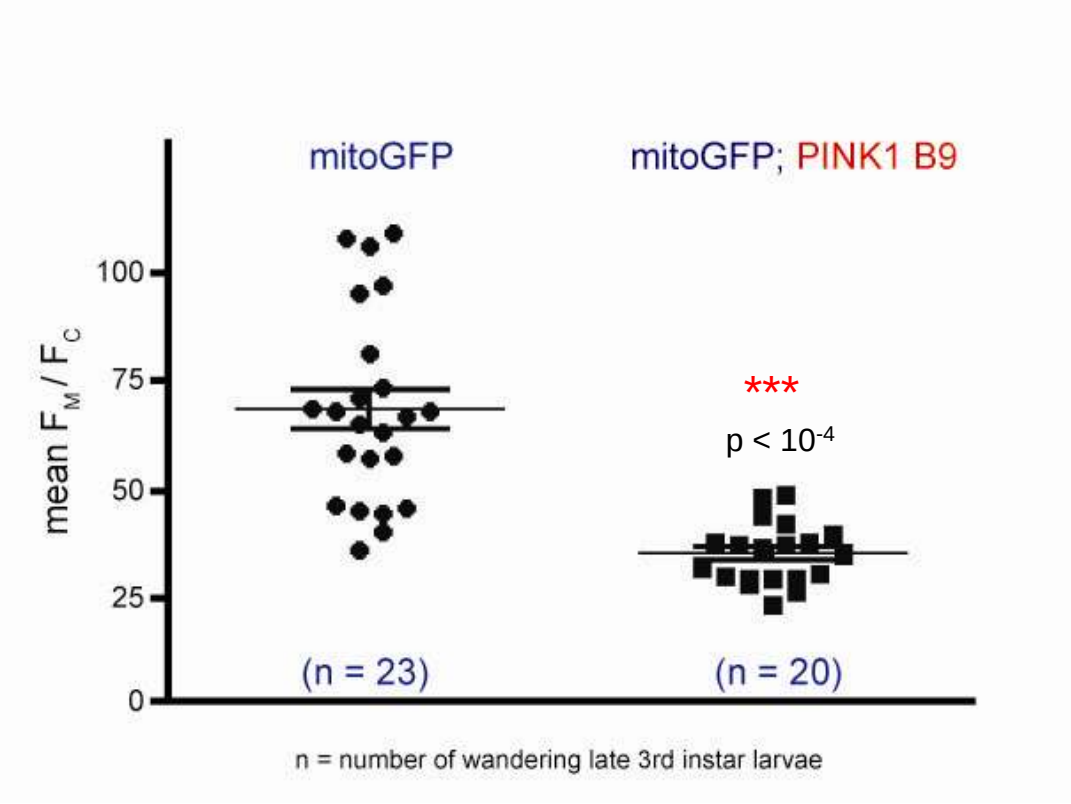
altered fission-fusion balance



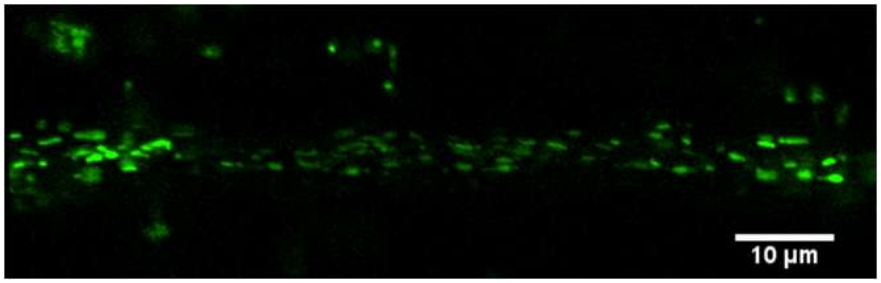
PINK1^{B9} mutants show diminished axonal mitochondrial membrane potential ($\Delta\psi_m$).

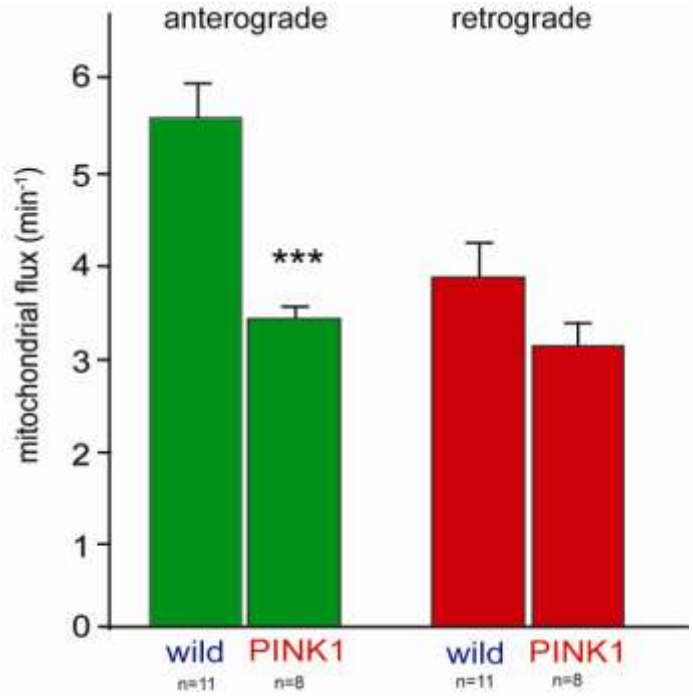


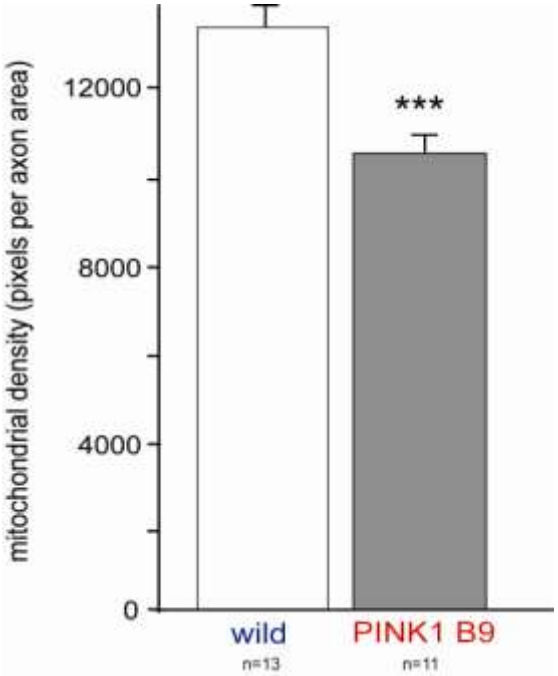




PINK1^{B9} mutants: mitochondrial movement & density in axons



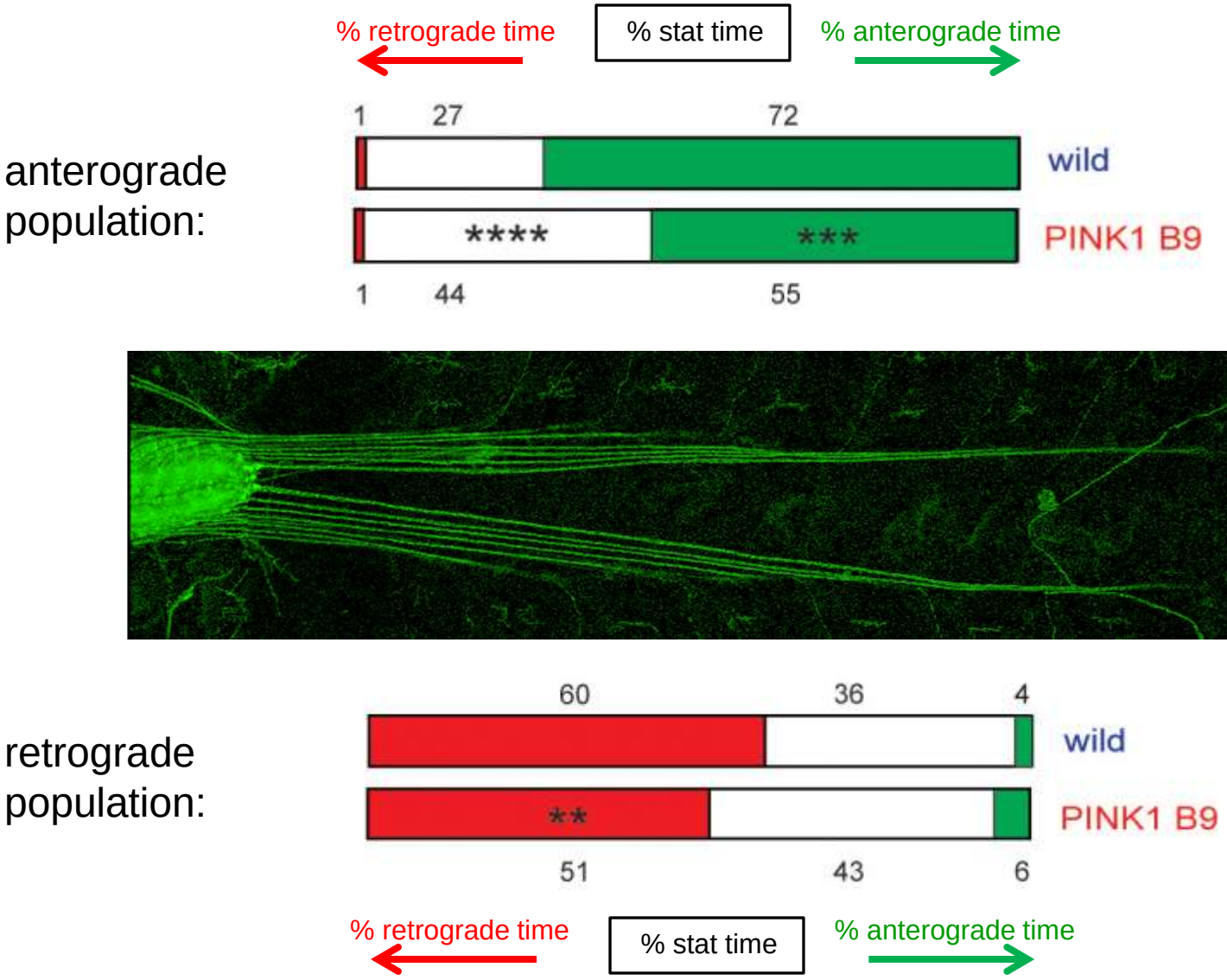




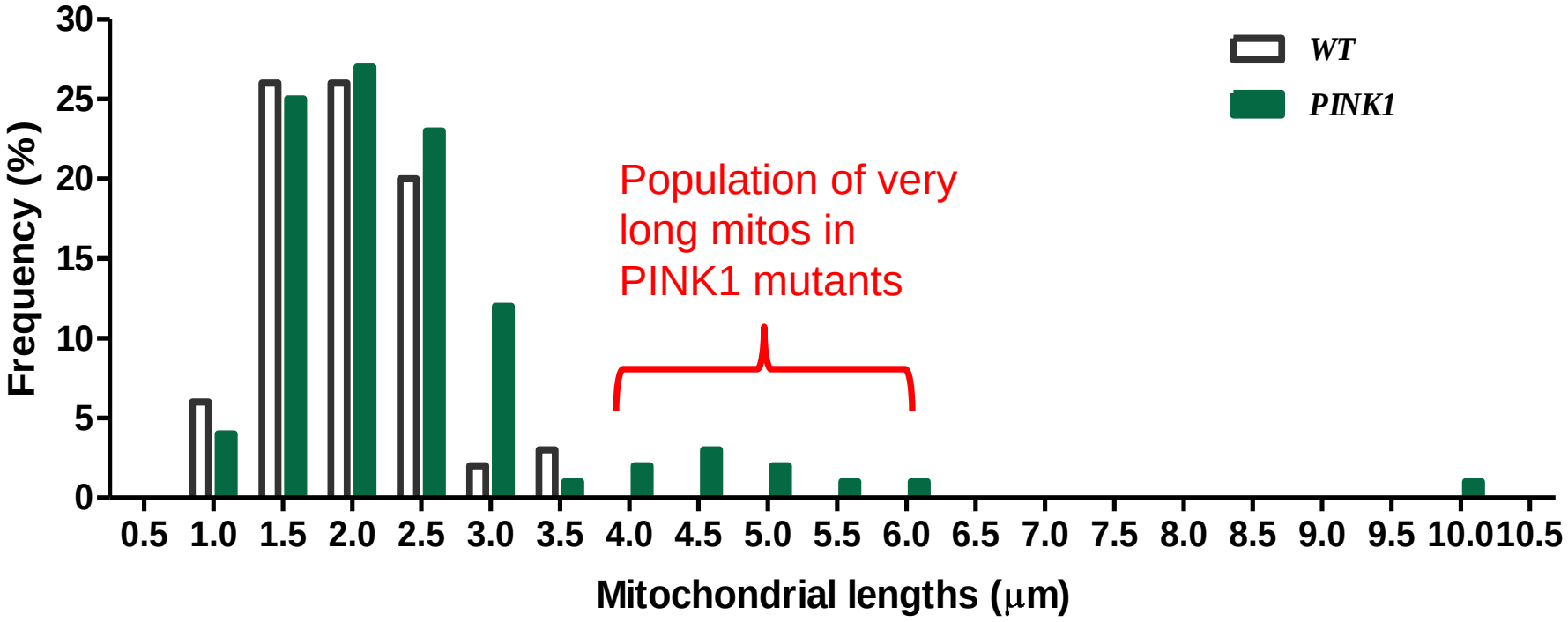
anterograde flux diminished

axonal mito density diminished

PINK1^{B9} mutants: altered duty cycle for axonal mitochondria



PINK1^{B9} mutants show an increase in the lengths of stationary axonal mitochondria



Parkinsons disease models: fly *PINK1* mutants

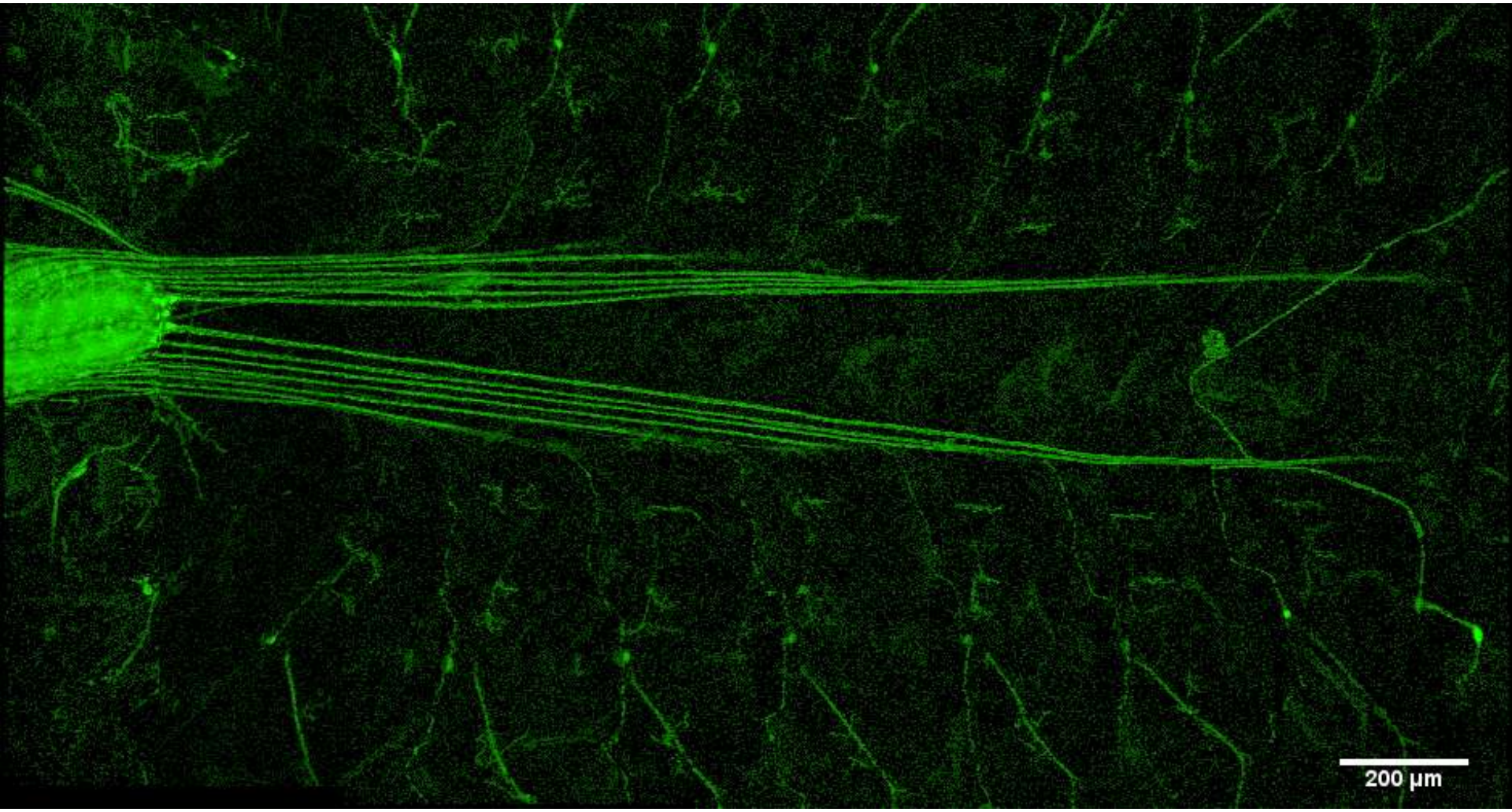
Axonal mitochondria show:

(1) Decreased anterograde delivery of mitochondria to the axon, and lower mitochondrial density

(2) Greatly reduced mitochondrial membrane potential

(3) A sub-population (~10%) of very long (stationary) mitochondria

Fission-fusion balance?
Rate and location of turnover?


200 µm

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Gone, not forgotten:
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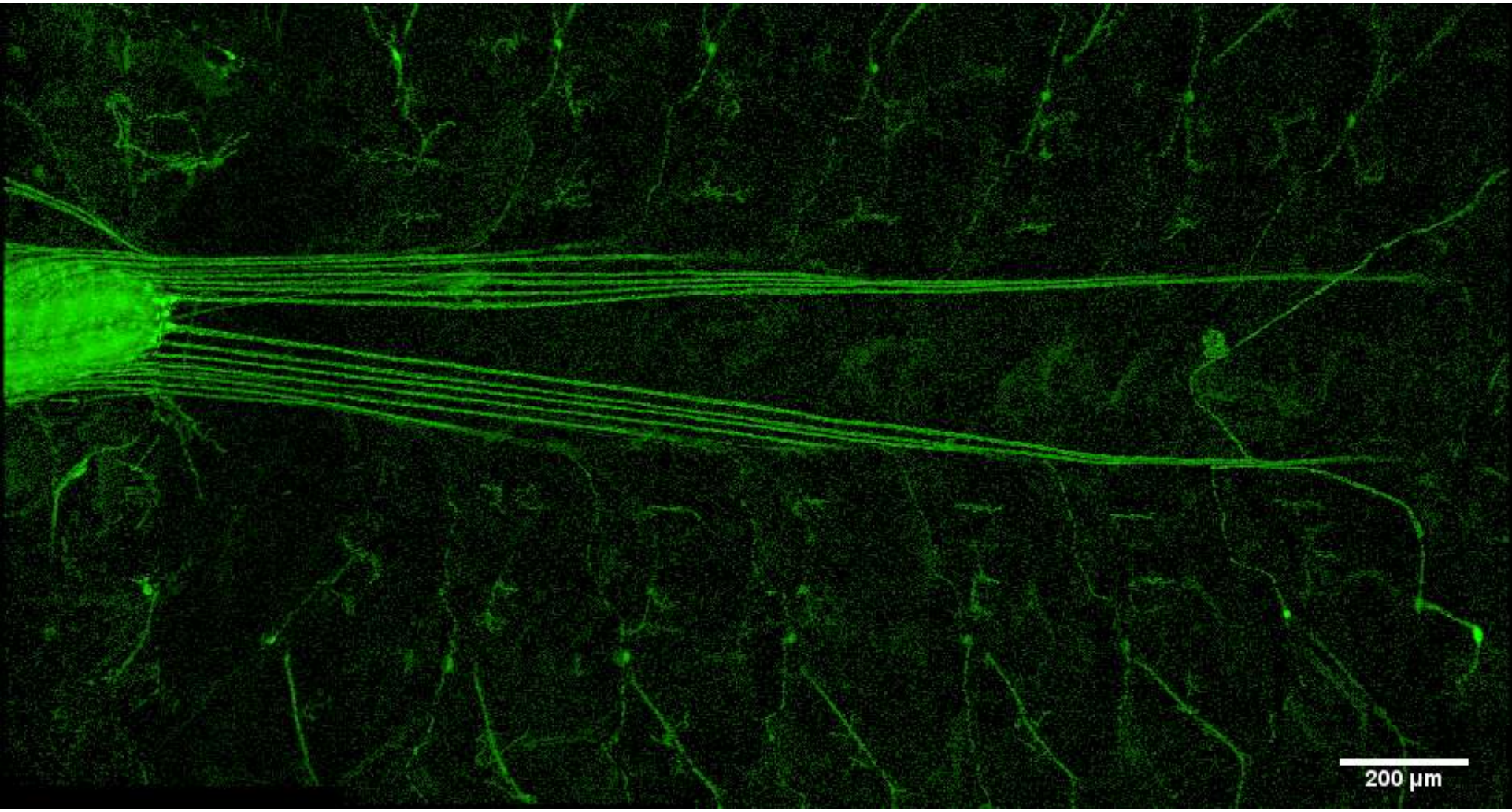
Funding from NINDS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