Mitochondria associate with the cytoskeleton

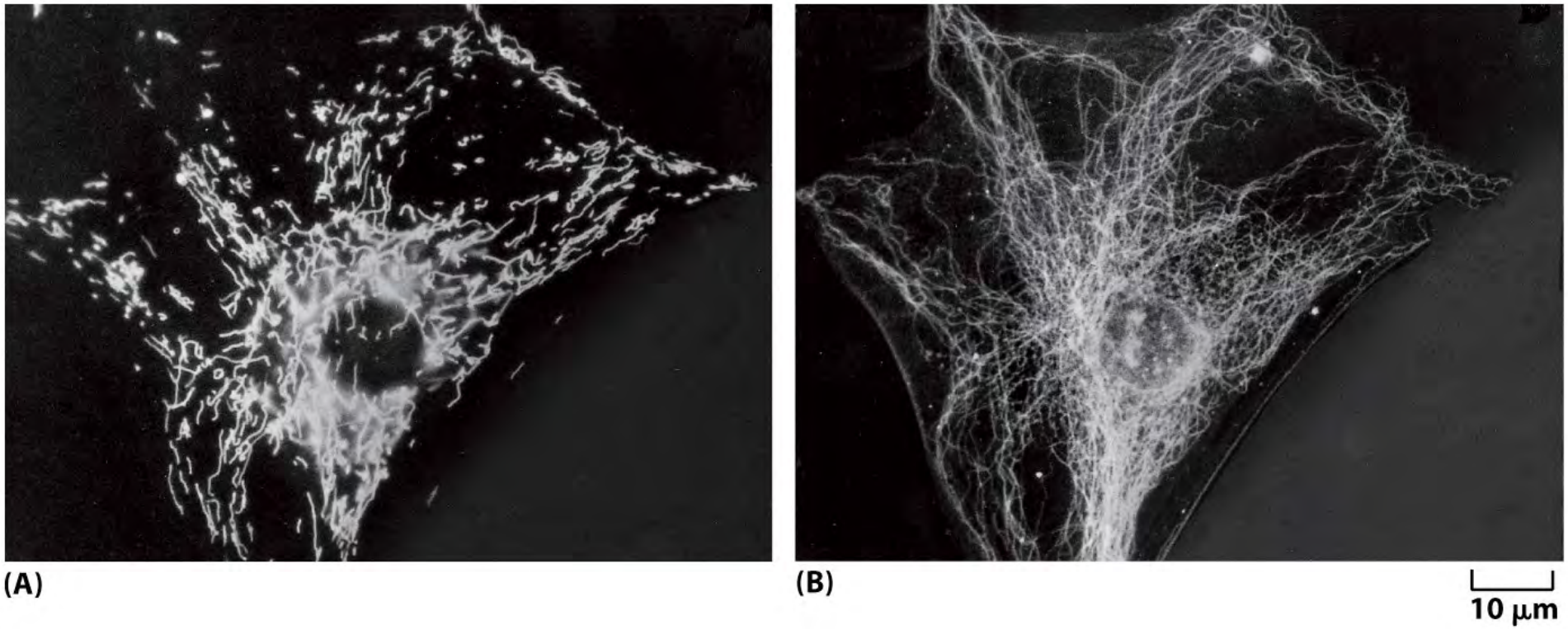


Figure 14-5 *Molecular Biology of the Cell* (© Garland Science 2008)

Special arrangements of mitochondria

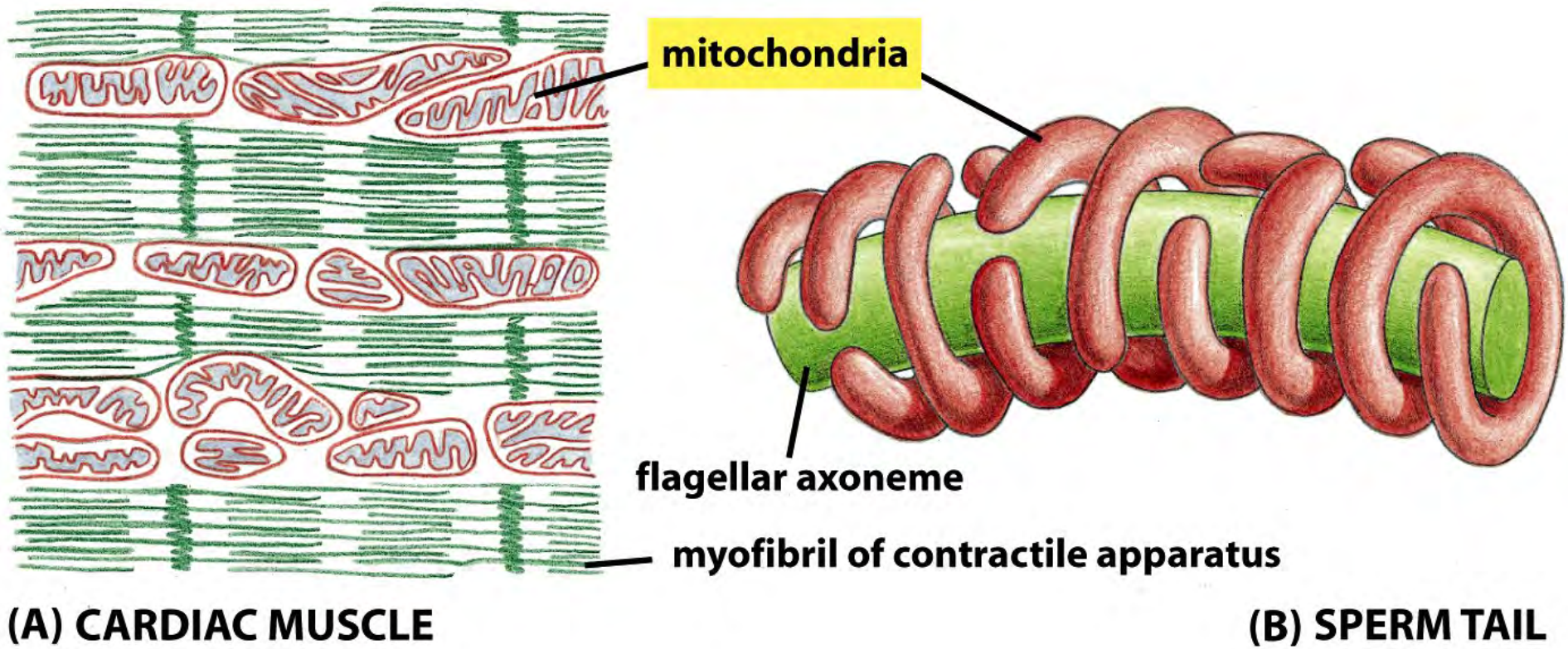
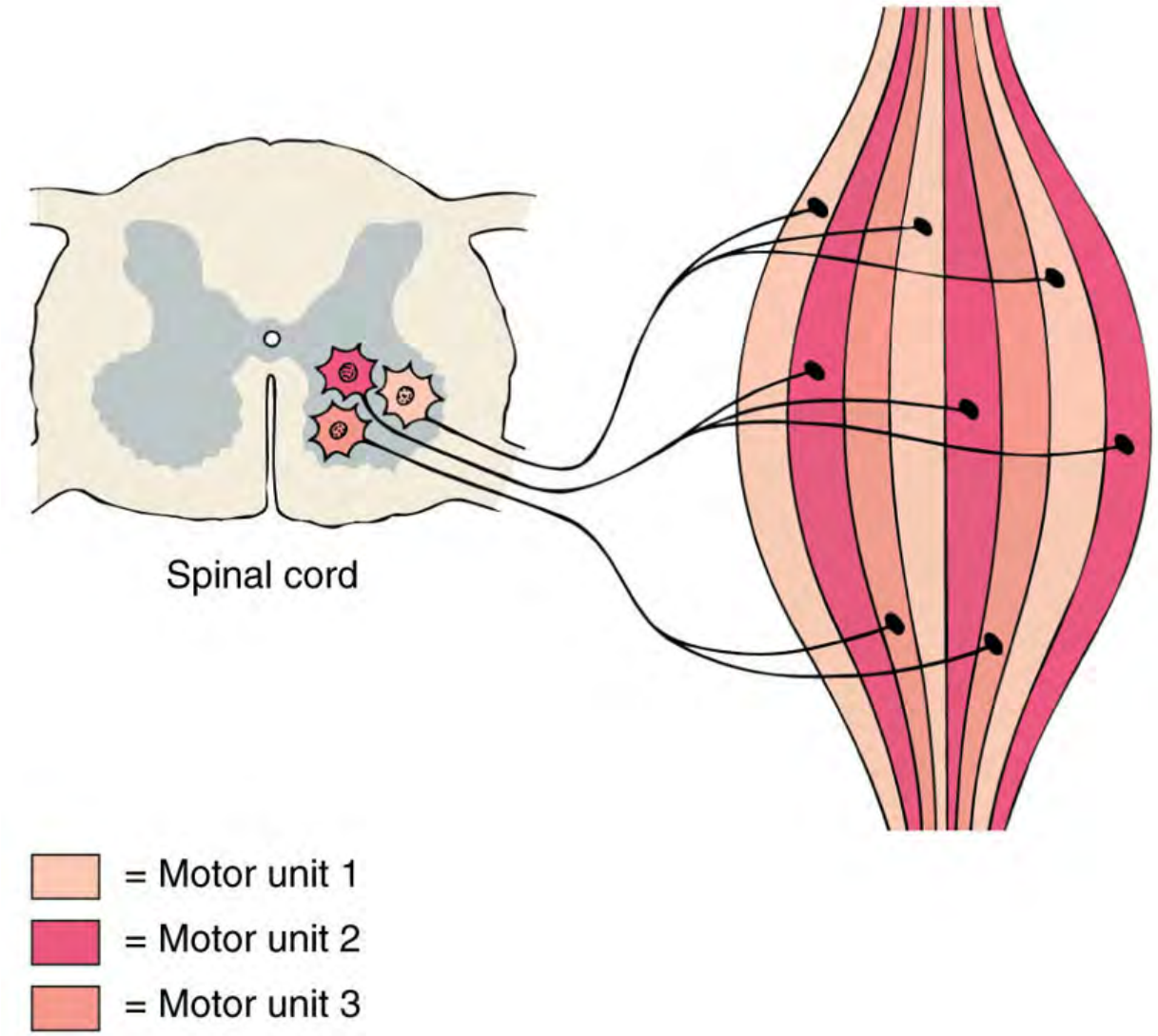


Figure 14-6 *Molecular Biology of the Cell* (© Garland Science 2008)

Relationship of motor neurons to muscle fibers



• Each muscle innervated by N motor neurons.

• A single motor neuron enters muscle, branches (unmyelinated), and supplies many dispersed muscle fibers ("one to many").

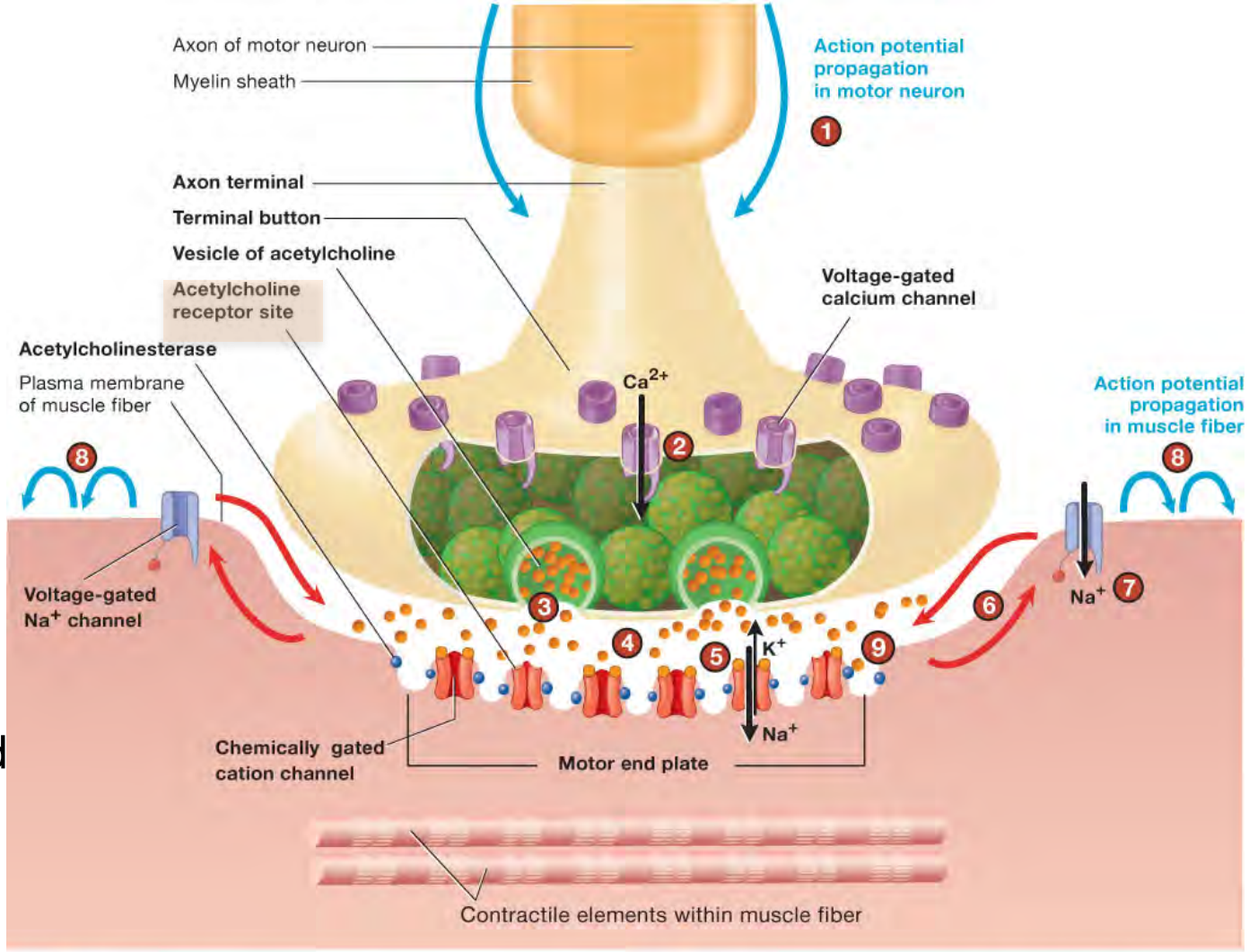
• One motor neuron plus all the muscle fibers innervated= **motor unit**. Depending on muscle, a motor unit can have 10-2000 muscle fibers.

• Each muscle fiber innervated by a single motor neuron.

• The nerve terminal at the neuromuscular junction (NMJ=nerve terminal + motor end plate) has a specialized organization of mitochondria.

• In adult mammals, one muscle fiber has only 1 NMJ.

Acetylcholine receptors are a marker for the motor end plate.



1. An action potential in a motor neuron is propagated to the terminal button.
2. The presence of an action potential in the terminal button triggers the opening of voltage-gated Ca^{2+} channels and the subsequent entry of Ca^{2+} into the terminal button.
3. Ca^{2+} triggers the release of acetylcholine by exocytosis from a portion of the vesicles.
4. Acetylcholine diffuses across the space separating the nerve and muscle cells and binds with receptor sites specific for it on the motor end plate of the muscle cell membrane.
5. This binding brings about the opening of cation channels, leading to a relatively large movement of Na^{+} into the muscle cell compared to a smaller movement of K^{+} outward.
6. The result is an end-plate potential. Local current flow occurs between the depolarized end plate and adjacent membrane.
7. This local current flow opens voltage-gated Na^{2+} channels in the adjacent membrane.
8. The resultant Na^{2+} entry reduces the potential to threshold, initiating an action potential, which is propagated throughout the muscle fiber
9. Acetylcholine is subsequently destroyed by acetylcholinesterase, an enzyme located on the motor end-plate membrane, terminating the muscle cell's response.

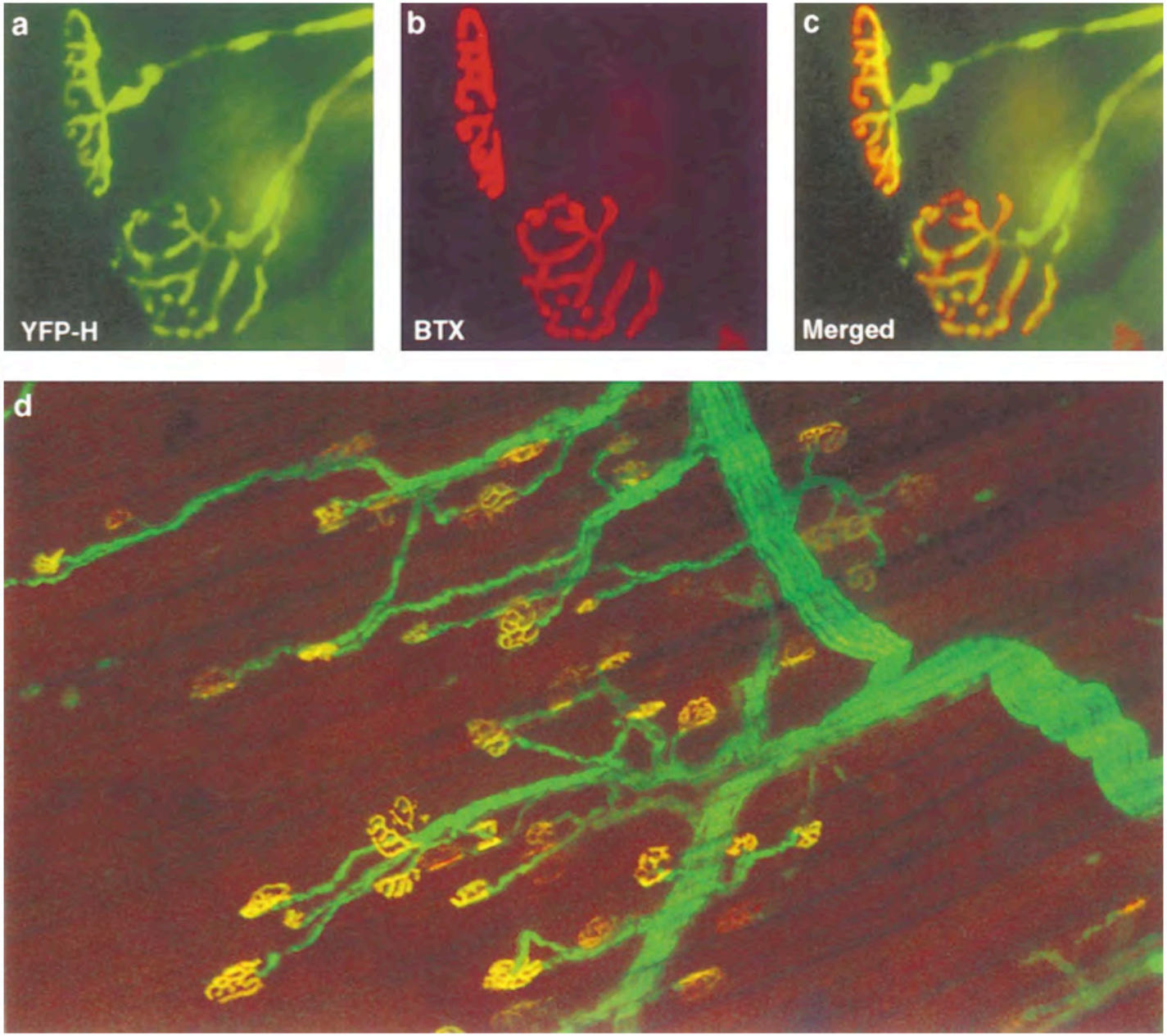
Thy1-YFP highlights motor neuron, including terminals

a, b, c: sternomastoid muscle with water immersion lens

d. Fixed gluteus muscle. All AChR-positive sites are apposed by YFP-positive nerve terminals.

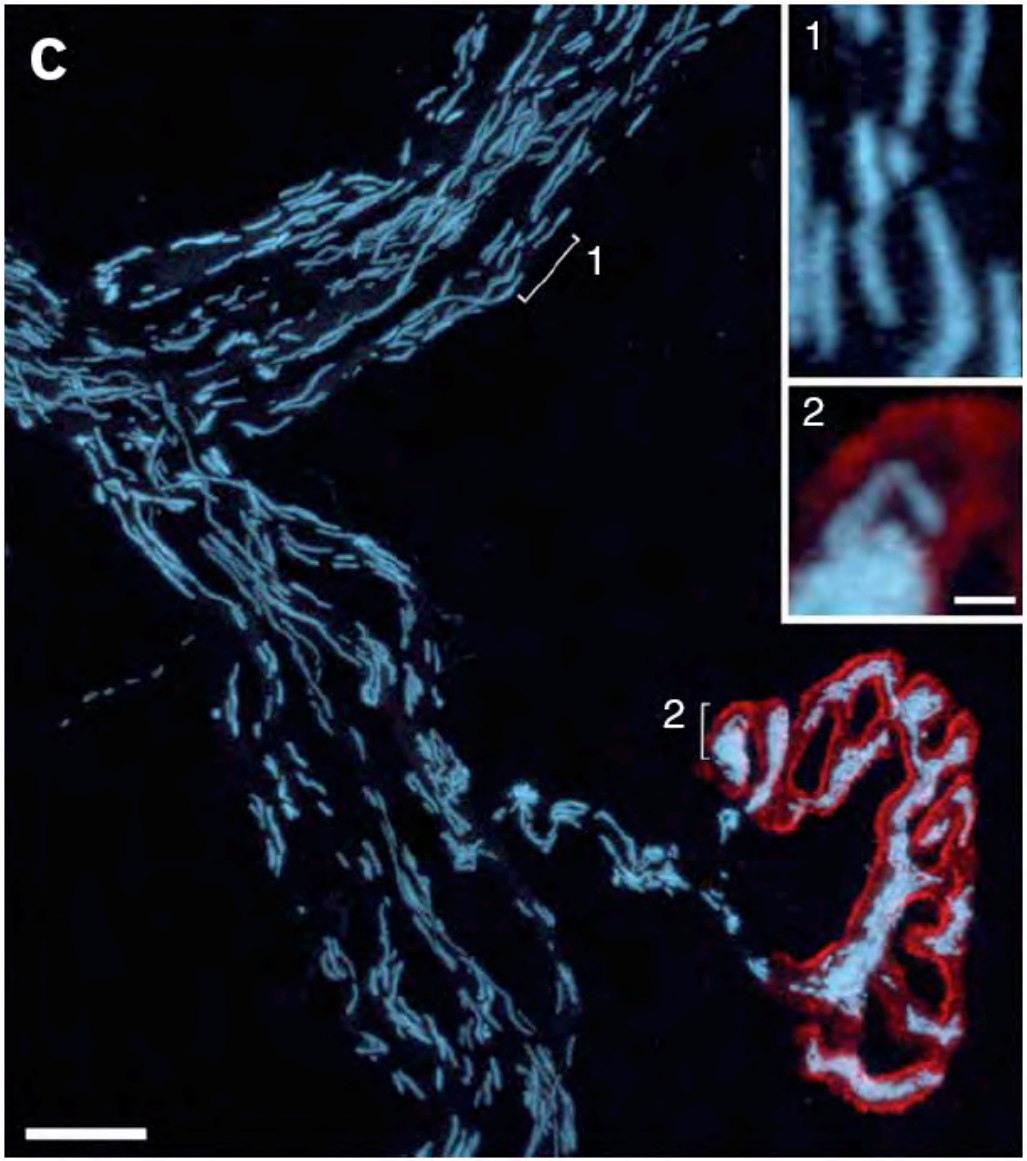
α-Bungarotoxin: a component of a snake venom that binds to acetylcholine receptors at the NMJ; causes paralysis, respiratory failure

Feng et al., Neuron, 2000



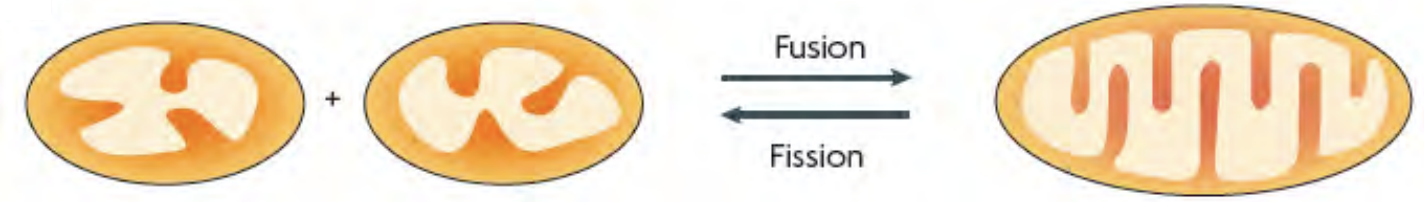
Mitochondria at NMJ of Thy1-mitoCFP mice

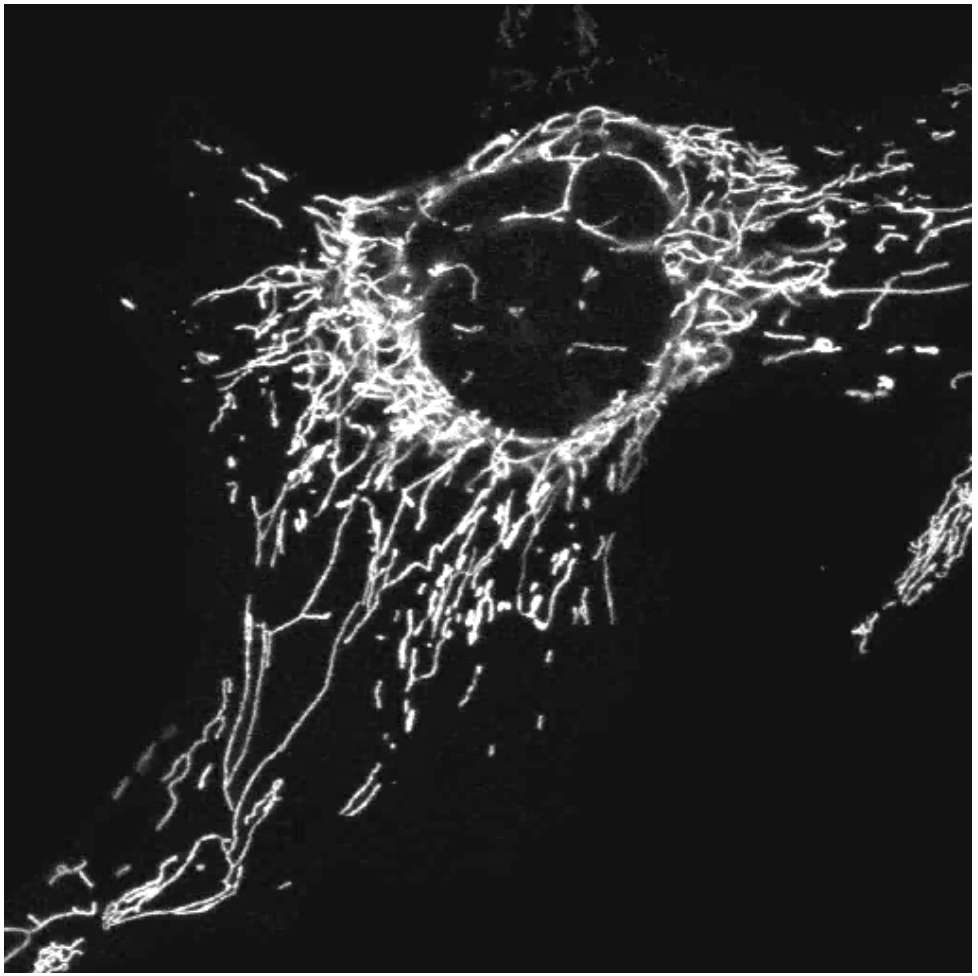
Fluorescence intensity suggests ~300 mitochondria in a single NMJ



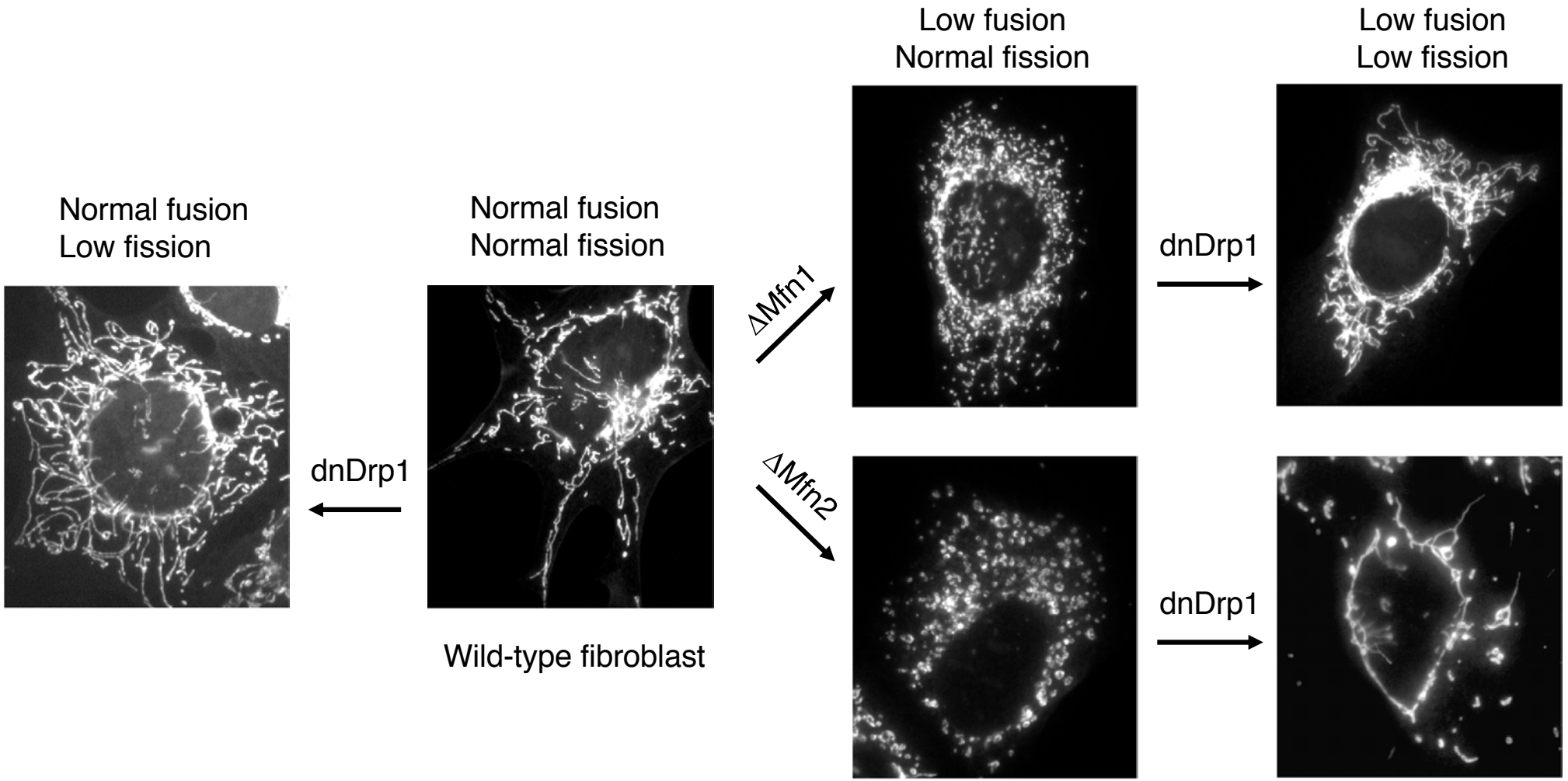
Misgeld et al, Nature Methods, 2007

Mitochondria are dynamic organelles: fusion and division



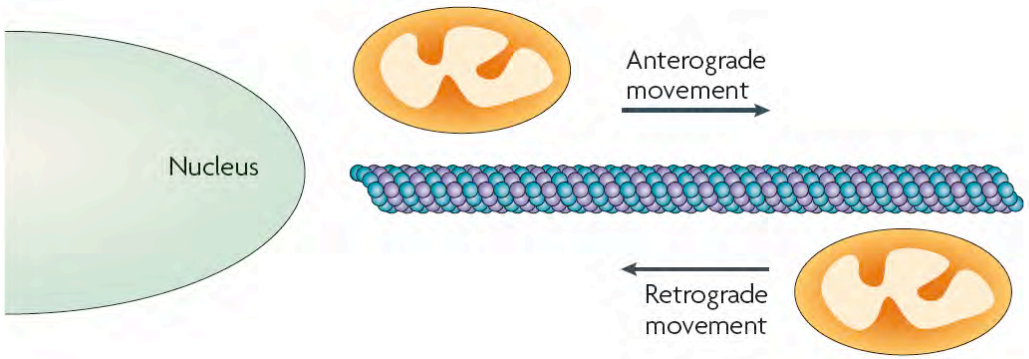


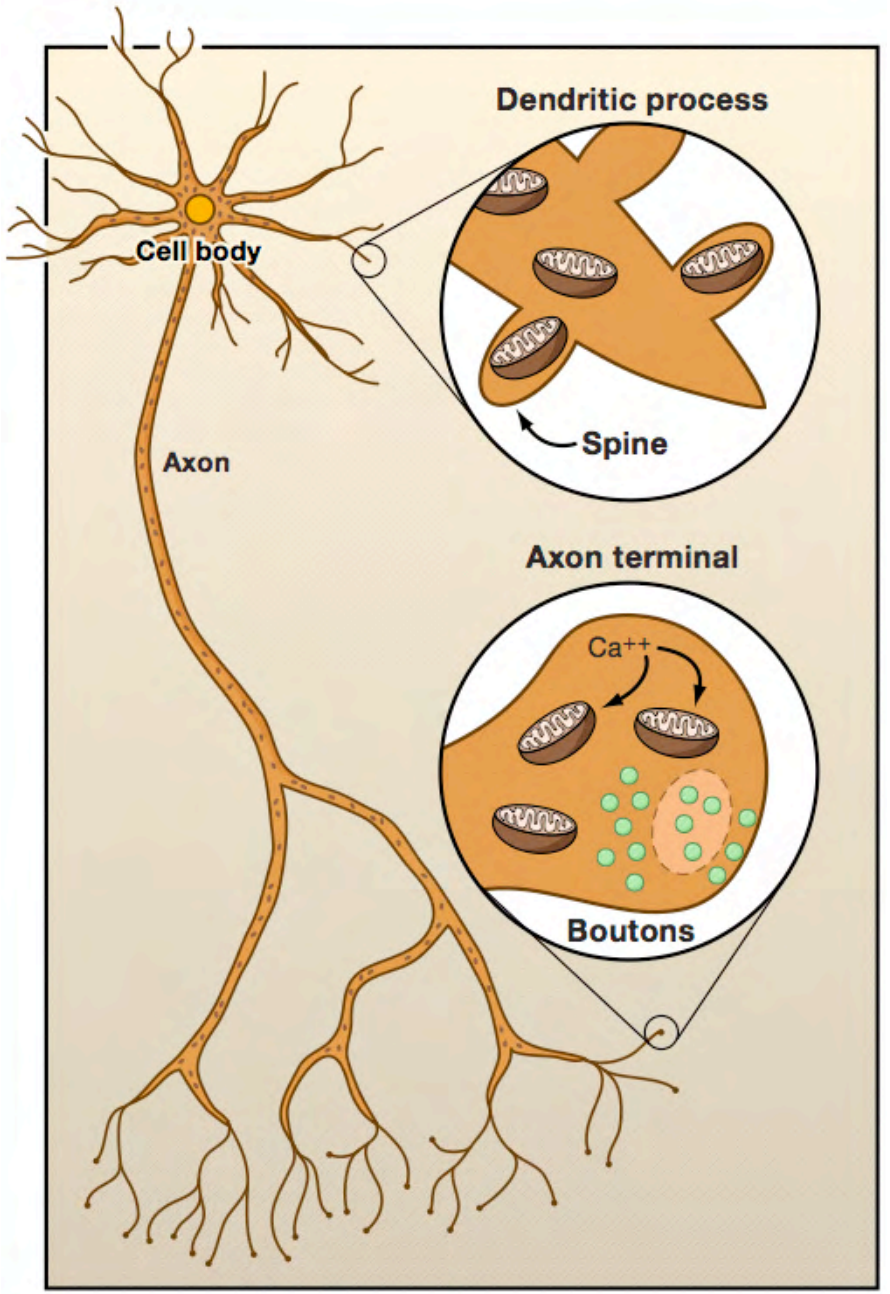
Manipulation of mitochondrial morphology by control of fusion/fission ratios



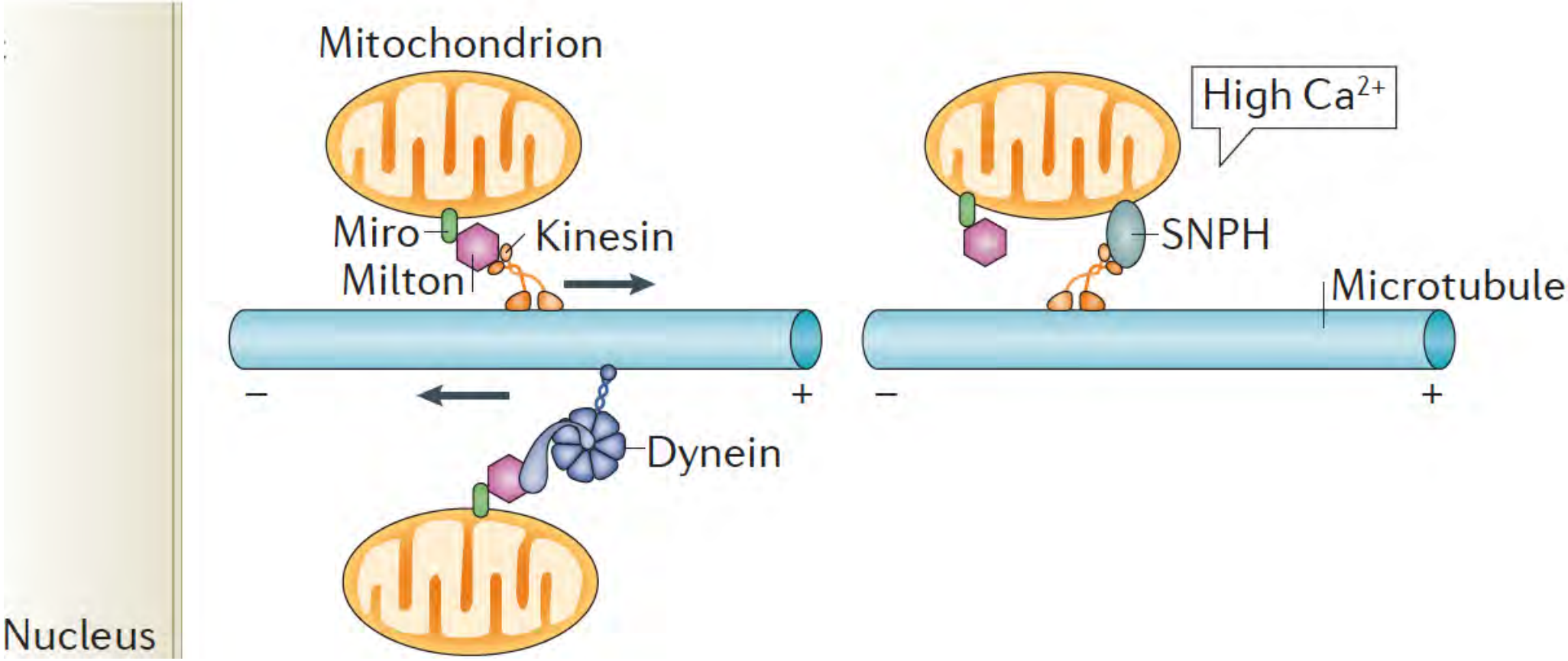
Chen and Chan (2004) Curr. Topics Dev. Biol.

Mitochondria are dynamic organelles: II. active transport



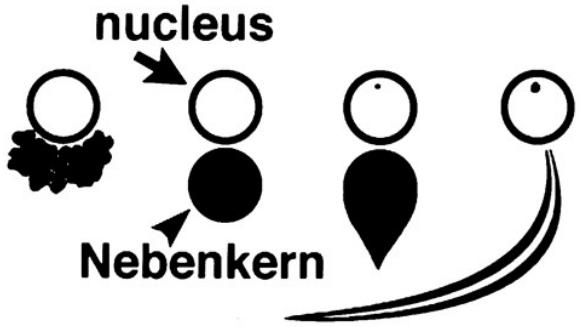


Transport of mitochondria along microtubules

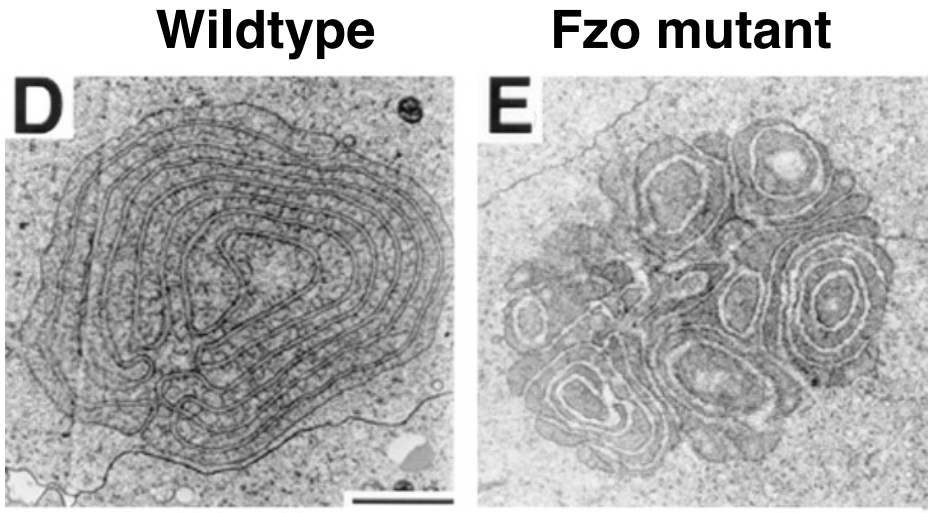


Mishra & Chan (2014) Nat Rev Mol Cell Biol

Drosophila Fzo is essential for developmentally regulated mitochondrial fusion during spermatogenesis

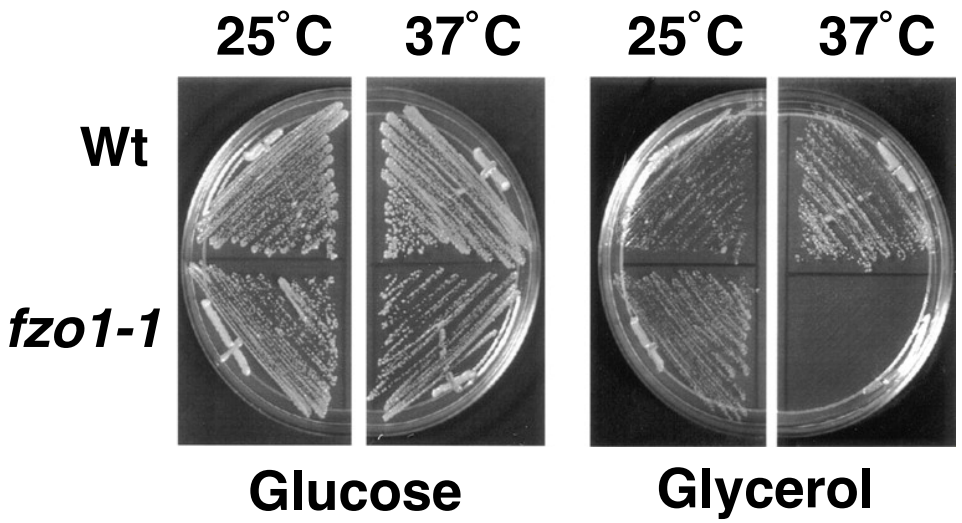


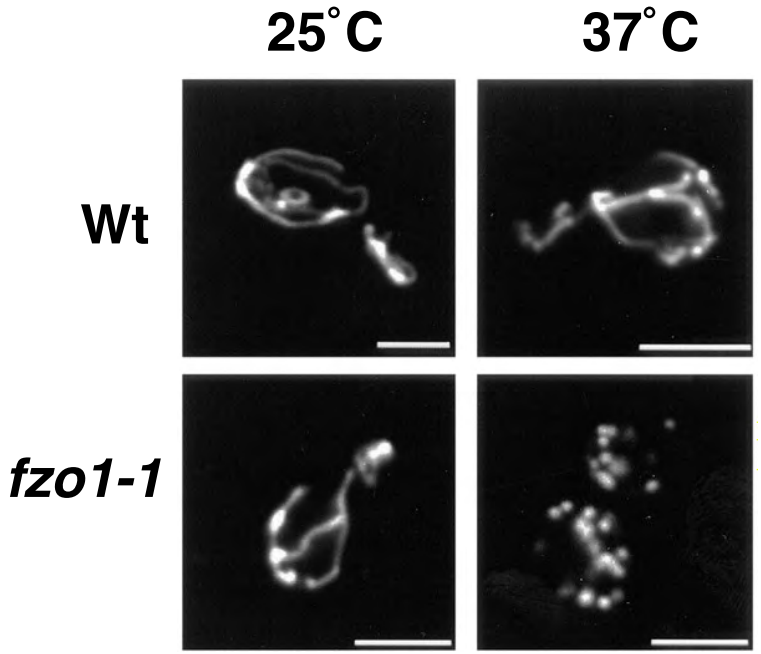
Fzo mutant was found in a collection of male sterile files.



Hales and Fuller, 1997

Yeast Fzo is essential for mitochondrial fusion and function





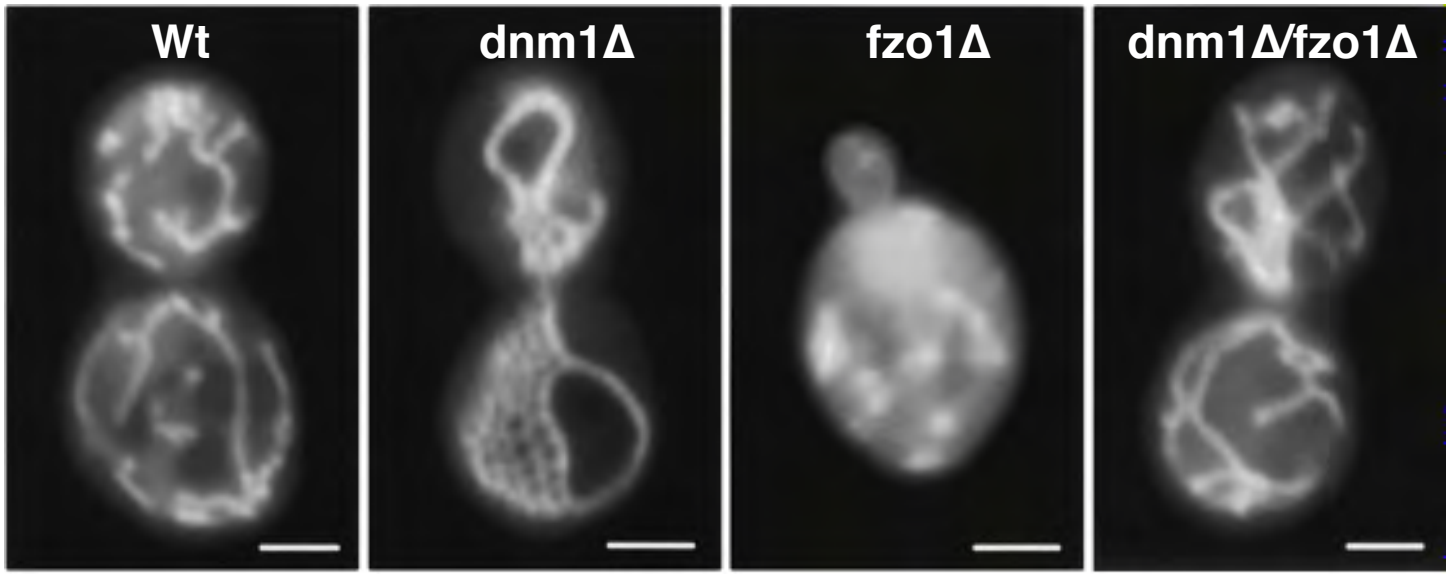
Opportunity for a genetic screen:

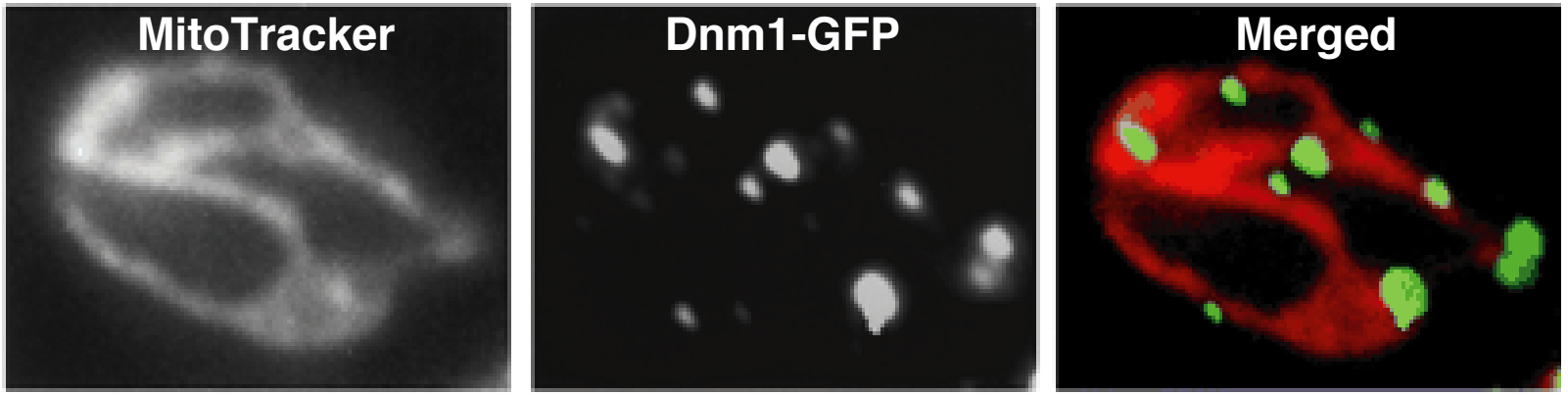
- Temperature sensitive allele with a conditional growth defect.
- Can loss of mtDNA be bypassed?

Hermann et al, 1998

Fzo and Dnm1 antagonistically regulate mitochondrial dynamics

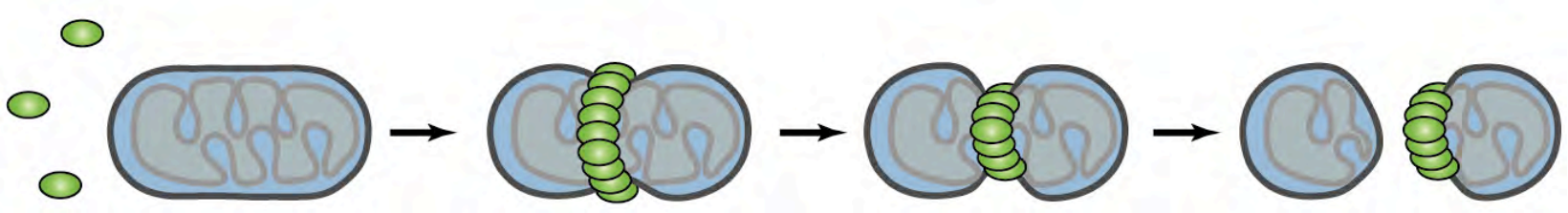
Dnm1 was found as a suppressor of the growth defect phenotype of fzo1 mutants





Sesaki & Jensen, 1999

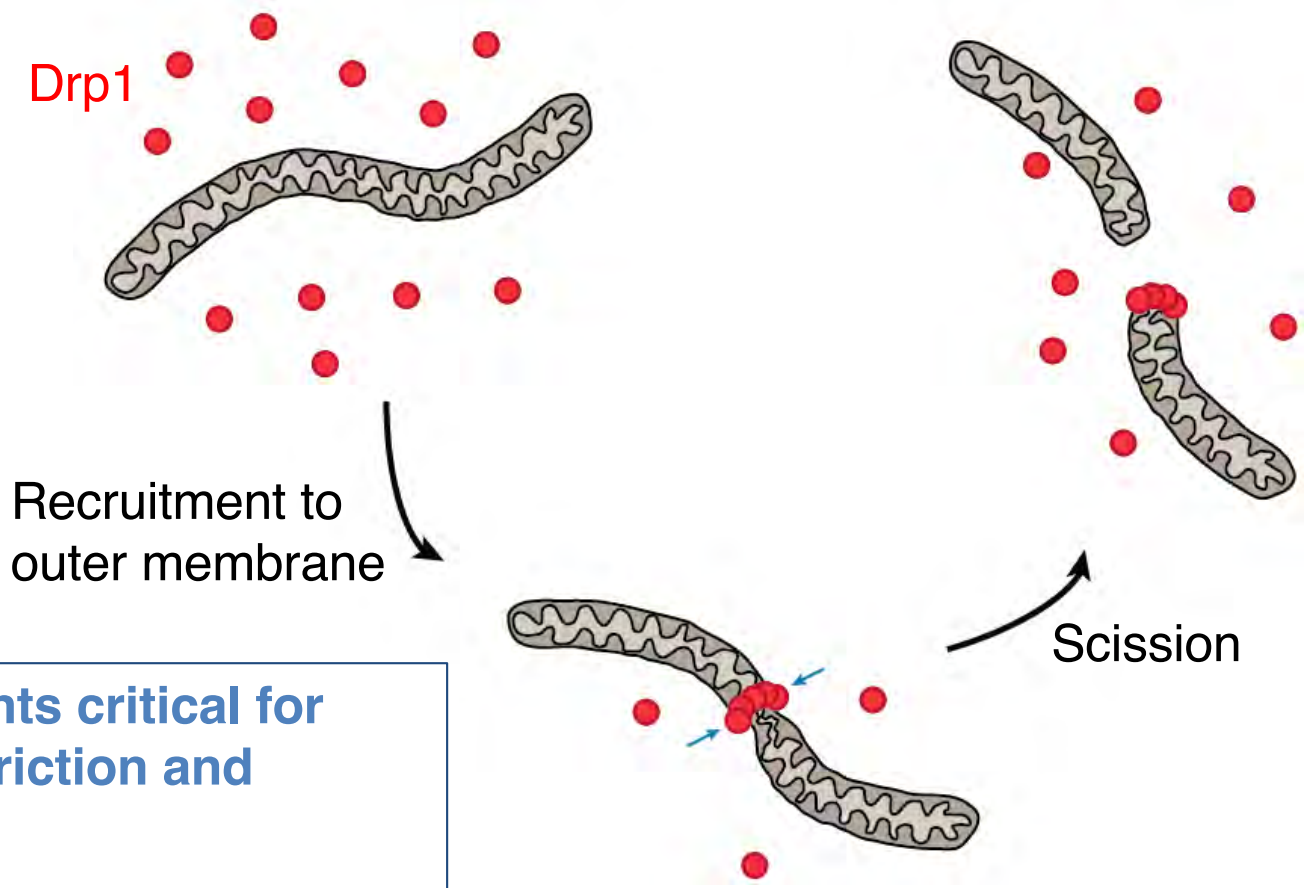
Drp1/Dnm1 constricts mitochondrial membranes to mediate fission



How does Dnm1 get recruited to mitochondria?

- Fis1 essential for Dnm1 recruitment
- Either Mdv1 or Caf4 essential for Dnm1 recruitment

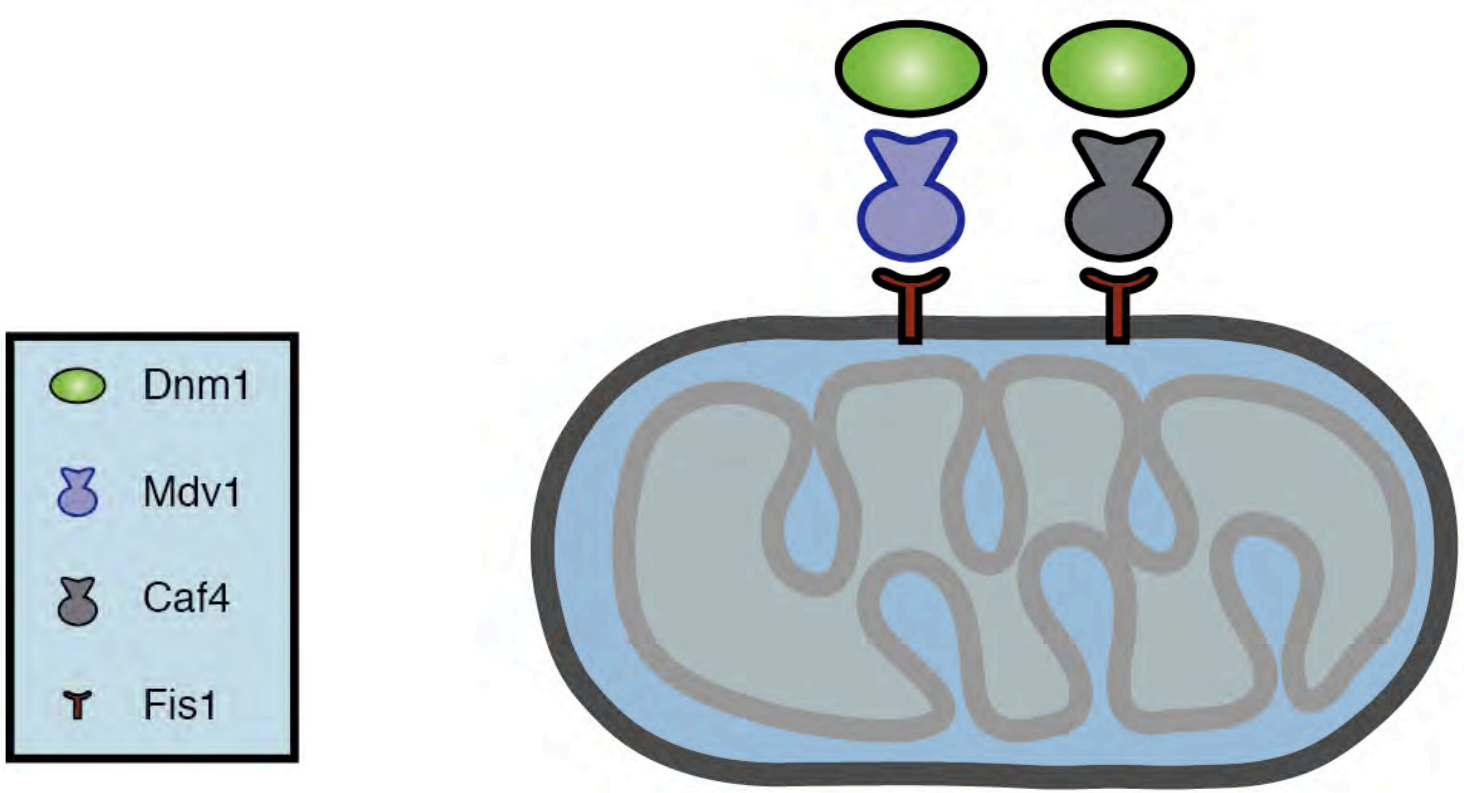
Activation of mitochondrial fission



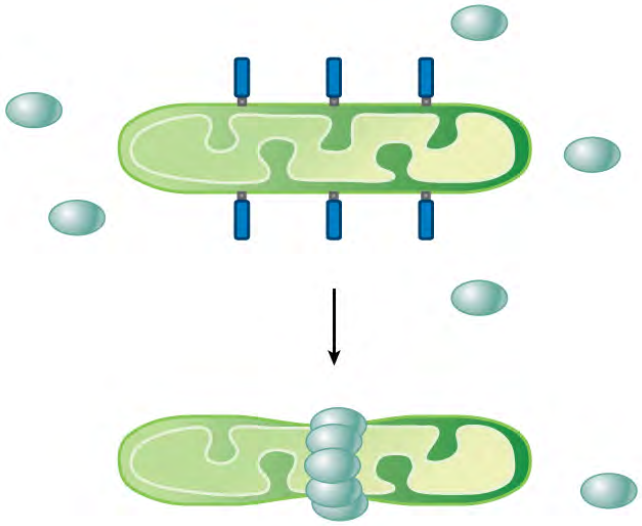
Two key events critical for tubule constriction and scission:

- Assembly of Drp1 into helical spirals
- Enhanced GTP hydrolysis

Mdv1 and Caf4 are molecular adaptors that recruit Dnm1p to mitochondria



In mammals, multiple receptors on the mitochondrial outer membrane can recruit Drp1

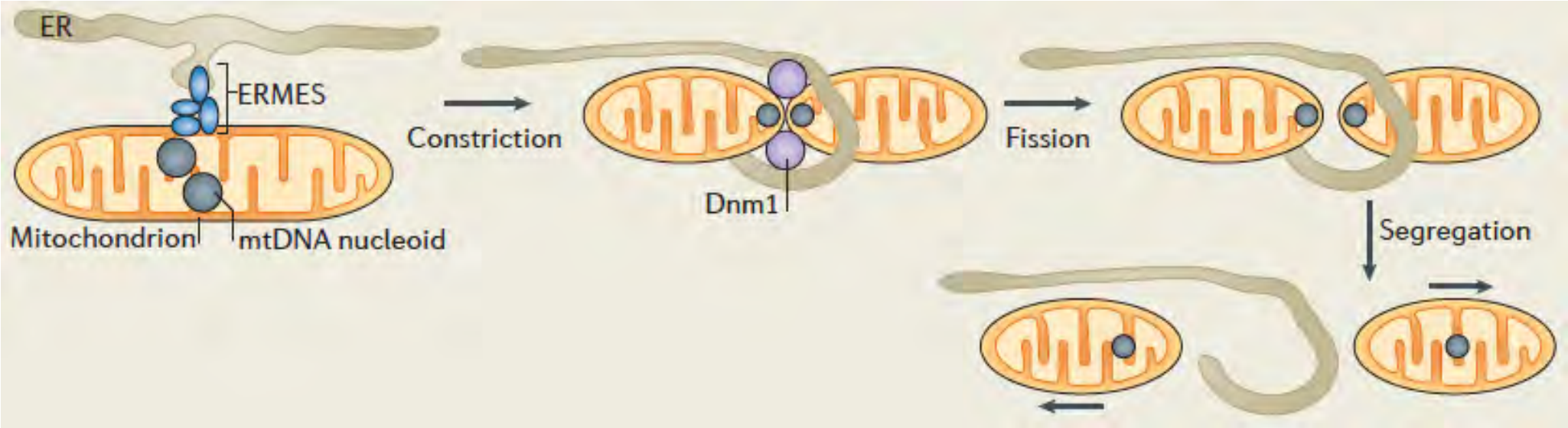


- Fis1, Mff, MiD49 and MiD51 can all recruit Drp1 to mitochondria.
- In fibroblasts, Mff and MiDs have more important roles than Fis1.

Mff: van der Bliek; MiD51: Ryan and Nister

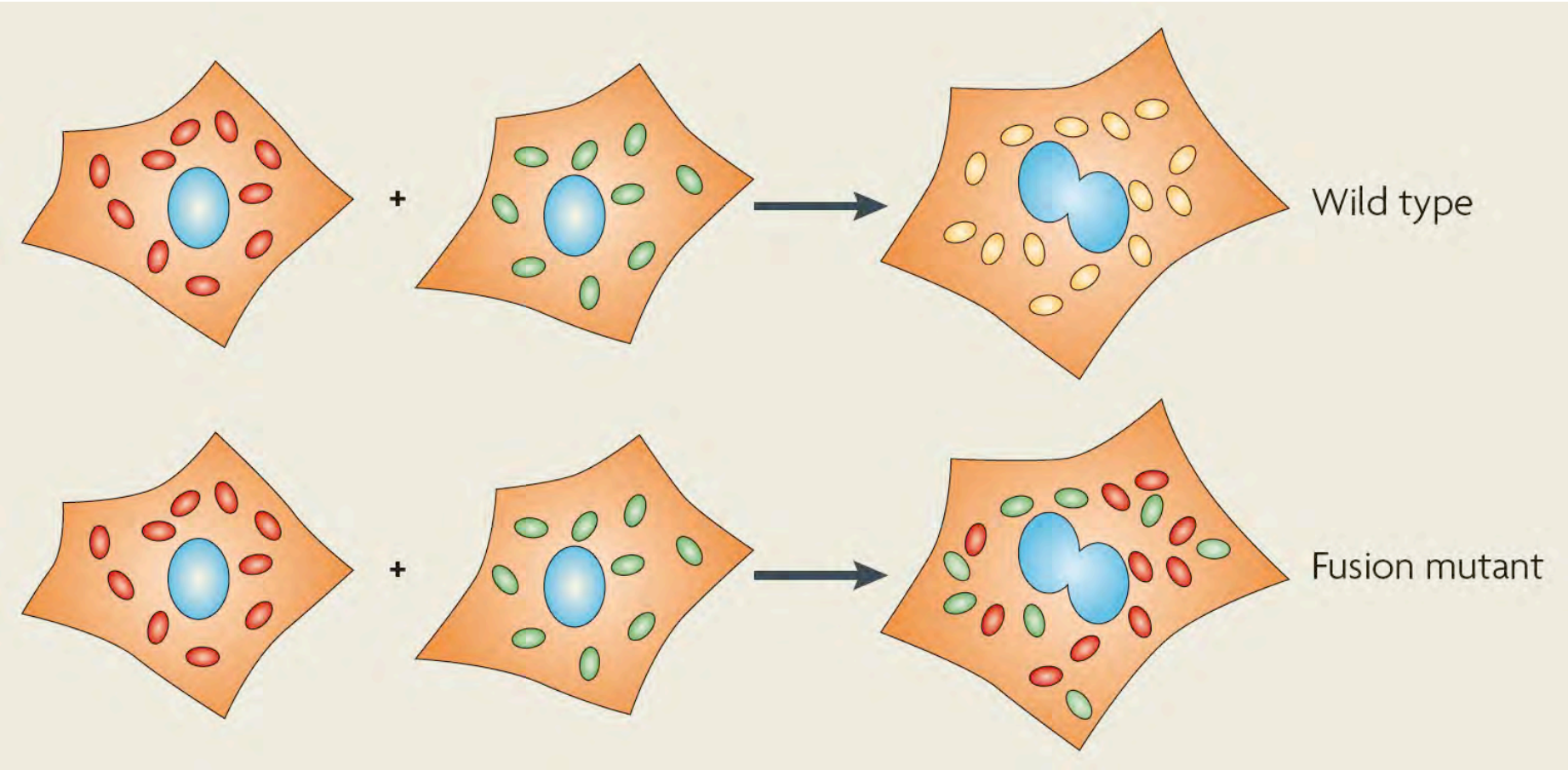
Chan (2012) Ann Rev Genet

ER interactions often accompany fission sites

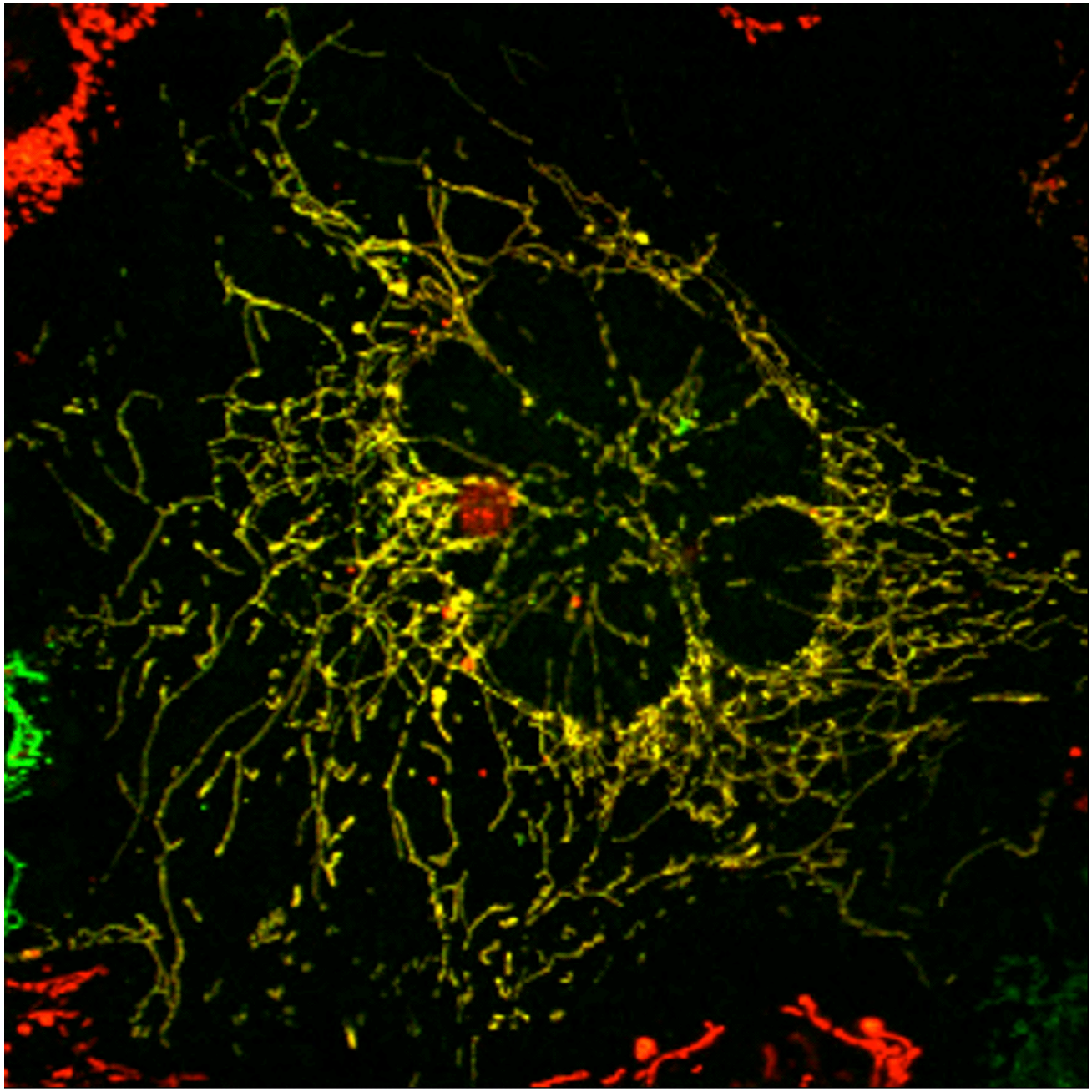


Mishra & Chan (2014) Nat Rev Mol Cell Biol

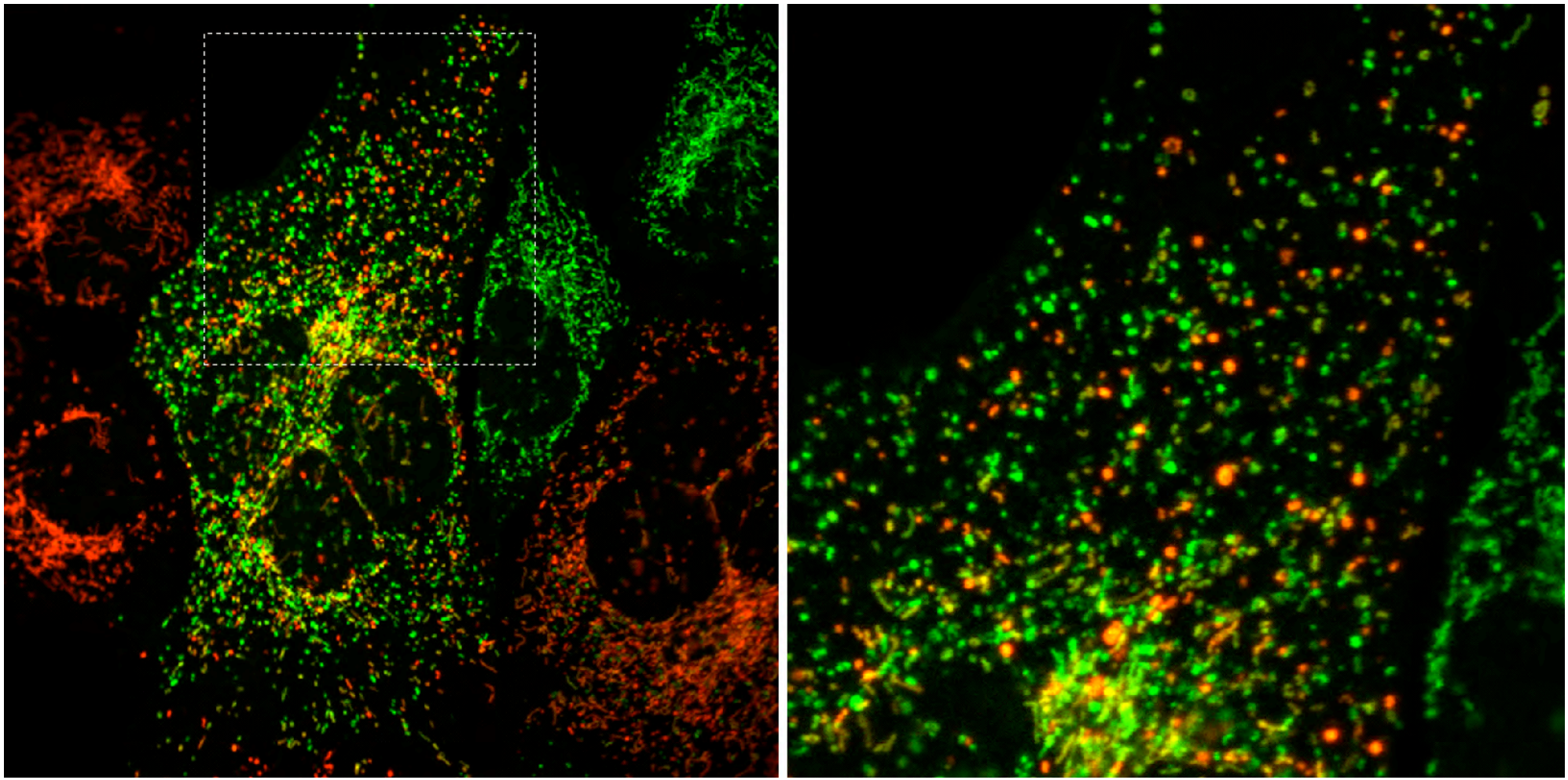
Mitochondrial fusion assay



Wildtype cells show extensive mitochondrial fusion

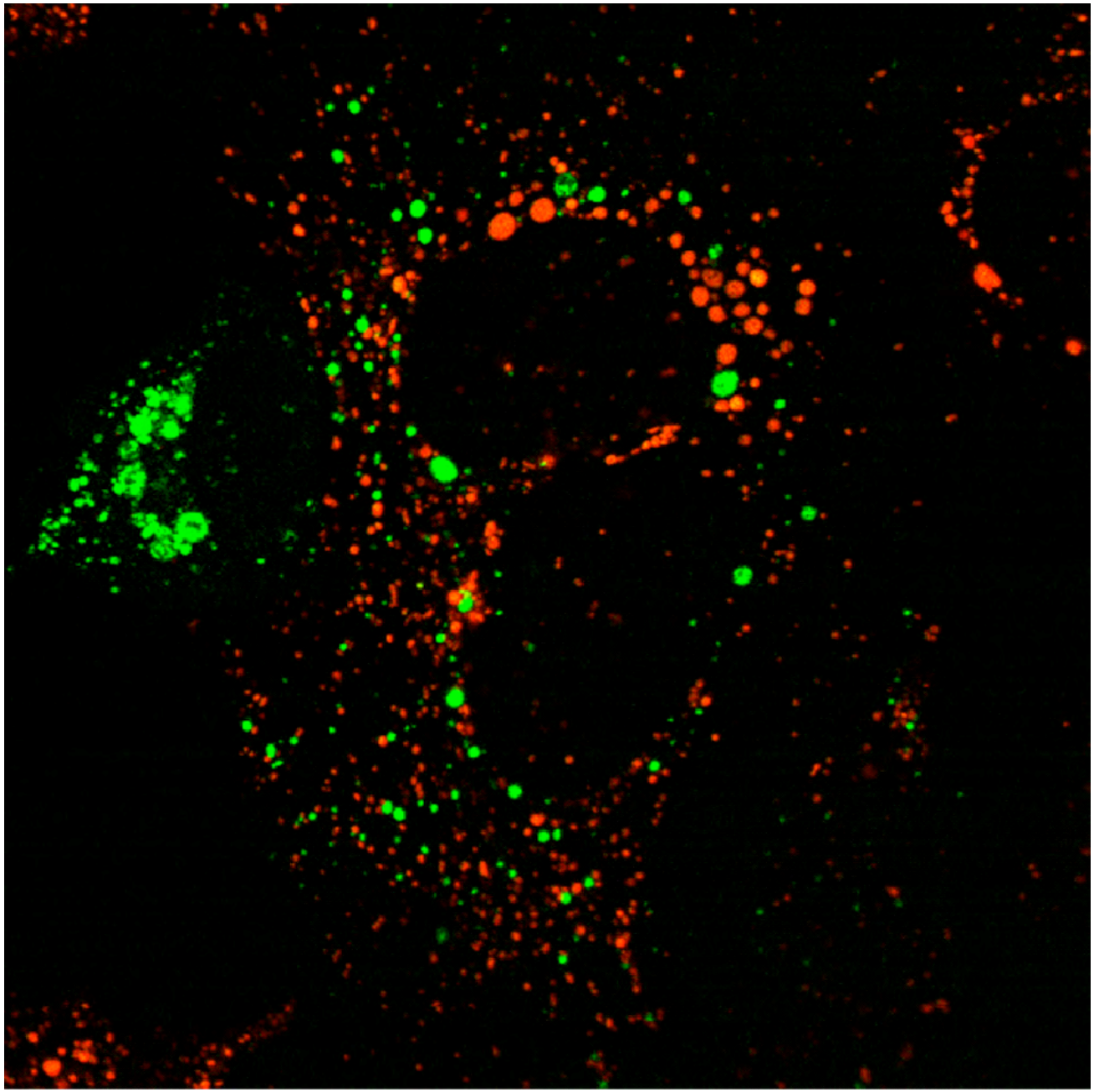


Mfn1 mutant cells have reduced mitochondrial fusion

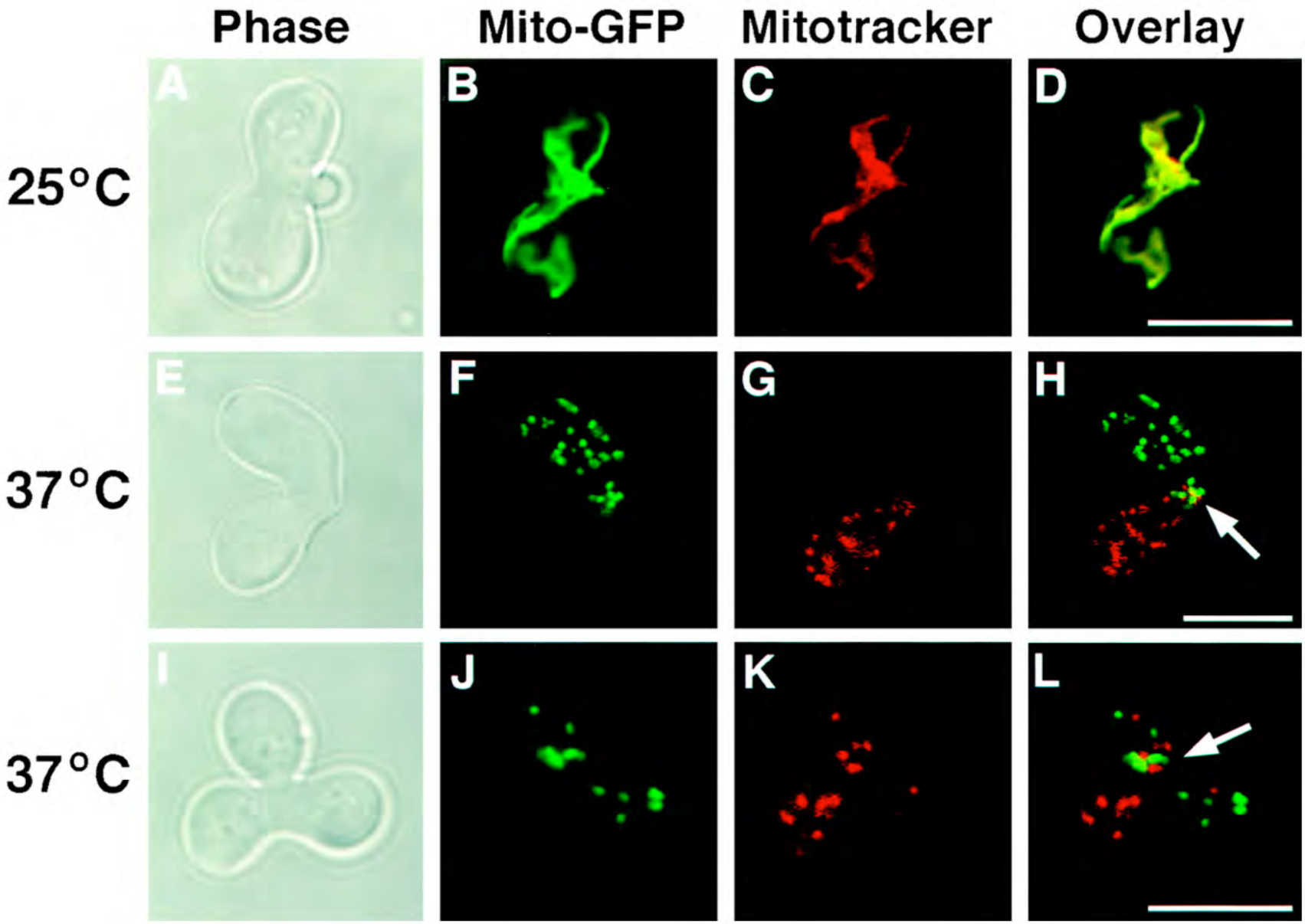


Double Mfn-null cells have no mitochondrial fusion

Mfn1 and Mfn2 are partially redundant molecules with similar biochemical functions.



Yeast mating provides an assay for mitochondrial fusion



Hermann et al. (1998) JCB

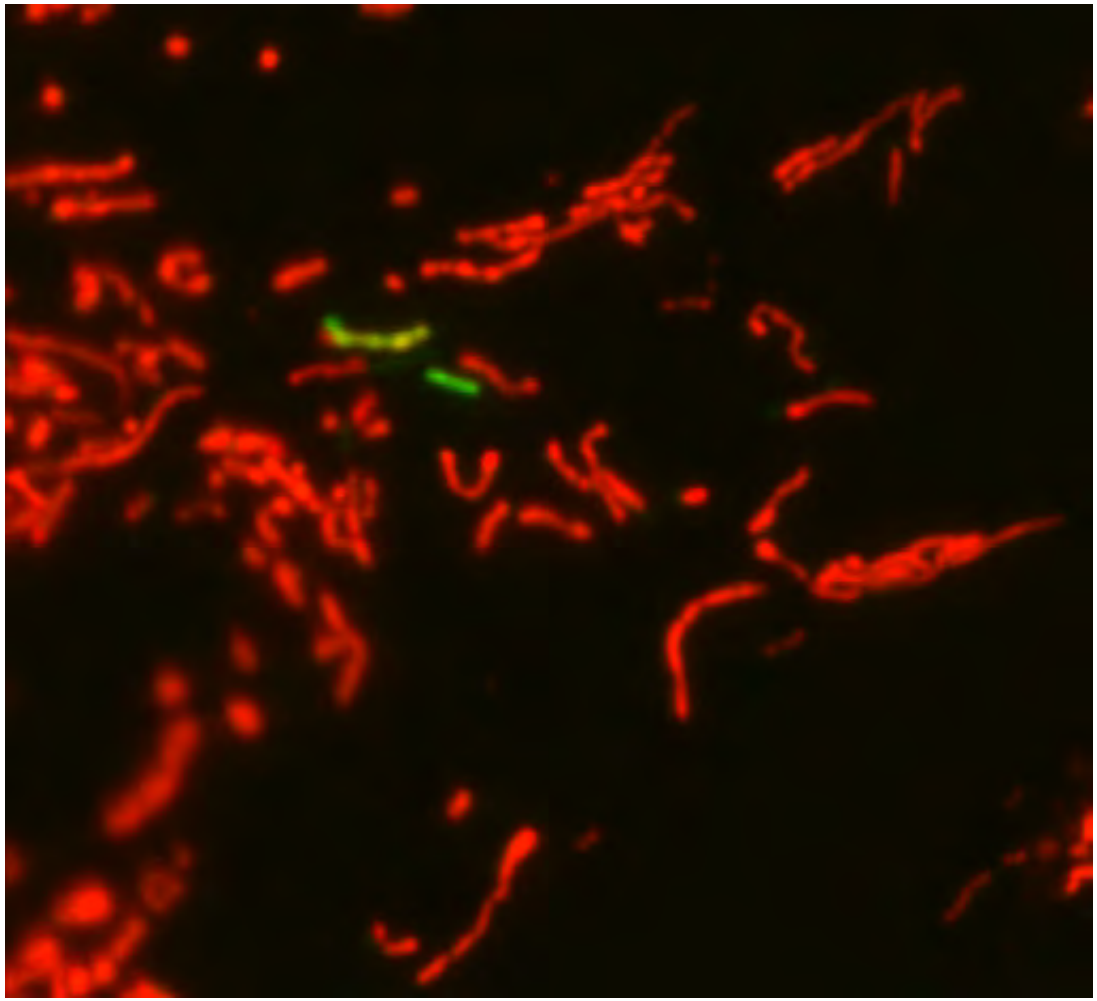
PA-GFP allows real-time analysis of mitochondrial fusion

Mito-DsRed

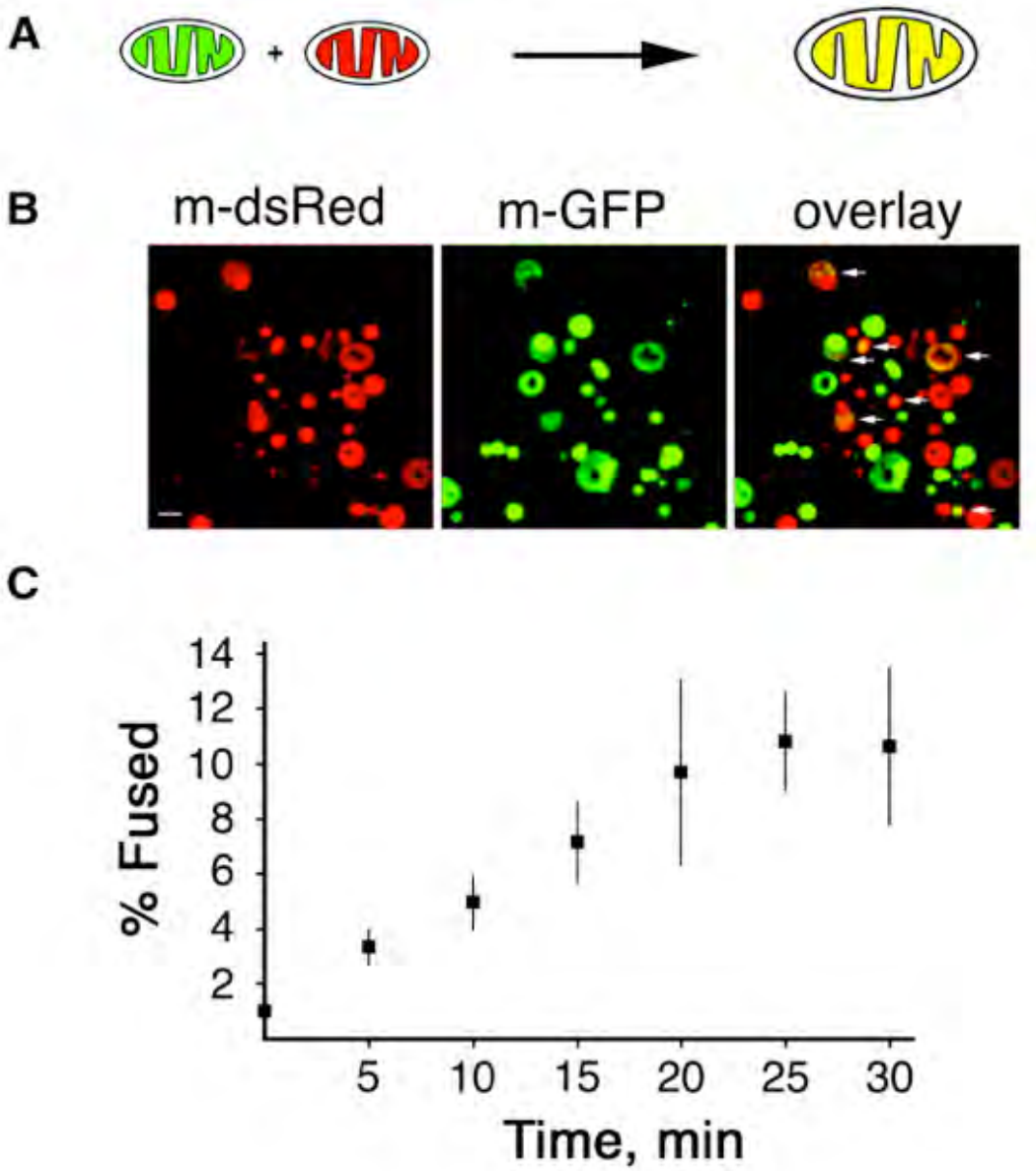
Mito-PA-GFP

gift of R. Youle

PA-GFP by J. Lippincott-Schwartz

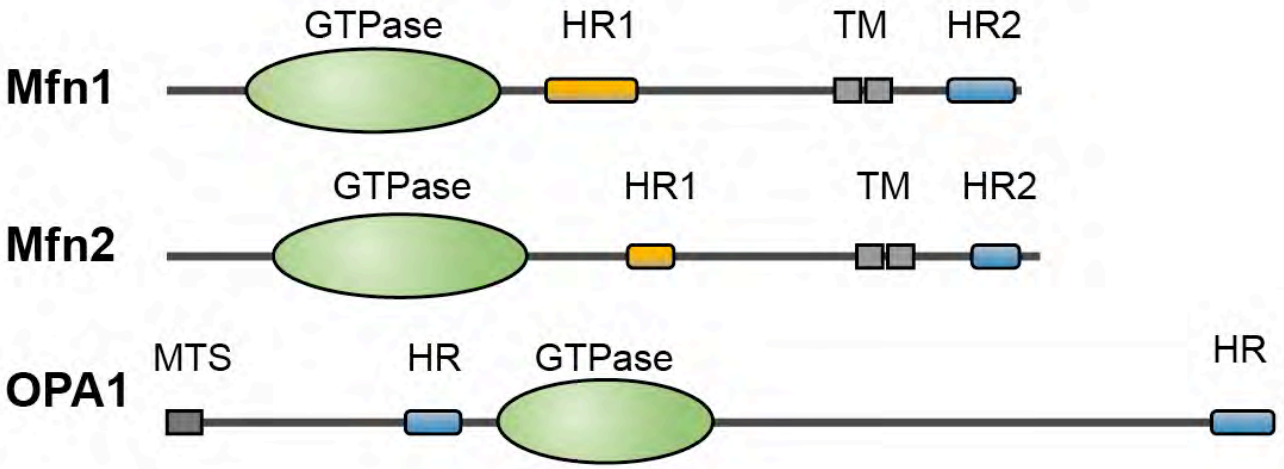


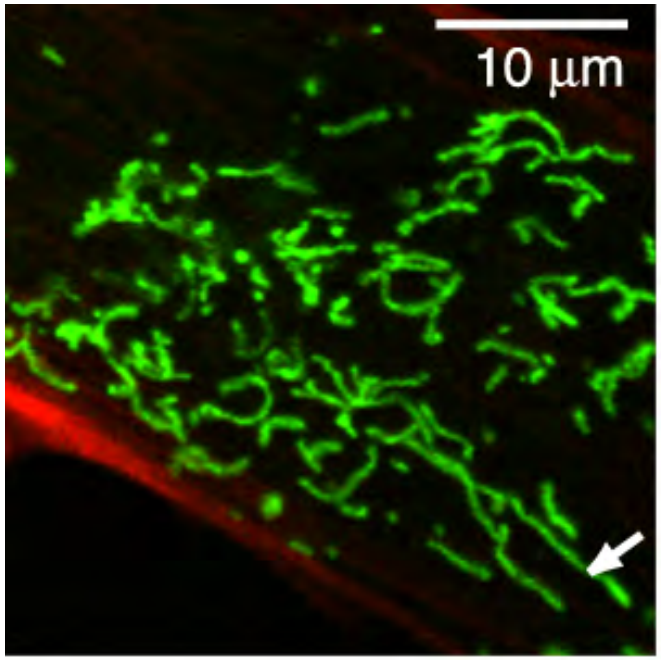
An *in vitro* mitochondrial fusion assay in yeast

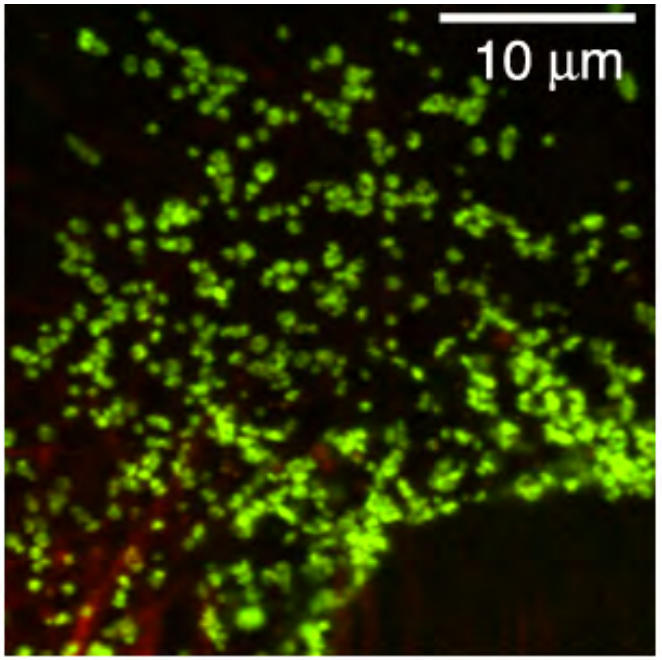


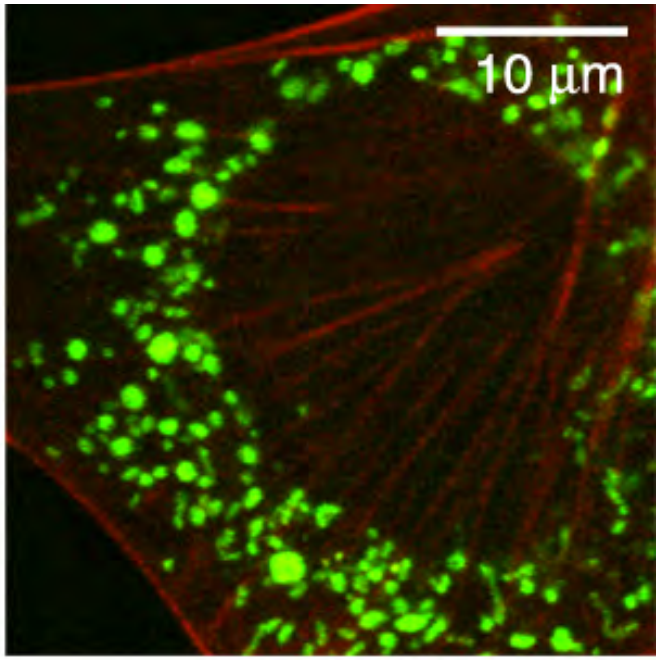
Meeusen et al. (2004) Science

Molecules necessary for mitochondrial fusion









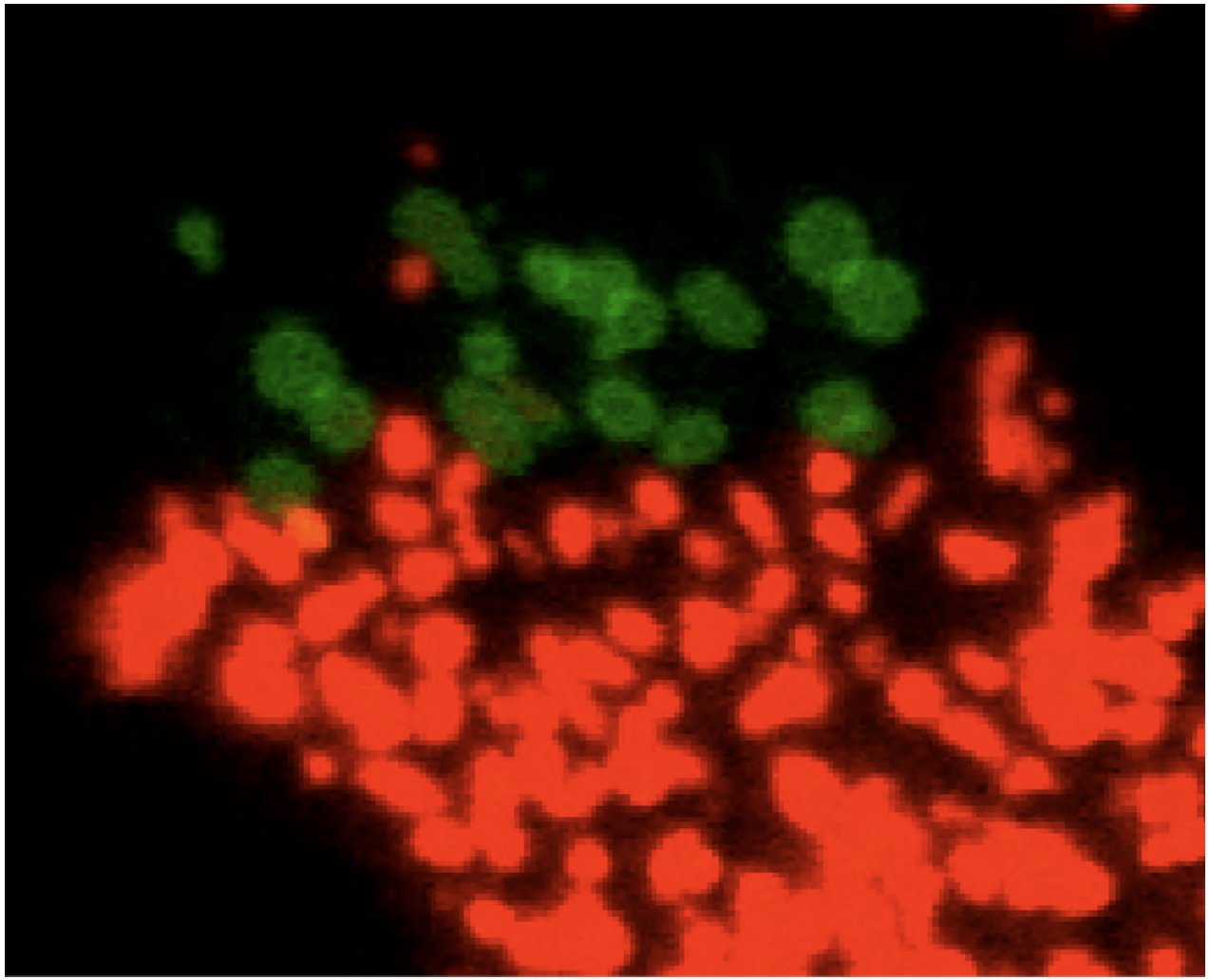
Fusion and fission control mitochondrial size, shape, and number.

Chen et al (2003) J. Cell Biol

Tracking membrane fusion with mitochondrial PA-GFP

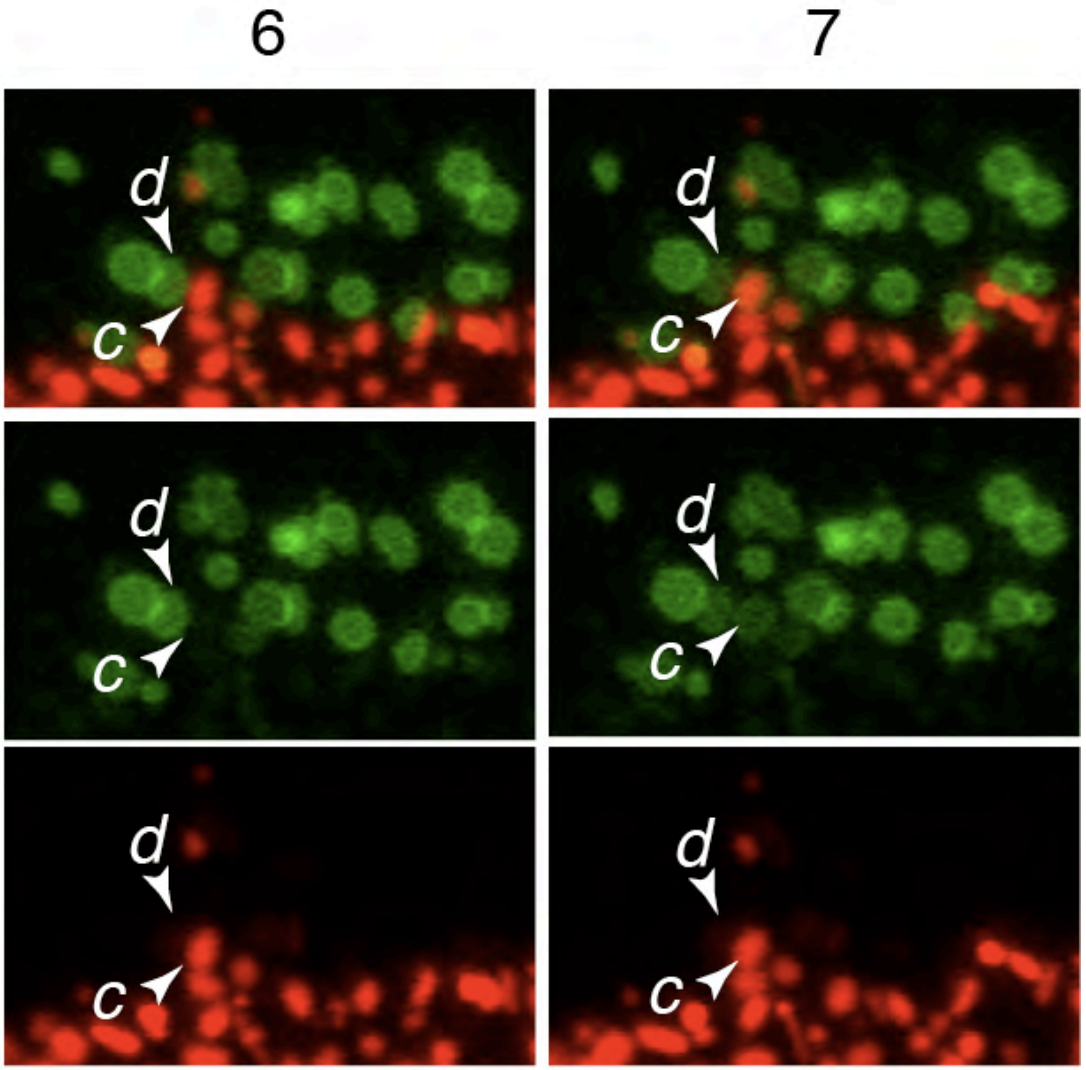
To distinguish between full fusion vs outer membrane fusion:

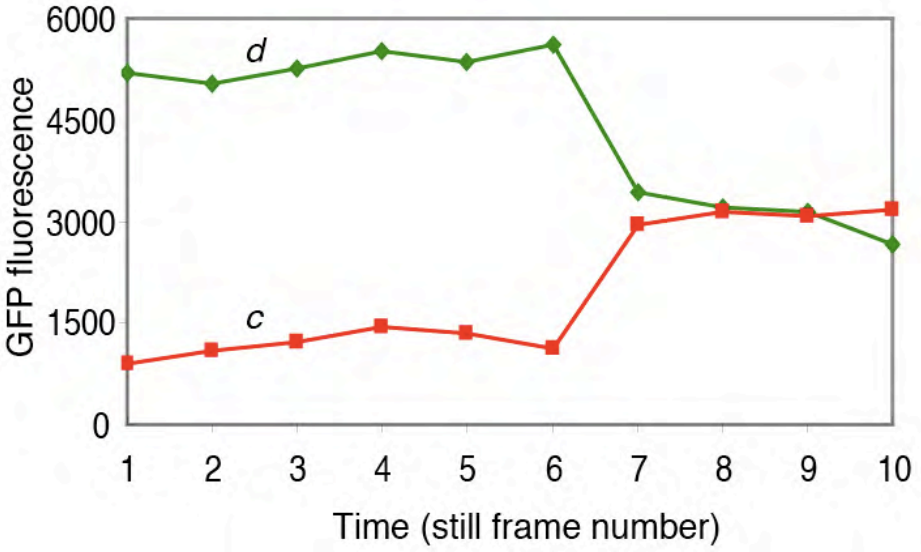
PA-GFP can be targeted to the mitochondrial matrix or the outer membrane.



Zhiyin Song

Visualizing outer membrane fusion events in OPA1-null cells

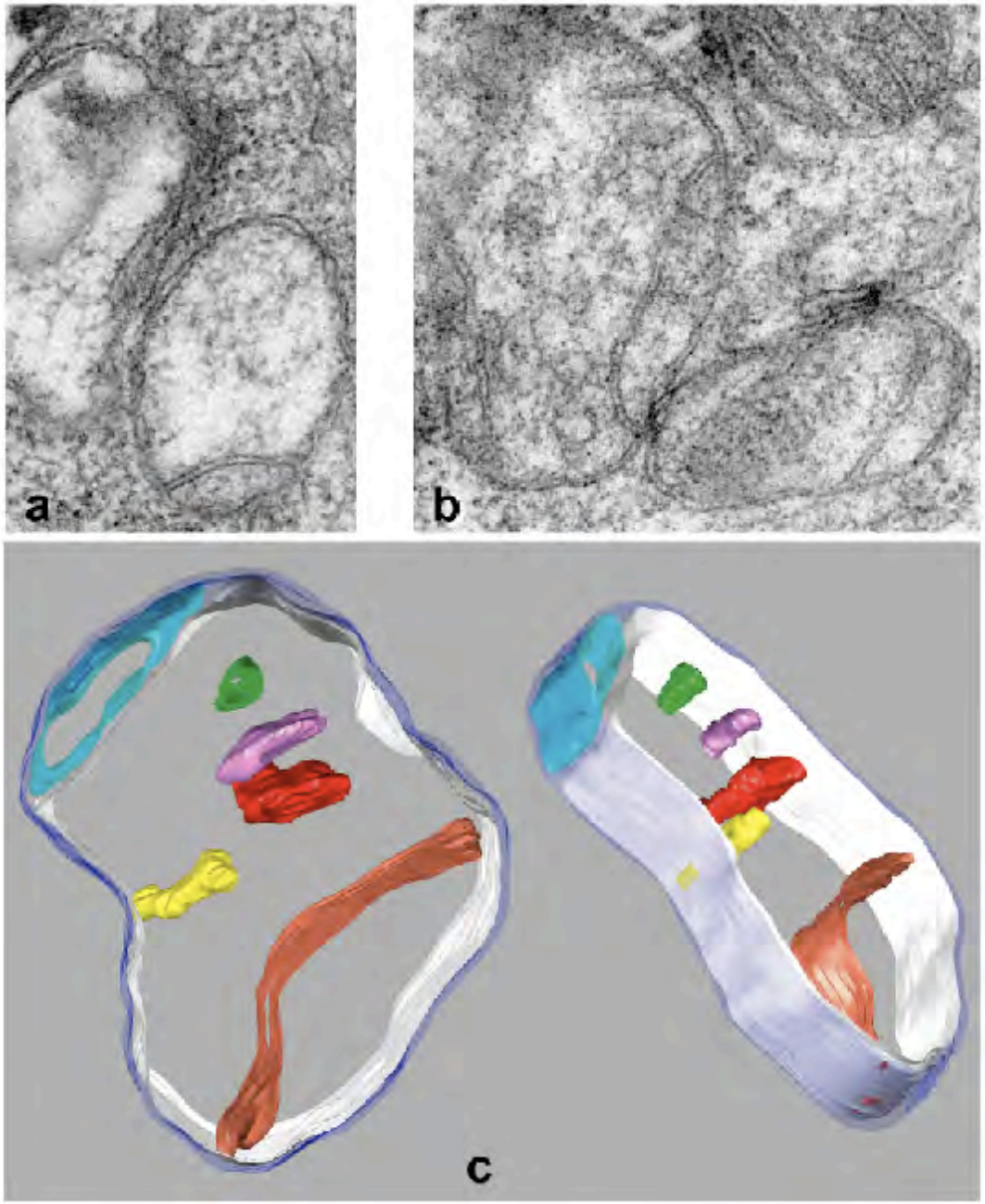




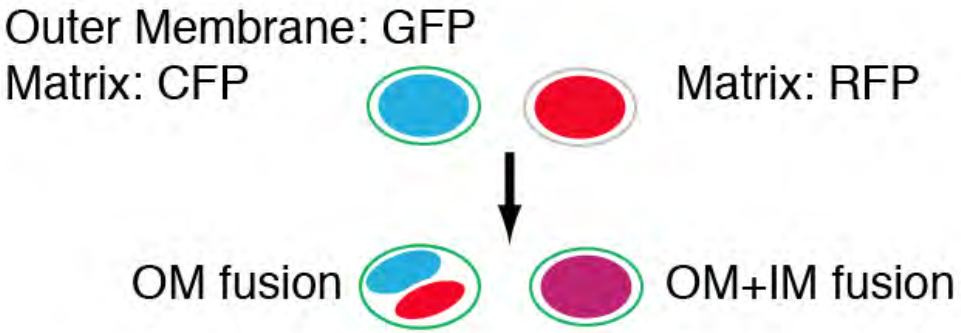
- Stepwise transfer of PA-GFP
- No transfer of DsRed
- OM fusion uncoupled from matrix mixing

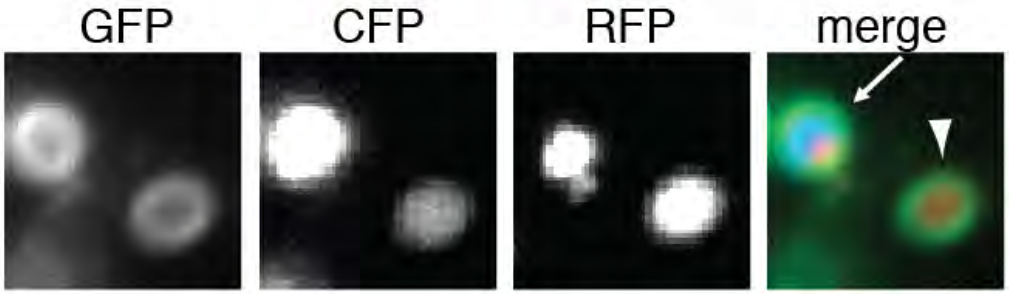
Song et al. (2009) MBC

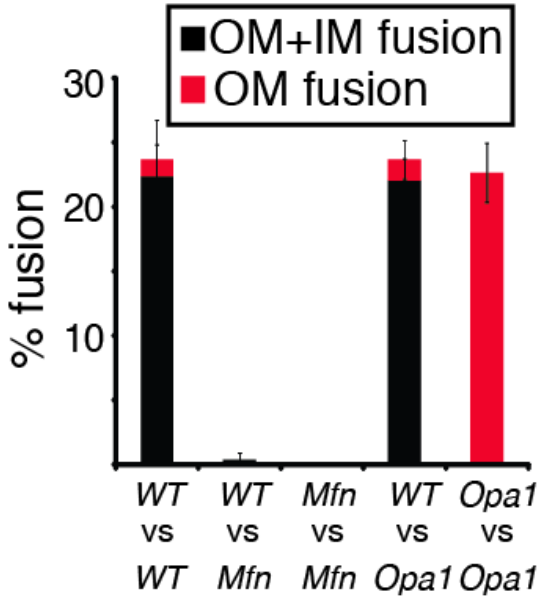
Multiple matrices in mitochondria of OPA1-null cells



An *in vitro* assay to distinguish outer membrane versus inner membrane fusion





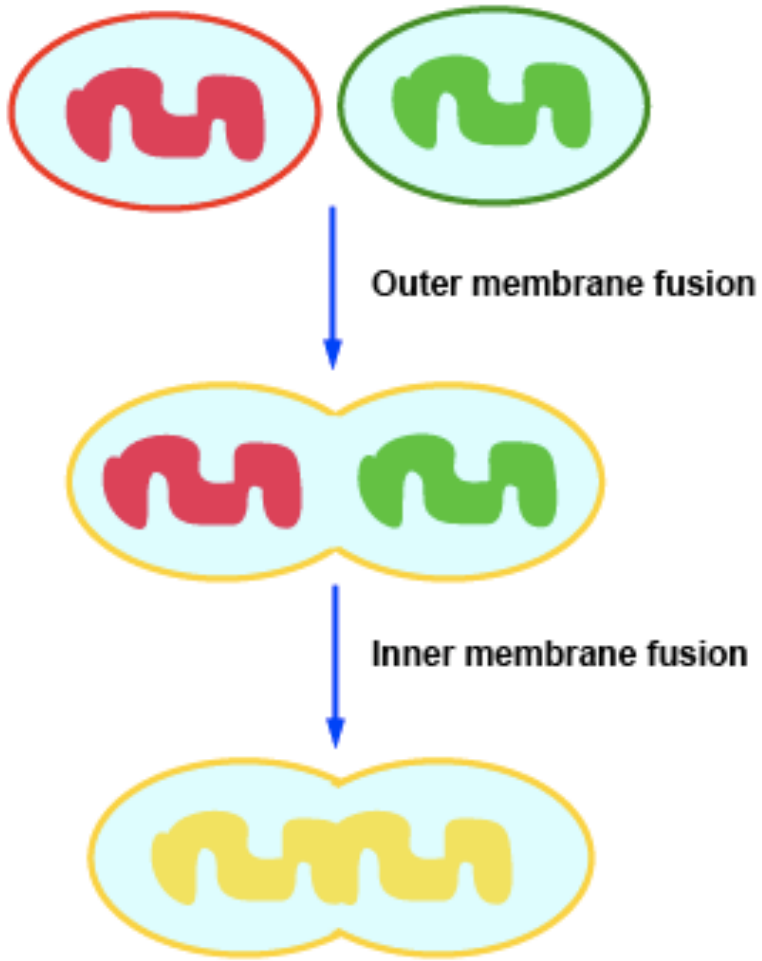


- OM fusion requires mitofusins
- IM fusion requires OPA1
- Respiratory substrates stimulate IM fusion.

Mitofusins and OPA1 act at distinct steps in mitochondrial fusion

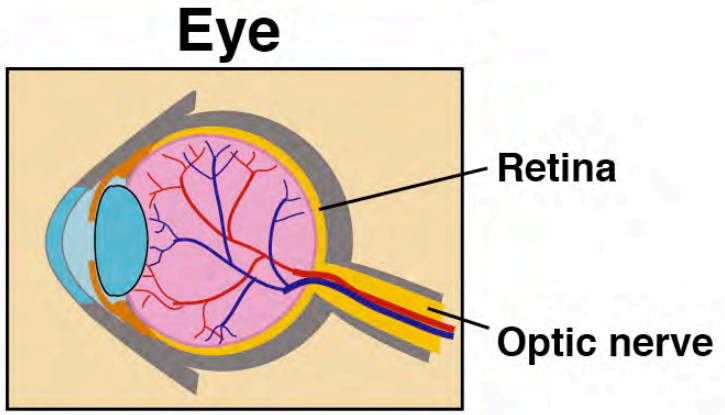
• Mitofusins are involved in outer membrane fusion, consistent with a role in mitochondrial tethering.

• OPA1 is only required for inner membrane fusion.

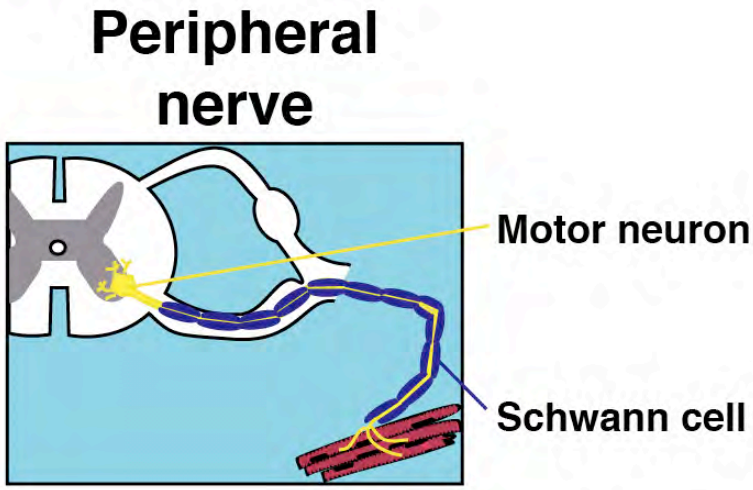


Perturbations in mitochondrial dynamics cause neurodegenerative disease

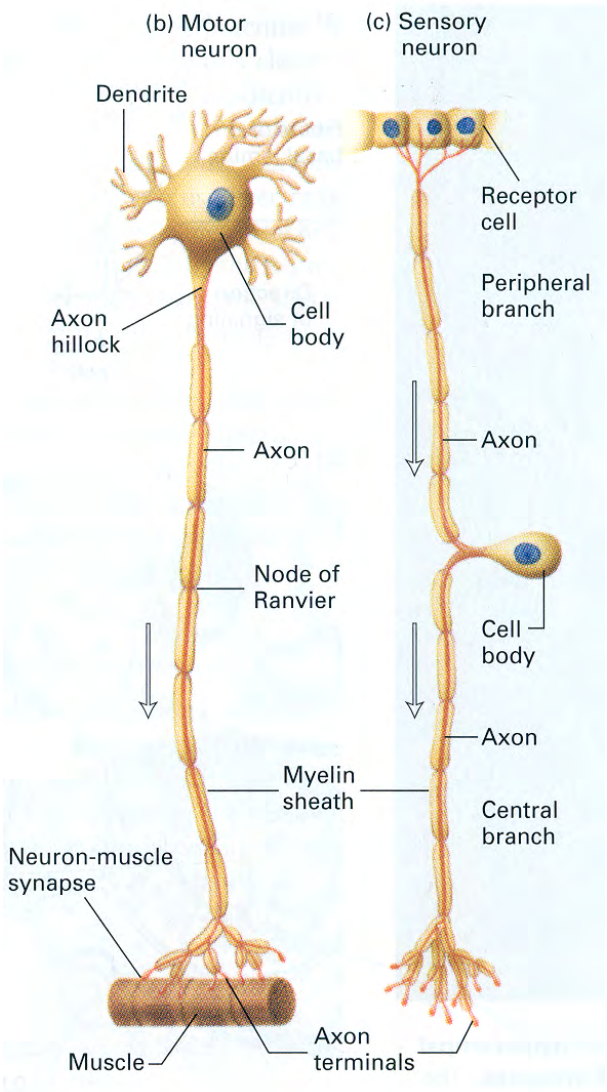
Mutations in OPA1 cause **dominant optic atrophy**
- most common inherited optic neuropathy
- degeneration of retinal ganglion cells



Mutations in Mfn2 cause **Charcot-Marie-Tooth disease 2A**
- neuropathy of long peripheral nerves
- degeneration of axons (versus demyelination)



Charcot-Marie-Tooth Disease (CMT)
Hereditary Motor Sensory Neuropathies (HMSN)



(Mol. Cell Biol., 4th Ed.)

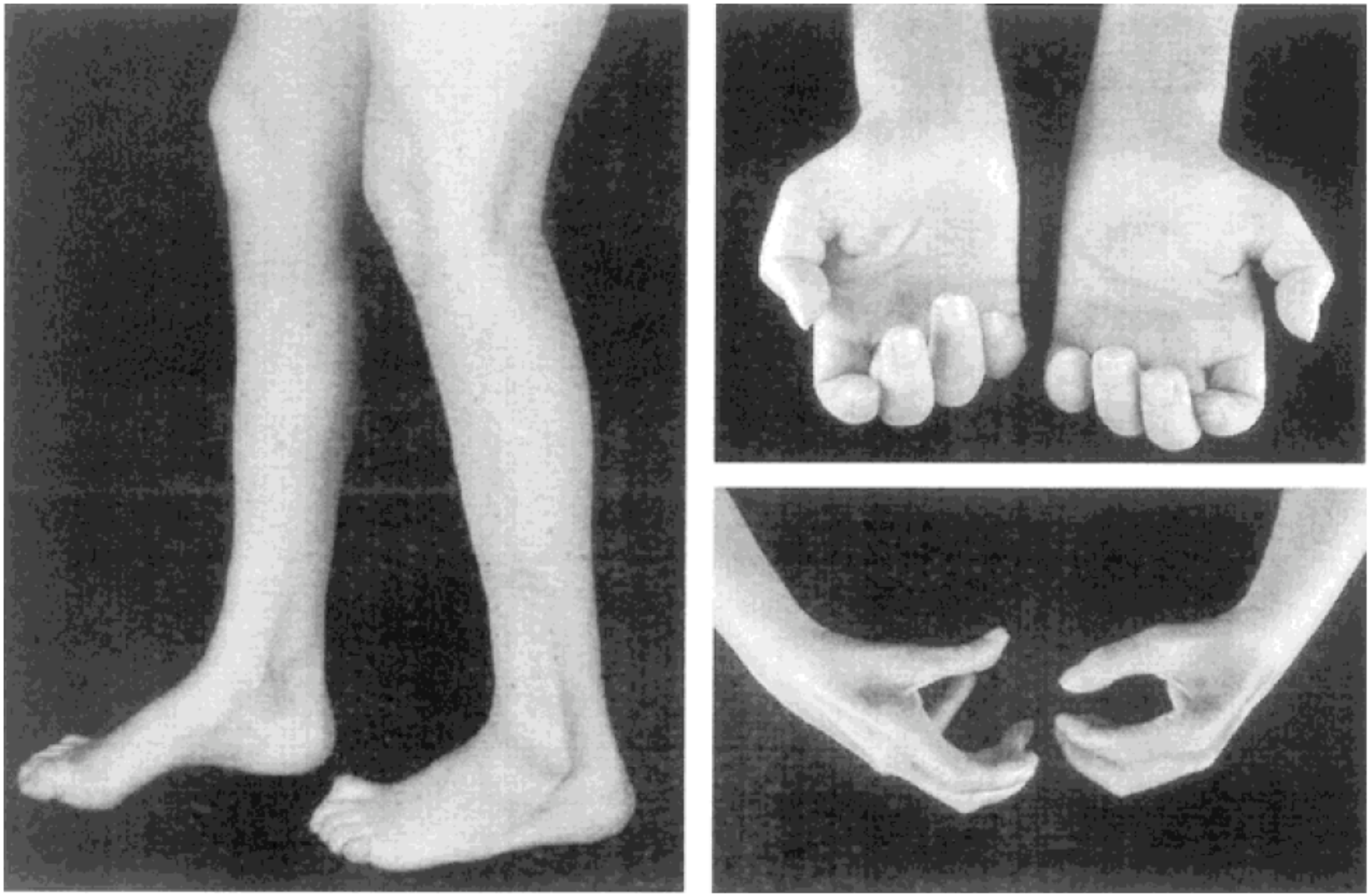
Most common inherited neuropathy, ~1/2,500

Peripheral nerve dysfunction (not affecting CNS)

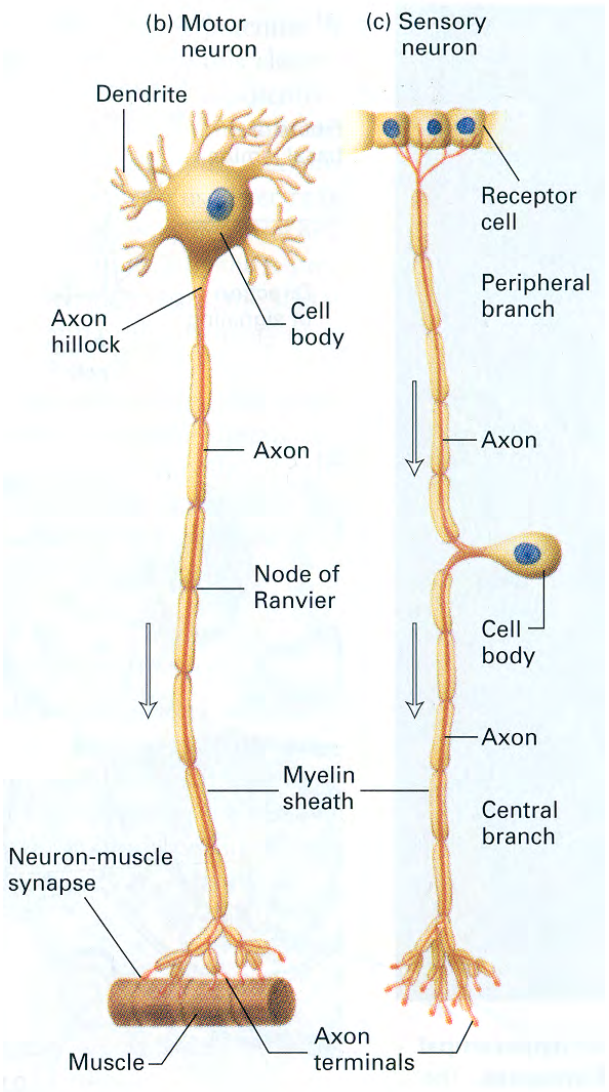
<u>Symptoms</u>

- distal muscle weakness and atrophy, primarily foot and peroneal muscles; hands and forearms later in life
- foot deformities (claw toes, drop foot) leading to gait impairment
- distal sensory defects
- age of onset variable, most commonly 20-30 years old
- normal lifespan

Distal limb defects in CMT disease



Charcot-Marie-Tooth Disease (CMT)
Hereditary Motor Sensory Neuropathies (HMSN)



(Mol. Cell Biol., 4th Ed.)

Demyelinating and axonal subtypes

CMT1: demyelinating disorder of peripheral nerves
- decreased NCV (nerve conduction velocity)
- onion bulb myelin structures

CMT2: axonal defect
- normal NCV
- myelination normal
- Type 2A caused by Mfn2 mutations

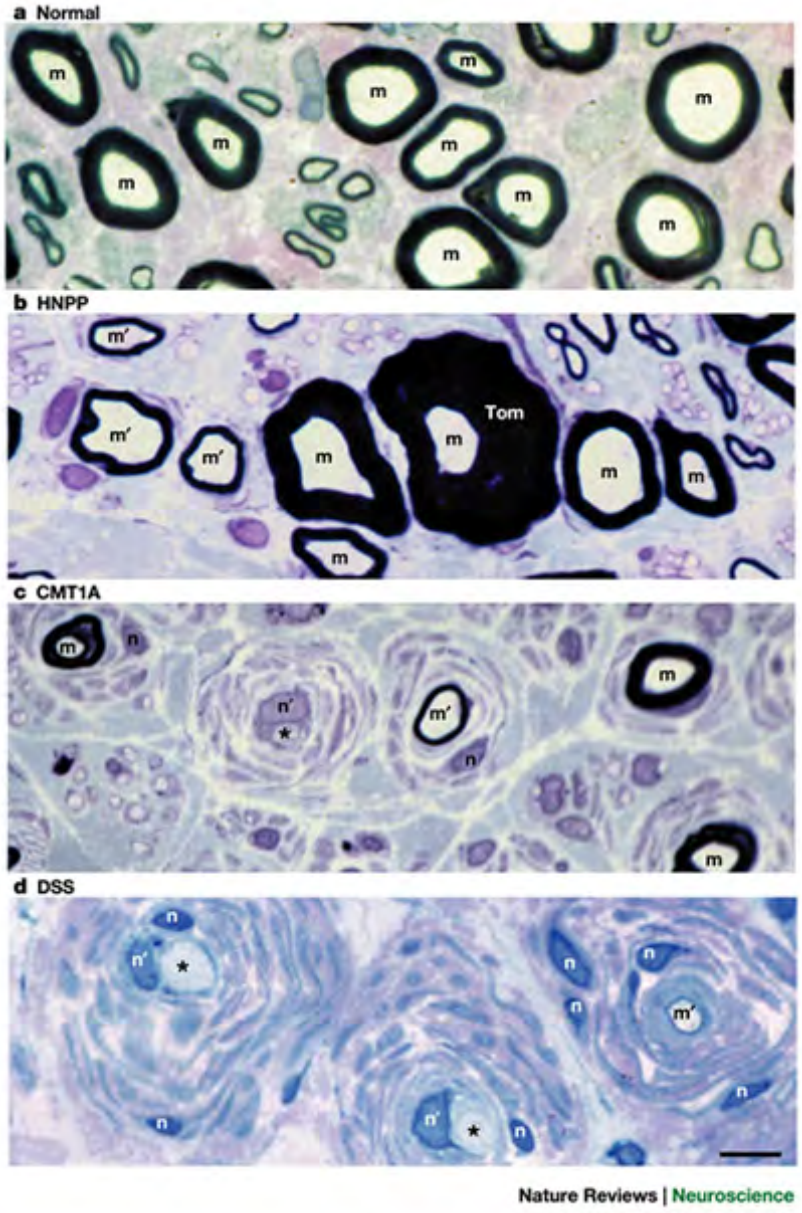
Both types have selective loss of long peripheral axons

Nerve defects in CMT patients

Hereditary neuoropathy with liability to pressure palsies

CMT1A: mutation in PMP22, note thinning in myelin, supernumery Schwann cell processes and nuclei; onion bulbs

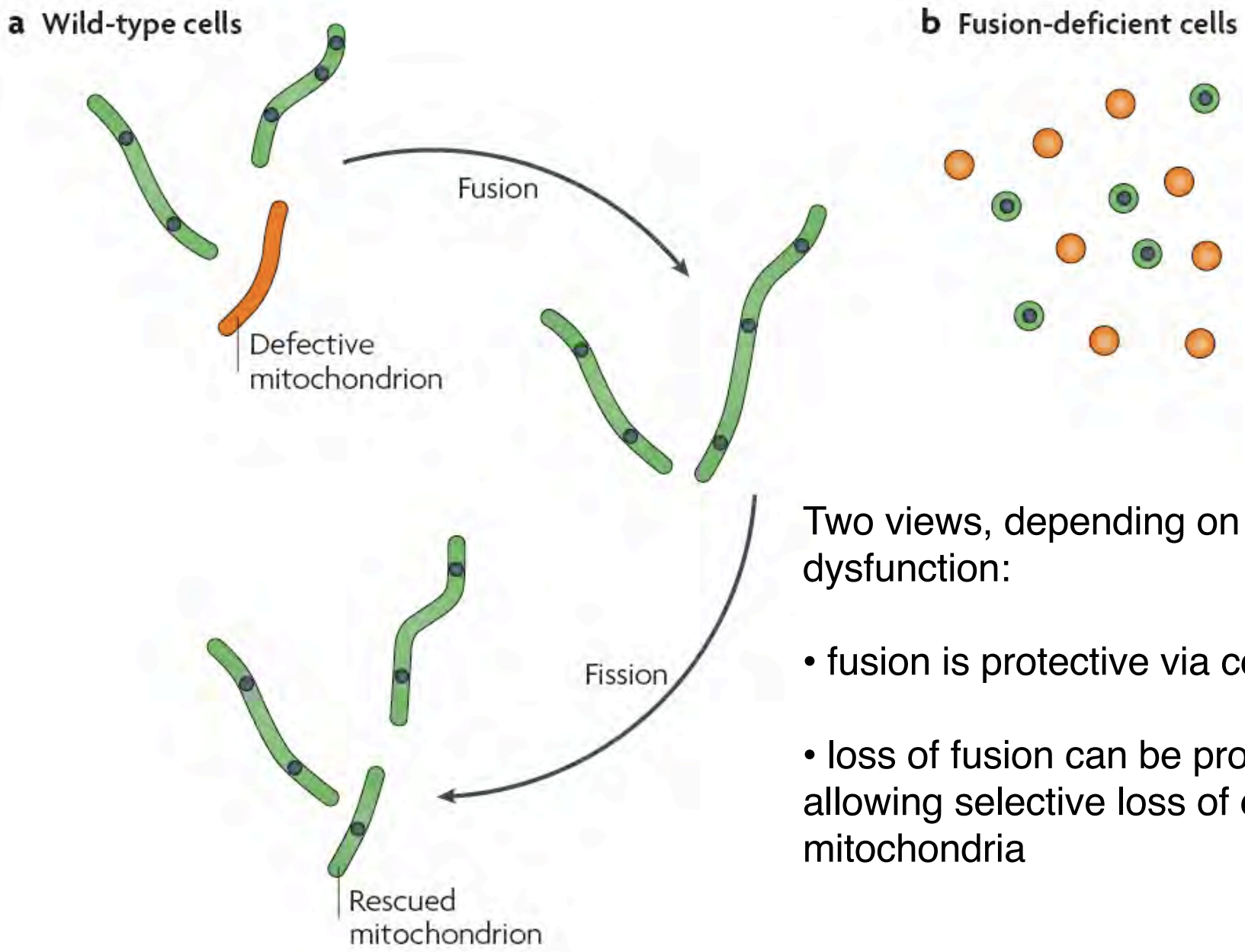
Dejerine-Sottas syndrome



M=myelinated axons m'=thinly myelinated axons

Suter & Scherer (2003) Nature Neuroscience

Fusion enables cooperation between mitochondria



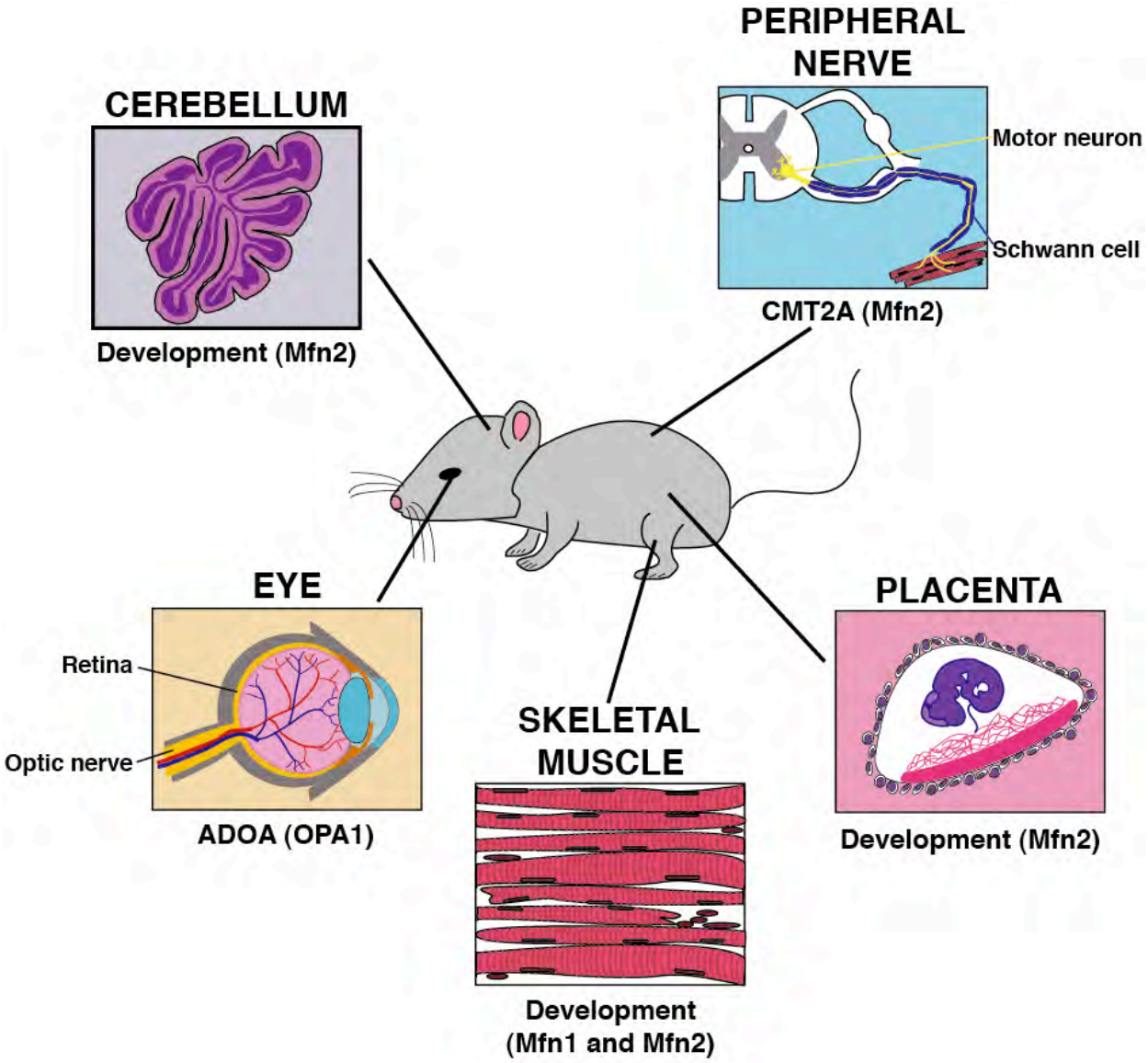
Two views, depending on extent of dysfunction:

- fusion is protective via content mixing
- loss of fusion can be protective by allowing selective loss of dysfunctional mitochondria

Detmer and Chan (2007) Nature Reviews

Mitochondrial fusion is important in many tissues

Fusion:



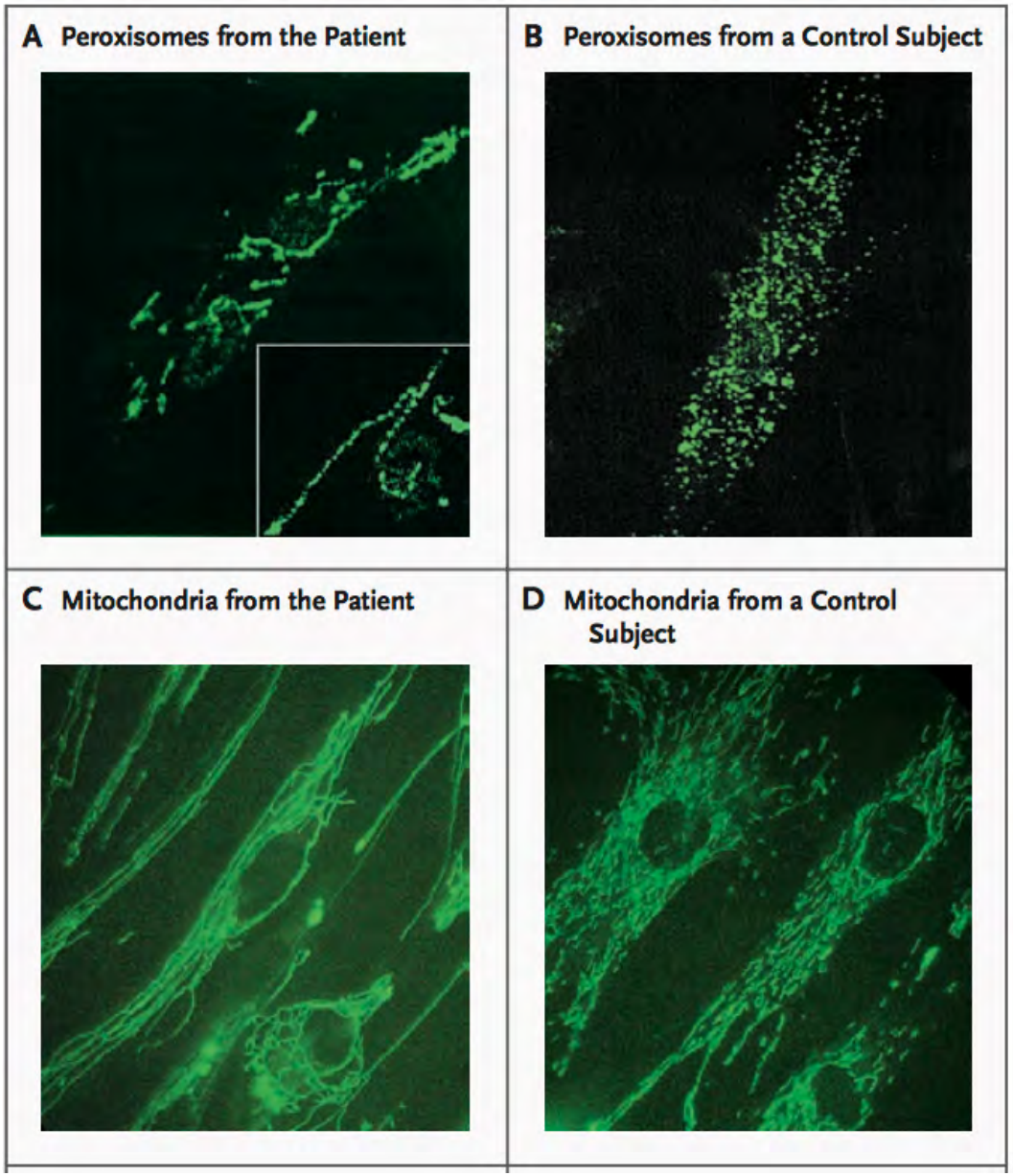
Links to Huntington's, Parkinson's, and Alzheimer's disease

Chen and Chan (2005) Hum. Mol. Genet.

Mitochondrial fission is also important in many tissues

- Neonatal lethality in newborn girl (1 month)
- Microcephaly, poor feeding, hypotonia, no tendon reflexes, multiple neurological signs
- MRI: abnormal brain structure, dysmyelination
- Elevated lactate
- Skin fibroblasts had elongated mitochondria and peroxisomes
- Heterozygous Drp1 A395D mutation (in middle domain), dominant-negative mode of action; tetramers formed but had impaired higher order assembly.

- Neuronal defects in Drp1 knockout mice



Waterham et al (2007) N Engl J Med