Regulation of Cellular Energy: mitochondria and autophagy

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Cell Biology
Mitochondria contain two lipid bilayers.

1. Reminder of mitochondria structure.
2. Generates ATP.
3. Other roles.
4. Several prominent diseases thought to be caused in part by defects in mitochondrial function.
Mitochondria buffer intracellular calcium during signaling reactions.

1. Cells need to keep cytosolic Ca low.
   1.1. Resting Ca concentration = $10^{-7}$ M
   1.2. After signaling event, Ca concentration rises 10 to 20 fold.
   1.3. Need to remove Ca from cytosol to prevent over stimulation and potential cell damage.
2. Mitochondria contain Ca pumps that take up Ca from cytosol.
3. Experimental evidence.
   3.1. Label mitochondria with GFP-tagged protein and Ca-sensitive fluorescent dye.
   3.2. Stimulate signaling event that involved Ca release.
   3.3. Mitochondria become fluorescent $\rightarrow$ increase in Ca.
   3.4. Quantify amount of Ca in cytosol versus mitochondria.
      3.4.1. Initial burst of Ca in cytosol.
      3.4.2. Slow increase in mitochondria Ca as cytosolic Ca levels fall.
4. Cells can position mitochondria.
   4.1. Keep Ca levels low in one part of cell during signaling event.
Mitochondria harbor protein that initiates apoptosis.

1. Mitochondria sequester factors that trigger apoptosis if released into cytosol.
2. Upstream signal causes protein Bid to traffic to outer mitochondria membrane where it assembles into pore forming complex.
3. Pore large enough to let cytochrome C to leak out.
4. Cytochrome C binds to Apaf and induces conformational change.
5. Apaf assembles into scaffold complex that activates caspases.
6. Caspases are proteases that initiate cell death.
Mitochondria localize to regions of high energy consumption in cells.

1. Subcellular localization of mitochondrial transport is critical for cell function.
   1.1. Mitos localize to regions of high energy use.
      1.1.1. Epithelial cells areas where there are pumps actively removing or taking up material.
      1.1.2. In neurons, mitos localize throughout dendrites and axons to provide energy for release of synaptic vesicles at axon terminus or processing of signals in dendrites.
   1.2. Given the size of cells, especially neurons, there must exist some mechanism to transport mitos.
   1.3. $ATP \ D = 0.15 \times 10^{-5} \ cm^2/sec$
      1.3.1. 3.5 days to reach end of 10 mm axon.
      1.3.2. One year to reach end of 1 m axon.
Mitochondria move bidirectionally in axons.

1. Microtubule based motility.
2. Move bidirectionally along microtubules.
3. Cells position mitochondria where needed.
   3.1. Axon terminus to supply with ATP.
   3.2. Return to cell body to regenerate.
Kinesin moves mitochondria toward synapse in axons.

1. Kinesin ferries mitochondria toward end of axon.
2. Mitos with higher membrane potential (sign of ATP synthesis) more likely to move toward plus end.
Dynein moves mitochondria toward cell body in axons.

1. Mitos return to cell body to replenish proteins encoded in nucleus.
2. Mitos with low membrane potential (sign of weak ATP synthesis) more likely to move toward minus end.
Intracellular calcium inhibits transport of mitochondria in dendrites.

1. Experiments show that mitos move toward sites of high energy need.
2. Highest energy consumption in neurons is restoring ion gradients after axon potential or signaling event.
3. Need mechanism to end transport mitos to location that has undergone some signaling event or action potential.
   3.1. Calcium reduces transport of mitos.
   3.2. Actin likely acts as anchor.
4. Kinesin linked to mitos via two proteins.
   4.1. Miro is calcium sensor.
      4.1.1. Binds calcium → conformational change.
      4.1.2. Dissociates from kinesin.
Mitochondria undergo fusion and fission.


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1. Mitochondrial undergo show another interesting dynamic phenotype.
   1.1. As the move along filaments, fuse and the sever.
   1.2. Don’t sever into same size mitos.
2. Interesting phenomena. Proteins involved have been identified.
Drp1 mediates fission of mitochondria.

1. Drp1 binds and polymerizes.
2. Fis1 recruits Drp1.
3. Undergoes conformational change to squeeze and divide mitos into two.
   3.1. Similar to dynamin.
4. Other proteins likely involved but have yet to be identified.
Mitofusins and Opa1 mediate mitochondrial fusion.

1. Fusion takes place in two steps.
   1.1. Outer membrane
   1.2. Inner membrane
2. Mitofusins on outer membrane mediate membrane fusion.
   2.1. Mitofusin1 and mitofusin2 -> can both mediate fusion.
   2.2. Structure similar to SNAREs -> extended helical regions.
3. Inner membrane fusion driven by Opa1 that resides in inner membrane.
4. Complete fusion allows for mixing of proteins and DNA
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Mitofusin mutants fail to fuse and have reduced size.

1. Mutations in mitofusins result in smaller mitochondria.
2. Fuse two cells with differently labeled mitos.
   2.1. Wild type: mitos fuse and colors overlap
   2.2. Mutant: mitos remain distinct with little overlap of colors.
Mitochondrial fusion mutants associated with neurodegenerative diseases.

1. Mitofusin mutants inhibit movement of mitos into axons.
   1.1. Reduced energy production along length of axons.
   1.2. Severe consequences on neuron function, especially neurons with longer axons.
2. Mitofusin mutants lead to more low functioning mitochondria.
   2.1. Reduce energy generating capacity of cell.
3. Several neurodegenerative diseases associated with mitochondria fusion defects.
   3.1. Mutation in mitofusins one cause of Charcot–Marie–Tooth
       3.1.1. Start with tingling in extremities.
       3.1.2. Leads to neurodegeneration of motor neurons and muscle wasting → weakness in hands and forearms.
   3.2. DOA.
       3.2.1. Loss of visual acuity.
       3.2.2. Degeneration of neurons in retina.
   3.3. Mutant gene associated with Parkinson’s encodes mito proteins and causes larger mitos with reduced activity.
   3.4. Mitos in Alzheimers and Huntingtons much smaller → reduced fusion?
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Disruption of mitochondrial fusion leads to neurodegeneration.

1. Test importance of mito fusion by making knockout mouse.
   1.1. Complete knockout = lethal.
   1.2. Conditional knockout in cerebellum.
2. Mito fusion mutants smaller and lethargic \( \rightarrow \) lack ability to move and coordinate movement.
3. Mutant mice show atrophied cerebellum over time.
4. Mutant mice show loss of cellularity in cerebellum.
5. Stain for apoptosis reveals increase numbers of cell deaths in mutant cerebellum.

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Inhibiting mitochondrial fusion leads to defect in transport of mitochondria.

1. One effect of mitofusin mutation is clustering of mitos around nucleus.
   1.1. Reduced numbers of mitos in axons and dendrites.
   1.2. Transport failure?
2. Mitos have reduced activity -> selective transport of functioning mitos.
3. Mitos smaller and more numerous -> less efficient transport.
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Each mitochondria contains several copies of its own genome.

1. Why would lack of mitochondria fusion lead to less active mitochondria?
2. Mitochondria contain multiple copies of genome.
   2.1. DNA in nucleoids 5 – 10/mito.
   2.2. Some contain mutations, some wild-type.
   2.3. Number of mutations may affect performance.
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Mitochondrial genome contains genes essential for function.

1. Over 600 proteins encoded in nuclear genome.
2. Mitochondria contain own DNA.
   2.1. Several copies.
   2.2. Encode rRNAs, tRNAs and 13 proteins involved in oxidative phosphorylation and ATP synthesis.
   2.3. Genes not replaceable by nuclear genes, so essential for functioning mitochondria.
   2.4. Higher mutation rate in mitochondria.
      2.4.1. Higher exposure to free radical due to electron transport chain.
      2.4.2. Less robust repair system.
   2.5. Several diseases result from mutations in mitochondrial DNA.
      2.5.1. Mitos with several copies of genome.
         2.5.1.1. How many mutated before phenotypic effects?
         2.5.1.2. Inheritance complicated.
      2.5.2. Need way to ensure fidelity of genome -> multiple copies?
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Inhibiting mitochondrial fusion leads to loss of mitochondrial DNA.

1. Mitofusin mutant mitos have reduced or no DNA.
Disruption of mitochondrial fusion leads to muscle atrophy.

1. mitofusin mutants have reduced skeletal muscle.
2. Smaller muscle -> atrophy.
3. Mutant has more, smaller mitos.
4. Muscle cells change of physiology.
   4.1. Normal muscle have high level of cytochrome C oxidase -> complex IV.
       4.1.1. Stain brown.
       4.1.2. Encoded in mito genome.
   4.2. Mutant mice show increased amount of succinate dehydrogenase -> complex II.
       4.2.1. Stain blue.
       4.2.2. Encoded in nuclear genome.
   4.3. Cytochrome C encoded in mito genome, sd in nuclear genome -> defect in mito DNA?
5. Reduced size of muscle cells -> reduced muscle size.
6. Lack of fusion lead to defects in expression of mitochondria proteins.
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Fusion and fission may help mitochondria handle mutations in their DNA.

1. Mito fusion allows for complementation between mutant mitos.
2. Mitos with two different mutations in genome.
3. Fusion allows mixing of genomes and complementation.
Fusion of mitochondria with genetic mutations restores respiration.

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1. Cell fusion experiment.
2. Look at COX activity.
3. Wild-type has high activity.
4. Mitos with no DNA have low COX.
5. Two cells with mutations in separate mito genes that reduce COX activity.
6. Fuse celldddds
Inhibition of mitochondrial fusion increases mutation rate in mitochondrial DNA.

1. Mito fusion may help maintain integrity of mito DNA.
2. High rate of mutations, some mitos may accumulate significant number of mutations that affect performance.
3. Fusion between mitos would allow exchange of genomes, so that both mitos have enough wild-type DNA to ensure proper function.
4. Replication of wild type genome and degeneration of mutant would restore large number of wild type genomes.
5. Without fusion → mitos with mutant DNA would lose activity can’t regenerate wild type DNA.
6. Allow for repair of mutated mito genome
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Mitophagy engulfs and then delivers mitochondria to the lysosome.

1. Cells can target and destroy low functioning mitos by delivering them to lysosome.
2. Mitophagy.
   1. Related to process called autophagy.
   2. Membrane structure grows around defective mitos.
   3. Engulfs mitos and fusion encloses mitos in membrane-bound vesicle.
   4. Vesicle fuses with lysosome and mitos destroyed.
PINK1 and Parkin target damaged mitochondria for mitophagy.

1. Mitochondria targeted for mitophagy through protein Parkin that binds outer membrane.
2. Parkin is E3 ligase that ubiquitinates proteins on mito surface.
   1. Different linkages than ubiquitins for proteosome degradation.
   2. Adapter protein links ubiquitins to autophage machinery.
3. Parkin accumulates on mitos with low membrane potential → defective mitos.
4. Parkin recruited to mitos via Pink.
5. Pink constantly binds mitos but is degraded by proteases in active mitos.
6. Inactive mitos don’t degrade Pink so it accumulates and recruits Parkin.
7. Genetic mutations in Pink and Parkin linked to Parkinsons disease.
8. Parkinsons patients more likely to accumulate mitos with damaged DNA
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Autophagy in cell starvation and beyond
Autophagy allows cells to recycle macromolecules and organelles.

1. Autophagy observed 50 years ago by EM -> self eating.
2. Steps.
   2.1. Collection membrane or vesicles in cytosol.
      2.1.1. Origin unclear -> ER, TGN?
   2.2. Begin to coalesce around portion of cytosol -> phagophore.
      2.2.1. Non-selective.
      2.2.2. Recruitment of more membrane increases size of phagophore.
   2.3. Fusion of membrane encloses cytosolic contents in two lipid bilayers -> autophagosome.
      2.3.1. Not SNARE mediated.
   2.4. Fuse with lysosome to digest macromolecules -> SNAREs
   2.5. Lysosome channels release degraded components into cytosol.
3. Recycle old material.
Atg proteins mediate formation of autophagosome.

1. Proteins involved first identified in yeast → ATG.
2. Initiate phagophore formation and completion of autophagosome.
Starvation triggers autophagy in cells.

1. Starvation increases number of autophagosomes.
2. Recycle old material to be used for essential cell functions.
TOR inhibits autophagy in the presence of nutrients and growth factors.

1. Autophagy tightly regulated.
2. TOR.
   2.1. Activated by growth factors and high concentration of nutrients \(\rightarrow\) amino acids.
   2.2. Kinase.
   2.3. Phosphorylates and inactivates Atg proteins that initiate phagophore formation.
3. As nutrient levels fall, TOR is inactive \(\rightarrow\) phagophore formation increases.
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