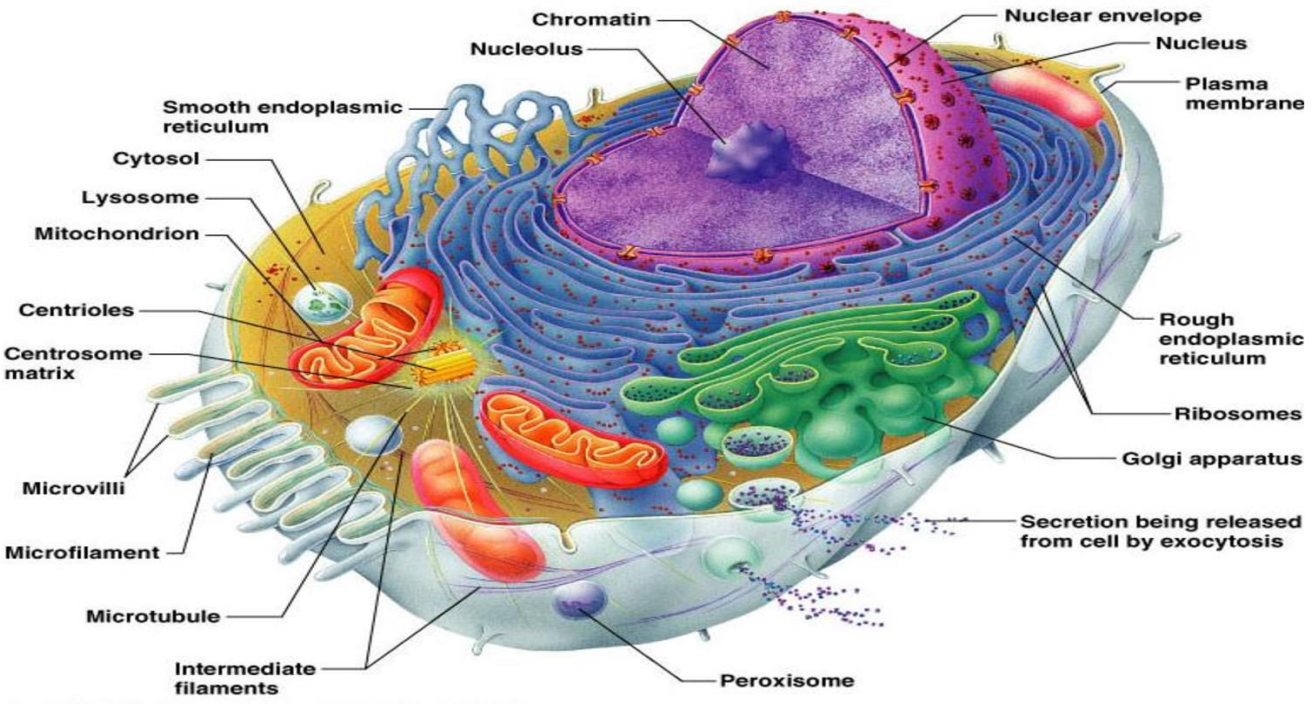
Lecture 1:
Mitochondria: what they are, what they do and what can go wrong

Rachel Nicoll PhD

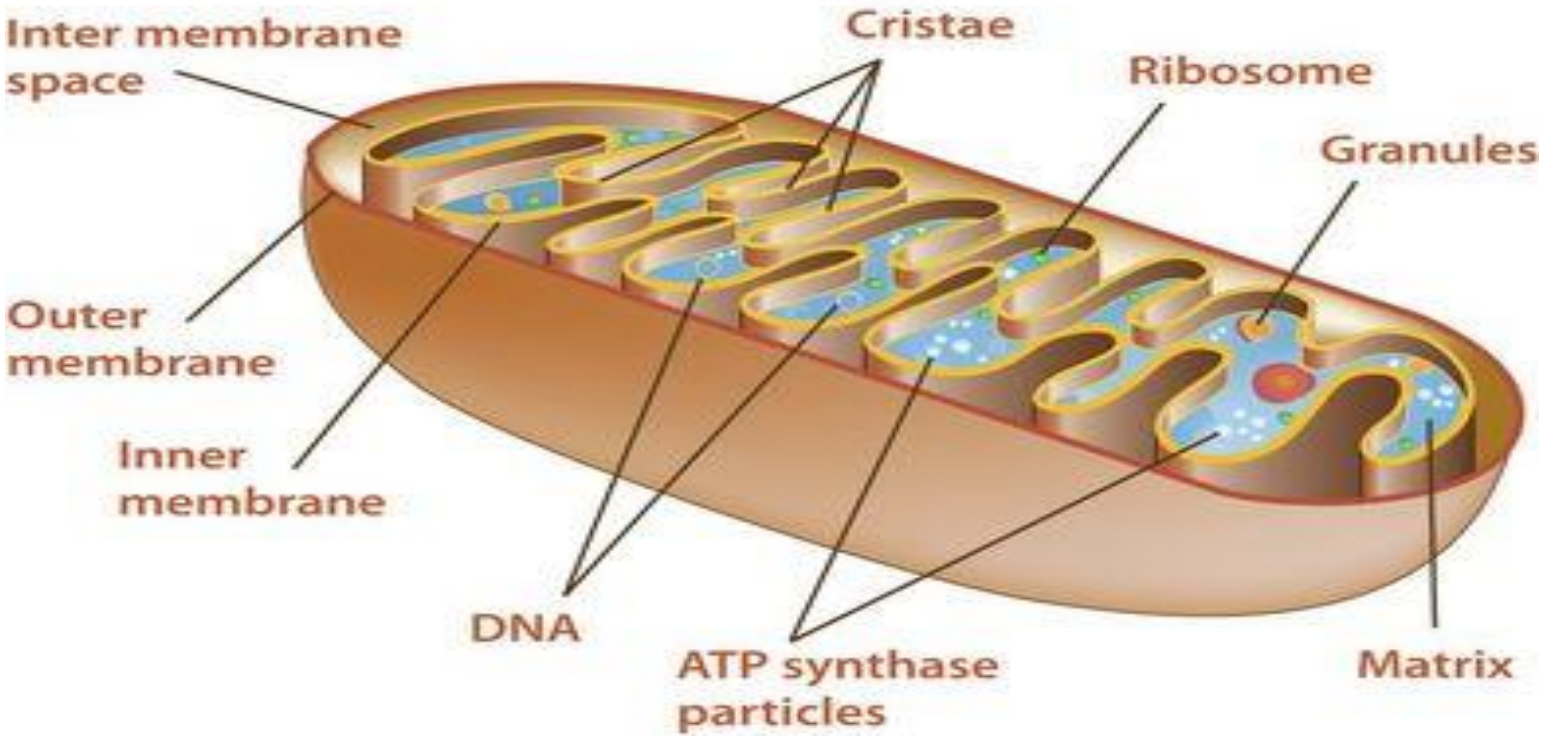
What are mitochondria?

- Mitochondria (singular: mitochondrion) are **cellular organelles** (specialised sub-units within a cell) whose **main function is to produce cellular energy** through oxidative phosphorylation (OXPHOS), i.e. cellular respiration.
- **There can be up to 10,000 mitochondria in each cell but the number depends on energy demand.** Neurons and cells in cardiac and skeletal muscle, liver and brown adipose tissue (BAT) have the greatest number. Red blood cells and skin cells have no mitochondria. Egg cells (oocytes) have hundreds of thousands of mitochondria.
- The eyes also have a very high concentration of mitochondria. Mitochondrial dysfunction causes the loss of ability to perceive subtle shades of grey (apparently there are around 50!).
- The mitochondrion was given its name in 1898 by microbiologist Carl Benda. It derives from the Greek:
  - Mitos = thread
  - Chondros = granule

The mitochondrion's place in a typical cell



Mitochondrion



Mitochondrial structure

- **Mitochondria** are the only organelles in the cell with a **dual-walled membrane**. This is a **phospholipid bilayer**, comprising phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine. These are unsaturated fatty acid derivatives, which are highly susceptible to oxidation.
- The **outer membrane is relatively permeable**. Two important channels may form in the outer membrane: the **mitochondrial apoptosis-induced channel (MAC)**, allowing triggers of apoptosis to flow out into the cytosol, and the **mitochondria-associated ER-membrane (MAM)** mainly allowing transfer of intracellular calcium.
- The **inner membrane** is characterised by **complex folds (cristae),** which expand the surface area to maximise energy production. The inner membrane is **highly impermeable** but the **mitochondrial permeability transition pore (mtPTP)** may form, which facilitates flow of calcium ions, acting as a valve on a pressure cooker. The inner membrane is rich in cardiolipin (diphosphatidylglycerol), an essential phospholipid required for the proper functioning of the ETC. There is a membrane potential across the inner membrane, formed by oxidative phosphorylation in the electron transport chain.
- The **matrix** (lumen) is the space enclosed by the inner mitochondrial membrane which contains the **mitochondrial DNA** (5-10 copies), tRNA, special mitochondrial ribosomes to manufacture proteins and several other enzymes, including those for the oxidation of pyruvate and fatty acids and the TCA cycle. These comprise about 2/3 of the total protein in the mitochondrion.

Mitochondrial structure: The outer membrane

- The smooth, continuous outer membrane is relatively permeable, allowing the passage of numerous small molecules, facilitated by membrane proteins called porins. Larger proteins can pass through the outer membrane with help of translocases (translocator proteins). The outer membrane also allows fatty acid transport into the matrix.
- The outer membrane contains the fatty acid desaturases and elongases, enzymes for triglyceride synthesis and phospholipases that hydrolyse phospholipids. The protein-to-phospholipid ratio is similar to the plasma membrane. The outer membrane also contains enzymes involved in oxidation of adrenaline and degradation of tryptophan.
- The mitochondrial apoptosis-induced channel (MAC) can form in the outer membrane, allowing triggers of apoptosis to flow out into the cytosol.

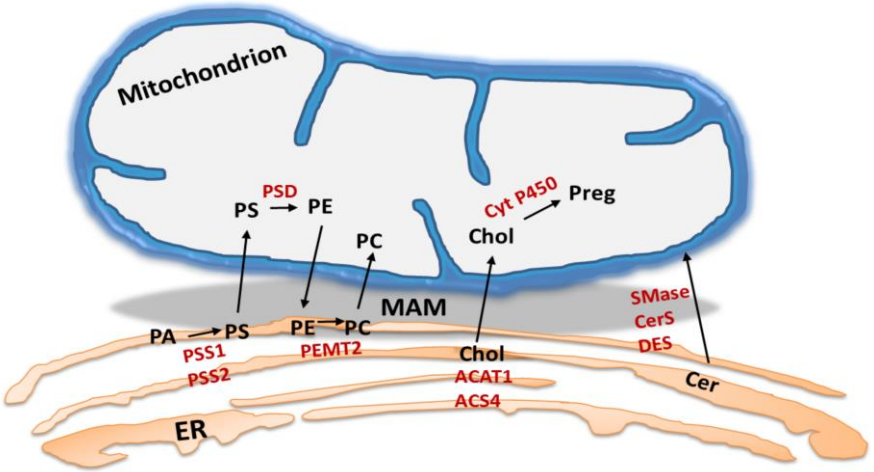
Mitochondrial structure: The inner membrane and inter-membrane space

- The inner membrane is characterised by complex folds and tubules (cristae), which extend into the matrix and expand the surface area to maximise energy production. In the liver, the area of the inner membrane is about 5 times larger than that of the outer membrane, due to the christae. The inner membrane contains 151 different proteins, 1/5 of the total mitochondrial proteins. These proteins are involved in OXPHOS, ATP synthase, nucleotide metabolism, transamination of amino acids, transporters for passage in and out of the matrix, protein import and mitochondrial fission/fusion.
- The inner membrane is highly impermeable except to certain fats, O2, CO2 and H2O; other substances can only cross the inner membrane in special membrane transporters such as translocases. The inner membrane regulates the uptake of substrates into the matrix for oxidation and provides a transport protein to take ADP into the matrix and ATP out of the matrix. There is a membrane potential across the inner membrane, formed by oxidative phosphorylation in the electron transport chain.
- There is a very high protein-to-phospholipid ratio. The inner membrane is rich in cardiolipin (diphosphatidylglycerol), required for the proper functioning of the ETC and which is thought to help make the inner membrane impermeable. Cardiolipin is a bacterial feature and is an essential phospholipid that serves as a cofactor for a number of critical mitochondrial transport proteins and retains cytochrome c at the inner mitochondrial membrane through the electrostatic interaction.
- The mitochondrial permeability transition pore (mtPTP) acts like a valve on a pressure cooker and is a channel through which excess mitochondrial calcium can be released through activation of cyclophilin D. But the opening of the PTP leads to loss of mitochondrial control, and if the mtPTP is open too long, the mitochondria will swell up, the membrane potential will be dissipated, ROS production will increase, ATP production will reduce and cytochrome c will be released, triggering apoptosis.
- The intermembrane space concentration of small molecules such as ions and sugars is similar to the cytosol, as would be expected because the outer membrane is permeable to small molecules. The protein content is different because of the need for translocase to import certain proteins. In a healthy mitochondrion, cytochrome c is always found in the intermembrane space.

The mitochondria-associated endoplasmic reticulum (ER) membrane (MAM)

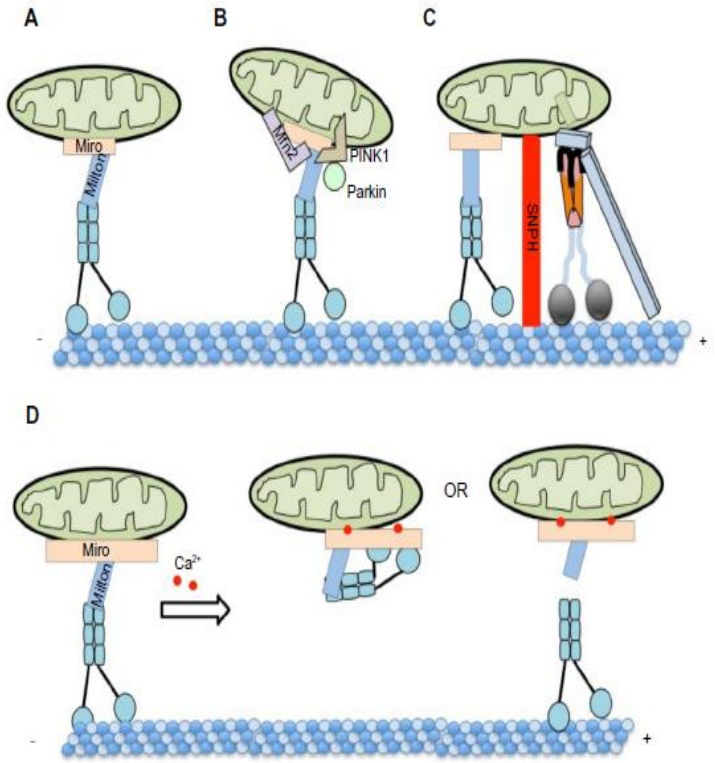
- The outer membrane can associate with the membrane of the endoplasmic reticulum (ER), another cell organelle, forming a structure called the mitochondria-associated ER-membrane (MAM), which can comprise up to 20% of the outer mitochondrial membrane.
- The MAM facilitates intracellular calcium exchange, with the MAM regulating the precise amounts.
- The MAM is enriched with enzymes involved in lipid biosynthesis, such as phosphatidylserine, and facilitates phospholipid exchange to ensure a constant supply of phospholipids during fission and fusion.

(Raturi A, Biochim Biophys Acta, 2013



Mitochondrial movement (motility)

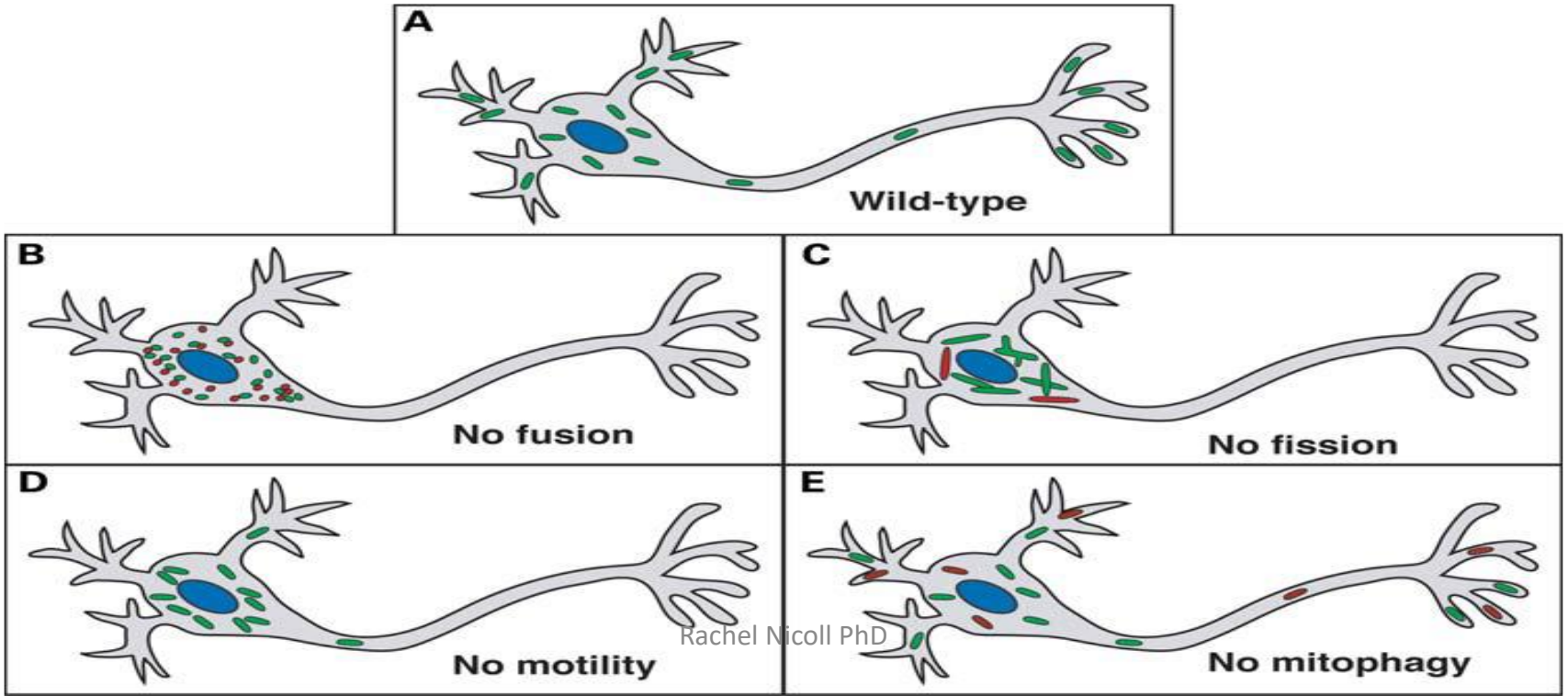
- Mitochondria are dynamic, self-moving (motile) shape-shifters, in a continual cycle of fission and fusion.
- Microtubules (**cytoskeletal tracks**), which also support the cell structure, are utilised by the mitochondria to facilitate their movement, **with the help of the proteins kinesin and myosin. Kinesins have 2 feet (globular heads) that literally walk the mitochondria to their new location.**
- The cytoskeletal tracks are similar to rail tracks, powered by protein motors (kinesin and dynein) to deliver mitochondria to the parts of the cell most in need of energy. Kinesin and myosin can also help rebuild microtubules.
- This is particularly important in **neurons**, as they enable the mitochondra to reach the entire perimeter of the cell, including the length of the axon and into the synapse.



Watch a video of this at: https://evolutionnews.org/2020/06/the-workhorse-of-the-cell-kinesin/

Defects in mitochondrial dynamics that lead to neuronal dysfunction

(A) In wild-type neurons, mitochondria travel long distances from the cell body out to dendritic and axonal termini. (B) In the absence of fusion, the mitochondrial population fragments and a subset show ultrastructural defects and dysfunction (red). The mitochondria secondarily have transport defects that prevent proper distribution to the periphery. (C) In the absence of fission, the mitochondrial population is excessively long and interconnected, and a subset shows dysfunction (red). These large mitochondria cluster within the cell body and are not efficiently transported to the periphery. (D) Primary defects in mitochondrial motility prevent distribution of mitochondria to the periphery. (E) In the absence of mitophagy, abnormal mitochondria (red) accumulate. (From Chen H, Hum Mol Genet, 2009)



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Mitochondria have a bacterial origin and retain many bacterial properties

- All animals and plants are eukaryotes (multi-cellular organisms containing a nucleus and organelles), which originated 2 billion years ago.
- There are several theories of mitochondrial development; the currently trending hypothesis is ‘endosymbiosis’, the fusion of:
  - archaea (single-celled organisms without a nucleus or any organelles), the most ancient organisms so far discovered, which engulfed
  - α-proteobacteria (ditto), which were aerobic, enabling the host to thrive in an oxygen-rich environment.
- This degree of co-operation, with such remarkable evolutionary consequences, has only been seen once and enabled the development of eukaryotes and eventually human life, with the α-proteobacteria becoming our mitochondria.
- Mitochondria retain many bacterial properties, for example the presence of cardiolipin in the inner mitochondrial membrane.

Mitochondria retain many bacterial properties

- Mitochondrial structure, function and genome are similar to those of bacteria.
- Both mitochondria and bacteria have a relatively impermeable cell membrane, just inside the cell wall, which they use for energy production.
- Bacterial and mitochondrial genes are not protected by histones ('naked DNA').
- Abundance of cardiolipin in the inner membrane.
- Both contain an electron transport chain that pumps protons across the inner mitochondrial membrane with the resulting proton motive force driving ATP synthesis via ATP synthase,
- Both the matrix of mitochondria and the cytosol of bacteria contain DNA, tRNA, ribosomes and numerous soluble enzymes,
- Both reproduce by binary fission,
- Both bear a N-formylmethionine start residue in their proteins,
- The ribosomes encoded by mitochondrial DNA are similar to those from bacteria in size and structure.
- Both share some pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), so that the innate immune system recognises mitochondrial bacterial motifs.

Mitochondrial evolution

- In nature, one cell engulfing another is fairly routine – so what was special about this? And why was the α-proteobacteria not 'digested' and discarded as waste, as normally occurs with bacterial phagocytosis?
- But then the α-proteobacteria had the energy while the archaea did not, and α-proteobacteria could consume oxygen at a time when oxygen levels were rising on earth, while archaea were methanogens and could not survive in an oxygenated environment. Did they strike a deal?
- The archaea/α-proteobacteria combination are known as 'symbionts', with both benefitting from the presence of the other. This appears to contradict Darwin's theory of evolution (incremental species change through natural selection) because endosymbiosis is a process of co-operation rather than competition and it occurred only once instead of gradually. But late 20th century evolutionary biology expanded the concept of evolution to encompass co-operation as well as competition (Margulis L, Bostonia, 1987).
- Initially, each organism was totally autonomous and contained all the genes necessary for independent life. But over time, trial and error produced the perfect working relationship: the α-proteobacteria specialised in energy production and became the mitochondria, while the archaea specialised in other areas. Both retained their own DNA.
- This triggered a quantum leap in evolution, with the development of a cell with a nucleus. So if not for the acquisition of mitochondria, we might never have evolved beyond a single-celled organism.

Mitochondria statistics

- There are approximately 10 million billion mitochondria in an adult human, representing around 10% of total body weight and around 40% of cellular weight.
- Instead of counting the number of angels dancing on the head of a pin, we could count the number of mitochondria that would fit on the head of a pin:
- Answer: >1 billion
- Mitochondria range in size from 0.5-10µm. They are not visible under a microscope unless stained.

More mitochondrial statistics

- The search for the mechanism involved in the manufacture of ATP has given rise to not one but two Nobel prizes, in 1978 and 1997.
- 90% of the oxygen we breathe in is used by the mitochondria.
- A single cell consumes around 10 million molecules of ATP every second. With 40 trillion cells in the body, this gives a total turnover of ATP of 60-100 kg/day. This is roughly our own body weight in ATP, every day. Every molecule of ATP is recharged once or twice a minute. By contrast, E. coli synthesises about 4 times the amount of ATP per cell as we do.
- A mitochondrion generates 150-200 millivolts across its 5 nanometre membrane. If this were translated to metres, it represents 30 million volts. It has been estimated that, size for size, a mitochondrion produces as much energy as a bolt of lightning.
- Egg cells (oocytes) have hundreds of thousands of mitochondria, followed by neurons, cardiac and skeletal muscle, liver and brown adipose tissue (BAT), as these are organs with the greatest energy demands. Red blood cells and skin cells have no mitochondria, while immune cells have the lowest content. BAT is brown because of the high concentration of mitochondria, which are brown in colour because of the iron content.
- Mitochondrial half-life varies from a few days to a few weeks, depending upon their location in the body. The half-life of mitochondria in the liver is 1-2 days, heart 5-6 days, brain and kidneys c24 days.

Mitochondrial energy production

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How the substrates for energy production get into our body

- Sunlight: in plants, light energy (photons) effectively catalyse molecules of H_2O and CO^2 to produce glucose and O_2.
- Whether we eat the plants, eat the animals that eat the plants or animals that eat the animals that eat the plants, the energy from sunlight is passed up the food chain and into our bodies as proteins, carbohydrates and fats.
- Our bodies then absorb the digested proteins, carbohydrates and fats, which are taken into our cells in their most basic form and converted to adenosine triphosphate (ATP) in a series of catabolic reactions.
- But there is a long road between absorbing nutrients and the creation of ATP!

Mitochondrial fuels

- Glucose (from carbohydrates): used first (but is not necessarily best!)
- Fatty acids: a more efficient fuel
- Ketones: made in the liver from fats. Produce >20% more ATP than pyruvate and with much less free radical damage.
- Protein: used as emergency starvation fuel but usually involves muscle catabolism

The end product of energy production: adenosine triphosphate (ATP)

- **Adenosine triphosphate (ATP) is a nucleotide composed of 3 major chemical compounds:**
  - **Adenine** – a purine base
  - **D-ribose** – a pentose (5-carbon) sugar (adenine + ribose = adenosine)
  - **3 inorganic phosphate groups** (i.e. triphosphate)
- ATP is synthesised by the addition of an inorganic phosphate group (Pi) to adenosine diphosphate (ADP).
- The chemical energy stored in ATP is released by breaking the high energy bond to release Pi, reducing it to ADP again. $ATP + H_2O \longrightarrow ADP + Pi$
- ATP synthesised from OXPHOS exits the mitochondria via translocator proteins (translocases) into the cytosol.
- The chemical energy released is then used to drive processes requiring energy, including biosynthesis, muscle contraction or transport of molecules across cell membranes.
- There are around 1 billion molecules of ATP in the average cell and each is recycled around 3 times per minute. Each mitochondrial ATP cycle can create about 600 ATP molecules per second at maximum demand.

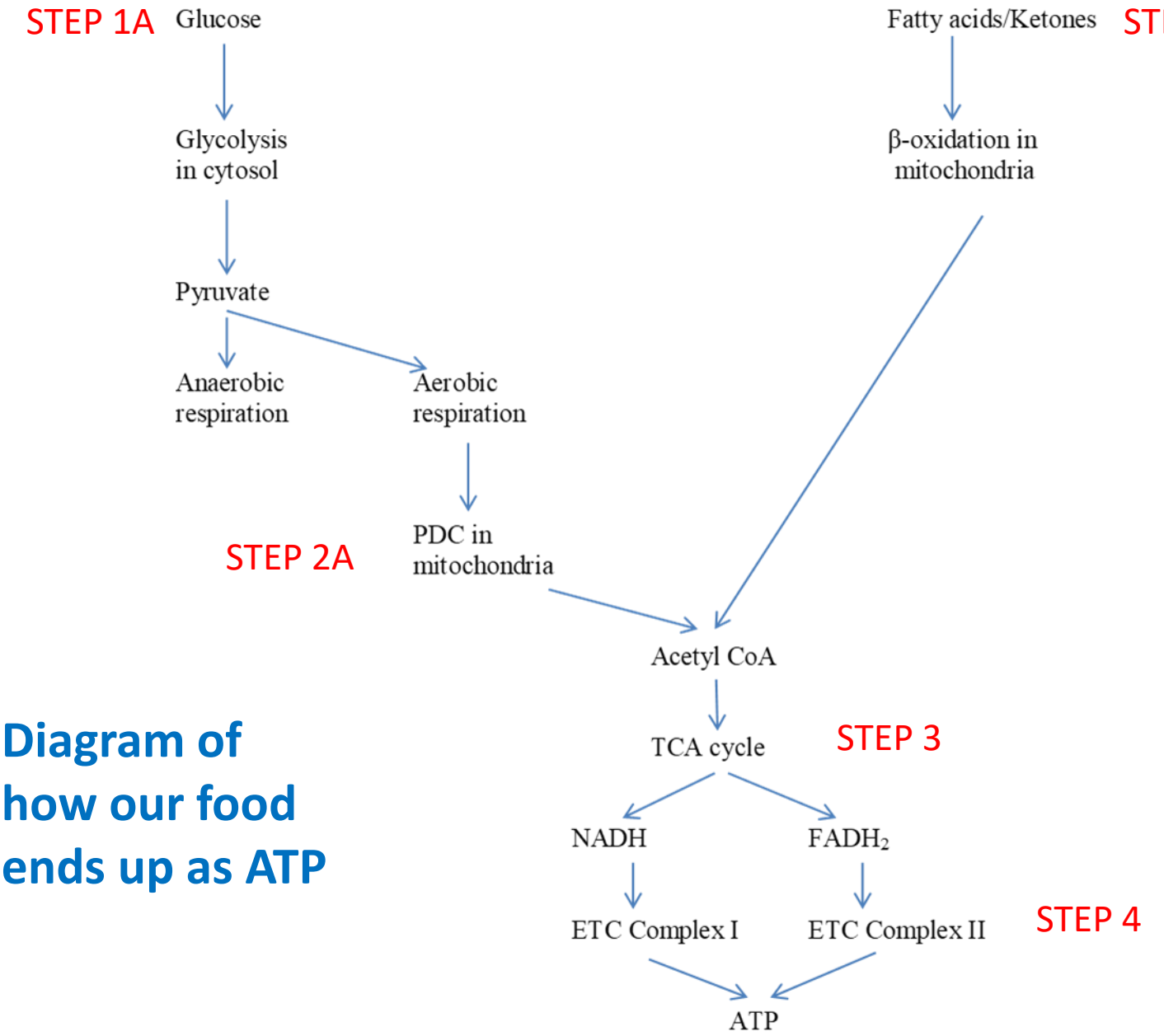
More on ATP, ADP and AMP

- ATP can also be broken down to adenosine monophosphate (AMP), releasing pyrophosphate (Ppi), which can convert into 2 molecules of inorganic phosphate (2Pi).
- ADP can be made from breaking the high energy bond in ATP or from AMP by adding a phosphate.
- AMP comprises adenine, ribose and just 1 phosphate group; it is used to make ADP or to synthesise RNA. AMP can itself be made from the hydrolysis of ATP and ADP or when RNA is broken down.
- AMP can also be made by adding a phosphate group to adenosine, which can be made in most cells and is present in some foods.
- How does this relate to cAMP (cyclic AMP)? cAMP is a derivative of ATP, a second messenger, used for intracellular signal transduction, such as transferring into cells the effects of several hormone which cannot pass through the plasma membrane. It is also involved in the activation of protein kinases.

Energy production can be aerobic or anaerobic

- **The mitochondria produce 90-95% of the ATP of the organism through aerobic respiration (using oxygen), aka oxidative phosphorylation (OXPHOS),** and themselves require large amounts of energy to function and support respiration, structure, regeneration and growth.
- 'Oxidative' = requires oxygen; 'phosphorylation' = adds a phosphate group.
- **The remaining 5-10% of the cell's energy comes from anaerobic respiration (without using oxygen), aka substrate-level phosphorylation. This takes place in the cytosol without the involvement of the mitochondria.**
- Substrate level phosphrylation (ADP is the substrate) generally only occurs in the absence of oxygen, which is important for certain tissues such as contracting skeletal muscle, but in cancer it can also take place aerobically.
- Glycolysis can begin either aerobic or anaerobic respiration. In fact, glucose is most commonly used for aerobic respiration and is always utilised first.
- When glucose is in short supply, fatty acids are used. Amino acids may be oxidised as a last resort. However, fatty acids and amino acids cannot undergo glycolysis; that is reserved for glucose.
- The cell has the ability to be both aerobic and anaerobic, depending upon need.

Diagram of how our food ends up as ATP



Step 1a: Glucose broken down to pyruvate

- **Glycolysis is the breakdown of glucose to produce pyruvate in the cytosol** through the action of pyruvate kinase. 1 molecule of glucose is transformed into 2 molecules of 3-carbon pyruvate + 2 hydrogen ions (H^+).
- **The pyruvate then follows one of two paths:**
  - **It undergoes anaerobic respiration, which produces only net 2 molecules of ATP, so less efficient than…**
  - **It is taken into the mitochondrial matrix for aerobic respiration, producing a net >30 molecules of ATP from glucose**
- Although it seems as though mitochondrial aerobic respiration would always be the preferred option, **there are occasions when anaerobic respiration is appropriate**:
- Glycolysis is stimulated when blood insulin levels are high relative to blood glucagon levels (i.e. after a carbohydrate meal).
- Some cells, such as red blood cells, lack mitochondria, so that glycolysis is their only means of producing energy.
- Also, in the absence of oxygen or when oxygen demand cannot keep up with supply (such as in exercising skeletal muscle), anaerobic respiration ('emergency energy production') will take place to provide all the cell's ATP needs until oxygen supply is restored.

Pyruvate undergoes anaerobic respiration

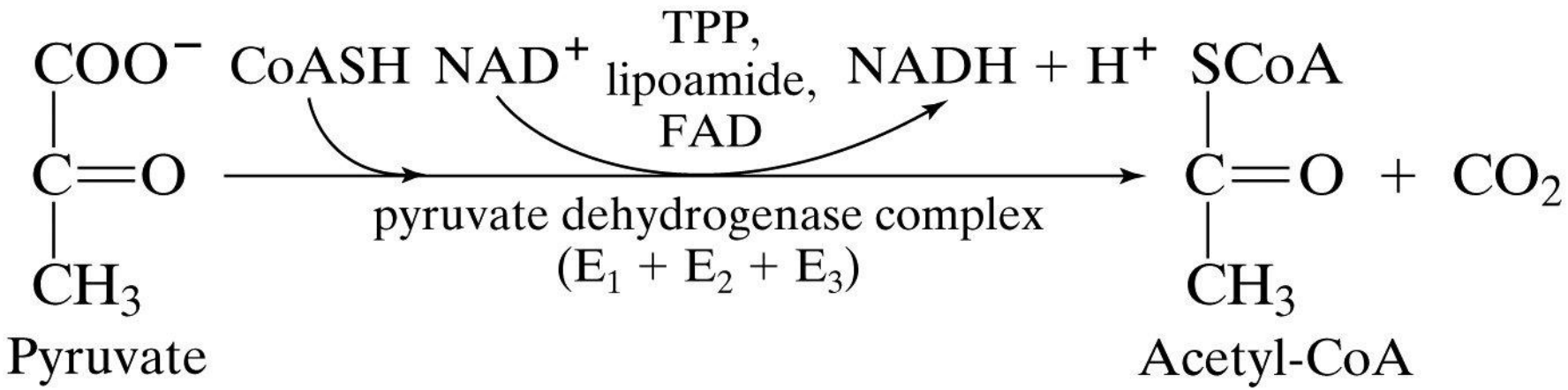
- **Pyruvate + 2Pi + 2ADP → 2ATP + 2H_2O + 2 lactate**
- The pyruvate then produces 4ATP + 2NADH (electron carriers) but 2ATP are consumed in the process (hence net 2).
- The waste product lactic acid is produced via lactate dehydrogenase using NADH to reduce pyruvate. **Lactic acid build-up during anaerobic respiration can result in lactic acidosis: excessively low pH (high cellular acidity).**
- **This is the root cause of muscular pain, including exercise-induced angina.** It arises because hypoxia (lack of sufficient oxygen for the mitochondria to carry out OXPHOS) causes anaerobic respiration and lactic hence acidosis.
- So exercise-induced angina is a sign that the body is respiring anaerobically and more oxygen is required.
- The low pH (increased lactic acid) is in fact a very useful signal to the cell that energy is being produced anaerobically and not aerobically, which triggers mitochondrial biogenesis to produce more ATP aerobically.
- Eventually the liver will extract the circulating lactate and oxidise it back to pyruvate via lactate dehydrogenase.

Step 2a: Pyruvate undergoes aerobic respiration

- **Pyruvate enters the pyruvate dehydrogenase complex (PDC), located in the mitochondrial matrix, where it combines with coenzyme A to produce Acetyl Coenzyme A (Acetyl CoA).**
- Pyruvate undergoes pyruvate decarboxylation (losing a carbon atom) and binding with coenzyme A. The conversion is dependent upon:
  - lipoic acid,
  - thiamine pyrophosphate (needs vitamin B1),
  - coenzyme A, which is dependent on pantothenic acid (vitamin B5),
  - flavine adenine dinucleotide (FAD) and
  - nicotinamide adenine dinucleotide (NAD^+)
- The PDC operates a negative feedback loop: when there is sufficient acetyl CoA, it inhibits itself to avoid waste, but when there is a shortage, it turns itself back on.
- Other activators of the PDC include insulin, calcium, magnesium and exercise.
- With age and development of insulin resistance, we reduce our ability to activate the PDC. But unless the PDC is activated, pyruvate has nowhere to go and so is converted in the cell to lactic acid, which can lead to lactic acidosis, muscle pain and fatigue. All this can be avoided with fat burning instead of glycolysis!

(Pietrocola F, Cell Metab, 2015; Stacpoole PW, Aging Cell, 2012)

Equation for the production of Acetyl CoA from pyruvate



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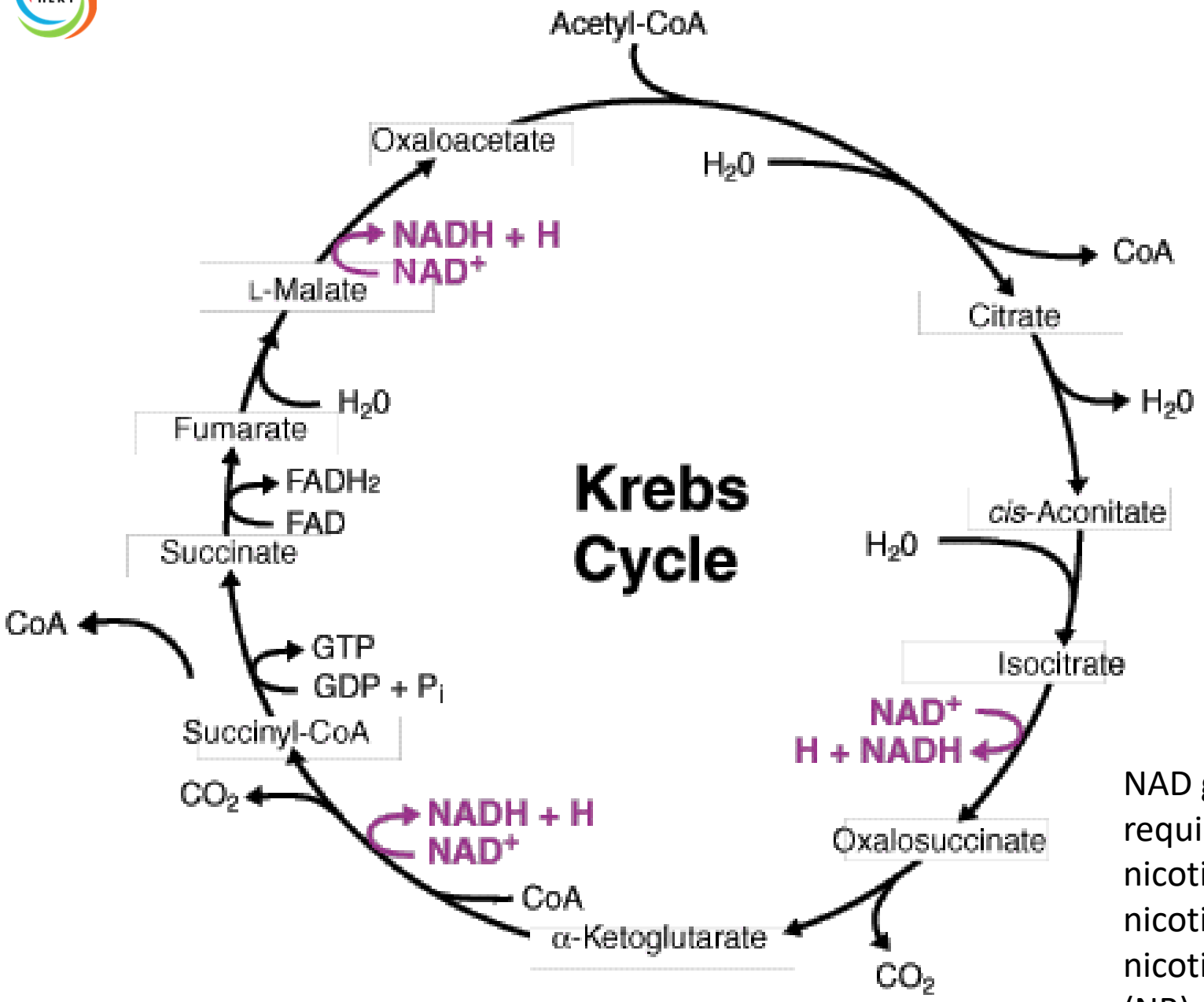
Step 1b: Fatty acids undergo β-oxidation

- **β-oxidation takes place in the mitochondrial matrix** of many tissues but principally liver and muscle. The brain and RBCs are unable to oxidise fatty acids because they lack the necessary enzymes (and RBCs lack mitochondria).
- **How do the different types of fatty acids enter the mitochondrial matrix?**
  - **Medium chain fats** (palm oil or coconut oil) **and short chain fats** (propionate, butyrate, acetate) can **diffuse into the matrix without a carrier protein**.
  - **The inner mitochondrial membrane is impermeable to long chain fats (e.g. ω3), so they enter the mitochondrial matrix with the help of the 'carnitine shuttle'** (carnitine palmitoyl transferase 1 - CPT 1), This is the rate-limiting enzyme in fatty acid β-oxidation, so it's important to have sufficient carnitine.
- **The oxidised fatty acids then combine with coenzyme A to produce acetyl CoA**; they lose 2 carbons in the process.
- Fatty acids derive from:
  - Free fatty acids from the diet circulating in the bloodstream, or
  - Excess glucose stored in adipose tissue as triglycerides and released during exercise or fasting.

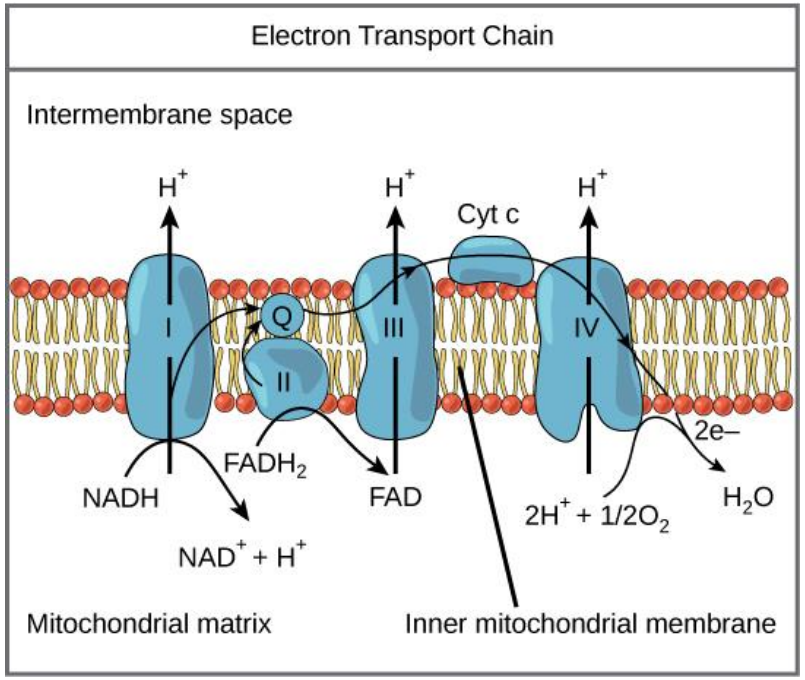
Step 3: The Tricarboxylic Acid (TCA) cycle (aka Krebs or citric acid cycle)

- **Acetyl CoA feeds into the tricarboxylic acid (TCA) cycle in the mitochondrial matrix**, where acetyl CoA is successively converted by various enzymes and the addition of O_2 (because it's aerobic) to CO_2, which we exhale.
- The TCA cycle **catalyses the conversion of acetyl CoA to the electron carriers:**
  - nicotinamide adenine dinucleotide (NAD^+) to its reduced form **NADH**
  - flavin adenine dinucleotide (FAD) to its reduced form **$FADH_2$.**
- NADH has gained 2 electrons and a hydrogen ion (proton), while $FADH_2$ has gained 2 electrons and 2 hydrogens (protons).
- Hence, acetyl CoA can be made from pyruvate, the β-oxidation of fatty acids or ketones or deaminated amino acids, depending on availability. If ketones are the fuel substrate, they are converted to acetyl CoA through oxidation in the mitochondrial matrix, as with fatty acids. Acetyl CoA is, in effect, reactive vinegar!
- Both glucose and fatty acids produce $FADH_2$, which can produce ROS, but ketone bodies produce only NADH, which produces less ROS. Hence ketone bodies are considered a cleaner fuel.



NAD generation requires tryptophan, nicotinamide and nicotinic acid and nicotinamide riboside (NR).

Step 5: The Electron Transport Chain (ETC)



- **The ETC comprises 5 Complexes which carry out oxidative phosphorylation (OXPHOS).**
- **NADH and $FADH_2$ from the TCA cycle enter the ETC and give up their electrons,** to form NAD+ and FAD, respectively.
- The ETC takes place in the cristae of the inner mitochondrial membrane. **One mitochondrion contains tens of thousands of copies of ETCs.**

The ETC Complexes I-IV

Complex I: NADH coenzyme Q reductase accepts **electrons from NADH**, producing NAD^+, passes the electrons to the **electron carrier ubiquinone** (coenzyme Q, oxidised coQ10). **4 protons** (4 x hydrogen ions (H^+) are pumped across the inner mitochondrial membrane (IMM) into the inner membrane space. The **ubiquinone/electron complex is passed to Complex III**.

Complex II: Succinate dehydrogenase accepts **electrons from $FADH_2$**, producing FAD, passes them through **iron/sulphur clusters to ubiquinone.** The **ubiquinone/electron complex is passed to Complex III. Complex II does not pump protons over the IMM**, and hence contributes less energy than Complex I. Succinate dehydrogenase is the only element that is common to both the TCA cycle and the ETC.

Complex III: **The ubiquinone/electron complex gives up its electrons to the electron carrier cytochrome c**. **4 protons** are pumped across the IMM. **The cytochrome c/electron complex is passed to Complex IV.**

Complex IV: **The cytochrome c/electron complex gives up its electrons to cytochrome c oxidase. They combine with molecular oxygen (O_2), reducing it to water (H_2O). 2 protons are pumped across the IMM to create a proton gradient (transmembrane electrical potential).**

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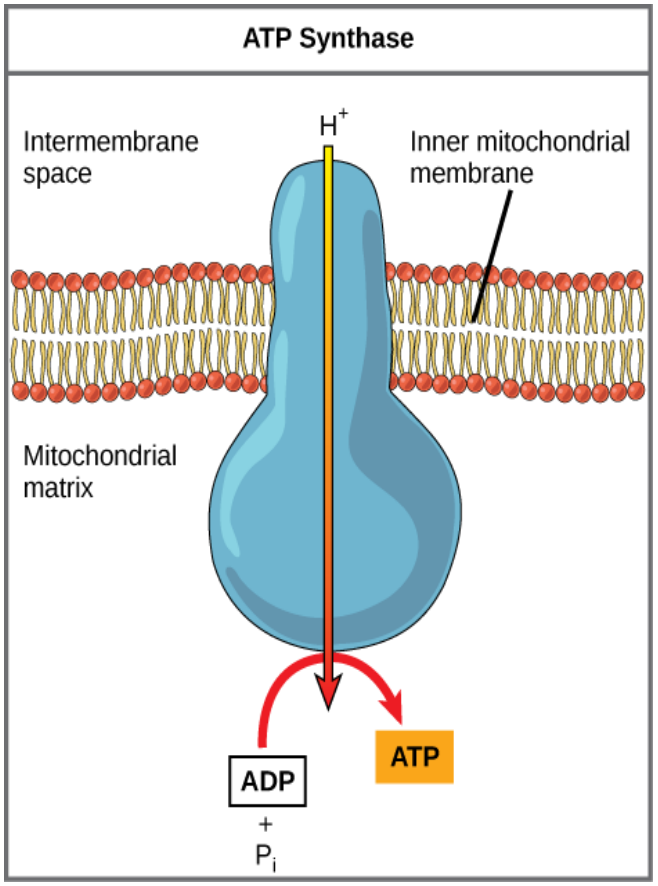
The ETC proton gradient

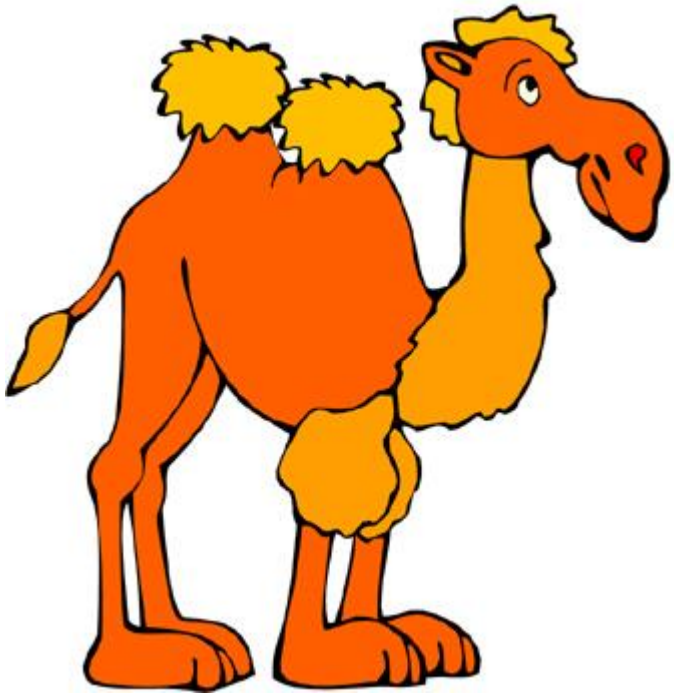
- The electron movement down the ETC releases sufficient energy to pump protons (hydrogen (H) ions) across the inner mitochondrial membrane (IMM) into the intermembrane space.
- This creates an electrochemical gradient across the membrane, known as the mitochondrial transmembrane potential ($\Delta\psi$ (m)), which reflects the energy stored in the electrochemical gradient across the inner mitochondrial membrane.
- In the trillions of ETCs in the 40 trillion cells in the body, more than 10^{21} protons are pumped over the IMM, *every second*. This generates a huge electrical potential (150-200 millivolts), which taken as a whole is equivalent to a bolt of lightning or 1000 times the capacity of household wiring. This potential energy is known as the proton motive force.
- Proton gradients have become known as the ultimate sensors of cellular health.
- The IMM effectively acts as a dam, preventing flow of protons back to the matrix until the proton channel in Complex V opens.

The ETC Complex V (ATP synthase)



- **Complex V (ATP synthase, aka ATPase)** is a large enzyme complex containing **a proton channel through which the protons re-enter.**
- ATP synthase is the smallest known machine. It is a **turbine composed of 2 linked rotary motors** (F0 and F1) **powered by the proton flow.** The influx of protons powers F0, which then turns F1, in a manner similar to a hydroelectric turbine; the head can spin at >100 revolutions per second.
- **A full rotation requires all 10 protons** and **carries out the conversion of ADP to ATP adding a phosphate (Pi),** releasing 3 molecules of ATP.

How can camels live for weeks without water?

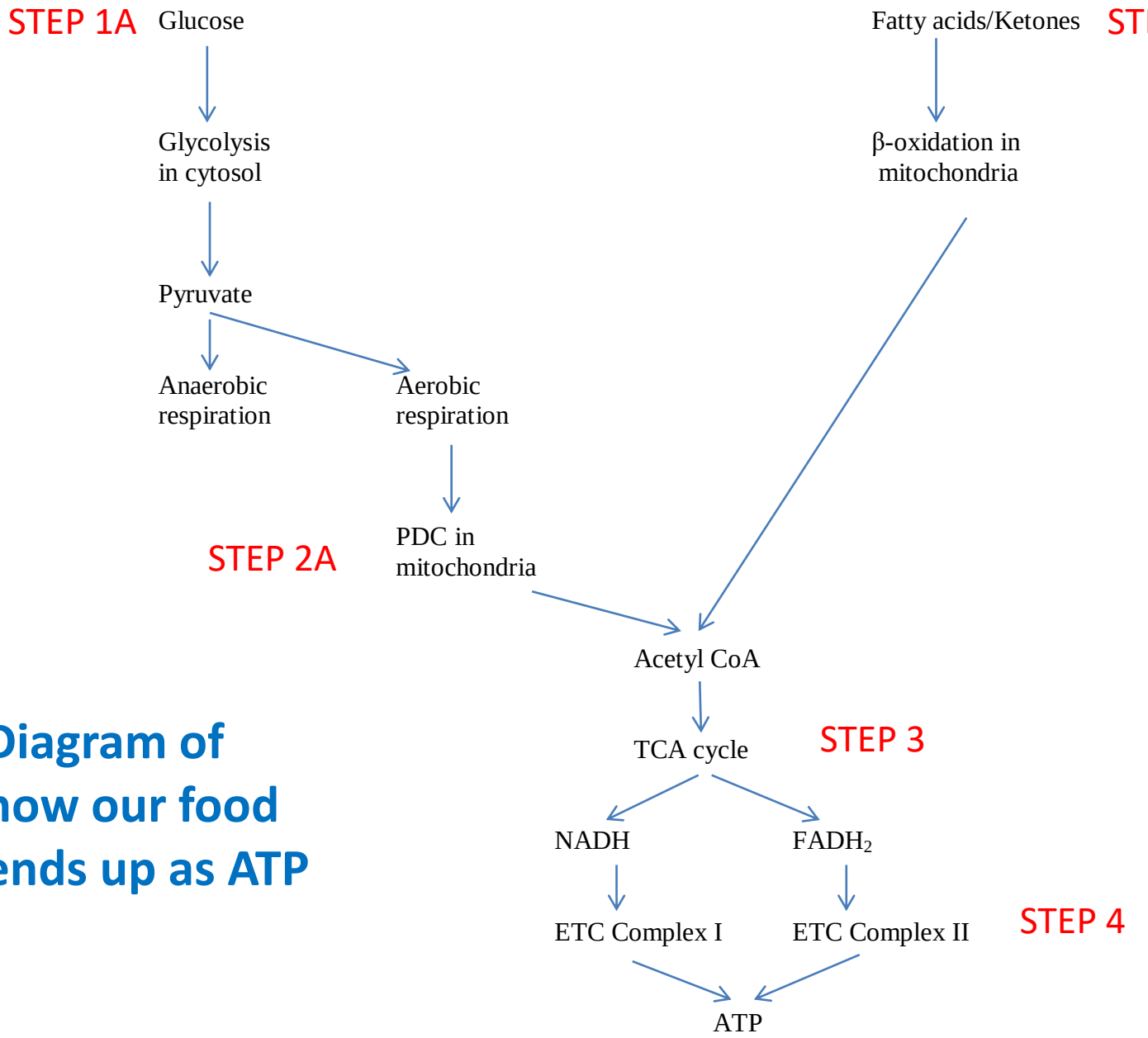


- No, they do not store water in their humps; humps contain fat.
- But because there is so much fat for a camel to burn, there is a very high rate of OXPHOS. The water produced from this sustains the camel for weeks.

Complex V and the proton gradient

- Complex V converts ADP to ATP using the proton gradient to power the conversion. Without the proton gradient, there is no ATP produced; any energy not converted to ATP is released as heat.
- If the proton pumps are blocked, no protons cross the membrane, the membrane gradient collapses and ATP production ceases. This is the means by which bacteria commit suicide when infected with a virus: they perforate their IMMs, this allows protons to flow back into the ETC, collapsing the gradient and signalling to the genes to trigger apoptosis.
- Proton gradients have become known as the ultimate sensors of cellular health.

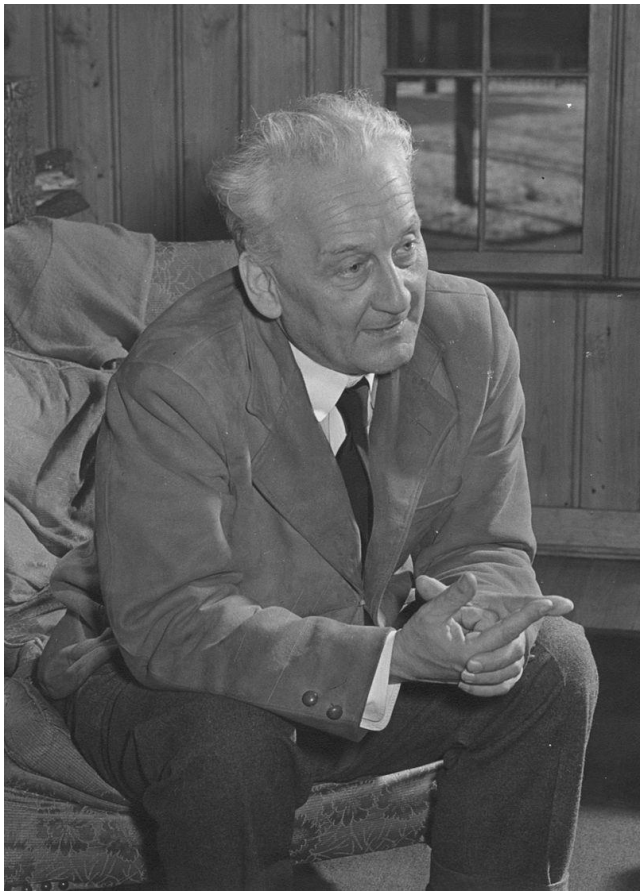
Diagram of how our food ends up as ATP



The ETC as a redox (reduction and oxidation) mechanism

- OXPHOS functions as a redox mechanism: by oxidising (removing an electron) or reducing (donating an electron), with each of the electron carriers being successively reduced (gaining electrons) and oxidised (losing electrons).
- The Complexes are arranged in order of increasing redox potential and increasing electron affinity. Each component of the ETC is alternately oxidised and reduced as electrons pass down the chain.
- OXPHOS proceeds most efficiently when there are equal numbers of oxidised and reduced electron carriers.

Albert Szent-Györgyi



Albert Szent-Györgyi , Hungarian biochemist, discovered vitamin C and the components and reactions of the TCA cycle, for which he won a Nobel prize.

‘Life is nothing but an electron looking for a place to rest’.

Importance of NAD^+

- NAD^+ is one of the most important coenzymes in the body and is known as the 'molecule of youth', as levels decline with age.
- Its main functions include:
  - Supporting healthy metabolism.
  - It is the primary oxidising agent of glycolysis but is in limited supply. Yet glycolysis cannot continue without it.
  - It is required by polyADP ribose polymerases (PARPs), the DNA repair enzymes.
  - It stimulates sirtuins, particularly SIRTs 1 and 3, for longevity and mitochondrial biogenesis.
  - It is essential for an adaptive response to hypoxia through hypoxia-inducible factor 1α (HIF- 1α).
- NAD^+ declines significantly with age, particularly over the age of 40. It is also lowered in chronic inflammation, disrupted circadian rhythms, low oxygen levels, over-consumption, excess alcohol, high blood sugar and insulin levels and extensive DNA damage.
- Symptoms of low NAD^+ include fatigue, brain fog, increasing weight gain, moodiness and depression, poor circulation, infections that linger, rising cholesterol and frailty in old age.
- A reduced supply of substrates (oxygen, ADP, NAD^+, FAD) for the ETC exerts over-riding control of the TCA cycle and can shut it down. In particular it will be inhibited by high ATP:ADP or high NADH:NAD^+.

(Katsyuba E, EMBO J, 2017; Canto C, Cell Metab, 2015; Houtkooper RH, J Cell Biol, 2012; Srivastava S, Clin Transl Med, 2016)

The importance of NADH

- The ratio of NAD+/NADH is critical for energy production. It is part of a negative feedback loop, with inhibition of NADH production in the TCA cycle if the ratio is too low.
- Nicotinamide adenine dinucleotide hydride (NADH) is the biological form of hydrogen. It reacts with the oxygen in every living cell producing energy and water.
- Liquid hydrogen and liquid oxygen are used as rocket fuel; when blended they create a huge explosion. How does our body prevent this happening in the cell? By coupling the a small amount of H to a larger molecule of NAD+, so that it is sufficiently reactive when it encounters oxygen to create energy and water but will not create an explosion.
- Dietary sources of NADH: principally meat and fish. Much is destroyed during cooking. We rely on the NADH components and recycling from NAD+ to ensure adequate NADH levels.
- In the TCA cycle, hydrogen is taken from the food source and transferred to NAD+ to make NADH.
- DNA damaged by doxorubicin can be repaired by NADH. If administered prior to exposure, NADH can also protect liver cells from radiation damage and protects cells from other toxins. (Zhang JR, J Tumor Marker Oncol, 1998; Fa-Quan L, World J Gastroenterol, 2003; Meng X, J Tumor Marker Oncol, 1998; Reibnegger GJ, J Tumor Marker Oncol, 2003).

The importance of nicotinamide phosphoribosyltransferase (NAMPT)

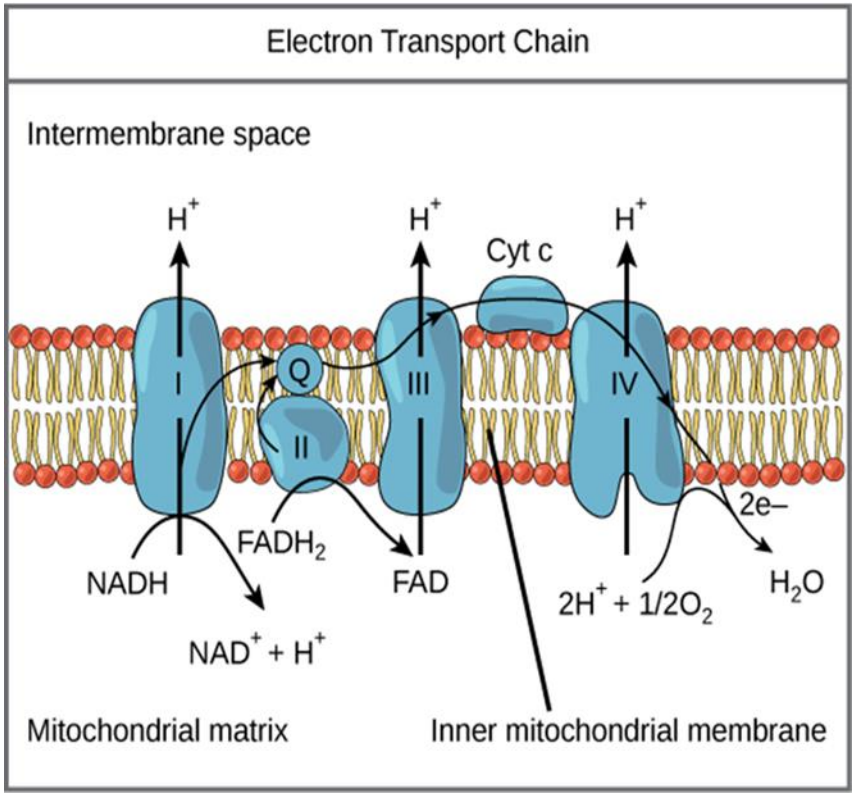
- The regeneration of NAD+ is constrained by the rate-limiting enzyme nicotinamide phosphoribosyltransferase (NAMPT), which converts nicotinamide to nicotinamide mononucleotide to enable NAD+ biosynthesis. It also directly regulates SIRT1 activity.
- Caloric restriction, exercise and stress activate the NAMPT gene, increasing mitochondrial energy production.
- NAMPT is under strong circadian control; any disruption to the circadian cycle compromise the body's ability to create NAD, thus radically limiting its ability to repair DNA damage.

The importance of nicotinamide adenine dinucleotide phosphate (NADP+) and NADP hydrogen (NADPH)

- NADP+ is a coenzyme that functions as a universal electron carrier, accepting electrons and hydrogen atoms to form NADPH. NADP+, in its turn, is created from NADPH in anabolic reactions, particularly the synthesis of cell components. NADPH donates the hydrogen (H) and associated electrons, oxidizing the molecule to create NADP+. NADP+ differs from NAD+ in the presence of an additional phosphate group.
- NADPH is the reduced form of NADP+ and is an essential electron donor, effectively providing a reservoir of electrons for exhausted antioxidants; a key role of NADPH is to recharge glutathione once it becomes oxidised. NADPH is also used in reactions such as lipid and nucleic acid synthesis, where it acts as a reducing agent.
- The only thing that determines the antioxidant status of a cell is the redox ratio of NADPH/NADP+. The regeneration of NADPH is rate-limiting in these reactions and can be increased in ketosis. NADPH can be harmful in excess.
- NAPDH oxidase (NOX) breaks down NADPH. When activated, NOX generates superoxide and is upregulated in a number of pathological conditions and chronic diseases that generate large amounts of oxidative stress. Activation of NOX is a key mediator of pro-inflammatory microglial activation and can induce insulin resistance in adipocytes. However, the useful functions of NOX include aiding the immune system and ensuring that T-cells function properly.

Why do we need $FADH_2$?

- Since there is more NADH than $FADH_2$ and it produces more ATP in the ETC, why do we need $FADH_2$ at all?
- Because in the succinate step of the TCA cycle, the reducing power of succinate is insufficient to reduce NAD^+ so FAD is reduced instead to $FADH_2$. Furthermore, this step takes place in the inner mitochondrial membrane as part of Complex II of the ETC, instead of the mitochondrial matrix as in other steps.
- So since we need FAD here, the mitochondria may as well utilise the resultant $FADH_2$ in the ETC.



Do we live only to support our mitochondria?

- So as long as the mitochondria are provided with 2 basic ingredients, electrons from food and oxygen from the air, this cycle continues millions of times per second in every cell of the body.
- So effectively, we take in oxygen and nutrients to power ATP production, suggesting that we live only to support our mitochondria!

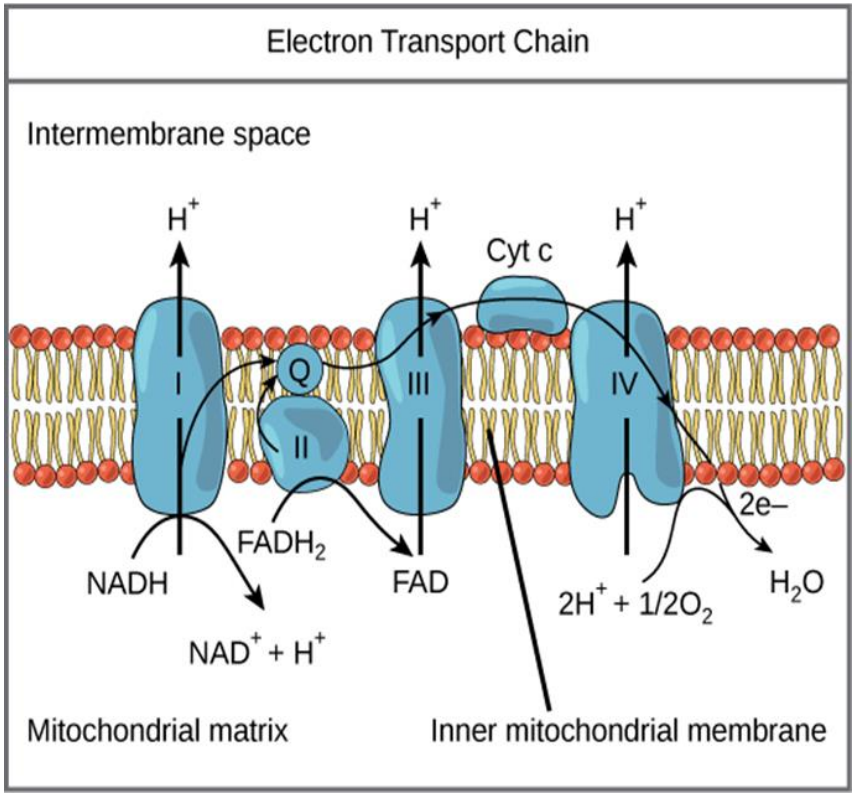
Comparison of glucose and fatty acids as energy producers

- **1 molecule of oxidised fatty acid can produce >100 molecules of ATP, whereas 1 molecule of glucose can only produce around 30 molecules of ATP.** So 1 molecule of a fatty acid yields >3 times the energy of 1 molecule of glucose. (These precise ATP yields are still a matter of debate but the general principal holds.)
- **The oxidation of fatty acids yields 9kg/g of energy, compared to 4kg/g for glucose and protein**; ketones produce approximately the same amount of energy as glucose.
- Glucose is in fact the least efficient fuel for energy production. nevertheless, **glucose is always selected first.**

Reverse electron transport (RET)

(just when you thought it couldn't get any more complicated!)

- RET is produced when electrons from Complex III are transferred back to Complex I, reducing NAD+ to NADH.
- It's impact may be either beneficial or detrimental, depending upon context.
- This process generates a significant amount of ROS, particularly superoxide.
- Reverse electron transport is a property shared with bacteria.



When might RET occur?

- RET could take place with highly reduced ubiquinone pools, high mitochondrial membrane potential, high transmembrane pH, blocked Complexes III or IV, or accumulated metabolic substrates. It can also be caused by excess linoleic acid in the diet.
- It might help to think of it as being like Reverse T3: it can be pathogenic but can also serve a useful purpose in slowing things down.
- It's impact may be either beneficial or detrimental, depending upon context.
- RET is associated with mitochondrial diseases and has also been seen in ischaemia/reperfusion injury following heart attack or stroke.
- RET has been shown to be instrumental for the activation of macrophages in response to bacterial infection, re-organization of the electron transport chain in response to changes in energy supply and adaptation to changes in oxygen levels. In fruit flies (Drosophila melanogaster), stimulating RET extends lifespan.

(Onukwufor JO, Antioxidants, 2019; Komlodi T, J Bioenerg Biomembr, 2018; Robb EL, J Biol Chem, 2018; Scialo F, Front Physiol, 2017)

What happens to the ATP produced?

- **Most of the ATP formed in the mitochondria is moved into the cytosol where it can be utilised by other cellular structures. In the process it loses a Pi and reverts to ADP.**
- Meanwhile, **ADP in the cytosol must be moved into the mitochondria where it can be recycled to ATP.**
- But the mitochondrial membrane is impermeable to ATP and ADP. What to do?
- The **enzyme ATP-ADP translocase (aka translocator protein) exchanges ATP for ADP across the membrane.** This enzyme keeps up the flow of ATP to the cytosol and the flow of ADP back to the mitochondria for recycling in OXPHOS.
- **A magnesium ion ($Mg2^{+}$) is usually attached to ATP** to facilitate its activation and movement to wherever it is needed in the cell.
- ATP is recycled approximately every 10 seconds in a healthy person, but slower recycling indicates slow cellular processes.

Mitochondrial Supercomplexes

- The ETC Complexes exist as single free-moving proteins but are also able to assemble into Supercomplexes, comprising particularly Complexes I, III and IV but it can occur with any of them.
- A Supercomplex of 1 unit of Complex I, 2 units of Complex III and 1 unit of Complex IV is known as the respirasome, but Supercomplexes may have other combinations.
- A Supercomplex decreases the distance between one Complex and the next, allowing electrons to flow more efficiently and decreasing ROS generation.
- Supercomplexes allow OXPHOS to take place even in the absence of electron carriers such as CoQ10 or cytochrome c and provide stability and increased efficiency, while decreasing ROS production. Their assembly appears to depend upon membrane lipids and lipid peroxidation, although oxidative stress can disassemble Supercomplexes.
- Mitochondria can also exist in an interconnected network which allows them to rapidly communicate and distribute energy throughout the body. But if part of the mitochondrial grid stops working, a mechanism acts like a circuit breaker, cutting off the faulty section at intermitochondrial junctions (IMJs) from the rest of the grid so that the rest can continue functioning without problem.

(Sergi D, Front Physiol, 2019; Rigotto G, Oxid Med Cell Longev, 2019; Lenaz G, Adv Exp Med Biol, 2012; Bleck CKE, Nat Commun, 2018)

What can go wrong with energy production?

The principal problems that can occur include:

1. Energy demand but no supply
2. Energy supply but no demand
3. Excessive electron leak and free radical production
4. Lack of magnesium ions (Mg^{2+})

Every mitochondrial development is either good or bad, depending upon context.

1. Energy demand but no supply: principal causes

- **Lack of substrate from the TCA cycle**: insufficient NADH and FADH2 produced; various causes of this.
- **Lack of oxygen**: due to altitude, stroke, MI, COPD etc, causing the electrons to back up, slowing OXPHOS and energy production. The body switches to anaerobic respiration.
- **Imbalanced availability of Complexes**: due to mutations in mitochondrial and nuclear genes, either inherited or acquired.
- **Lack of ETC cofactors**: CoQ10, B vitamins etc.
- **Excessive glucose levels in the cell** prevents the conversion of NADH to NAD+, causing electron accumulation at Complex I, increased ROS and depletion of iron storage.
- **Imbalanced ratio of AMP:ADP:ATP;** a constant ratio is required for optimum cellular function.

ETC: absence of cofactors

- The ETC is a ‘just in time’ operation. Continued flow is critically important: if Complex I has an electron, it can’t take on another until the first electron has been passed on.
- But if there is an absence of CoQ10, oxygen and the various other co-factors, the ETC will be blocked and energy production will revert to anaerobic respiration, producing only 2 molecules of ATP from glycolysis and none at all from fatty acids.
- Note that 2 of the subunits of Complex II (succinate dehydrogenase), also operate in the TCA cycle. This allows the ETC to monitor production of nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) in the TCA cycle as a ‘substrate early warning system’.

Imbalanced ratio of AMP:ADP:ATP

- The cell needs to maintain a constant ratio of AMP:ADP:ATP. Ideally the production of ATP from ADP and its recycling to ADP when energy is released should occur smoothly, with no imbalance in any ingredient.
- This is facilitated by the phosphotransferase enzyme adenylate kinase (ADK). ADK catalyses the interconversion of the adenine nucleotides (ATP, ADP, and AMP) and constantly monitors the nucleotide levels inside the cell, adjusting the levels as necessary.

  ATP (A+3Pi) + AMP (A+1Pi)  ADP (A+2Pi) + ADP (A+2Pi)

- ADK also shuttles ATP to sites of high energy consumption and removes the AMP generated.

Emergency measures when there is an ATP shortage

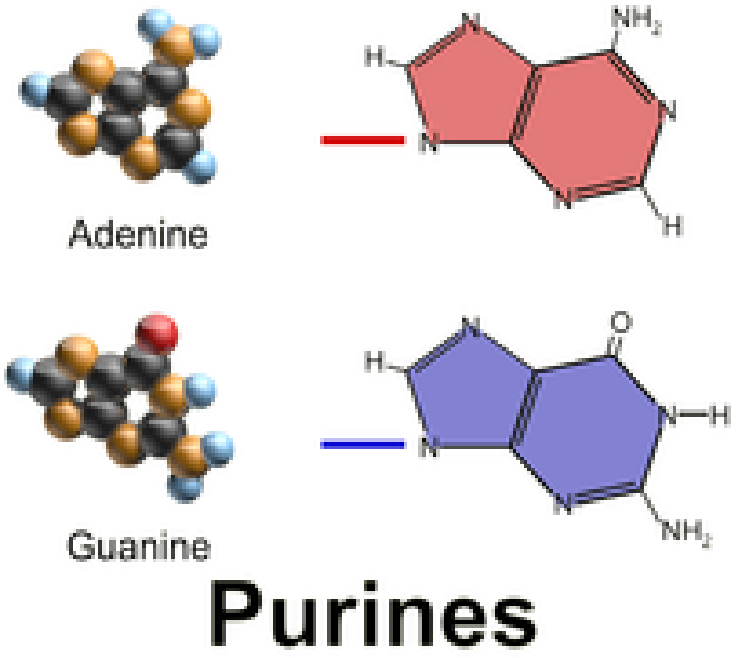
- When ATP is in short supply, the cell will combine two ADP molecules to produce one ATP molecule and one AMP molecule - this is not sustainable in the longer term because the cell will run out of ADP.

  ADP (A+2Pi) + ADP (A+2Pi) ⟺ ATP (A+3Pi) + AMP (A+1Pi)

- This will reduce ADP and increase ATP. But AMP rises whenever the ATP:ADP ratio falls; this will eventually lead to excess AMP as well as deficient levels of ADP.
- So a critical indicator of energy stress is a high AMP:ATP ratio. This activates the energy/nutrient sensor AMP-activated protein kinase (AMPK), which alters enzyme activity and gene expression to lower the AMP:ATP ratio and switch cellular metabolism from anabolic to catabolic mode to generate more ATP (Hardie DG, Biochem Soc Trans, 2011; Hardie DG, Endocrinology, 2003).
- Eventually any excess AMP will be converted to uric acid and excreted in urine as it cannot be recycled.

Rebuilding energy molecules

The building blocks of energy molecules are purines (better known for producing gout!)



- Rebuilding the stock of purines can be a slow process and requires the 5-carbon sugar D-ribose, which is synthesised through 2 possible pathways:
  - De novo purine building – creating D-ribose from glucose from scratch via the pentose phosphate shunt. The slow pathway: it would take the human heart over 100 days to make all its energy molecules this way. And in sick patients, this pathway will be even slower.
  - Purine salvage – instead of eliminating the end products of AMP degradation, the cell retains them as building blocks to accelerate the manufacture of D-ribose.
- Or you can supplement D-ribose to help things along.

2. Energy supply but no demand

- **If not all the ATP is being utilised, it is not converted back to ADP and protons are no longer needed to drive ATP synthase.**
- This causes:
  - **electron flow in the ETC to slow down and stop.**
  - **uncoupling proteins to be activated,** with proton leak.
- **Only increased energy demand can restore the flow, allowing some of the excess ATP to be used up.** This is why exercise is so important for optimum mitochondrial function.
- The proton reservoir in the intermembrane space fills up; when the proton gradient is too high, the small number of electrons produced by the ETC are no longer sufficient to pump the protons against the gradient.

3. Excessive electron leak and resultant free radical production

- **Electron leak occurs when electrons passing down the ETC leak out at Complexes I or III. 1% leakage from Complex I is normal, with a smaller amount from Complex III. Electron leak increases with age.**
- **Electron leak can also occur from excess protons being pumped across the membrane**, which can occur with over-consumption.
- **The leaked electrons react with oxygen to form superoxide.** Superoxide can travel through cell membranes because of its negative charge and in excess can cause free radical propagation in the cell membrane itself and contributes to the ageing process.
- Superoxide is then dismutated to hydrogen peroxide in the mitochondrial matrix and intermembrane space. Hydrogen peroxide is a very destructive free radical.
- Mitochondria are themselves extremely vulnerable to oxidative damage, which can result in mitochondrial dysfunction and contribute to cellular senescence.

(Valez V, Arch Biochem Biophys, 2013; Gustafsson AB, Cardiovasc Res, 2008; Zhao RZ, Int J Mol Med, 2019)

4. Lack of magnesium ions (Mg^{2+})

- **ATP must bind to a magnesium ion (Mg^{2+}) in order to be biologically active.** The active form of ATP is therefore technically a Mg-ATP complex but it is rarely called that.
- Consequently, an **adequate magnesium intake is critical for proper functioning of ATP molecules**.
- The Mg^{2+} ion reduces the electrochemical charge of ATP, helps it move around within the cell and helps it bind with the various cellular structures that require energy.

Other functions of mitochondria

Other than energy production, there are additional mitochondrial functions which are important, and include:

- **Production of reactive oxygen species (ROS) and mitochondrial antioxidants**
- **Heat production and uncoupling**
- **Apoptosis**
- **Storage and regulation of calcium ions (intracellular calcium)**
- Synthesis of haem and iron-sulphur clusters
- Regulation of innate and adaptive immunity
- Stem cell regulation: maintenance, differentiation and reprogramming of pluripotent stem cells (PSCs)
- Synthesis of pregnenolone, the mother of all other steroid hormones
- Synthesis of nitric oxide
- Conversion of ammonia to urea
- Production of ketones in liver mitochondria
- Microbiota cross talk
- Forming contact sites with the nucleus to signal the mitochondrial retrograde response to initiate nuclear stabilisation of pro-survival transcription factors.
- There are several others, including synthesis of melatonin in pineal mitochondria, hormonal signalling through mitochondrial oestrogen receptors, cell signalling, cellular differentiation, controlling the cell cycle, with more possibly not yet discovered.

Mitochondrial production of reactive oxygen species (ROS)

- Oxygen utilised in mitochondrial OXPHOS is the main source of reactive oxygen species (ROS) within a cell, which can occur from 2 sources:
  - **electrons escaping the ETC (electron leak) and joining with oxygen.**
  - **iron from the iron/sulphur clusters, mainly in Complex II.**
- **A healthy level of ROS production is required for important signalling functions.** At higher levels, they can induce an inflammatory response and at extremely high levels they activate apoptosis and autophagy pathways, with a cascade of tissue injury and degradation. Of particular concern is damage to membrane phospholipids (particularly cardiolipin), DNA, enzymes and other proteins.
- Iron can act as a catalyst, transforming H_2O_2 into OH^+ through the Fenton reaction. This is one of the most dangerous reactions that can occur in the body since OH^+ destroys mtDNA, proteins and membranes, contributing to increased systemic inflammation.
- The rate of ROS production is affected by subsequent energy expenditure, the highest rate occurring when ATP demand is low and the proton motive force is high, a scenario which can be caused by excess calorie intake and low ATP demand.
- Up to 5% of the oxygen consumed in mitochondria is converted to ROS: superoxide, hydrogen peroxide (H2O2) and the hydroxyl radical (OH+). A mitochondrion can produce 200,000 free radicals per second.

Prevalence and effect of excess mtROS production

- Mitochondria are themselves extremely vulnerable to oxidative damage to proteins, lipids and DNA, all of which can result in mitochondrial dysfunction and contribute to cellular senescence.
- DNA suffers a free radical attack between 10,000-100,000 times per day; this works out to around 1 attack per second. Not all free radicals derive from the mitochondria; approximately 10% are produced elsewhere, but that still leaves 90% from the mitochondria. Damaged DNA induces mutations, which increase oxidative stress.
- Electron leakage and superoxide generation in human mitochondria is around 1% at birth, increasing to 2-3% in older mitochondria. This causes approximately 10,000 mutations per cell per day and contributes significantly to the ageing process.
- Approximately 1-3% of total electrons flowing through the ETC leaks and reacts prematurely with oxygen at Complexes I and III before reaching Complex V. During normal OXPHOS, generation of partially-reduced oxygen molecules comprises 0.4-4% of the oxygen consumed. ROS production is increased when the electron carriers early in the ETC harbour excess electrons.

(Lee S, Cell Res, 2011; Bao L, J Neuroscience, 2009; Murphy M, Biochem J, 2009; Drose S, Adv Exp Med Biol. 2012; Jin H, Biochim Biophys Acta, 2014; Valez V, Arch Biochem Biophys, 2013; Gustafsson AB, Cardiovasc Res, 2008; Bunkar N, Front Biosci. 2016; Zhao RZ, Int J Mol Med; 2019)

Effects of different types of excess ROS

- Superoxide: Because of its negative charge and poor membrane permeability, superoxide is relatively unreactive, although in excess it can cause free radical propagation in the cell membrane itself and contributes to the ageing process. It can react rapidly with nitric oxide (NO) to form the potent oxidant and nitrating agent peroxynitrite and subsequently other reactive nitrogen species (RNS). Moreover, it is able to damage some mitochondrial iron-sulphur cluster-containing proteins. Superoxide is then dismutated to hydrogen peroxide in the mitochondrial matrix and intermembrane space.
- Peroxynitrite: Peroxynitrite can inhibit Complexes I and II of the ETC, as well as blocking ATP synthase and causing mutations in mtDNA. Whenever Complex I is inhibited, more ROS are produced, contributing to a positive feedback loop.
- Hydrogen peroxide (H2O2) in excess is a very destructive free radical, that is stable, membrane permeable and has a relatively long half-life enabling diffusion within the cell. As a redox active species, H2O2 can inactivate some enzymes by oxidizing their thiol groups, although it is unable to oxidize DNA or lipids directly; it can also react with metals. Hydrogen peroxide can be decomposed by cytosolic and mitochondrial antioxidant systems such as glutathione peroxidase (GPx), catalase and thioredoxin reductase. If not removed, it can further produce the highly reactive hydroxyl radical in the presence of Fe2+ cations via the Fenton reaction.
- The hydroxyl radical has a strong oxidising potential and can damage virtually every type of macromolecule close to their site of origin, making it an extremely dangerous compound. Furthermore, unlike superoxide and hydrogen peroxide, which can be detoxified by an enzymatic conversion, no enzymatic routes are known for eliminating hydroxyl radicals. Non-enzymatic mechanisms for scavenging peroxyl radicals include several antioxidants such as vitamin E and glutathione.

Mitochondrial antioxidants

- **Mitochondria produce their own endogenous antioxidants: MnSOD in the matrix and CuZnSOD in the intermembrane space**. Their principal job is to **protect the lipid bilayer and mtDNA against the superoxide radical**; low levels of SOD are associated with many health conditions.
- In a healthy cell with normal levels of ROS production, our antioxidant defences are adequate to keep normal ROS levels low but **these antioxidants are in short supply and will be inadequate if ROS levels rise.**
- Most of the **endogenous and exogenous antioxidants found in the cytosol generally cannot penetrate the outer mitochondrial membrane**.
- Furthermore, in the ETC CoQ10 and lipoic acid help prevent electron loss, while the cellular enzymes GPx, glutathione reductase and catalase help prevent oxidative damage.
- When the number of free radicals outnumbers our endogenous antioxidant enzymes, oxidative damage occurs in the mitochondria, particularly to membrane phospholipids (particularly cardiolipin), DNA, enzymes and other proteins.
- An imbalance in ROS and antioxidants, particularly from excess superoxide and H2O2, is associated with CVD and can cause impaired mitochondrial-nuclear cross-talk in endothelial cells and neutrophils.

Heat production and uncoupling

- **When there is over-supply of ATP and not enough demand, uncoupling occurs if the individual does not start using up the excess ATP** (exercise or caloric restriction)**.**
- **Coupling is where the proton gradient powers ATP synthase to produce ATP; uncoupling is where the OXPHOS is uncoupled from ATP production as protons do not flow back to activate ATP synthase.**
- **Instead the protons re-enter the mitochondrial matrix through the membrane mediated by uncoupling proteins (UCPs). This is known as proton leak**.
- Uncoupling can be pathogenic or it can be beneficial, depending upon context. For example, MDMA (aka 'Ecstasy') is known for inducing excessive heat production, a sure sign that uncoupling is occurring.
- In practice there is an element of both depending upon conditions; no ETC is perfectly coupled. In resting mammals, up to 25% of the proton gradient is naturally dissipated as heat.
- Explaining uncoupling: www.youtube.com/channel/UC67CzGY-98LwOAWca450UjQ

(Ledesma A, Genome Biol, 2002; Cadenas S, Biochim Biophys Acta Bioenerg, 2018; Rousset S, Diabetes. 2004; Nicolson GL, Integrative Medicine: A Clinician's Journal. 2014)

Uncoupling proteins (UCPs) and proton leak

- **UCPs are effectively proton channels**, since they allow unused protons to leak back into the mitochondrial matrix.
- In total there are **5 uncoupling proteins (UCPs 1-5)**.
- Today we will mostly be concerned with **UCP1** (found in **brown adipose tissue**). UCP1 allows the **protons re-entering the mitochondrial matrix to be dissipated at heat.**
- UCP 1 is what keeps **hibernating mammals** alive during winter.
- Similarly, there the Inuit, who need heat but not much energy, have much higher levels of BAT (and UCP1) than African populations, who need energy but little heat.
- Proton leak decreases the electrochemical proton gradient despite continuation of OXPHOS.

Uncoupling proteins (UCPs 1-5)

- UCP1 is expressed in brown adipose tissue (BAT), where it generates heat by non-shivering thermogenesis (endothermy).
- UCP2 is present in most cell types but importantly in pancreatic β-cells, the cardiovascular system, hippocampal cells and neurons. Its main function appears to be protction against ROS.
- UCP3 is present in skeletal muscle and white adipose tissue (WAT) - may not always induce proton leak.
- UCP4 and UCP5 are found in neurons of the CNS.
- UCP gene expression is upregulated by thyroid hormone, noradrenaline, adrenaline, leptin and other ligands for nuclear receptors, including peroxisome proliferator-activated receptors (PPAR) and retinoid-X receptors.
- The entire scope of functions is not yet known.

(Saito M, Nihon Yakurigaku Zasshi, 2001)

UCP1 in humans: ethnic differences

- As we saw with mitochondrial genes, people who lived in tropical Africa need more energy and less heat, while the Inuit need more heat but not so much energy.
- As might be expected, the Inuit population have very high levels of BAT and their mitochondria do not leak electrons to the same extent as those in warm climates. Consequently they have less ROS production and a lower incidence of degenerative disease.
- But African populations have much smaller quantities of BAT as more of the proton gradient is used to produce energy but there is a high rate of electron leakage, ROS production and incidence of degenerative disease. Physical activity for those of African descent is critical to avoid electron leakage (high ATP supply must be matched by demand).

(Mishmar D, Proc Natl Acad Sci USA, 2003)

How polar bears keep warm

- Polar bears can survive in temperatures as low as -55°C and wind speeds of up to 50km/hr.
- The thick layer of fat under their skin, known as blubber, has a very high proportion of BAT, hence large amounts of UCP1.
- The majority of the food they eat goes not in producing energy but in keeping warm.
- As with the camel, polar bears rarely need to drink water; they can get all they need from Complex IV.
- With global warming, bears are suffering the loss of their habitat, requiring more energy to go further afield to find food, leaving fewer protons for heat generation.

Inducing UCP1 as a therapeutic target

- Inducible uncoupling is an obvious therapeutic target for metabolic disease, particularly upregulating of UCP1 (thermogenin) in brown adipose tissue (BAT) through cold exposure, exercise, fasting or pharmacological manipulation. BAT has a large number of mitochondria and many UCPs.
- UCP1 controlled directly by sympathetic nerves, principally through the beta-adrenergic action of norepinephrine, and is also involved in fatty acid mobilisation and oxidation. UCP1 synthesis is increased proportionally to temperature and duration of cold exposure.
- 2,4-dinitrophenol (DNP) was found to be an activator of UCP 1 and was developed as a weight loss drug in the 1930s. In rats with diet-induced obesity, the controlled release of DNP reduced hypertriglyceridaemia, insulin resistance, hepatic steatosis and diabetes. However, its use in high doses came with severe side-effects, including hyperthermia and a number of deaths due to lack of tissue selectivity.
- But this demonstrates that deliberate activation of UCP 1 could help in metabolic diseases. Other pharmaceuticals are under development.
- Both salicylic acid and E (ecstasy) act as uncoupling agents and will decrease production of ATP and increase body temperature if taken in excess.

(Larson CJ, Handb Exp Pharmacol, 2019; Goldgof M, J Biol Chem, 2014; Perry RJ, Science, 2015; Ost M, Biochimie, 2017; Ricquier D, Front Endocrinol, 2011; Fromme T, Am J Physiol Regul Integr Comp Physiol, 2011)

More on UCP2

- The main function of UCP2 appears to be the reduction of mitochondria-derived reactive oxygen species (ROS) within a negative feedback loop. In pancreatic β-cells, UCP2 is considered a metabolite transporter rather than a strict UCP. UCP2 is also found in hippocampal cells and neurons, where it stimulates mitochondrial biogenesis.
- Activators of UCP2 include fatty acids and certain ROS by-products that are also reactive. Current scientific consensus states that UCP2 and UCP3 perform proton transportation only when activated by ROS.
- Increased UCP2 activity is associated with cell degeneration, decreased insulin secretion and T2D.
- UCP2 could be neuroprotective by reducing mitochondrial Ca^{2+} uptake and preventing mitochondrial accumulation of reactive oxygen species (ROS) following cerebral ischemia.
- So there are a number of apparent contradicitions concerning the functions of UCP2. No dobut they will become clear in time.

(Broche B, Diabetes, 2018; Paradis E, Trends Mol Med, 2003; Tian XY, Front Physiol, 2018; Sreedhar A, Mitochondrion, 2017)

More on UCP3

- UCP3 is found mainly in adipocytes and skeletal and cardiac muscle cells.
- Its function is still not well understood, mainly because it appears not to cause proton leak or physiological thermogenesis.
- Studies show a role for UCP3 in energy metabolism, particularly fatty acid metabolism, with UCP3 being upregulated when fatty acid supply to the mitochondria exceeds the capacity to beta-oxidise them and downregulated when oxidative capacity is improved. Thus UCP3 protects mitochondria against lipid-induced oxidative stress.

(Garvey WT, J CLin Invest, 2003; Nabben M, Physiol Behav, 2008; Costford SR, Appl Physiol Nutr Metab, 2007)

More on UCPs 4 and 5

- UCP4 and UCP5 are found principally in the neurons of the CNS, where they demonstrate different characteristics to other UCPs, suggesting different roles, although there is limited scientific evidence as yet.
- Both UCP4 and UCP5 can uncouple OXPHOS, which reduces oxidative stress. They also exert a protective influence against environmental toxins but are implicated in neurological conditions. UCP4 increases astrocyte pH, which acidify as a consequence of glutamate uptake, and reduce the efficiency of mitochondrial ATP production, allowing glycolysis to compensate. This spares neurons the damaging H_2O_2 production.
- Nevertheless, UCP4 overexpression can also increase ATP synthesis by interacting with Complex II to protect neurons against energy crisis.

(Perreton Lambert H, J Biol Chem, 2014; Ho PW, PLoS One, 2012; Ramsden DB, Brain Behav, 2012)

Apoptosis: programmed cell death

- **When cells become worn out or damaged beyond repair, apoptosis is triggered in order to preserve the integrity of tissues and organs by killing defective cells.** It is a deliberate and beneficial action, carried out for the greater good of the whole, so that the damage is not replicated in daughter cells.
- Apoptosis can be beneficial or pathogenic, depending upon context. Defective apoptotic processes have been implicated in a wide variety of diseases. Excessive apoptosis causes the loss of neurons in neurodegenerative disease and the decline in brain function with ageing, whereas an insufficient apoptosis results in uncontrolled cell proliferation, as seen in cancer.
- Normal embryonic development: 80% of all neurons formed in the early months are destroyed before birth. This allows the brain to be 'wired' with greater precision, facilitating functional connections between specific neurons and the formation of neuronal networks. It has been suggested that autism may be due to failure to eliminate redundant neurons.
- Apoptosis: a term first ascribed to Hippocrates to mean the leaves falling in Autumn.

(Kinally KW, Apoptosis, 2007; Kinally KW, Biochim Biophys Acta, 2011; Ryu SY, Biofactors, 2010)

Principal regulators of apoptosis

- Among the principal regulators of apoptosis, Bax, Bad, Bid and Bak are pro-apoptotic, while Bcl-2 proteins and Bcl-xl may be either pro- or anti-apoptotic.
- The pro-apoptotic Bcl-2 proteins normally reside in the cytosol, where they act as sensors of cellular damage or stress. They translocate to the outer mitochondrial membrane following death signalling. The anti-apoptotic proteins reside in the outer mitochondrial membrane and inhibit cytochrome c release.
- **The balance of mitochondrial pro- and anti-apoptotic proteins determines whether apoptosis will take place.**
- **If the pro-apoptotic proteins predominate,** they will cause the **opening of the mitochondrial permeability transition pore (mtPTP) in the inner membrane and the mitochondrial apoptosis-induced channel (MAC) in the outer membrane.**
- This will allow **cytochrome c release into the cytosol,** together with other pro-apoptotic molecules. In the cytosol, **cytochrome c** binds with other proteins to **activate caspase 3**, the apoptosis effector protein.
- Note that whether cytochrome c acts as the **Complex III electron carrier** in the inner mitochondrial membrane (IMM) or **apoptosis-inducer** through activation of caspase 3 is **purely down to location (IMM or cytosol)**. This is why cytochrome c is not available as a supplement to support the ETC: because it would kill the cell since it would have to enter the cytosol before the mitochondria, where it would immediately activate apoptosis.

Cell death

- Cells can undergo 2 forms of cell death:
  - Necrosis: violent, unexpected, swift, provokes an inflammatory reaction. 'Blood on the carpet'.
  - Apoptosis: premeditated and silent : 'The cyanide capsule'.
- Necrosis: Traumatic cell death that results from acute cellular injury. Following the opening of the mitochondrial permeability transition pore (mtPTP) in the inner membrane, the cell swells to abnormal size. It then ruptures and organelles disintegrate. Associated with loss of control of ionic balance, uptake of water, swelling and cellular lysis.
- Apoptosis: Programmed cell death that occurs in multicellular organisms. Events leading to apoptosis include cell shrinkage, nuclear fragmentation, chromosomal DNA fragmentation, chromatin condensation and global mRNA decay. The average adult human loses between 50 and 70 billion cells each day due to apoptosis.

The apoptosis pathways

- The apoptosis decision is made in the mitochondria through 2 possible pathways:
  - Extrinsic: extracellular ligands bind to cell surface 'death receptors', which activate caspases. The ligands include inflammatory cytokines such as TNF-alpha. This is a non-mitochondrial pathway.
  - Intrinsic: cell damage, particularly DNA damage, signals the mitochondria to activate caspases. Causes can be environmental toxins, pharmaceuticals, physical stresses, oxidative stress, viruses and bacteria. These intrinsic triggers almost invariably cause loss of the electrochemical gradient across the inner mitochondrial membrane, followed by a burst of oxygen free radicals, which is always a trigger for apoptosis.
- Both extrinsic and intrinsic pathways activate initiator caspases, followed by effector (executioner) caspases, which then kill the cell by degrading proteins indiscriminately.
- There is cross-talk between these two signalling pathways, indicating that they are not independent of each other.
- So the mitochondria integrate signals arriving from different sources, and if the balance of signals indicates that the cell is damaged beyond repair, apoptosis is activated.

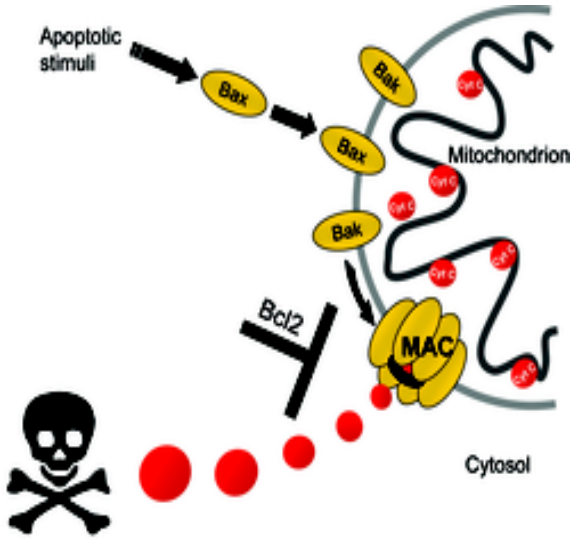
Mechanisms of apoptosis

- Permeabilisation of the outer mitochondrial membrane is a critical development in apoptosis and triggers release of apoptogenic factors, such as cytochrome c, from the mitochondrial intermembrane space into the cytosol where they ensure propagation of the apoptotic cascade and execution of cell death.
- Two mechanisms have been proposed as triggering this permeabilisation: the opening of the mtPTP in the inner membrane and the mitochondrial apoptosis-induced channel (MAC) in the outer membrane. mtPTP can facilitate both apoptosis and necrosis.
- Activation of MAC is regulated by the Bcl-2 family of proteins. MAC provides specific pores in the outer membrane for the passage of intermembrane proteins, such as cytochrome c, into the cytosol.
- There is cross-talk between the MtPTP and MAC.

(Kinally KW, Apoptosis, 2007; Kinally KW, Biochim Biophys Acta, 2011; Ryu SY, Biofactors, 2010)

How the mitochondrial apoptosis-induced channel (MAC) works

- The opening of the mitochondrial apoptosis-induced channel (MAC) in the outer mitochondrial membrane causes it to become highly permeable, so that it loses its proton gradient and hence its electrical charge.
- Activation of MAC is regulated by the Bcl-2 family of proteins. MAC provides specific pores in the outer membrane for the passage of intermembrane proteins, such as cytochrome c, into the cytosol.
- There is cross-talk between the mtPTP and MAC.
- An apoptotic signal causes release of cytochrome c and other pro-apoptotic factors through the MACand mtPTP into the cytosol.



Apoptosis via the mtPTP

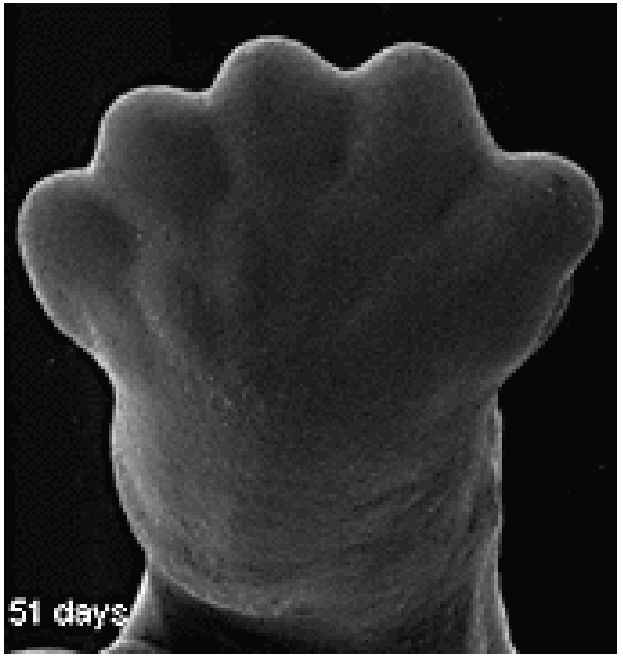
- The opening of the mtPTP triggers the dissipation of the proton gradient created by electron transport, causing the uncoupling of oxidative phosphorylation and increasing the permeability of the mitochondrial membrane.
- It also enables water to enter the mitochondrial matrix, resulting in swelling of the intermembrane space and rupturing of the outer membrane, causing the release of apoptogenic proteins.
- Cyclophilin D, located in the mitochondrial matrix, is a modulatory component of the mtPTP. The permeabilising of the membrane allows influx of cyclophilin D from the cytosol into the mitochondrial matrix, increasing the matrix volume and disrupting the outer membrane, inducing apoptosis.
- Cyclophilins are a family of proteins named after their ability to bind to cyclosporin, an immunosuppressant which is usually used to suppress rejection after organ transplants.

Cytochrome c

- Cytochrome c is normally loosely bound to lipids, particularly cardiolipin in the inner mitochondrial membrane, and is released when the lipids are oxidised by free radicals through the actions of the proteins Bax and Bak.
- But cytochrome c is a protein and is too large to penetrate the outer membrane to escape into the cytosol. There are 2 possible pathways:
  - The mitochondrial permeability transition pore (mtPTP) opens as a result of oxidative stress to release cytochrome c and/or
  - Proteins of the bcl-2 family migrate to the mitochondria and form a similar pore in the outer membrane. But some bcl-2 proteins protect against apoptosis, so it depends which are more numerous.
- Cytochrome c then binds with various proteins, creating the apoptosome, which activates the apoptosis effector caspase 3. With the addition of ATP, the cell is then broken down into apoptotic bodies wrapped in a membrane, which are phagocytosed.
- The link between life (cytochrome c is the Complex III electron carrier in the ETC) and death (it activates caspase 3) is purely down to location (inner mitochondrial membrane or cytosol). This is why cytochrome c is not available as a supplement to support the ETC: because it would kill the cell since it would have to enter the cytosol before the mitochondria, where it would immediately activate apoptosis.

Beneficial apoptosis example

- Foetal fingers and toes are initially webbed. Apoptosis causes the webbing to disappear, leaving us with 10 separate digits.
- It seems in general that the shaping of the body is by subtraction (removal of redundant tissue), not addition.
- “It is normal to give away a little of one’s life in order not to lose it all” (Albert Camus, Carnets: 1935-1942)



Storage and regulation of calcium ions

- **Intracellular calcium (Ca^{2+}) is an important signalling molecule and can also trigger cell death, so cytosol levels must be kept within tight limits.**
- **To facilitate this, excess calcium may be stored in either the endoplasmic reticulum (ER), the principal storage site, or the mitochondria,** increasing calcium uptake via specialised calcium transporters to buffer cytosolic levels and promote cellular homeostasis.
- **The mitochondria-associated ER membrane (MAM) facilitates regular cross-talk between the endoplasmic reticulum and the mitochondria to inform on calcium levels.**
- Intracellular calcium can be beneficial or pathogenic, depending on context. It can act as an accelerator to drive greater ATP synthesis, as it controls 3 mitochondrial enzymes: pyruvate dehydrogenase, isocitrate dehydrogenase and oxoglutarate dehydrogenase. This enables the mitochondria to match ATP production to the required cellular energy levels. So intracellular calcium regulates and is regulated by the mitochondria.
- Calcium ions are taken up into the matrix by the mitochondrial intracellular calcium uniporter (MICU) on the inner mitochondrial membrane, primarily driven by the membrane potential.
- Calcium overload in the mitochondria will dissipate the proton gradient (membrane potential), triggering apoptosis, increasing ROS binding to cardiolipin and loss of the mitochondrion. Calcium homeostasis is frequently lost in chronic disease.

(Raturi A, Biochim Biophys Acta, 2013; Cali T, Cell Calcium, 2012; Guardia-Laguarta C, Mov Disord, 2015; Schon EA, J Alzheimer's Dis, 2010; Tubbs E, Diabetes, 2014; Chaudhari N, Front Cell Neurosci, 2014; Yi M, J Cell Biol, 2004; Phillips CB, Elife, 2019)

Rachel Nicol PhD

Calcium signalling in the cell

- Intracellular calcium is perhaps the most important 2nd messenger within a cell. A cell receives external stimuli such as hormones or neurotransmitters (the 1st messenger) and passes on the signal to calcium as the 2nd messenger.
- Calcium signalling operates by increases and decreases in intracellular content and by calcium waves and pulses. A calcium wave is a momentary increase in cytosolic calcium succeeded by another momentary increase. They may occur in just one cell or across many cells simultaneously. Calcium waves in cardiac myocytes can enable contraction, while their mitochondria help conserve cardiac energy by dampening excessive heart muscle contraction.
- Excess intracellular calcium can also lead to increased nitric oxide synthase (NOS), a calcium-responsive enzyme producing nitric oxide, which can impair mitochondrial function and the ETC, on its own and with its more toxic by-product peroxynitrite, leading to lower ATP output. Healthy mitochondria can increase ATP production and absorb the excess calcium to overcome this, but unhealthy mitochondria in high calcium environments cannot absorb the excess calcium.

(Yi M, J Cell Biol, 2004; Phillips CB, Elife, 2019; Bonora M, Nat Rev, 2019; Brookes PS, Mitochondrion, 2004)

Iron, haem and iron-sulphur clusters (ISCs)

- Iron is essential for mitochondrial respiration as it transfers oxygen from haem molecules into the ETC.
- There are 2 important iron-related processes that take place in the mitochondria: the construction of both haem and iron-sulphur clusters. Mitochondria also posses their own ferritin (an iron storage protein).
- Of the 8 steps required to produce haem, the 1st and last 3 steps take place in the mitochondria, with the remainder occurring in the cytosol. The first step is vitamin B6 dependent. In the final step, iron is inserted into the haem molecule by ferrochelatase.
- Cells require mitochondrial ISC synthesis not only to ensure the biogenesis and function of a large number of ISC-dependent enzymes but also to maintain a balance between iron uptake and iron utilisation. The cell responds to reduced mitochondrial ISC synthesis with a rapid increase in cellular iron uptake and accumulation in mitochondria.
- However, the body has a limited ability to excrete iron, so it can easily build up. Excess iron can encourage oxidation and tissue damage, particularly in the heart, liver and brain. It reacts with hydrogen peroxide in the inner mitochondrial membrane, forming hydroxyl radicals.

(Paul BT, Expert Rev Hematol, 2017; Isaya G, Front Pharmacol, 2014; Richardson DR, Proc Natl Acad Sci, 2010; Atamna H, Arch Biochem Biophys, 2002)

Iron-sulphur clusters

- Iron-sulphur clusters (ISCs) are located in the mitochondria, cytosol, endoplasmic reticulum and nucleus, where they contribute to various core cellular functions. ISCs are also a bacterial property.
- Iron-sulphur cluster functions:
  - essential components of tricarboxylic acid (TCA) cycle enzymes and enzymes for DNA synthesis and repair.
  - enable electron transfer in Complexes I, II and III of the ETC. There are more than a dozen iron-sulphur clusters in each respiratory chain.
  - make up ferrochelatase, the enzyme that inserts iron into haem.
- Absence of functioning iron-sulphur clusters is seen in several mitochondrial diseases including Friedreich's ataxia, where there are low levels of frataxin, a protein that help to assemble iron-sulphur clusters in mitochondria.

(Alfadhel M, Neurosciences (Riyadh). 2017; Gakh O, J Biol Chem. 2016)

Regulation of innate and adaptive immunity

- In immune cells, mitochondria participate in signalling through ROS production, metabolite availability and by physically acting as scaffolding for protein interaction. Mitochondrial signals appear to be necessary for the immune cell to fulfil its specific role in the innate and adaptive immune response. (Weinberg SE, Immunity, 2015)
- Mitochondria lie at the heart of immunity. Amongst other immune functions, extracellular ATP and mtDNA act as a danger-associated molecular patterns (DAMPs), and the outer mitochondrial membrane is a platform for signalling molecules and for the inflammasome. Mitochondrial biogenesis, fusion and fission have roles in aspects of immune-cell activation. Most important, TCA cycle intermediates such as succinate, fumarate and citrate engage in processes related to immunity and inflammation, in both innate and adaptive immune cells. (Mills EL, Nature Immunity, 2017)
- Mitochondria regulate innate immunity at multiple levels and can serve as DAMPs themselves, forming platforms for downstream signalling and responding as effectors, primarily through the generation of ROS, to facilitate the antimicrobial host-cell response. (Tait SWG, J Cell Sci, 2012)
- In immune activation, mitochondria shift from ATP production to immune support, so we have less ATP. This is why we feel tired with an infection.

Mitochondrial involvement in stem cells

- Our body's ability to renew tissue throughout our lifetime is dependent on reservoirs of stem cells. Stem cell populations do not decline with age but they do lose their restorative potential; functional stem cell decline is associated with eventual organ malfunction and failure.
- Mitochondria play a crucial role in the maintenance, differentiation and reprogramming of pluripotent stem cells (PSCs), which can self-renew and differentiate into any tissue. Increased anaerobic glycolysis is required for PSC differentiation, because it produces energy at a faster rate with lower ROS generation, despite the lower ATP levels. Mitochondrial homeostasis in the pluripotent state relies on mitochondrial fission and fusion, as well as degradation through mitophagy.
- Stem cells, can rescue stressed cells by providing healthy mitochondria. The stressed cells grow 'tunnelling nanotubes' (TNTs) to invite healthy cells to donate mitochondria. This is particularly important in slow-growing or post-mitotic cells such as neurons and cardiac myocytes. However TNTs may also rescue cancer cells from apoptosis.
- Differentiated stem cells display a more developed and functional mitochondrial network and rely heavily on OXPHOS. Accordingly, mitochondrial function directly regulates stem cell differentiation through various mechanisms involving ROS production, metabolomic modifications, and modulation of the redox and energy status, with cross-talk between the various pathways. A 2015 study followed the fates of old and young organelles during stem cell division and found that in aged stem cells mitochondria were distributed asymmetrically between daughter cells, while daughter cells that received fewer mitochondria maintained fewer stem cell traits compared to young stem cells.
- Inhibition of mitochondrial fission disrupted various processes and caused loss of stem cell properties in daughter cells.
- Hence it appears there may be built-in mechanisms for stem cells to asymmetrically sort young and old mitochondria, allowing at least one daughter cell to maintain a large pool of healthy mitochondria, while another receives the defective mitochondria in an attempt to maintain its stem cell properties for as long as possible.

(Wang X, Cell Death Differ, 2015; Katajisto P, Science, 2015; Xu X, Cell Metab, 2013; Wanet A, Stem Cells Dev, 2015)

Synthesis of pregnenolone

- Synthesis occurs in the mitochondria by first importing cholesterol and then converting it to pregnenolone by the haem-containing enzyme CYP11A1, assisted by the iron-sulphur cluster enzyme adrenodoxin.
- Both enzymes are dependent on mitochondrial iron metabolism for their own synthesis.
- Prenenolone synthesis can only occur if there is a correct balance between mitochondrial fission and fusion, because mitochondria need to be fused and elongated. Researchers have found a direct correlation between levels of fused mitochondria and progesterone concentrations.
- Since insulin resistance is one of the main reasons why mitochondria fail to fuse appropriately, insulin resistance can have a major impact on steroid hormone synthesis.
- Oestrogen, in turn, can increase Nrf1, which stimulates mitochondrial biogenesis.

(Strushkevich N, Proc Natl Acad Sci USA, 2011; Duarte A, PLoS One, 2012; Mattingly KA, Mol Endocrinol, 2008)

Synthesis of nitric oxide (NO)

- Mitochondria produce nitric oxide (NO) via Ca^{2+}-sensitive mitochondrial NO synthase (mtNOS).
- This NO regulates mitochondrial oxygen consumption and transmembrane potential via a reversible reaction with cytochrome *c* oxidase.
- The reaction of this NO with the superoxide anion yields peroxynitrite, which irreversibly modifies susceptible targets within mitochondria and induces oxidative and/or nitrosative stress.
- Mitochondria may play a role in metabolising nitrite, which in turn can be a regulator of mitochondrial function.

(Ghafourifar P, Trends in Pharmacol Sci, 2005; Shiva S, Nitric Oxide, 2010)

Conversion of ammonia to urea

- As protein is deaminated, ammonia is released.
- But ammonia is toxic to the body, particularly the brain astrocytes, located near synapses, and the nervous system as a whole by initiating an inflammatory response via activation of neutrophils (white blood cells).
- So ammonia is converted to urea in the urea cycle. As with haem production, the synthesis of urea is divided between the mitochondria and cytosolic enzymes and occurs mainly in the liver. The urea is then flushed out by the kidneys.
- Patients with cirrhosis of the liver can develop psychiatric symptoms due to poor ammonia clearance, while high ammonia levels are commonly seen in autism.

Production of ketones in liver mitochondria

- The liver produces ketones because it cannot metabolise large amounts of dietary fat.
- β-oxidation breaks the long carbon chains of fatty acids into multiple 2-carbon acetyl CoA molecules.
- But these cannot all fit into the liver TCA cycle, so they are packaged into ketone bodies.
- Brain, kidneys, heart and muscles can convert them to acetyl CoA so they can enter the TCA cycle.

(Akram M, J Med Food, 2013)

Mitochondrial/microbiota crosstalk

- New discoveries in metagenomics and clinical research have highlighted the importance of the gut microbiota for human health through the regulation of the host immune response and energetic metabolism.
- The microbiota interacts with host cells in particular by intermingling with the mitochondrial activities. This mitochondria microbiota crosstalk is intriguing because mitochondria share many common structural and functional features with the prokaryotic world. Several studies reported a correlation between microbiota quality and diversity and mitochondrial function
- The mitochondrial production of reactive oxygen species (ROS) plays an important role during the innate immune response and inflammation, and is often targeted by pathogenic bacteria. Data suggest that excessive mitochondrial ROS production may affect ROS signalling induced by the microbiota to regulate the gut epithelial barrier.
- Finally, the microbiota releases metabolites that can directly interfere with the mitochondrial respiratory chain and ATP production, while short chain fatty acids have beneficial effects on mitochondrial activity. All these data suggest that the microbiota targets mitochondria to regulate its interaction with the host. Imbalance of this targeting may result in a pathogenic state as observed in numerous studies.

(Saint-Georges-Chaumet Y, Pathog Dis. 2016)

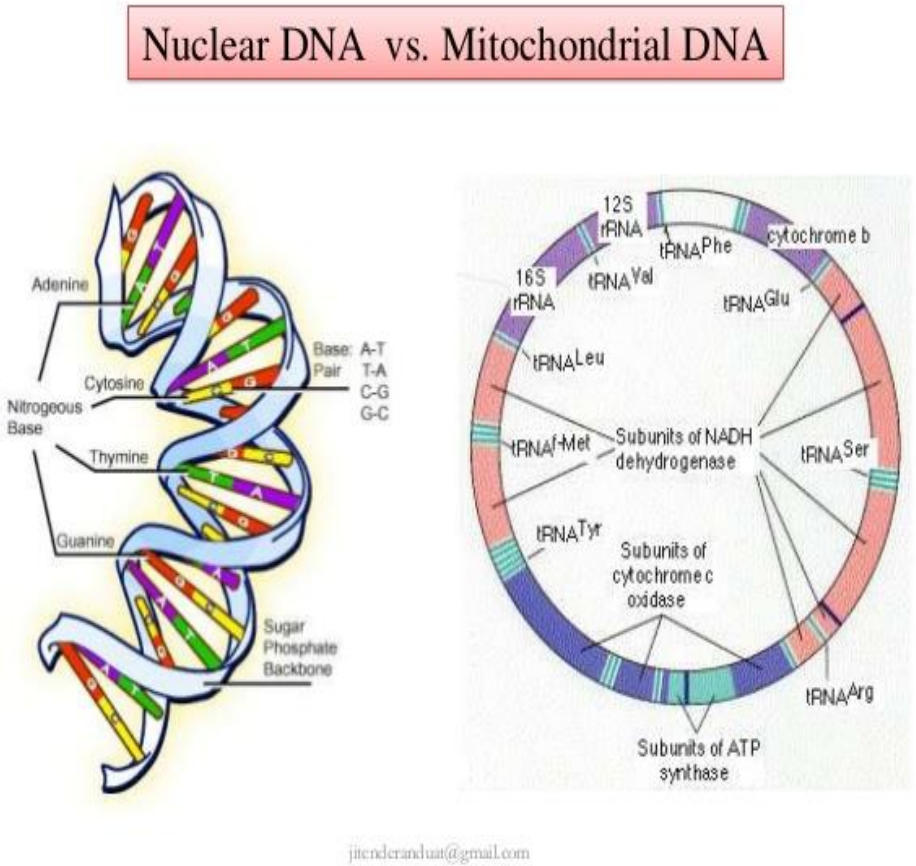
Mitochondrial genes

- Over time, α-proteobacteria, the mitochondrial ancestor, shed a number of its genes to the nucleus of the host organism. In today's terms this means that **many of the genes controlling mitochondrial function have been outsourced to the nucleus of the cell.**
- **Within the human mitochondrion are now only 37 genes:** 22 encoding tRNA, 2 encoding rRNA and 13 encoding proteins for the ETC. The remaining 1,145 ETC proteins are encoded by the outsourced nuclear genes. The full genetic sequence can be seen in MitoCarta on the Broad Institute website: https://www.broadinstitute.org.
- **This carries an important advantage. With histone coating, nuclear genes have better protection** than mitochondrial genes from the massive free radical production that occurs within the mitochondria. **MtDNA has no protective histones and limited mtDNA repair capability and so is far more susceptible to oxidative injury as it is situated at the source of ROS** and therefore has a 17-fold higher mutation rate than nuclear DNA**.**
- **Mitochondria have 5-10 copies of each gene within each mitochondrion, compared to the 2 copies in the human cell nucleus**, likely in case of free radical damage to mtDNA. In a human cell there can be 100–10,000 separate copies of mtDNA.
- Our mitochondrial genes have several thousand times more power per gene than those of our bacterial ancestors, which has significantly contributed to our evolution.

(Lane N, Nature, 2010; Fosslien E, Mitochondrial medicine, 2001; Lee HC, Exp Med Biol, 2007; Taanman JW, Biochim Biophys Acta Bioenerg, 1999; Bunkar N, Front Biosci. 2016)

Nuclear and mitochondrial DNA (mtDNA)

- **Mitochondrial DNA is circular, in contrast to nuclear DNA, with the double helix.**
- Bacterial DNA is also circular.
- The mitochondrial genome is evolving more than 50 times faster then our personal nuclear genome.
- Whereas nuclear DNA can barely distinguish between chimpanzees and humans, mtDNA can be very specific on race, family and era.



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Why are any mitochondrial genes retained in the mitochondria if they are so much better off in the nucleus?

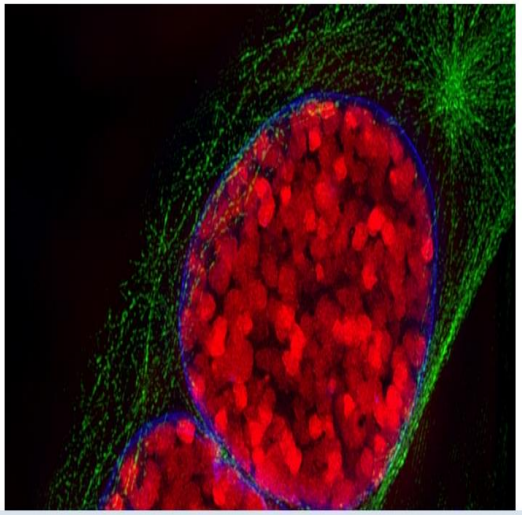
- **Because cells need to respond quickly to energy demands** and adapt their energy production on an individual mitochondrial basis to keep up. This means that the **mtDNA must be near the site of energy production, so that it can respond quickly and effectively to make new proteins.**
- If the **mitochondrial ROS had to signal to the nucleus**, some distance away, to produce additional proteins for the ETC, **it would take much longer**. Also the histones protecting nuclear DNA can slow down arriving signals, whereas mtDNA is not histone-protected.
- However, once mitochondrial function is reduced, there is an ever-increasing cycle of damage, as free radicals further damage mitochondrial proteins and DNA in a PFL.
- Also, if the nuclear genes make a protein for a particular mitochondrion, a marker needs to be attached to direct it to that specific mitochondrion, otherwise the nucleus would over-produce the protein and all mitochondria would receive it; adding the marker takes further time and energy in attaching it. So the mitochondrion in need will not receive enough proteins and those with sufficient will receive too many. This will generate a new signal to the nuclear DNA to stop producing that particular Complex proteins. So none of the mitochondria has what it needs.
- So the mitochondria have to be in control of their own destiny with respect to the ETC Compexes and sub-units. Although there are only 13/74 genes for ETC Complexes controlled directly by the mitochondrion, they are the genes that are critical for Complex construction and proton pumping, without which there would be no electron flow and no ATP.

Artificial mitochondrial gene transfer

- Researchers are currently working on **transferring more genes from the mitochondrion to the nucleus in an attempt to prevent ageing.**
- But this assumes that there is **no good reason for retaining mitochondrial genes in in the mitochondria.**
- It will result in the mitochondria losing control of the membrane potential, leading to huge amounts of free radicals being loosed into the cell – surely a development which will promote ageing and apoptosis?
- Have scientists forgotten that our mitochondrial genes evolve up to 50 times faster than nuclear genes. And what if there is incompatibility between nuclear and mitochondrial genes?
- What could possibly go wrong?

SEPTEMBER 10, 2016

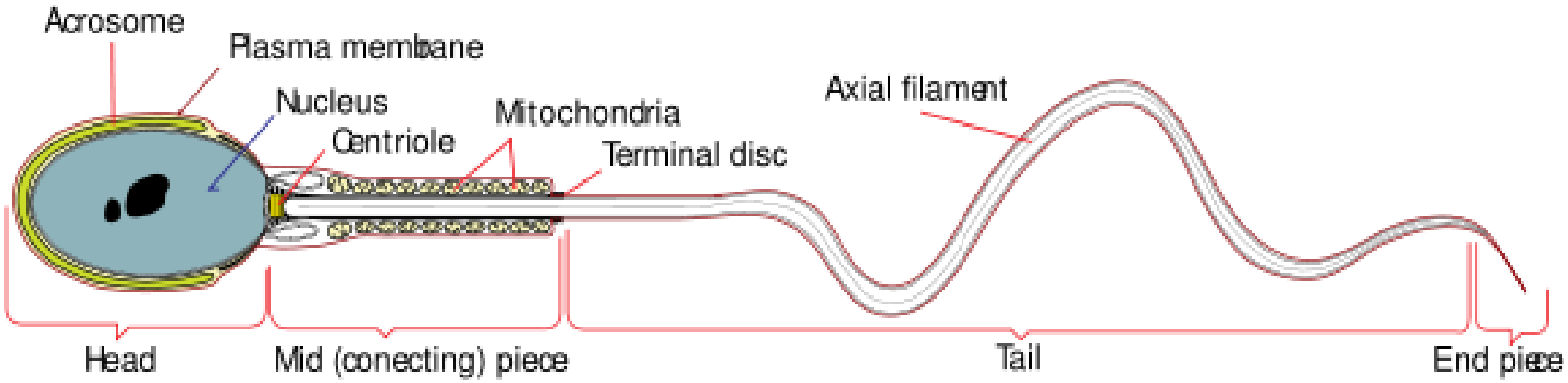
Breakthrough In Transferring Mitochondrial Genes To The Nucleus



Mitochondrial gene mutations

- MtDNA mutations can be both advantageous and disadvantageous, but usually the latter. **MtDNA mutations can be heritable and/or acquired (somatic);** acquired mutations are usually induced by oxidative damage over time.
- **Heritable mtDNA mutations can induce the rare but very serious mitochondrial diseases**, mostly involving cardiac or skeletal muscle, causing blindness and dementia. These often first appear in children but sometimes they do not become obvious until later in life, as the ROS of ageing increase the damage to mitochondrial functioning.
- The female egg cell (oocyte) will pass on more than 100,000 mitochondria to the next generation, whereas sperm cells typically contain fewer than 100. **Mitochondrial DNA is primarily maternally inherited, but occasionally some paternal DNA may also appear in the offspring.**
- **Where both female and male mtDNA are present in a mitochondrion, this is known as heteroplasmy**; only 1 type of DNA is known as homoplasmy.
- **Mitochondrial diseases have generally been found to be heteroplasmic.**
- Heteroplasmy exists in 10-20% of humans and is common is other species. Not all heteroplasmy gives rise to mitochondrial diseases.

What happens to the mitochondria in sperm?



The mitochondria are located in the tail, which drops off once the egg is fertilised and undergoes phagocytosis.

So in mitochondrial terms, the male is a genetic dead end!

Mitochondrial diseases

- Mitochondrial diseases are long-term, genetic, often inherited disorders that occur when mutated mitochondria fail to produce enough energy for the body to function properly. One in 5,000 individuals has a genetic mitochondrial disease.
- Typical symptoms include seizures, fatigue, vision and hearing loss, cognitive disabilities, heart problems, respiratory problems or poor growth.
- Often it is not possible to find a genetic reason for a mitochondrial disease; diagnosis is made on biochemical and/or morphological findings.
- Nevertheless, they are mainly maternally inherited defects of mtDNA replication through absence of proof-reading of replication of the enzyme polymerase gamma, disabling cells to repair incorrect base pairs. There may also be impaired cross-talk between nucleus and mitochondria.
- Disorders of CoQ10 biosynthesis are relatively common, when oral supplementation will be required (30mg/kg/day in childhood). Primary CoQ10 deficiency disease should always be the differential diagnosis of all mitochondrial disease presenting in childhood. It can be determined by muscle biopsy to investigate the ETC.
- Other potentially beneficial therapies include B vitamins, particularly thiamine (vitamin B1) and riboflavin (vitamin B2), and a ketogenic diet with carnitine and lipoic acid to bypass disorders in the pyruvate dehydrogenase complex.

(Bunkar N, Front Biosci. 2016)

Mitochondrial Eve

- So in theory, there should be a pure line of mtDNA stretching from our common maternal ancestor (known as Mitochondrial Eve, thought to have lived 170,000 years ago in East Africa) all the way down to every modern human.
- Studies of heteroplasmy in anthropology have shown that Mitochondrial Eve lived around 6,000 years ago. But we know that *homo sapiens* has been around a lot longer than that – so what has happened?
- An Australian fossil, so far the world's oldest mtDNA at 40,000 years old, shows that the original mtDNA line became extinct, despite containing the nuclear DNA of modern humans. 40,000 years ago coincides with a period of severe climate change.
- So it appears that natural selection is at work in mitochondrial genes as well, with those that fail to adapt dying out. The corollary of this is that different mutations have been found to thrive in different areas: there are strains of African mtDNA that are not seen elsewhere.

Ethnic differences

- It appears that the balance between two main functions of the mitochondria (energy production and heat production) will vary depending upon geographical location. The proton gradient across the IMM can either power ATP production (coupling) or generate internal heat by dissipating the gradient (uncoupling). People who lived in tropical Africa benefitted from less heat and more energy for hunting, while the Inuit benefitted from more heat but did not need so much energy for fishing. (Mishmar D, Proc Natl Acad Sci USA, 2003)
- Dissipating the gradient as heat also has the benefit of lowering free radical formation. But in those of African origin, unless the gradient is dissipated in exercise to use up the ATP, free radical formation will be high. It will be made much worse by overeating, as the ETC will be full of electrons from food with nowhere to go because the ATP is not being used up. This makes them more prone to diseases of free radical damage such as diabetes and heart disease, as seen repeatedly in studies of African Americans. Conversely, we also find that the Inuit are much less prone to diabetes and heart disease, despite eating a high animal fat diet.
- We also see this in infertility (asthenozoospermia). Because the male sperm have very few mitochondria, a lot of ATP production per mitochondrion is required for the sperm to fertilise the egg. If much of the proton gradient is dissipated in heat, fertility should be lower. And indeed this is seen in a study of Northern Swedes vs southern Europeans.
- All this demonstrates that mitochondrial genes are also subject to natural selection.

Mutations in the mitochondrial control region

- Mitochondrial DNA (mtDNA) has two major parts, the control region and the coding region.
- The mtDNA control region is the area of the mitochondrial genome which controls RNA and DNA synthesis, i.e. non-coding DNA.
- Mutations in the control area of mtDNA accumulate with age and can spread to all the mitochondria in the cell and all cells in the tissue by lateral gene transfer. They can affect the binding of transcription or replication factors but do not affect gene sequence.
- Any particular mutation in the control area can make the mitochondria quicker or slower to replicate when signalled to divide. If slower, then the number of defective mitochondria would continually decrease and eventually die out. If faster, then the mutated DNA would proliferate and eventually displace the normal mitochondria in the cell.

Mutations in the mitochondrial coding region

- The mitochondrial coding region is the part of the mtDNA genome that contains genes which code for proteins.
- Because it contains genes, the coding region is believed to be slower to mutate than the control region. Often, the mutations that are found in the coding region are used to define haplogroups.
- Where a mutation occurs in the coding region, it can be amplified within particular cells but tends not to spread beyond 1% of the cells in a tissue.
- Mutations in the coding region are likely to affect mitochondrial respiration, particularly if they code for some protein sub-unit of the ETC. This will result in increased electron leakage but the resulting ROS signal to make new sub-units would not correct the problem as they would all have the same mutation.
- This is not a national disaster. The defective mitochondria send a special signal, known as the retrograde response, to the cell nucleus which allows the cell to adapt.
- Retrograde signalling switches energy production to anaerobic (without oxygen and mitochondria), and the anaerobic respiration itself acts as a signal to initiate mitochondrial biogenesis.

Lateral (horizontal) gene transfer: bacteria and mitochondria

- Our mtDNA is more similar to bacterial DNA than our nuclear DNA. Bacterial DNA can undergo lateral gene transfer: the movement of genetic material between bacteria other than by reproduction. It is an important factor in the evolution of many organisms.
- Lateral gene transfer is the primary mechanism for the spread of antibiotic resistance in bacteria: 1 bacterium becomes resistant and passes its genes through lateral transfer to 26,000 others.
- The precise mechanism is unclear but one theory is that transfers may be facilitated by viruses, which inject their genomes into host cells to replicate and can be horizontally transmitted between their hosts.
- Lateral gene transfer has also been seen in mitochondria, where the age-related mutations in the control area of mtDNA can spread to all the mitochondria in the cell and all cells in the tissue.
- The ability to undergo lateral gene transfer has implications for GM foods, since our microbiome (and also possibly our mitochondria) could incorporate 'Monsanto genes' into our bodies, just through eating GM crops or animals that have eaten GM crops.

(Gilbert C, Science Direct, 2017)

Mitochondrial quality control

- **The number of mitochondria in each cell is not fixed but varies according to tissue energy requirements**; with high energy demand, mitochondria are increased to meet it and vice versa.
- **Mitochondrial quality control comprises fission, fusion, biogenesis and mitophagy** (the mitochondrial equivalent of autophagy).
- Damaged mitochondria can be toxic by generating excessive amounts of ROS, by consuming ATP through reverse electron transport or by interfering with other metabolic processes. Low levels of damage are reparable but badly damaged mitochondria will contaminate other mitochondria unless they are eliminated.
- Mitochondria divide and replicate on their own timetable, distinct from that of the cell.

(Mishra P, J Cell Biol, 2016; van der Bliek AM, Cold Spring Harbour Perspect Biol, 2013; Sergi D, Front Physiol, 2019; Burchell VS, Expert Opin Ther Targets, 2010; Mozdy AD, J Cell Biol, 2000; Liesa M, Cell Metab, 2013; Archer SL, NEJM, 2013; Parra V, J Bioenerg Biomembr 2011)

More on mitochondrial quality control

- If there is a mild energy deficit, the cell will allow the least damaged mitochondria to replicate, while the most damaged mitochondria will be allowed to die out.
- Damaged mitochondria left to die will be broken down by fission and the components recycled (mitochondrial fusion).
- By continually correcting the mitochondrial deficits, cells can in theory extend their lifespan almost indefinitely.
- Mitochondria fuse together and split apart in continual cycles of fission and fusion; the balance between fission and fusion determines the health of the cellular mitochondrial network.
- The outer mitochondrial membrane potential is the litmus test for mitochondrial quality. If the charge is too low (the membrane is depolarised below a certain $\Delta\psi_m$) the decision is made that the components should go to fission/mitophagy rather than fusion/ biogenesis.

Fission and fusion cycles

- Mitochondria fuse together and split apart in continual cycles of fission and fusion, where worn out mitochondrial fragments undergo mitophagy and viable fragments fuse with other mitochondria to maintain peak efficiency.
- A healthy cellular mitochondrial network depends on the balance between fission and fusion, not necessarily the absolute rate of fission and fusion. Losing this dynamic connection between the two can be a major factor in accelerated ageing, poor health and chronic disease.
- Frequent mitochondrial fission and fusion is necessary for cell survival, mitosis, mitochondrial mobility and repair.
- Fission and fusion also aid structural modification. Fusion forms long filamentous mitochondria, while fission generates small spherical mitochondria.
- Elongation of mitochondria results in more coupled respiration and increased energy efficiency. The extent of mitochondrial fission and fusion varies greatly between cells, making them difficult therapeutic targets.

(Sergi D, Front Physiol, 2019; Burchell VS, Expert Opin Ther Targets, 2010; van der Bliek AM, Cold Spring Harbour Perspect Biol, 2013; Mozdy AD, J Cell Biol, 2000; Liesa M, Cell Metab, 2013; Archer SL, NEJM, 2013; Parra V, J Bioenerg Biomembr 2011)

Mitochondrial fission

- **Fission is the process of fragmenting mitochondria to allow fusion for the healthy fragments and mitophagy for the damaged fragments (to prevent replication).**
- Although mitochondrial fission and mitophagy are essential to prevent damaged mitochondria being replicated, **excessive fission is thought to be a mechanism underlying many chronic diseases**, particularly neurodegenerative diseases. It can result in smaller and less functional mitochondria that are more likely to generate excessive ROS and undergo apoptosis.
- Fission leads to a higher proportion of smaller, shortened and rounded mitochondria, resulting in decreased energy efficiency.
- In mammals, mitochondria replicate their DNA and divide mainly in response to the energy needs of the cell, rather than in phase with the cell cycle.
- Mitochondria divide by binary fission, similar to bacterial cell division.

(Sergi D, Front Physiol, 2019; Mozdy AD, J Cell Biol, 2000; Liesa M, Cell Metab, 2013; Friedman JR, Science, 2011)

Mechanism of mitochondrial fission

- **Mitochondrial fission sites often occur at MAMs**, where the endoplasmic reticulum is in contact with mitochondria.
- Here **the endoplasmic reticulum tubules wrap around the mitochondrion, constricting it to break apart the outer and inner membranes.**
- Dynamin-related protein 1 (Drp1) is then recruited to divide the mitochondrion. Drp1 is also involved in apoptosis, as the outer mitochondrial membrane is fragmented by the translocation of Drp1 from the cytosol to the mitochondria. Drp1 then promotes the cytochrome c release.

Mitochondrial fission: pros and cons

- Benefits of mitochondrial fission:
  - Improved mitochondrial mobility, particularly important in elongated neurons where more mitochondria are required at synapses and nerve terminals.
  - If fission/mitophagy does not occur, damaged mitochondria will reproduce the damage, leading to disease.
- Problems with excessive mitochondrial fission:
  - Results in smaller and less functional mitochondria, that are more likely to generate excessive ROS and undergo apoptosis. Excessive fission has been found in many chronic diseases: neurodegenerative (PD, AZ, Huntingdon's), T2D, cancer.
- Curiously, upregulated AMPK both triggers fission and protects against excessive fission by inhibiting the fission-initiating protein Drp1, suggesting that it may be adaptongenic.
- The fact that the process is deliberate and not random demonstrates the innate intelligence possessed by mitochondria.

Fission in cell division (mitosis)

- Cell division (mitosis) increases the number of cells in a growing body, but what happens to mitochondria when cells divide? Do they divide too, or are they allocated to daughter cells?
- Mitochondria do not undergo division in the same way that cells divide. Instead they undergo mitochondrial fission with the purpose of redistribution.
- Mitochondrial fusion then ensures that mitochondria are shared equally between the 2 daughter cells. Failure to do so could result in the whole cell being compromised through insufficient ATP production.

Mitochondrial fusion

- Following fission, the damaged mitochondrial fragments should be removed via mitophagy. **The healthy mitochondrial fragments fuse with other mitochondria,** creating long filamentous mitochondria.
- **Fission and fusion ensure that during cell division, mitochondria are divided equally between daughter cells** and ensures that the number of mitochondria are sufficient for the cell's energy needs.
- Mitochondrial fusion is principally regulated by mitofusins (Mfns). A drop in ATP levels or an increase in demand can trigger mitochondrial fusion, facilitated by mitofusins 1 and 2 for the outer mitochondrial membranes and optic atrophy protein (Opa) 1 for the inner membranes.
- Fusion increases energy efficiency and ATP production, exchanges damaged mtDNA for intact mtDNA and allows the dilution of superoxide and mutated mtDNA, as well as the repolarisation of membranes.
- Mitofusins have other roles in the mitochondria, including expression of ETC Complex subunits, regulation of contact sites with the endoplasmic reticulum and promoting creation or maintenance of a mitochondrial network.

(Sergi D, Front Physiol, 2019; Chan DC, Annu Rev Genet, 2012; Simula L, Sem Cancer Biol, 2017; Parra V, J Bioenerg Biomembr 2011)

Mitochondrial fusion: pros and cons

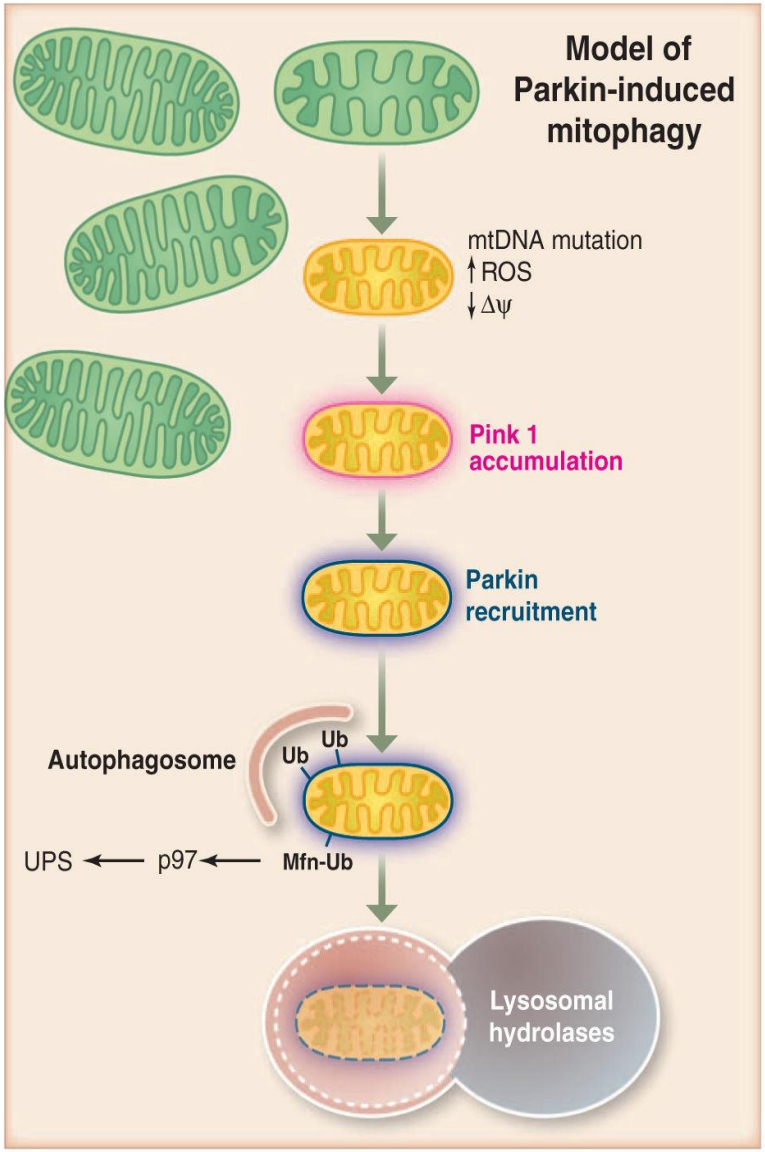
- Benefits of fusion:
  - Larger fused mitochondria are less likely to experience excess cellular stress and premature degradation.
  - OXPHOS is likely to be more stable, improving β-oxidation of fatty acids.
  - There is greater mtDNA integrity and protection of against mutations through dilution.
- Problems with fusion:
  - If very damaged mitochondria fuse with healthy mitochondria the health of the whole can be undermined (mitochondrial contagion). Mitochondrial contagion is thought to play a role in development of familial Parkinson's disease.

Mitophagy (mitochondrial autophagy)

- **Mitophagy is the removal of defective mitochondria or their parts by autophagosomes and catabolism by lysosomes**. It prevents accumulation of dysfunctional mitochondria which can lead to cellular degeneration.
- Mitophagy occurs following damage or stress, and in response to changes in metabolic state, redox state and nutrient availability. It ensures the efficient and smooth running of the cell.
- Mitophagy is regulated by PINK1 and Parkin proteins. With depolarisation of the mitochondrial membrane, PINK1 accumulates on the outer mitochondrial membrane and signals Parkin to translocate to the mitochondria. Parkin then initiates the formation of the autophagosomes.
- Mitophagy is particularly important in neurons, as mitochondria are required to travel large distances and accumulation of dysfunctional mitochondria prevents motility. Neurons cannot survive without mitophagy.
- Mitophagy naturally reduces with age. If mitophagy does not take place, there will be reduced energy production and increased ROS and cellular inflammation, as occurs in many chronic health conditions.

(Fujimaki S, Int J Mol Sci, 2017; Montgomery M, Endocrine Connect, 2015; Ding WX, Biol Chem, 2012; Valdinocci D, Front Neurosci, 2019)

PINK1 and Parkin mechanism



1. PINK1 is constitutively degraded by inner mitochondrial membrane proteases and maintained at low levels on healthy mitochondria.
2. When a mitochondrion becomes damaged to the point of depolarising the potential across the inner membrane, PINK1 import to the inner membrane is prevented, thereby sequestering it on the outer mitochondrial membrane so it cannot be degraded.
3. PINK1 accumulates there and recruits Parkin from the cytosol via PINK1 kinase activity. Parkin conjugates ubiquitin to a variety of proteins on the outer mt membrane and mediates the proteosomal elimination of mitofusins 1 and 2.
4. Lastly, Parkin induces autophagic elimination of the dysfunctional mitochondria. This pathway may constitute a quality-control mechanism to eliminate damaged mitochondria.

(From Youle RJ, Science, 2012)

Key: Ub = ubiqionone; UPS = ubiquitin proteasome system.

AMPK and mTOR effect on mitophagy

- Mitophagy can be increased by exercise, ketogenic diet, fasting and caloric restriction through upregulation of the nutrient/energy sensor AMP-activated protein kinase (AMPK), which detects healthy energetic stress (low ATP, NADH and acetyl CoA reserves). When ATP/NADH are low, AMP/NAD^+ are high. Mitophagy is only activated when the body is in catabolic mode, i.e. AMPK is upregulated and mammalian target of rapamycin (mTOR) is downregulated.
- AMPK activates healthy levels of mitochondrial fission/mitophagy and initiates biogenesis and β-oxidation, while decreasing all non-essential ATP-consuming processes such as fatty acid synthesis. Upregulated AMPK causes ATP to be replenished by putting mitochondria on high alert and triggering anti-ageing processes.
- AMPK also decreases the half life of mitochondria, i.e. mitophagy is increased in frequency, improving mitochondrial efficiency and integrity. Over-consumption increases the half-life, decreasing mitochondrial efficiency.

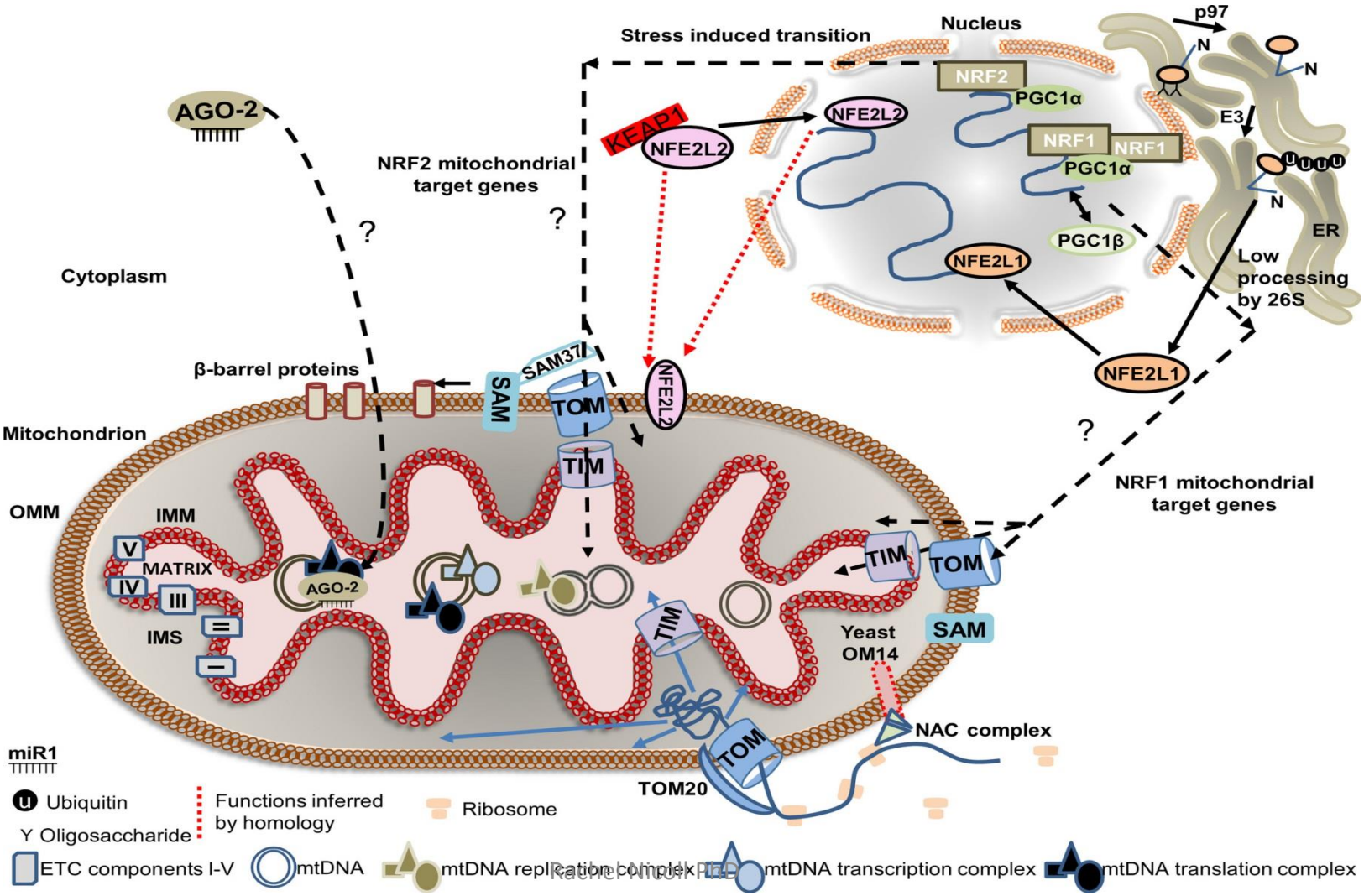
(Zhang CS, Cell Metab, 2016)

Mitochondrial biogenesis

- **Mitochondrial biogenesis can take place through the transcription of genes in both the nuclear and mitochondrial genome to make new mitochondrial proteins.**
- **The master co-ordinator of mitochondrial biogenesis is peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α),** which has to relocate from the cytoplasm to the nucleus to trigger mitochondrial biogenesis. PGC-1α itself is regulated by AMPK and SIRT1.
- Nitric oxide (NO) and calcium/calmodulin-dependent protein kinase IV (CaMKIV) can also stimulate PGC-1α gene transcription.
- Mitochondrial biogenesis requires the co-ordinated synthesis and import of c1000-1500 proteins encoded by the nuclear genome and synthesised on cytosolic ribosomes.
- Mitochondrial biogenesis declines with age and development of metabolic disease through loss of function of mitochondrial transcription factor A (TFAM).

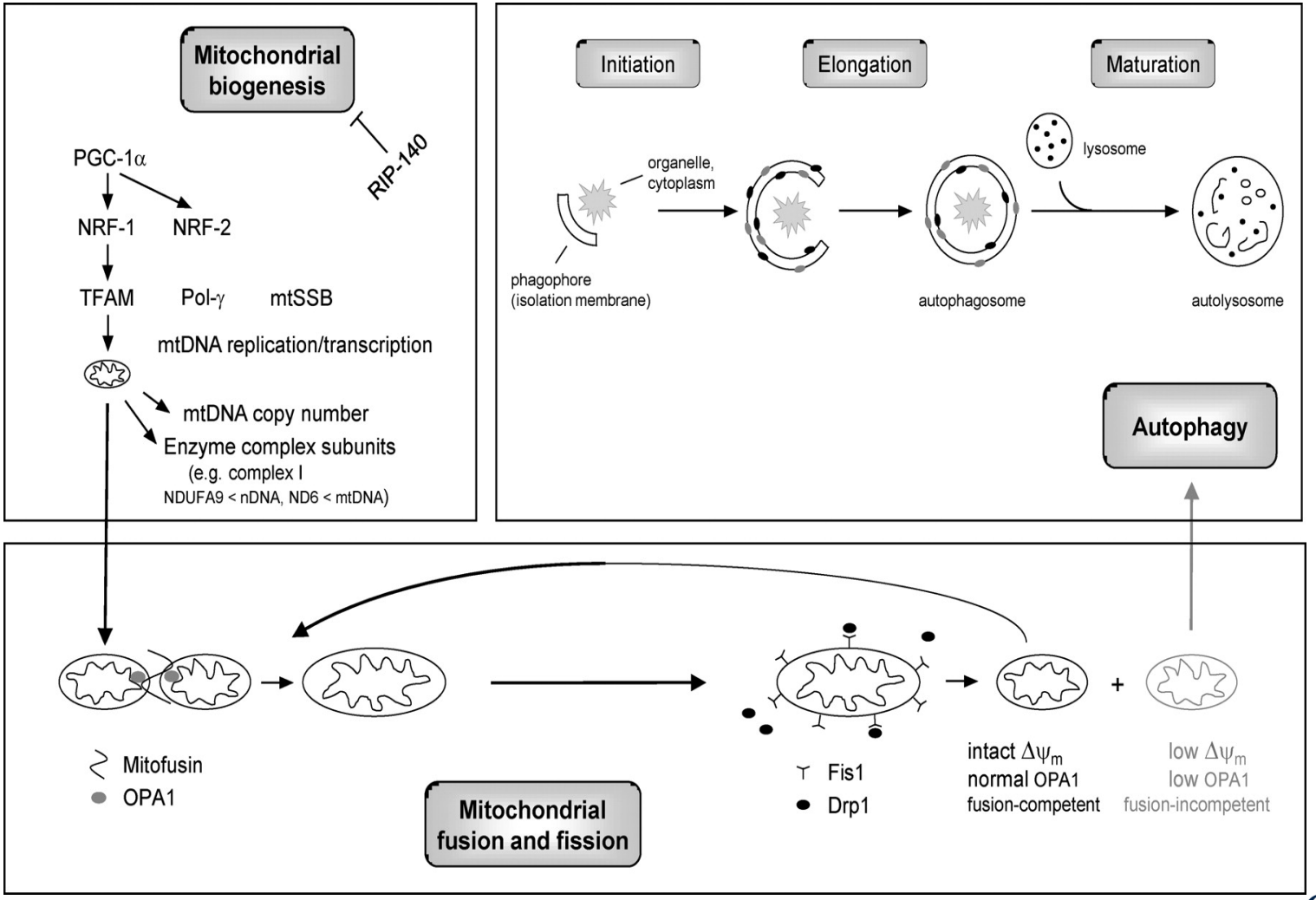
(Jornayvaz FR, Essays Biochem, 2012; Jornayvaz FR, Essays Biochem, 2010; Valero T, Curr Pharmaceutical Design, 2014; Sanchis-Gomar F, Curr Pharmaceutical Design, 2014; Ritov VB, Diabetes, 2005; McCarty MF, Med Hypotheses, 2004; Bunkar N, Front Biosci. 2016)

Mechanisms that contribute to mitochondrial biogenesis (Ploumi C, FEBS J, 2016)

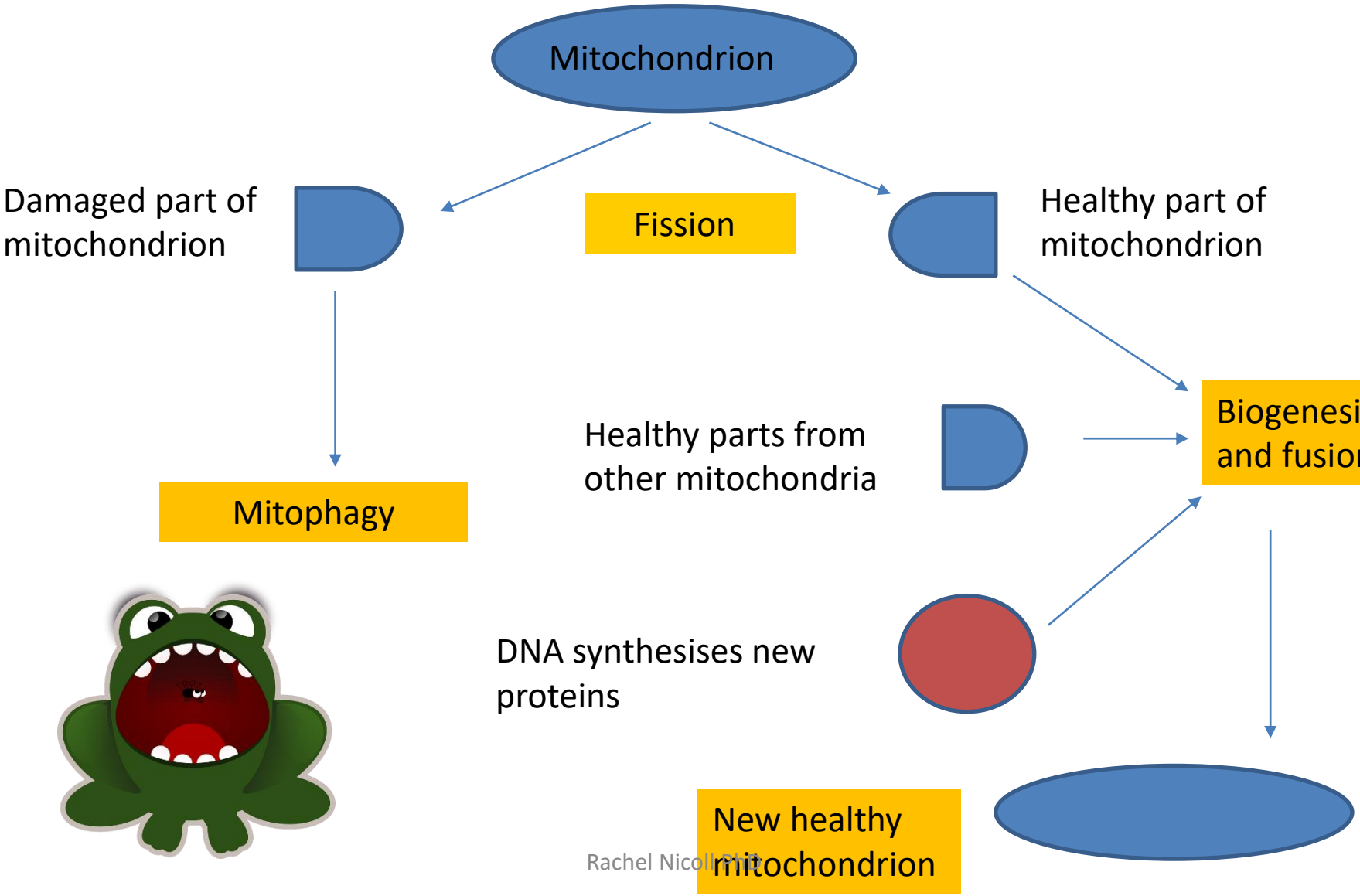


Rachel Amon PhD

Simplified scheme of the mitochondrial repair mechanisms and their interaction.



The mitochondrial quality control mechanism



When quality control mechanisms fail: the last resort

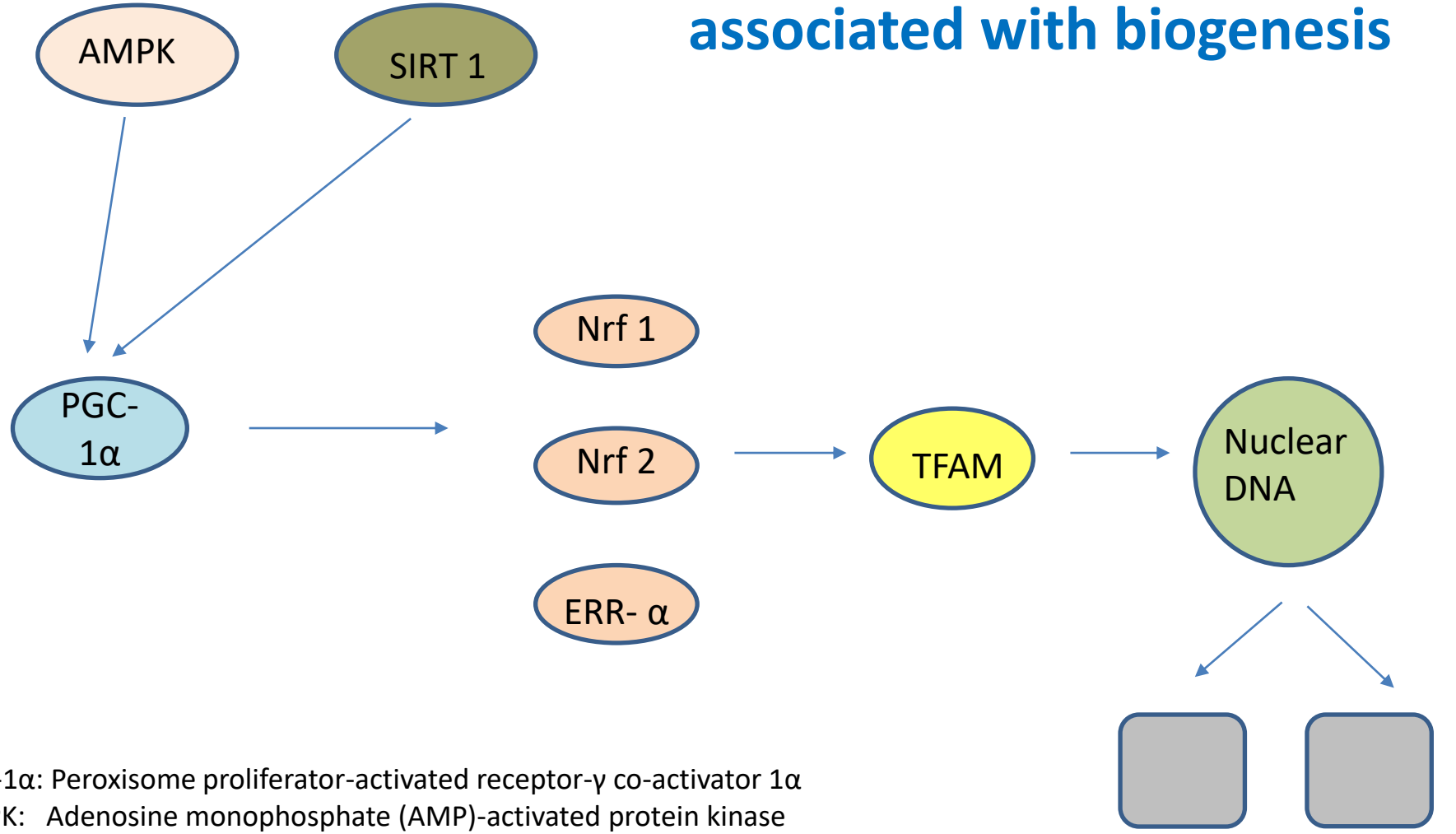
- When the cell detects that mitochondria are becoming dysfunctional, for example by the loss of mitochondrial membrane potential, and normal quality control mechanisms are insufficient, it will institute retrograde signalling.
- The normal cell signalling is nucleus to mitochondria (anterograde) but retrograde signalling reverses this as the mitochondria send SOS signals to nuclear DNA to upregulate nuclear genes which help rebuild the TCA cycle and ETC, import proteins into the mitochondria and activate mitochondrial biogenesis.
- The retrograde response can extend the replicative life span of the cell by interacting with several other signalling pathways, such as mammalian target of rapamycin (mTOR) and ceramide signalling. All of these pathways respond to stress, including metabolic stress.
- The retrograde response is also linked to both autophagy and mitophagy at the gene and protein activation levels.
- The existence of retrograde signalling indicates that mitochondrial quality control constitutes a complex network of processes, which are not yet fully understood.

(Butow RA, Mol Cell, 2004; Jazwinski SM, Biochim Biophys Acta, 2013)

Mitochondrial regulators

- Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α)
- Adenosine monophosphate (AMP)-activated protein kinase (AMPK)
- Nuclear respiratory factors 1 and 2 (Nrfs 1 and 2)
- Transcription factor of activated mitochondria (TFAM)
- Sirtuins
- **Mammalian/mechanistic target of rapamycin (mTOR)**
- **Heat shock proteins (HSPs)**
- **Reactive oxygen species (ROS)**
- **The microbiome**
- Nitric oxide (NO)
- Serotonin
- MicroRNAs

Mitochondrial regulators associated with biogenesis



PGC-1α: Peroxisome proliferator-activated receptor-γ co-activator 1α
AMPK: Adenosine monophosphate (AMP)-activated protein kinase
SIRT 1: Sirtuin 1
Nrf: Nuclear respiratory factor
ERR-α: Oestrogen-related receptor-alpha
TFAM: Transcription factor of activated mitochondria

Activation and regulation of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α)

- Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) is the master co-ordinator of mitochondrial biogenesis, which itself is regulated by AMPK and SIRT1. AMPK regulates biogenesis by phosphorylating and activating PGC-1α whenever it senses an energy deficiency.
- PGC-1α activates nuclear respiratory factors 1 and 2 (Nrfs 1 and 2) and oestrogen-related receptor-alpha (ERR-α) which induce transcription factor of activated mitochondria (TFAM). TFAM passes information for gene transcription of ETC subunits directly to the DNA.
- Expression of PGC-1alpha increases with higher cellular ATP demand. It can be upregulated by caloric restriction, a ketogenic diet, exercise and certain supplements.
- PGC-1α is not needed for normal functioning of the mitochondria but only when under physiological stress.

(Sergi D, Front Physiol, 2019; O'Neill HM, Mol Cell Endocrinol, 2013; Craig DM, Front Physiol, 2015; Hardie DG, Nature Rev Mol Cell Biol, 2012; Davinelli S, Immun Ageing, 2013; Zhang Y, Biochem Soc Trans, 2016; Bough KJ, Ann Neurol, 2006; Nisoli E, Science, 2003; Reznick RM, J Physiol. 2006; Leary SC, Bioessays, 2003; Ruan H, Proc Natl Acad Sci USA, 2002; Bunkar N, Front Biosci. 2016)

Other peroxisome proliferator-activated receptors (PPARs)

- PPARs are a group of nuclear receptor proteins that function as transcription factors, regulating gene expression. PPARs play an important role in the regulation of mitochondria, oxidative stress and neuroinflammation, the major causes of neurodegenerative disease pathogenesis.
- Three isoforms (α, δ, and γ) have so far been found. They act on DNA response elements through the nuclear retinoic acid receptor and play a major regulatory role in energy homeostasis and metabolic function.
- PPAR-α is present in liver, heart, and, to a lesser extent, skeletal muscle. When activated, it promotes fatty acid oxidation, ketone body synthesis, and glucose sparing. Fibrates, which are used as hypolipidemic drugs, are ligands of PPAR-α. PPAR-α activates genes involved in fatty acid uptake and β-oxidation but this may exceed the capacity of downstream mitochondrial respiration and lead to an accumulation of toxic lipid metabolites, which can worsen insulin resistance.
- PPAR-δ is ubiquitous and could also favour fatty acid oxidation in tissues in which PPAR-α is absent or less expressed.
- PPAR-γ is expressed in adipose tissue, the large intestine and immune cells. Activation of PPAR-γ induces the differentiation of preadipocytes into adipocytes and stimulates triglyceride storage.

Rachel Nicoll PhD

(Ferre P, Diabetes, 2004; Tyagi S, J Adv Pharm Technol Res, 2011; Lee, J Biomed Sci, 2017)

Adenosine monophosphate (AMP)-activated protein kinase (AMPK)

- AMPK is a cellular energy sensor, which is activated by a decrease in the ATP/AMP ratio within the cell. Because ATP is in part broken down into AMP, AMP levels will generally rise as ATP is used up.
- AMPK restores energy (ATP/AMP) balance in the cell by phosphorylating specific enzymes and growth control nodes to increase ATP generation. It sets the body in catabolic mode (breaking down), instead of anabolic (builidng up).
- AMPK initiates mitochondrial biogenesis by phosphorylating and activating PGC-1α whenever it senses an energy deficiency.
- It also promotes mitophagy and increases fatty acid oxidation in the liver and skeletal muscle to provide a more plentiful source of ATP production compared to glucose metabolism.
- In skeletal muscle, AMPK senses changes in the creatine to phosphocreatine ratio. At the start of exercise, phosphocreatine is used as an immediate source of energy to power muscle contraction, and is converted to creatine, increasing the ratio. As ATP is broken down to replace the phosphocreatine, ATP levels also fall, activating AMPK.
- AMPK activity has been shown to decrease with age, which may contribute to decreased mitochondrial biogenesis and function with ageing.

(Hardie DG, Cell Metab 2007; Hardie DG, FEBS Lett 2008; Misra P, Expert Opin Ther Targets 2008; Jornayvaz FR, Essays Biochem 2010 Herzig S, Moll Cell Biol, 2018)

Nuclear respiratory factors (Nrfs)

- Nrf1 and Nrf2 mediate the coordination between nuclear and mitochondrial genomes by directly regulating the expression of several nuclear-encoded ETC proteins, and by indirectly regulating the three mitochondrial-encoded cytochrome oxidase subunit genes.
- Nrfs encode transcription factors including transcription factor of activated mitochondria (TFAM), proteins which activates the expression of key metabolic genes regulating cellular growth and nuclear genes required for respiration, haem biosynthesis and mitochondrial DNA transcription and replication.
- If the cellular oxidation state is high, Nrf1 becomes a major transcriptional regulator of mitochondrial biogenesis, initiating the necessary gene expression by stimulating mitochondrial fission in an attempt to dilute the oxidative state. Disruption of Nrf1-mediated mitochondrial biogenesis results in impaired mitochondria and a slow, progressive cellular degeneration.
- Nrf2 regulates the gene expression of antioxidant, anti-inflammatory and detoxification enzymes, as well as proteins that assist in the repair or removal of damaged macromolecules. Nrf2 has a crucial role in the maintenance of cellular redox homeostasis by regulating the biosynthesis, utilisation, and regeneration of glutathione, thioredoxin and NADPH and by controlling the production of ROS by mitochondria and NADPH oxidase. It also affects the mitochondrial membrane potential, fatty acid oxidation, availability of substrates (NADH and FADH2/succinate) for respiration, and ATP synthesis. Activation of Nrf2 counteracts increased ROS production in mitochondria via upregulation of UCP3 and influences mitochondrial biogenesis by maintaining the levels of Nrf1 and PGC-1α.

(Kiyama T, Mol Neurodegener, 2018; Dinkova-Kostova AT, Free Radic Biol Med, 2015)

Transcription factor of activated mitochondria (TFAM)

- TFAM is a DNA-binding protein that activates transcription at the two major promoters of mtDNA—the light strand promoter (LSP) and the heavy strand promoter 1 (HSP1). Equally important, it coats and packages the mitochondrial genome.
- TFAM plays a critical role in maintaining copy number and structure of mtDNA, and is hence crucial for efficient transcription of mtDNA genes such as cytochrome c oxidase subunits. TFAM binding to mtDNA is regulated via phosphorylation. It is also important for embryo development and its loss is implicated in mitochondrial diseases.
- The nuclear DNA-encoded TFAM is synthesised in the cytosol and transported into mitochondria, where it enhances both transcription and replication of mtDNA.
- PGC-1α, Nrfs and SIRT1 promote TFAM expression, which upregulates mitochondrial biogenesis.

(Le Moine C, J Exp Biol, 2010; Gabrielson M, PLoS ONE, 2014)

Sirtuins

- Sirtuins are deacetylases (enzymes) which can influence a wide range of cellular processes, as well as mitochondrial biogenesis, where they act with PGC-1α.
- They are dependent on NAD+ signalling to remove acetyl groups from other proteins.
- There are at least 7 different sirtuins (SIRT 1-7), their location depending largely on tissue type. SIRTs 1 and 3 are the only sirtuins we will be concerned with here. Their roles are becoming gradually clearer with time but generally involve reducing inflammation and oxidative stress and improving mitochondrial function.
- SIRT1 is found in the nucleus and cytosol and helps to modulate transcription factors.
- SIRT3 is located in the mitochondria and is important for mitochondrial biogenesis, repair of DNA damage and as a regulator of ATP production, controlling overall energy homeostasis.

(Kitada M, Front Endocrinol, 2019; Ansari A, Aging Cell, 2017; Kane AE, Circ Res, 2018; Zhu Y, J Clin Invest, 2018; Rahman S, Cell Commun Signal, 2011; Dai H, Pharmacol Ther, 2018)

Rachel Nicol PhD

Sirtuins 1-7

- SIRT1 is found in the nucleus and cytosol and helps to modulate transcription factors such as p53, NF-κB, PGC-1α and DNA repair proteins such as PARP1.
- SIRT2 is a cytosolic sirtuin, present in a wide range of tissues and organs, particularly brain, muscle, liver, adipose tissue, pancreas, kidney, testes.; expression is much higher in the brain than all other organs. Its exact function not yet clear.
- SIRT3 is located in the mitochondria and is important for mitochondrial biogenesis, repair of DNA damage and as a regulator of ATP production, controlling overall energy homeostasis. Some tissues with high energy demand, such as the heart, kidney, and liver, normally express high levels of SIRT3. It also upregulates antioxidant enzymes, triggered by increased ROS levels, and plays a role in oocyte development.
- SIRTs 4 and 5 are located in the mitochondria, with roles in oxidative stress and lipid metabolism.
- SIRT6 and 7 are nuclear sirtuins with roles in gene expression and DNA repair.

(Kitada M, Front Endocrinol, 2019; Ansari A, Aging Cell, 2017; Kane AE, Circ Res, 2018; Zhu Y, J Clin Invest, 2018; Dai H, Pharmacol Ther, 2018)

Energy/nutrient-sensing pathways

- **The principal energy sensors are AMPK, mTOR, insulin and insulin-like growth factor (IGF).**
- **AMPK opposes mTOR, insulin and IGF.**
- **AMPK triggers mitochondrial biogenesis when it senses low energy.** It sets the body in **catabolic (breaking down) mode.**
- **Fasting upregulates AMPK.**
- They play a crucial role in survival by closely matching growth to nutrient availability. If there are no nutrients, mTOR is dormant and growth stops; if there are nutrients available, mTOR, insulin and IGF increase and growth increases.
- But continual growth does not lead to longevity; there are periods for growth (anabolism) and periods for breakdown and clearance (catabolism).
- Insulin is stimulated by carbohydrates and, to a lesser extent, protein; mTOR is stimulated only by protein.

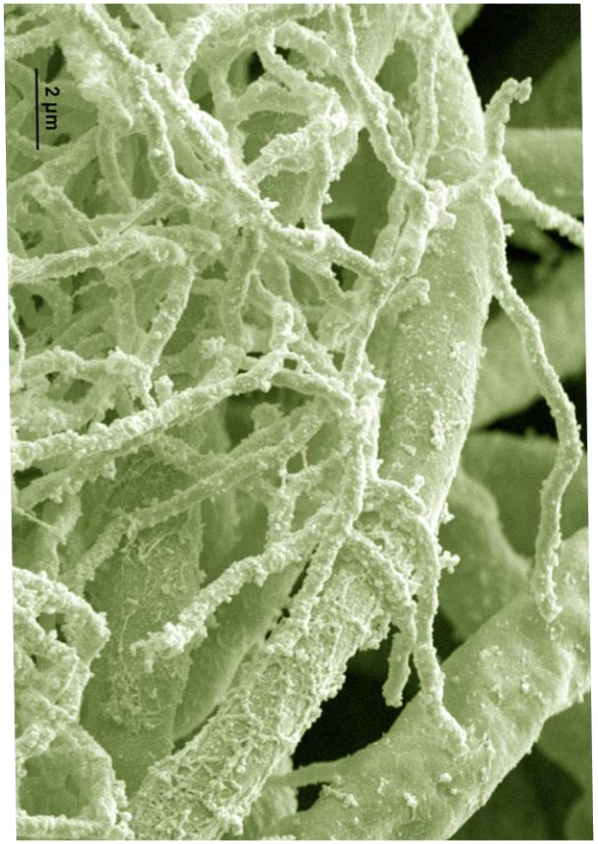
Mammalian/mechanistic target of rapamycin (mTOR)

- **mTOR opposes AMPK and sets the body in anabolic (building up) mode.** It integrates the input from upstream pathways, including insulin, growth factors such as IGF-1 and amino acids. **Eating upregulates mTOR**; mTOR activation by insulin is longer lasting than protein activation.
- So low mTOR stimulates autophagy and mitophagy but high mTOR stimulates protein synthesis and mitochondrial biogenesis and upregulates ATP production. **A balance between mTOR and AMPK is required**.
- mTOR is inhibited by fasting/caloric restriction and particularly by protein restriction, essentially anything that downregulates insulin and IGF1. Protein, particularly branch chain amino acids (BCAAs) and casein, stimulates IGF-1 and insulin release almost as much as carbohydrates. BCAAs are known to inhibit glucagon production, while leucine upregulates mTOR, increasing cell division and proliferation, and has been found to reduce sensitivity to tamoxifen in breast cancer patients.
- The mTOR pathway is a central regulator of metabolism and physiology, particularly in the liver, muscle, brain, WAT and BAT. It is dysregulated in diseases such as diabetes, obesity, depression, ageing and certain cancers.
- mTOR is a kinase that adds a phosphate (PO4) group during phosphorylation.

(Morita M, Cell Metab, 2013; Saito Y, Nature, 2019; Watson K, Sem Cell Dev Biol, 2014)

More on mammalian/mechanistic target of rapamycin (mTOR)

- Rapamycin is so named from an antifungal compound secreted by *Streptomyces hygroscopicus*, a bacterium found uniquely on Easter Island (Polynesian name '*Rapa Nui*'). Its target in the human body had not previously been discovered and was therefore named mammalian (now mechanistic) target of rapamycin.
- The compound, named rapamycin (or Sirolimus in the UK) was later developed as an anti-microbial, immunosuppressive and antiproliferative drug. Amongst other functions, it is used to coat coronary stents and prevent organ transplant rejection. It inhibits activation of T cells and B cells by reducing their sensitivity to IL-2.
- Unlike other immunosuppressive drugs, it decreases, rather than increases cancer risk, dissolving solid tumours and preventing proliferation. Nevertheless, a suppressed immune system does lead to increased infections and long-term use leads to insulin resistance, T2D and dyslipidaemia.
- Rapamycin acts by inhibiting mTOR, even in the presence of nutrients. It has the potential to benefit many conditions and promote longevity and is being considered as an anti-ageing drug. It can double the lifespan of yeast and increase the lifespan of mice by up to 14%, regardless of the age at which treatment was begun. It can also improve many chronic diseases such Alzheimer's disease, possibly by increasing neuronal autophagy. It reduces appetite and body fat and is therefore the original fasting mimetic.



Heat shock proteins (HSPs)

- **Heat shock proteins (HSPs) are molecular chaperone proteins which are formed in cells under stress to help protect the cell and prevent cell death.** They are responsible for increased tolerance and survival during and after heat and other stresses and help restore cellular homeostasis.
- **Many cellular proteins need to be folded and they are assisted in this by chaperone proteins such as HSPs**. **HSPs can also help reverse or degrade misfolded proteins** which are formed as a **cellular stress response**.
- There are several different HSPs, with **HSP60 acting as a mitochondrial chaperone protein,** facilitating the correct folding of proteins in the mitochondrial matrix**.** It is also involved in the replication and transmission of mtDNA.
- HSP70 is activated with depolarisation of the mitochondrial membrane and can induce Parkin and mitofusins.
- Extracellular HSPs can be sensed by the immune system as DAMPs.
- HSPs can help prevent development of metabolic disease. Low levels of HSP70 was shown to lead to higher insulin resistance in humans, while in rats HSPs interacted with insulin signalling, leading to lower insulin resistance.
- Some HSPs have also been implicated in the development of cancer and atherosclerosis.

(Jovaisaite V, J Exp Biol, 2014; Miova B, Curr Pharm Des, 2016; Drew BG, Diabetes, 2014)

Misfolded proteins

- Proteins misfold as a cellular stress response, such as oxidative stress, excess inflammation, impaired cellular protein degradation and free iron.
- Where mitochondrial misfolded proteins are detected, the mitochondria signal to the nuclear DNA for assistance (retrograde signalling).
- The nucleus upregulates production of the mitochondrial chaperone protein HSP60, which facilitates the correct folding of proteins in the mitochondrial matrix. It is also involved in the replication and transmission of mtDNA.
- Misfolding usually occurs in the endoplasmic reticulum and can trigger an immune response. Misfolded proteins have been implicated in the development of many chronic diseases, including type 2 diabetes, Alzheimer's and Parkinson's.

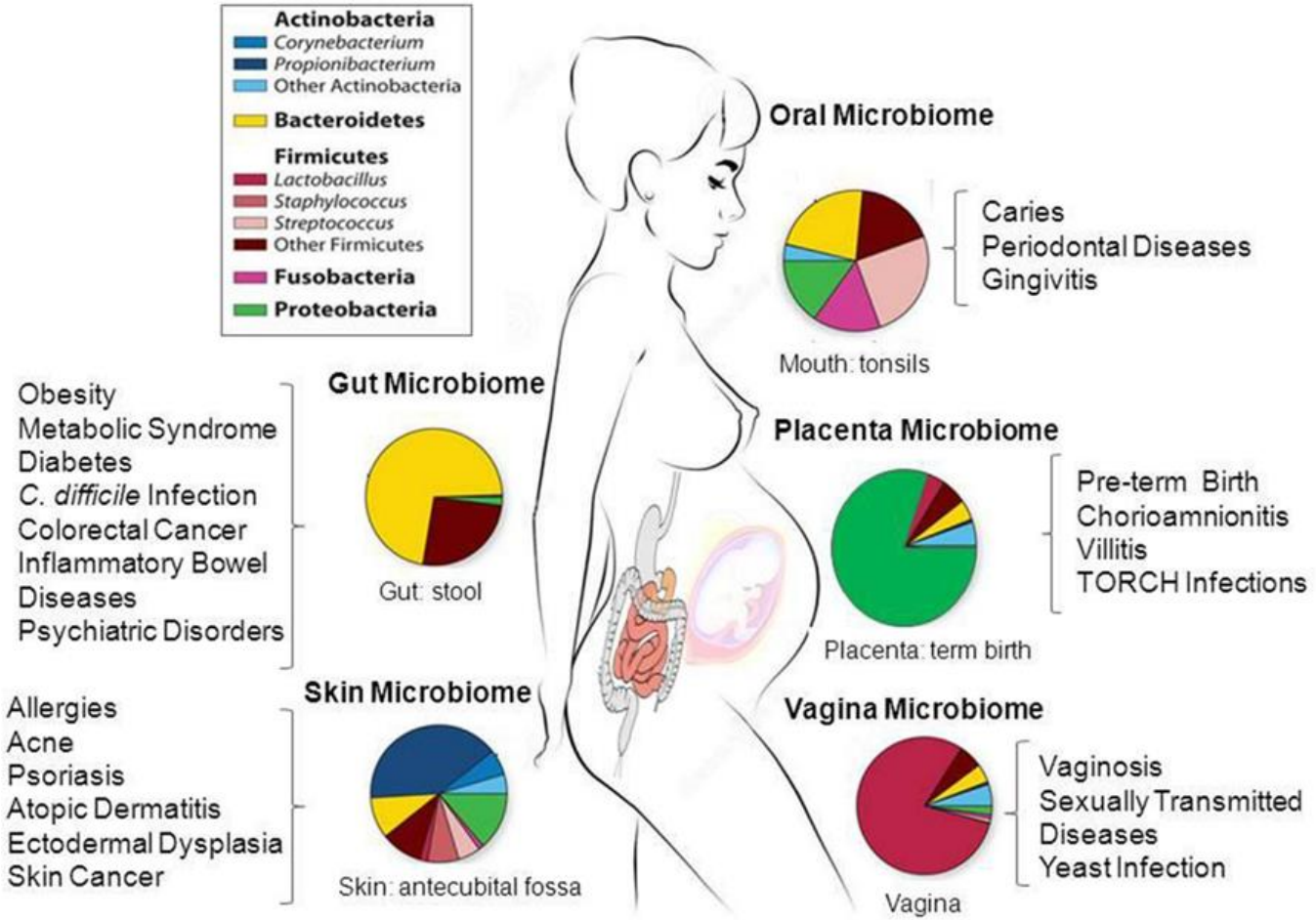
Reactive oxygen species (ROS)

- It is one of the functions of the mitochondrial ETC to produce ROS, and this is in part because they have a **cellular and mitochondrial signalling role**, particularly in the brain where they regulate dopamine transmission through glutamate.
- **ROS in the mitochondria trigger production of ETC sub-unit proteins** in the mitochondrial ribosomes to increase ATP production.
- They can also **trigger production of antioxidants and signal the time for mitochondrial fission and mitophagy**.
- Other regulatory ROS may be produced by the TCA cycle enzymes α-ketoglutarate dehydrogenase and aconitase, pyruvate dehydrogenase, dihydroorotate dehydrogenase and glycerol-3-phosphate dehydrogenase and the mitochondrial outer membrane proteins.
- At higher levels, they can induce an inflammatory response and at extremely high levels they activate apoptosis and autophagy pathways, with a cascade of tissue injury and degradation.

The microbiome

- Several studies reported a **correlation between microbiota quality and diversity and mitochondrial function and there is evidence of cross-talk between the two.**
- The gut microbiota and mitochondria have a common bacterial ancestral heritage.
- In addition, short chain fatty acids fermented from fibre by the microbiota are extremely beneficial to mitochondria, while bacterial pathogen-associated molecular patterns (PAMPs) increase the production of mitochondrial ROS. Mitochondrial ROS and dysfunction have been associated with intestinal permeability.
- The mitochondrial production of reactive oxygen species (ROS) plays an important role during the innate immune response and inflammation and is often targeted by pathogenic bacteria. Data suggest that excessive mitochondrial ROS production may affect ROS signaling induced by the microbiota to regulate the gut epithelial barrier.
- It is thought that mitochondrial diseases are linked to an imbalanced microbiome.
- Finally, the microbiota releases metabolites that can directly interfere with the mitochondrial respiratory chain and ATP production. (Saint-Georges-Chaumet Y, Pathog Dis, 2016)

Should the mitochondria be considered part of the microbiome?



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Nitric oxide (NO)

Nitric oxide (NO) is produced from L-arginine and oxygen by endothelial nitric oxide synthase (eNOS). NO mediates many mitochondrial functions, including:

- Stimulation of PGC-1α and mitochondrial biogenesis, suggesting that NO is itself a master regulator of mitochondrial number.
- Controls the supply of oxygen and respiratory substrates to mitochondria through regulation of the flow of blood to tissues, as well as the binding and release of oxygen from haemoglobin.
- Dissipates the heat generated from the ETC.
- Involved in different mitochondrial signalling pathways that control respiration and apoptosis.

In skeletal muscle, NO effects on PGC-1α expression are mediated by AMPKα1, with a likely synergistic interaction between AMPK and NOS that is critical for maintenance of metabolic function and mitochondrial biogenesis in skeletal muscle cells.

Both of these two cellular sensing systems (AMPK and NO) allows the cell to respond to increased energy demand by stimulating production of additional mitochondria, although any exercise-induced benefits accrue largely to skeletal and cardiac muscle.

Increased NOS can impair mitochondrial function and specifically the ETC, both alone and with its more toxic by-product peroxynitrite. This will lead to raised ROS and reduced ATP output.

(Naseem KM, Mol Aspects Med 2005; Borniquel S, FASEB J, 2006; Lira VA, J Physiol, 2010)

Serotonin

- Serotonin plays a key role in inducing mitochondrial biogenesis in cortical neurons via the serotonin receptor, SIRT1 and PGC-1α. Serotonin also increases mitochondrial function, particularly cellular respiration and ATP production.
- It also reduces cellular ROS and is strongly neuroprotective against stress via SIRT1.
- Serotonin (via administration of fluoxetine, an SSRI) greatly enhances mitochondrial movement in the axons of rat hippocampal neurons in vitro.

(Fanibunda SE, PNAS, 2019; Chen S, Mol Cell Neurosci, 2008)

Micro RNAs

- Mitochondrial functions are regulated by the products of both nuclear and mitochondrial genomes, which include micro RNAs, the 18- to 24-nucleotide non-coding RNAs that provide post-transcriptional inhibition of target gene expression.
- Recent studies have shown that micro RNAs are expressed in mitochondria and regulate mitochondrial energy metabolism, apoptosis, and biogenesis.

(Goud et al. Role of microRNA in the regulation of mitochondrial functions. Sci Lett. 2015 3;2:83-88)

What can damage the mitochondria?

- **Pharmaceutical drugs,** particularly statins and antibiotics.
- **Environmental toxins**
- Bacterial endotoxins/lipopolysaccharides
- Viruses
- Alcohol
- Elevated blood glucose
- Both hypoxia and excessive oxygen consumption
- Excessive reactive oxygen species (ROS) and lipid peroxides
- Food additives
- Inadequate haem synthesis
- Deuterium

Pharmaceutical drugs: Medicine safety testing does not test for mitochondrial safety

- **Antibiotics**
- Statins and fibrates (**covered under CVD**)
- Anti-epileptic drugs
- Antidepressants and antipsychotics
- Thiazolidinediones (glitazones for T2D)
- Non-steroidal anti-inflammatories: aspirin, paracetamol
- Antiviral nucleoside analogues and anti-retrovirals
- Corticosteroids,
- Beta-blockers,
- Some anaesthetics,
- Some muscle relaxants,
- Cholestyramine,
- Psychotropic drugs
- Chemotherapy, tamoxifen.
- And probably many more!

(Wang X, Bioessays, 2015; Finisterer J, Handb Exp Pharmacol, 2017; Nadanaciva S, Toxicol Appl Pharmacol, 2013; Scatena R, Adv Exp Med Biol, 2012; Pinti M, Biochim Biophys Acta, 2006; Nadanaciva S, Toxicol Appl Pharmacol, 2007; Finsterer J, Drug Chem Toxicol, 2010; Sandoval-Acuña C, Chem Biol Interact, 2012; Li Y, Toxicol Sci, 2012; Kumar P, Nature Sci Rep, 2019)

Antibiotics

- **Because mitochondria are bacterial in origin, antibiotics that we use to target bacterial DNA are also highly toxic to mtDNA.**
- **Fluoroquinolone antibiotics,** such as Cipro and Levaquin, **not only contain fluoride but also damage mtDNA and prevent its replication, interfere with the ETC function and increase ROS formation**, including peroxynitrite, leading to cellular apoptosis.
- **The term 'floxxed' has been used for the side effects of these antibiotics, which include extreme fatigue, joint and muscle pains.** Does this sound like anything we know?
- Tetracycline and minocycline reduce ATP production, through depletion of cytochrome c and NAD+, and altered mitochondrial calcium retention capacity in rat hepatocytes.
- The antibiotics piperacillin, metronidazole, tigecycline, azithromycin and clindamycin trigger mitochondria-induced apoptosis in neurons.
- However, antibiotics can induce apoptosis in various forms of cancer through inducing mitochondrial dysfunction and oxidative stress and enhances the efficacy of chemotherapy, suggesting that this property can be turned to advantage!
- Note that antibiotics can also be present in drinking water and non-organic animal food, where they are used as growth-promoters.

(Wang X, Bioessays, 2015; Schonfeld P, FEBS J, 2013; Wang J, Biochem Biophys Res Commun, 2018; Cao C, J Bioenerg Biomembr, 2017; Tan Q, Med Sci Monit, 2017; Jiang S, Can J Physiol Pharmacol, 2018; Hsaio CJ, Chem Biol Interact, 2010; Xiao Y, J Biochem Mol Toxicol, 2019)

Environmental toxins

Virtually all environmental toxins impact mitochondria but research on this is still fairly new. So far there is evidence that the following categories of toxins cause damage:

- Pesticides and herbicides
- Toxic metals
- Persistent organic pollutants (POPs)
- Volatile organic compounds (VOCs)
- Air pollution
- Smoking
- Disinfectant byproducts
- Fluoride
- Ionising and non-ionising radiation

The damage is normally caused by increased ROS.

Pesticides and herbicides

- Glyphosate (herbicide): Inhibits the ETC and ATP production and lowers membrane potential.
- Dieldrin: disrupts mitochondrial protein production in the CNS
- Malathion: reduction in activity of ETC Complexes with elevation in lactate production; downregulation of apoptotic signalling
- Methoxychlor: inhibits mitochondrial respiration, induces ROS production and decreases antioxidant expression
- Fipronil: increased ROS production and apoptosis; decreased membrane potential
- Chlorpropham: increased apoptosis, reduced ATP production
- Paraquat: reduction in Complex I activity and increased superoxide production
- Rotenone: inhibits Complex I and induces apoptosis through increased ROS production.

(Bayley DC, Environ Toxicol Pharmacol, 2018; Cowie AM, Data Brief 2017; Karami-Mohajeri S, Hum Exp Toxicol, 2014; Gupta RK, Toxicol Appl Pharmacol, 2006; Zhang B, Pestic Biochem Physiol, 2015; Nakagawa Y, Toxicology, 2004; Cocheme & Murphy, 2008; Li N, J Biol Chem, 2003)

Glyphosate (herbicide)

- Glyphosate: Inhibits the ETC and ATP production and lowers membrane potential.
- Glyphosate is now the most common global herbicide, legal in the EU and widely used by farmers and gardeners, despite being labelled a ‘probable carcinogen’ by IARC.
- Claimed to be safe for humans and animals because it kills weeds via the shikimate pathway, which is not present in mammals.
- But it is present in plants and bacteria.
- That means it is damaging our gut microbiome and therefore almost certainly our mitochondria.
- Could this be the mechanism of the cancer incidence giving rise to hundred of million dollars being awarded in lawsuits?

Toxic metals

- Iron (excess and deficiency): haem synthesis and iron/sulphur clusters
- Mercury: mitochondrial swelling, membrane potential loss, increased cytochrome c release
- Cadmium: reduced ATP production, excess ROS
- Copper: membrane potential loss, reduced ATP production
- Aluminium: reduced mitochondrial membrane potential and increased ROS production in lymphocytes
- Arsenic: increased mitochondrial biogenesis and oxidative stress lead to mitochondrial DNA damage and mutation in arsenic induced cancers
- Lead: reduced ROS production, altered membrane potential and increased mitochondrial mass
- Nickel: increased ROS production and apoptosis, reduced mitochondrial membrane potential
- Tributyltin: decreased ATP production, induced mitochondrial fragmentation and reduced mitochondrial fusion

(Paul BT, Expert Rev Hematol, 2017; Ma L, Toxicol Res, 2018; Gobe D, Toxicol Lett, 2010; Borchard S, Toxicol in Vitro, 2018; Skarabahatava AS, J Trace Elem Med Biol, 2015; Lee CH, Front Biosci, 2016; Mani MS, Mitochondrion, 2019; Wang YF, Toxicol Appl Pharmacol, 2012; Yamada S, Toxicol in Vitro, 2016)

Persistent organic pollutants(POPs)

- Dioxins: degradation of the mitochondrial aryl hydrocarbon receptor and altered oxygen consumption rate and respiration
- Furans: loss of mitochondrial enzymes involved with the TCA cycle and ATP production
- Polychlorinated biphenols (PCBs): reduction in ATP production and downregulation of the mitochondrial aryl hydrocarbon receptor
- Phthalates: lowered mitochondrial membrane potential, promoted ROS generation, lowered mitochondrial antioxidants and activated caspases
- Bisphenol A (BPA): induces hypermethylation of PGC-1α, contributing to cardiomyopathy; induced excessive ROS production and apoptosis, downregulated all ETC Complexes, dissipated mitochondrial membrane potential
- Polycyclic aromatic hydrocarbons (PAHs): decrease sperm mtDNA copy number
- Polybrominated diphenyl ethers (PBDEs): depolarised the mitochondrial membrane potential and downregulated ATP production, reducing mt fusion
- Perfluorooctanoic acid (PFOA): induced mitochondria toxicity in foetal brain, liver and heart

(Hwang HJ, Toxicol Appl Pharmacol, 2016; Moro S, Toxicol Sci, 2012; Park WH, Sci Rep, 2017; Rosario-Berrios CA, Toxicol in Vitro, 2011; Jiang Y, Toxicology, 2015; Ling X, Environ Pollut, 2017; Shan A, Chem Res Toxicol, 2019; Salimi A, Environ Toxicol, 2019; Fu G, Environ Toxicol, 2017; Quan C, Environ Toxicol, 2017; Wang C, Environ Toxicol, 2017; Khan S, Environ Toxicol, 2016; Singh RP, Environ Toxicol Chem, 2015)

Volatile Organic Compounds (VOCs)

- Formaldehyde: induced oxidative stress, reduced ATP production, ETC enzymes (Complexes I and IV) and membrane potential,
- Polycyclic aromatic hydrocarbons (PAHs): induced oxidative damage
- Ethanol: induced Parkin over-expression and mitophagy
- Benzene: induced mtDNA mutations affecting cytochrome c oxidase (Complex IV)
- Naphthalene: decreased activity of several mitochondrial enzymes and increased oxidative stress
- Ammonia: inhibited mitochondrial dehydrogenase activity, induced dissipation of mitochondrial membrane potential, induced mitochondrial swelling and increased ROS production

(Zerin T, Cell Biol Toxicol, 2015; Pardo M, Sci Total Environ, 2019; Eid N, Cells, 2019; Wang D, J Thorac Dis, 2018; Vijayavel K, Chem Biol Interact, 2006; Niknahad H, Clin Exp Hepatol, 2017)

Air Pollution

- Nitrogen dioxide: decreased respiratory complexes and ATP production, increased ROS production, loss of membrane potential, inhibition of biogenesis
- Carbon monoxide: reduced mtDNA copy number, displaces oxygen from haem
- Particulate matter: decreased mitochondrial oxygen consumption rate and mtDNA copy number, increased mtDNA oxidation
- Sulphur dioxide: decreased mtDNA content, membrane potential, biogenesis and expression of Complexes IV and V
- Carbon dioxide: reduces biogenesis and increases apoptosis
- Hydrogen sulphide: inhibits OXPHOS
- Ozone: induces mitochondrial oxidative damage

(Yan W, Environ Res, 2015; Kaali S, Int J Environ Med Public Health, 2018; Breton CV, Mitochondrion, 2019; Qin G, Environ Sci Pollut Res Int, 2017; Takeda D, PLoS One, 2014; Buckler KJ, Pflugers Arch, 2012; Xu M, Free Radic Res, 2019)

Non-ionising radiation

- Mobile phone radiation: induced oxidative damage, apoptosis and depolarisation of the mitochondrial membrane
- Professor Martin Pall showed that low frequency microwave radiation (mobile phone, wireless router etc) opens voltage-gated calcium channels in the cell membrane, allowing an enormous influx of calcium ions into the cell.
- This activates nitric oxide (NO) and superoxide (O_2), which react to form peroxynitrite ($ONOO^-$), one of the most damaging of the reactive nitrogen species (RNS) to the mitochondria. Downstream responses to EMF exposures may be mediated through calcium/calmodulin stimulation of nitric oxide synthesis.
- Peroxynitrite causes huge amounts of oxidative stress, leading to inflammation, mitochondrial dysfunction and DNA damage. The European REFLEX study 2004 showed that the non-thermal effects of 2G and 3G radiation are similar to the effects of X-rays in terms of DNA damage (but can be repaired through PARPs, see previous slide).
- Also the mitochondrion is paramagnetic because it contains iron atoms. This means it can be polarised in a magnetic or electric field.

(Kahya MC, Biol Trace Elem Res, 2014; Pall ML, J Cell Mol Med, 2013)

Other

- Tobacco smoke: induced mitochondrial oxidative damage, including in cardiolipin
- Disinfectant byproducts (organohalogens) in water: mtDNA damage, depolarisation of the membrane, protein aggregation
- Fluoride: inhibited mitochondrial fission and mitophagy
- Ionising radiation: reduced mitochondrial membrane potential and increased ROS production.
- High energy frequencies of ionising radiation (principally scanners) cause single and double DNA strand breaks. These can be repaired but require poly-ADP ribose polymerases (PARPs), a family of enzymes that function as DNA damage sensors and bind strand breaks. The primary fuel for PARP is NAD+, so DNA repair results in depletion of NAD+ and mitochondrial membrane potential and decreases mitochondrial oxygen consumption. This slows the rate of ATP production, upregulates inflammatory pathways and eventually leads to functional impairment or cell death.

(Dikalov S, Am J Physiol Heart Circ Physiol, 2019; McMinn B, J Toxicol, 2019; Zhao Q, Arch Toxicol, 2019; Fernandez-Gil BI, Oxid Med Cell Longev, 2019; Chen Z, Tumour Biol, 2016; Virag L, Pharmacol Rev, 2002)

Bacterial endotoxins/lipopolysaccharides

- Endotoxins are lipopolysaccharides (LPS) and are found as components of the exterior cell wall of Gram-negative bacteria, such as E. coli, salmonella etc. They are secreted by bacteria within the cell and comprise a lipid and a polysaccharide, with cell wall antigens.
- While the bacterium may not be pathogenic, its endotoxin is a cell-associated toxin. LPS elicits a variety of inflammatory responses and activates complement and coagulation pathways, likely contributing to the pathology of Gram-negative bacterial infections.
- Levels of endotoxins are associated with many forms of critical illness, particularly sepsis, where its presence correlates with severity of disease and outcome. It may also be related to altered gut permeability.
- The endotoxin hypothesis of neurodegeneration states that endotoxins cause or contribute to neurodegeneration, inducing microglial activation, memory deficits and loss of brain synapses and neurons, promoting amyloid β and tau aggregation and neuropathology. This may be mediated in part by mitochondrial dysfunction.
- Endotoxins inhibit leydig cell steroidogenesis via perturbation of mitochondria and reduce mitochondrial membrane potential and cellular ATP content in liver and skeletal muscle. Mitochondrial DAMPs, including mtDNA, induce endotoxin tolerance in human monocytes in myocardial infarction.

(Brown GC, J Neuroinflammation, 2019; Guzman-Cottrill JA, 2012; Zhao W, J Neuroinflammation, 2019; Allen JA, Endocrine, 2004; Jeger V, Biomed Res Int, 2015; Fernández-Ruiz I, PLoS One, 2014)

Viruses

- Professor Lewis Thomas in 'The lives of a cell' (1974) worried about his mitochondria catching a virus.
- And he was right to worry! Pederson showed that some viruses (known as mitoviruses) used mitochondrial machinery for replication and found viral particles living within mitochondria.
- Viruses either induce or inhibit various mitochondrial processes in a highly specific manner so that they can replicate and produce progeny. Many regulate the balance between the anti- and proapoptotic proteins or modulate the mtPTP, thereby increasing their own survival within the host cell.
- Viruses such as Herpes simplex virus 1 deplete the host mtDNA, while HIV hijacks the host mitochondrial proteins to function fully inside the host cell.

(Pederson PL, Prog Exp tumor Res, 1978 ; Ohta A, Mitochondrion, 2011; Reshi L, Mitochond Dis, 2018; Anand SK, Adv Virol, 2013)

Alcohol

- Alcohol can increase blood acetate levels up to 30-fold after one drink. This presents an enormous challenge to cells and mitochondria, as there will be massively increased protein acetylation, which will deactivate sirtuins.
- Alcohol is metabolised to acetaldehyde by losing a hydrogen ion, which is taken up by NAD^+ to create NADH. Acetaldehyde is then metabolised to acetate by losing another hydrogen ion, which is again taken up by NAD^+. Acetaldehyde is extremely toxic to the ETC.
- Alcohol impairs hippocampal processing and induces hepatocyte injury through mitochondrial dysfunction and its cardiac toxicity is mediated through increased opening of the mtPTP. Foetal alcohol syndrome is also mediated through excessive mitochondrial NADH production.

(Manzo-Avalos S, Int J Environ Res Public Health, 2010; Mira RG, Drug Alcohol Depend, 2019; Manzo-Avalos S, Int J Environ Res Public Health, 2010; Hajnóczky G, Alcohol Clin Exp Res, 2005)

Elevated blood glucose

- Mitochondria are particularly sensitive to raised blood glucose. Their NADH production can overwhelm the ETC, leading to damaging levels of mitochondrial ROS.
- High glucose concentrations can also suppress retrograde signalling (where the mitochondria send SOS signals to nuclear DNA).
- Glycation, brought about by both oxidative and non-oxidative reactions, attaches a sugar molecule to a protein or a lipid. This can cause serious malfunction to the metabolic pathway and destabilise the mitochondrial membrane, impairing the shuttle of fatty acids into the mitochondria for β-oxidation, as well as other metabolic substrates that help sustain ATP levels. If lipids in the membrane are glycated, lipid peroxidation can occur and the fluidity of the membrane increases.

(Yan LJ, J Diabetes Res, 2014; Pun PBL, Int J Cell Biol, 2012)

Hypoxia and excessive oxygen consumption

- Oxygen levels must be maintained within strict limits to avoid mitochondrial mutation and ROS production.
- Hypoxia causes a wide spectrum of alterations in mitochondrial structure, dynamics and genome stability, resulting in reduced respiration, excessive ROS production, oxidative damage and accumulation of mtDNA mutations.
- In the absence of sufficient oxygen, OXPHOS is reduced in favour of glycolysis, leading to the build-up of lactic acid. In excess, lactic acid decreases the ability of muscles to contract. There will also be purine leakage, causing build-up of uric acid and development of gout.
- Hypoxia also results in reduced haemoglobin and ferritin levels, which may cause elevated nitric oxide (NO) levels by induction of inducible NO synthase (iNOS). This causes loss of mitochondrial membrane potential and fragmentation of mitochondrial DNA, eventually resulting in apoptosis.
- Excessive consumption of oxygen in the ETC leads to damage to surrounding genetic material, proteins and lipids that are responsible for membrane structure and transport.
- Uncoupled protons can induce increased levels of oxygen consumption while redirecting protons away from ATP production. This causes serious damage to lipids in the mitochondrial membrane.

(Nicolson G, J Am Nutraceutical Assoc, 2010; Lane RS, Arthritis Res Ther, 2015)

Reactive oxygen species (ROS) and lipid peroxides

- The mitochondria are not only the major site of ROS production but they are also the first site to be compromised by prolonged exposure to ROS, particularly the cristae and mtDNA.
- During normal OXPHOS, up to 4.0% of all the oxygen consumed is converted in the mitochondria to the superoxide radical and other ROS, while peroxynitrite can also be formed by the combination of ROS with RNS via nitric oxide. Superoxide has been shown to damage the TCA cycle enzyme aconitase, an iron/sulphur cluster electron carrier. This exposes iron, which reacts with H_2O_2 to produce hydroxyl radicals.
- All ROS can generate lipid peroxides, nitrogen and sulphur oxide free radicals.
- Cardiolipin is highly sensitive to oxidative damage due to its high content of PUFAs and location near the site of ROS production. Consequently, pathological remodelling of cariolipin has been implicated in the aetiology of mitochondrial dysfunction commonly associated with diabetes, obesity, heart failure, neurodegeneration and ageing.
- Oxidative damage to mtDNA occurs at 5-10 times the rate of damage to normal DNA.
- Lipid peroxides interacting with neurotoxic proteins can reduce ATP synthesis (by 30% in Alzheimer's disease). Lipid peroxides have a strong affinity for mitochondrial protein binding, particularly cardiolipin, and will oxidise them and inhibit their function.

(Witztum JL, J Clin Invest, 1991; Shi Y, J Biomed Res; Aufschnaiter A, Cell Tissue Res, 2017; Zhong H, Redox Biol, 2015)

Food and beverage additives

- Food colouring (E numbers) can significantly inhibit mitochondrial energy production.
- Silver and titanium nanoparticles induced mitochondrial swelling and uncoupling in hepatocytes and upregulated ROS production.
- Stevia acted as an uncoupling agent in liver mitochondria, while aspartame upregulates mitochondrial ROS production and apoptosis.

(Reyes FGR, J Food Add Contam, 2009; Pereira LC, J Trace Elem Med Biol, 2018; Kelmer Bracht A, Biochem Pharmacol, 1985; Qu D, Molecules, 2019)

Inadequate haem synthesis

- Haem biosynthesis occurs partially in the mitochondria.
- Inadequate haem synthesis disrupts mitochondrial cytochrome c oxidase (Complex IV) and the integrity and function of mtDNA and causes oxidant release. Other effects of haem deficiency include low haemoglobin and impaired Phase I detoxification, hormone synthesis and vitamin D metabolism.
- It has been found to trigger many of the signs and symptoms of Alzheimer's disease, activating nitric oxide synthase and amyloid precursor protein and disrupting iron and zinc homeostasis, suggesting that failure of mitochondrial haem synthesis may drive neurodegenerative disease and brain ageing.
- Biotin deficiency caused haem deficiency in lung fibroblasts, which led to loss of Complex IV, triggering oxidative damage.
- One cause of haem deficiency may be exposure to lead and aluminium, as both can block synthesis. Other inhibitors of haem biosynthesis include salicylic acid, which induces mitochondrial injury.

(Atamna H, Arch Biochem Biophys, 2002; Atamna H, Proc Natl Acad Sci, 2002; Atamna H, J Nutr, 2007; Fiorito V, Pharmaceuticals, 2018; Gupta V, Mol Pharmacol, 2013)

Deuterium

- We normally only hear of deuterium in connection with heavy water production for the hydrogen bomb during WW2. Deuterium is hydrogen with an extra neutron (known as 'heavy hydrogen'); it has the same properties as hydrogen but is twice the weight. It occurs naturally in small proportions, when it can be beneficial.
- The ATP synthase motor in Complex V should by powered by the proton motive force of pure hydrogen ions. Excess deuterium will reduce the power of the motor, thereby reducing the amount of ATP produced and increasing ROS production.
- We acquire deuterium through food, particularly processed foods, fluids and air. Our ability to process and excrete deuterium decreases with weight gain, age, illness and lack of quality sleep. Levels can be measured by a saliva test kit.
- One way to reduce deuterium in our bodies is to supplement molecular hydrogen (see later). Another way is to drink specially treated water (expensive) or to eat a ketogenic diet. Fat burning through β-oxidation ensures that water produced by OXPHOS is deuterium-free. Grains have particularly high deuterium levels, so any grass-fed animal has a lower level of deuterium. Pesticides/herbicides carry a high load of deuterium; in fact any non-organic food will contain a level that is too high for optimum health.
- Deuterium-depleted water can protect against cancer, inhibiting proliferation and inducing apoptosis (Yavari K, Nutri Cancer, 2019; Gyongyi Z, Nutr Cancer, 2013).

Are mitochondria alive?

- There is a vigorous online debate about this.
- **The expert consensus seems to be that mitochondria are not independently alive as they cannot exist outside the cell.**
- However, parasites and viruses cannot survive without a host, yet they are definitely alive.
- But mitochondria are alive in the sense that they respire, move around independently and reproduce, just not in the way that we do!
- Although mitochondrial evolution has led them to this point, there is no technical reason why they should not evolve further, which could involve independent life again.
- The mitochondrial genome is evolving more than 50 times faster then our personal nuclear genome.

Professor Nick Lane, Imperial College

- A bizarre intelligence that has been on earth for around 2 billion years.
- And their genome is evolving more than 50 times faster then our personal nuclear genome.
- ‘Clandestine Rulers of the World’?