

Oxidative phosphorylation

Cell energetics in a nutshell

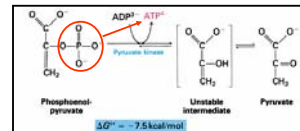
- Majority of the processes in living cells such as
 - Biosynthesis
 - Active transport (ATP pumps)
 - Motility
 require energy
- Energy comes from the "food" molecules that are degraded

Where is energy coming from?

- Breaking bonds in glucose and fatty acids
- The energy from glucose (or fatty acids) is used to produce ATP (from ADP and Pi) in two ways
 - Substrate level phosphorylation
 - Oxidative phosphorylation

Substrate level phosphorylation

- Phosphorylation because phosphate group is added (in this case to ADP)
- Substrate level because phosphate group is transferred directly from phosphorylated intermediate to ADP
- Happens in glycolysis and Krebs cycle



Oxidative phosphorylation

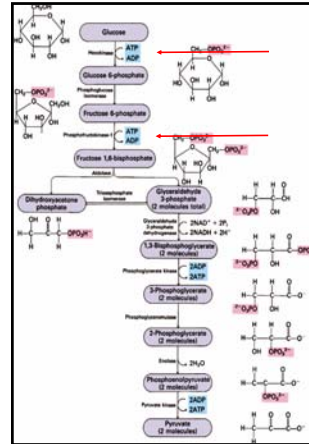
- Oxidative
- Happens only in aerobic conditions (molecular oxygen is an acceptor of electrons)
- Two steps
 - Citric acid (Krebs) cycle
 - ATP synthesis in electron transport chain
- Happens in mitochondria

Oxidation of glucose

- Before glucose can enter Krebs cycle it has to be "prepared" in the process of **glycolysis**
 - Glucose is broken to 2 molecules of the 3-carbon compound pyruvate and a small amount of energy
 - Pyruvate is transported to mitochondria where it forms acetylCoA
 - AcetylCoA enters Krebs cycle

Glycolysis

- Initial stage of glucose metabolism
- Does not involve O_2 - **anaerobic process**
- Takes place in the cytosol
 - Carried out by the cytoplasmic enzymes
- Glycolysis produces a small amount of energy and the 3-carbon compound pyruvate

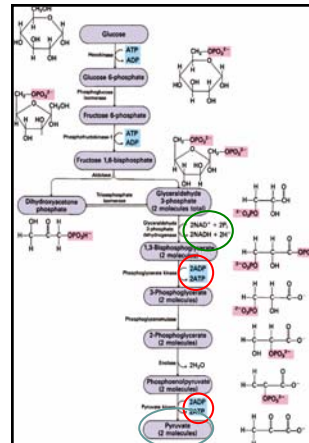


Glycolysis

- Substrate is D-glucose (hexose)
- Has nine steps (enzymes)
- First reactions of glycolysis involve energy investment (2 ATP)
- All intermediates between glucose and pyruvate are phosphorylated compounds

Glycolysis

- In the remaining steps the energy is harvested
 - 4 ATP molecules are formed in substrate level phosphorylations
 - Net gain is 2 ATP molecules because 2 ATP were used in early steps of glycolysis
 - Some extra energy (proton and 2 electrons) are transferred to 2 molecules of $NAD^+ = NADH$ that is stored for later oxidation



Products of glycolysis

- Three major products of glycolysis are:
- Chemical energy in the form of ATP
 - Chemical energy in the form of NADH
 - Pyruvate

Products of glycolysis

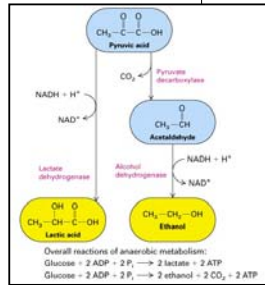
- 2 ATP molecules represent only 2.2% of the potential energy of glucose (glycolysis is not very energy efficient process)
- 2 molecules of pyruvate that can be further processed to produce more energy (ATP)
- Some energy in 2 in molecules of NADH that can be recovered later in electron transport chain

What happens next?

- In aerobic cells, the pyruvate is transported into mitochondria, where it is metabolized to H_2O and CO_2 (oxidized)
- Oxidation in mitochondria is coupled to ATP synthesis \Rightarrow more energy

Metabolism of glucose in anaerobic conditions

- In anaerobic cells, the pyruvate is metabolized to lactate or ethanol and CO₂ and some ATP

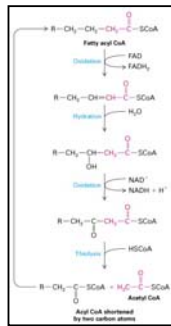


Oxidation of other "food" molecules

- Most fatty acids are metabolized in peroxisomes
 - Oxidation is not coupled to production of ATP
 - Energy is converted to heat
- Fatty acids can also be oxidized in mitochondria
 - Oxidation is then coupled to ATP production

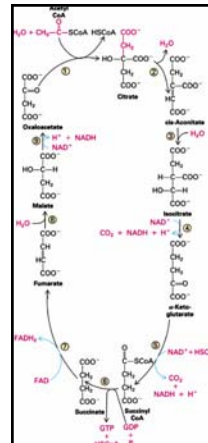
Oxidation of fatty acids

- Fatty acids enter mitochondria as fatty acyl CoA
- In mitochondrial matrix they are broken into 2 carbon pieces attached to CoA (acetyl-CoA) that enter Krebs cycle



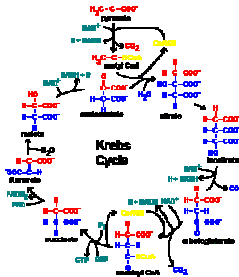
Krebs cycle a.k.a. tricarboxylic acid cycle

- Aerobic process**— needs oxygen
- Takes place **ONLY** in mitochondria
- All enzymes are located in mitochondrial matrix



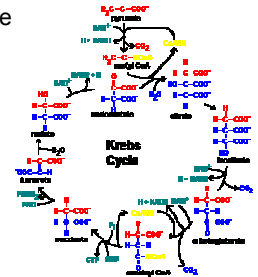
Krebs cycle

- First enzyme joins acetyl group of acetyl CoA to oxaloacetate to produce citrate
- The remaining enzymes catalyze a series of rearrangements, decarboxylations and oxidation - reductions

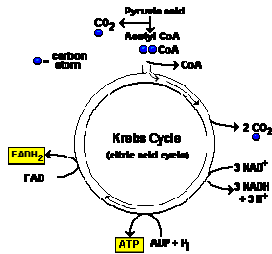


Krebs cycle

- Products of this cycle are CO₂ and oxaloacetate (the molecule we began with), ATP and stored energy



Krebs cycle – energy production



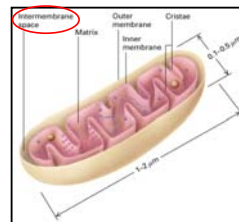
Now we ended up with

- 4 molecules of ATP
 - 2 from glycolysis
 - 2 from Krebs cycle
- And a lot of stored energy in reduced nucleotides NADH and FADH₂

Processing of the stored energy

- Electrons stored in NADH and FADH₂ are losing their energy as they travel through **electron transport chain**
 - Finally they are transferred to O₂ to form H₂O (that's why we call the process **oxidative phosphorylation**)
- “Lost” energy is used to pump protons to intermembrane space (!!! Create proton gradient)
- Proton gradient is used by F₀F₁ pump to synthesize ATP

Structure of mitochondrion

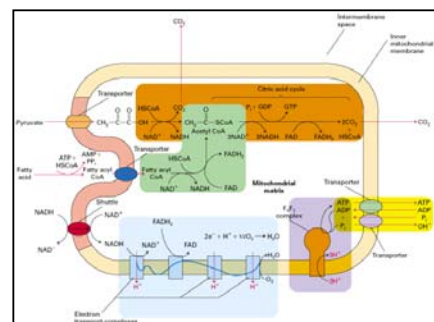


- Mitochondria possess two structurally and functionally distinct membranes and two separate spaces
- Intermembrane space - hydrogen ion reservoir

Reactions in mitochondria

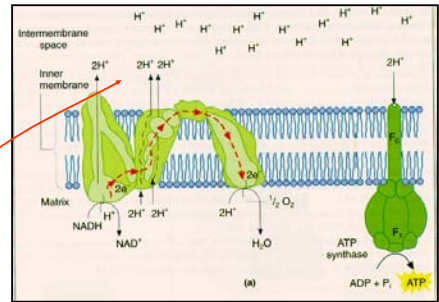
- Oxidation of pyruvate or fatty acids to CO₂ coupled to the reduction of NAD⁺ and FAD – **in the matrix**
- Electron transfer from NADH and FADH₂ to O₂ - **inner membrane** - generation of proton-motive force
- ATP synthesis - **ATP synthase in the inner membrane** - uses the energy stored as proton gradient

Reactions in mitochondria



Electron transport chain

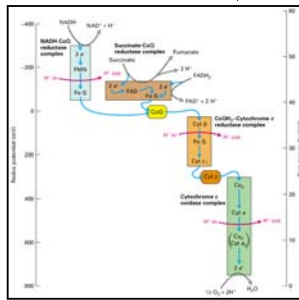
- Four multiprotein complexes in the inner mitochondrial membrane
- Transfer electrons to O_2 (redox reactions)
- Energy is used to pump H^+ into intermembrane space (create a gradient)
 - Redox driven pumps



Transfer of electrons causes release of energy that pumps protons from matrix to intermembrane space - this produces high-energy reservoir

Electron transport chain a.k.a. respiratory chain

- Allows the large amount of free energy produced by the transfer of electrons to O_2 to be released in small increments
- This energy is conserved in proton gradient

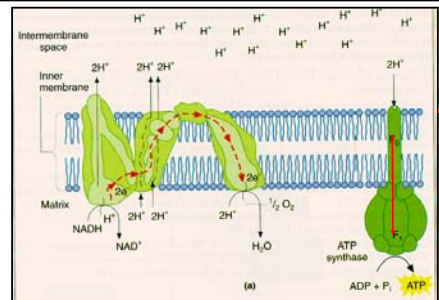


Where the energy is?

- Stored in chemical and electrical gradients
- Chemical - proton gradient (pH)
- and voltage gradient (transmembrane electric potential)
 - Voltage gradient is possible because the inner mitochondrial membrane is poorly permeable to other ions than H^+

Final step – synthesis of ATP by a F_0F_1 pump (ATP synthase)

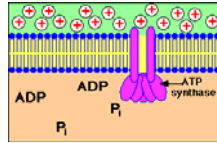
- Protons accumulated in the intermembrane space flow back into the mitochondrial matrix through a pore in the ATP synthase (because of the gradient)
- Conformational change of the F_1 subunit catalyzes the phosphorylation of ADP to ATP
- Gradient is changed into energy (ATP)



Protons flowing from intermembrane space back to matrix power an ATP synthase

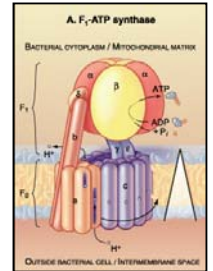
Mitochondrial ATP synthase or F_0F_1 type pump

- Moves protons from intermembrane space of mitochondria to the matrix
- **Protons flow down the gradient !!!**
- Movement of protons powers the synthesis of ATP
- In fact this is an opposite of the pump – it makes ATP !!!



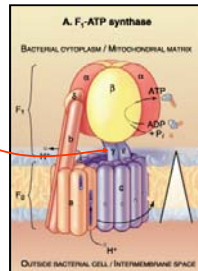
Structure of ATP synthase

- F_1
 - Mitochondrial matrix
 - Enzyme – synthesizes ATP
- F_0
 - Transmembrane
 - Proton channel

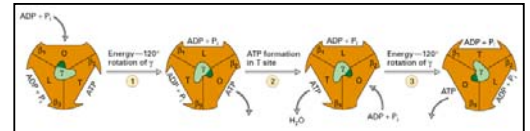


Model of ATP synthesis

- Energy released by movement of protons down the concentration gradient powers rotation of γ subunit
- This changes the conformation of β subunit and ATP synthesis



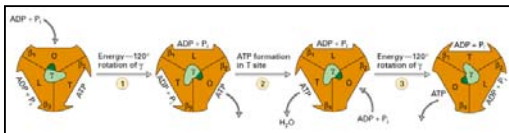
Model of ATP synthesis



- Rotation of the protein shaft couples proton flux to ATP synthesis

Model of ATP synthesis

- Catalytic sites on β subunits assume different conformation every 120° turn
 - One of them binds ADP and P_i
 - One of them catalyses addition of P_i to ADP
 - One of them releases ATP



Energy conversion of reduced nucleotides (stored energy)

- Each NADH from glycolysis (substrate level phosphorylation) yields 2ATP molecules
- Each NADH from conversion and citric acid cycle yields 3 ATP
- Each $FADH_2$ yields 2 ATP molecules