

The Energy Generating Machinery of the Cell

Professor Alfred Cuschieri
University of Malta
Department of Anatomy



Objectives:

By the end of this session, the student should be able to:

- Describe the structure and compartments of mitochondria
- Interpret the structure of mitochondria on electron micrographs
- Correlate differences in mitochondrial morphology and number with function
- Outline how cells synthesise ATP by glycolysis, anaerobic respiration and mitochondrial or aerobic respiration
- Name the sites where the molecules involved in glycolysis and the Krebs cycle are located
- Outline the sites and functions of the electron transport complexes
- Outline the structure and function of F_1F_0 complexes in mitochondria
- Outline the location and function of mitochondrial DNA

Recommended Reading

The World of the Cell: Becker WM, Kleinsmith LJ, Hardin J. 4th Edition

- Chapter 13 Chemotropic Energy metabolism: Glycolysis and Fermentation
- Chapter 14 Chemotrophic Energy Metabolism: Aerobic Respiration

Note: This book describes the biochemical details of respiration, which are not required for the purposes of this part of the medical course. The biochemical aspects of respiration will be dealt with fully in the biochemistry.

Structure of mitochondria

Mitochondria consist of two compartments enclosed by two membranes:

- a) The outer membrane
- b) The outer compartment is enclosed by outer and inner membranes
- c) The inner membrane is thrown into folds or cristae
- d) An inner compartment containing the matrix and including:
 - i. DNA fibrils
 - ii. Ribosome particles
 - iii. Particles in matrix



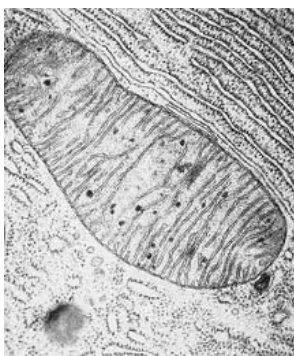
Negative staining demonstrates F1 particles projecting from the inner membrane into the inner mitochondrial compartment.



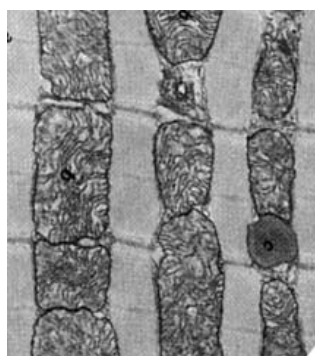
Mitochondria vary greatly their morphology.

- Vary in shape: filamentous to spherical
- Vary in size: 0.5 to 7.0 μm
- Vary in number from a few in spermatozoa to 300,000 in oocytes
- Vary in number and shape of their cristae
- Are often clustered in certain regions of cells

The following examples show the variable mitochondrial morphology in different cells



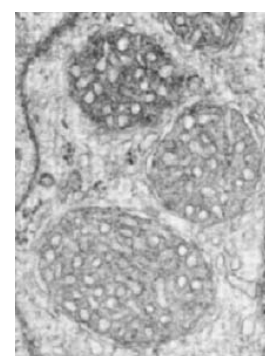
a.



b.



c.



d.

- a) Liver cell - oval mitochondria with numerous cristae
- b) Skeletal muscle - rows of mitochondria
- c) Kidney - elongated mitochondria situated between basolateral invaginations of the plasma membrane
- d) Steroid secreting cell - mitochondria with tubular cristae

In general mitochondria are most numerous in cells that have a high metabolic activity. They are often concentrated around the sites of high ATP utilization. For example, mitochondria in sperm are concentrated around the basal body. In renal tubular cells, mitochondria are located between baso-lateral membrane invaginations, which contain ATPase pumps for establishing concentration gradients.

The inner mitochondrial compartment contains:

- a) The mitochondrial matrix
- b) Ribonucleoprotein particles (mitochondrial ribosomes)
- c) Mitochondrial DNA

The cristae carry the F1 particles facing the inner compartment

F1 particles are :

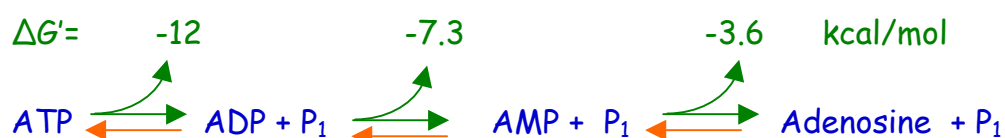
- a) 8.5 nm in diameter
- b) Regularly spaced at 10 nm intervals
- c) Attached to the membrane by a stalk
- d) Involved in the coupling of oxidation and phosphorylation

Most energy requiring or endergonic reactions in cells utilise adenosine triphosphate (ATP) in the following reaction:



Structure of the ATP molecule

Adenosine is a nucleoside consisting of the adenine and ribose. Addition of one, two or three phosphate groups form adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) respectively. The phosphate groups are linked by **phosphoanhydride bonds**, which are broken down by hydrolysis releasing a phosphate group and free energy. The actual free energy released at each step is shown in the diagram below. **Hydrolysis** of ATP to ADP releases the maximum amount of energy per molecule.

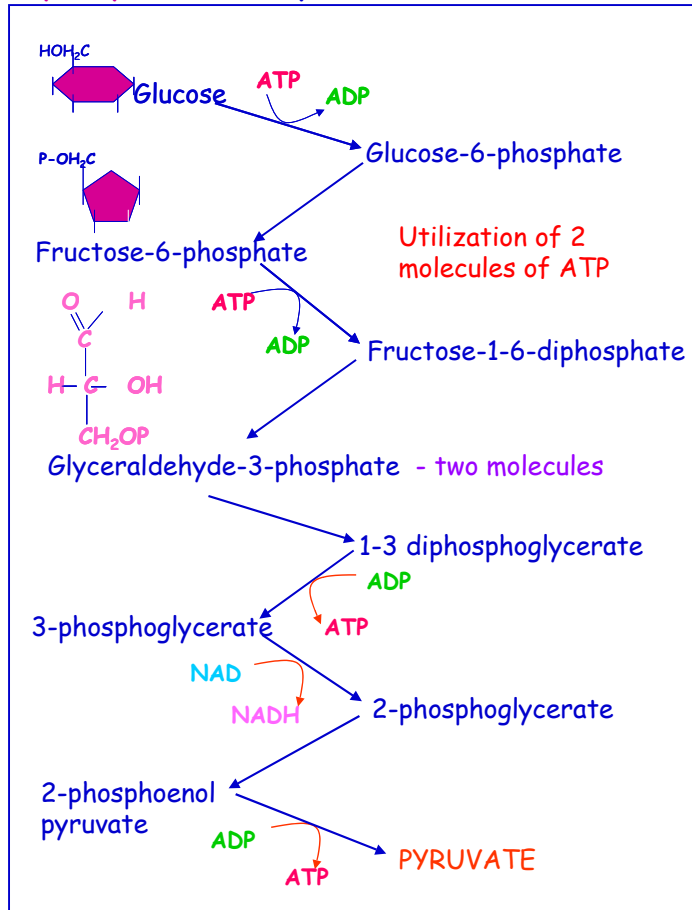


For the **synthesis** of AMP, ADP and ATP, the reactions run in the reverse direction (indicated in orange) and utilises approximately the same amounts of energy at each step. The energy for ATP synthesis is provided by oxidation.

Cells have evolved three major ways of producing ATP

1. Glycolysis is the breakdown of glucose to pyruvate generating ATP
2. Oxidative phosphorylation is the utilisation of atmospheric oxygen coupled with phosphorylation of ADP
3. Photosynthesis uses light energy to produce glucose and ATP.

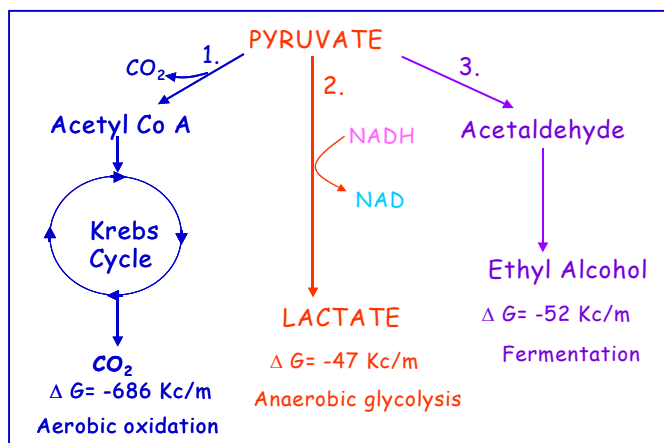
Glycolysis is the process of breakdown of glucose to pyruvate.



It involves a number of intermediates. The breakdown of glucose to glyceraldehydes-3-phosphate involves the utilization of two molecules of ATP. The further breakdown to pyruvate releases four molecules of ATP (because two molecules of glyceraldehydes 3-phosphate were produced in the first part of the reaction). This is a net release of 2 molecules of ATP.

Cells can break down pyruvate in three possible ways:

1. **Aerobic respiration** involving the utilization of atmospheric oxygen
2. **Anaerobic Glycolysis**
3. **Alcoholic fermentation**



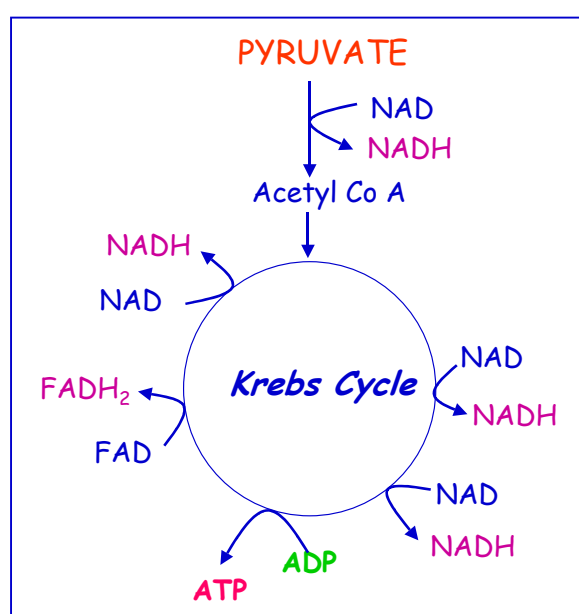
Aerobic respiration is almost 15 times as efficient in energy release as the other pathways. **Anaerobic glycolysis** is used in mammalian cells under conditions of temporary oxygen deprivation. This occurs in muscle following strenuous exercise when

the cells utilize oxygen faster than it is supplied. **Lactate**, produced in the reaction causes the temporary muscle pain until it is removed by the circulation to the liver to be converted back to glucose. Alcoholic fermentation occurs in some organisms but not in mammalian cells. The enzymes that are responsible for glycolysis are situated in the cytosol. Glycolysis does not occur in mitochondria.

The process of aerobic respiration involves a series of reactions that may be conveniently subdivided into 3 stages:

1) **Krebs cycle or tricarboxylic acid cycle (TCA)** involves

- a) The oxidation of pyruvate to CO_2 ,
- b) The reduction of 5 molecules NAD to NADH
- c) The reduction of 1 molecule FAD to FADH_2
- d) The formation of 1 molecule of ATP.



It does not involve oxygen utilization but is the first step in the process of aerobic respiration. NAD (nicotinamide adenine dinucleotide) and FAD (flavine adenine dinucleotide) are coenzymes that act as electron acceptors. Their reduced forms, NADH and FADH_2 will pass on their electrons through a series of reactions and ultimately to O_2 .

The intermediate molecules and enzymes of Krebs cycle (to be

dealt with in biochemistry) are situated in the mitochondrial matrix.

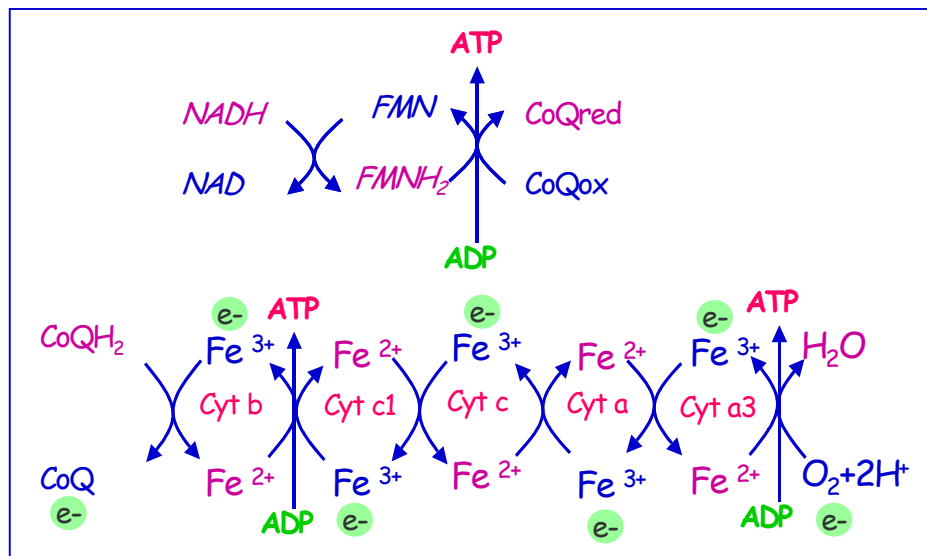
2) **Transfer of electrons to O_2 along the cytochrome chain.**

The process of electron transfer (or oxidation) from the reduced coenzymes NADH and FADH_2 to oxygen occurs stepwise through a series of intermediates or carriers, which include:

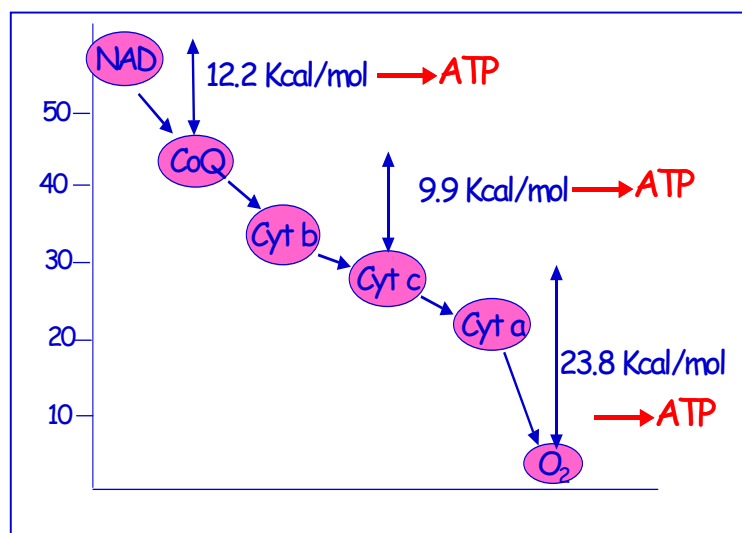
- a) **Flavoproteins** are membrane-bound proteins that have either FMN (flavine mononucleotide) or FAD (flavine adenine dinucleotide) as prosthetic groups that transfer electrons from molecules in the Krebs cycle to Coenzyme Q. They include:
 - i) NADH dehydrogenase, which has FMN as prosthetic group,
 - ii) Succinate dehydrogenase which has FAD as prosthetic group,

- b) **Iron-sulphur proteins** containing iron-sulphur centres in which iron is oxidised from Fe^{2+} to Fe^{3+} (the electron acceptor) or reduced from Fe^{3+} to Fe^{2+} .
- c) **Cytochromes** are intrinsic membrane proteins that contain iron as part of a porphyrin prosthetic group, and transfer electrons from Fe^{2+} to Fe^{3+} . Some cytochromes contain iron-copper centres in which electrons are transferred from Cu^+ to Cu^{2+} .
- d) Coenzyme Q is a non-protein molecule, which can be reversibly converted from quinone (CoQ) to dihydroquinone (CoQH_2).

The figure below shows the electron transport system

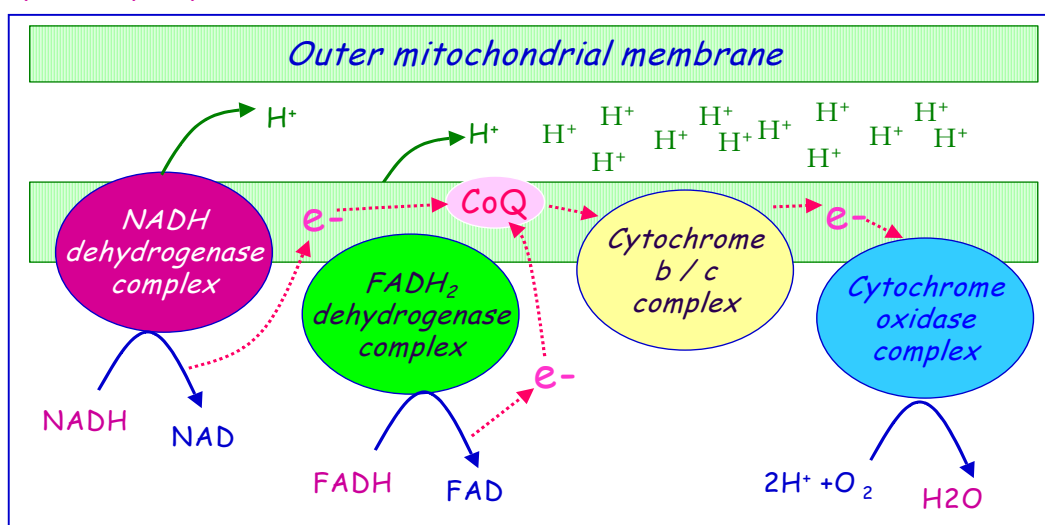


There is a **gradual decline in free energy** as electrons are transferred from NADH to O_2 along the respiratory chain. Enough energy is liberated to drive the synthesis of ATP at different steps as indicated in the following diagram.



The electron transfer system is located on the **inner mitochondrial membrane** as four major respiratory enzyme complexes as indicated in the following diagram.

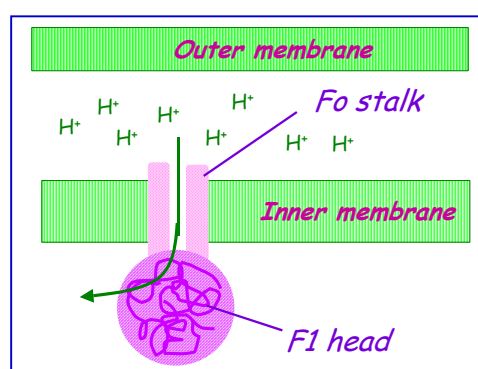
The process of electron transfer also involves a dehydrogenation of the reduced molecules NADH and FADH_2 . The H^+ (protons) released in the process accumulate in the outer mitochondrial compartment creating a strong **proton electrochemical gradient** across the inner membrane. The dehydrogenase complexes are therefore transmembrane proteins and act as **proton pumps**.



3) Phosphorylation of ADP to ATP (ATP Synthesis)

ATP synthase is a transmembrane protein on the inner mitochondrial membrane. It consists of the F_1F_0 complex consisting of:

- An F_1 particle (or head)
 - Situated on the matrix side of the inner membrane
 - Consists of 5 polypeptide units
 - Is the site for ATP synthesis
- An F_0 particle (or stalk)
 - Is embedded in the membrane,
 - Consists of 4 hydrophobic polypeptide subunits
 - Provides a proton channel from the outer to the inner mitochondrial compartments along the electrochemical gradient.



The energy derived from the flow of protons along the electrochemical gradient drives the ATP synthesis in the F_1 head.

In summary the locations of the molecules involved in energy release are:

- a) Glycolysis and anaerobic respiration in the cytosol
- b) Krebs cycle in the mitochondrial matrix
- c) The electron transport system in the inner mitochondrial matrix
- d) Proton pump and electrochemical gradient in the inner membrane and outer space respectively
- e) ATP synthesis (phosphorylation) in the F_1 particles
- f) Proton channels in F_0 stalk.

The outer mitochondrial membrane:

- a) Is permeable to most small molecules (< 5000MW), particularly to pyruvate and NADH (generate by glycolysis in the cytosol)
- b) Contains pores formed of the integral protein porin
- c) The pores are about 2nm diameter
- d) The pores are voltage gated to prevent proton from diffusing out of the outer compartment
- e) Contains monoamine oxidase - an enzyme that breaks down (inactivates) amines such as epinephrine, serotonin, dopamine and some other neurotransmitters

The inner mitochondrial membrane:

- a) Is impermeable to ions and small molecules
- b) Contains specific translocase proteins for transporting ions and other molecules, the most important of which are:
 - A specific Ca^{2+} ATPase pump transports Ca^{2+} into the matrix and regulates cytoplasmic Ca^{2+} levels
 - A proton pump (part of the dehydrogenase complexes) creates a proton gradient in the outer membrane
 - Phosphate carrier
 - Pyruvate carrier
 - Fatty acid carrier
 - ADP carrier
 - Specific carriers for molecules involved in Krebs cycle e.g. di- and tri-carboxylate, oxoglutarate, glutamate etc
- c) Contains the electron transport system

The outer mitochondrial space contains:

- A proton electrochemical gradient

The matrix contains:

- a) The molecules required for:
 - Krebs cycle
 - Fatty acid oxidation
 - Transamination and de-amination of amino acids
- b) DNA strands and ribonucleoprotein particles for the synthesis of:
 - Electron transport complexes including NADH reductase, cytochrome oxidase, and cytochrome c reductase oxidase
 - The F_0 protein channels
 - R-RNA and t-RNA for synthesis of the above proteins.
- a) Calcium phosphate particles - storage of Ca^{2+}

Clinical applications:

- 1) Genetic mitochondrial disorders are caused by mutations in the mitochondrial DNA resulting in defective proteins, usually of the electron transport complexes. Mitochondrial disorders:
 - a) Affect mainly skeletal muscle and neurons:
 - i) Mitochondrial myopathies include a variety of muscle disorders including muscle weakness and fatigue. A group of these myopathies is characterized by "ragged-red muscle fibres" due to aggregates of abnormal mitochondria. The defect is in one of the proteins in the four complexes of the electron transport system.
 - ii) Leber's optic atrophy (or neuropathy) causes blindness in young adults, mostly men due to optic atrophy. The defect usually affects NADH-Coenzyme Q oxidoreductase.
 - b) Shows mitochondrial inheritance - transmitted by females; affects both males and females
- 2) Chronic alcohol intake - ethanol is converted to acetaldehyde, which inhibits monoamine oxidase in the outer membrane and results in accumulation of serotonin
- 3) Psychoactive drugs, such as isocarboxazid and phenelazine, inhibit monoamine oxidase in the outer membrane, and are used in the treatment of depression.

- 4) Mitochondrial DNA sequences show racial and evolutionary differences. Mitochondrial DNA analysis has been used to trace ancestry. Africans have the highest frequency of mutations, which implies that Africans are evolutionarily the oldest race because they had more time to accumulate mutations. On this basis, and because mitochondrial mutations are transmitted only through females, it has been postulated that "mitochondrial Eve" lived in Africa about 200,000 years ago.