Programme: F.Y BSc
Subject: Microbiology
Paper Code: MIC 101
Paper Title: Microbiology and Biochemistry II
Unit: IV
Module Name: Electron transport chain; components
Module No: 19
Name of the Presenter: Rajani Prabhu

19_Notes

- The energy rich carbohydrates (such as glucose), fatty acids and amino acids undergo a series of metabolic reactions to finally get oxidised to CO2 and water.
- These reactions produce number of reducing equivalents. These reducing equivalents are transferred to coenzyme NAD+ and FAD+ to produce NADH2 and FADH2.
- The NADH and $FADH_2$ formed in glycolysis, TCA cycle and fatty acid oxidation are energy-rich molecules because they contain a pair of electrons that have high transfer potential.
- These reduced coenzymes pass through a series of reactions called electron transport chain or respiratory chain, finally reducing oxygen to water.
- This passage of electrons is associated with loss of free energy, a part of this free energy is used in ATP generation.
- Because energy generated by the transfer of electrons through the electron transport chain to O_2 is used in the production of ATP, the overall process is known as **oxidative phosphorylation.**
- The ETC takes place in **mitochondria** in case of **eukaryotic cells**. Since **bacterial cells** do not have mitochondria, all enzymes required for the electron transport chain of bacteria are membrane bound and is present in the **plasma membrane**. The hydrogen ion gradient, which drives ATP synthesis, is thus generated across the plasma membrane in case of bacteria.
- Since the structure of mitochondria is similar to a bacterial cell, we can discuss the structure of mitochondria and see all the components of ETC present in it.
- The components and working of ETC in eukaryotes are very similar to that of bacteria.
- Mitochondria has outer mitochondrial membrane, inner mitochondrial membrane (IMM) and the space between the two membranes is called intermembrane space (IMS). The IMM has infoldings called cristae.
- ETC components are located in the IMM
- The inner surface of IMM consist of specialised structures which look like lollipops called as phosphorylating subunits which are centres of ATP production.

- The matrix consists of enzymes responsible for Kreb's cycle, Beta oxidation and oxidation of amino acids.

Components of ETC

The ETC consist of:

A) Hydrogen and electron carriers
B) Membrane bound enzyme complexes

Hydrogen and electron carriers

1) **NAD+** :
   - It is a coenzyme that acts as a hydride carrier as it carries hydride ion (H^-).
   - It receives two hydrogen atoms (2H) from substrates as isocitrate, malate etc.
   - Its reduced form ($NADH+H^+$) passes its hydrogen to flavoprotein containing FMN and iron sulfur protein (FeS).
2) **Flavoproteins**:
   - FAD and FMN serve as hydrogen carriers, which are tightly bound to flavoproteins as a manner that prevents its reduced form from reacting with oxygen directly.
   - There are many types of flavoproteins that have a role in electron transport chain. Two main types are: FMN containing Flavoprotein receives two hydrogen atoms from reduced NAD^+ passing them to coenzyme Q. FAD containing Flavoproteins receive two hydrogen atoms from substrates as succinate, acyl CoA passing them to coenzyme Q.
3) **Ubiquinone (coenzyme Q):**
   - Ubiquinone's are a group of compounds containing quinone ring but vary according to number of isoprene units at the side chain.
   - The most common ubiquinone is coenzyme Q that has structural similarity to vitamin K.
   - It is a small, lipid soluble molecule, therefore it can freely diffuse in the inner mitochondrial membrane, colleting reducing equivalents from the more fixed component of the respiratory chain.
   - Ubiquinone can carry two hydrogen atoms forming ubiquinol (reduced coenzyme Q or QH2) or one hydrogen atom forming semiquinone (QH*). So, it forms a bridge between flavoproteins, which can carry 2 hydrogen atoms, and cytochrome b, which can carry one electron only.
   - Reduced coenzyme Q passes the electrons to cytochrome b and releases $2H^+$ into the mitochondrial matrix
4) **Cytochromes:**
   - They are electron carriers transferring electrons from coenzyme Q to oxygen.
   - They have given letter designation a, b and c according to their order of discovery.
   - All cytochromes are hemoproteins but they differ in redox potential.
   - The heme in cytochromes differs from that of hemoglobin as the iron atom oscillates between oxidation (Fe^{+3}; ferric state) and reduction (Fe^{+2};

ferrous state) during the physiological action of cytochromes, while the iron of hemoglobin remains in the reduced form during its physiological action.

- Cytochrome c is a water soluble, peripheral membrane protein. It is relatively mobile. It is associated with iron sulfur protein in addition to the heme group.
- Cytochrome a3 contains copper in addition to the heme group.

Thus, there are **two mobile components in the electron transport chain** -: **coenzyme Q and cytochrome c** which collect reducing equivalents from the other fixed components

5) **Iron sulfur protein**
   - It is an additional component found in the electron transport chain. It is also called FeS or non-heme iron.
   - It consists of a cluster of cysteine residues which complex iron through covalent bonds with the sulfur of cysteine.
   - It is associated with the flavoproteins and cytochrome b.
   - The sulfur and iron are thought to take part in the oxidation-reduction mechanism between flavoproteins and coenzyme Q as the iron atom in these complexes oscillates between oxidation and reduction that allows them to either give up or accept electrons.

Enzyme Complexes of the Electron Transport Chain

- The IMM contains 5 set of protein-enzyme complexes denoted as complex I-complex V (roman numerical)
- The enzymes of the electron transport chain are organized in the inner mitochondrial membrane in the form of four enzyme complexes.
- The five enzyme complexes of the electron transport chain are:

1) **Complex I**: NADH dehydrogenase (NADH-ubiquinone oxidoreductase)
   - It is a flavoprotein that contains FMN as well as FeS protein as coenzymes.
   - It contains at least 6 Fe-S centers.
   - It is L shaped complex with one arm of L in the membrane and other extending in matrix.
   - Function: It transfers hydrogen atoms from $NADH+H^+$ to ubiquinone. It acts as a proton pump and transfers 4 protons from matrix to IMS of mitochondria.
2) **Complex II:** Succinate dehydrogenase (succinate-ubiquinone oxidoreductase).
   - It is a flavoprotein enzyme complex that contains FAD as well as FeS protein as coenzymes and a binding site for succinate.
   - It is the only membrane bound enzyme in citric acid cycle.
   - Function: It transfers hydrogen atoms from succinate to ubiquinone. It doesn't act as proton pump.
3) **Complex III:** Cytochrome C oxidoreductase/cytochrome reductase/ bc1 complex/

Ubiquinol dehydrogenase (ubiquinol-cytochrome c oxidoreductase).

- Contains at least 8 polypeptide chains.
- Function: It transfers electrons from ubiquinol to cytochrome C using cyt b and cyt c1 as coenzymes. It also acts as a proton pump.
- The site for ubiquinone on this complex is in the middle of membrane whereas cyt C is on IMS side.

4) **Complex IV:** Cytochrome C oxidase (cytochrome-oxygen oxidoreductase).
   - This is a large enzyme complex (13 subunits).
   - It contains two copper ions (Cu A and Cu B) and two heme groups designated as cyt a and cyt a3 as coenzymes.
   - Function: It transfers electrons from cytochrome c to oxygen.
   - For every 4 e- passing through this complex, enzyme consumes 4 H+ from matrix and reduces oxygen to water. The energy of this conversion is used to pump 2 H+ into IMS
   - Inhibitors: cyanide, CO, azide
5) **Complex V**: ATP Synthetase.
   - This enzyme is also known as ATPase since it can hydrolyse ATP to ADP and Pi.
   - It is a complex enzyme consisting of two subunits F1 and F0.
   - F1 and F0 subunit coordinate with each other and couples proton movement with ATP synthesis.
   - F0 subunit is integral membrane components and it translocate proton's re-entry into matrix from IMS
   - The F1 particle is attached to F0 and is found in the cytoplasmic side of membrane.
   - It consists of 5 distinct polypeptides namely α, β, γ, ε.
   - This subunit catalyses formation of ATP from ADP and Pi by undergoing a conformational change.

Working of ETC

- The matrix of mitochondria consists of enzymes responsible for Kreb's cycle, Beta oxidation and oxidation of amino acids, the reducing equivalents produced by these pathways donates its electrons to the these set of enzyme complexes.
- If the reducing equivalent is NADH it will donate its electron to complex I and if it is FADH2 it will donate the electron to complex II.
- Complex I is called as NADH Dehydrogenase, complex II is called Succinate dehydrogenase, complex III is Cytochrome C Oxidoreductase, complex IV is Cytochrome C Oxidase and final complex is ATP Synthase enzyme. (The functions of these enzyme complexes can be deduced from their name itself).
- The electron will travel from complex I to IV (or II to IV)
- The electron transferred finally is taken up by Oxygen which acts as the final electron acceptor and converts H+ to water while doing so.
- The transfer of electron will generate energy. The energy generated will be utilised to pump H+ from the matrix region to the IMS of mitochondria via Complex V.
- Complex I, III, IV and V act as the proton pump.

- This pumping of H+ creates a concentration gradient leading to positive charge in IMS and negative charge in matrix region. Therefore, this gradient is called as electrochemical gradient.
- IMM is impermeable to ions like H+ and H+ cannot cross the membrane.
- Hence a special transporter is used to balance the gradient, enzyme ATP synthase. (complex V)
- ATP synthase transports H+ into matrix and uses the energy generated by flow of electrons to synthesise ATP using ADP from the matrix.
- Thus, overall ETC generate ATP and water.
- For every four H+ pumped, one ATP molecule is generated.
- Complex I and III pumps four H+ each and complex V pumps two H+.
- As discussed, NADH passes its electrons via complex I, thus each NADH molecules pumps 10 H+ and can generate 2.5 or approx. 3 molecules of ATP
- Whereas each FADH2 molecule passes through complex II pumping 6 H+ and liberating 1.5 or approx. 2 molecules of ATP.
- Using these calculations and depending on the amount of NADH and FADH2 produced by various metabolic reactions, we can estimate the total energy generated by various pathways.