INTERMEDIARY METABOLISM

Electron Transport Chain and Oxidative Phosphorylation

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Keywords

Electron transport; oxidative phosphorylation; Redox reactions; Redox potential; P:O ratios; Inhibitors and Uncouplers – Electron Transport System; ATP synthase; Proton pump

Introduction

Life on earth is sustained by harnessing energy from the surroundings, entrapping it into utilizable forms (metabolites) and utilizing it for various processes like growth, locomotion and propagation. Existence of every organism is thus associated with a colossal number of chemical reactions involving generation or utilization of energy. As we have learned in the previous chapters, nature has evolved one (but not the only one) high energy compound viz., ATP, that is readily and widely utilized as the source of energy in numerous biochemical reactions. Thus every organism, be it a simple prokaryote or highly complex mammal, has evolved efficient mechanisms of ATP generation and consumption. Therefore, in a way, ATP can be considered as the sole energy currency for the biological world.

The original source of energy for the biological kingdom is the sun. The solar energy (in the form of light) is first captured by photosystems in plants and cyanobacteria, leading to the hydrolysis of water and generation of NADPH, oxygen and ATP. NADPH and ATP are then utilized for the synthesis of glucose from CO_2 and H_2O, thereby entrapping the solar energy. The trapped energy in glucose is released upon its subsequent oxidation (glycolysis) producing CO_2, H_2O and ATP. Organisms (like human) which can not synthesize glucose, obtain it from plant sources, thereby meeting their energy requirements. Synthesis glucose and its breakdown thus lead to the ultimate entrapment of solar energy in the form ATP, the energy currency.

The breakdown to glucose in any cell type, involves two distinct processes. First it is converted to a three carbon compound i.e., pyruvate which then is fed to the TCA cycle, leading to the generation of NADH (and CO_2). In the second process, NADH transfers its hydrogen to molecular oxygen, generating H_2O and ATP. Synthesis of ATP from NADH and oxygen is known as Oxidative phosphorylation. The biochemistry of oxidative phosphorylation has been a fascinating subject of research for many decades and in spite of an enormous progress in our understanding of this complex phenomenon, certain aspects of it are yet to be fully understood. Nevertheless, studies done by numerous researchers have revealed that the entire process of oxidative phosphorylation is associated with a series of redox reactions. Therefore, understating of redox biology is a prerequisite for understanding oxidative phosphorylation.

Redox reaction and redox potential

Biochemical reactions occurring in an organism can be broadly divided into a number of categories *viz*., group transfer reaction (e.g., acylation, phosphorylation); rearrangement and isomerization (e.g., aldose-ketose intercoversion); creation and breakage of covalent bonds (e.g., fatty acid biosynthesis); oxidation-reduction reactions (e.g., synthesis of aldehydes from alcohols). Although there are plentiful of reactions belonging to each of these categories, oxidation-reduction reactions are one of the most widely occurring during metabolism. Oxidation-reduction reactions (also known as redox reactions) involve transfer of electrons from one electron donor to an electron accepter. As an example, in the following redox reaction, Fe^{3+} is the electron accepter (oxidant) getting reduced to Fe^{2+} while Cu^{+} is the electron donor (reductant), getting oxidized to Cu^{2+}.

$$Fe^{3+} + Cu^{+} \rightarrow Fe^{2+} + Cu^{2+}$$

Furthermore, the above reactions can be split into two half reactions as follows:

$Fe^{3+} + e \rightarrow Fe^{2+}$ and $Cu^{+} \rightarrow Cu^{2+} + e$

In both the reactions, electron is the common intermediate and both have to occur simultaneously (thereby coupling oxidation reaction with reduction). Oxidation-reduction can also occur for a compound consisting of different elements. As an example, during the breakdown of glucose to carbon dioxide and water, electrons are transferred from carbon to oxygen, resulting in the oxidation of the former. Noticeably, such transfer (or flow) of electron depends on the relative propensity (or tendency) of the donor as well as the recipient to do so. Also, based on the counterpart, an electron donor in one reaction may become a recipient in another. As an example, metals in general have tendency to loose electrons and become positively charged ions. Thus, both Mg and Zn can loose two electrons and become Mg^{2+} and Zn^{2+} respectively. However, when two are in equilibrium with their ions in a solution, Mg being more electropositive will loose electrons, while Zn^{2+} will receive it and become Zn metal. On the contrary, when Zn and Cu are in equilibrium with their ions, Zn being more electropositive will loose electrons and produce Zn^{2+} while Cu^{2+} will receive it and become elemental copper. So, in the first scenario, Zn is an oxidant while it is reduced but in the second scenario, it is a reductant as it is oxidized.

Therefore, for the understanding of redox reactions, it is necessary to know which one amongst the reactants is likely to be oxidized, and which one is to be reduced. Such anticipation is possible only if we know the "reduction potential" of each of the reactants. Reduction potential is a measure of the propensity of each substance (element or a compound) to be reduced. However, to have a better understanding of reduction potential, we need to understand "half cell". If we take a copper plate and immerse it in a solution of Cu^{2+} ions, Cu atoms will continuously loose electrons and form Cu^{2+} ions while Cu^{2+} ions from the solution will continuously gain electrons and deposit as Cu on the plate, thereby maintaining an equilibrium. Similarly, when a zinc plate is in equilibrium with Zn^{2+} ions, it will continuously loose electrons forming Zn^{2+} ions while Zn^{2+} ions in solution will gain electrons to become Zn. Now, if the Zn and Cu plates are connected by a salt bridge (conductor), electrons will continuously flow from Zn plate to Cu plate and Zn will be solubilized as Zn^{2+} while Cu^{2+} will deposit as Cu. If we connect the salt bridge through a voltmeter, it thus will give a potential difference between two plates that remains constant for specific concentrations of Cu^{2+} and Zn^{2+} ions and their temperatures. In this case, both Zn/Zn^{2+} and Cu/Cu^{2+} combinations form half-cells while two together form a cell and the potential difference between the two is a measure of the propensity of Zn to loose electrons (and to reduce copper). Conventionally, when a half-cell consists of hydrogen (in association with a platinum electrode) in equilibrium with 1M H^{+} at 25^{0}C, its potential is considered as zero and such hydrogen electrode is called standard electrode. Now, any half-cell when connected to standard hydrogen electrode will generate a potential difference that is considered as its reduction potential (as the potential of hydrogen electrode is zero). Furthermore, when a half cell is connected to hydrogen electrode, flow of electrons may be from hydrogen towards the half cell (if hydrogen is more electropositive than the constituent of the half cell) or it can be from half cell towards hydrogen (if the constituents of the half cell is more electropositive than hydrogen). Based on such directionality of flow of electrons, the redox potential of the former is considered negative while that of the latter is positive. Also, when the constituents of a half-cell are under standard condition i.e. the constituent ion is at 1M concentration and at 25^{0}C, its redox potential is considered as "standard redox potential". Therefore any two redox-reactants when come together, one which has higher "Standard reduction potential" will be the reductant (and thus will be oxidized) while the other with lower "Standard reduction potential" will be the oxidant (and

will be reduced). See Table 1 to have an understanding of standard reduction potentials of certain bio-molecules.

Na+(aq) + e− → Na(s)	−2.71
H_2 + 2e− → 2H−	−2.25
Fe2+(aq) + 2e− → Fe(s)	−0.44
2H+(aq) + 2e− → H2(g)	0.00
C(s) + 4H+ + 4e− → CH4(g)	+0.13
HCHO(aq) + 2H+ + 2e− → CH3OH(aq)	+0.13
Fe3+(aq) + e− → Fe2+(aq)	+0.77
O2(g) + 4H+ + 4e− → 2H2O	+1.23

Table 1: Standard reduction potentials of some biomolecules

Oxidative phosphorylation involves series of redox reactions

As mentioned in the dioxide and water. However, this oxidation process involves multiple steps which can be summarized into two half reactions as follows:

$$C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24H^+ + 24e^-$$

$$6O_2 + 24H^+ + 24e^- \longrightarrow 12H_2O$$

Furthermore, in the first reaction, electrons are not generated in their free form, rather they are produced as NADH ($NAD^+ + H^+ + e^-$) and FADH2, which are then fed into a series of electron transporting complexes and finally delivered to oxygen, leading to its reduction to water releasing energy. The released energy is then trapped as ATP. Furthermore, the transport of electrons and concomitant generation of ATP occurs in a unique organelle named mitochondria. However, before we go into further details how electron transport in mitochondria leads to ATP generation, it is necessary to have an understanding mitochondrial structure and organization.

Mitochondrial structure and organization

Mitochondria are found in almost all eukaryotes viz., plants, animals and fungi. They are visible under light microscope and their presence in eukaryotic cells was known almost for past two hundred years. However, their functions became apparent only when intact mitochondrion was isolated from host cells in 1950s. Mitochondrial constituents are encapsulated by two highly specialized membranes with a narrow inter membrane space. While the outer mitochondrial membrane act like a sieve that restricts free flow of molecules from the cytoplasm; the inner membrane is further selective in nature and play a critical role in electron transport and ATP generation. The inner mitochondrial membrane contains all the proteins involved in electron transport, ATP generation and transport of metabolites (both in and out of mitochondria). Under electron microscope, the inner mitochondrial membrane appears to be wrinkled (folded) and organized in layers called cristae (Fig. 1). Formation of cristae significantly increases the total surface area of the inner membrane. The number of mitochondria present in a cell depends upon its metabolic activities and may range from a single large mitochondrion to thousands of mitochondria of various sizes. The inner mitochondrial membrane encapsulates the

mitochondrial matrix that contains the TCA cycle enzymes, dissolved oxygen, carbon dioxide and various metabolic intermediates, especially those involved in energy generation. The mitochondrial electron transport chain catalyzes a reaction that can be represented as follows:

$2\ H^+ + 2\ e^- + \frac{1}{2}O_2 \longrightarrow H2O + \text{energy}.$

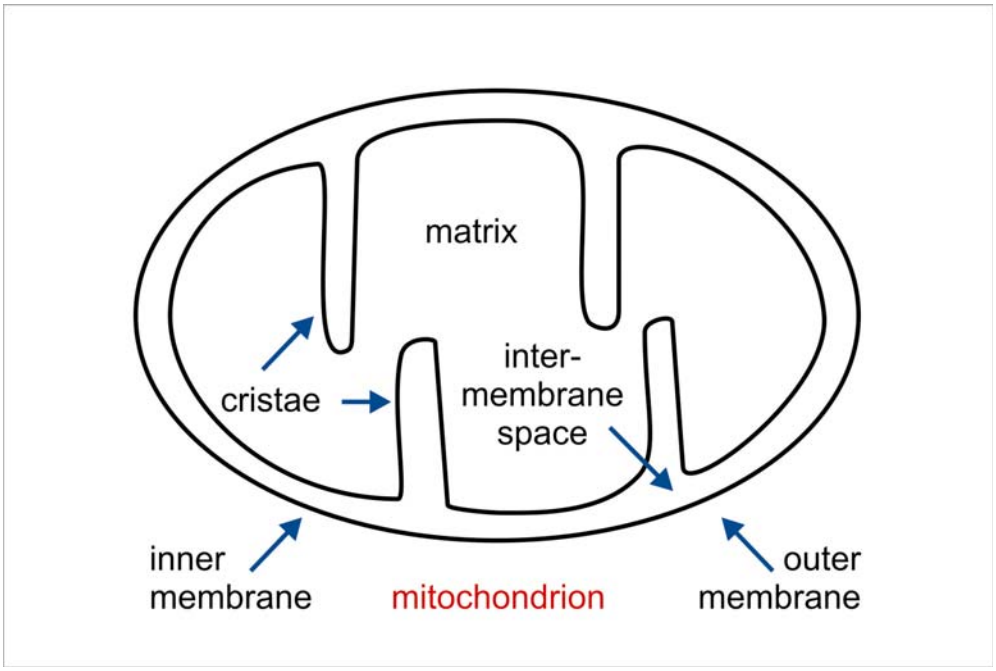

Fig. 1: A schematic view of mitochondrial structure. The respiratory chain is embedded in cristae of the inner membrane. Tubular cristae connect to the inner membrane via narrow passageways that may limit the rate of H+ equilibration between the lumen of cristae and the inter membrane space (and the cytosol)

One fundamental difference between mitochondria and other organelles is the presence of its own genome (DNA). Mitochondria thus reproduce independently of its host cell. It is hypothesized that millions of years ago small, free-living prokaryotes were engulfed by larger prokaryotes and the two organisms developed a symbiotic relationship (endosymbiosis). In course of evolution, the larger organism developed into the eukaryotic cell while the smaller one evolved in to mitochondrion. Mitochondria thus also contain ribosomes for synthesizing many proteins (including various enzymes) involved in mitochondrial function. Plant chloroplasts, the primary sites of photosynthesis are also like mitochondria. Like mitochondria, chloroplasts also contain their own DNA and are able to grow and reproduce independently within the cell.

The energy thus released is trapped in the form of ATP. However, as described below, the entire process is enormously complex and has been the subject of intense research over past fifty years. Furthermore, this process is not initiated by free electrons but by NADH which is produced in abundance from the TCA cycle and fatty acids oxidation. Mitochondrial electron transport chain consists of four large multi-protein complexes termed Complex I-IV. The constituents of each complex contain various prosthetic groups like flavin mononucleotide (FMN), iron sulfur centers (Fe-S), heme groups etc which renders onto them oxidation reduction function. Noticeably, although almost all these prosthetic groups contain iron ions in their redox centers (except Complex IV that that contains Cu^{++}), each has its characteristic redox potential. This is due to the fact that each protein has characteristic amino acid sequences that surround those prosthetic groups and thereby influencing their redox potentials. The redox potential of each protein collectively constitutes the redox potential of each complex and place them in a hierarchical order dictating who will be the oxidant and who will be the reductant when together (Fig. 2 for further explanation). In this context, remember that any redox reaction consists of two half reactions where one with more negative standard reduction potential acts as reductant while the other with more positive standard redox potential acts as an oxidant. The electron transport process is initiated by feeding NADH into the Complex 1 (also known as NADH reductase) of the electron transport chain. Since the redox potential of NADH/NAD+ is ~-4.0 while that of first rdox protein (FMN containing) of Complex I is ~3.0, NADH is oxidized to NAD^+ by releasing two electrons ($NADH \rightarrow NAD^+ + H^+ + 2e^-$) which are then transferred to the next redox protein in Complex I that is an iron-sulfur protein (FeS). Thereafter, the electrons are transferred to coenzyme Q (Q), the next in the hierarchy which is then reduced QH_2. Coenzyme Q is a small organic molecule that undergoes reversible oxidation reduction reaction and thus acts as an electron carrier. Furthermore, unlike the Complex I-IV which are embedded in the inner mitochondrial membrane, enzyme Q can diffuse through the membrane and carry the electrons to the next recipient i.e., Complex III (also known as cytochrome reductase, bc1). The electrons pass through Complex III in the following order: cytochrome b1 ➔ cytochrome b2 ➔ iron-sulfur protein ➔ cytochrome c1. Electrons are then finally transferred to cytochrome c which is a small diffusible protein molecule (like Coenzyme Q) carrying electrons to the final recipient in the hierarchy, that is Complex IV. In Complex IV, electrons passes through cytochromes a and a3; then finally delivered to oxygen. One oxygen atom reacts with two electrons and two hydrogen ions, generating one molecule of water ($O + 2H^+ + 2e^- \rightarrow H_2O$). During the passage of electrons through the electron transport chain, each complex acts as a proton pump and excludes H^+ from the mitochondrial matrix to the inter membrane space. A total of ~10 protons are ejected from the mitochondrial matrix per 2 electrons transferred from NADH to oxygen via the respiratory chain. Out of ten electrons, four are pumped out by each of Complex I and III while two are by Complex IV (Fig 3). It is believed that during the passage of electrons the carrier redox proteins undergo conformational changes that cause H^+ transport (as occurs with an ion pump). The Complex II of the electron transport chain is in a way little different from the other three described above (i.e., Complex I, III and IV). Complex II is the succinate dehydrogenase in the citric acid cycle that oxidizes succinate to fumarate. During this process it first reduces FAD to $FADH_2$ and then regenerates FAD by removing electrons that are passed to Coenzyme Q through its Fe-S centre. Therefore, Complex II is in a way a duel function enzyme complex that is part of TCA cycle (it is the only TCA cycle enzyme that is embedded in the inner membrane of mitochondria) as well as a part of the electron transport system. Complex II thus provides an alternative route of entry of electrons (from $FADH_2$ rather than NADH) into the electron transport chain. Complex II does not have any proton exclusion activity. Since Complex II

bypasses Complex I and directly sends the electrons to Complex III (via Coenzyme Q) it thus results in pumping out six instead of ten electrons.

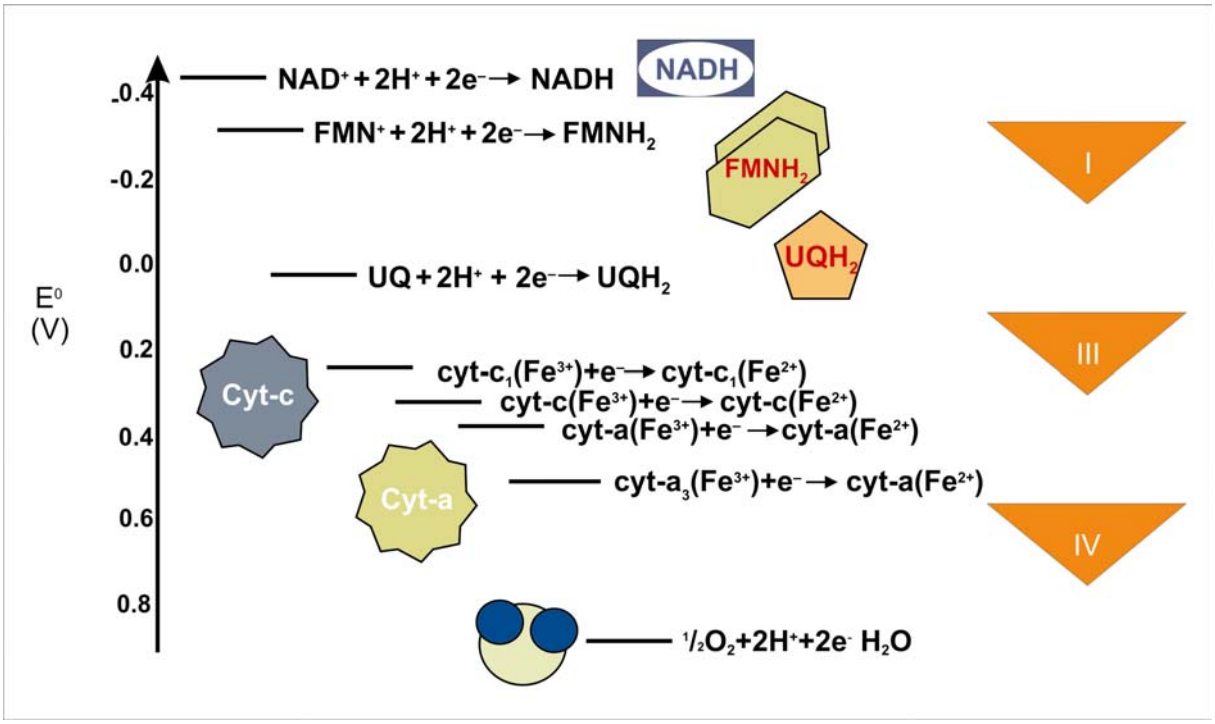

Fig. 2: A schematic representation of relative reduction potentials of various complexes in the mitochondrial electron transport system. Various complexes are identified by different shapes and are placed in the hierarchical order as they carry electrons. The direction of flow of electrons and the respective reactions are also shown

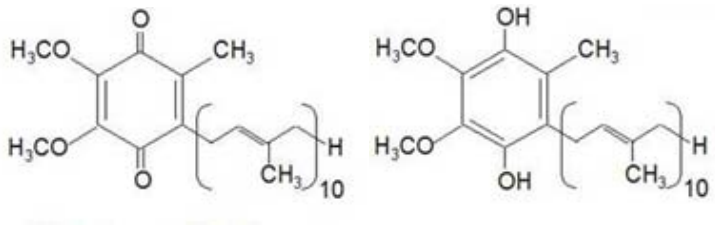

Fig. 3: The oxidized and reduced forms of coenzyme Q (left and right panels respectively). Ten repeating iso-prenyl chemical subunits make it highly hydrophobic and thus it is diffusible into the mitochondrial membrane

Pumping of H^+ from the matrix to the inter membrane space creates an asymmetric distribution of protons between the two compartments, generating a gradient of electric charge ($\Delta\psi$, negative in the matrix and positive in the inter membrane space) and pH (ΔpH, acidic in the inter membrane space and alkaline in the matrix) that is collectively called "electrochemical gradient" which acts as a reservoir of free energy. This electrochemical gradient then tries to push back the protons into the matrix. However, since the inner mitochondrial membrane is impermeable to

protons, the only way the protons can come back to the matrix is through Complex IV which is an ATP synthase. While transporting back the protons from the inter membrane space to the matrix, Complex IV harnesses energy of the electrochemical gradient and synthesizes ATP. A schematic representation of the electron transport and ATP synthesis in the mitochondria is shown in Fig. 4. Also, further details of the mechanisms of ATP synthesis by Complex IV are given below.

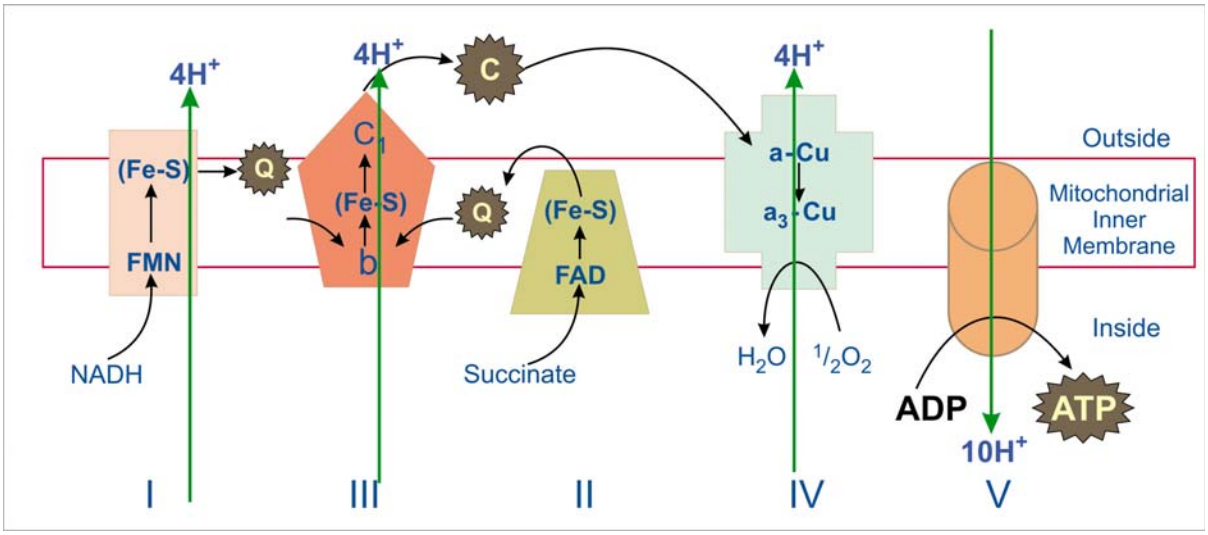

Fig. 4: Electron transport and ATP generation in the mitochondria. The electron carrier complexes and their associated prosthetic groups are shown. Two electrons generated by the oxidation of NADH passes through the chain and results in exclusion of ten protons from the matrix to the inter membrane space. The protons then return to the matrix via Complex IV/ATP synthase generating ATP

Complex V-ATP synthase

The Complex IV or the F1-Fo ATP synthase consists of two major subunits viz., F1: the catalytic subunit and Fo: a complex of integral membrane proteins acting as a proton pump. The F1-Fo complex harnesses the energy of electro chemical gradient by transporting H^+ from inter membrane space to the mitochondrial matrix and in the process by coupling it with ATP generation. Physicochemical analysis suggests that for the synthesis of one molecule of ATP, at least $3H^+$ are transported back to the matrix. ATP is synthesized by the F1 domain which an assembly of five different proteins viz., α, β, γ, δ and ε wih a stoichiometry of 3:3:1:1:1. The three α-subunits and the three β-subunits are alternately arranged around a central γ-subunit. The γ-subunit protrudes from the $\alpha_3\beta_3$ assembly while the δ- and ε-subunits are associated with its foot and connect it to a ring shaped assembly of c proteins (10-12 in number) of the Fo domain. Thus, the γ-, δ- and ε- subunits form a central "stalk" that links the $\alpha_3\beta_3$ sub-complex of the F1 domain to the barrel shaped c-assembly of Fo domain. The $\alpha_3\beta_3$ sub-complex is also connected to the Fo domain by another assembly called the "stator" made of one each of OSCP, b, d and F6 proteins. The protons pass through the c-ring assembly and the a protein (also called ATPase-6). The functions of other subunits like e, f, g and A6L are not understood as yet.

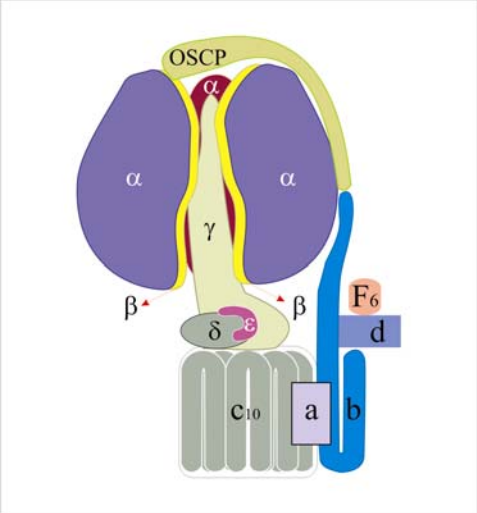

Fig. 5: The subunit structure of F1-Fo ATP synthase

Mechanism of ATP synthesis

The biochemical mechanism by which electron transport in the mitochondria leads to the generation of ATP has been a subject of intense research and debate for almost half a century. In early nineteen fifties, when various metabolic pathways were being discovered, it was believed that electron transport leads to the generation of a high energy intermediate that in turn is enzymatically converted to ATP and a energy deficient product. Such concept was called "Chemical coupling hypothesis" as it perceived the existence of an energy rich chemical compound that couples electron transfer to ATP generation. It was in fact in analogy with one step in the glycolytic pathway where the oxidation of 3-Phospho-glyceraldehyde into glyceric acid requires the generation of 3-Phospho-glyceric acid as an energy rich intermediate. 3-Phospho-glyceric acid then transfers the high energy phosphate to ATP and gets converted into energy deficient glyceric acid. However, in spite of extensive research for almost three decades, scientists could not isolate that high energy intermediate and the hypothesis was questioned. Another argument against this hypothesis was, such a reaction is likely to occur in solution. However, it became apparent during fifties and sixties that to generate ATP, the inner mitochondrial membrane must be intact. So, the "Chemical coupling hypothesis" was abandoned. Subsequent years another model was proposed by Paul D Boyer who proposed that the energy released during electron transport results in a conformational change of a protein (ATP synthase or another associated protein) that in turn transfer the stored energy to ATP and returns to its low energy state (or conformation). Therefore, "Conformational coupling hypothesis" is a revised form of "Chemical coupling hypothesis" with the exception that in this case, the energy is presumed to be stored in the form of an altered conformation of a protein rather than as an energy rich small molecule. The analogy of such model was the actin-myosin complex that first undergoes conformational change by the hydrolysis of ATP and then utilizes the stored energy to contract and returns to original conformation. This model was primarily based on the observation that upon addition of ADP, respiring mitochondria undergoes rapid changes in its ultrastructure (as seen under electron microscope). However, this model was never experimentally proved as the protein molecule that undergoes such conformational change was not identified and characterized. The third model was proposed by Peter Mitchel in 1961.

According to this model, when electrons move through the transport chain, it pumps out protons into the space between the outer and the inner mitochondrial membranes generating an electrochemical gradient between the matrix inside and the space outside (as already discussed in the previous sections and shown in Fig. 4). The protons then move back through the c-ring-assembly of F1-Fo ATP synthase providing it energy to rotate (clockwise) along with the attached central γ-subunit (the stalk or the rotor) at about 50-100 rotations per second. When the γ-subunit rotates, it changes the conformation of β subunits of the $\alpha_3\beta_3$ assembly in a sequential manner (remember that the β subunits are in contact with the rotating shaft). Each of the three β subunits can attain one of three possible conformation named L (Loose), O (Open) and T (Tight) representing three different stages of the catalytic cycle that synthesizes ATP. At any given time, three β subunits of the $\alpha_3\beta_3$ assembly will be in L, T and O conformation respectively. Furthermore, with the rotation of the γ-subunit, each one will undergo conformational changes attaining the other two conformations in the same order. Therefore, each β subunit will have all three conformations sequentially (while at a given time each β subunit in an assembly will have one of those conformations). Now, when the catalytic site (of which β subunit is a part) is in L conformation, it picks up ADP and Pi from the matrix and loosely binds it. Thereafter, when it changes its conformation to T, it immediately converts them into ATP that remains in close association with the catalytic site. Thereafter, the catalytic site switches to the O conformation, the bound ATP is released and a second round of conformational cycle begins. Although the binding change mechanism is widely accepted now, some structural details of this process are still investigated. Studies using various techniques have shown that the rotation of $\alpha_3\beta_3$ assembly occurs in discrete 120^o steps, with intervening pauses. Notably, although F1-Fo ATP synthase is characterized for its ATP synthesizing function, it can also hydrolyze ATP and use the released energy for rotating the shaft in opposite direction and pumping out proton from matrix to the inter membrane space. For this reason it is also called F1-Fo ATPase. The catalytic site in "L" conformation can perform both ATP synthesis and hydrolysis in a reversible fashion. Only when it undergoes conformational change to the T form, it pushes the reaction towards ATP synthesis. The O conformation has minimum affinity for ATP and thus it releases it immediately after attaining this conformation. This model is the culmination of work done by many researchers for over thirty years and at present it is the most widely accepted model of ATP synthesis. Mechanism of mitochondrial electron transport and ATP generation has been one of the most fascinating problem in biochemistry that has engaged numerous researchers over half a century and among them Paul Boyer and John Walker got the Nobel Prize in 1997 for their contribution in determining the structure of the F1-Fo complex.

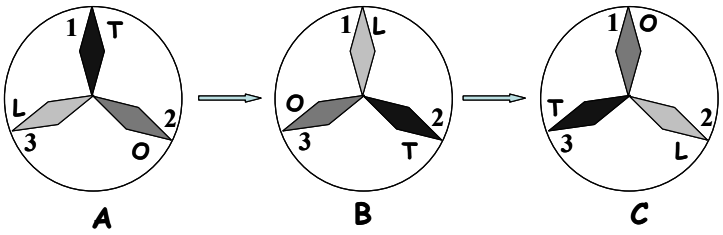

Fig. 6: The mechanism of ATP generation by ATP synthase. The three pairs of α-β subunits of the F1 part of ATP synthase is shown by diamond shapes and marked 1-2. The T, L and O conformations are shown by three different shades. Each of the pairs

sequentially undergoes conformational changes as the γ subunit rotates leading to the ATP generation

Stoitiometry of ATP generation

Since ATP synthesis does not occur by a simple phosphorylation of ADP by an enzyme, it has taken long and sustained efforts to understand the stoitiometry of ATP generation (that is how much ATP is produced from one molecule of NADH or glucose). It was shown in early fifties by A L Lehninger that that one mole of NADH can produce three moles of ATP. Thereafter, using different mitochondrial electron transport inhibitors and metabolites which can pass the electrons through the transport chain only partially (NADH is a metabolite that passes the electron through the entire chain), it was established that the electron transfer pathway can be segregated into three parts, each of which generates one molecule of ATP. The first segment is at the beginning of the chain till coenzyme Q, second segment is cytochrome b till cytochrome c and the third segment is from cytochrome a till the end point that is oxygen (Fig. 2 and 4 for their arrangement). Accordingly, since $FADH_2$ enters the chain after the first segment and passes electrons only through second and third segments, it produces only two molecules of ATP. This also means, each of those segments pump out just sufficient protons that can harness only one molecule of ATP upon coming back to matrix via complex V or ATP synthase. Now, each molecule of pyruvate oxidized in the TCA cycle generates four molecules of NADH and one molecule of $FADH_2$. Thus, one molecule of pyruvate will eventually generate 4 X 3 =12 plus 1 x 2 = 2, that is fourteen molecules of ATP. Also, in TCA cycle, one molecule of GTP is produced per molecule of pyruvate oxidized. Thus, from two molecules of pyruvate generated from one molecule of glucose, total ATP generation will be thirty (we are considering GTPs are equivalent to ATP as energy rich molecule). Since glycolysis also generates two molecules of NADH in the cytosol, which is then brought into the mitochondrial matrix as $FADH_2$, via a mitochondrial membrane associated shuttle system, that $FADH_2$ also contributes four ATP. Therefore, total ATP yield from one glucose molecule becomes thirty four. Finally, each molecule of glucose oxidized, two molecules of ATP are produced by substrate level phosphorylation, making the total yield thirty six ATP per molecule of glucose. However this calculation is based on thermodynamic and other parameters as ATP generation is not a direct process and based upon other cellular conditions, this yield might vary. As an example, mitochondria might also produce lesser amounts of ATP by a mechanism called uncoupling. During uncoupling, a protein (e.g., thermogenin), brings in protons from intermembrane space to the matrix, thereby bypassing the ATP synthase. As a result, the energy released by electron transport is not converted to ATP but is dissipated as heat (that is why the protein is called thermogenin). Certain synthetic compounds like dinitro-phenol has also been shown having function like thermogenin and they are collectively called uncoupler as they uncouple electron transport from ATP generation.

P:O Ratios and Respiratory Control

The P:O ratio is a measure of the number of high energy phosphates (i.e. amount of ATP) synthesized per atom of oxygen ($^{1/2}$ O_2) consumed or per mole of water produced. As mentioned earlier, the theoretical yield of ATP from one mole of NADH is about 8 moles; however by actual measurement with isolated mitochondria, the P:O ratio for oxidation of metabolites that yield NADH is 3 and the ratio for those that yield $FADH_2$ is 2. The P:O ratio can be calculated from the amount of ADP used to synthesize ATP and the amount of oxygen taken up by

mitochondria. For example, if 2mmol of ADP is added and 0.5 mmol of oxygen (1.0 atom of oxygen) is taken up the P:O ratio is 2.0.

Inhibitors of Oxidative Metabolism

Electron Transport System Inhibitors

Inhibitors of electron transport selectively inhibit complexes I, III or IV interrupting the flow of electrons through the respiratory chain. This results in a block in proton pumping, ATP synthesis and oxygen uptake. Several of the inhibitors are poisons that could be encountered in medical science. The sites of inhibitors are depicted in Fig. 7 and a short description follows:

1. ***Rotenone inhibits Complex I (NADH-Q reductase)***

   Rotenone is an insecticide that inhibits complex I of the electron transport system. Since malate and lactate are oxidized by NAD, their oxidation would be inhibited by rotenone. However, substrates yielding $FADH_2$ can still be oxidized because complex I is bypassed and electrons can be donated to ubiquinone through Complex II.

2. ***Antimycin A inhibits complex III (QH_2-Cytochrome C reductase)***

   The inhibition of complex III by antimycin A prevents transfer of electrons from either complex I or complex II to cytochrome c. Hence components preceding complex III in the pathway become reduced and those after it become oxidized. The stimulation of respiration by ADP is inhibited by antimycin A, but the addition of succinate does not relieve the inhibition. Ascorbic acid can reduce cytochrome c and addition of ascorbic acid restores respiration showing that complex IV is unaffected by Antimycin A.

3. ***Cyanide and Carbon monoxide inhibit complex IV***

   Both cyanide and carbon mono oxide bind to and inhibit complex IV (cytochrome c oxidase). Since complex IV is the terminal electron transfer complex, its inhibition is of significance and cannot be ignored. All components preceding complex IV become reduced and as it is not possible to pump protons, uncouplers such as Dinitrophenol have no effect.

4. ***Inhibitors of ATP Synthase***

   The proton channel of ATP synthase is Fo protein, named so as it is sensitive to oligomycin that blocks the transport of protons back into mitochondria. Oligomycin inhibits respiration but as against to electron transport inhibitors, it is not a direct inhibitor of the electron transport system. It causes a build up of protons outside the mitochondrion, as the proton pumping system is still intact, but the proton channel is blocked.

Uncouplers of Electron Transport Chain & Oxidative Phosphorylation

These compounds dissociate oxidation in the respiration chain from phosphorylation and allow mitochondria to use O_2 regardless of whether or not there is any phosphate ADP available i.e. when uncoupler is added there is marked increase in O_2 uptake.

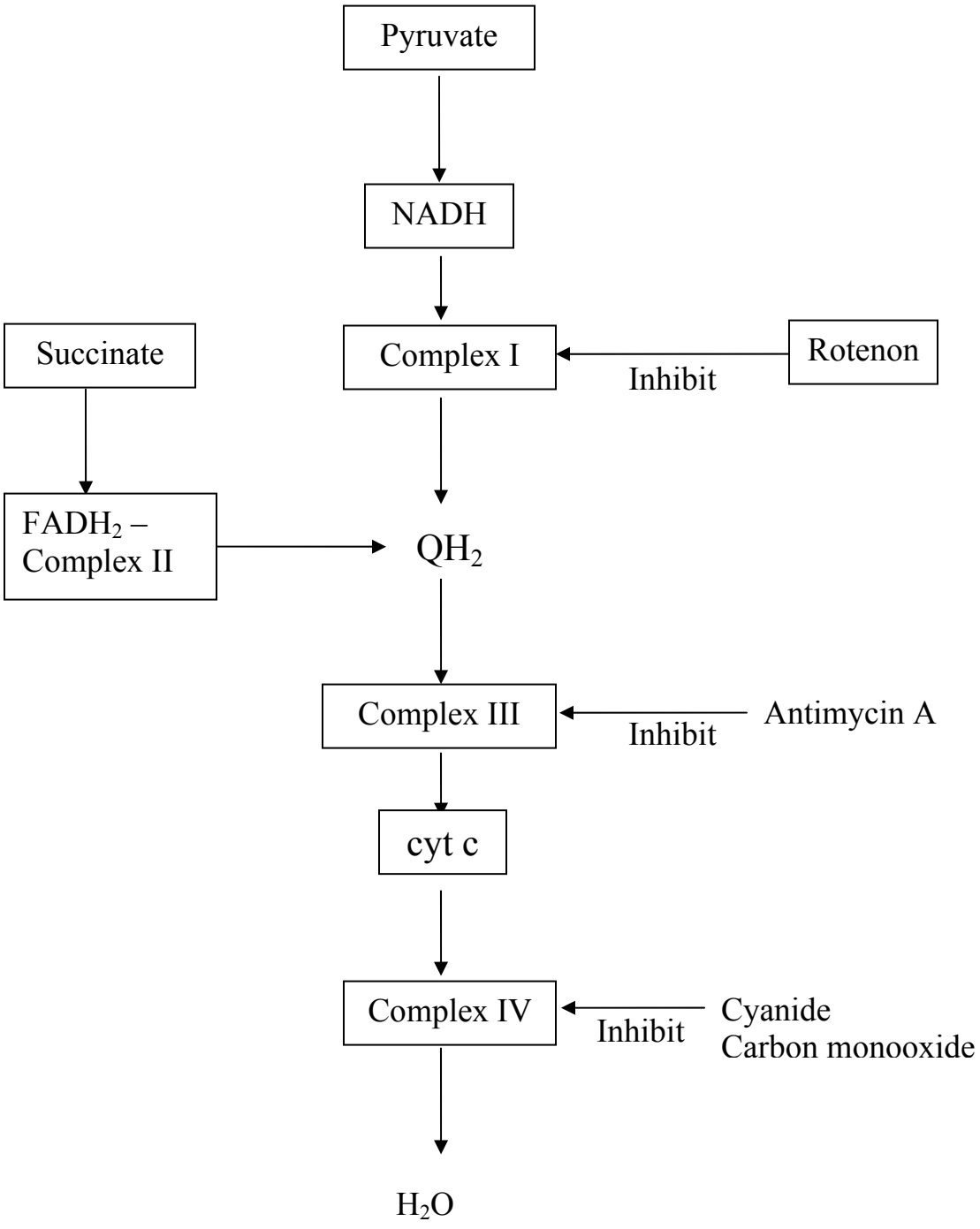

Fig. 7: Inhibitors of electron transport chain inhibiting specific complexes

2, 4-Dinitrophenol is a classic uncoupler which is lipophilic proton carrier that readily diffuse through the mitochondrial membrane. Uncouplers of oxidative phosphorylation dissipate the proton gradient by transporting protons back into mitochondria by passing the ATP synthase. This stimulates respiration, since the system makes a futile attempt to restore the proton gradient by oxidizing more fuel and pumping more protons out of mitochondria. Hence, a typical uncoupler, such as 2, 4-dinitrophenol (DNP) dissociates (uncouples) ATP synthesis from oxidation. Therfore while oxidation proceeds normally, the ATP synthesis doesn't occur.

Suggested Reading

1. Boyr, P.D. The ATP synthase: A splendid molecular machine. *Ann. Rev. Biochem*. (1997) 66, pp. 717-49.
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3. Bhattacharya, J. and Anubhuti. Biological oxidations: Electron transport chain – oxidative phosphorylation *In* Concepts of biochemistry edited by L. M. Srivastava. Delhi: CBS Publishers, 2004. 1st ed. Pp. 323-42.