ATP Synthesis

8

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Adenosine triphosphate (ATP) is the energy currency of the cell and is required in cell metabolism of all living beings including bacteria, plants, and animals. It is utilized for the endergonic reactions and various energy-requiring processes in the cell. Various energy-requiring processes of the cell include transport of ions and biomolecules across plasma membrane against concentration gradient, movement of substances within the cell, cell growth including cell division, anabolic reactions of the cell, and various other cellular processes. ATP synthase catalyzes ATP synthesis during oxidative phosphorylation and photophosphorylation. Structure of ATP synthase and the mechanism of ATP synthesis have remained conserved through evolution. ATP was discovered in 1929 by German chemist Karl Lohmann. In 1941, it was demonstrated by Fritz Albert Lipmann that ATP was the universal energy carrier of the cells. Later on, Lohmann shared Nobel Prize with Hans Krebs in 1953 for his work on citric acid cycle. Lipmann was the first to use the squiggle (~) symbol and the term "high-energy bond" for the compounds having high phosphate group transfer potential. In 1948, Alexander Todd undertook chemical synthesis of ATP and deciphered its structure, for which he was awarded Nobel Prize in 1957. The standard free energy of ATP hydrolysis is 30.5 $kJ.mol^{-1}$. However, under cellular conditions, the free energy value is around -50 $kJ.mol^{-1}$. Thus, synthesis of 1 mole of ATP requires around 50 kJ of energy. There are two ways by which ATP can be synthesized in a cell. One of the ways is known as substrate-level phosphorylation, in which high-energy bond of the substrate is hydrolyzed and the energy released is utilized for ATP synthesis. Another mechanism involves coupling of energy of proton gradient with the synthesis of ATP. Proton gradient is created by electron transport chain either during oxidation of $NADH/FADH_2$ or during the light reaction of photosynthesis in mitochondria and chloroplasts, respectively.

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8.1 Proton Gradient Coupled ATP Synthesis

ATP synthesis in mitochondria and chloroplasts is coupled with dissipation of proton gradient which is the result of electron transport during oxidation of substrates or during light reaction of photosynthesis. Energy required for movement of electrons is derived from oxidation of substrates in mitochondria, while in chloroplasts light energy is responsible for movement of electrons. ATP synthesis during electron transport in mitochondria is known as **oxidative phosphorylation**, while in chloroplasts it is known as **photophosphorylation.** In 1961, Peter Mitchell proposed **chemiosmotic model** for ATP synthesis according to which an electrochemical gradient is established across mitochondrial membrane when electrons move downhill during oxidation of the substrate through electron transport chain. Energy so released is utilized in the movement of protons across the inner mitochondrial membrane from matrix to intermembrane space, resulting in accumulation of protons. Thus, an electrochemical potential is developed which is the result of chemical potential energy (due to gradient in pH (ΔpH) created as a result of accumulation of H^+ in the intermembrane space) and electrical potential energy which contributes to membrane potential (ΔΨ) (intermembrane space being more positive in comparison to the matrix because of accumulation of H^+) (Fig. 8.1).

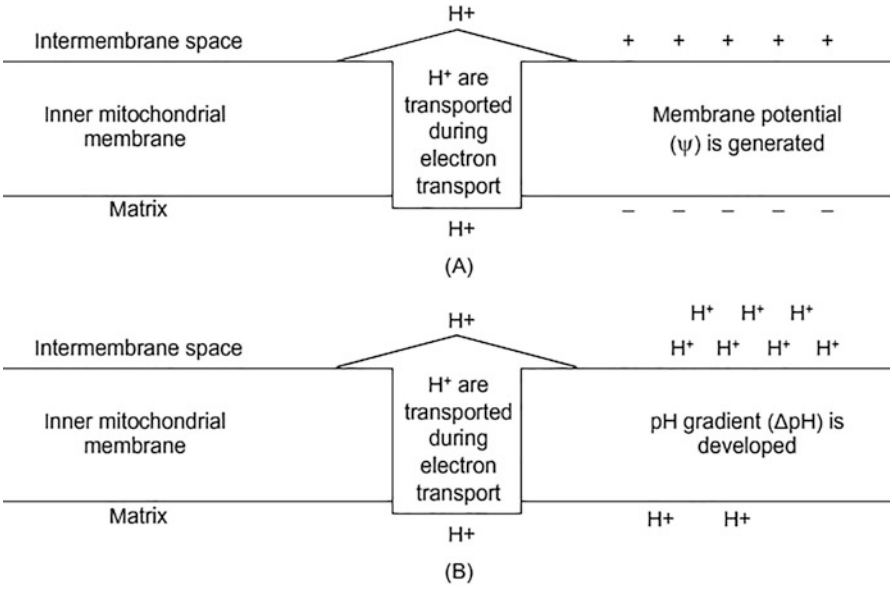


Fig. 8.1 Two components of PMF. (**a**) Membrane potential due to charge difference. (**b**) pH gradient

NADH oxidation in mitochondria through electron transport chain involves removal and transport of two electrons from NADH to O_2. This results in conservation of around 200 kJ energy in the form of 10 H^+. Mitchell called this energy due to electrochemical gradient as **proton motive force (PMF)**, which is measured in volts (V). This energy is the result of transmembrane potential (Ψ) of about ~160 mV and a ΔpH of about 1 unit, which is equivalent to ~60 mV.

$$\text{PMF}\ (\Delta p) = \Delta\Psi - 59\ \Delta pH\ (\text{pH}_\text{i} - \text{pH}_\text{o})$$

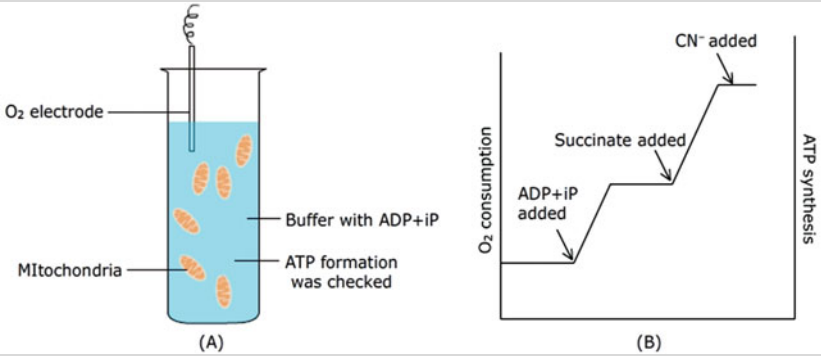
where ΔΨ refers to potential gradient across the inner mitochondrial membrane while ΔpH is the difference in pH across the membrane between matrix (pH_i) and intermembrane space (pH_o). This value will be negative, in case pH_o is lesser because of accumulation of H^+ in comparison to pH_i. 59 mV per pH unit is the constant of proportionality, which demonstrates that a transmembrane pH difference of 1 unit will contribute to a transmembrane potential of 59 mV at 25 °C. Thus, PMF refers to the energy responsible for the passive movement of protons from outside the mitochondria (intermembrane space) to the inside (matrix). Since the inner membrane of mitochondria is impermeable to protons, proton movement in response PMF occurs only through **ATP synthase** localized in the membranes, which provides the channel for proton movement. ATP synthase, which is also known as Complex V, is responsible for utilizing energy of proton movement for ATP synthesis.

$$\text{ADP} + \text{P}_\text{i} + \text{nH}_\text{p}^+ \rightarrow \text{ATP} + \text{H}_2\text{O} + \text{nH}_\text{N}^+$$

nH_p^+ and nH_N^+ refer to H^+ concentration on the positive (intermembrane space) and negative sides (matrix of mitochondria). Mitchell proposed **chemiosmotic** model in 1961. However, its significance was realized later, and Mitchell was awarded Nobel Prize in Chemistry in 1978 for his contribution. "Chemiosmotic" term used by Mitchell reflects a link between the chemical bond-forming reactions that generate ATP ("chemi") and membrane transport processes ("osmotic"). Sometimes it is referred as chemiosmotic coupling, where coupling refers to coupling of ATP synthesis and flow of electrons through electron transport chain in the inner mitochondrial membrane. Initially a mechanism involving formation of high-energy bond, such as in substrate-level phosphorylation, was favored. However, no intermediates with high-energy bonds could be isolated. Various experimental evidences are proposed in support of chemiosmotic model (Boxes 8.1 and 8.2). In chloroplasts, protons are pumped from stroma to the lumen of thylakoids. As a result, lumen becomes more acidic in comparison to stroma. Since thylakoid membrane is permeable to anions such as Cl^-, ΔpH plays more significant role in comparison to ΔΨ for generating PMF in chloroplasts. In case of mitochondria, it is the ΔΨ which contributes more to PMF (Figs. 8.2 and 8.3).

Box 8.1: Evidence in Support of Chemiosmotic Hypothesis

Coupling of ATP synthesis with electron flow from a substrate (succinate) to O_2 can be demonstrated in an experiment by measuring change in O_2 concentration in the medium containing ADP and P_i.

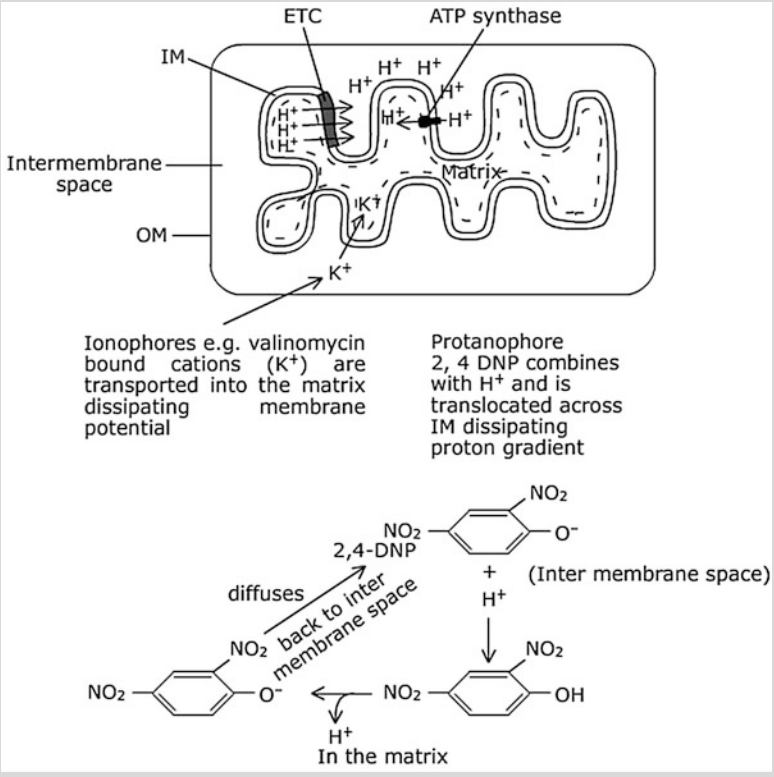


(**A**) Isolated mitochondria are suspended in buffer. Change in O_2 concentration is monitored using O_2 electrode. Reduction of O_2 is indicative of electron transport since on accepting electrons, O_2 will be converted to water. Addition of ADP and P_i alone does not result increase in O_2 consumption. However, an increase in O_2 consumption was observed on addition of ADP + P_i along with succinate, which stops after some time indicating that ADP and P_i have been consumed. This is validated by checking ATP synthesis. Addition of CN^- blocks electron transfer. (**B**) Graphical representation of the experiment.

Use of uncoupling agents: Uncouplers are the amphiphilic compounds which are soluble both in water and lipids. As a result, they can permeate through the membranes by diffusion, transferring H^+ or OH^- across, and resulting in dissipation of proton gradient without affecting electron flow, but ATP synthesis does not occur. Uncouplers provide pathway for proton movement, dissipating proton gradient bypassing ATP synthase activity. During the experiment addition of uncouplers results in continuation of electron transport and proton pumping, without generation of any proton gradient. ATP synthesis does not occur without affecting uptake of oxygen. In the absence of proton gradient, however, protons are transported in reverse direction through ATP synthase at the expense of ATP. Uncouplers which transfer protons across the membrane are known as protonophores. These include FCCP (carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone) and 2,4-dinitrophenol (DNP). Protonated DNP (a weak acid) diffuses from high proton concentration side of the membrane to low proton concentration side where it gets dissociated to generate protons resulting in dissipation of proton gradient. Membrane is permeable to both protonated and anionic forms of these

(continued)

Box 8.1 (continued)
uncouplers. The anionic form diffuses back and combines with more protons and carries them to the other side of the membrane creating short circuiting for protons. As a result, no ATP is synthesized. However, electron transport is not affected, and oxygen will be reduced to water in the experimental conditions. Another class of uncouplers, known as ionophores, transfer alkali cations (e.g., K^+ ions) along with them across the membrane. As a result, membrane potential is dissipated. These include valinomycin.



Studies with reconstituted membrane vesicles: Coupling of proton gradient with ATP synthesis was also demonstrated by Efraim Racker and colleagues (Cornell University in the mid-1960s) in an experiment with isolated mitochondria. Besides supporting coupling of proton gradient with ATP synthesis, the experiment also demonstrated that H^+ / ATP synthase of mitochondria consists of two parts, an oligomycin-sensitive membrane-bound factor, F_o, which is required for proton translocation, and a soluble fraction F_1 which is required for ATP synthesis. Racker called these particles F_1 ATPases. Mordechai Avron from Israel demonstrated the existence of similar particles in

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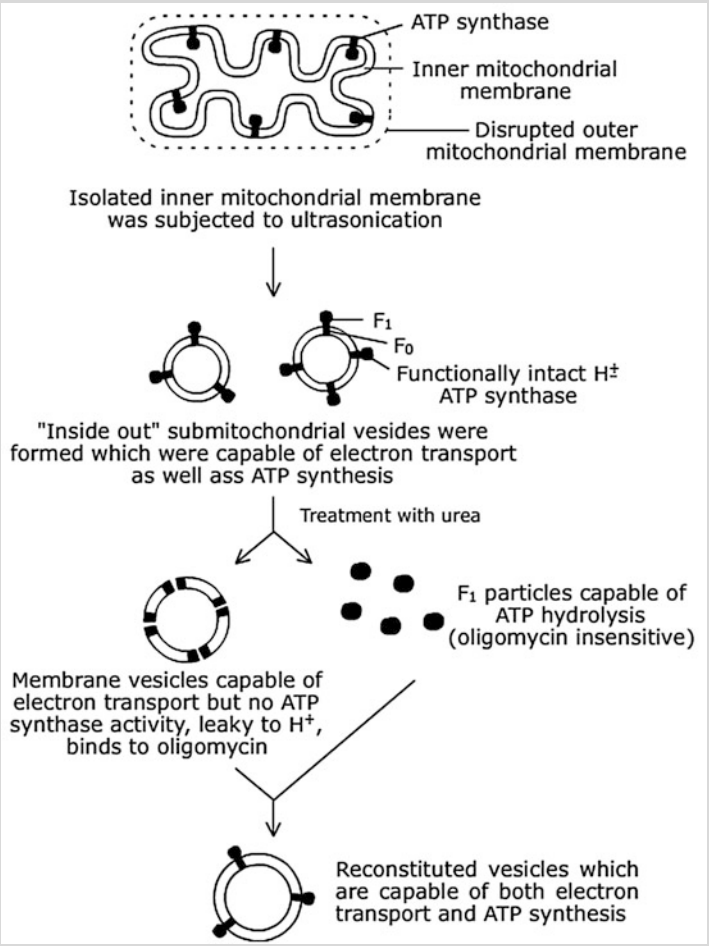
Box 8.1 (continued)
chloroplast membranes which also had ATPase activity. Though H^+ ATP synthase of chloroplasts is not inhibited by oligomycin, membrane-bound part of these is designated as F_o. Similar particles are also reported in bacteria, and these collectively are called F-ATP synthases or F-ATPases or F_o-F_1 ATP synthases or F_o-F_1 ATPases.



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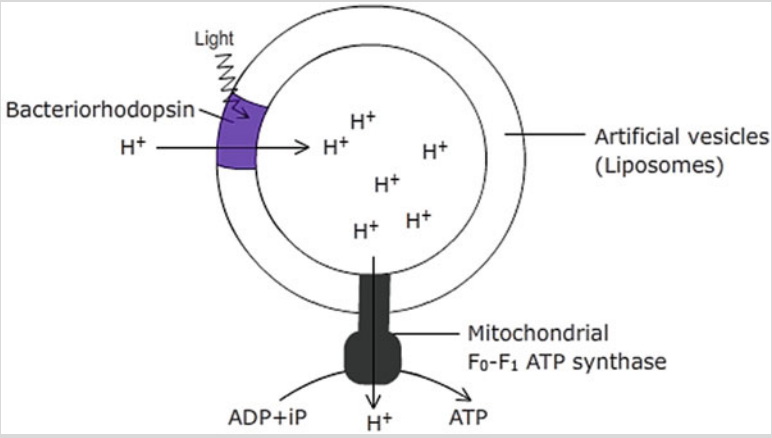
Box 8.1 (continued)

Experiment was performed with artificial vesicles prepared from purified phospholipid (liposomes) to demonstrate coupling of light-driven proton gradient with ATP synthesis. Mitochondrial F_o-F_1 particles and bacteriorhodopsin were incorporated in the membranes of liposomes. In the presence of light, bacteriorhodopsin pumped H^+ into the lumen of the vesicles, which resulted in synthesis of ATP from ADP and P_i. Photosynthetic archaebacteria utilizes bacteriorhodopsin to generate proton motive force.



Jagendorf's experiment: Daniel Arnon (Berkley) had observed in 1954 that ATP was synthesized from ADP and P_i when suspended thylakoid membranes are illuminated. This process of ATP synthesis in the presence of light was called photophosphorylation. In earlier observations ATP synthesis had been found to be coupled with oxidation of NADH in mitochondria. Photophosphorylation, however, was coupled with NADPH generation during light reaction. American scientist Andre Jagendorf carried out an experiment in which he experimentally demonstrated that by subjecting thylakoids to a proton gradient ($\Delta pH = 4$), ATP could be synthesized from ADP and P_i, even in the absence of light. The experiment demonstrates that during light reaction of photosynthesis, the role of light is to create proton gradient. ATP synthesis in thylakoid could occur even in dark, if pH gradient could be established across thylakoids. This observation further supported chemiosmotic hypothesis.

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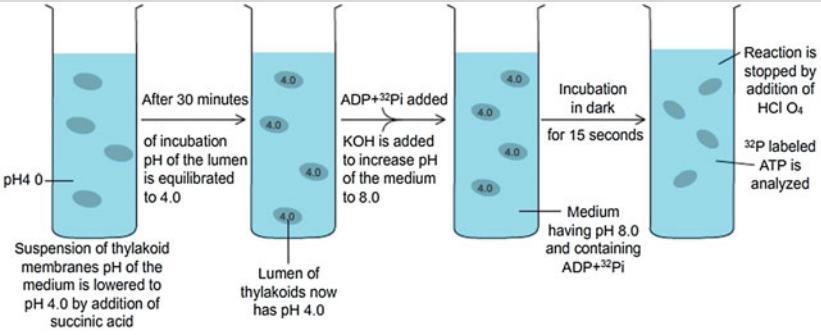
Box 8.1 (continued)



Box 8.2: Bacteriorhodopsin

Halobacteria, e.g., *Halobacterium salinarum*, contain a purple-colored protein bacteriorhodopsin which absorbs green wavelength of light in the range of 500–650 nm. Bacteriorhodopsin is the example of simplest proton pumping mechanism where a single protein is responsible for generation of proton gradient. These bacteria grow in salt lakes where the salt concentration exceeds 5 mM. These are aerobes and use organic fuel for energy production. However, at times availability of O_2 becomes limiting because of which the oxidative mechanism of energy production needs to be supplemented by capturing light energy. On absorbing photons, bacteriorhodopsin can transport protons from inside of the cell to extracellular side resulting in generation of proton gradient across the plasma membrane. Protons flow back in response to the proton gradient through ATP synthase localized in the plasma membrane resulting in ATP synthesis. Bacteriorhodopsin consists of a light-sensitive cofactor retinal which is an aldehyde derivative of vitamin A and a protein bacteriopsin. The protein has seven transmembrane helices. On absorbing proton, retinal undergoes photoisomerization. This results in conformational change of the surrounding protein bacteriopsin followed by proton pumping action toward extracellular side of the membrane. ATP generation by this means in these bacteria is not associated with any release of O_2 or any CO_2 assimilation.

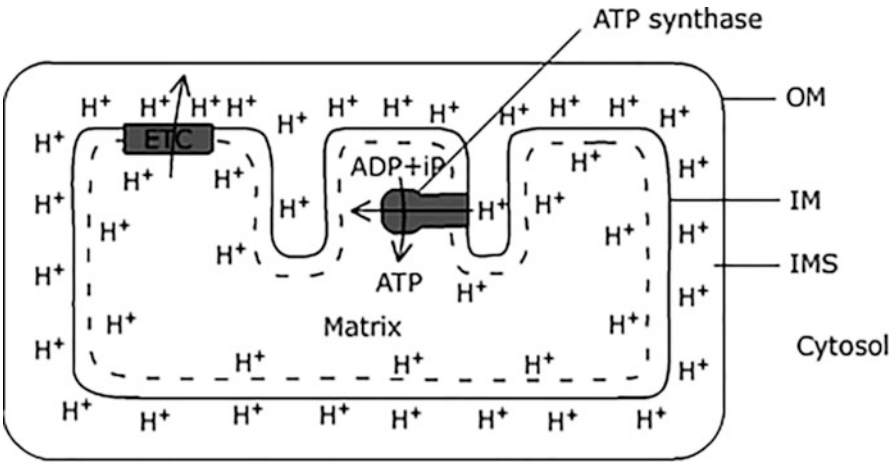


Fig. 8.2 Oxidative phosphorylation in mitochondria. Membrane potential ($\Delta\Psi$) is the major component of proton motive force in mitochondria. OM, outer mitochondrial membrane; IM, inner mitochondrial membrane; IMS, intermembrane space

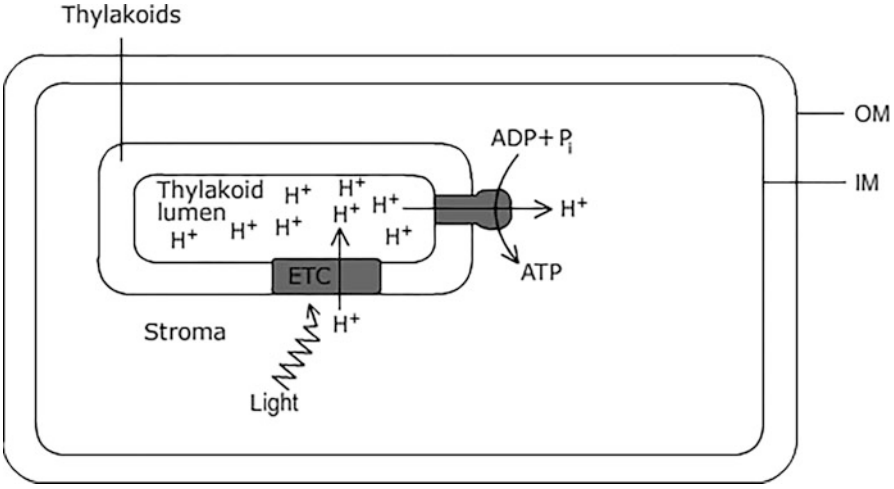


Fig. 8.3 Photophosphorylation in chloroplasts. Δ pH in between stroma and lumen of thylakoid is the major component of PMF. OM, outer membrane of chloroplasts; IM, inner membrane of chloroplasts; ETC, electron transport chain localized in thylakoid membrane. Light energy is used for pumping H^+ from stroma to thylakoid lumen

8.2 ATP Synthase

Parsons and Fernández-Moran first demonstrated elementary particles in 1963–1964 in their preparations of animal mitochondria using negative staining method. These "elementary particles" were present along the inner membrane of mitochondria and consisted of a spherical headpiece attached by a stalk to the membrane inner surface. However, a relationship among these subunits with oxidative phosphorylation was subsequently demonstrated by Efraim Racker. It was demonstrated in the experiment that during oxidative phosphorylation, the headpiece of these subunits acts as a coupling factor (F_1) for ATP synthesis. F_1 was so called since it was the first factor found essential for ATP synthesis. These structures did not have any electron transport capacity. These are of ancient origin, and similar structures are also found in chloroplasts and bacteria. These are F-type ATPases since these were demonstrated to catalyze ATP hydrolysis when isolated and these were called **F_1 ATPases**. Alan Senior and Harvey Penefsky purified bovine F_1 complex. Penefsky demonstrated it to be an assembly of five different kinds of polypeptides, which he called α, β, γ, δ, and ε. These were found to be having a mass of more than 500,000 Daltons. In 1994, John Walker and his colleagues at Cambridge carried out crystallographic determination and gave the three-dimensional structure of F_1 part of ATP synthase isolated from beef heart mitochondria (Fig. 8.4). Though amino acid composition of the three β-subunits is identical, these three subunits differ in their conformations (β-ATP, β-ADP, and β-empty). γ-subunit is associated with one of them having β-empty conformation. Membrane-bound factor F_o is associated with

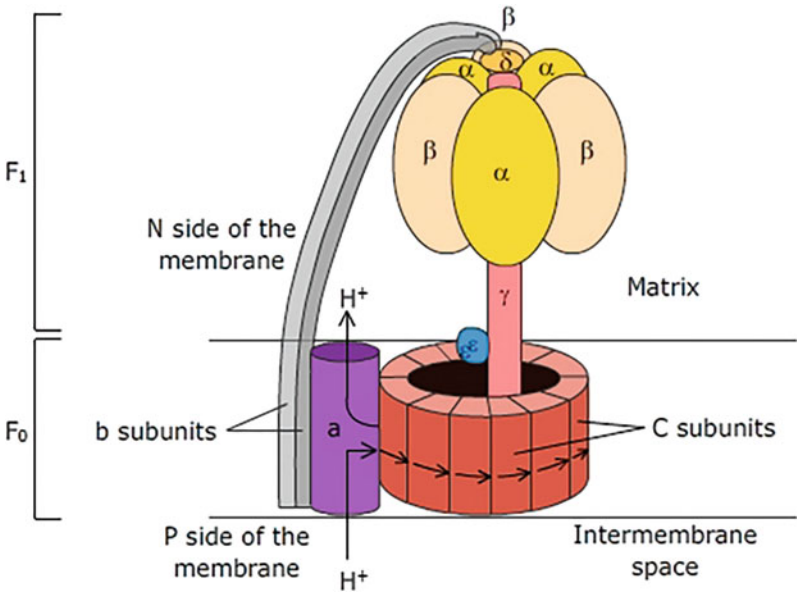


Fig. 8.4 A model depicting ATP synthase structure

proton translocation, which is inhibited by adding oligomycin; thus, it is called as F_o. "c" is a small hydrophobic protein which has two transmembrane-spanning helical regions with a loop extending in the matrix of mitochondria. The two transmembrane helices of c-subunits run perpendicular to the plane of membrane and are arranged in two concentric rings. The inner ring consists of N-terminus of the polypeptide, while the outer ring consists of C-terminus. The outer circle has a diameter of 55 Å. The c-ring makes a rotatory movement associated with proton movement. Hence F_o is also called "rotor." The two b-subunits anchor F_o-F_1 to the membrane through binding with a-subunit of F_o and δ-subunit on the other side, holding α- and β-subunits in place. Thus, two b-subunits along with a-subunit and F1 headpiece (consisting of $\alpha_3\beta_3$) form "stator." The ε-subunit along with γ-subunit forms leg and foot that projects from the bottom of the F_1. Subunit "a" of F_o consists of several hydrophobic transmembrane helices and is in close association with one of the c-subunits of the c-ring (Fig. 8.4). These provide a transmembrane channel for protons. Structures of ATP synthase in mitochondria and chloroplast differ significantly in their F_o domain though there is similarity in the structure of F_1. Orientation of the enzyme is similar in both the cases. F_1 is present toward the alkaline side (N) of the membranes. ATP synthase in chloroplasts is known as CF_o-CF_1. It is a 400 kDa complex consisting of 9 different subunits, some of which are encoded in chloroplast genome while others in nuclear genome. Similar to F_1, CF_1 also consists of three large subunits of α and β (three copies of each) and three small subunits, i.e., γ, δ, and ε (one copy each). δ-subunit links CF_o to CF_1, γ-subunit appears to control proton gating through the enzyme, while ε-subunit blocks catalysis in the dark as a result of which breakdown of ATP is prevented. γ-subunit may also participate in the regulatory mechanism by the Fd/thioredoxin system. As a result, ATP synthase may be activated in light at low PMF, and enzyme may be deactivated in dark. CF_o consists of four types of subunits, i.e., I, II, III, and IV. Except III all other subunits are present in single copy. There may be 14 copies of subunit III per complex in plant chloroplasts, which form the pathway for translocation of protons from luminal space to stroma.

8.3 Mechanism of ATP Synthesis

Paul Boyer proposed **rotational catalysis** as a key to **binding change mechanism** for ATP synthesis for which he was awarded Nobel Prize in 1997 together with John E. Walker. According to this model, the major energy-requiring step is not ATP synthesis; rather energy is required for change in the affinity of β-subunits of the enzyme complex for the substrates (ADP + P_i) and release of the product (ATP), which occurs due to change in conformation of the protein subunits. The active sites of F_1 are responsible for catalyzing ATP synthesis. It is also proposed that these affinity changes are coupled with rotation of a shaft (consisting of γ- and ε-subunits) associated with rotation of c-ring which itself is associated with proton translocation.

Mechanism of ATP synthesis, both in chloroplasts and mitochondria, is believed to be identical. ATP synthesis by rotational catalysis will be dealt as (i) ATP synthesis due to rotation of γ- and ε-shaft of F_o-F_1 complex (ii) rotation of γ- and ε-shaft due to proton movement in response to electrochemical gradient.

8.3.1 Rotatory Model (Binding Change Mechanism)

F_1 headpiece is a hexamer consisting $\alpha_3\beta_3$. The subunits constituting headpiece do not move since it is bound to two "b" polypeptides through δ-subunit, and the two "b" polypeptides are bound to static a-subunit of F_o constituting "stator." The active sites for catalysis of ATP synthesis are localized in the β-subunits of F_1 at α and β interfaces. There are three possible conformations for each of β-subunits, i.e., β-ADP (loose), β-ATP (tense), and β-empty (open). In β-ADP (loose) conformation, the subunit can loosely bind with ADP and P_i, followed by change in its conformation to β-ATP (tense) resulting in ATP synthesis. Change in conformation of β-tense to β-empty results in release of ATP. Each of the β-subunit changes from β-ADP (loose) to β-ATP (tense) to β-empty (open). The adjacent β-subunits are not in similar conformations. The β-subunit prior to β-ADP (loose) will be in β-empty (open) conformation, while the β-subunit prior to β-empty will be in β-ATP (tense) conformation. Thus, on one side of β-empty subunit, it is β-ATP and on the other side β-ADP. Change in conformation of β-subunits occurs due to the rotation of γ-polypeptide. The γ-subunit brings about the conformational change in β-subunit so that ATP is released. Thus γ-subunit is linked with β-empty conformation. Rotation of γ-subunit occurs in anti-clock direction (Fig. 8.5). With each anti-clock rotation of 120°, γ-subunit gets attached to different β-subunits, which is forced to change its conformation from β-ATP to β-empty. One complete rotation of γ-subunit (360°) will cause each of the three β-subunits to acquire all the three possible conformations. The rotation is not smooth rather occurs in three discreet steps of 120° each. It is not the binding or synthesis of ATP rather it is the release of ATP which is energy-requiring process. Direction of the rotation of γ-subunit determines the enzymatic nature of the complex. In the absence of proton gradient, the isolated enzyme complex catalyzes ATP hydrolysis. This model was confirmed in an experiment conducted by Masasuke Yoshida and Kazuhiko Kinoshita in Japan. They attached a fluorescent molecule to the upper end of γ-subunit contained in an F_o particle present in the membrane. Using a special video microscopy documentation, they were able to demonstrate that during ATP hydrolysis, γ-subunit rotates in the opposite direction, i.e., in a clockwise manner. However, ATP synthesis due to movement of γ-subunits in anti-clockwise manner could not be demonstrated in the absence of proton gradient. Movement of shaft consisting of "γ" and "ε" occurs due to rotatory movement of c-ring of F_o.

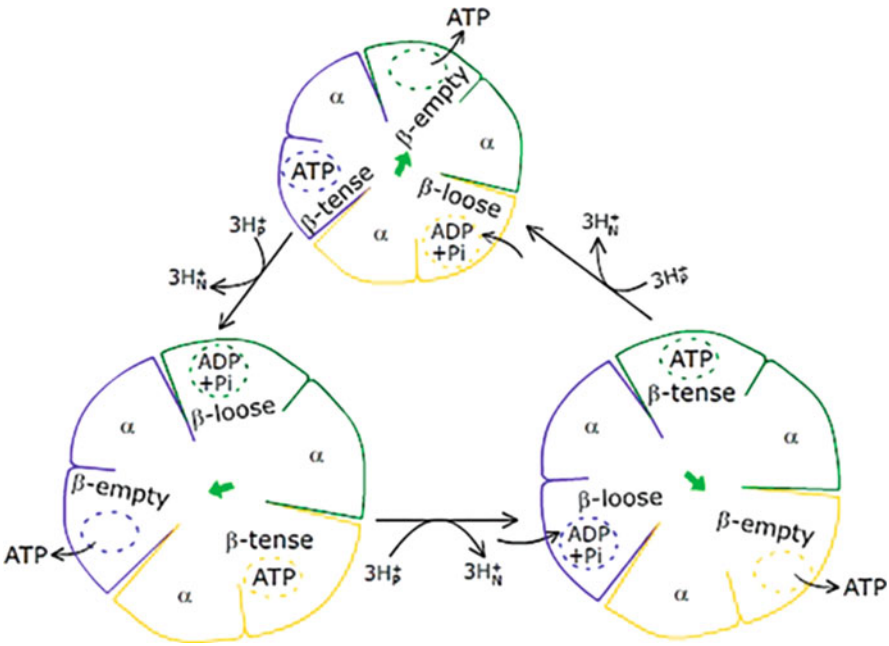


Fig. 8.5 Rotational model for binding change mechanism proposed by Paul Boyer for ATP synthesis

8.3.2 Rotatory Movement of c-Ring of Fo

How is the movement of shaft, consisting of γ- and ε-subunits, coupled to proton translocation through proton channel? Proton movement occurs in response to electrochemical gradient created by proton transport. F_o consists of two types of subunits, single a-subunit and 8–15 subunits of "c." The number is variable depending upon the source of ATP synthase. In animal mitochondria, the number of c-subunits is 8, in yeast mitochondria it is 10, in chloroplasts the number is 12–14, while it can be as high as 15 as in cyanobacterium *Spirulina platensis*. F_o functions like a nanomotor. In chloroplasts, its velocity of rotation has been estimated to be 160 revolutions per second. It is the c-ring which rotates causing the movement of shaft consisting of γ- and ε-subunits, which are arranged in leg and foot formation, while the a-subunit does not move and is part of "stator" along with two b polypeptides, a δ-subunit and F_1 headpiece. The hydrophobic helices of c-subunits are arranged perpendicular to the membrane around the axis forming c-ring. The two transmembrane helices of c-subunits which run perpendicular to the plane of membrane are arranged in two concentric rings. The outer circle has a diameter of 55 Å and consists of C-terminus of the polypeptide, while the inner ring consists of N-terminus of the polypeptide. Wolfgang Junge from Germany developed a model for explaining how proton gradient drives the nanomotor. Two hydrophilic half channels for protons are localized in the a-subunit, one is leading from "P" side of

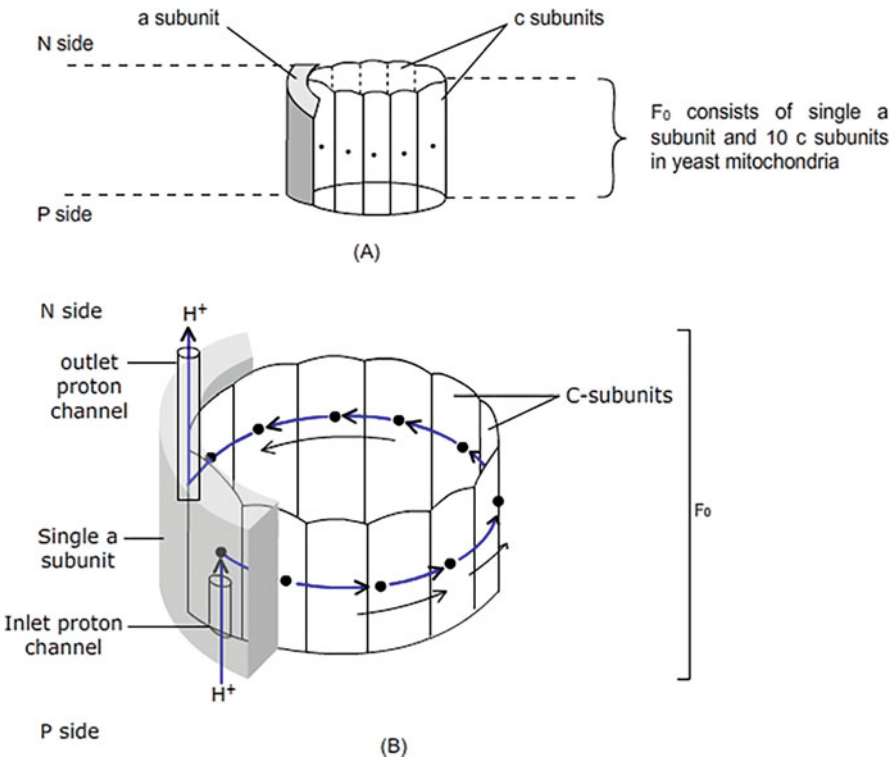


Fig. 8.6 (**a**) F_o of ATP synthase. (**b**) Figure depicting a model demonstrating rotation of c-ring. Rotation of c-ring is driven by proton translocation from acidic to alkaline side of the membrane in response to electrochemical gradient

the membrane to the middle of the membrane, while the other is leading from middle of the membrane to "N" side (Fig. 8.6). The a-subunit is believed to have five hydrophobic membrane-spanning regions and has positively charged arginine (Arg^{210}) which plays active role in channeling protons across. Subunit "b" is anchored in the membrane by a single N-terminal α-helix. Each of c-subunits has a critical aspartate in the middle (Asp^{61} in *E. coli*) or glutamate buried in the membrane. The side-chain carboxyl group of the residue plays a critical role in proton translocation. This carboxyl group is conserved throughout in all known subunits of "c" and is required for transmembrane proton transport. The c-subunits form a ring by interactions through their C-terminal α-helices with the N-terminal α-helices outside the ring. The annular structure of c-ring has been visualized by **atomic force microscopy**. The proton (H^+) travels from "P" side to the middle of the membrane through the half channel where it protonates carboxylate group (COO^-) of Asp^{61} of c-subunit. As a result of this, electrostatic attraction between the protonated carboxyl (COOH) group of Asp^{61} of c-subunit and positively charged Arg^{210} of a-subunit is weakened, and the positively charged Arg^{210} side arm swings and displaces the

proton of carboxyl group of its Asp^{61} of c-subunit present on the other side. This results in rotation of c-ring because of Brownian movement. The c-ring subunit (having deprotonated carboxylate group) now faces the other half channel of the stationary a-subunit. The displaced proton enters the half channel and is released toward "N" side of the membrane (Fig. 8.6). The half channel of a-subunit facing "P" side is ready to receive another proton from "P" side, and the cycle is repeated again. Proton translocation occurs as long as the proton gradient is there. In the absence of proton gradient, ATP synthase will act as ATPase and will generate proton gradient at the expense of ATP. Why does Brownian movement occur only in one direction? This may be due to the presence of two half channels, and possibly the repulsion between the positively charged Arg^{210} residue of a-subunit and the proton loaded c-subunit prevents the backward movement of the rotor. ATP synthase may form a complex, known as **ATP synthasome**, with adenine nucleotide translocase (ADP/ATP symporter) and phosphate carrier (P_i/H^+ symporter). A complete rotation of c-ring with 10 subunits will involve translocation of 10 H^+. This will result in rotation of γ- and ε-shaft by 360°. As a result of this, all the three β-subunits will undergo changes in their three conformations, resulting in synthesis of three ATPs. This indicates that translocation of 10 H^+ across the membrane will result in synthesis of three ATPs. Transport of a pair of electron from NADH to ½ O_2 results in the release of 214 $kJ.mol^{-1}$ free energy, which is conserved as gradient of 10 H^+ across the membrane. Free energy (ΔG) required for ATP synthesis is 50 $kJ.mol^{-1}$. Energy conservation equivalent to 214 $kJ.mol^{-1}$ justifies that. The rest of the energy may be released as heat.

8.4 Stoichiometry of O_2 Consumption and ATP Synthesis (P/O Ratio)

P/O ratio or $P/2e^-$ refers to the number of moles of ATP synthesized per ½ O_2 reduced (i.e., per pair of electron transferred to O_2) during oxidative phosphorylation. Generally, the experimental value varies from 2.5 in case of NADH oxidation to 1.5 in $FADH_2$ oxidation. The value also depends upon the number of c-subunits which constitute F_o of the F_1-F_o complex of mitochondria. One full rotation of the c-ring results in translocation of 8 H^+ and synthesis of 3 ATPs, in case there are 8 subunits. In addition, three P_i would need to be translocated for ATP synthesis. Thus, that a total of 11 H^+ would need to be translocated for synthesis of 3 ATP molecules, i.e., 3.7 protons (11 H^+ and3 ATP) are required for generation of one ATP. Oxidation of one NADH is coupled with generation of a gradient of 10 H^+, which indicates that the number of ATPs produced coupled with oxidation of one NADH will be 10/3.7, i.e., 2.7 ATPs. In yeast mitochondria, a complete rotation of c-ring will result in translocation of 10 H^+ (since there are 10 subunits) and in the synthesis of 3 ATP molecules. In addition to this, three H^+ are translocated along with three P_i. It makes a total of 13 H^+ translocated for synthesis of 3 ATP molecules, i.e., $13/3 = 4.3$ H^+ are translocated for the synthesis of one ATP. Thus P/O ratio of NADH during oxidative phosphorylation in yeast will be $10/4.3 = 2.3$ ATPs. Since a

gradient of 6 H^+ is created in case of oxidation of $FADH_2$, P/O ratio will be 6/3.7 = 1.6 or 6/4.3 = 1.4, respectively, if c-ring has 8 or 10 subunits. The experimental values of P/O ratio for NADH and $FADH_2$ oxidation generally used are 2.5 and 1.5, respectively. In case of chloroplasts (photophosphorylation), 14 copies of subunit III (c) appear to be present. Hence, translocation of 14 H^+ will be required for complete rotation of c-ring, resulting in the synthesis of three ATP molecules. Thus H^+/ATP ratio will be 14/3 ATP, i.e., 4.67.

8.5 Substrate-Level Phosphorylation

ATP can also be synthesized directly by coupling bond energy of high-energy compounds with ATP synthesis. For example, there are two steps of glycolysis and one step of TCA cycle during which ATP is synthesized directly without involvement of any electron transport. Energy released during the hydrolysis of high-energy compound during these reactions is more than that required for ATP biosynthesis. In one of the glycolytic reactions, phosphate group is transferred from 1, 3-bisphosphoglycerate to ADP, resulting in ATP synthesis. The reaction is catalyzed by the enzyme **glycerate kinase**.

$$1,3-\text{Bisphosphoglycerate} + \text{ADP} \rightarrow 3-\text{Phosphoglycerate} + \text{ATP}$$

The standard free energy change during hydrolysis of 1,3-bisphosphoglycerate is -49.3 kJ.mol^{-1}, and for ATP synthesis, it is +30.5 kJ.mol^{-1}. Reaction is favored by net $\Delta G^{\circ\prime}$ of the reaction being -18.8 kJ.mol^{-1}. Another reaction of glycolysis involves synthesis of pyruvate from 3-phosphoenolpyruvate, which is also coupled with synthesis of ATP. The reaction is catalyzed by **pyruvate kinase**. Phosphoenolpyruvate is a high-energy compound with high group transfer potential.

$$\text{Phosphoenolpyruvate} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP}$$

Free energy of hydrolysis PEP (-61.9 kJ.mol^{-1}) is more negative than that required for ATP biosynthesis (+30.5 kJ.mol^{-1}). The two reactions are coupled, and the net reaction that occurs is the sum of hydrolysis of PEP and ATP synthesis with $\Delta G^{\circ\prime} = -31.4$ kJ.mol^{-1}. Substrate phosphorylation also occurs in TCA during synthesis of succinate from succinyl-CoA. Free energy of hydrolysis of high-energy **thioester** bond of succinyl-CoA is -33.4 kJ.mol^{-1}, while standard free energy requirement for ATP synthesis is 30.5 kJ.mol^{-1}. Overall reaction will be slightly exergonic with a net $\Delta G^{\circ\prime}$ is -2.9 kJ.mol^{-1}.

$$\text{Succinyl} - \text{CoA} + \text{ADP} + P_i \rightarrow \text{Succinic acid} + \text{CoA} + \text{ATP}$$

The reaction is slightly exergonic and is catalyzed by the enzyme **succinyl-CoA synthetase** (SCS), also known as **succinic thiokinase**. The mammalian SCS consists of two subunits, α and β, which have molecular weight 32,000 Daltons and 42,000 Daltons, respectively. α-subunit has got the binding site for CoA and a phosphate-binding site, i.e., histidine (His^{246}). The β-subunit confers specificity to either GDP or ADP. Active site of the enzyme is present at the interface of the two subunits. Reaction occurs in three steps: (i) In the first step of the reaction catalyzed by SCS, the –CoA group of succinyl-CoA is replaced by a phosphate group, resulting in formation of high-energy acyl-phosphate. (ii) In the second step, high-energy phosphate group of acyl phosphate is transferred to His^{246} of the α-subunit, resulting in phosphorylation of the residue, and succinate is released free. (iii) The third step involves transfer of this high-energy phosphate group to ADP/GDP, resulting in the synthesis of ATP/GTP, respectively, and the enzyme is released free (Fig. 8.7). This reaction is the only reaction of TCA cycle which is the source of ATP generation in the absence of functional electron transport chain, especially when O_2 is absent. Equilibrium constant of the reaction is nearly 1, so that the ratio of nucleotide present (ADP/ATP in plants or GDP/GTP in animals) will determine the direction of the reaction. Net result of the reaction is conservation of energy as ATP, without involvement of any electron transport.

8.6 Electrochemical Gradient-Driven Transport of Various Metabolites Across Inner Mitochondrial Membrane

Inner mitochondrial membrane is impermeable to most of the biomolecules carrying charge. However, the metabolic pathways which operate in mitochondria require exchange of various biomolecules across the membrane. Majority of the cell's requirements are met by ATP generated in mitochondria which needs to be exported, while ADP and P_i need to be transported inside. Since the membranes are impermeable for ATP and ADP, their transport is facilitated by ADP/ATP transporters. ATP $^{4-}$ carries four negative charges, while ADP^{3-} carries three negative charges. Movement of ATP^{4-} is driven by membrane potential generated due to proton transport across the membrane. Net charge in the intermembrane space is positive due to accumulation of H^+, while toward the matrix side, it is negative. The transport is facilitated by **adenine nucleotide translocases**, which are antiporters. The same protein moves ATP^{4-} out and ADP^{3-} in. **Phosphate translocases** are symporters responsible for transporting one P_i into the matrix, coupled with transport of one H^+. This transport is also favored by the transmembrane proton gradient (ΔpH). A complex of ATP synthase along with the adenine nucleotide and phosphate translocases is known as **ATP synthasome**, which has been isolated from mitochondria by gentle treatment with detergents. Pyruvate is also cotransported along with H^+ into the matrix of mitochondria in response to proton gradient.

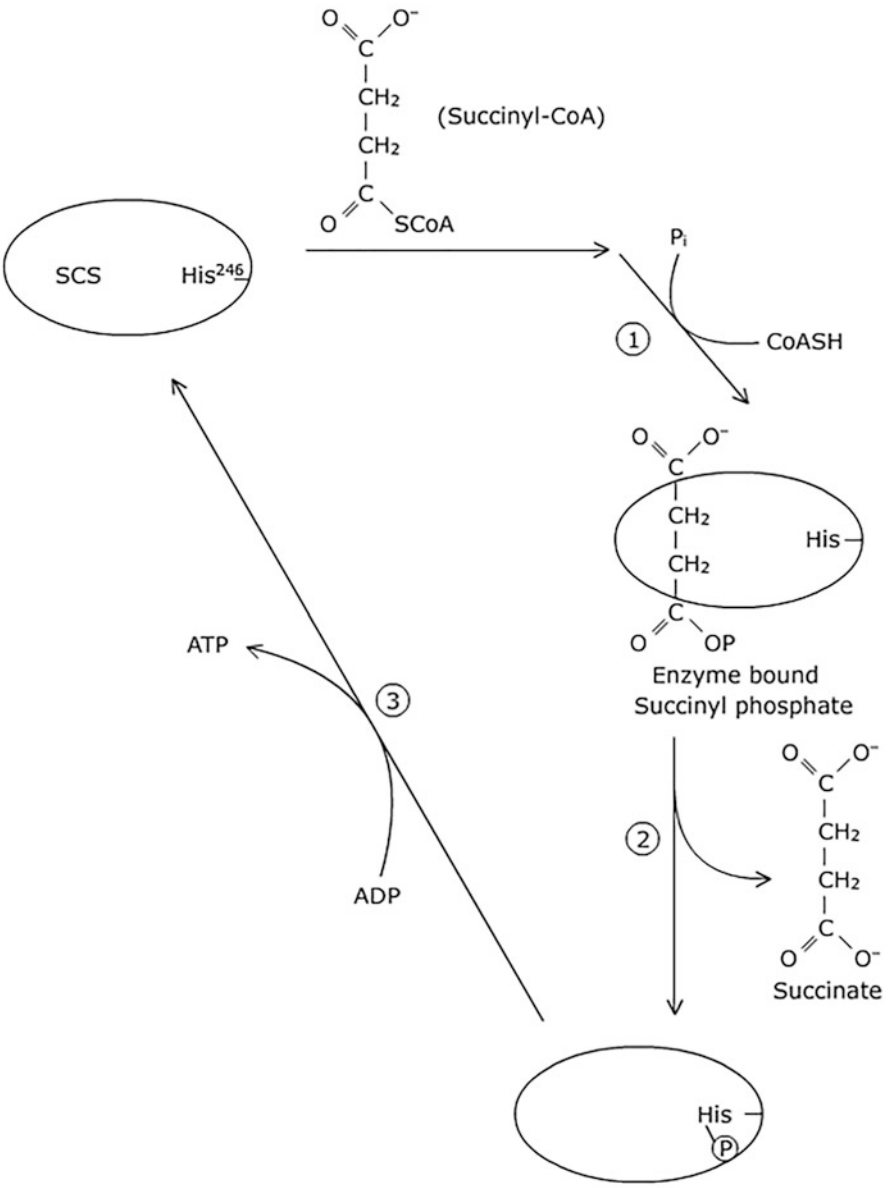


Fig. 8.7 Role of succinyl-CoA synthetase (SCS) in substrate-level phosphorylation. Reaction occurs in three steps. Step 1, SCS catalyzes replacement of CoA of succinyl-CoA with P_i resulting in enzyme-bound succinyl phosphate; Step 2, P_i-group of succinyl-P is transferred to His^{246} of the enzyme protein resulting in formation of N-phosphohistidine and succinate is released free; Step 3, finally, phosphoryl group is transferred from phosphorylated His^{246} to ADP resulting in synthesis of ATP

8.7 Oxidative Phosphorylation and Photophosphorylation: A Comparative Account

Unlike in animals, ATP synthesis in plants occurs both in chloroplasts and mitochondria during photophosphorylation and oxidative phosphorylation, respectively. The two phosphorylation processes differ from each other in relative contributions by the two components of PMF. In mitochondria, accumulation of H^+ in the intermembrane space will lower cytosolic pH adversely, affecting activity of many cytosolic enzymes since intermembrane space is in continuation with cytosolic space. Thus, membrane potential ($\Delta\psi$) plays more significant role in building up the electrochemical gradient instead of ΔpH in mitochondria. On the contrary, in chloroplasts, H^+ are accumulated in the enclosed space of lumen. As a result, the activity of the enzymes present in stroma will not be affected. Membrane potential of thylakoids is negated due to the movement of many anions such as Cl^- to the lumen. Thus, in chloroplasts, it is the pH gradient which is the major component contributing to the electrochemical gradient. A comparison of oxidative phosphorylation, photophosphorylation, and substrate-level phosphorylation is given in Table 8.1.

Summary

- ATP is the universal energy currency of the cell in all living beings. Both its structure and mechanism of synthesis have been conserved during course of evolution. Synthesis of ATP occurs by oxidative phosphorylation, photophosphorylation, and substrate-level phosphorylation. Peter Mitchell had proposed chemiosmotic model for ATP synthesis which states that the energy released during electron transport is conserved as electrochemical gradient across inner membrane of mitochondria which contributes to proton motive force. There are two components of proton motive force, i.e., pH gradient (ΔpH) and membrane potential ($\Delta\Psi$). Since the membrane is impermeable, the protons flow back in response to proton motive force through ATP synthase, the enzyme localized in the inner mitochondrial membrane. Evidence which support the hypothesis include experiments conducted using uncouplers of proton gradient, with reconstituted membrane vesicles and isolated thylakoids.
- ATP synthases are F-type ATPases, known as F_o/F_1 in mitochondria or CF_o/CF_1 in chloroplasts. These consist of a knob-like structure – F_1, which is projecting out, and F_o embedded in the membrane. The two are joined together through a stalk. F_1 consists of eight subunits of five different types of polypeptides, i.e., $\alpha_3\beta_3\gamma\delta\varepsilon$. The headpiece consists of three α- and three β-subunits which alternate with each other and are arranged like pieces of an orange. δ-subunit holds α- and β-subunits in place and is connected with two b-subunits. F_1, along with two b- and one a-subunits, forms "stator," while c-subunits of F_o along with γ-subunit is the "rotor" of ATP synthase. γ-subunit is connected with c-ring through ε-subunit in leg and foot manner.
- Paul Boyer had proposed binding change mechanism for ATP synthesis. According to this model, catalytic site for ATP synthesis is present in β-subunits

Table 8.1 Comparison of oxidative phosphorylation, photophosphorylation, and substrate-level phosphorylation

Characteristics	Oxidative phosphorylation	Photophosphorylation	Substrate-level phosphorylation
Site of occurrence	Mitochondria	Chloroplasts	Cytosol and matrix of mitochondria
Electron transport, resulting in proton gradient across membrane	Proton gradient is created across inner mitochondrial membrane	Proton gradient is created across thylakoid membrane	Energy of high-energy bond of the substrate is coupled with synthesis of ATP. Electron transport is not required rather
Metabolic pathway associated with ATP generation	During respiration. Reduced cofactors NADH, $FADH_2$ are produced	During light reaction of photosynthesis	During two reactions of glycolysis and one reaction of TCA cycle
Electron transport chain			Not involved
Location	Inner membrane of mitochondria	Thylakoid membrane	
Components	Immobile components consist of Complex I, Complex II, Complex III, and Complex IV; mobile carriers are UQ and Cyt c	Immobile components are PSII, $cytb_6f$, and PSI; mobile carriers are PQ and PC	
Source of energy for ATP synthesis	Oxidation of NAD^+, $FADH_2$	Light energy	Energy released from hydrolysis of high-energy bonds
Electron donor	NADH/NADPH/ $FADH_2$	H_2O	
Electron acceptor	O_2	$NADP^+$	Not involved
Main contributor to PMF	Voltage gradient across inner mitochondrial membrane and intermembrane space. pH in intermembrane space is only 0.2–0.5 units lower than that of matrix	pH gradient across thylakoid membrane. Lumen being acidic and stroma alkaline	Not involved
ATP synthase	F_o–F1 in inner mitochondrial membrane,	CF_o-CF_1 in thylakoid membrane	Not involved
	F_1 is located toward alkaline side (N) of the membrane, i.e., the matrix of mitochondria,	CF_1 is located toward alkaline side of the membrane, i.e., stroma of chloroplasts	
	F_1 consists of $\alpha_3\beta_3\gamma\delta\varepsilon$ and OSCP[a]	CF_1 consists of $\alpha_3\beta_3\gamma\delta\varepsilon$	
	F_o consists of a, b, and c, i.e., ab_2c_n where $n = 8–15$.	CF_0 consists of a, b and b' (I and II), and c (III), 14 subunits of c (III) are present	

(continued)

Table 8.1 (continued)

Characteristics	Oxidative phosphorylation	Photophosphorylation	Substrate-level phosphorylation
Ratio of number of ATP molecules formed to electrons transferred (P/O ratio)	High since number of c-subunits present in F_o are less; high P/O ratio is predicted	Low since 14 c-subunits are present in the F_o complex; low P/O ratio predicted	
Inhibition of proton translocation	Proton translocation is inhibited by oligomycin, since it binds with OSCP which limits O_2 uptake	Proton translocation is not inhibited by oligomycin	

[a]*OSCP* Oligomycin-sensitivity conferring protein

which can exist in three possible conformations, i.e., loose, tight, and empty. These conformations of β-subunits differ in their affinity for ATP. In loose conformation, β-subunits are loosely bound with ADP and P_i, while in tight conformation, ATP synthesis occurs, and in empty conformation, ATP is released. There is a change in conformation of these subunits due to rotation of γ-subunit which occurs due to rotation of the "rotor" in response to proton movement from positive side of the membrane to the negative side. The energy of proton movement is coupled with the movement of rotor.

- Thermal movement of "rotor" occurs due to interaction of positively charged group of a-subunit with the c-subunits because of the periodic protonation and deprotonation of carboxyl group present in residues of c-subunit polypeptides. Protonation and deprotonation of the carboxyl group occur due to movement of protons through two half channels present in a-subunit, the inlet channel and an outlet channel.
- ATP synthesis also occurs by substrate-level phosphorylation during which ATP is synthesized directly due to transfer of high-energy phosphate bond of the substrate without involvement of any electron transport. The reactions are mediated by kinase enzyme.

Multiple-Choice Questions

1. Proton motive force across the membrane is created due to:
   (a) pH gradient
   (b) Membrane potential
   (c) Both of them
   (d) None of them

2. Uncouplers are chemical agents which:
   (a) Interfere with electron transport across ETC.
   (b) Interfere proton movement across membrane.
   (c) Electron transport occurs but interfere with proton movement.
   (d) Electron transport occurs but proton gradient is dissipated.
3. Protonophores are the uncouplers which dissipate proton gradient due to the following:
   (a) Their acidic nature combines with protons on the acidic side and diffuses to the other side of the membrane.
   (b) They make the membrane permeable to protons.
   (c) They interfere with electron transport as a result proton gradient is not formed.
   (d) They allow the movement of other cations.
4. Energy released during proton movement in mitochondrial membrane in response to PMF is required by ATP synthase:
   (a) For binding of ADP and P_i to β-subunits of F_1
   (b) For ATP synthesis
   (c) For release of ATP
   (d) For transport of electrons
5. The "stator" component of F_1–F_o particles consists of:
   (a) c-Ring
   (b) γ- and ε-shaft of the particle
   (c) 3 α- and 3 β-subunits of F_1
   (d) 3 α- and 3 β-subunits, δ-subunit, b-subunit of F_1 attached to "a" of F_o
6. PMF in mitochondria is because of:
   (a) Gradient of pH and membrane potential
   (b) Mainly pH gradient
   (c) Mainly membrane potential gradient
   (d) None of the above
7. Jagendorf's experiment demonstrated that:
   (a) Light is required for ATP synthesis.
   (b) ATP synthesis can occur even if light is not present.
   (c) ATP synthesis can occur when thylakoids are suspended in buffer of pH 4.0.
   (d) ATP synthesis in thylakoid can be promoted by addition of KOH.
8. Chemiosmotic hypothesis for ATP synthesis was proposed by:
   (a) Paul Boyer
   (b) John Walker
   (c) Peter Mitchell
   (d) Charles Darwin

Answers

1. c 2. d 3. a 4. c 5. d 6. c 7. b 8. c

Suggested Further Readings

Boyer PD (1997) The ATP synthase-A splendid molecular machine. Ann Rev Biochem 66:717–749

Junge W, Nalson N (2015) ATP synthase. Ann Rev Biochem 84:631–657

Prebble JN (2012) Contrasting approaches to a biological problem: Paul Boyer, Peter Mitchell and the mechanism of the ATP synthase, 1961–1985. J Hist Biol 46(4):699–737

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