ELECTRON TRANSPORT CHAIN

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SYNTHESIS OF ATP

ATP can be synthesized in two ways

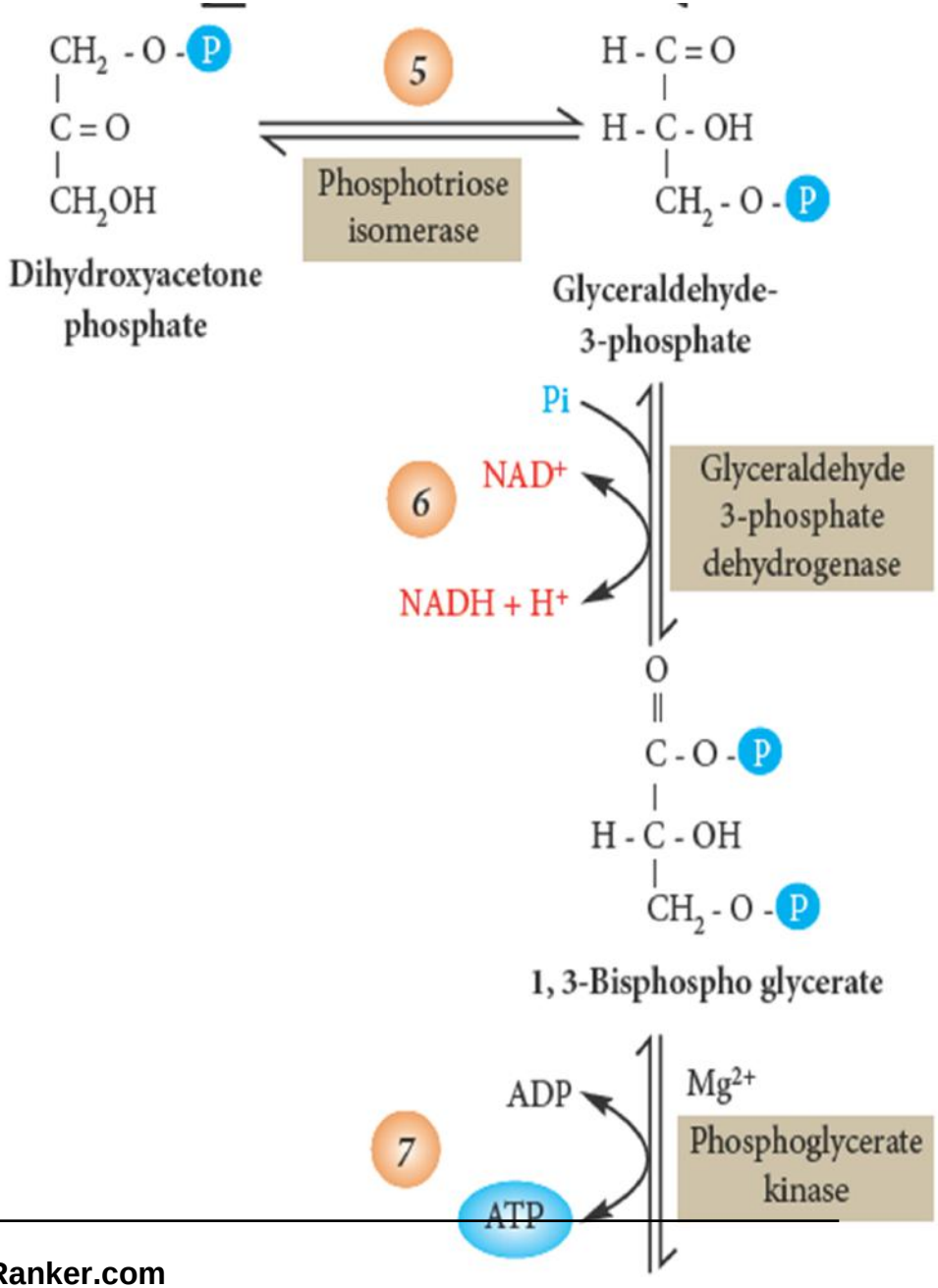
1. Oxidative phosphorylation:

Major source of ATP in aerobic organisms.

It is linked with mitochondrial ETC.

2. Substrate level phosphorylation:

When the energy of high energy compound is directly transferred to nucleoside diphosphate to form a triphosphate without the help from ETC.



The high-energy compounds such as

- PEP
- 1,3-bisphosphoglycerate
- Succinyl CoA

can transfer high-energy phosphate to ultimately produce ATP.

STORAGE FORMS

- Phosphocreatine (creatine phosphate)
- Provides high energy reservoir of ATP to regenerate ATP rapidly, catalyzed by creatine kinase.
- Stored mainly in Muscle, Heart & Brain.

BIOLOGICAL OXIDATION

The transfer of electrons from the reduced coenzymes through the respiratory chain to oxygen is known as biological oxidation.

Energy released during this process is trapped as ATP.

This coupling of oxidation with phosphorylation is called **oxidative phosphorylation.**

TRANSPORT OF REDUCING EQUIVALENT :SHUTTLE PATHWAY

- The inner mitochondrial is impermeable to NADH.
- Therefore, the NADH produced in the cytosol cannot directly enter the mitochondria.
- Two pathways

A. Glycerol-phosphate shuttle- In muscle and brain

B. Malate-aspartate shuttle - In liver and heart

GLYCEROL-PHOSPHATE SHUTTLE

- Cytosolic glycerol 3-phosphate dehydrogenase oxidizes NADH to NAD^+
- The reducing equivalents are transported through glycerol 3-phosphate into the mitochondria.
- Glycerol 3-phosphate dehydrogenase-present on outer surface of inner mitochondrial membrane – reduces FAD to FADH2.

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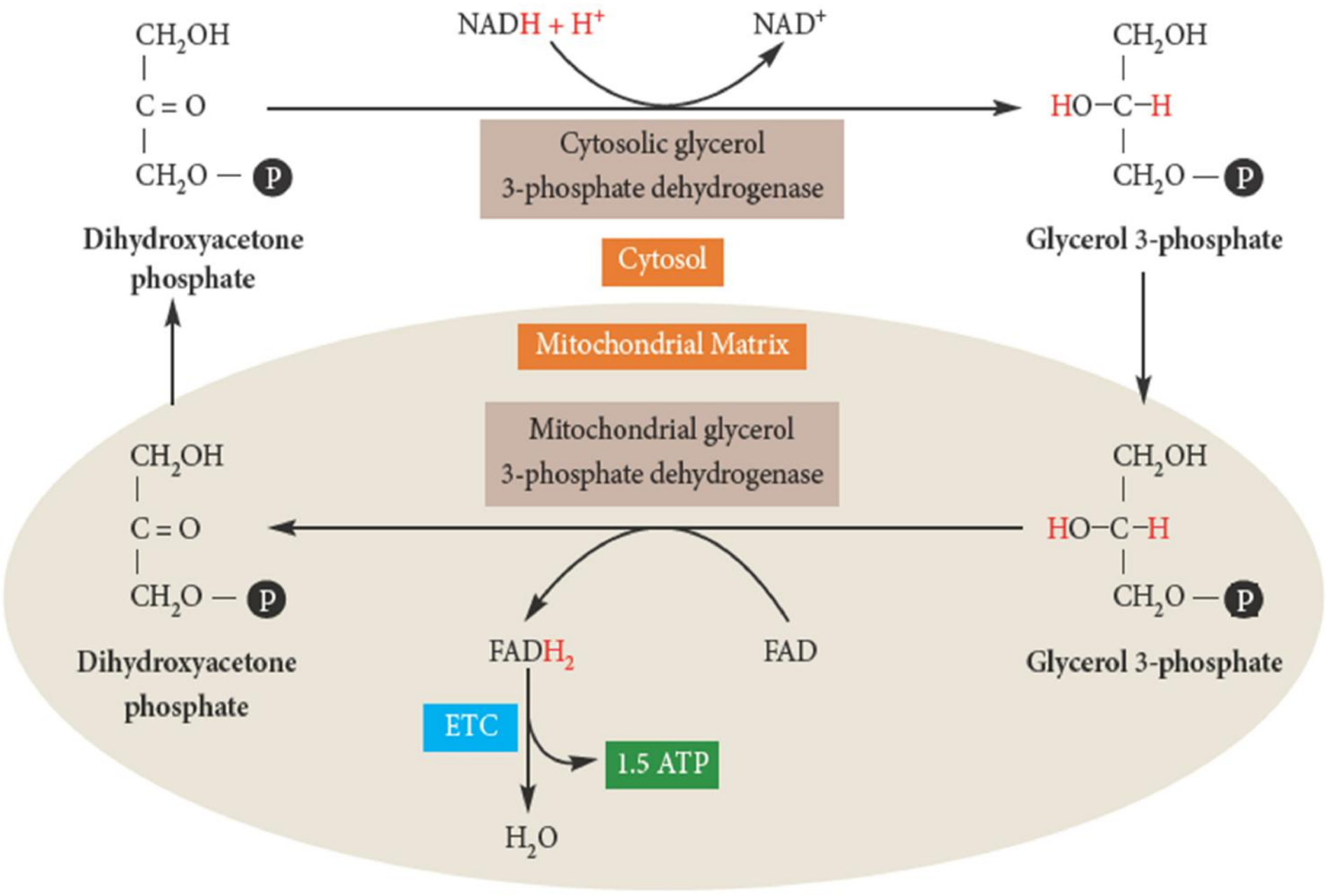
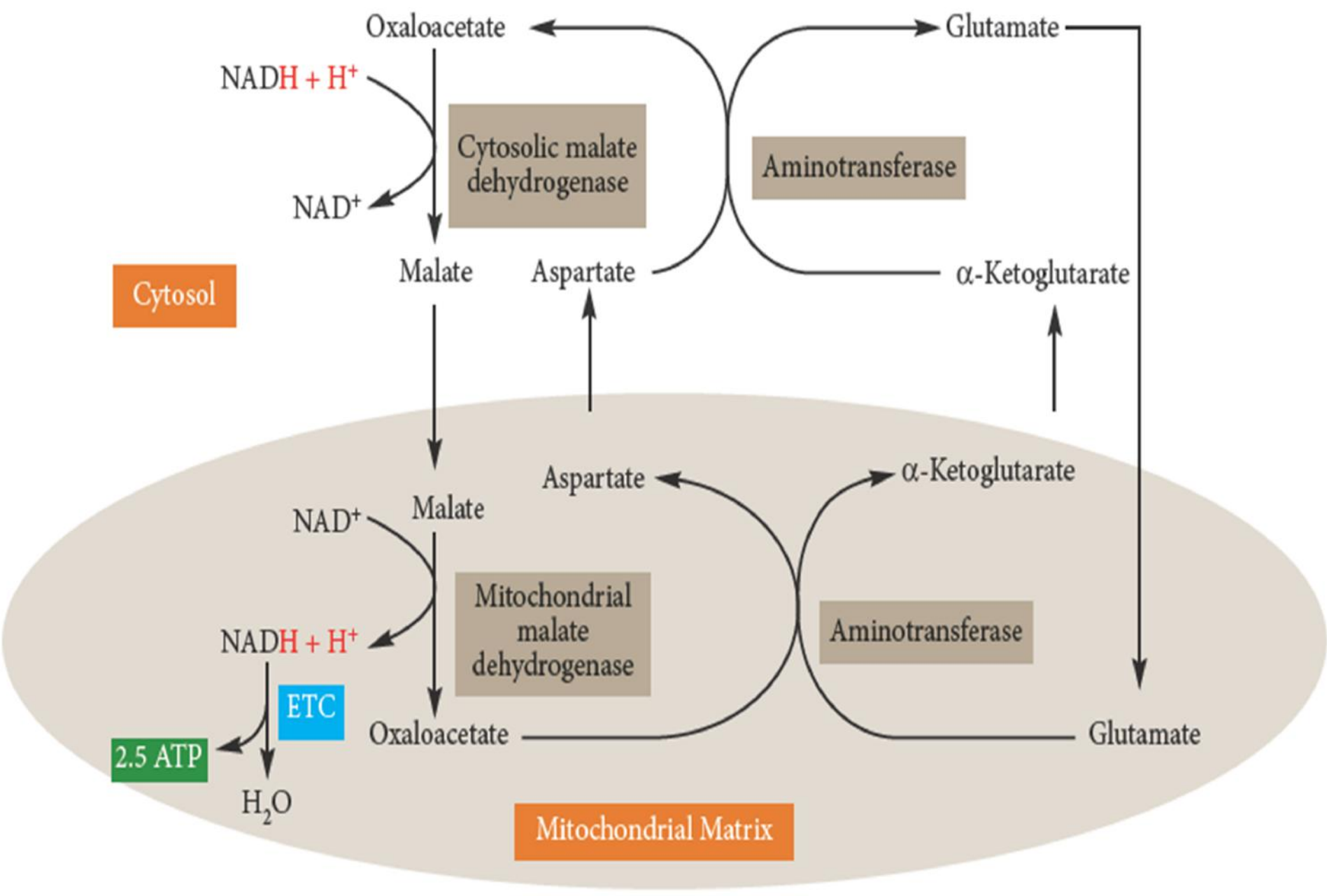
- Dihydroxyacetone phosphate (DHAP) escapes into the cytosol & the shuttling continues.
- FADH2 gets oxidized via ETC to generate 1.5ATP



GLYCEROL PHOSPHATE SHUTTLE

MALATE-ASPARTATE SHUTTLE

- In the cytosol, oxaloacetate accepts the reducing equivalents (NADH) & becomes malate.
- Malate enters the mitochondria where it is oxidized by mitochondrial MDH
- In this reaction, NADH & oxaloacetate are regenerated.
- NADH gets oxidized via ETC & 2.5 ATP are produced.



MALATE–ASPARTATE SHUTTLE .

- In the mitochondria, oxaloacetate participates in transamination reaction with glutamate to produce aspartate & α ketoglutarate.
- The aspartate enters the cytosol & transaminates with α-ketoglutarate to give oxaloacetate & glutamate.

REDOX POTENTIAL

Oxidation:

- Oxidation is defined as the loss of electrons and reduction as the gain in electrons.

- When a substance exists both in the reduced state & in the oxidized state, the pair is called a redox couple.

Redox potential(E_0):

- The oxidation-reduction potential or redox potential, is a quantitative measure of the tendency of a redox pair to lose or gain electrons.
- The redox pairs are assigned specific standard redox potential at pH 7.0 & 25^0C

- The more negative redox potential represents a greater tendency to lose electrons.
- A more positive redox potential indicates a greater tendency to accept electrons
- The electrons flow from a redox pair with more negative E_0 to another redox pair with more positive E_0
- The redox potential (E_0) is directly related to the change in the free energy (ΔG^0)

Standard redox potential (E_0) of some oxidation-reduction systems

ETC Component	Redox Potential (volts)
NAD^+	–0.32
↓	
FMN	–0.12
↓	
CoQ	+0.04
↓	
Cyt b	+ 0.07
↓	
Cyt c_1	+0.23
↓	
Cyt c	+0.25
↓	
Cyt a	+ 0.29
↓	
Cyt a_3	+0.55
↓	
O_2	+0.82

ELECTRON TRANSFER CHAIN

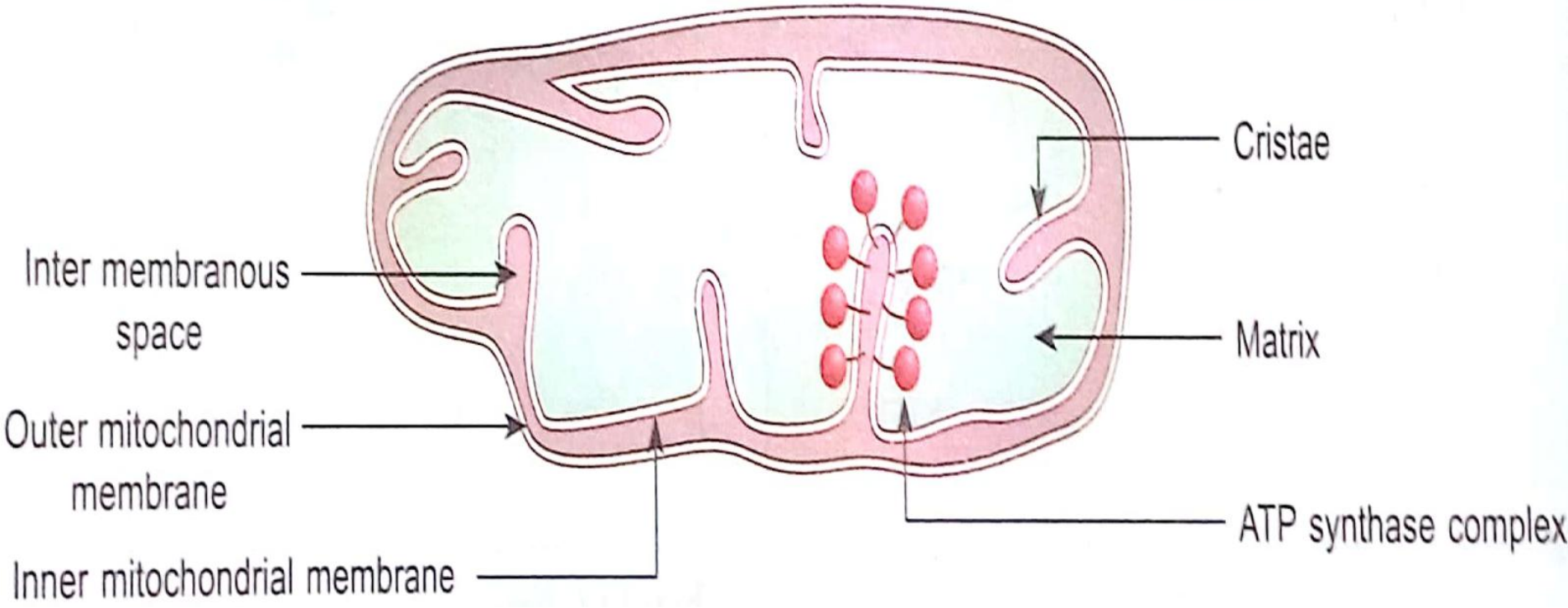
- The flow of electrons occurs through successive dehydrogenase enzymes in mitochondria , together known as the ETC. (the electrons are transferred from higher to lower potential.)

Significance:

- The free energy released during the transport of electrons is utilized for the formation of ATP

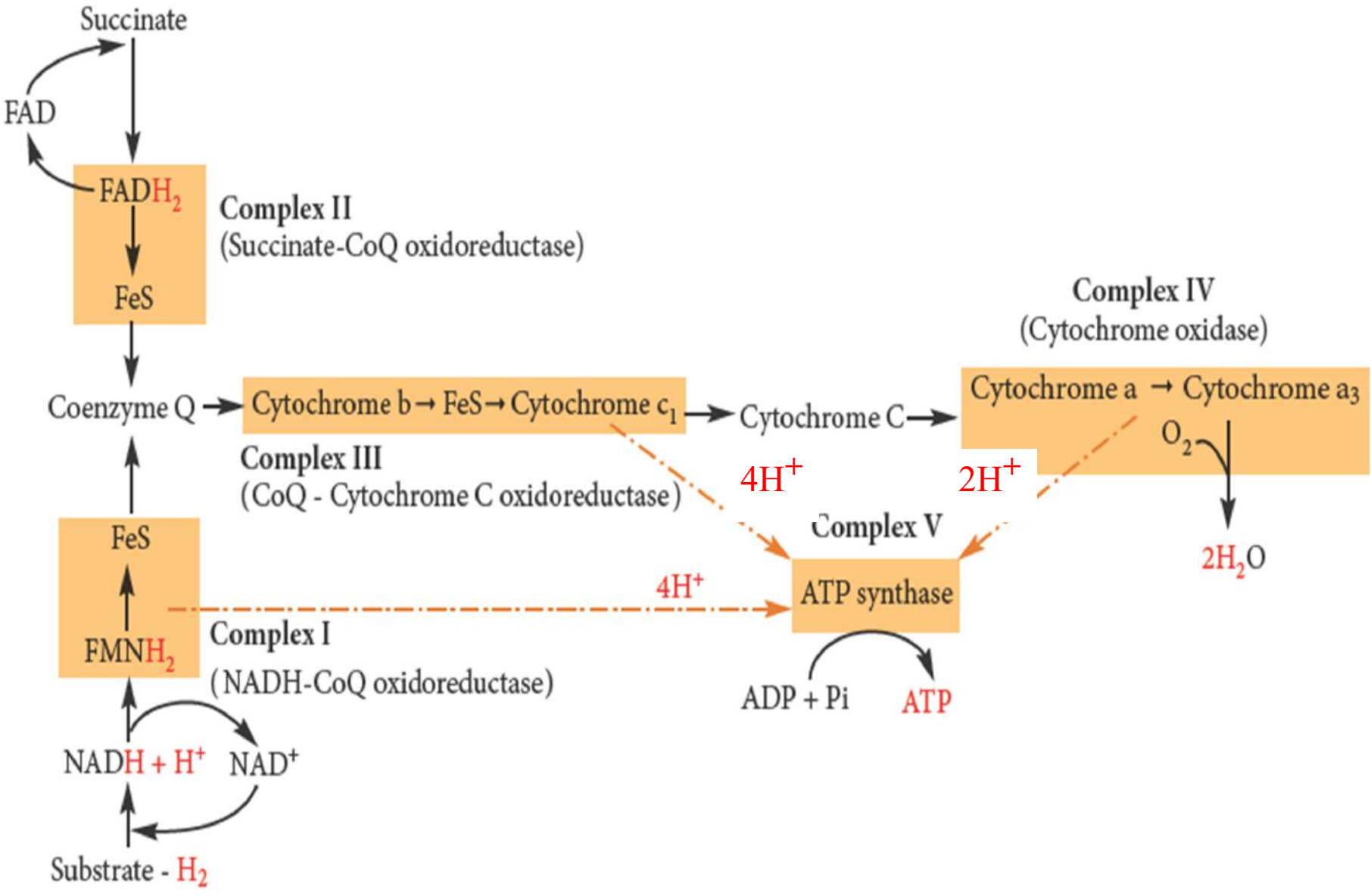
MITOCHONDRIAL ORGANIZATION

- Mitochondria consists of five distinct parts
- Outer membrane, inner membrane, intermembrane space, cristae & matrix

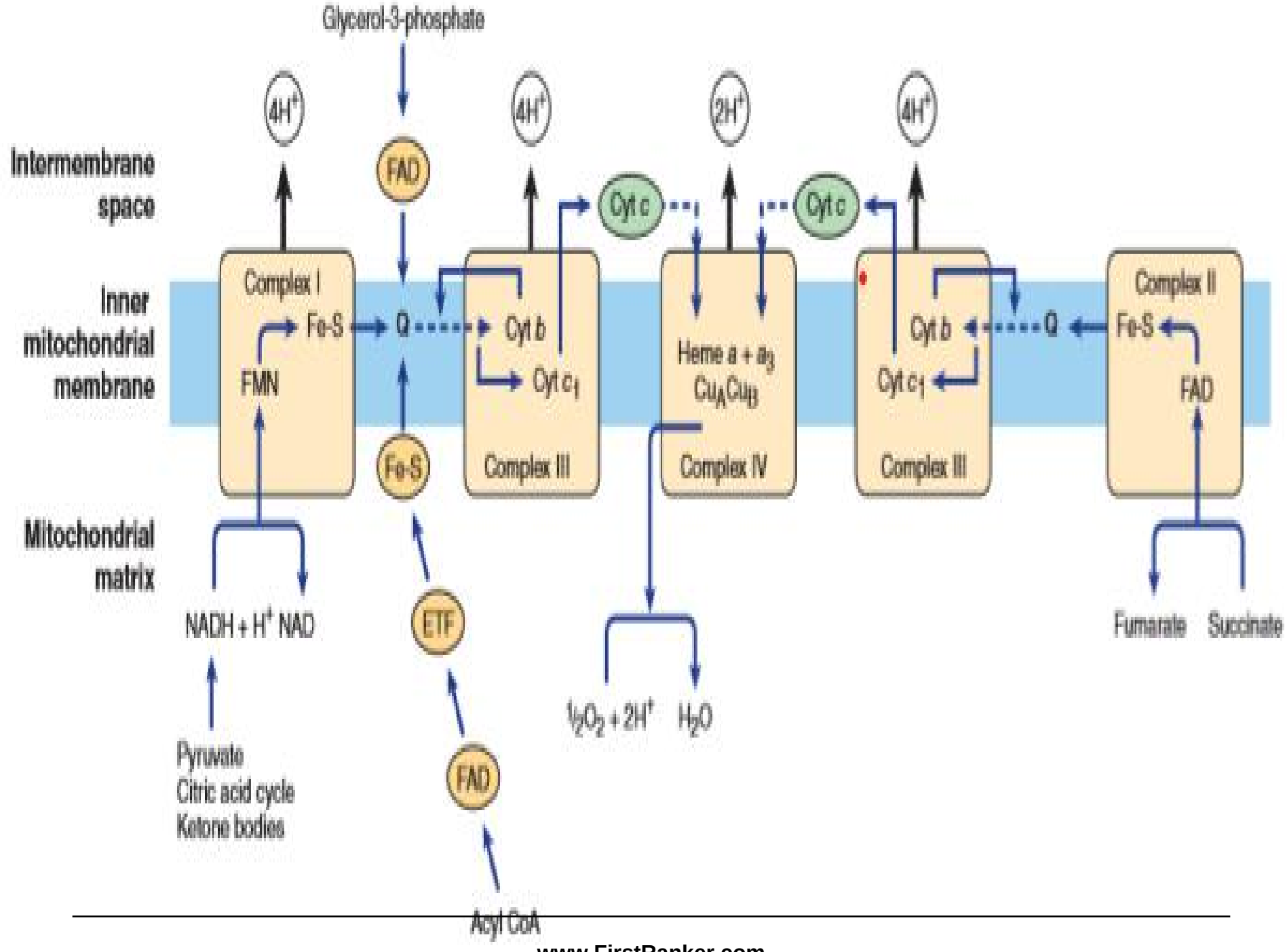


Inner mitochondrial membrane:

- The ETC & ATP synthesizing system are located on inner mitochondrial membrane, which is specialized structure, rich in proteins
- Inner membrane is highly folded to form cristae.
- Surface area of inner mitochondrial membrane is increased due to cristae.
- The inner surface of inner mitochondrial membrane possesses specialized particles, the phosphorylating subunits which are centres for ATP production.



Organisation of electron transport chain and route-map of electron flow through ETC.



ETC consists of four enzymes complexes & two free electron carriers

Complex I: NADH-ubiquinone oxidoreductase

Complex II: Succinate dehydrogenase

Complex III: Ubiquinol cytochrome oxidoreductase

Complex IV: Cytochrome oxidase

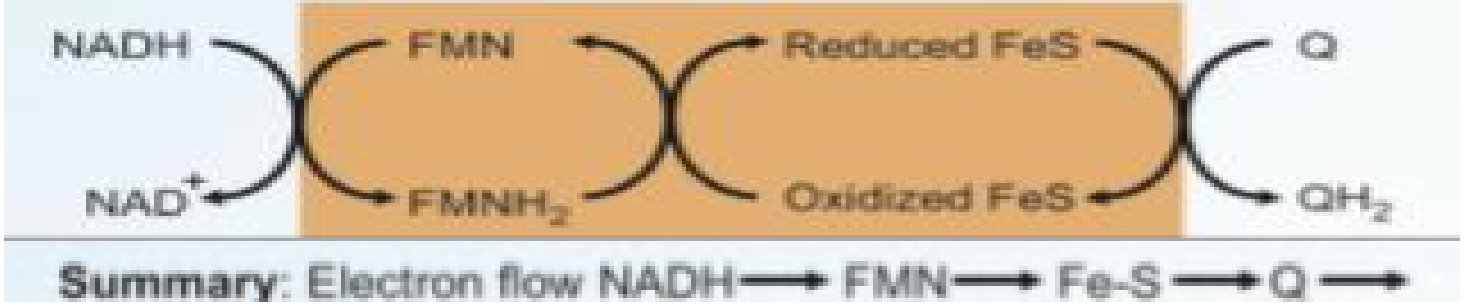
- Two free electron carriers are coenzyme Q & Cytochrome C.
- Complex V: It is ATP synthase.
- The complexes I-IV are carriers of electrons while complex V is responsible for ATP synthesis.

- The enzyme complexes & mobile carriers are collectively involved in the transport of electrons which, ultimately, combine with oxygen to produce water.
- Largest proportion of O_2 supplied to body is utilized by mitochondria for the operation of ETC.

Complex I

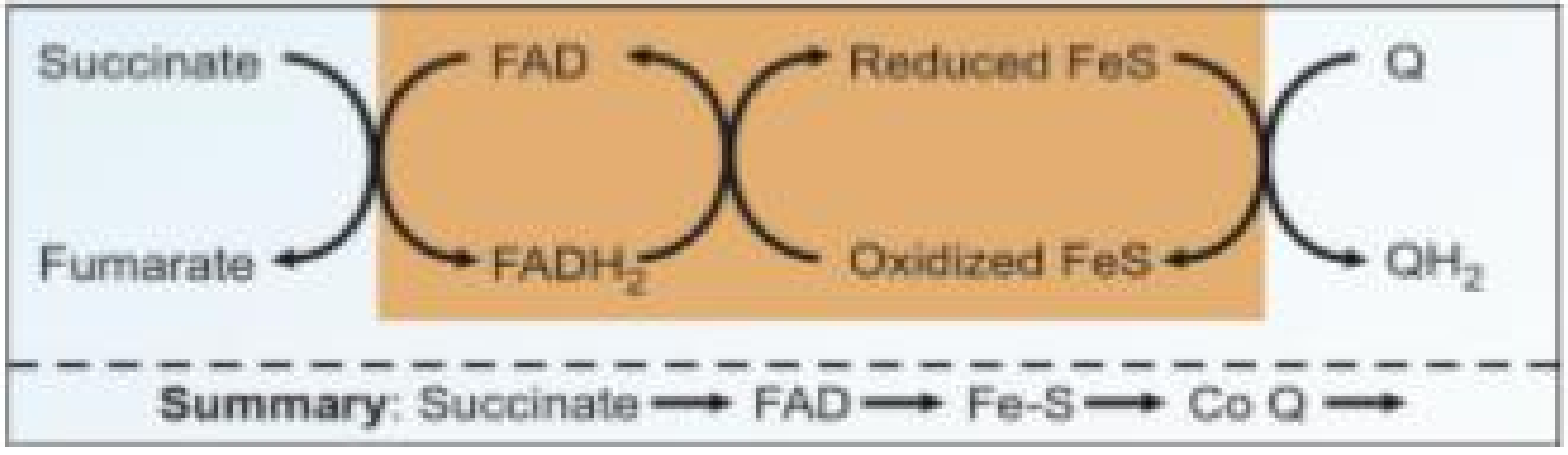
- Of the two coenzymes NAD^+& $NADP^+$, NAD^+ is more actively involved in ETC.
- Tightly bound to the inner membrane
- NAD^+ is reduced to NADH + H^+ by dehydrogenases with the removal of two hydrogen atoms from the substrates, the substrates includes pyruvate, gly-3-P. etc.
- NADPH is more effectively utilized for anabolic reactions - fatty acid synthesis, cholesterol synthesis.

- The enzyme NADH dehydrogenase (NADH coenzyme Q reductase) is a flavoprotein with FMN as the prosthetic group.
- The coenzyme FMN accepts two electrons & a proton to form $FMNH_2$.
- NADH dehydrogenase is a complex enzyme closely associated with non- heme iron proteins or iron-sulfur proteins.
- In this, 4 protons are pumped out from mitochondria.
- $NADH + H^+ + FMN \longrightarrow NAD^+ + FMNH2$



Complex II – Succinate - Co Q- Reductase

- The electrons from FADH2 enter ETC at the level of Co Q.
- Succinate DH is an enzyme found in inner mitochondrial membrane.
- It is also a flavoprotein with FAD as coenzyme.
- The 3 major enzyme systems that transfer their electrons directly to ubiquinone are:
    a. Succinate dehydrogenase
    b. Fatty acyl CoA dehydrogenase
    c. Mitochondrial glycerol phosphate dehydrogenase.

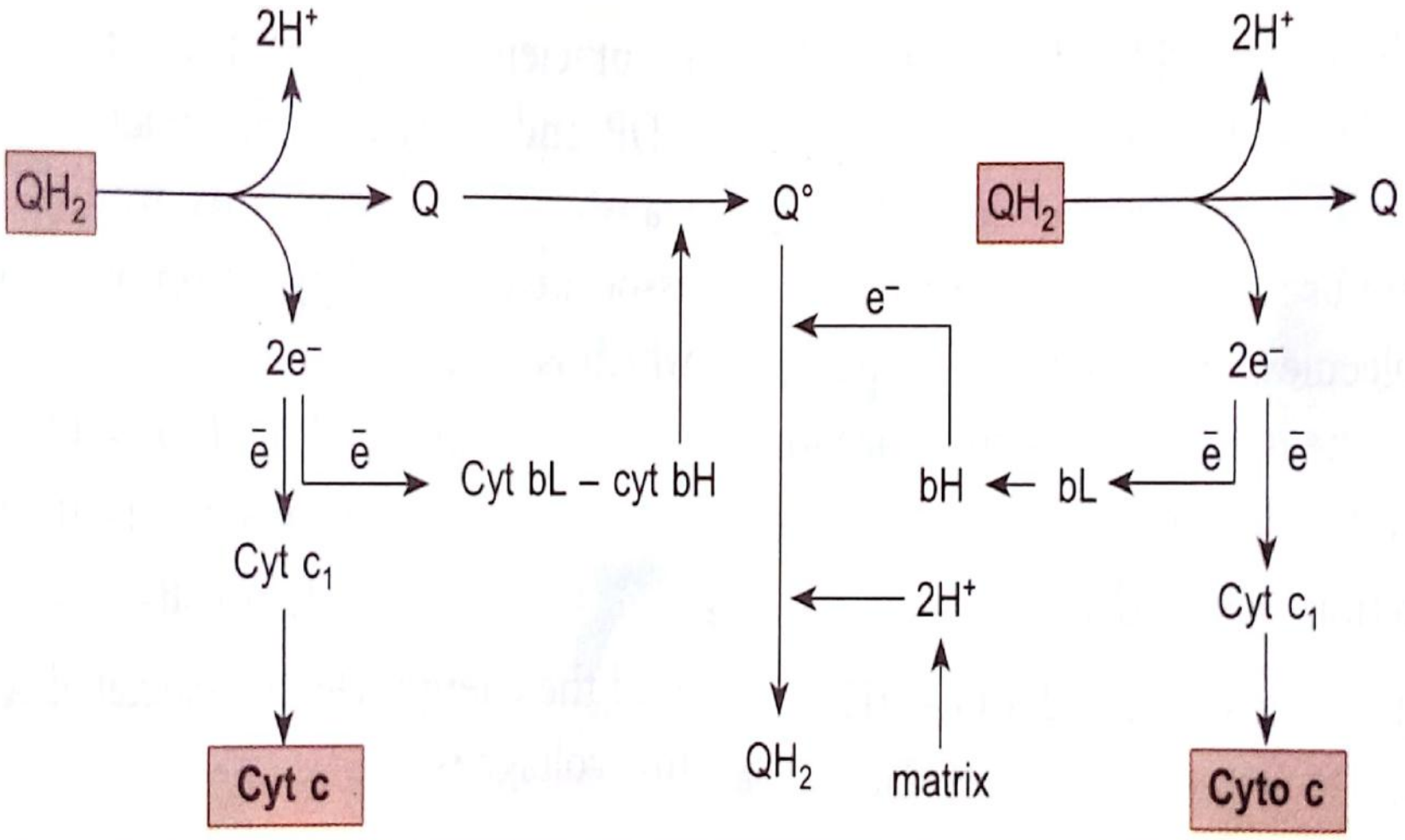


Iron-sulfur centers

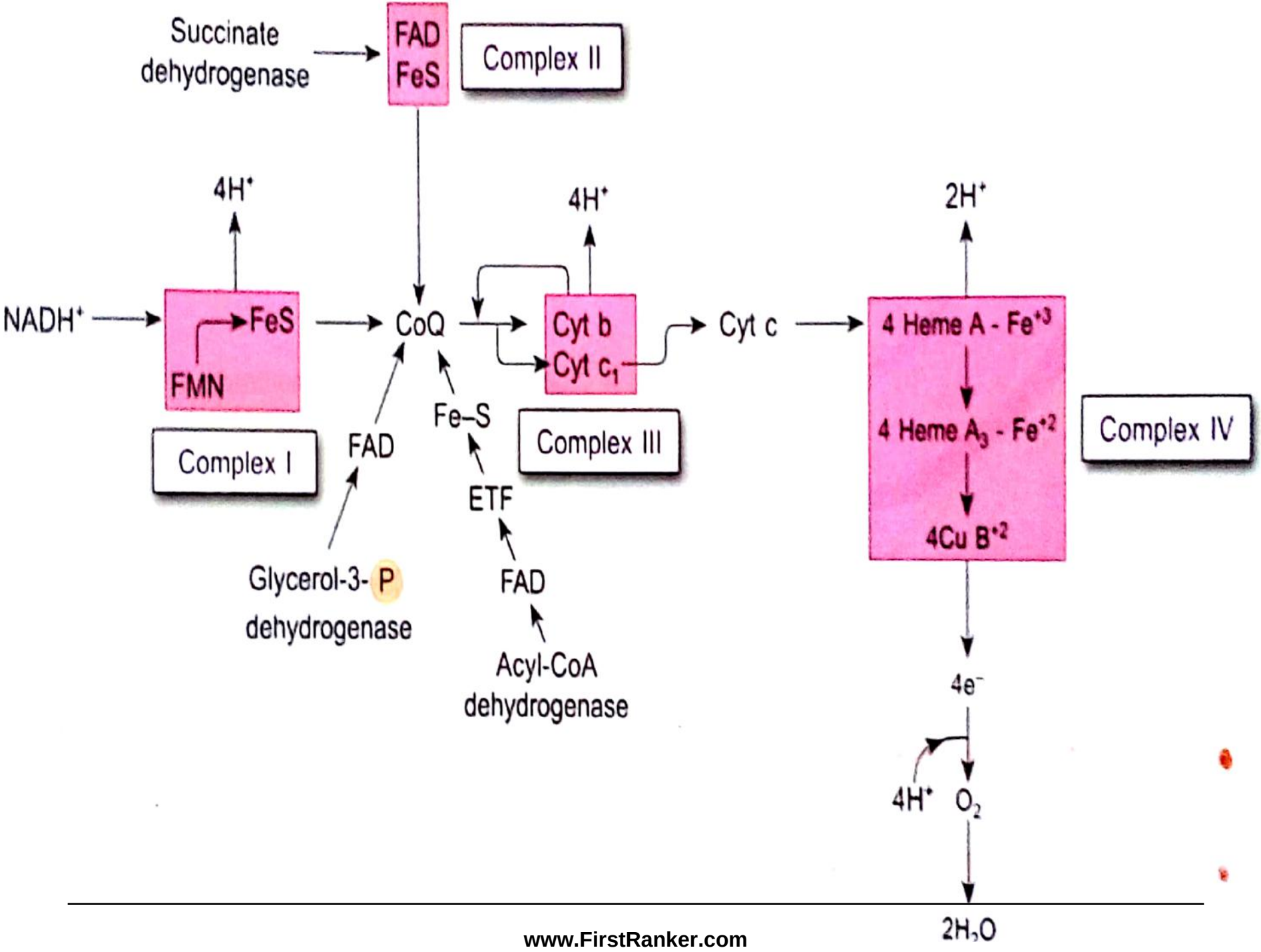
- Iron-sulfur centers (Fe-S) are prosthetic groups containing 1-4 iron atoms
- Iron-sulfur (Fe-S) proteins exist in the oxidized (Fe^{3+}) or reduced (Fe^{2+}) state.
- Iron-sulfur centers transfer only one electron, even if they contain two or more iron atoms
- Fe-S participates in the transfer of electrons from FMN to coenzyme Q.
- Other Fe-S proteins associated with cytochrome b & cytochrome c1 participate in the transport of electrons.

Coenzyme Q

- It is also known as ubiquinone.
- It is a quinone derivative with isoprenoid side chain
- The ubiquinone is reduced successively to semiquinone (QH) & finally to ubiquinol (QH2)
- It accepts a pair of electrons from NADH or FADH2 through complex I or complex II respectively.
- 2 molecules of cytochrome c are reduced.
- The Q cycle facilitates the switching from the 2 electron carrier ubiquinol to the single electron carrier cytochrome c.
- This is a mobile carrier.



The CoQ cycle. Q = CoQ; QH2 = CoQH2



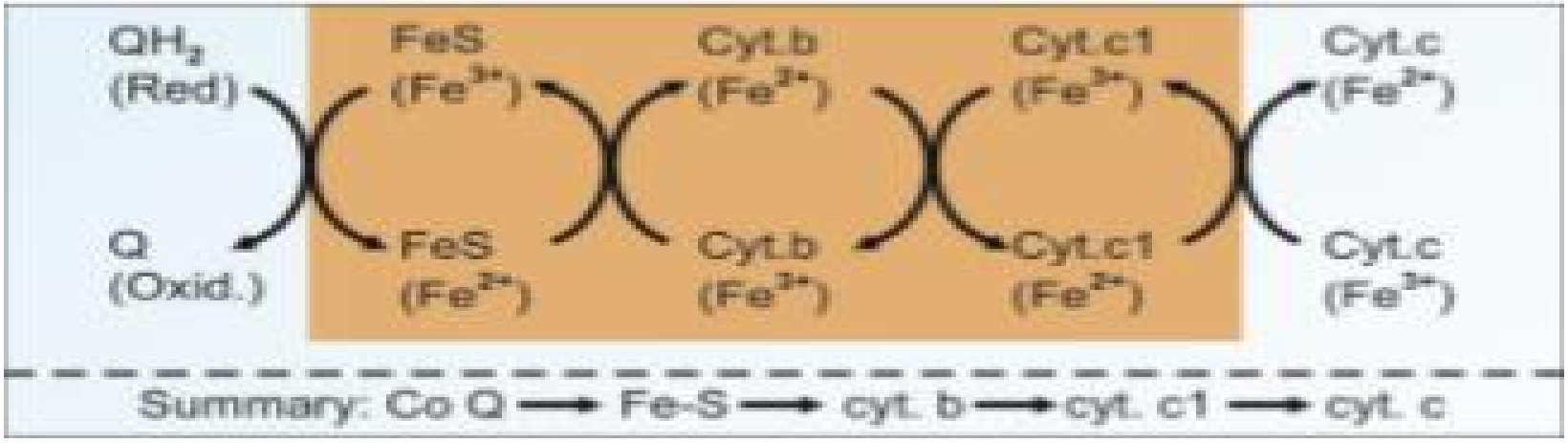
Complex III Cytochrome - Reductase

- This is a cluster of iron-sulphur proteins, cytochrome b & cytochrome c1, both contain heme prosthetic group.
- Consists of a porphyrin ring with iron atom.
- The iron of heme in cytochromes is alternately oxidized (Fe^{3+}) & reduced (Fe^{2+}) which is essential for transport of electrons in the ETC.
- In this, 4 protons are pumped out.
- This complex transfers 2 electrons to cytochrome c from 2 molecules of CoQH2 along with the vectorial movement of $4H^{+}$ from mitochondrial matrix to intermembranous space.

- The property of reversible oxidation reduction of heme iron present in cytochromes allows them to function as effective carriers of electrons in ETC.
- Cytochrome C:

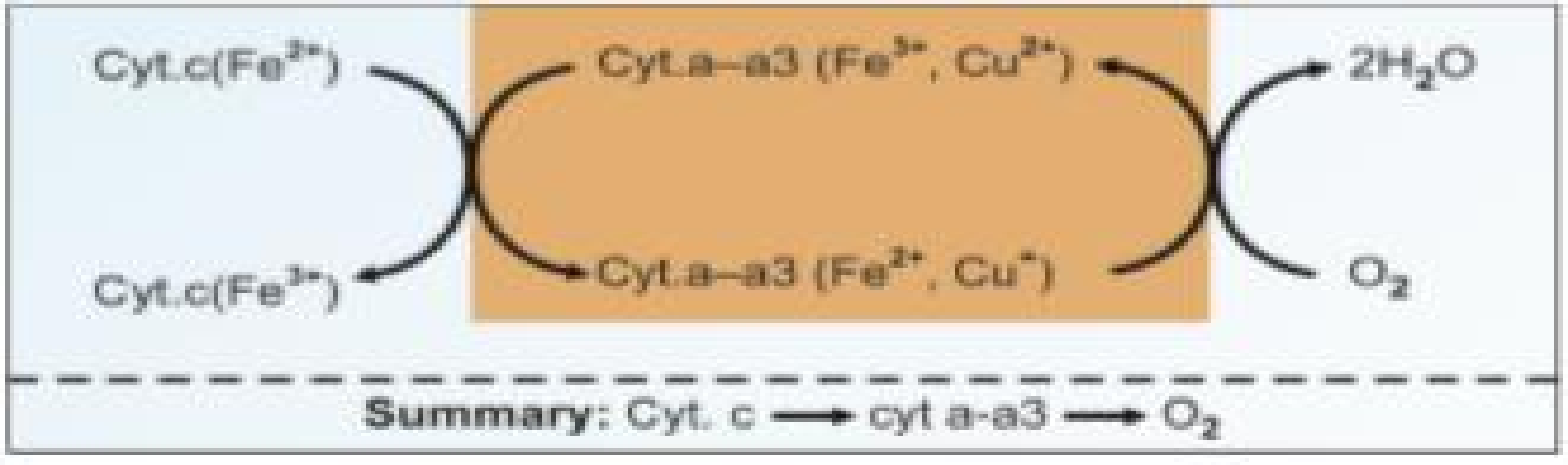
  It is a small protein containing 104 amino acids & a heme group.

  It is a loosely bound to inner mitochondrial membrane & can be easily extracted.



Complex IV Cytochrome - Oxidase

- Contains cytochrome a and cytochrome a3 which is the terminal component of ETC
- Tightly bound to inner mitochondrial membrane.
- Cytochrome oxidase is the only electron carrier, heme iron of which can directly react with molecular oxygen.
- It also contains copper that undergoes oxidation reduction during transport of electrons.
- 2 protons are pumped out.
- In the final stage of ETC, the transported electrons, the free protons & the molecular oxygen combine to produce water



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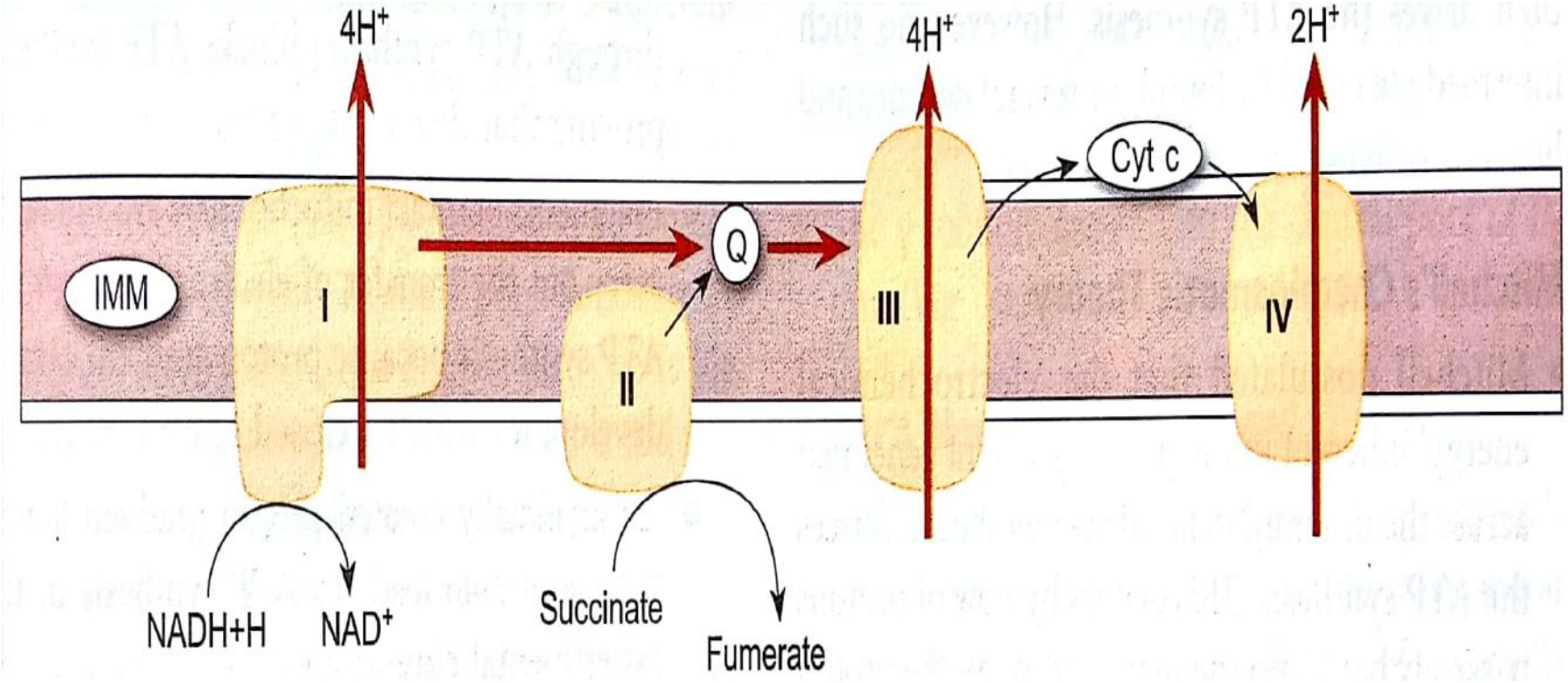


INHIBITORS OF ETC

- The inhibitors bind to one of the components of ETC & block the transport of electrons
- This causes the accumulation of reduced components before the inhibitor blockade step & oxidized components after that step.
- The synthesis of ATP is dependent on ETC.
- All the site-specific inhibitors of ETC also inhibit ATP formation.

Complex I: NADH & coenzyme Q

- Fish poison rotenone, barbiturate drug amytol & antibiotic piercidin A inhibit this.

Complex II:

Carboxin inhibit this site.

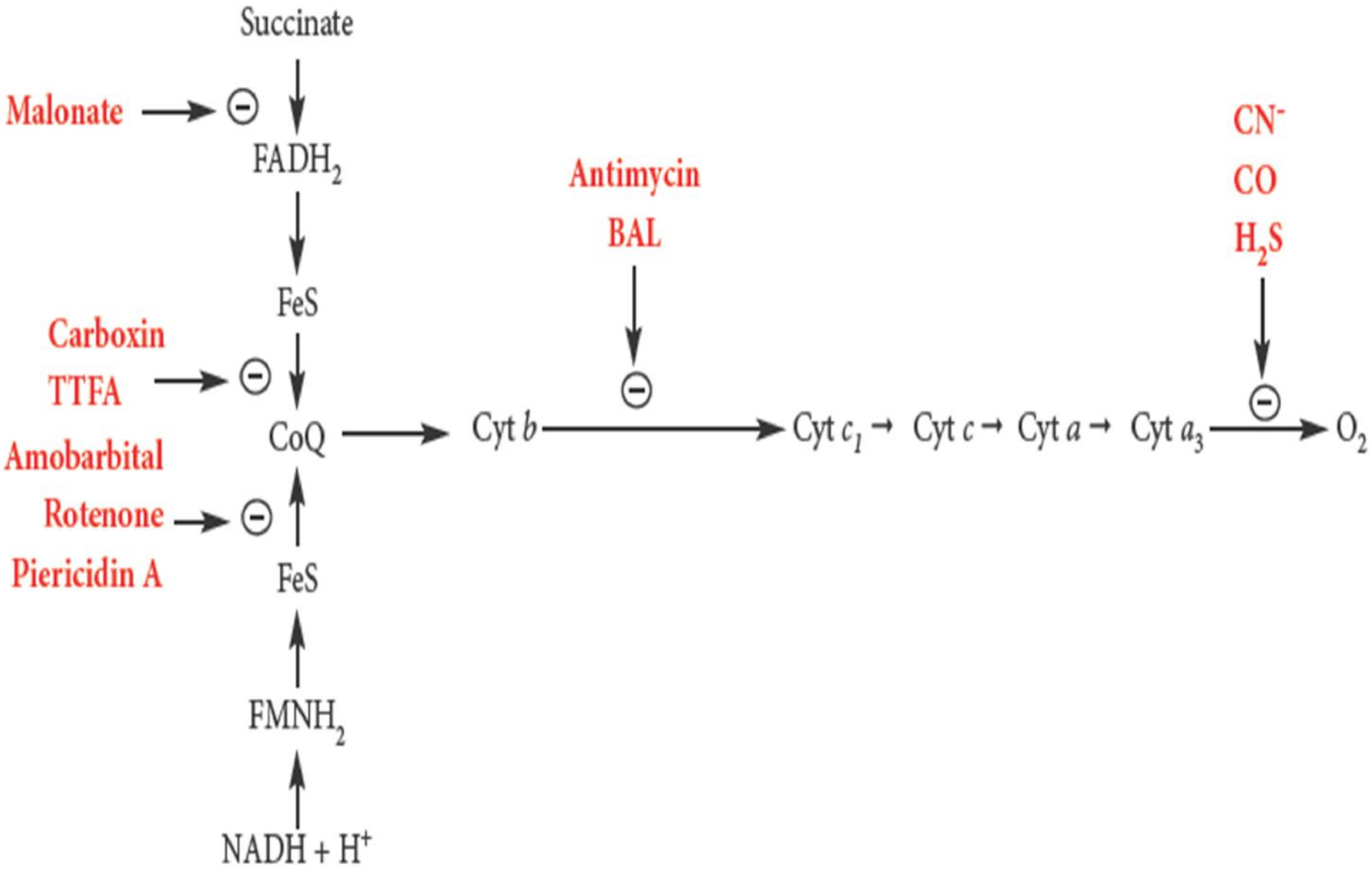
Complex III Between cytochrome b & c1

- Antimycin A –an antibiotic,
- British antilewisite (BAL) –an antidote used against war-gas
- Naphthoquinone are important inhibitors of the site between cytochrome b & c1.

Cytochrome oxidase (Complex IV):

Carbon monoxide, cyanide, hydrogen sulphide & azide

- Effectively inhibit cytochrome while cyanide & azide react with oxidized form of cytochrome.
- Cyanide is most potent inhibitor of ETC
- It binds to Fe3+ of cytochrome oxidase blocking mitochondrial respiration leading to cell death.
- Cyanide poisoning causes death due to tissue asphyxia (mostly of CNS)



Site specific inhibitors of ETC.

Biological Oxidation:

- The transfer of electrons from the reduced co enzymes though the respiratory chain to oxygen is known as biological oxidation.
- Energy released during this process is trapped as ATP.
- This coupling of oxidation with phosphorylation is called as OXIDATIVE PHOSPHORYLATION.
- Complex V of the inner mitochondrial membrane is the site of oxidative phosphorylation.

PHOSPHAGENS

- Phosphagens act as storage forms of high energy phosphate and include creatine phosphate, which occurs in vertebrate skeletal muscle, heart, spermatozoa & brain.

- Arginine phosphate, in invertebrate muscle.

- When ATP is rapidly being utilized as a source of energy for muscular contraction, phosphagens permit its concentrations to be maintained, but when the ATP/ADP ratio is high, their concentration can increase to act as a store of high-energy phosphate.

SITES OF OXIDATIVE PHOSPHORYLATION IN ETC

- There are 3 reactions in the ETC that are exergonic,

  Where the energy change is sufficient to drive the synthesis of ATP from ADP and Pi.

- Site1:

  Oxidation of FMNH2 by coenzyme Q.

- Site2:

  Oxidation of cytochrome b by cytochrome c1

- Site3:

  Cytochrome oxidase.

ENERGETICS OF OXIDATIVE PHOSPHORYLATION

- $\frac{1}{2} O_2 + NADH + H^+ \longrightarrow H_2O + NAD^+$

The redox potential difference between these two redox pairesis 1.14V, which is equivalent to an energy 52 Cal/mol

3 ATP are synthesized in ETC when NADH is oxidized which equals to 21.9 Cal.

(each ATP=7.3 Cal)

The efficiency of energy conservation is calculated as

$$\frac{21.9 \times 100}{52} = 42\%$$

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When NADH is oxidized, about 42% of energy is trapped in the form of 3ATP & remaining is lost as heat.

The heat liberation is not a wasteful process, since it allows ETC to go on continuously to generate ATP.

This heat is necessary to maintain body temperature.

MECHANISM OF OXIDATIVE PHOSPHORYLATION

- Two important hypothesis to explain the process of oxidative phosporylation.
- Namely

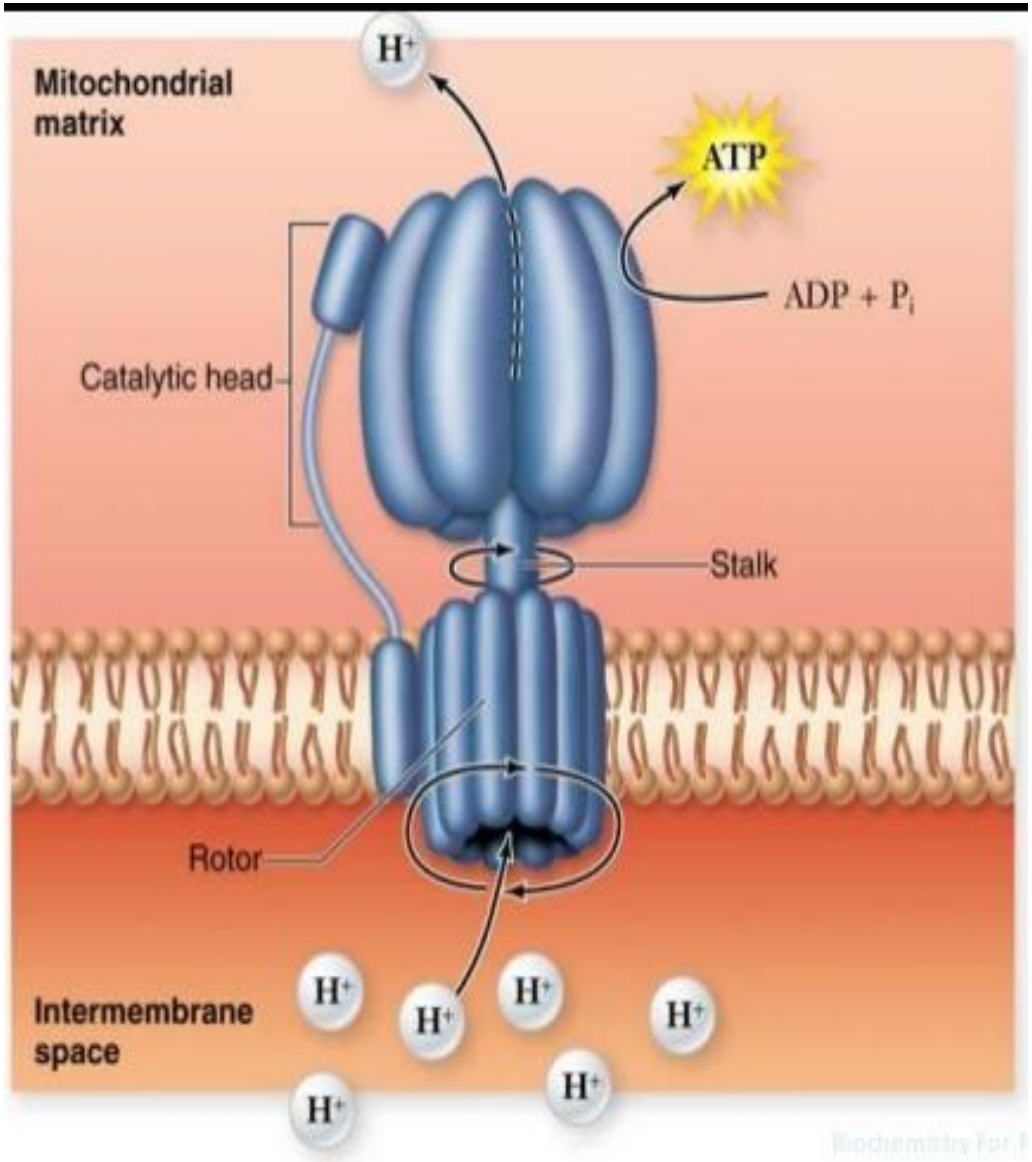
  Chemical coupling &

  Chemiosmotic

Chemical coupling hypothesis:

- This hypothesis was put forth by Edward Slater (1953)
- According to this, during the course of electron transfer in respiratory chain, a series of phosphorylated high-energy intermediates are first produced which are utilized for the synthesis of ATP.
- These reactions are believed to be analogous to the substrate level phosphorylation that occurs in glycolysis or citric acid cycle.
- This hypothesis lacks experimental evidence.

CHEMIOSMOTIC THEORY



Chemiosmotic theory, proposed by Peter Mitchell in 1961, postulates that the two processes are coupled by a proton gradient across the inner mitochondrial membrane so that the proton motive force caused by the electrochemical potential difference (negative on the matrix side) drives the mechanism of ATP synthesis.

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- The transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi.
- PROTON GRADIENT:

  The inner mitochondrial membrane, is impermeable to protons (H+) & hydroxyl ions (OH-).

  The transport of electrons through ETC is coupled with the translocation of protons (H+)across the inner mitochondrial membrane from the matrix to the inter membrane space.

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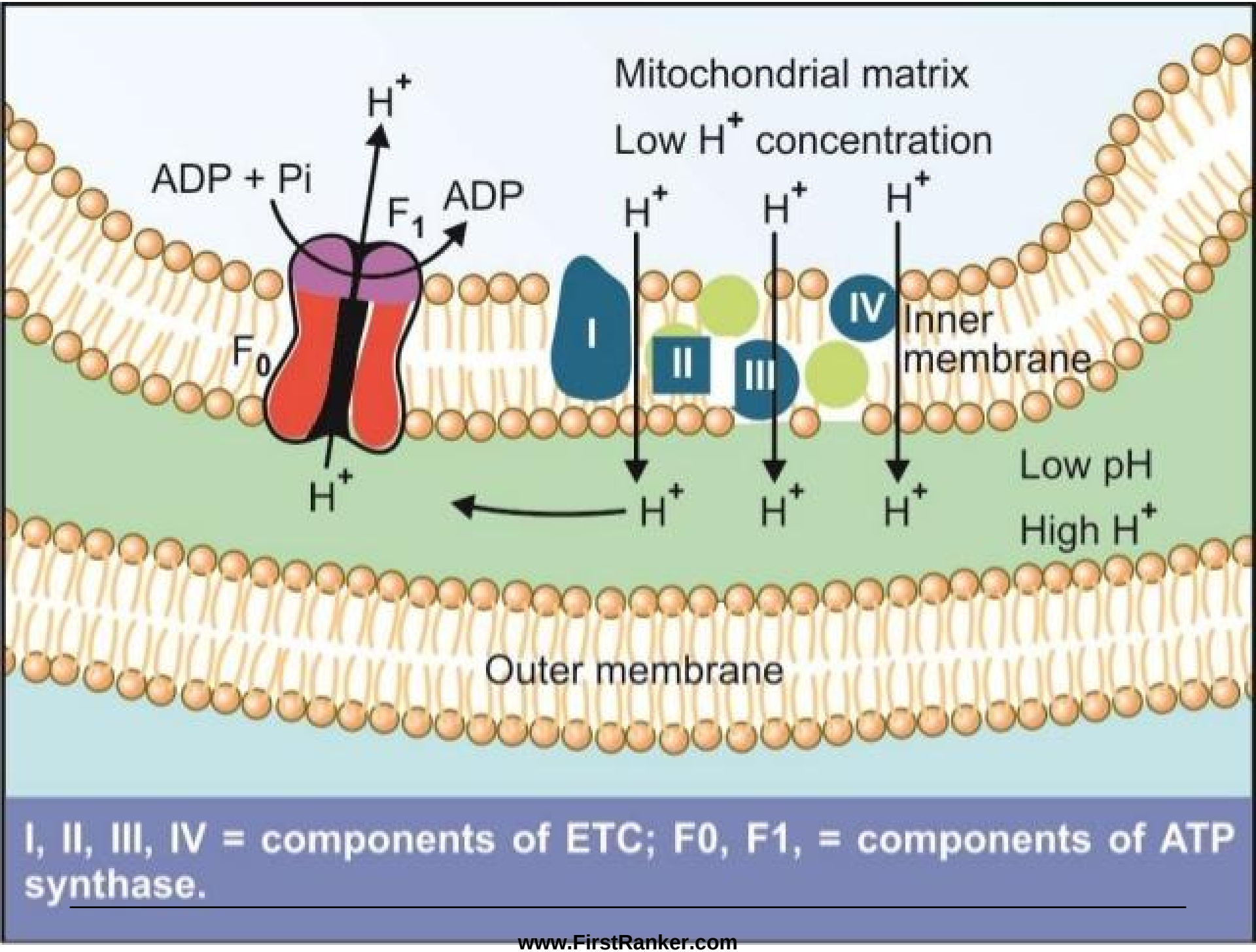
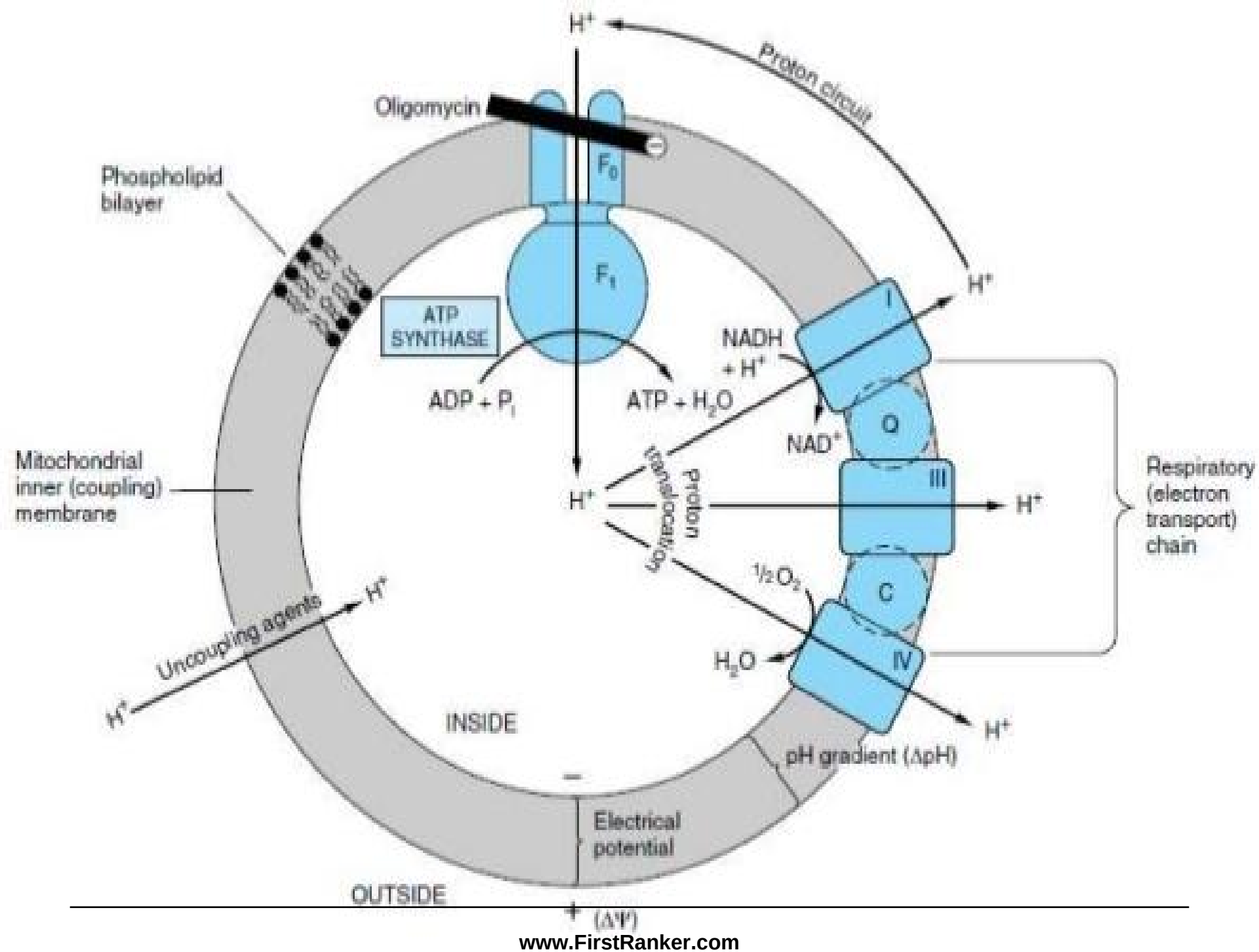
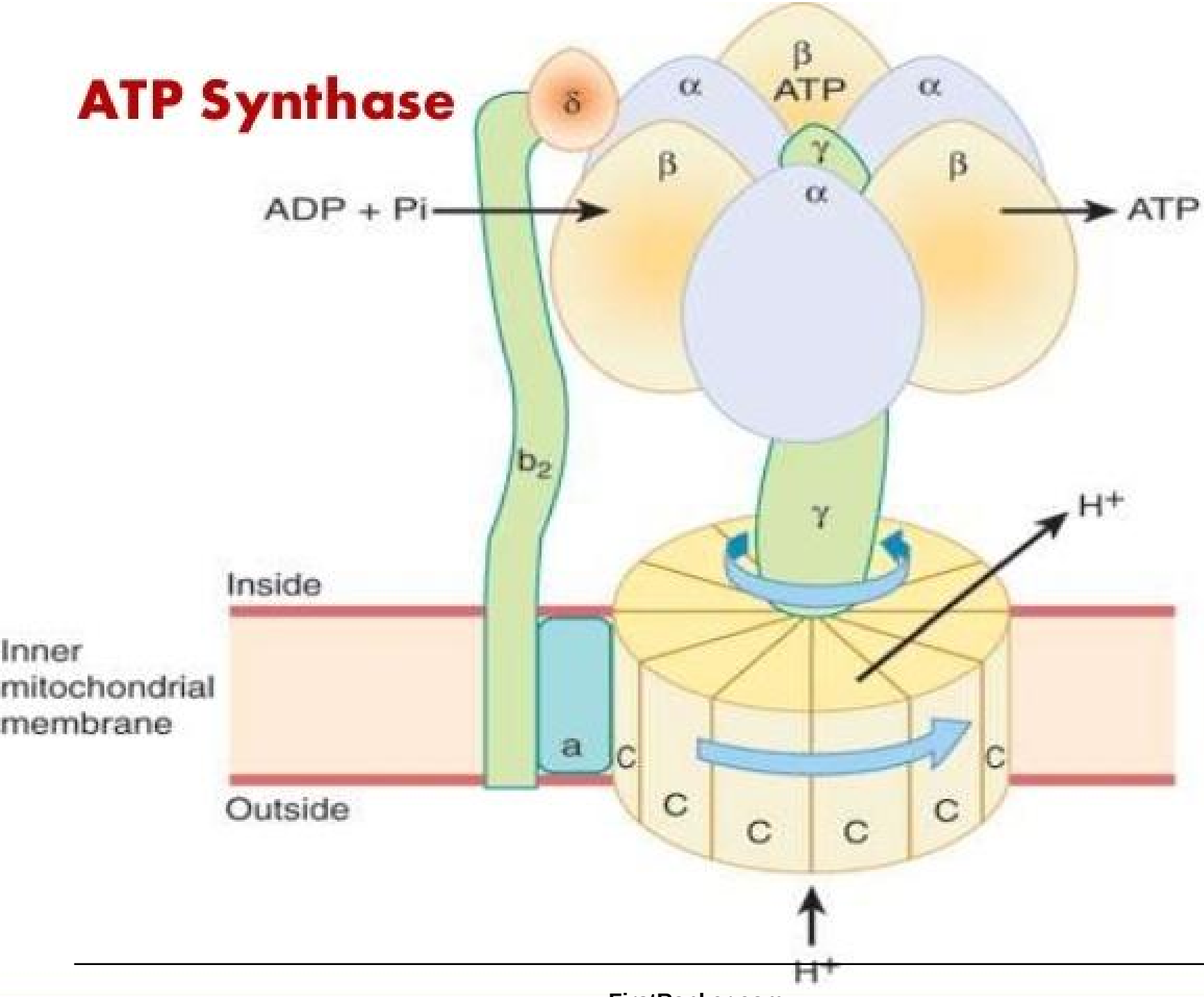
- The pumping of protons results in an electrochemical or proton gradient
- This is due to the accumulation of more H^+ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side.
- The proton gradient developed due to the electron flow in the respiratory chain is sufficient to result in the synthesis of ATP from ADP +Pi.



I, II, III, IV = components of ETC; F0, F1, = components of ATP synthase.



ATP Synthase

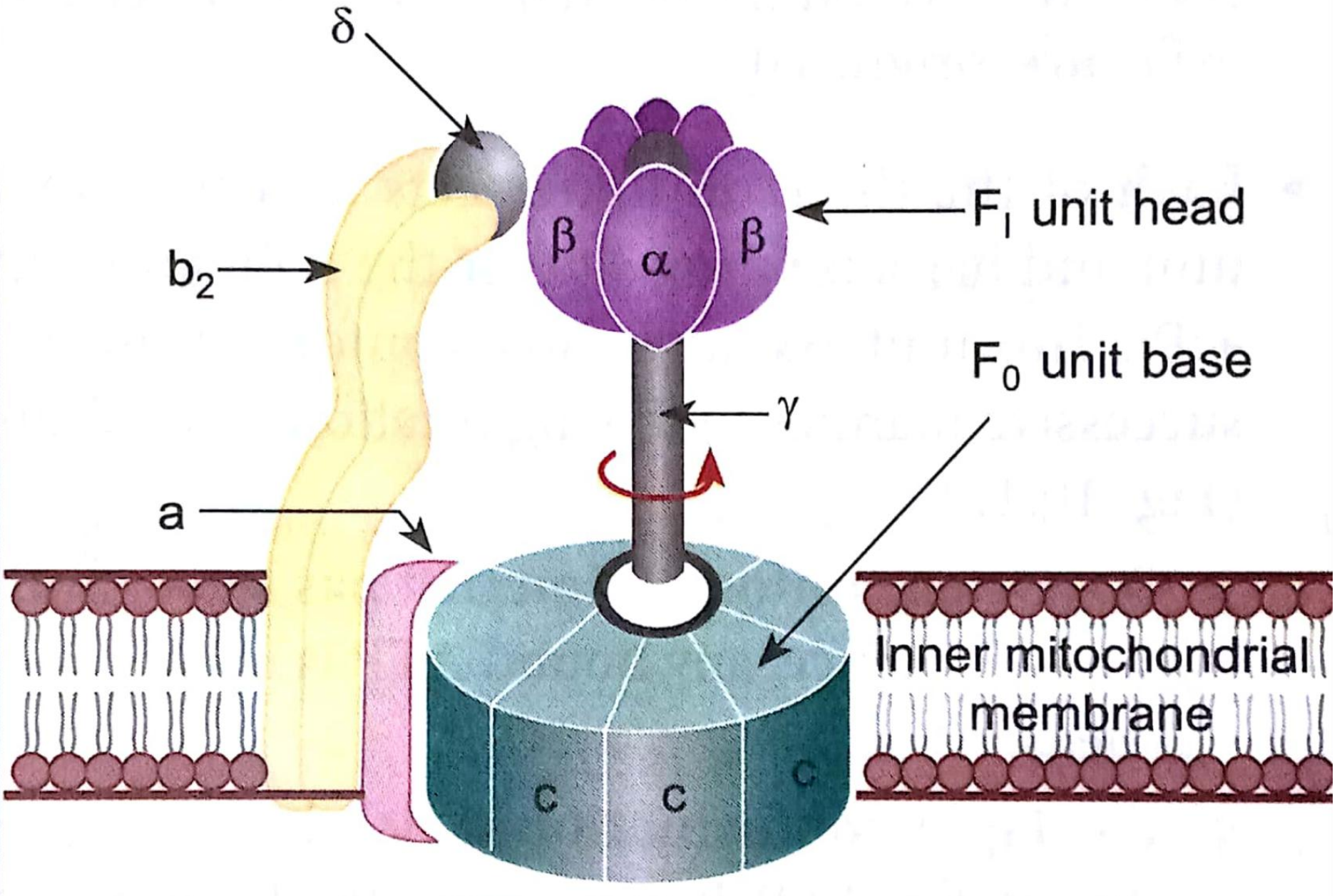


Enzyme systems for ATP synthesis

- ATP synthase, present in the complex V, utilizes the proton gradient for the synthesis of ATP.
- This enzyme is also known as ATPase, since it can hydrolyze ATP to ADP + Pi.
- ATP synthase is a complex enzyme & consists of two functional subunits, namely F1 & Fo.
- Fo unit: O stands for oligomycin,
- Fo inhibited by oligomycin.
- Fo spans inner mitochondrial membrane acting as a proton channel through which protons enter the mitochondria
- Fo unit has 4 polypeptide chains & is connected to F1

.F1 UNIT

- F1 unit: It projects into the matrix.
- F1 has 9 polypeptide chains, (3 alpha, 3 beta, 1 gamma, 1 delta, 1 epsilon)
- The α chains have binding sites for ATP & ADP & **beta chains have catalytic activity**.
- ATP synthesis requires Mg +2 Ions.
- Its structure is comparable with lollipops.
- The protons that accumulate on the intermembrane space re-enter the mitochondrial matrix leading to the synthesis of ATP



ROTOR MOTOR MODEL FOR ATP GENERATION

- Paul Boyer in 1964 proposed that a conformational change in the mitochondrial membrane proteins leads to the synthesis of ATP
- This is now considered as rotary motor/engine driving model or binding change model.
- widely accepted for the generation of ATP.
- The enzyme ATP synthase is Fo & F1 complex
- The Fo sub complex is composed of channel protein ‘C’ subunits to which F1-ATP synthase is attached.

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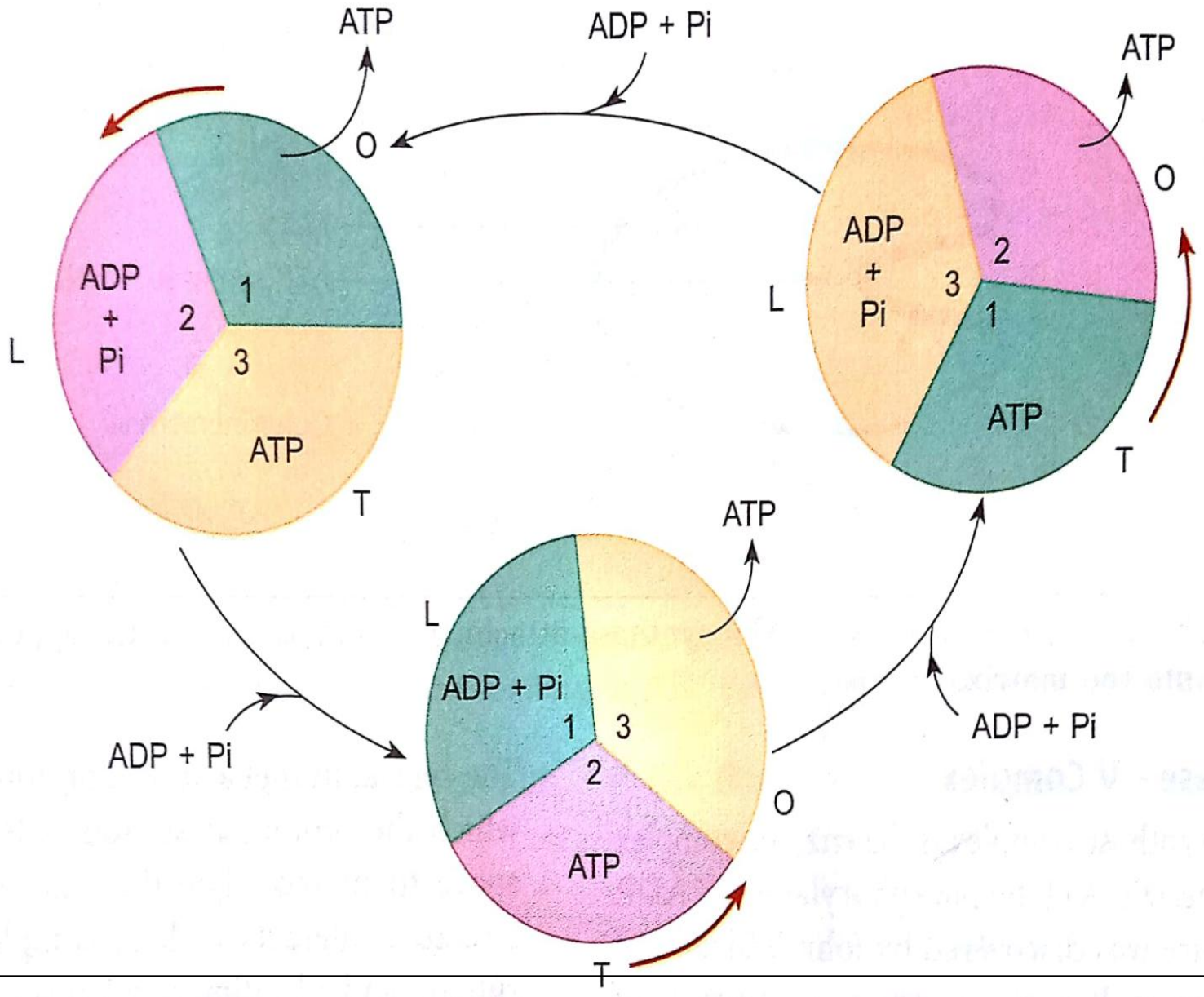
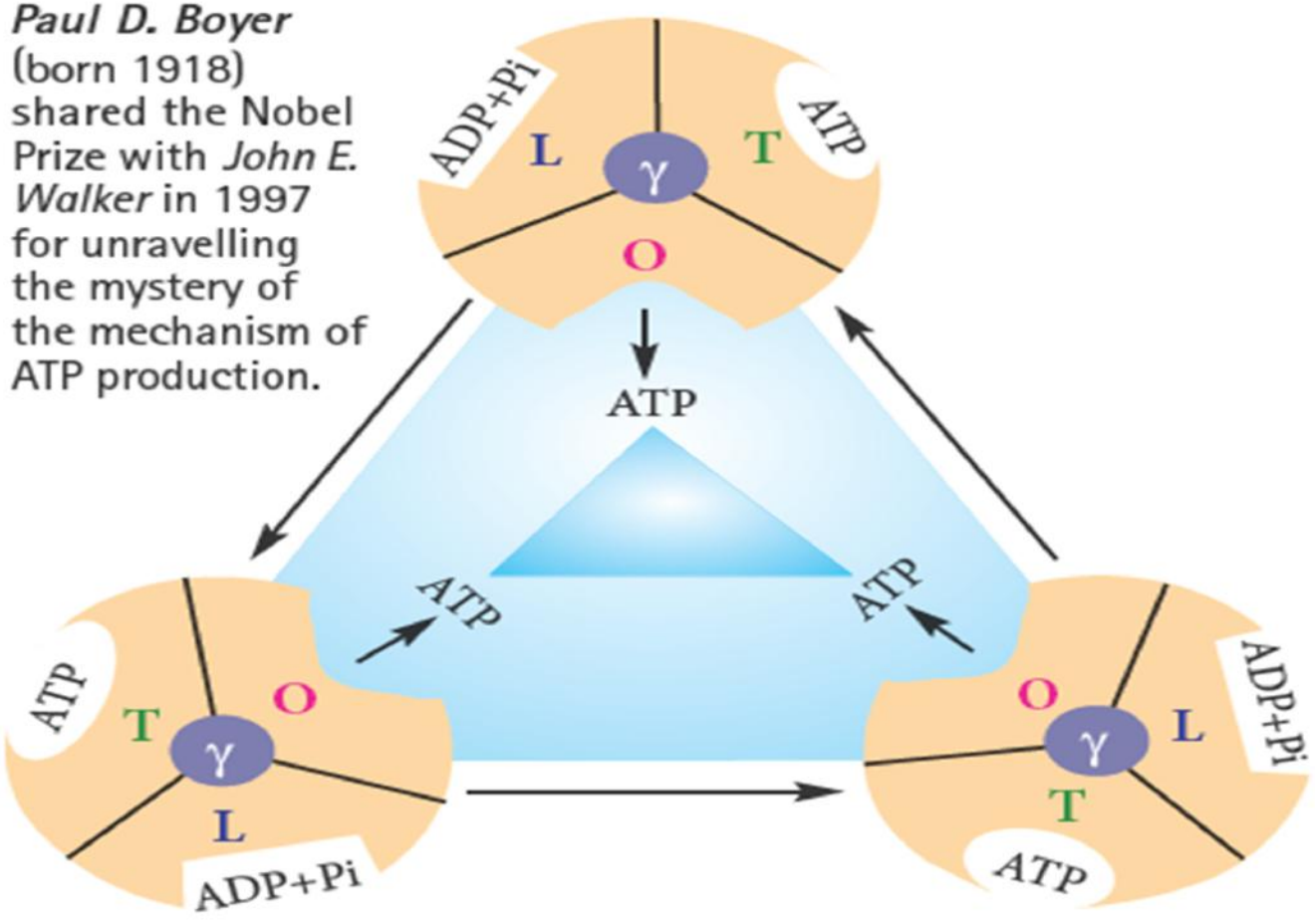
- F1-ATP synthase consists of a central gamma subunit surrounded by alternating alpha & beta subunits (α3 & β3).
- In response to the proton flux, the gamma subunit physically rotates.
- This induces conformational changes in the β3 subunits that finally lead to the release of ATP.
- According to the binding change mechanism, the three β subunits of F1 - ATP synthase adopt different conformations.
- One subunit has Open (O) conformation, the second has loose (L) conformation while the third one has tight (T) conformation.

- By an known mechanism, protons induce the rotation of gamma subunit, which in turn induces conformation changes in β subunits,.
- The substrates ADP & Pi bind to β subunit in L conformation.
- The L site changes to T conformation, & this leads to the synthesis of ATP.
- The O site changes to L conformation which binds to ADP + Pi.
- The T site changes to O conformation & releases ATP.
- This cycle of conformation changes of βsubunits is repeated.
- Three ATP are generated for each revolution.



Paul D. Boyer (born 1918) shared the Nobel Prize with *John E. Walker* in 1997 for unravelling the mystery of the mechanism of ATP production.



BOYER'S BINDING CHANGE MODEL FOR ATP SYNTHESIS BY ATP SYNTHASE.

INHIBITORS OF OXIDATIVE PHOSPHORYLATION

- The mitochondrial transport of electrons is tightly coupled with oxidative phosphorylation.
- Oxidation & phosphorylation proceed simultaneously.
- There are certain compounds that can uncouple (or delink) the electron transport from oxidative phosphorylation.
- Such compounds are known as **uncouplers,**
- Causes increase in the permeability of inner mitochondrial membrane to protons (H+).
- The result is that ATP synthesis does not occur

.

- The energy linked with the transport of electrons is dissipated as HEAT.
- The uncouplers allow (often at accelerated rate) oxidation of substrates (via NADH or FADH2) without ATP formation
- Examples:
- 2,4-dinitrophenol (DNP):

  It is small lipophilic molecule.

  DNP is a proton – carrier & easily diffuse through the inner mitochondrial membrane.

  Others –dinitrocressol, pentachlorophenol, trifluorocarbonylcyanide, phenylhydrazone.

PHYSIOLOGICAL UNCOUPLERS

- Certain physiological substances which act as uncouplers at higher concentration.
- These are thermogenin, thyroxine and long chain fatty acids & unconjugated bilirubin

Significance of uncoupling:

The maintenance of body temperature is particularly important in hairless animals, hibernating animals & the animals adopted to cold

- These animals possess a specialized tissue called brown adipose tissue in the upper back & neck portions.

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- The mitochondria of brown adipose tissue are rich in electron carriers & are specialized to carry out an oxidation uncoupled from phosphorylation.
- This causes liberation of heat when fat is oxidized in the brown adipose tissue.
- The presence of brown adipose tissue in certain individuals is believed to protect them from becoming obese.
- **Thermogenin** is a natural uncoupler located in the inner mitochondrial membrane of brown adipose tissue
- It acts like an uncoupler, blocks the formation of ATP, & liberates heat.

IONOPHORES

- Ionophores: These are lipophilic substances that are lipid soluble and increases the permeability of inner motochondrial membrane to ions and thereby destroy the proton gradient leading to inhibition of ATP synthesis.
- By either forming channel or
- By binding an ion and then diffusing into membrane.
- Valinomycin (binds with K^+) & Nigercin also act as uncouplers

INHIBITORS OF OXIDATIVE PHOSPHORYLATION

- Oligomycin: This antibiotic binds with enzyme ATP synthase & blocks the proton(H+) channels.
- Thus it prevents the translocation (re-entry) of protons into the mitochondrial matrix and prevent ATP synthesis
- Atractyloside: It is a plant toxin & inhibits oxidative phosphorylation.
- It blocks the adequate supply of ADP by inhibiting ADP/ATP transporter

INHERITED DISORDER OF OXIDATIVE PHOSPHORYLATION

- 100 polypeptides are required for oxidative phosphorylation.
- Of these, 13 are coded by mitochondrial DNA & synthesized in the mitochondria, while the rest are produced in the cytosol (coded by nuclear DNA) & transported.
- mtDNA is maternally inherited since mitochondria from the sperm do not enter the fertilized ovum.

- Mitochondrial DNA is 10 times more susceptible to mutations than nuclear DNA.
- mtDNA mutations are commonly seen in tissues with high rate of oxidative phosphorylation (e.g. CNS, skeletal & heart muscle, liver).
- **Diseases:**

  Lethal infantile mitochondrial opthalmoplegia

  Leber's hereditary optic neuropathy (LHON)

  Myoclonic epilepsy

  Mitochondrial encephalopathy lactic acidosis stroke like episodes (MELAS)

Oxidative Phosphorylation Diseases

Syndrome	Feature
Laber's heriditory Optic neuropathy (LHON)	Complex I defect, Blindness, cardiac conduction defects.
Myoclonic epilepsy ragged red fiber disease (MERRF)	Myodonic epilepsy, myopathy, dementia.
Mitochondrial encephalopathy lactic acidosis stroke like episodes (MELAS)	Complex I defect; Lactic acidosis, stroke, myopathy, dementia.
Leigh's syndrome	Complex I defect, Movement disorders.

John E. Walker (born 1941)
The biochemist who determined the structure of ATP synthase, *the smallest molecular motor on earth.*

STRUCTUTRE OF ATP SYNTHASE.