Metabolism 2

Electron transport chain

ETC present in mitochondria, and it use redox reaction to release the energy in nutrients

Oxidation reduction reactions (Redox reactions)

- Commonly the oxidation reactions are accompanied by reduction reactions and they are called redox reaction.
- Redox reactions are accompanied by energy liberation, necessary for the cells.
- In the redox reaction. H_2 is oxidized while, O_2 is reduced, and if occurs it will be accompanied by a massive energy explosion.

$$2H_2 + O_2 \longrightarrow 2H_2O + \text{energy}$$

Terminal reaction in ETC between hydrogen and oxygen to produce water and energy accompanied by liberation of massive amount of energy in multistep to avoid the dangerous effect

- Instead of massive energy is liberated, hydrogen must be transferred to oxygen in gradual steps. Thus, small fractions of energy are liberated and stored for further use

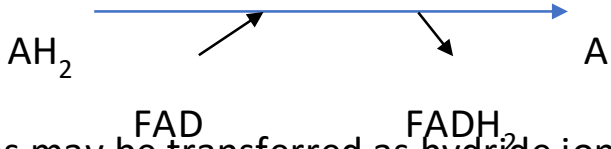
Where there is oxidation reaction there will be reduction reaction to prevent free H+ ions conc, to increase in the cell and thus disturb the enzymatic activity in the cells

Electrons are transferred from one molecule to another in one of four different ways

- They may be transferred directly as electrons as the Fe 2+ (ferrous) / Fe 3+(ferric) redox pair.

If electrons leaves ferrous it will be oxidized to ferric form , if it receives electron, it will be reduced

- Electrons may be transferred as incorporated in hydrogen atoms as in case of FAD



Oxidation of A and reduction of FAD

- Electrons may be transferred as hydride ions (H+)

- Electron may be transferred as a direct combination of an organic reductant with oxygen (to produce alcohol)

$$R\text{-}CH_2 + \tfrac{1}{2}\ O_2 \longrightarrow R\text{-}CH_2OH$$

Redox Potential (electron affinity)

- Oxygen has the highest electron affinity i.e. highest redox potential.
- Hydrogen has the lowest electron affinity i.e. lowest redox potential.

The molecule that has the highest electron affinity located at the end of the chain (oxygen) and the molecule at the beginning of chain has the lowest electron affinity (hydrogen)

Redox chain:

- It is a chain of different compounds of increasing redox potentials between hydrogen and oxygen.

A → B → C → D…..

- Each components of redox chain has a redox potential higher than hydrogen and lower than oxygen.

- During hydrogen (H^+ and electron) transfer through different components of the redox chain, energy is liberated in steps and in small amounts to be utilized.

If there is 4 components , the first one has the least affinity to electrons and the second has higher .. , so the H+ will jump from the first to the second , and then to component number 3 , so it will depend on the organization of different components in ETC

Respiratory chain (ETC)

- It is a system of electron carriers located in the inner-mitochondrial membrane, oxidizes the reduced cofactors by transferring electrons in a series of steps to O2 (the terminal electron acceptor).
- Free energy released by these oxidation reactions is used to drive the synthesis of ATP.
- Each component of the chain can accept electrons from the preceding carrier and transfer them to the following one.
- A variety of substances (carbohydrates, fatty acids and amino acids) can use respiratory chain as a final pathway as they give electrons to the oxidized NAD^+ and FAD^+ to form the energy rich reduced coenzymes $NADH+H^+$, $FADH_2$.

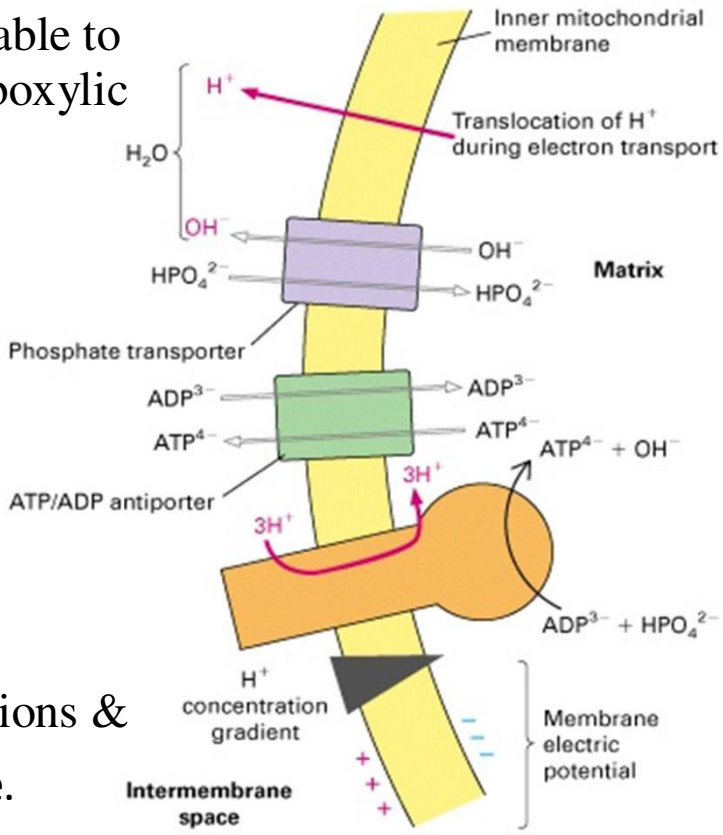
NADH+H+ and FADH2 transfer the electrons to first or second components and then followed by oxidation- reduction reactions and liberation of energy

- $NADH+H^+$ and $FADH_2$ give hydrogen and a pair of electrons to electron carriers collectively, called the respiratory chain components.

In mitochondria there is inner and outer membrane and the matrix inside the inner membrane , that has enzymes of multiple metabolic pathway which will produce the reduction equivalence to be used in ETC

- Outer mitochondrial membrane is permeable to most ions as O_2, CO_2, NH_3 and monocarboxylic acids.
- Di- and tricarboxylic acids need special transporters.
- ATP and ADP need special transporter to allow ADP in and ATP out of mitochondria.

(to prevent accumulation thus preventing feedback inhibition of ATP because it should be in continuous state , so there should be transporter and enzyme (ATP/ADP translocase enzyme) to get ATP out mitochondria and ADP in)



- Inner membrane is impermeable to most ions & molecules: H^+, Na^+, K^+, ATP, ADP, pyruvate.
- Matrix contains enzymes for oxidation of pyruvate., A.A.s , F.A.s and TCA.

Main sources for producing NADH+H+ and FADH2 to join the ETC

Organization of respiratory chain

- The inner mitochondrial membrane contains four enzymatic complexes (I, II, III, IV) and complex V catalyzes ATP synthesis, arranged in order of increasing electronegativity (weakest to strongest)
- Each complex accepts or donates electrons to relatively mobile electron carriers as coenzyme Q and cytochrome C.

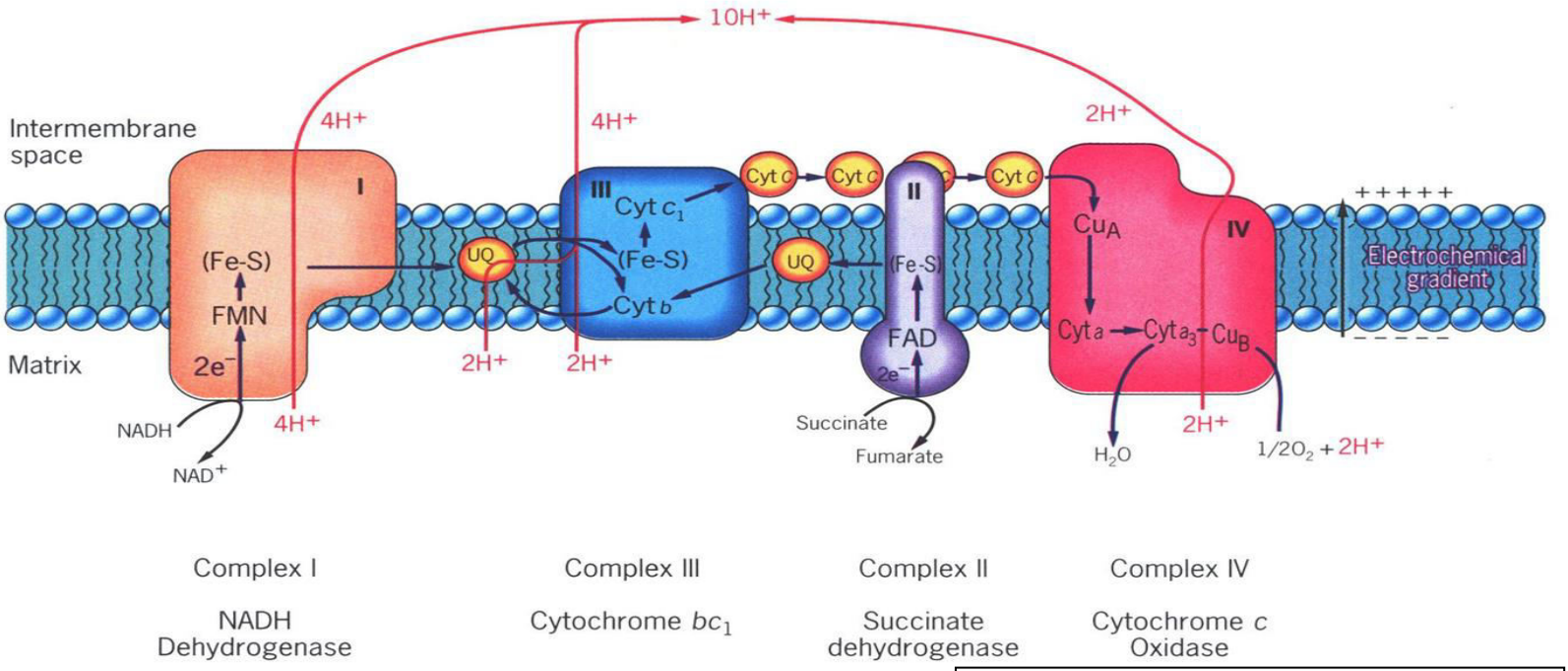
Once electron inside the chain it will move from complex 1 to 3 by coenzyme Q and then to 4 by cytochrome c .

- Oxidative phosphorylation starts by entry of electrons into the respiratory chain.

- Most of these electrons arise by the action of dehydrogenases that collect electrons from catabolic

pathways and pass them to the electron acceptors NAD and FAD.

- As electrons are passed down the respiratory chain, they lose much of their free energy.



The oxygen is the final electron acceptor.

- Part of this energy can be captured and stored by the production of ATP from ADP and inorganic phosphate (Pi).
- The process is called oxidative phosphorylation.

-The remainder of the free energy not trapped as ATP is released as heat.

Components of the respiratory chain

- With the exception of coenzyme Q (vit. K derivative / fatty in nature) , all members of this chain are proteins.
- All are embedded in the inner mitochondrial membrane. /except complex 2 is embedded in the matrix .

** each complex has enzyme + coenzyme and prosthetic group

Complex I:

- Contains an enzyme called NADH dehydrogenase.

- Its coenzyme is FMN – flavin mono nucleotide - (can accept two hydrogen atoms to become $FMNH_2$)

- It contains several iron and sulfur atoms (iron sulfur protein) -> prosthetic group . (almost 9)

- NAD+ is reduced to $NADH+H^+$ by dehydrogenases that remove hydrogen atoms from their substrates.

There will be substrate with electron has lower electron affinity than complex one came from different reactions and give the electron to complex 1

Complex II: (succinate dehydrogenase complex)

- The entry point of $FADH_2$ (its coenzyme is FAD).
- Contains an enzyme called: flavo - protein dehydrogenase e.g. succinate dehydrogenase of TCA and acyl CoA dehydrogenase of β oxidation of fatty acids.
- It contains iron and sulfur atoms (iron sulfur protein).

H+ enter the ETC to complex 1 **will not** jump to complex 2 /

صح انه نفس ال Q بنقل الالكترون من ال both complexes بس ب طرق مختلفة بينهم

Complex III:

- It is cytochrome reductase complex, or cytochrome bc1 complex"
- Transfers electron from QH_2 to cytochrome C.
- Contains an enzyme cytochrome b.

Complex IV: (cytochrome oxidase)

- This complex contains cytochrome a, a3 and 2 copper atoms.
- Complex IV catalyzes the transfer of electrons from reduced cytochrome C to molecular oxygen.
- The copper atoms are crucial for such a transfer.

In complex 3 and 4
Coenzyme -> iron بدخل في تركيب الانزايم
Prosthetic group -> iron sulfur protein

The most collecting point in ETC is ubiquinone.

TWO mobile component

Ubiquinone "Coenzyme Q"

- It is a lipid soluble vitamin K derivative
- Coenzyme Q can accept hydrogen ions both from $FMNH_2$, produced by NADH dehydrogenase (complex I) and from $FADH_2$ which is produced by (complex II).
- It is freely diffusible between the lipid bilayer of inner mitochondrial membrane.

Cytochromes (hem-proteins)

- Cytochromes are proteins that contain an iron-containing heme group. This iron oscillates between ferric form (Fe^{+++}) when it losses an electron, and ferrous form (Fe^{++}) when it accepts an electron.
- All are integral membrane proteins with the exception of cytochrome C, a soluble free protein.

TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

Enzyme complex/protein	*Mass (kDa)*	*Number of subunits**	*Prosthetic group(s)*
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome $c^{\dagger}$	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

- Cytochrome a3 contains copper in addition to iron and called cytochrome oxidase, it is the terminal component of the ETC.

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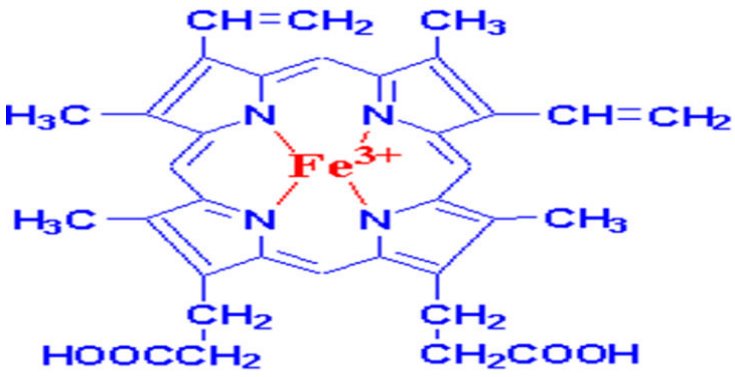

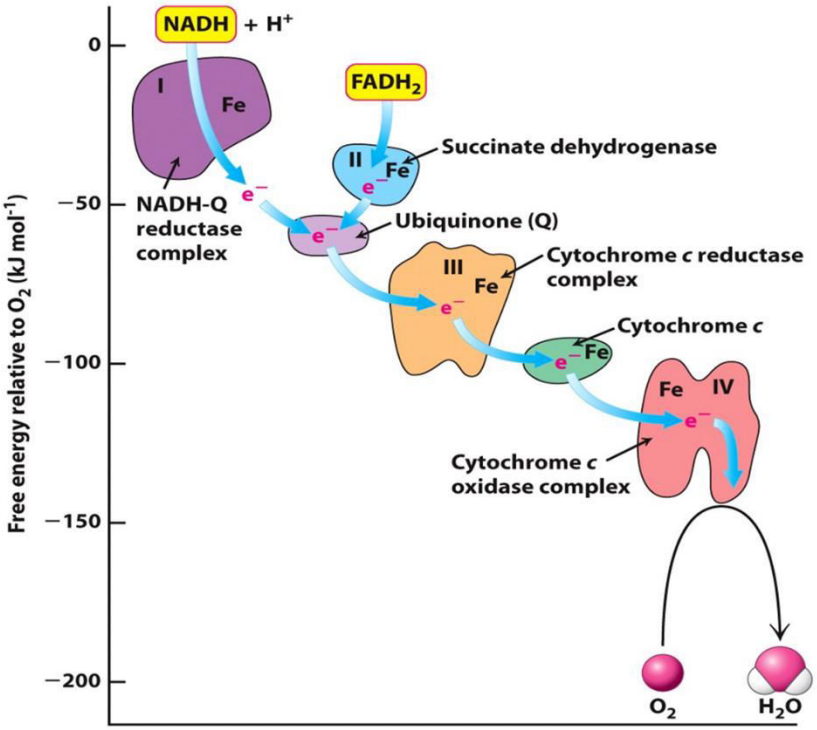

Figure 20.6
Biochemistry: A Short Course, Second Edition
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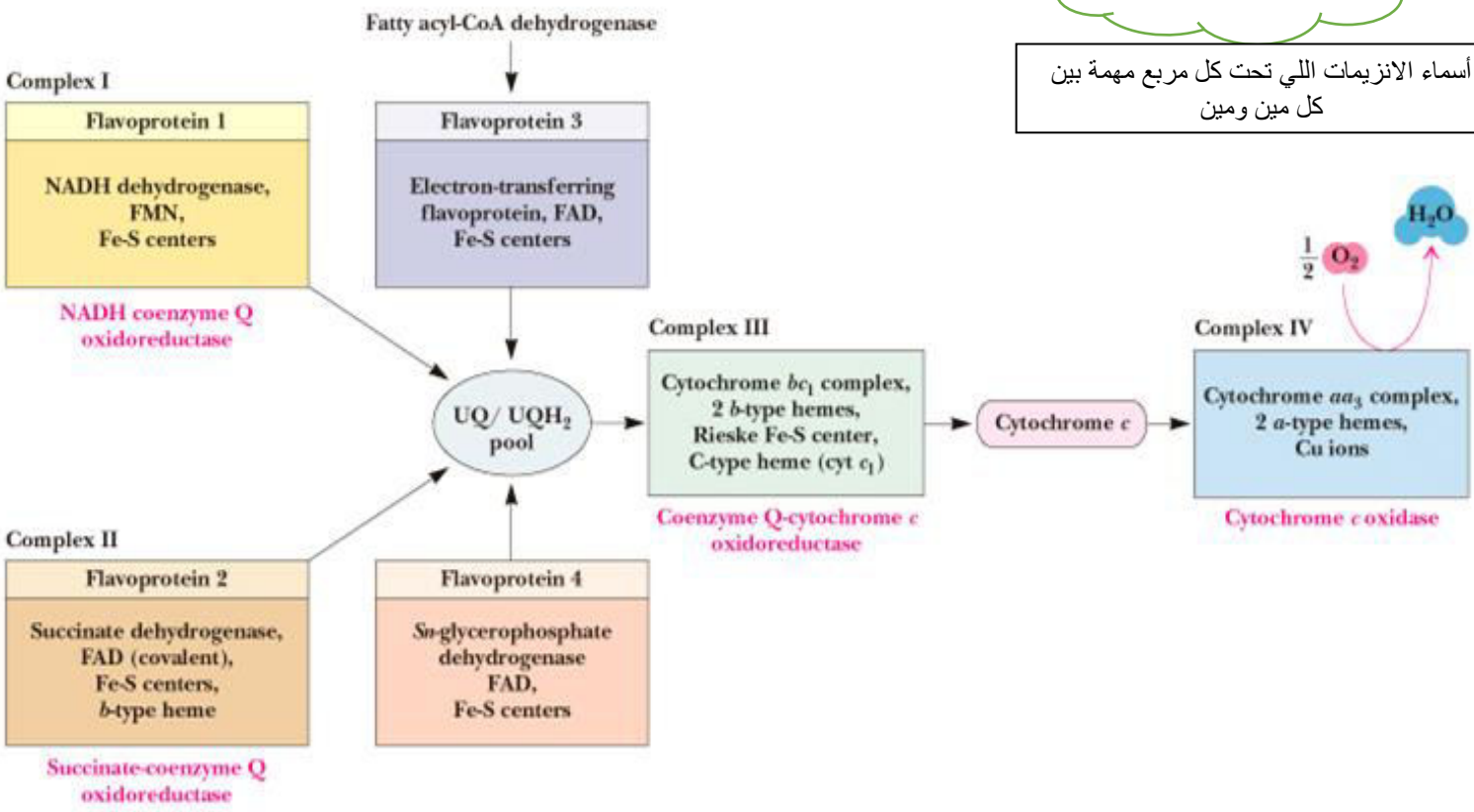
Garrett & Grisham: Biochemistry, 2/e
Figure 21.4

هاي شرحها، وشرحها
إعادة لكل اللي فات

أسماء الانزيمات اللي تحت كل مربع مهمة بين كل مين ومين



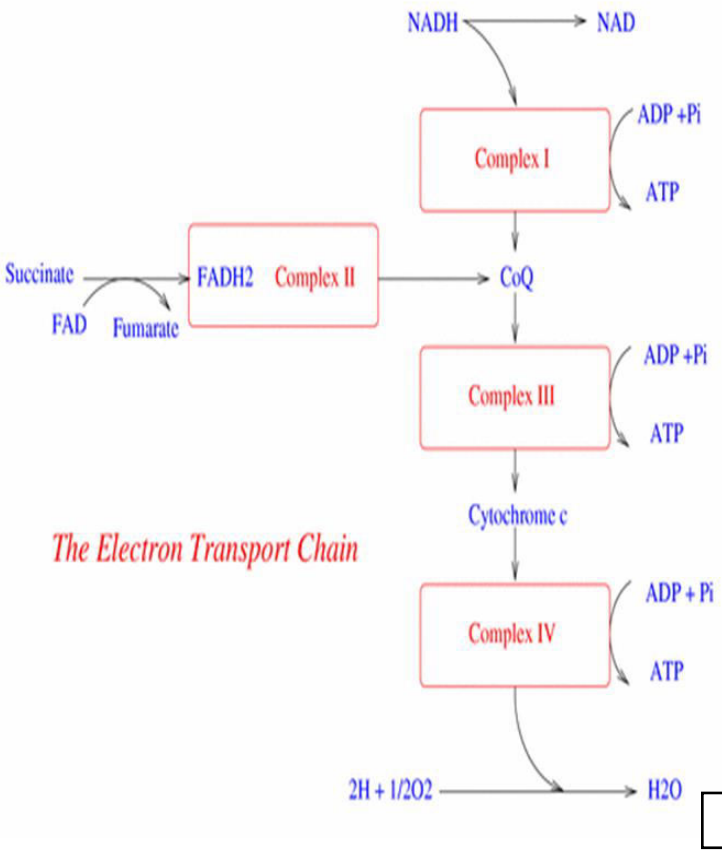
There are some reactions take the complex 2 pathway because it has redox potential higher than complex 1 and lower than 2

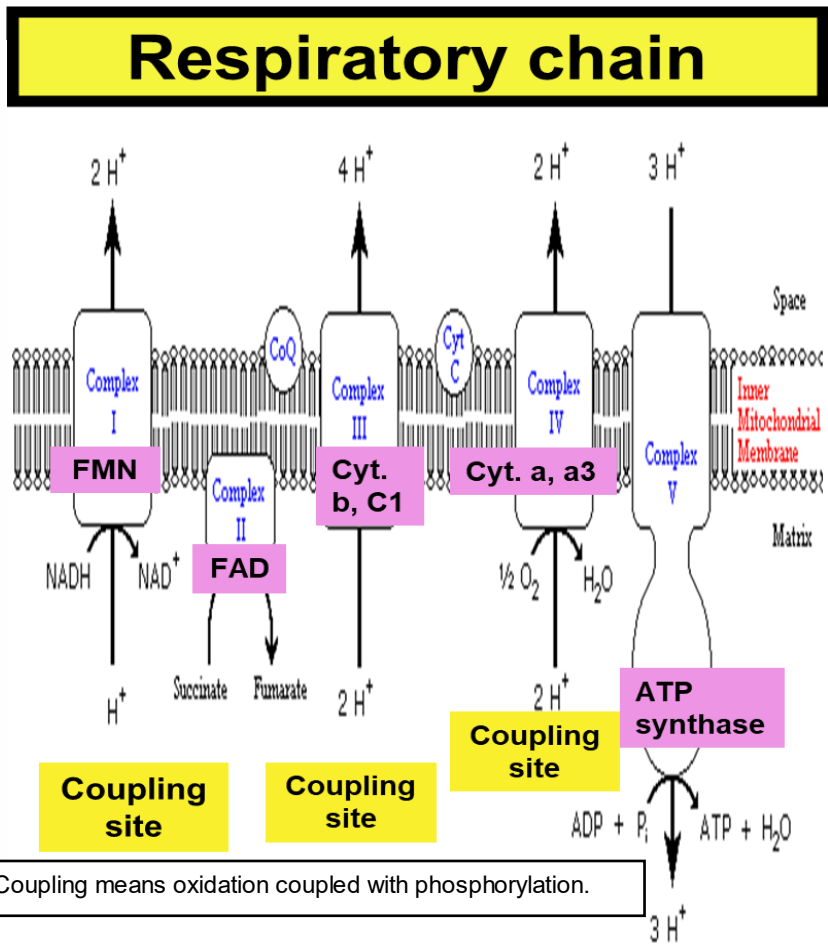
- As electrons pass down the respiratory chain, they lose much of their free energy.
- Part of this energy can be captured and stored as ATP from {ADP and inorganic phosphate (Pi)}.
- The process is called oxidative phosphorylation.
- The non trapped free energy as ATP so, released as heat.

Energy produced in ETC by redox reactions (liberation), and then by complex 5 (ATP synthase) pick up the energy f

Oxidative phosphorylation

- Oxidative phosphorylation is a coupling process of oxidation and phosphorylation.
- The flow of electrons from NADH to oxygen (oxidation) results in ATP synthesis by phosphorylation of ADP with inorganic phosphate (phosphorylation), therefore, there is coupling between oxidation and phosphorylation. (oxidation to liberate energy and then phosphorylation this energy into ADP to form ATP)
- Two theories explain the ATP synthesis; chemiosmotic hypothesis and membrane transport system.





Coupling means oxidation coupled with phosphorylation.

We have 3 phosphorylation(coupling) sites in ETC , first site produce ATP is **complex 1** because there is enough liberated energy to form 1 ATP.
Complex 2 (site 2) not produce energy enough to form ATP .
Site 3 in complex 3 and site 4 in complex 4 also participate in ATP production as 1

Inhibitors of respiratory chain:

- Are compounds prevent the passage of electrons to bind a component of the chain (the three sites responsible for electrochemical potential difference), blocking the oxidation reduction reaction.

- There are specific sites for binding inhibitors:

Site I: binding with complex I as barbiturates, rotenone (an insecticide) and piercidin A (an antibiotic).

So it will inhibit ATP production through pathway of complex 1 , so there will be only ATP production from complex 2 / or inhibition of complex 2 there will be ATP production from 1 . **** Less ATP but still present . ****

Site II: binding with complex III as antimycin A and dimercaprol.

Inhibition of 3 lad to loss of ATP production completely

Site III: binding with complex IV as H_2S, cyanide (CN), carbon monoxide (CO) and sodium azide.

Inhibition of 4 also will lead to lose of ATP but its more dangerous

- Because electron transport and oxidative phosphorylation are tightly coupled, inhibition of the respiratory chain also inhibits ATP synthesis.

4- ADP/ATP transporter **inhibitors** as atractyloside. -> **lead to accumulation of ATP inside the mitochondria**

N.B. Malonate which acts as competitive inhibitor of succinate dehydrogenase inhibits ETC through complex II.

Cyanide poisoning (the most dangerous one that inhibit complex 4) / irreversible

- Cyanide is one of the most potent and rapidly acting poisons.

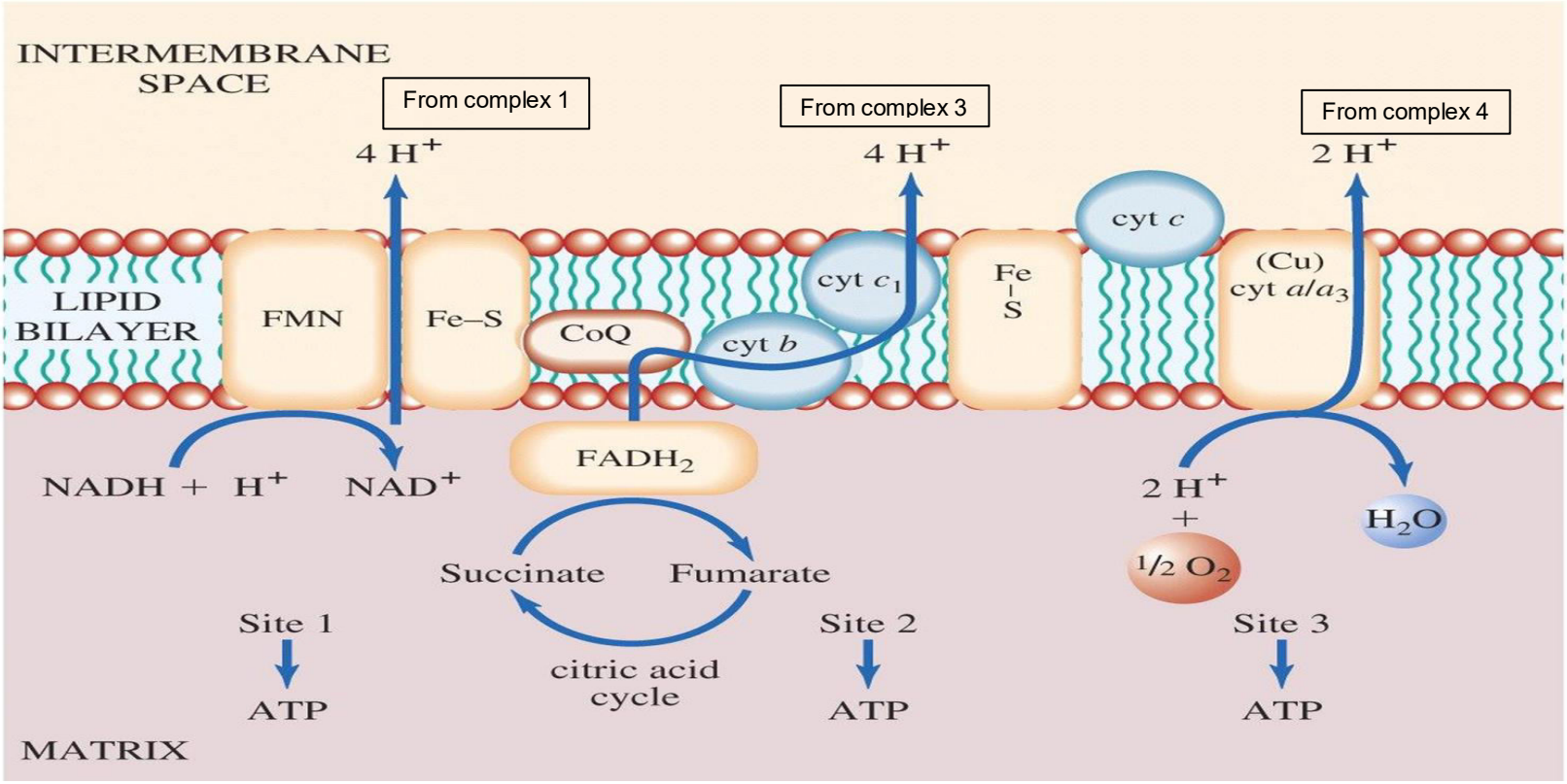
- Cyanide binds to cytochrome aa3 so, inhibits the oxidative phosphorylation at level of cytochrome oxidase complex (complex IV).

- The energy production of cells will be blocked resulting in tissue asphyxia especially of central nervous system leading to death.

Any reaction (as example : oxidative decarboxylation of pyruvate) will produce H+ , and it should be ejected outside and prevent its accumulation , so it leaves mitochondria by three complexes , Complex 1 , 3 ,4 -> by ejecting H+ from matrix to the inner space

complex 2 don't participate (for second time)

This result in electro chemical potential difference (inner space will have a higher change positive charge and lower PH)

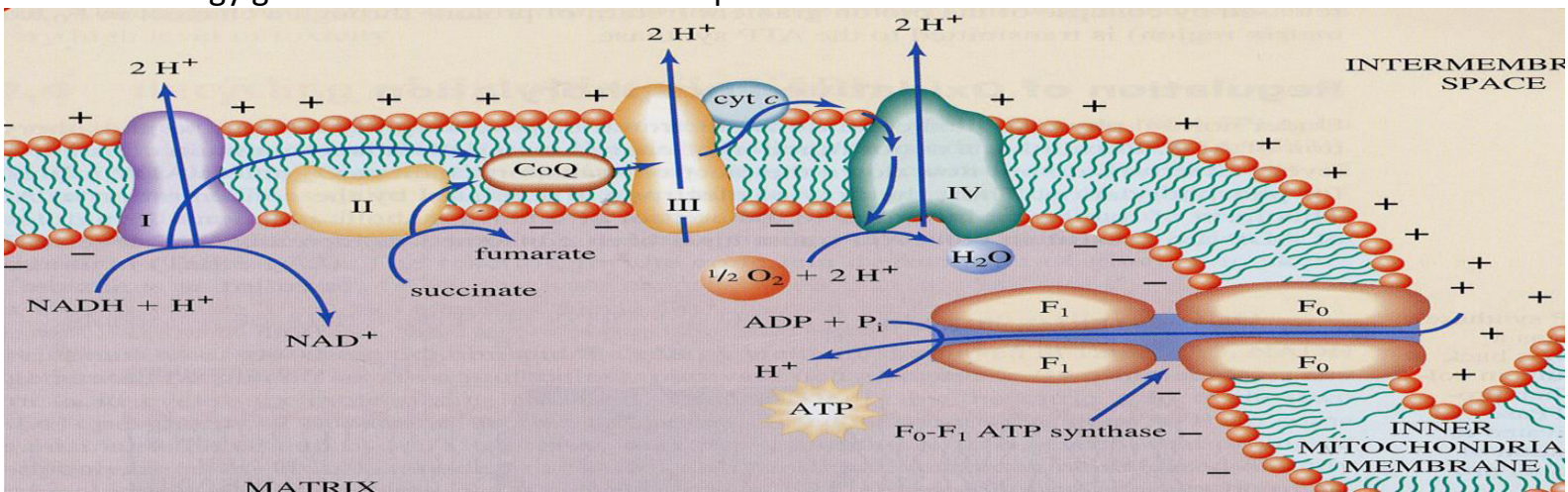


Chemiosmotic hypothesis:

- This hypothesis postulates that the transfer of electrons along the respiratory chain is accompanied by outward pumping of protons across the inner mitochondrial membrane.

Proton pump

- The transport of electrons down the respiratory chain creates an energy which is used to transport H+ from mitochondrial matrix across inner mitochondrial membrane → inner mitochondrial space.
- This process is carried out by complexes I, III, IV to create across the inner mitochondrial membrane:
  - An electrical gradient with more **positive changes** on the outside of the membrane than on the inside. **./ and chemical potential difference**
  - A pH gradient as the outside of the membrane is **at lower pH than the inside.**
  - The energy generated is sufficient for ATP production.



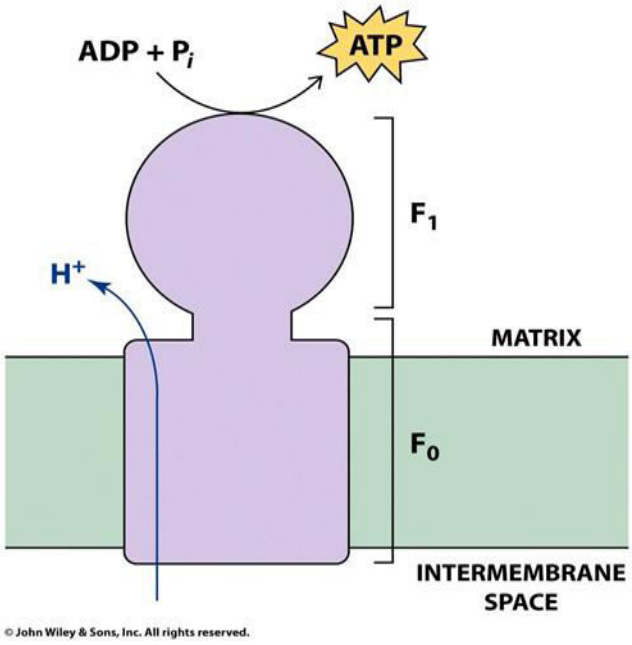
ATP synthase (complex V)

- ATP synthase enzyme presents in the inner mitochondrial membrane, it is a phosphorylating enzyme complex and it is formed of 2 subunits:
  - **F_1** subunit which protrudes into matrix**.**
  - **F_0** subunit which presents in the membrane.
- The energy stored in the electrochemical gradient is used to drive the synthesis of ATP by the movement of protons down the electrochemical gradient using **ATP synthase**.
- The protons outside the inner mitochondrial membrane can re-enter the mitochondrial matrix by passing through channel (**F0- F1** complex) to pass by ATP synthase enzyme which is present in F1 subunit.
- This results in the synthesis of ATP from ADP + Pi.
- At the same time decreases the pH and electrical gradients.

ATP Synthase

- **F_0F_1 ATP Synthase** uses the proton gradient energy for the synthesis of ATP
- Large transmembrane protein complex
- Faces into the mitochondrial matrix – spans the IMM
- Composed of a "knob-and-stalk" structure
- **F_0 (stalk) has a <u>proton channel</u> which spans the membrane.**
- Forms a proton pore
- Membrane-spanning portion – integral membrane protein
- Made up of 4 different subunits
  - F_0 subunit composition: $a_1 b_2 c_{9\text{-}12}$

(c subunits form cylindrical, membrane-bound base)



Inside matrix we have ADP and inorganic P that continuously exchange with ATP , and the redox reaction play its role on ETC at the same time , and there is enzyme to collect ADP with inorganic P and the energy produced from ETC reactions to form ATP -> this enzyme is **<u>ATP synthase enzyme</u>** and its activated by H+ that present in the intermembrane space which enter by F0 ATP synthase enzyme to activate it

- **F_1 (knob) contains the <u>catalytic (ATP-synthesizing) subunits</u>**
- Where ATP synthesis takes place
- F_1 knobs: inner face of the inner mitochondrial membrane
- (subunit composition: **$\alpha 3\beta_3\gamma\delta\varepsilon$**)
  - $\alpha_3\beta_3$ oligomer of F_1 is connected to catalytic (C) subunits by a multi subunit stalk of γ and ε chains.

Protons passage through F_0 into the matrix is coupled to ATP formation

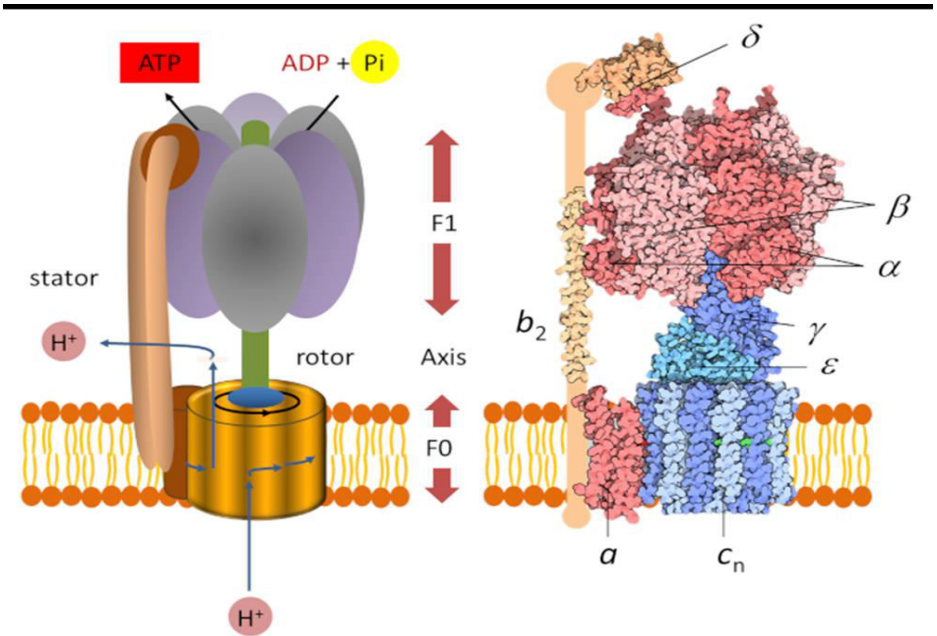
- Estimated passage of **3 H+ / ATP** synthesized
- F_0 is sensitive to **oligomycin**, it binds in the channel and blocks H^+ passage, thereby inhibiting ATP synthesis

The role of F0 is to allow H+ inter to activate the catalytic subunit of the enzyme

- For each ATP molecule there will be ejection of 4 H+ from matrix / 3 for ATP formation for activation of the enzyme and 1 for exchange with inorganic phosphate to release H+ outside and facilitate the joining between inorganic phosphate and ADP to form ATP in the matrix

Mechanism of ATP Synthase

- F_1-F_0 complex serves as the molecular apparatus for coupling H+ movement to ATP synthase.
- There are 3 active sites, one in each β subunit
- Passage of protons through the F_0 channel causes the rotor **<u>(part of F0)</u>** to spin in one direction and the stator **<u>(part connecting F0 to F1)</u>** to spin in the opposite direction **<u>(one to the right side and the other to opposite site)</u>**
- Proton flow → C unit rotates **<u>(part of F0)</u>** → γ rotates **<u>(part of F1)</u>** → conformation changes → ATP synthesized



Regulation:

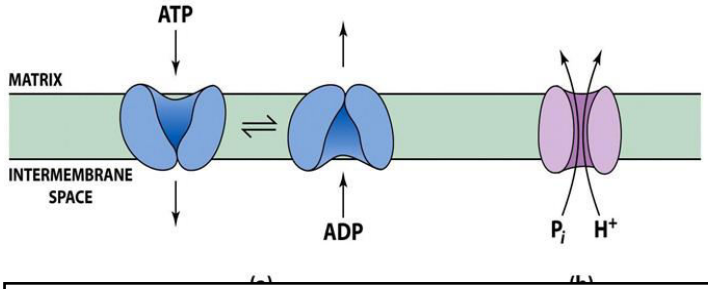
Availability of ADP is what stimulate the action of ETC

- Electrons do not flow unless ADP is present for phosphorylation
  - Increased ADP levels cause an increase in catabolic reactions of various enzymes including:
    - Glycogen phosphorylase - PFK-1 (Phosphofructokinase-1) - Citrate synthase

ATP, ADP and Pi active transport across the inner mitochondrial membrane

- ATP is synthesized in the mitochondrial matrix
- ATP must be transported to the cytosol in exchange with ADP and Pi
- ADP/ATP carrier exchanges mitochondrial ATP^{4-} for cytosolic ADP^{3-}
  - The exchange causes a net loss of -1 in the matrix (draws some energy from the H+ gradient)
  - Adenine nucleotide translocase: unidirectional exchange of ATP for ADP (antiport)
  - Symport of Pi and H^+ is electroneutral

ATP formed by ETC , then pass outside by ADP/ATP translocase that exchange ATP to ADP + Pi = this help in keep maintenance of ETC all the time



The P:O Ratio

Ratio between ATP produced to oxygen molecules consumed (amount of oxygen to be reduced)

P:O ratio = molecules of ADP phosphorylated / atoms of oxygen reduced.

- Translocation of $3H^+$ required by ATP synthase for each ATP produced
- 1 H^+ needed for transport of P_i, ADP and ATP
- Net: 4 H^+ transported for each ATP synthesized

Calculation of the P:O ratio

Complex	I	III	IV
#H^+ translocated/2e^-	4	4	2

- Since 4 H^+ are required for each ATP synthesized:

For NADH: 10 H^+ translocated / O (2e^-)
So, P/O = (10 H^+ / 4 H^+) = 2.5 ATP/O
(by complex 1 pathway)

For succinate substrate = 6 H^+ / O (2e^-)
So, P/O = (6 H^+ / 4 H^+) = 1.5 ATP/O
(by complex 2 pathway)

It equals zero in presence of uncouplers.

Uncouplers of oxidative phosphorylation

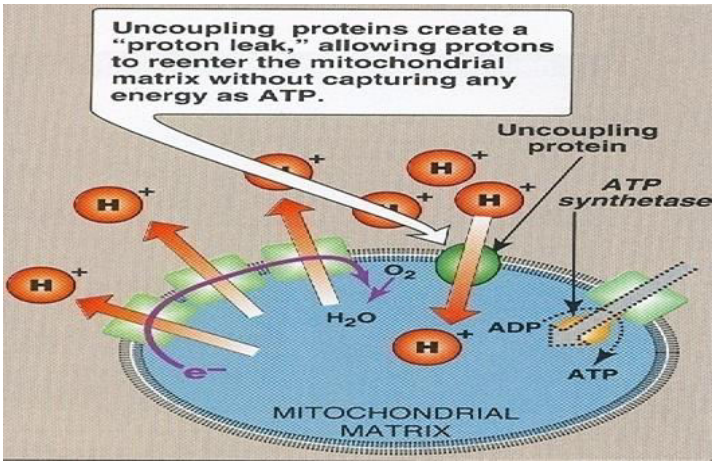
- Uncouplers are a group of substances that interrupt (uncouple) oxidation and phosphorylation i.e. oxidation will proceed building proton gradients but will not result in ATP synthesis ,so, energy released by electron transport will be lost in the form of heat.
- This explains the hotness sensation after these substances intake.

1- Oligomycin: This drug binds to the stalk of ATP synthase(complex 5) , closes the H channel and prevents re-entry of protons to the mitochondrial matrix.

2- 2,4 dinitrophenol : it increases the permeability of the inner mitochondrial membrane to proton causing decrease in the proton gradient.

H+ not inter by hydrogen channel so there is no activation of ATP synthase

3- Calcium and high doses of aspirin : this explains the fever that accompanies toxic overdoses of these drugs.



4- Ionophores : e.g.

Valinomycin and Nigericin.

- They are lipophilic substances and they have the ability to make a complex with cations as potassium "K" and facilitate their transport into mitochondria and other biological membranes.
- They inhibit phosphorylation because both electrical and pH gradient.

Once potassium and other ions inter mitochondria there will be chemical potential difference but not electrical difference

5- High level of Thyroxine: as in thyrotoxicosis and bilirubin.

6- Snake venoms.

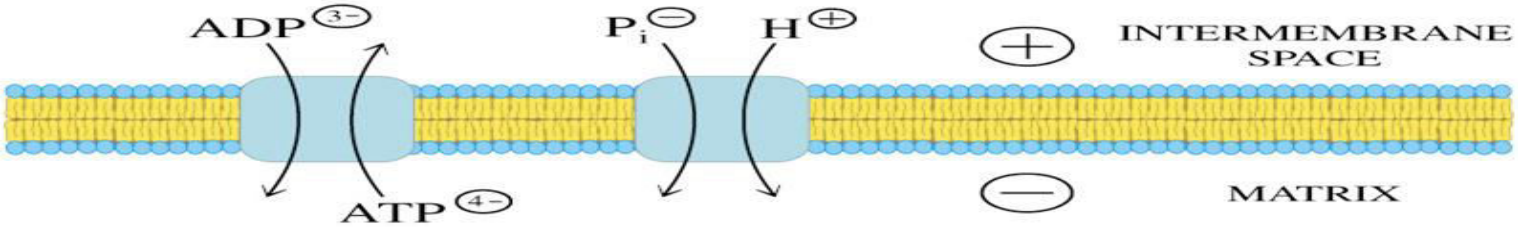
N.B. Uncoupling proteins (UCP) = separate oxidation from ATP synthesis (the synthesis is interrupted) → energy from H+ gradient is released as a **heat**

7- Thermogenin: (brown adipose tissue)

- Thermogenin also called uncoupling protein 1, or UCP1 is an uncoupling protein found in the mitochondria of brown adipose tissue.
- It is used to generate heat by non-shivering thermogenesis.
- Non-shivering thermogenesis is the primary means of heat generation in hibernating mammals and in human infants.
- The molecular mechanism of UCP1-mediated uncoupling is reasonably well understood; UCP1 allows protons to reenter the mitochondrial matrix without passing through F0-F1 complex (ATP synthase), allowing respiration (and hence heat production) to proceed in the absence of ATP synthesis.
  - UCP1 is restricted to brown fat, where it provides a mechanism for the enormous heat-generating capacity of the tissue.

Membrane transport chain

- The inner mitochondrial membrane contains numerous transport proteins (carriers) that permit passage of specific molecules from the cytosol to the mitochondrial matrix e.g. ADP-ATP carrier (adenine nucleotide translocase) which carries ADP from cytosol into mitochondria, while, carrying ATP from the matrix back to cytosol.



What about NADH from glycolysis? (reaction number 6 in cytosol / catalyzed by glycerol 3 – phosphate dehydrogenase enzyme (GPDH))

- NADH made in cytosol
- Can't get into mitochondrial matrix

By 2 mechanisms:

A- In muscle and brain (Glycerol phosphate shuttle)

- Each NADH converted to $FADH_2$ inside mitochondrion

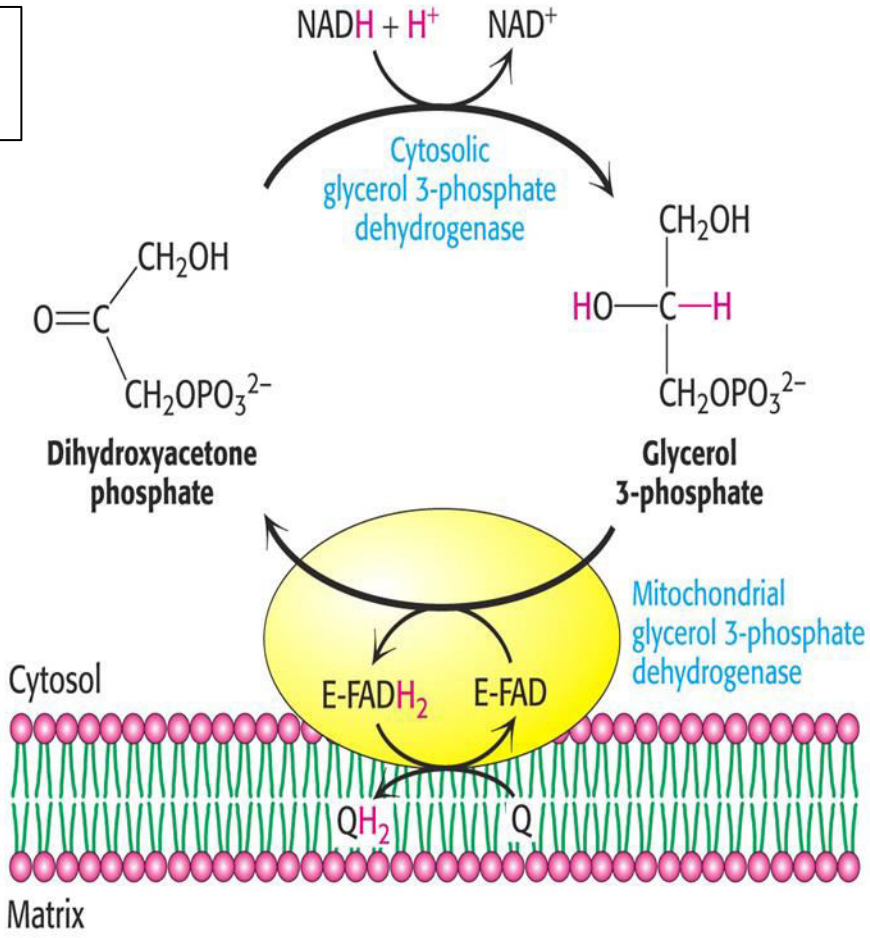
- $FADH_2$ enters later in the electron transport chain

- Produces 2 ATP

Because H+ is not allowed to penetrate the inner mitochondrial membrane

There will be reaction between NADH (resulted from type 6 reaction glycolysis) with dihydroxyacetone phosphate in present of cytosolic GPDH -> glycerol 3 phosphate which give H+ to another component FAD -> FADH2

The aim of this reactions to bring H+ result in the cytosol to join in the ETC inside mitochondria



B- In liver and heart (Malate / aspartate shuttle)

- NADH oxidized while reducing oxaloacetate to malate by malate dehydrogenase

- Malate crosses membrane **(by carrier called malat-alpha ketoglutarate carrier)**

- Malate reoxidized to oxaloacetate
- Malate dehydrogenase
- NAD+ reduced to NADH
- NADH via electron transport yields 3ATP

Glutamate aspartate carrier to allow except of aspartic acid and alpha ketoglutarate to regenerate oxaloacetate outside mitochondria again

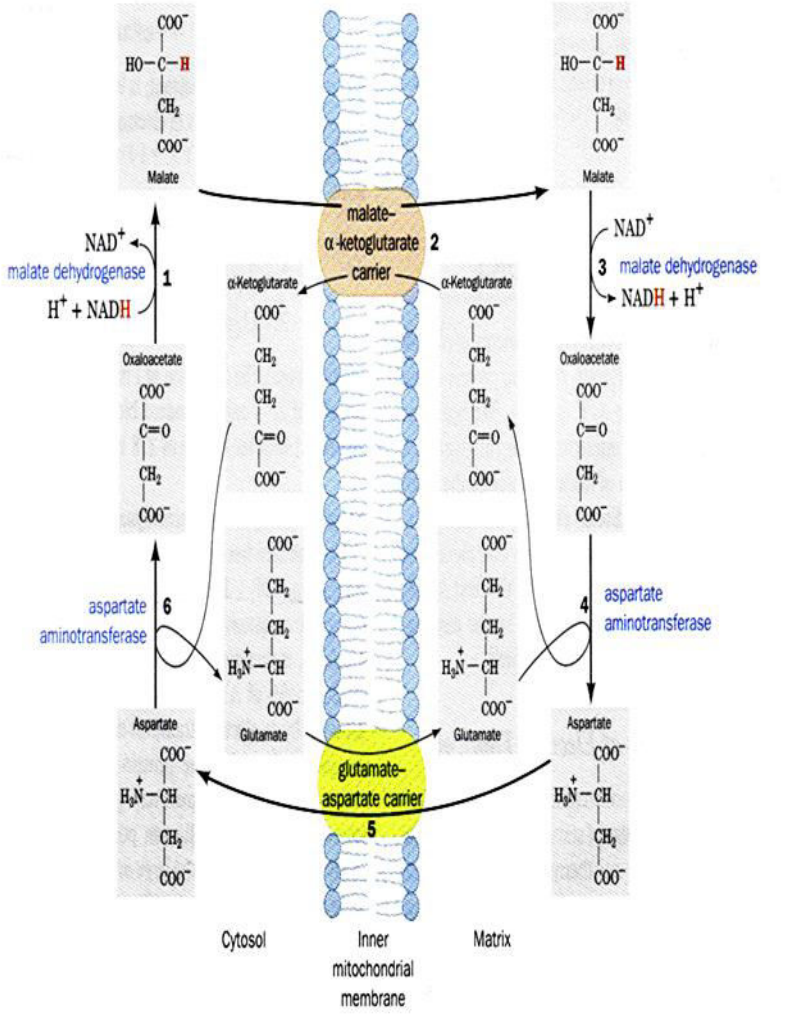


FIGURE 20-7. The malate–aspartate shuttle. The electrons of cytosolic NADH are transported to mitochondrial NADH (shown in red as hydride transfers) in Steps 1 to 3. Steps 4 to 6 then serve to regenerate cytosolic oxaloacetate.

Inherited defects in oxidative phosphorylation

- **Mitochondrial DNA (mtDNA) (37 genes)** is maternally inherited as mitochondria of sperm cell do not enter the fertilized ova.
- Mitochondrial DNA (mtDNA) codes for **13 polypeptide** (of total 120) required for oxidative phosphorylation, 22 tRNA and 2 rRNA.

(while the remaining are synthesized in the cytosol & are transported into the mitochondria).

- Defects of oxidative phosphorylation usually results from **alteration in mtDNA** (mutation rate 10 times more than that of nuclear DNA).
- **Tissues with greater ATP requirement** (as CNS, skeletal muscles. & cardiac muscle, kidney & liver) are most affected by defects in oxidative phosphorylation.

Examples for diseases caused by mutations in mtDNA:

1- Mitochondrial myopathies (defective energy production → muscle cramping, weakness and severe fatigue).

2- Leber hereditary optic neuropathy (bilateral loss of vision due to optic nerve damage).

Substrate Level Phosphorylation:

- Very small amount of ATP molecules are produced

- Few reactions can form ATP at substrate level: e.g.

1. Glycolysis (phosphoglycerate kinase and pyruvate kinase)
2. TCA cycle (succinate thiokinase)

Respiratory control:

- There is no mechanism for storage of ATP and ATP present at any moment is only enough to meet the need of our cell for only few seconds.

- For this reason, there must be an efficient and controlled way for the production of ATP.

1- Availability of ADP (ATP/ADP transporter may rate limiting at certain times.

2- Availability of electrons (↑ NADH/NAD and/or ↑ FADH2/FAD).

3- Availability of O2.

4- Insulin