فريق طوفان الأقصى

METABOLISM

Modifide N.8

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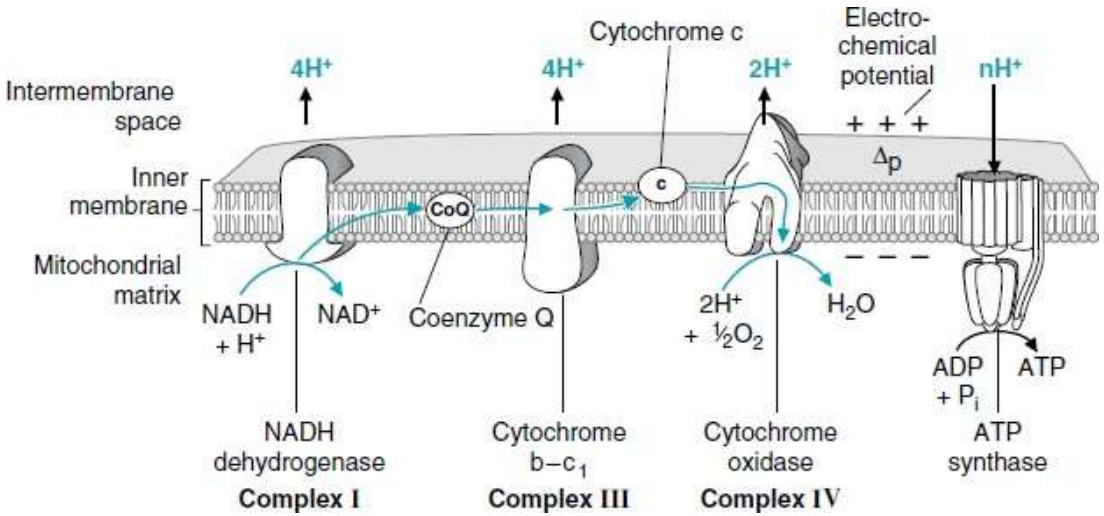
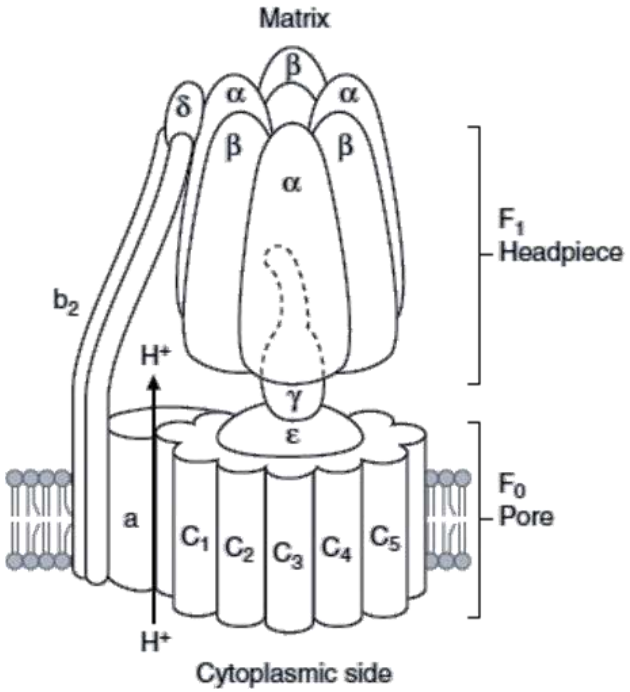
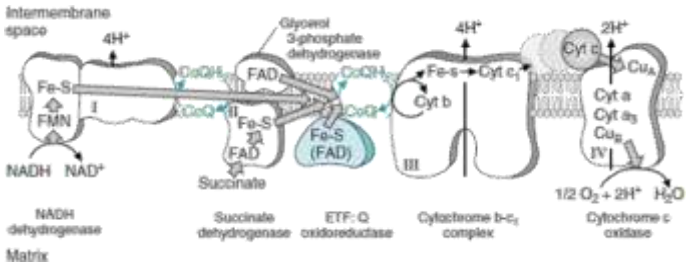
Requirements of OxPhos

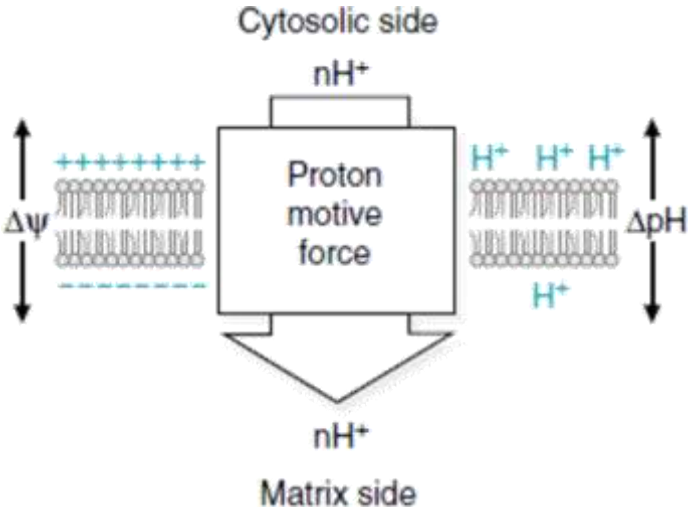


- Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2)
- An intact IMM
- ETC of proteins: 4 complexes+1 soluble protein +CoQ
- ATP synthase

The complement in this slide: Each one of those complexes contain many prosthetic groups , complexes (1,3,4) and co-enzyme Q which isn't a protein , it is a lipid in nature and we have a carrier* small soluble protein cytochrome c* that could move in the membrane , while complexes can't because they are integral .



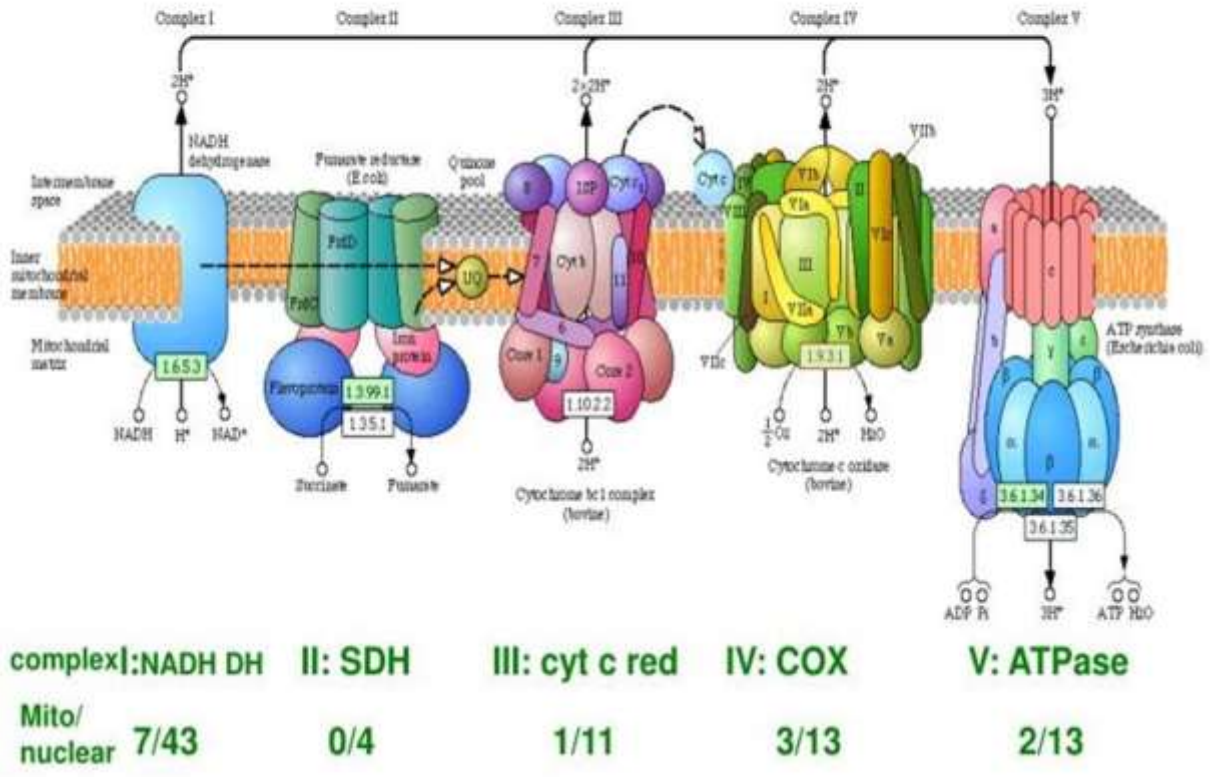




ET to O2, how does the process occurs?
"The chemi-osmotic theory"

- **The complement in this slide:** now in order for oxidative phosphorylation to happen (phosphorylation of ADP producing ATP) , in order to do this process , the cell requires an enzyme called ATP synthase ‘ATPase’ , this enzyme is highly complexed , it is the site of ATP synthesis.

ANATOMY OF THE RESPIRATORY CHAIN



1. Complex I: NADH Reductase.

NADH + e-> CoQ

2. Complex II: Succinate-CoQ Reductase,

Succinate+ e →CoQ.

3.Complex III, Cytochrome C Reductase,

CoQ +e →Cyt c

4. Complex IV, Cyt Oxidase,

Cyt c+e → Oxygen

5. Complex V: ATPase

- **The complement in this slide:** first we will talk about protein complexes that participate and electron transferring from NADH + H and FADH2 till those electrons reach oxygen and reduce one oxygen atom to produce water .
- now as you see in complex 1 , complex 1 is NADH dehydrogenase and it is a very big protein complex , that the NADH will take the electrons and protons from the NADH and transfer them to co-enzymeQ *co-enzymeQ is another electron transporter in the ETC but it is not a protein , its not among the protein complexes that participate in electron transferring
- Q:What is the nature of co-enzymeQ(ubiquinone)?
- ANS : it is lipid in nature
- And it is important because once it collects the electrons from the protein complex 1 , it will diffuse in the lipid layer till it reaches the complex 3 ,NOT COMPLEX 2, IT IS MOVABLE IN THIS MEMBRANE(WE ARE TALKING ABOUT CO-ENZYMEQ)
- Something is important about protein complex 1 that it is considered as proton pump so it will pump protons from the matrix to the inner membrane space , as the electrons pass from complex 1 to co-enzymeQ they will form an electrical current , and that electrical current because of the energy releasing , some protons will be pumped from the matrix to the inner membrane space

- **The complement in this slide:** The second PROTEIN COMPLEX is succinate reductase , it is a protein complex and it contains the succinate dehydrogenase enzyme , in which FADH2 is covalently bound to succinate dehydrogenase , which is part of the complex 2 which is called succinate reductase .
  Succinate reductase will accept the electrons from FADH2 and transfer them to co-enzymeQ , co-enzymeQ will diffuse till it binds to complex 3 , and it will donate its electrons , REMEMBER THAT COMPLEX 2 ISN'T A PROTON PUMP (doesn't pump any protons from the matrix to the inter membrane space)

The complement in this slide: Complex 3 will receive the electrons transferred from complex 1 and complex 2 , the electrons which were taken from co-enzymeQ , co-enzymeQ will bind to complex 3 and donate the electrons collected from complex 1 and complex 2 , the name of this complex is Q-cytochrome C oxi-reductase , it is composed of multi subunits as complex 1 , but complex 1 is larger in size , this complex is also considered as proton pump , its function is pumping protons from the matrix to the inner mitochondrial membrane , it has many of the cofactors that we have talked about specially cytochrome b and other types of cytochromes , as well as iron sulfur centers in which they will assist in electron transferring from this complex into cytochrome c , cytochrome c is a small soluble carrier protein , all these protein complexes (1,2,3) are integral in the inner mitochondrial membrane while cytochrome c is a soluble protein that could diffuse within the membrane to bind to complex 4 , once it binds it will give its electrons to complex 4.

- **The complement in this slide:** complex 4 is a highly complexed structure it has a different types of cytochromes , those prosthetic groups which compose complex 4 will help in transferring electrons from cytochrome c to oxygen , complex 4 is also considered as proton pump but it pumps only 2 protons , oxygen will be reduced into water by the electrons taken by cytochrome c , and the reduction of oxygen will take place in a complex form which is called cytochrome c oxidase .

- To sum up: complex 1 and complex 3 each one pumps 4 protons , while complex 4 pumps only 2 protons
- Complex 2 pumps 0 protons (its not a proton pump).

The complement in this slide: **Complex 5 which is called ATPase , is a special protein that has an enzymatic activity which synthesize ATP by oxidative phosphorylation , once the protons which were collected pass through ATPase (details later) some activation will take a place and the ADP will be phosphorylated .**

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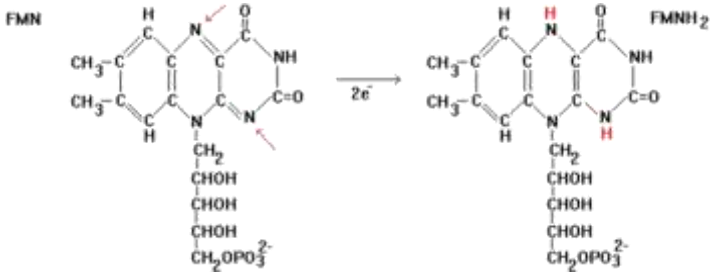
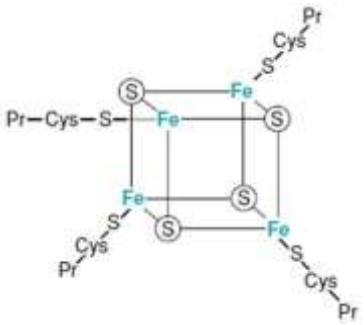




Oxi–Red Components of the ETC “NADH Dehydrogenase” OR oxidase – Complex I

NADH-Q oxidoreductase

More than 25 polypeptide chain

A huge flavoprotein membrane-spanning complex (**that uses FAD and FMN)**

The FMN is tightly bound

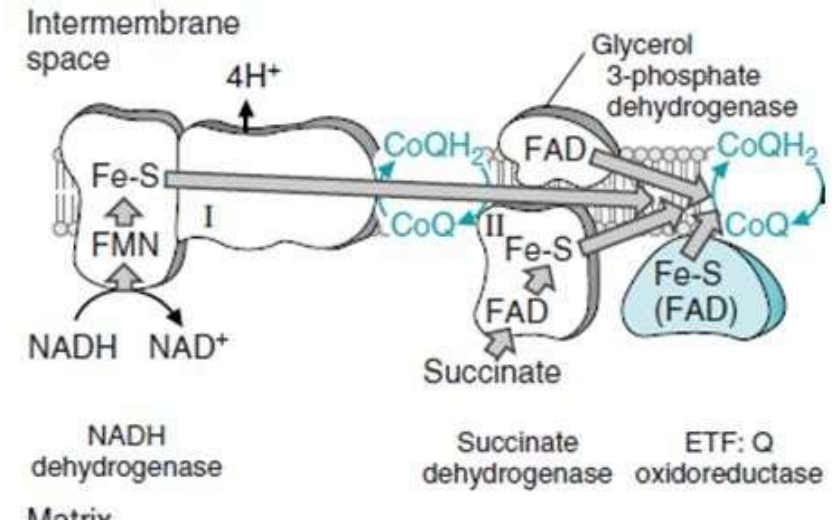
Seven Fe-S centers of at least two different types

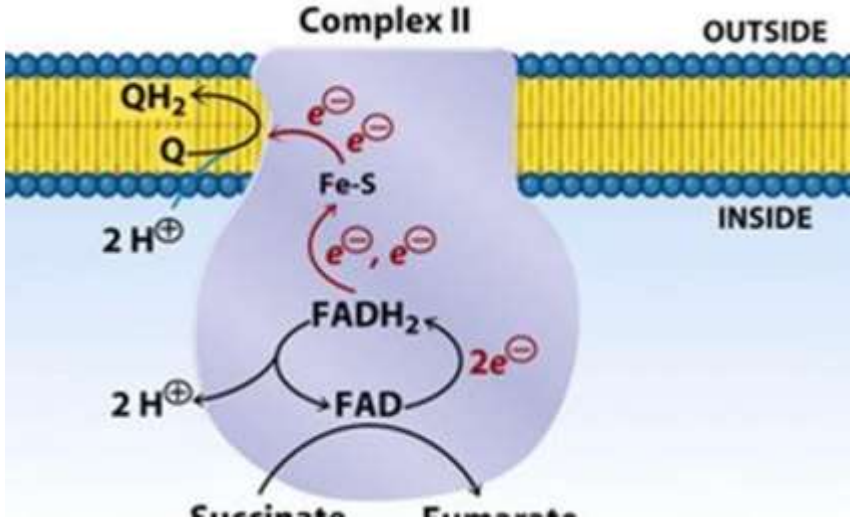
Binds NADH & CoQ

4 H+

- **The complement in this slide:** taking electrons from NADH, oxidizing NADH so it is dehydrogenase and it is an oxidase and it is an oxidation for NADH and a reduction of coenzymeQ that's why we call it NADH-Q oxido-reductase

- **NOTE :** Don't memorize the details just you should know that it is a complicated protein structure .
- More complicated than pyruvate dehydrogenase complex .



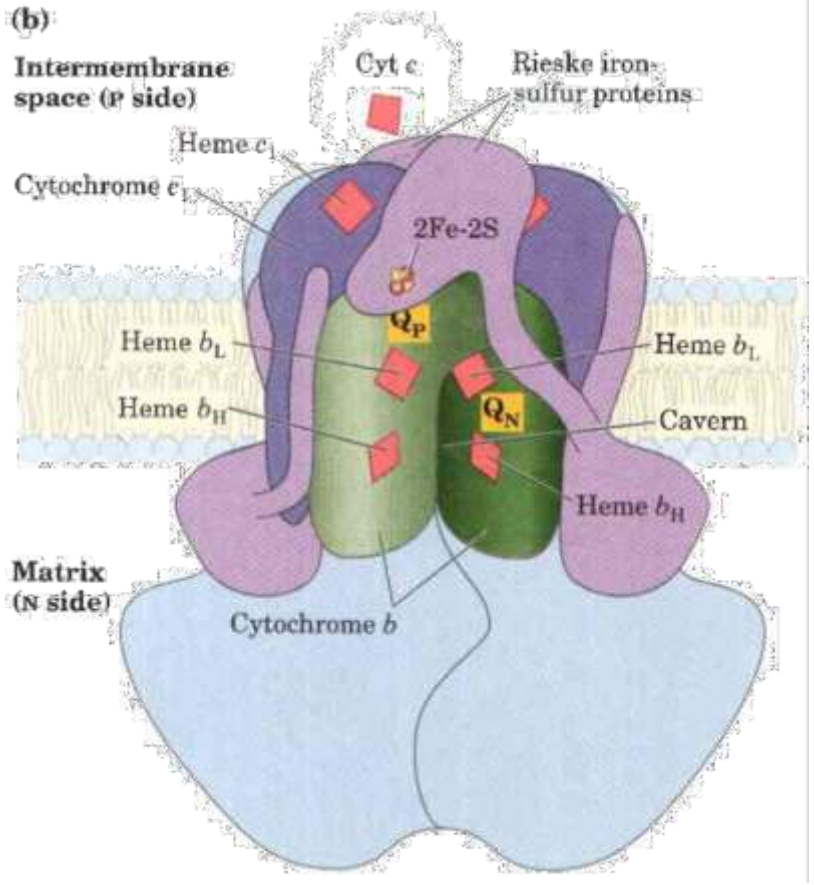


Oxi–Red Components of the ETC "Succinate Dehydrogenase" – Complex II

- **Succinate Dehydrogenase (a part of complex 2 which is called succinate reductase) & other flavoproteins**
- **TCA cycle**
  - **ETF-CoQ oxidoreductase (ex. fatty acid oxidation)**
  - **≈Substrates oxidized by FAD-linked enzymes bypass complex-I**
  - **Three major enzyme systems:**
  - **Succinate dehydrogenase**
  - **Fatty acyl CoA dehydrogenase**
  - **Mitochondrial glycerol phosphate dehydrogenase**
  - 0 **kcal, H+?**

The complement in this slide: Those are the enzymes that produce FADH2 and donate the electrons to this complex .

It has many prosthetic groups (these prosthetic groups will help this complex to donate electrons to co-enzymeQ) , and some iron-sulfur centers .



Oxi–Red Components of the ETC "Cytochrome bc1" – Complex III

- Also called: Q-cytochrome c Oxidoreductase **(oxidizing co-enzymeQ and reduce cytochrome c)**
- Catalyzes the transfer of electrons from QH2 to cytochrome c
- 11 subunits including two cytochrome subunits
- Contains iron sulfur center
- Contain three heme groups in two cytochrome subunits
- Contain two CoQ binding sites
- 4H+

NOTE: Prosthetic groups varies from complex to another , some of them will have heme some of them don't

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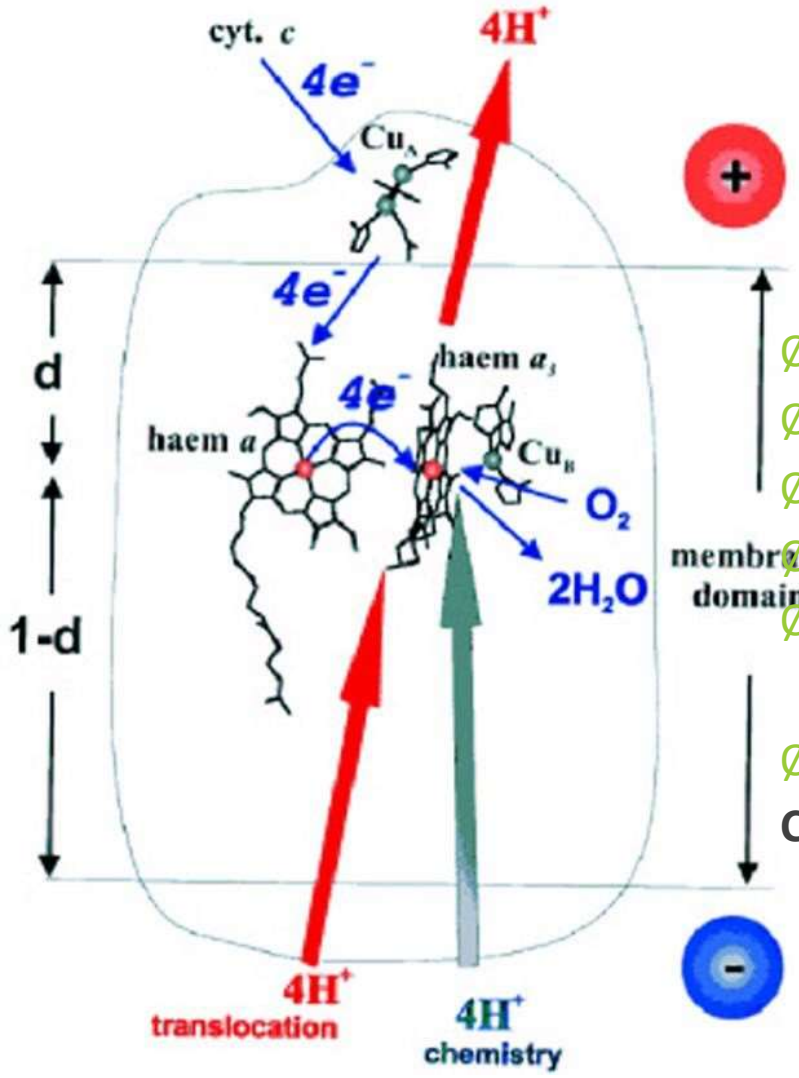
Cytochrome contains heme

Oxi–Red Components of the ETC
"Cytochrome c oxidase" – Complex IV



- Passes electrons from Cytochrome c to O2
- Contains cytochrome a and a3
- Contains two copper sites
- Contains oxygen binding sites
- O2 must accept 4 electrons to be reduced to 2 H2O (2H+/2e-)
- Cytochrome c is one electron carrier

$Cyt\ c_{red} + 4H^+ + O2 \rightarrow Cyt\ c_{ox} + 2H2O$

- What is the difference between cytochrome a and a3?
- 1-the heme structure
- 2-the side chains
- So their absorption and structure are different
- But what's may be similar between them is that each one of them has at least one heme group .

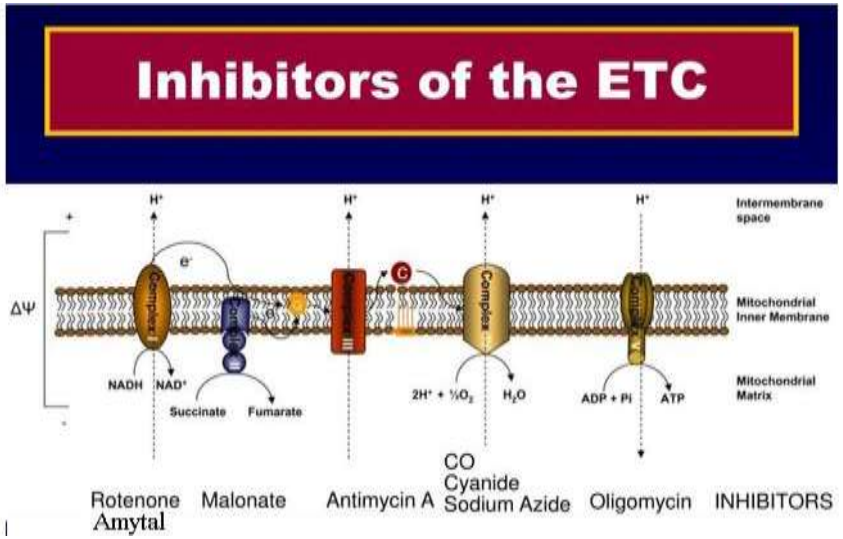
- ATPase=ATP
- synthase
  Because it could
  hydrolyze ATP

- Remember when the electrons are passing from one complex to the next complex , the direction of the flow of electrons depend on the redox potential (from the small redox potential to the large redox potential), NADH has the lowest redox potential, and oxygen has the highest redox potential, while the electrons are passing an electrical current will be formed and some energy is also released, that energy is used to pump protons from complex 1 , 3 and 4 ,those protons are collected in the inter membrane space forming two things

- 1-charge gradient between the outside of the membrane and inside it, positively charge outside and negatively charge inside (the matrix region)

- 2-PH gradient

- The gradient of charge and PH will form what is called the electromotive force (delta PSI/delta PH) that will help protons to cross the membrane again from the inter mitochondrial space to the matrix via ATPase, so this membrane will act as a battery and the power in that battery will help to pump protons within gradient into the matrix via ATPase.

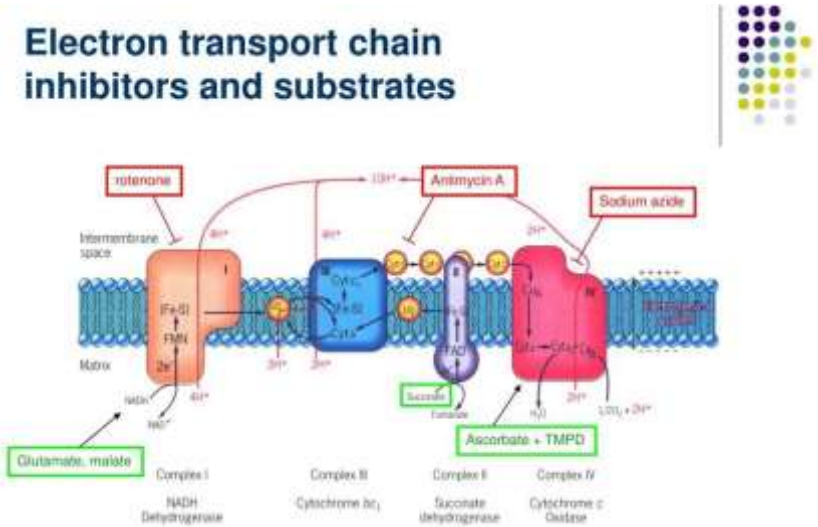


Usually those electron carriers under normal conditions are found in a partially oxidized form

1. Amytal.(sedative)-inhibits NADH-Q Oxireductase
2. Rotenone.(insecticide)-inhibits NADH-Q Oxireductase
3. Antimycin A: inhibits electron flow **(in complex 3)** between cyt b and c1, which prevents continued ATP synthesis at sites I and II as the carriers. InhibitsQ-cyttochromr c oxireductase,once reduced can not be oxidized. **A very few ATP could be formed .**
4. CO. –inhibit cytochrome c oxidase
5. Sodium Azide . –inhibit cytochrome c oxidase
6. Cyanides. –inhibit cytochrome c oxidase
7. Oligomycin—inhibits ATP synthase

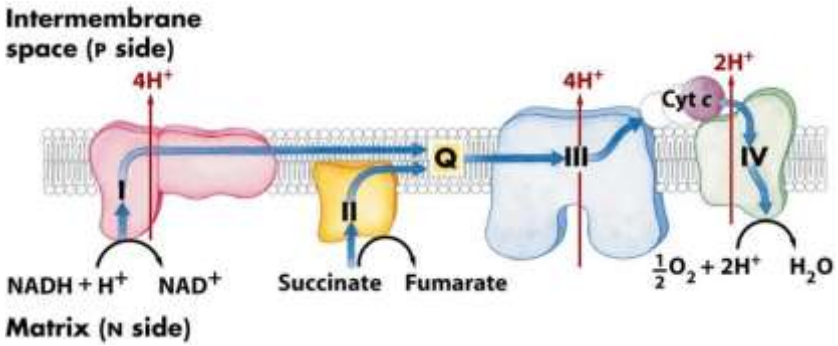
- There is a different types of inhibitors
- 1-Amytal: which is a (sedative) agent that inhibit complex 1 so complex 1 will be unable to oxidize NADH and electron transfer will be blocked (no ATP will be formed).
- 2-Rotenone: (insecticide) same as amytal .
- Amytal and Rotenone are lethal inhibitors.
- 3-co, 4-sodium azide,5-cyanides: these are highly poisonous because they inhibit cytochrome oxidase(complex4),and as a result , oxygen will not be reduced, and ATP will not be formed, so they are lethal if someone is exposed to them
- 7-Oligomycin

- These inhibitors could be used to determine the sequence of any electron transport chain because once the electron flow is blocked all the receptors before the blocking site will be reduced and all the carriers will be more oxidized than the ones before the blocking site because they are close to oxygen so by doing these experiments they could determine the sequence of which comes first and which is the next and which is the last .



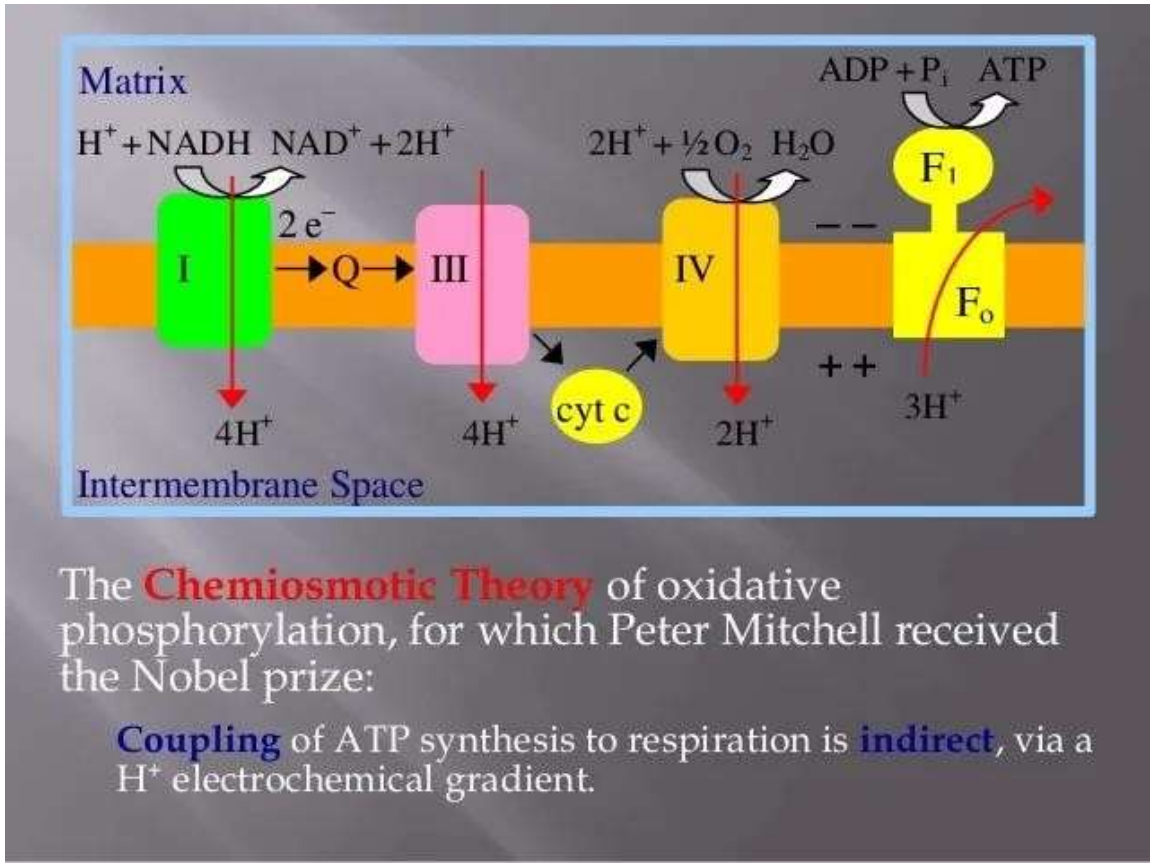
1. Complexex I, III, IV all have large enough E0 for the transfer of 2 electrons to support the synthesis of one ATP.
2. Complex I, III, IV are recognized as phosphorylation sites I, II, and III.
3. Oxidation of 1 molecule NADH+H+ or FADH2 corresponds to the synthesis of 3 or 2 molecules of ATP, respectively, and the reduction of one atom of oxygen.
4. Oxidation of NADH + H+ and FADH2 occurs with P/O ratio of 3 and 2, respectively.
5. Using ascorbate as substrate and TMPD as artificial electron carrier, a P/O ratio =1.
6. P/O ratio is the number of moles of Pi incorporated into ATP per atom of oxygen utilized.
7. P/O for malate=3, succinate=2, ascorbate=1

Pumping of Protons



- For every 2 electrons passing:
- $4H^+$ (complex I); $0H^+$ (complex II); $4H^+$ (complex III), $2H^+$ (complex IV

- When NADH is completely oxidized without any interruption there will be 10 protons produced ,it was calculated that every 4 protons will cause a synthesis of one ATP , so 10 protons will produce 2.5 ATP
- FADH2 will start from complex 2 so it will pass 4 protons and what remains is 6 protons , and the 6 protons will produce 1.5 ATP.



Chemiosmotic hypothesis:

- a proton gradient is generated by a proton pump in the inner membrane of the mitochondria.
- The proton pump is operated by electron flow and causes protons to be expelled through the membrane from the matrix space.
- Protons flow back into the matrix down their electrochemical gradient and the energy released is used to drive the synthesis of ATP.

The difference in PH is caused by the transferring of protons pumped from the matrix to the inter membrane space .

V2:

Inter instead of inner in pages:7,17,17,22 in yellow