Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 11

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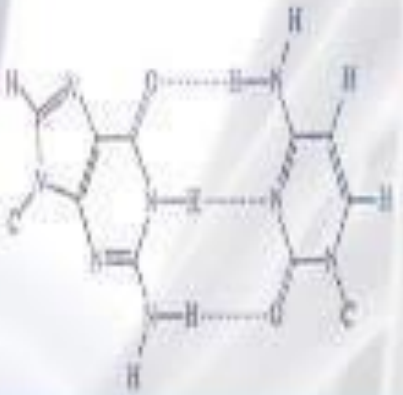
Biochemistry



Majida Al-Foqaraa'

Oxidative phosphorylation

- Revision: The last thing we talked about was the anaplerotic reactions, we said that pyruvate carboxylase is the main anaplerotic enzyme which converts pyruvate to oxaloacetate. It requires biotin (vitamin B7) as a coenzyme (since it is a carboxylase). It is activated by acetyl CoA because it produces oxaloacetate from pyruvate , the more acetyl CoA we have, the more oxaloacetate we need for the citric acid cycle to move.
- It is present in a high concentration in the liver and kidney because gluconeogenesis happens there, so malate is taken out (at the expense of the oxaloacete formation) which means that we have less oxaloacetate, and this anaplerotic reaction compensates for this low concentration of oxaloacetate.

Now we'll talk about the oxidative phosphorylation process (the last stage of energy metabolism , as we know that the stages are: Digestion; Acetyl-CoA formation ; TCA; Oxidative phophorylation.)
) .we'll start with electron transport chain.

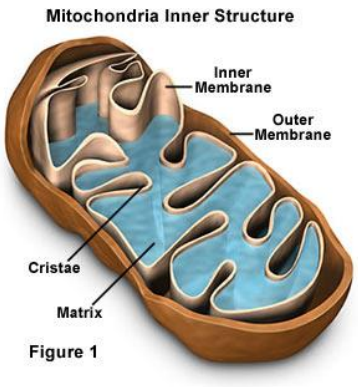


Figure 1

What does oxidative Phosphorylation mean?

Oxidative : the redox reactions that occur in the ETC .

Phosphorylation : It is the process of ATP production by Phosphorylating ADP through ATP synthase .

The mitochondrial structure

- An outer mitochondrial membrane
- Inner mitochondrial membrane that has the matrix within it and t is extensively convoluted (forming cristae) in order to increase the
- An intermembranous space that lies between the two membranes.

• The matrix is where all the oxidation pocesses occur (reactions that degrade macromolecules (like :carbohydrates,proteins and lipids) to produce energy) , except for glycolysis which occurs in the cytosol.

The inner mitochondrial membrane is impermeable to anything even to H+ which is the smallest ion (since it is only a proton, a nucleus and no electrons around it). So if you want to get anything out of the mitochondrial matrix or bring anything into it, you need a carrier, a channel protein that transports it , but the outer mitochondrial membrane is permeable to small molecules of a molecular weight of 5 kilo Dalton or less (anything <5kd can exit through the outer mitochondrial membrane).

• **When does oxidative phosphrylation process happen?**

By digestion and processes of breaking down ,all proteins, carbohydrates and fats will eventually give acetyl-coenzyme A that gets into the Kreb's cycle. From this cycle we'll get the electron carries NADH, FADH2, that will be used in the electron transport chain so that at the end we will get ATP . So, stages of energy metabolism: Digestion; Acetyl-CoA; TCA; Oxidative phophorylation.

- The oxidative phosphorylation process works through the *(chemiosmotic) theory*, described by Peter Mitchell (1961).

• It involves three major things:

1) Flow of electrons (that are extracted through citric acid cycle) from NADH, FADH2 to the protein complexes through the electron transport chain.

2) Reduction Potential is what makes electrons move from one complex to the other, from a high energy point to a low energy point, from a more negative reduction potential to a more positive reduction potential.

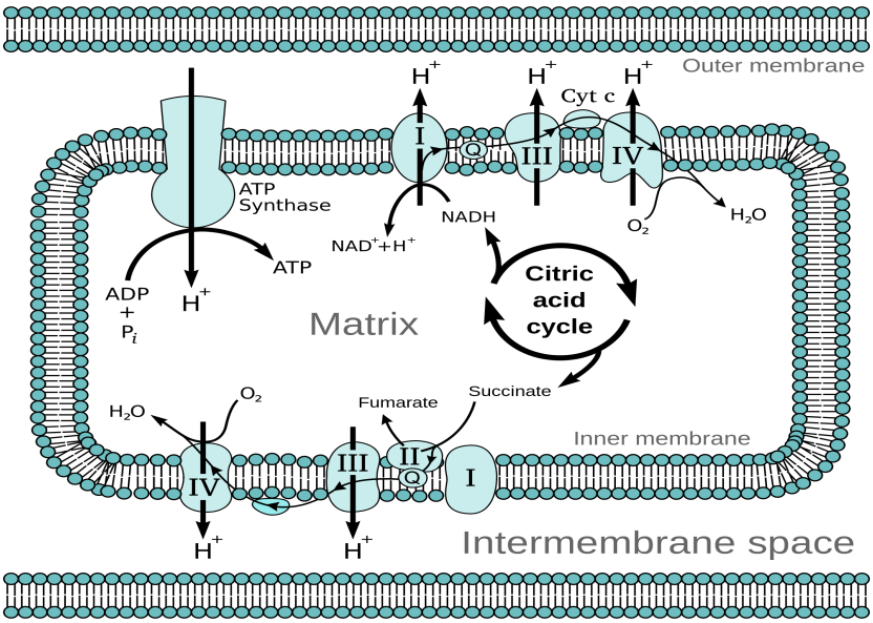
And here the doctor says that what he said about the reduction potential was wrong, and that it is the ability to **accept** electrons (not donate). *And he tells us to stop asking him on facebook about it :P*

3) The difference in energy produced due to the movement of electrons from a higher energy point to lower energy point *will be used not lost* (because according to the first law of thermodynamics ; energy can't be lost or destroyed , it is converted from one form to another).

- This energy is used to pump protons from the matrix to the intermembranous space, since the inner membrane is impermeable so there should be carriers (pumps) and those need energy for pumping. The protons will accumulate in the inter membranous space. They are not allowed to get back to the matrix because of the impermeable inner membrane, so they start exerting pressure on the membrane because of their charge (more positive outside than inside).And there will also be a concentration gradient from outside to inside, so the concentration gradient and the electrical (charge) gradient due to the same molecule /ion ,and we call this the (electrochemical gradient) or sometimes the (proton motive force) which is the force due to protons' movement .
- They are only allowed to come back to the matrix through one point ,which is the *ATP synthase* according to their electrochemical gradient (going with the flow) so they will give energy which is used to make ATP.

And this is the whole concept of OxPhos.

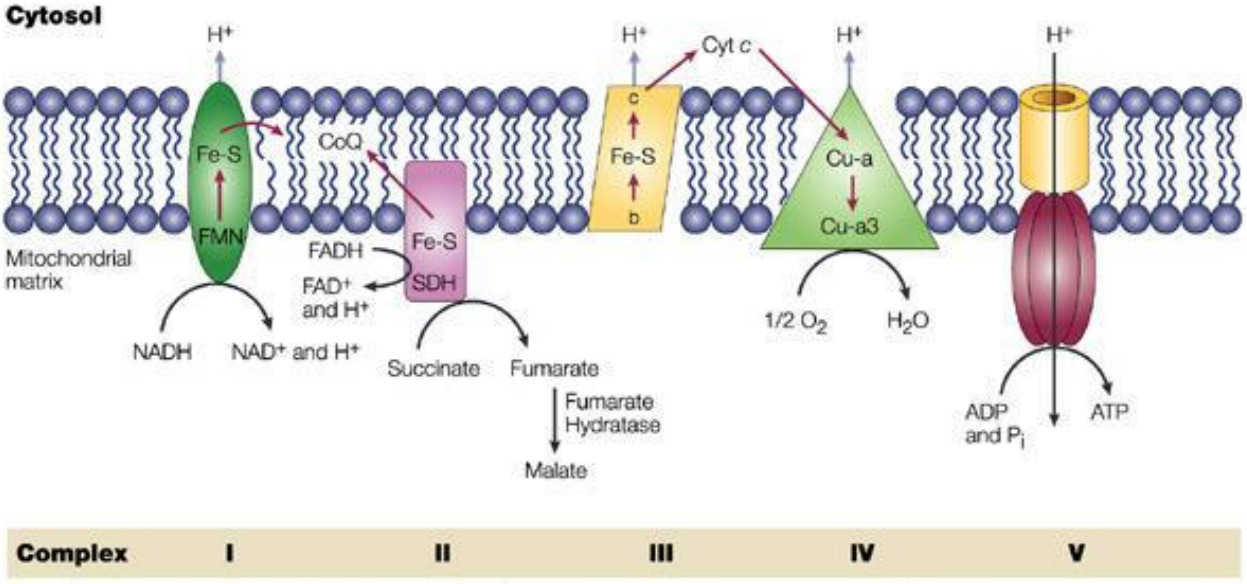
- Now the electrons that come from the citric acid cycle by NADH and FADH2 should not have the same acceptor. Logic wise, two different materials have two different binding sites.
- There are 5 complexes (the fifth is the ATP synthase) in the ETC.. Complex 1 is an acceptor for NADH electrons and NADH binds to it , Complex 2 is not an acceptor for FADH2; it is the one which contains FADH2.
- Imagine the citric acid cycle which occurs in the matrix, it is connected to ETC through step 6 of the cycle which is the transformation of succinate to fumarate catalyzed by succinate dehydrogenase, and this enzyme makes complex 2.
- Complex 2 is the only direct physical connection between the citric acid cycle and the electron transport chain (they are all connected functionally). So when electrons come out from succinate ,they will be loaded on FAD within the succinate dehydrogenase and will stay there in complex 2.



- Now Complex 1 is an acceptor for NADH, it will get the electrons (with the hydrogen) out of NADH and oxidize it into NAD+. It takes the hydrogen, and the substrate is NADH ,so the complex is named <u>NADH dehydrogenase.</u>
- Complex 2 is <u>succinate dehydrogenase.</u>
- So those are the entry points for electrons loaded on NADH and FADH2 (two different complexes that are not connected at all, not functionally nor physically.)

- Then , the electrons from both complexes 1 &2 have a common acceptor which is Coenzyme Q (quinone or Ubiquinone). After complex 1 takes electrons from NADH, it will donate them to this coenzyme Q. And complex 2 that receives them from FADH2 will donate them too.
- Then Coenzyme Q will donate them to complex 3.
- All the complexes are proteins (enzymes) fixed within the membrane, they don't move. So how will electrons move between these far away proteins? They need a carrier which will carry electrons from one complex to the other .
- One of the characteristic of this coenzyme is that it should be lipid soluble in order to travel through the membrane (proteins are water soluble). So from complex 1 and 2 to complex 3 there is the ubiquinone, and from complex 3 to 4 there is cytochrome C. Cyt c is a protein (water

soluble) and is not included within the membrane, it moves on the outer surface of the membrane, takes the electrons from complex 3 and give them to complex 4.



• In complex 4, oxygen, which is the last electrons acceptor and with the highest positive reduction potential, will be converted to water.

• **<u>Can electrons be added directly to oxygen?</u>**

-No, in order to maintain life since oxygen is present everywhere . For electrons to be added on oxygen, O2 should be activated, it cannot accept electrons directly although it's the final and best electron acceptor. The simplest way for activating oxygen is to bind the O2 molecule with another thing so as the bonds between the 2 oxygen atoms will be weakened , and now the ability to accept electrons is there .
-A common way to bind oxygen in order to activate it is to bind it to heme. Within complex 4 there is a heme structure. Heme cannot bind oxygen except when it is reduced (which means that iron within the heme should be reduced not oxidized, it should be in the ferrous state Fe^{+2}). This is why we need electrons to move, so that they will reduce heme when they reach complex 4, and when heme is reduced it can bind oxygen, and when oxygen is bound to heme it is activated as its electronic status will change ,

then electrons move from heme to the oxygen easily making it more negative , so it will bind hydrogen ions and form water .

- Until this step when water is formed ,there is no ATP generation, so the process of electron movement (through the ETC until oxygen reduction) does not involve ATP generation (this is only the Oxidative part of the process oxidative phosphorylation). Because of the electron movement there will be pumping of protons using the energy difference each time, those protons will exert a pressure against the inner mitochondrial membrane and then they will come back through ATP synthase complex that makes ATP. *The process of making ATP is different from electron movement ,however both processes are coupled to each other*, if you are moving electrons you should generate ATP at the end. The electron movement includes oxidation-reduction reactions, for example :when electrons move from NADH to complex 1 they reduce it and NADH is oxidized, then from complex 1 to CoQ which will be reduced and so on until the end of the chain.

- The phosphorylation part occurs at complex 5, the generation of ATP is due to the flow of energy (flow of protons upon their electrochemical gradient), and it is not an oxidation-reduction reaction.

- This oxidative phosphorylation process cannot logic wise be uncoupled , because as long as you have a flow of electrons there should be ATP generation at the end, however it can be uncoupled using uncouplers that we'll talk about in the next lectures.

How electrons are moving within proteins? How electrons are moving within complex 1 for example?

They need certain carrier to hold them within the complex itself, so there are carriers in every complex(1,2,3,4)

-The electron carriers we know within proteins are:

1) FADH2 and it is present in complex 2 (succinate dehydrogenase)

2) FMN **in complex 1** (flavin mono nucleotide) without the adenine part that Is present in FAD (refer to the structures) , so we have only one nucleotide which is riboflavin, it does the same function of FAD; electrons will come to FMN from NADH.

3) iron- sulfur clusters either 2 sulfurs with 2 irons, 4 sulfurs with 2 irons (we won't talk about them, just be familiar with the name). (found in complex 1,2 and 3)

4) Heme structure can move electrons within proteins. Iron in the Heme will be reduced then oxidized, then reduced then oxidized (ferrous, ferric , ferrous , ferric)…moving just one electron at a time .

So those structures are found in proteins and responsible for the movement of electrons within proteins.

Note :

NADH is an electron carrier but **not** within proteins, it works freely in solution

Types of electron transfer:

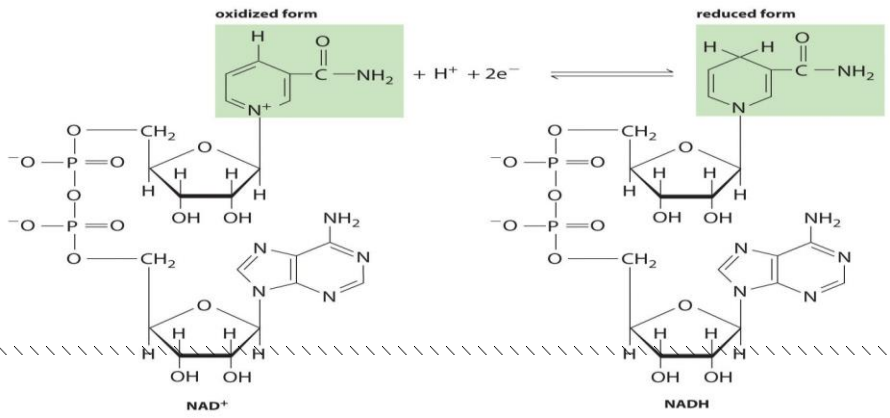
How electrons are moving from one structure to the other?

1) Direct electron transfer: and this occurs in heme where there is only one electron moving by itself directly to the heme without any other structure added.

2) Hydrogen atom**:** such as what happens in FMN and FAD where 2 hydrogen atoms (with 2 electrons) are transferred .

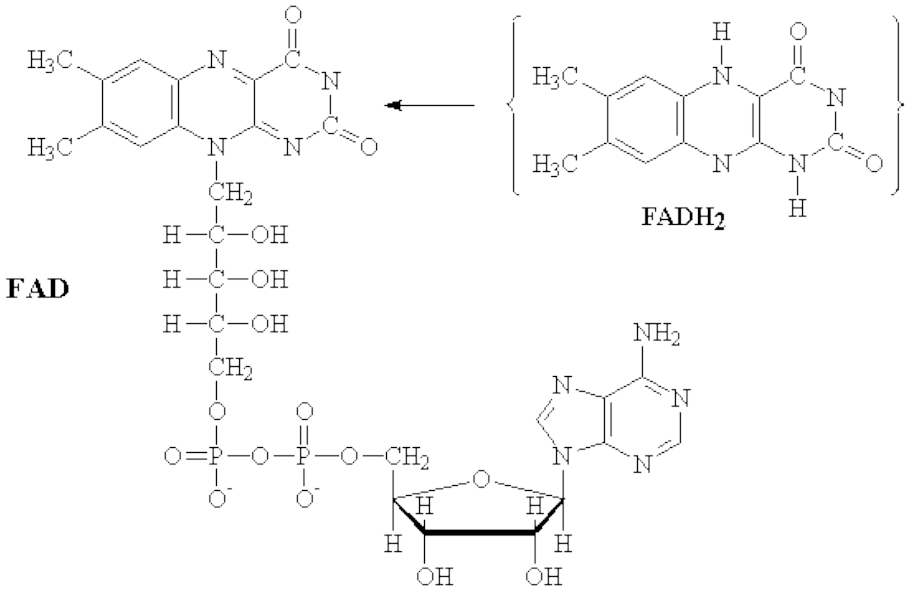
3) Hydride ion: and this occurs in the case of NAD+ where a hydride ion (hydrogen with 2 electrons) is transferred, and NAD+ becomes NADH.

** Look at NAD+ structure (niacin and adenosine) and notice that



when it becomes NADH, electrons are added as a hydride ion on the **Nicotinamide ring.**

**Notice the structure of FAD (flavin and adenine) and the attachment of 2 electrons as 2 hydrogen atoms on the nitrogens of the flavin ring.



*We talked about the difference between NADH and FADH2, now we'll talk again about the difference between NADH and NADPH.

- The difference is in the attachment of a phosphate to carbon 2 of the ribose ring (it has nothing to do with the addition of electrons to that structure ,as electrons are not added on the ribose), if there was a phosphate group then it is NADPH and *it is usually used for anabolic reactions (lipid metabolism especially)* . if it was a hydrogen atom then it is NADH and it is used in energy metabolism. And neither of them can cross the inner mitochondrial membrane , both of them are electron carriers but differ in localization for better regulation .

***Flavoproteins:**

- **FAD** has 2 nucleotides while FMN has only one nucleotide (doesn't have adenine)
- As the flavin ring is present in both , they transfer electrons using the same mechanism.

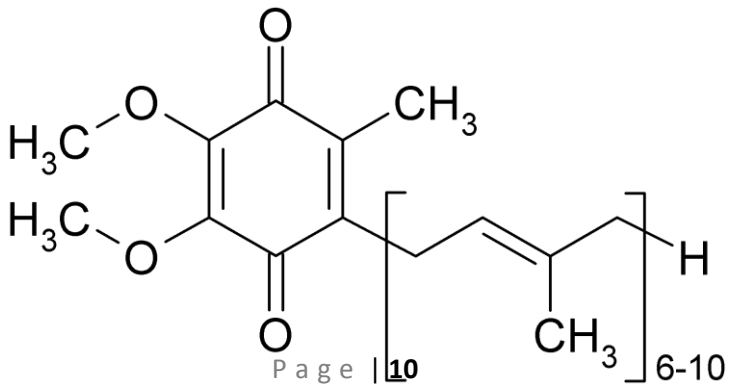
***Ubiquinone (or quinone or Coenzyme Q) are** coenzymes, all synonyms for the **same structure.**

To name any molecule as a (quinone) ,the structure should have a ring and two double bonds with oxygen (C=O), cyclic diene structure.

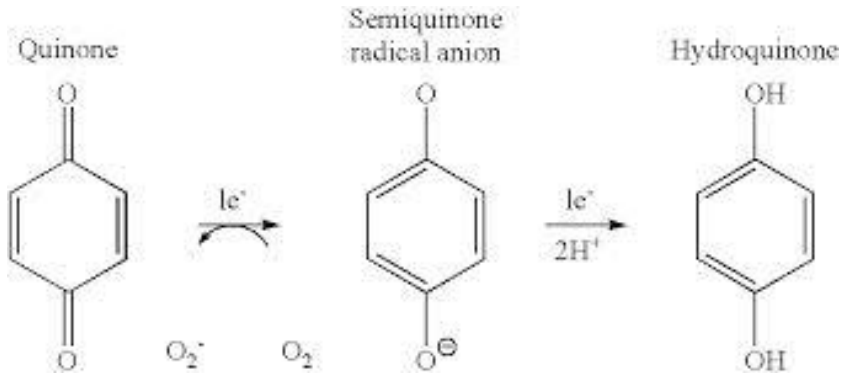
- The function of ubiquinone is to carry electrons from complex 1 to 3 and from complex 2 to 3, so the structure should have the ability to accept and donate electrons so it should be water soluble. However it should move in the membrane so it should be lipid soluble at the same time.

• So when we look at the structure of CoQ ,we see the ring with the 2 oxygen double bonds (2 ketone groups)which make it hydrophilic. And there is a part attached to it which is called isoprene unit repeated from 6 to 10 times (a long unit), and this makes the structure lipophilic , so that it can move through the membrane.

*So : the part that carries electrons is the *ring* , and the part that attaches it to the membrane is *the repeated isoprene unit* .



- The 2 oxygen atoms are the place of electrons attachment. When there are no electrons and they are just oxygens it is called a **Quinone** (ketone , fully oxidized).
- When only one electron is added to the structure, it is a **semiquinone.** If you look to the structure of the semiquinone , you will find one of the oxygens is reduced to OH (the electron is added to it making it more negative and able to bind protons, so it becomes OH) which causes changes in the electronic status of the ring making the other oxygen a free radical . It is the dangerous one which has free radical oxygen. So the structure of quinones is similar to FAD since both of them can generate free radicals with one electron reduction.
- When 2 electrons are added, the structure is reduced and the ketone is converted back to alcohol, so it is then called a **Quinol** (From the word alcohol).



Best wishes ☺