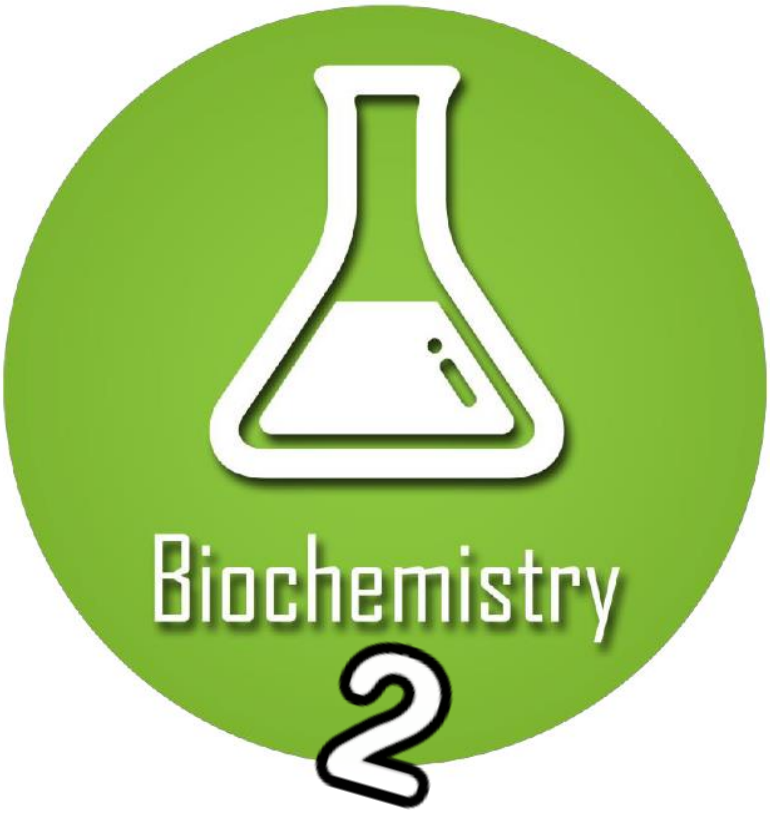
2014 2020
Medical Committee
The University Of Jordan



◉Sheet ○Slides

Number: 11

Done by: Murad Dweik YakBIII

Corrected by: Noor Isbeih

Subject: Oxidative Phosphorylation

Doctor: Nafith Abu Tarboush

22/10/15

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In today's lecture we're going to continue studying about the oxidative phosphorylation process. But first let's quickly recap what oxidative phosphorylation is:

It is the process by which ATP is formed as a result of the transfer of electrons from NADH/$FADH_2$ to O_2 by a series of proteins and coenzymes. This process takes place at the Inner Mitochondrial Membrane (IMM) and is responsible for about 90% of the total ATP synthesis in cells.

Complex II: "Succinate Dehydrogenase"

- It's a flavo-protein and it's the only link between Kreb's Cycle and the Electron Transport Chain (ETC)
- FAD is tightly bound (By Covalent bonds)
- Entry site for $FADH_2$
- A member of the Oxido-reductase family (Oxidizes FADH2 and reduces Co-Q).
- It's the only peripheral protein with no transmembrane channel for proton pumping, therefore ZERO protons are pumped to the intermembrane space.
- Transfers two electrons from $FADH_2$ to CO-Q, converting it to QH_2.
- It contains : i) Fe-s clusters (non-heme protein)
  ii) Coenzyme Q (Free in membrane)

Complex III: "Cytochrome BC1"

- Contains 2 Cytochromes (Hemes): B (containing BH & BL) and Cytochrome C_1
- Also a member of the Oxido-reductase family (Oxidizes QH_2 and reduces cytochrome c)
- Receives electrons from Complexes I and II through Ubiquinone, which comes as reduced molecule with 2 electrons (QH_2) and donates them to Cytochrome C.
- It has 2 binding sites for Co-Q, one close to intermembranous space, and the other close to the matrix.

- Qh_2 binds to the site with affinity to the reduced Ubiquinol, donating 2 electrons, meanwhile 2 protons (H+) will be pumped to the intermembranous space.
- The 2 electrons will go through 2 different pathways which will be explained next in the Q-cycle:

The Q-cycle:

The Co-Q comes to complex III (from complex I and complex II) in its reduced state as ubiquinol (QH2). It gets oxidized to Q and loses two H+ and two electrons.

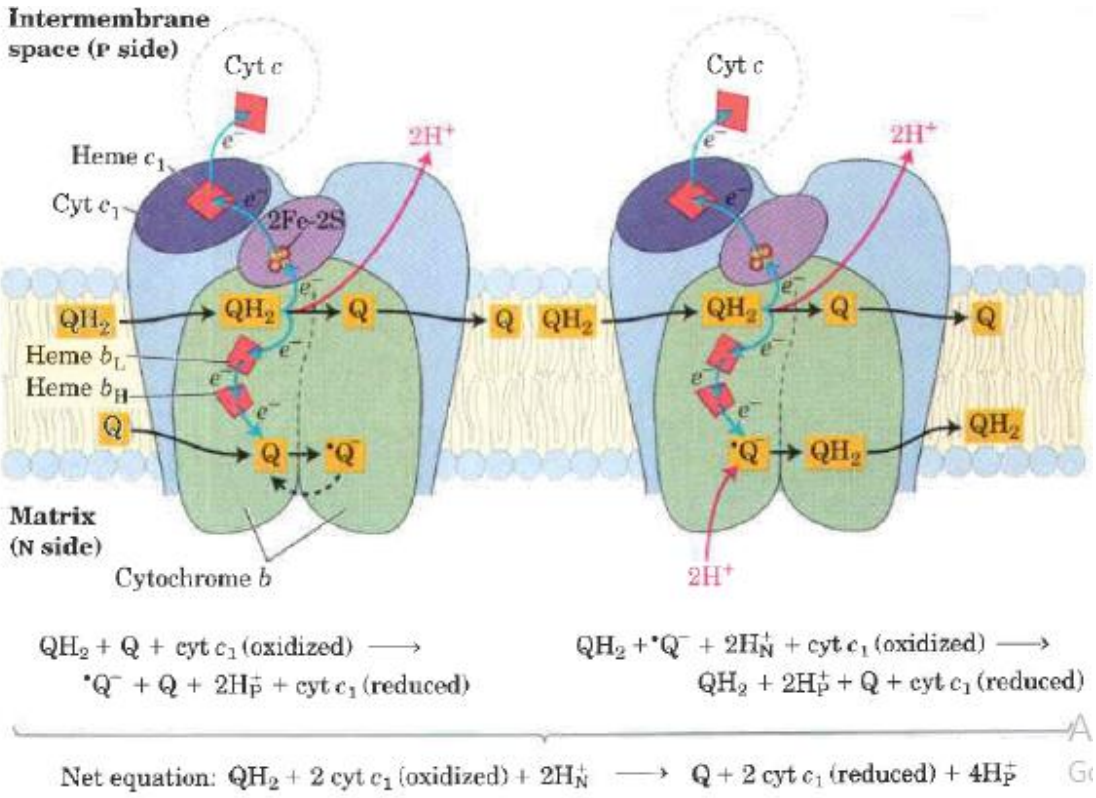
- The two protons from the QH2 will be released into the intermembrane space directly.

- One electron is transferred to the Fe-S cluster, then to cytochrome heme c1 then to cytochrome c (which is capable of carrying only one electron) then it will transport the electron to Complex IV.

The other electron from the reduced Ubiquinol will go downward in the complex to cytochrome heme BL then to heme BH, reaching a site with high affinity for oxidized Quinone (which has no electrons). It then finally reaches the oxidized ubiquinone (Q) at the cytoplasmic side of the membrane, converting it to a semi-reduced (semi-oxidized) Quinone (.QH). This is the first part of the cycle.

The second part of the cycle is very similar to the first part, again another QH_2 molecule reaches the outer side of the complex, and 2 protons get pumped to the intermembrane space. The QH_2 gets oxidized to Q, one electron goes through the Fe-S cluster, to cytochrome C_1, and then to Cytochrome C, which transports it to complex IV. And the other electron is transferred down the complex to cytochrome b containing heme BH to heme BL and finally reaching the semi-reduced Ubiquinone (QH) converting it to a reduced ubiquinol (QH_2).

- (Note that the oxidized Q and the semi-oxidized Q acquire protons from the matrix in order to be converted to QH and QH_2.)



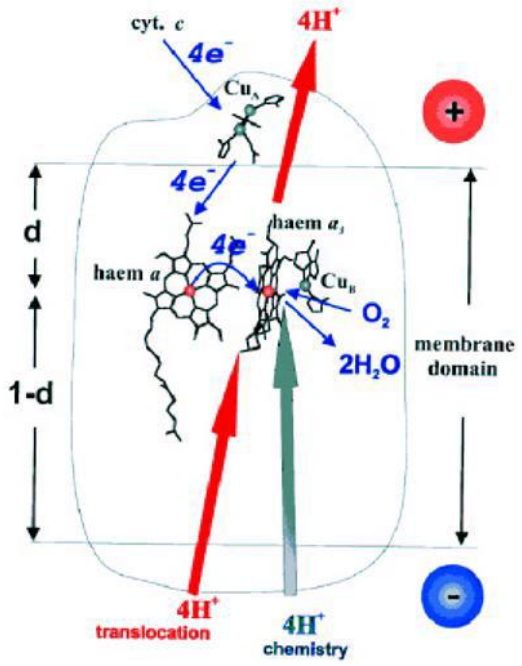
The net result of Q-cycle is:

- ✓ Two QH2 were converted to Q and one Q was converted (regenerated) to QH2 at the end of the second cycle, so the total is that **one QH2 was converted to Q.**
- ✓ **Four protons** were pumped to the intermembrane space; two protons per QH2.
- ✓ Four electrons were used in the cycle, Two electrons were transferred to complex IV in a sequential manner (each electron is carried by one cytochrome C), while the other two electrons were used to reduce the Q to QH_2 in order for it to be used again in the cycle. So therefore we have a net of **Two electrons** to complex IV

Complex IV: "Cytochrome C oxidase"

- It's the enzyme which oxidizes cytochrome C.
- Remember that each cytochrome contains Heme/s and cytochrome C contains Heme c which is able to carry one electron only. (So 2 electrons = Two Cyt c).
- Complex I =FMN and Fe-S clusters.
- Complex II=Fad.
- Complex III=Fe-S and hemes C and B (two Bs).
- Complex IV=Contains 4 coenzymes/co-factors that are able to get oxidized or reduced : Cytochrome hemes a & a3 (the electron acceptors) , and 2 copper atoms (Like Iron, copper can go into 2 oxidation states Cuprous and Cupric, Cu^{+1} and Cu^{+2}).
- Complex IV also has a site to bind oxygen.

Electron movement through complex IV:



CuA → heme a →heme a3+ CuB

After cytochrome c donates its electron to Cu_a, the electrons are transferred to Heme a, but since these two enzymes are not so near to each other, the electrons move through them in a sequential manner, whereas heme a3 and CuB are very close to each other therefore they form one unit and act as one centre in order to share the electrons with each other.

So both of CuB and heme A3 work as one oxidation-reduction center.

NOTE #1: Both copper and heme can accept only one electron. Therefore since we have 4 coenzymes we need $4e^-$ (electrons)

NOTE#2: The heme needs to be fully reduced in order to bind the Oxygen, since oxygen can't bind to an oxidized heme. There is an enzyme surrounding the heme which keeps the heme reduced. If this enzyme is absent for any reason (such as genetic, or cases of poisoning), the heme won't be reduced anymore and therefore oxygen can't bind to it. This will result in a case of anemia known as Methemoglobinemia (which means oxidized hemoglobin which is unable to bind to oxygen).

How does the oxygen reach complex IV?

The oxygen initially comes from **respiration** (as free O_2) and gets in the lungs, then binds to **hemoglobin** (in the circulation), then to **myoglobin** (in the cytoplasm of tissue cells) and finally to **mitochondria** (cytochrome c oxidase "complex IV")

- This order takes place due to affinity differences, Haemoglobin has higher affinity than free oxygen therefore it binds to it.
- Myoglobin has a higher than haemoglobin therefore it binds oxygen.
- And finally the final oxygen acceptor complex IV has the highest affinity (higher than myoglobin) therefore oxygen binds to it.

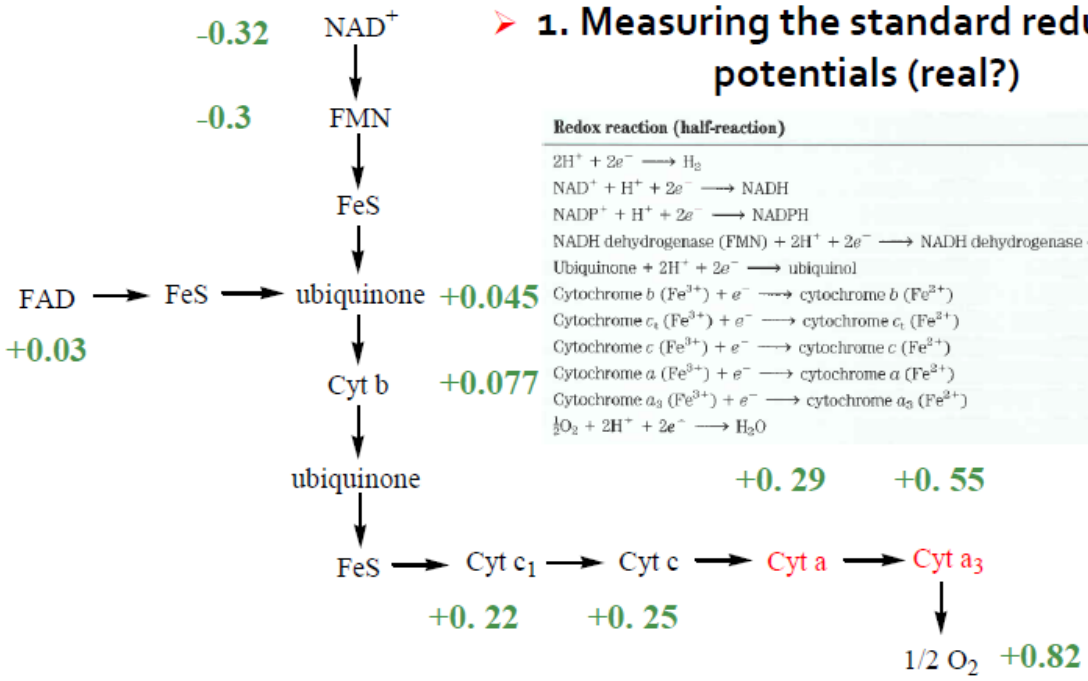
NOTE#3: Km works in an opposite way to affinity, as we go from respiration to mitochondria the km decreases. Therefore km changes from higher to lower until the oxygen reaches its final acceptor, complex IV.

The arrangement of the ETC:

Electrons are continuously moving NAHD in complex I to Co-Q, to Cytochromes c and c1 in complex III, to cytochromes a and a_3 in complex IV, and finally reaching the oxygen which is the final electron acceptor, but how do we know that this arrangement is right? We've got 3 methods:

1. First method is by measuring the **standard reduction potentials.** This is done by measuring the reduction potential of each component in ETC starting from NADH at complex I and ending at the oxygen at complex IV. From numbers that had been obtained, it was found that electrons move from the most negative reduction

potential to the most positive reduction potential. It is not a must for cells to follow this arrangement, since the values we have were obtained under standard conditions in labs, we don't really know how the conditions in the Mitochondria are therefore we can't really tell if these values are true or not. That's why we need other methods to find out whether this arrangement is correct or not.



➢ 1. Measuring the standard reduction potentials (real?)

Redox reaction (half-reaction)	E'° (V)
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.8166

2. Second method is by the reduction of the entire ETC with no O_2. What do you think will happen if an electron source reaches the cycle, but under anaerobic conditions (No O_2)??
   Well the cycle would go on, but only once. Each complex would get oxidized and reduced as electrons pass through it, except complex IV which can't get oxidized after being reduced, since there is no O_2. And that means the cycle won't be able to happen again.

NOTE#4: how do we know if the proteins (complexes) get oxidized or reduced?
Each protein has certain spectroscopic features. All the proteins contain electron transfer centers (such as heme, iron-sulfur clusters or flavoproteins) within their structure, and this center when subjected to light on the spectrophotometer shows a certain band, and this band is changes whenever the center is oxidized or reduced.

3. Third method is by the addition of inhibitors:

<u>NOTE#5:</u> There is a specific inhibitor for each complex.

- What do you think would happen if an inhibitor was added to complex I???
  The answer is that electrons won't be able to pass through complex I which means that complexes III & IV will remain oxidized as there are no electrons passing to reduce them, (there are some restrictions to this as we have another entry point for electrons which is $FADH_2$ through complex II), but if the source of electron is only NADH (in the experiment) then the whole cycle will stop.

- If an inhibitor was added to complex III what would happen?
  The answer is that electrons pass through complexes I & II and reduces them, but complexes III & IV remain oxidized.

They kept trying this experiment by inhibiting a different structure each time, anything coming before the inhibited point should be reduced, anything coming after should be oxidized, and eventually it was proven by both methods 2 & 3 that the standard reduction potential in this case was informative about the right arrangement of ETC.

Pumping of protons:

- ✓ For every two electrons transported by NADH we pump 4 protons (4H+) from complex I and 4 protons (4H+) from complex III and 2 protons (2H+) from complex IV.
- ✓ And for every two electrons transported by $FADH_2$; 4 protons (4H+) will be pumped out from complex III and 2 protons (2H+) from complex IV, so the total is 6 protons (6H+).

ATP Synthase:

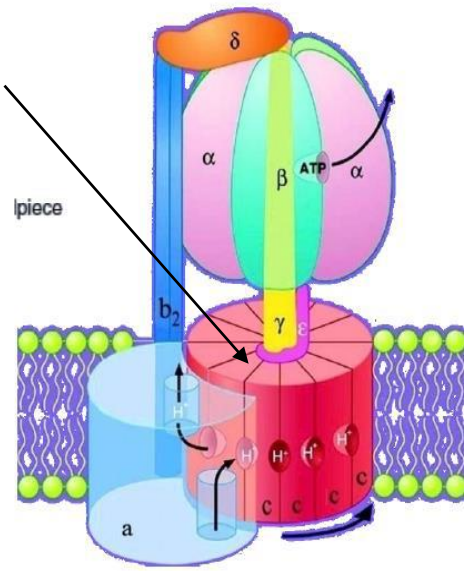
- ➢ It is a complex protein composed of too many subunits that we must be familiar with.
- ➢ Each subunit has an important function. ATP synthase is a transmembrane protein that is composed of 2 parts :

1. One is within the membrane we call it (F0).
2. The other piece is objected toward the matrix and we call it (F1) or (headpiece).

- The piece which is within the membrane looks like a cylinder (C-subunits) and it rotates inside the membrane (there are 12 C-subunits), attached to it from one side is a C-like shaped domain (a - subunit), both of these components are within the membrane.
- In the middle of the cylinder (C-subunits) there is stalk that contains a polypeptide chain called (ɣ-subunit) this subunit is **curved** (not straight), it passes through the headpiece (F1).
- The headpiece (F1) is composed is 6 subunits (3α + 3β) and the sequences (α, β, α, β, α, β), the (3α) are for structural reasons they function as a support to keep the protein in its shape, (β-subunit) are the ones responsible for the catalytic process of forming ATP.

What happens is that the a-subunit has a pore at intermembranous space, where the concentration of H^+ is very high, so there will be a concentration gradient that drives the proton towards the pore. The proton will then face one of the C-subunits, at the C-subunit we have a glutamic acid residue which is negatively charged (COO^-), which will result in the neutralization of the charge, this will cause a conformational change resulting the rotation of C-subunit facing the opening (it moves away, letting another C-subunit with negatively charged glutamic acid residue face the opening). This cycle is repeated until all the 12 C-subunits have been neutralized and rotated.

On the a-subunit there is another pore/opening for exiting protons into the matrix. When a proton reaches that point through its rotation, the **pKa** there is different so it will cause the release of that proton to the exit point, bound glutamic acid will be back to its negative charge form, and this is how the cycle continues.



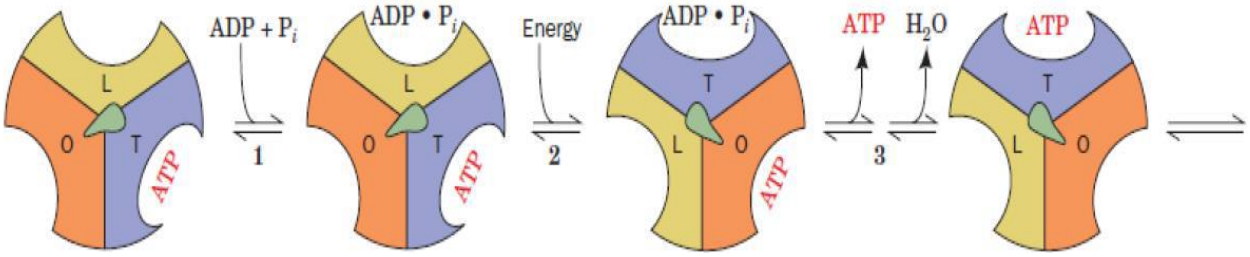
How is ATP generated?

When the C-subunits rotate within the membrane the ɣ-subunit will start rotating as well (since it is attached to them), and because it's angled; through the rotating it starts hitting the β-subunits.

NOTE#6: if the stalk was straight there will be No use of it as it wouldn't be able to hit the β-subunits.

Every hit to one of the β-subunits causes conformational change in all the β-subunits (it's a protein and hitting it will cause conformational changes which change its shape), every β-subunit can go into 3 states of conformational changes: it can be open (O), loose (L) and tight (T).

- When it's loose it can **accept** ADP+Pi and when the ɣ-subunit hits it, it causes a conformational change to make it tight.
- Tight conformation will **enclose** the ADP+Pi to make ATP, once ATP is formed the ɣ-subunit hits the β-subunit again converting it to be open.
- The open conformation **releases** the ATP.one more hit will make it loose again and bind to ADP, another hit will make the active site of β-subunit smaller so it will enclose the ADP+Pi it will be tight forming ATP, one more hit it will be open and it will release the ATP, and this is how the cycle continues this way.



Here's a brief summary to what happens in ATP Synthase:
(C-subunits rotate → ɣ-subunit rotates → ɣ-subunit hits β-subunit because it's angled→ rotation will result in hitting the first, second & third β-subunit → causing each β-subunit to go into 3 conformational changes (Loose, Tight, and Open).

<u>Note#7:</u> for every 4 protons coming through, we produce 1ATP molecule. And since we have 12 protons going into ATP synthase causing 12C-subunits to rotate, therefore we produce a total of 3ATP per cycle.

(For each 4 protons→ 4 shifts/rotations in the c-subunits → rotation of the ɣ-subunit **once** to hit one β-subunit causing conformational changes in **all** of the 3 β-subunits → release one ATP)

As any other enzyme, ATP synthase needs to be able to work backwards, it can catalyse the forward and backward reaction. Which means if you have high concentration of ATP within the mitochondria, and low concentration of H+ in the intermembranous space then the enzyme will run backward? How does this process take place? Simply the protons will

start to come back through the a-subunit pore causing the c-subunit to rotate in the other way around and protons will come out from the other side. It will start degrading the ATP, so it will be called **ATPase.** By breaking down the ATPs, protons will be pumped back to the cycle, so they can come into the intermembranous space.

Energy Yield from the ETC:

- Transfer of electrons from NADH to the oxygen results in: 53 kcal
- Transfer of electrons from FADH2 to the oxygen results in: 41 kcal
- Energy efficiency of TCA cycle was 90%.

Efficiency for ETC:

- Each NADH gives → 2.5 (ATP) → 2.5 x (7.3) → 18.25

Calculating the efficiency: (18.25÷53) = 0.34 = 34%

- Each FADH2 gives → 1.5 (ATP) → 1.5 x (7.3) → 11

The efficiency: (11÷41) = around 0.25 = 25%

- This shows that the efficiency of the ETC is much lower than Kreb's cycle (which is the best machine).

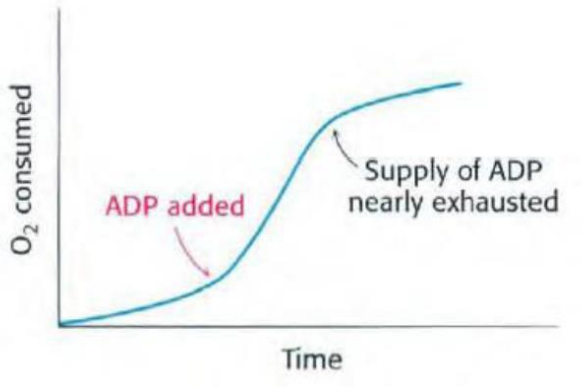
* Where is the lost energy from ETC used?

It's used to exchange ions/ADP/protons across the inner mitochondrial membrane (such as Ca^{2+}) since the membrane is **impermeable** to anything. If you want to transfer anything across the membrane outside or inside, you will have to use energy. So this is where loss of energy takes place. Generation of heat also requires energy.

How is the ETC regulated?

1) The need for ATP

The main and most important regulator of the citric acid cycle is the **ADP** and maintaining its concentration is known as: **respiratory control**.
ADP is the only molecule that can allosterically activate the enzyme **Isocitrate dehydrogenase**.
We should monitor the oxygen consumption (the final e- acceptor)

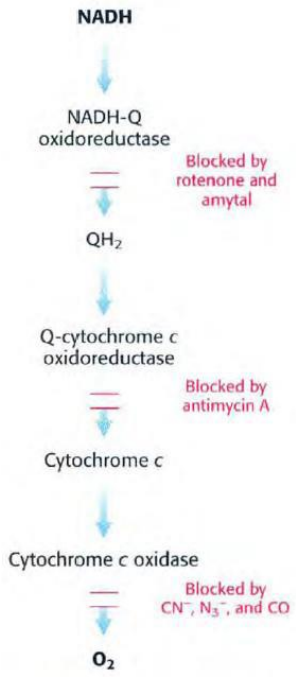


The more O2 consumption → the more the ETC is working
With decreased ADP conc. you will find the Consumption of oxygen getting slower, if you add excess ADP you will get a sharp increase in the oxygen consumption, thus in the generation of ATP. Once the supply of ADP ends, the consumption will come back to its slow process.

2) Inhibition

HINT: (The doctor said there will be a question on this for sure in the exam so watch out YakBIII)

There are certain inhibitors for each complex (I, II, III, IV, V "ATP-synthase")



- Rotenone (insecticide) + Amytal (sedative material "drug") : both are inhibitors for complex I
- Antimycin A (antibiotic) : inhibitor for complex III
- Cyanide (CN-), Azide (N_3^-), CO, all bind to complex IV and inhibit it, it's the most dangerous complex cause its responsible for respiration and it has the highest affinity toward the O2 which by default has the highest affinity for CO, that's why CO can bind to it and inhibit it.
- CO: competes with O2 (higher affinity for CO to bind and stays), haemoglobin, myoglobin and cytochrome C oxidase (complex IV).
- Oligomycin (antibiotic): inhibits the influx of H+ from the cytoplasmic side toward the matrix. (ATP Synthase).

Cyanide: there's a compound called cyanide glycoside found in fruit seeds like peach and Apricot. It can be toxic if taken in high concentrations, so you have to be careful of mixtures that contain them. In Jordan the father of hanin and hani put cyanide in their milk and killed them within 10 mins☹, Also Hitler and his girlfriend committed suicide using cyanide. (LOL☺).

If you stop the e- movement, you will stop both pumping of protons and ATP synthesis.
Electrons can still move without generating ATP- this called Uncoupling.
But you can't stop the e- movement and still make ATP. So when you stop the e^- movement
Through any of these inhibitors you will result in stopping the process of ATP generation.

3) Chemical uncouplers:

If we have a certain molecule which can pass through the membrane to bring back H+ from outside to the inside without passing through the ATP-synthase, the ETC will keep working because there is e^- movement and oxygen will be reduced to water.

- The phosphorylation and oxidation are coupled together to make ATP
- Mechanism of Uncoupling: Protons are being pumped out through a leak in the membrane without ATP generation.

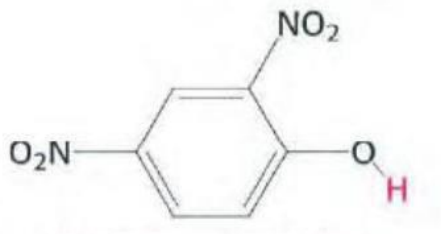
If you are eating carbohydrates, lipids, proteins they get broken down in your body at the end to produce ATP to build up your body.

In the case of uncoupling you are pumping out H+ and its coming back through the membrane without passing through the ATP-synthase (through uncoupling proteins) so there will be NO ATP, (instead of ATP generation there will be heat generation) so the person won't build up his body instead he will start degrading the compounds he/she ate, so uncoupling process works as anti-obesity.

- Note that the uncoupling proteins differ from the inhibitors, as the inhibitors stop the oxidation process while the UCPs don't do that they only uncouple the oxidation from the phosphorylation.

Dinitrophenol:

This is used commercially in drug production. Ex: Dinitrophenol (DNP) drug was used in the America and in 1930 this drug was prevented, it was used by girls in order to be thin and by soviet soldiers in world war II in order to be able to Telerate the snow in Siberia.

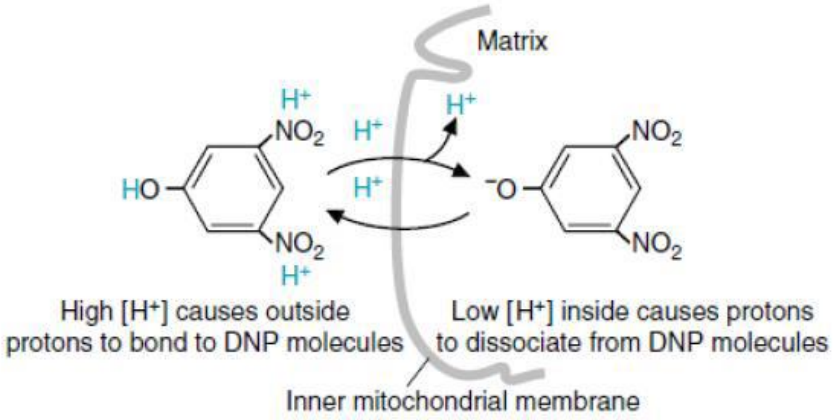


2,4-Dinitrophenol (DNP)

The drug was banned in 1938 in America, because it started causing malignant hyperthermia, problems in eyes and death by causing very high fever (high body temperature). The problem was that the increase in temperature wasn't related to the dose of the drug or the machinery process (process within the human) so you don't know specifically what is the dose that is within the normal rate so they banned it.

*** How does DNP work?**

DNP contains OH if we remove H it will be O^-.
When it's close to the **outer side** of the inner mitochondrial membrane it will attach to H+ (form the intramembranous space) becoming OH, it passes through the IMM. When it's close to **inner side** of the inner mitochondrial membrane it will donate H+ (to the matrix) and it will continue in this cycle (take H+ from outside to in → this won't cause ATP generation)



❖ There are natural (physiologic) uncoupling proteins in our bodies.

Uncoupling proteins (UCP) have several types: 1,2,3,4 and 5. They have tissue localization;

-**UCP 1** is called thermogenin that is found in brown adipose tissue; mostly within:

1-The **infants** (neck, breast, around kidneys) mainly to generate more heat because babies cannot cover themselves when they're cold.

2-Brown adipose tissue, non-shivering thermogenesis

- Fatty acids directly activates UCP1

- **UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)**

Mitochondrial genetic diseases (OXPHOS diseases) (Self Study)

- The most commonly encountered degenerative diseases.
- The clinical pathology may be caused by gene mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) that encodes proteins required for normal oxidative phosphorylation.

Mitochondrial DNA (mtDNA):

General Features:

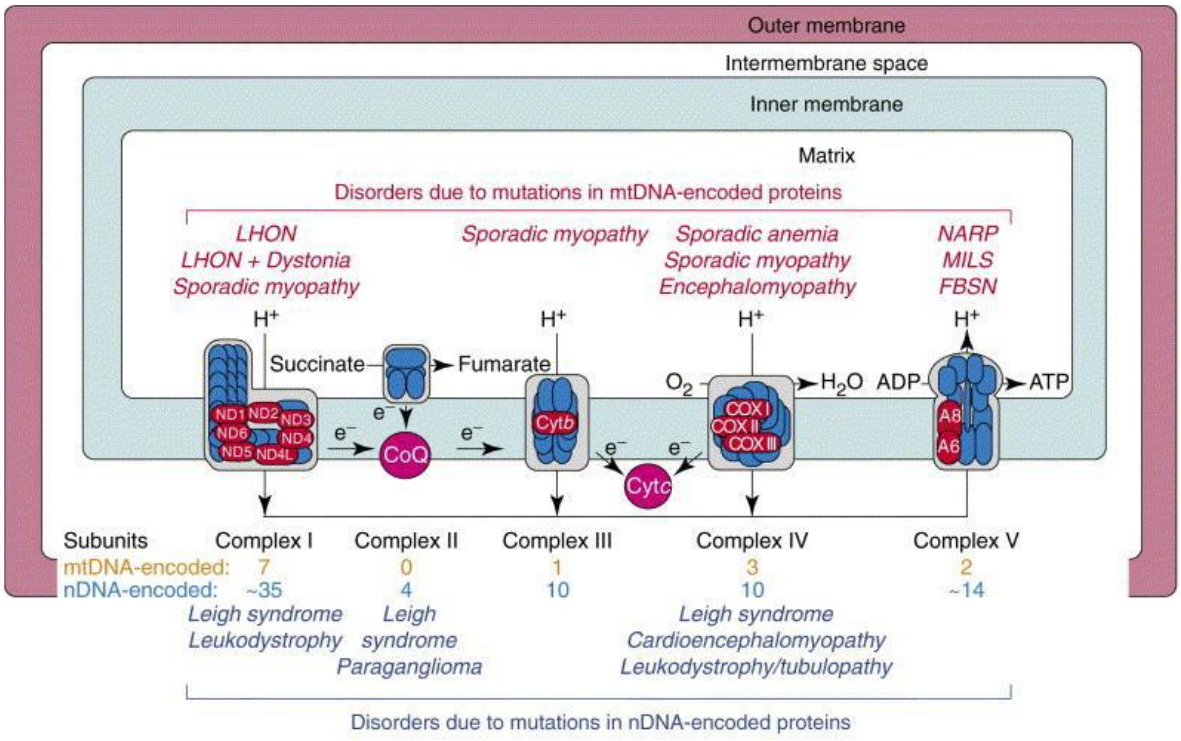
- Small (16,569) base pair, double-stranded, circular DNA
- Encodes 13 subunits: 7 (I) , 1 (III), 3 (IV), 2 (F0)
- Also encodes necessary components for translation of its mRNA: a large and small rRNA and tRNAs.
- Maternal inheritance, replicative segregation & heteroplasmy
- Accumulation of somatic mutations with age
- Threshold expression: at some stage, the ATP-generating capacity of a tissue falls below the tissue-specific threshold for normal function due mutations.
- Symptoms and defects appear in the highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver.

Replicative segregation & heteroplasmy?

As cells divide during mitosis and meiosis, mitochondria replicate by fission, but various amounts of mitochondria with mutant and wild-type DNA are distributed to each daughter cell (replicative segregation). Thus, any cell can have a mixture of mitochondria, each with mutant or wild-type mtDNAs (heteroplasmy). The mitotic and meiotic segregation of the heteroplasmic mtDNA mutation results in variable oxidative phosphorylation deficiencies between patients with the same mutation and even among a patient's own tissues.

Nuclear DNA (nDNA):

- Most of the estimated 1,000 proteins required for oxidative phosphorylation are encoded by nDNA.
- Usually autosomal recessive
- Expressed in all tissues
- Phenotypic expression with high ATP demand.

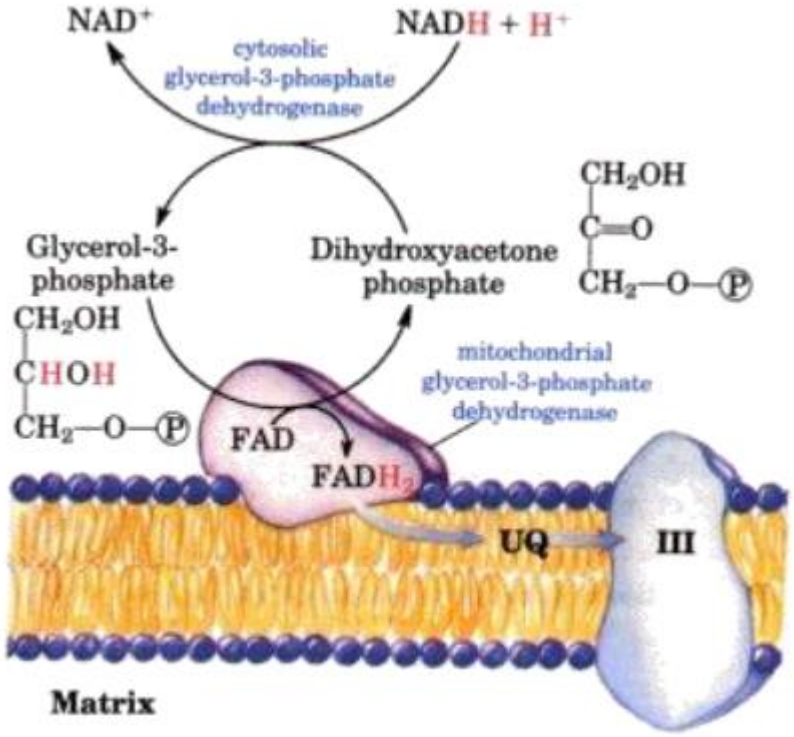


Shuttling systems:

The electrons in the cytosol there is no use for them, unless they are moved into the mitochondria through shuttling system.
There are two shuttling system:

1. glycerol-3-phosphate shuttle:

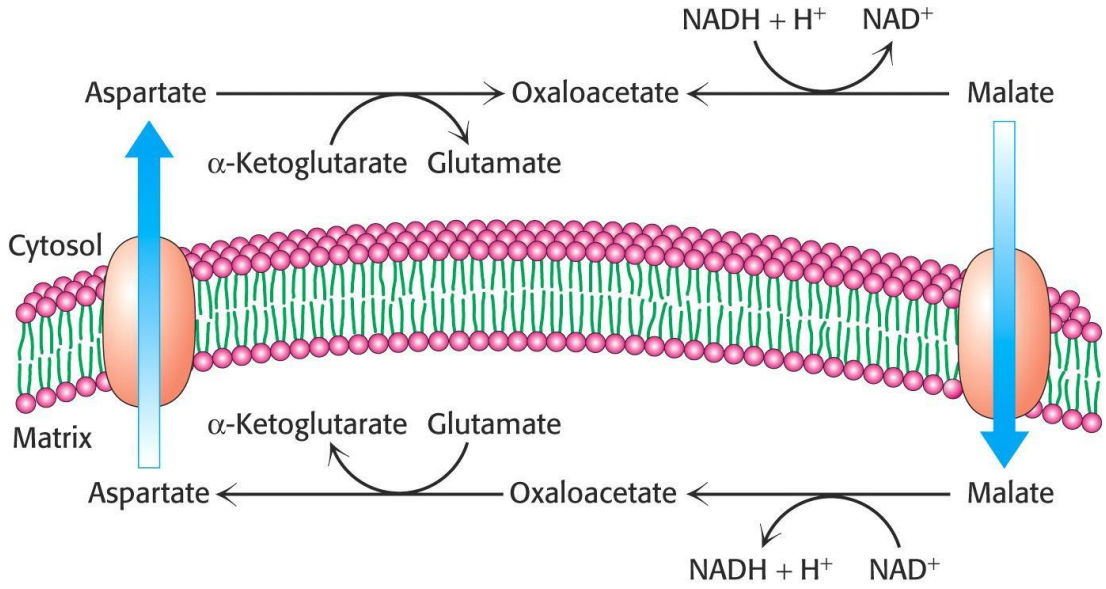
We have an enzyme called glycerol-3-phosphate dehydrogenase, it have two copies:
-Cytosolic copy
- Mitochondrial copy (which is found on the outer surface of IMM).
This enzyme contains FAD (flavo-protein).
We have NADH in cytosol, the cytosolic copy of the enzyme converts [dihydroacetone phosphate → glycerol-3-phosphate] by consuming the electrons in the NADH. Now glycerol-3-phosphate can pass through the outer mitochondrial membrane reaching the inner mitochondrial membrane, where there is the mitochondrial copy of the enzyme, which can convert it back (in a reversible reaction) to dihydroacetone phosphate generating back the two e- The form of $FADH_2$. $FADH_2$ will pass the e- to Ubiquinone (UQ) and Then to complex III and IV. So the NADH from the cytosol if it Comes through the glycerol-3-phosphate shuttle it will be converted to $FADH_2$ to Ubiquinone to complex 3 and 4, so it'll give you 6 protons only, because it used a FADH source.



2. malate-aspartate shuttle:

Malate has a certain shuttle to pass it from the cytosol to the matrix of mitochondria. Aspartate can pass from the matrix toward the intermembranous space. We said in TCA cycle; malate is converted to oxaloacetate (this converting gives NADH) so if you have excess NADH in the cytosol, you can form malate and transport it to mitochondrial matrix where it will be converted to oxaloacetate resulting in a NADH molecule.

So if you get the NADH which is in the cytosol through the aspartate-malate shuttle you are regenerating this NADH within the matrix and thus generating **10 protons**.



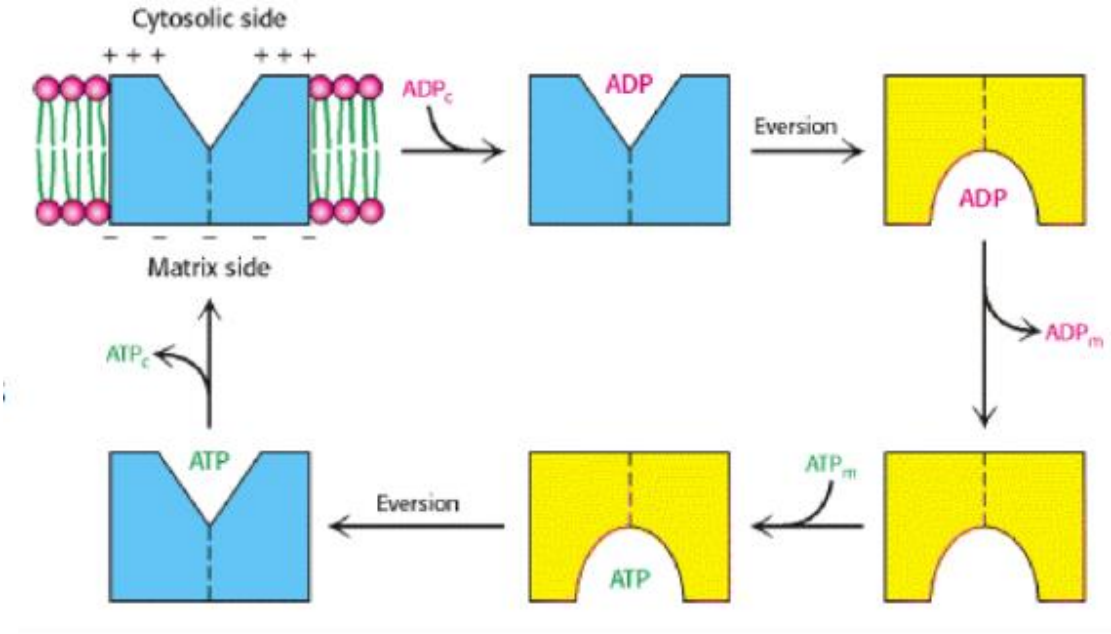
3. ADP/ATP translocase:

It's called translocase or adenine nucleotide translocase (ANT). When the ATP is synthesized it's located in the matrix of the mitochondria, in order to it to work in anabolic processes within the cytosol you have to translocate it (move it from matrix to cytosol). Energy is needed to get it out.

Because you don't want to disturb the ratio between ADP and ATP, the shuttle shape is opened first to inside and second time to the outside. Inside you have ATP; it will enclose the ATP and open it in the outside, releases ATP and gets ADP from outside

And open it inside.

- Per every ATP going out there should be one ADP getting in (1:1 ratio).
- This method is found in high amount to get ATP outside.
- 14% of the proteins of the inner mitochondria membrane are ANT.



This is it for today's lecture...

Excuse me for any mistakes as this is the first sheet I make, good luck to everyone and you're all YakBIII ☺