Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 13

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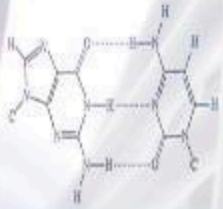
Biochemistry



Majida Al-Foqaraa'

بسم الله الرحمن الرحيم

Oxidative Phosphorylation 3

* Complex IV:

It's called Cytochrome C oxidase→ it oxidizes cytochrome C

*** Structure:**

Two copper atoms: - Cu_a : electrons come to it firstly

- Cu_b : electrons come to it secondly

- There are **two** oxidation states for copper; Cu^{1+} (cuprous) and Cu^{2+}(cupric).

Two heme groups: - heme a

- heme a3

- Both copper and heme can accept one electron only.

Cytochrome a donates its electrons at first to Cu_a, then they move to heme a. Cu_a and heme a are far from each other to some extent, so electrons have to translocate through these sights sequentially. On the other hand, Cu_b and heme a3 are close to each other (they can share the electrons).

Complex IV reduces O_2 converting it into two H_2O molecules, this conversion process requires: 4 electrons ($2e^-$ for each oxygen atom).

Through the ETC, each electron carrier (NADH, $FADH_2$) gives off two electrons only, so the cycle must repeat itself twice with two electron carriers in order to reduce oxygen into water.

*** How does oxygen bind to complex IV?**

The oxygen initially comes from **respiration**; lungs → hemoglobin (in the circulation) → myoglobin (it's found in the cytoplasm of tissue cells) → mitochondria (cytochrome c oxidase "complex IV")

- Myoglobin has higher affinity toward oxygen than that of haemoglobin; that's why oxygen moves from haemoglobin to myoglobin.
- Complex IV has the highest affinity upon all of them, so it can be the final oxygen acceptor.

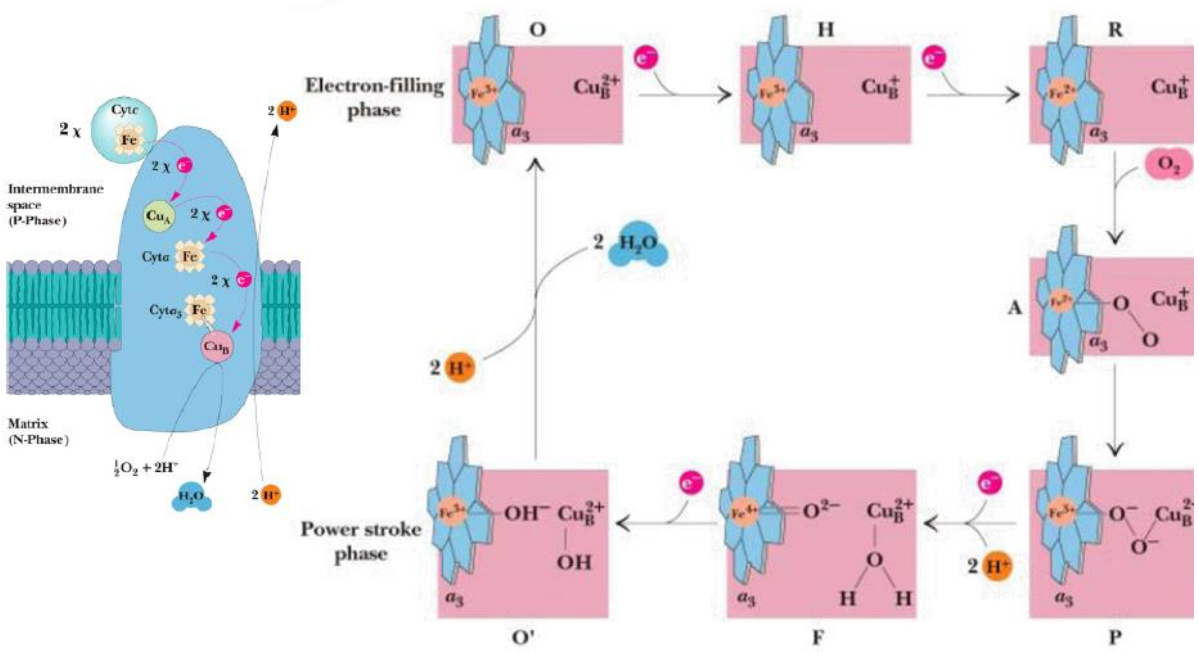
* heme a3 & Cu_b:

At first the heme group has oxidized iron (Fe^{3+}), and the copper is also oxidized (Cu^{2+}). The first electron reduces the copper (Cu^{1+}), the second electron reduces the iron (Fe^{2+}); reduced iron can bind oxygen (O_2). The electronic status of O_2 changes, and because the copper and heme are close to each other, they'll both share the electronic status between them.

Iron is converted back to Fe^{3+} and copper to Cu^{2+} , the two oxygen atoms have partial negative charges; which indicates that they have accepted the electrons.

Another two electrons enter the complex, bringing the atoms back to their reduced form and so on..

The partial negative charges on the oxygens allow them to get protons from the solution in order to form water molecules. The bond between the two oxygen atoms breaks, resulting in two H_2O; one is bound to iron and the other to copper.



* The arrangement of the ETC:

Electrons are moving from NADH to complex I to CoQ to Cytochrome bc1 (complex III) to Cytochrome c oxidase (complex IV) and ending to an oxygen to make water.

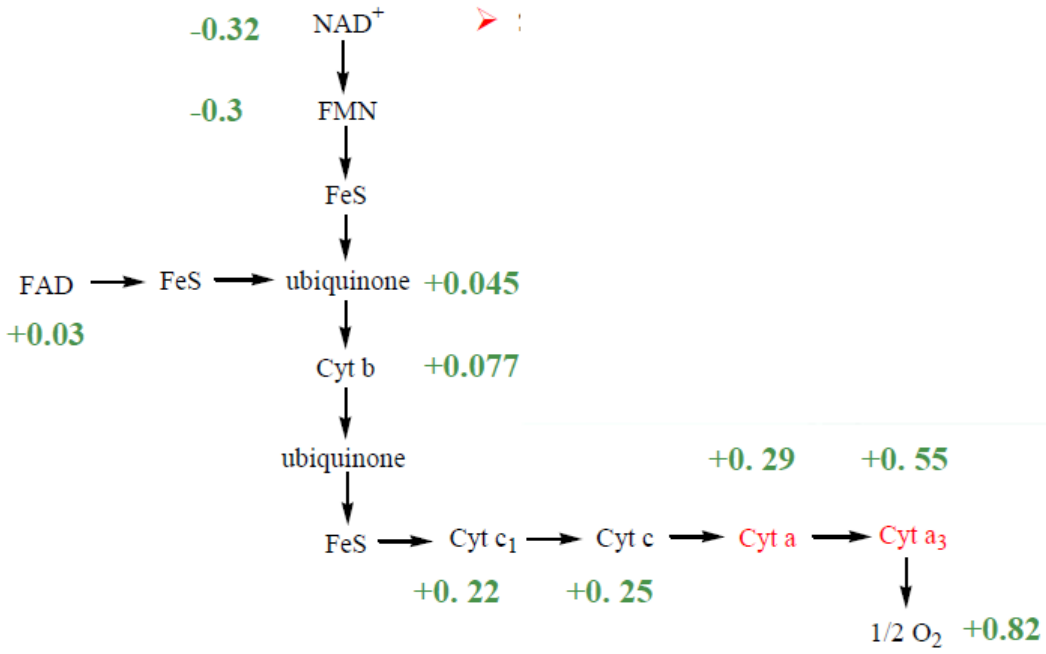
- How did we know the right arrangement of electron movement?

By 3 methods; the first method is according to the reduction potentials of the ETC components.

- How to measure the reduction potentials?

We can measure the reduction potentials of all the components within the electron transfer chain (ETC), then we can know the right sequence of electrons' movement through the chain. They started measuring at **standard reduction potentials**; cytochrome b, iron-sulfur clusters and cytochrome c1 are inside complex III, cytochrome C, cytochrome A and A3 in complex IV and last comes the oxygen.

According to the numbers they got they found that electrons are moving from the most negative standard reduction potential to the most positive. So, this is how they knew that arrangement; from more negatively to the more positively.



- Is this arrangement right?

Nobody knows, cause these are standard reduction potentials that occur at *standard conditions* and the conditions within the mitochondria are different. However, this method can be considered as an indicator that electrons can go into that sequence. In order to confirm, they did other procedures.

- Method 2: reduction of the entire ETC with no O_2
- When a source of electrons reaches the cycle, what happens?

Electrons move from one complex to the other by oxidizing the donor complex and reducing the acceptor complex until they reach oxygen and reduce it to water. So each

complex must be oxidized after it's reduced in order to be able to accept another pair of electrons (become reduced again) and this is facilitated by the presence of oxygen which is the final electron acceptor.

- If you supply it by source of electrons (NADH), and do the experiment under anaerobic conditions (NO oxygen), what will happen?

The electron is going to move and go into <u>one cycle</u> only. Electrons will move from complex I → CoQ →complex III → complex IV and they will stop there. Complex IV will remain reduced because there is NO oxygen to take electrons from VI to be converted to water, and every protein will be reduced and oxidized only once.

> They said that each protein has an electron transfer center (heme, iron-sulfur clusters or flavoproteins), and this center when subjected to light on the spectrophotometer shows a certain band, and this band is different when the center is oxidized or reduced.

You have to put a source of electrons and keep watching them and see which one complex will be reduced first and it's the first one which will take the electrons, and which one will be reduced second…

- If they put oxygen it will take the electrons and the cycle continues, then complex III and IV might be reduced at the same time cause the cycle is continuous and oxygen is expelling the electrons. But when there is no oxygen the cycle won't continue except once and at the end complex IV will remain reduced.

- Method 3: Addition of inhibitors

To confirm these results they said we have a certain inhibitor for each complex.

- If I put an inhibitor for complex III, everything **before** the complex should be **reduced** cause it has the ability to take the electrons and anything *after* complex III will be *oxidized*.
- If I put it at complex I, then all of them will be oxidized (there is some restriction to this cause we have another entry point for electrons which is $FADH_2$), but if the source of electron is only NADH (in the experiment) then the whole cycle will stop.
- If I put an inhibitor for complex IV, then III and I will be reduced and IV won't be reduce.

So every time we put an inhibitor on a place and see what is before and after, to get to know the arrangement of electrons' movement.

- The arrangement through inhibition and through measuring oxidation reduction without the oxygen, matches what they got in the standard electron potentials.

Every two electrons moving of 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 moving of $FADH_2$; 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:

- How ATP-synthase works?

ATP-synthase is transmembrane protein, it has two pieces:

1) One is within the membrane we call it (F_0)
2) The other pieces is objected toward the matrix and we call it (F_1) or (headpiece).

The piece which is within the membrane looks like cylinder (C-subunits) and its rotating inside the membrane, 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 stock that contains a polypeptide chain called (ɣ-subunit) this subunit is **angled** (not straight), it passes through the headpiece (F_1).

The headpiece (F_1) is composed is 6 subunits (3α + 3β) and the sequence is (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.

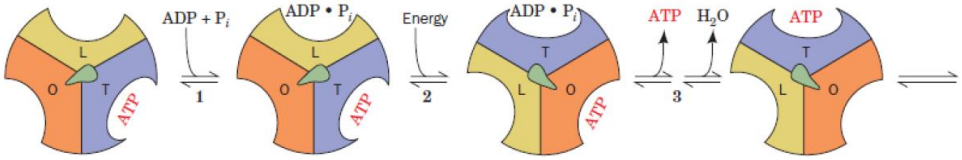
- How ATP is generated?

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.

- if it was straight there will be No use of it (this is why its angled)

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+P_i and when the ɣ-subunit hits it, it causes a conformational change to make it tight.
- Tight conformation will **enclose** the ADP+P_i 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+P_i 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 work occurs.

(C-subunits rotate → ɣ-subunit rotates → ɣ-subunit hits β-subunit because it's angled → more rotation it will hit the first & second & third β-subunit → causing each β-subunit to go into 3 conformational changes (Loose, Tight, and Open).

* a-subunit:

The a-subunit has two points; one for entry of protons and one for exit of the protons.

In the intramembranous space we have more H^+ (more pressure, electrochemical gradient) when the protons find a leak within the a-subunit they will enter it and face one of the C-subunits (the a-subunit is not open all the way through the IMM, it's projecting toward the C-subunit).

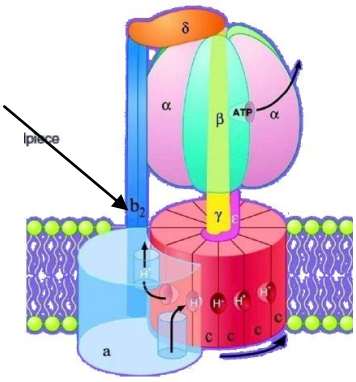
When H^+ is coming through the a-subunit, each H^+ will bind to a glutamic acid residue on the c-subunit and neutralize the charge on it {a glutamic acid residue is negatively charged (COO^-)}

When a c-subunit is neutralized due to binding of a proton, its binding with the a-subunit will change and it will get apart from its site, it will be apart to one side projecting the C-subunit after it to come to that position and one more H^+ will bind to the glutamic acid and neutralize it, causing more movement.

With repetitive entrance of protons through the a-subunit, the c-subunits will keep rotating.

- We have **12 C-subunits** rotating within the membrane.
- How many protons will get into the C-subunit? 12 protons, one for each subunit.

On the a-subunit there is another point 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, band glutamic acid will be back to its negative charge form, and this is how the cycle continues.



Per every4 protons moving through, we can make 1 ATP

- 4protons → 1ATP
- 12 protons → 3 ATP

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

The 3 β-subunits can't have the same conformation state at the same time; there is always one in the L-form, another in the T-form and another in the O-form.

So with every hit by the ɣ-subunit, one β-subunit (which was in the tight state) will become in the open state and release an ATP.

ATP-synthase can run **backward** as any enzyme; which means if you have high concentration of ATP within the mitochondria, low concentration of H^+ in the inter membranous space→ the enzyme will run backward.

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 intermembranes space.

* Energy yield from the ETC:

- Transfer of electrons from NADH to the oxygen results in: 53 kcal
- Transfer of electrons from $FADH_2$ to the oxygen results in: 41 kcal
- Energy efficiency of TCA cycle was 90%.

For ETC:

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

Calculating the efficiency: (18.25÷53) = around one third to one fourth

- Each $FADH_2$ gives → 1.5 (ATP) → 1.5 x (7.3) → 11

The efficiency: (11÷41) = around one fourth (0.25)

- Much lower efficiency when compared to kreb's cycle (which is the best machine).

* Where is the lost energy from ETC used?

It's used to exchange ions across the inner mitochondrial membrane because it is **impermeable** to anything. If you want to transfer anything across the membrane outside or inside, you will have to spend energy. So this is where loss of energy is spent.

* Regulation of ETC:

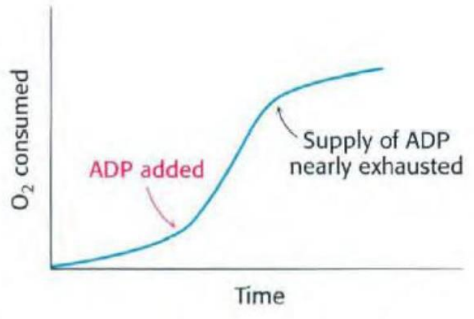
1) The need for ATP

The main and most important regulator of the cycle is the ADP concentration. This method is called: **respiratory control**.

We should monitor the oxygen consumption (the final e- acceptor)

- The more O_2 consumption → the more 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. When the supply of ADP ends, the consumption will come back to its slow process.



2) Inhibition

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

inhibition is a classical question every year in the exam, which material is an inhibitor and for which complex

- Rotenone (insecticide) + Amytal (sedative material "drug") →both are inhibitors for complex I
- Antimycin A (antibiotic) → inhibitor for complex III
- Cyanide (CN^-), Azide, 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 O_2 which by default has the highest affinity for CO,that's why CO can inhibit it.

✓ CO: competes with O_2, hemoglobin, myoglobin and cytochrome C oxidase (complex IV).

✓ Cyanide: there's a compound called cyanide glycoside found in fruit seeds (بذر الفواكة) like peach and Apricot. It can be toxic if taken in high concentrations, so you have to be careful of mixtures that contain them. Hitler and his girlfriend used cyanide to suicide, and in Jordan the father of hanen and hani put cyanide in their milk and killed them.

- Oligomycin (antibiotic)→ inhibits the influx of H^+ from the cytoplasmic side toward the matrix.

If you stop the e- movement, you will stop pumping out of protons and ATP synthesis.

❖ Electrons can 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 then you will stop the process of ATP generation.

3) Chemical uncouplers

If we have certain material 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.

Protons are being pumped out through the leak in the membrane without ATP generation.

If you are eating carbohydrate, lipids, proteins they are broken down in your body at the end to produce ATP to build up your body – in ETC you are pumping out H+ and its coming back through the membrane without passing through the ATP-synthase then 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 compound he ate.

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.

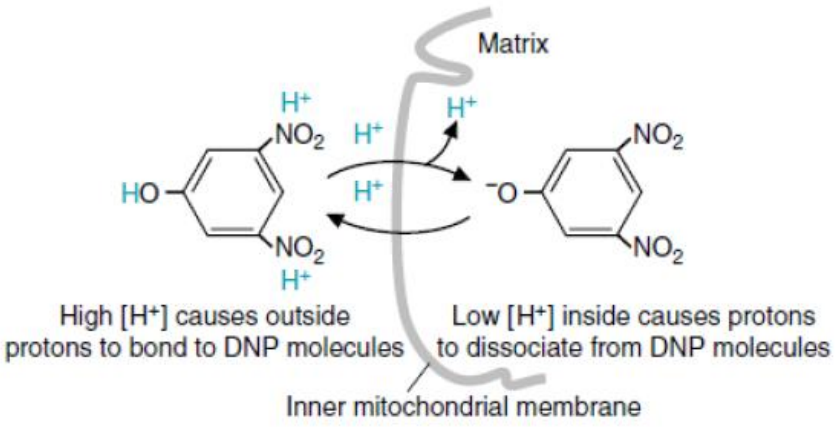
The drug was banned in 1938 in America, because it started causing *malignant hyperthermia*, problems in eyes and death by causing very high fever (high body temperature). The problem was that the increase in temperature wasn't related to the dose of the drug or the machinery process (process within 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?**

2,4-Dinitrophenol (DNP)

DNP contains OH if we remove H it will be O^- .

When it's close to the **outer side** of the inner mitochondria 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 uncoupling as well as natural uncoupling proteins in our bodies.

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

-Tissue 1 is called thermogenin that is found in *brown adipose* tissue; mostly within the **infants**. To generate more heat because babies cannot cover themselves when they're cold.

* 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:

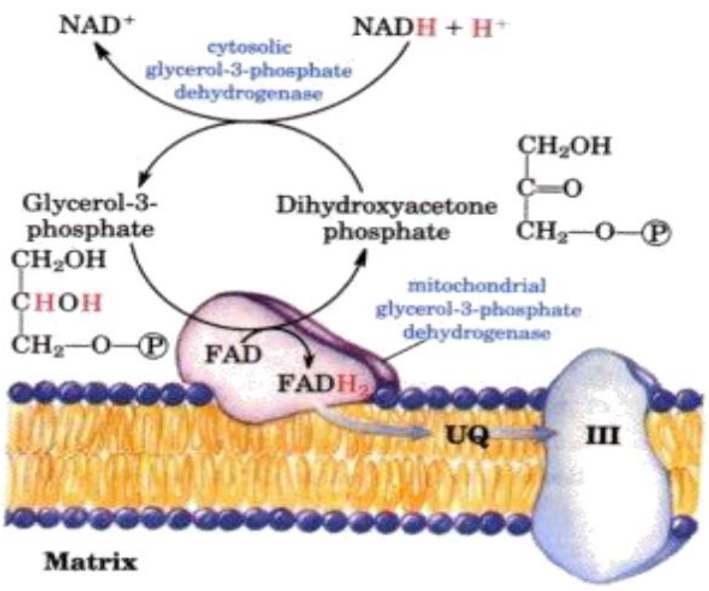
1. **glycerol-3-phosphate shuttle:**

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).

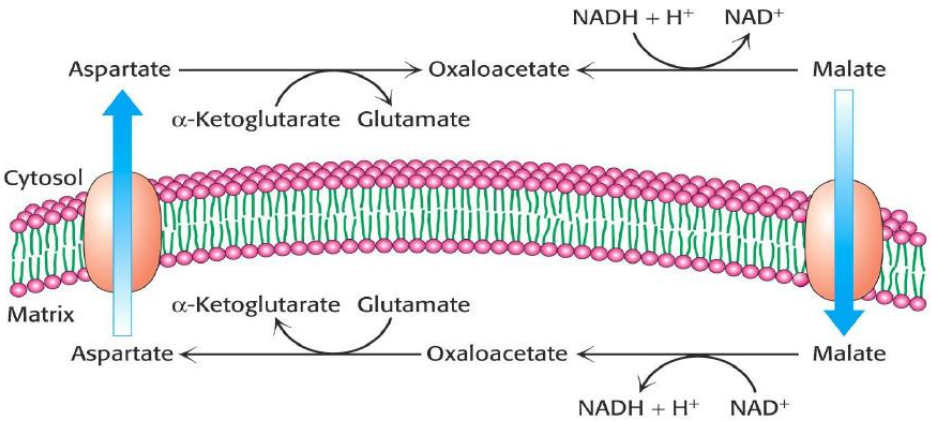
This enzyme contains FAD (flavo-protein).
We have NADH in cytosol, the cytosolic copy of the enzyme converts [dihydroacetone phosphate → glycerol-3-phosphate] by consuming the electrons in the NADH. Now glycerol-3-phosphate can pass through the outer mitochondrial membrane reaching the inner mitochondrial 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- in 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).

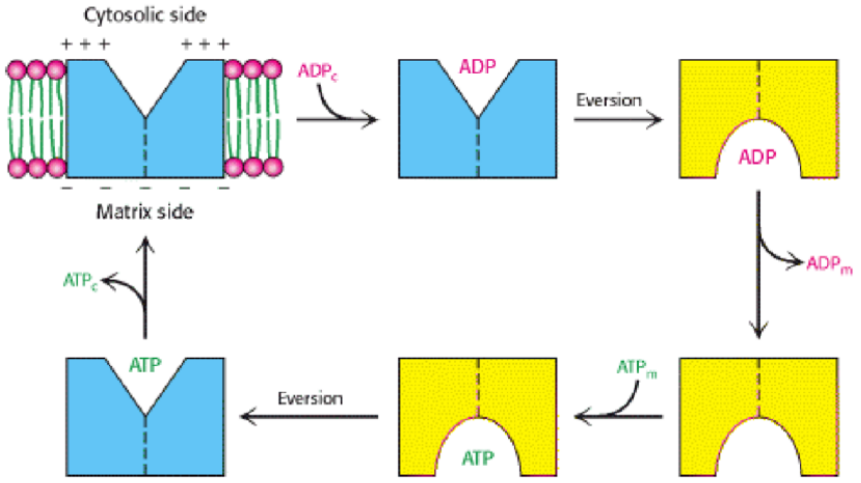
When the ATP is synthesized it's located in the matrix of the mitochondria, in order to it to work in anabolic processes within the cytosol you have to translocate it (move it from matrix→cytosol). Energy is needed to get it out.

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.

-25% of the energy spent during the ETC is going to pump this ATP outside and get ADP inside.



"مهما توارى الحلم في عيني وأرقني الأجل مازلت ألمح في رماد العمر شيئا من أَمَل" #فاروق_جويدة

Good luck for all ..

sorry for any mistakes

This table was not complete in Sheet #10 at page 7, it has been edited in the online copy of the sheet but was not edited in the Raed copy

α- ketoglutarate dehydrogenase complex – a summary :

- Catalyzes an oxidative decarboxylation reaction to convert α-ketoglutarate to succinyl CoA
- Is composed of three enzymes E1, E2 and E3
  - E1 is a decarboxylase
  - E2 is a tranacylase
  - E3 is a dehydrogenase

- Each of the three enzymes is attached to one or more coenzymes
  - E1's coenzyme is Thiamine
  - E2's coenzymes are Lipoic acid and CoA
  - E3's coenzymes are FAD and NAD+
- Each of the three enzymes will give one product
  - E1 will give CO_2
  - E2 will give succinyl CoA
  - E3 will give NADH

  Together, these three products constitute the products of α-ketoglutarate dehydrogenase enzyme.