◉Sheet ○Slides

Subject :	ATP synthase
Done by :	Obada Zalat
Corrected by :	Omar Yahya
Number :	8

Requirement for the oxidative phosphorylation process:

1-Electron source as NADH and FADH2

2-Electron acceptor as O2

3-Electron carriers to make electrons flow in chain

4-ATP synthase; to convert the electrochemical gradient of hydrogen protons into energy which produces ATP

5-Intact membrane; if the membrane wasn't intact the protons would return back to the matrix; so we would lose the gradient.

Brief Discussion about the Complexes:

1) Complex (1)

It called NADH dehydrogenize, it has FMN, Iron-sulfur clusters and binding site for NADH and coenzyme Q (Ubiquinone). Oxidized Ubiquinone , which the complex has high affinity to, binds with the complex and takes electrons from it, forming Ubiquinone in the reduced state which is called ubiquinol (an alcohol) or QH2 as it gains electron. ubiquinol has low affinity so it will be released from the complex, and the same mechanism goes for NADH, the complex has a high affinity for NADH and low affinity for NAD+.

2) Complex (2)

It is called succinate dehydrogenase (part of TCA cycle), it contains FAD and iron-sulfur clusters. The energy difference of the electron movement from complex (2) to Ubiquinone is almost Zero. So there is no pumping of protons; that why it's a peripheral protein.

3) Complex (3)

It called complex b-c1, it contains iron-sulfur cluster, two types of heme B and one type of heme C

*Note: every enzyme complex contains coenzymes to help in electron transferring as the proteins themselves cannot perform this task.

4) Complex (4)

It is also called cytochrome C oxidase; because it oxidizes cytochrome c, it has 4 redox centers: heme a, heme a3 and two Cu atoms

*cytochrome c transfers electrons from complex 3 to complex 4, one at a time; as it contains heme.

* This complex is responsible for reducing oxygen into water

Pumping of protons

Complex 1 pumps 4H+, complex 2 does NOT pump any proton, complex 3 pumps 4 H^+ and complex 4 pumps 2H+ and all these protons will be pumped out per 2 electrons.

The protons will pumped out of the matrix to the intermembrane space and then they will return back to the matrix via channel called ATP synthase to produce ATP.

*NADH results in pumping of 10 protons while FADH2 results in pumping of 6 protons only.

ATP synthase

-ATP synthase compose of two domains, the first domain is called F0 and it is found inside the membrane, the second domain is called F1 and it projects towards the matrix.

-F0 domain is a cylinder that can rotate within the membrane, and there is an angled polypeptide chain (called gamma chain) projecting out of it, the cylinder is composed of 12 polypeptide chains (each chain is called C subunit), they are partially covered by a subunit. Once the C subunits rotate, they rotate the gamma subunit with them.

-F1 domain is composed of 6 polypeptide subunits (3 alpha and 3 beta subunits in alternating way…alpha, beta, alpha, beta and so on) the alpha subunits preserve the enzyme structure, while beta subunits are the catalytic subunits (join ADP with phosphate to make ATP). But from where do we get the energy for the reaction?
-The energy comes from the protons that enter the ATP synthase, the protons will lose their energy to be used to produce ATP.

**minutes 0-10:00

* refer to the slides for the ATP synthase structure or Lippincott's Biochemistry Fig. 6.14 P78.

The mechanism:
-The entry point for proton is from a subunit ,a subunit has two openings; one towards intermembranes space and one towards the matrix, so protons will start to pass through the opening (that faces the intermembrane space), once they enter they face the C subunits, each subunit at the level of opening has glutamic acid portion (which has a negative charge),when the proton come and face the glutamic acid it will pick up the proton and lose its charge; this will cause conformational change that will make the subunit move , once the subunit moves, another subunit will face the opening and another proton will bind causing the movement of the subunit and so

on to all subunits. The subunit will move until it face the other opening (that faces the matrix), in that side the pKa will change causing the subunit to lose the proton.

-The rotation of the C subunits causes the angled gamma subunit to rotate, every time the gamma subunit rotates, it hits one of the beta subunits (only beta because alpha subunits don't evolve in the reaction), causing conformational change in the beta subunit, the angle causes gamma subunit to hit the beta subunit, and if it was straight this won't happen.

At the beginning, beta subunit will be widely open, then the first hit will cause the binding of the ADP and phosphate (P_i) to the subunit (open conformation), the second hit will initiate the reaction to produce ATP (tight conformation) and the third hit will cause the release of ATP (loose conformation).

-ATP synthase can run backward as other enzymes, so when we have more protons and ATP in the matrix, than the intermembrane space, the protons will come from the matrix and enter the opening of ATP synthase and make it rotate in the reverse direction so there will be more protons on the intermembranous space. Also, the conformation of the beta subunit will change in the reverse direction to hydrolyze ATP to ADP and phosphate, so we can name this enzyme ATP synthase or ATPase, because it produce ATP and at the same time it breaks down ATP.

Energy yield from the oxidation phosphorylation process

-For every 4H+ one ATP will be synthesized, we have 12 C subunit so we have 12 H+ in each full turn and 3 ATPs will have to be synthesized.
-NADH causes 10 H+ to be pumped out and then enter the ATP synthase, so NADH has the capacity to produce 2.5 ATPs (10H+/4).

-FADH2 causes 6H+ to be pumped out, so it has the capacity to produce 1.5 ATP (6H+/4)

*In some books you will find them 3 ATPs for NADH and 2 ATPs for FADH2, these values are approximate numbers, but the actual values are what we mentioned before.

-What is the efficiency of oxidative phosphorylation process?

The entries are NADH and FADH2 and the output is ATP.
The energy of NADH is 53 kcal and it produces 2.5 ATPs, if we multiply the ATP molecules (let's say it 3 not 2.5) by 7(the energy of one ATP molecule), so we will have 21 kcal, at the end divide 21/53 and the efficiency will be **30%**.
The energy of FADH2 is 41kcal and it produces 1.5 ATP, use the same calculation above and we will have 14/41 which results in **30%.**

*In the exam give exact values if you are asked to do calculations.

-the efficiency of oxidative phosphorylation is very low than TCA cycle. Where the rest of the energy has gone?
NOT all the energy goes to produce ATP, the cell doesn't need all energy to produce ATP, and it uses this energy to produce heat. Also, we need energy to insert some molecules (that found out of the mitochondria) such as ADP, phosphate and Ca (we need Ca to activate enzymes), and to release some molecules such as ATP.

- Regulation of oxidation and phosphorylation

-The main regulator is ADP (it is called respiratory control), so we deal with the effect of ADP level on the O2 consumption. Why O2? Because it's the parameter that will indicate how ETC is working. The O2 is the final electron acceptor in the ETC; so when we have more O2 converted to H2O the ETC is working more efficient.

When we add more ADP, consumption rate will increase, so ADP is an activator for ETC.

ETC refers to the electron transport chain

There are other types of regulation; by inhibitors or by uncoupling. Before discussing these two mechanisms of regulation we must know that oxidative phosphorylation couples ETC with phosphorylation process.

** minutes 10:00-20:00

Regulation by inhibitors:
-actually if we inhibit the ETC we inhibit the complexes and ATP synthase (usually called complex 5)

-if we inhibit complex (1) we inhibit most of the chain, but there is still movement of electrons (from FADH2) from complex (2) to complex (3).

-if we inhibit complex (2) the ETC will still running

-if we inhibit complex (3) there will be inhibition for the movement of electrons.

-if we inhibit complex (4) we will stop the chain completely.

-There are many drugs and toxic materials that inhibit the complexes, such as:

1-at the level of complex (1) there is Rotenone (insecticide) & Amytal (sedative).
2-at the level of complex (3) there is Antimycin A (antibiotic)
3-at the level of complex (4) there is Cyanide (CN-), Azide (N3-), & CO.

4-at the level of ATP synthase there is Oligomycin (antibiotic), so there is no influx of protons toward the matrix.
*CO causes suffocation because it binds to complex (4) and prevents the binding of O2.
*if we inhibit the complexes we inhibit the movement of electrons, so we inhibit the producing of ATP.

***all the toxic materials must be memorized.**

We will discuss one toxic material which is CN (cyanide):
-CN binds to complex (4) then binds to the iron in the heme, so the O2 can no longer bind to the complex, at the end there will be no production of ATP; causing death.
-CN is found mainly in the seeds of fruits like apples and peaches, but we need high amount of fruit seeds to have CN toxicity.

Regulation by uncoupling
Can we make the electrons flow without generation of ATP?
Yes, we can by making electron flow between the complexes (No inhibitors on the complexes), so there will be pumping of protons, but if we make these protons return to the matrix without reaching ATP synthase there WON'T be a generation of ATP; instead there will be a generation of heat.

But what is the effect of that?

We use ATP for anabolic processes to build up our bodies, so if we found a material that allows protons to return to the matrix without generation of ATP, we will find a drug for obesity.

One example of these drugs is Dinitrophenol, this drug consist of phenol ring connected to OH, because it has phenol ring it can swim through the membrane, so the drug takes the H+ from outside to the matrix. But the production of the drug stopped due to side effects such as; excess heat (malignant hyperthermia), eye bleeding and death.

-Brown fat tissue (found in high concentration in young children) has high concentration of mitochondria and special proteins called UCP1 (UCPs-uncoupling proteins- have many types and UCP1 is one of these types), this protein makes uncoupling process and moves H+ back to the matrix without going through ATP synthase, so there will be generation of heat instead of ATP. That is why this tissue is a source of heat in young children. On the other hand, white fat tissue has less amount of UCPs; that why it doesn't produce heat.

*UCPs concentration may be affected by genes, so people from different countries have different concentrations of UCPs.
* Children has more brown fat than adults

*minutes 20:00-30:00

Oxidative Phosphorylation Diseases

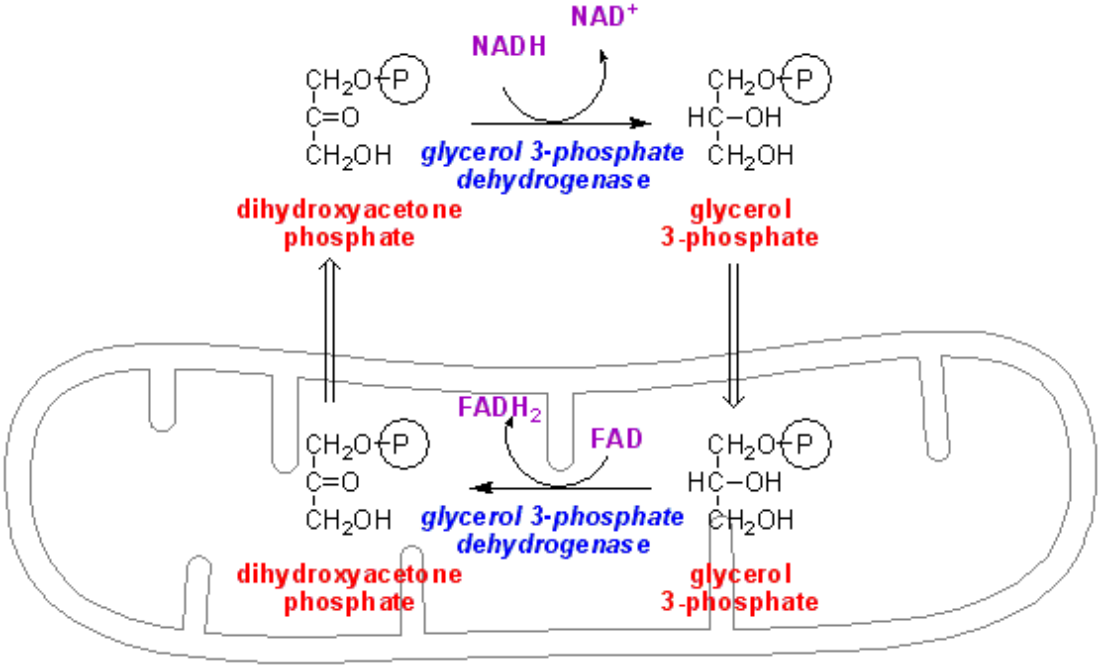
Mitochondria contains high concentration of proteins, but the DNA found in the mitochondria is little, so proteins that will be synthesized will have little concentrations (7 subunits of complex (1), 1 subunit of complex (3), 3 subunits of complex (4) and 2 subunits of F0 portion of ATP synthase) and the other proteins come from the nucleus' DNA. So diseases of mitochondria may result from mutation of mitochondrial or nuclear DNA.
*Nuclear DNA mutation might cause mutation in all body tissues, where mitochondrial mutation might cause mutations in one or more type of tissues.
* Mitochondrial diseases have only maternal inheritance and no paternal inheritance.

Electrons Recourses for Oxidative Phosphorylation

-TCA is not the only recourse of electrons for Oxidative phosphorylation. Glycolysis (that occurs in the cytoplasm) produces NADH to be used in ETC.

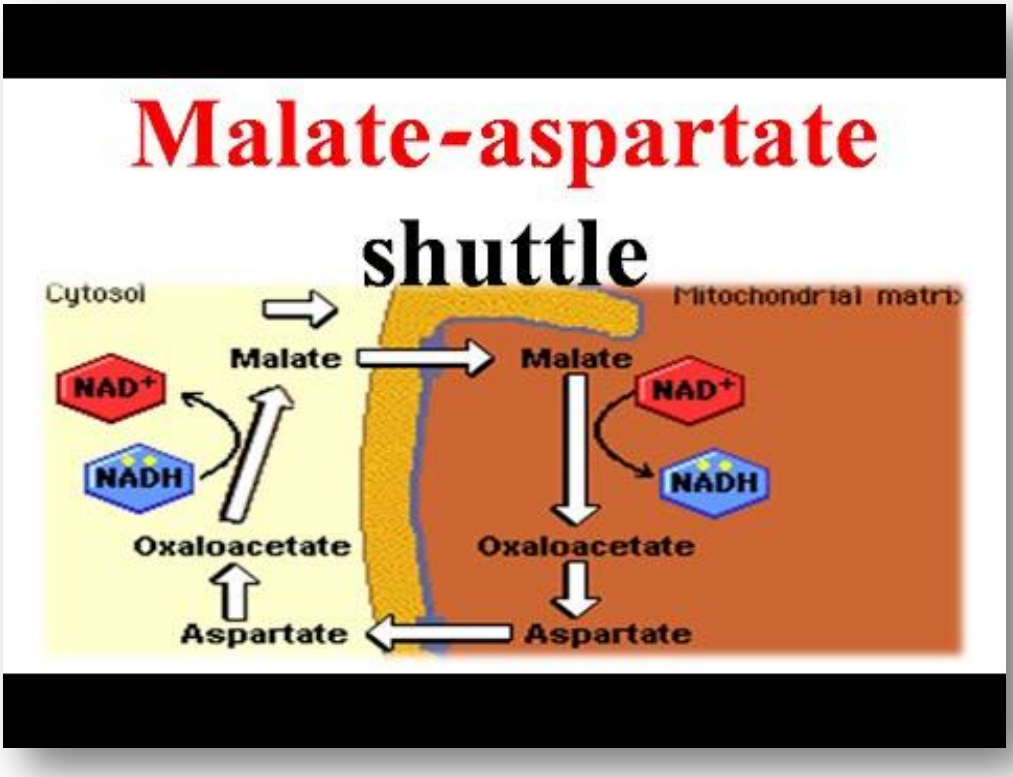
But, how does it enter the mitochondria?
-by shuttles (mitochondrial shuttling) that transfer molecules from outside to the inside and in the reverse direction, but we don't have specific shuttles for NADH, so we shuttle the electrons of NADH by two ways (two types of shuttling):

1) The first shuttle enzyme is glycerol-3-phosphate dehydrogenase, we have two copies of this enzyme:
Cytosolic copy and mitochondrial copy (on the outside portion of inner mitochondrial membrane).It converts dihydroxyacetone phosphate to glycerol-3-phosphate (the cytosolic copy), it uses electrons of NADH in this reaction and convert it to NAD+, once glycerol-3-phosphate passes inside the mitochondria ,it becomes a substrate for the mitochondrial copy, and converted to its original form (dihydroxyacetone phosphate), inside the glycerol-3-phosphate dehydrogenase there is FAD, once it accepts electron it converts FAD to FADH2, so we will have 6H+ and 1.5 ATP at the end.



2) Malate aspartate shuttle, in the TCA malate conversion to oxaloacetate and production of NADH occurs. If we reverse the reaction (oxaloacetate to malate) it will consume NADH, so when we

have NADH in the cytosol the enzyme converts oxaloacetate to malate and NADH to NAD+, then malate will enter the mitochondria through a specific shuttle. Once the malate is in the mitochondria, it will be converted to oxaloacetate and produce NADH.



*If NADH that found outside the mitochondria is shuttled (actually the electrons of NADH) by malate aspartate shuttle mechanism; it will pump 10H+ once it enters the mitochondria. But if it is shuttled by glycerol-3-phosphate dehydrogenase, 6H+ will be pumped and less ATP will be produced.

However, we need to transfer ATP outside the mitochondria; because most of the metabolism reactions happen in the cytoplasm, so the cell uses ADP-ATP translocase enzyme to do this mission, the enzyme has an opening towards the matrix, once ATP is produced it binds to the enzyme, then the enzyme encloses and opens to the outside and releases ATP, then it binds to ADP, then the enzyme will close and open to the matrix and release ADP. For every ATP translocated to the outside ADP must come in (the ratio is 1:1,so the reaction is highly regulated).This enzyme is found in a high amounts

in the mitochondria and consumes high energy for shuttling, the energy comes from the ETC, so it affects the efficiency and makes it low as mentioned before.
*14% of proteins on the inner mitochondrial membrane are ADP-ATP translocases (which is a high amount).

How scientists knew the order of complexes in the oxidation phosphorylation process?

-Experiment 1: they measured the standard reduction potential values for all components (it may be equal to the actual values in the cell), then noticed that electrons flow from the most negative to the most positive component.

-Experiment 2: they used source of electrons (NADH) and did the ETC without a final acceptor (O2), so the complexes reduced and oxidized until complex (4) which only reduced, then they followed which of the complexes reduced and oxidized first; to know the correct order.

-Experiment 3: we know that there is an inhibitor for each complex, so we do many experiments, in each one we inhibit one of the complexes, for example if we inhibit complex 1 it will be reduced, but it will <u>not</u> oxidize, so every complex reduced will be before the inhibitor, and any complex still oxidized will be after the inhibitor, with multiple experiments with different inhibitors they knew the correct order.

** minutes 30:00-45:00

Sorry for any mistakes.

"Nothing is impossible; the word itself says 'I'm possible!"

Good Luck