We talked about the Q-cycle in the last lecture, we talked about complexes .

As a revision complex 3 is a dimer that Q-cycle occurs in it.

It consists of cytochrome B and cytochrome C1.

Complex 4:

 the electrons go from complex 3 to complex 4 through an intermediate electron carrier (cytochrome C)

cytochrome C will shuttle the electron from complex 3 to complex 4.

How many electrons cytochrome C can carry?

One electron at time.

Per 2 electrons moving from complex 3 to complex 4 we need two cytochrome C molecule .

The electrons come to complex 4 , complex 4 ( cytochrome C oxidase) that’s mean that it oxidize cytochrome C . when cytochrome C comes carrying electrons it will be in the reduced state , cytochrome C oxidase will remove the electrons from cytochrome C, so it will become oxidized ( that’s why it’s called cytochrome C oxidase)

How the oxidation mechanism occurs it’s not included , however you need to know that there is two copper sites (copper A , copper B) , also there is two structure of the heme ( heme A and heme A3) . electrons will pass through these Coenzymes ,or tightly attached metals to this enzyme either copper or heme. When the electrons reach the heme , the heme will become reduced ,( as we know reduced heme can bind oxygen ) binding of oxygen occurs at heme A3 , it bind the oxygen so it will be reduced by electrons coupled to protons and it will produce a molecule of water .

To convert O2 to water how many protons I need ? how many electrons I need ?

4 protons . 4 electrons

Explanation : electrons when come to bind oxygen it comes as hydrogen , every single oxygen needs 2 hydrogen to become H2O ,so it needs 4 electrons . (the electrons that in the reducing equivalents (NADH , FADH2 each of them is a 2 electrons equivalent). In order to reduce oxygen you need 2 molecules of NADH or 2 molecules of FADH2 or one molecule of NADH and one molecule of FADH2)

Per 2 electrons moving you can reduce on oxygen atom . **you can’t reduce the oxygen molecule except by 4 electrons.**

To reduce oxygen how many protons will exit ?

 First 2 electrons pass ,2 proton will exit to the inter membrane space from complex 4, and for the second set of electrons 2 protons will exit also , the total is 4 .

 **Just a revision for the number of protons exit from each complex :**

(at complex 3 will exit 4 and at complex 1 also 4 )

The affinity of heme A3 with in the complex 4 (the affinity for oxygen) , is it high or low ?

Its very high .

is it logical or not ?

its logical ( the oxygen comes through the lunges , hemoglobin passes through blood , hemoglobin will extract oxygen from the lungs and goes through the blood , then it goes to the tissues and myoglobin takes oxygen from hemoglobin , because the affinity of myoglobin to oxygen is higher than the affinity of hemoglobin to oxygen ,within tissues it goes to the cells , which have mitochondria and in the mitochondria cytochrome C oxidase will take the oxygen from myoglobin , so the affinity must be higher than myoglobin which is higher than hemoglobin , that’s why the Km is less.

Redaction of oxygen must be full all the time , it can’t be partial redaction , because partial reduction is hazardous , it will make reactive oxygen species which can generate free radicals , free radicals are bad .

How can we know the right arrangement?

We said the electrons exit from the citric acid cycle as NADH and FADH2 where they hit first ?

We said complex 1 and complex 2, then complex 3 and complex 4 till oxygen.

How we can prove it?, if this is the right sequence ?

By energy difference ( ΔG) which is based on( ΔE) reduction potential , but we can’t measure reduction potential , you can ,measure it for each one separate , when you have reference for all of them , you can measure the standard redaction potential out side of the mitochondria .

 You measure standard reduction potentials for all of the molecules ( NAD+ , “FMN, Fes” from complex1 , “FAD” from complex 2, ubiquinone, Cytb, Cyt c1, Cyt c,Cyt a,Cyt a3 then to oxygen).

 Notice that it’s a ascending process until we reach the highest reduction potentials which is the best electron accepter . this process is it right or wrong ? its standard, but is it proved or not ?

It’s not proved it’s just an indication , because process in human body doesn’t occur at standard condition .

How can we prove it more?

1. We make reduction by putting excess of NADH or FADH2 , and we don’t put oxygen inside the mitochondria . what will happen? We are making an experiment under anaerobic conditions ( no oxygen) what will happen ?

Complex 4 will stay reduced it won’t be oxidized because it didn’t donate it electrons to the oxygen , and the others are oxidized ,electrons will move from one complex to another ( from one set to another ).

Each complex when you put it in a solution and measure it under spectrophotometer , it will produce certain bands according to the position of heme or if you have another complexes such falvines , each one got certain spectral features .(complex 1 have different spectral features than 2 different than 4,, COQ is different than cyt c ).

So u put the source of electrons and you don’t have oxygen , and notice which one is the first one , because the shape of oxidized will be different than the shape of the reduced, as we said in the heme ( the oxidized heme is different from the reduced heme ) and the heme in each protein give a different band , close to each other but still different in the nanometer. So u notice which one has been oxidized and which one has been reduced , (the first have been oxidized and the second one … and etc .) so you can know the range.

1. Also we can inhibitors : for certain steps in the sequence u got it before ( which was an indication ) you put inhibition the molecule before it must be reduced and the one after it must be oxidized .

After applying these methods they knew that it’s the same arrangement they got through the prediction.

**Pumping of protons :**

Per NADH molecule it will pump 10 protons

Per FAFH2 molecule it will pump 6 protons

**ATP synthase :**

It consist of two pieces ( piece with in the membrane at the inner mitochondrial membrane , and piece projecting towards the matrix of the mitochondria )

These two pieces they have a rotation between each others .

The cylindrical circular consist of 12 piece all of them are called Cs ( C subunit) ( C1,C2,C3…..C12) it contain glutamate ( there structure almost identical to each others ) each one on a certain place you find that it has glutamate

The circle is linked to the A subunit , the A subunit is fixed within the membrane

The C subunits can rotate in the circle that is made by the A subunit

The C subunit is linked to what is called the ( γ subunit) the γ subunit is attached to the F0 piece,and enter to what is called the F1 piece . the circle inside the membrane is called the F0 portion .

The upper circle is consist of 6 sub unit ( 3 α and 3β)

The β subunits are the places where producing of ATP occurs

The α subunits are presents for structural reasons ( to conserve the structures of enzymes units place )

The stock (γ subunit ) it’s not strait its bended

What is the advantage of being not strait ?

When the C subunits rotate every time it (γ subunit )will hit β subunit

When the γ subunit hits the β subunit it will cause conformational change in the protein ( it will change the shape of the protein )

There is three conformations that the protein can change to when its hit by the γ subunit

1. Loose
2. Open
3. Tight

If the γ subunit was strait it would give symmetrical movement that’s why its bended to give asymmetrical movements.

The C subunit rotated it hit the first β subunit ( it was loose conformation then its converted to tight subunit )

Loose conformation can accept the ADP presenting in the matrix with the inorganic phosphate, because its slightly opened.

Tight conformation closes ADP and the inorganic phosphate to each other initiating the reaction which gives ATP

It hits again converting the tight conformation to open conformation ( ATP released ) and the cycle will continue

What makes the C subunits move ?

The flow of protons ( as we said before the flow of protons from outside to inside generates ATP)

**The flow of protons :**

The protons enter from the A subunit ( the A subunit is fixed in the membrane ) ( its look like semi circle ) its surrounds the C subunits .

When the protons enter they have certain pathway , when the protons enter they find the C subunit contains glutamic acid residue ( glutamate ( negatively charged ) ) as soon as the protons entered its bind the glutamate ( the glutamate is not negatively charged anymore ) it was fixed because it was charged , when the glutamate becomes not charged it causes a deflect and if moves a little , after it moved another proton will enter and bind the second glutamic residue that presented on another C subunit , and it keeps moving .

When you have full rotation.The exit of the protons is different than the entry, it found a different place inside the A subunit and has a space where the proton can be released .

So the movement of proton is continuous from the outside to the inside of the matrix .

Every 4 protons enter they can generate one ATP molecule after the first rotation ( **you must have a full rotation at the beginning )**

**Important information : ATPsynthase can run backward which means that you can name the enzyme either ATPsynthase or ATPase , it can hydrolyze ATP or synthesize ATP.**

**Same story with the conformations :**

**The γ subunits rotate clock wise but when the conc. Of ADP changed and you have more H+ inside than the outside ,the H+ goes out again by ATPsynthase and the γ subunit will rotate counter clock wise instead of rotating clock wise , every time it hit β subunit it will reverse the confirmation , so it converts the ATP to ADP and inorganic phosphate .**

The energy yield from the electron transport chain:

Each NADH molecule when enters citric acid cycle till it reaches the oxygen the difference in reduction potential results in ΔG=53 kcal in excess

FADH2 presented inside the succinate dehydroginase =41kcal.

We generate 2.5 ATP molecule from NADH and 1.5 molecule of ATP from FADH2

Each molecule of ATP results in how many kcal ? 7.3

7.3 \*2.5=17.25kcal for each NADH.

Efficiency 17.25/53 \* 100% = 32.5 % yield is very low in comparison with citric acid cycle that may reach 90 %

ΔG is so negative so its irreversible although theoretically they are reversible reaction .

The other energy where does it go (THE EFFICIENCY IS LOW) ?

It pump ( anion , Ca+2 ), produce heat .energy used to keep the oxidative phosphorylation running to produce heat .

**Regulation of the oxidative phsphorylation process**

Most important regulator in the oxidative phosphorylation process is ADP conc.(ADP/ATP) ratio when the ADP ratio is low the oxidative phosphorylaton won’t go well

The graph illustrate how much i use oxygen , because the redox reaction is coupled to the phosphorylation . (slide 25)

If you added excess ADP you’ll notice that the oxygen consumption is becoming very high till the ADP is used it will go back within the normal range

The regulation of the rate of oxidative phosphorylation by the ADP is called respiratory control.

**Other regulation that occur to the oxidative phosphorylation process :**

1. **Inhibition** : you can inhibit the oxidative phosphorylation at complexes (1,2,3,4 also ATP Synthease (complex 5)

If you inhibit the electron transport chain will you inhibit ATP synthesis ?

It depends where you are making the inhibition if you are making the inhibition complex 1 it will decrease but it won’t stop , because there is another entry for electrons at complex 2 and there no relation between complexes 1 and 2 so it will keep running .( the same thing for complex 2 )

If you inhibit complex 3 or complex 4 you are stopping the process of electron transfer, there will be no ATP generated because the process is coupled

Is there any inhibitors ?

Yes , complex 1 🡪 rotenone (insecticide) &amytal(sedative)

Complex 3 🡪 antimycin A ( antibiotic)

Complex 4 🡪 cyanide (CN), Azide (N3), &(Co) the inhibition occurs when they bind to the iron on the heme in complex 4, so they inhibite the binding of oxygen , so there will be no reduction and no ATP synthesis .

Complex ATP Synthaes🡪 oligomycin(antibiotic) it prevebts the entry of protons , so it doesn’t generate ATP.

Please check the table slide # 26

Cyanoglycosides as the name implies they contain cyanide, they presenting in high amount in fruits seeds.

If u consumes a lot of cyanide it will be harmfull.

They believes that Hitler died from cyanide

Inhibition stops the synthesis of ATP ,it stops moving of of electrons.

1. **Uncoupling : if I have movement of electrons and I found a leak through the membrane that the protons are coming back inside through it , the movement of electrons will continue but there is no ATP generation .( it may caused by medicines or physiologically .**
2. By medicines 🡪 2,4-dinitrophenol(DNP) ( it’s a phenol ring ( which is a benzene ring with (OH) this H can be lost or accepted )

When its near the inter membranous space it accept the proton , when its near the matrix it lose the proton ( so it move the protons from the inter membranous space to the matrix ) so we lost the electrochemical fore we have made but still there is a movement for electrons

What is the benefits from the continuation of the electrons movement ?

You didn’t generate ATP, that’s mean that all the energy from braking down carbohydrates, lipids and protein you are converting it to NADH,FADH , and the electrons move and reduces oxygen but you are not making ATP ( Why do we need ATP ? to build up our bodies ),so people who wants to lose weight, they take dinitrophenol,its very dangerous because the energy in this case converted to heat,,and that’s lead to increase in body temperature and death.

B) Physiologically : we have proteins called uncoupling protein there function is to make uncoupling , there present in the membrane , they take the protein and return it back inside , in order to give heat to the body.

There are uncoupling protein (1,2,3,4,5) everyone has tissue localization

Uncoupling protein 1 (UCP1) it’s called thermogenin it presents in the brown adipose tissue , around the kidneys in infants.

Why there is brown adipose tissue in infants ?

In order to give them heat .

So it makes uncoupling , when it makes uncoupling it produce heat

**Oxphos diseases :**

Genetic diseases affecting oxphos ,can result from 2 sources:

1. DNA presenting in the mitochondria
2. DNA presenting in nucleus

Around 50% of the mitochondria is proteins .cuz all reactions of energy production occure there.

DNA in the mitochondria is small ,double stranded, circular,Its look like the bacterial one ,it incodes 13 subunits only.

 If you have genetic mutation it may be from the mitochondria or from the nucleus

Heteroplasmy there are some tissue having the problem and other doesn’t have it.

If you have a disease in the mitochondria it will progresses badly with age why ?

Because you will have somatic mutation in the DNA from the nucleus more protein will become ruined , and more subunits will be ruined , the problem will increase specially in tissues that has highest ATP demand , why ? because it needs more energy so the problem will appear clearly .

2)nuclear DNA:

It produces around 1,000 protein within the mitochondrial

The problem occurs its usually autosomal recessive , it will show up in every cell ( because it divides by mitosis), so the problem will be distributed to all daughter cells equally.

Also here the tissue that needs high ATP demand it will face the same problem as the mitochondrial mutations , the disease will appear more.

The diseases name slide #31 are **NOT** important **.**

Just know the differences between nuclear and mitochondrial genetic disorder and their properties.

Citric cycle its not the only place where NADH produced , it also produced in other places such as cytosol by glycolesis ( converting glucose to pyruvate ) its produces NADH molecule ., it also give ATP

In order to have continues glycolesis process NAD+ is required . if i don’t have high NAD+ conc. Sure NADH con. Will be high , so how can I use NADH that presents in the cytosol ? it must go to the mitochondria in order to be used in the oxidative phosphorylation process ( NADH can’t cross the inner mitochondrial membrane ), so how to get it inside to be able to bind complex 1.

We have shuttles so NADH is converting to NAD+ it gives its electrons and converted to what called glycerol-3-phosphate through the enzyme which called cytosolic glycerol-3-phosphate dehydrogenase (this enzyme take 2 eleectrons from NADH and produces glycerol 3-phosphate ) this process occurs in the cytoplasm .

Now I have glycerol-3-phosphate , it will enter the inter membranous space cuz the outer membrane is permeable, I have another enzyme same as the cytosolic glycerol-3-phosphate dehydrogenase called mitochondrial glycerol-3-phosphate dehydrogenase present in the outer surface of the inner mitochondrial membrane, it contains FAD , which accept the electrons from cytosolic glycerol-3-phosphate and convert it to Dihydroxyacetone phosphate

It took the electron from the glycerol -3-phosphate and gave them to the FAD so it becomes FADH2.

If the NADH entered the mitochondria ( the matrix) from the cytosol through glycerol-3-phosphate dehydrogenase , it will give its electrons to the FADH2 , an the FADH2 will give the electrons to the ubiquinone then complex 3 , which will pump 6 proton NOT 10 .

So the NADH that comes from the cytosol if it took the root of glycerol-3-phosphate dehydrogenase it will give 1.5 ATP not 2.5 ATP

This enzyme present in the skeletal muscle and the brain ( because there is no high need to ATP as the heart and liver )

\*in heart and lever we have there is another shuttle called malate –aspartate shuttle .

Malate and aspartate can cross the inner mitochondrial membrane through carrier

You have NADH presented in the cytosol , it will give electrons to the oxaloacetate converting it to malate ( opposite to what had happened in the citric acid cycle ).

Malate can cross the membrane , now we are inside the matrix , inside the matrix and through the enzyme malate dehydrogenase it will oxidize this malate and converting it to oxaloacetate again , and produce NADH inside the matrix which can go to complex 1 .

So if the cytocolic NADH enter the mitochondria through malate –aspartate shuttle it will give u 10 protons and 2.5 ATP ( so it depends on the root where NADH will choose .

Oxaloacetate goes through trans amination process and gives aspartate ( we will take them in the amino acid metabolism ). And we said in the anaplerotic reactions , and the intermediates of citric acid cycle the oxaloacetate converted to aspartate , and the asparet can be converted to oxaloacetate if the amino group moves between them .

Aspartate can cross , aspartate goes through transamination in the cytosol and it becomes oxaloacetate , and it takes NADH again , and the cycle continuous

**ATP-ADP translocase**

Inner mitochondrial membrane is not permeable to anything , it needs certain carrier to carry ATP to the outside to be used in building up of molecules , it goes out by ATP-ADP Translocase .

ATP-ADP Translocase: it carry through the membrane , every ATP goes out another ADP enters in a ratio of 1:1 .

When the ATP Leaves the matrix and bind there (ATP/ADP translocase) will cause conformational change and it pump the ATP to the outside, and the conformational change bind the ADP from outside,binding of ADP will cause another conformational change, and it opens to the inside

This protein is very important, without it oxidative phosphorylation wont occurs why ?

Because ADP in the matrix will be consumed,and the ADP is the most important regulator in the oxidative phosphorylation process , and the oxygen is low .

When you pump ATP to the outside and let ADP to enter this process is endergonic process , it needs energy .

25% of the energy produced in the oxidative phosphorylation is used in the pumping of ATP and entering of ADP ( its highly endergonic )

If we inhibite the ATP-ADP translocase what will happen?

It will lead to subsequent inhibition

When you pump ATP and take ADP you are giving one more negative charge to the outside ,ATP have 3 phosphate ADP has 2 phosphate so one more negative phosphate will get out side

Outside must be positive , inside must be negative that what makes the proton motive force .

When you pump more negative to the outside that’s mean that you are reducing the proton motive force .

