**TCA cycle intermediates:**

These intermediates are precursors for biosynthetic pathways, for example:

-Citrate ---- fatty acid synthesis.

-α-ketoglutarate ----amino acid synthesis & neurotransmitter “GABA”.

-succinyl CoA----heme synthesis.

-malate----gluconeogenes is pathway and it has a role in the carbohydrate metabolism .

-oxaloacetate ---- amino acid synthesis.

***Anaplerotic reactions:***

They are reactions that replenish the intermediates of the TCA cycle. (Reactions that compensate the intermediates that are taken away).

All the compounds in the citric acid cycle are intermediates, every compound is a product of a reaction and also it’s a substrate for the second reaction.

-***pyruvate carboxylase*** is a major anaplerotic enzyme. (it’s an enzyme that adds carboxyl group to pyruvate ) ( pyruvate got 3 carbon atoms and when we add the pyruvate carboxylase they become 4 carbons which is oxaloacetate)

• It requires biotin (vitamin B7) as a cofactor.

•it is activated by acetyl CoA. (Why?) To complete the citric acid cycle, so if the acetyl CoA conc. increases, then oxaloacetate conc. increases , together they form citrate .

•very high conc. in liver and kidney. (due to high gluconeogenesis there ).

gluconeogenesis uses oxaloacetat and malate.

Other anaplerotic reactions :

-all form acetyl CoA & citrate.

Glutamate can make alpha ketoglutarate

***Oxidative phosphorylation :***

-mitochondria has two membranes, outer and inner with inter membrane space between them .

- All energy metabolic procedures occur in the matrix of mitochondria except for glycolysis ( occurs in the cytosol)

-oxidative phosphorylation is the 4th step & the last stage where we generate ATP through it .

**Substrate level phosphorylation:**

Generation of ATP without O2.

e.g: formation of succinate from succinyl CoA ,which gives energy used to transfer GDP toGTP.

***Oxidative phosphorylation :***

Generation of ATP aided by O2 .

**Chemiosmotic theory:**

-electron carrying molecules from Krebs cycle are :

•NADH

•FADH2

-both of these carries donate their electrons to complexes of protein “ enzymes “, these complexes need to have a structure that undergoes reduction and oxidation ( accept and donate electrons)

- They are oxidoreductase enzymes , conjugated enzymes .

**Note that :**

**-**not all proteins can serve (function ) that way ,e.g: amino acids .

- heme can donate and accept electrons .

- metals as well, such as zinc,copper and lead.

\* \* \*

-NADH from krebs cycles has the highest ability to donate electrons , in terms of reduction potential its charge is –ve and its high .

-other carries should be more +ve

-O2 has the highest reduction potential & more +ve than the others .

- electrons are moving according to gradient potential in terms of ΔG .

- difference in energy between complexes will be used to pump protons (H+)outside the inner mitochondrial membrane , these protons form a force ,”concentration force “ : atoms outside the membrane more than in ,& “ electrical gradient “: more +ve charge outside than in ,so there will be an electrochemical gradient across the inner mitochondrial membrane.

Inner mitochondrial membrane is impermeable to anything in this life even to protons,

So it needs a carrier or transporter allowing protons to pass through the membrane, which is ATP synthase .

these protons can’t go out .

There will be electrochemical gradient this gradient will push the inner mitochondrial membrane in order to get inside but it’s not allowed except through transporters .

-these protons move according to:

•Their chemical gradient .

•Difference in energy. Will be used to phosphorelate ADP to make ATP

**Steps :**

**-**there are 5 complexes (1,2 ,3,4.

-NADH donates its electrons to complex 1 .

-Complex 1 donates its electrons to complex 3.

-then complex 3 donates to complex 4 then to O2 ,converting O2 to H2O.

**Note :**

There are two entry points for electron transport chain :

•complex 1

•complex 2

-both of these complexes donate their electrons to complex 3.

- complex 1 has no relation to complex 2 . (there is no direct connection between them )

\* \* \*

-the complexes are enzymes “ proteins”, oxidoreductases .( when they accept electrons they become reduced ,and when they donate electrons they become oxidized .

-also ,these complexes are integral proteins – transmembrane proteins ;spanning the membrane , all except complex 2 .

-these proteins can’t move ,so there should be carries which have the ability to transport electrons from complex to another , a carrier is called” Co Q” ,Co enzyme Q , Ubiquinone .

- this CoQ carry electrons from complex 1 & 2 to complex 3 .

-what transport the electrons from complex 3 to complex 4 is “cytochrome C”; a protein that has heme C , used in electron transport.

-difference in energy used in transferring electrons from complex 1 to CoQ used in pumping protons

-NADH donates 2 electrons to complex 1 becoming NAD+ .

Per 2 electrons moving from complex 1 to CoQ there will be four protons pumped out of complex 1 .

-complex 2 donates its electrons to CoQ but no protons pumped out. (Why?)

Because difference in energy resulted from transferring electrons from complex 1 to CoQ is 4 protons , while the resulted energy from transferring electrons from complex 2 to CoQ is almost zero, so complex 2 is designed not to span the membrane .

-when complex 3 donates its electrons to cytochrome C , there will be enough difference in energy to pump 4 protons out .

-when complex 4 donates its electrons to O2 , there will be enough difference in energy to pump 2 protons out .

- entry point of NADH is complex 1 , now the number of protons pumped out through complexes :

• 4 protons from complex 1 . •4 protons from complex 3 . •2 protons from complex 4.

So total number of protons pumped out is 10 protons .

-now for these protons to move back inside through ATP synthase , the equation is ( per 4 protons passing through ATP synthase there will be 1 ATP generated .)-----so, the amount of ATP generated is 10/4=2.5 ̴3 ATP.

- FADH2 is not swimming in the mitochondria , it’s always protein bound to complex 2 , which is succinyl dehydrogenase (from krebs cycle, step 6),this is the only direct link between krebs cylcle in the matrix and electron transport in inner mitochondrial membrane

- entry point of the electrons form the FADH2 is complex 2 , now the number of protons pumped out through complexes:

•no protons from complex 2 •4 protons from complex 3 •2 protons from complex 4.

So total number of protons pumped out is 6.

- the amount of ATP generated is 6/4=1.5 ̴2 ATP.

**Note:**

-For 2 electrons moving from NADH there will be a generation of 2.5 ATP.

-For 2 electrons moving from FADH2 there will be a generation of 1.5 ATP.

 SO, the amount of energy ATP generated from NADH is more than FADH2.

\* \* \*

-How to transfer electrons :-

There are three ways to transfer electrons

•direct electron transfer like in heme : oxidized from Fe+2 to Fe +3.

•transfer as a hydrogen atom .(like in FADH2)

•transfer as a hydride ion .(like in NADH)

**flavins :-**

**-**they are a group of co enzymes as in FAD & FMN.

-FAD = flavin adenine dinucleotide ( has two nucleotides in its structure ).

-FMN= falvin mononucleotide( has one nucleotide only ).

-both can accept two electrons in the form of hydrogen atoms , the addition is at the nitrogen atoms in the cycle

-FMN is found in complex 1.

**Note: check the structures of FMN &FAD in the slides.**

**CoQ :-**

**-**structure:its like the benzene ring with two oxygen atoms with double bonds ( go back to the slides and check the structure ). Attached to this ring we have long hydrocarbon chain

 -the function of the ring is to accept electrons from complex 1 or complex 2

-the function of the hydrocarbon tail is to pass freely within the membrane . ( due to the hydrophobic structure )

-the fully oxidized form is called Ubiquinone

-the fully reduced form is ubiquinol. (alcohol)

-semi oxidized or semi reduced form is called semiquinone (it can go through radical state).

 **CoQ in medicine :**

-sometimes prescribed to patients after MI ( myocardial infraction ) , to speed up the electron movement from complex 1 to complex 3 or from complex 2 to complex 3 , there for the occurrence of the electron transports chain is more frequent and more ATP IS generated , this is beneficial to patients who suffered from MI , as the heart muscle has become weaker , so you need more effort to transport electrons.

Ubiquinone structure can accept one or two electrons and its enter the free radical state

**Other electron-carrying molecules :**

**Cytochrome**

-transfer electron and uses heme to transfer electrons .

Heme behavior is just like the falvins they can’t present by themselves in the solution

-Heme can go into other oxidizing states like iron 4 (Fe+4),very oxidized state of the iron

Iron can bind oxygen and the problem is after oxidization or reduction of the heme it can bind oxygen , and oxygen can generate reactive oxygen species it may make (OH) with free radical or supra oxide (oo-) molecule ( all these molecules are very bad for health they can attack molecules because of their free radicals

-Its always attached to a protein , so, its reduction potential is depended on the protein attached to it .like FADH2

**Requirements of oxidative phosphorylation:-**

* Redox reactions.
* Complexes (proteins).
* Intact inner mitochondrial membrane.
* IF the membrane is not intact, Transporting of electrons will occur but without generating ATP.
* **the process has two parts** :
* Oxidative part (oxidation-redaction reactions occur there )& Phoshorylation part , this process is always coupled .
* Coupled means that as long as you have electron movement there must be generation of ATP .
* This coupling is lost when the membrane is not intact; oxidative part occurs while the phosphorylation part doesn’t occur.
* ATP synthase.

**Cytochrome C:-**

-Cytochrome C can bind only one electron , because it has a heme that can go into one oxidation state ( Fe+2 to Fe+3 when it loses that electron ).

-Cytochrome b-c1 is another name for complex 3 ( contains heme b & heme c1)

-NADH dehydrogenase is another name for Complex 1( dehydrogenation of NADH to NAD+)

**Complex 1 :- (NADH –Q oxidorductase).**

-complex protein( more than 25 polypeptide chain )

-contains FMN .( falvin mononucleotide ) the first one that accepts the electrons from the NADH

-FMN tightly bound to complex 1( because it can go into free radical state )

Transporting electrons from complex 1 to Co enzyme Q generate difference in energy = 16 kcal

Here 13kcal are enough to pump 4 protons out side complex 1

Ubiquinone can accept 2 electrons.

**Complex** **2**

Converting succinate into fumarate

The electrons from the convergence of succinate to fumarate will be loaded on FADH2 and from FADH to Ubiquinone and Ubiquinone will release it’s electrons to complex 3

**Complex 3:-**

-dimer, very big complex.

-contains heme b &c1.

The Q-cycle: (occurs on complex 3).

 -complex 3 accepts electrons ( 2 electrons ) from CoQ, each one of them will go to a heme (b &c1).

- one electron will pass to an electron carrying molecule that has a sulfur cluster,then to heme C1.

- the second electron will go to the other heme ( heme b ), heme b1 then to heme b2 ,within complex 3 there is a place that bind the Ubiquinone (oxidaized one Ubiquinone binding site ) the uquinone that haven’t been oxidized yet will bind it , now I have ubiquinol( has two electrons) one electron will go to heme C and the other will go to heme b then to ubiquinone binding site . the electron when reach the uniquinone it will has an extra electron . uniquinone can accept 2 electrons and enter the free radical state , and the electrons that went to heme C will go to cytochrome c

-now, another molecule of ubiquinone which also has 2 electrons , enter the same cycle.

- 2 molecules of the reduced ubiquinonol (QH2) through two repetitive cycles& one molecule exits, so the amount of QH2 used is (the net ) is one .

-we moved 2 electrons in the first cycle & and another two in the second cycle =4 electrons, and we formed 2 electrons so the net is 2 electrons ,

- With the passage of these 2 electrons , 2 protons are pumped out from the first molecule and another 2 from the second molecule , total is 4 protons.(the ratio is 4H+/2e-).

