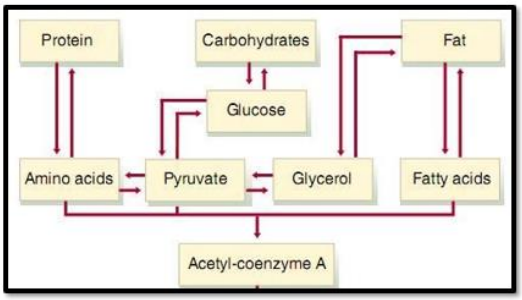
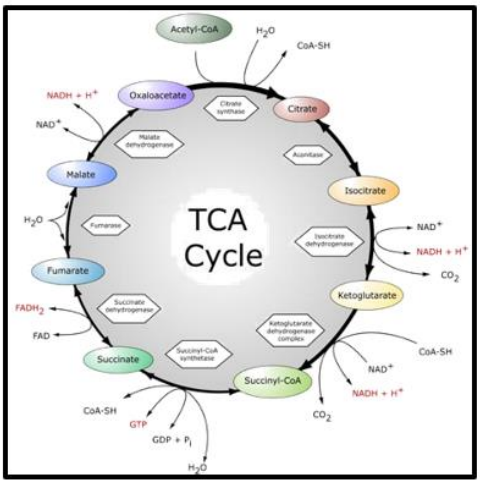
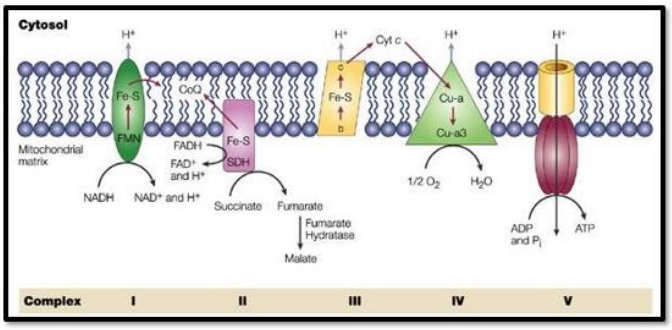
Oxidative Phosphorylation

The oxidative phosphorylation, Where are we?





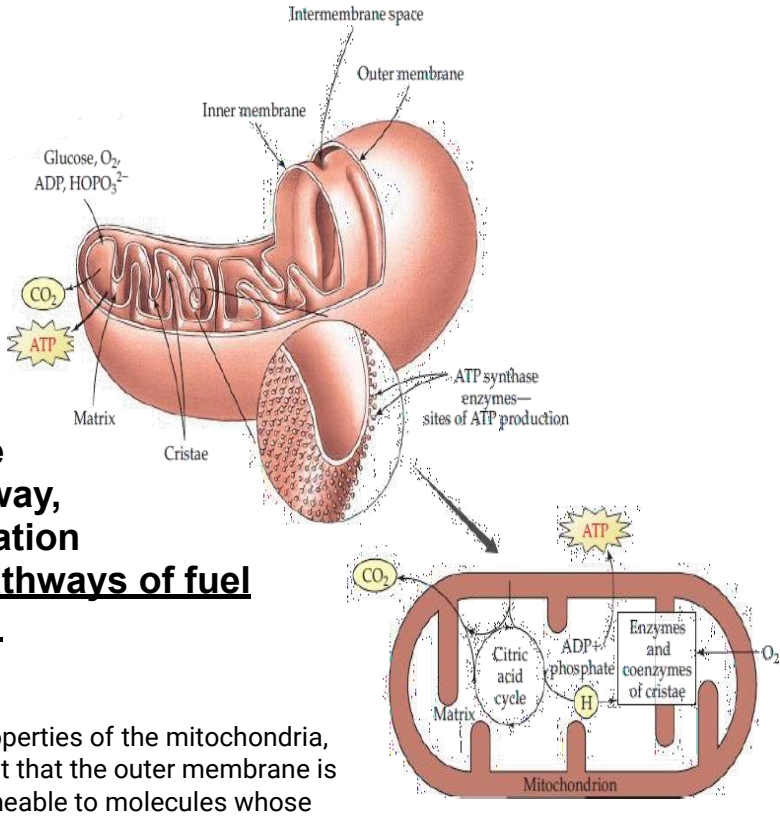


where does oxidative phosphorylation fit? It is the last process in energy metabolism. oxidative phosphorylation carries two different concepts: oxidation reduction reactions, which is highlighted by the oxidative part and phosphorylation as another process, those are two different processes, firstly, oxidation- reduction reactions occur, and as a result of these reactions, phosphorylation will occur, So those are the two different processes and those two different processes are coupled to each other (one of them cannot happen without the other).

The Mitochondria

What was mentioned in the slide:

- **OMM: <u>permeable</u> to small molecules (MW<5,000) & ions, <u>porins</u> (transmembrane channels)**
- **IMM: <u>impermeable</u> even to H+; specific transporters**
- **IMM bears the components of the respiratory chain and the ATP synthase**
- **Matrix: contains pyruvate dehydrogenase complex & TCA cycle enzymes, fatty acid β-oxidation pathway, and the pathways of amino acid oxidation**
- **In other words: <u>matrix contains all pathways of fuel oxidation except glycolysis (cytosol).</u>**



What was mentioned in the lecture:

Now starting with the mitochondria, those are all the properties of the mitochondria, which we've discussed them before, we want to highlight that the outer membrane is permeable to things just like other membranes, it's permeable to molecules whose molecular weight is than 50,000 Daltons (five kilodaltons), However, the inner membrane of the mitochondria is impermeable to anything in this life, Even H^+ which is the smallest thing in the life, so anything that would cross the inner membrane should have a specific transporter.

Remember: a mitochondrion has an inner mitochondrial membrane (IMM), outer mitochondrial membrane (OMM), intermembrane space between them, and the area enclosed by the IMM is called the mitochondrial matrix

What was mentioned in the textbook:

- The electron transport chain of the mitochondrion (except for cytochrome c) is located in the inner mitochondrial membrane and is the final common pathway by which electrons derived from different fuels of the body flow to oxygen (O_2).

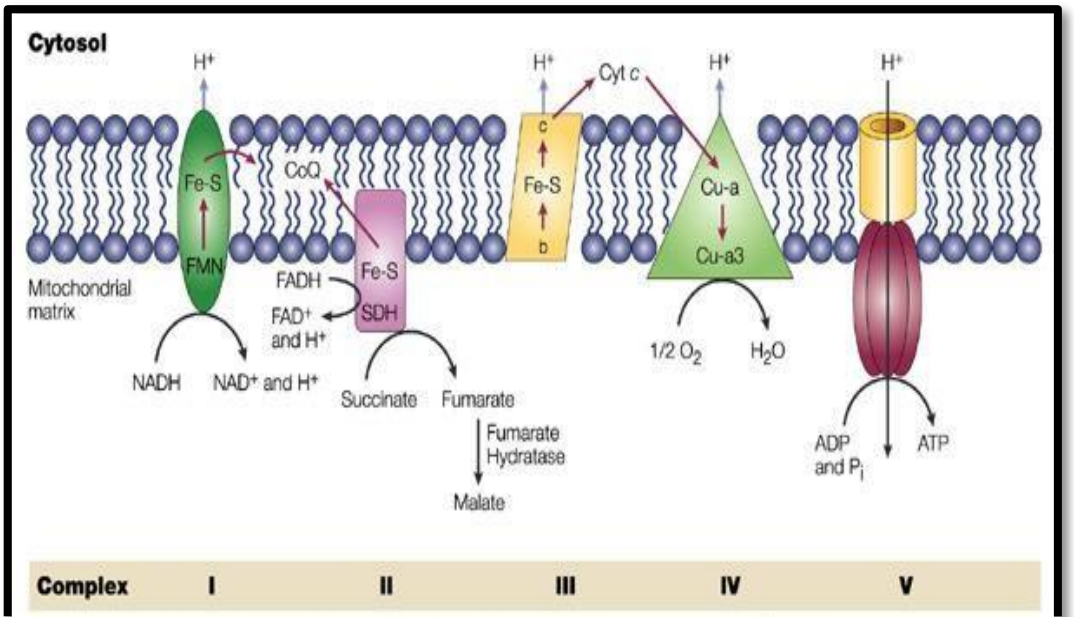
1. Membranes of the mitochondrion: The mitochondrion contains an outer and an inner membrane separated by the intermembrane space. Although the outer membrane contains special channels (formed by the protein porin), making it freely permeable to most ions and small molecules, the inner membrane is a specialized structure that is impermeable to most small ions, including protons and small molecules such as ATP, ADP, pyruvate, and other metabolites important to mitochondrial function (Figure 6.7). Specialized carriers or transport systems are required to move ions or molecules across this membrane. The inner mitochondrial membrane is unusually rich in protein, over half of which is directly involved in oxidative phosphorylation. It also is highly convoluted. The convolutions, called cristae, serve to greatly increase the surface area of the inner membrane.

2. Matrix of the mitochondrion: This gel-like solution in the interior of mitochondria is also rich in protein. These molecules include the enzymes responsible for the oxidation of pyruvate, amino acids, and fatty acids (by β-oxidation) as well as those of the tricarboxylic acid (TCA) cycle. The synthesis of glucose, urea, and heme occurs partially in the matrix of mitochondria. In addition, the matrix contains NAD^+ and FAD (the oxidized forms of the two coenzymes that are required as hydrogen acceptors), and ADP and Pi, which are used to produce ATP. [Note: The matrix also contains mitochondrial DNA (mtDNA) and RNA (mtRNA) and ribosomes.]

Oxidative phosphorylation (OxPhos)

What was mentioned in the slide:

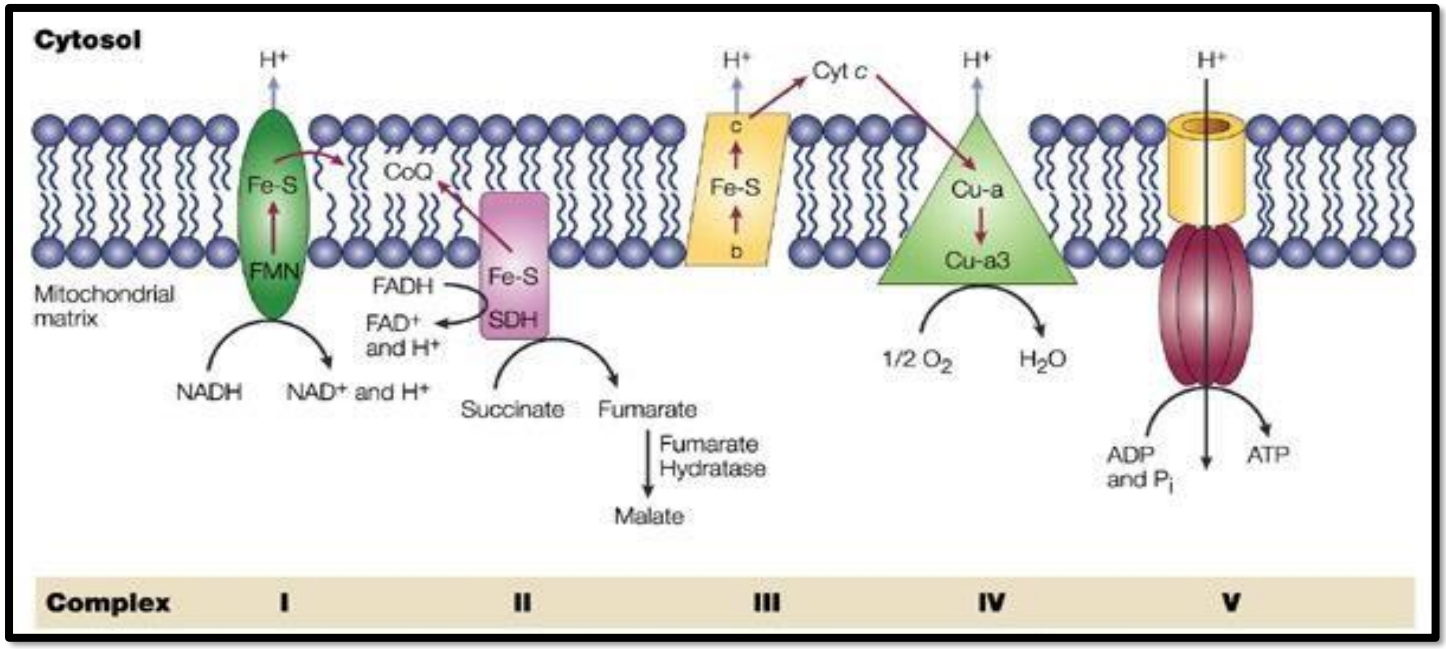
- **Generation of ATP aided by the reduction of O_2**
- **Peter Mitchell (1961): the chemiosmotic theory**
- **Oxidative phosphorylation has 3 major aspects:**
- ✓ **(1) It involves <u>flow of electrons</u> through a chain of membrane-bound carriers (<u>prosthetic groups</u>)**
- ✓ **(2) The free energy available (exergonic) is <u>coupled to transport protons across</u> a proton-impermeable membrane**
- ✓ **(3) The transmembrane <u>flow of protons</u> down their concentration gradient provides the free energy for synthesis of ATP (ATP synthase)**



What was mentioned in the lecture:

Peter Mitchell came in 1961 and explained the chemiosmotic theory, which explains how the oxidative phosphorylation occurs, it involves three major themes. What happens exactly inside the oxidative phosphorylation process? We've got electrons, and those electrons has to move, and as we've studied before, if we have electrons, Those electrons will move according to the potential difference, So there must be a difference in energy, there is a difference in ΔG and otherwise they won't move, that difference in energy is used to take protons from inside of the mitochondria to the outside of the mitochondria, If we need energy to take molecules from a place to a place, that means that we're moving them against their potential, whether it's a concentration difference (gradient), whether it's an electrical difference (potential) or whether it's both, in the case of H^+ (protons), we are working against both, we call it electrochemical gradient because H^+ has a charge and a concentration, So that excess of energy released from the movement of electrons will be used to pump protons out from the matrix of the mitochondria to the intermembrane space, and H^+ protons will not be allowed to come back, because -as we've just said- the inner mitochondrial membrane is impermeable to anything in this life, even H^+ , we need specific transporters to move it, and there are no specific transporters to move it again, that will make the pressure on the inner mitochondrial membrane to increase, when protons are allowed to come back according to their potential, which means there will be release in energy, that energy will be used to couple inorganic phosphate with ADP to make ATP. Those are the three major themes, electron movement, proton pumping and generation of ATP.

Oxidative phosphorylation (OxPhos)



What was mentioned in the lecture:

Now after the three major themes have been defined, we're going to explain how the process occurs exactly, we will start by the oxidative part (oxidation reduction reactions), Then we will explain how phosphorylation occurs, and then we will explain how the coupling occurs.

how oxidation-reduction occurs? What are the substrates for the electron transport chain? oxidation reduction reactions occur in a pathway called electron transport chain, electron transport chain does not produce ATP by itself, What produces ATP is oxidative phosphorylation because it involves two processes, the oxidative part which is the electron transport chain, So what are the substrates for the electron transport chain? They include NADH and $FADH_2$, considering NADH, three molecules will come, swimming in the matrix, the receptor for NADH exist in the inner mitochondrial membrane, Now NADH will come from the citric acid cycle which occurs inside the matrix of the mitochondria and it donates its electrons to an oxidoreductase enzyme, it will take electrons from NADH converting it to NAD^+ and storing these two electrons within itself. Now, how electrons move within enzymes or proteins?
What makes a protein able to attract electrons, to obstruct electrons and release electrons while the other protein is not? there should be something inside this protein that the other protein lacks and this is what we call redox centers, Heme is one of them, because some hemes work in electron transfer, Metals are one of them like iron, copper, etc. and some molecules derived from the protein itself are one of them like FAD, FMN, And also we have structures that look like crystals, which are designed in a nice way, We call them iron sulfur clusters, where Iron is connected to sulfur in a nicely designed crystallized way, for example, 2-iron-2-sulfur clusters where we have two irons and two sulfurs connected to each other, also we have four-iron-four-sulfur clusters, etc. those molecules have the ability to carry one electron at a time because what carries electrons is the iron and iron can switch in between two oxidation States (Fe^{+2}/Fe^{+3})
Likewise, the heme can carry only one electron at a time, Why? Because it has iron and iron can switch in between two oxidation States, copper can carry one electron at a time. FAD can carry two electrons at a time, FMN can carry two electrons at a time, etc. So those are the different molecules which work inside these enzymes.
going back, NADH Will donate its two electrons, Those two electrons will be caught by FMN inside this enzyme (the ellipsoidal green one in the figure), And then they will be loaded to iron sulfur clusters, we have seven of these iron sulfur clusters inside this complex till the electrons reach this point, what do we call this enzyme? It is the first one that takes the electrons from NADH, NADH is the substrates
And what exactly this enzyme does is that It takes out the hydrogens with the electrons, so we call it. NADH dehydrogenase, so it can be called NADH dehydrogenase, NADH coenzyme Q, which is the after molecule, NADH coenzyme Q oxidoreductase or because of the sequence they've called it historically as complex I.
we have now two electrons, the oxidative phosphorylation process all occur inside the inner membrane of the mitochondria, Those enzymes and proteins are embedded inside the inner mitochondrial membrane, Now we have two electrons, Can electrons move within membranes and why? No, because there are charged and the membrane is made of lipids (lipids are not electricity conductors), even integral membrane proteins cannot move laterally within the membrane, except for a short distance, especially proteins, Complex I doesn't have any relation to complex II, electrons from complex I has to cross all that distance to complex III, how they can pass distance? there should be a transporter and it happens for that transporter to be called coenzyme Q, It is also called ubiquinone, we will come to its structure after a while, coenzyme Q can carry two electrons at a time, with each electron in a different place, so It can generate free radicals (the same as FAD), it will deliver these electrons to complex III.
Complex II's substrate is $FADH_2$, but $FADH_2$ cannot swim inside the mitochondrial matrix, therefore, the enzyme that catalyzes the sixth step in Krebs cycle which is called succinyl dehydrogenase (it is the only one that generates $FADH_2$ in the cycle) is embedded in the mitochondrial membrane!!
So when it converts succinate to fumarate, it takes the two electrons and load them on FAD to become $FADH_2$, and now the electrons are here, So complex II of the electron transport chain is the same exact one as succinate dehydrogenase which was present in the TCA cycle. the only direct link in between Krebs cycle and electron transport chain is, that is a smart way (سبحان الله), So now $FADH_2$ is inside the enzyme, who will abstract those two electrons from succinate dehydrogenase (complex II)? It is coenzyme Q again, it will take those two electrons and it will deliver them to complex III.

complex III has different names, It can be called complex III, It was named as this because of the order within the inner mitochondrial membrane. Also it can be named after the substrate and the product. We call it coenzyme Q cytochrome C oxidoreductase, It does oxidation for coenzyme Q and it reduces cytochrome C, also it has another name which is called cytochrome BC1 complex. What do we means by cytochrome BC1?
It is named cytochrome BC1 complex because it has two types of heme, it has heme B and it has heme C, it has two heme Bs and it has one heme C. when coenzyme Q reaches complex III, it has two electrons, so it is in the reduced form, (the oxidized coenzyme Q is different than the reduced one), there is a place high up on the cytosolic site complex III which has high affinity for the reduced coenzyme Q, so coenzyme Q (the reduced one) will bind there, It will give two electrons each time. The output of complex III is cytochrome C, cytochrome C carries one heme C molecule, so cytochrome C can carry one electron at a time, So one of the electrons that comes from coenzyme Q will pass to a heme B on complex III and then it will be passed to cytochrome C, what about the other electron? The other electron will pass to another heme B and let's stop here, after the coenzyme Q releases its two electrons, The affinity will not still be high for it to be attached to complex III, so it will released, but there is a place on the matrix side of complex III that has high affinity for oxidized coenzyme Q, after the detachment of coenzyme Q from the cytosolic area, it will bind on matrix area and the electron which is passed through the complex will come and semi-reduce the coenzyme Q again, then, another reduced coenzyme Q molecule will come again repeating the cycle, When it repeats the cycle, there will be one electron passing to heme B --> cytochrome C's heme C, And the second electron will pass there on the complex III to heme B and then to the semi-reduced coenzyme Q making it fully reduced, So we regenerated another molecule of coenzyme Q with full reduction which can do the cycle again, How many electrons are coming to complex III? They are four electrons, how many cytochrome C molecules came out of the complex III? They are two molecules, each one carrying one electrons, therefore two electrons had left complex III, and the other two was used to generate a reduced coenzyme Q molecule to repeat the cycle, this cycle is called Q cycle, (Q is for quinone).

So we have ended up with two cytochrome molecules, cytochrome C is a protein, it's different than coenzyme Q, coenzyme Q is a molecule, which can be within the membrane, but cytochrome C is a protein, it cannot swim freely within the membrane, it is soluble in water, So it will be on the outer side of the inner mitochondrial membrane. it comes and takes the electrons from complex III and deliver them to complex IV, each time it delivers an electron.

Complex IV contains electron carriers, What are the electron carriers which are present in complex IV? It has two copper ions named according to the site: copper A site and copper B site, it also has two heme groups known as heme A and heme A3, copper can only switch between two oxidation states (Cu^{+}/Cu^{+2}), therefore it can only accept one electron, and so is heme by its iron, So this electron, which is coming from cytochrome C, it will go to the heme A or to the copper site? this single electron will be shared in between the copper and the heme !!!!!!, they share the electron such as a covalent bond!!, When another cytochrome C comes and donates the electrons, the first electron will transfer from copper A and heme A to copper B and heme A3, , the second electron which is coming will be shared between heme A and copper A site , So when we will have two electrons, each one will be shared in between copper and heme.

Electron transport chain at the end will convert O_2 to H_2O, that needs four electrons, because we have two atoms of oxygen ($O_2+H_2 \rightarrow 2H_2O$), So each single oxygen has two hydrogens with their electrons, So for O_2 two to be converted, it will produce two molecules of water.

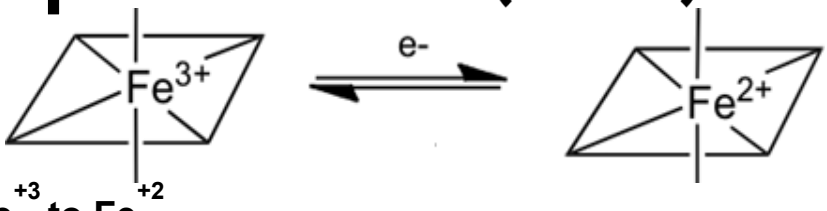
this is the purpose of oxygen, why do we need oxygen in breathing? to go to the mitochondria at complex IV, getting reduced by the electrons to become water, So most of the oxygen that we do breathe gets converted to water, and this how we keep the homeostasis of water within the body.
Always remember that oxygen has nothing to do with carbon dioxide within the body, just in the lungs Oxygen will be inhaled and CO_2 will get out, but within the metabolism each one behaves differently, Oxygen that we breathe binds on the iron in heme of hemoglobin, but CO_2 bind H_2O to form carbonic acid that constitute the buffering system in the blood (summer semester), and also it sometimes binds hemoglobin at the free N termini of chains forming the carbamate, so these two have different binding forms, when they'll go out in the energy metabolism, CO_2 gets out through Krebs cycle while O_2 get out through electron transport chain, two processes have different from each other.
So far we have explained electron transport chain in detail, but we haven't make ATP, ATP is made through ATP synthase which is different, it is sometimes called complex V, ATPase, and ATP synthase. Complex V after is position in the chain, ATPase because it degrades ATP, and ATP synthase because it synthesizes ATP, because electrons are moving from a molecule to another, they move according to a difference in electrical potential, which is translated into ΔG .
So that difference in potential when the two electrons from NADH are passed to coenzyme Q (through complex I) is translated to difference in energy, which is enough to pump four protons out, And when those electrons reaches cytochrome BC1 complex which passes those two electron to two molecules of cytochrome C, it results in a difference in energy, which is able and enough to pump four protons, and when those two electrons reach complex IV and donated to one of the oxygens, that difference in energy is capable of pumping two protons, So the total is 10, So for the two electrons, which are coming from NADH, when they pass along the electron transport chain, there will be 10 protons pumped outside of the matrix to the intermembranous space.
now for the case of the $FADH_2$, two electrons pass through complex II into coenzyme Q, that difference in energy is not able (enough) to pump any proton out, this is why complex II is not a transmembrane protein, it is not because it is not transmembrane protein so it cannot pump protons out, but because the difference in energy is not enough to pump any proton then why to make the protein transmembrane?!!!, this difference of energy is expressed as heat, there is a lot of energy in ETC than is wasted as heat.

A proton need an energy of 4 kilocalories to be pumped, the energy released by travelling of electrons from $FADH_2$ to coenzyme Q is less than 4 kilocalories, therefore, there will not be pumping of protons

Coenzyme Q will reach complex III and the passage of electrons from complex III to cytochrome C will make a difference in energy capable of pumping four protons, and at the level of complex IV there will be pumping of two protons, So the total is six protons, if NADH is the source there will be ten protons, pumped out, but if $FADH_2$ is the source, then there will be six protons pumped out.
We are pumping protons out so we are increasing the proton gradient across the IMM, if we suppose that we don't have pores in the membrane, will the electrons keep moving? Yes ,but will the protons will keep being pumped outside? No, because the outside pressure on the membrane will become high and high and high, therefore, leakage of protons through ATP synthase is important for the process to continue, The electrochemical gradient outside of the inner mitochondrial membrane is pressurizing the membrane more and more and more. And this pressure is the one which drives the proton to come through the ATP synthase, the difference in energy from outside to inside when protons are moving is capable of joining inorganic phosphate with ADP to make ATP.

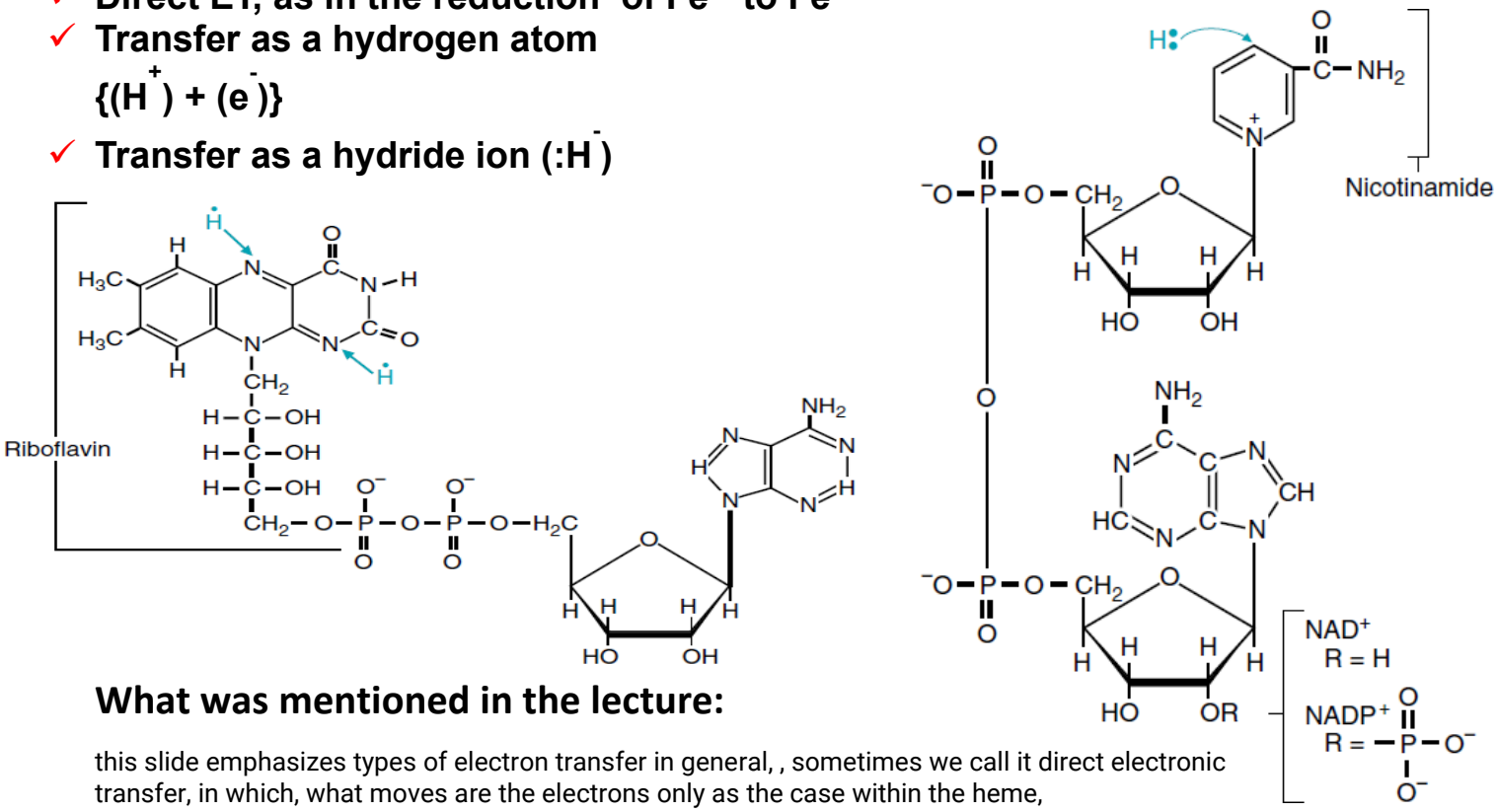
for each ATP generated, how many protons do I need for them to pass through the ATP synthase? I need four protons for each molecules of ATP, because NADH gives two electrons and it pumps 10 protons out, How many ATP molecules can be generated out of those? two and a half, for $FADH_2$, its one and a half molecule, Is there such a thing in science? No. When they discovered the electron transport chain and how it goes, they assumed that to be three ATPs per NADH molecule and two ATPs per $FADH_2$ molecule. What happens actually is that each two NADH gives 5 ATPs while each two $FADH_2$ gives 3 ATPs.

Types of electron transfer (ET) through the electron transport chain (ETC)



What was mentioned in the slide:

- **3 types of ET occur in OxPhos:**
- ✓ **Direct ET, as in the reduction of Fe^{+3} to Fe^{+2}**
- ✓ **Transfer as a hydrogen atom $\{(H^{+}) + (e^{-})\}$**
- ✓ **Transfer as a hydride ion ($:H^{-}$)**



What was mentioned in the lecture:

this slide emphasizes types of electron transfer in general, , sometimes we call it direct electronic transfer, in which, what moves are the electrons only as the case within the heme,

for heme to switch in between Fe^{+2} and Fe^{+3} what changes is only the electrons, which means that there is movement of electrons as direct electron transfer, Also electron can be moved by coupled to H^{+} as a hydrogen atom and this is the case that we see with FAD and FMN, We have two electrons each one coupled on an H^{+} , which means two hydrogen atoms, and also electrons can be moved as a hydride ion and this is the case that we see with NAD^{+}, So three types of movements with three examples.

Electrons are funneled to a universal electron acceptors

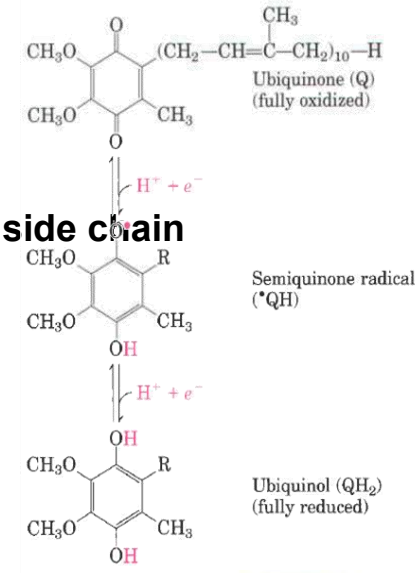
What was mentioned in the slide:

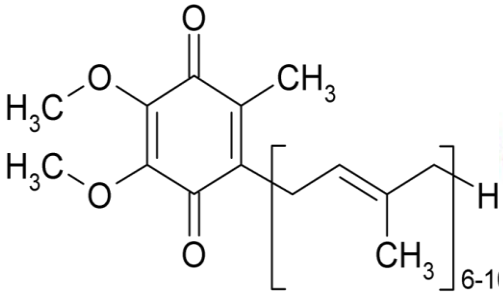
COENZYME	AS OXIDIZING AGENT	AS REDUCING AGENT
Nicotinamide adenine dinucleotide	NAD^+	$NADH/H^+$
Nicotinamide adenine dinucleotide phosphate	$NADP^+$	$NADPH/H^+$
Flavin adenine dinucleotide	FAD	$FADH_2$
Flavin mononucleotide	FMN	$FMNH_2$

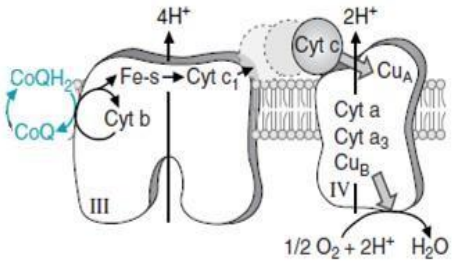
Other electron-carrying molecules "Ubiquinone"

What was mentioned in the slide:

- **Also called coenzyme Q, or Q**
- **Lipid-soluble benzoquinone with a long isoprenoid side chain**
- **Small & hydrophobic (freely diffusible)**
- **Carries electrons through the IMM**
- **Can accept either 1 e- or 2 e-**
- **Act at the junction between a 2-electron donor and a 1-electron acceptor**
- **Sometimes prescribed for recovering MI patients**







What was mentioned in the lecture:

considering the Ubiquinone, the ubiquinone can swim freely within the membrane, that seem to Doesn't make sense, it can swim freely within the membrane but at the same time, it can carry electrons, to be able to get oxidized and reduced, It should be water-soluble and to move through the membrane, it should be lipid soluble., So how can this combination occurs? You can see from this structure that the structure of the ubiquinone or coenzyme Q contains two major aspects, it has a ring, which we call a cyclic diene structure, diene corresponds to di (two) and ene (double bonds) it has two alkene groups (double bonds), and it has two keto groups, upon the reduction by one electron, One of the keto groups will become -OH, and upon the reduction by the second electron, the second one will be -OH, this is (in the figure) the structure of the oxidized one and we call it ubiquinone., as you can see here from the name (-one), which is in reference to ket(one) and when it is reduced by one electron (so it has one -OH), we call it semiquinone or semiquinol, with respect to the alcohol but it's semi because one of the oxygens is getting reduced, when it is fully reduced (two electrons are coming), As you can see in this structure, we call it the ubiquinol because it's all in the alcoholic structure.

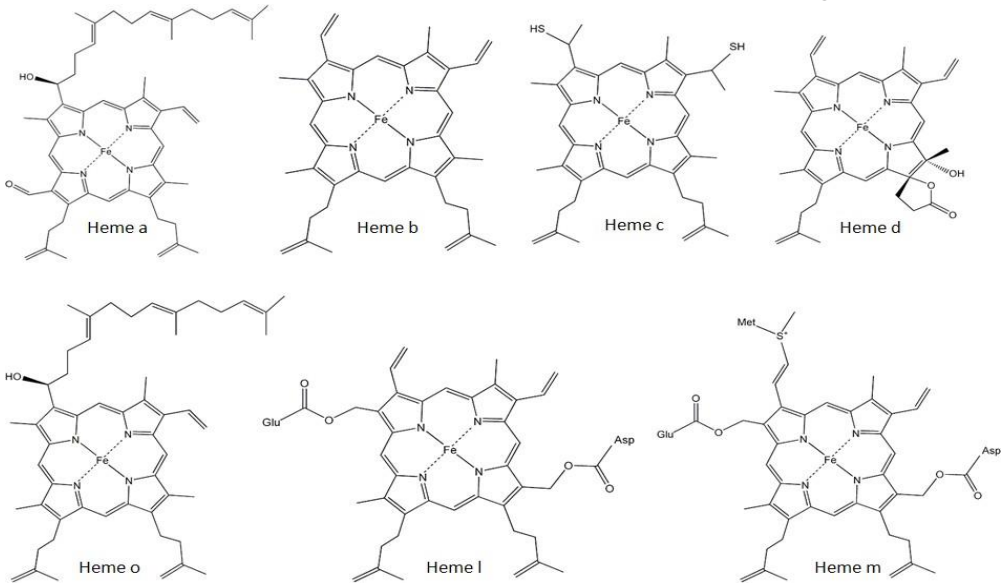
it can carry the electrons and pass them, and when it passes the electrons, it comes back to its original structure as an oxidized one, now what makes it able to pass through the membrane freely is the connection over here with the ring (the five carbons attached to the ring) this structure, which can be repeated 6 to 10 times (we can have 20 to 50 carbon atoms attached to the ring), depending on the organism and the place. It is a hydrocarbon chain, so by this huge structure which can be repeated over and over again, it will make the molecule lipid soluble and can swim freely within the solution.

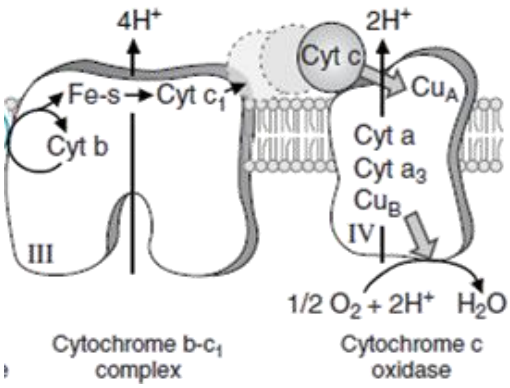
for patients who are affected by myocardial infraction, sometimes coenzyme Q is prescribed to them, what is the purpose of that? because Some of the tissue in the heart is dead and now it cannot contract as usual because of ischemia, . Accordingly, for the heart to work better, you need the amount of ATP, which is being generated by other cells to overcome the deficiency from this tissue and this is where extra coenzyme Q can help. But only to certain limit, we cannot give the patients another component of the ETC because they’re all proteins and therefore will not affect if given orally.

Other electron-carrying molecules "Cytochromes"

What was mentioned in the slide:

- Proteins with characteristic strong absorption of visible light (Fe- containing heme prosthetic groups)
- Classification based on light absorption
- Mode of binding (a, b, c)
- Mitochondria contain three classes of cytochromes (a, b, & c)





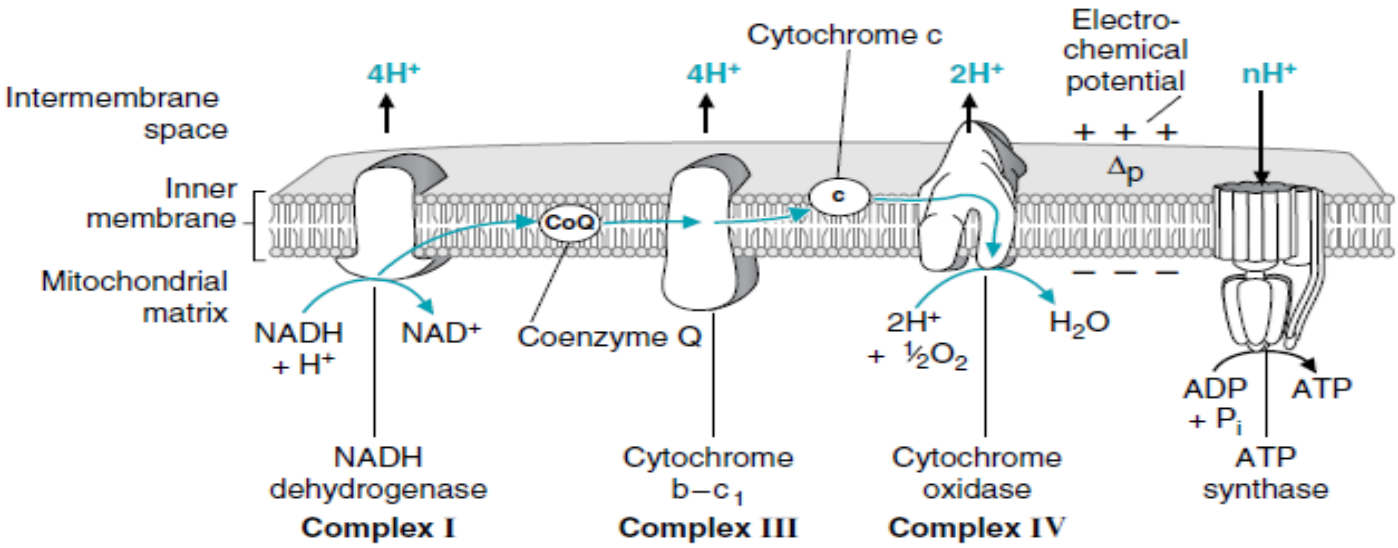
What was mentioned in the lecture:

Heme is the prosthetic group responsible for carrying electrons in cytochromes, we have a lot of types of hemes, all of them share one feature that they can carry one electron at a time because of the presence of the iron.

Requirements of OxPhos

What was mentioned in the slide:

- **Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2)**
- **An intact IMM**
- **ETC of proteins**
- **ATP synthase**



What was mentioned in the lecture:

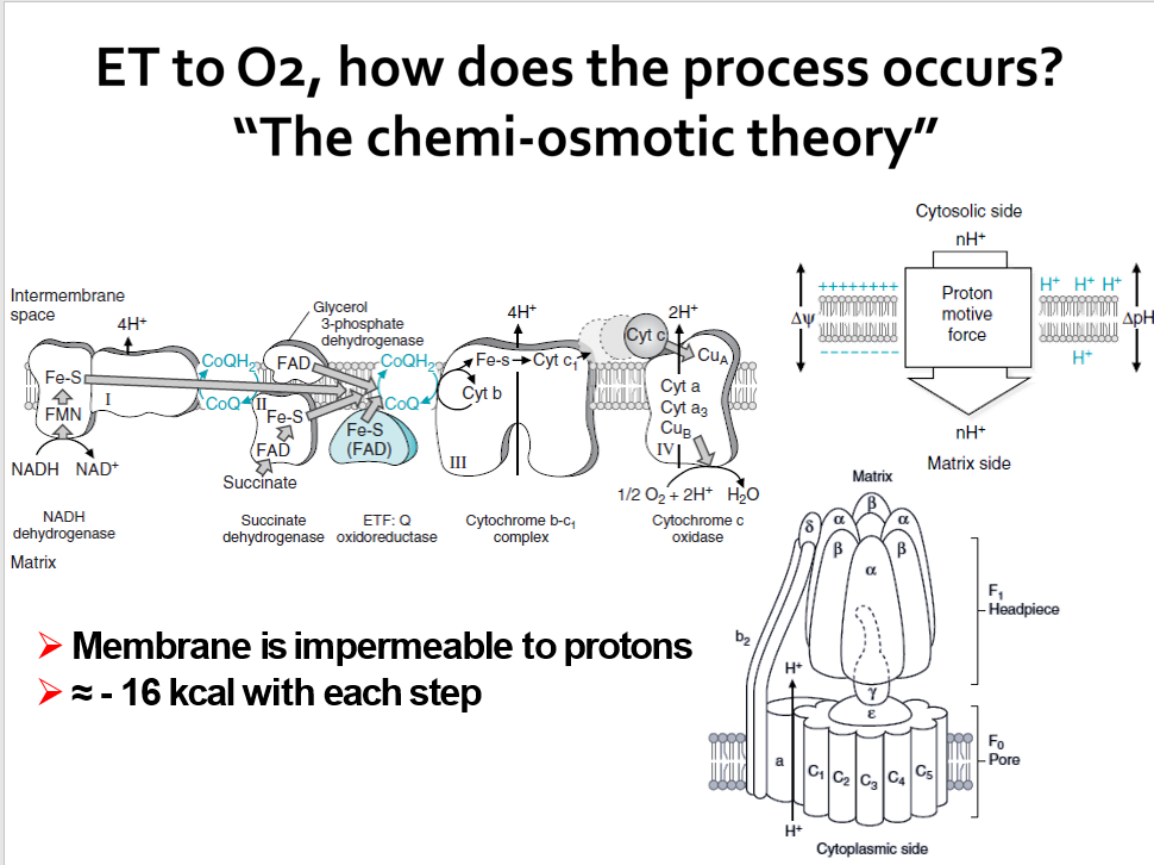
for the oxidative phosphorylation process to occur, What do we need? We must have a source of electrons which are expressed as NADH and $FADH_2$, and I must have a final electron acceptor which is oxygen, that why tissues which have deficiency in oxygen, like when someone is exercising too much will have fatigue, why? Because there is no enough oxygen in the muscular tissue to induce the oxidative phosphorylation, so pyruvate is getting converted to lactate, we also need carriers to carry the electrons between them, which are the enzymes: complex I,II,III,IV. Also ATP synthase is required, and the last most important thing that must present, is the intact inner mitochondrial membrane, what happens if that membrane has become leaky? Electrons are moving, protons are being pumped out and then this gradient should be expressed, If the membrane is leaky in some pores, some of the protons will come through these pores and Some of them will come through the ATP synthase what will happen to the process of ATP synthesis? ATP efficiency will be less, so I'll make less ATP. what happens to the energy difference that comes from coming back of the protons from outside to inside through these leaks? It will be wasted in the form of heat. The inner mitochondrial membrane does not allow anything to pass through it except through a carrier, leakage of protons through the inner mitochondrial membrane (not through the ATP synthase) is important in thermogenesis and it is carried by uncoupling proteins, uncoupling proteins have different types and differ from one type of tissue to another, and differ in their amount and type from a human to another.

how much uncoupling proteins do you have within the inner mitochondrial membrane? the more you have uncoupling proteins, less ATP will be made, accordingly the more the heat is being generated and the less obese you are. We need ATP for anabolic pathways, So if I'm having lower amounts of ATP from the food,

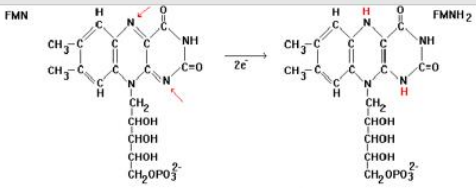
Then I will eat much more freely and I won't get fat. Studies claim that societies that are more obese have more frequency of mutations in uncoupling proteins, some people eat a lot but do not get fat, while other people eat less and get fat faster, this is one of the reasons but not the only reason.

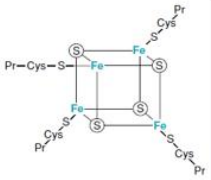
Nowadays, there are researches that focuses on designing a molecule that can be inserted in the inner mitochondrial membrane that can pick up protons from out and deliver then to the matrix through the membrane, that will increase the heat content, so you need it to be regulated, if not, it will cause what it called malignant hyperthermia, which an uncontrolled increase in the body temperature that will cause death, so you something that is controlled so a certain amount of protons that are pumped out will get back in, that will increase the heat content and make a person slimmer.

Uncoupling can be carried by drugs or naturally by uncoupling proteins.

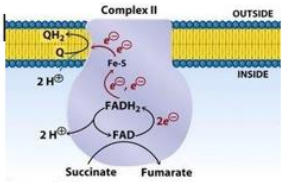


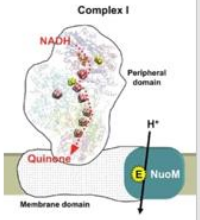
Oxi–Red Components of ETC



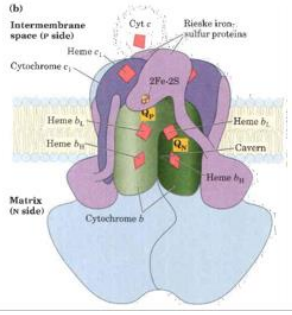


- "NADH Dehydrogenase" – Complex I
  - NADH-Q oxidoreductase

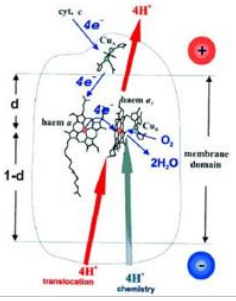




- "Succinate Dehydrogenase" – Complex II
  - ≈ 0 kcal, H+?
- "Cytochrome bc1" – Complex III
  Q-cytochrome c Oxidoreductase



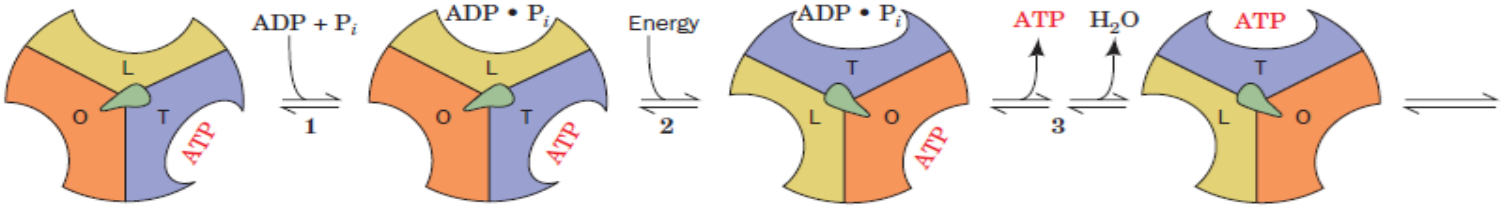
"Cytochrome c oxidase" – Complex IV

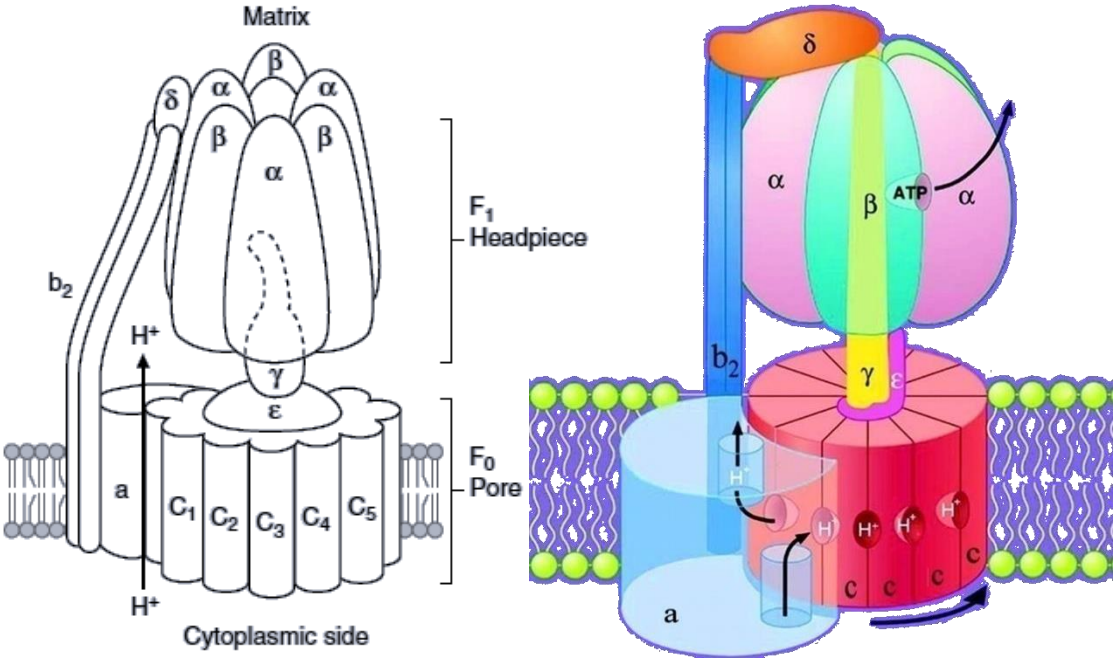


ATP Synthase

What was mentioned in the slide:

- **F1:**
  - **"γ" subunit: rotates**
  - **"β" subunit: binds**
  - **"α" subunit: structural**
  - **3 conformations: tight (T), loose (L), open (O)**
  - **F0:**
  - **"a" subunit: point of entry & exit**
    - **"c" subunit rotates**
    - **4H+/ATP**
  - **Can run backwards**





What was mentioned in the lecture:

ATP synthase is composed of two parts: the F0 portion which is inserted within the membrane, and the F1 headpiece which is directed toward the matrix, this complex has many subunits, we have the a subunit which is shaped like a hook and is within the membrane, it surrounds a cylinder inside it partially from one side, this inner cylinder in composed of 12 subunits which are called the C subunits, the a subunit is fixed within the membrane, but the c subunits can rotate in place, connected to the C subunits is this rod-like yellow γ subunit, γ subunit is angled, on the top of the angle, there is something that looks like a hat, this hat is composed of 6 subunits, those six subunits are in sequence: α,β,α,β,α,β the α subunits are there for structural purposes, to conserve the enzyme's shape and location, The β subunits are the catalytic ones, they are the ones which catalyze the reaction to join ADP and inorganic phosphate to produce ATP.

How does the mechanism occur exactly?

In the cytoplasmic side (the intermembranous space) there is a high concentration of protons, when protons pass through an opening (there is an opening, protons do not pass all the way through in one single pathway, they'll face of the C subunits, specifically, it will face a certain amino acid in this subunit which is the glutamate amino acid, glutamate has a negative charge in its carboxyl group, So when the H^+ binds the negative charge, there will be no charge, the amino acid will be neutralized, this will cause a conformational change in the C subunit causing it to move from its place, it will move from its place, bringing another C subunit to the opening, Another H^+ will come binds to another glutamate acid causing another displacement, And this is how the cylinder moves With one C unit at a time. We have 12 C subunits, so for a full turn of C subunits, 3 ATP molecules will be generated, when the C subunits complete a full rotation, there will be another opening facing the H^+, So the H^+ will get detached at this area because of the difference in pH, then it should be in fact, (the pH difference causes the H^+ to attach or detach), so H^+ will leave to the matrix and every time it will face this opening, the H^+ gets in or gets out.

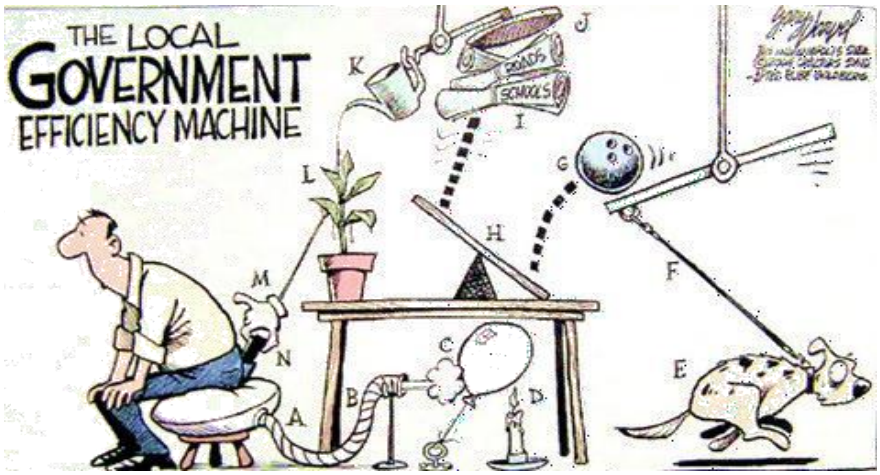
Until now we haven't generated ATP, what is the importance of this rotational movement of the C subunits? This movements affect the gamma subunit, the most important thing in this gamma subunit is because it's angled, it's angled and there are three beta subunit, Now, when it is angled and rotating, every time it will hit one of these beta subunits, when it hits that beta subunit, it causes a conformational change and when it gets detached from it, it causes another conformational change, The beta subunits goes into three different confirmations, tight, loose, and open, regardless of each one what it does, but for you to know that there will be different confirmations in the beta subunits with each hit and release from the beta subunits when the gamma rotates, So these conformational changes, one of them is open to release the ATP, loose conformation will attach itself to ADP and inorganic phosphate (It has high affinity for ADP and inorganic phosphate), and the tight conformation encloses itself on the ADP and inorganic phosphate for the reaction to occur.

We've studied before that most enzymes can catalyze the forward and the backward reaction, this applies over here also, Which means what that ATP Synthase can run backward, It means that it can catalyze the breakdown of ATP to produce ADP and inorganic phosphate if it rotates in the opposite direction, What will make the C subunits to rotate in a different way? It is H^+ , if its gradient is going from inside to outside. (When the electrical chemical gradient is flipped back) that happens when a lot of ATP is produced, so you use some of this ATP to regenerate the gradient.

Energy yield from the ETC

What was mentioned in the slide:

- NADH, -53 kcal,ATP?
- FADH2, -41 kcal,ATP?
- $\Delta G^{o'}$ is so negative, never reversible
- ATP machine efficiency, (anions, Ca^{+2}, heat, phosphate, substrates)
- Electron transport chain is our major source of heat



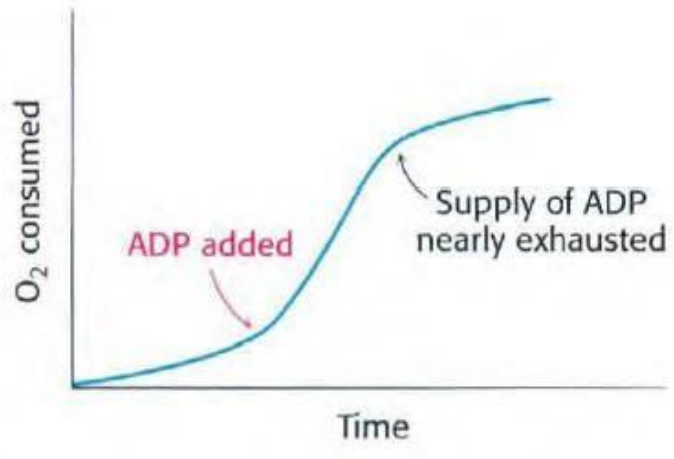
What was mentioned in the lecture:

How much energy do I get outside of the electron transport chain? for the two electrons, When they pass from NADH to oxygen, there will be 53 Kilocalories generated, in $FADH_2$ to oxygen, it generates 41 Kilocalories, How many ATPs are coming out with NADH? It is two and a half or three,
How much kilo calories are there? If we calculated it 3, they are 21 kilocalorie, and if we calculated 2.5 it is almost 18 kilocalories, 18 over 53, the efficiency of the electron transport chain is approximately 35%
And if I want to discuss over here, the $FADH_2$ gives 41kilo calories, one and a half ATPs, approximately 10.5 kilo calories, which means around 25% efficiency so It's very low regardless of the electron source, It's either 25% in case of $FADH_2$ or approximately 35% in case of NADH, it's very low, Where does this energy go? It goes to generate heat. as we said before, and for calcium, you bring calcium in by active pumping through the inner mitochondrial membrane, for anions to exchange in or out of the outside of the mitochondria because the inner mitochondrial membrane is impermeable to anything. everything should have a channel. And if you are going against concentration, you need the ATP to be exploited, for the getting out of ATP You need energy, to bring ADP in You need energy ,The phosphates are the same, So a lot of things you spend energy on, including heat.
So this is why the efficiency of the electron transport chain is lower compared to the Krebs cycle.

Regulation – the need for ATP

What was mentioned in the slide:

- **What OxPhos needs? (NADH, O2, ADP, and Pi)**
- **In skeletal muscles, 20% drop in ATP concentration**
- **In the heart, Ca^{+2} activates TCA enzymes for extra push (NADH, ATP), no drop**
- **ET is tightly coupled to phosphorylation (simultaneously)**
- **<u>ADP is the most important factor</u> in determining the rate**
- **The regulation of the rate of oxidative phosphorylation by the ADP level is called <u>respiratory control</u>**



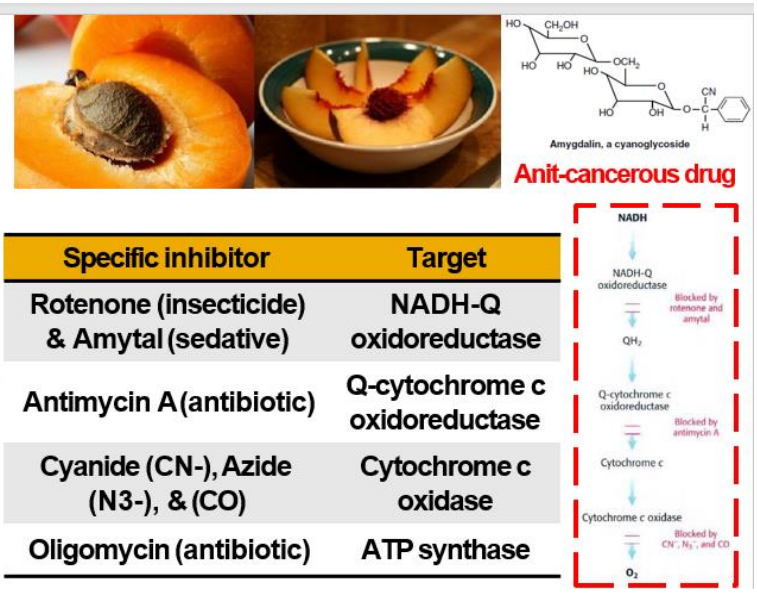
What was mentioned in the lecture:

ADP is the most important factor in determining the rate of the electron transport chain, the plot in the figure depicts oxygen consumption, which is the parameter that you depend on of how's the rate is going (Am I consuming a lot or less of O_2?), So when the electron transport chain is functioning, Once you add ADP, there will be a sharp increase in oxygen consumption and when the source of ADP Is going out then there will be a plateau again. (meaning that the rate of O_2 consuming will become almost fixed when there is low ADP), So ADP is the most important factor in determining the rate of the electron transport chain, they call it as a molecule the respiratory control, it is the molecule which controls the respiratory process.

Regulation – inhibition (coupling)

What was mentioned in the slide:

- ➢ Can occur at any stage
- ➢ Specific inhibitors:
- ✓ Cyanoglycosides such as amygdalin are present in edible plant pits
- ✓ Oligomycin prevents the influx of H+ through ATP synthase (tight coupling)



Specific inhibitor	Target
Rotenone (insecticide) & Amytal (sedative)	NADH-Q oxidoreductase
Antimycin A (antibiotic)	Q-cytochrome c oxidoreductase
Cyanide (CN-), Azide (N3-), & (CO)	Cytochrome c oxidase
Oligomycin (antibiotic)	ATP synthase


أشهر جرائم القتل العائلية في المملكة

جراسا نيوز -

جراسا -نعرض فيما يلي قائمة بأشهر جرائم القتل العائلية التي حدثت في الاردن خلال السنوات الماضية ، والتي كان لكل منها وقع الصدمة حين وقوعها لما تمثله من فعل غريب على المجتمع وأعرافه ، فضلا عن مخالفتها الشرائع السماوية والقوانين النافذة والطبيعة الإنسانية بعامة.

قضية السيانيد

أول جريمة من نوعها يرتكبها أب ضد ولديه ، اذ قام الاب بوضع مادة السيانيد في كأس الحليب وطلب من طفليه ان يشربا منه ، حيث فارقا الحياة بعد 10 دقائق من مغادرة الام المنزل لتعود وتجدهما جثتين هامدتين.

وقد ادين الاب بعقوبة الاعدام شنقا الا ان والده اسقط الحق الشخصي كونه وليا عن الطفلين وحكم عليه بالاشغال المؤبدة.

What was mentioned in the lecture:

in the discussion of controlling and regulation of the electron transport chain and the oxidative phosphorylation process in general, there are what we call inhibitors, inhibitors can inhibit enzymes, there are certain inhibitors for complex I, II, III, IV, and the ATP synthase, which is complex V, look at the table rotenone which is an insecticide and amytal which is a sedative are inhibitors for complex I, Antimycin which is an antibiotic is an inhibitor for complex III, we have also cyanide (CN^-), azide (N_3^-), and carbon monoxide, carbon monoxide is the cause for most suffocation accidents form heaters in the winter, it binds complex IV, carbon monoxide is an analog for oxygen (it is like the oxygen's antagonist), it always coupled in mechanism to oxygen , it can bind where oxygen binds with higher affinity, it can also bind hemoglobin, cyanide also binds where oxygen binds, carbon monoxide and cyanide binds complex IV preventing oxygen from converted to water, if anything inhibits one of the things in the electron transport chain, If you inhibit any point at it, The whole chain will be inhibited, anything before the point of inhibition will be loaded with electrons and anything after the point of inhibition will be deficient of electrons. Oligomycin inhibits ATP synthase at the FO portion, actually the piece from the ATP synthase, which resides within the membrane, was named FO after Oligomycin.

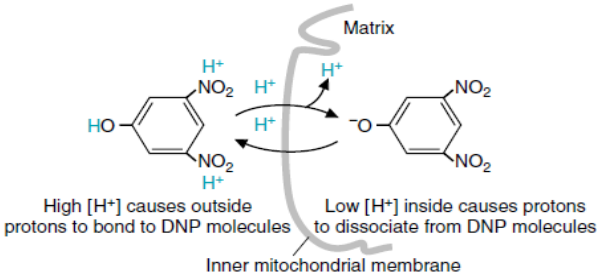
Cyanide presents in high concentration in apple, apricots and plum seeds, for human these concentration won't reach any thing toxic but we must be careful.

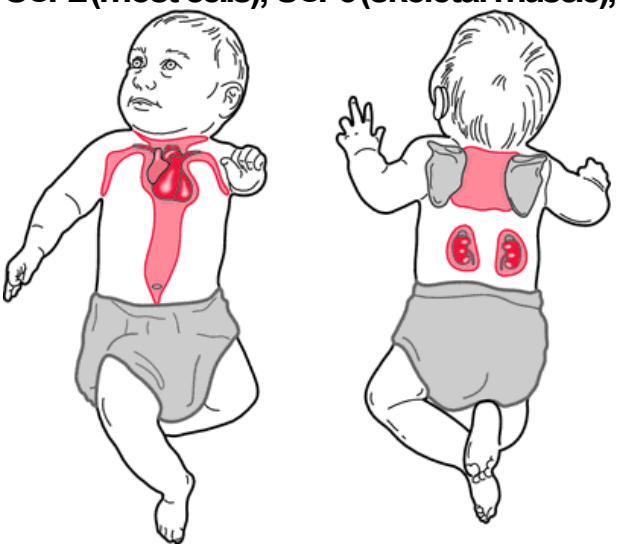
Regulation – Uncoupling
Unregulated – chemical uncouplers

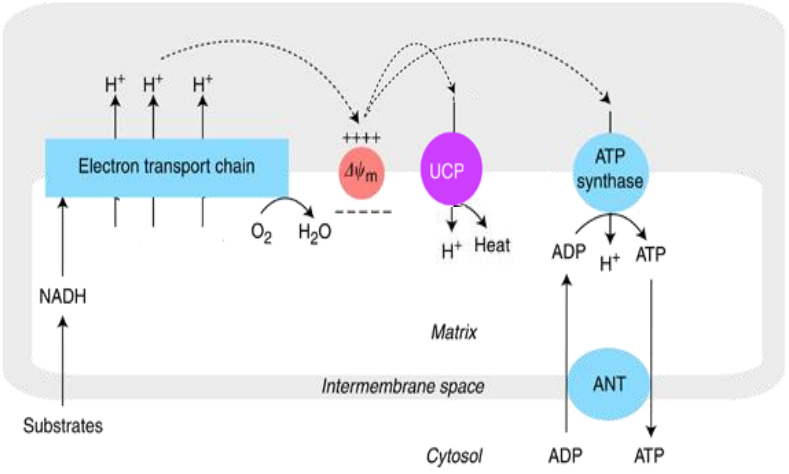
What was mentioned in the slide:

2,4-Dinitrophenol (DNP)

- What is uncoupling?
- How does it occur? Dissipation of PMF
- What is the result?
- Is it physiological or not?
- 2,4-dinitrophenol (DNP) & other acidic aromatic compounds
- What changes happen? ↑ O2 consumption, ↑NADH oxidation
- Soviet soldiers were given DNP, FDA banned DNP (1938)
- Short-circuiting ATP synthase
- UCP1 (thermogenin):
- ✓ Brown adipose tissue, non-shivering thermogenesis
- ✓ Infants: neck, breast, around kidneys
- ✓ Fatty acids directly activates UCP1
- UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)
-







What was mentioned in the lecture:

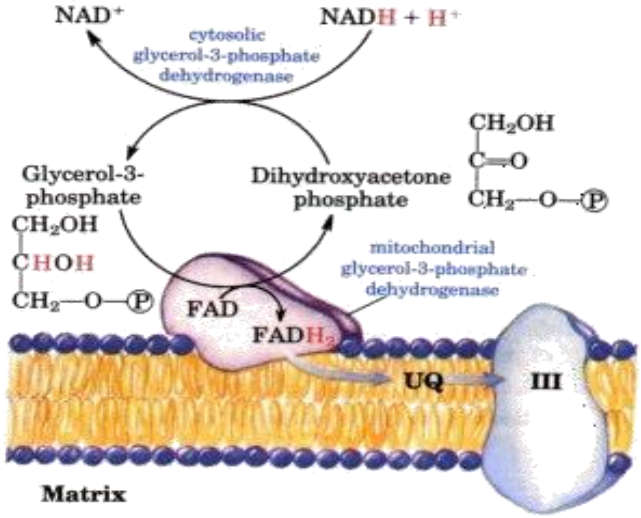
some of the drugs that have been used in the past were working in the same the issue of uncoupling, in the figure is very simple molecule that has two nitro groups and a hydroxyl coupled to the benzene ring, it is called dinitrophenol, When this molecule comes closer to the inter membranous space, it gets attached to the H^+, and when it comes closer to the inner side of the inner mitochondrial membrane at the matrix, it

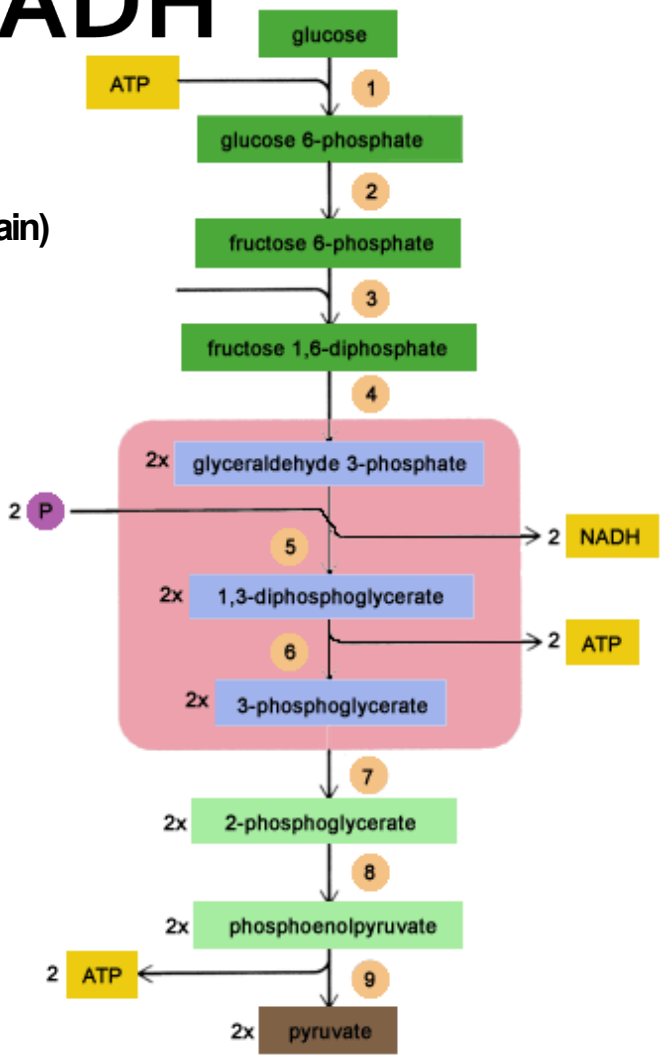
releases the H^+, then comes back through the membrane and takes the H^+ and then releases it, so it dissipates the electro chemical gradient.
Dinitrophenol was popular in the past, but it was linked to cases of malignant hyperthermia and eyes bleeding, therefore it was panned,

Mitochondrial shuttling systems "Cytosolic NADH"

What was mentioned in the slide:

- **Glycerol 3-phosphate shuttle (Sk. muscles &brain)**
- **Glycolytic pathway as an example**
- **How NADH passes?**
- **ATP yield?**

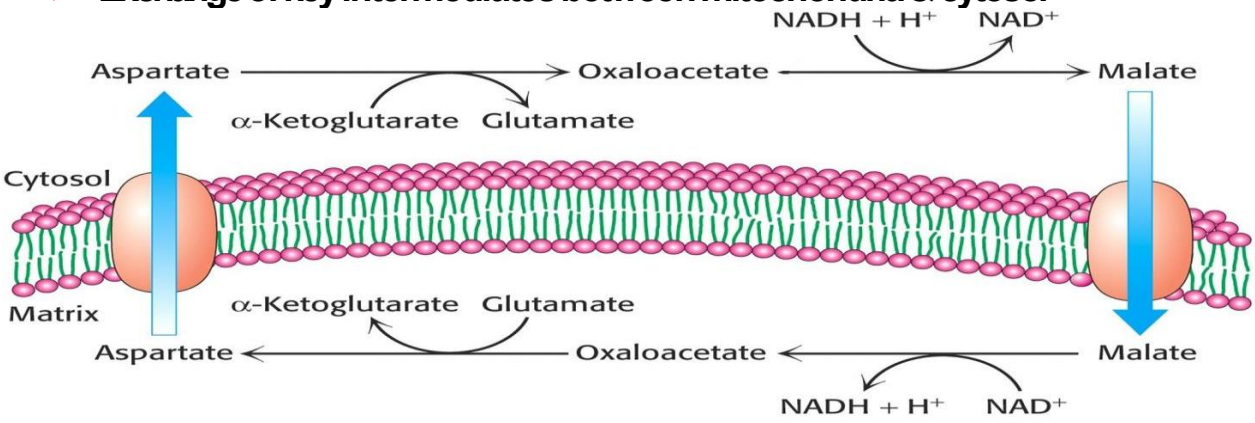




What was mentioned in the lecture:

so far we've studied energy metabolism as if it happens in this sequence: acetyl-CoA -> Krebs cycle -> oxidative phosphorylation, citric acid cycle is not the only provider of NADH and $FADH_2$, actually, a good percentage of these two molecules comes form other sources other than TCA cycle, for example, glycolysis pathway which occur in the cytosol provides some NADH, how to bring these into the mitochondria provided that there is no specific channel for them? (if there were a specific channel for them, the ones that are produced from krebs cycle will go out of the mitochondria) (to be continued)

- **Malate-Aspartate shuttle**
- **Heart &liver**
- **2membrane carriers & 4enzymes**
- **Readily reversible (vs. Glycerol 3-phosphateshuttle)**
- **NADH can be transferred only if the NADH/NAD+ ratio is higher in the cytosol than in the mitochondrial matrix**
- **Exchange of key intermediates between mitochondria & cytosol**



What was mentioned in the lecture:

There is oxaloacetate outside the mitochondria, oxaloacetate can be converted to malate by a reaction that is opposite to the one that converts malate to oxaloacetate in the Krebs cycle (therefore consuming NADH rather than producing it), and by a different cytosolic isoform of the same enzyme that carry that reaction in Krebs cycle, malate can exit and enter the mitochondria freely (it has a specific transporter, it has a role in gluconeogenesis), then malate that has been produced in the cytosol return to the mitochondria, it can be converted again to oxaloacetate producing an NADH, so it is a process of going around the NADH to produce it again in the mitochondria to be able to use it in electron transport chain.

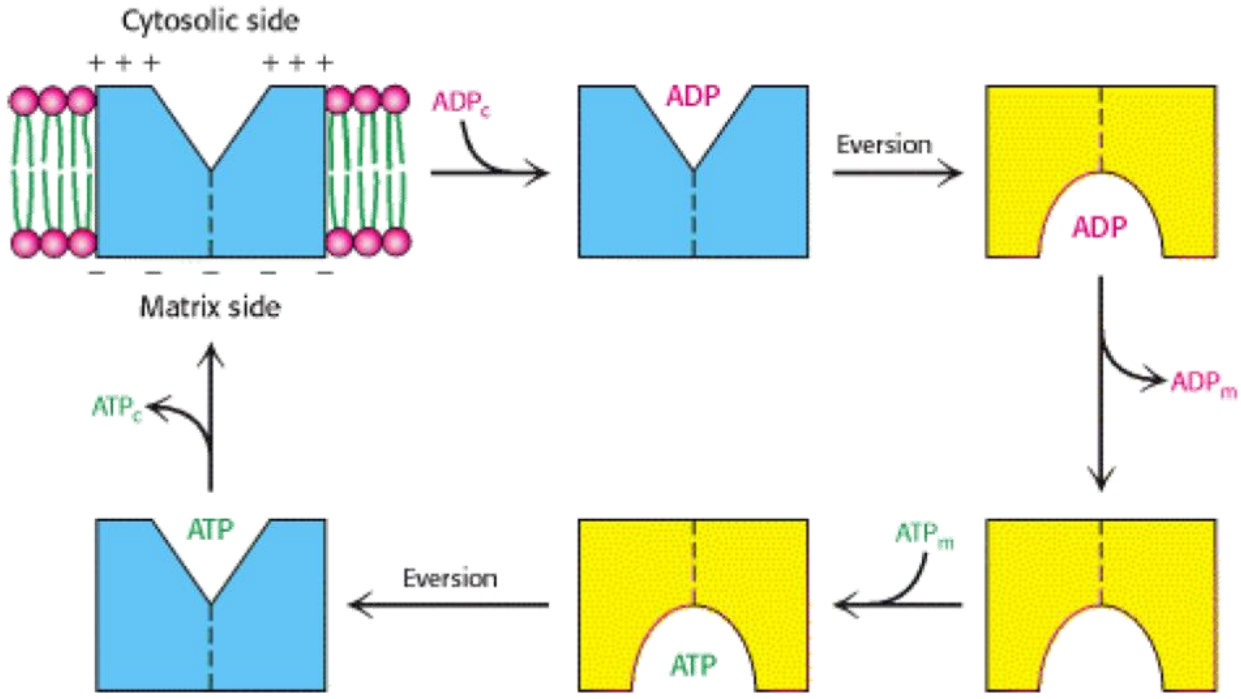
NADH can pass also through another source which is the glycerol 3-phosphate shuttle, Dihydroxyacetone phosphate can get converted to glycerol 3-phosphate using the two electrons on NADH from outside, through the cytosolic copy of glycerol 3-phosphate dehydrogenase, glycerol 3-phosphate can also be converted back to dihydroxyacetone phosphate, but by loading the electrons on the mitochondrial form of glycerol 3-phosphate dehydrogenase which has FAD inside it and closer to the outer side of the inner mitochondrial membranes, $FADH_2$ then delivers them to the ETC.

The main difference between them is that if NADH gives it electrons to the oxaloacetate-malate shuttle, that will cause pumping of ten protons, but if it gives them to the glycerol 3-phosphate shuttle, that will cause pumping of six protons because they will transduced to FAD and they will not pass through complex I.

Mitochondrial shuttling systems "ATP/ADP"

What was mentioned in the slide:

- ATP-ADP Translocase (also called adenine nucleotide translocase or ANT)
- The flows of ATP and ADP are coupled (ADP enters only if ATP exits, and vice versa)
- Highly abundant (14% of IMM proteins)
- Contains a single nucleotide-binding site (alternates)
- Similar affinity to ATP and ADP
- Endergonic (25% of ETC)
- Inhibition leads to subsequent inhibition of cellular respiration



What was mentioned in the lecture:

This the last transporter which is very important, ATP is generated inside the mitochondrial matrix, when ATP is generated, it changes its conformation so that its cytoplasmic domain has a high affinity for ATP, when it binds ATP, it changes its conformation again so the ATP (the binding site) is directed toward the intermembranous space and it releases the ATP, when it binds ADP from the outside, it changes its conformation again so its binding side will be directed toward the matrix and ADP will be released inside

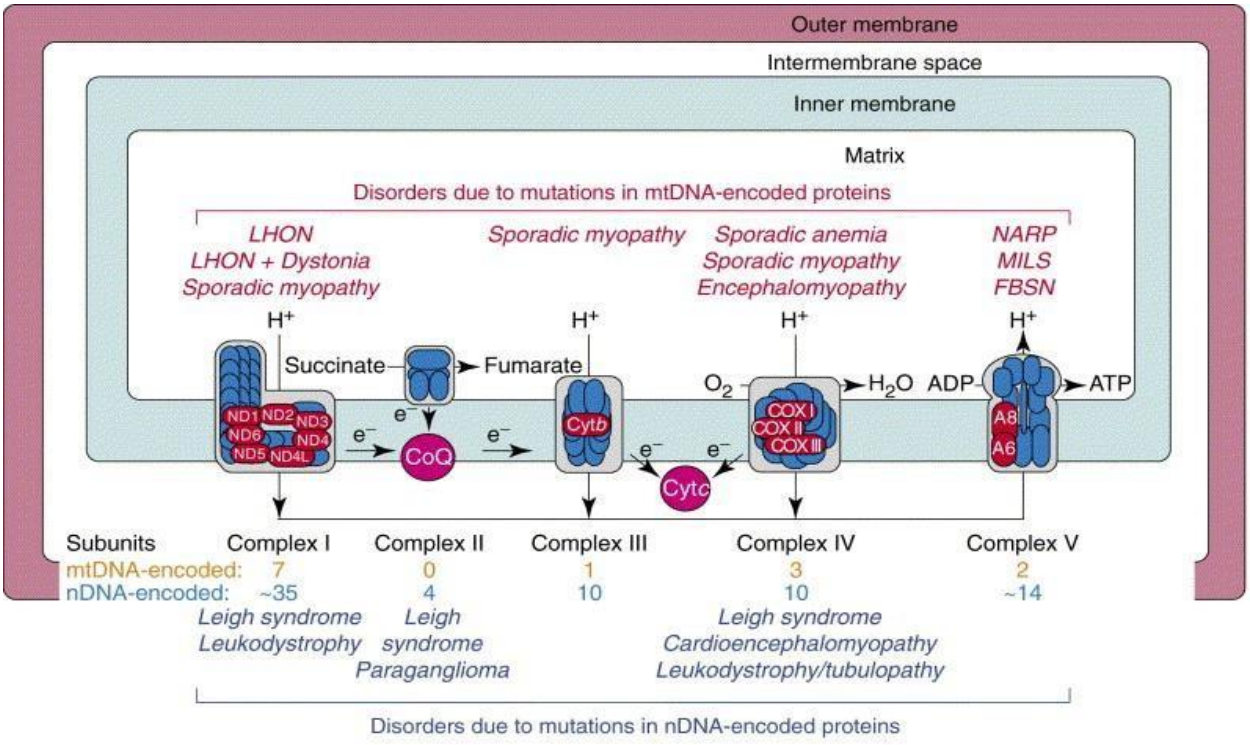
and the cycle repeats itself. This is what makes the ratio of ATP to ADP is 1:1 , every ATP pumped must be accompanied with an ADP, this process is endergonic, 25% of the energy produced by the ETC is spent on this translocation alone. ATP/ADP translocase is the most abundant protein in the inner mitochondrial membrane, it accounts for 40% of protein content in that membrane

OxPhos Diseases (Genetic)

What was mentioned in the slide:

- ➢ **A. Mitochondrial DNA and OXPHOS Diseases**
- ✓ **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 own mRNA: a large and small rRNA and tRNAs**
- ✓ **Maternal inheritance, heteroplasmy**
- ✓ **Accumulation of somatic mutations with age**
- ✓ **Highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver**

- ➢ **B. Nuclear Genetic Disorders of Oxidative Phosphorylation**
- ✓ **1,000 proteins (50% of the mitochondria is protein)**
- ✓ **Usually autosomal recessive**
- ✓ **Expressed in all tissues**
- ✓ **Phenotypic expression with high ATP demand**



What was mentioned in the textbook:

Thirteen of the approximately 90 polypeptides required for oxidative phosphorylation are coded for by mtDNA and synthesized in mitochondria, whereas the remaining proteins are coded for by nuclear DNA, synthesized in the cytosol, and transported into mitochondria posttranslationally. Defects in oxidative phosphorylation are more likely a result of alterations in mtDNA, which has a mutation rate about 10 times greater than that of nuclear DNA. Tissues with the greatest ATP requirement (for example, central nervous system, skeletal and heart muscle, and liver) are most affected by defects in oxidative phosphorylation. Mutations in mtDNA are responsible for several diseases, including some cases of mitochondrial myopathies, and Leber hereditary optic neuropathy, a disease in which bilateral loss of central vision occurs as a result of neuroretinal degeneration, including damage to the optic nerve. [Note: mtDNA is maternally inherited because mitochondria from the sperm cell do not enter the

fertilized egg.]

refer to page 17/18 of bioenergetics handout for more