Bio-energetics of Life Processes
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Lecture - 19
ATP Synthesis

Welcome back to the 4th lecture of the 4th week, on bioenergetics of life processes. So, the last lecture when we concluded we talked about how the manganese cluster works and the charge accumulator model, where 4 proton gradients are being generated 4 protons are being generated; and 4 electrons are being ejected out while at the cost of two water molecules.

Where the two molecule water molecules get entrapped into the manganese cluster; where 4 as of now what we know 4 to 6 manganese atoms are clustered in a way and sitting at different oxidation state, which can unzip the water molecule and eject out 4 protons and 4 electrons another byproduct a strong oxidant which is water. Now the story if you go back. So, on the so, we talked about this strong reductant.

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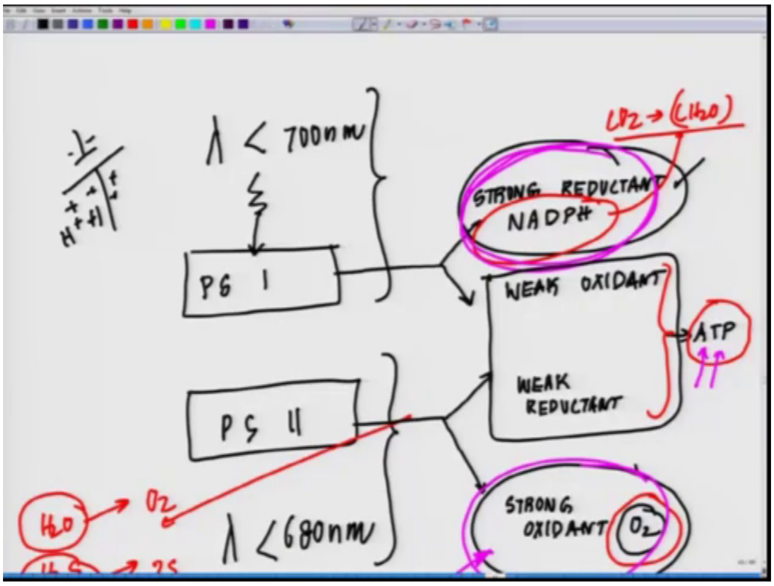

So, which eventually leads to the formation of the dark reaction; which we are not dealing here which is the Calvin cycle, where the carbohydrate synthesis happens, but end of the day what is most important from the bioenergetics perspective for you to understand is that you need it perennial electron source.

And in this case since water is the perennial electron source we are not only generating as a byproducts oxygen this process also leads to generation of lot of protons; and that leads to a proton gradient. So, whenever you talk about a gradient we talked about it could be either a pH gradient. So, whenever we talk about a proton gradient that essentially means H plus ion concentration is higher. So, when you talk about a higher H plus sign concentration the first thing which is striking is the pH gradient, which is pH is what? essentially H plus ion concentration right?

So, we have a neutral pH where H plus o H minus is at equilibria then if the H plus increases then we as the lower pH from 7, 6, 5, 4, 3, 2, 1, 0. Your H plus ion concentration goes on rising whereas, if you go on the other direction from 7, 8, 9, 10 o H minus ion concentration increases ok. So, any form of gradient for any kind of energetics you have to realize you always remember this, you have to have a gradient some form of a gradient; that gradient could be created by any form of ions.

It could be you know H plus ions H plus ions or you know something or an electron gradient or a proton gradient, but you will have to have in gradient or you could have any gradient. So, you could have gradients like it here you could have a gradient of ions, some positively charged ions on one side, negatively charged ion on other side or higher concentration of positively charged say for example, if you think of the cell when we write a cell.

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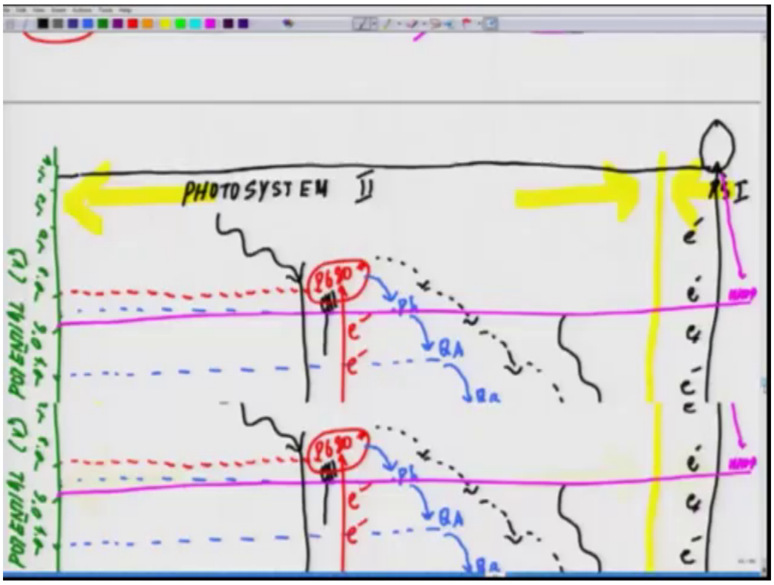

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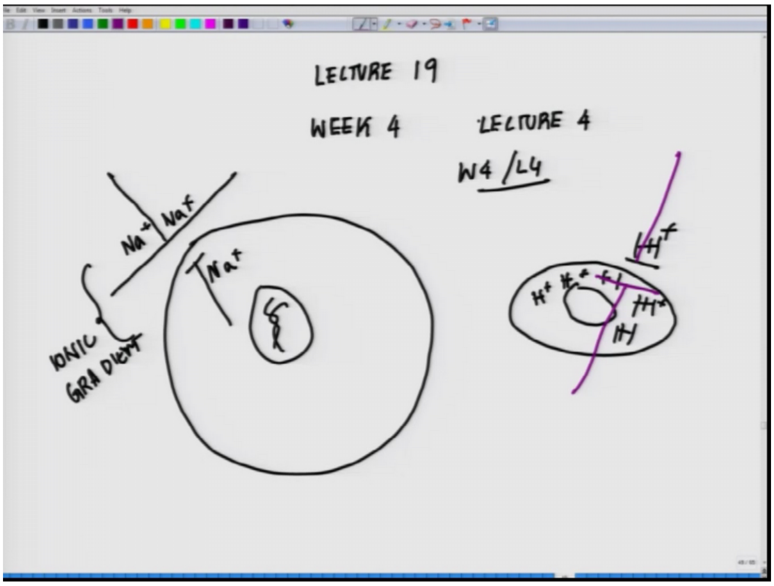

So, this is lecture 19 which is the 4th lecture of today. So, this is week 4 lecture 4 W 4 L 4. So, when we see a cell say for example, this is a biological cell with a nucleus and everything. So, you have sodium which is higher outside and sodium lower inside. So, essentially if you if I have an equivalent circuit model for sodium it will be something like this. So, there is a sodium gradient; in other words, this is you can call it as an ionic gradient ok.

So, say for example, similarly this ionic gradient could be utilized to generate a force ok. Similarly, if we have say just for a hypothetical situation if I have something like this, where I have a lot of protons higher on the inside as compared to protons outside. So, what will happen? There will be a proton gradient something like this ok. There will be and such gradients could be utilized to generate energy because as we know by the basic thumb rule from the higher potential things flows towards the lower potential.

And during that process many useful work can be accomplished; it is just like say for example, water is falling from higher altitude to lower altitude, you can utilize to produce hydro hydle energy by hydroelectricity; exactly by the same way as long as you can create an gradient of anything; you can always do some useful work. And today we will be talking about a fundamental concept which was first proposed by a gentleman called Peter Mitchell. Peter Mitchell is known in history for one of the boldest hypothesis called Chemiosmotic hypothesis.

In the next class we will talk a little bit more about Chemiosmotic hypothesis or Peter Mitchell, but today we will talk about this basic concept that ADP synthesis is driven.

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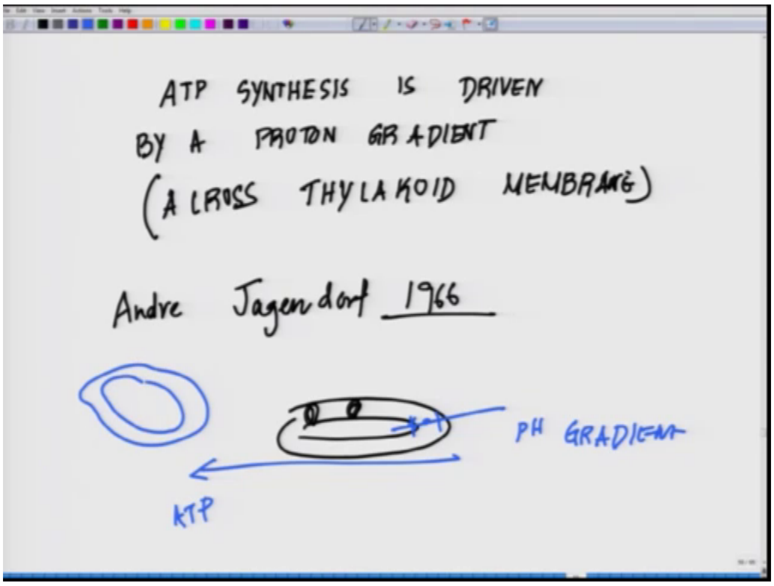

ATP synthesis is driven by a proton gradient across, now this part is important across thyla koid membrane. So, in 1966 there is a gentleman called Andre Jagan Dorf

Show that chloroplast synthesize ADP in the dark when an artificial pH gradient is imposed across the thylakoid membrane. So, you remember the thylakoid membrane I told you the 4 systems are lies there and if you could imposed a pH gradient across it. So, for example, somewhere other he create some kind of across this membrane you have a pH gradient. Then this could lead to ADP synthesis. To create this transient pH gradient chloroplast first were soaked in a buffer of pH 4 for several hours. So, you take isolated chloroplast and soak them in a buffer of pH 4 ok.

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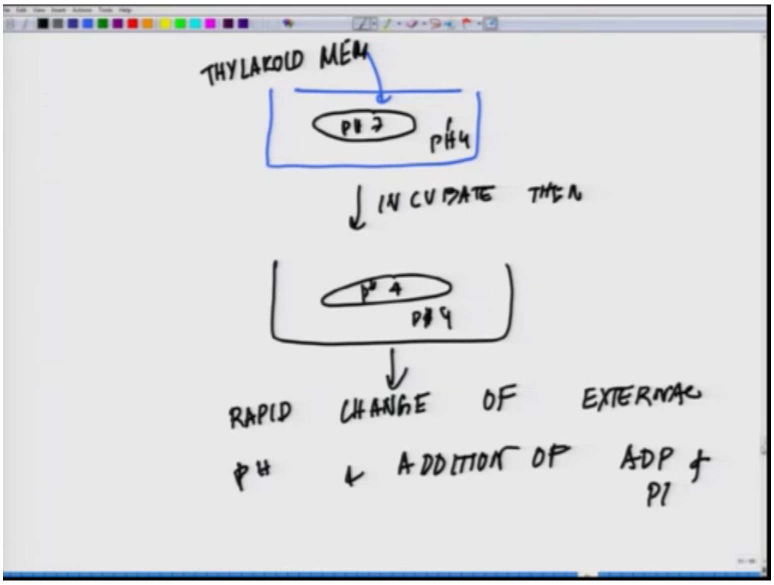

So, something like this you take a thylakoid membrane thylakoid membrane and you know pH 7 pH 4 ok. So, to create a transient pH gradient chloroplast where first soaked in a pH 4 buffer for several hours. These chloroplast were then rapidly mixed with a pH 8 buffer containing ADP and pi ok. Then what you do? Thylakoid membrane, you are keeping them at 4 that you incubate them. And then you incubation for several hours and then what you do? Rapid change of external pH and addition of ok. So, to create the transient pH gradient the chloroplast our first soaked in a pH 4 buffer for several hours, this chloroplast were then rapidly mixed with a pH 8 buffer containing ADP and pi ok.

So, this is what you do pH 4 pH 4 ok. Now followed by this here is a rapid change of external pH and addition of ADP and PI which is the ingredient to make ADP ok.

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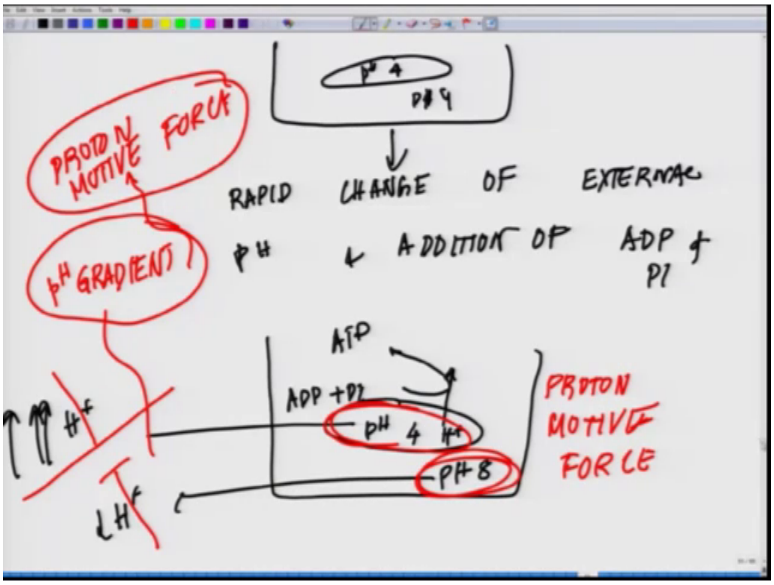

Then what you observed here is that; here you have the pH 4 H plus ions they move out and what they do is outside ADP plus PI making ADP and outside the pH is 8. So, what you are observing out here H plus ion concentration is extremely higher as compared to outside where H plus concentration is lower.

So, essentially if I had to put a signature here it will be something like this. So, here you have a pH gradient like a pH battery ok. So, the pH of the stroma suddenly increased to 8 whereas, the pH of the thylakoid space remained at 4. So, it remain at 4 and here you are increasing it to pH 8 whereas, the pH of the thylakoid space remain at 4 a burst of ADP synthesis then accompanied the disappearance of pH gradient across the thylakoid membrane. This incisive experiment was one of the first to unequivocally support Peter Mitchells hypothesis that ADP synthesis is driven by what we call as proton motive force. So, there is a proton motive force generated because of the pH gradient ok.

So now proton motive force. So, this proton motive force which is generated because of the pH gradient is being supported here the ADP synthase of chloroplast closely resembles.

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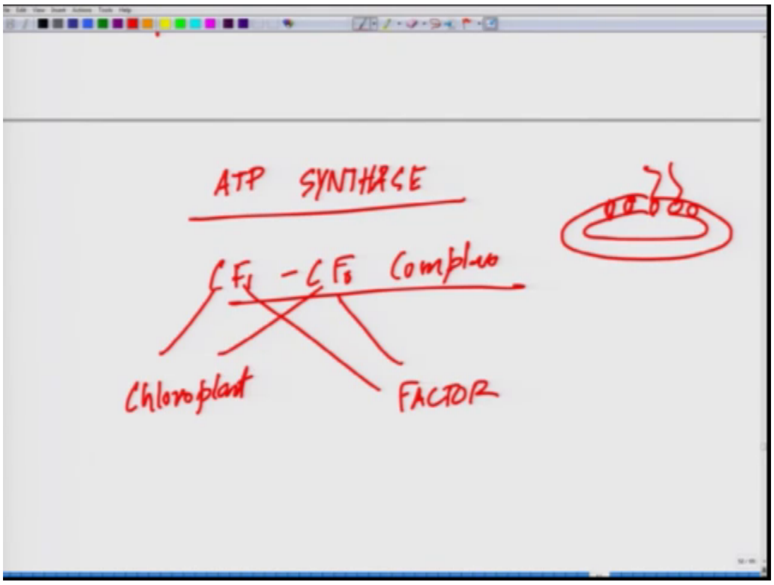

So, there is an enzyme which is involved in it membrane ADP synthase, which is an membrane bound organelle which is something like a mechanism of ADP synthesis in chloroplast is very similar to that of the mitochondria which will coming in the next class. ADP formation is divin driven by a proton motive force in both photophosphorylation which is happening in chloroplasts and oxidative phosphorylation which is happening in mitochondria.

Furthermore, the enzyme assembly catalyzing ADP formation in chloroplast is very similar to that of mitochondria and bacteria the ADP synthase of chloroplast is also called C F 1 C F 0 complex and this complex, where C stands for Chloroplast and F stands for the Factor Chloroplast Factor.

They are again these are all those if you remember thylakoid membrane I told you photosystem one photosystem 2 they are all sitting there ok. So, if you if you just for your recollection 1 second let me yes.

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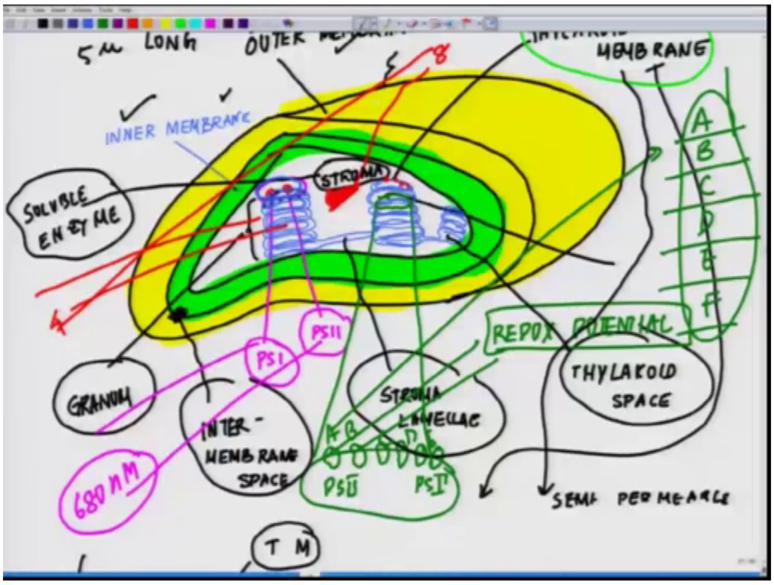

So, out here those are all sitting and this is the space what I was talking; the inside and the outside this stroma we the outside space ok. So, this is where pH 8 and this is where inside pH 4. So, you are creating a gradient between inside and outside and that is the gradient I was trying to show you in this picture; out here, that is the kind of gradient you are creating here.

So, closer example at similar what you see here C as in the next class we will move on to my talk on day you will see F 1, F 0 which is essentially nothing but for mitochondria for oxidative phosphorylation. So, C F 0 consists of 4 subunits which conducts proton across thylakoid membrane and C F 1, like F 1 which is the counterpart for mitochondria catalyzes the formation of ADP from ADP and PI ok.

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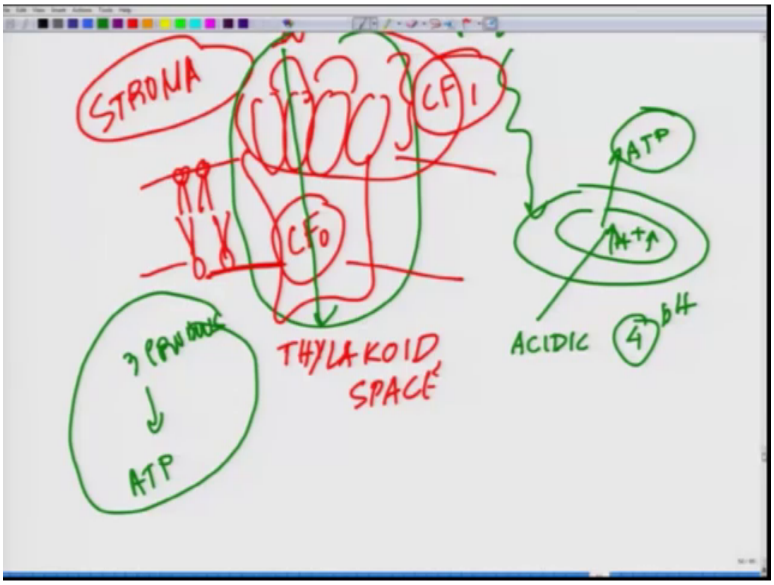

So, the way this protein is arraigned is if this is the membrane of the thylakoid. So, this is how this protein is arranged in multiple loop something like this ok. And this part is C F 1 and this is C F 0 ok. And this is the thylakoid space and this is the stroma, which is outside the thylakoid space and as I mentioned that ADP the knobs of the external surface. So, these are the knobs of the external surface of thyroid membrane are C F 1 unit of ADP synthase.

And C F 1 has subunit composition there are multiple subunits, which are alpha gamma, beta, which you do not need to really bother the electron transfer through asymmetrically orient. So, this is an asymmetric orientation of this particular ADP synthase oriented for system 1 and 2 and the cytochrome B F complex produces a large proton gradient across the thylakoid membrane the thylakoid space between Merkley acidic.

So, this part inside so if you look at the thylakoid space this part is acidic, which is essentially a lot more proton concentration in site and with a pH approaching around 4. So, pH is around 4 as the Gandalf experiment and the light induced trans membrane proton gradient is about 3.5 unit. So, because of the light the pH gradient is around 3.5-minute light induce proton gradient.

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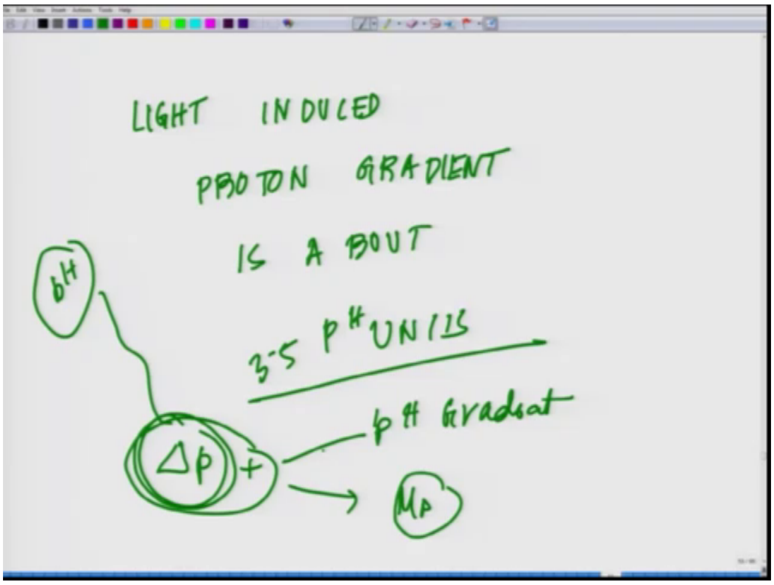

When the light is falling this is and the proton gradient is created it is called light induced proton gradient is about pH 3.5 units and if you look at it the proton motive force or delta p which is the proton motive force consists of a pH gradient contribution. And membrane potential contribution which is MP, which is membrane potential these to sum up to the proton motive force in chloroplast nearly all delta p. Proton motive force arise from the pH gradient purely a pH gradient which is created; on the contrary as we will read about mitochondria will observe the contribution from membrane potential is larger.

So, you have to realize this delta p which is the proton motive gradient, proton motive force is generated purely on pH gradient in chloroplasts ok. And about 3 proton flows through this C F 0 C F 1 complex ok. 3 protons flows through this C F 1 and C F 0 complex per ATP synthesis for one ATP molecule ok.

Interestingly, ATP and ADPH the products of the light reaction of photosynthesis are appropriately positioned for the subsequent dark reaction in which CO 2 is converted into carbohydrate. And C F 1 is on the stromal surface of the thylakoid membrane. And so, the newly synthesized ADP is released into the stromal space. So, the ADP which is now synthesized is comes out into the stroma ok. So, if I have to put it in perspective of how all these 3 reactions looks like it will be something like this, now i will draw the thylakoid membrane.

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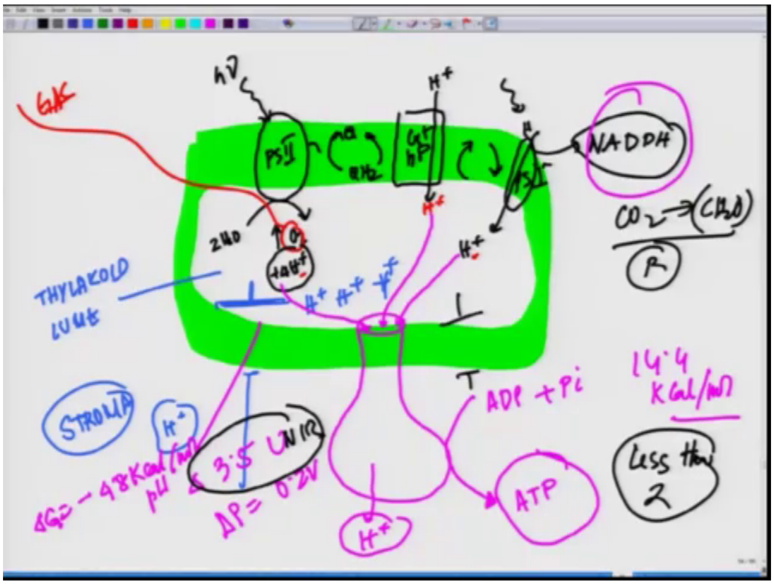

Now here is see for example, this is the thylakoid space what we are talking about ok.

Now, on this we have we have PS 1 sitting out here sorry PS 2 ok. So, PS 2 receives the light and what PS 2 is doing 2 H 2 O plus oxygen, which is going out into the air and plus 4 H plus in the thylakoid space ok. Now this electron is traveling through QH 2 refer to the previous lecture Q and it is coming to cytochrome B F complex cyt B F complex. If you remember and there also i told you that there is a proton gradient which has being created there is a proton gradient created here, there is a proton gradient created out here ok.

Then here through plus to sign in and everything the electron moves to photosystem 1, PS 1 at the PS 1 also there is a proton gradient which is created. So, if you look inside there is lot of and of course, this is the one which is creating the NADPH which is taking part in the dark reaction for CO 2 to C H 2 O ok. The reduction reaction the major reductant and here is the major oxident which is going away as the gas coming out ok.

So now you see there are 3 sources of proton gradient here here and here. So, inside the thylakoid membrane if you look at it inside the thylakoid lumen your thylakoid lumen you have very high concentration of H plus.

So, essentially if I had to put a pH gradient it will be something like this, as a contrary outside it is much more less now which is the stromal side stroma. Now here sits that wonderful ADP synthase here is the knob outside, which drawing i did if you remember the drawing which i shown you earlier out like this there is a knob like structure outside. So, this is that knob like structure which is facing the stromal side here also is exactly the same; here is the knob like structure facing. So, this one is the one where all the H plus ions are being funneled. The protons are funneled through this. And at this ADP synthesis what happens and now you correlated with the (Refer Time: 20:42) and off experiment, outside in the stromal cire surface you have lot of ADP and pi and because of the proton gradient it leads to the formation of ATP out here and proton comes out.

So, this is essentially, the vectorial arrangement of photosystem 1 and 2 and cytochrome B F complex and ADP synthase in the thylakoid membrane. Light induced proton pumping makes the inner space acidic and the flow of protons through C F 0 to the stromal side led to the synthesis of ADP by C F 1 and NADPH is also formed on the stromal side.

So, all the byproduct are coming on the stromal side. So, this is that most fundamental reaction a pH gradient so remember one more thing a pH gradient of 3.5 unit across the thylakoid membrane corresponds to a proton motive force of a the pH gradient is the difference is delta differences 3.5 unit. The corresponding proton motive force delta p what we talk about is 0.2 volt and the delta G for such situation is minus 4.8 kilo calorie per mole, which is a spontaneous reaction to happen.

About 3 proton flows from C F 1 to C F 0 complex per ADP synthesis which corresponds to free energy input of 14.4 kilo calorie per mole of ADP. No ADP here is the important part a take home message no ADP is synthesized if pH gradient is less than 2 unit. So, your most optimal is 3.5, 3.5 units, but if it is less than 2 remember, less than 2 if across this the pH gradient is less than 2 then there would not be any ADP synthesis, because the driving force is then too small.

So, you are realizing this whole dynamics is all a function of your gradient and flow as long as you can maintain the gradient you can always attain a flow. So, I will close in here next class we will move on to now we have an idea about ADP synthesis we will talk about mitochondria and we will talk about chemiosmotic hypothesis.

Thank you.