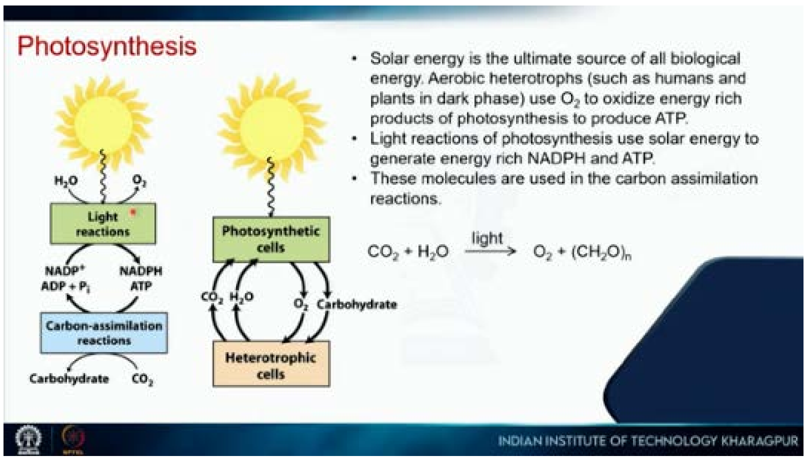
Introduction to Complex Biological Systems
Professor Dibyendu Samanta and Professor Soumya De
Department of Bioscience and Biotechnology
Indian Institute of Technology, Kharagpur

Lecture 34
Photosynthesis

Welcome to the course Introduction to Complex Biological Systems. This is the fourth lecture of Week 7. Today, I am going to discuss photosynthesis. So, this week, I am discussing metabolism and the harnessing of energy in living systems.

Today, we are going to see how light energy is harnessed into chemical energy in living systems via photosynthesis. So, this is something that we have already seen: heterotrophic cells, which are cells that we have, such as humans. So, our cells use carbohydrates and other high-energy molecules as energy sources. We oxidize those into carbon dioxide and water and trap that energy in the form of ATP, which is further used for other functions. So, we have already seen this. We have seen the process of glycolysis, the citric acid cycle, and oxidative phosphorylation, which is this part.



It turns out that these carbohydrates are made by plants. So, what they do is they trap light energy and use that energy to produce these high-energy molecules, NADPH and ATP, which are further used in carbon assimilation reactions to produce carbohydrates. So, you can see this complete cycle: carbohydrates to carbon dioxide and water, and here, carbon dioxide and water to carbohydrates. Ultimately, what happens is light energy is passed via light reactions, which is photosynthesis. We are going to see this into this chemical energy, and this chemical energy is used by both plants and us for various functions. So, ultimately, solar energy is the ultimate source of all biological energy.

Aerobic heterotrophs, such as us and other plants, use the dark phase. So, plants also use these reactions in the dark phase. They use oxygen to oxidize energy-rich products of photosynthesis. So this photosynthesis produces carbohydrates. We will see that there are two parts.

One is a set of light reactions, which produce high-energy molecules and then these high-energy molecules are used to produce carbohydrates, which do not need light. They are also called dark reactions. The light reactions of photosynthesis use solar energy to generate rich NADPH and ATP molecules.

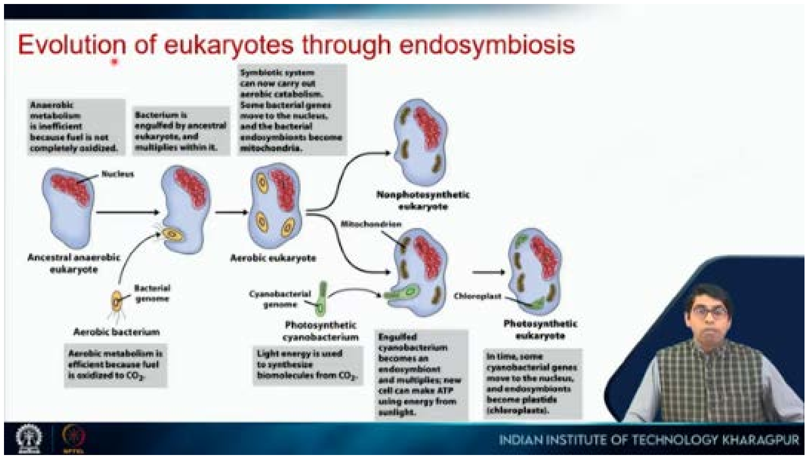
So these are the energy-rich molecules, and they are used in the carbon assimilation reactions. Today, we are going to see the first half, the light reactions, and in the next lecture, which will be the last lecture for Week 7, we will see the other reactions. These reactions are typically called the light reactions, and these reactions are typically called the dark reactions. Before I get into that, I want to take a step back and talk a little bit about the important organelles that carry out these functions. We have already seen mitochondria, and today we are going to see chloroplasts.

So I want to give you a historical context, and this is something that I will discuss in more detail in next week's lecture when I talk about evolution. So here is a timeline of life on Earth. So we are here. This is zero. So this is the present, and this is the past and the scale is millions of years ago. So 4500 million years means 4.5 billion years. So that was the time when Earth was formed. So that's the formation of Earth 4.5 billion years ago, 4 billion years ago, the formation of oceans and continents occurred. So 3.5 billion years ago, the appearance of photosynthetic sulfur bacteria and methanogens occurred. So these are organisms that use these inorganic sources. Almost 3,000 million or 3 billion years ago, the first photosynthetic oxygen-producing cyanobacteria appeared. So now this is where things start to change. So before this, the atmosphere was reducing. Now, these cyanobacteria appeared, and they started splitting water and producing oxygen, which means that they started releasing oxygen into the atmosphere and slowly converting Earth's atmosphere into an oxidizing environment and since you have oxygen, it becomes feasible for these microbes or new microbes that evolve to use that oxygen to further metabolize these rich carbon sources. So now, aerobic respiration or aerobic bacteria appear and now you have this development of a more oxygen-rich atmosphere. So oxygen is coming from here and that oxygen is used by these aerobic bacteria. So almost another billion years have passed. So this is 1.5 billion years ago.

That is the appearance of the first eukaryotes. Now these are primitive eukaryotes where the nucleus is there. So the DNA is sort of enveloped in the nucleus. 1 billion years ago, something very interesting happened, which is called endosymbionts or endosymbiosis. So, we will, I will discuss this in more detail in the next lecture and this is where these organelles, mitochondria and plastids, they appear and then the appearance of red and green algae, 500 million years ago, multicellular organisms appear and then they start diversifying and then we are here. So, this is the present. So let's look at this step.

Evolution of eukaryotes through endosymbiosis. So, what is endosymbiosis? This is the ancestral or primitive anaerobic eukaryote. It is a larger cell than the bacterial cell, and it has a nucleus. The DNA is segregated in this nucleus.

It has anaerobic metabolism, but it is insufficient because the fuel is not completely oxidized. We have already seen that when you do glycolysis, you produce pyruvate and only two molecules of ATP. But if you go through aerobic respiration, you can produce 32 molecules of ATP. That's 16 times more ATP molecule production from one glucose. This is the ancestral anaerobic eukaryote.



Now, something interesting happens. This is an aerobic bacterium. If I go back, If I go back, we are looking at this. Now, aerobic bacteria had already appeared almost 1.5 billion years ago.

So there is aerobic bacteria, and there are the eukaryotes. So between this time, this aerobic bacterium is taken up by the eukaryotes. So it can be a case of infection, where this bacterium infects this eukaryote, but then it survives in this high-energy-rich environment and then some sort of symbiosis happens in some cells. So that results in the evolution of aerobic eukaryotes. So this aerobic bacteria, its metabolism is far more efficient.

This bacterium goes inside. The bacterium is engulfed by the ancestral eukaryote, and the bacteria multiply inside it. So now you have these multiple bacteria, but now they are not killing each other. They are living together. So this is symbiosis.

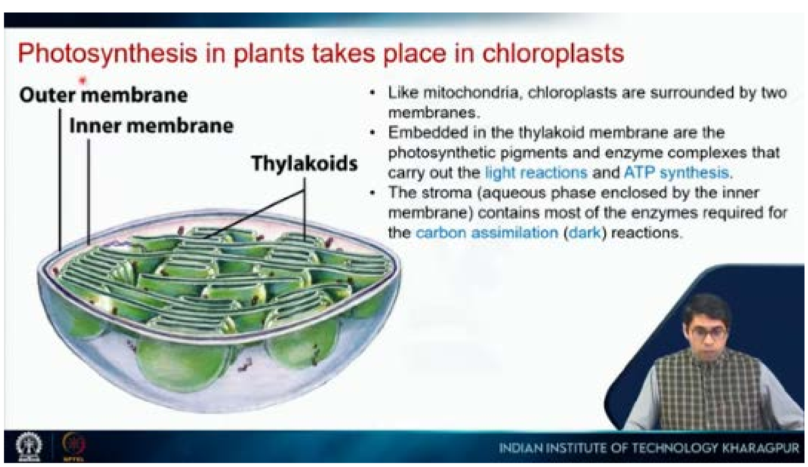
The symbiosis system can now carry out aerobic catabolism because that is present in this bacteria. Now, some of the genes which were present in the bacteria are transferred from the genomic DNA of the bacteria to the genomic DNA of the eukaryote. So some of the bacterial genes move to the nucleus, and the bacterial endosymbionts become mitochondria. So they lose some of their genes, but ultimately they do still keep some of the DNA.

So there is mitochondrial DNA and these bacteria ultimately become mitochondria. So this is the modern eukaryote, which is a non-photosynthetic eukaryote, and it has mitochondria and we have already seen how these cells, this type of cell, metabolizes glucose or catabolizes glucose to produce ATP. Another set of these aerobic eukaryotes took up the cyanobacteria.

So, if I go back, cyanobacteria appeared here. So, they took up these cyanobacteria, which can do photosynthesis. So, the engulfed cyanobacterium becomes an endosymbiont and multiplies. So, this will also multiply, and it will again lose some of its genes to the nucleus, but it will retain some other genes.

Now it can make ATP using energy from sunlight. So, this cyanobacterium becomes a chloroplast. So, this is a photosynthetic eukaryote, which has both chloroplasts and mitochondria and these are present in the plant cells, in the leaves of plants where photosynthesis occurs. So, in time, some cyanobacteria genes move to the nucleus, and the endosymbionts become plastids or chloroplasts.

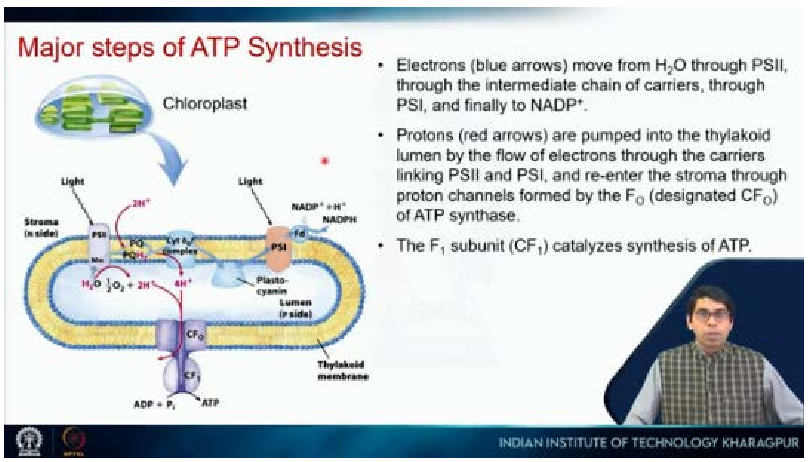
So, we are going to look at these chloroplasts in more detail today because this is where photosynthesis happens. So photosynthesis in plants takes place in chloroplasts. We have already seen mitochondria. Like mitochondria, chloroplasts are surrounded by two membranes, and you can see them here. So there is an outer membrane and an inner membrane and within the inner membrane, we have these structures, which are called thylakoids. Embedded in the thylakoid membrane are the photosynthetic pigments and enzyme complexes that carry out the light reactions and ATP synthesis. So we are going to see both. We are going to see the light reactions where electrons are extracted from water and then they will be ultimately transferred to NADP to form NADPH. While that happens, a proton gradient is established, and that proton gradient will be used to synthesize ATP.



So that last part is very similar to what we have seen in the case of mitochondria. So we are going to discuss this in detail today. In the next lecture, what we are going to see is carbon assimilation. So the stroma or the aqueous part which is enclosed by the inner membrane.

So the stroma is here, the aqueous part that is here. It contains most of the enzymes required for carbon assimilation reactions, which are also called the dark reactions because you don't need light. But ultimately, the energy is coming from light so you can call it a misnomer. But typically, this is referred to as the dark reaction.

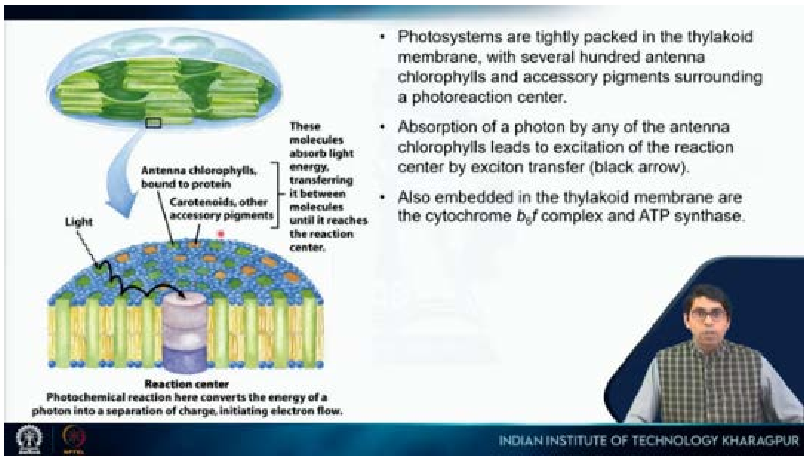
So these are the major steps of ATP synthesis. So this is the chloroplast. Electrons move from water. So, this is water.



So light comes in, and that energy is used to split water, and water becomes oxygen plus hydrogen ions. So electrons move from this to photosystem, this is PSII, which means photosystem II, through an intermediate carrier. So we will see all of these in detail. Ultimately, it ends up in NADPH, so $NADP^+$ is reduced to NADPH. So the electrons from water end up in NADPH. Protons, while this happens, are pumped in this direction. So protons are pumped into the thylakoid lumen by the flow of electrons to the carriers linking photosystem 2 and photosystem 1. So this is photosystem 2, this is photosystem 1, and in between there is this cytochrome b_6f. So this system, the electron flows through this, and while this happens, protons are pumped into the lumen of the thylakoid and these protons, they are then, so there is a proton gradient here.

So now these protons will spontaneously pass out and when that happens, that energy is used to synthesize ATP. So this is very similar to what we have seen in mitochondria. So in this case, the ATP synthase has again two parts. The CF_0 and the CF_1.

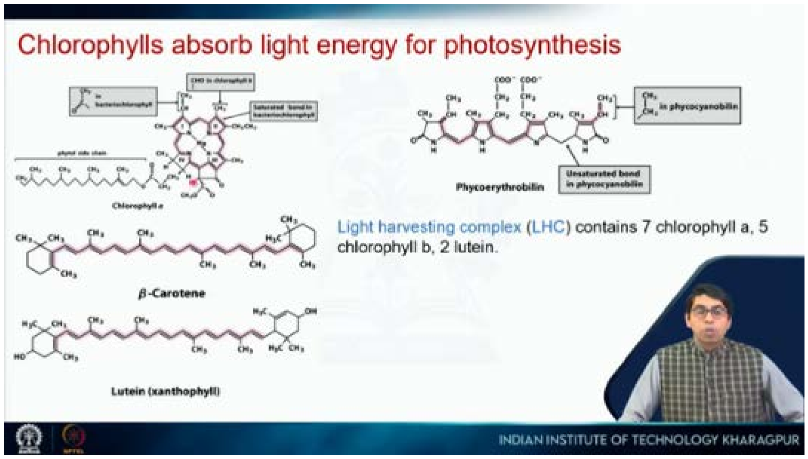
So C is prefixed here to indicate that this is in chloroplast. So now let's look at the light reaction. The photosystems are tightly packed in the thylakoid membrane with several hundred antenna chlorophylls and accessory pigments surrounding a photoreaction center. So this is the photoreaction center and this photoreaction center is surrounded by all these antenna pigments.



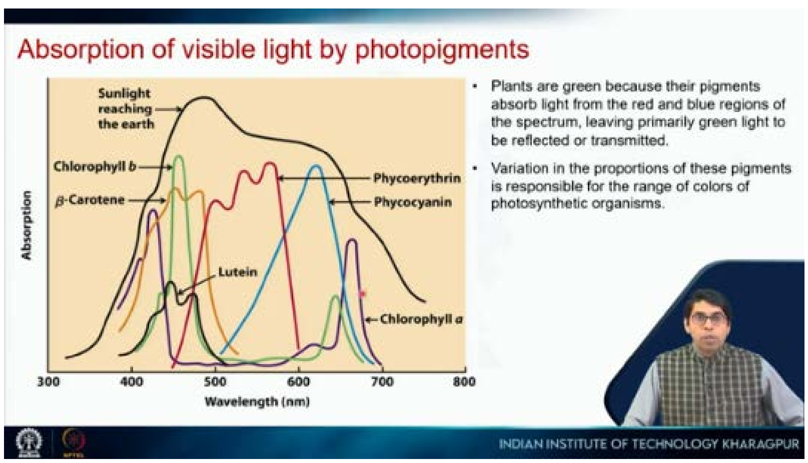
So they can be chlorophyll, or they can be other pigments. We will see the structure of these pigments in the next slide. Absorption of a photon by any of the antenna chlorophylls. So it can be absorbed here, it can be absorbed here, or it can be absorbed here. So it can be absorbed anywhere.

Once they are absorbed, they are passed on from one pigment to another pigment. Ultimately, the excitation reaches the reaction center. So the exciton is transferred to the reaction center. Also embedded in the thylakoid membrane are the cytochrome b_6f complex and ATP synthase. So we will look at those later.

So these are the pigments of photosynthesis. So this is the structure of chlorophyll. So, this is chlorophyll A, this is beta carotene, this is lutein, and this is phycoerythrobilin. So, these are the different pigments that are present here.



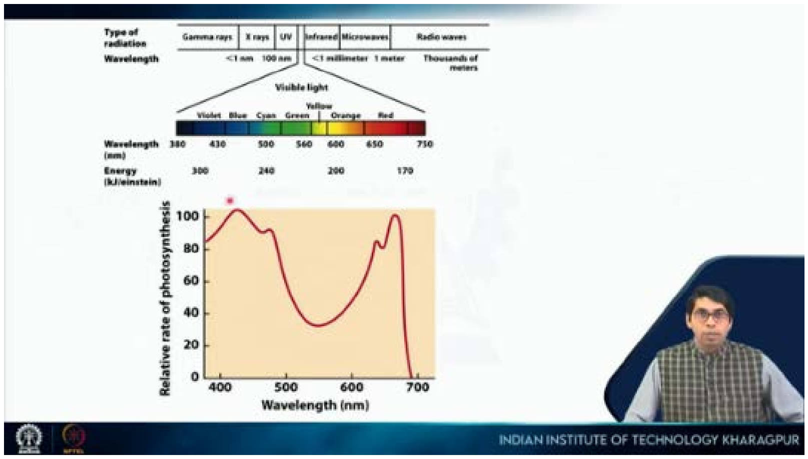
Now, the light-harvesting complex, we will see that in detail. It contains 7 chlorophyll *a* and 5 chlorophyll *b*, and 2 molecules of this. So all these pigments, why do we need so many pigments. So this is the visible range of light, 300 nanometers to 800 nanometers and sunlight, its typical spectrum will look like this. So this is the absorption spectrum.



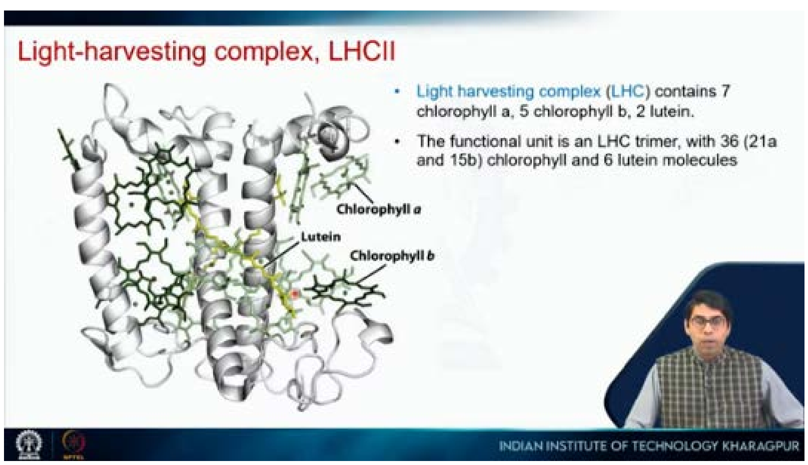
Now, each of these pigments absorbs light at different wavelengths. So you need all these different pigments so that you can cover most of this range. Now, it turns out that plants are green because their pigments absorb light mostly from the red and blue regions. So the red region and the blue region. So this is the blue region, and this is the red region, which means that there is not much absorption in the green region.

So it leaves this green light either reflected or transmitted. So that is why we see that green light. Now, variation in the proportion of these pigments is responsible for the range of colors of photosynthetic organisms. Even in plant leaves, we see different colors. That is because of this variation in the pigments.

So the same thing is shown here. This is the relative rate of photosynthesis as a function of the wavelength of light. So you can see that for the blue region. So this is the blue region. For the blue region and for the red region, the rate of photosynthesis is very high.

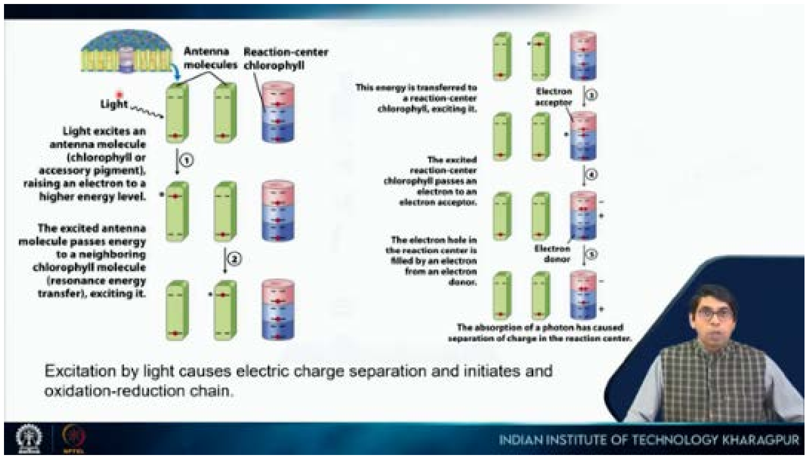


But for the green region, the rate of photosynthesis is very low. It means that it does not absorb much of this light. It absorbs mostly this light and this light, which is why the green light is either transmitted or reflected, and we see our leaves as green. So the light-harvesting complex contains 7 chlorophyll *a* and 5 chlorophyll *b*. But it turns out that this light-harvesting complex is actually a trimer.



So, which means that you have to multiply these numbers by 3. So, there are 21 chlorophyll *a*, 15 chlorophyll *b*, and 6 lutein molecules in a functional unit of a light-harvesting complex trimer. So this is just showing you the light-harvesting complex. So light is absorbed by any of these antenna chlorophylls, and then it is passed on to this reaction center. So let us look at what happens here.

So, light is absorbed by the antenna molecules. So, these are the antenna molecules. So, light excites an antenna molecule. It can be chlorophyll or beta-carotene or any other pigment and it raises the electron to an excited state.

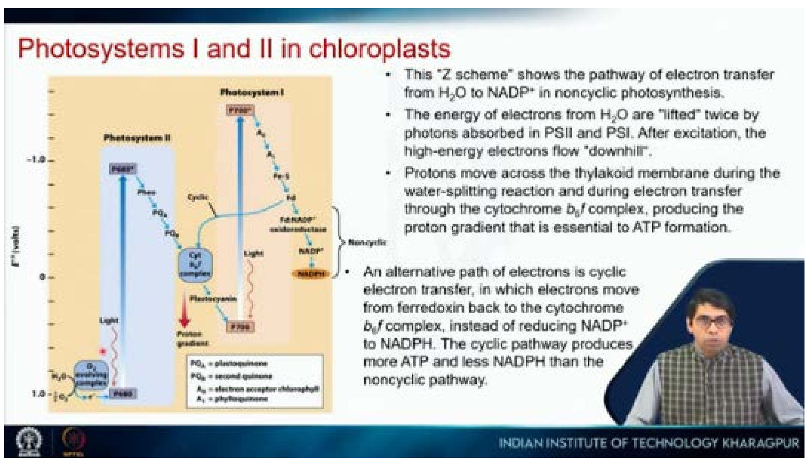


So, this is, let us say, the ground state, and this is the excited state. So, when light comes in, *hv* will excite this electron to the excited state, and that is shown here by this star. Now, this chlorophyll is excited; there is another chlorophyll which is right next to it physically. So, this excitation will be transferred to the next chlorophyll, which means that this electron will come down to the ground state, and this electron will go up to the excited state. So, the excitation is passed on from one antenna molecule to another antenna molecule like this.

Then, the energy is transferred to the reaction center chlorophyll. So, now this is a chlorophyll which is in the reaction center. So, this energy is passed on to this chlorophyll. So, it goes from the ground state to the excited state. The excited reaction center chlorophyll passes an electron to an electron acceptor.

So, there are some molecules; we will see those molecules. It will pass an electron to the electron acceptor, which means that an electron hole is created. The electron hole in the reaction center is filled by an electron from an electron donor. So, there will be an electron donor that will pass the electron. So, we will see what the electron donor is. Ultimately, the electron acceptor will be $NADP^+$ where this electron is passed on, and the electron donor will be water, which will split and produce oxygen. So, in essence, what happens here is that the excitation by light. So this is the excitation by light, it causes electric charge separation that we see here and initiates an oxidation-reduction chain. So, light energy results in this electric charge separation, and then, since an electron is given off, it triggers this oxidation-reduction reaction. So, water is oxidized to oxygen, and the electron is passed on through various channels, ultimately to $NADP^+$, which becomes NADPH.

So, here is a summary of what happens. So, we will see the physical structure of this. This is a schematic diagram. So, this is the oxygen-evolving complex. This is where water is split into oxygen and electrons. Now, this electron is then excited in Photosystem II. So, Photosystem II excitation lifts this electron to a higher energy and then it is passed on through all these carriers, ultimately to photosystem I. Now, while it is handed down by all these carriers, it passes through this cytochrome b_6f complex, and when it passes through this, it results in the pumping of protons.

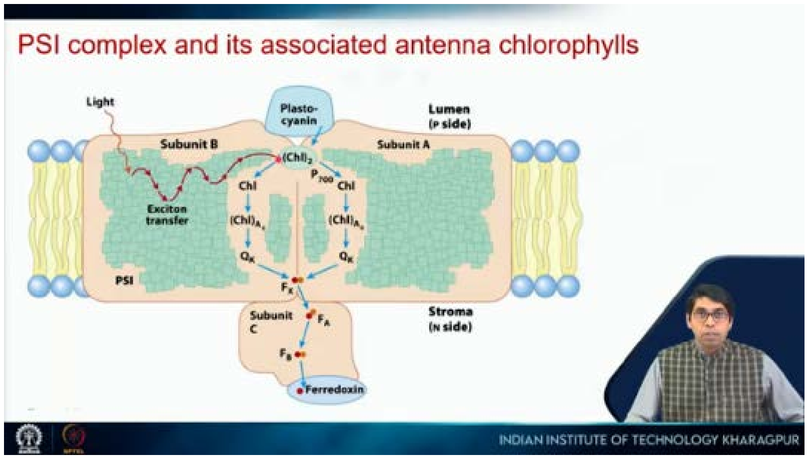


So, some of the energy is used to pump out some proton ions. We will see exactly how many in the next slides. So, this electron has lost its energy. In Photosystem I, another photon of light is used to further excite it.

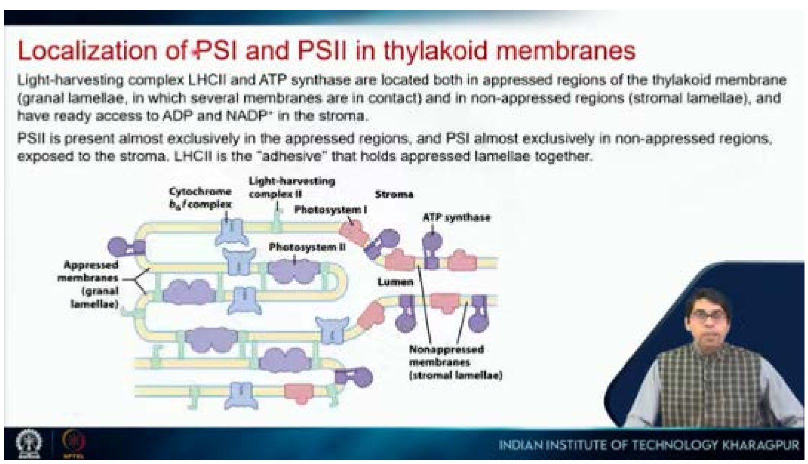
So now it goes even higher, and this electron is finally handed down and used to reduce $NADP^+$ to NADPH. So, the electron that originates here from a water molecule ends up in NADPH, and this NADPH will be used for ATP synthesis. This is called non-cyclic reactions. Another event happens where this ferredoxin hands over the electron back to this cytochrome b_6f complex. So, instead of passing it on to $NADP^+$, it hands over the electron to this complex, where it passes it to plastocyanin and again to photosystem 1, resulting in the pumping of more protons, and then again it goes here. So the electron originates here and goes through this, comes back up, goes to the ferredoxin, comes back to this, again goes to photosystem I, comes back up, and again it comes back to this. So in this case, it just moves in a cyclic fashion. So this is a cyclic pathway and in this cyclic pathway, you keep on pumping more and more protons. So the energy that is derived here is used for the pumping of protons. So in the cyclic pathway, you are not creating any NADPH, but ultimately this proton gradient will be used to create ATP. So the cyclic pathway will create more ATP since it is not producing any NADPH.

So all of this is summarized here. This is called the Z scheme or the Z scheme because if you look at it, it looks like a Z. So the Z scheme shows the pathway of electron transfer from water to $NADP^+$ in non-cyclic photosynthesis. The energy of electrons from water is lifted twice, so you see it is lifted once and then again lifted by the two photosystems, in photosystem two and one. After excitation, the high-energy electrons flow downhill. So once it is excited, it just goes downhill like this. It is exciting again, and then again it goes downhill. Protons move across the thylakoid membrane during the water-splitting reaction and during electron transfer through the cytochrome b_6f complex. So when water splitting happens, protons are released, and when the electron is passing through the cytochrome b_6f complex, again protons are pumped and this proton gradient is used for ATP formation. An alternative path of electrons is the cyclic electron transfer, where it goes up to this ferredoxin and then comes back to the cytochrome, in which electrons move from ferredoxin back to the cytochrome b_6f complex. So instead of producing $NADP^+$ to NADPH, the cyclic pathway produces more ATP and less NADPH than the non-cyclic pathway. So, the tuning of this non-cyclic versus cyclic pathway ultimately results in the ratio of ATP and NADPH, and that is something that will become important in the next lecture. Again, let me just go back here.

So what you see here is the cytochrome b_6f complex. This is plastocyanin, and plastocyanin is the one which is handing over the electron to Photosystem I. So this step is shown in the next slide. So this is plastocyanin. It is handing over the electrons to the Photosystem I complex.



Now, Photosystem I complex also has its own antenna chlorophylls where light is absorbed, and then this excitation will be passed on by all these pigments into this chlorophyll. Where the second excitation happens, and then ultimately it is handed over to ferredoxin. The light, Photosystem I and Photosystem II in the thylakoid membrane, are physically separated. So this schematic diagram shows it, the light-harvesting complex LHCII and the ATP synthase are located in both apperessed regions of the thylakoid. So, what is an appressed region?

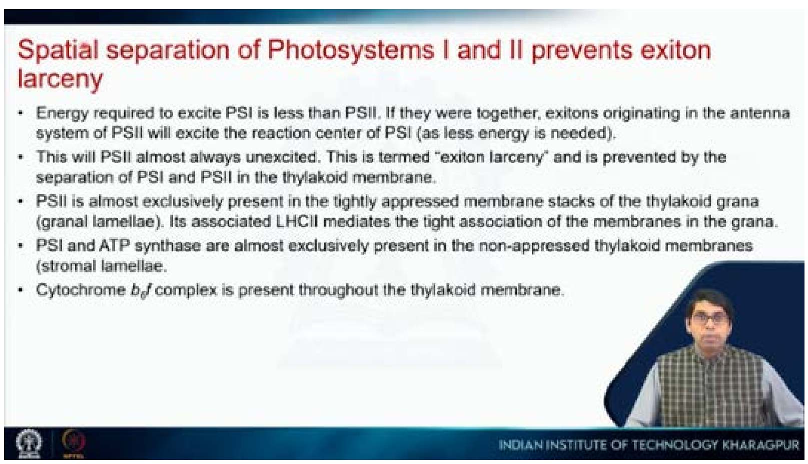


This is an appressed region where the membranes are sticking together. So, in this appressed region of the thylakoid membrane, which is also called the granule lamellae, several membranes are in contact. So, they are in contact with each other and non-appressed regions are this. So, this is the stromal lamellae.

So, light-harvesting complex II and ATP synthase are present in both the appressed membranes as well as the non-appressed regions. However, Photosystem II is present almost exclusively in the appressed regions. So, these are the appressed regions, and this is where you see Photosystem II is present. Whereas photosystem I is almost exclusively present in the non-appressed regions. So, this is the Photosystem I showed in red.

They are present in these non-appressed regions. So, these two are physically separated from each other via this mechanism and what triggers this compression of these membranes or the formation of these appressed membranes is done by this light-harvesting complex II, which is shown in green. So, this LHCII is the adhesive that holds the appressed lamellae together.

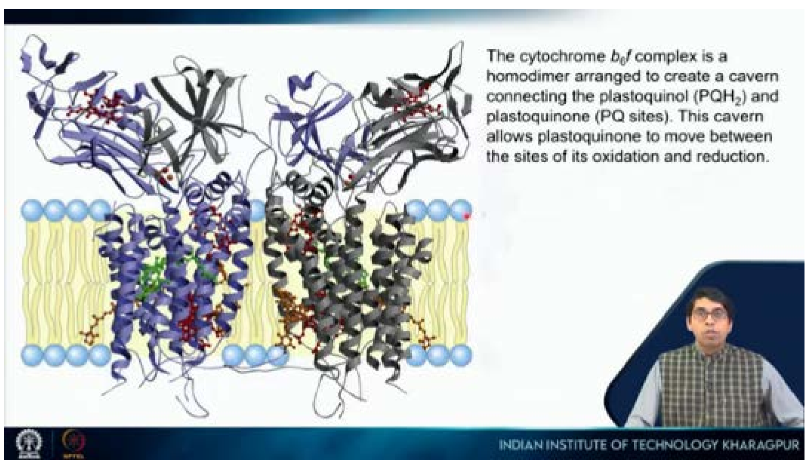
So, you see that this is holding these two membranes together. So, it is the light-harvesting complex 2 which does that, and it can trigger between this and this, and this is done by phosphorylation. So, a phosphate group is added, and the light-harvesting complex can switch between these two conformations. So, why is this special separation needed? Special separation of Photosystems I and II prevents exciton larceny.



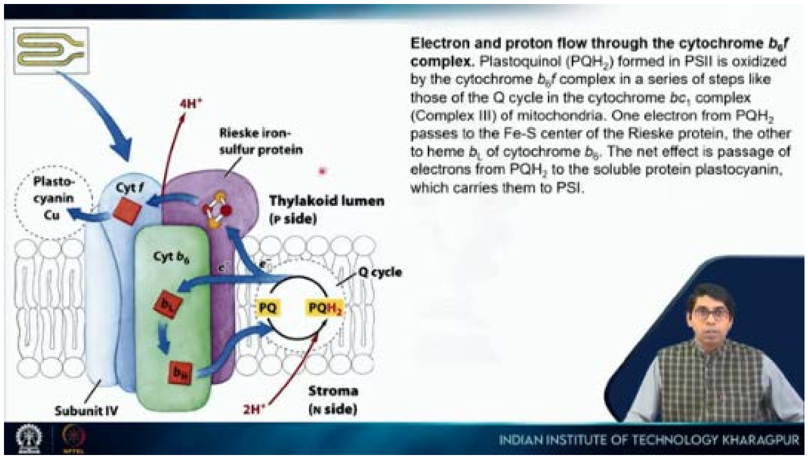
So, what is larceny? Larceny is basically stealing. So, what does that mean? The energy required to excite photosystem 1 is less than that for Photosystem II. Now, if both work together, then the excitons originating in the antenna system of Photosystem II will excite the reaction center of Photosystem I, because less energy is needed for Photosystem I. So, instead of exciting Photosystem II, all the excitation will happen for Photosystem I. So, this is where Photosystem I will actually steal the excitons from Photosystem II, which is the larceny and what will result is that it will result in the unexcited state of Photosystem II. So, Photosystem II will almost always remain unexcited. So, this is termed exciton larceny and is prevented by the physical separation of these two photosystems in the thylakoid membrane. Photosystem II, as we have seen, is almost exclusively present in the tightly appressed membrane stacks of the thylakoid grana, which is also called granal lamellae.

So, these are some of the terms that you should be familiar with. Its associated light-harvesting complex II is the one that mediates this tight association of the membranes in the grana, like this one. On the other hand, Photosystem I and ATP synthase are almost exclusively present in the non-appressed thylakoid membranes, which are the stromal lamellae. The cytochrome b_6f complex is present throughout the thylakoid membrane. So, the cytochrome b_6f complex is shown here.

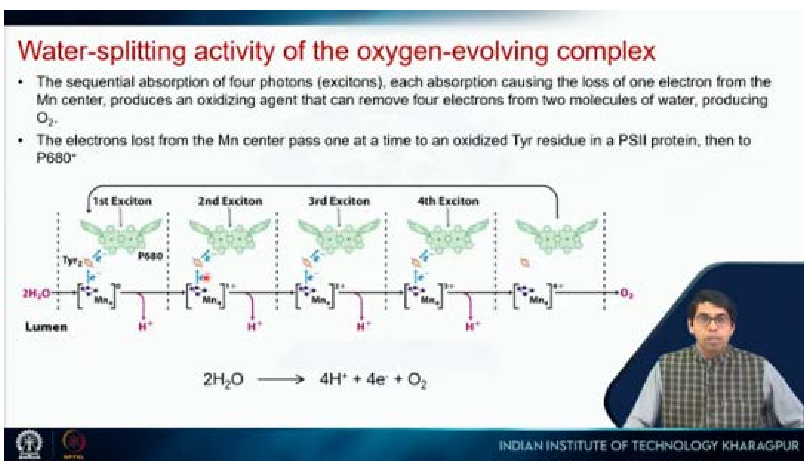
So, it is present both in the non-appressed as well as the appressed part of the membrane. So, this is the structure of the cytochrome b_6f complex. It is a homodimer arranged to create a cavern.



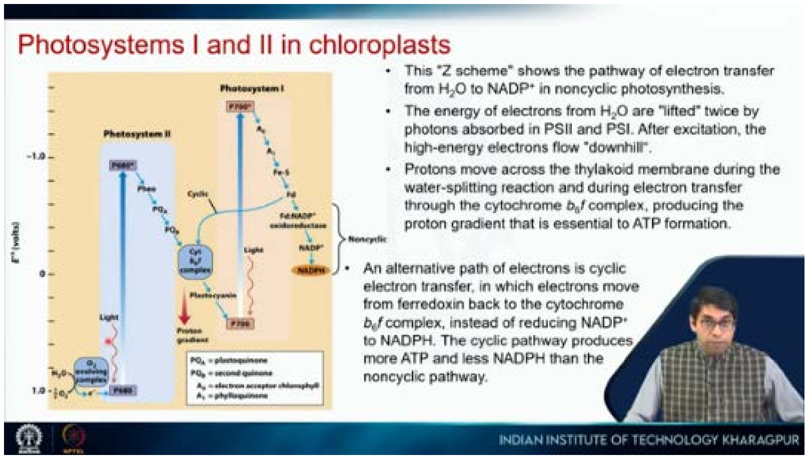
So, that is a cavern connecting the plastoquinol PQH_2 and plastoquinone. So, this cavern allows plastoquinone to move between the sites of its oxidation and reduction. So, this schematic diagram shows it in more detail. So, this is the thylakoid membrane, and in that membrane, we have this.



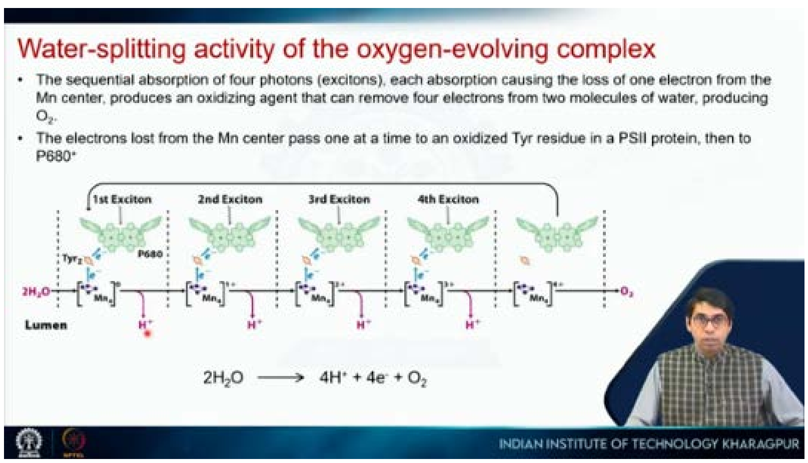
So the net effect is the passage of electrons from this reduced plastoquinol to the soluble plastocyanin, which carries these electrons to Photosystem I. That is shown here. So this is your plastocyanin so electrons are handed over to the plastocyanin, which carries them to photosystem I and there, the second absorption of a photon excites that electron further and then again, it goes through these downhill processes. It can end up in NADPH or it can be handed back to this complex. So let's look at this. oxygen evolving complex. So we have already seen Photosystem II, Photosystem I, the cytochrome b_6f complex.



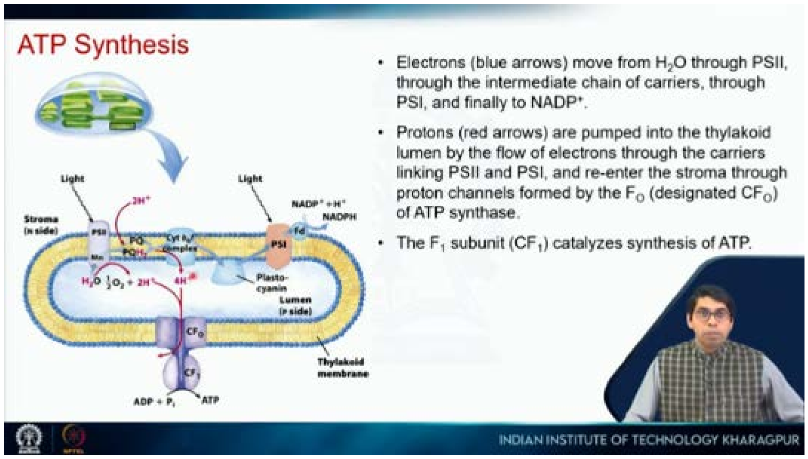
Now let us look at this oxygen evolving complex. So here, water splitting activity of the oxygen evolving complex happens. So water is split, resulting in the formation of hydrogen ions and oxygen. So it can be written like this: two molecules of water will result in 4 hydrogen ions.



So, 1, 2, 3, 4, and the passage of 4 electrons. So, you see one electron goes, second, third, and fourth, and ultimately the formation of one molecule of water. So, the sequential absorption of 4 photons. So, 4 photons are absorbed here in each step. Each absorption causes the loss of 1 electron from the manganese center.

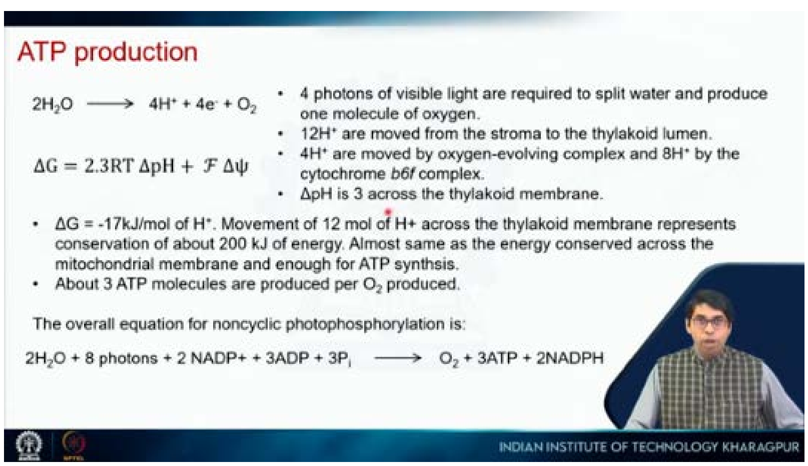


You can see that it goes from 0 to +1 to +2 to +3 to +4. So it goes through these different oxidation states and it produces an oxidizing agent that can remove four electrons from two molecules of water, producing oxygen. So four electrons are removed from two molecules of water, resulting in the production of oxygen, one molecule of oxygen, and four hydrogen ions. The electrons lost from the manganese center pass one at a time to an oxidized tyrosine residue, which is shown here. So it passes from this manganese to the tyrosine residue and ultimately to the Photosystem II protein. Then it is passed on, as we have seen earlier. So four photons can oxidize two molecules of water and produce one oxygen molecule and pump four protons. So that is something that happens here.



So now let us look at these calculations. So four photons of visible light are required to split water and produce one molecule of oxygen. This is what we just saw. So it turns out that 12 hydrogen ions are moved from the stroma to the thylakoid lumen then this happens, 4 protons are moved by the oxygen-evolving complex.

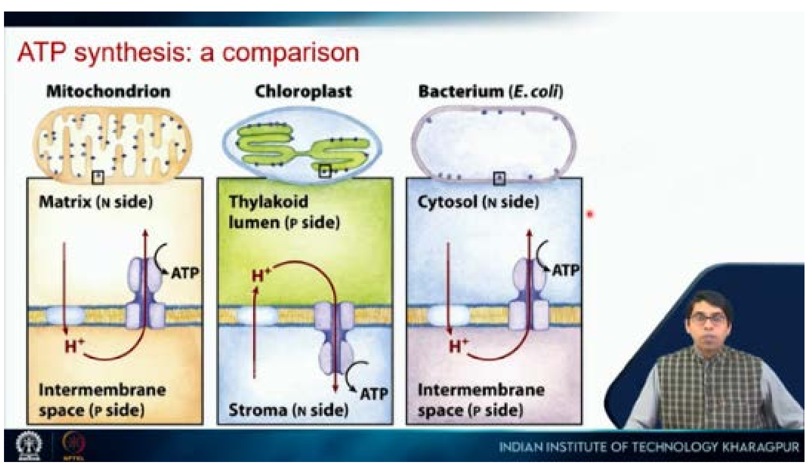
So we just saw that these are the 4 protons. So these are the 4 protons which are moved by the oxygen-evolving complex, and 8 protons are moved by the cytochrome b_6f complex. So the cytochrome b_6f complex will move 8 protons. So, total 4 plus 8 is 12. So, 12 protons are moved when 4 photons of light are absorbed to split water, producing 1 molecule of oxygen, which is this reaction.



So, when these protons are transferred, there is a proton gradient created, and the proton gradient, so on one end, the proton concentration is three times more than the other end, which means that the delta pH is 3 across the thylakoid membrane. Now, if we plug in this number here, it turns out that delta psi does not contribute much because of the passage of counter ions. So, if we plug in this number, we get ΔG as minus 17 kilojoules per mole of protons. So, the movement of 12 moles of protons across the thylakoid membrane will produce, so you just multiply these two, you get around 200 kilojoules of energy. So, this much energy conservation happens when four photons of visible light are used to split water, producing one oxygen molecule. If you remember from the last lecture, this 200 kilojoules of energy is almost the same energy that is conserved across the mitochondrial membrane, and it is enough for ATP synthesis. So, if you do your calculations right, it turns out that about three molecules of ATP are produced per oxygen molecule produced. So, four photons are absorbed to produce one oxygen molecule, and that energy can produce three ATP molecules.

So if we add up all of these things, the overall equation for non-cyclic photophosphorylation will be two molecules of water plus 8 photons plus $2NADP^+$ plus 3ADP and 3 inorganic phosphate will produce 1 oxygen molecule, ADP becomes ATP, so 3ATP molecules and $NADP^+$ will become NADPH, so 2 molecules of NADPH. But remember, this is from non-cyclic photophosphorylation. From cyclic photophosphorylation, we are not going to get any NADPH molecules, so we will only get ATP molecules. So now we can compare the synthesis of ATP in mitochondria, chloroplasts, and also in bacteria.

$$2H_2O + 8\ \text{photons} + 2NADP^+ + 3ADP + 3P_i \rightarrow O_2 + 3ATP + 2NADPH$$



So this is mitochondria. This is chloroplast in plant cell plant leaves, and this is a bacterium. Now this membrane we have on one side the matrix and the intermembrane space protons are pumped out by these various reactions that we have seen the TCA cycle and then oxidative phosphorylation A proton gradient is established across this membrane and when the protons pass through, this is spontaneous because it is flowing along the gradient, that energy is used to synthesize ATP. Similarly, in the case of thylakoid, light energy is used to develop or create this proton gradient. So this is the lumen or the P side, and this is the stroma or the N side. So more protons here, less protons here, ΔpH is almost 3. When these protons pass in this direction, which is the spontaneous reaction, that energy is used to produce ATP.

Now, in the case of bacteria, there are no mitochondria or chloroplasts. So, the only membrane that is present is the plasma membrane. So, on this side, this is the cytosol, and on the other side, this is the intermembrane space. So, this is the inner membrane, and then there is an outer membrane. So, it is that periplasmic space.

So, in that space, a proton gradient is established. So, again, the pH here will be around 7.4 or something, and this will be more acidic. So, more proton concentrations here. When these protons pass through the ATP synthase, this will be a spontaneous reaction, and that energy will be used to produce ATP. So, we see that ATP synthesis is almost exactly the same in all these three different organelles. So, the bacteria, chloroplasts, and mitochondria in eukaryotes. So, again, you can go through any biochemistry book, especially Lehninger Principles of Biochemistry, for the topics that I have covered today. Thank you.