Environmental Chemistry and Microbiology
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Module - 11
Lecture - 56
Metabolic Diversity - I

Welcome everyone. We are going to be starting a new topic today and it is called Metabolic Diversity. As the name suggests, this is an extremely large topic and it has been spread out over 2 modules. It is actually split into 6 parts. The first 3 parts are part of module 11, and the remaining 3 parts are part of module 12. So, we will start with part 1. This is lecture 56 of module 11.

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So, this particular topic is actually one of the biggest topics in this course. We are going to really understand how different species of bacteria have adapted themselves to different conditions in terms of electron acceptors, electron donors and so on. So, these are real examples of environmental microbiology, and you will be able to appreciate the enormous diversity in terms of microbial abilities in this particular topic. So, in today's first part, we are going to look at phototrophy. And within phototrophy, there are 2 major types of reactions: light and dark reactions.

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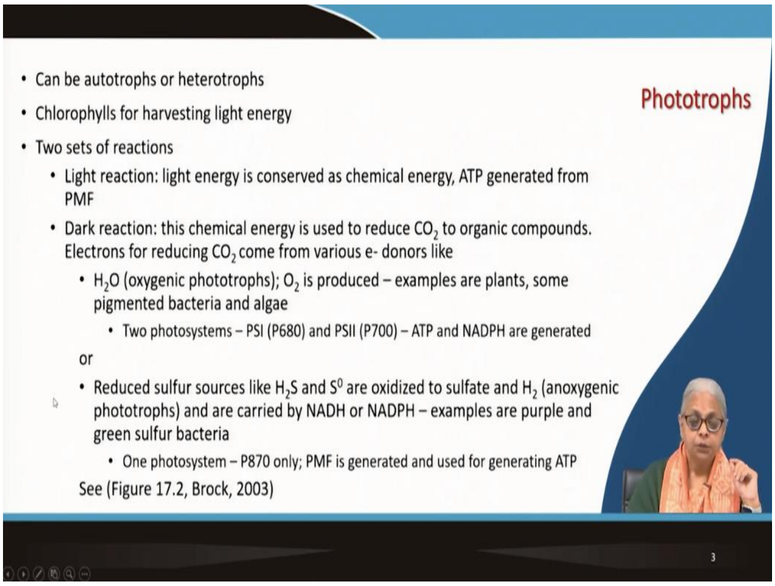

Now, we have by now a fairly good understanding of the fact that there are certain species and certain groups of bacteria that are capable of utilizing light energy, photo energy; and they may be autotrophs or they may be heterotrophs. So, these are organisms which get their energy from light, and they will convert that to chemical energy, and they will use CO_2 as their carbon source, in which case they are autotrophs; or they will use organic carbon as their carbon source, in which case they would be called photoheterotrophs.

So, you have 2 types; you have photoautotrophs and photoheterotrophs. They are all light harvesting species, and therefore they need chlorophyll for doing that. And that is something that you are already familiar with. Now, there are 2 sets of reactions like I mentioned, there are light reactions and dark reactions. So, the light reaction is basically utilising the light energy and converting it to chemical energy and ATP is generated from the proton motive force. And we will go into a little detail about this as we go along.

The second set of reactions are called dark reactions. Here, the chemical energy that has been harvested from light, is going to be used to reduce CO_2 to organic compounds. So, there are 2 ways of doing that. Now, electrons for reducing CO_2 from different electron donors can be done in different ways.

So, here we have 2 examples, we have what are called oxygenic phototrophs and we have anoxygenic phototrophs. So, under oxygenic phototrophs, the electron donor is water; oxygen is produced. Remember, this is the reverse of the respiration reaction. In the past, we have looked at aerobic respiration, where glucose plus oxygen are converted to CO_2 and water. Now, here in this case, what we are doing is, we are reversing that reaction.

CO_2 plus water are being converted to oxygen and sugar or other organic compounds. So, this is the normal photosynthesis reaction that we are all familiar with. Back from high school biology and science, whatever you have learned, this is the one that is most common. So, that is called oxygenic photosynthesis or oxygenic phototrophic organisms are capable of doing that.

All higher plants, all the green plants that you see around you, small ones, large ones, whatever size they are, some pigmented bacteria as well as algae are responsible for oxygenic photosynthesis. Oxygenic photosynthesis depends on 2 photosystems. They are called photosystem 1, which is based on harvesting light at the wavelength of 680 nanometers and the second one is photosystem 2, where 700 nanometer wavelength is harvested.

So, light energy at these wavelengths is what is being utilized. And in the process, ATP as well as reducing power are generated. And if you remember again back from aerobic respiration, you remember that reducing power can be used to generate ATP. So, that remains the same thing.

We also have another way of photosynthesis and that is called anoxygenic photosynthesis or the species that are responsible for this reaction are called anoxygenic phototrophs. So, they are capable of using reduced forms of sulphur like hydrogen sulphide and elemental sulphur and oxidising them to sulphate and hydrogen. Now here, the reducing power is carried by NADH or NADPH. And the examples of these bacteria are purple and green sulphur bacteria. Only 1 photosystem is used and that is called P870. Proton motive force is generated and then utilized for generating ATP. So, again it is similar to the previous one. You can also refer to the figure in the text for this.

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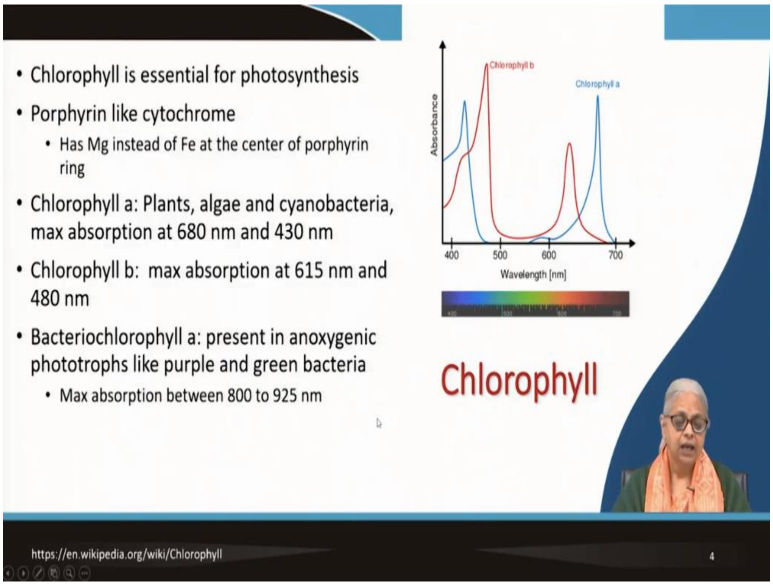

Let us take a little closer look at chlorophylls. We all know that chlorophylls are essential for photosynthesis. That much is known to all of you. But how do they function? So, if you remember again from cell chemistry as well as biology, I was talking about cytochromes and cytochromes are porphyrins. So, similarly, chlorophylls are also porphyrins. And instead of having iron at the center of the porphyrin ring, we have magnesium at the centre. So, that is the basic difference between cytochrome and a chlorophyll molecule. And now you have any number of examples of different types of chlorophylls. So, plants, algae, cyanobacteria, they have chlorophyll a, and some of them have chlorophyll b. Bacteria have what is called bacteriochlorophyll a, and it goes all the way to g; so, a, b, c, d, e, f, g.

I am not going to go into any of these details. I have been saying this often, very frequently throughout the lectures that this is, your assignments are all open book by definition. So, you can always refer to the textbook for the greater details and answer the questions in the assignments. So, here we have the absorption spectra for chlorophyll a and chlorophyll b. Like I said, whenever you are looking at an object and you see it as a particular colour, it means it is reflecting light of that colour and absorbing all the other colours.

Remember that black is something that absorbs all colours and white is when all colours are reflected. So, same thing here. When we see a plant as green, that means it is reflecting green. There is no absorption over here. It is reflecting green. The highest absorption is in the blue region and the red region. Both chlorophyll a and b are absorbing light in the blue and red regions. So, the maximum absorption for chlorophyll a is at 680 nanometers as well as 430 nanometers and chlorophyll b has maximum absorption at 615 nanometers followed by 480 nanometers. Bacteria chlorophyll is quite similar. It is present in anoxygenic phototrophs like purple and green bacteria. Maximum absorption is kind of off the scale, it is towards the infrared region, and that is 800 to 925 nanometers.

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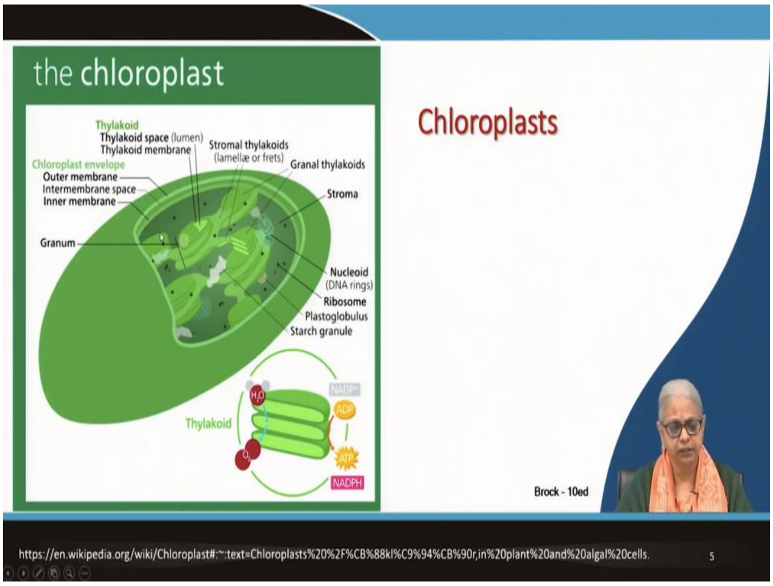

Where are the chlorophyll molecules located? I will refer you to the text again where there are several schematics explaining where the chlorophyll molecules are located. So, here is the chloroplast. The chloroplast contains chlorophyll molecules. And we have already seen in previous topics; we have seen where the chlorophyll is located. So, it is located in the thylakoids. So, here is the chloroplast. It has an inner and an outer membrane. So, you have the inner membrane and the outer membrane. And between them, there is the inter-membrane space. So, this is the envelope, the chloroplast envelope. And the thylakoid is basically these stacks. The stacks are the grana or the granum. And the empty spaces between the grana would be called the stroma.

So, these convoluted membranes of the thylakoid is where the chloroplasts are located. The nucleoid is present in these thylakoids. You also have ribosomes in the stroma. So, the nucleoid is freely floating throughout the stroma, as are the ribosomes. And you also have storage granules like starch granules and so on.

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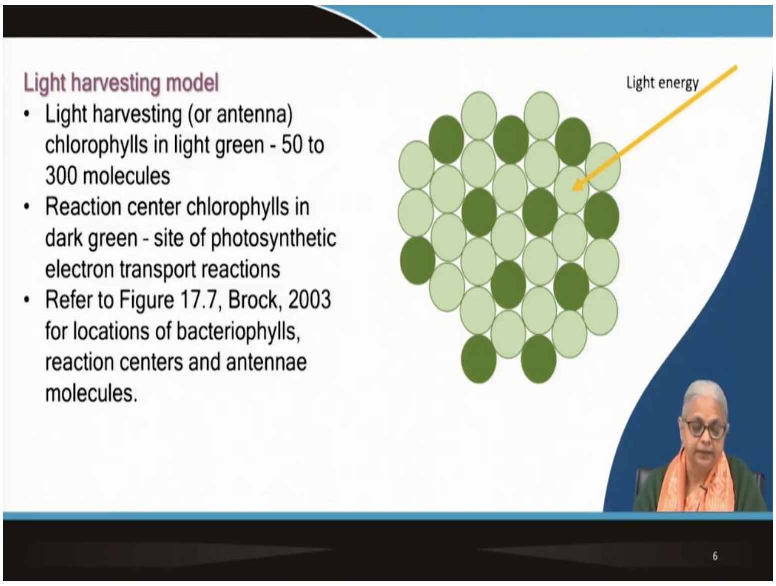

Now, we come to the light-harvesting model. So, how does the plant or the bacteria that is photosynthetic; algal cells, bacteria, plants, how are they harvesting light? There are 2 types of molecules that are present. The reaction centres are proteins that are fixed in the cytoplasmic membrane. The antennae chlorophylls are shown over here in light green colour, the reaction centres are shown in dark green colour. What you see here are that for every reaction centre, there may be 50 to 300 molecules of the light-harvesting chlorophylls. And again I will refer you to figure 17.7 in the Brock textbook. This is a very simplistic schematic of how light energy is taken up by the antenna molecules and channelled. So, all these 50 to 300 molecules are channelling this light energy to the reaction centre.

The reaction centre contains chlorophylls and that is shown in dark green. And this is the site of the electron transport reactions, which we will be looking at in the subsequent slides. So, the site of photosynthetic activity where electron flow is started or initiated happens at the reaction centre. So, for the locations of the bacteria chlorophylls, reaction centres, antenna molecules, all of these are shown very clearly in the text; and you can refer to that.

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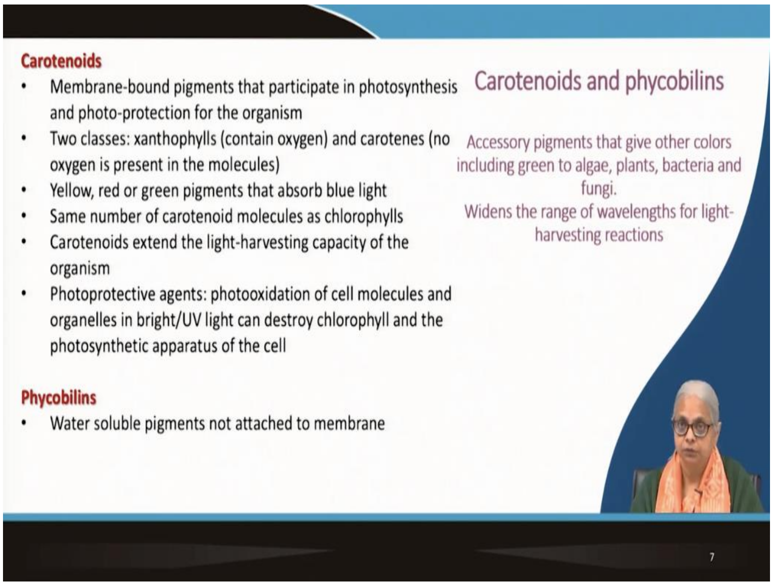

Let us now come to some other pigments. So, after chlorophylls, you can see that the chlorophylls have 2 basic maxima in the absorption spectra. But it kind of limits the plant or the photosynthetic organism to a very small range of the visible light spectrum. Now, there are other accessory pigments which are called carotenoids and phycobilins and these are accessory pigments.

So, when you see plants with yellow, green, red, these kinds of colours, these are accessory pigments that are giving these plants those colours. You can also find them in algae. You can find red algae; you can find pink algae; all these colours are there in both microorganisms as well as higher plants. And these accessory pigments have 2 major functions to perform. The first function is they increase the absorption spectrum of that organism.

So, instead of just absorbing in 2 major bands, they extend it. So, if it is a red-colored pigment, then again it will reflect red light and absorb green, blue and yellow. So, these are accessory pigments that enhance the ability of the organism to harvest light throughout the visible spectrum. So, you have yellow, red or green pigments that can absorb blue light and remember that blue light has higher intensity compared to red light.

So, this really makes a difference in terms of the light-harvesting capacity of the organism and therefore its ability to survive. Now, these are membrane-bound pigments that participate in photosynthesis. I have also mentioned a second thing and that is photoprotection. Remember again that beyond the visible light spectrum, you have UV light on the blue side and infrared light on the red side.

Now, this UV light is high-intensity light and it is capable of causing mutations in the DNA. So, it can cause adverse problems; it can damage the genetic code, it can damage the cell organelles and so on. You all know that UV light in many cases has been blamed for higher incidence of skin cancers and so on in human beings. So, similarly, for plants and other pigmented organisms, it can cause severe damage to the genetic material of that organism.

So, photoprotective agents are required; and these carotenoids perform that function by absorbing blue light. So, simply by absorbing blue light, they are protecting the rest of the material of the organism from damage. In general, the number of carotenoid molecules is equal to the number of chlorophylls. And I have already mentioned all the other points.

And then we come to phycobilins. Phycobilins are also similarly pigmented molecules, but they are water-soluble pigments that are not attached to the membrane. So, that again extends the capacity of the organism to harvest light energy.

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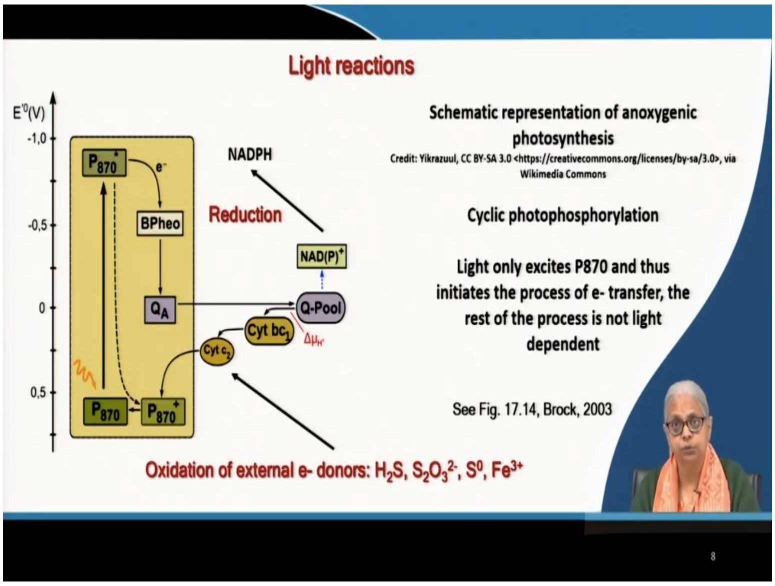

So, coming to how the electrons are transported and how energy is generated. Let us take photosystem 1 which is present in anoxygenic photosynthetic organisms like the purple bacterium. So, here we have a particular molecule; it is a pigment and it is capable of absorbing light at 870 nanometers. So, that is why it is called P870. Now, 870 nanometers is in the infrared range. It is not necessarily visible light, it is somewhere between visible and infrared. So, when light falls, when this wavelength of light falls on this molecule, there is some excitation of the electrons. The electrons are bumped up to a higher energy level. So, you can see that the electron potential increases. And then, these electrons are passed through the electron transport chain.

So, you have bacteriopheophytin; you have a series of quinones, quinone A, quinone B - quinone pool; and then cytochrome bc 1 and cytochrome c 2. This is a series. If you remember the electron transport chain in aerobic respiration where we are generating reducing power as well as ATP; and remember that reducing power is used for generating the proton motive force and the proton motive force is in turn used for generating ATP.

So, the same thing is happening over here. The electron, the excited electron is passed through this series of electron carriers. And in the process, reducing power is generated. It is an energy-consuming reaction, and that is what light energy is all about and you have external electron donors like hydrogen sulfide, thiosulphate, elemental sulphur and ferrous iron.

Now, all these electron donors after having donated; remember these electron donors are giving their electron here at P870. And when that gets excited by the light energy, it is being passed through these electron carriers. So, the oxidation of the electron donors is happening over here and reducing power is generated, which later on will be used for generating ATP.

Now, it is important to remember that light is doing only one part of this process. So, the only thing that light energy is being utilized for is for exciting the electrons. And once the electrons are excited, they will pass spontaneously through the electron carrier chain and the rest of the reactions are no longer light-dependent.

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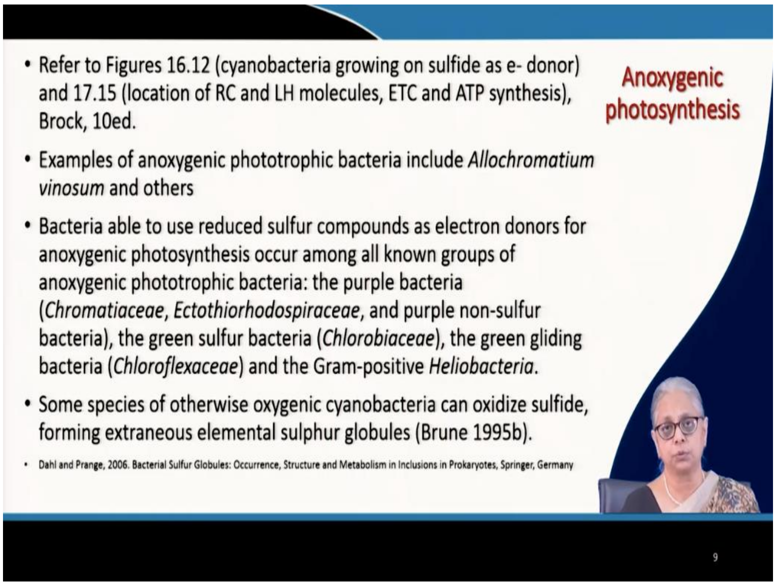

So, now that we know that cyclic photophosphorylation can happen leading to anoxygenic photosynthesis, let us also take a look at some examples of various types of bacteria or various bacterial species that are capable of anoxygenic photosynthesis. If you have access to Brock's biology, you can refer to the tenth edition or even subsequent editions and look at figures 16.12 which shows cyanobacteria growing on sulphide and utilising sulphide as the electron donor.

They also have a figure, figure 17.5 which shows the locations of the reaction centres, the light harvesting molecules, the electron transport chain and the ATP synthesis process. I will talk about that in a little bit in the next slide.

So, before we go too far, let us just take a look at some of the anoxygenic phototrophic bacteria. So, one of the most common ones or the most studied one is *Allochromatium vinosum.*

There are several other examples. Bacteria that are capable of using reduced sulphur compounds as electron donors, they are all capable of anoxygenic photosynthesis. So, you have the purple bacteria that is…..; there are several species that are mentioned here, purple non-sulphur as well as sulphur bacteria; there are green sulphur bacteria, *Chlorobiaceae*; the green gliding bacteria *Chloroflexaceae*; and the gram-positive *Heliobacteria*.

There are several other oxygenic cyanobacteria that can oxidise sulphur and they are the ones that generate elemental sulphur globules. And these globules, as I mentioned, I think in a previous slide; these sulphur globules or elemental sulphur globules can be found either inside the cell of the bacteria or outside the cell. So, there are any number of examples in the reference shown here at the bottom of the slide as well as in the textbooks.

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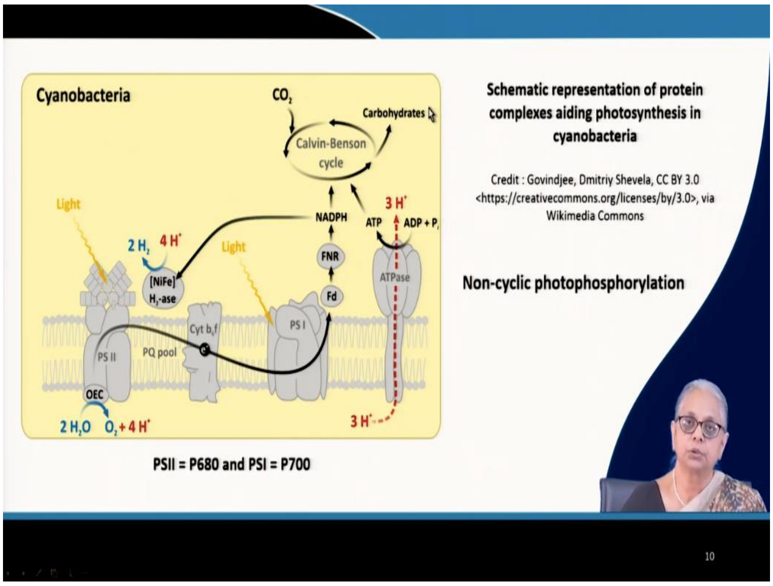

So, up to this point, we have looked at anoxygenic photosynthesis. Now, anoxygenic photosynthesis happens by cyclic photophosphorylation. But what we are all more familiar with is oxygenic photosynthesis which happens by non-cyclic photophosphorylation. Now, non-cyclic photophosphorylation is based on 2 photosystems instead of 1.

We have photosystem 2 and photosystem 2 is also based on P680. The 680 stands for the wavelength of light that is utilised by photosystem 2, and the electrons in this are bumped up to a higher energy level, from where they enter the electron transport chain which you see over here in the graphic. And they are transferred from P680 to P700, which is photosystem 1. Now, this transfer of electrons is associated with the development of the proton motive force as well. So, while the electrons are being transferred through the quinones and the cytochromes, the protons are also being pumped out of the cytoplasm into the periplasmic space, which you can see over here. So, a proton motive force is generated. And then, subsequently, this proton motive force is going to be utilised to generate ATP. So, that is how ATP is generated. And the only major difference between, let us say, aerobic respiration where you see a similar kind of process. The difference here is that ATP as well as reducing power in the form of NADPH are utilised in the Calvin–Benson cycle to convert CO_2 to carbohydrates and other organic compounds. We will take a look at all these processes in a little bit.

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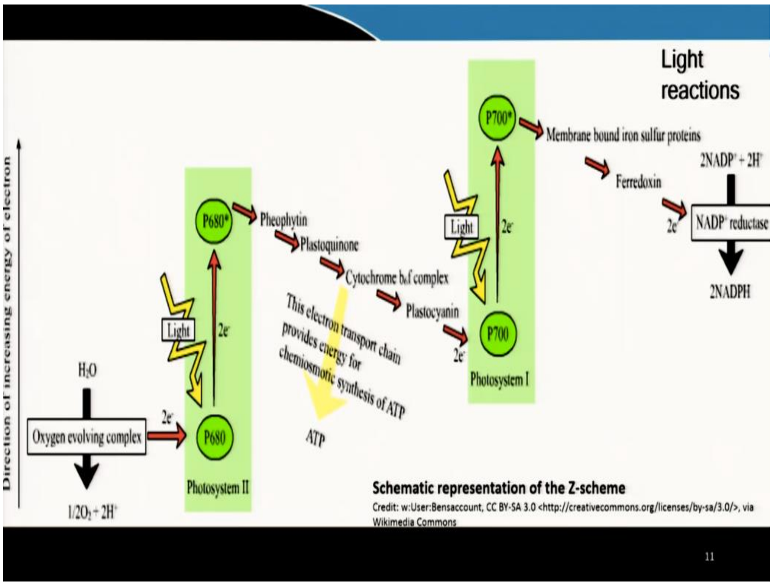

So, I think I can just go through this graphic one more time to explain the same thing. So, we have the generation of oxygen. When water is converted to oxygen (and hydrogen) gas, electrons are released and these electrons enter photosystem 2. Because of the light energy, they get bumped up to a higher energy level. They pass through all of these cytochromes and quinones. They are all collectively called the quinone pool.

And these electrons then hit P700, photosystem 1. Again, more light energy is utilised at this point. The electrons are further excited. And unlike anoxygenic photosynthesis, they are not recycled back to photosystem 2. Instead, they are transferred to $NADP^+$ to form NADPH. So, these are membrane-bound iron-sulphur proteins, ferredoxin and so on. Now, there is one step which is not shown over here but is shown in the textbook.

And that is where the electrons from ferredoxin are transferred to cytochrome bf complex. And that is where the cycle is completed. So, there is a transfer back of the electrons as well. But that is not shown in this particular figure, you can refer to Brock's textbook for that.

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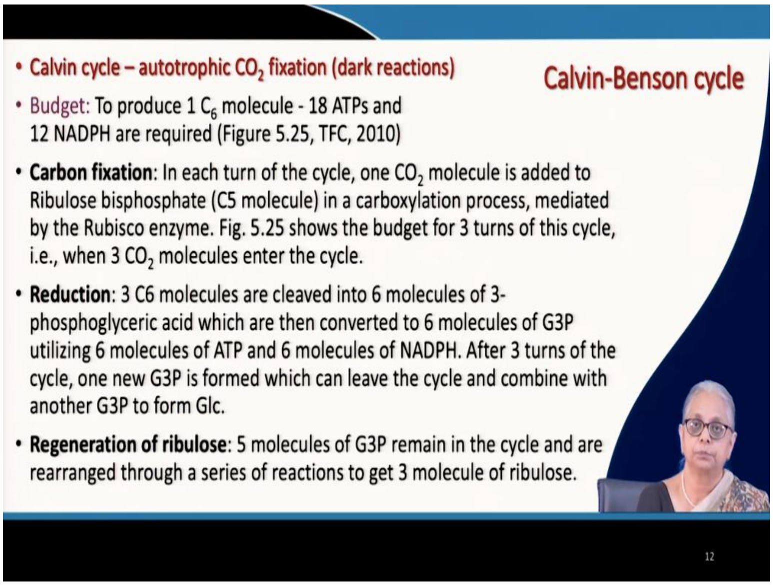

We then come to the Calvin-Benson cycle. So, like I said, the last part of photosynthesis is where you have fixation of CO_2 into organic matter. So, the conversion; what we have seen up to this point is the utilisation of water and the generation of oxygen or elemental sulphur and so on; so, in oxygenic as well as anoxygenic photosynthesis.

Now, we will take a look at Calvin-Benson cycle which is common to both types of photosynthesis processes. And this Calvin-Benson cycle happens in terms of carbon dioxide fixation. So, this is autotrophic carbon dioxide fixation. And these reactions are not, they do not require light. The only thing that is required is ATP and reducing power in the form of NADPH. So, let me just see if I have anything; no. So, I would refer you to figure 5.25 in the recommended textbook for this course, Tortora, Funke, and Case.

So, there is a figure; 5.25 shows you a very simple schematic which gives you some information about how CO2 is taken into the Calvin-Benson cycle and then utilised to form what are glyceraldehyde 3-phosphate molecules and 2 of these G3P molecules are then combined to form glucose. So, that is an example of how carbon dioxide is fixed by photosynthetic organisms.

So, to produce 1 C_6 molecule, 18 ATPs and 12 NADPH are utilised. So, we have 3 steps in the process: carbon fixation, reduction and regeneration of Ribulose. So, let us start with the very first step. In the first turn of the cycle, 1 carbon dioxide molecule is added to Ribulose bisphosphate, which is a C_5 molecule. And this is a carboxylation process. It is mediated by what is called the Rubisco enzyme. And you can refer to the figure.

Now, the figure in the textbook shows the budget for 3 turns of this cycle. So, in 1 cycle, you do not get much. So, in 3 turns of the cycle, you will get G3P which is glyceraldehyde 3-phosphate. And it takes another; so, 2 of these; so, 6 turns of the cycle will give you 1 glucose molecule. So, in the figure in the text, you have 3 carbon dioxide molecules that enter the cycle, one at a time by being added to Ribulose bisphosphate.

Now, in the reduction part, the second step. let me back up here a little bit. 1 carbon dioxide, when it is added to Ribulose bisphosphate, generates a C6 molecule. So, 3 of these C6 molecules are cleaved into 6 molecules of C3 compounds. Now, these C3 compounds are 3-phosphoglyceric acid, which is then converted to 6 molecules of G3P. And in the process, 6 molecules of ATP and 6 molecules of NADPH are utilised.

With 3 turns of the cycle, 1 new glyceraldehyde-3-phosphate is formed. And this is, after a few turns, this is excess glyceraldehyde 3-phosphate. So, that can leave the cycle. And there are still 2 glyceraldehyde 3-phosphate that are remaining. To regenerate Ribulose, you need 5 molecules of G3P to remain in the cycle. So, G3P is a C3 molecule and 5 molecules means you have 15 carbons that are available.

Now, these carbon compounds are rearranged through a whole series of reactions and the end result is 3 molecules of Ribulose, Ribulose being C5. So, you get 3 molecules of the original Ribulose compound. And that is the full Calvin-Benson cycle. So, that is, it is only when you get this excess G3P that is formed, that can leave the cycle. And when you have 2 molecules of G3P, that will allow you to generate 1 glucose molecule. So, I will end this part of metabolic diversity here. Thank you.

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REFERENCES

- Tortora, Funke and Case (TFC, 2010) Microbiology: An Introduction, Pearson Education.
- Madigan MT, Martinko JM, and Parker J (2003, 2015) Brock Biology of Microorganisms. 10th and 14th ed., Prentice Hall