

Indian Institute of Technology Kanpur

National Programme on Technology Enhanced Learning(NPTEL)

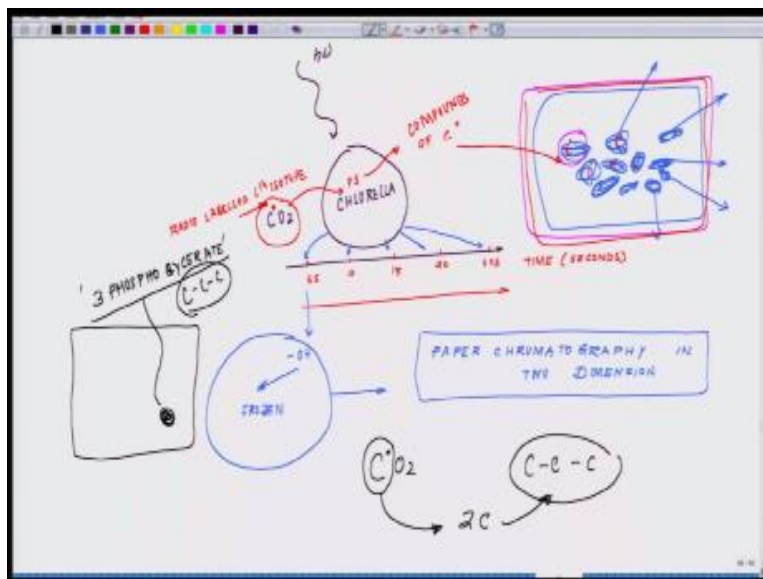
**Course Title
Bioenergy**

**Lecture -13
From CO₂ to 2 Molecules of 3 Phospho Glycerate by RUBISCO**

**By
Prof. Mainak Das
Biological Sciences & Bioengineering &
Design Programme
IIT Kanpur**

Welcome back to the lecture series in bioenergy so we are in module 3 on the phosphor so in the first lecture I started with you people about the carbon dioxide fixation and I give you a brief historical sketch when Melvin Calvin started his research on how carbon-dioxide is fixed in the chlorella, so the first break which he got so you remember in the last class and one of the slides I showed the get back to the slide in the previous slide.

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I showed you this autoradiograph so one thing you forgot to tell you in the last class so once you get these moieties on the paper trimmerography you put it in the radioactive processing which is essentially called autoradiograph and that is where you get all those spots so those were CO₂ got incorporated into organic molecules with radioactive labeled. So in other words and in these spots what will you see you will have carbons with radioactive levels which essentially means that these carbons have come from the medium from the surrounding injected medium into the photosynthetic assembly and got incorporated or may got reduced or for msome certain compounds of carbons, okay.

So what essentially we are seeing here are different compounds of carbon we where the carbon has been radio labeled, so this stuff what you see out here was done after 60 seconds now at five seconds when he froze it by putting the whole complex in alcohol Calvin observed only one single spot or something like this in autoradiograph if you see in that autoradiograph see at that specific spot there is only one and which he identified as 3-phosphoglycerate and while I am writing this 3-phosphoglycerate this will remind you in the very, very first sessions when we talked about photosynthesis I talked about this 3-phosphoglycerate which is form.

So3-phosphoglycerate is a carbon it is a three carbon okay, so what you are having here is something a molecule like this so essentially what people started thinking very first thought which came to mind was so now if the product is 3 carbon and you are starting with a single carbon which is of course like this okay, which is coming from single CO₂ so is it this carbon from CO₂ is attaching to some to carbon molecule and making a three carbon molecule is it clear to you people think of it.

So what I get is a molecule 3-phosphoglycerate and three carbons and you started with a one carbon molecule CO₂ carbon single carbon right, so from one to three you have only two options right, so if this one is coming here so you already have something which is two carbon and this one which is coming here and form a three carbon simplistic thought this is how it should work interestingly that is exactly not how it worked to pick surprise so there was no 2 carbon substrate so one more to add upto have a recall back so you already have the reduction assembly in the form of NPT which is produced from the process system one, okay.

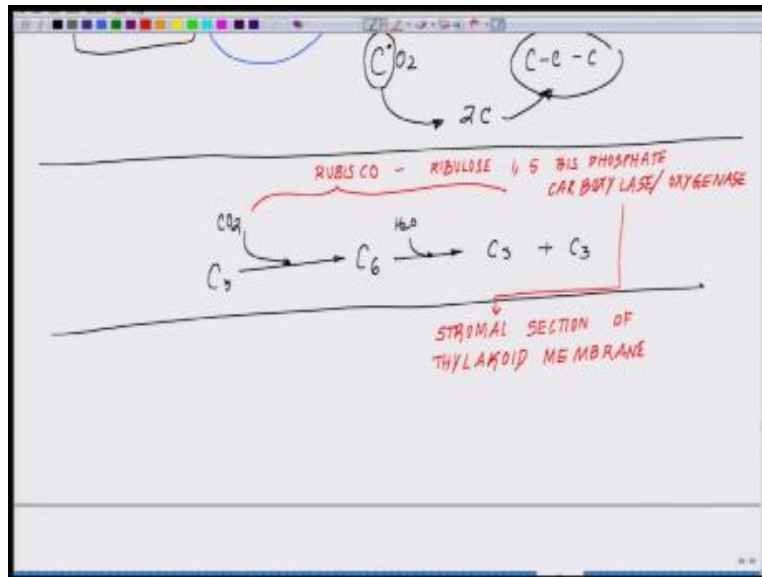
So very simplistic and one specific spot telling that it is 3-phosphoglycerate is form because one carbon coming from carbon dioxide and you have some to carbon molecule something which you do not know okay, so it forms three but apparently that was not the story a story or something else and that is where lies one of the most beautiful journey of Calvin and the Calvin cycle which involved lot of post docs lot of research a lot of collaborators likewise and if you read through is a Nobel lecture which in last reserves kind of built confused it was in 1961 and that this lecture was delivered on December 11, 1961.

And stock and there is clearly mentioned that this was not something which happened overnight it was 45, 55 like 15 years of rigorous research you know but by spot figuring out the intensity of the spot the molecular nature of it and the structure of it the long journey, so what we will do I will not go through that journey anyway I will give you a hand out to read through that Nobel lecture it will get really fascinated by to see how this first how the so called bio-fuels in nature is being made which now we are employing techniques to convert it but what was found out was interesting the first acceptor of carbon dioxide is not a two carbon compound instead it is a five carbon compound.

So five carbon compound so try to visualize it first this after that I will write down each of the formula okay that is not important partners to visualize how nature function, so it is a five carbon attached to one carbon or rather one carbon attached to five carbons so it makes us six carbon C₆ then through a series of process this fixed carbon gets split into three, three now river back get that cock block back to yourself so what we talked about 3-phosphoglycerate right, three carbon so one carbon coming from carbon dioxide five carbon coming from that XYZ molecule they add up together kind of a condensation or adding together and make it six carbon.

That six carbon now gets split up into three, three molecule of phosphoglycerate how that happen and who made it happen, the enzyme which made it happen walls one of the most abundant enzyme and which is so let me just write it down what I just talked to you people.

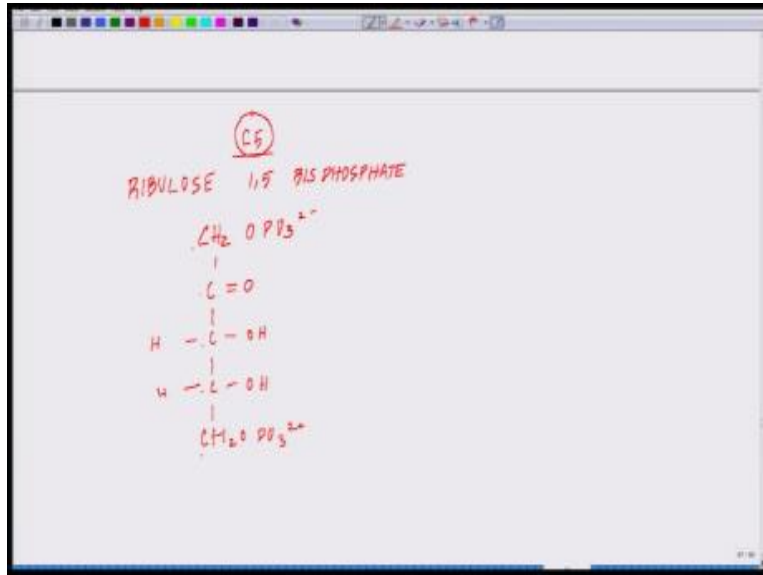
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So that it kind of engraved so the first thing was a C₅ okay, on the C₅ and not identifying the molecule at this stage you are adding the CO₂ or your carbon dioxide okay this makes it C₆ from the C₆ you are making a C₃ + C₃ and this whole thing happens in the presence of or is being orchestrated by an enzyme called RUBISCO whose full form is Ribulose-1,5-bisphosphate carboxylase oxygenase and I will come to that why I am mentioning like that carboxylase/oxygenase this is one of those enzymes which is located on then if you know the location if you remember the structure it is located on the stromal section of thylakoid membrane.

We will little later we will talk more about it, so now the main question which must have been modeling what is that C₅ molecule what we are talking about.

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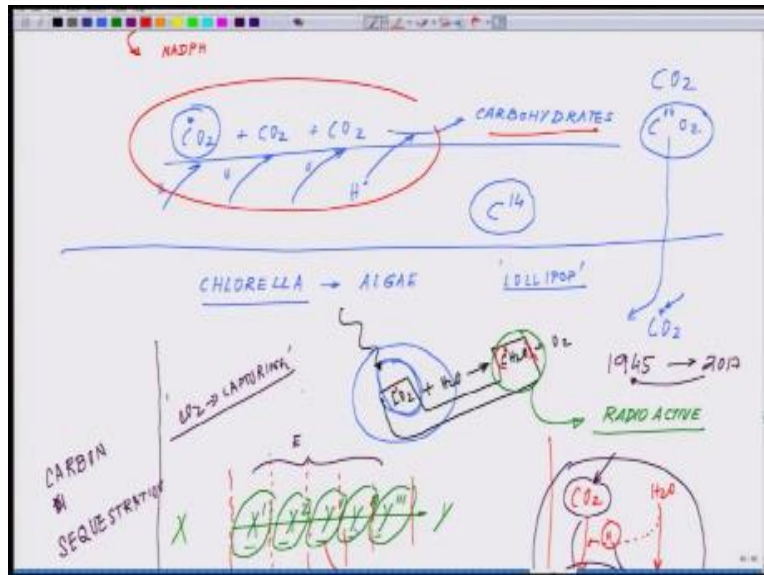


So now what we will do we will talk about that C5 molecule or the very first carbon-dioxide acceptor and remember it we still have not talked about the NADPH which will come later and we will move slowly because this cycle just the way you have understood the light cycle is very essential that it engraved in your mind map exactly how each one of these reactions are taking place there are some of the most beautiful reactions, okay.

So to start off with the C5 what is that C5 molecule is, so C5 is essentially a molecule called Ribulose 1,5-bisphosphate how this molecule looks like so this is the name of the molecule and the molecule looks like this $\text{CH}_2\text{OPO}_3^{2-}$ you have ketone group out here COH ever hydrogen here you have another OH group on this side hydrogen on this side and you have CH $\text{CH}_2\text{OPO}_3^{2-}$ so let us number them 1 2 3 4 5 so this is that first substrate which is the starting point of accepting carbon.

So ruling out all speculation so if you remember where we started this journey so I will show that very in the previous class I told you that it could be possible like this with a lot of carbon dioxide is coming in and forming carbohydrate.

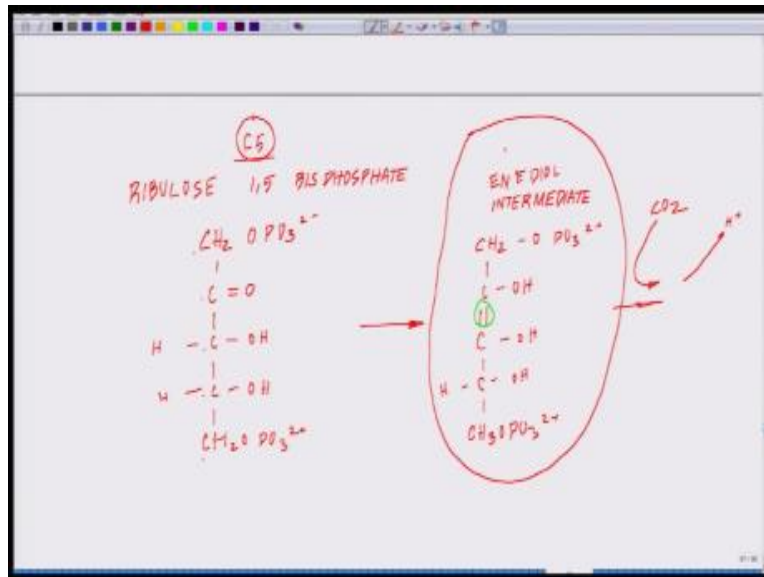
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Then we talked about in today's class we started off with this reaction possibly carbon dioxide coming and attaching your to carbon and making a three carbon then we moved on to deciphering it and this process was not easy just imagine from all those a sport figuring out possibly which one is the first one and this all went back to the process of freezing the moment by putting it in alcohol we just throws that moment hold that moment and doing a paper chromatography then autoradiography the long drawn process of step by step to figure out the process.

So you have Ribulose 15 days fasting Reba lose 15 bisphosphate under the action of the enzyme which you have already talked about action of Rubilose come there is a modification which happens in it which is called the enediol complex.

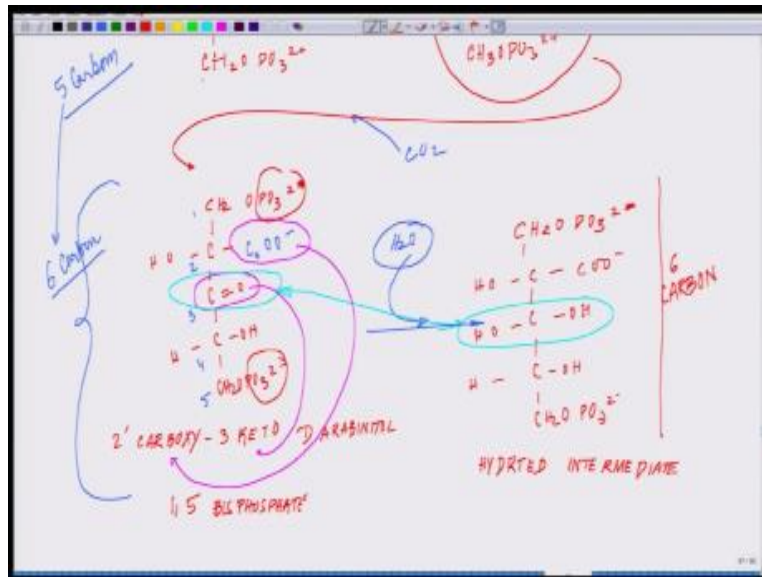
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Which is call enediol intermediate or in radical complex, so any deal is essentially your $\text{CH}_2\text{OPO}_3^{2-}$ and you have COH so here you see and then here is a change so the double bond which got introduced out here I am just highlighting for your understanding what is the difference.

So the double bond which is coming and then and is CHOHPO_3^{2-} okay, so this enediol intermediate we talk about you talk about this later how this enzyme acts on it. Now out here after this is where CO₂ is adding as of now there is no addition of CO₂ okay, CO₂ is adding and there is a proton which is formed so now we will talk about after the CO₂ addition how the molecule looks like.

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So after the CO₂ addition so I am picking up the reaction from there what do you see CH₂OPO₃²⁻ okay then you have see Oh and this is where COO⁻ minus this is you 2 coming through and see ketone group again the OH, Hand CH₂PPO₃²⁻ okay, so this inter me this after the addition this is called two Prime carboxy three ketone to the carboxy group you could see that to Prime carboxy three key to so 3 ketone when you talk about this is the three ketone group and here you have the carboxy group okay, it is a ketone group D arabinital 15.

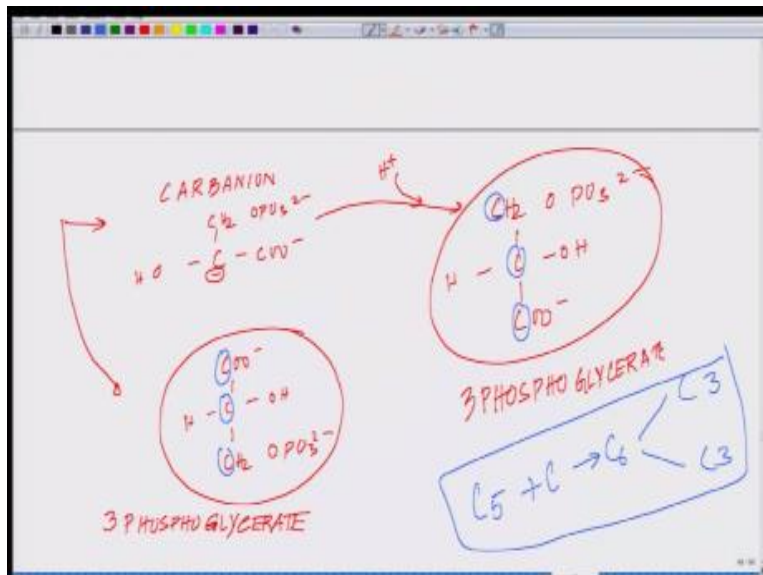
So these are the phosphate coming through one and five so your numbering is going like this 1,2,3,4,5 okay so that is why you see the three jetone and which already number it okay 15 base phosphate this is after the addition of CO₂ to this site gym so now what you have if you count one two three four five and there is a six carbon so from five we want to 5 carbon to six carbon, so this was the next development.

Now at this stage there is a hydrolysis taking place okay, so there is a water molecule which is added in the beginning I did not explain why that water molecule is coming in the beginning you must have seen now I explaining it so there is an hydrolysis reaction taking place and a hydrolysis reaction leads to this following product what you have is CH₂OPO₃²⁻ sorry 2- C COO

carboxylate carboxylic group out there then you have OH OH now you could see the change the ketone group is no more there and here for those what I would follow look at here and compare it with here you will see the difference, okay.

Just compare it so this is the change which is taking place after the hydrolysis reaction then you have the OH you have the H and then you have again the $\text{CH}_2\text{OPO}_3^{2-}$ so this is your hydrated intermediate and followed by once you get this hydrated intermediate this hydrated intermediate which is again a six carbon.

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Now this six carbon gets split up into two carbon product and the products are like this what you have is a carbon ion which is C^- over there $\text{CH}_2\text{OH PO}_3^{2-}$ and an OH and the other product so this is one product which is formed other product which is formed is after the hydrated intermediate getting dissociated into two three carbon compounds is the OPO_3^{2-} so this is your 3-phosphoglycerate. Whereas this molecule further after addition of another proton so you remember there are lot of protons which played a critical role so this form the same thing OPO_3^{2-} COOH and CO another three so the carbon ion form 3 phosphoglycerate.

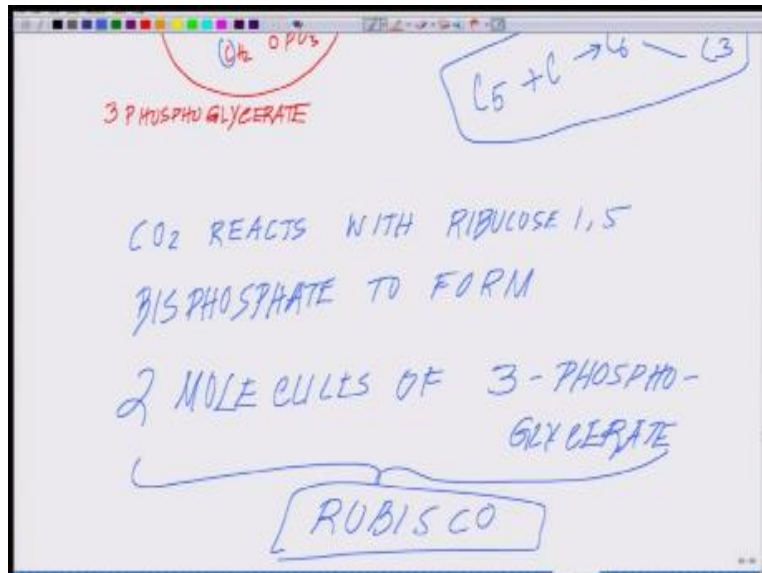
So what you see out here if you label them blue one carbon take in carbon third carbon and 1 carbon second carbon third carbon so you have a $C_5 + C$ forming a C_6 and then splitting up into C_3 C_3 so last reaction what I just now drew in front of you a C_5 plus CO_2 and then forming a C_6 and then splitting up into two compound of C_3 C_3 , so this is the core initial reaction where CO_2 gets accepted so contrary to all the beliefs earlier of CO_2 is adding together forming carbohydrates or CO_2 adding to two carbon making a three carbon contrary to all the beliefs Kelvin showed that it is a five carbon attaching to another carbon from carbon dioxide and converting it into a six carbon molecule.

And that six carbons from 3-phosphoglycerate and how this 3-phosphoglycerate so in the in the process the enzyme which got involved was enzyme called rubisco so Rubisco is a very, very interesting enzyme will talk in the next class in detail about rubisco but just give you an idea it is also called carboxylase because it is adding carbon dioxide is also called oxygen is it reacts with oxygen.

So in other words or not it reacts with oxygen it promotes the reaction of oxygen after binding to it so in a way the Rubisco is a very peculiar enzyme which assists in oxygenation as well as carbon dioxide addition and what is the implications of it how it really act, so in the next class what we will do will talk about the basic mechanism and then we will talk about it rubisco other side the oxygenation and what it leads to the photorespiration which is kind of the beginning of understanding C_3 and C_4 .

And then in the meantime what we will do we will talk about how these phosphoglycerate are fed into the Calvin cycle to make the other carbohydrate molecules, okay so I will close in here and we will pick up our discussion from this point on since now we have finished our journey of essentially what we have done today is if I to summarize it.

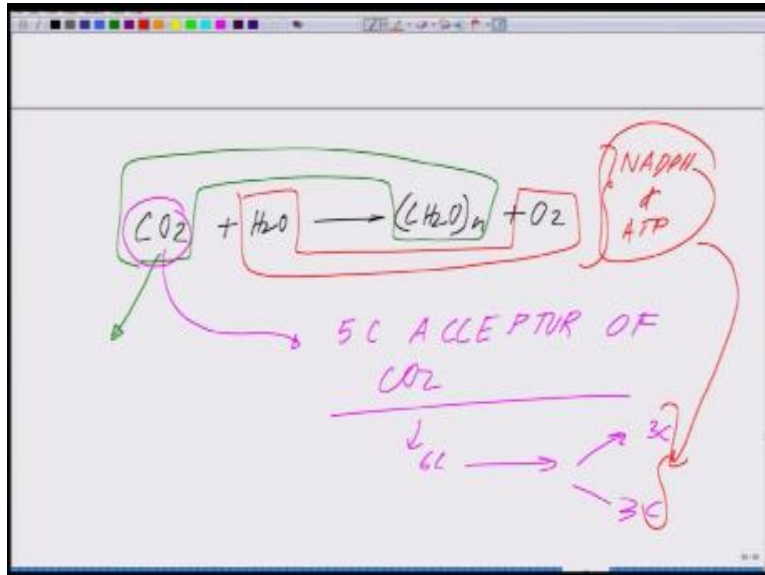
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So this will fall under CO₂ reacts with rib lows15 biphosphate to form two molecules of 3 phophoglycerate and the influence of or it is being carried out by through this scope, so in the next class we will initiate our journey with the structure of rubisco and how the Rubisco carries out this reaction and what are the drawbacks will be school have and what are the current research and rubisco and then from there we will work with the Calvin cycle this is the first part of it we have a complete cyclic reaction which happens after this and still we have in please keep tab we still we have not talked about where NADPH is coming to place.

Because NADPH is still the product which is coming from the coming from the for system one from the light reaction okay, so now we have taken care so if I had to do a kind of in economics then this is what all we have done as of now.

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CO₂ so now we know where CO₂ is binding we have talked about H₂O we have talked about now CH₂N plus oxygen so we have talked about this reaction earlier and today we have initiated in last two classes this part of the reaction and we have first thing what we have kind of learned together is the 5 carbon acceptor of CO₂ making it 6 carbon and then splitting up into three carbon and three carbon and how these three carbons and still as I was trying to tell you we have not accounted for NADPH and ATP what are their uses and how they are clubbing in out here okay, so I will close in here, thank you and we will follow up in the next class.

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Prof. Satyaki Roy

Co-ordinator, NPTEL IIT Kanpur

NPTEL Team

Sanjay Pal

Ashish Singh
Badal Pradhan
Tapobrata Das
Ram Chandra
Dilip Tripathi
Manoj Shrivastava
Padam Shukla
Sanjay Mishra
Shubham Rawat
Shikha Gupta
K. K. Mishra
Aradhana Singh
Sweta
Ashutosh Gairola
Dilip Katiyar
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