Applied Environmental Microbiology
Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

Lecture – 11
Microbial Metabolism I

Hello students. In the previous lectures we talked about the microbial energetics and how gibbs free energy is the main driver for any biochemical reaction to happen. And for microbes to harness that chemical energy and assimilated for either sustenance or growth. Today in this lecture we will complete the complete what we started in the previous lecture. And talk mostly about respiration and biosynthesis.

So, if you remember in the previous lecture we talked about fermentation. Which is anaerobic utilization of glucose, and today we will talk about the aerobic one which is respiration. In short, the key for this lecture and the few lectures before has been how microbes convert this chemical energy into biological life force which allows them to carry on their day to day operations, all right. So, first of all if you remember this slide from the previous lecture we talked about glycolysis, where we had 2 options.

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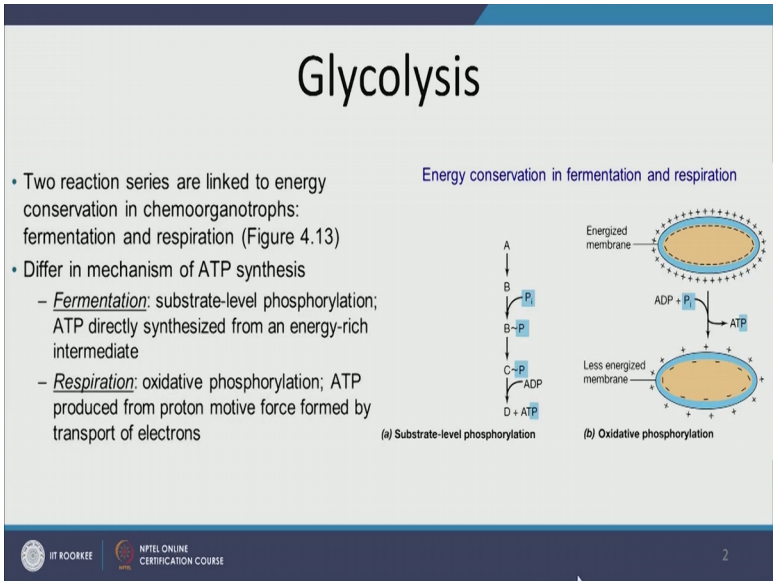

Either have substrate level phosphorylation as is mostly the case with anaerobic metabolism, or have oxidative phosphorylation which is the case with aerobic phosphorylation.

Today we are going to talk about this the oxidative phosphorylation which is an example of respiration. Now if you remember from one of the first lectures in this course, we talked about how cellular membrane also serves another purpose of acting like a battery which stores charge. So, positively charged on the outside and negatively charged on the inside this potential difference drives many biological processes. And when the cell utilizes this this potential differences it is referred to as oxidative phosphorylation and respiration.

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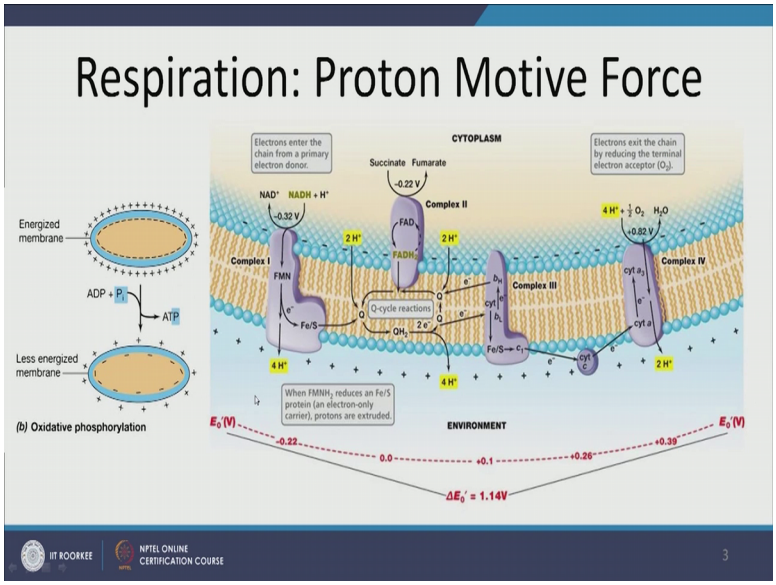

So, respiration or oxidative phosphorylation where the proton motive force, because remember these are the protons that have assembled outside cellular membrane creating this potential difference. So, this proton motive force is utilized for microbes to carry energy in or carry energy out. So, let us look at this cellular membrane diagram, and notice here there are 4 different complexes. Now these purple integral membranes some of them are peripheral, some of them are integral. Notice that this side the upper side is cytoplasm. So, it faces inside of the cell. And the lower side does environment. So, it faces the outside of the cell.

Now these purple protein complexes are referred to as complexes because they have many many microbe many many proteins inside them. For example, complex one in itself has at least 10 proteins, and all of them are very well characterized by the way. So, let us look at complex one. In complex one we have NADH, it utilizes a proton and it

creates NAD plus. So, it gives away it is negative charge here. And as such fmn been carries a charge to the q cycle reaction, and the 4 protons are evicted out to the cellular membrane.

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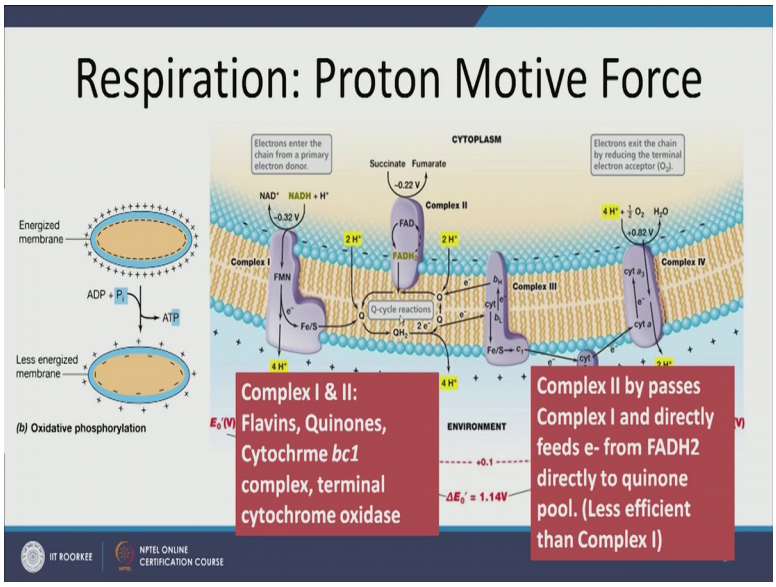

Now complex one and complex 2 have proteins such as flavins quinones cytochrome bc one complex, and terminal cytochrome oxidases. It is bc one complex is very, very important, this is where most of the chemical reactions take place. Now in complex 2 what it does is it bypasses the complex one step where it actually takes a proton from here consumes it and then evicts it out. And it directly throws electron into the quinone pool which is with this a 2-cycle reaction.

So, the electrons are directly sent here, and then here we have protons for protons. And here we have negative charge in complex 3 and complex 4 we have slightly different chemistry.

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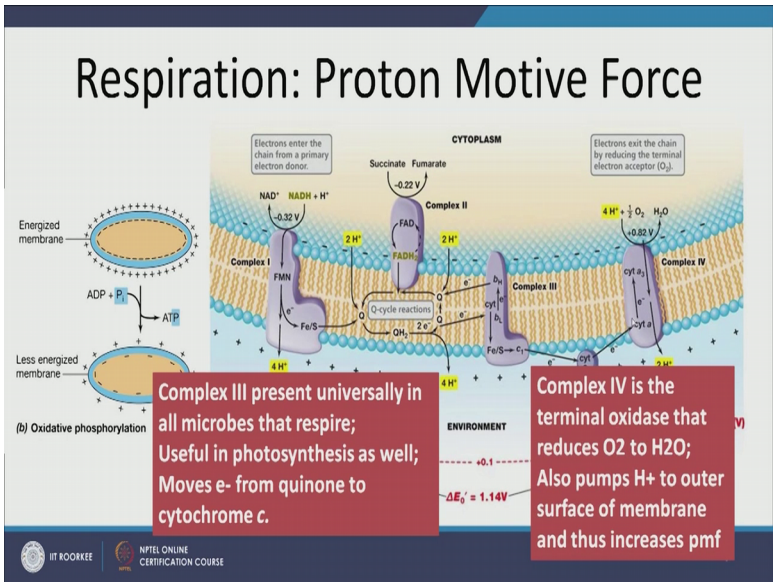

In, but note here that complex 3 is present in all microbes that respire. So, any aerobic microorganism be it cyanobacteria be it us be it plants we probably will have complex 3 most likely. And it is very useful in photosynthesis. What it does is that it moves electron from quinone to cytochrome c where most of the reactions take place. And acts like a channel through which proton can assemble on the outside of cellular membrane and thus increase their potential difference.

Complex 4 does the same thing, but if it is the terminal oxidase that reduces oxygen to water. That reduces oxygen to water and it pumps hydrogen to the outside of the cell, and thus increases the proton motive force. So, note the common thing between all the 4 complexes is that they are complex of proteins. And their primary function is to pump proton outside and have a nice strong PMF. Now what is the use of having this PMF? How does this proton motive force convert into energy for the cell? How can cell use this potential difference of proton motive force and make energy out of it?

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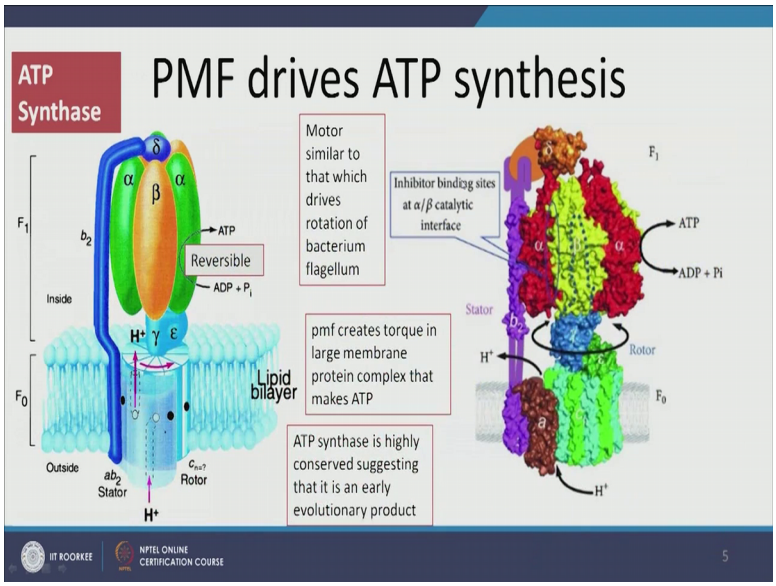

Remember in the previous lecture I mentioned that ATP adenosine triphosphate is a molecule that cell uses as it is energy currency. So, whenever energy is required ATP will come it will convert into ADP. I get phosphorylated give away energy. Whenever there is excess energy that needs to be tapped in ADP will go convert into ATP and store energy. Thus, ATP is the currency of energy in the cell. Now how is ATP produced? ATP is produced by using this proton motive force. Now this proton motive force is a member on the cellular membrane. So, if this is our outside of the cell, and this is the cytoplasmic interior of the cell, then here we have protons. And here we have negative charged assembled.

Now, this is the actual protein looks something like this. But the picture on the left panel is more easy to understand and it explains the biophysics behind ATP synthesis. And this by the way this assembly is referred to as ATP synthase, it is a very complex proton protein with beautiful multiple domains and each of them act together to form one of the smallest motor in life in biology. Now the way it works is this motor works very similar to how bacterial flagellum works and how bacteria couples itself.

So, basically the proton motive force which is positive charge on the outside negative charge on the inside creates a torque in this motor. So, torque is created because protons from outside our channeling in and they are go here, but there is a potential difference created. So, there it rotates and it creates a torque. Now this torque will drive the

formation of ATPs and ATP. And in case of bacterial flagellum this similar torque drives the movement of bacteria.

So now, here is another important thing that this ATP synthase is common in all life forms. So, most all ATP synthesis are very similar they have similar amino acid sequences similar structure. All primary secondary co ordinary tertiary, all kind of structures are similar. Thus, implying that very early in evolution ATP synthesis had been developed. And evolutionary process decided that this is the best way to form ATP.

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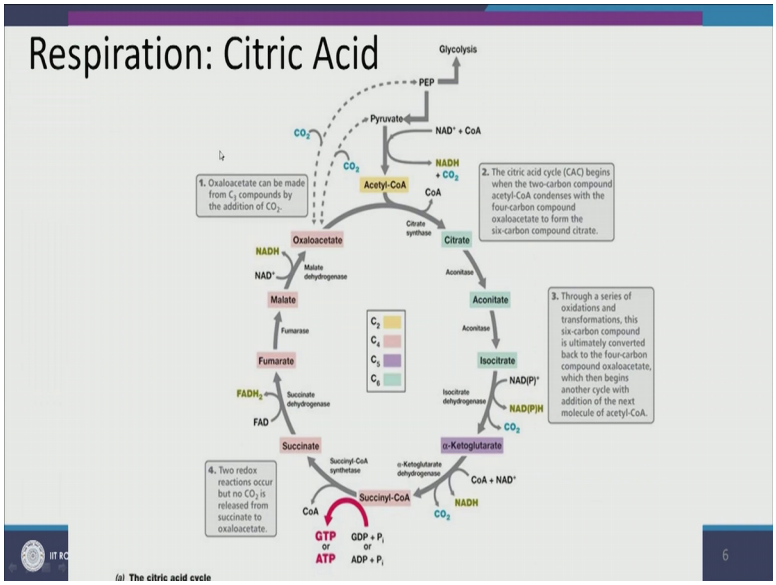

So now coming back to a respiration; so remember we talked about fermentation and respiration.

So, fermentation is what we covered earlier where the substrate is directly phosphorylated, but in respiration all this has to happen. Somehow, we have to make sure that we increase the PMF of cellular membrane which can be converted into ATP. So, if you remember from the last time we talked about how glycolysis will ensure that glucose gets broken down into pyruvate. And we mentioned about how many ATPs are generated, how many NADH are consumed and released.

Now, what happens to this pyruvate? It is not the end product of glycolysis, which is degradation of glucose to produce energy. Now pyruvate undergoes a very famous cycle the citric acid cycle. And in this cycle pyruvate produces energy undergoes many

chemical reaction. And finally, it gives back, it allows the it leaves this cycle leave allowing it to continue forever. So, let us look at this. Pyruvate is oxidized by NAD plus and coenzyme A and is converted into acetyl coenzyme A. Which is a 2 carbon molecule, and this 2 carbon molecule is how the citric acid cycle gets triggered.

So, once the citric acid cycle has begun acetyl COA will can turn into citrate will turn into aconitate. Now notice here this is a 2 carbon molecule. And it releases coenzyme A and form citrate which is a 6 mole carbon molecules molecule. Sorry, 6 carbon molecule. So, notice so, some smaller molecule you are making a bigger one. And then it again breaks down into aconitate isocitrate of the chemical reactions under in presence of specialized enzymes and then after this it is finally, broken down into 5 carbon alpha ketoglutarate.

Now, in this process converting from citrate to alpha ketoglutarate it has consumed one a NAD, and it has released carbon dioxide. Now alpha ketoglutarate which is a 5-carbon molecule converts into a 4-carbon molecule succinyl coenzyme A, by again releasing NADH. Now this succinyl breaks down to succinate to fumarate to malate and to oxaloacetate. And in this process, it releases the ATP fda h 2 and NADH which are all energy rich molecules. Now this oxaloacetate what it does it is what is required for the citric acid cycle to continue. And thus, oxaloacetate except acetyl COA from another pyruvate molecule and the same cycle continue that is why it is referred to a citric acid cycle.

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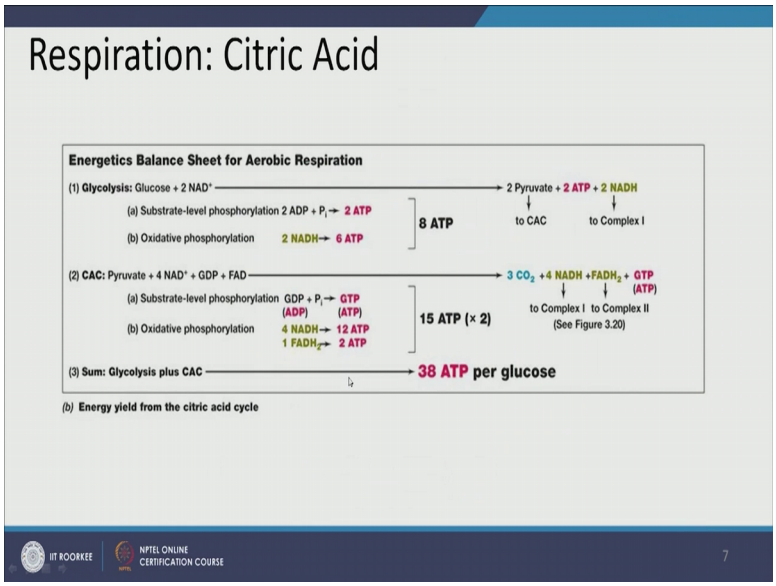

Now let us look at the energetic balance sheet for the citric acid cycle first we have glycolysis there are 2 possibilities we can have substrate level phosphorylation within a oxidative phosphorylation. So, this is fermentation and this is respiration. Now in either case we generate some ATP's. Now this and we also generate pyruvate. So, glucose plus 2 NAD plus, we will get 2 pyruvate 2 ATP and 2 NADH already. Now what happens to this pyruvate? We mentioned earlier it undergoes this beautiful citric acid cycle. Now the in citric acid cycle it consumes 4 NAD plus 1 gdp one fad all energy demanding molecules and once they reduce they make energy rich molecules.

So, and it pyruvate breaks down into carbon dioxide, which is the highest oxidized form of carbon possible. And notice here that if we are undergoing through a substrate level phosphorylation which is fermentation. Or oxidative phosphorylation, we generate certain ATPs and GTP's and other energy rich molecules. Overall, we notice that glycolysis plus citric acid cycle we can make 38 ATP per glucose which is a pretty good deal for microbes.

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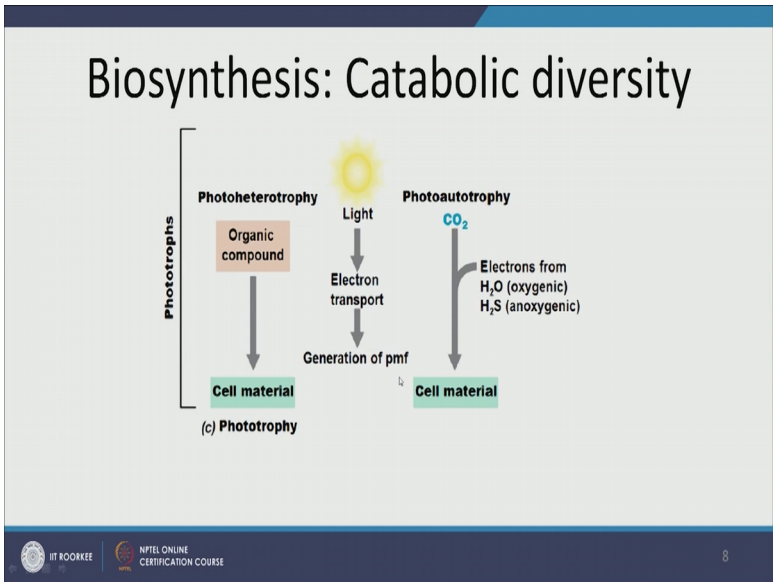

Now, we know how glucose converts into ATP. And we have mentioned briefly about the different kinds of energy sources that are used by different kinds of microbes, and also different sources of carbon.

So, let us go through this briefly and see where in our understanding of a energy being transferred within the cell from a chemical reaction and being consumed for driving biochemical processes, this understanding lies. So, first we talk about photoautotrophs and photoheterotrophs. So, so energy source is light. And if the source of carbon is carbon dioxide we call them photoautotrophs and we will go in little bit more detail in this and other lectures. They use electrons and water and what are the nutrients they require from environment, and then they make cell material. And then again energy is light, but organic compounds service source of carbon that is why it is called photoheterotroph.

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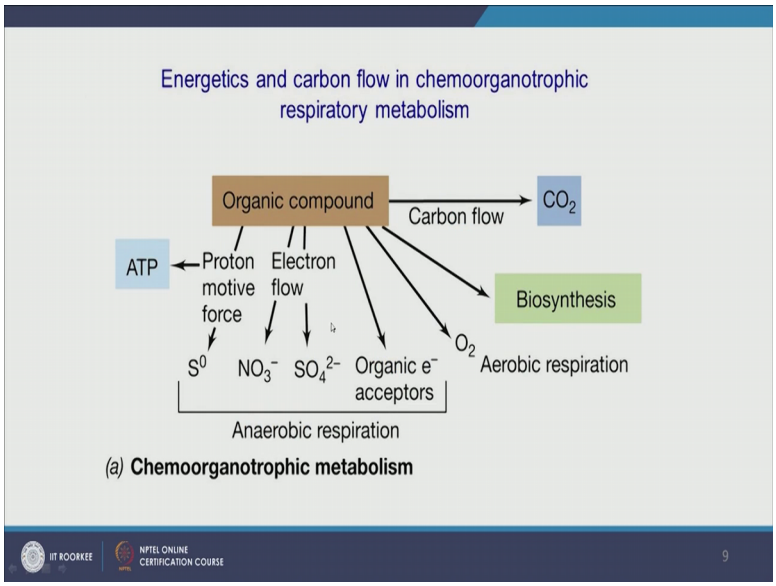

And then we have chemoorganotroph. So, in chemoorganotrophs what they do is they use organic material to allow to go to carbon cycle and maybe even used as a carbon source may not be. But definitely organic compound drives the energy reactions and the need of energy. So, organic compounds creates the motive proton motive force, which will either reduce different ah inorganic electron or organic electron acceptors and make ATP. And remember how will PMF make ATP; by ATP synthase which we studied just few minutes ago.

Now, this organic compound with service electron donor and this will help reduce many unreduced organic or inorganic electron acceptor. Overall this is referred to as an aerobic respiration, because we are not talking about oxygen. If you are talking about oxygen then this is aerobic respiration.

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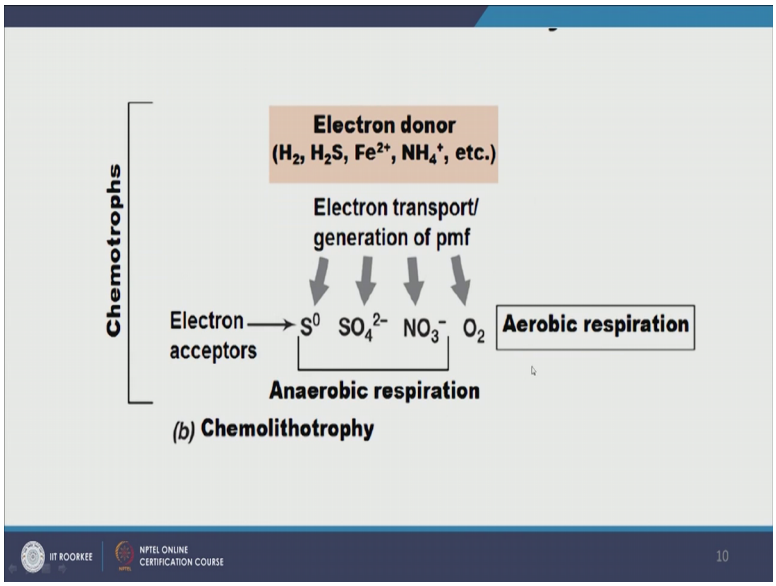

Now, let us look at chemolithotroph. In chemolithotroph the electron the different compounds they serve as source of energy.

So, we can have electron acceptors, such as sulfur, sulfide, nitrate, oxygen and in case of oxygen it is aerobic respiration, other cases it is anaerobic respiration.

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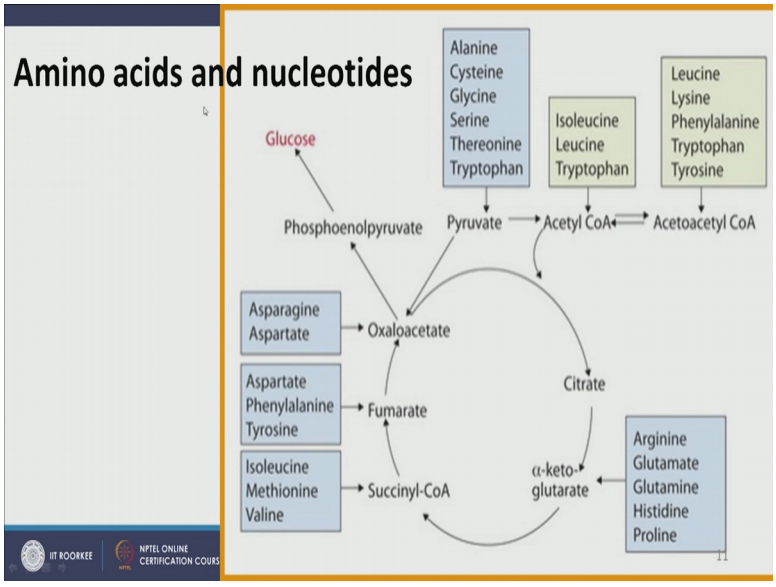

So, not only so, as we saw here the electron donation and carbon these are 2 different parallel reasons for classifying microbes into different types such as chemolithotrophs or

chemoorganotrophs. And they use energy for either producing biomass or for driving their a biochemical reaction.

Now, the same energy and carbon can also be used for generating or synthesizing other important chemicals that life requires such as amino acids and nucleotides and if you remember nucleotides that are important for life in terms of genetic material atgc or adenine, thiamine, guanine and cytosine, and there are in if it is RNA then we have uracil instead of thymine. And in amino acids we have nearly 20 essential amino acids and some other related amino acids.

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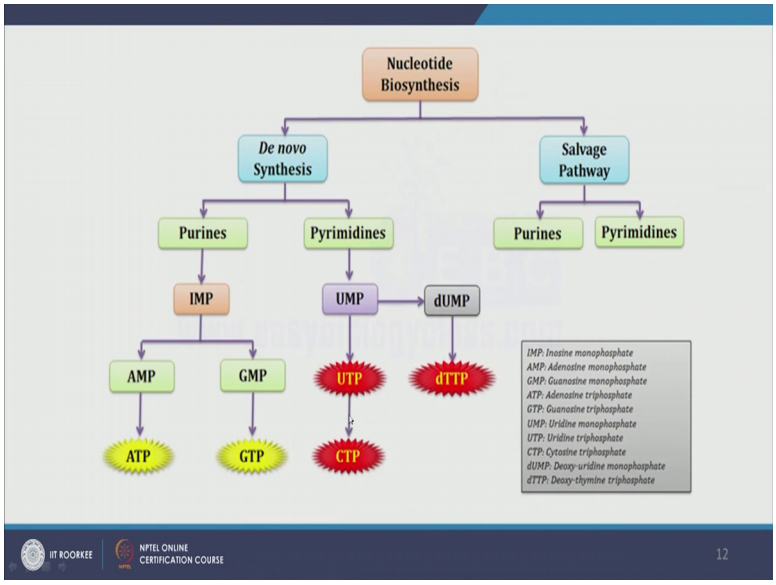

Now, these amino acids have to be synthesized within the cell. So, how we will cell use glucose? How we will cell use the byproducts of glycolysis to produce these amino acids? Now here is a list of different amino acids and this diagram suggest how we know the different parts of our citric acid cycle will give us daughter not daughter parent products for production of these amino acids. Now let us look at nucleotide biosynthesis which we are just mentioning. There are 2 ways they can be made De novo synthesis or salvage pathway.

The difference between De novo and salvage pathway is this. The De novo synthesis is when the microbes use essential organic compounds like very basic organic compounds to build what they require like purines and pyrimidines. Salvage pathway is when they salvaged the organic compounds of dead and utilized purine purines and pyrimidines. So,

let us say we have atgc that have been used and that have had some chemical degradation or some modification, and they are no longer required. So, the cell starts breaking them up.

Now, when the cell has started breaking them up, the salvage pathway what it can do is it can pick up these broken purines and pyrimidines and make them a new. And thus, this salvage pathway is a recycling pathway of the cell, De novo synthesis is using the essential organic compounds that are required for making purines and pyrimidines and then making them out now.

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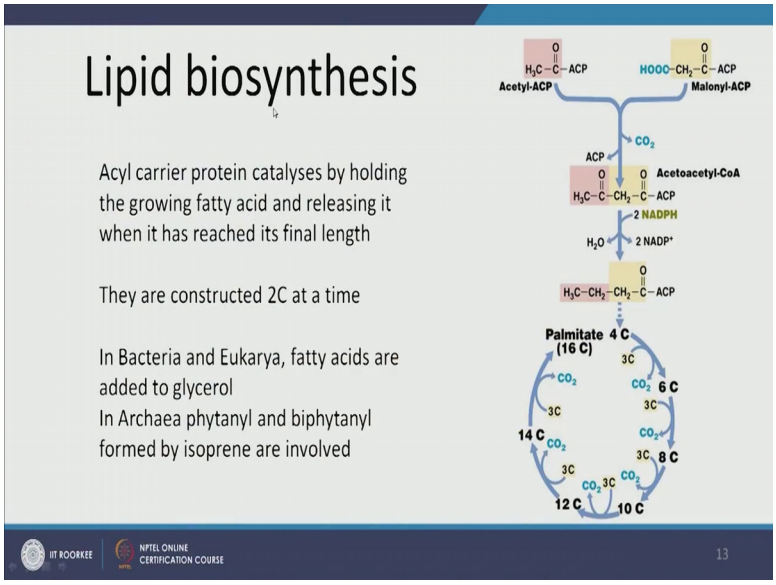

Let us look at lipid biosynthesis. And this is one of the this is one of the most important and also perhaps the last portion of this biosynthesis and microbial energetic spot that we are going to cover here today.

So, how does cell make fat now there is common mis conception that bacteria do not store fat which is not true they do even eukaryotes of course, we store fat. So, do prokaryotes. Now fat cells are not containers of fat that just float around in the body. In terms in cases of higher order of life, but in fact, they are stored within the cell. So, let us look at how fat is made from the from the essential organic compounds or from the food that microbes consume. I want to mention here that lipid is not only a way of storing energy for using it later. But lipids are also essential part of cellular membrane, many other proteins and parts of the cell.

Thus, when cell makes lipid it is not only to store energy in future, but also to maintain it is integrity and to have healthy proteins in it. So, one of them very important for a catalyst in lipid biosynthesis is ACP or acyl carrier protein; this acyl carrier protein what it does is it holds on to the initial template for making fatty acids for making lipid and then as fatty acids come and join. It keeps on holding it until it has reached the right length and only then it will drop it. So, this is the first step acetyl ACP comes here, malonyl acp. So, ACP is holding acetyl and it is holding malonyl, they will combine one ACP will be released because now malonyl and acetyl groups have joined to each other. And one carbon dioxide it is also released.

And now we have acetoacetyl COA one ACP remains attached, because this has to ensure that the chain continues. And one ACP is released and then in the next step we have converted this into a fatty acid chain. And similarly, it will keep growing keep growing until it makes the it makes a right size of a fat lipid. Now notice here that these fatty acids are joined and they are added by 2 carbon at a time. So, we in so, here we have 2 carbon, now here we have 4 carbon. Next time we will have 6 carbon and so on 8 carbon 10 carbon 12 14 and 16 which is palmitate. In palmitate as some of you might know is also used from making soap, and it is used for many other industrial purposes.

So, this is how microbes will take very essential compounds and make them into long chain fatty acid. Now as you can note that this process where we use smaller compounds to make bigger compounds, it requires lot of energy. So, we are reducing the order when we are making bigger complex compounds, and this energy input in cells is catalyzed by enzymes and thus our energy demand is reduced. Energy barrier is reduced, and even at room temperature microbes can make long fatty acids.

Now, there is an essential difference between fats lipids that are made in bacteria eukarya and in archaea. Now if you remember from the first few lectures we talked about how bacteria and eukaryote they have their cellular membrane, that have glycerol as one component and then fatty acids are attached to it in archaea; however, we had phytanyl and biphytanyl which are found by isoprene. And thus, we know that archaea lipid synthesis will be very different from bacterial and eukaryotic lipid synthesis.

Now, dear students I hope you remember we mentioned how cellular membrane how cellular membrane is essentially a lipid bilayer. And does you know that this is essential

for any cell replication to happen at the even at the beginning. So, today we talked about different processes in respiration. And to recap I want to mention that respiration is the second half of fermentation, where fermentation happens and aerobically mostly respiration happens oxidatively. One of the very key draw point that I want you to take it home is that in respiration it is proton motive force which is our objective.

We want to use the energy that is gained by degrading any organic compound which is the food, and convert it into proton motive force. Now this proton motive force can be tapped by ATP synthase to make ATP and as we have studied earlier, ATP is what microbes use for driving all their biochemical reactions, and all the movements and the work that the cell needs to do at any given time. So, the proton motive force once it has been generated and this solid PMF. Then this can make ATP and it can also be used for transportation if you remember from the previous lecture. Where there are 3 different forms of assisted transportation, and at least 2 of them require PMF to support it to allow things or biomolecules to go outside the cell or come inside the cell.

Now, when we are generating PMF from the cell the food or the energy that we have gained from food, lots of hydrogen iron and OH minus ions are produced. Now one of the obvious question that you must ask is; why do these a OH minus ions and H plus ions do not just diffuse across the cellular membrane and neutralize the PMF I mean; obviously, if you are having a capacitor with it has to have some separation or some insulation that does not allow these electrons or protons to move across the membrane. And interestingly because they are polar, just because they are polar they will they are not allowed to move around the cellular membrane.

And this brings us back to transportation lecture, where we talked about transportation across cellular membrane. And I encourage you to go ahead and review this lecture and notice not only how transportation happens, but also how bacterial and eukaryotic membrane are share some similarities, versus how archaea is very different in terms of cellular membrane which gives another important insight that you must remember, that respiration process which is developing PMF using degradation energy from degrading degraded electron donors and energy donors, would be different for bacteria eukarya and for archaea. Because archaea their cellular membrane structure is very different they use different compounds for example, fraternal bi fraternal.

So, all of these things that we studied here this the respiration and developing the proton motive force would be very different in archaea. Because they would not have complex one complex 2 complex 3 and complex 4 and just because they do not have fatty acids linked to glycerol and instead they have fraternal and bi fraternal. So, in your homework for this week you will notice that I have given you questions regarding archaea cellular membrane, how and the question asked you to calculate how many NADH are consumed and how many are utilized.

And also, to explain what is what are the differences between bacteria and archaea respiration. This is all for this lecture. And in the next lecture we will be talking about different kinds of we will be actually continuing this topic and talking about how different kinds of trophies, or different kinds of consumption of food processors exist in our earth and how they drive our nutrient cycle across the globe. So, that is all for today.

Thank you.