Environmental Chemistry and Microbiology
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Module - 8
Lecture - 39
Cell Biology - II

Welcome everyone. We will now start with lecture 39 of module 8. This is the second part of Cell Biology.

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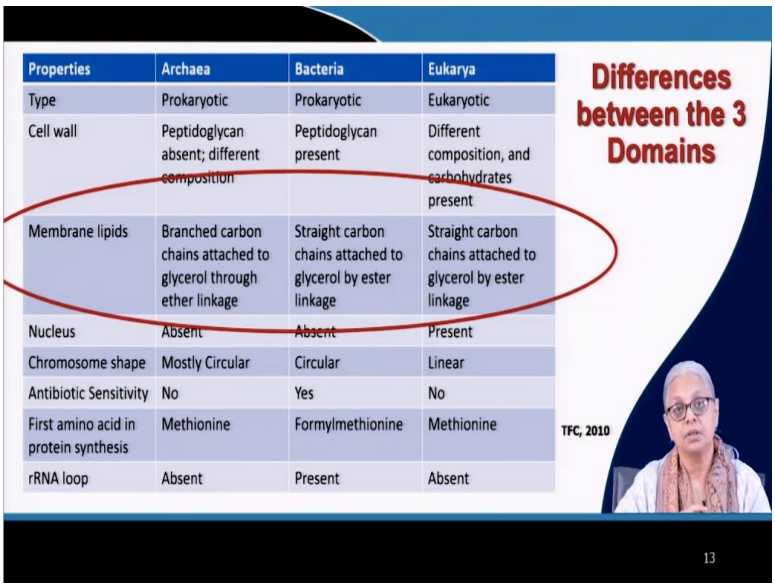

Properties	Archaea	Bacteria	Eukarya
Type	Prokaryotic	Prokaryotic	Eukaryotic
Cell wall	Peptidoglycan absent; different composition	Peptidoglycan present	Different composition, and carbohydrates present
Membrane lipids	Branched carbon chains attached to glycerol through ether linkage	Straight carbon chains attached to glycerol by ester linkage	Straight carbon chains attached to glycerol by ester linkage
Nucleus	Absent	Absent	Present
Chromosome shape	Mostly Circular	Circular	Linear
Antibiotic Sensitivity	No	Yes	No
First amino acid in protein synthesis	Methionine	Formylmethionine	Methionine
rRNA loop	Absent	Present	Absent

So, as I said, the world is divided these days into 3 domains: archaebacteria, bacteria, and eukarya. So, if we were to focus on just the membrane lipids; people have seen biomarkers, people have observed that there are biomarkers that determine the differences between these 3 domains. So, archaebacteria have branched carbon chains attached to the glycerol, through an ether linkage. So, I already mentioned to you that ester versus ether is a biomarker that separates bacteria and eukaryotes on one side; and archaebacteria, the more primitive organisms on the other side. So, this is one biomarker. And look at the similarity, what do Bacteria and eukaryotes have in common; the more modern bacteria and the eukaryotes have something in common, and that is straight carbon chains attached to the glycerol by ester linkages. So, what I showed you in the previous slides is exactly that.

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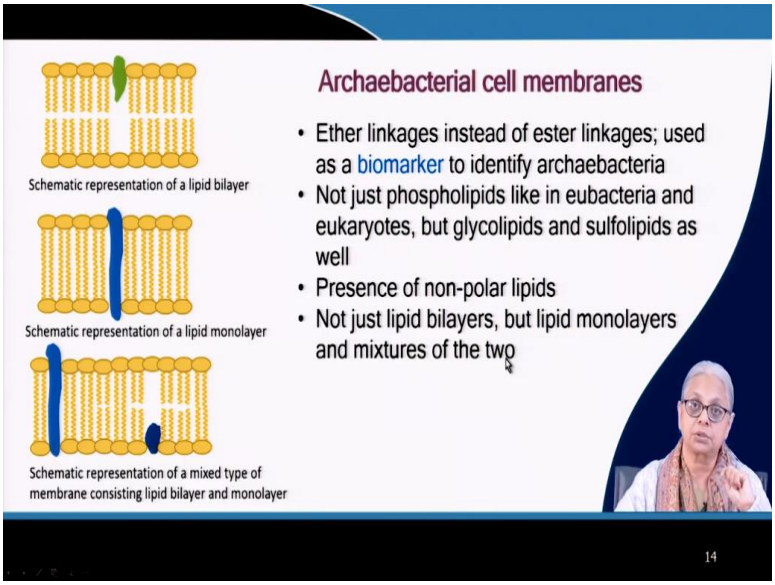

Then we come to archaebacterial membranes. And you can see, there are significant differences. So, the modern bacteria, or what is just called bacteria, have a bilayer. And the bilayer is formed out of phospholipids only. And the archaebacterial cell membrane may be formed out of a bilayer of lipids. This is one possibility but they also have something very interesting. They have lipid monolayers. So, if you have a molecule that has hydrophilic heads on both sides and a sort of hydrocarbon chain linking these hydrophilic heads, then that is what you get, a lipid monolayer. So, you see this lipid monolayer. It has got the same structure. The basic structure is more or less the same. But they are not 2 different layers, it is 1 layer.

And then you also have different types of cell membranes, where you have a mix. So, you see monolayers, like this one; and you see bilayers. So, you have a combination of the two. And I think all of these are markers that tell us how life has evolved. You know, it may have started with monolayers and then become bilayers. And it is just speculation for us at this point. But it is a very interesting part of microbiology. And another point that is mentioned over here is that modern bacteria as well as eukaryotes, all have only phospholipids.

And it is the archaebacteria that may have phospholipids, glycolipids, and sulfolipids. So, again we see that whole evolutionary tangent. And then we have the presence of non-polar lipids. And another difference is that you have lipid bilayers, monolayers, and mixtures; which you do not see in modern bacteria.

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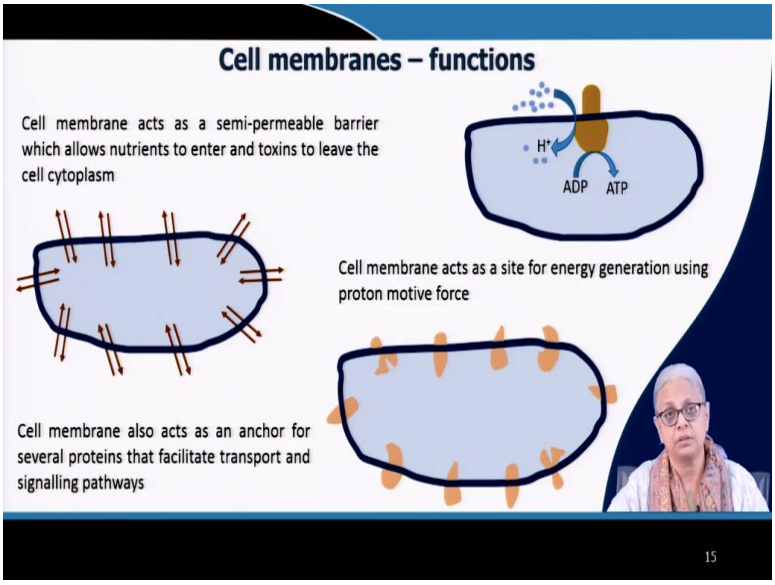

What are the functions of the cell membrane? So, the cell membrane acts as a semi-permeable. I use the word semi-permeable barrier because it is the phospholipid bilayer that would not allow anything to come in and out. It has become completely clear that nothing can pass through literally. And maybe only gases like oxygen and other gases may be the only things that can pass through this phospholipid bilayer.

So, cell membranes are semi-permeable barriers that allow nutrients to enter and toxins to leave the cytoplasm. And they are all mediated; all this is mediated. The transport of all these things, except perhaps the gases, is mediated by proteins. I have already mentioned to you that it is the site for energy generation using the proton motive force. So, we will go into some detail about that in this topic, but in the subsequent lectures.

So, here we have ATP synthase. And this is the site at which ATP is generated and to generate ATP, you need a proton motive force. So, just like your dry cell which has electron motive force; in the bacterial cell, you have what is called a proton motive force. So, the protons inside the cytoplasm are pumped out of the plasma membrane. So, they go into the periplasmic space. And here, you get a higher concentration of protons. And utilizing this proton motive force, that energy is utilized to create ATP. As I said, we will cover that in the next lectures. And finally, the cell membrane is an anchor for several proteins that facilitate the transport and signaling pathways. So, the site, you have already seen that this is ATP synthase, which is another transmembrane protein. And you have several other proteins, which will determine where the water goes in and out; which will determine whether glucose goes in and out; what is the form in which glucose will go in and out. So, all these things are transported in and out of the membrane by specific proteins. So, for all practical considerations, you may say that the

plasma membrane is either completely impermeable; or at the least, it is semi-permeable; it is not a very permeable layer.

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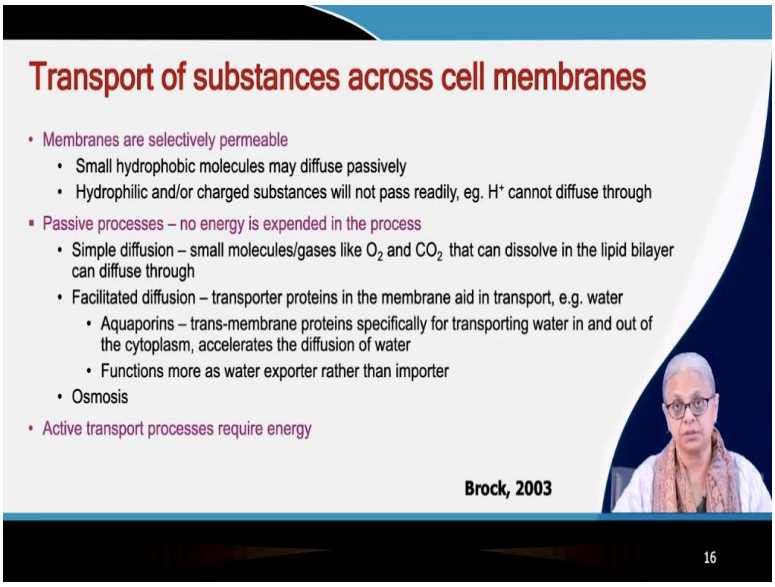

So, let us come to the next thing. How does any substance go through that cell membrane? As I said, these membranes; the better word for describing the nature of these membranes is, selectively permeable. Because whether it is passive transport or active transport, they are very selective in allowing different substances to pass in or out. The textbook that I am referring to mentions that small hydrophobic molecules may pass passively in and out of the membrane.

And I will show you some examples in a little bit. You can also have hydrophilic or charged substances which will not pass readily. Now, remember that we are dealing with an oily layer. This oily layer is a non-polar solvent. It will not allow anything like an ion to pass through; even a proton cannot diffuse through. So, that is how impermeable it is. So, it is not just the size of the molecule that will determine whether it goes in or out. It is also the charge that will determine whether it goes in or out. So, hydrophilic or charged substances cannot pass in and out of the cell through the membrane.

There are 2 types of transport processes. We have passive transport and active transport. In passive transport, no energy is utilized by the cell. So, there are 3 types of passive processes that we are going to look at.

One is simple diffusion; the second is facilitated diffusion, and the third is osmosis. So, we will take a look at each one of them. And the last one is active processes which require energy. So, active means, the cell has to utilize energy for pushing things through, in, or out. And passive

means, no energy is utilized. So, let us take a look at the passive processes first. And then we will take a look at active processes.

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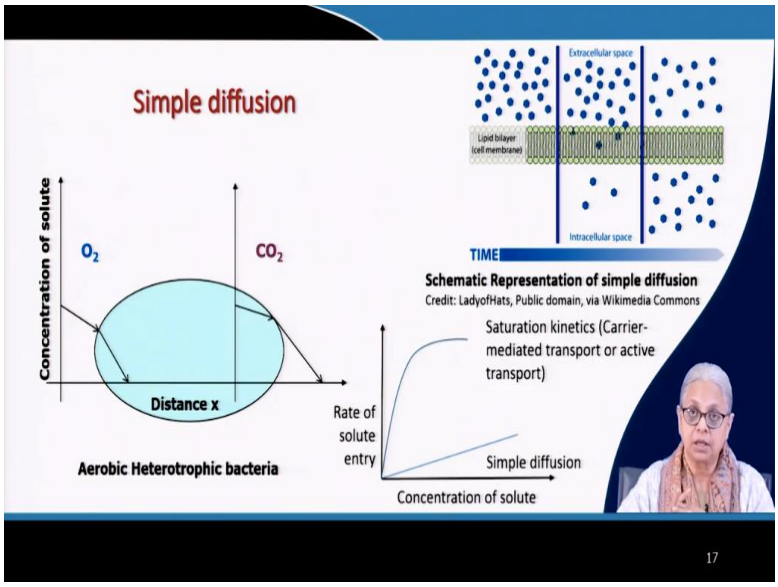

So, here we have simple diffusion. Let us take a look at this schematic that describes simple diffusion. So, you have your phospholipid bilayer. And let us assume it has some substrate, some nutrients, some food that is there in the environment, which needs to be brought into the cell. So, over time what will happen? This substance, if it is perhaps a gas, like either oxygen or CO_2; we know that water cannot do this; the only possibilities these days seem to gases. So, it is either oxygen or CO_2. These gases can perhaps, in response to a concentration gradient, pass through the plasma membrane. And I will come to this graphic next. And eventually, when the concentration is equal on both sides of the bilayer, at that point, you get this dynamic equilibrium between the inside and the outside. So, that is the simplest one. So, let us see how passive diffusion would work.

Simple diffusion; how will that work? We know that hydrophilic molecules, charged molecules; no chance for them to get in and out, even by diffusion; not possible. For simple diffusion to happen, we know that gases like oxygen and CO_2 are capable of diffusing in and out of the membrane without the organism spending any energy in doing this. So, how does it work?

Let us take our normal aerobic heterotrophic bacteria. Our normal aerobic, the one that we see everywhere around us; it is in the water; it is in the soil; it is everywhere; inside us, outside us; they are all aerobic heterotrophic bacteria. What are they doing? They need to take up oxygen and they need to throw out CO_2. CO_2 is the end product of this process. So, let us say they are utilizing organic matter. We use the example of glucose. Glucose is just the simplest organic

compound that aerobic heterotrophic bacteria can use. It can be any other organic molecule. So, let us say glucose plus oxygen is what is required by the cell. Glucose cannot be transported into the cell without some amount of energy expenditure. We know that the oxygen level in the water is generally higher because the bacteria is utilizing oxygen; so, the oxygen inside the cytoplasm is going to be less than the oxygen in the water. So, the dissolved oxygen (DO) in the water is a source of oxygen for the cell. So, as long as this concentration outside is higher than the concentration inside, Fick's law will be the operational principle. So, it will diffuse into the cell.

What is the end product? The end product is carbon dioxide. Carbon dioxide concentration inside the cell is building up because that is the nature of the metabolic process. So, the carbon dioxide concentration inside the cell is higher than the carbon dioxide concentration in the water. As long as that is true, the carbon dioxide from the cell will diffuse out of the cell into the water. And so, this is simple diffusion.

And we know that perhaps this is true for gases. We know that it is not true for dissolved compounds. Then let us come to another part of what I was talking about. Let us take our simple example of glucose plus oxygen going to CO2 and water. Now, if that is the case, then, along with oxygen, the cell requires this organic compound, whether it is glucose, acetate, any other compound. Why cannot that be diffusing in?

First is; I have already mentioned that something like glucose is hydrophilic. It is not hydrophobic. And it will not pass through the plasma bilayer, through the phospholipid bilayer on its own; simple diffusion will not happen. So, you have protein-mediated transport. We would not call it diffusion, because it is no longer diffusion. Diffusion is a process that happens in response to a concentration gradient.

So, we are not going to look at diffusion. We are now looking at what is called protein-mediated transport. And when you have carrier-mediated; carrier meaning proteins, the transmembrane proteins are the carriers; and these carriers mediated transport or active transport will allow a much higher rate of solute entry. So, the solute which may be glucose or any other organic molecule will have a very high rate of solute entry.

So, this is the graph that you are likely to see. And this is saturation kinetics. So, those of you who know what saturation kinetics are, you know that there is a limitation. And the limitation is the concentration of the carriers. How many carrier proteins are there in the cell? And how much load can they take literally in terms of the organic compounds? How much of the organic compound can they transfer as quickly as possible into the cell?

So, that is carrier-mediated transport or active transport. And you can see how high the rate is compared to simple diffusion. Simple diffusion has a very slow and low rate. The carrier-mediated transport or active transport; yes, you have to utilize energy for that. But you also get a much higher rate of solute entry. So, now we come to the second point and that is facilitated diffusion. Now, what has facilitated diffusion?

It is still diffusion driven by a concentration gradient. But in this case, there are transporter proteins in the membrane that help in the process of transport. So, we today know that there are aquaporins. There are aquaporins which are transmembrane proteins, specifically for transporting water in and out of the cytoplasm. It accelerates the diffusion of water. Remember that water is not going to diffuse through this hydrophobic layer of the plasma membrane. So, these proteins, the aquaporins are shown over here.

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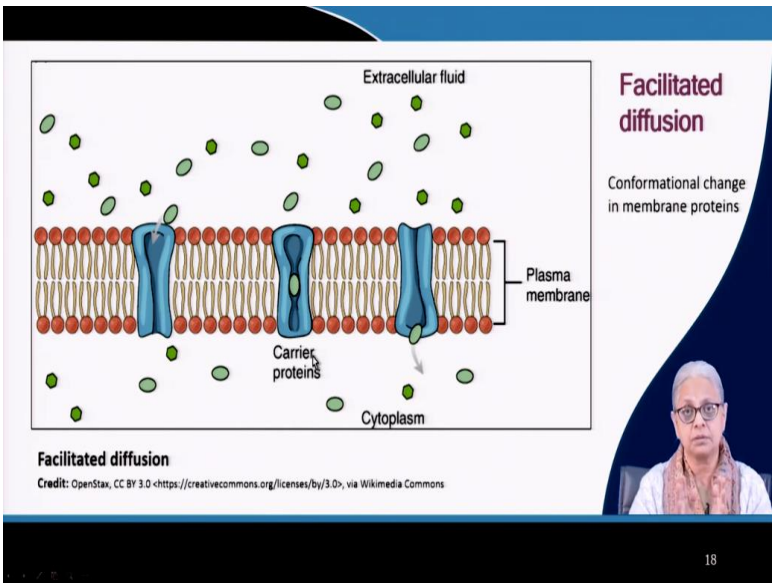

So, here you have these transmembrane proteins. You can see one of them. And any material, whether it is water or any other kind of substance, will come in. There will be a conformational change. The shape of the protein will change. So, it will open up on the side that the substrate is; and then it will slowly push the substrate into the cell or the cytoplasm.

So, you have the cytoplasm at the lower end; and you have the extracellular fluid on the other side. So, these are carrier proteins that are pushing through. It is still based on concentration gradient, and they are pushing through by changing the shape of the protein.

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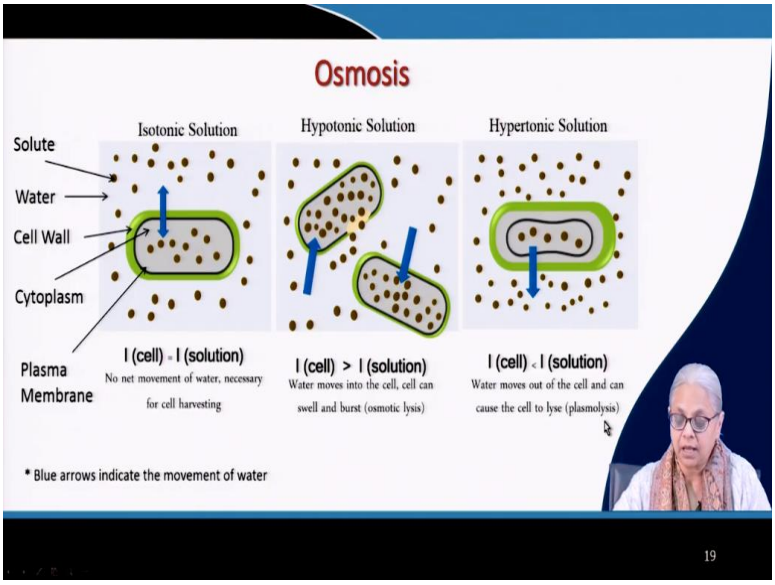

We then come to the next process, which is again a passive process; no energy is utilized by the cell, and it is called osmosis. Now, you are all familiar with osmosis. You have learned it way back in your high school. And it is of enormous importance in determining, especially the transport of water in and out of the cell. So, here we have our cell. And I can tell you from experience again, that when you are harvesting cells, you need to make sure that you harvest and transfer them to a media that is at the same ionic strength.

So, over here, I stands for ionic strength. Ionic strength inside the cell should be equal to the ionic strength of the solution. If they are not equal; a little bit here and there is okay. But if they are not equal, what will happen is, you will get the other 2 situations. You will have a situation where the ionic strength may be higher than the ionic strength of the solution; or you may have a situation where the ionic strength of the cell, the cytoplasmic solution, or the cytosol is less than the ionic strength of the solution.

So, we will take a look at all 3 scenarios. So, as I said, if I am harvesting cells and I am transferring them from one media to a fresh media, I have to ensure that the ionic strength remains the same. Otherwise, you will get the transfer of either nutrients or water in and out of the cell. And you can cause damage to the cells if you do not take care of that. So, this kind of solution or scenario is called an isotonic solution. So, it has enormous implications for doing microbiological work; enormous implications for when you are trying to culture bacterial cells. So, you have your solute molecules, which are in the solution. And they are being either taken up or released into solution. And this is your cell wall, the water, and so on. So, there is no net movement of water in or out of the cell.

Now, let us take a situation where the ionic strength inside the cell is much greater than the ionic strength outside the cell. So, based on the gradient, the concentration of water in the

solution is much greater than the concentration of water in the cell. So, water will push into the cell and the cell will swell and burst. And those of you who are familiar with raisins, kishmish; this is the word we use in Hindi. So, you know that what happens. These are dehydrated grapes. So, the last one is what you get when you have dehydration. You have the dehydration of the grape. But then, when you put it in water; if you imagine that the grape is a cell; when you put it in water, it absorbs water, because there is very little water inside the grape. So, it is the same principle over here. It will absorb water and it will continue to absorb water to the point that it may swell and burst. So, you may get osmotic lysis, which does not happen with raisins.

So, that is one possibility. So, when we are doing cell harvesting, when we are doing cell cultures, we have to make sure that we have this situation (isotonic solution) and not the other 2. So, this is called a hypotonic solution. Then you can have a hypertonic solution, where the ionic strength of the solution is greater than the ionic strength of the cell. So, in this case, water inside the cell will move out and it will cause the cell to lyse. And that is called plasmolysis.

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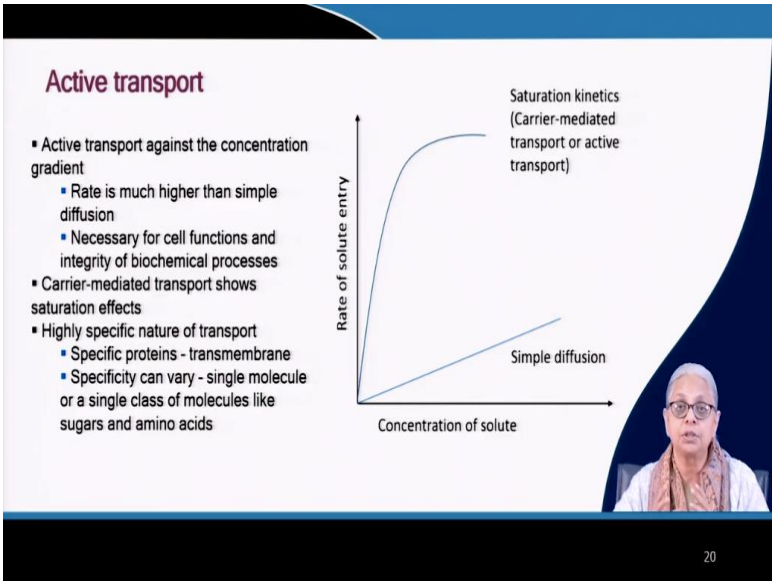

Then we come to active transport.

So, here we have active transport. I have already mentioned that saturation kinetics are much faster, but there is a limit. And in simple diffusion, the limit is only when equilibrium is reached. So, just like is shown over here, unless the 2 sides are equal in terms of concentration, there will continue to be diffusion of the solute. Active transport happens against the concentration gradient, which cannot happen for diffusion.

So, passive transport is based on diffusion or osmosis; and that can never happen against the concentration gradient. The rate is much higher than simple diffusion in the case of active transport, and it is necessary. You will find in all subsequent examples, that cell functions and the integrity of most biochemical processes are based on active transport. So, carrier-mediated transport shows saturation effects.

And then you have a highly specific nature of the transport. You have specific proteins; these may be transmembrane proteins. And, a big point over here is, that each type of molecule or group of molecules has to have specific proteins. You cannot have one protein doing all the work. So, the cell has any number of different types of proteins which will mediate the transport of different groups of molecules or even single molecules like water.

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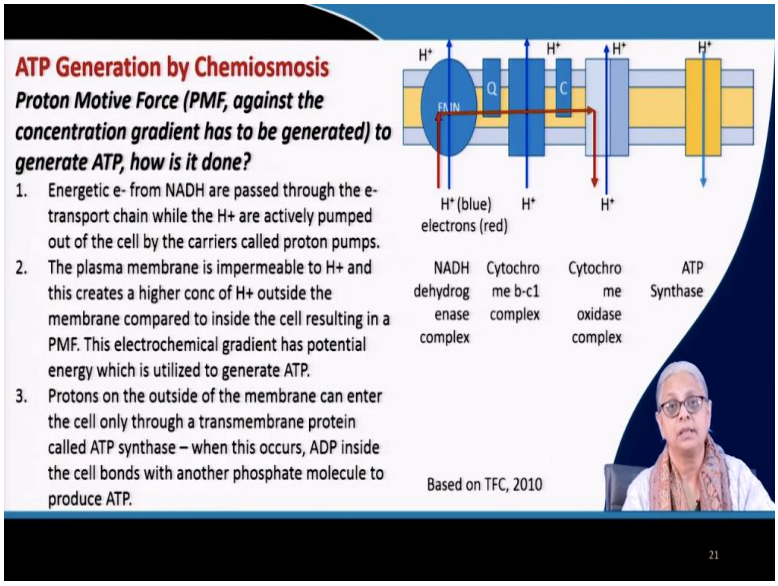

So, now we come to the next point; and that is ATP generation by chemiosmosis. So, how does the cell generate ATP? So, from our point of view, we just want to know how is ATP generation happening. So, this is a very simplistic way of doing things. So, we have a proton motive force. Now, we know that proton motive force has to be generated. And then, that force will be dissipated to get energy for the cell to store. So, the first thing is to generate a proton motive force. And the second thing is to generate ATP, utilizing that proton motive force. So, we have a fairly complex set of proteins, which are part of the electron transport chain. And if you are wondering what the electron transport chain is, we are way ahead of that. And when we come to that, you will be able to understand how complex all these life processes are.

But let us start with a simple introduction to how proton motive force and ATP are generated. And when we come to the electron transport chain, we will go into all the details. So, for simplicity, we have 3 complexes and 1 ATP synthase. So, we have NAD dehydrogenase complex; we have cytochrome b-c1 complex, and we have cytochrome oxidase complex. And finally, we have ATP synthase. So, these are the protein groups. That is why they are called complex. Because, they are not a single protein, there are several proteins attached. And they are the ones that are going to mediate the transfer of both electrons as well as protons.

So, NADH is the starting point. So, the NADH that is going to be transporting both; gives up a proton and an electron; and both of them are transported. So, the red lines show you the transport of electrons; and the blue lines represent protons. And in the first 3 complexes, you get the pumping of protons out of the cytoplasm. So, the bottom part is the cytoplasm and the top part is the outside of the cell. And these complexes, these protein complexes are pumping the protons out of the cytoplasm. So, you can see in all 3 cases, protons are being pumped out. And that will give you a higher concentration of protons at the outside, rather than the inside of the cell.

Where are the electrons? The electrons are together with the protons here. And as the protons are being pumped out, the electrons are passing from one complex to the other in the electron transport chain. I am not going to go into any details; we will come to them later. They are being passed from one complex to the other. And eventually, for let us say aerobic heterotrophic bacteria, which have an organic compound as a substrate and oxygen being converted to water. In that case, water inside the cytoplasm will pick up the electrons. And that is the final terminal electron acceptor. So, we have our oxygen which is the terminal electron acceptor. It will pick up all these electrons and be converted to water. And in the final process, we have ATP synthase. The proton motive force has been created because of the concentration gradient of protons outside the cell (membrane) versus inside the cell (membrane).

And like I said, the plasma membrane is not passively permeable. It is impermeable to protons. It has a higher concentration of protons. And the protons on the outside of the membrane will enter the cell only through this ATP synthase. So, this proton motive force will now be dissipated by ATP synthase. And ADP that is already there in the cytoplasm; it can be AMP; it can be ADP; along with phosphate, the molecule will utilize the energy that is being dissipated by the proton motive force.

That will be utilized to generate ATP. So, it sounds very complicated; and it is. But that is what the life process of ATP generation is. It is a very complex process.

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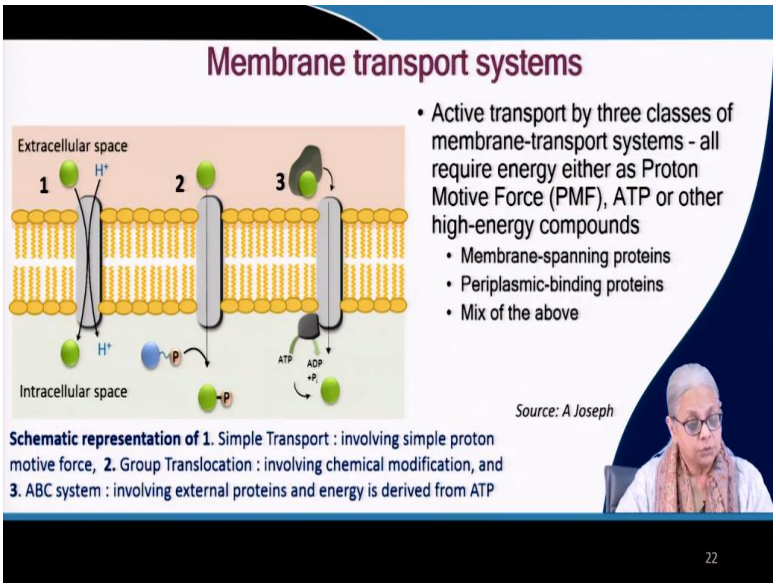

So, we have membrane transport systems. And these are all part of the active transport systems. Here we have 3 classes of proteins. We have proteins which are membrane-spanning proteins. We have periplasmic-binding proteins. So, we have the quinone group, the cytochromes. All these are periplasmic-binding proteins and some of them are a mix of the two. So, some of the transport processes are where you have a mix of membrane-spanning proteins and periplasmic-binding proteins. And you can see this process of ATP generation is a mix of all these processes.

So, the first one is a simple process, simple transport, which involves a simple proton motive force. So, here, you have a compound that has to be brought into the cell. And we already have the proton motive force in place. So, simple transport is defined here as, a proton being brought in along with the substrate. So, that is, along with the dissipation of the proton motive force, you have transport of the substrate.

Then you have group translocation. So, in this case, the best example is glucose 6 phosphates. So, when glucose has to enter the cell, it cannot just freely diffuse into the cell. It comes in; it gets what we call phosphorylated; it is converted to glucose 6 phosphate, and then it comes in. So, that is group translocation.

And then the final one is the ABC system. The ABC system is short for ATP-binding cassette. It involves external proteins. So, there is an external protein here. It binds to the substrate that needs to be brought in. The entire thing does not; the entire complex does not come into the protein or the cell. This extracellular protein will bring the substrate to the transmembrane protein.

It will release the substrate here, for the transmembrane protein to pick it up. And it will bring it into the cell. In the process, ATP inside the cell is being utilized by binding to the substrate. So, this is the ATP-binding cassette.

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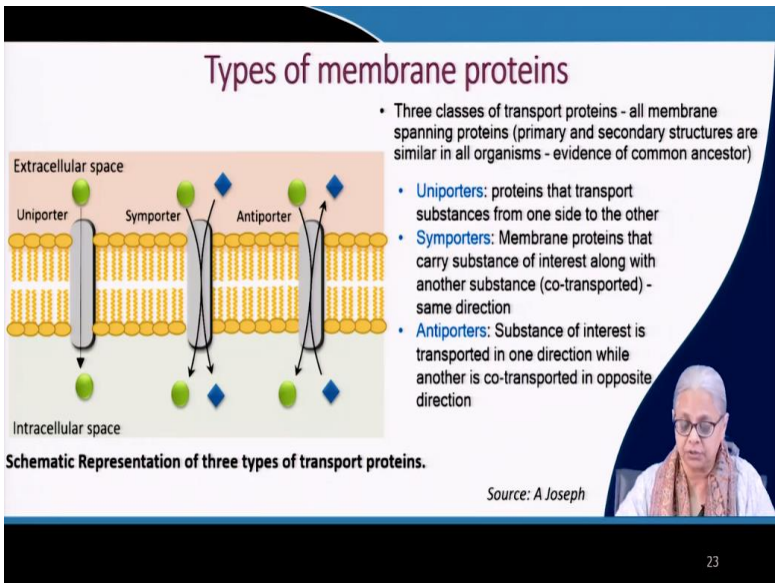

In terms of types of membrane proteins, there are 3 types of membrane proteins: uniporters, symporters, and antiporters. One interesting fact, again part of evolution is that primary and secondary structures of all transport proteins are the same. So, if you remember when we looked at proteins, we looked at primary, secondary, tertiary, and quaternary structures. So, at the primary and secondary levels, all organisms have the same proteins. So, that is what people are saying, is the evidence of a common ancestor for all life forms. So, when you see the abbreviation LUCA; so, the Last Universal Common Ancestor, which is at the root of the phylogenetic tree. And all life forms that existed at any point in time, including today, are all considered to have evolved from that common ancestor.

So, let us take a look at uniporters. Uniporters are proteins that transport substances from one side to another. So, simple transport is mediated by the uniporter protein. Then you have a symporter. So, what I just showed you over here is a symporter. So, simple transport or symporter is the same thing. So, 2 substances are brought into the cell together. I will show

you examples of all of this. And finally, we have the antiporter. Antiporter means, one substance comes into the cell and another substance has to be thrown out of the cell.

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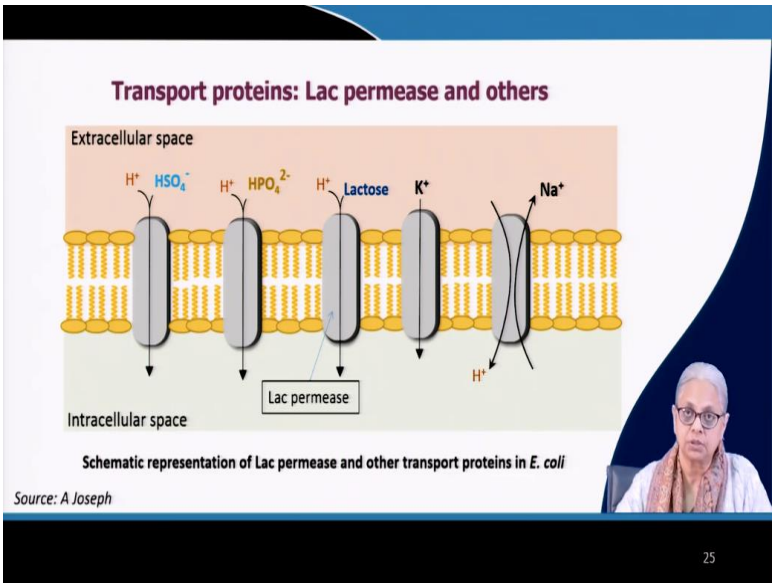

Here is an example of Lac permease. So, here we have several different types of membrane-spanning proteins, antiporters, uniporters, symporters; all of them are there. Lac permease is a protein that exists in *E. coli*. And several other examples are shown in this graphic. So, you have a symporter that is transporting protons as well as sulfate into the cell. Another symporter here, lactose. The Lac permease is also a symporter. So, they are utilizing the proton motive force and bringing the different substrates sulfate, phosphate and lactose in. The uniporter is a protein that is pumping potassium into the cell and the antiporter is sodium that has been pumped out of the cell, while the protons are being brought into the cell. So, again, there is the dissipation of the proton motive force along with the pumping out of sodium.

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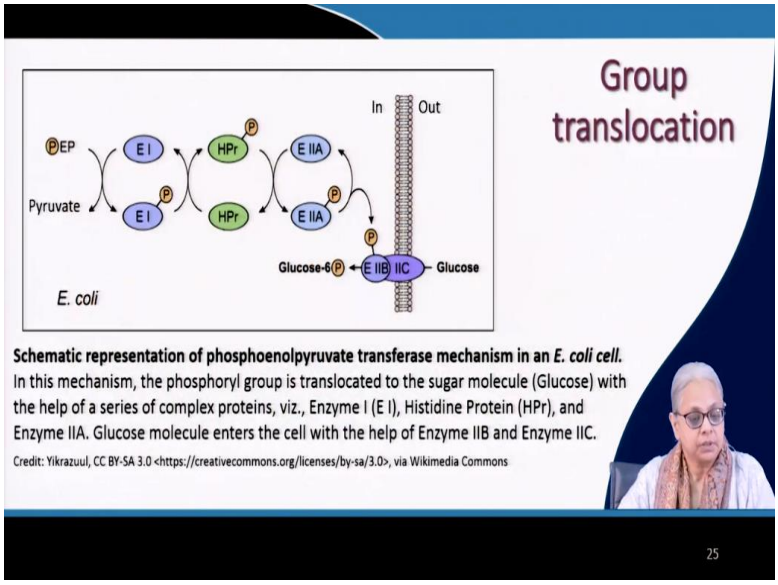

So, then we have group translocation. So, we have phosphoenolpyruvate, which is being converted to pyruvate; and it is mediated by the first complex, the enzyme I complex. And then you have the histidine protein, which will pass the phosphate from I complex to the histidine protein to the second enzyme complex and the; IIA, E IIA, and E IIB, E IIC. All of these are how the phosphate is being transported from one compound to glucose. Remember, when glucose has to come in, it has to be phosphorylated and glucose 6 phosphate has to be generated. And this is how the phosphate is attached to the glucose (molecule) that is then brought into the cell. So, that is group translocation.

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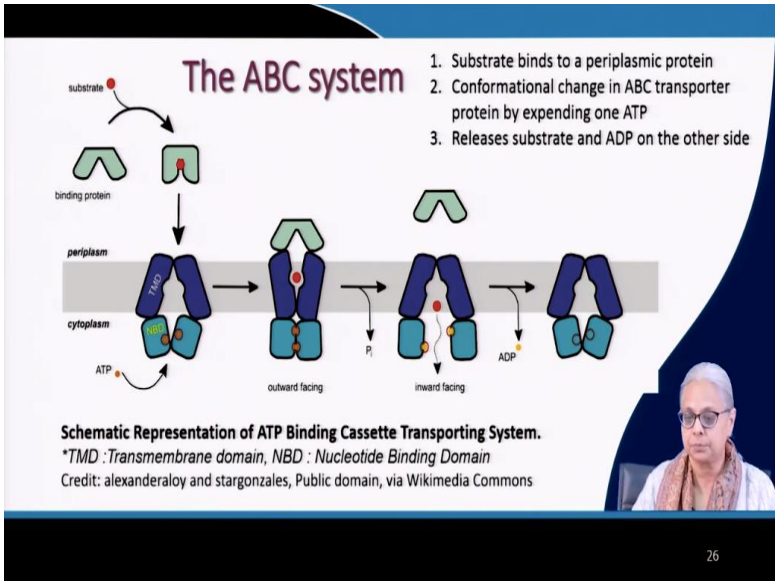

And then we have the last one, which is the ABC system. As I said, it is the ATP-binding cassette. And what it does is, you have a substrate in the periplasmic space that is going to be bound by a particular protein, which is also in the periplasmic space. And this substrate will be released to the transmembrane protein. There will be a conformational change. So, if you can see this, **(Video Starts: 34:11)** so, you have a conformational change.

The substrate is taken in it; in a sense, the gate closes; the substrate is inside the transmembrane protein; the other gate inside the cell is opened; **(Video Ends: 34:26)** the substrate is entering the cell; and the entire protein structure, the entire transmembrane protein goes back to its original state. But what is important in this process is that ATP has to be utilized to change the form of the protein, so that it can allow this substrate to come into the cytoplasm. So, I will stop at this point.

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Thank you.