Metabolic Engineering
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Lecture - 08
Review of Cellular Metabolism - Part C

In today's lecture, we are going to review the cell metabolism with respect to the cellular transport process.

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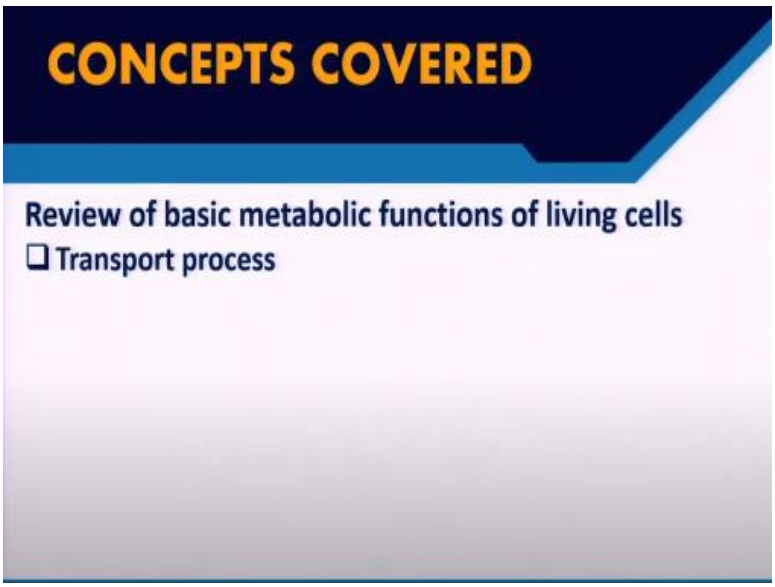

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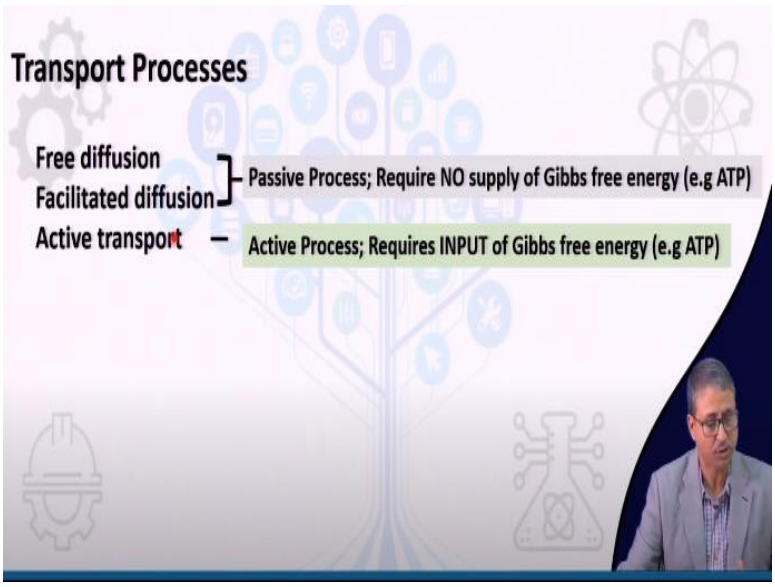

Now cellular transport processes are divided into two main types and subsequently into subtypes. The first one is the free diffusion and this is followed by the facilitated

diffusion and both this free diffusion and facilitated diffusion are considered as passive transport since they do not depend on the supply of free energy like ATP.

And the next one is the active transport which requires input of free energy for example, from the ATP or other energy sources within the cellular systems. So there are certain other types also which are not included in this type of list like they cannot be differentiated between passive or active processes. So we will talk about them in this lecture also.

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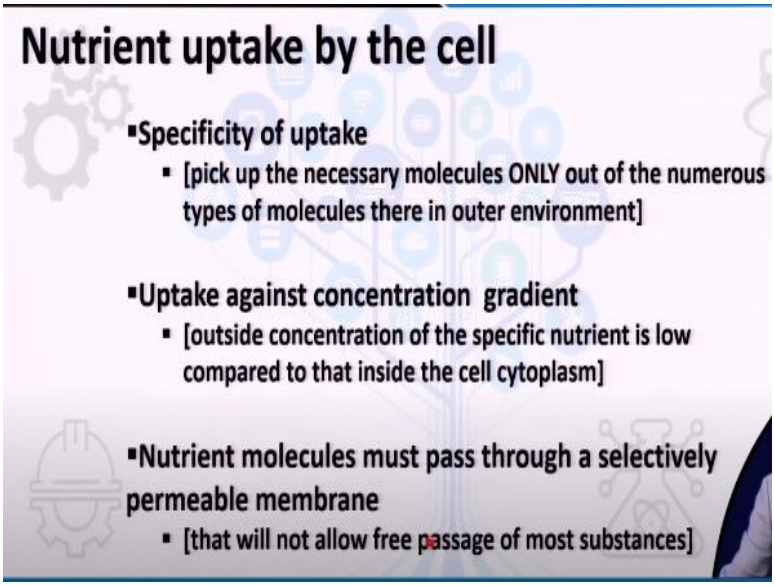

Now nutrient uptake by the cell has certain very specific properties. The first and foremost is the specificity of the uptake process. So most of the cellular uptake or cellular transport processes are developed, so as to pick up the necessary molecule only out of the numerous types of molecules there in the outer environment.

As the cellular system has evolved within the natural environment, where the number of molecules present in the outer world could be very high in terms of both the types of the molecule present and their chemical properties and their requirements. So the cellular transport process has been evolved with a very specific nature of transporting the required molecule only inside the cells and other non required molecules may be excluded from the transport process.

The next one is the uptake against concentration gradient. The concentration of the solute or the molecule which is to be transported inside the cells might be very low

outside and compared to the inside of the cell, yet the cell might be requiring that particular solute molecules.

So solute transport would be against the concentration gradient. And otherwise, if the outside concentration is very high and inside concentration of the solute molecule is low, then the transport might be occurring through the concentration gradient and we will be discussing both this type of through the concentration gradient or the down the concentration gradient transport as well as against the concentration gradient transport as well.

But the main point of focus would be that the transport could be against the concentration gradient because in cellular systems, often many of the required metabolites are required solute molecules, nutrient molecules are present in relatively higher concentration inside the cell compared to outside environment. But still, the transport process allows the cell to move the molecule inside the cell only.

The next one is the characteristic property of the membrane through which the transport occurs. So that is nutrient molecules must pass through a selectively permeable membrane. The membrane could be the cellular membrane, it could be the inner membrane or plasma membrane, or it could be the outer membrane in case of gram negative bacteria. So we generally refer these as membrane permeability.

It is the characteristic property of the cellular membrane that will allow only selected molecules to pass through it. Others will not be allowed to pass through freely. So when we say that the some molecules will be allowed to pass freely and some molecules would not be allowed pass freely, we refer to the selective nature of the membrane.

So for the molecules which are generally not allowed to pass through freely, for them cellular system has evolved certain mechanisms by which such molecules, which are not allowed by the membrane under normal circumstances would be made available inside the cell. So we will be discussing briefly about those processes as well.

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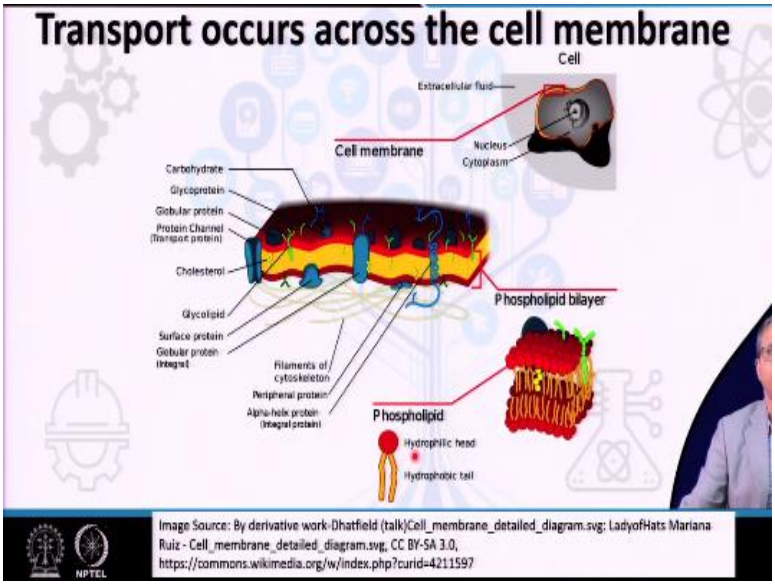

Now here, let us look at the cellular structure particularly the cell membrane structure. This will be essential for us to understand the membrane transport process and cellular acquisition of different metabolites including the nutrients etc. So one of the most important components within the membrane is the phospholipid bilayer.

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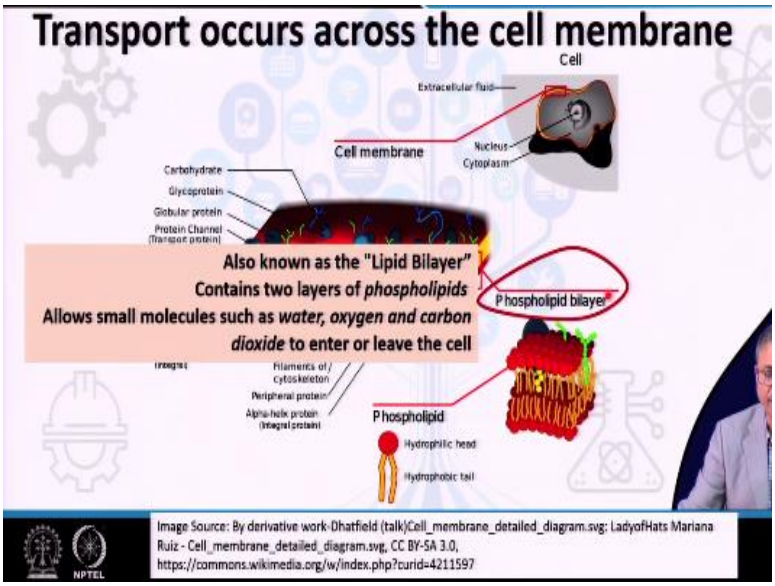

Now this phospholipid bilayer is also referred as a lipid bilayer because, characteristically it is having two layers of the lipid molecules and these two layers of phospholipid molecules because they have a phosphate group attached to it, it provides a kind of both hydrophilic and hydrophobic nature of the membrane structure.

It allows the small molecules such as water, oxygen and carbon, particularly the molecules which are having molecular mass less than 600 Dalton or so to enter or leave the cell easily. Now if we look carefully within this membrane bilayer structure, the two outer surfaces, this surface and the surface over here is considered as hydrophilic because they are having the groups which are like phosphate groups and the glycerol moieties which are available for interaction with the water readily.

Whereas, the internal portion of the membrane which is composed of mainly the lipid molecules which are representing the hydrophobic part of the membrane. So the lipid bilayer structure represents both the hydrophilic part and hydrophobic part.

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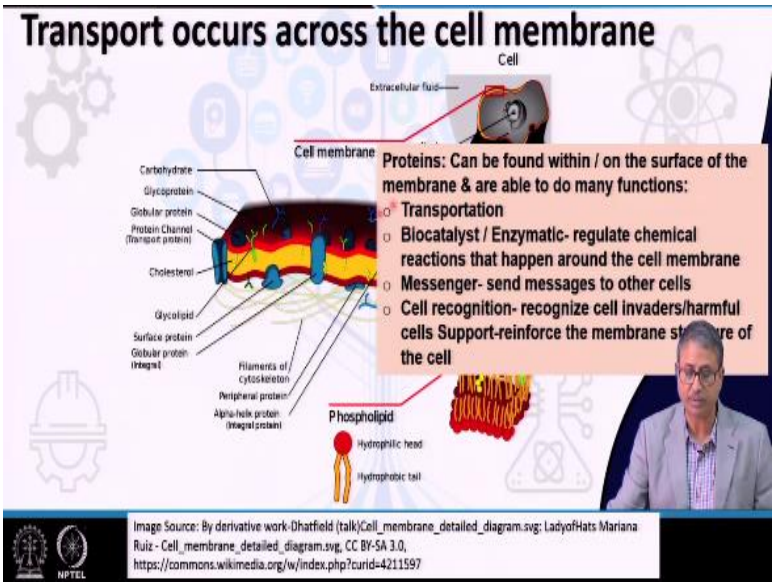

Now in association with the lipid membranes or the bilayer structure, there are protein molecules present within the membrane. These proteins can be found within a surface of the membrane like they may be bound to the surface either the inner surface or the outer surface or they may be across the membranes which are called transmembrane proteins.

These proteins are involved in a number of processes which are related to the cellular transport and other essential functions like the transportation of different molecules, as we will see. They are involved in often different enzymatic functions. They are also involved in as a messenger to send messages to other cells and as a cell recognition system. Thereby, they help the cells to recognize the invader molecules or harmful cells or molecules and also provide supports to the cellular membrane structure.

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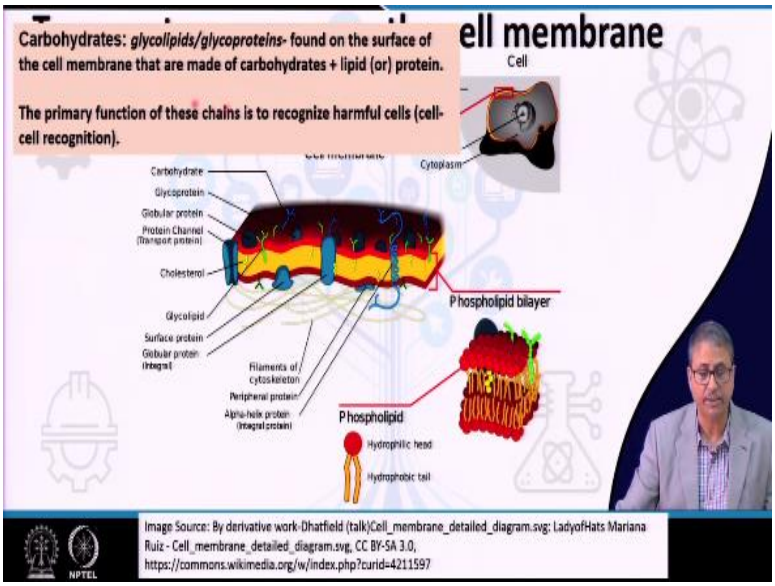

So there are also carbohydrate groups attached to the membrane like the glycolipids or glycoproteins, which are found on the surface of the cell membrane and made up of carbohydrate plus lipids or carbohydrate plus proteins. And the primary function of these carbohydrate moieties or carbohydrate chains of different kinds is to recognize the harmful cells. So basically they help in the cell-cell recognition system.

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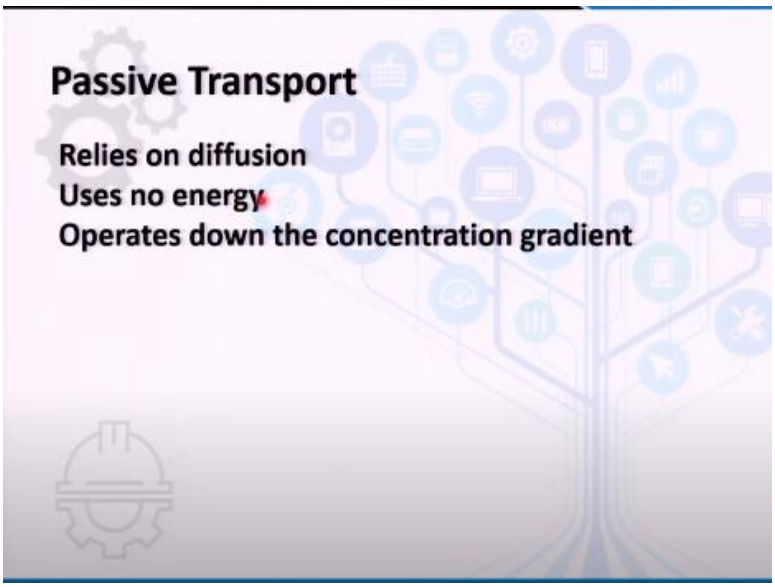

So now, we move on to the first type of transport which is called the passive transport. So we mentioned earlier that there could be two major types and there are actually subtypes. So total three types of transport we mentioned in the initial discussion. So the first one is the passive transport which will be having two specific type.

One is the simple diffusion type and other is the facilitated transport or we call facilitated diffusion also. So the broad characteristics of the passive transport are that it relies on diffusion only. That means, from a higher concentration to the lower concentration regime, the transport of the molecules or the transport of the solutes occur. It uses no energy because it is down the concentration gradient, the energy requirement is not there.

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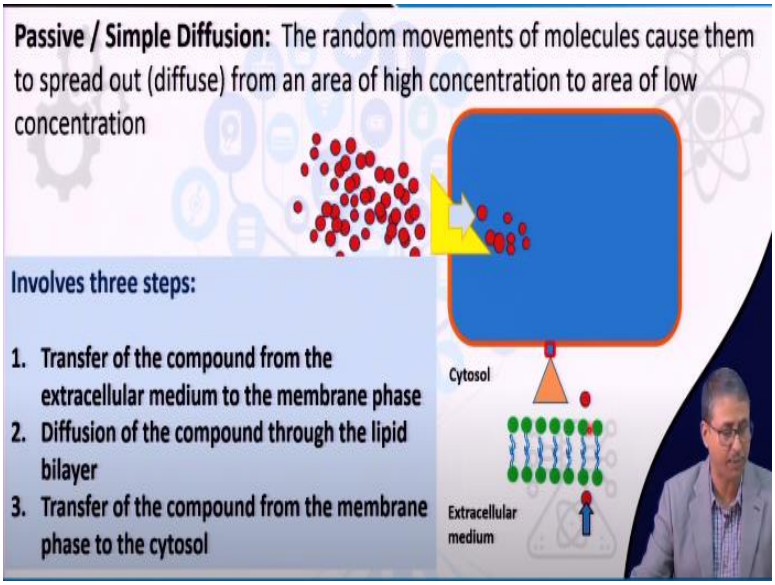

Now let us look at the processes involved in this passive or simple diffusion, which is basically the random movement of the molecules, which allow them to spread out or diffuse out from an area of high concentration to an area of low concentration. Like if you have a higher concentration outside and the lower concentration inside these molecules will tend to diffuse.

And if they are allowed by the membrane like they are soluble in the membrane or membrane permeability is there for these molecules, the molecules may move inside the cell unless and until the equilibrium is attained. And it basically involves three steps. So step number one is the transport of the compound from the extracellular medium to the membrane phase.

So the first one was the transfer of the compound from the extracellular medium like the extracellular medium is over here, from that to the membrane phase, which is the hydrophobic phase. So from the hydrophilic site to the hydrophobic phase, that is the first step.

The second step is the diffusion of the compound through the lipid bilayer like through the hydrophobic zone, which is within the membrane itself, membrane bilayer itself, the movement of the molecule occurs, that is step two. And the step three is the transfer of the compound from the membrane phase that is the hydrophobic phase to the cytosolic phase.

So we could identify easily three phases within this passive or simple diffusion across the lipid bilayer, which is the step 1, step 2, and step 3.

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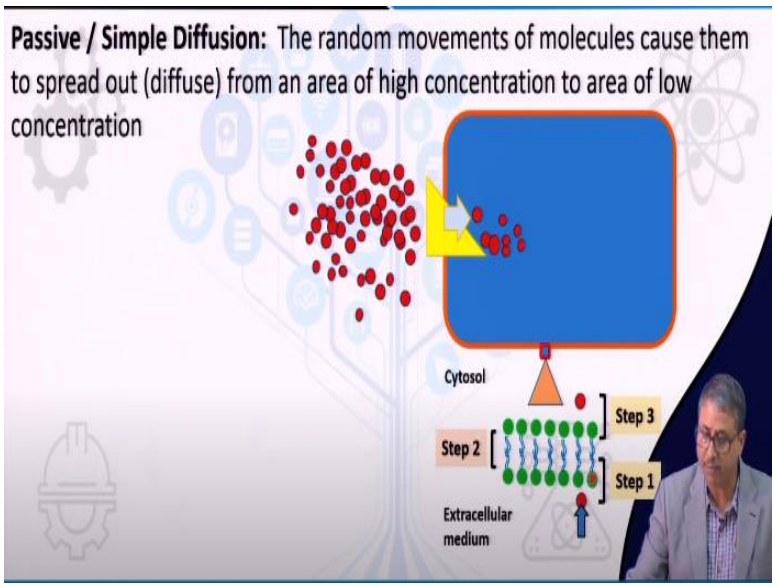

Now ideally, these steps are well marked over here, the step 1, step 2, and step 3.

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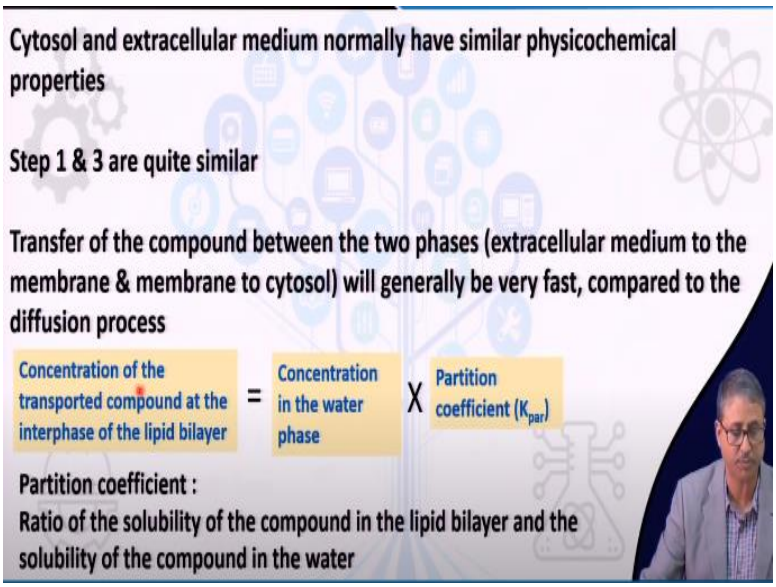

So step 1 and 3 are basically quite similar, because the cytosol and the extracellular medium normally have the same physicochemical properties and they are basically hydrophilic in nature, lot of water molecules are there. So step 1 and step 3 are quite similar. Now the transfer of the compound or the solute molecule between the two phases like extracellular medium to the membrane, and membrane to the cytosol will generally be very fast.

Like the step number 1 and step number 3 will be very fast compared to the diffusion process, which is basically the diffusion through the trans membrane region or the internal part of the membrane, which is the hydrophobic part of the membrane.

Now overall, we could derive that the concentration of the transported compound at the interface of the lipid bilayer that is the internal hydrophobic zone of the lipid bilayer is dependent on the concentration in the water phase, which is which can be easily measured, multiplied by the partition coefficient.

And the partition coefficient is the ratio of the solubility of the solute molecule or the compound in the lipid bilayer and the solubility of the compound in the water phase.

So if we know the, this partition coefficient of a solute molecule or the compound which we are trying to investigate or trying to study, and if we can determine by some analytical method, the concentration of the solute within the aqueous phase either in the cytosol or in the outer environment, we will be able to determine that how much could be the expected concentration within the membrane hydrophobic zone.

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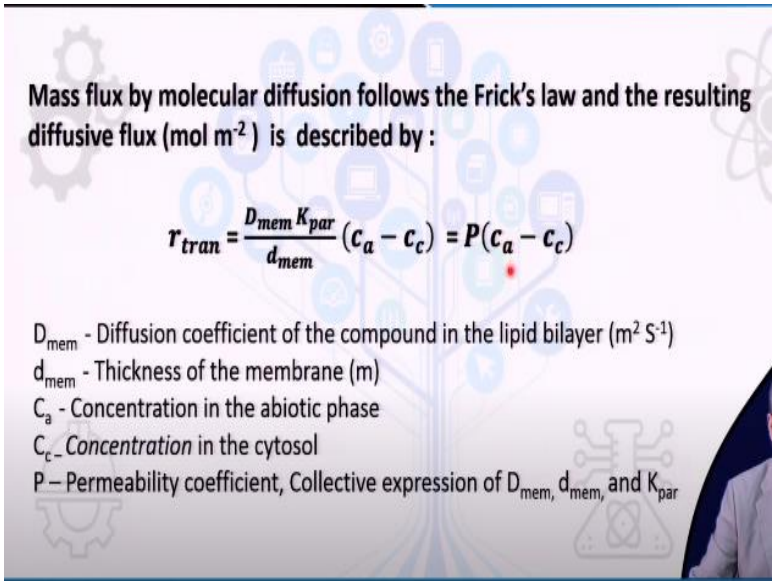

$$r_{trans} = \frac{D_{mem} K_{par}}{d_{mem}} (c_a - c_c) = P(c_a - c_c)$$

Now we can also determine the diffusive flux, which can be actually described by the mass flux or molecular diffusion following the Frick's law, that it will be represented as r tran and which is equal to the D membrane K par divided by small d mem. Now this D mem is the diffusion coefficient of the compound in the lipid bilayer and small d mem is the thickness of the membrane and K par is the partition coefficient.

And it is multiplied by the concentration in the abiotic phase and concentration in the cytosol. So if we know the concentration in the abiotic phase, and concentration in the cytosol, we will be able to determine the diffusive flux of the particular metabolite. Now this entire portion of the equation is considered as the permeability coefficient and it is actually collectively expressed as P.

So r tran or the diffusive flux of the any particular molecule which is trying to enter inside the cell or under study is equal to the permeability coefficient multiplied by the concentration of the metabolite into the abiotic phase minus the concentration in the cytosolic phase.

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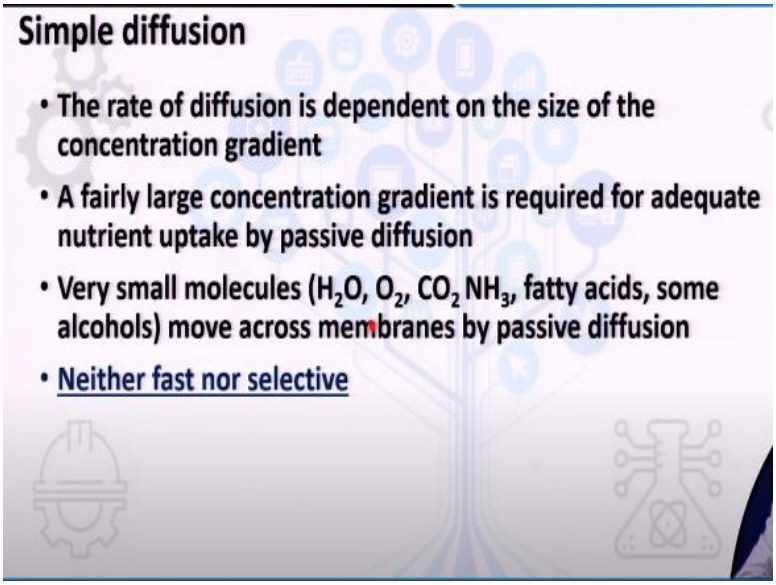

Now overall, the simple diffusion is basically the rate of diffusion which is dependent on the size of the concentration gradient. Larger the gradient means higher the concentration outside or lower the concentration inside the cytosol, the rate of the diffusion will be faster. And a fairly large concentration gradient is generally required for adequate nutrient uptake by passive diffusion.

And the higher the gradient, larger will be the transport process or the rate of the diffusion process, because cell has generally no control on this type of transport process, except the cell might consume the internal metabolites which the cell intends to transport through this kind of simple diffusion and thereby try to maintain the concentration, cytosolic concentration of the solute or the metabolite always very low inside the cell.

Very small molecules for example, water, oxygen, carbon dioxide, ammonia, small fatty acids and alcohols, some organic acids are generally move across the membrane by passive diffusion. And it is neither a very fast process nor a very selective process. The question of selective does not come over here because there is no selection.

It has to be permeable through the membrane like only these kinds of small molecules will be permeable through the membrane. So they will pass through as long as the gradient is allowing them to move through. As soon as the gradient is over like it is it has attained the equilibrium, the transport process will stop.

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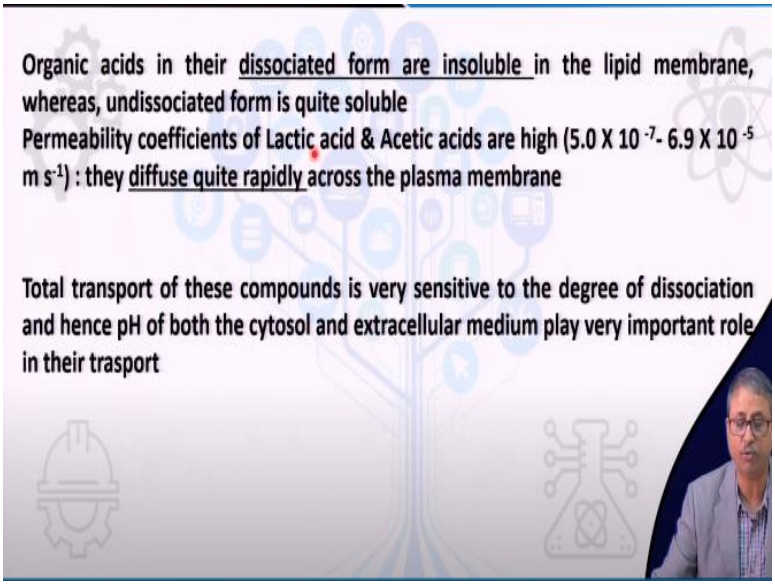

Now let us talk briefly about that about the organic acids and the role of medium pH or environmental pH in organic acids. Because from a metabolic engineering point of view, this could be very relevant. Now organic acids in their dissociated form are insoluble, okay. So however, in their undissociated form, they are quite soluble in the lipid membrane.

That is the internal hydrophobic part does not allow the dissociated forms of any organic acid to pass through. Now the permeability coefficient of for example, the lactic acid, acetic acid etc., are very high unless and until they are undissociated. The moment they are dissociated like they release the proton out of that, they will be considered to be insoluble and they will not be able to move through the hydrophobic membrane inner part very readily, very rapidly.

Now the total transport of these compounds, so basically the transport of these acids either from the inside to the outside or outside to the inside both depends on their level of dissociation and it is very sensitive to the degree of dissociation and the degree of dissociation is connected to the pH of the environment.

So maintenance of the proper pH is important because, if we are working on some cells, which are our systems where high amount of lactic acids are produced or acetic acid are produced inside the cell and compared to outside the concentration is very high inside the cell and we would be expecting a simple diffusion that the lactic acid

or acetic acid will move out from the cell cytosol to the outer environment. In that case, we need to monitor the pH of the cytosol very carefully.

Because, if the pH is not suitable, the lactic acid or acetic acid will undergo dissociation and the moment they are dissociated, they would not be able to pass the lipid bilayer.

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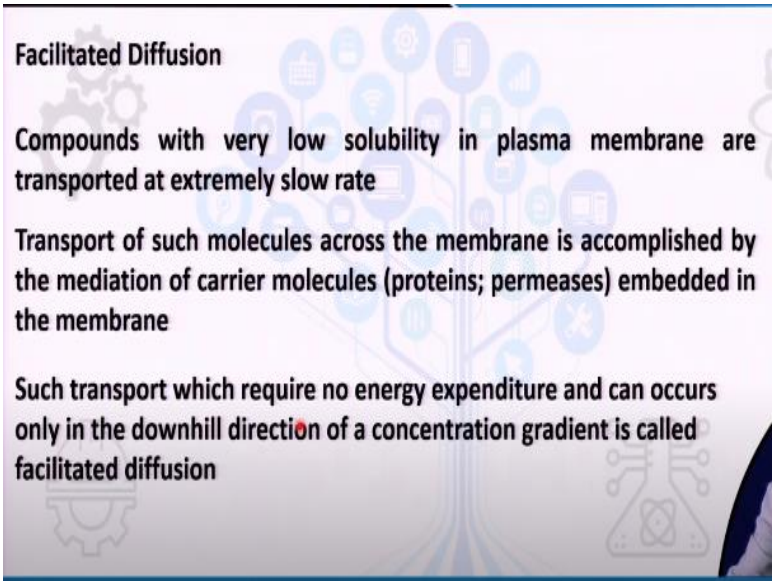

So facilitated diffusion is characterized by movement of the molecules or solute molecules, which with very low solubility in plasma membrane. So we are now moving towards the facilitated diffusion part where, which is little different from the simple diffusion and the word facilitated means that some facilitator molecules or facilitation is required. Somebody should be there to help in the diffusion process.

The diffusion cannot be achieved or cannot continue without the participation of a particular facilitator or a carrier molecule. Now compounds with very low solubility particularly, which are having very low solubility, we are talking about the organic acids also. The compounds with very low solubility in plasma membrane particularly the hydrophobic part of the membrane part or lipid bilayer are transported extremely slowly, because they are not soluble in the membrane.

Now transport of such molecules, which are having a solubility issue within the membrane, across the membrane is accomplished by the mediation of a carrier

molecules or a facilitator molecules. These facilitated molecules could be proteins or permeases. Sometimes we call them embedded in the membrane.

So this is again a diffusion only like from high concentration to the low concentrations the molecules will move or the solutes will move, but only difference over here is that that they are not readily diffusible or permeable through the membrane because of their chemical characteristics. So they need some kind of support from the cellular system that some carrier molecules must be there to help them.

This transport obviously, does not require any energy because it is down the concentration gradient and occurring only in the downhill direction of the concentration, it is called the facilitated diffusion.

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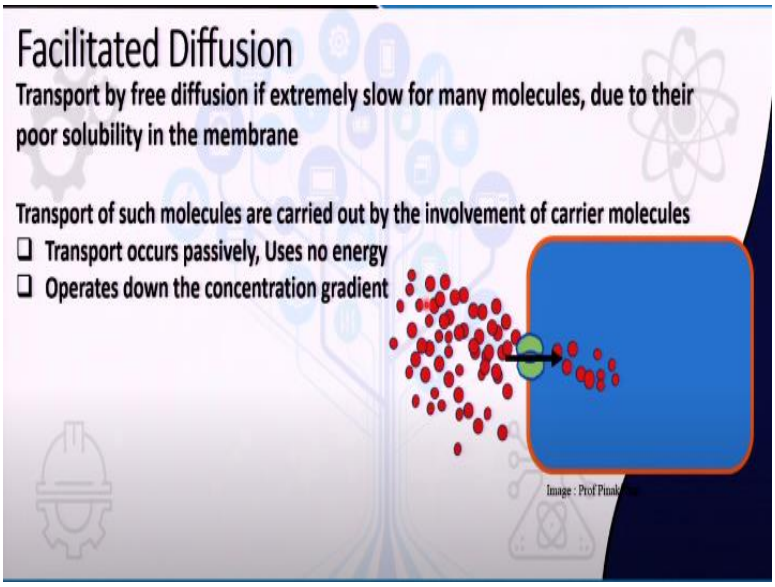

Like as you can see over here you have a higher concentration outside and in the inside the cell we have a very low concentration. So you can engage a very or the cell can engage a very specific carrier molecules for this particular solute only or the compound only and they will allow the movement of the molecule inside the cell. So these transport by free diffusion is extremely slow for many molecules and that is why it is required that a carrier molecule must be in place.

Now the transport of such molecules, such molecule means the molecules which are not readily movable inside the or through the plasma membrane are carried out by the

involvement of the carrier protein. So these are specific protein molecules which are called carrier proteins or sometimes we are also referring them as permeases. Transport occurs passively because no energy input is required except the concentration gradient.

So their concentration gradient must be established and it operates down the concentration gradient.

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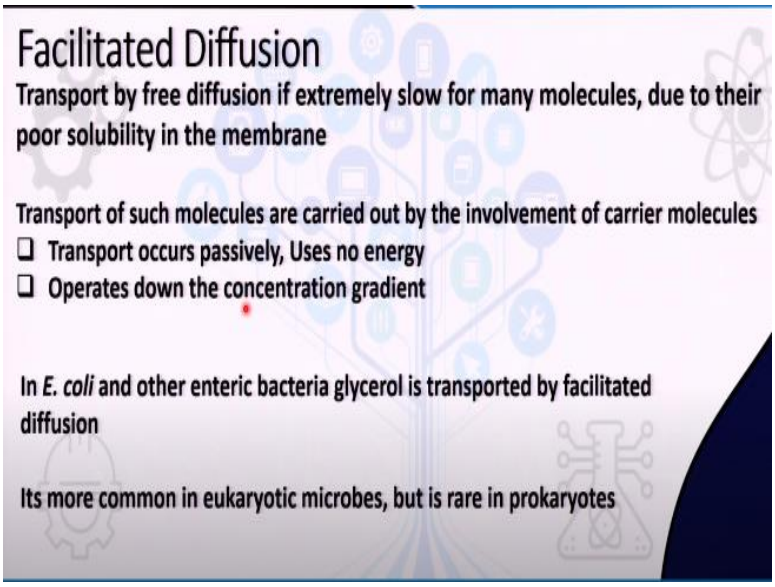

So in *E. coli* and other enteric bacteria, the glycerol transport is mainly observed to be achieved by facilitated diffusion. And it is more common in eukaryotic microbes, but is generally rare in prokaryotic system. In case of facilitated diffusion, we see that it is more alike in yeast system or so. We will be able to see that the facilitated diffusion is more commonly occurring.

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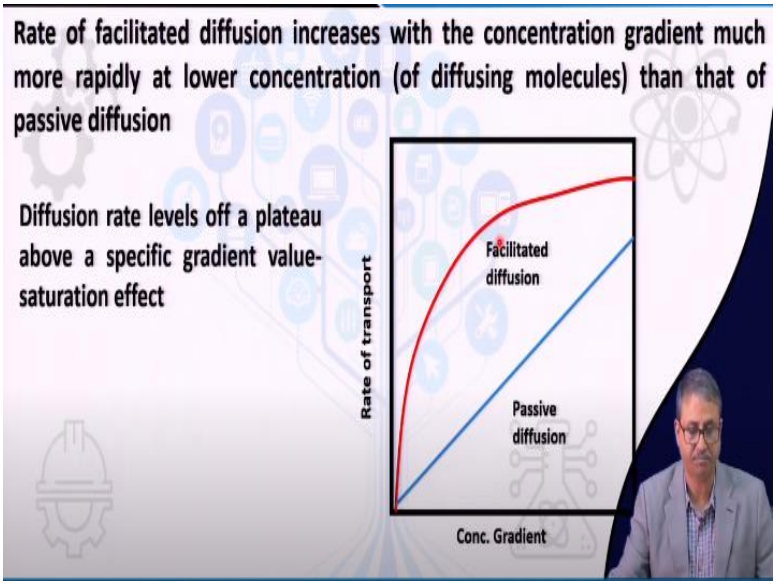

Now the rate of facilitated diffusion or the kinetics of the facilitated diffusion demonstrates a very interesting property which somehow somewhat resembles the Michaelis–Menten kinetics of enzyme substrate reaction. The rate of the diffusion, here both the rates are plotted for the passive diffusion and the facilitated diffusion. Now in case of facilitated diffusion what we can see that it increases.

This is the rate of transport. The rate of transport increases with the concentration gradient much more rapidly at the lower concentration of the diffusing molecule, than that of the passive diffusion. Why it is so, because passive diffusion is entirely dependent upon the concentration gradient, okay.

There is no involvement of carrier molecules. But in case of facilitated diffusion, we have the involvement of carrier molecules and in the initial phase of the transport when there are enough of carrier molecules present in the membrane, then it is slight rise or slight increase or a minor increase in the concentration of the solute molecules, there will be lot of carrier molecules which are which will be engaged with the transport process and those will be transported very rapidly.

As we increase the concentration, we will find that more and more carrier molecules are able to bind to these solute molecules and able to transport it. So the rate of the transport will be very rapid in the initial phase. But very soon this will level off or this will reach to a saturation and this saturation level will be indicated or will be

connected to a kind of condition when almost all the carrier proteins are engaged in transporting the concentration gradient.

So suppose we have 50 or 100 transporters so initially there were only one molecule of solute and 2, 3, 4, 5 then as soon as it has reached to 50, 50 to 70 every active molecule is actively transported by this carrier molecules. But as soon as the concentration of the solute molecules it reaches to 100 then there are only 100 carriers. So some incoming solute molecules need to be waited for some time because not that all the carrier molecules are free.

So they need to wait for some times unless the carrier molecule is free, then only once they are free then they will be able to bind the solute molecule and will be able to transport it. So it shows a high degree of saturation pattern.

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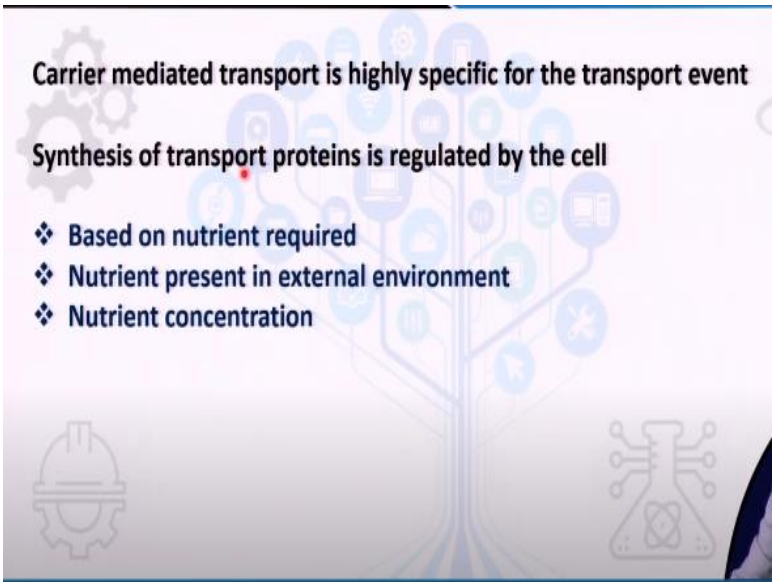

But one interesting point over here compared to the normal diffusion is that carrier mediated transport is highly specific for the transport event. Because the carrier proteins or carrier molecules highly specific cell produces these kind of carrier molecules only in order to transport specific solute molecules inside the cell.

Now the synthesis of this transport proteins because as I mentioned earlier these carrier molecules are proteins and they are considered as a broad category as a transport proteins. So these transport proteins are synthesized within the cells and their synthesis process is tightly regulated.

And this regulation of their expression of those genes and the translation of those mRNA to active proteins are again dependent on several factors like based on the nutrients required what type of nutrients required when a particular system or a particular cell is growing under a kind of condition. So because the cellular requirement of nutrients vary from condition to condition.

Nutrients present in the external environment, what type of nutrients are existing in outside environment. Do we need a facilitated diffusion or normal diffusion would be enough, okay and the nutrient concentration, because ultimately it is going to be or it is down the concentration gradient. So if it is down the concentration gradient, the concentration of the nutrient to be transported plays a very important role.

So the kind of nutrient molecules that also regulates the expression of these proteins and presence of these proteins as well as the concentration of these nutrient molecule. If the cell realizes that the concentration is not enough in respect to the concentration gradient that means, down the concentration gradient, it may not be able to transport the molecule for a long time or some time at least, the cell may not invest lot of energy and efforts to produce the transport proteins to ferry these molecules inside the cell.

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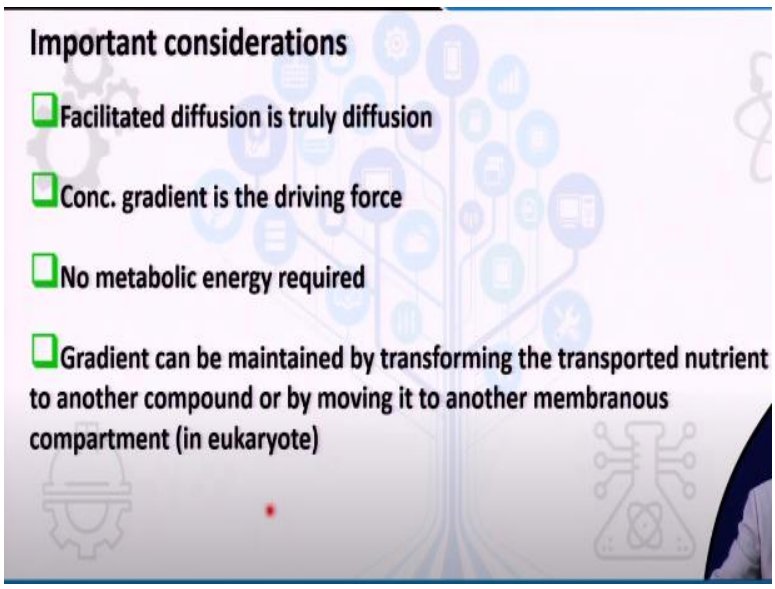

Some other considerations which are also important are the facilitated diffusion is a truly diffusion process. No matter whether it is involved with a carrier protein bound

or not all diffusion processes are based on only on the concentration gradient. So concentration gradient is the major driving force. Larger the gradient or the bigger the gradient the faster the transport. However, with respect to facilitated diffusion, there is a caution.

It is dependent on the concentration gradient only at the when it is on the lower side. As soon as the concentration gradient rises to some level, number of carrier molecules become limited and these limiting carrier molecules lead to saturation of the transport process with respect to the facilitated diffusion. No metabolic energy is required because it is driven only by the concentration gradient.

And gradient can be maintained by transforming the transported nutrients to another compound or by moving it to another membranous compartment. Particularly what we observe in eukaryotic cellular system or eukaryotic cells, that even though some molecule or solute molecule is transported inside the cell, cell cytoplasm, the cellular system immediately translocate that internalized solute molecule to another compartment so that the actual concentration in the cytoplasm remains always low.

So as it remains low the transport gradient or the concentration gradient remains active so as to support the diffusion readily. Now we are going to talk about the active transport.

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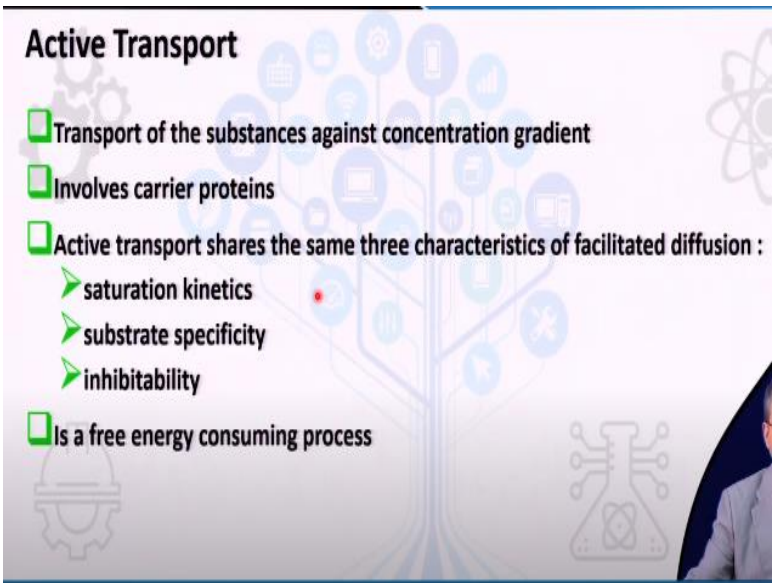

Active transport is the transport of substance or the solute molecules against the concentration gradient. So this is the transport molecule where outside we have a very low concentration. Inside we have a relatively higher concentration in the cellular environment of the cytosol. Still, we would like to transport the solute molecule. It involves carrier proteins, because it is against the concentration gradient.

And it shares three characteristic features with the facilitated diffusions which are easy to understand because it also involves the carrier protein. So obviously a saturation kinetics would be expected and substrate specificity is also expected because the carrier proteins are highly specific to their substrate. And it is it can be inhibited by some inhibitor molecules which is also a property of the facilitated diffusion.

Anywhere carrier proteins are involved. Any surrogate molecule or any analog molecules analog to the substrate molecule, which adversely affect the carrier protein structure or function or ability to transport the molecule acts as the inhibitor. So in active transport process also the transport process can be inhibited. And finally, it is a free energy consuming process because it is against the concentration gradient.

So we need to spend energy to transport this type of molecules to this transport type of mechanisms.

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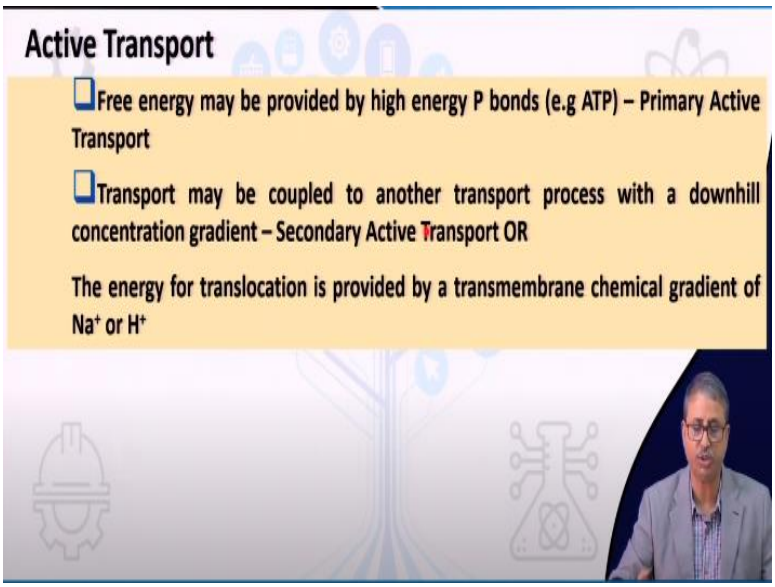

Now since we are talking about involvement of energy into it, because it is against the concentration gradient. So if it is against the concentration gradient it must be supported with the free energy. Now the free energy for this transport process may be provided by high energy phosphate bond like the hydrolysis of ATP.

So if it is, the energy source is the ATP hydrolysis, then this kind of active transports are called as a primary active transport. The transport process might get the energy by coupling the transport of the specific solute molecules or the molecules of interest to another transport process with a downhill concentration gradient.

Downhill concentration gradient means the second molecule which is transport co-transported might be having a favorable situation with respect to its concentration regime. So it might be transported down the concentration gradient. So the other molecule which is actually on the, or against the concentration gradient can be achieved by coupling these two transports.

The energy for translocation is provided by the transmembrane chemical gradient like sodium ions or protons. So we are going to discuss these points briefly.

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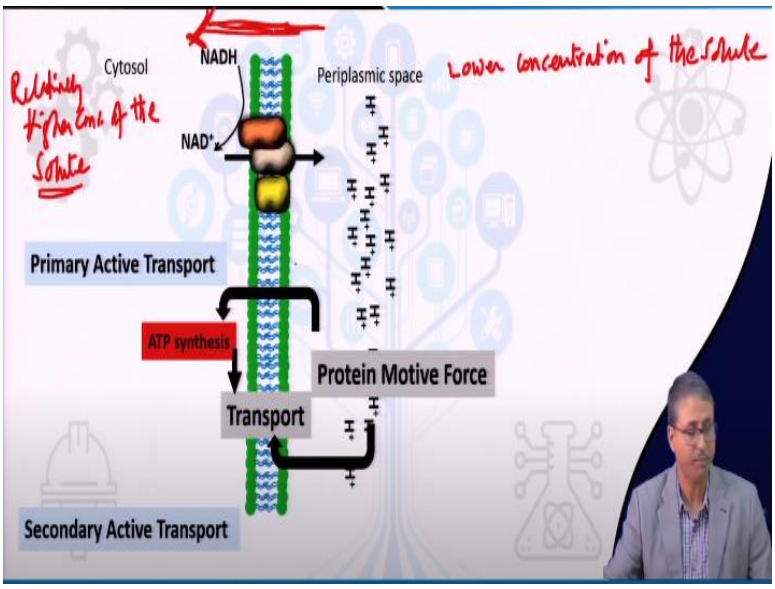

Now let us assume that or let us consider that this is the membrane bilayer and these are the hydrophobic portion of the internal portion of the membrane and this part is not readily permeable for many molecules and of course, for active transport the

concentration of the molecules might be very low outside and concentration of the molecules might be still higher this side but the transport might go on, okay.

So if we wish to mention that over here, so we have a lower concentration of the solute and here we have relatively higher concentration of the solute. But still we want to transport this because this is this solute is of high demand. So the cell is going to or trying to metabolize these particular solute molecules readily. So the cell is not in a situation to do any kind of compromise the cell want.

The solute molecules should be transported inside the cell. So if it is so there could be two strategies. One we just mentioned about the primary active transport and other is the secondary active transport. So now, over this membrane, we have already learned that there are electron transport processes which are coupled with the oxidation of reducing electron carriers like NADH could be oxidized to NAD+.

And this electrons can be flow up to the electron acceptor, terminal electron acceptor, and some of these carrier molecules or the electron transport complexes are also they also act as a efflux pump, proton flux pump. So protons are thrown outside the cytoplasm and particularly in case of gram negative bacteria if we consider this will be the periplasmic space. So a concentration of proton builds up.

Now this concentration of proton which is building up is actually developing the proton motive force, okay. And this actually disequilibrium or inequality in concentration of the protons or it may be sodium ions sometimes, so that the disequilibrium of these ions okay causes a kind of a pressure to the membrane and that is generally referred as the proton motive force.

Now this proton motive force enables the either to drive some kind of pump or functional units to utilize these disequilibrium to their favor. Now this as we have briefly discussed that this could be connected to an ATPase, ATP producing enzyme system that the protons will be allowed to move in and ATP will be produced.

Now this ATP, which is produced can be utilized to transport a particular molecule like in this case, the solute molecule that was we were referring to earlier. So the

solute molecule can be transported by utilizing the ATP molecule which is produced by utilizing the proton motive force. And this is considered or called as the primary active transport.

On the other hand, the proton motive force can directly be used to transport some other molecule like galactose or lactose etc., which is directly related to the proton transport. Because the proton will be transported down the concentration gradient because it is outside high concentration inside low concentration.

So proton transport will be down the concentration gradient, but the lactose or galactose or similar other solute molecules, which are waiting to be transported inside the cell might be transported through those type of transporters. So now, I must mention over here this the type of the transporters are very specific.

Whenever we are using a ATP based transporters, which are involved in primary active transport, so there are entirely different set of transporter molecules, because lot of conformational change and ATP hydrolysis and all these events are included. So there are specific set of transporter proteins which are involved in primary active transport and also specific set of protein molecules or carrier protein molecules are there which are involved in the secondary active transport process.

So for each of the type of the molecules or each of the type of the metabolites or solutes to be transported, and whether ATP will be utilized or whether proton motive force or an ion motive force will be utilized, based on that all these carrier transports transporters are produced by the cells and cell controls the synthesis of all these carrier molecules considering as we mentioned earlier, the availability, the requirement and the concentration of all these molecules.

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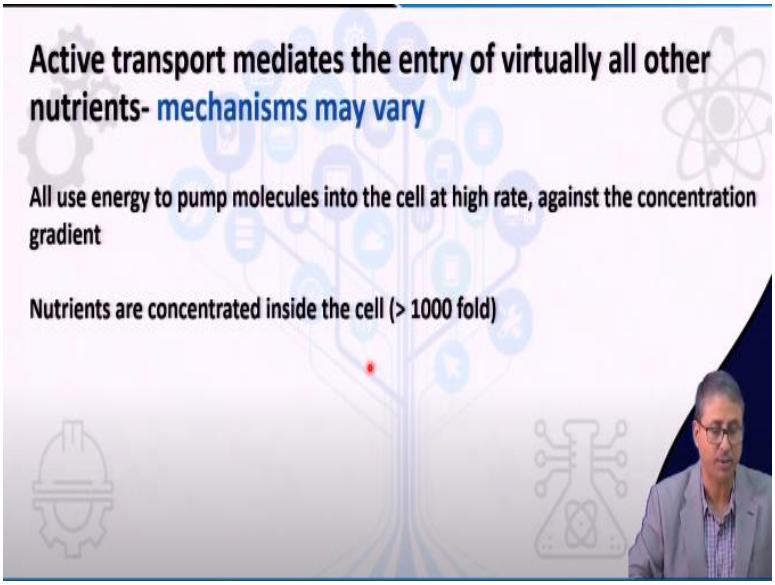

Now active transport mediated mediates the entry of virtually all other nutrients and the mechanism however, may vary. So there might be two or three broad mechanisms by which the active transports occur. Now all of these different mechanisms by whether it is utilizing ATP hydrolysis or it is utilizing the iron motive force or proton motive force, everyone is using energy to pump molecules into the cell at a very high rate and it is against the concentration gradient.

So there are, these are the two important aspect of this transport, active transport that they transport the molecules at very high rate because the carrier molecules are very efficient and once they are present and they are able to recognize the solute molecules they will be transporting the molecules in a very high rate. And of course, against the concentration gradient.

Sometimes even it is several orders of magnitude the concentration difference might be there. So from so they can still transport very efficiently. And nutrients are concentrated inside the cell even up to 1000 folds. So within the cell high and high concentration will be built up compared to the outside environment.

But the still the cell will try to acquire the nutrients because maybe the cell is under the impression that the demand for the metabolism is so that those kind of nutrients will be readily metabolized like the galactose or lactose etc. The carbon and energy sources are so demanding the cell would always like to take them up and inside the cell they would like to utilize them.

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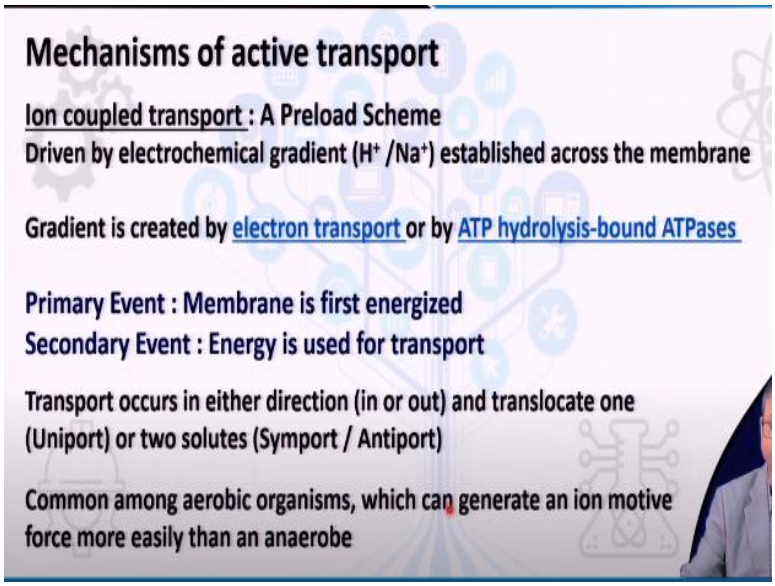

Now the mechanisms of active transport. So we will be discussing two main mechanisms first. The first one is the ion coupled transport, which is considered to be a preload system. So it is called ion coupled transport, because it is driven by electrochemical gradient. We were referring to this earlier, that is either a proton gradient or a sodium gradient is established across the membrane.

And it is a kind of a prior requirement that this gradient is established. Since we are talking about a preexisting membrane gradient or gradient which is first established, it is called a preload system. So because if we want to transport a particular solute through ion coupled transport, first we need to establish this electrochemical gradient by transporting proton or sodium.

Now this gradient of sodium or proton can be created by electron transport, which is coupled to the electron transport system or oxidative phosphorylation or by ATP hydrolysis bound ATPases. So there are actually a number of transport systems, active ion coupled systems are there where ATP hydrolysis is used to transport sodium for example, outside the cell, so that a sodium gradient is created.

And then the sodium gradient will be utilized to transport the actual or the targeted metabolites. So the primary event is the membrane is energized. Membrane is first energized through electron transport based or ATP hydrolysis based creation of the

gradient, gradient of proton or sodium. And the secondary event is this energy is used for the transport.

Now once the gradient is established, this gradient is utilized for the transport to happen. Now there are two important characteristic properties of this kind of ion coupled transport system. The one is the transport occurs in either direction. So it can be cytosolic side to the outer environment or from the outside environment to the cytosolic site and it can translocate either one solute at a time that is called uniport.

Or it can transport two solute molecules, either in the same direction is called symport or in the opposite direction that is called the antiport system. It is very common among the aerobic microorganisms, aerobic organisms also which can generate an ion motive force more easily than anaerobic system because you need to spend lot of energy to generate this gradient across the membrane.

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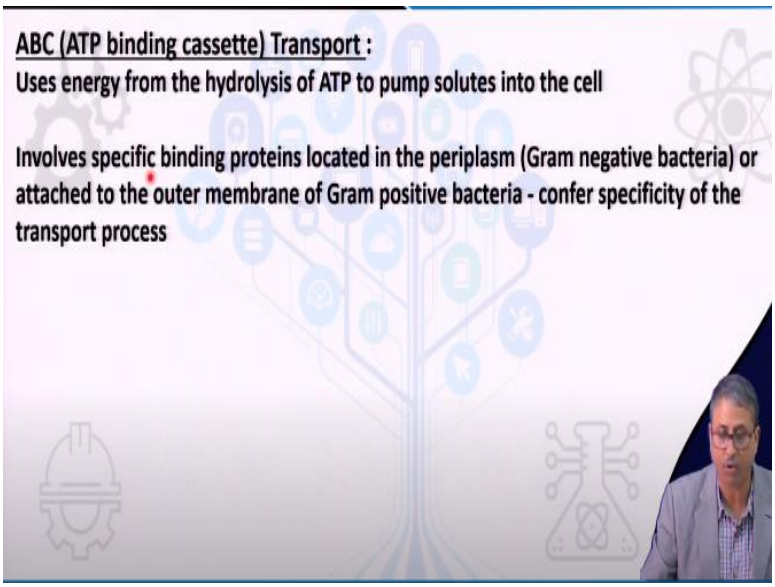

The next one or the second one is the ATP binding cassette transport or ABC transport. ABC transport as it says it involves ATP hydrolysis, and it uses the energy from the hydrolysis of ATP to pump the solutes. It is more like a direct event. It involves the specific binding proteins located on the periplasm.

So it has multiple steps. So let us just go through the brief steps that it has some binding proteins located on the periplasm, particularly in the Gram negative bacteria

or attached to the outer membrane of Gram positive bacteria that confer the specificity of the transport process.

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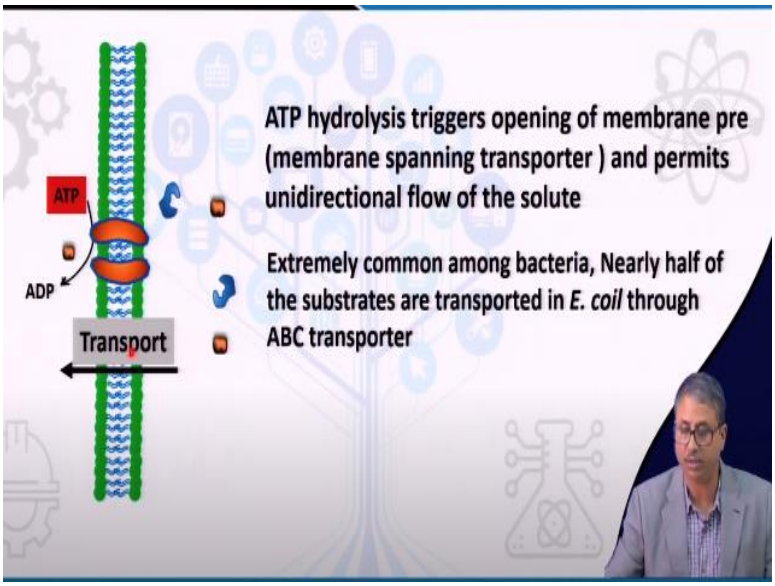

So here is the membrane and here are the carrier transmembrane carrier proteins, which are called ATP binding cassette. So these are referred to as ABC transporter. So entire setup is ABC transporter. It has three components. So component number one is this protein molecule, which is a kind of extra to the membrane. Either it is present in the periplasm or maybe on the outside of the outer membrane.

So it is responsible for binding the substrate very specifically and then bringing this whole complex to the transmembrane carrier complex. So that the molecules of solute molecule can be available for the transport. The one of the binding, this protein carrier protein complex is also having a ATP binding site where the ATP molecules are able to bind and hydrolyze and thereby providing energy to the system.

Now let us see how ATP hydrolysis can trigger the opening of this membrane. Now as soon as this binds to the binding protein, which is an extraneous component of the system, so these enable the particular solute to close or available to the carrier complex or the carrier molecules and then the hydrolysis of ATP occurs. As soon as the ATP hydrolysis occurs, the membrane channel gets open and the solute molecule is able to transport within the membrane.

And then this binding molecule is again free and it is able to bind another molecule and then the same way the process goes on. Now without the ATP hydrolysis, this channel will be remain closed or the transport occurs only in one direction. Because these are highly specific transporter, because the entire system is dependent on both the three factors.

One is the binding molecule and other is the ATP hydrolysis component and third one and very important one is the transmembrane domain. So since all these components are highly organized, the direction of the transport is highly specific. It is unidirectional only. Extremely common among bacteria. Nearly half of the substrates transported in *E. coli* are through these ABC transporters.

So ABC transporters are extremely popular within the microbial system.

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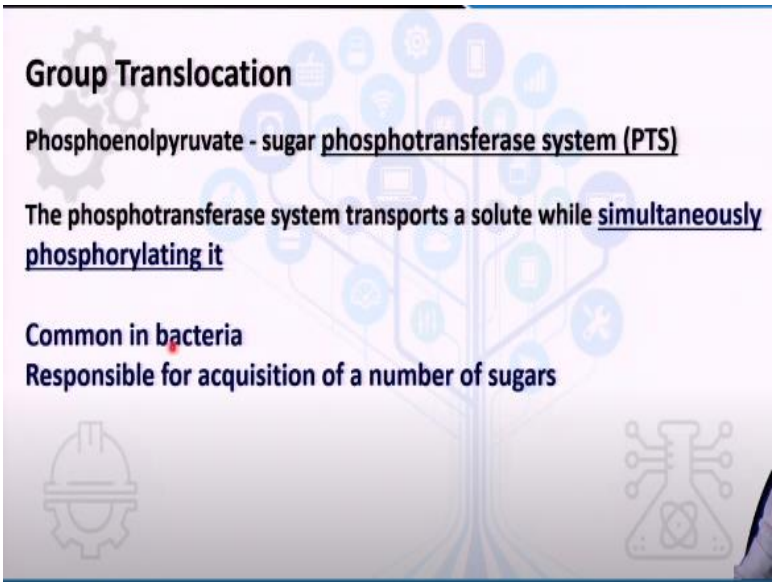

The next one is the group translocation, which is a very interesting transport process, which is also called as phosphoenolpyruvate sugar phosphotransferase system or PTS system. So the involvement of a phosphoenolpyruvate, one of the important metabolites within the glycolysis or Embden-Meyerhof-Parnas pathway is present in this transport system.

The phosphotransferase enzyme system transport a solute like a glucose or mannitol or some kind of carbon substrate like this, while simultaneously phosphorylating it. So the point of interest over here is the molecule, sugar molecule is transported inside

the cell. But as soon as it is transported inside the cell, it is phosphorylated. It is very common in bacteria and it is responsible for acquisition of a number of sugar.

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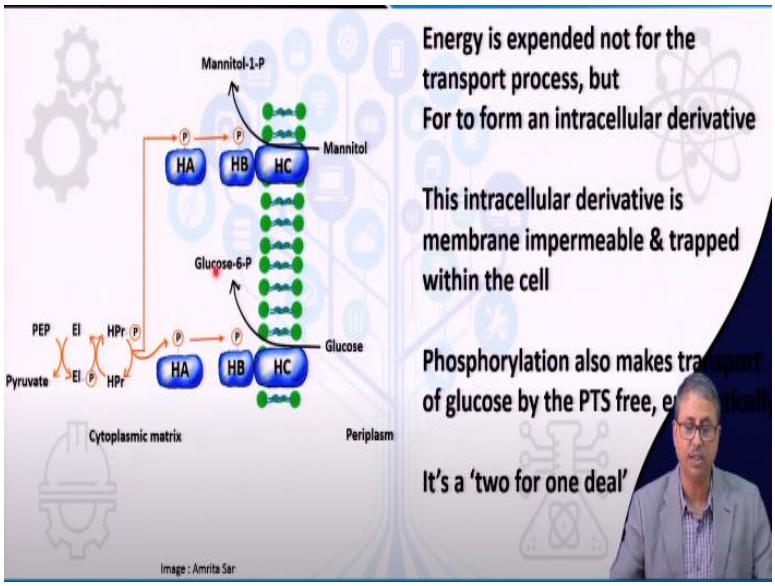

So let us see how it works. So it has actually multiple components as we can see. Here are the phosphoenolpyruvate, which is hydrolyzed and releasing the phosphate group, activating some enzyme complex like E1, then HPr. And then the phosphate group is transferred to these other complexes. So we are not going into the detail of the complexes.

But the main point over here is that cytosolic hydrolysis of the phosphoenolpyruvate and transfer of the phosphate group is connected to a series of protein complexes and eventually the carrier molecule is activated. As soon as the carrier molecule is activated the glucose or mannitol or this kind of sugar molecules are allowed to be transported inside the cell.

That means the channel is open only when it is activated and the activation of this particular last protein complex, which is a transmembrane domain is achieved only when the associated cytosolic site or counterpart of the protein complex is activated through the phosphorylation. Now the energy is expended not for the transport process directly, but to form an intracellular derivative.

So here derivative means we see that glucose is transported and converted to glucose 6-phosphate. Mannitol is converted to mannitol 1-phosphate. So the energy which is

available here like phosphoenolpyruvate is given finally to the carrier, but the carrier eventually transfer it to the solute molecules which is entering like glucose is converted to glucose 6-phosphate.

Now this is called intracellular derivative of the solute molecule or the molecule which is transported inside the cell. The sugars are mostly the candidate molecules. Now this intracellular derivative, so mannitol is converted to mannitol phosphate or glucose is converted to glucose 6-phosphate, this intracellular derivative is membrane impermeable and trapped within the cell.

So one very interesting strategy developed by the cell is the cell will continue taking up glucose and once the glucose is coming inside it is converted to 6-phosphate and this is, the 6-phosphate molecule is unable to leave it. So they are considered to be trapped inside the cytosol unless and until the cell is going to utilize them. Now phosphorylation also makes the transport of glucose by the PTS free energetically.

So ideally it is considered to be energetic energetically free and it is like two for one deal. So how about two for one deal? Like we are spending one energy over here in the form of phosphoenolpyruvate, but we are getting two things done. One we are getting these transport done which is which may be against the concentration gradient also or the second one is we are converting glucose to glucose 6-phosphate.

Now what is benefit in glucose to glucose 6-phosphate that glucose to glucose 6-phosphate conversion is a very important energy consuming step otherwise in glycolytic reaction or Embden-Meyerhof-Parnas pathway or even the pentose phosphate pathway. Everywhere we see the transported cytosolic glucose is converted to a glucose phosphate molecule.

So that phosphorylation otherwise would require an ATP molecule. So that is the ATP molecule which is now would not be required, because the group translocation allowed the formation of a phosphorylated glucose itself within the cytoplasm. So that is why it is considered as a two for one deal. So by spending one ATP or ATP equivalent high energy phosphate compound, we are gaining two functions done.

That is one is a transport, another is the first step of the glycolytic reaction is completed.

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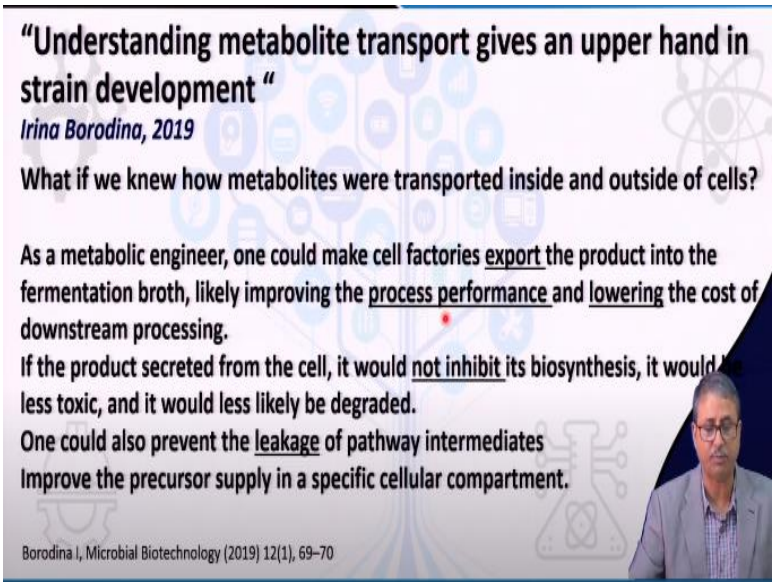

Now let us briefly discuss or talk about or give some example of understanding the metabolic metabolite transport, its importance in the metabolic engineering or strain development because it has been considered to be giving an upper hand in microbial strain development or microbial cell factories.

Now there is a nice introductory remark about this that what if we knew how metabolites were transported inside and outside the cell. What we can do if we know that how the metabolites are transported inside and outside the cell, like the details of the process. Like how the cells are exporting or importing the molecules would help us to improve the process performance and lower the cost of downstream processing. So we will look at some of the examples.

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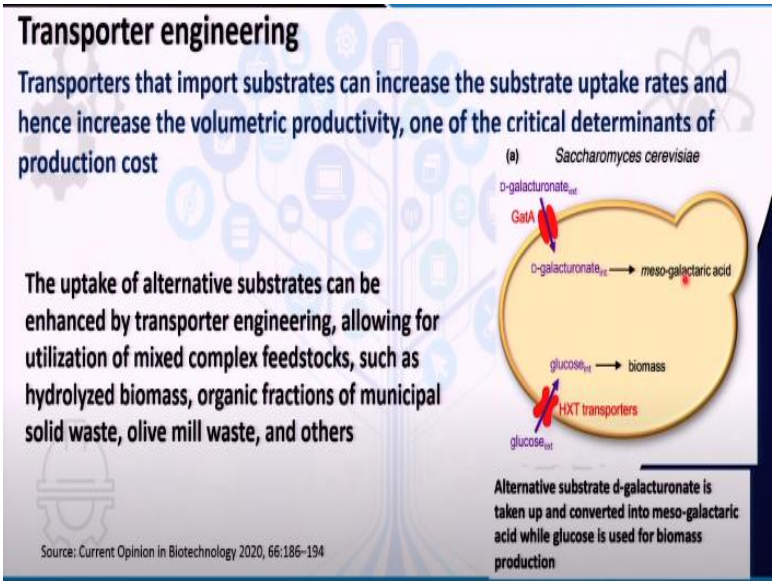

So these are called actually transporter engineering in terms of the cell factory production or the advanced level of metabolic engineering and transporters that import substrates can increase the substrate uptake rates and hence increase the volumetric productivity. So enhanced transport of metabolites or substrates inside the cell.

Now the uptake of transport or uptake of the molecule or transport of the molecule which are substrate molecule for the entire biochemical reactions are understood as they will be able to channelize more flux towards the desired reaction. But we can do this thing by multiple ways. So one is utilizing the existing and efficient transporters like the glucose transporters, which are more efficient and allowing more glucose to be transported inside the cell.

But at the same time, using some unique transporter like GatA, which is capable of transporting galacturonate inside the cell. Now the galacturonate is a very important component coming out of the kind of pectin residues present in the plant biomass.

And if we are able to engineer our cells during the metabolic engineering program, the galacturonate which is entered inside the cell through this type of specific transporter can be processed towards formation of meso-galactaric acid and meso-galactaric acid has found to be very important advanced biotechnological applications.

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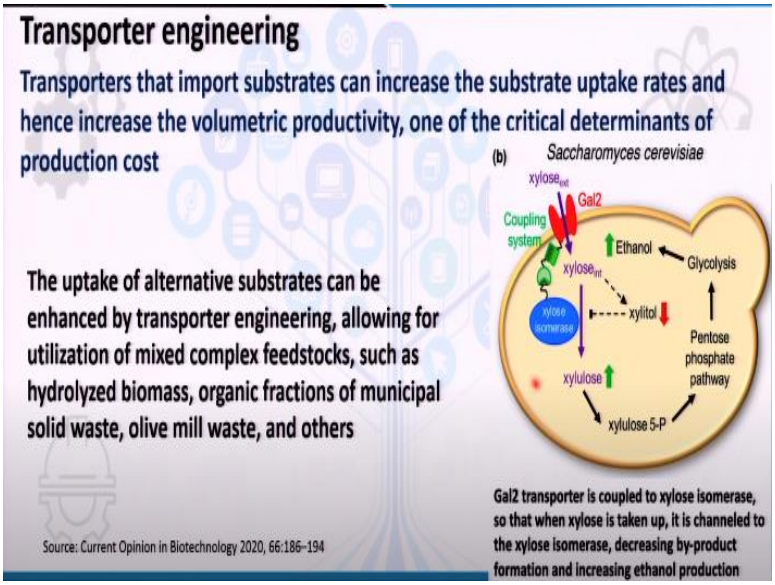

Similarly, the use of xylose a pentose sugar which is again present in abundance within the plant derived biomass into ethanol production. Engineering a xylose transporter like Gal2 and with a xylose isomerase keeps the internalized or cytoplasmic xylose close to the xylose isomerase and avoiding its use in other pathway.

So the internal of cytosolic xylose can be converted to xylulose and then xylulose will be eventually converted to ethanol very effectively.

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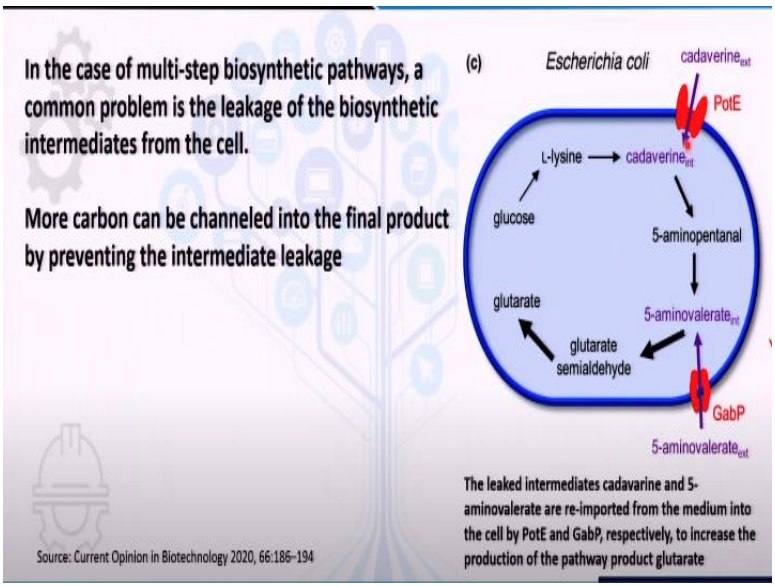

There are also cases where we see that there are leakage within the metabolic reactions. So particularly in multi-step biosynthetic pathways like lysine production or anti the amino acid production, there are problems with leakage of some of the

intermediates. Like as you can see here, the cadaverine or the aminovalerate, these are commonly found to be leaking from the cellular environment.

So lowering the flux towards the desirable the production compound, okay, like in this case from towards the glutarate production or so sometimes we see that the leakage the production is severely disturbed. So the engineering the particular transporters for the cadaverine or for the aminovalerate like the GabP or PotE for cadaverine helped the metabolic help in the metabolic engineering towards the improved performance of these biochemical reactions, because the leakage of the substrates or intermediates are stopped.

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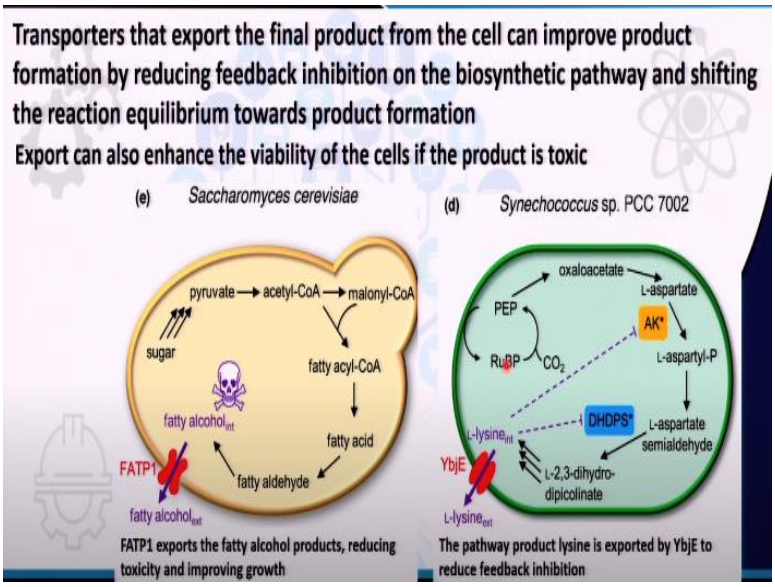

So similarly, within the cell there could be some toxic products produced and these toxic products need to be removed from the cell like in the fatty acid biosynthesis, we often see there are certain aldehydes produced. So engineering the cells with the transporters which are specific to the fatty alcohol transporter make transporting these fatty alcohols from cytosol to the outside environment.

Or the efficient transport of lysine which is produced inside the cell to the outside the cell which is highly desirable because in lysine producing strain, if we can readily take out the lysine then otherwise, the lysine might act negatively because lysine production lysine biosynthesis is very tightly regulated.

If we have accumulation of lysine inside the cell cytoplasm, then feedback inhibition would implement and the production of the lysine or the reaction towards lysine synthesis would be inhibited. So by rapidly efluxing or exporting lysine from inside the cell to the outside actually helped the metabolic engineers to efficiently manage the entire set of problems.

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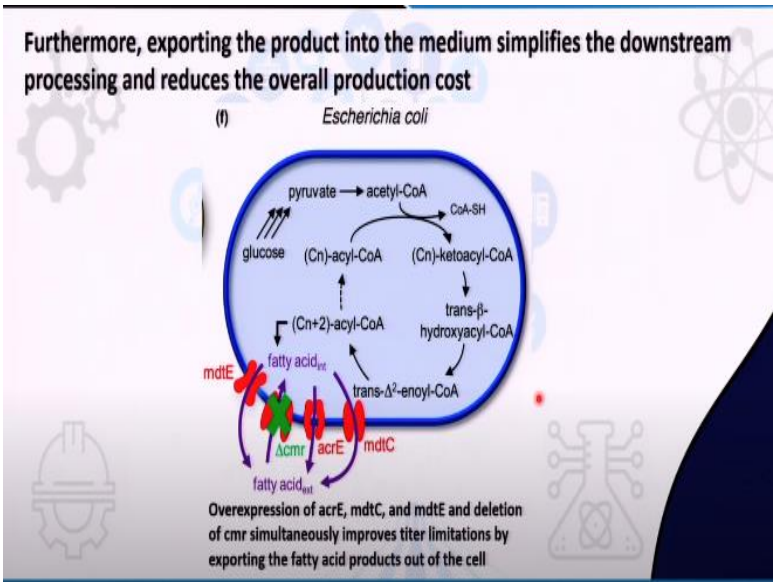

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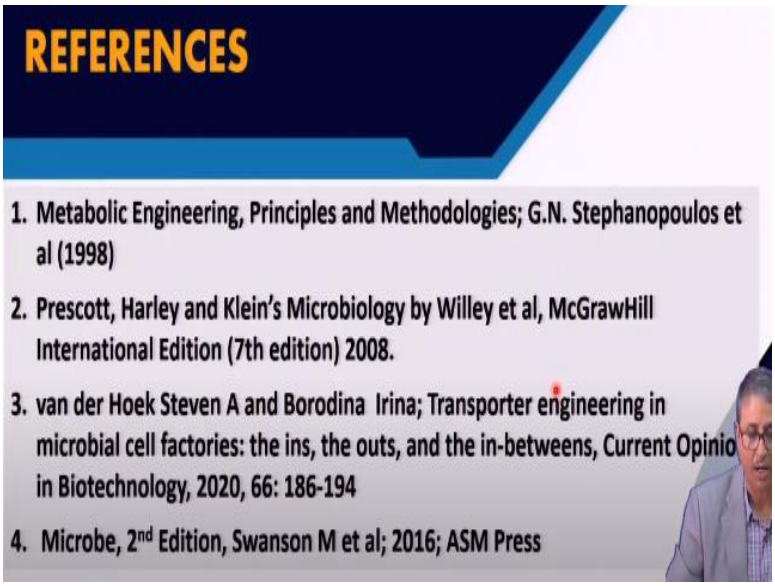

So after talking of all these things, so we will be concluding today's class and for today's class, we have used the following references, the metabolic engineering textbook, along with this the Prescott microbiology and an interesting review on transporter engineering and the Microbe second edition.

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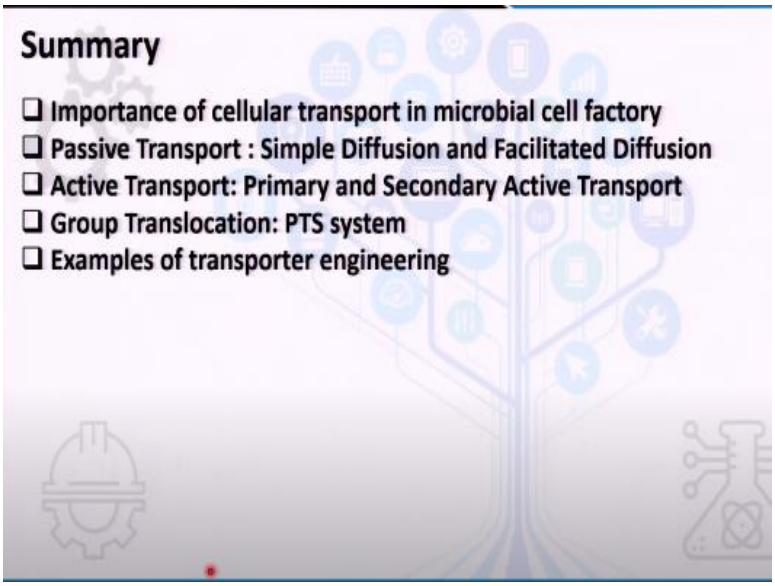

So to summarize today's lecture, the importance of cellular transport in microbial cell factory or advanced metabolic engineering is emphasized. The passive transport, simple diffusion and facilitated diffusion, active transport, group translocation and PTS system are all mentioned. Thank you.