Cell and Molecular Biology
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Week 04
Cellular Transport
Lecture - 16
Transport in Eukaryotic Cells (Part 1)

Hello, everyone. This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And what we were discussing was the transport of material into the cell. So what we have discussed so far in the previous lecture is the transport mechanisms in the prokaryotic system. So we have discussed passive diffusion.

We have discussed the active transport. And within active transport, we have discussed the different classes of molecules like carriers, transporters, and so on. So, with these discussions, it is clear that you require very well-organized machinery for facilitating the transport of materials. Now, if you see the different types of material, what you see here is that some of the materials are very easy to transport, such as gases like oxygen or carbon dioxide, which are being transported to the cell surface or the lungs in the case of the human system.

And some other molecules, like. Hydrophobic molecules like benzene and small molecules like ethanol or water are also readily taken up. There is no system required to take up these molecules. Then apart from that, some of the molecules are charged molecules, or they are also molecules for which you actually require special machinery for transport. So, in the previous lecture, we discussed the prokaryotic system.

Now, in today's lecture, we are going to discuss the eukaryotic system and how the machinery is different in the eukaryotic system. If you recall, in the previous lecture, I also said that these are the processes that are occurring in the prokaryotic system. But these are not the exclusive methods. These could actually happen in the eukaryotic system as well, right? So, when you talk about the transport of these materials across the plasma membrane, you are actually going to have different mechanisms as per the requirements of that particular organism or as per the machinery of that particular organism. If you talk about the eukaryotic system, it is much more complicated, and the eukaryotic system requires a much more organized structure so that you can take up the material more precisely, right? So these molecules, which we talk about, like the gaseous molecules or the hydrophobic molecules or the polar molecules, even like water and the other molecules, could cross the plume.

So the permeability of these molecules is very much related to that. But if you talk about the ions, for example, the sodium ions, potassium ions, chloride ions, and the polar molecules that are unable to cross the membrane by diffusion, most of these molecules cross the membrane using transport proteins. And these proteins are important for the selective permeability of the plasma membrane. Remember that the plasma membrane

present in the eukaryotic system is semi-permeable. So, it allows for the selective transport of the molecules.

The proteins have multiple membrane-spanning regions that allow the charge or the polar molecule to affect the membrane by forming a protein pore through the molecule interacting with the hydrophobic chains of the phospholipid in the membrane, right? So as for the transport is concerned, first you have to understand what the requirement of energy is in terms of energy. Remember that when we're talking about diffusion and passive transport, we said that it actually does not require energy, whereas that. In the case of active transport, it requires energy because you are taking up the material against the concentration gradient. So, as far as the thermodynamics of the transport is concerned, we have two different types of processes. We have passive diffusion, or we can have active transport.

Passive diffusion is the process in which you are actually required, you know, it is going to be driven simply by the concentration of the molecule. So it goes from high concentration to low concentrations. Now, if you actually understand the thermodynamics of this particular transport, what you're supposed to do is take a chamber. So, you will take a chamber, then you split this chamber into two sections with the help of a membrane. You can consider that this is a plasma membrane.

On this side, you are actually going to have the molecules, and on this side, it is actually going to have no molecules. Which means if I open this like this, this is actually the cell, this is going to be the external media, and this is going to be the internal. And this is actually the external; this is the internal environment of the cell, which is separated by a membrane. When you do so, the molecules will passively diffuse through this plasma membrane. If the molecules are hydrophobic, along with other characteristics like those of gas molecules, they will transfer.

And in this case, the concentration outside versus the concentration inside is actually going to play a driving role. So, when the two aqueous compartments with different solute concentrations are separated by a permeable membrane. The solute will diffuse from one side of the higher concentration to the side with the lower concentration, continuing until both compartments have the same concentration, right? So, it is actually going to maintain equilibrium. So, initially, there will be zero molecules on this side, and you will have the maximum number of molecules on that side. So, slowly, slowly, slowly through the diffusion, these molecules will transport like this, and then ultimately you will reach a situation where the concentration outside and inside is going to be the same.

So, the equation for the passive diffusion of a molecule across the membrane is going to be determined by the concentration outside versus the concentration inside. So, when A is an uncharged molecule, remember that we are talking about passive diffusion. If you are talking about active diffusion, then there are many more factors that are going to participate in this reaction. There is no energy required for transportation. There is no energy required for this.

Right. And it is moving in this direction. So the chemical potential, what it is actually going to generate is GA, which is GA naught plus RTL in the concentration of A, which is actually outside. So, here the bar represents the quantity per mole, and GA is the chemical potential of A, and the GA is the standard chemical potential of A. So, when you use this equation, you will be able to calculate the chemical potential, and according to the chemical potential, it will actually tell you about the many parameters and the many ways in which you can produce energy. So, due to the concentration difference across the membrane, it generated a chemical potential, which is delta GA, and this is going to be delta GA in versus delta GA out.

So, which is equivalent to the RT lin concentration of A inside divided by the concentration of A outside. If A outside is higher than the inside, delta G will be less than 0 because this value is going to be, you know, this value will be negative, right? And because of that, it will cause the spontaneous diffusion of A from outside the membrane to the inside. So, remember that if you calculate the delta G, and it comes out negative, which is actually the case here, it is going to give you facilitated transport, so it is not going to oppose it. If the delta G is positive, then it is actually going to say, "Okay, I will not allow you to transport," because then you require energy to facilitate the transport. If the a in is higher than the a out, then da will be more than zero, which means it is going to be positive, causing the spontaneous net diffusion of a from inside the membrane to outside, enabling the flow to occur from outside to inside, and energy-releasing processes like ATP hydrolysis must be coupled to make the overall free energy change negative and allow the inward movement of a.

Basically, you have to calculate the delta GA, and delta GA is RT times delta A in divided by delta A out. So, this is the concentration of A inside; this is the concentration of A outside. If this value is higher, then the total value of delta GA would be negative, and when it is negative, it will allow the spontaneous movement of A from outside to inside. But if the delta G is positive, like it is more than zero, then it will not allow it; it will actually oppose it. It will say, "Okay, the molecule can go from inside to outside, but if you want the molecule to come from outside to inside, then you have to work against the concentration gradient, and in that case.

.." You have to couple that with the energy source so that you can use that energy for pumping sinks. Right, so this is exactly as simple as if you are filling the water at the hilltop. Right, and you have a person here, then the water will spontaneously come from that side to this side. Right, but if you want this water to go back, then you have to spend energy into the system. Right, and that's why we require a lot of pumps and all those kinds of systems to.

You know to deliver the water right because then you are working against gravity exactly the same way you are working against the concentration gradient if the concentration inside is higher than the concentration outside. So when the molecules' membrane separates, the ions having opposite charges and electrical gradients form across the membrane. This difference in charge creates a membrane potential denoted as psi or Vm, and it is measured in volts. The membrane potential creates a force that

reduces the ion movement that would increase the potential further and encourages the movement that would reduce it. Therefore, the spontaneous movement of a charged solute across a membrane is determined by both its concentration gradient and the electrical potential difference.

So, in the previous examples, if you remember, we have assumed that the molecule is uncharged. But if the molecule is charged and if the charged molecule is going to be delivered into the cell, then it will not only change the concentration of that particular molecule, it is also going to bring additional charges. And when it brings the additional charges, it is actually going to change the membrane potential, right? Because initially, the membrane potential would be 0 volts. But if there is 1 proton that is going to enter the cell, then the electrode potential would be -1, right? And 2, 3, 4 like that, right? So because of that, the cell has to work against the two different processes. One, it is actually going to work against the concentration gradient, and the other is that it has to work against the electrode potential difference, which means it has to work against the charges as well.

And if you combine these two values, right, if you combine the effect of the chemical and electrical gradients, then it is going to be called the electrochemical gradient or the electrochemical potential. In that case, the delta G would be G_in minus G_out plus zAf_in minus A_out, right? So basically, you are bringing in one additional factor for measuring the electrical gradients, right? And in that case, delta GA would be like RTlin delta A in by delta A out plus ZAF and the difference of the charges or electrode potentials. So, if the delta is delta in minus out, the delta GA is the electromagnetic potential, ZA is the ionic charge of A, and F is the Faraday constant. So, this is the Faraday constant; ZA is the ionic charge of A, which means, for example, if it is a proton, then ZA is going to be 1, and so on. So, this moment of the solute follows the second law of thermodynamics.

We state that the molecule naturally moves towards a state of maximum randomness and minimum free energy. So, this is the situation for passive diffusion, right? In this case, we assume that the concentration outside is going to be high. So, if there is passive diffusion, there are two factors that will participate. One is the concentration; the other one is the charge. Both of these factors are going to determine on which side the molecule will move.

And we have taken an example of an uncharged molecule. We have taken an example of when the molecule is going to be charged and does not require the receptors. It does not require transporters. So, presume that these are the additional conditions, correct? If you are actually having a charged molecule but it requires additional transporters or channels, then this equation will not hold. Then actually you require additional parameters to be considered because the movement of those molecules and the functioning of those molecules also require an additional amount of energy.

So what will happen if there is active transport, right? So what will the energy be and what will the thermodynamics of the active transports be? So active transport is not

thermodynamically favorable. It is endergonic, right? So, when the concentration of A is higher inside the cell than it is outside, delta G is positive. This means A cannot move inward on its own and needs to be coupled to a favorable process like ATP hydrolysis to drive the process. So basically, thermodynamics is only studying the spontaneous process; non-spontaneous process is that if your concentration is high inside and low outside, in that case, also if you require the material that is outside, then you actually have to work against the concentration gradient. And in that case, you're supposed to spend a lot of energy.

Remember that what we have discussed delta GA. If it is negative, then the process is spontaneous, smooth, and does not require energy. But if delta G is positive, or, you know, more than zero, then it actually requires some sort of energy. Now, there are different types of processes that are occurring in the eukaryotic system. We have discussed some of these processes in the prokaryotic system.

So we discussed passive transport and we have also discussed exotransport, right? So, in passive transport, the first process is diffusion, right? And remember that the last time we discussed the diffusion when we were discussing the types of transport processes in the prokaryotic system. So, we are not going to discuss the diffusion. Now, we will discuss facilitated diffusion. So, remember that facilitated diffusion is different from normal passive diffusion, right? So, passive diffusion is where the concentration gradient works, but facilitated diffusion is something different, right? So, what is facilitated diffusion? Facilitated diffusion is a type of passive transport in which the molecules move across the membrane according to their relative concentrations. So, this is true even for normal passive diffusion as well.

It does not require external energy and transports along the concentration gradient or per charged molecule in response to the membrane's electric potential. The transport of molecules does not occur by dissolving into the bilayer but by the protein that enables the molecules to cross the membrane without exposure to the hydrophobic interior. So this actually makes a difference between normal diffusion and facilitated diffusion. So normal diffusion occurs because the molecules dissolve into the lipid bilayer and then cross the bilayer.

But in this case, it is not. It actually requires a protein. It requires a transporter. It requires some kind of channels or those molecules that will enable the transport. So it is actually going to use that particular pathway, right? But still, there's no need to spend energy because it is a diffusion process. So it is actually going to be the concentration that is going to decide the transport.

Polar and charged molecules like ions, carbohydrates, and amino acids can cross the membrane without any expenditure of energy. As long as you are not spending energy, all those processes are passive processes, whether it is diffusion or something else. The movement across the membrane is mediated by the transport proteins, which expand the entire membrane and enable specific molecules or ions to pass through by diffusion. So we have already discussed this when we were discussing the procreative system, that you

have the molecules on this side, and then they will actually use a transporter by different types of mechanisms. So this is going to involve inward and outward movements.

Of the molecules, and that's how they will be transported to the site. So it can have the protein channels that are actually going to have a track, and utilizing this track, these molecules will enter into the cytoplasm. Whereas in the case of carriers, it is going to perform the flipping moments and all that. So facilitated diffusion is typically carried out by two main types of proteins, which are called carrier proteins and channel proteins. And we discussed a lot about carrier proteins and channel proteins in our previous lectures.

Now the carrier protein transports the molecule by binding it on one side of the membrane, leading to conformational changes that allow the molecule to move across the membrane and be transported to the other side. On this side, you have the molecules. This is a carrier protein; it is actually going to bind the molecule, and then there will be, you know, this movement—remember when we were talking about that? So it is actually going to have the Inward and outward movement, and because of that, the molecule will come here, and then it will open on this side, and that's how this molecule will be present in the cytosol. Sugar, amino acids, and nucleosides are transported across the membrane using carrier proteins in most cells, right? This is just a general mechanism; we have not taken the examples right. How we are going to happen? One of such examples is the glucose transporters, or GLUTs, which are examples of the carrier proteins involved in the uptake of glucose.

So, this is the structure of the GLUT protein, where you have the 12 transmembrane helices containing the intracellular amino and carboxyl terminals, and they are classified into GLUT1, GLUT2, GLUT3, GLUT4, and so on. These are the 12 transmembrane domains, and their job is to transport glucose from outside to inside, right? So in class 1, you are going to have GLUT1, GLUT2, GLUT3, and GLUT4. Whereas class 2 includes GLUT5, GLUT7, GLUT9, and GLUT11. Class 3 includes GLUT6, GLUT8, GLUT10, GLUT12, and GLUT13. And how is it actually going to be transported? So mechanism is some studies suggest that the GLUTs generally work by changing their conformations.

The first conformation the transporter has the glucose binding site facing outside the cell, so it is actually going to work like this, right? So it is going to work like this. So the molecule has an active site that can actually bind the glucose, but it is facing outward. Once it binds, there will be conformational changes, and it's actually going to open the cavity on this side, and that's how the glucose will be transported. So, upon binding the glucose, it undergoes another round of conformational changes that cause the glucose molecule to phase inside the cell, releasing the glucose into the cell. The transporter then returns to its original conformation, and as soon as the glucose is removed again, it will revert back to this particular conformation where it can actually receive the glucose from outside.

In most of the cells, glucose enters through facilitated diffusion because its extracellular concentration is very high; inside the cell, glucose is quickly metabolized or

phosphorylated with the help of the enzyme called hexokinase, and glucose is converted into glucose-6-phosphate. So all the glucose molecules are either present as glucose-6-phosphate or will be converted into glucose-1-phosphate. And then glucose-1-phosphate is going to be used as a substrate by an enzyme called glycogen synthase. And that's how it is going to be converted into glycogen, but this reaction is not occurring in all the cells; it is only occurring in the muscle or liver cells. However, this reaction is occurring in all the cells, so as soon as glucose enters the cell, it does not remain as glucose.

It is going to be phosphorylated, and that is how it is going to produce glucose 6-phosphate, and then it will enter into the metabolism; it is going to be utilized. But some portion of the glucose is going to be converted into this form, and that is how it is actually going to be used for storage, so that you can use this particular glucose in starvation conditions. So, keep the intercellular level low and allow for continuous uptake. However, since the glucose transporter is reversible, glucose can also exit the cells, such as liver cells, that produce and release glucose into the blood. So since these glutes, all the proteins can actually have the conformational changes.

They can actually deliver the glucose on this side and on that side. The same protein can be used even for releasing glucose, right? So basically that happens when there is a starvation condition. When there is a starvation condition, glycogen will be broken down and will actually produce glucose, right? And that is going to happen within the liver cells. And then this glucose is going to be utilized by Glut4 or Glut3, whatever the transporter, and then it is going to be used for releasing the glucose into the blood, and that's why there is always a dynamic equilibrium between the blood glucose level and what is present in the liver, and that's why it is always said that if you actually have a functional liver, right? Then you will actually be going to maintain the blood glucose level very nicely. Most of the time, when people are suffering from diabetes or glucose-related disorders, it is actually linked to problems with the liver rather than the pancreas.

So channel proteins allow the diffusion of specific molecules through the membrane by forming open pores. Many cells have aquaporins, which are water channel proteins that allow the movement of water at a faster rate than simple diffusion through the membrane. So, facilitated diffusion is. Actually, you know, it is very rare that it is fast, right? Compared to normal passive diffusion, right? Because passive diffusion is only going to occur through normal concentration gradients. Another example of the channel proteins is ion channels that allow the movement of ions through the membranes.

They allow the rapid movement of ions and are highly selective for ions of appropriate charge and size. Most ion channels are regulated by GATs that open in response to a specific signal to allow transport. Remember that we discussed the voltage-gated ion channels and other things. And then we have the ligand-gated channels, which are opening in response to the signaling molecules. So, that is also what we discussed in the previous lecture, right? Examples are nicotinamide and all these receptors that are present in the brain and their cation-selective channels, which are involved in neurotransmission in the central and peripheral nervous systems.

And this particular receptor requires the four or five transmembrane subunits. Each contains the subunit having the four transmembrane domains, which are M1 to M4, and an intercellular loop and extracellular N-terminus having the cysteine loop and the C-terminus. The ligand-binding site is near the N-terminus. They can be homopentameric or heteropentameric based on the subunit assembly. The acetylcholine receptors primarily regulate the flow of sodium, potassium, and calcium across the membrane, and examples of the ligand for this particular receptor are acetylcholine.

When acetylcholine exists in different conformational states, the receptor mostly has two acetylcholine binding sites; ligand binding causes the stabilization of the open state, which allows the movement of cations for a few milliseconds before closing back to a resting state. Then we have the voltage-gated channels open in response to the electrical potential across the membrane. So, for example, these voltage-gated potassium channels are mostly operative in neurons and other types of brain cells. And they consist of the four subunits that are identical, and each subunit has the six transmembrane domains S1 to S6. S4 is the voltage sensor, while S5 and S6 form support containing a pre-loop that confers ion selectivity.

So it is not that the voltage-gated channels are going to work against a voltage channel, right? Against the change in voltage. It is actually going to work against a particular ion and change the voltage by the particular channel, right? What is the mechanism? So, according to some studies, membrane depolarization leads to conformational changes in the voltage-gated potassium channels, which allow the open conformation to be energetically favorable, leading to the activation of the channel. So, this is what it is going to say, right? So, this is the intercellular side, this is the extracellular side, and you have the S4, you know, the transmembrane domain. And when there is, you know, the entry of the molecule, this particular charged molecule or this particular, you know, voltage-gated channels are going to change the structures, right? Or change the charge, right? And because of that, it is actually not going to allow the moment, right? So it is going to be closed. But when there is, it is going to open, right? When there is a depolarization, it will open, and that is how it will allow the moment.

So, under the negative membrane potential, the S4 is pulled down because of the electrical attraction as the inner membrane is negatively charged, changing the conformation of the channel to a closed state. So when there is a negative charge on the inner side of the membrane, it is actually going to attract the positive charges present on the receptor, and because of that, it is going to close the receptor or close the channel, and there will be no movement of molecules. But then, when there is a depolarization and there is a positive charge inside, there will be a repulsion, right? So, there will be a repulsion, and because of that, the channel is going to open. Upon depolarization, the negative membrane potential is decreased or reversed, S4 is now pulled to the outer membrane, changing the conformation to an open state. Now, let's talk about filtration, right? So, filtration is another way of passive transport.

So it is also called the method of fatigue transport that relies on the pressure gradient. So molecules are transported from a region of high pressure to a region of low pressure.

Filtration is non-specific, meaning molecules pass through based on their size and the pressure gradient rather than being selected. Thus, molecules that are small enough to pass through the membrane can be transported.

The force driving this movement is called the hydrostatic pressure. One example is capillary exchange. So hydrostatic pressure drives fluid transport between the capillaries and the tissue. Capillary hydrostatic pressure specifically refers to the pressure exerted by blood against the capillary wall. CHP pushes the fluid out of the capillaries and into the tissue. So this is actually a way in which the lymph flows, right? Remember that we have lymph fluid, right? Lymph is actually getting into the blood and coming out of the blood, and you know that lymph is circulating throughout our body so that it can clean the body, and that's how it collects all the debris and those kinds of molecules.

On the other hand, lymph is also providing nutrition. In the same way, when blood flows in with high pressure within the capillaries and when it reaches the end of the capillaries, The fluid that is present in the blood comes out and then goes into the neighboring tissue. And that's how it provides nutrition to the neighboring tissue. In the same way, when the pressure is applied, it is also taking up the molecules from this particular site. So, from the tissue site, it provides nutrition and oxygen. And then, at the same time, it is collecting carbon dioxide, waste material, and so on.

As the fluid moves into the tissue, the interstitial fluid pressure rises, opposing the CHPs or opposing this movement. CHP from the arterial pathway is usually higher than the FHP because the lymphatic vessels continuously absorb excess fluid from the tissues. This fluid moves out of the capillaries into the tissue, right? Filtration is one of the very important mechanisms through which the body is giving some molecules simply by diffusion through the blood capillaries, and one such mechanism is used for delivering oxygen while taking up carbon dioxide from the tissue sites. Then we have osmosis, which is also a passive transport. Osmosis is the movement of water molecules through a semipermeable membrane from an area of high water potential to an area of lower water potential.

An example is capillary exchange; osmotic pressure, also known as oncotic pressure, drives the reabsorption by moving fluid from the interstitial fluid back into the capillary. It is determined by the concentration gradient of solute between the blood and the tissue fluid. A region with higher solute and lower water draws water from the region with low solute and high water concentration. So osmosis is following these reverse mechanisms.

It is a movement of water molecules from high concentration to low concentration. Which means it is a movement of solute from high concentration to low concentration. So when the blood colloidal osmotic pressure, or B COP, is generated by the plasma protein that cannot cross the capillary membrane, this creates a high solute concentration in the blood and then in the interstitial fluid, drawing water back into the capillaries. This is actually a mechanism through which the kidneys are working, as the kidneys are absorbing nutrients from the waste material and so on; they are actually using the mechanism of osmosis. Since the interstitial fluid has very few proteins, these osmotic

differences drive the reabsorption and pull the dissolved molecules with the water. So, this is how you can understand that the nephrons work: when the blood is flowing properly, it actually delivers all the molecules to the kidneys, and that's how they go through a process of filtration and reabsorption.

So, in the reabsorption, it is actually going to be taken up again back into the blood, right? Then we'll talk about the active transport. So in active transport, we have primary active transport and secondary active transport. So let's talk about active transport. So active transport moves the molecule against its concentration gradient using energy commonly derived from ATP hydrolysis. So remember that ATP is the most preferred molecule that you can use for producing energy.

But it is not always ATP. You can use alternatives as well. It can also utilize the energy from light or the electrochemical gradients. Active transport is classified as primary or secondary based on the energy source you are going to use. And remember this particular type of movement that is actually going to happen because of active transport. where the ATP is actually going to induce the conformational changes of inward and outward movement in reverse fashion. And that is how even the molecules are more here and less there; the movement would not be from here to here; the movement will be from here to here.

Although this is against the concentration gradient, that is why you require a significant amount of energy. So, what is the primary mode of transport? So, primary active transport directly uses energy, commonly from ATP, photons, or the electrochemical gradient, for the movement of molecules from low to high energy across the membrane. So, you are going to use the ATPases that catalyze the ATP-driven transport of molecules. Hydrolysis by the enzyme causes the loss of phosphate from ATP, leading to the formation of ADP. This causes the conformational changes of the transporters, leading to the inward or outward movement of the molecule to be transported.

Another classical example of where the ATP is going to be used is needed. So you have the molecule on this side, and then when the ATP binds, there will be inward and outward conformational changes, and that's how the molecule will be delivered into the cell. And then we have the ion pumps that contain the ion gradient across the cell membrane as examples of primary active transport by ATP hydrolysis. Sodium-potassium ATPase is a common example of an ion pump. So sodium-potassium ATPase is going to utilize the ATP, and that's how it is actually going to throw the sodium outside, whereas the sodium is high on this side, right? So high is on this side, and it is actually going to take up the potassium.

And this is what is going to happen in the neurons. And that's how there will be conduction of an electrical pulse, right? So this is all we have discussed in detail in one of my MOOC courses called Basics of Biology. The concentration of sodium outside the cell is 10 times greater than inside the cell. In contrast, the concentration of sodium is higher inside than outside. But the sodium-potassium ATPase, or the sodium-potassium pump, is going to maintain the concentration gradient, which means sodium is more

outside and potassium is more inside; it is going to take up the sodium from outside and throw the sodium outside, actually. So, ion pump transport occurs against their concentration gradient by using the energy provided by ATP hydrolysis.

For every ATP consumed, the ATPase pumps 3 sodium ions outside and 2 potassium ions inside, so basically one molecule of ATP is going to be utilized, and in that process, it is actually going to throw three molecules of sodium outside. So remember that the concentration of sodium is going to be very high outside. But even then, it is working against the concentration gradient. And it is going to take up the two molecules of potassium, although the potassium will be very high inside.

And because of this, there will be a loss of positive charge. And because of that alone, it is actually going to induce the electrical pulse, and that is going to be conducted across the neurons. So the structure consists of the catalytic alpha subunit and the beta subunits. And the alpha subunit has 10 transmembrane helices containing the ion binding site. So, this is the general structure of the sodium-potassium ATPase.

The beta subunit has a single transmembrane domain and is highly glycosylated. So, this is the beta domain. And the pump has three sodium binding sites and two potassium binding sites. So basically this has 10 transmembrane helices. You can have one, two, or something like that, right? And the 10th one, then you have the beta subunit, which is actually going to have the heavy glycosylated transmembrane domains. How is it going to transport the sodium? So it is actually going to have the conformational changes between the two main states.

One is called the E1 state, the other one is called the E2 state. So E1 state has a high affinity for sodium and it is open to the cytoplasm, right? Whereas E2 has a high affinity for potassium and is open to the extracellular side, remember that it will have two states: one is E1, and the other is called E2. E1 has a very high affinity for sodium, which is outside, while E2 has a very high affinity for potassium, which is actually from inside. Sodium ions first bind to the high-affinity side inside the cell, stimulating the ATP hydrolysis and phosphorylation of the form.

What happens is that sodium is going to bind. And so this one is going to bind the sodium from within. And as soon as it binds, it is actually going to induce the hydrolysis of ATP, right? And once there is hydrolysis of ATP, it is actually going to induce the conformational changes. And because of that, the sodium is going to be pumped outside. When the sodiums are going to be pumped outside, it is actually going to acquire the E2 state, right? This causes conformational changes that expose the sodium binding site to the outside, reducing their affinity for sodium and releasing it into the extracellular space. Once this happens, the sodium is going to be released, and at the same time, it is also going to expose the potassium binding site, or K plus binding site, which means it is going to acquire an E2 state, right? And the E2 has a very strong binding to potassium, right? So, as soon as it acquires the E2 site, right? It is going to bind potassium.

And then simultaneously, high potassium binding sites are exposed on the cell surface.

The binding of extracellular potassium causes the hydrolysis of the phosphate group that was attached to the pump. And this causes another conformational change that exposes the sodium to acetosol and releases the potassium into the cytosol, so as soon as the potassium binds, that binding induces the conformational changes, and because of that, the phosphorylated group is going to be removed from this sodium-potassium ATPase. And then these, it is going to induce another round of conformational changes. And because of that, the sodium will be delivered. And then it is again reaching back to the even state where it will again bind another molecule of potassium, right? So, it will again bind the three molecules of potassium.

Again, there will be a binding of ATP, and then the same cycle continues; basically, it is in the E1 state where it will actually bind the sodium from inside, and once it binds, there will be conformational changes. Because of that, the sodium is going to be transported outside, and the molecule is going to be converted into the E2 conformation, where E2 will actually bind the potassium. And the potassium is again; this will reverse, right? E2 is going to form the E1 conformation, and potassium is going to be delivered inside.

In this process, one molecule of ATP is going to be consumed. Then we have the secondary active transports. So, secondary active transport moves the molecule from low concentration to high concentration using energy from an ion gradient instead of ATP. It functions through the electrochemical energy generated by the ion pumps and can include symporters and antiporters. Symporters utilize the molecule of one molecule in the direction of its concentration gradient for the transport of another molecule against its concentration gradient. Both particles move in the same direction. So, symporters are moving in the same direction, whereas the antiporters are moving one molecule down its concentration gradient, and that energy is used to transport another molecule up its concentration gradient.

So, it moves the molecule in a different direction, which means one molecule is moving in this direction while the other one is going in that direction. Then we have these importers, so we'll take an example: the sodium gradient created by the sodium-potassium pump serves as an energy source commonly used to drive the active transport of sugar, amino acids, and iron in mammalian cells. Epithelial cells in the intestine use active transport driven by the sodium gradient to absorb dietary sugars and amino acids; for example, glucose uptake is mediated by a transporter that simultaneously moves sodium ions and glucose molecules into the cell. The energy for the glucose uptake comes from the flow of sodium down its electrochemical gradient, so remember that the secondary transporters are where it is actually going to draw the energy from the electrochemical gradient or electrochemical potential rather than from ATP or any of the other perforated molecules.

You have primary active transport and secondary active transport. Primary active transport uses energy in the form of ATP, while secondary active transport uses energy in the form of the electrochemical gradient. So a family of glucose transporters called sodium glucose-linked transporters, or SGLTs, is involved in the active transport of glucose. Two members of the protein families are SGLT1 and SGLT2, both functioning

as symporters. Both proteins have 14 transmembrane helices. SGLT1 facilitates glucose absorption in the small intestine, while SGLT2 is involved in glucose reabsorption in the kidney.

SGL2-1 co-transports two sodium ions and one glucose molecule into the cell. In contrast, the SGL2 co-transports one sodium ion with one glucose molecule. So, this is the structure of the SGLT2 and where you have the 14 transmembrane helices. Then, what is the mechanism? The model suggested for glucose uptake by SGL2 is the alternating access mechanism. So, upon binding a single sodium ion, the transporter undergoes conformational changes to an outward-facing conformation. The conformational change promotes the binding of glucose depending on the concentration of sodium across the membrane.

Glucose and sodium are then released into the cell by an inward-facing conformation. Then we have the antiporters. Antiporters are where you have the molecules moving in opposite directions. So sodium calcium exchangers are a family of membrane proteins that mediate the entry or exit of calcium across the cell membrane. The exchangers typically export one calcium in exchange for three sodium, contributing to the depolarization because there will be a change in the charge. When you bring in one calcium, you bring in two positive ions, but when you are exchanging for three sodium, you are actually losing three plus, right? So this means there will be a deficiency of one electron. So NCX is present in many cell types, like cardiac cells and neural cells, and plays an important role in removing calcium in the resting state, thereby maintaining the low concentration of sodium in the cytoplasm.

Normally, the sodium gradient produced by the sodium-potassium ATPase is the driving force of the exchanger. At resting membrane potential, the exchanger transports calcium outside of the cell due to the high concentration of sodium outside the cell. However, elevated intracellular sodium levels can reverse the exchanger's direction, leading to calcium entry and sodium export. The direction of ion flux is regulated by many factors, such as ion transport, stoichiometry, membrane polarization or depolarization, and the transmembrane gradient of sodium and calcium ions. One of the examples of this is the, you know, the structure that consists of 10 transmembrane helices, TM1 to TM10, right? So, this is the transmembrane structure.

And it has two hubs, like TM1 to TM5 and TS6 to TM10, that are oriented inversely. Alpha 1 and alpha 2 are highly conserved repeats and make the four-helix structures, like TM1 and TM3, TM7, and TM8. Creating an ion channel passing for the binding site is correct, so this is the ion channel binding site; the two hubs are connected to TM5 and TM6 by a cytosolic F loop, and the loop contains many regulatory sites. The regulatory sites present in this loop are the XIP, which is called the auto-inhibitory XIP domain.

Then we have the sodium potential in the sodium inhibitory domain. Then we have a THB. This is a THB, right? It is called a 2-helix bundle domain. Then we have CBD1, which is a calcium-binding regulatory domain 1. Then we have a CBT2, which is a calcium-binding regulatory domain 2. Then we have a CAL, which is a calmadoline

binding domain.

And then we also have PAL, which is called a permutylation domain. What is the mechanism? How are the antiportals working? So a proposed model explains the process of inactivation of the cardiac FCS1 induced by sodium and its activation by intercellular calcium. Inactivation of the exchanger occurs while operating in reverse mode with sodium efflux and low concentrations of intracellular calcium. The exchanger is sodium-loaded with the inward-facing state. When there is a high concentration of intracellular calcium, its binding induces conformational changes that cause the activation of the exchanger for ion transport. Now, what is the difference between the, you know, mediated and non-mediated transport? So, you have mediated transport, and you have non-mediated transport.

The transport of the molecule does not occur by diffusion through the membrane but by means of membrane proteins like pores, channels, carrier proteins, and pumps. In contrast, a non-mediated transfer occurs through simple diffusion across the membrane. So it is, you know, where the concentration gradient will work. The transport protein spans the transmembrane and provides an aqueous environment for the transport of molecules that are not suitable for the phospholipid.

The molecule does not require the transport proteins. It can include both active mediated and passive mediated transport, and it only includes passive transport. So in non-mediated transport, you can only have passive transport. Examples are glucose transporters, ion channels, and ATP-dependent pumps. Here the examples are gases like oxygen, carbon dioxide, and small nonpolar molecules like benzene, and so on. Now, in terms of uptake kinetics, the differences are that in the graph, like if you are looking at solute flux, how the transport is occurring, right? Remember that non-mediated transport is thermodynamically more favorable than mediated transport because mediated transport could be linked to energy, requires particular additional transporters, and so on.

So basically, in mediated transport, what happens is that if you observe the flux of the molecules, the flux is going to increase and then it is actually going to reach a saturation point because you require the protein, right? You require a protein, the carrier, or the transporters to facilitate. So once the transporters become saturated, there will be no additional uptake, right? Then there will be no additional uptake of the molecules. Whereas non-mediated transport is completely governed by the concentration of the molecule. So it is actually going to be linear with the concentration of the molecule.

So the rate of flux is also going to be linear with the concentration. So as the concentration is very high to high, the molecules will be transported across the cell membranes. So, for non-mediated transport, the flux increases linearly with the concentration gradient. Whereas in mediated transport, there is a saturation effect caused by the limited number of transport proteins. Once enough proteins are present to constantly occupy all binding sites, maximum flux is attained, and an increase in concentration would not overcome the limit, right? So non-mediated transport is going to be, since it is following the saturation kinetics, it is actually going to be more specific.

So, it is actually going to be regulated. Whereas in non-mediated transport, it cannot be regulated. If you have a large quantity of glucose outside, or if you have a large quantity of, you know, the XYZ molecule, and if it can passively diffuse, then all the molecules will be delivered into the cell irrespective of whether the cell wants them or not, right? So the membrane transport facilitators or the membrane transport proteins are specialized proteins that consist of the movement of a molecular substance across the cell membrane. These proteins can function in different ways, including passive transport and active transport, which require energy such as ATP hydrolysis. They can also include the channels, carriers, and pumps, which help to move the ion molecules and other substances that cannot diffuse freely through the lipid bilayers. Then we'll talk about the specific classes, or we'll take some examples.

So we can take one example of calcium ATPase, and the other example we'll take is the proton pumps. Just to explain how the transport phenomena occur in both of these cases. So, in the case of calcium ATPase, the calcium ATPase is the pump located in the plasma membrane and the membranes of many organelles, such as the endoplasmic reticulum, right? And they actively transport calcium ions against their concentration gradient. One example is the plasma membrane calcium ATPase, PMCA, which belongs to the P-type ATPase family. These pumps utilize ATP by forming a transient phosphorylated intermediate during the transport cycle; it works like calcium ATP stoichiometry. Thus, hydrolysis of one ATP is required for each calcium ion transported, so it's a very energy-driven process, and the pump has a high affinity for calcium.

So this is the structure of calcium ATPase. It is proposed to have the 10 transmembrane domains and two large intracellular loops. So this is the intracellular loop, right? What you have is a short loop linking the domain on the external side. The cytosolic loop between domain 2 and domain 3, like this one, right, is termed the transfuser domain, having many important sites such as the acidic phospholipid-binding domain. These phospholipids are responsible for increasing the calcium affinity of the pumps. Then we have the cytosolic pump loop between domains 4 and 5, which also has a significant site, such as the ATP binding site.

The loop contains the catalytic center. The carboxy-terminal tail sites, such as the calmodulin binding domain or CMBD, are present. CMBD is an autoinhibitory domain, and the binding of caldoidiline removes the autoinhibition from the pump. Is the mechanism, so the mechanism is explained by the E1 and E2 model. Remember that what we have discussed in the past is that it is going through a cycle from E1 to E2, right? So the pump alternates between the two conformations, where E1 has more affinity for sodium and E2 has potassium. So, exactly the same way here, it is also going to follow the E1 conformation. So in the E1 conformation, the calcium sites are oriented towards the cytoplasm, and in the E2 conformation, the calcium sites are facing outside the cell.

So, upon binding the calcium, the ATPS activity of the pump is stimulated, resulting in the formation of a phosphorylated intermediate, which is the E1P, and the occlusion of the bound calcium ions. And then what happens? The calcium ion is transported across

the membrane simultaneously with the release of stored energy as the pump shifts from E1 to E2 conformation. This conformation has low affinity for calcium and thus releases the calcium on the extracellular side. Afterwards, the phosphoenzyme undergoes hydrolysis and the pump transitions from the E2 to E1.

So, this will restore the conformation so that it can go for one more round of calcium transport. The outward movement of the calcium, which carries the positive charge, is at least partially balanced by the inward movement of protons to help maintain charge neutrality across the membrane. Now let's talk about the proton pump. So the proton pump is very important. It is being utilized very extensively in the mitochondria and the chloroplasts for producing energy.

So proton pumps are membrane proteins that move protons across the membrane. thereby establishing and maintaining an electrochemical proton gradient and being involved in primary active proton transport. So the proton pump can drive energy from various processes like light energy, energy-rich metabolites, etc. Then we have the VATPHs, or the vacuolar-type H+ ATPase, that is present in the lysosomes and is important for maintaining, or you know, producing the low pH within the lysosomes. And they can also be found in the endomembrane of organelles like vacuoles, lysosomes, the Golgi apparatus, etc. And this is a typical example of the, you know, VATPase, although we have also discussed the other ATPase as well, right? So ATP synthase, when we were discussing it, is like having a similar kind of structure.

So, it contains the two major functional domains that are called V1 and VO. And V1 is a soluble domain. It contains eight different subunits and has three catalytic sites formed by subunits A and B. For ATP hydrolysis, the subunits are present in the stoichiometry of A3, B3, CD, E3, F, G3, and H. The VO is a membrane-embedded domain.

It contains up to five subunits. The subunits are present in the stoichiometry of this, and they play a role in the proton translocations. Then the VATPS functions as a rotary motor, right? Consisting of both stationary and rotating components. So this is a stationary component, and this is a rotary component. So this consists of the A_3B_3 hexamer with three ATP binding pockets, three peripheral EG stalks, and the subunit THA. Whereas the rotor consists of the central stock of subunit D and F linked to the protolipid ring of subunit D and A of the CNV.

Subunit E is possibly a stator subunit. What is the mechanism? A conformational change in the catalytic hexamer due to ATP hydrolysis causes the rotation of the central stalk and the proteolipid rings. The rotation of the ring relative to subunit A is important for the transport as the subunit has hemichannels. One is the cytosol-facing hemichannel that enables the entry of protons. The other is a lumen-facing hemichannel that enables the exit of protons. The stator subunit component stabilizes the complex and prevents the rotation of the stator subunit; the C termini of EG bind to the N termini of the B subunit, and the N termini of EG interact with the subunit CH and N-terminal region of the subunit C and H.

consists of two globular domains termed as the C head by C foot and CNT by CT, which are linked by the flexible connector. VATP's activity is highly regulated, right? Primarily through the reversible assembly into the V1 and V0. Remember that V1 is the rotatory domain and V0 is the stationary domain, which is present in the task membrane by a process known as regulated assembly. In the dissociated state, both ATP hydrolysis by V1 and proton transport are inactive. During disassembly, there are conformational changes in the subunit H causing the HCT to interact with the subunit F to possibly prevent the rotations.

So, this is all about what we discussed in today's lecture. What have we discussed? We have discussed the moment of the molecule across the membrane for its different types of cellular functions and how it is regulated. The movement of the molecule is regulated across the membrane. We discuss passive transport. We have discussed the thermodynamics of passive transport. And we understand that it is the concentration gradient that is actually going to drive it, and it will make the free energy changes right; if you want movement across the concentration gradient, then you are supposed to spend energy in the form of ATP, or you can actually derive energy from the gradients or electrochemical gradients.

So based on these energy sources, the transport could be the primary active transport or the active transport. We have taken a couple of examples from each category and each example so that it would be easy for you to understand the mechanisms of these molecules, the mechanisms of the transport processes, and so on. So, with this, I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss some more aspects related to the transport of molecules across the membrane. Thank you.