Cell and Molecular Biology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati
Week 04
Cellular Transport
Lecture - 17
Transport in Eukaryotic Cells (Part 2)

Hello, everyone. This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering at IIT Guwahati. And what we were discussing was the uptake of molecules or the movement of molecules within the cell. So far, what we have discussed is the different processes that are required for the transport of material into the cell. In this context, in the previous two lectures we discussed, we focused mainly on the transport of what is happening from outside the cell.

 Right? So if you see the transport right, the transport is very essential for many types of functions, like transport being essential for uptake and taking out nutrition; transport is important for driving cell signaling and so on. So far, we have focused only on the uptake of material from outside, and in that process, we have discussed the diffusion, osmosis, active transport, passive transport, and so on. Now, in today's lecture, we are going to focus on the transport of material inside the cell, right? And within the cell as well, right? Because you are not only going to have transport from outside, but you're also going to have transport within the cell and within the different organelles as well. So when you talk about a cell, it actually requires the uptake of the molecule, which is from outside; so when it is crossing the outside, it is actually crossing this particular barrier.

 Depending upon the chemical property or the structural property of this molecule and the chemical property of the barrier, there are different methods that the molecule or the organism is going to adopt so that this molecule will get inside the cytosol. Now, once it gets into the cytosol, then it has to be distributed. To the different parts of the cell. Then it has to be distributed to its final destination. Its final destination could be the mitochondria, the lysosomes, the nucleus, and so on.

 And it could be a final destination that it has to be in the fat cells. So in today's lecture, we are going to discuss the intracellular distribution of the molecule. So how is the transport occurring inside the cell, how is this molecule transporting to the mitochondria, how is this molecule transporting to the nucleus, and so on? So, the transport of the material within the cell is actually being considered; when the molecule is going to be internalized, it is going to be utilized for these kinds of intracellular transports. So, endocytosis is a process by which the eukaryotic cell internalizes molecules such as plasma membrane components, fluids, solutes, micro molecules, and particulate substances. An example is if you are supposed to take the glucose, if you are taking the particulate matter like bacteria and so on.

 The internalized cargo may include receptor-ligand complexes, various nutrients and

their transporters, components of the extracellular matrix, cellular debris, microbes such as bacteria and viruses, and, in specialized cases, even the whole cell. For example, if one cell is undergoing apoptosis and another cell is going to take up the whole cell so that it can actually digest that particular cell and then redistribute the nutrients that are present. This process also allows the cells to regulate the composition of their plasma membrane in response to the changing external conditions. Endocytosis can generally be divided into two broad categories: phagocytosis and pinocytosis. Phagocytosis means eating, and pinocytosis means drinking.

So further various classifications of pinocytosis have been proposed, and the classification of endocytosis is that it is broadly classified into two processes: one is called phagocytosis, and the other is called pinocytosis. Phagocytosis means eating the particles, right? We are going to discuss this phagocytosis in detail when we discuss the immune responses. Then we have pinocytosis. Pinocytosis means drinking, or drinking the fluids. Now, pinocytosis could be of two types.

It could be a clathrin-dependent process or a clathrin-independent process, right? So, clathrin is a protein, right? And within the clathrin-dependent process, you can have clathrin-mediated endocytosis, right? And then within the clathrin-independent process, you can have the caveolae-mediated processes, the caveolae and clathrin-independent process, and then you have macropinocytosis. And within the caveolae and clathrin-independent processes, you can have the ARF-associated endocytosis; you can have the CLIC or GEEC pathway; you can have the flotillin-associated endocytosis; or you can have the RhoA-dependent endocytosis. Now, let's first talk about endocytosis. Endocytosis is the transport of molecules within the cell, right? So the cargo that is being transported is enclosed by a small portion of the plasma membrane through invagination and then pinching off to form an endocytic vesicle that contains the cargo. So when the molecule enters, it is actually going to form a vesicle, right? It has to be trapped within the vesicles.

And this vesicle is called the endosome, right? Most of the endocytic vesicles then fuse near the periphery of the cell with the receiving compartment termed the early endosome. It is the primary sorting station where the internalized cargo is sorted. The endosomal system is complex and consists of many tubular and vesicular membrane-bound compartments. The tubular region of the early endosomes forms the vesicles that return internalized cargo to the plasma membrane directly or by a recycling endosome. The rest of the cargo is mapped for degradation and directed to form the late endosomes.

Endosome maturation is the process by which late endosomes are formed from the vacuolar portion of the early endosome. Thus, as early endosomes mature into late endosomes, their tubular extensions disappear. So this is what it shows here, right? You are actually going to have the internalization, and that's why you're going to form the early endosome. And then this early endosome will go through definite steps of processes, and that's how it is actually going to form, you know, late endosomes, and then late endosomes are actually going to fuse with the lysosomes to form the endolysosomes. Because the lysosomes have degrading enzymes, they contain digestive enzymes and

have a low pH, which allows them to degrade all the material taken up from the outside.

That's how this material is now ready for redistribution within the cytosol or the other organelles. So the maturing late endosomes, also known as the multivesicular body, are transported along the microtubule towards the cellular interiors, and when the endosome matures, it stops recycling material to the membrane, and all the remaining content is designated for degradation. Late endosomes fuse with the existing lysosomes to form endolysosomes. Lysosomes are the membrane-bound organelles containing the hydrolytic enzymes that break down the micromolecules. The degradation of the cargo takes place in the endolysosome.

And endolysosomes mature into endolysosomal hydrolysis, finishing the digestion of their contents, including the intracellular vesicles. Now, all the stages of endosome maturation are linked via the bidirectional vesicular transport process to the trans-Golgi complexes. They are connected by the transport vesicles with the Trans-Golgi network. And the transglutaminase network sorts the proteins based on their final destinations. These are actually part of intracellular tracking, right, and we are not discussing this in detail, right, because we briefly discussed it when we were talking about the cellular structure.

And now we are also not getting into the molecular details of these kinds of processes. But if you are interested, there are so many excellent courses about cell biology where the instructors are focused solely on these aspects, so there are many hardcore cell biology courses available that can be followed. These pathways deliver the newly made lysosomal enzymes from the ER back to the early secretory pathways. Now, from endocytosis, it can be divided into phagocytosis or pinocytosis. Let's talk about phagocytosis very briefly because we are going to discuss phagocytosis in detail when we take up the immune system.

So phagocytosis is the uptake of large particles like bacteria. It is also called cell eating. It occurs primarily in a specialized cell type. So you know that these are going to happen in all those, you know, antigen-presenting cells like macrophages, dendritic cells, and B cells. It is a type of endocytosis where the cell utilizes large endocytic vesicles termed phagosomes, so phagocytosis is a process through which you are actually engulfing material that is very large in size from outside.

Mostly, phagocytosis is used to elicit immune responses or protect cells from infectious organisms like bacteria, fungi, viruses, and so on. And they are being used for, you know, eliciting the immune responses, and that is why the vesicle that is going to be formed after the phagocytosis is known as the phagosome. They are used to digest particles such as microorganisms and dead bodies. It plays a role in ingesting the elemental particles which are larger than 0.5 micrometers, and if the molecules are bigger than this, then it is going to be called phagocytosis.

So, upon the binding of the particle to the receptor on the surface of the first, basically, phagocytosis could be receptor-mediated phagocytosis or non-receptor-mediated

phagocytosis. Mostly, receptor-mediated phagocytosis is more efficient, whereas non-receptor-mediated phagocytosis is less efficient, just like we discussed about facilitated diffusion versus normal diffusion, right? Even the passive processes are passive, but facilitated diffusion is much more efficient compared to normal diffusion, so the extension of the pseudopodia is induced, which in turn affects the actin-based movement of the cell surface. Pseudopodia surround the particle, and then the membrane fuses to form a phagolysosome. So basically what happens is that you have a particle, right? And so what happens is the cell will actually approach first; if it is receptor-mediated phagocytosis, it is actually going to bind the particles with the help of the receptors. So there will be a ligand that is present on this particular object.

For example, if there are bacteria, then they may have the, you know, bacterial-associated antigens. And these antigens are going to be recognized by the receptor that is present on the cell surface. It could be the toll-like receptors. It could be S-receptor gamma R, and it could be IL receptors. It could be interferon-gamma receptors and so on.

So once it recognizes, then it is actually going to start spreading its pseudopodia; right, it will start spreading the pseudopodia across this, right? So it is going to start spreading the pseudopodia from both sides, right? And ultimately, these pseudopodia, which are coming from the cell, are actually going to be fused. And then this particle will come in. And that's how it is actually going to form a phagosome. So this is exactly what is going to   happen   here.

Right. So the cell is actually spreading its cytoplasmic particles around the particles. Right. And then the particle cannot move around because it has only been bound to the receptor that is present on the cell surface. And then ultimately, these pseudopodia are going to be fused. And that's how it is actually going to be presented in a bilayer membrane.

Right. And that has been called the phagosome. Now this phagosome will go through a process of maturation just like we discussed for the endosomes, and then it is actually going to fuse with lysosomes. And then it is going to form the phagolysosomes, and these phagolysosomes are then going to be used for digesting the material, right? So it is going to digest the bacteria into small pieces, and these small pieces, which are the peptide fragments, are then going to be expressed along with the MHC. and that's how it is actually going to give the signal to the body that how the if there is an infectious organism we should actually now start you know sending the immune responses. So phagosomes fuse with lysosomes to form phagolysosomes, where the ingested particles are digested by the hydrolytic enzymes present in the lysosomes, and phagosome maturation then recycles some of the membrane proteins back to the plasma membrane.

So many amoebae use phagocytosis as a feeding mechanism by capturing food particles such as bacteria or protozoans. Large particles engulfed into the phagosomes are delivered to the lysosomes where they are digested, resulting in breakdown products in the cytosol for use as nutrients. So phagocytosis is a generalized mechanism through which you are actually taking up large particles from the surroundings. Now let's move

on to the next process, and the next process is pinocytosis, which means drinking. Right, so remember that drinking is always done for the fluid, not for the particulate matter.

If you are dealing with particulate matter, then it is going to be for phagocytosis. This means the size of these particles has to be less than 0.5 micrometers. Pinocytosis is the uptake of fluid or macromolecules in a small vesicle.

It is also called cell drinking. It can occur in all eukaryotic cells and include various different mechanisms. Nearly all eukaryotic cells constantly take in small parts of their plasma membrane through pinocytosis or endocytic vesicles. A process in which the extracellular fluid or small particles are enclosed in a membrane-bound vesicle and internalized into the cell. So basically, you have the extracellular fluid, right? If this fluid could contain very small particles like glucose and fructose, all those kinds of molecules, then what happens is the cell is going to, you know, become invaginated right inside. And then it is going to be trapped, right? And because of this, this particular vesicle will be formed.

And this vesicle has actually taken up the material that is present here, right? What is present in the environment, right? So you see this green-colored particle, right? It is being taken up. So this green-colored particle is going to be taken up. And then it is actually going to form the endosomes, or they are going to be called endocytic vesicles. And then these vesicles again go through the process of maturation. So, pinocytosis could be of different types.

It could be clathrin-mediated endocytosis, caveola-mediated endocytosis, clathrin- or caveola-independent endocytosis, or micropinocytosis. So, this is micropinocytosis, this is clathrin-mediated pinocytosis, this is caveolae-mediated, and this is a case of clathrin and caveolae-independent processes. Right, so in this case, you are actually going to use the clathrin proteins; here you are going to use the caveolae, and here you are not using anything. So first, talk about clathrin-dependent endocytosis; it is involved in the selective uptake of specific macromolecules. It is involved in cargo transport in virtually all setups, including the turnover of plasma membrane components, internalizing nutrients like LDL and iron-saturated transferrin, and the endocytosis of many growth factor receptors after they are activated.

Pathogens like influenza viruses and bacterial toxins such as Shigella toxins exploit clathrin-mediated endocytosis to enter the cell. So basically, clathrin-mediated endocytosis or pinocytosis is used for taking up small molecules, growth factors, viruses, toxins, and bacterial toxins, right? So, all of these are part of the fluids. All these parts are considered fluid, right? So, for example, the viruses also. Viruses are very small particles. So, they are not going to be taken up by phagocytosis.

They are going to be taken up by the phagocytosis. So the cargo binds to a specific cell surface receptor. Most of this cargo is present in a specialized region of the plasma membrane termed the clathrin-coated pits. Dynamin, a GTP-binding protein, helps the pit bud from the membrane to form small clathrin-coated vesicles that contain the receptor

with the bound ligand that is the cargo. The clathrin-coated vesicles fuse with the early endosomes, which leads to the sorting of their contents for either recycling to the plasma membrane or transport to the lysosomes.

Clathrin-mediated endocytosis selectively captures the ligands using the receptors. This increases their internalization efficiency of ligands more than 100-fold, leading to the uptake of large amounts of even minor components of the excess cellular fluid. And examples of clathrin-mediated endocytosis are the uptake of cholesterol in mammalian cells. Through this endocytosis, they can acquire the cholesterol needed to make the new membrane. And in this process, you are actually going to use the LDL receptors and all of that.

So the transport of most cholesterol in the blood is in the form of cholesterol esters within lipid protein particles known as low-density lipoprotein or LDL. You remember that we have three different types of fats, right? High-density lipoproteins, low-density lipoprotein, and very low-density lipoprotein. So we have the LDL, we have the HDL, and we also have the VLDL, right? The diagrams on the right side show one of the LDL particles, right? So the core has approximately 1500 cholesterol molecules. So within this, you see that these are the cholesterol molecules that are esterified to the long-chain fatty acid.

So, these are the cholesterol molecules. These are the cholesterol molecules. The core of the cholesterol ester is surrounded by a lipid monolayer consisting of 800 phospholipid molecules and 500 phospholipid molecules that are not identified. You have a lipid bilayer, which contains phospholipids. And then you also have the cholesterol molecules that are present in this particular membrane. And then you also have a protein part, which is called apolipoprotein B, playing an important role in the organization of the particle and the binding of LDL to the LDL receptor on the cell surface.

And this core is being, you know, all these lipid particles are organized around the protein part, which is called apolipoprotein B, right? And that's how this is the kind of complex particle that is going to be formed, and all these particles are, you know, being classified as the LDL particles, HDL particles, or VLDL particles depending upon the composition and the type of cholesterol present in them. So, what is the structure of the LDL receptor? So you see this is the structure of the LDL receptor where you have, on the N-terminus, the LDL binding domain. So this is the LDL binding domain, which is actually extracellular, right? And then we have N-linked glycosylation. Then we have O-linked glycosylation. All these we are not discussing, but N-linked glycosylation, where the, you know, the N is going to be utilized for linking the sugar molecules.

Whereas O-linked glycosylation involves the oxygen present on the amino acids being utilized to attach glucose molecules. Then we have the plasma membrane. So this is the bilayer, right? And this is the transmembrane region. And then you also have the cytoplasmic tail. The cytoplasmic tail is going to be used for driving the downstream signaling so that it can facilitate uptake.

As soon as the LDL particle comes and binds to this, the whole LDL and LDL receptor complex will be internalized. By the process of endocytosis. And then it is actually going to deliver the LDL into the cytosol. And then this receptor is going to be recycled and will reappear on the surface. So it consists of 700 extracellular amino acids, a transmembrane alpha helix containing 22 amino acids, and a cytoplasmic domain with 50 amino   acids.

So, a large number of our more than 90% protein is present outside; a very small fraction is present inside. The N terminal forms the LDL binding domain. So, this is the LDL binding domain. The internalization of the signal is present in the cytoplasmic tail. So this is actually a cytoplasmic tail, which is going to bind the scaffolding proteins and downstream   machinery.

And that's how it is actually going to pull the whole LDL and LDL receptor complex into the vesicles. So the cells synthesize the LDL receptor when they require cholesterol, right? And these receptor proteins are transmembrane proteins. And when they are inserted into the plasma membrane, they diffuse until they are associated with the clathrin-coated pit, which is in the process of forming. So what happens is that you are actually going to have the LDL receptor, correct? And then the LDL receptor is going to be coated with the clathrin molecule. So you are going to have the aggregation of the clathrin molecules around this particular pit where the LDL receptors are gathering.

And you see the other side; the LDL is already bound. So this is the LDL molecule that is bound. And then we are actually going to have the clathrin-coated vesicles. So these vesicles, which are actually individual clathrin molecules, are coming and coating this particular part, and that's how they are actually going to make a vesicle. Now this vesicle is going to be pulled downwards, so when it is actually pulled, it is not only going to pull the vesicles or the protein, but it is also going to remove this portion as well. So this whole thing is going to be internalized, and that's how it is actually going to form the vesicles where the LDL receptor and the LDL molecule are going to be facing downward.

Right. And then what happened is that all these LDL particles are going to be delivered into the endosomes, whereas the receptor is going to be removed once this happens. And then the LDL receptor is going to be recycled back to the plasma membrane. And that's how it is actually going to deliver the LDL into the endosomes. So upon receiving an endocytosis signal, its cytoplasmic tail, the receptor binds to the membrane-bound adapter   protein,   which   is   called   AP2.

Clathrin is then recruited by AP2 to start the endocytosis process. Upon forming clathrin-coated vesicles, the LDL that is bound to the LDL receptor is internalized in these vesicles. The clathrin coat is shed and the contents of the vesicle are then sent to the early endosome. The low pH in the endosome causes the release of LDL from the receptor, which is then delivered to the lysosome through a late endosome. The cholesterol esters present in LDL are hydrolyzed to form free cholesterol, which can then be used by the cell to synthesize a new membrane. So, once it has been present in the

early endosomes, the early endosomes will mature and form late endosomes.

The late endosome is going to, you know, fuse with the lysosomes, and that's how the material is going to be recycled, and cholesterol is going to be released into the cell, and then this cholesterol is going to be used as a building block to synthesize the new plasma membrane. If there is an excess of free cholesterol inside the cell, the cell stops its own cholesterol synthesis along with LDL receptor synthesis. So basically, if the cell is synthesizing a large quantity of cholesterol, then it will actually downregulate the amount of LDL receptors that are present on the cell surface. And that's how it is actually not going to remove the circulating cholesterol, isn't it? So we have discussed clathrin-mediated   endocytosis.

Now we'll talk about the clathrin-independent process. So in the clathrin-independent process, the first process is the caveola-mediated process. So it's a clathrin-independent process, but it is a caveola-mediated process. So in the caveolae-mediated endocytosis, in many cell types, the plasma membrane is covered by a small pit, which is called a caveola. They are small, bulb-shaped plasma membrane invaginations, typically 70 to 100 nanometers in diameter, and extend into the cytosol. They are present in many cell types, such as adipocytes, endothelial cells, muscle cells, fibroblasts, and astrocytes.

The caveola coat includes the protein that plays a role in the formation and stabilization of the membrane invagination. Some of the proteins include caveolin, which has three orthologs in humans: caveolin 1, 2, and 3; and caveins, which include caveins 1 to 4. And then we have the bar protein domain containing synaptin or the pasticine 2. And then we have the dynamin-radiated ATPases, which are called EHD2. So this is the general structure of a caveola, right, where you have the caveolin protein.

We have a cave-in. We have the bar protein domain containing it. And then we also have dynamine-related ATPases. Caviolins are oligomeric cholesterol-binding integral membrane proteins. They can range in size from 17 to 24 KDA and play an important role in the invagination of the caveolae from the plasma membrane. The cavioline protein family is encoded by three genes. They consist of six cavioline subtypes: cavioline 1 alpha, cavioline 1 beta, cavioline 2 alpha, cavioline 2 beta, cavioline 2 gamma, and cavioline   3.

The structure of Kevulin-3 consists of 151 amino acids, which form a different domain, including the intracellular domain, Kevulin scaffolding domain, oligomerization domain, and the signature motif. So these are some of the things that are present, right? We have the intercellular domains. We have the scaffolding domain. Then we have the oligomerization domain, and we also have the signature motif. OD, which is the oligomerization domain, contains the CSM, and the SM is shown by a yellow box in the diagram.

So this is what it shows here, right? Whereas the intramembrane domain plays a role in the insertion of clavulin oligomers into the membrane with the N and C terminals facing the cytoplasm, triggering the membrane structure and the mature clavulin formation

along with the other proteins. So the clavulin plays a role in membrane association, cholesterol binding, oligomerization, and the regulation of central conduction. The domain does play a role in the interaction of the scaevulin with other signaling molecules. Scaevulins are linked to each other by the oligomerization domain and form a disk-shaped oligomeric complex on the membrane.

The C-terminal can spiral assemble into barrel-like channels. Some studies have suggested more domains in the protein, such as pin motifs, spoke regions, and beta strands. These domains possibly play a role in forming the disc-shaped oligomeric complexes of the cavulins on the plasma membrane required for the cavulins' function. Kaolins play a role in lipid and fatty acid uptake, neurovascular coupling, viral internalization, melanocyte pigmentation, and many other functions. Animal viruses like SV40 and the papillomavirus can enter the cell in vesicles derived from kaolin. They are first transported to the early endosome and then, through the transport vesicles, move to the ER lumen.

The genome of the viruses then leaves the ER, goes to the cytosol, and is then transported to the nucleus, where it can initiate the infection. Suggested mechanism for the cabular internalization. So, cabuli is our dynamic membrane domain that can move literally into the plasma membrane. Both the EHDTU and fascism play a role in stabilizing the cabulin in the plasma membrane.

Cabulin detachment from the plasma membrane is possibly caused by the removal of EDH2. It is possible that dynamin might play a role here. After detachment, the cabulin is internalized and trafficked to the particular organelle. It is suggested that the various tissues and cell-specific signaling events or ligands may induce the internalization of substances such as albumin, cholesterol, and SV40, the virus, right? So this is all about the caveolae-mediated endocytosis. Now let's talk about the caveolae and clathrin-independent processes.

So clathrin- and caveolae-independent endocytosis. The endocytotic pathway that does not use clathrin and the associated molecular machinery is termed the clathrin-independent endocytic pathway or CIES. The pathway can be constituted or induced by a specific signal or exploited by pathogens. These pathways differ in the kinetics and mechanism of formation of endocytic vesicles, the molecules involved, and the destination of the transported cargo. Now, within this glycerin-independent endocytosis, you can have either the dynamin-independent process or the dynamin-dependent process. So, dynamin is a row-dependent protein, isn't it? So, within the dynamin-independent process, you can have ARF6-associated endocytosis, the GECC pathway, or flotilline-associated endocytosis.

Now, as long as the ARF6-associated endocytosis, ADP ribosylation factor 6, or ARF6, is a GTP-binding protein. It is present on the cell surface in mammalian cells. It is also present on the endosomes that have the cargo proteins from the clathrin-independent pathway. Proteins that utilize this pathway include GLUT1, CD98, LAT1, CD44, and CD147. Although the internalization is independent of clostridine and dynamine, it is

dependent   on   cholesterol.

Then what is the mechanism? So ARF6 has two states: the inactive GDP-bound state and the active GDP-binding state. The active state in the plasma membrane helps in the internalization of cargo by utilizing the energy from GDP hydrolysis mediated by PIP2. Inactive state is then associated with the tubular recycling endosome. Guanine nucleotide exchange factors like the GRP-1 associated scaffolding protein can cause the activation of ARF6. So basically, in the basal inactive state, it has always been in the GDP binding state, right? ARS6 GTP then returned to the plasma membrane and led to the formation of a pure TUDing actin-rich structure, which helps in recycling the membrane back to the cell   surface.

As this endocytic route requires cholesterol, the ARS6-associated endosomes are rich in cholesterol and PIP2. It is suggested that ARS6 activation is not required for endocytosis, but plays a role in recycling. Endocytosis has a role in the regulation of cell adhesion, migration, tumor invasion, and cytokinesis. NEDD9 is a marker as well as a target for metastatic   breast   cancer.

It inactivates ARF6 and thus inhibits the recycling of MMP14 to the plasma membrane. This increases MMP14 activity and facilitates tumor invasion. These are all things we are not discussing regarding how MMP 14 is, you know, helping tumor invasion and all that. And then we have the GECC pathway. So in the GECC pathway, we have the glycosyl phosphoenolphthalein-anchored protein used as a dynamin-independent pathway for endocytosis.

They are endocytosed into a specialized early endosomal compartment. The endocytic structures that contain the endocytes, the GPI-anchored proteins, are called the GECC or the GPI-anchored enriched early endosome compartment. They are formed by the fusion of primary uncoated clathrin-independent tubular waste carriers, called CLIC, which are derived from the surface of the cell. The pathway is then termed the JLIC-GE3C pathway. Suggested mechanisms, a guanine nucleotide exchange factor, or GEF, for ARF1,   GBP1   are   brought   to   the   cell   surface.

It activates the ARF1, which then recruits a Rho-GTPase, activating the protein to the cell surface. The complex plays a role in maintaining the Rho-GTPase TDC42 in its cycling state. TDC42 alternates between the active state and the inactive state at the plasma membrane, and this cycling is essential for recruiting the actin polymerization machinery. GTPase regulator associated with focal adhesion kinase 1 has the BAR domain,   SH3   domain,   and   the   ROJAP   domain.

It is another molecule possibly involved in GEEC formation. The rho-gap domain can inactivate CTC42. With the loss of the ARH-gap 10x20 activity, the bar domain is possibly involved in generating membrane curvature. It is suggested that after the CLICs are formed, they bud quickly from the plasma membrane, mature to the GEC stage, and then fuse with the early endosomes. Then we have flotillin-associated endocytosis. So flotillin is the protein that localizes to the specific microdomain or the lipid rafts in the

plasma membrane. There are two flotillin genes, flotillin 1 and flotillin 2, which are possibly involved in inducing membrane invagination in a dose-dependent manner.

Flotillin-mediated endocytosis is regulated by the soft family kinases like Fin. Many studies suggest the role of flotillins in the endosomal starting process and the formation of exosomal vesicles in the endosome. Some studies also suggest their role in cargo recycling. So, upon receiving the stimulus, the dynamin flotillin can oligomerize, which then recruits transmembrane proteins like the EGFR, DAT, and the ARP. So when you have any stimulation, when you are getting these stimulations, these proteins are actually going to be recruited onto the cell surface and then they will form a pre-endocytic clustering.

So they are actually going to form a cluster. Then the cargo is going to be internalized by endocytosis without the involvement of Ritalin, for example, by clathrin-mediated endocytosis. So, then this whole thing is going to invaginate with the help of clathrin-mediated endocytosis, and that is how this vesicle is going to form the endosomes. Flotillin is a cellular cargo sorting protein. So, flotillin microdomains are found not only on the plasma membrane, but also in early, late, and recycling endosomes, as well as in exosomes. In the plasma membrane, Plutalins help the transmembrane cargo proteins during cluster formation before endocytosis, as described in the previous slide.

So in endosomes, flotillin possibly plays a role in sorting the cargo for recycling to the plasma membrane, transport to the Golgi and ER, or to the intercellular vesicle of the multivesicular body that later fuses with the plasma membrane to release the vesicle as the exosome. Then we have the rho-dependent IL-2 receptor endocytic routes. IL-2 receptors are concentrated and internalized by small invaginations that are not coated. Various cytokine receptors and their compartments, like the beta chain of the IL-2 and IL-15 receptors and the common cytokine gamma chain of the receptor for the internalization of 2, 4, 7, 9, 15, and 21, utilize these endocytic routes.

The endocytosis regulated by dynamin here is cholesterol-sensitive. So, IL-2 receptors undergo endocytosis. Endocytosis is clathrin- and caveolae-independent and dependent on dynamin. So this is the dynamin, right? And the pathway depends on the rho small gTPS, which is rho A, and it is regulated by the PI3 kinase and the lipid kinase. IL-2 activates PI3 kinase due to its conversion into regulatory P85 subunits. This causes the production of PIP3 at the membrane.

PIP-3 then induces WAF-2, which is a guanine-nucleotide exchange factor for RAC. This causes the activation of the RAS. The RAC GTP then binds to the P85 and gets recruited to the sites having the IL-2 receptor. The downstream target of RAC1, P21-activated PAC1, is activated. So from this IL-2 receptor, what is present in the vesicles is actually going to be presented in the early endosomes, then it forms the late endosomes and so on.

And then it is actually going to force the lysosomes to act. From lysosomes, it is going to be degraded, and that's how the material is going to be recycled. The cascade facilitating

the recruitment and activation of the complex with corticine and WAPs, ARP 2, 3, leads to the generation of F-actin during IL-2 receptor endocytosis. Upon internalization, they are suggested to sort into early and then late endosomes, lysosomes, and either be degraded or recycled to the plasma membrane. We are already done with the clathrin or caveolae-independent processes; now we'll move on to micropinocytosis.

Micropinocytosis is another process that is also a clathrin-independent process. So, what is macropinocytosis? So macropinocytosis involves a non-specific uptake of large volumes of extracellular fluid, solutes, and membranes into a large endothelial vesicle, which are called micropinosomes. Right. So it is actually going to take up a large amount of water or a large amount of extracellular material.

Large vesicles of the size of 0.15 to 5 micrometers in diameter are involved in the uptake process. So these are the micropinosomes that are going to be formed after taking up all these fluids, which are extracellular fluids. It occurs in virtually all cells, but in most cell types, it does not occur continuously; rather, it is induced for a limited time. It is induced by the activation of a cell surface receptor by growth factors, integrin ligands, apoptotic cell remnants, and some viruses. The ligand activates a signaling pathway that causes a change in actin dynamics and leads to the formation of cell surface protrusions, which are called ruffles.

The collapsing of the ruffle back in the cell leads to the formation of macropinocytosis. So this is the first step where you are actually going to activate the cell signaling receptors. Then it is actually going to form the ruffles. And then these ruffles are actually going to rebound. And that's how it is actually going to form the macropinocytosis and pinocytosome. This temporarily increases the bulk fluid uptake of the cell; micropinosomes non-specifically trap the extracellular fluid along with other particles and micro molecules.

Micropinocytosis is a degradative pathway where the micropinosomes acidify. And then fuse with the light endosome or the endolysosome; they do not recycle their cargo back to the plasma membrane, so basically, micropinocytosis is a method by which you can remove some of this fluid from the extracellular media. And that's how you are actually going to, it's a degrading pathway where all these molecules are going to be degraded by fusing with the lysosomes or the late endosomes so that the digestive particles or digestive enzymes from these particles will digest. Then we have exocytosis. So far, what we have discussed is how the particles are getting inside and how this particle is being distributed to the different organs with the help of a process called endocytosis.

Now, what we discussed was the reverse. How a particle that the cell has is actually going to be expelled in this process, which is called exocytosis. So exocytosis, the fusion of a transport vesicle to the plasma membrane, is a fundamental process in all eukaryotic cells. It includes the transport of large molecules out of the cell to the cell exterior, right? So, because of exocytosis, you can even recycle the receptors. You can actually throw the antibody molecules and so on. So, transport vesicles from the transglial gene network move towards the plasma membrane, carrying the membrane proteins and lipids to

provide   the   new   components   for   the   plasma   membrane.

The vesicles also carry soluble proteins that are secreted into the extracellular space. For example, the cell secretes components of the extracellular matrix, such as proteoglycans and glycoproteins, through exocytosis. Exocytosis can include the transport of newly made proteins from the ER to the Golgi to the plasma membrane. It can also include the recycling of internalized materials from the endosome back to the cell surface.

So, basically, exocytosis is used for many purposes. It has been used for secretion. It has been used for, you know, placing the receptor back onto the cell surface. And it has also been used for removing waste material. And in some cases, it has also been used for signaling because the molecules are produced from the start, go to the outside, and then induce the signaling in the neighboring molecules. So exocytosis can be constitutive or regulated.

Constitutive exocytosis occurs in all cells via the trans-Golgi network-derived vesicles. The pathway occurs continuously. Many soluble proteins are continuously secreted via this pathway, which also delivers the newly made membrane lipids and proteins to the plasma membrane. So basically, exocytosis is required for delivering the cargo to the plasma membrane or even outside the cell. Then, what is the mechanism? The empty vesicles actively loaded neurotransmitters into their lumen using an electrochemical gradient created by the proton pump. Once they are filled, these vesicles are transported to   the   cell   surface   for   release.

Translocation could possibly be diffusion-limited or dependent on the molecular motor. Exocytosis is triggered by the calcium influx to the voltage-gated calcium channels; the presynaptic action potential causes these calcium channels to open. So, before releasing their content, the synaptic vesicles dock at the presynaptic membrane at the active zone and undergo a priming step to prepare for exocytosis. Secretion is then triggered by the opening of voltage-gated calcium channels in response to the membrane depolarization caused by the action potential in neurons or the receptor potential in sensory cells. Vesicle fusion and pore formation are derived from the strong interaction between the conserved proteins, such as SNAREs, right? They're called the N-ethylaminate-sensitive fusion   protein   attachment   proteins.

Snares are the vesicles called V-snares, while those on the presynaptic membrane are called T-snares, such as syntaxin and SNAP-25. So basically, vesicles actually have the V-snares. The target side has the T-snares, and these two are cognate pairs to each other. So that's why vesicles will go directly to this particular target site, and that's how they will   deliver   the   cargo.

After exocytosis, the vesicles are retrieved via endocytosis. There are at least three known mechanisms of SV endocytosis. You have the kiss-and-run endocytosis. You have diffusion pores and closures allowing the vesicle to be quickly retrieved and reused. And the pathway is fast, requiring one to two seconds. So basically, what happened is that you are actually going to have the vesicles which will go for exocytosis; they will fuse with

the plasma membrane, release the content, and then these vesicles are going to be retrieved because they are actually going to be filled with the material again, and then this continues, right? So this will continue, and it will keep releasing the neurotransmitter, and these neurotransmitters will then be received by another neuron, and that's how the signal from this neuron will go to that neuron.

So what we have discussed so far is the intracellular trafficking or intracellular transport of the material. And we have discussed phagocytosis. We have discussed penocytosis. We have discussed the detailed mechanism of phagocytosis. It's relevant in terms of eliciting the immune responses and how, post-phagocytosis, you are actually forming the phagosomes, which are fusing with the lysosomes to form the phagolysosomes. Because the lysosome has hydrolytic enzymes, it is actually digesting the material that has been engulfed, and then it is going to be presented along with the MHC class molecules so that it can actually trigger immune responses and so on.

As far as this is concerned, uh, we have also discussed decluttering mediated. We have discussed decluttering independent mediated. And then we also discuss the cable independent process, cable independent process. And then lastly, we also discuss the micro. Subsequent to that, at the end, we also discussed exocytosis, through which the cell is actually expelling material outside the cell. So far, we have discussed internalization and the distribution of the molecule within the cell. And then we also discussed how the cell is going to throw the molecule outside; these outside molecules could be for neurotransmission, they could be utilized for giving the signal to the neighboring cells, or they could be for recycling the receptors onto the plasma membrane.

So, with this detailed discussion about the transport of material within the cell or outside the cell, I would like to conclude today's lecture. In our subsequent lecture, we will discuss some more aspects related to this particular course. Thank you.