Basics of Biology
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Lecture 35
Vesicular Transport

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing, we were discussing about the living organisms and so, far what we have discussed, we have discussed about the classification of the living organisms, evolution of the living organisms. And then we have also understood the structure and function of the prokaryotic as well as the eukaryotic cell.

And then we have also discussed about the structure and function of the different types of biomolecule. So, we have discussed about the proteins, carbohydrates and lipids and as well as nucleic acids. And in the previous module, we have also discussed about the central dogma of molecular biology or the central dogma of life, where we have discussed about the replication, transcription and the translations. And in the current module, we were discussing about some of the basic cellular processes.

And in the previous lecture, we have discussed about the immunology or the immune defense responses and how the body or the cells are actually producing the different types of reference responses. And in the previous lecture, we have also discussed about the cell cycle, cell growth and as well as the apoptosis. Now, in today is lecture, we are going to discuss about how the cell is distributing the material within the cells like so that the cell is made up of the different types of organelles.

We can have the mitochondria, chloroplasts, endoplasmic reticulum, Golgi bodies and so on even nucleus also. So, these alternates are getting the proteins and other biomolecules utilizing a very well developed vesicular trafficking system. So, in today's lecture, we are going to discuss about the vesicular trafficking system and how that is happening within the cell.

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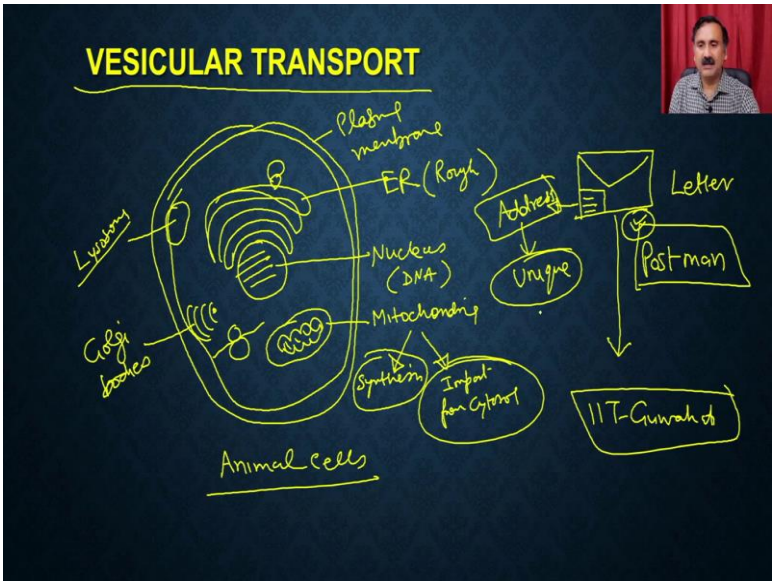

So, what is mean by the particular tracking system? So, that in the cell for example, it is the animal cell or the plants. So, if for example, if this is a animal cell. So, in the animal cell, what are the things we going to have? We are going to have a nucleus where we are going to have the, so, this is going to be a nucleus where you are going to have the DNA as a genetic material. Then outside this nucleus, you are going to have the ER, so, you are going to have the ER like this. So, you are going to have the ER then you are going to have the Golgi bodies. So, you are going to have the Golgi bodies.

So, then you are going to have the mitochondria. So, these are the different types of organelles what are present and in a typical eukaryotic cell in a plant cell also your instead of mitochondria, you are also going to have the chloroplast as well. Apart from that you can also have the lysosomes, you can have the and this and you can also have the double layer like plasma membrane, you can also have the plasma membrane. Now, what is mean by the vesicular trafficking is that the proteins which are going to be produced by the ribosomes, so you are going to have the ribosomes also, so the protein is going to be produced by the ribosomes.

And that ER is going to have the ribosomes docked on door on their surface and that is why it is actually going to be called as the rough ER. And the protein is going to be produced to the ribosomes and then these proteins are actually going to be distributed to the different organelles. For example, you can have the proteins which are present in the mitochondria. So, mitochondria is actually going to have the protein from the 2 sources. For example, it can have its own synthesis, it can have its own synthesis, like utilizing the genome, but is present

within the mitochondria and it can also have the import of the protein from the cytosol. So, these are the 2 sources.

Now, the protein which are going to be imported or from the cytosol is actually going to go through a systematic delivery system and that delivery system is nothing but a vesicular transport system. Now, when you want to send the any packet, for example, if I want to send a packet to my home, or if I sent a packet to my college, what I am going to do is I am going to have a packet. So, I am going to have a packet like this, so this is going to be a letter which I want to send to IIT Guwahati.

So, how I how and the person who is going to send this packet is going to be a postman. So, if you have all the postal services, but how the postman will know that you are actually going to send the package to the IIT, Guwahati. So, for that, what I am going to do is, I am going to write the address here. This means if you want to do a vesicular trafficking, and if you want to do the vesicular transport, you require the 2 materials, one, you require a person who can actually carry the packet, which is going to be the postman in this case, and then you also require the address.

So, this address is going to be a unique address, which means if I want to send a packet to the IIT, Guwahati, even if I want to send a packet to my laboratory, it is going to have a unique address, which is going to be pasted on this particular letter, and then only this packet is going to reach to my laboratory, how we are going to achieve in our daily life, we are actually going to be achieved that by the having a postman and then going to have an address and you see a very systematic the address.

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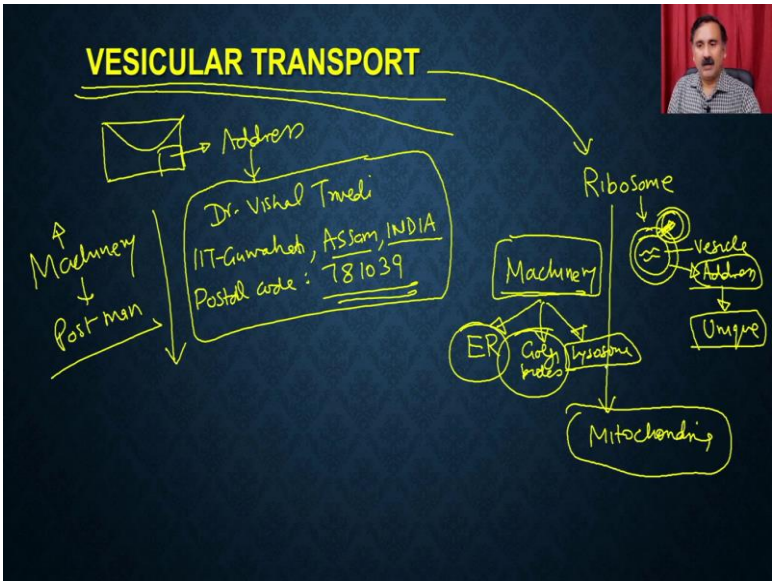

Like if you send a packet, you are going to add the address. And if you see the details of the address, what you are going to see is I am going to write my name. So, that is going to be a unique, but there could be which also vary in other places as well. So, what I will do is I will then I am going to write the address away, it is going to be like IIT Guwahati, then I am going to define which place so I am going to write Assam, and then I am going to write India otherwise, so that is not a still be unique, what I am going to do is I am going to write the postal code.

If I write the postal code, for example, 781039 then that has been a defined address a very, very well-defined address. So, that is what going to happen and then you also require a machinery. So, you are going to have a machinery which is actually going to carry so in this case, it is going to be the postman, which is going to carry this packet and the machinery is also going to have like a complete postal department where you are going to have the many people who are going to facilitate this process.

Similar to that when you want to send a packet for example, a protein is being synthesized from the ribosome. So, if the protein is being synthesized from the ribosomes and if it has to go to the mitochondria, then what you are required is you are going to require an address. So, you have to attach address to this particular protein which is going to be present in a bicycle. So, or t, so this is a this is vesicle is actually going to contain the protein of your interest which you are going to send to the mitochondria.

Now to this vesicle, we have to attach 2 things we have to attach first the address. So, in this case, if you see this case, we are actually writing each and everything in a in alphanumeric codes whereas in the case of address, you are actually going to put some kind of tag here you can put a tag onto this vesicle so that this tag is going to be recognized by the machinery and that the address is going to be unique.

Similarly, you are going to have a unique tag which is going to be only for the mitochondria and within the mitochondria also, you can actually define whether you want to send the protein to the outer membrane of the mitochondria or to the inner membrane of the mitochondria or whether you want to send the protein to the stroma or, the Christi and all other places. So, you can actually make the unique microcode unique address. Apart from that, you also require the machinery.

So, in the machinery, here, we have the machinery in terms of the postal services like the postman, in this case, you are going to have the machinery which has the multiple types of organelles, which are going to facilitate this process, what are these organelles, you can actually require the help of the endoplasmic reticulum, you require the Golgi bodies, and you also require the lysosomes. So, these are the 3 organelles, which are actually going to participate into the vesicular transport. And that is why these organelles are also being considered as the organelles of the vesicular trafficking.

So, if you see it is actually be very similar to what we actually practice in our daily life. And the same is going to happen in the cell as well, except that the, the machinery and as well as the way, you are actually going to put the address is going to be different. Now, let us, so, if you want to understand a particular trafficking, we have to understand the 2 aspects, one, we have to understand about the machinery and the organelle what are involved into this machinery, and then we also have to understand how this particular unique address is being generated, and how that unique address is helping vesicle to reach to the mitochondria.

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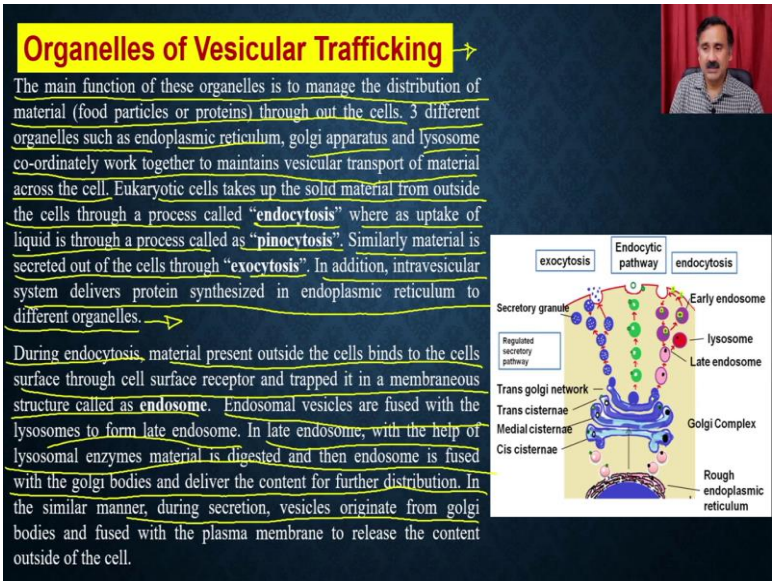

These organelles of the vesicular trafficking we have already discussed when we are discussing about the structure of the eukaryotic cells. So, I am not going to go into the detail of the each and every detail of this particular process. So, the main function of the organelles of the organelle trafficking is to distribute the material throughout the cell, 3 different organelles such as endoplasmic reticulum, Golgi apparatus, and the lysosomes coordinately work together to maintain the vesicular transport of material across the cell.

Eukaryotic cells take up the solid food from the outside through the process, which is covered as the endocytosis whereas the uptake of the liquid is also called as the pinocytosis. This is also we have already discussed when we were discussing about the phagocytosis. Similarly, the material is secreted out of the cell which is called as the exocytosis. In addition, the intravascular system delivers the proteins synthesized in the endoplasmic reticulum to the different organelles.

During Endocytosis, the material present outside the cell bind to the cell surface to the cell surface receptor and trapped in a membranous structure which is called as the endosome. The endosomal vesicles are fused with the lysosome to form the late endosome and in the late endosome, with the help of the lysosomal enzyme material is digested and then the endosome is fused with the Golgi bodies and deliver the content for the distribution.

In the similar manner during secretion the vehicle originate from the Golgi bodies and fuse with the plasma membrane to relieve the content outside the cell. So, this is what is showing here. So, this is the endocytosis where the food material which is present outside is going to

be trapped into the membrane structure earlier it is going to form the early and assume that it is going to form the it is going to fuse in lysosome. And that is how it is going to form the late endosomes and then it is actually going to release the content.

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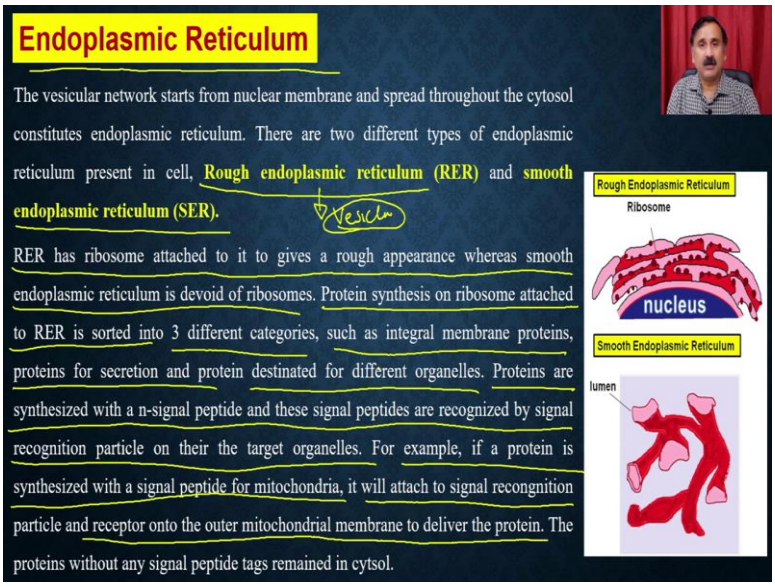

So, the 3 organelles, so you can have the endoplasmic reticulum, so endoplasmic you can have 2 different types of endoplasmic reticulum, the rough endoplasmic reticulum and the smooth endoplasmic reticulum. Rough endoplasmic reticulum is only the reticulum ER which is going to participate into the vesicular trafficking. So, rough endoplasmic reticulum has a ribosome to attach to its and gives a rough appearance where the smooth endoplasmic reticulum is devoid of the ribosome.

So, these are the rough endoplasmic reticulum where the ribosomes are attached on to the ribosome onto the endoplasmic reticulum. The protein synthesis on the ribosome attached to the RER is sorted into the 3 categories such as the integral membrane proteins, proteins for secretion and the protein destinated for the different organelles. So, you can have the protein which are of 3 different types, it can be a protein of the integral membrane proteins, which means it will go to the plasma membrane.

Then you can have the protein of the secretory pathways or the secretory proteins and then you can also have the protein which is meant for the other organelles. Proteins are synthesized with the n terminal signal peptide and these signal peptides are recognized by the signal recognition particle on their target organelles. For example, if a protein is synthesized with a signal peptide for the mitochondria, it will attach to the signal like mentioned particle

and receptor onto the outer membrane to deliver the proteins. Now, protein without any signal peptide is actually going to remain within the cytosol.

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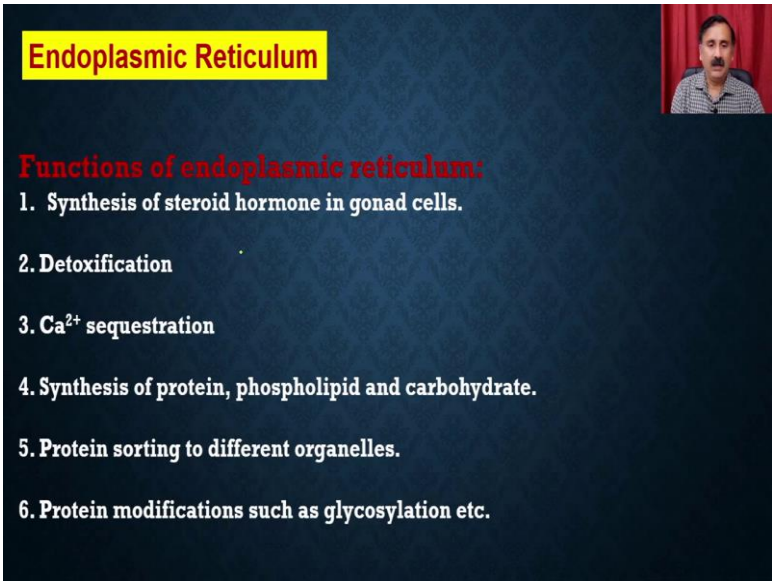

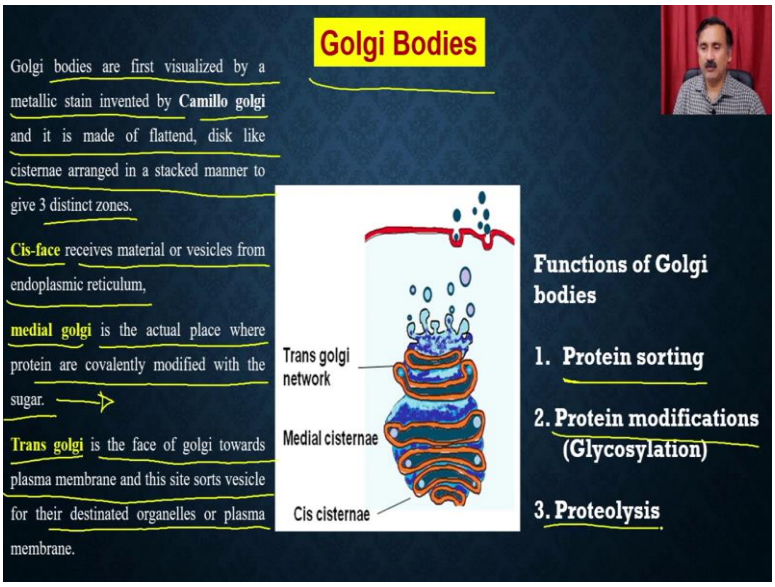

These are the different functions of the endoplasmic reticulum. Then, we have the Golgi bodies. So, Golgi bodies are first visualized by a metallic stain invented by the Camillo golgi. And it is made up of the flattened dislike cisternae arranged in a staggered manner to give 3 distinct zone. So, it can have the cisf golgi, medial golgi and the trans golgi. The cisf golgi receives the material or vesicle from the endoplasmic reticulum. So, these are the cisf golgi, medial golgi and transport golgi.

Medial golgi is the actual place where the proteins are covalently modified with the sugar. This is the way we are going to discuss and we are going to discuss about the preparation of

the label and then we have the trans golgi and trans golgi is the face of the golgi towards the plasma membrane and this is this site sorts the vesicle for their designated organelle for the plasma membrane.

So, these are the different functions of the golgi it can actually help in the protein sorting, it can help in the protein modifications and it can also help in the proteolysis.

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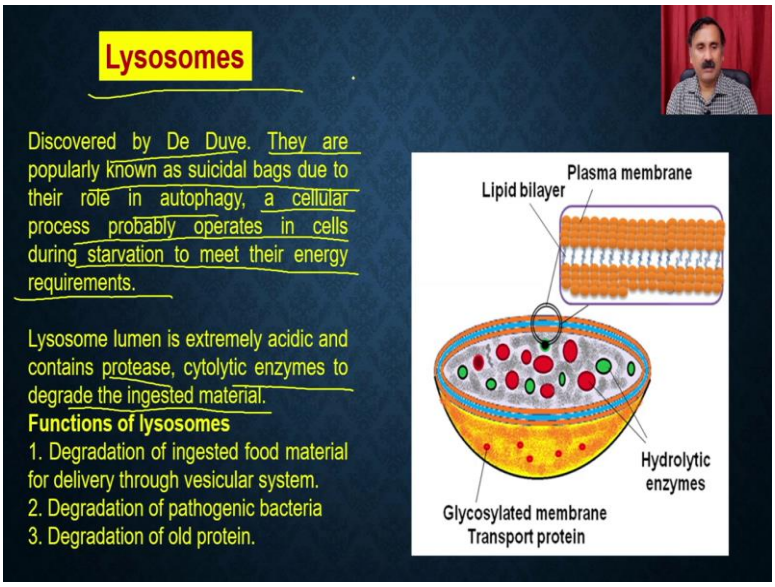

Then we have the lysosomes. So, lysosomes are actually going to be discovered by the Dude De Duve do they are properly known as suicidal bags due to their role in autophagy or cellular process probably operates in the cell during starvation to meet their energy requirement. Lysosome lumen is extremely acidic and it contains proteases cytosolic enzyme and integrates the ingested material. So, these are the functions of the lysosomes.

Now, since we have discuss about the machinery, let us talk about the how we can be able to generate the level.

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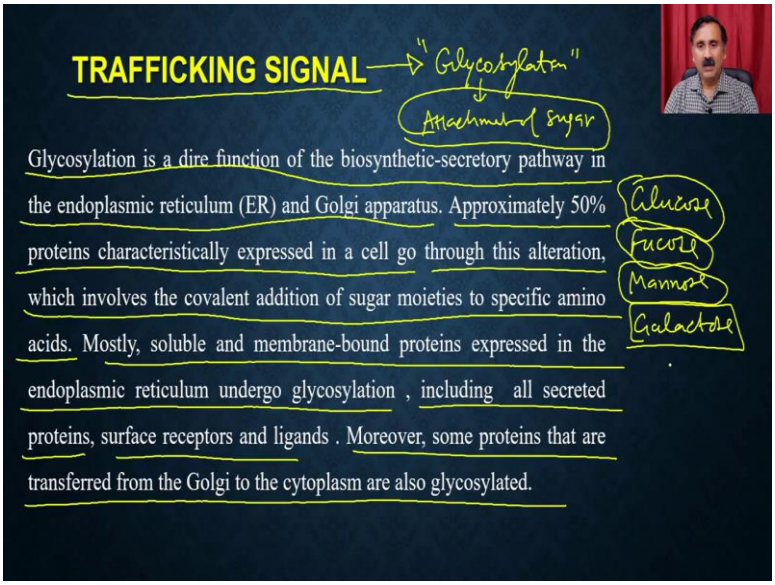

So, trafficking signals, so the glycosylation is a direct function of the biosynthetic secretory pathway in the endoplasmic reticulum and the Golgi apparatus, approximately 50 percent of the protein characteristically expressed in a cell grow through this alteration which involves a covalent addition of the sugar moiety to the specific amino acids. Mostly soluble and membrane bound protein expressed in the endoplasmic reticulum undergo glycosylation, including all secreted protein, surface receptors and the ligand.

Moreover, some proteins are being transferred from the Golgi to the cytosol are also glycosylated. So, one of the easiest way of generating the trafficking signal is that you are actually going to modified the protein with the help of a glycosylation and glycosylation means the attachment of the attachment of sugar to the protein. And it is not only a single sugar, it is going to be a complex sugar molecule, you will know that we have the different types of sugar, we can have the glucose, we can have the fucose, we can have the mannose, we can have the even the galactose.

So, all these different types of sugar actually can come together either the individually or in a computation and so, it is actually going to make a complex structure and complex function and these complex carbohydrate molecules are actually going to be recognized by the receptor which are present onto the target organelles. And so therefore they are actually going to provide the address.

So, you can actually have a these 3 sugar can combine with each other and they are actually going to give you a polysaccharide or a complex sugar. And so, they are actually going to

help you to generate the different types of organelles different types of tracking signal sequences, and that is why they can be targeted to the specific organelles.

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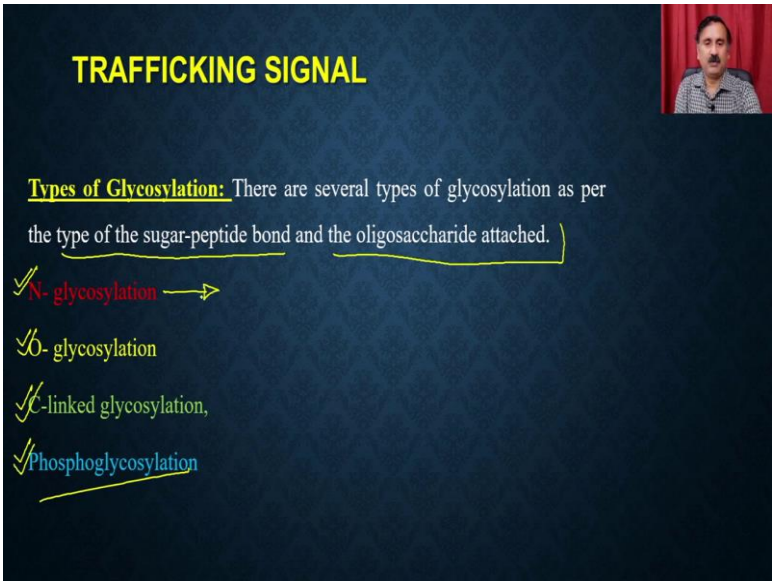

So, the glycosylation is concerned the glycosylation can be of 4 different types it can be N-linked glycosylation, it can be O-linked glycosylation, it can be C-linked glycosylation or it can be a phosphoglycosylation. All these glycosylation types are being done by the whether they are our type of sugar peptide bond they are sharing and what kind of oligosaccharide are attached. So, based on this, the glycosylation could be of 4 different types. So, let us discuss about the N-linked glycosylation.

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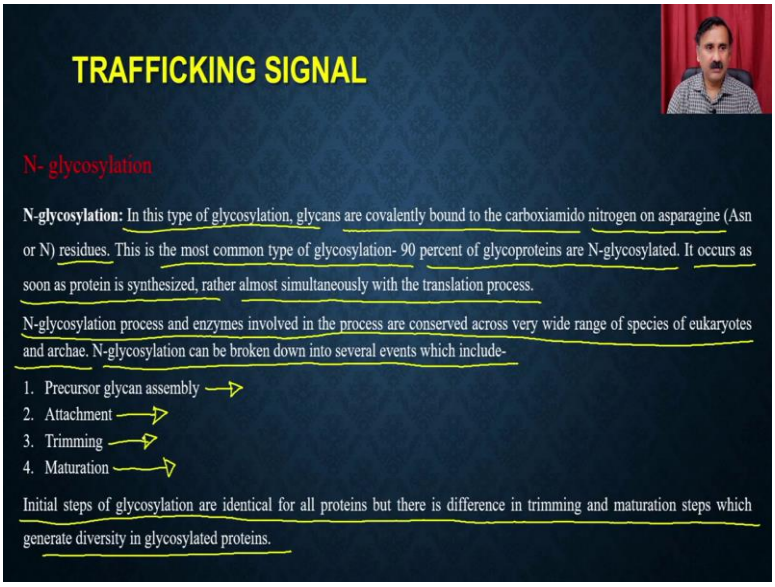

So, as the name suggests, N-linked glycosylation is in this type of glycosylation. The glycans are covalently attached to the nitrogen on the asparagine or residues this is the most common type of glycosylation. Approximately 90 percent of the glycoproteins are N-linked glycosylation. It occurs as soon as the protein is synthesized rather, almost simultaneously with the translation process. N-linked glycosylation process and the enzyme involved in this process are conserved across a wide variety of the species of the eukaryotes and the archive.

N-linked glycosylation can be broken down into several events. So, N-linked, how the N-linked glycosylation is going to happen. First is first event is the precursor glycan assembly, then you are going to have the attachment, then you are going to have the trimming, which means it is going to, do the final adjustments and then there will be a maturation. So, the initial step of glycosylation is identical for all protein there is a difference in the trimming and maturation step, which generate the diversity in those glycosylated proteins.

This means, all these steps are actually going to be performed for the different types of protein which are going to be the N-linked glycosylation except that the in the final stage, there the trimming and the maturation step is going to be different and because of that, it is actually going to generate the different types of trafficking signal.

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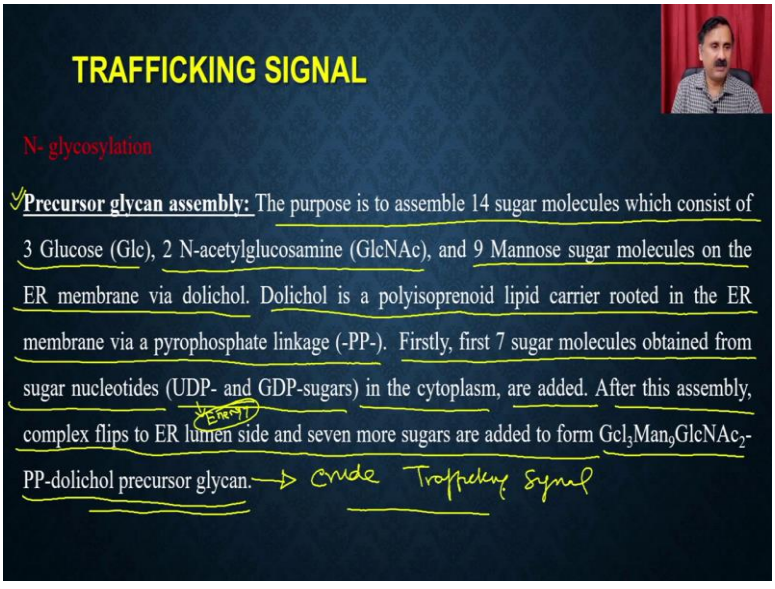

So, the first step is that you can actually be able to produce the precursor glycan assembly which means you are actually going to bring the different types of sugar and then you are going to put them together to generate the a crude glycan assembly. The purpose is to assemble the 14 sugar molecule which consists of the 3 Glucose molecules, 2 N-

acetylglucosamine and 9 Mannose sugar on the ER membrane via the dolichol. So, dolichol is a phosphoisoprenoid lipid carrier routed into the ER membrane by a pyrophosphate linkage.

First the seven sugar molecules obtained from the sugar nucleotides like the UDP and the GTP sugars in the cytosol are added. After this assembly, the complex flip to the ER lumen side and the 7 more sugars are added to form this complex sugar and it is going to be attached to the dolichol. And that is how it is actually going to be considered as the precursor glycan which means this is going to be a crude trafficking signal. So, first you are going to have the crude trafficking signal which is going to be generated and where you are going to have the 3 Glucose molecules, 2 N-acetylglucosa molecule and 9 Mannose sugar and how it is actually going to be done.

All these sugars molecules are going to be carried in the form of the UDP or the GDP sugar conjugates which means the UDP and a GDP is actually going to provide the energy into the system and that is why they are actually going to be carrier be a work as a carrier. And after they are going to be assemble they will be present into the ER lumen and they are then they are going to be formed a crude attacking signal.

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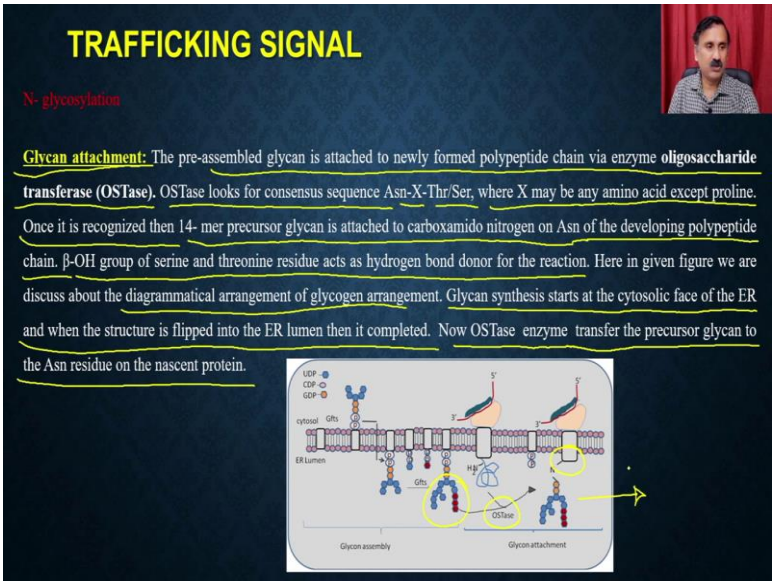

What the crude is generated then you are actually going to have the attachment of this crude sugar is on to the protein molecules. So, the pre-assemble glycan is attached to the newly formed polypeptide chain why the enzyme which is called as the oligosaccharide transferase or OSTase. OSTase look for the consensus sequence like the asparagine X threonil Serene,

where X can be any amino acid except the proline. Once it is recognizing then the 14 mark precursor glycan is attached to the through the carboxamido nitrogen on the asparagine of the developing polypeptide chain.

Beta hydroxyl alcohol group of serine and threonine residue act as a hydrogen bond donor for the reaction. So, what is going to happen? So, once the crude this sugar is going to be generated they are actually going to be present on to the human side and then it is actually going to generate the crude sugar molecules and then these sugar molecules are going to be transferred on to the protein with the help of an enzyme which is called as the oligosaccharide transferase.

And the diagrammatical arrangement of the electron element the glycans synthesis starts at the cytosolic phase of the ER and when the structure is flipped into the ER lumen then it is complete. Now the oligosaccharide transferase enzyme transfer the precursor glycan to the Asn on nascent protein. So, this is what is going to happen. Now, this is actually a crude glycosylation. So, after this is actually going to be trimmed so that you can actually remove some of the sugar molecules and you can actually also do the maturation.

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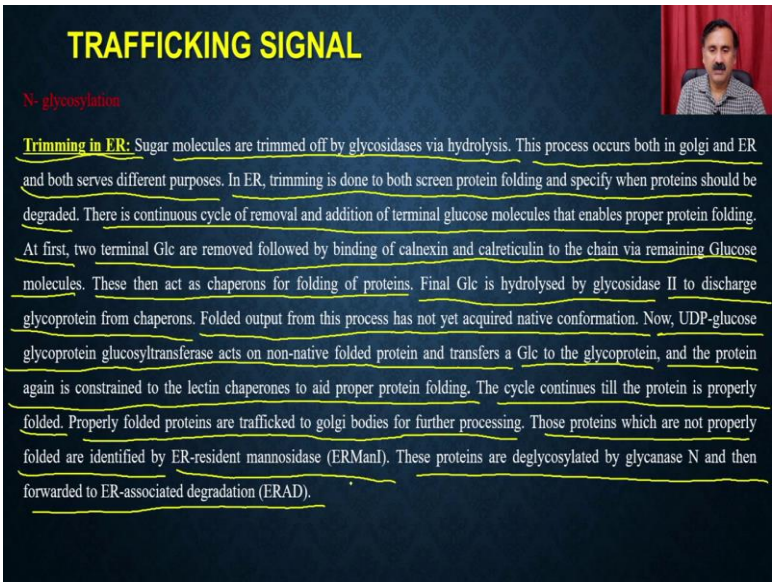

So, the next step is that you are going to have the trimming. So, sugar molecules are trimmed up by the glycosidases via the hydrolysis. This process occurs both in the Golgi and the ER and both cells at a different function. In the ER, the trimming is done both to screen the protein folding and the specify when protein should be degraded, there is a continuous cycle of removal and addition of terminal glucose molecule that enable the protein folding.

At first the 2 terminal glucose are removed followed by the binding of calnexin and calreticulin into the chain while remaining glucose molecules. These then act as a chaperone for the folding of proteins. Final glucose is hydrolyzed by the glucosidase II to the discharge of the glycoprotein from the chaperones.

Folding output from this process has not yet acquired native conformation. Now the UDP glucose glycoprotein glucosyltransferase act on the non-native folded protein and transfer of glucose to the lack of protein and the protein again is constrained to the lectin chaperones to add the protein folding. The cycle continue till the protein is properly folded, the properly folded proteins are traffic to the golgi bodies for the further processing.

So, those proteins which are not properly folded are identified by the ER resident mannosidases and these proteins are D glycosylated by the glycan and then forwarded to the ER associated degradation pathway.

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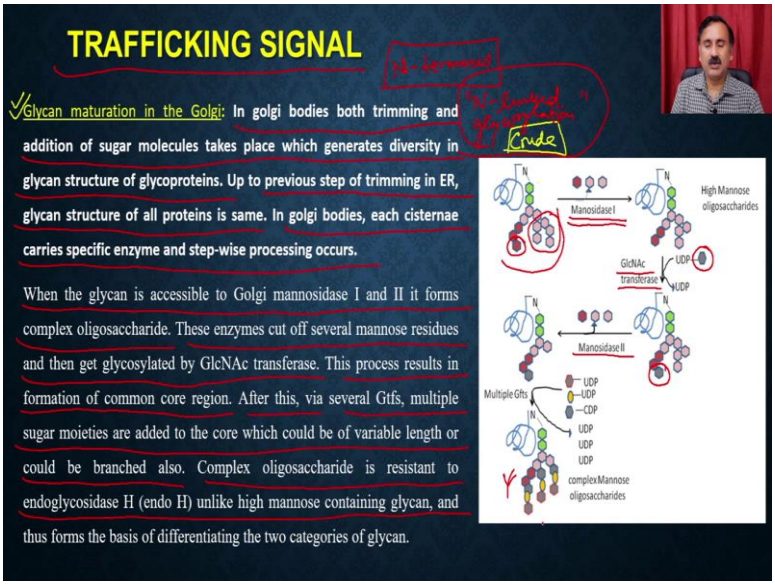

Apart from that, then they will be a glycan maturation which means after that there will be trimming and all that so this is what you are going to happen here. You are going to have a crude the signal and that type of crude trafficking signal is actually going to be matured by the different types of the different types of the enzyme so you can have the manosidase, you can have the manosidase II and then you can also have the different types of processes and through which this particular crude glycan assembly is actually going to be arranged and are going to form a mature like an assembly.

So, what are the things are going to happen? In the golgi bodies both trimming and the additional sugar it is take place with generate diversity in the glycan structure of the glycoprotein. Up to previous step of trimming in the ER the glycan structure of all protein is same. In golgi bodies, each system carries a specific enzyme and the step wise process occurs. When the glycan is accessible to the Golgi bodies monocyte is one and two it form the complex oligosaccharides. These enzyme cuts off the several mannose residues and then get glycosylated by the glucose and N acetylglucosamine transferase.

So, in the first event what you are going to do is the manosidase I is actually going to remove with so many mannose from the this complex crude sugar and then it is actually going to have the transfer of the glucose and a style glucoseamine onto this by the help of enzyme which is called as glucose N acetylglucosamine transferase. And the UDP is actually going to function as a carrier. So, you can see that if the mannose is going to be removed in some of the places and N acetylglucosamine is going to be transferred.

The same is going to happen when the manosidase II is actually going to act and that is why it is actually going to remove these mannose residues and again some more molecules of the glucosamine N acetyl glycan and all other sugars are going to be replaced his process replaced. This process results in deformation of a common core region. After this the several Gtfs multiple sugar moieties are added to the core which could be of variable length or could be of branched also.

The complex oligosaccharide is resistant to the endoglycosidases like the glycan and that is formed the basis of the differentiating the 2 category of glycan. So, this is the internal glycosylations where you are going to have the different types of steps and that is actually going to so this is going to be called as N-linked glycosylation which is all mostly happening into the more than 90 percent proteins where the first you are going to have a crude sugar moiety, we are going to have a crude sugar moiety and then these crude sugar moieties are actually going to be trimmed and mature into the ER and the Golgi bodies.

And that is how it is actually going to form a complex sugar, complex and that complex sugar molecule is going to be function as the trafficking signal. And that could be different for the different types of proteins and that is how it is actually going to be targeted to the different organelles.

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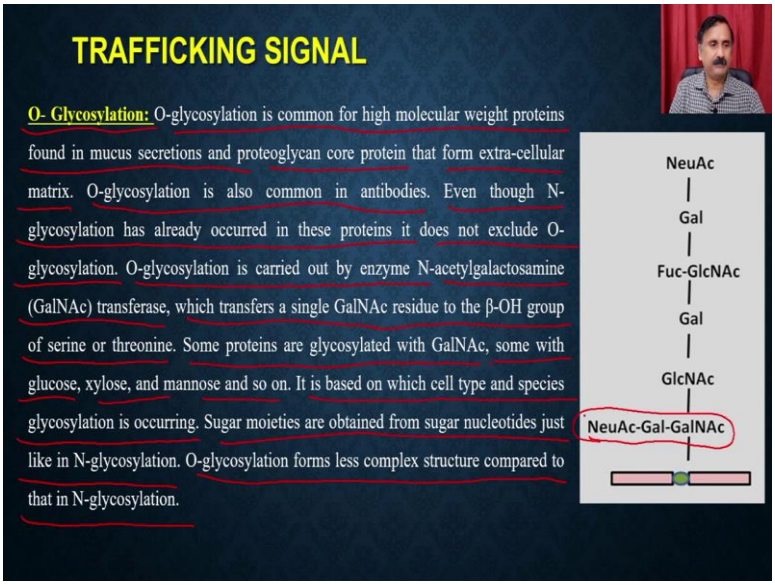

Then we have the O-linked glycosylation. So, the O-linked glycosylation is common for both the high molecular weight protein found in a mucus secretion and the proteoglycan core kind and then form the extracellular matrix. O-glycosylation is also common in the antibodies, even though the N-linked glycosylation has already occurred in those protein it does not exclude the O-linked glycosylation. O-glycosylation is carried out by the enzyme which is called as the N acetylglucosamine or transferase, which transfer a single galactosamine N acetylglucosamine this dive to the beta hydroxyl group of the serine or the threonine residues.

Some proteins are glycosylated with the galactose N acetylglucosamine, some with the glucose, xylose and mannose and so on it is based on which cell type and species glycosylation is occurring, the sugar moieties are obtained from the sugar nucleotides just like in N-glycosylation. And the O-glycosylation forms a less complex structure compared to the N-glycosylation. So, you can have the different types of sugar which are going to be come together and that is how it is actually going to form a complex sugar which is going to be attached on to the protein molecules.

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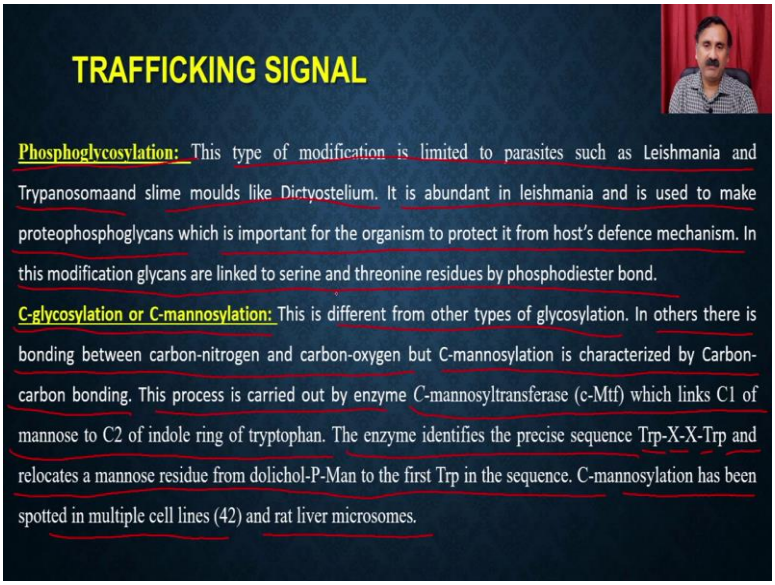

Then we have the phosphoglycosylation, so, this type of modification is limited to the parasites such as the Leishmania and Trypanosoma and slime moulds like the Dictyostelium, it is abundant in the Leishmania and it is used to make the proteophosphoglycans, which is important for the organism to protect it from the host defense response. In this modification, the glycans are linked to the serine and threonine residue by the phosphodiester bond.

Then we have the C-linked glycosylation. This is different from the other type of it occurs in other there is a bonding between the carbon-nitrogen and the carbon-oxygen but the C-glycosylation is characterized by the carbon-carbon bonding. This process is carried out by an enzyme which is called as the C-mannosyltransferase which links the C1 of the mannose to the C2 of the indole ring or the tryptophan. The enzyme identify the precise sequence like the tryptophan-X-X-tryptophan and the relocates a mannose attitude from the dolichol.

P-mannose to the first tryptophan in the sequence, C-mannosylation has been spotted in the multiple cell lines and the rat liver microsomes. So, with this glycosylations it is actually going to achieve the vesicular trafficking, how it is going to happen?

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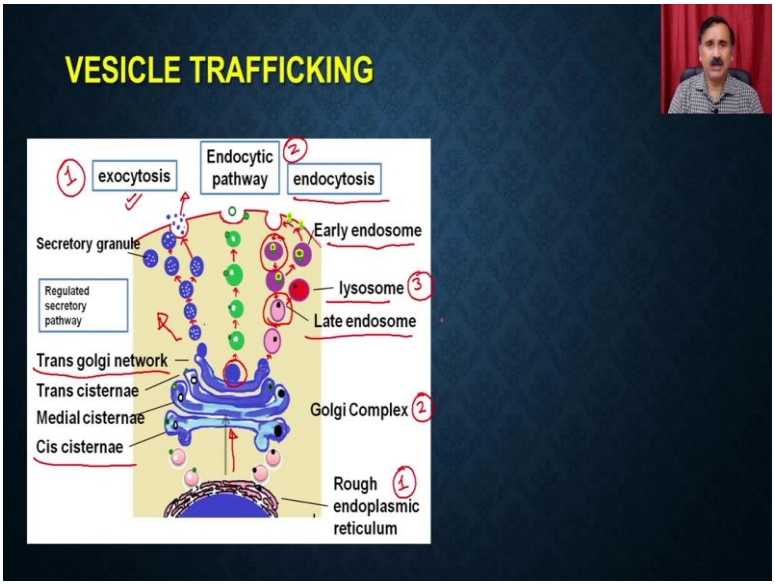

So, in a vesicular trafficking, you can have the 3 different pathways, you can have the exocytosis, you can have the endocytosis and then you can also have the distribution of the molecules within the cell types. And the 3 organelles which are going to participate into this, we are going to have the rough endoplasmic reticulum, which can be put to create in the Golgi complex and it is going to form the lysosomes. So, in the endocytosis, the food material which are actually going to be trapped into the membrane bound structure and these membrane bound structures are going to be called as the early endosomes.

And these early endosomes are then going to be fused with the lysosomes and that is how it they are actually going to form the late endosomes and within the late endosomes with the help of the enzymes from the lysosomes it is actually going to degrade the food material into the constituents monomers and that is how it is going to be released. Apart from that in the protein which are going to be synthesized by a ribosome which is attached onto the ER is actually going to be modified with the help of the different types of glyco sedations and these glycosylated proteins are actually going to enter into the Golgi bodies.

So, the CIS golgi, once it enters into the golgi, the golgi is going to receive these vesicles and they are actually going to be further modified by the glycol trimming and as well as the maturation and what they are going to be mature then these vesicles are actually going to go out from the trans golgi complex and this from the trans golgi complex, it is going to be packed into the vesicle with a signal for a particular organelle.

So, if the signal is for the mitochondria, it is actually go into the mitochondria, if signal is for the plasma membrane, the vesicle will fuse to the plasma membrane and then it is actually going to release the content and that is going to be exocytosis. So, some of the, for example, some of the antibody which are going to be produced within the cell is actually going to be fused with the plasma membrane and therefore, they are actually going to release the antibodies into the circulation and that is how they are actually going to participate into the immune responses.

If the vesicles are going to have no tag, like so, if the vesicles are not going to have any tag, then the vesicles are actually going to release the content into the cytosol. Same is true for the if the vesicle has the signal for the mitochondria, they are actually going to release the content into the mitochondria. So, this is all about the vesicular trafficking, what we have discussed, we have discussed about the machinery. So, we have discussed about the endoplasmic reticulum, we have discussed about the Golgi complex and we have discuss about the lysosomes.

And then we also discuss about the how the vesicular trafficking is utilizing the different or how it is actually going to generate the addresses like so, that the address is actually going to be unique for a particular cell organelles. And in that process, we have discussed about the ending the glycosylation, we have discussed about the different steps in which the N-linked glycosylation is going to happen and how the ER and the Golgi complex are actually going to participate into these reactions and how they are actually making a particular glycosylation pattern which is going to be unique for a particular cell organelles.

Apart from that, we also discuss about the O-linked glycosylation, C-linked glycosylation and phosphoglycosylation. Now, if you are interested and if you are planning to read more about the vesicular trafficking, you can actually be able to generate or you can be able to see that one of the different types of the vesicular trafficking signals are being generated by the ER and the Golgi complexes.

So, you can actually go to the web and you can actually be able to see the glycosylation pattern for the mitochondrial signals or the ER signals or the chloroplastic and then you will understand that how the cell is actually generating these different types of complex sugar signals and how specific it is. 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 living organism. Thank you.