Fundamentals of Protein Chemistry
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Module - 10
Protein Macromolecule Interactions I
Lecture - 46
Protein Carbohydrate Interactions - I

In the next two modules, we will be looking at protein macromolecular interactions. These form a major part of all the types of interactions and all processes that go on in our body. We had looked at specific protein ligand interactions and enzyme substrate interactions. In this case, we will be looking at larger macromolecules. We will have two such modules; module 10 and module 11. We start off with protein carbohydrate interactions.

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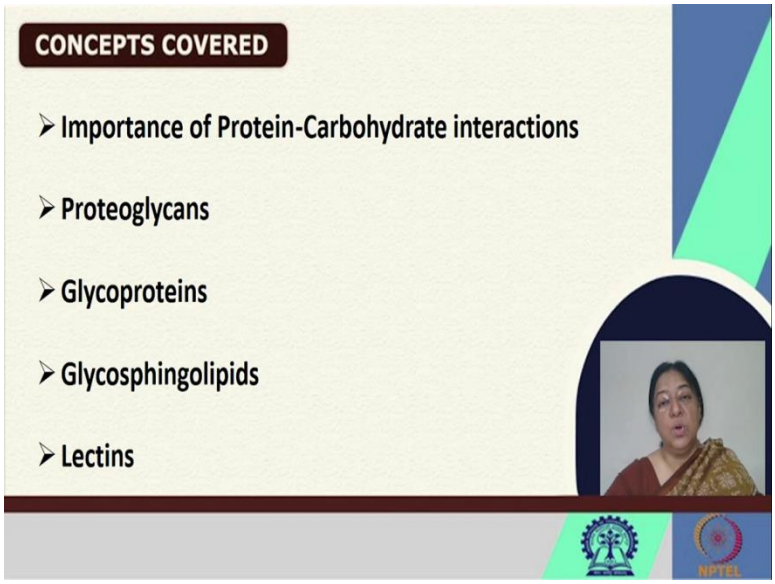

What we want to look at is the importance of protein-carbohydrate interactions. What we mean by proteoglycans, glycoproteins, glycosphingolipids, which are parts of the lipids that we have studied in the membrane proteins and we will look at what are lectins.

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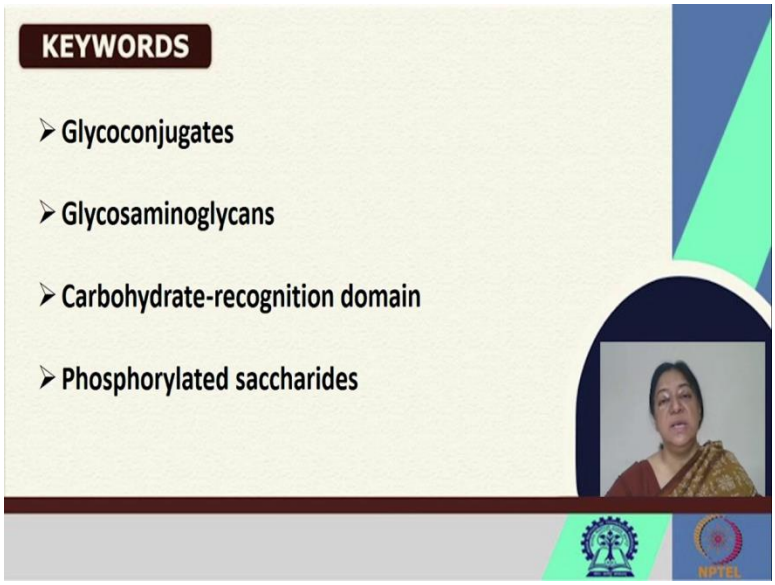

When we consider the glycoconjugates. The carbohydrates are conjugated to the proteins, that we saw in the membrane proteins mostly and their roles are extremely important in the processing, in signal transduction and several such reactions.

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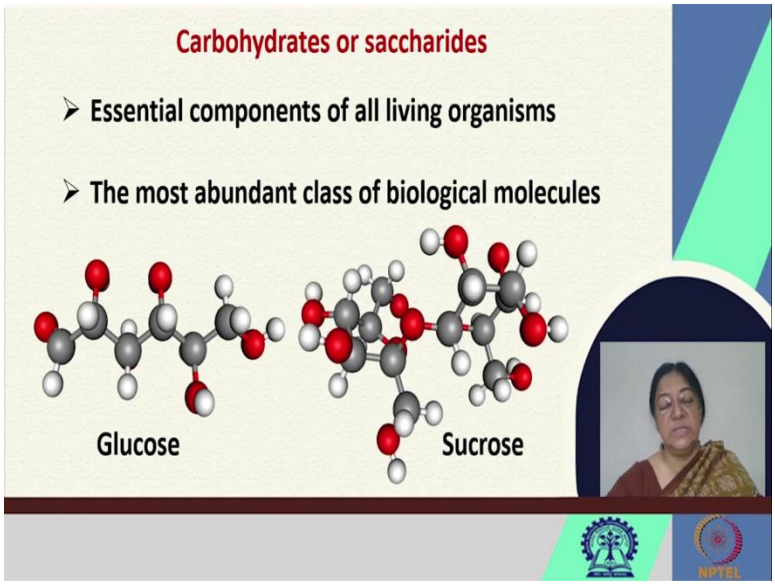

When we look at carbohydrates or saccharides in general, they are the essential components of all living organisms and they are the most abundant class of biological macromolecules. We have glucose and we have sucrose; two very common carbohydrates.

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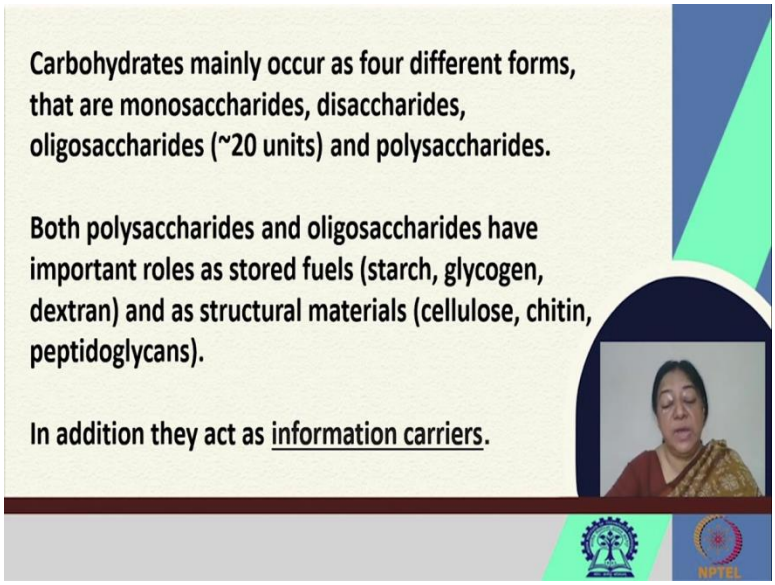

However when we look at them in different forms, the carbohydrates occur mainly in four different forms. They are the monosaccharides, the disaccharides and we have oligosaccharides which are about 20 units and then the polysaccharides.

The polysaccharides and the oligosaccharides have very important roles as stored fuels say as starch, as glycogen, as dextran and also as structural materials such as cellulose, chitin and peptidoglycans. Now, in addition they also serve as information carriers.

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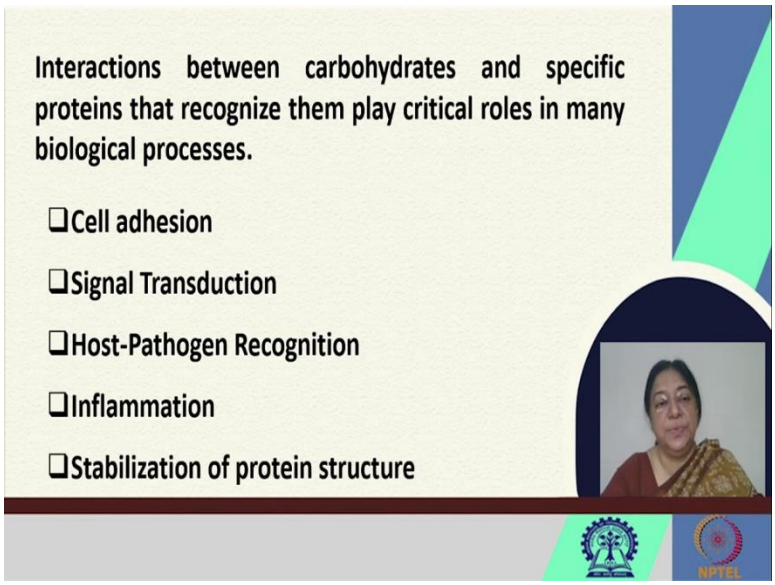

When we look at the interactions between the carbohydrates and specific proteins that recognize them, they play a very critical role in biological processes. When we look at protein carbohydrate interactions, we will see in most of the cases there are covalent linkages to the protein unit. However, we also have specific cases where the carbohydrates are recognized by the proteins in non-covalent interactions. Similarly, we will see how we will have specific recognition domains for the other macromolecule of interest.

So, each of these proteins have has their own domain of interest. If we look at the important processes that protein carbohydrate interactions are involved in, we have cell adhesion, signal transduction, host pathogen recognition, inflammation and the stabilization of protein structure.

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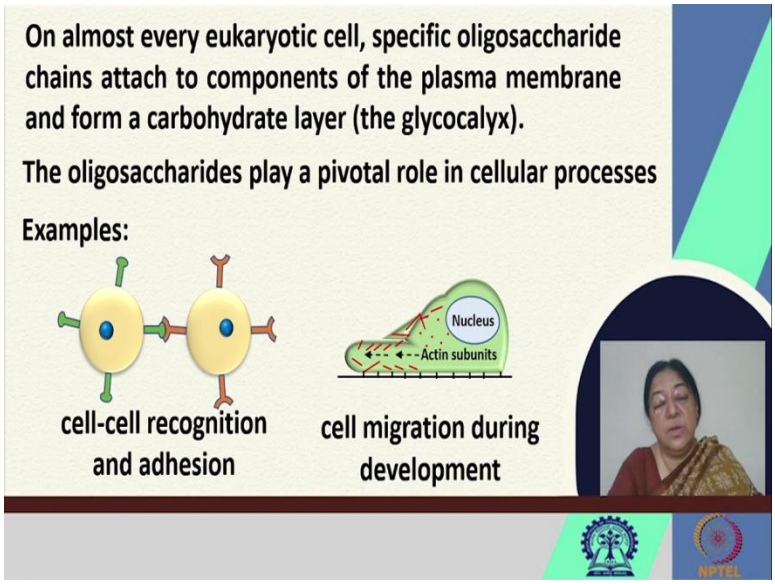

When we look at eukaryotic cells, we see on almost every eukaryotic cell, there is a specific oligosaccharide chain that is attached to the components of the plasma membrane, that forms a carbohydrate layer.

The oligosaccharide plays a very important role in this cellular process. For example, if we look at cell adhesion, cell-cell recognition occurs due to the presence of the saccharides the oligosaccharides on the surface. We have the specific cell migration during development, that is the protein carbohydrate interactions.

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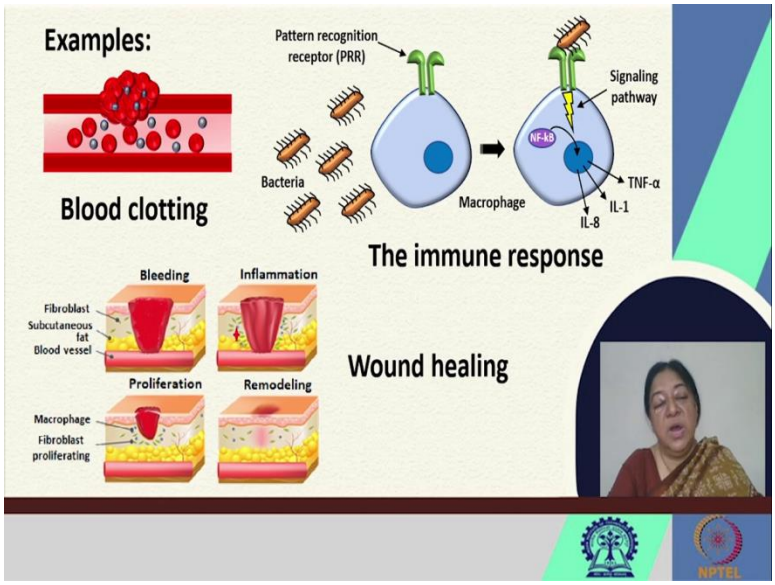

We have blood clotting, we have wound healing, the immune response in the signaling pathway; all of these are very important examples of our protein carbohydrate interactions.

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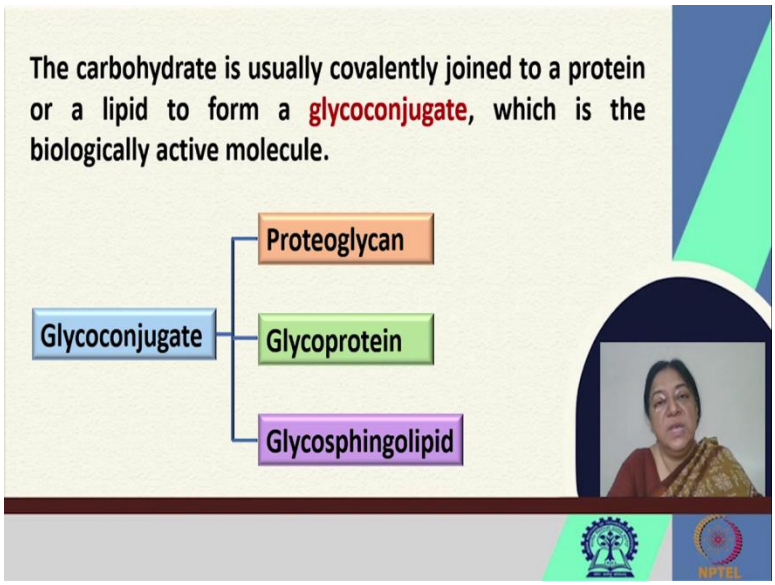

We will look at it in a bit detail, where we have the carbohydrate that is usually covalently joined to a protein or a lipid, to form what is called a glycoconjugate. We had seen some of these glycoconjugates when we looked at the lipid interactions or the proteins that were part of the membrane; the integral and the peripheral types of proteins.

When we form a conjugate with a carbohydrate, we call this a glycoconjugate. This glycoconjugate can be a glycoprotein, it can also be a glycolipid and this is the biological function active. So, if we look at the glycoconjugate, we can have what is called a proteoglycan. We can have a glycoprotein and we can have a glycosphingolipid, in the terms of the connection to a lipid.

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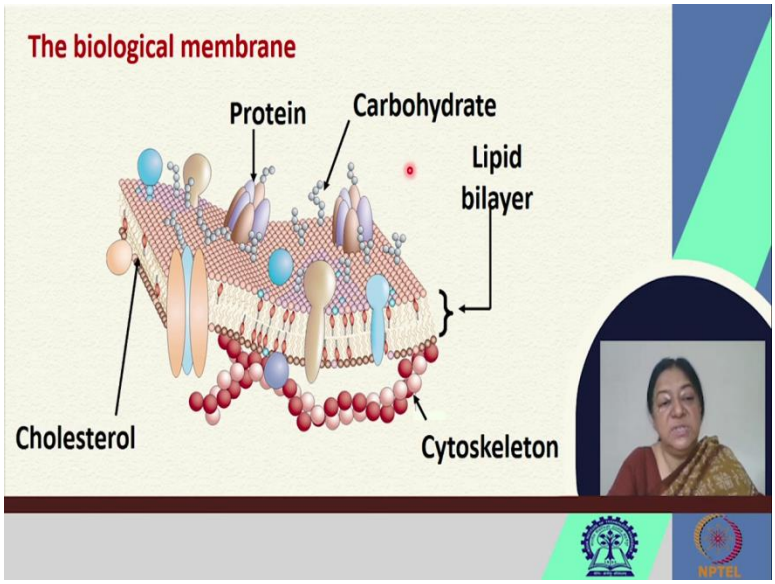

When we look at the biological membrane as we saw in the previous lectures, we looked at the specific lipid bilayer and then the combination of the lipid bilayer with the different types of proteins.

When we look [refer to slide] at these specific types of interactions where we are trying to see the integral protein membranes and so on and so forth. Then we try and understand that the lipid bilayer, the cytoskeleton, the protein and the carbohydrates that are linked to the protein by several amino acid residues. We will see what these are in a moment.

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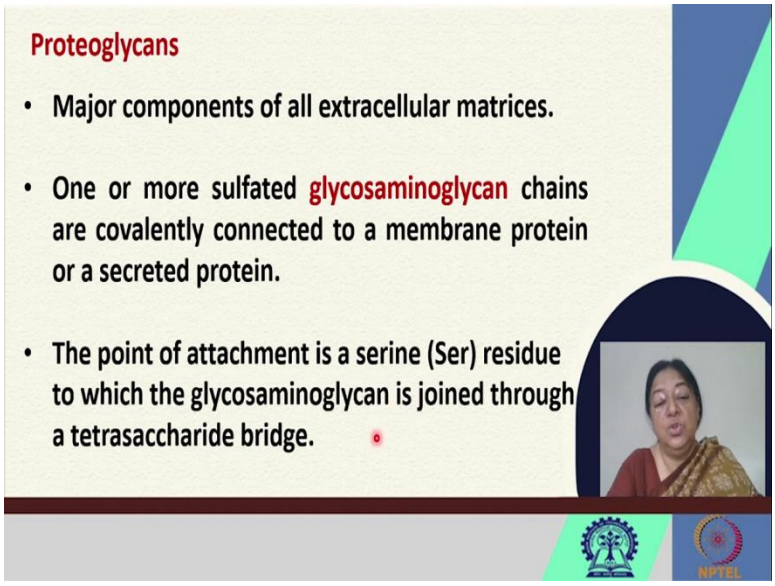

When we look at proteoglycans, these are the major components of all cellular matrices and they are one or more sulfated glycosaminoglycan chains that are covalently connected to the membrane protein or the secreted protein. Now, this is important in the way they function and the point of attachment at this case, is a serine residue to which the glycosaminoglycan is joined through a tetrasaccharide bridge.

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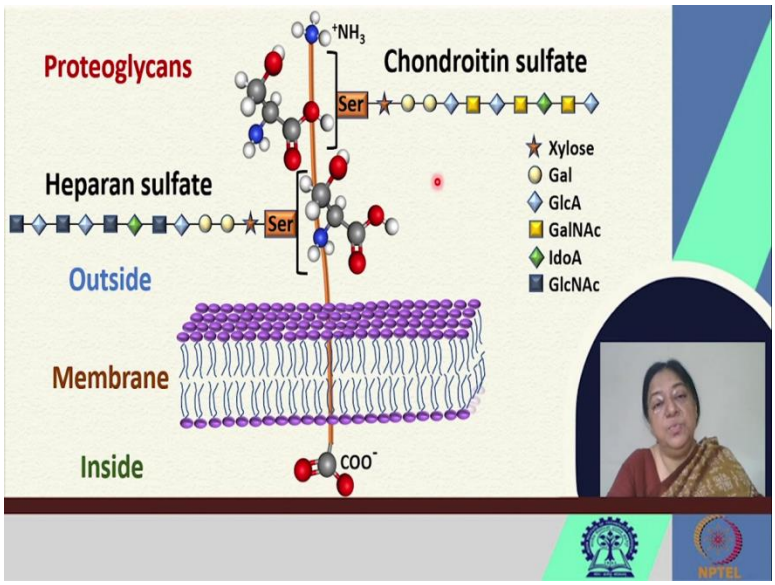

If we look [refer to slide] at the structural aspects, we have the proteoglycans and we have the lipid bilayer that is part of the membrane. This is the outside, this is the membrane part and this is the inside of the cell. Now when we have a specific protein chain a polypeptide chain, we have the amino part and we have the carboxylic acid part the C terminal and the N terminal given here.

Now, there may be serine residues that bind to a specific carbohydrate component. So we have chondroitin sulfate and heparan sulfate in this example of a proteoglycan.

These are the different ways in which they can interact and can form what is called this conjugate. This is required for the recognition of several components of the cell that may need to be transferred, connected and involved in cell-cell adhesion.

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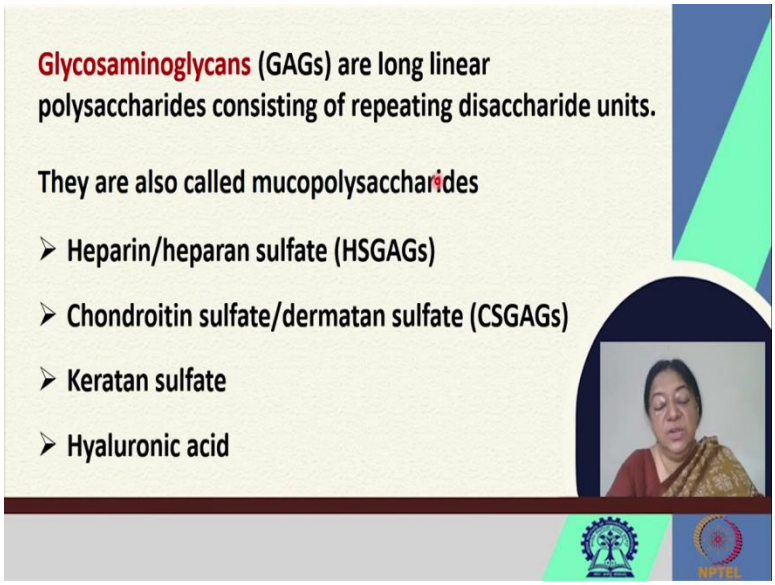

The glycosaminoglycans or GAGs are long linear polysaccharides that consist of repeating disaccharide units. They are also called mucopolysaccharides and they are important like we saw in the previous slide where we had heparan sulfate, chondroitin sulfate, keratan sulfate and hyaluronic acid.

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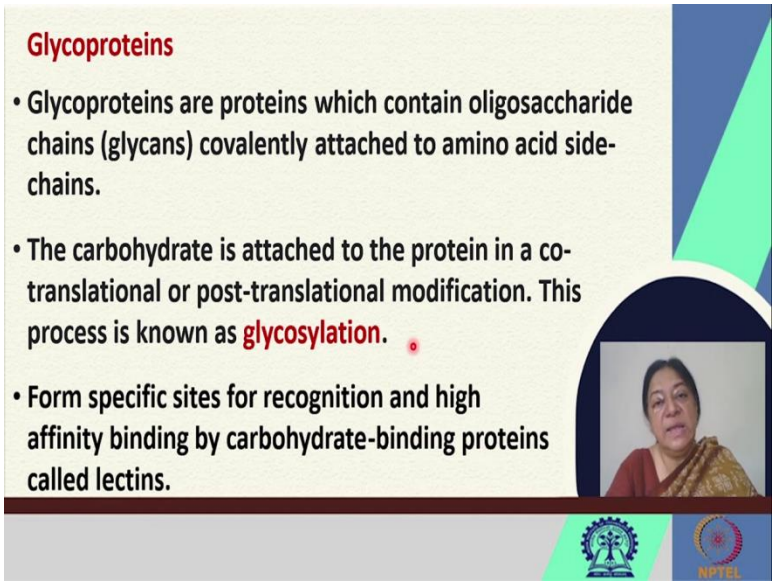

When we look at these specific types of glycoproteins, these are proteins which contain the oligosaccharide, the glycan. Again these are covalently attached to the amino acid side chains. So, we do not have a non-covalent interaction in these specific examples of protein carbohydrate interactions.

The carbohydrate is attached to the protein in a co-translation or post-translational modification. This process is called glycosylation, where the carbohydrate is attached later in a co-translation or a post-translational modification and they form specific sites of recognition and high affinity binding, by the carbohydrate binding proteins.

So, we have to remember that the association of a carbohydrate protein there, is one where we have covalent association in these types of examples and the others, where we have the carbohydrate binding proteins that are called lectins, that we will see as we go along in this lecture.

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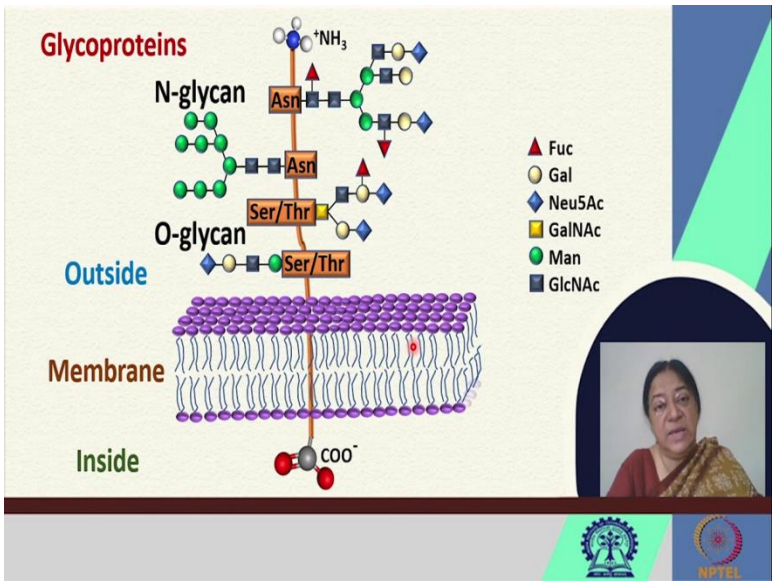

When we look [refer to slide] glycoproteins, we have again our lipid membrane; the outside, the inside of our specific cell and we have our protein chain. To this we have specific amino acid residues the asparagine, then we have another asparagine. This is now the recognition site for the N-glycan or the O-glycan that would have serine, threonine, asparagine.

The linkages that occur here are through the serine, through the asparagine and we have the specific carbohydrates associated with these amino acids, that will form or rather be covalently connected to the polypeptide chain.

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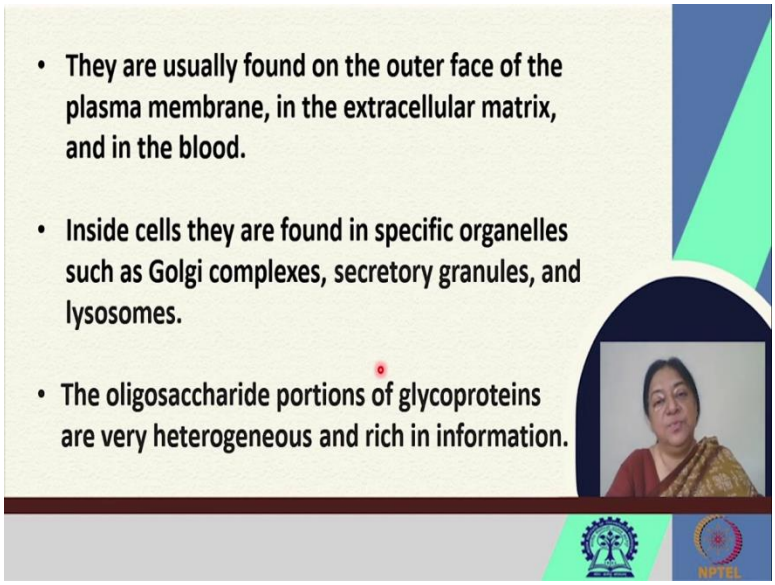

They are usually found on the outer surface of the plasma membrane as we just saw, in the extracellular matrix and in the blood and inside the cells they can be found in specific organelles such as Golgi complexes, secretory granules and also lysosomes. These oligosaccharide portions

of the glycoproteins are very heterogeneous, they are rich in information and given their heterogeneity, they are able to attract and bind to different kinds of molecules in our system.

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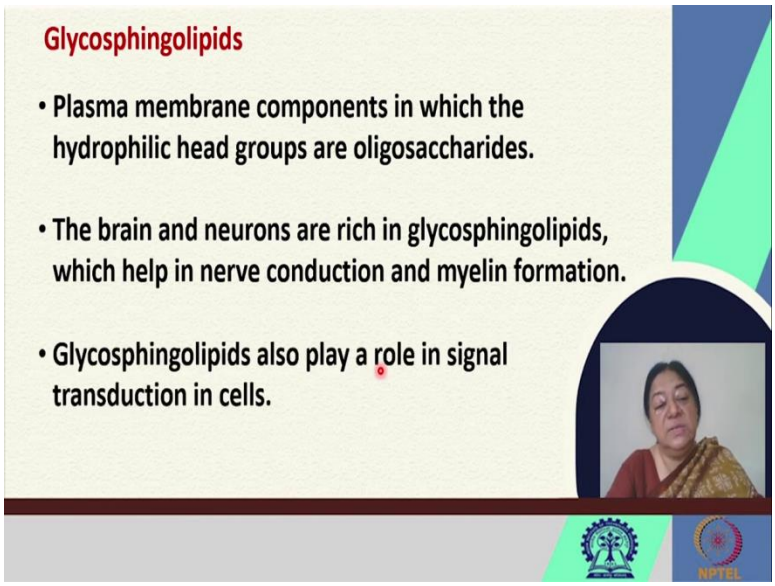

The glycosphingolipids, have the plasma membrane which we looked at in the previous lectures on our membrane proteins module, where we looked at the plasma membrane components in which the hydrophilic head groups are the oligosaccharides themselves. The brain and the neurons are rich in these glycosphingolipids, which help in nerve conduction and myelin formation and these are the ones that play a very important role in the signal transduction in cells.

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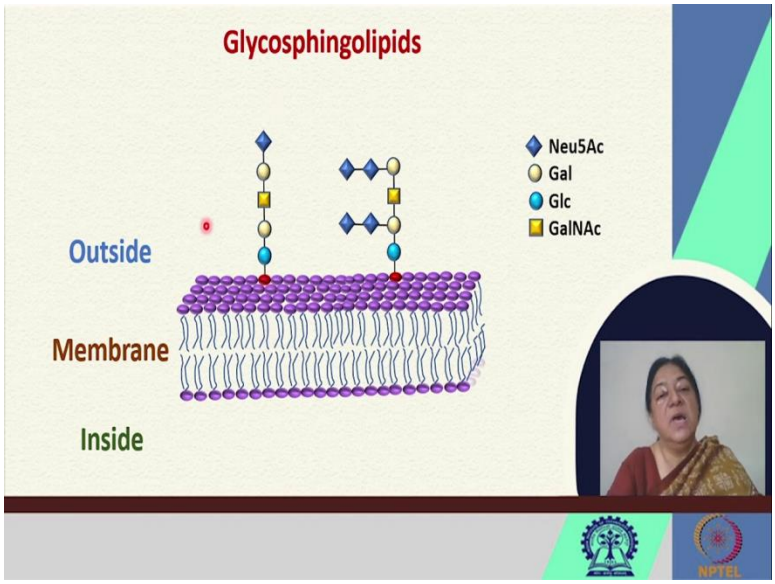

When we look [refer to slide] at examples of this, we have our specific lipid bilayer at the outside and the inside of the cell and we have the glycoconjugate directly relaying to the polar or the head group of our lipid and this linkage allows the specific transport of material through the cell.

We have these glycol conjugates to the sphingolipids themselves, forming the polar head group of this, involved in the specific type of recognition.

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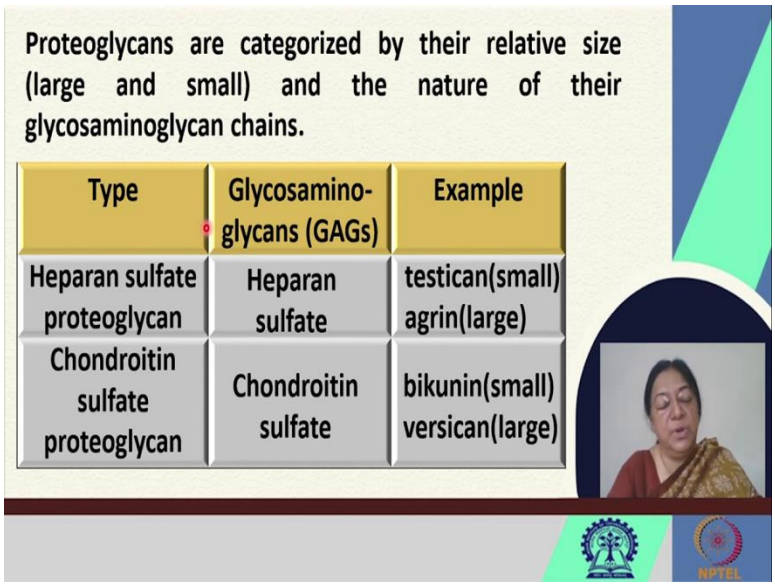

Proteoglycans are categorized by their relative size. They can be large and small depending on the nature of their glycosaminoglycan chains. We have the heparan sulfate proteoglycan that has its heparan sulfate and an example of a large type is agrin and a smaller type is testican

For chondroitin sulfate proteoglycan, we have chondroitin sulfate as the specific glycan and we have bikunin as the smaller example and versican as the larger example. We will look at some of these specific examples.

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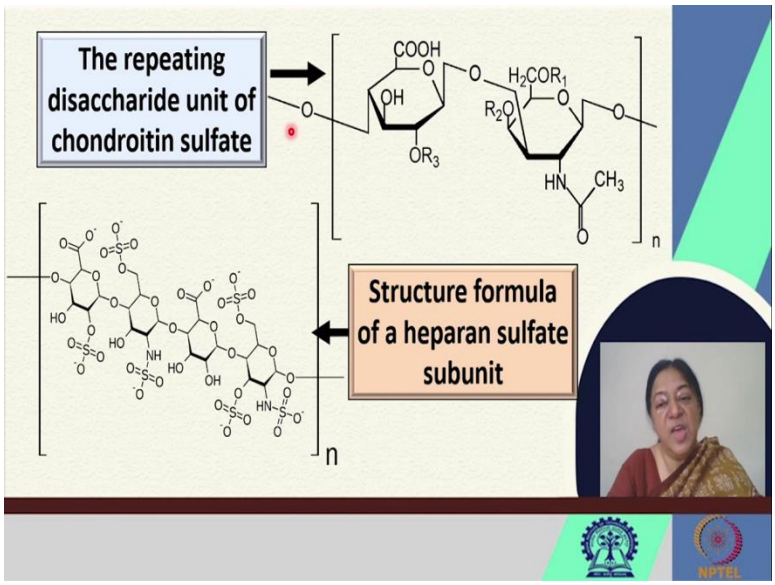

Now, if we look [refer to slide] at the repeating unit of chondroitin sulfate. This will be repeated in the way it interacts with the specific amino acid in forming the conjugate. Here, we have the structural formula for a heparan sulfate subunit.

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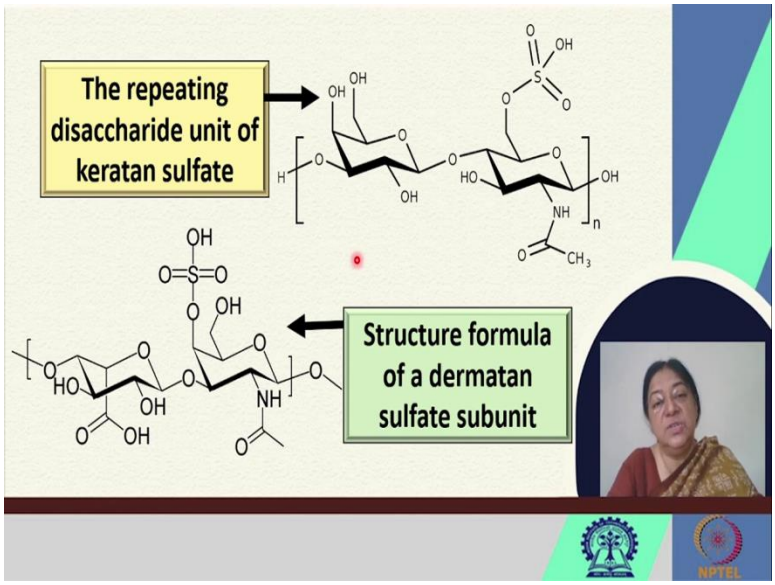

Next, we have the example of a keratin sulfate subunit and an example of a dermatan sulfate subunit. The idea here is to understand that these are the repeating units that are connected with the protein, the specific amino acids that are used in these linkages are serine or threonine or asparagine. So, these are the smaller polar amino acid residues that are involved in this linkage.

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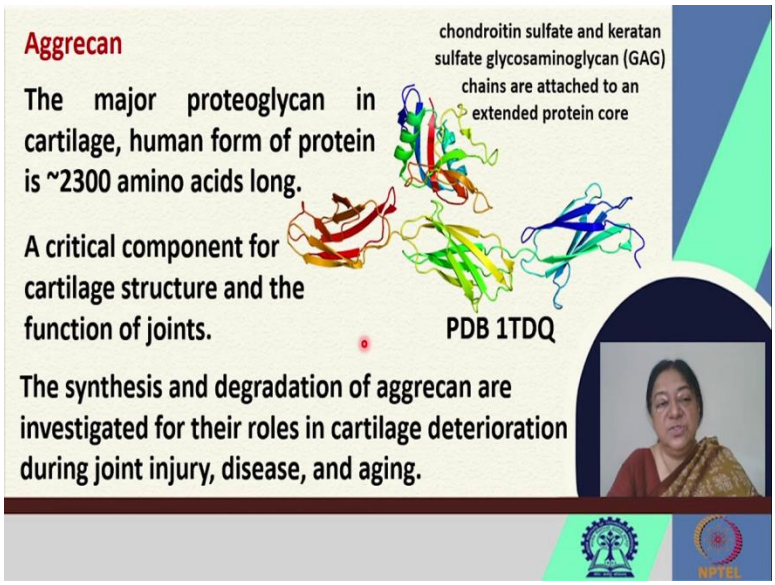

If we look at the structures and the specific roles of some of these examples, we have aggrecan. This is a major proteoglycan in cartilage and the human form of the protein is approximately 2300 amino acids long. Given that it is involved with the cartilage formation, the presence of the carbohydrate in the formation of the proteoglycan gives its strength as well. So, this is a critical component for cartilage structure and the function of joints.

So, we have examples of the chondroitin sulfate, the keratan sulfate glycosaminoglycans that are chains that are attached to an extended protein core. This attachment gives the strength to the cartilage, which is why it functions in the specific formation of the cartilage structure.

The synthesis and degradation of this proteoglycan aggrecan are investigated for their roles in cartilage deterioration and during joint injury, disease and aging. The cartilage deterioration results from the proteoglycan disruption in its way of interaction.

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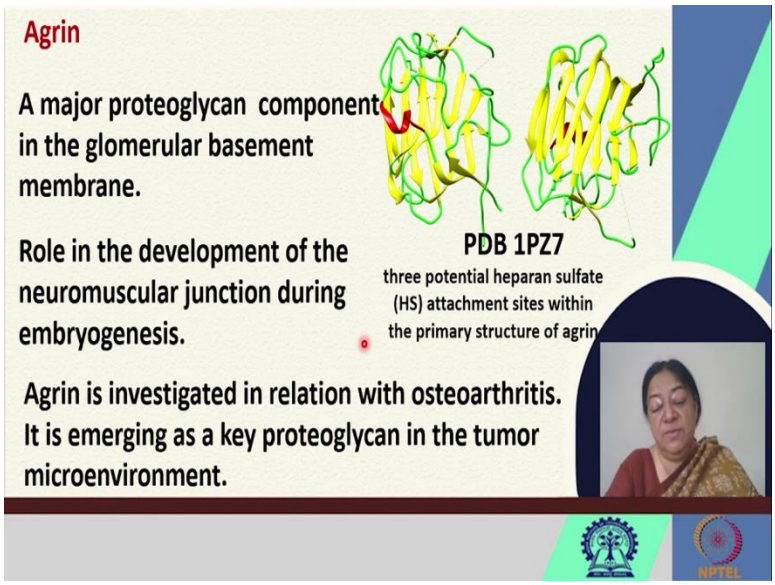

Another large type protein, a major proteoglycan is in the glomerular basement membrane. This has a role in the development of the neuromuscular junction during embryogenesis.

So there are heparan sulfate examples here, within the primary structure of agrin and this is investigated in relation to osteoarthritis and it is also now in investigations, where it is emerging as a key proteoglycan in the tumor microenvironment.

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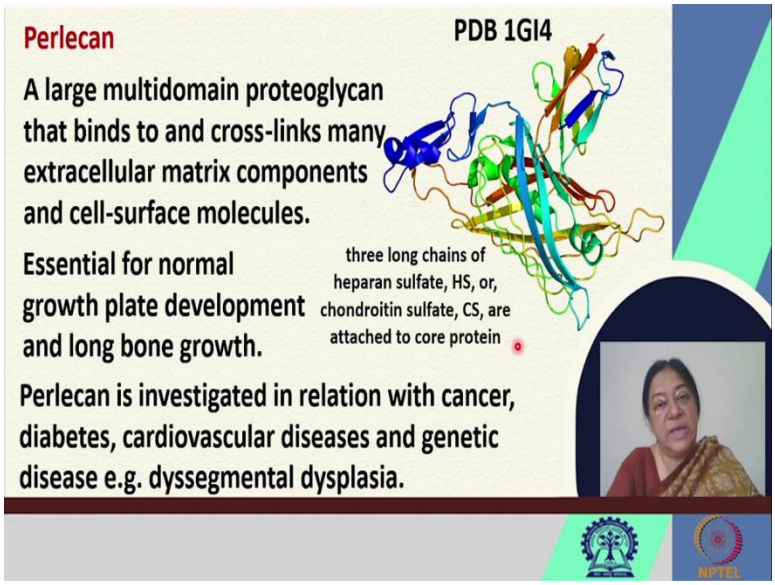

Now we look at another example, perlecan. Here also we have a very large multidomain proteoglycan, that binds to and cross-links many extracellular matrix components in cell-surface molecules. We realize that some of these interactions are important for the different types of processes that we mentioned; be it wound healing, blood clotting, cell-cell adhesion or even migration.

This is essential for normal growth plate development and long bone growth. And here also, we see three long chains of heparan sulfate HS, chondroitin sulfate which is marked CS attached to the core protein.

This connection gives these molecules their strength. In this case, perlecan is investigated in relation to several diseases such as cardiovascular diseases and some genetic diseases in addition to cancer and diabetes.

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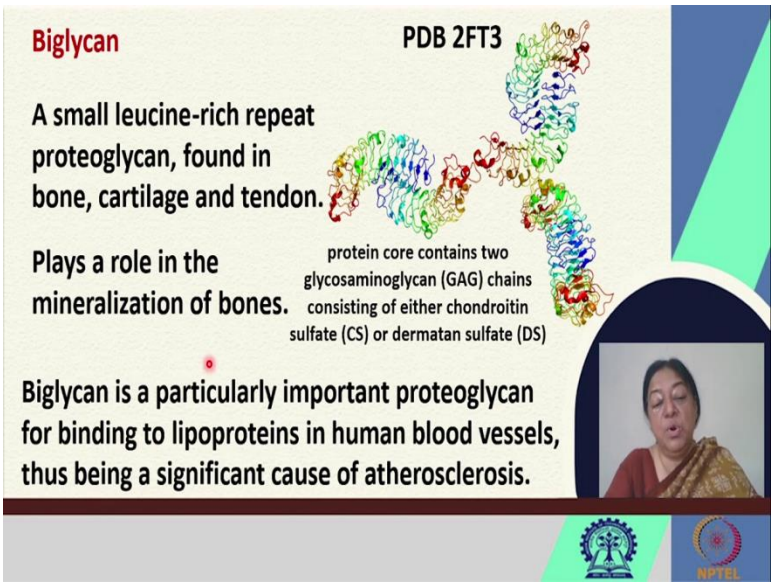

In a biglycan unit, that is a small leucine-rich repeat proteoglycan we see that the leucine is involved in a lot of hydrophobic interaction that again give the strength. So that it could be found in bone, cartilage and tendon.

This also plays a role in the mineralization of bones and there is a protein code that contains two GAG chains that is either consisting of chondroitin sulfate or dermatan sulfate.

So we can have heparan sulfate, chondroitin sulfate, keratan sulfate, dermatan sulfate; that are the repeating units of these carbohydrates that are linked to the proteins to form the proteoglycans. This biglycan is a particularly important proteoglycan, for binding to lipoproteins in the human blood vessels. So, this is an important protein and has again a role in disease.

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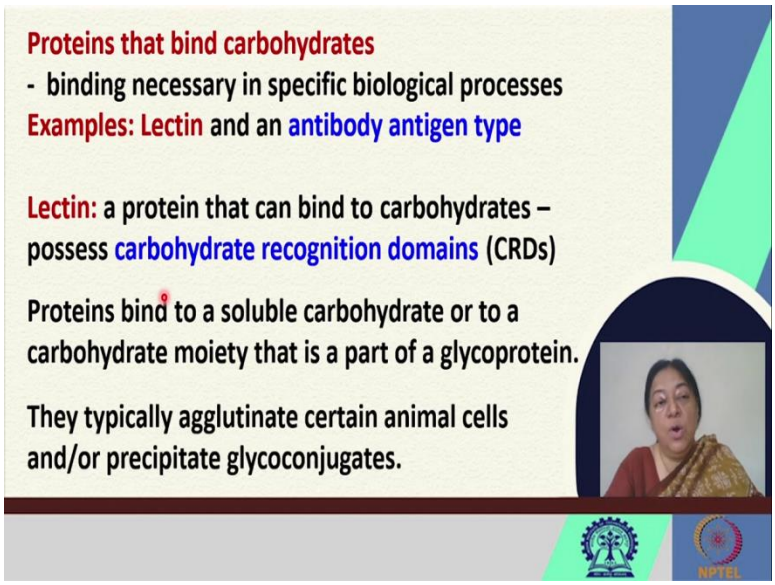

So far what we have looked at is, we have looked at the proteins that are covalently attached to the carbohydrates which is by far a very important aspect of protein carbohydrate interactions, but if we look at proteins that bind carbohydrates, these are of a different nature.

The binding is necessary in specific biological processes. If we have a proteoglycan where we have a carbohydrate linked to the protein and after we have the protein linked to the carbohydrate for the cell adhesion process or any such process to occur that involves a protein carbohydrate interaction, a non-covalent type of interaction, this is when these types of proteins come into the picture; where they have a specific carbohydrate recognition domain.

The examples of such are lectins and there are specific antigen antibody types. The lectin is a protein that can bind to the carbohydrate and it contains carbohydrate recognition domains on the surface of the protein. The protein binds to a soluble carbohydrate or it can bind to a carbohydrate moiety that is already a part of a glycoprotein.

The glycoprotein is one where we have a glycoconjugate of a sugar with a protein and these proteins for example, a lectin can bind to either a soluble carbohydrate individually or it could bind to a carbohydrate moiety that is already part of a glycoprotein. They typically agglutinate certain animal cells and/or precipitate glycoconjugates in several types of reactions.

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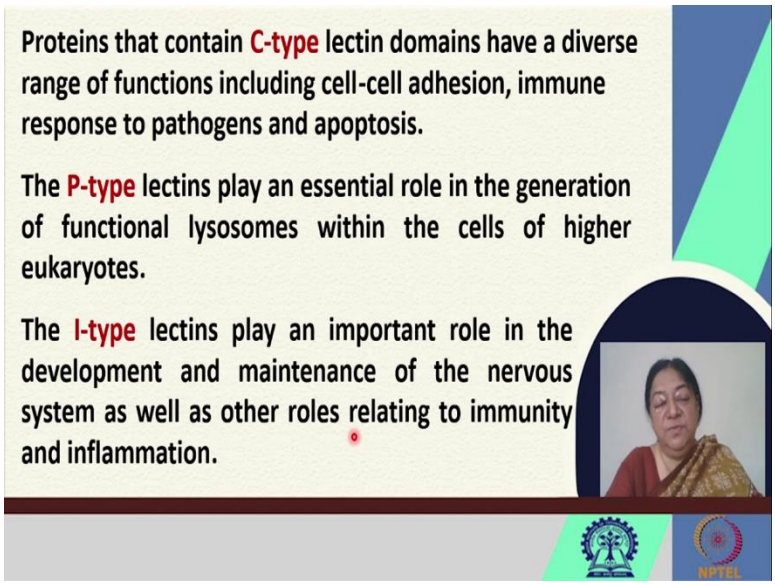

There are different types of lectins. Proteins that contain C-type lectins, have a diverse range of functions as we mentioned cell-cell adhesion, immune response to pathogens and apoptosis.

We know that our cell surface has a glycoconjugate where the sugar is attached to a protein in the cell membrane. Now we have a protein that is going to attach to this sugar, that is already covalently connected to a protein.

The P-type lectins play an essential role in the generation of functional lysosomes, within the cells of higher eukaryotes and then, there is the I-type. These play an important role in the development and maintenance of the nervous system, as well as other roles that relate to immunity and inflammation.

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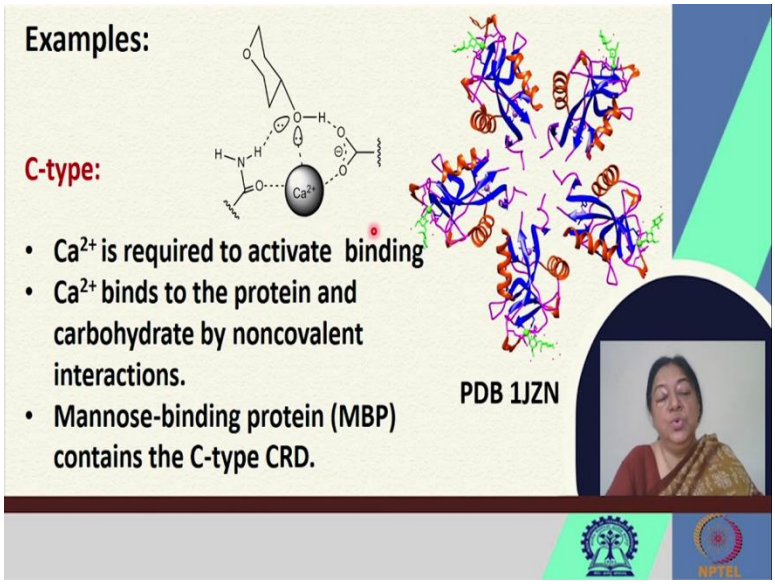

We look at the specific examples now. In the C-type, we have a specific protein molecule and we have a connectivity. Here [refer to slide], in this case there is a calcium ion present. Here we see an acidic amino acid being involved and an amide type being involved, like we saw in the previous cases of the glycoconjugates where we had the connection between an asparagine.

This calcium is required to activate the binding process. It binds to the protein and the carbohydrate by non-covalent interactions. So, here is our connection with the carbohydrate and these are the connections with the protein and example is a mannose-binding protein, that contains a typical C-type carbohydrate recognition domain.

This is the CRD, a carbohydrate recognition domain and we have the C-type indicating that we have this specific connection to the calcium ion here, that is required for the binding.

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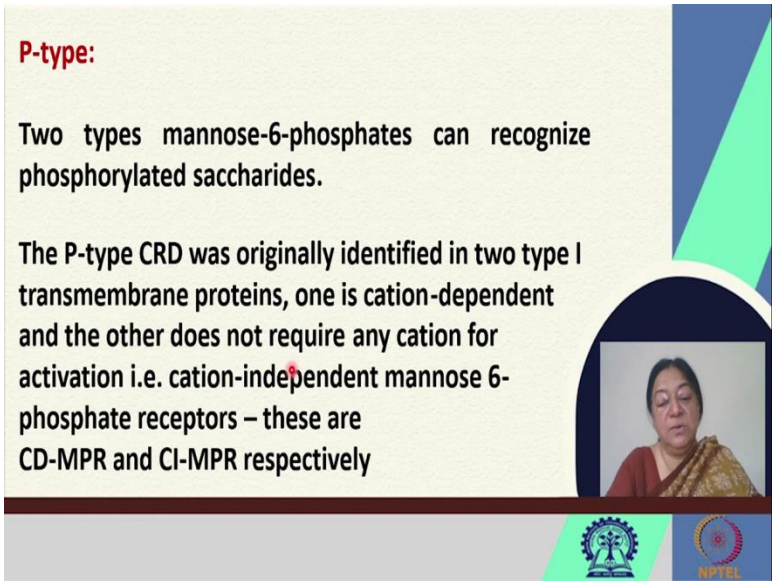

In the P-type, we have two types. The mannose-6-phosphates can recognize the phosphorylated saccharides, and in this type there are two types; one that requires a cation. So it is cation-dependent, called the CD the cation-dependent mannose protein.

Type two that is the cation independent type the CI- MPR, which is the mannose phosphate receptor. So, we have a cation-dependent mannose phosphate receptor and a cation-independent mannose phosphate receptor.

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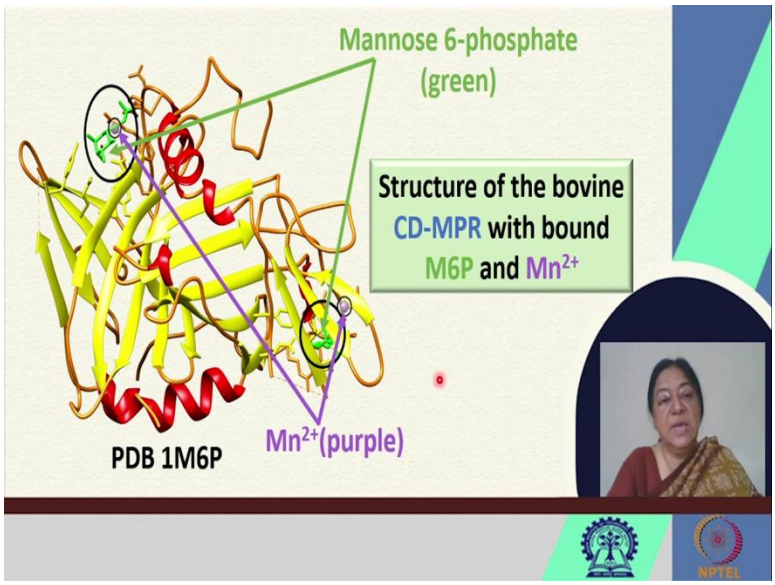

We will look [refer to slide] at the specific structure of the mannose phosphate receptor. This is the structure of the bovine cation-dependent mannose phosphate receptor, that has the bound M6P. These are the mannose 6-phosphates, the two sugars and we have the cation-dependent, where we have manganese as the particular cation in this case.

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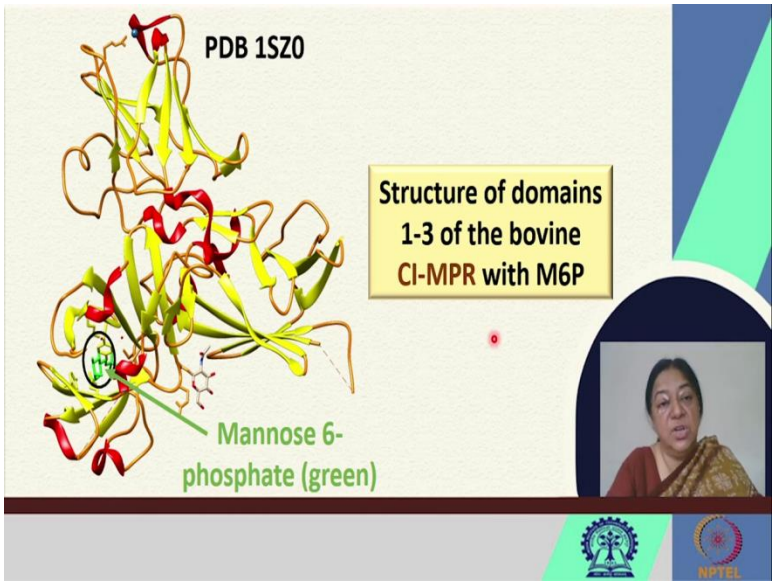

If we look at the cation-independent type, it would mean the cation-independent mannose phosphate; the receptor in this case has the mannose 6-phosphate only and does not require a cation for function.

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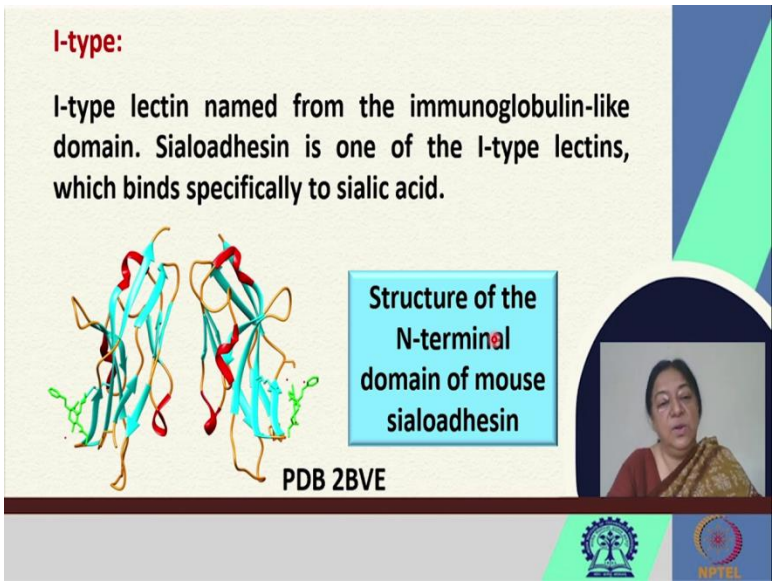

In the I-type ones where the lectin is named from the immunoglobulin-like domain, we have a sialoadhesin as an example of an I-type which binds specifically to sialic acid. So, this is an example of the N-terminal domain of mouse sialoadhesin, involved in specific types of inflammation or other such processes. We see an immune response type, we see a specific cell-cell adhesion type; so these are the interactions that occur.

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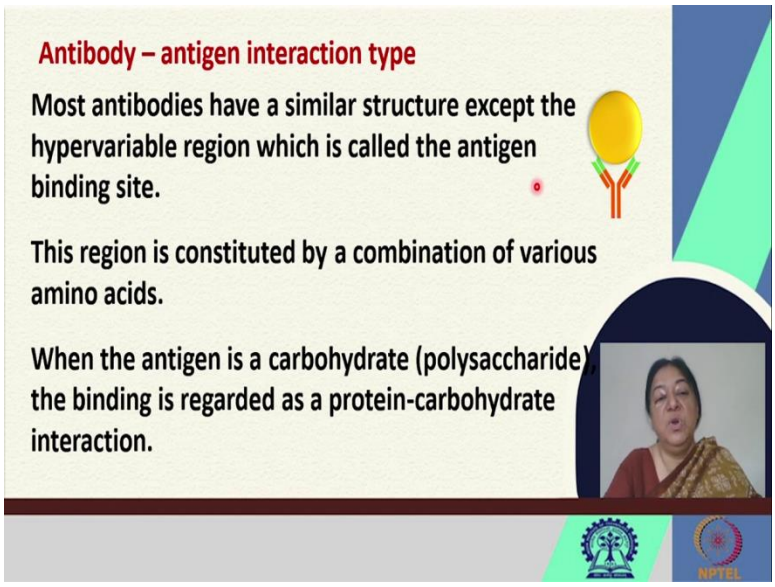

Another type is the antibody-antigen interaction type. In this case, we know that most antibodies have a similar structure except they vary along their hypervariable region that is called the antigen. So this [refer to slide] is the antigen binding site.

They have an overall structure that looks like a Y-shaped structure, that is typical of an antibody and the region is constituted by a constitution of various types of amino acids that are present.

Now, when the antigen that attaches to the antibody at this site is a polysaccharide, we call this a protein carbohydrate interaction in a specific type, where we have the carbohydrate attached to the antibody in a specific antibody-antigen interaction type.

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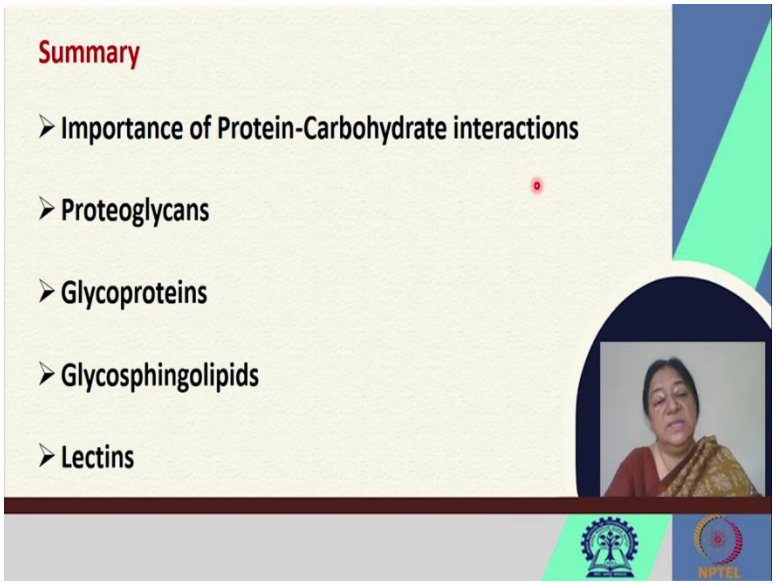

So what we looked at here, is the importance of protein-carbohydrate interactions. Specific examples of them, where we realize that we have a protein, a glycoconjugate, in the forms of glycoproteins, proteoglycans and what is important here is a direct covalent linkage of the protein with the carbohydrate in the specific processes that are involved.

However, there are cases such as lectins and the antibody-antigen type. There are very large number of lectins that recognize carbohydrates themselves, have carbohydrate recognition domains in the protein and these lectins can recognize carbohydrates individually or be attached to carbohydrates that are already attached to other proteins such as the glycoproteins.

So any sugar, any saccharide, oligosaccharide, polysaccharide attached to a protein could also be recognized by the protein that binds to the carbohydrate, the lectins and we could also have the antigen-antibody interactions.

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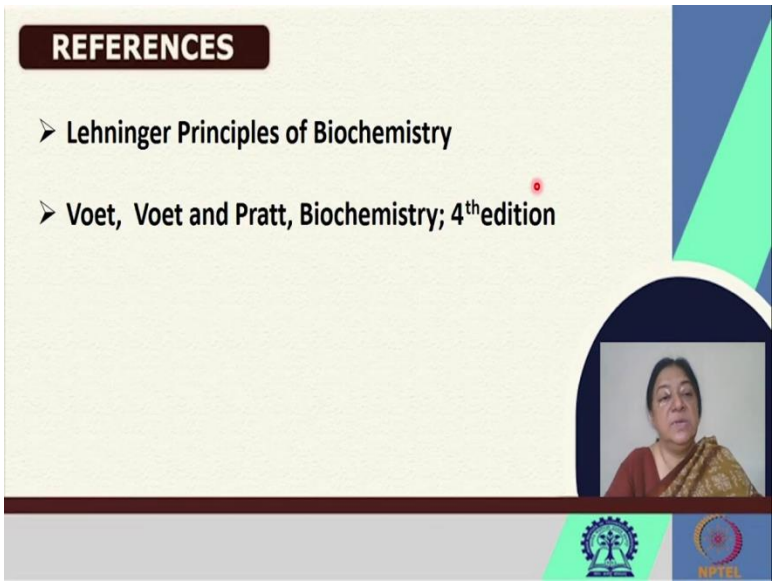

These [refer to slide] are the references.

Thank you.