Fundamentals of Protein Chemistry
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Module - 10
Protein Macromolecule Interactions I
Lecture - 49
Protein Nucleic Acid Interactions - II

In the first module of protein macromolecular interactions, we have beenlooking at protein nucleic acid interactions.

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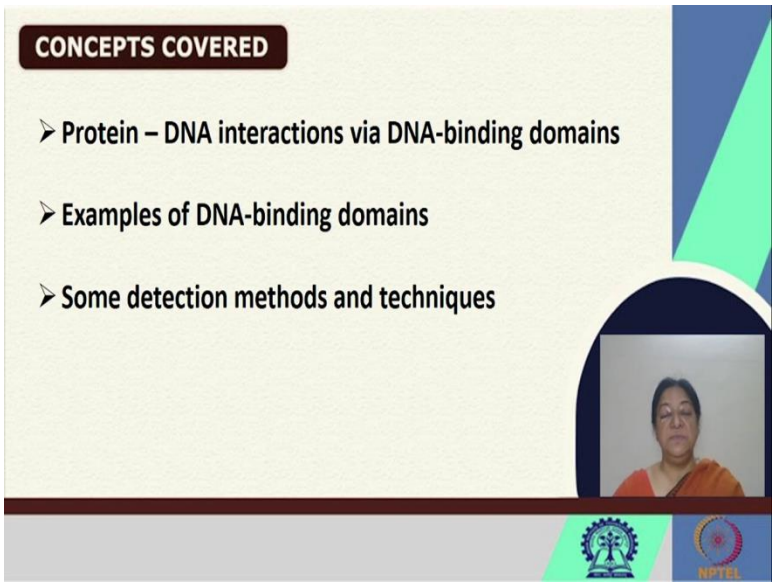

In the previous lecture, we understood why we need these interactions and what are important in RNA and DNA, in their binding characteristics, the specific types of interactions that may be possible and what we mean by specific and non-specific interactions. In this lecture we will be looking at protein DNA interactions in particular. For the protein DNA interactions, there are specific DNA binding domains and their specificity or their mechanism of action is important, based on the structure that they adopt to bind to DNA.

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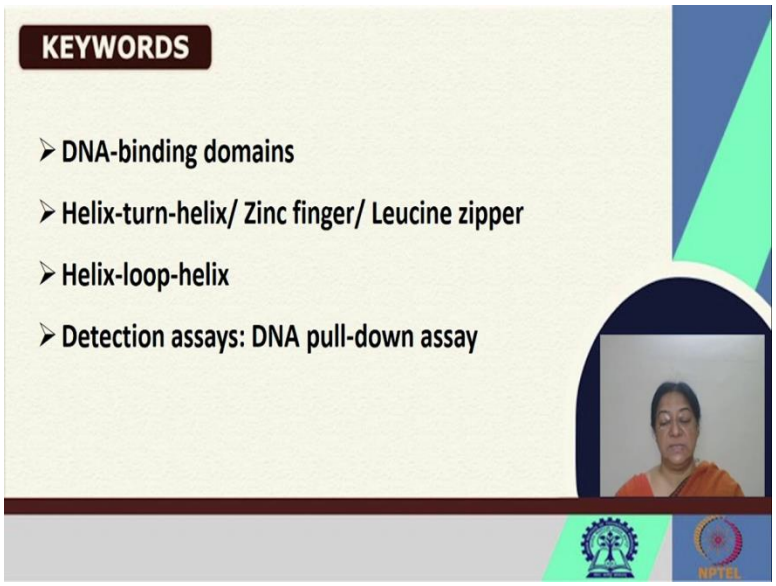

So we will be looking at DNA binding domains, these are helix-turn-helix, zinc finger, leucine zipper, helix-loop-helix and in the detection assays in this particular lecture, we will be looking at the DNA pull-down assay. In the previous lecture, we looked at the electrophoretic mobile shift assay.

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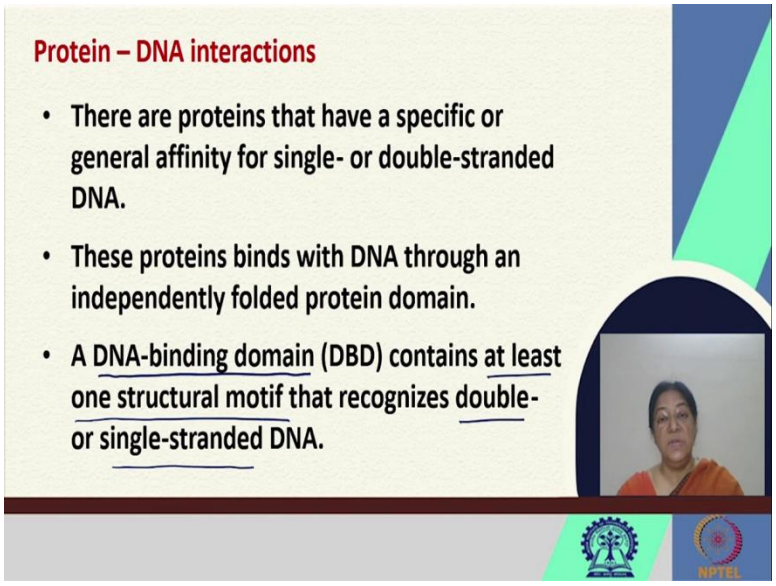

Now when we consider protein DNA interactions, we know that these proteins have a specific or general affinity for single or double stranded DNA. The proteins therefore bind through two DNA, by the use of an independently folded protein domain. This is important because the specificity is important in several cases, depending upon the function and the mechanism of action.

What we have here is a specific DNA binding domain. This DNA binding domain contains at least one structural motif, that is involved in the recognition process of either double stranded or single stranded DNA. We looked at protein ligand interactions, we understood that the recognition is most important in any such process of protein ligand binding.

In this case, our ligand are the nucleic acids. We looked at the specific interactions, we are going to look at now, protein with DNA. We have understood the basic structure of DNA in terms of the sugar phosphate backbone and the specific bases that form the DNA structure.

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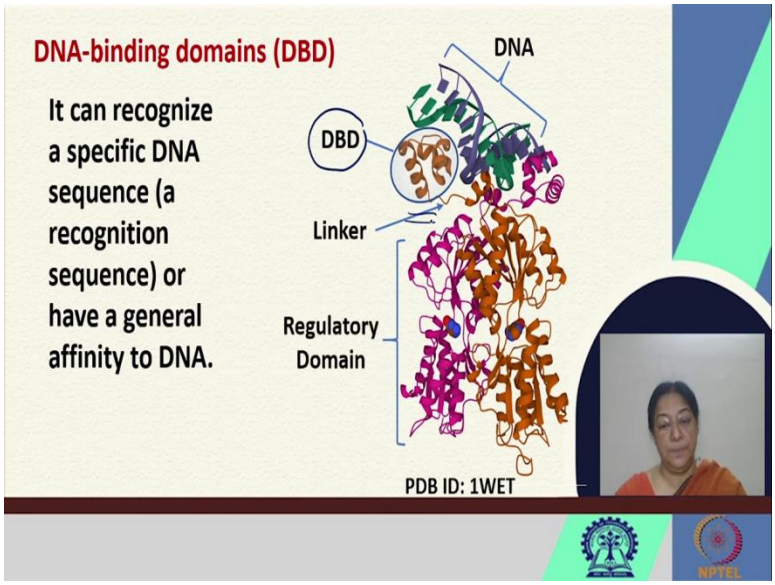

When we look at DNA binding domains, this means that there are special characteristics to these domains. They have to recognize a specific DNA sequence, which is known as the recognition sequence or in general should have an affinity for DNA to facilitate binding of the protein to DNA. So if we look [refer to slide] at the specific structure here, which we will revisit again later in this lecture, we have our DNA bound.

In this region there is a DNA binding domain marked by this circle here, that has a specific structural characteristic and a specific chemical characteristic in terms of the amino acid residues that are formed or are present in this region. There is a linker and here is a regulatory domain, in this specific example of a DNA binding domain.

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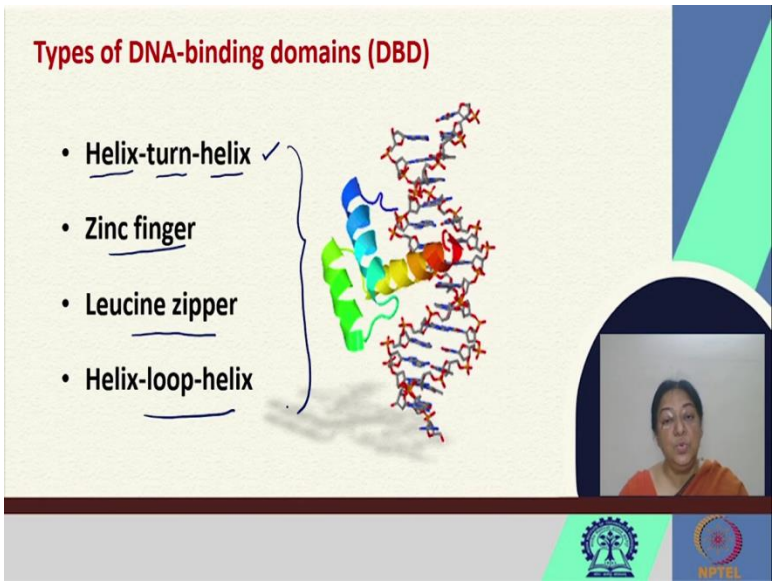

If we look now at the different types of DNA binding domains, we have a helix-turn-helix, that from the name itself suggests what kind of structural aspects we can expect. A helix followed by a turn and then again followed by another helix. When we look at the zinc finger, we will see specific characteristics associated with this.

Another is the leucine zipper and the helix-loop-helix. Each of these comprise a DNA binding domain, which means that their structural characteristics are such that they are going to be binding to the DNA molecule for specific structural and functional reasons.

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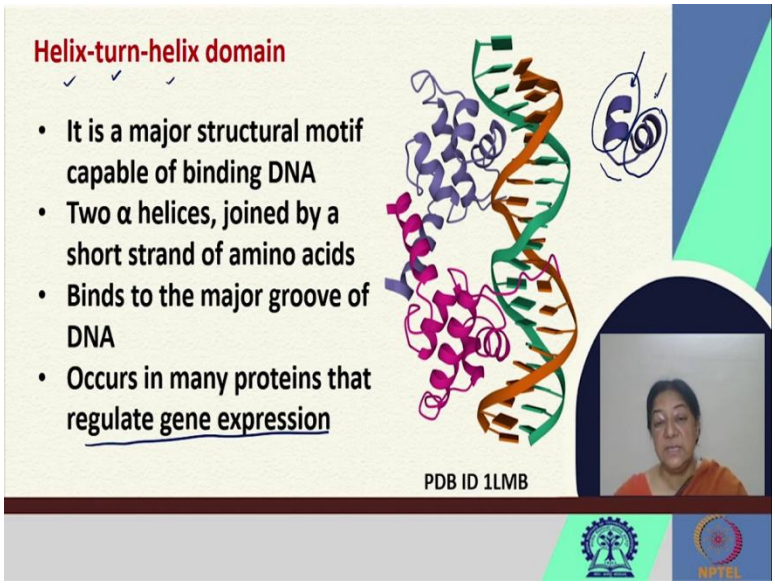

The helix-turn-helix domain as the name suggests, has a specific helix-turn-helix association. It is a major structural motif that is capable of binding DNA and this [refer to slide] is where we have a helix, a turn and again a helix. If we now look at the specific structural aspects, the two α

helices are connected by a short strand of amino acids forming a turn. This can bind to the major groove of the DNA and it occurs in proteins that regulate gene expression. So, the binding is to the major groove of DNA. The helix-turn-helix DNA binding domain is such that there are two helices that are connected by a short term.

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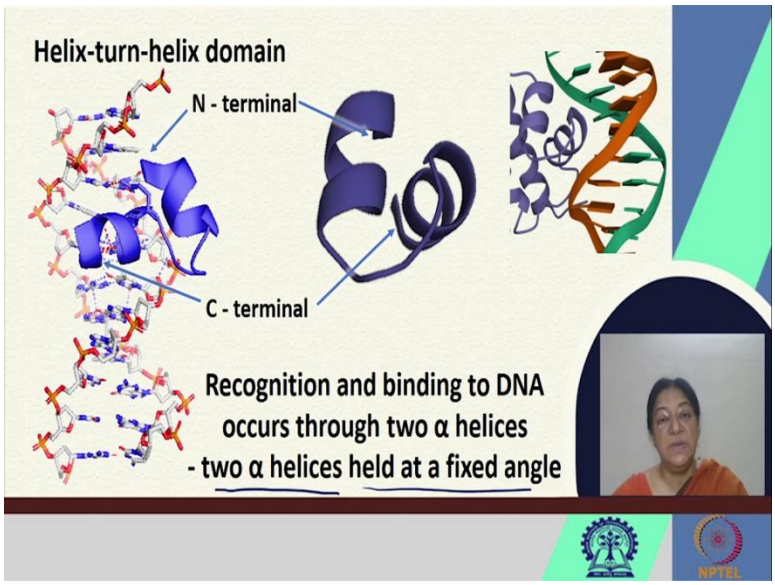

If we now look at this [refer to slide] in a bit more detail, the recognition and binding to the DNA occurs through these two helices and interestingly, these two α helices are held at a fixed angle to facilitate the geometrical interaction with the DNA molecule. So we have the specific angle, the specific recognition and the specific structural characteristics, that are going to allow the DNA to interact with this specific DNA binding domain.

We have the N-terminal part of the domain, we have the C-terminal part of the domain and we realize that the recognition and binding occurs in the major groove, in a manner where the two α helices are going to be held at a specific angle.

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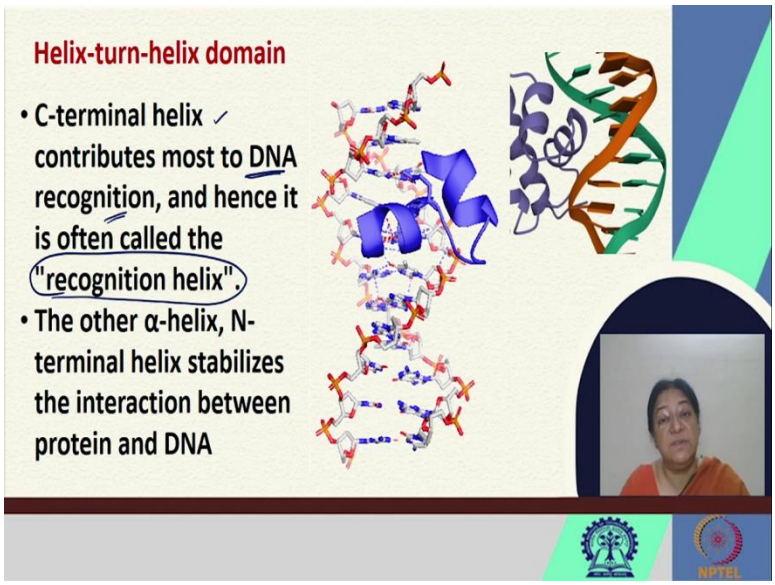

So there are two helices that are connected by a turn. The C-terminal helix in this case, contributes most to DNA recognition because the C-terminal helix is often referred to as the recognition helix. So, the C-terminal residue helix is the recognition helix, the other helix that is the N-terminal helix, stabilizes the interaction between the protein and the DNA and because of the fixed angle, it is able to position the C-terminal helix in a manner that facilitates the recognition of the protein domain with the DNA.

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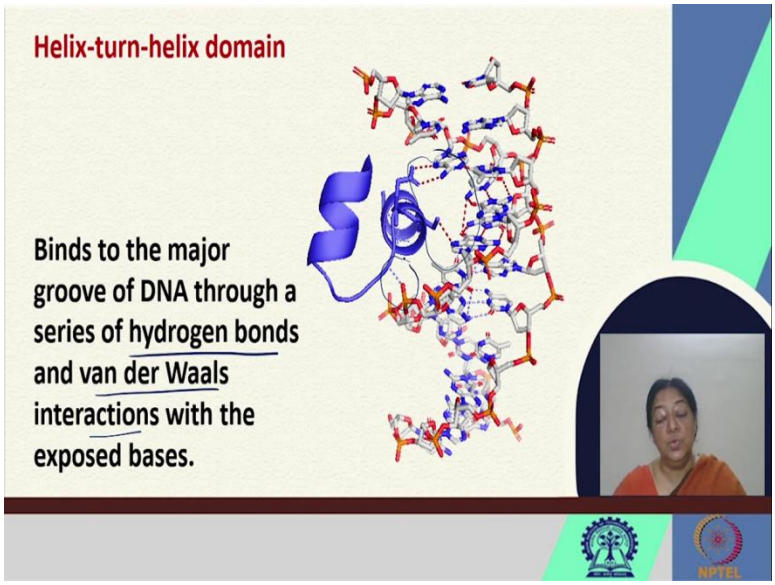

This binds to the major groove as we saw and the groove binding is through a series of hydrogen bonds and van der Waals interactions, with the exposed bases. So, here [refer to slide] are the specific interactions that have been shown with dotted lines. These are the specific interactions that occur due to the C-terminal helix and the positioning due to the N-terminals.

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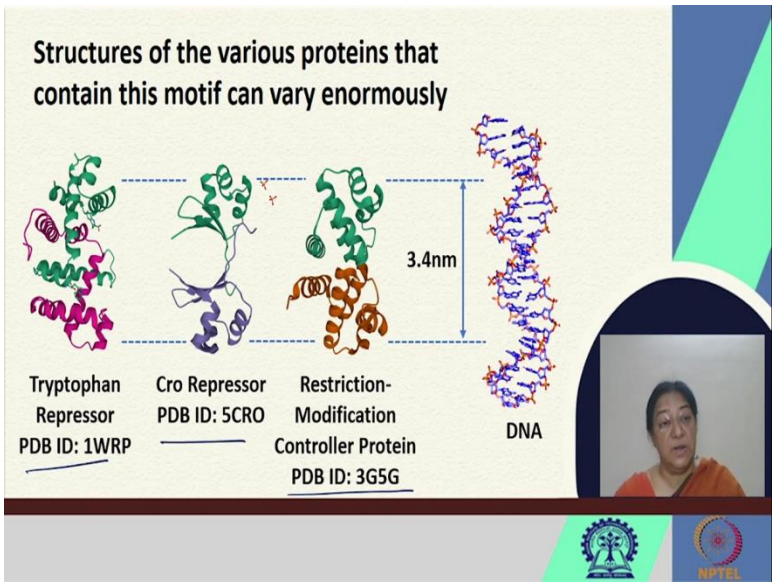

Structures of the various proteins that contain this motif, can actually vary enormously. So, we are looking [refer to slide] at a helix followed by a turn followed by another helix. The beauty of this is that each of these have very structural variations, but their binding motif, the helix-turn-helix motif, is very similar.

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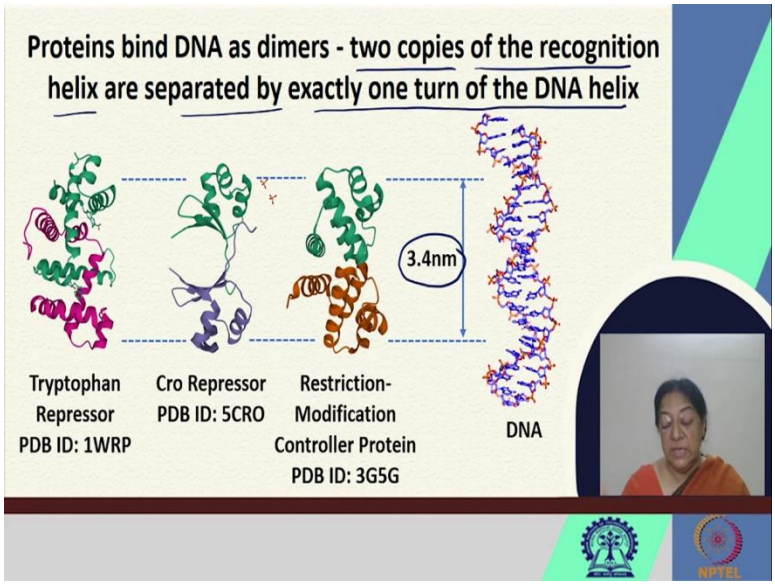

When we look at this, we see that these proteins actually bind to DNA as dimers and there are two copies of the recognition helix, that are separated by exactly one turn of the DNA helix, that is 3.4 nm. So, the beauty of the recognition lies in the fact that these occupy the major groove and the complete turn gives them the recognition required for the binding to occur, where the protein bind DNA as dimers.

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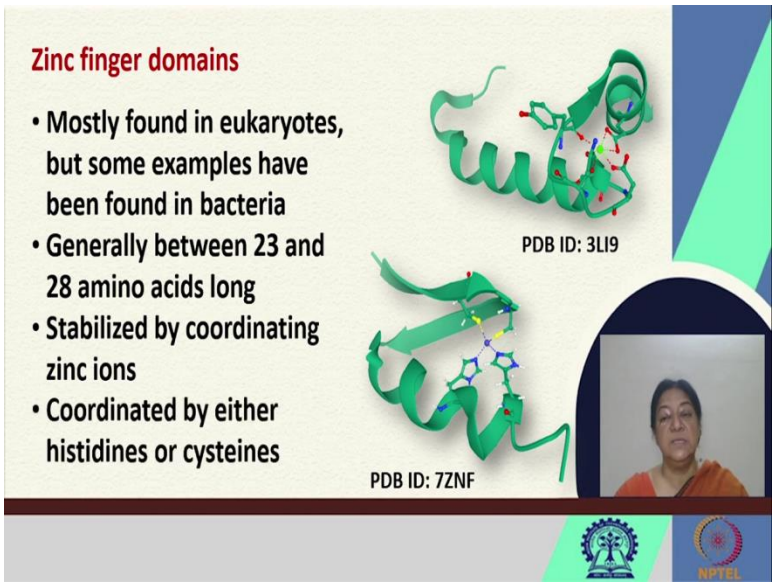

In the zinc finger domains, the second DNA binding domain that we are going to discuss, we have a specific structural motif and as the name implies, we have a zinc ion. This is mostly found in eukaryotes, but there are some examples that have been found in bacteria. This is generally 23 to 28 amino acid residues long and it is stabilized by coordinating zinc ions and the zinc ions are coordinated to histidines or cysteines.

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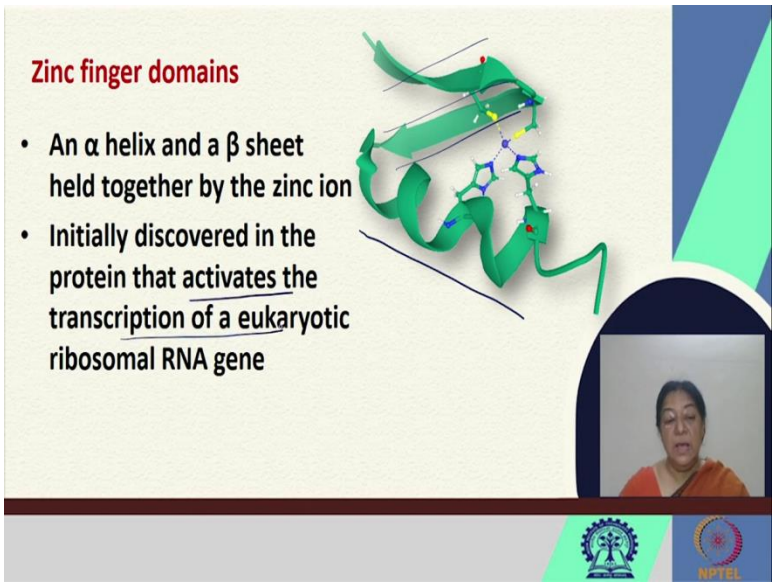

When we look [refer to slide] at these zinc finger domains, we have in this case as we can see an α helix and a β sheet here, we can see the strands of the β sheet that are held together by the zinc ion and this was initially discovered in the protein that activates the transcription of a eukaryotic ribosomal RNA gene. So we understand that the importance of this particular domain lies in the

activation of the transcription of the ribosomal RNA gene, that requires the recognition in the proper fashion.

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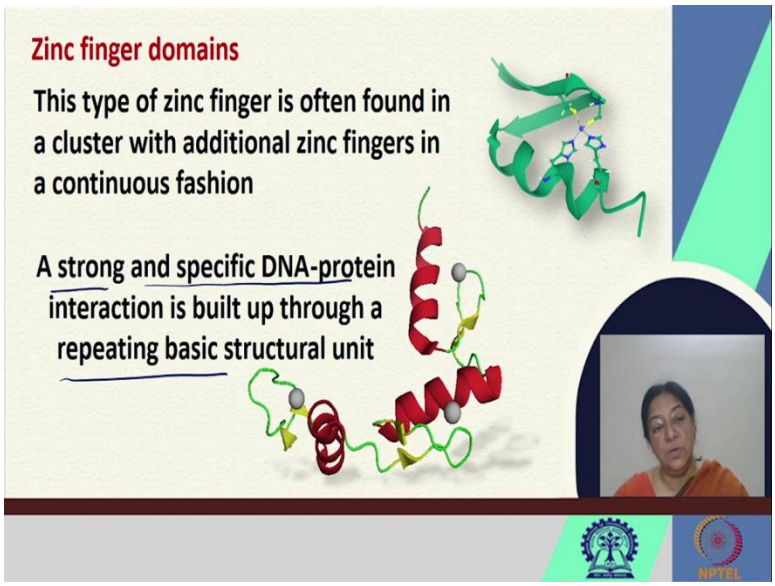

This type of zinc finger is sometimes again found in a cluster, with additional zinc fingers that occur in a continuous fashion to facilitate again recognition. Like we saw in the previous case where we have the helix-turn-helix case, we found out that this protein interacts as dimers.

In this case also, we will see [refer to slide] that there are additional cluster units here which allow a strong and specific DNA protein interaction to occur because of the repeating units that interact with the DNA. The zinc finger domains that we see have repeating units that are going to interact with the DNA form.

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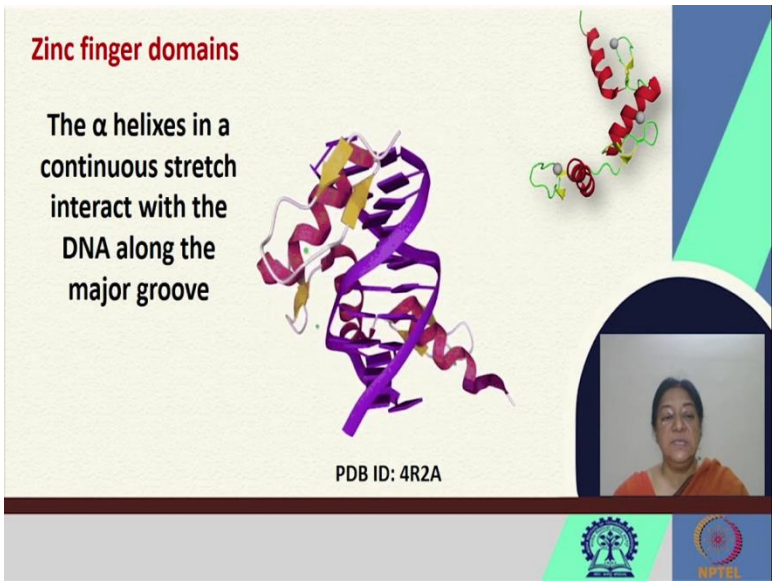

The α helices in the continuous stretch, interact with the DNA along the major groove of DNA and the strength and the specificity depends on the number of these zinc finger repeats that we have in the recognition site.

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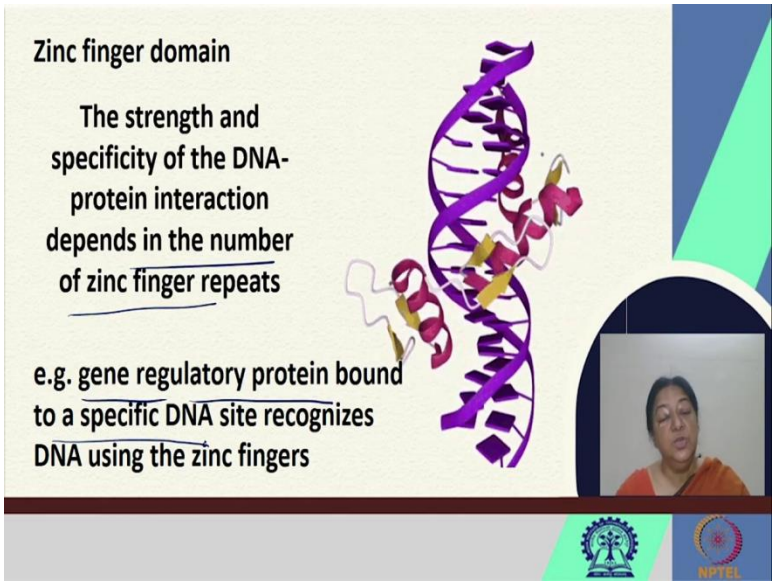

For example, a gene regulatory protein that is bound to a specific DNA sequence using these zinc fingers. So these are found in gene regulatory proteins.

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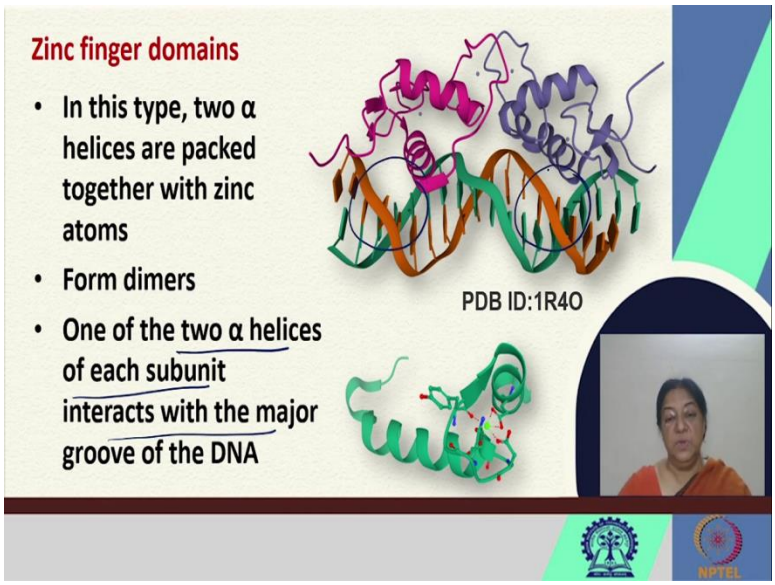

The zinc finger domains in this case, have two α helices, packed together with the zinc atoms and again they form dimers in the way they interact with the DNA. One of the two α helices on each of these subunits, interacts with the major groove of DNA in the formation of this structure and the recognition is such that the dimer interacts with the major groove as we can see here [refer to slide]. The helix interacting with the major groove giving a specific recognition in a geometric fashion and in a chemical fashion as well.

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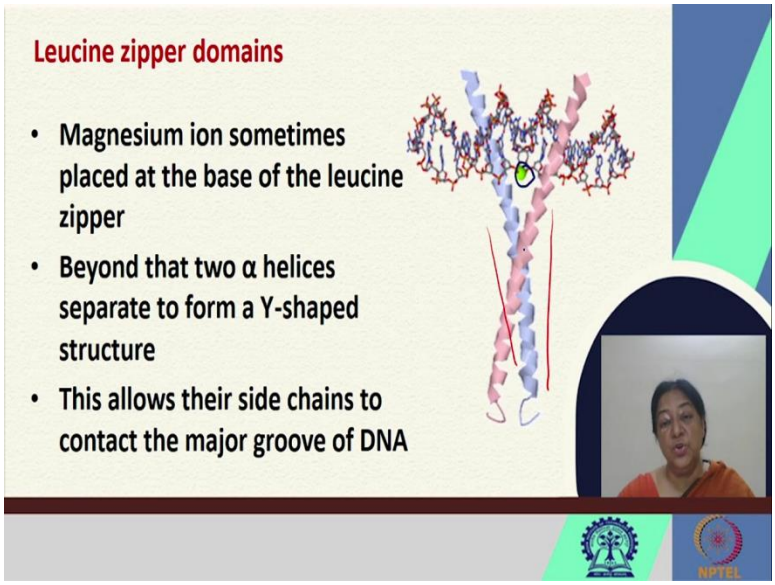

So what we see [refer to slide] in the leucine zipper domains, is an interaction between the specific types of amino acids that are present in the two helices that form this zipper domain. Now the magnesium ion that is present here is interesting because it interacts with the sugar phosphate backbone. The sugar phosphate backbone being negatively charged, this magnesium

ion is sometimes placed at the base of the leucine zipper and it facilitates the interaction with the DNA and allows the recognition of the helices.

Beyond that as we see, the two helices form a Y shaped structure and the interactions between the two helices allowing a hydrophobic type of interaction because of the presence of the leucine residues, recognizes or looks like a zipper which is why it is called a leucine zipper domain that interacts with the DNA. So the side chains now, can interact with and recognize the major groove of the DNA.

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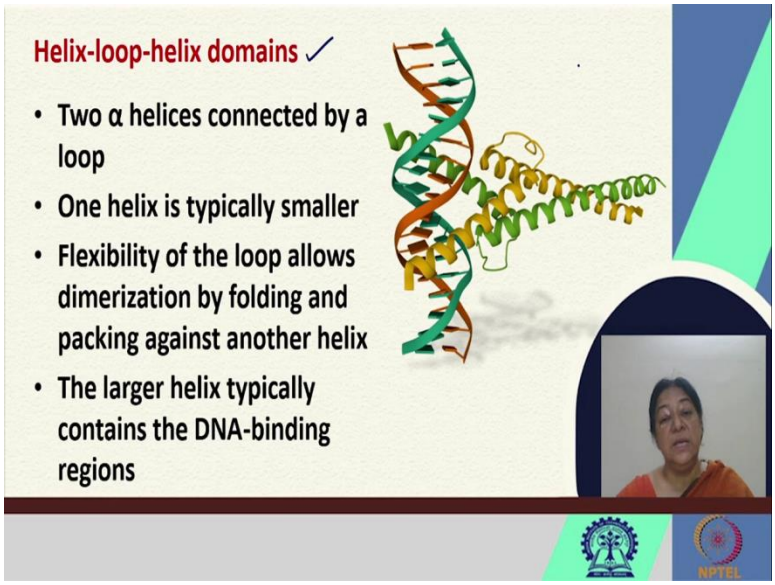

One of the domains that we will be looking at now is the helix-loop-helix domain. We looked at the helix-turn-helix domain and as the name specifies here, this is a helix-loop-helix domain. In this case the two α helices are connected by a loop. One helix is typically smaller than the other one and the flexibility of the loop allows a dimerization by folding, that packs against the other helix unlike the helix-turn-helix motif, where we saw specific fixed angle that allows the recognition.

In this case, we have one small helix and one relatively larger helix and because of the flexibility of the loop, it allows for a different type of folding and packing against the other helix and the larger helix typically contains the DNA binding region, which recognizes the DNA in the binding process of these types of proteins.

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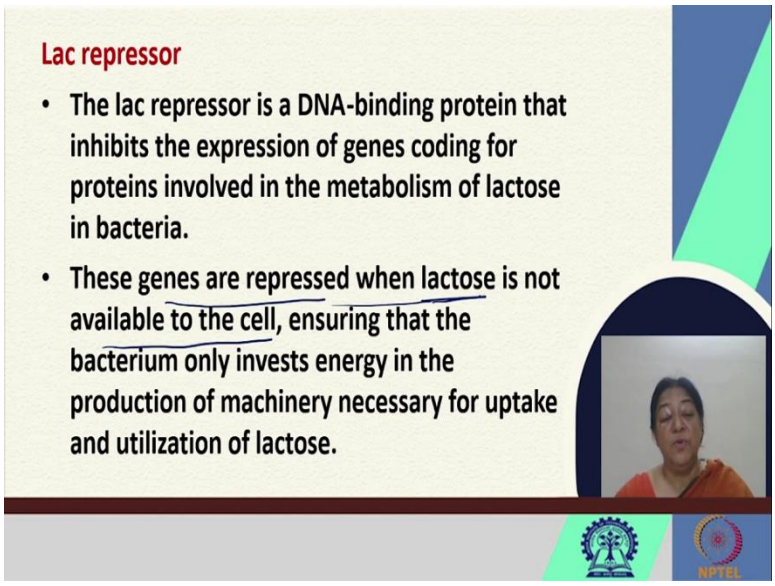

The lac repressor protein is a DNA binding protein, that inhibits the expression of genes that code for the proteins involved in the metabolism of lactose in bacteria. Now these genes are repressed when lactose is not available to the cell. This ensures that the bacteria only invests energy in the production of the machinery necessary for the uptake and utilization of lactose.

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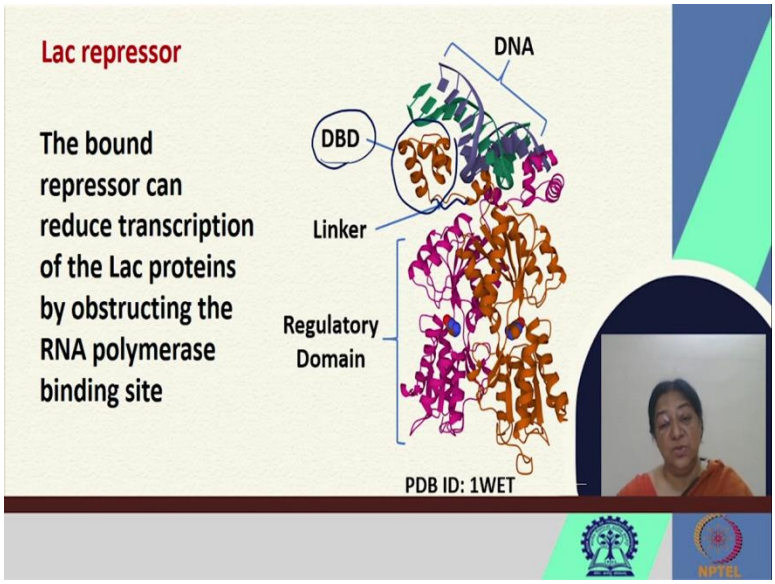

The bound repressor can reduce transcription of the lac proteins, by obstructing the RNA polymerase binding site. This is interesting in the fact that we have [refer to slide] a DNA binding domain here, we have a linker that can associate or regulate the DNA binding domain to bind with the DNA and we have a regulatory domain in addition to this.

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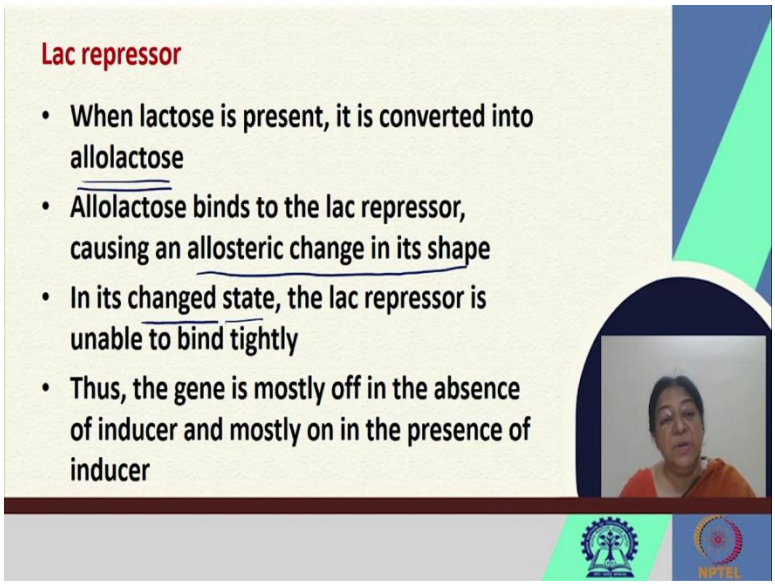

When the lactose is present, it is converted to allolactose. Now allolactose binds to the lac repressor in an allosteric fashion and it causes an allosteric change in its shape and in this changed state, the lac repressor is unable to bind tightly. The gene is off and on depending upon the absence or presence of the inducer. So the regulation that we see here [refer to slide] of the allostery associated with this, is because of the presence of the inducer.

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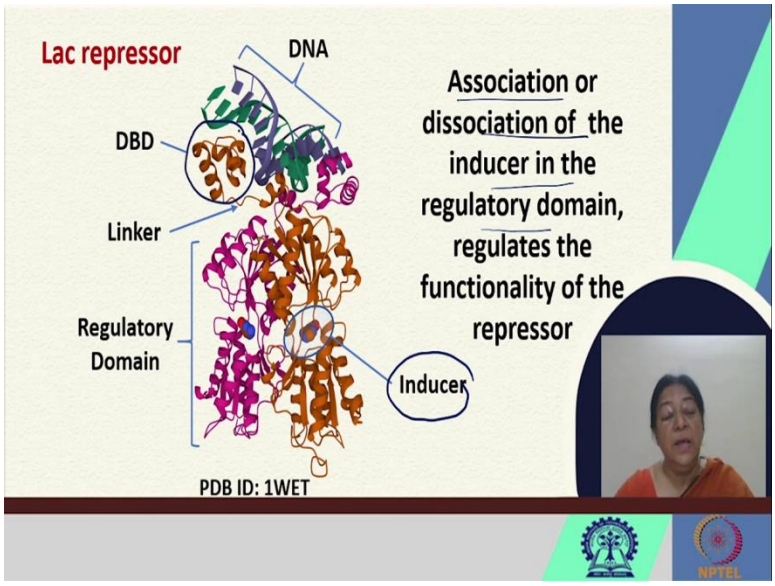

The association or dissociation of the inducer in the regulatory domain, regulates the functionality of the repressor and the location of the DNA binding domain can be altered in a manner, that affects the DNA binding of this particular protein.

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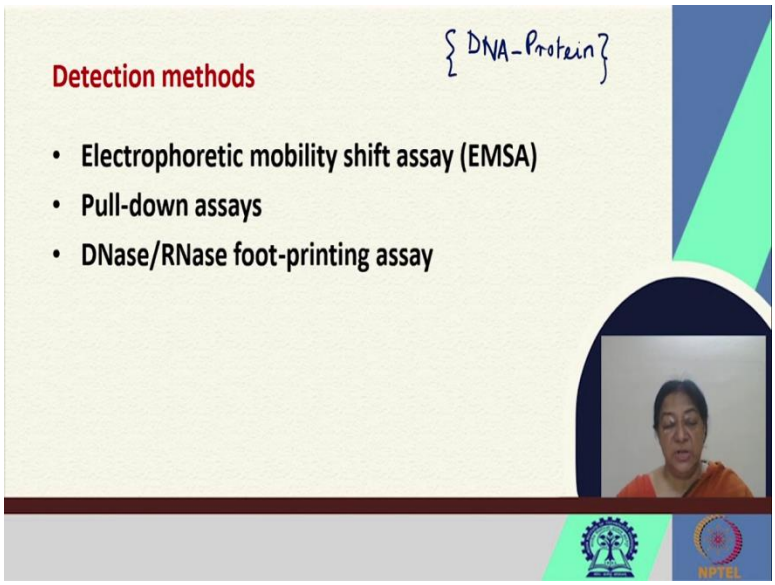

In the specific detection methods that can be used to determine whether we have our nucleic acid protein bound form in the complex form, there are specific methods that we can look at. There is the electrophoretic mobility shift assay which we discussed in the previous lecture, there is the pull down assays that can also identify a DNA protein complex formation and we have the DNase/RNase foot-printing assays.

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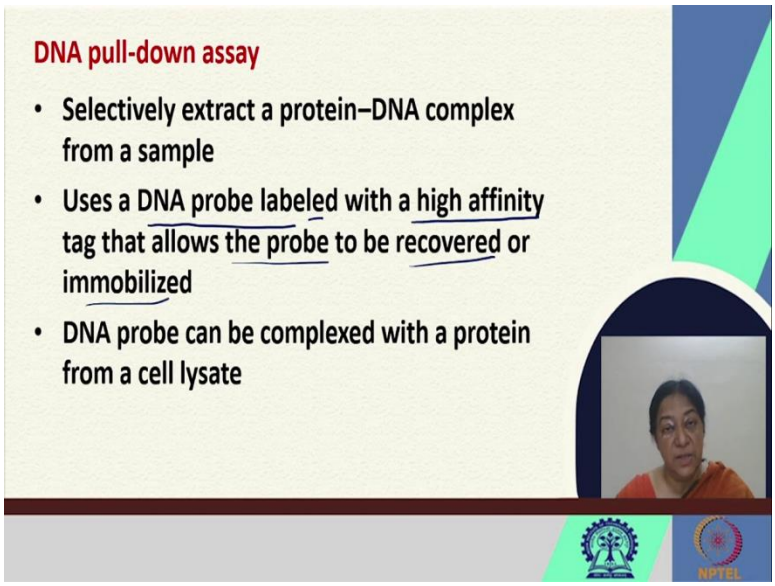

In the DNA pull down assay, there is a selective extraction of a protein DNA complex from a sample. In this case, there is a DNA probe that is labeled with a high affinity tag, that allows the probe to be recovered or immobilized. So, there is a DNA probe that is labeled with a high affinity tag.

There are several ways that this tag can be labeled and associated with the protein that is going to bind the DNA of choice. The DNA probe can then be complexed with the protein from a cell lysate and the protein that binds to the DNA, extracted in the process.

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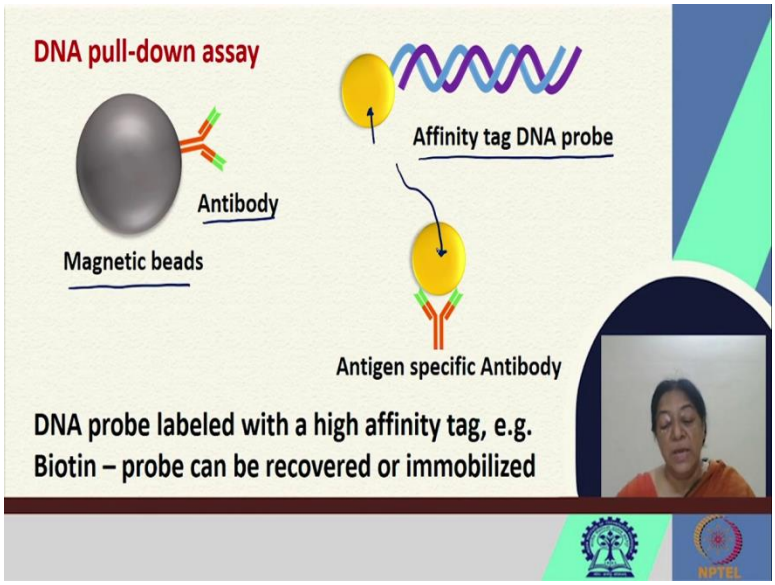

So here [refer to slide] is our affinity tag DNA probe, we have magnetic beads bound to the antibody and this is where we would have the antigen specific antibody. The process is such that the DNA probe labeled with the high affinity tag, for example in biotin, we can have the probe recovered or immobilized.

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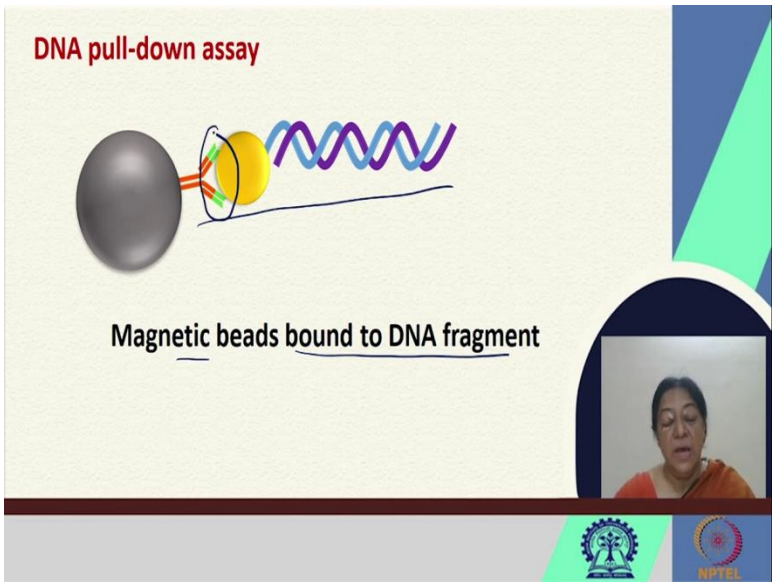

The way this works is, they are bound to the DNA fragment in this [refer to slide] fashion, where the recognition occurs in the antibody and affinity label site.

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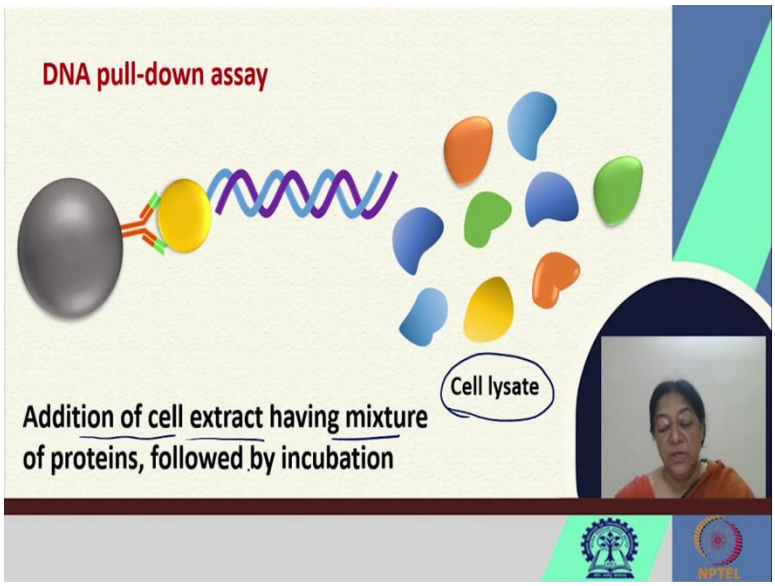

The specific proteins of interest now are brought through the cell lysate, which has a mixture of proteins. So, the addition of the cell extract occurs, that has the mixture of proteins. This is followed by incubation and during the incubation the DNA binding proteins would bind to the DNA present and the rest would be lost and not be bound to the DNA.

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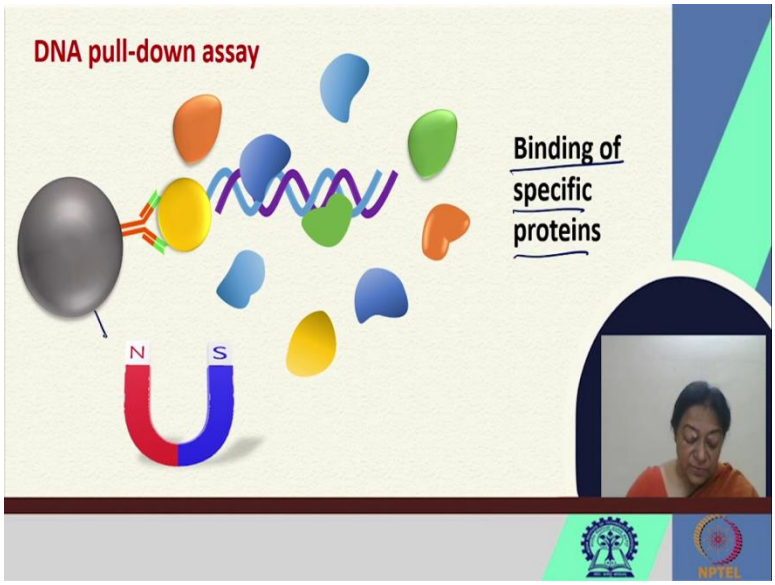

So as a result what happens because of the presence of the magnetic bead now, there is separation.

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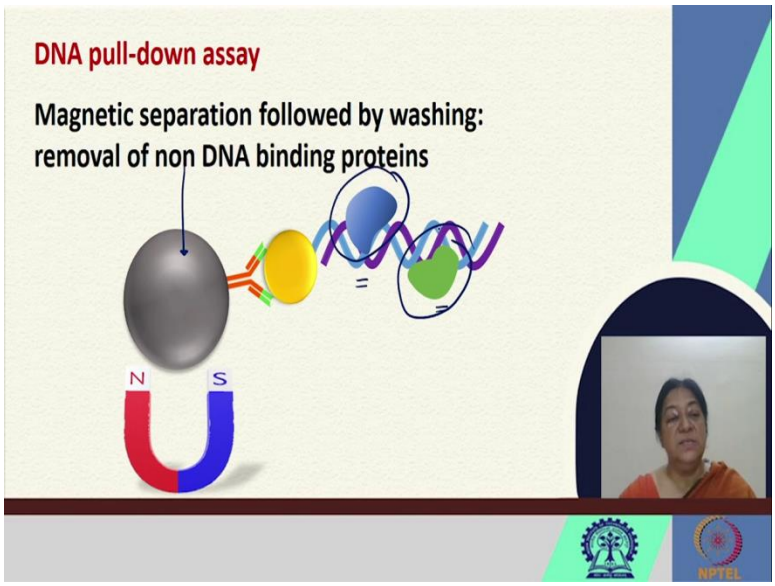

So the separation occurs where the DNA binding domain are bound to the DNA, that has been pulled down due to the magnetic attraction for the magnetic beads and now we have the protein bound to the DNA. This protein now has to be eluted or removed for analysis. So the magnetic separation occurs, this is followed by washing and in the event, there is a removal of the non DNA binding proteins.

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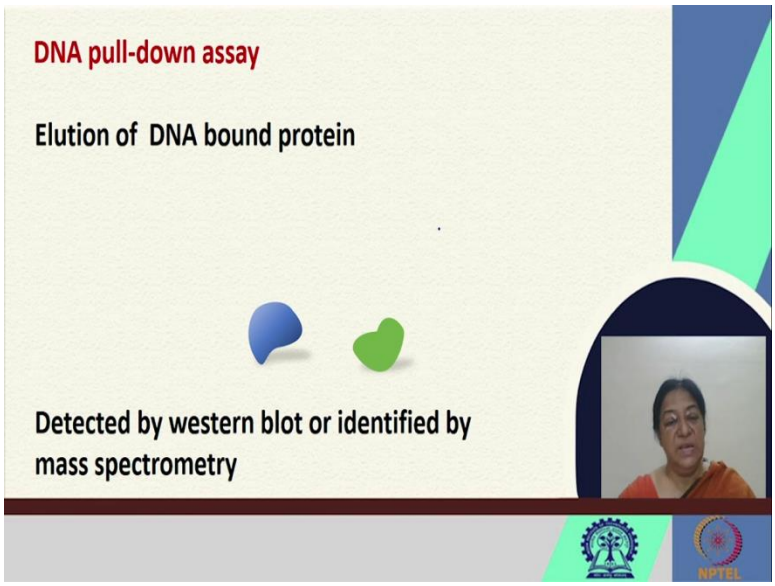

Now we have the elution of the DNA bound protein. This can be detected by western blot analysis or identified by mass spectrometry. In this fashion once we have our DNA bound protein, we can isolate this DNA bound protein, based on this specific acid.

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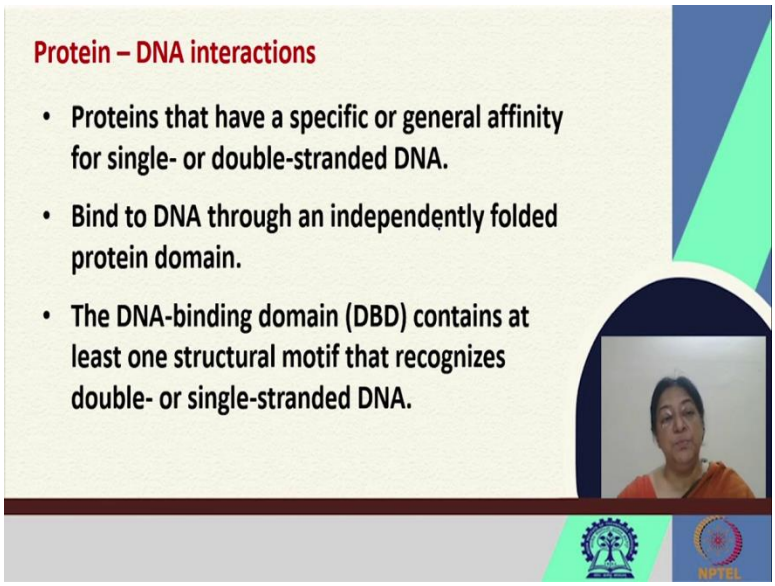

So in the protein DNA interactions what we did look at is, the specific or general affinity for a single or double stranded DNA. This occurs through specific independently folded protein domains, which we call the DBDs, the DNA binding domains and we found that these can be of different geometric representations in terms of the helix-turn-helix or a helix-loop-helix, a zinc finger binding domain or a leucine zipper type domain.

The DNA binding domain contains one structural motif which we saw in most cases was a helix, that recognized the double or the single stranded DNA and bound to the major groove of the DNA in a specific geometric and chemical fashion. So the recognition again as we see, is important for the protein DNA complex to form in the interactions that are observed.

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These [refer to slide] are the references.

Thank you.