Fundamentals of Protein Chemistry
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Module - 11
Protein Macromolecule Interactions II
Lecture - 54
Chaperone Proteins

In continuation of our discussion on protein molecule interactions; the second module that we are looking at, we looked at protein interactions and protein peptide interactions. In this lecture we will be looking at chaperone proteins; what we mean by these proteins and how they have an important role to play in the protein folding process.

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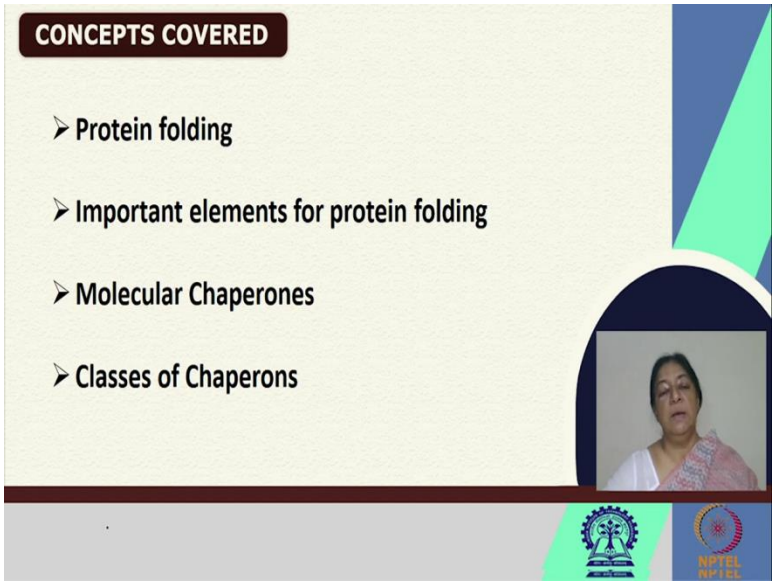

We have looked at the important elements of protein folding previously, which we will revisit here and what we mean by the classes and the chaperone molecules.

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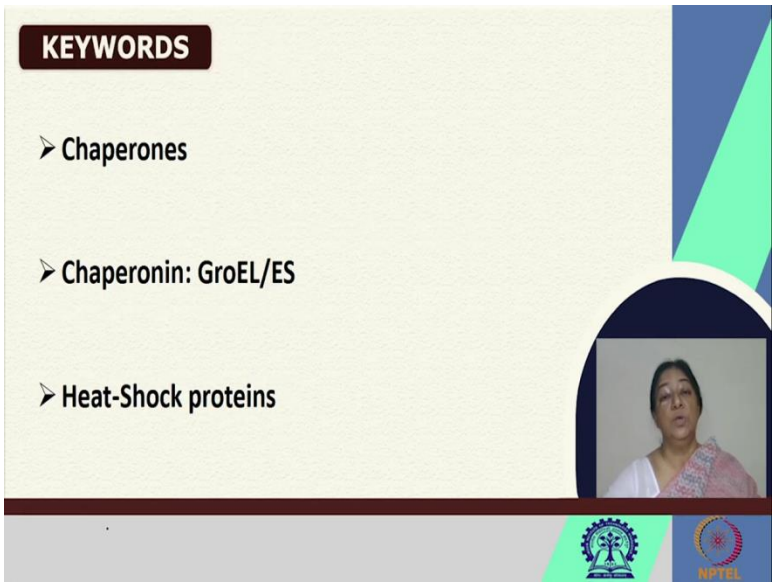

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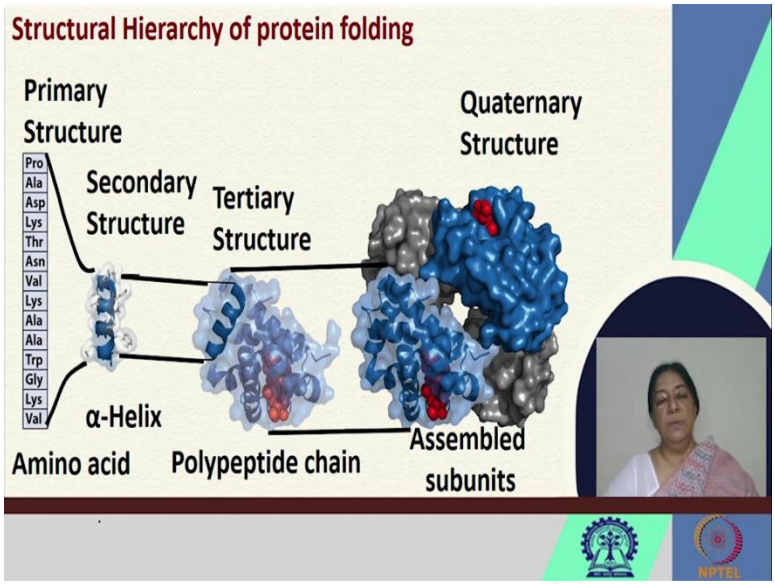

We have studied in the earlier classes related to protein structure; what we mean by the primary structure, the sequence of the amino acids followed by the secondary structure, the tertiary structure and the quaternary structure, which we have for the assembled subunits.

We also looked at protein-protein interactions, that would have these specific types of units put together in a complex formation. Now for a protein to function correctly, we realize that the final

folded form is extremely important because the structural aspects are related to the function of the protein.

If we do not have the active site residues in the enzyme in three dimensional space, in the specific scaffold that they are to be located in; then the substrate cannot bind. So the protein folding process is extremely important.

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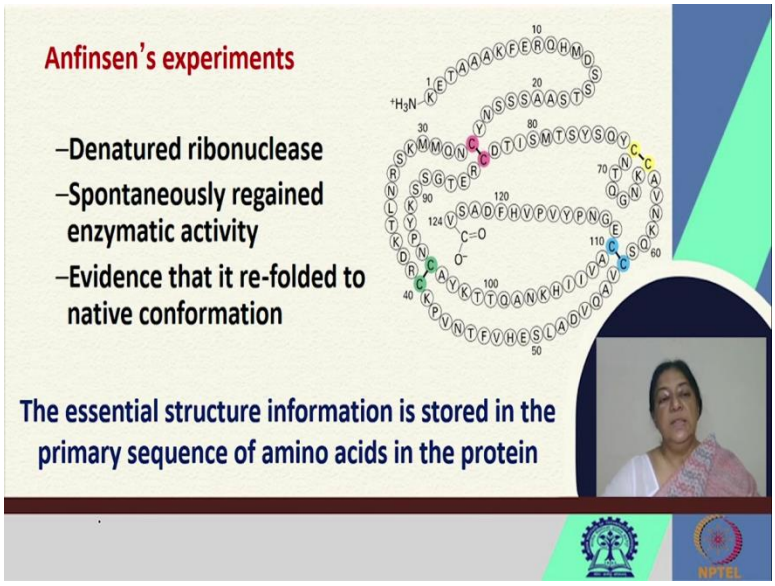

In Anfinsen's experiment, we came to know from the experimental details, that the essential structural information was stored in the primary sequence of the protein and this information was extremely important to understand that we have a folded protein.

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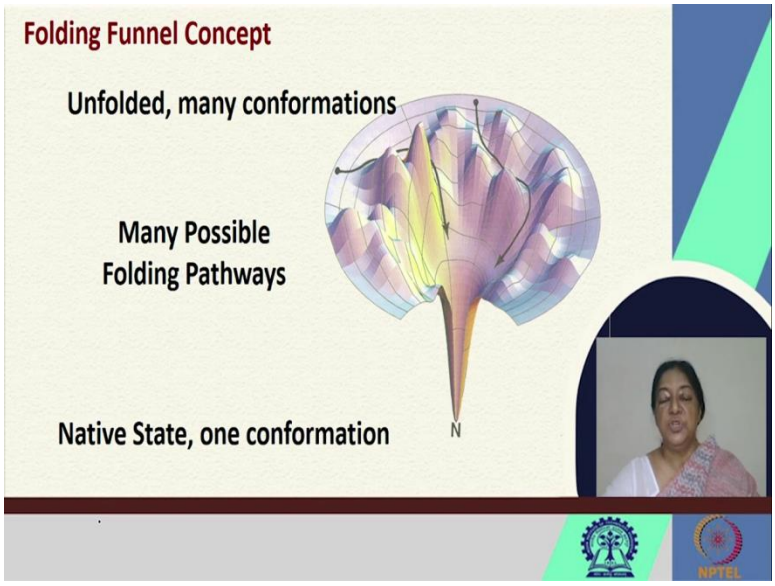

The elements of protein folding were discussed also in a folding funnel concept, where we looked at the many conformations that were possible when the protein was unfolded; looking at specific prediction pathways that would take us to the one confirmation - the native state.

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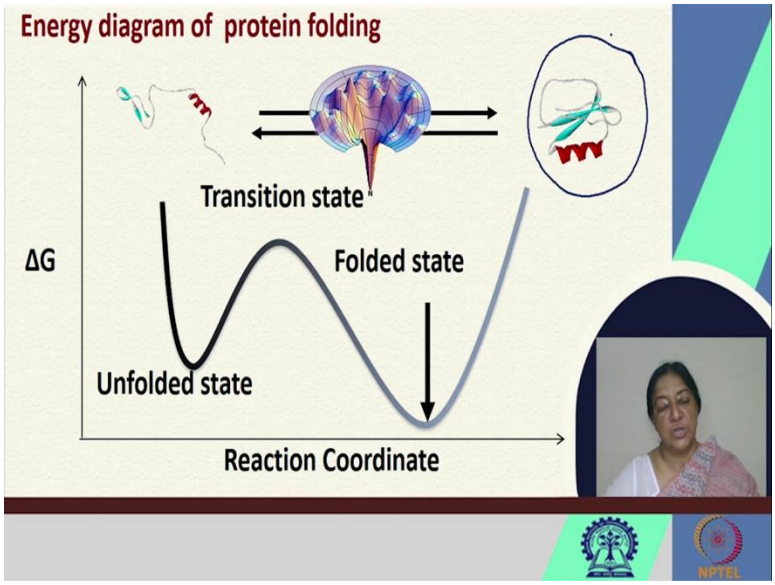

In an understanding of the unfolded state to the folded state we realized that there were many interactions, structure stabilizing interactions, that brought us to our final folded structure from an unfolded structure.

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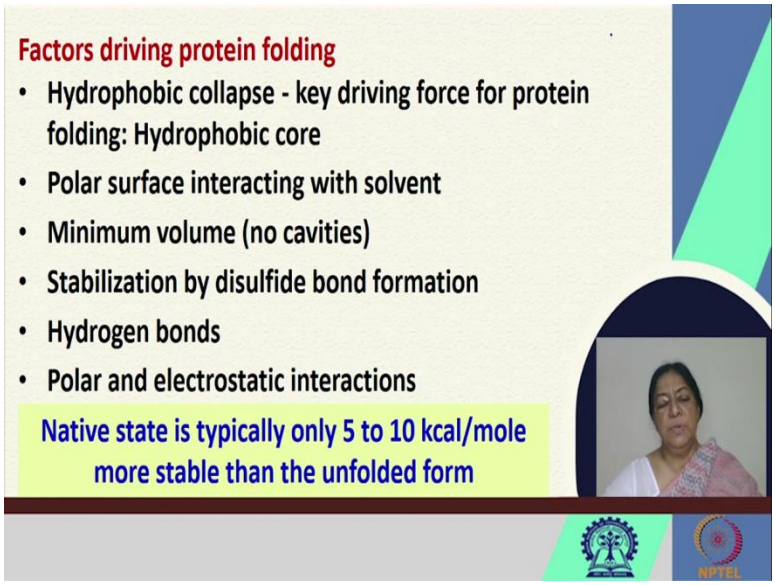

Looking at the factors that drive this protein folding, we realize that hydrophobic collapse is the key driving force for the protein folding to occur. In the regular globular protein, we see this as

the hydrophobic core of the protein. The polar surface that we see interacts with the solvent, there is a compact structure requiring minimum volume, so there are not very many cavities in the protein.

Additional stabilization by the disulfide bond formation, hydrogen bonds, polar and electrostatic interactions between the amino acid side chains. The native state is as we looked at earlier when we looked at protein folding, that the native state is just slightly stabler than the unfolded form.

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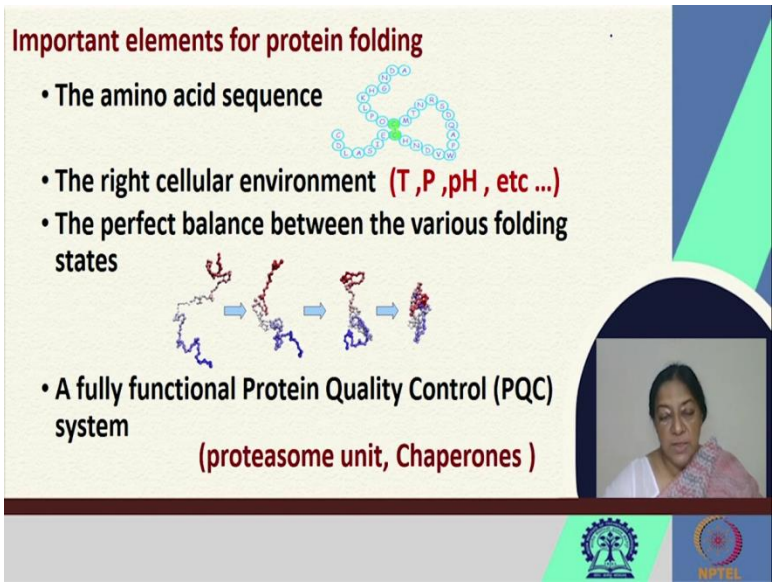

The elements for protein folding that are important, are the amino acid sequence. The right cellular environment - the temperature, the pressure, the pH and other balances between the various folded state, the various conformations and the proper interactions that are going to lead us to the folded structure of the protein, that is going to function correctly.

Once we have this functional protein, the idea is that there is protein quality control. What we need is, we also need a fully functional protein quality control system. In this lecture we will be looking at proteasome units and chaperones, that constitute the protein quality control.

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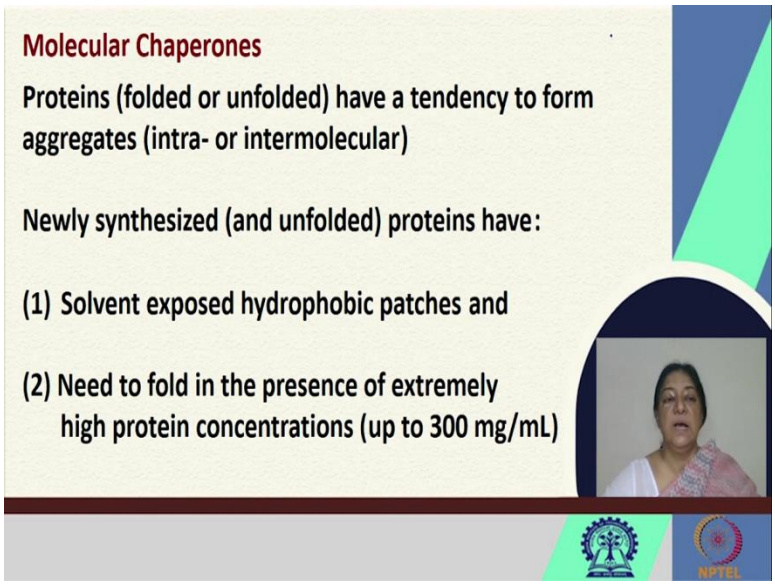

What are molecular chaperones? Molecular chaperones are proteins themselves, but what happens during protein synthesis, the folded protein or the unfolded protein has a tendency to form aggregates. We can have disorder proteins, which we will see in the next lecture, into what we mean by intrinsically disordered proteins that could form aggregates.

These aggregates could be intra or intermolecular in nature. The newly synthesized proteins, as well as unfolded proteins, have solvent exposed hydrophobic patches. Now these would rather be in the central core of the protein, in a regular globular protein and what has to happen is this unfolded protein or the newly synthesized protein, has to fold under high protein concentration. So how is this accomplished?

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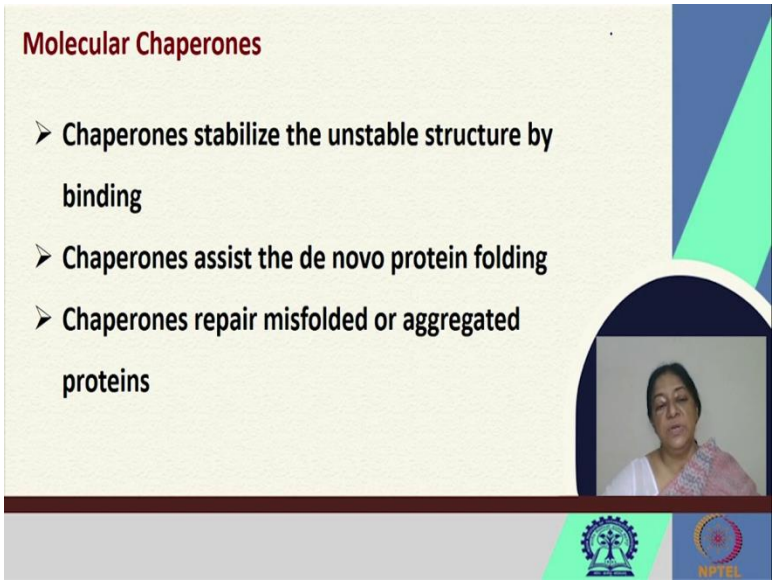

The chaperones stabilize the unstable structures by binding together and they assist in the de novo protein folding and what they can do is, they have the ability to repair misfolded or aggregated proteins.

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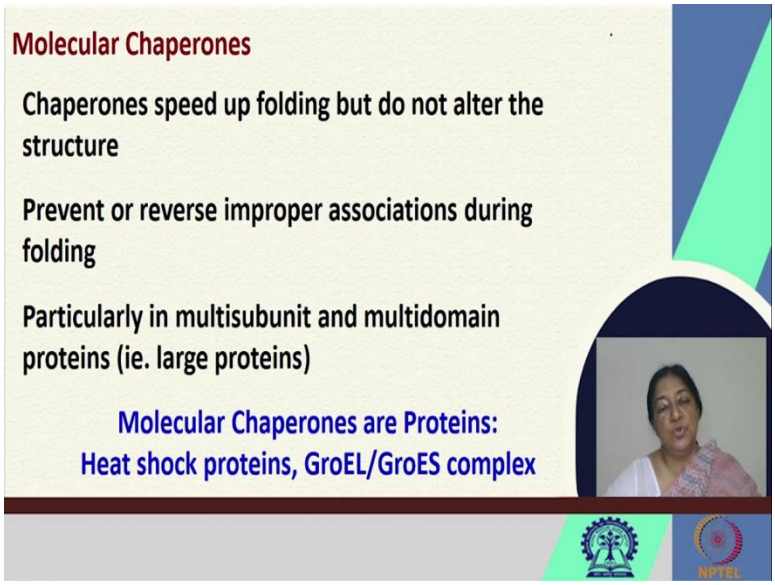

The molecular chaperones speed up the folding process, so that there is no sudden compactness or hydrophobic collapse in a manner that is going to result in a misfold in the protein. They prevent or reverse the improper associations that might occur during folding and this is particularly interesting in multisubunit and multidomain protiens. The molecular chaperones themselves are proteins and there are two very important types; one set is the heat shock proteins and the other is the GroEL/GroES complex. We will see in brief, how these are used or how these play a role in protein folding and how they can assist in protein folding and are true molecular chaperones.

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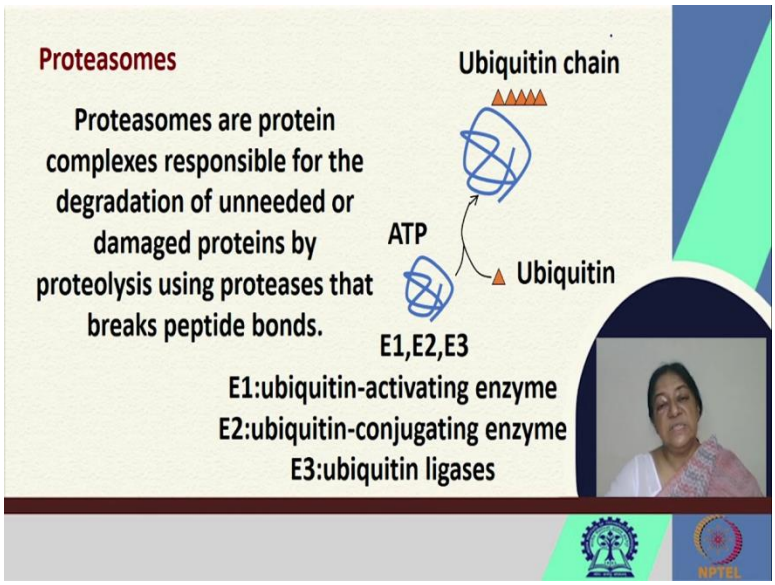

The proteasomes are protein complexes that are responsible for the degradation of unneeded or damaged proteins. So once a protein is realized as being damaged; then there is a process, a proteasome pathway, that would result in the fragmentation of this polypeptide chain.

In this case [refer to slide], a ubiquitin molecule is linked to the polypeptide chain that has folded incorrectly and there are specific enzymes involved in this process. There is a ubiquitin activating enzyme, a ubiquitin conjugating enzyme and a ubiquitin ligase, which we realize is going to link this to the protein chain.

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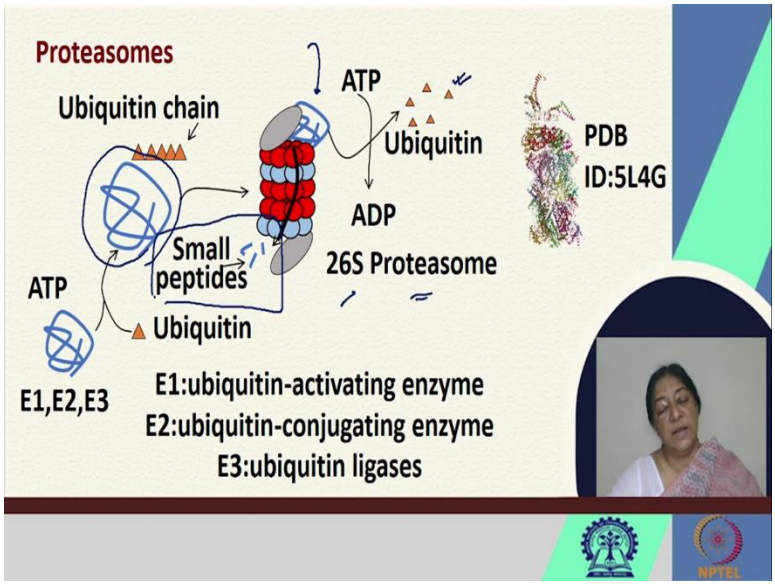

Once [refer to slide] we form this linked protein chain, we have the ubiquitin chain linked to our polypeptide chain. Then there is this 26S proteasome unit which takes the protein tagged with

ubiquitin. In an ATP involved process, it removes the ubiquitin and the polypeptide chain in the proteasome is broken up into smaller peptide units.

This is then utilized for other activities, but there is quality control here into not allowing the misfolded protein to go into the system.

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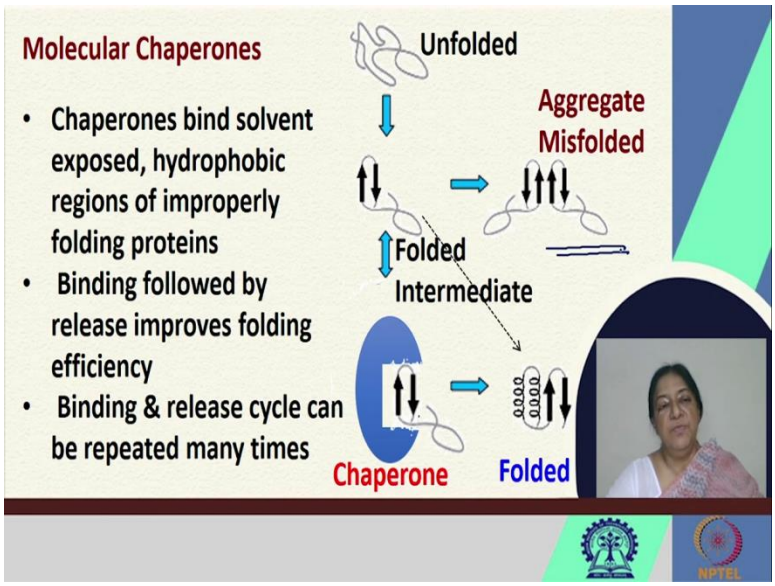

The chaperones on the other hand, bind solvent exposed hydrophobic regions of improperly folded proteins. So in the previous case, we looked at a proteasome system where a protease is present, that cleaves peptide bonds. In the molecular chaperone system, this will bind the solvent exposed hydrophobic regions of the improperly folded proteins.

Then they will create a folded intermediate, it will bind to the chaperone unit and a folded protein will be obtained from this; that would prevent any aggregation or misfolded protein to occur. So the hydrophobic portions that are exposed are bound to the chaperone, preventing any misfolded structure from occurring and a folded structure obtained. The binding is followed by a release that increases the folding efficiency and this cycle can be repeated a number of times.

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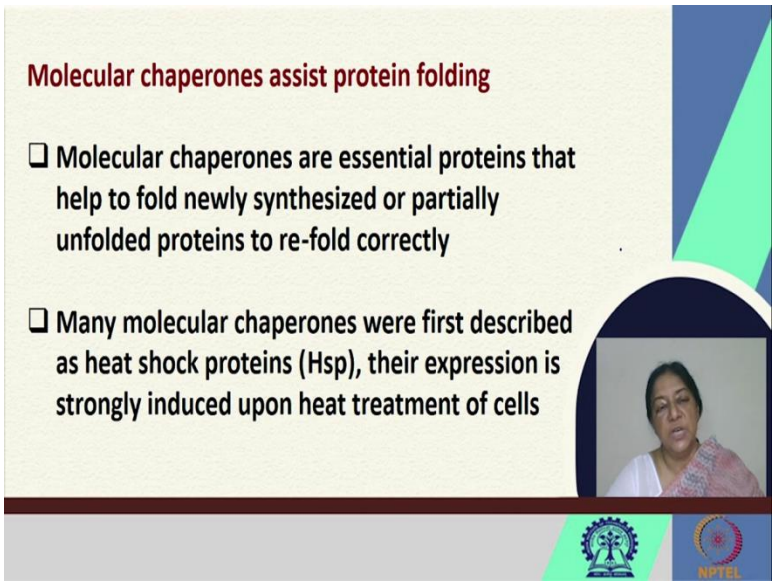

So in their assistance of protein folding, they can help to fold either newly synthesized or even partially unfolded proteins to refold correctly; because misfolded proteins could lead to a lot of degenerative diseases. The molecular chaperones, the ones that were first described, were the heat shock proteins and their expression is strongly induced upon heat treatment of cells.

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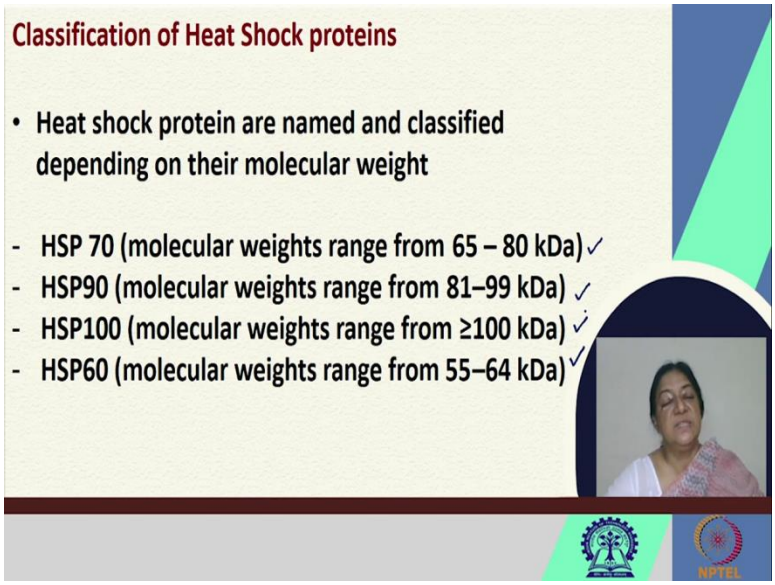

The heat shock proteins are named and classified, depending upon their molecular weight. We have a range that is from 60, 70, 90, 100. So HSP 70 indicates that these are heat shock proteins within the molecular weight range of 65 to 80; HSP 90, HSP 100 and HSP 60 giving us the classification of these heat shock proteins.

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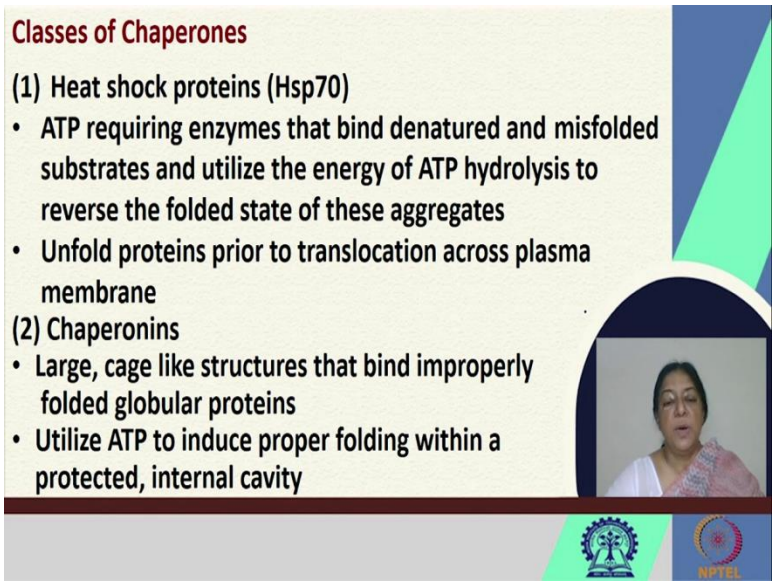

The classes of chaperones that we can see are heat shock proteins, for example the HSP 70. In this case, these are ATP requiring enzymes and as the chaperone function we saw, they bind denatured and misfolded substrates and utilize the energy of ATP hydrolysis to reverse the folded state of these aggregates, into trying to build up a system that is going to produce a correctly folded protein.

So the unfolded proteins prior to translocation across the plasma membrane, are therefore arrested. The chaperonins on the other hand, are large cage like structures that, can bind improperly folded globular proteins. They also utilize ATP to induce proper folding within a protected internal cavity, where the conditions are conducive for proper protein folding.

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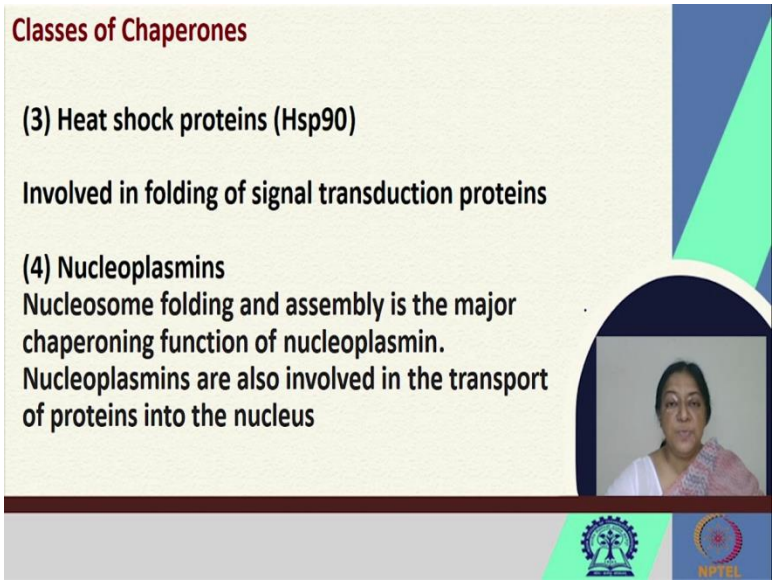

We have the heat shock proteins HSP 90. This is involved in the folding of the signal transduction proteins and we have the nucleoplasmins. The nucleosome folding and assembly is the major chaperoning function of the nucleoplasmin and the nucleoplasmin are also involved in the transport of proteins into the nucleus, for their specific function and activity.

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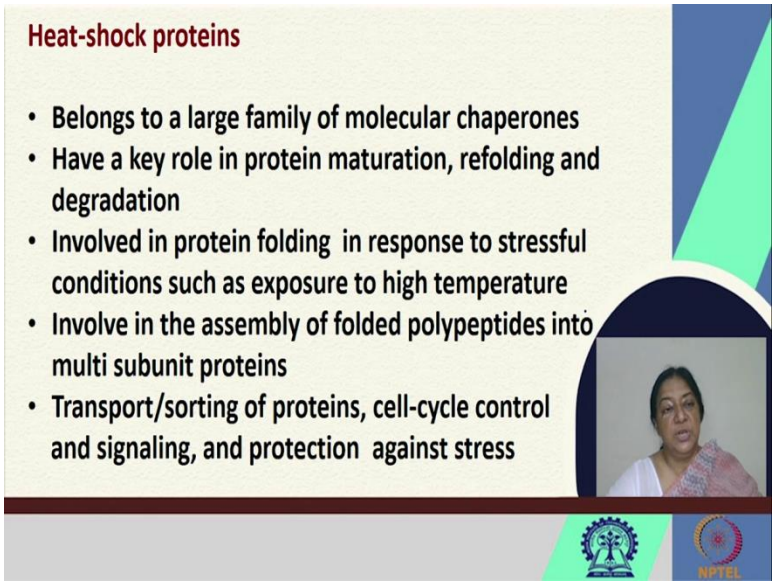

A discussion on the heat shock proteins, will show that they belong to a large family of molecular chaperones and they have a key role in protein maturation, refolding and degradation. They are involved in protein folding in response to stressful conditions, such as exposure to high temperature.

They are involved also in the assembly of the folded proteins into multi subunit proteins. So here we have the importance of the heat shock proteins, where they have responses to stressful conditions, such as exposure to higher temperature and they are involved in the transport sorting of proteins, cell cycle control and signaling and protection against stressful conditions.

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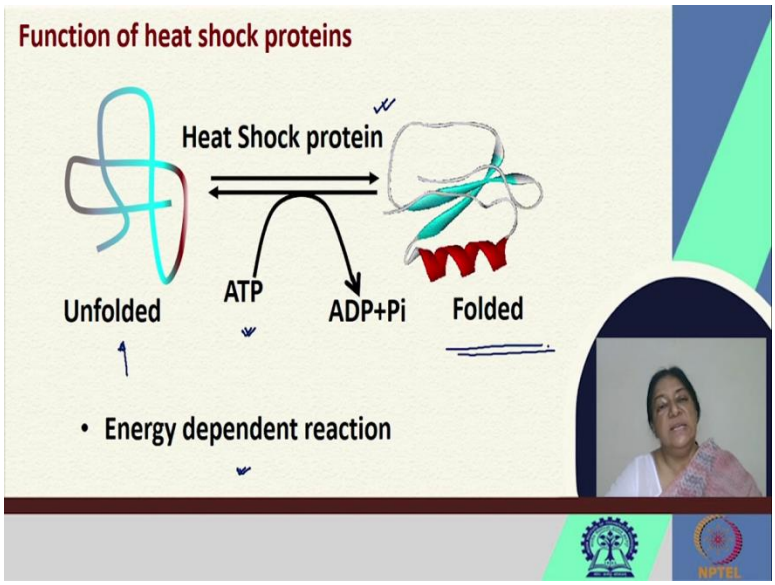

The function of the heat shock proteins is to take the unfolded polypeptide chain in the presence of the heat shock protein. Following ATP hydrolysis in an energy dependent reaction, we get the correctly folded protein that will have its native three dimensional structure, so that it can perform its correct function.

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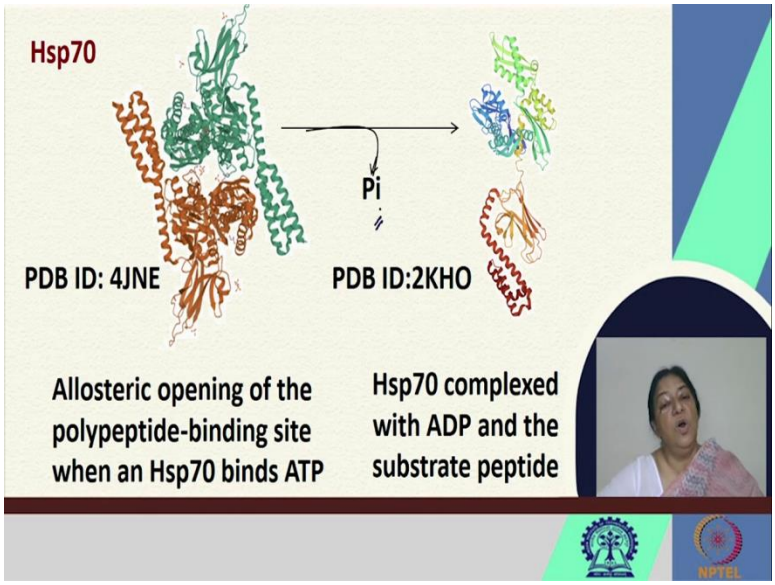

This [refer to slide] is the structure of HSP 70, where there is an allosteric opening of the polypeptide binding site, when an HSP binds ATP. Then, the HSP can be complexed with ADP and the substrate peptide on hydrolysis of the ATP.

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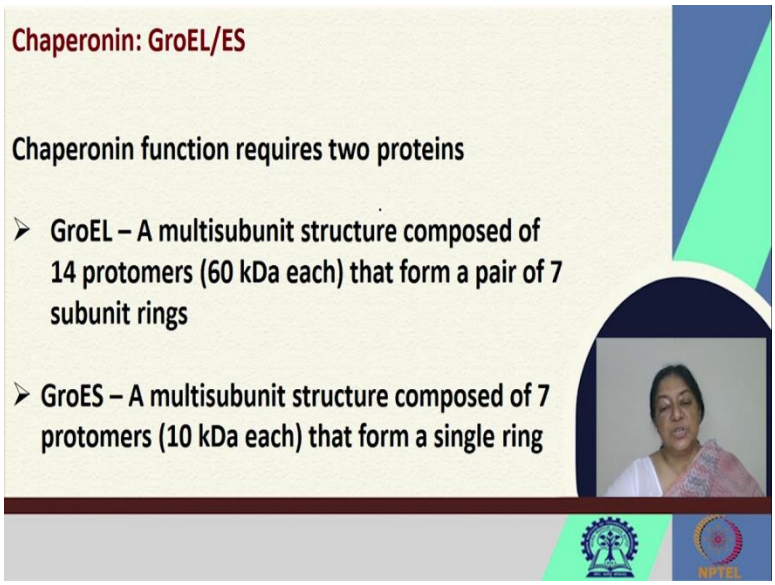

In the GroEL/GroES complex what we see is, this specific chaperone the GroEL/GroES complex requires two proteins. GroEL is a multisubunit structure, that is composed of 14 protomers of 60 kDa each, that form a pair of 7 subunit rings; and GroES is another multisubunit structure, that is composed of 7 protomers of 10 kDa each, much smaller than the GroEL one, that form a single ring.

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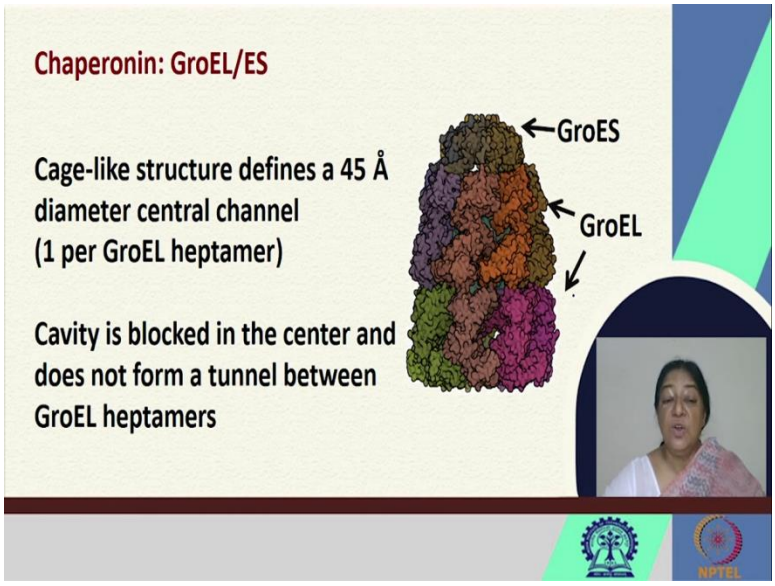

The beauty of the structure is in its assembly and how it acts on the misfolded protein, to create an environment within the cavity that is present and folding environment, so the misfolded protein can fold correctly. So this [refer to slide] cage like structure defines a 45 Å diameter central channel. There is one per GroEL heptamer; the 7 subunits that we spoke about and the

cavity is blocked in the center and it does not form a tunnel between the separate GroEL heptamers.

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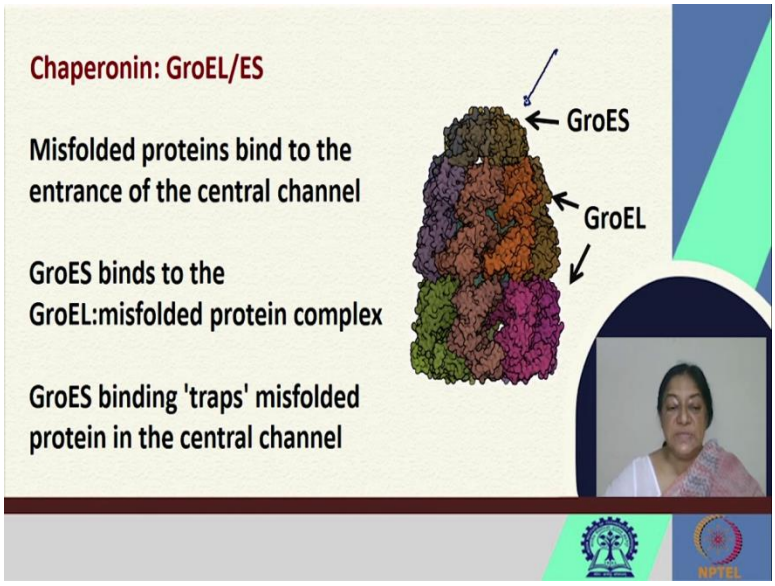

The misfolded proteins bind to the entrance of the central channel and then GroES binds to the GroEL misfolded protein complex and then it traps the misfolded protein in the central channel. The environment is made such that the misfolded protein is unraveled, protein folding conditions are induced and we have a protein folding environment, so that the misfolded protein can fold correctly.

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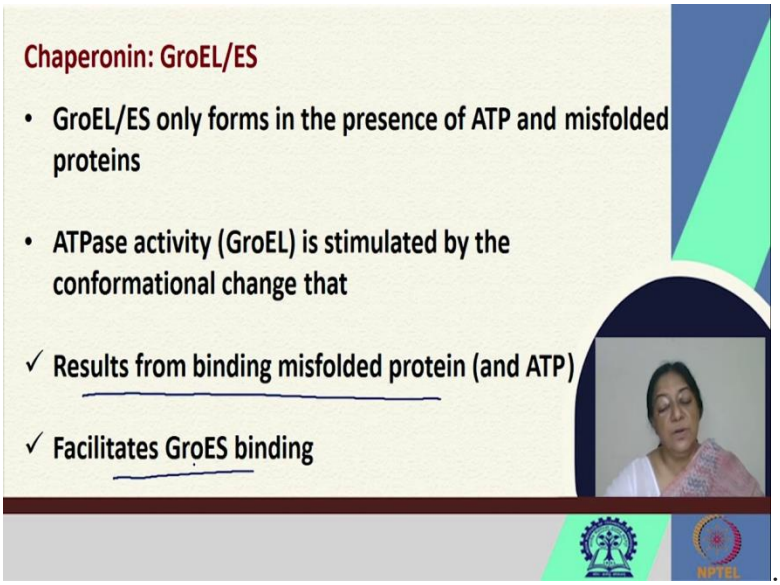

So the complex that we see of the GroEL/GroES will form in the presence of ATP and the misfolded proteins and the ATPase activity of the GroEL is stimulated by the conformational change that results from binding of the misfolded protein. Then this facilitates the GroES binding.

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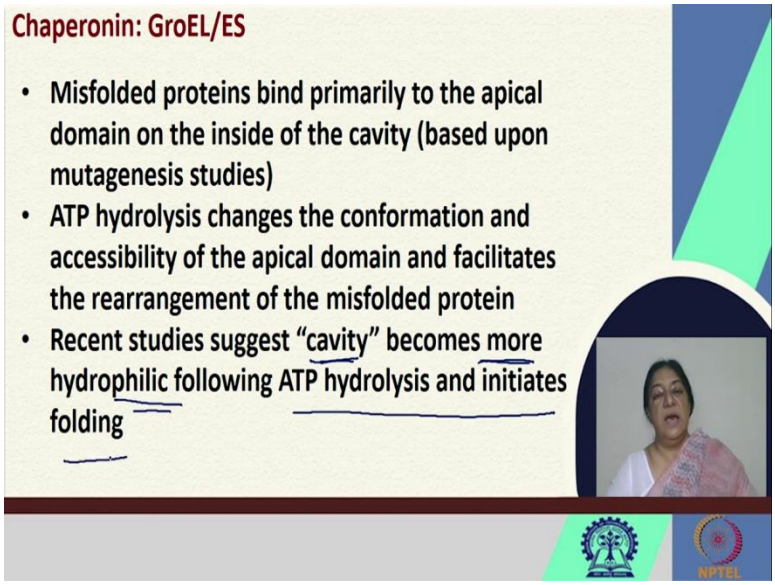

So the GroEL/GroES complex, the misfolded proteins bind primarily to the apical domain on the inside of the cavity. This has been obtained from mutagenesis studies and understanding of the way the GroEL/GroES complex works. The ATP hydrolysis then changes the confirmation, as well as the accessibility of the apical domain and facilitates the rearrangement of the misfolded protein. Now this cavity becomes more hydrophilic, following the ATP hydrolysis and initiates a folding environment, so that this misfolded protein can have an environment to fold in and try to fold correctly.

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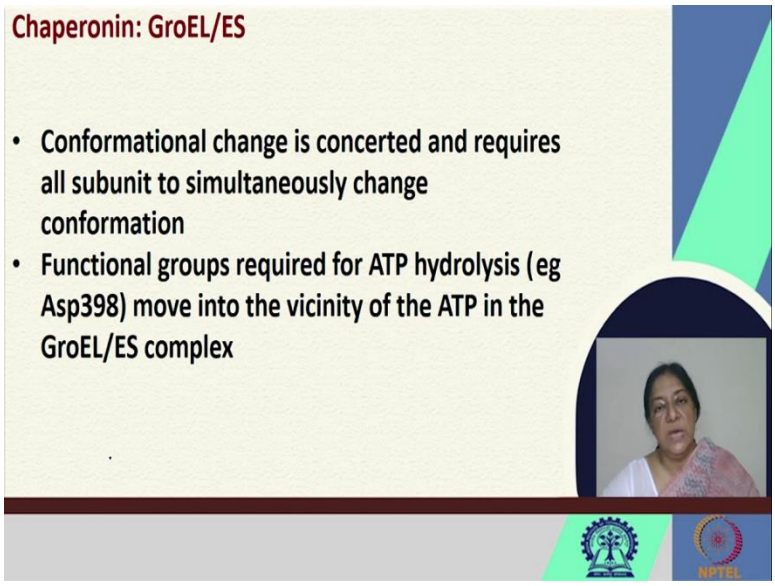

The conformational change is concerted and requires all the subunits to simultaneously change their confirmation and the functional groups that are required for the ATP hydrolysis, for example aspartic acid 398; they move into the vicinity of the ATP in this GroEL/GroES complex.

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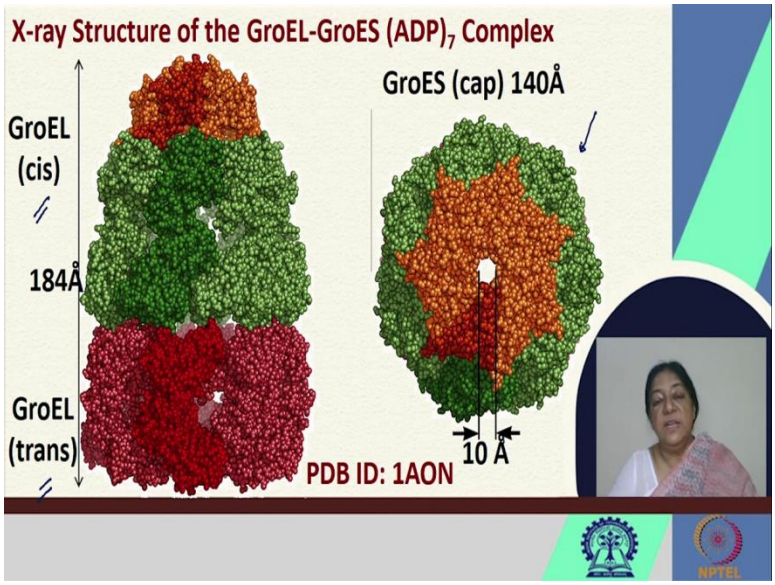

This [refer to slide] is the X-ray structure of the GroEL/GroES complex, where we have the GroEL in what is called a cis manner and one in the trans manner. This is the GroES cap that opens up to take in the misfolded protein, creates the environment due to the surface residues that are present here and the ATP hydrolysis that results in a conformational change, creating an environment so that the protein can fold correctly.

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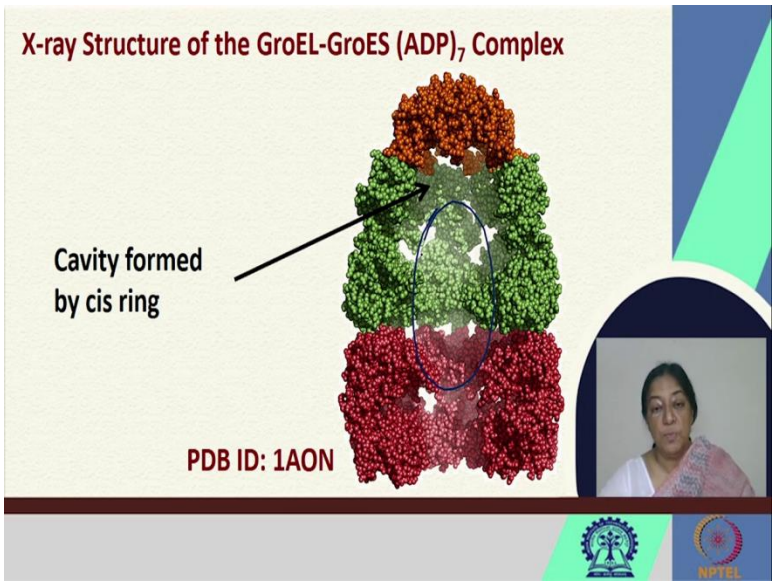

Then we have the cavity that is formed [refer to slide] by this cross sectional part of this ring where we have the huge cavity, where this cis ring allows the cavity to be formed.

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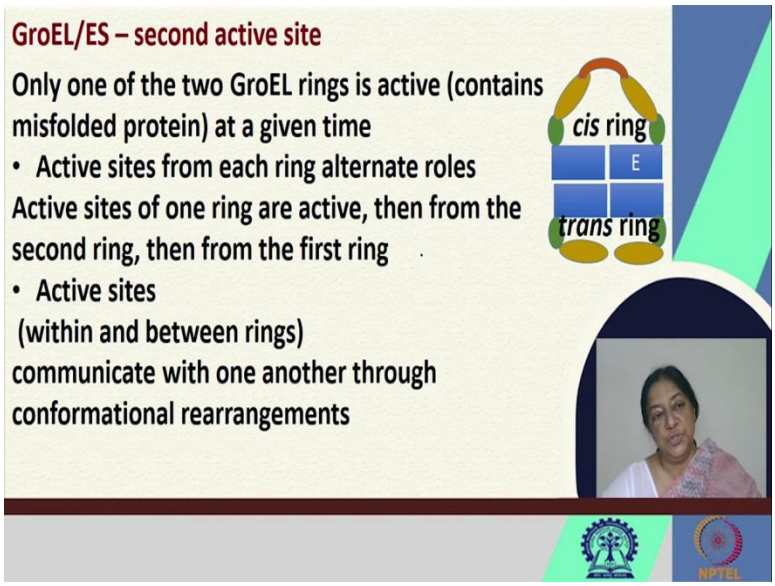

Now only one of the two GroEL rings is active, that is which contains the misfolded protein at a given time. Active sites from each of the ring alternate their roles, so the active site of one ring is active, then from the second ring and then from the first ring. So there is an alteration.

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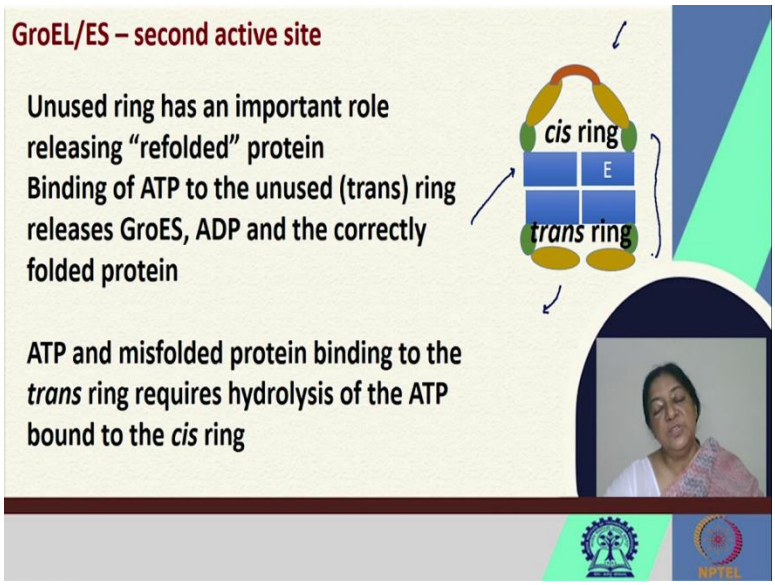

The active sites then communicate with each other through conformational rearrangements and what happens is the unused ring, the one that is not active at that point, has an important role that releases the refolded protein and the binding of the ATP to the unused, that is the trans ring releases the GroES, ADP and the correctly folded protein. So we have the entrance at a point, we have the release at a point of the folded protein and it takes place through ATP hydrolysis. The ATP and the misfolded protein binding to the trans ring, requires hydrolysis of the ATP that is bound to the cis ring.

So there is the cis ring and the trans ring that alternate their roles in the activity, in the binding of the specific polypeptide and what happens is, the ATP and the misfolded protein that are bound to the trans ring, require the hydrolysis of the ATP already bound to the cis ring, for this to occur. At the hydrophilic environment created due to this ATP hydrolysis, is conducive for the misfolded protein to refold and form its correctly folded protein and to be released from the GroEL/GroES complex.

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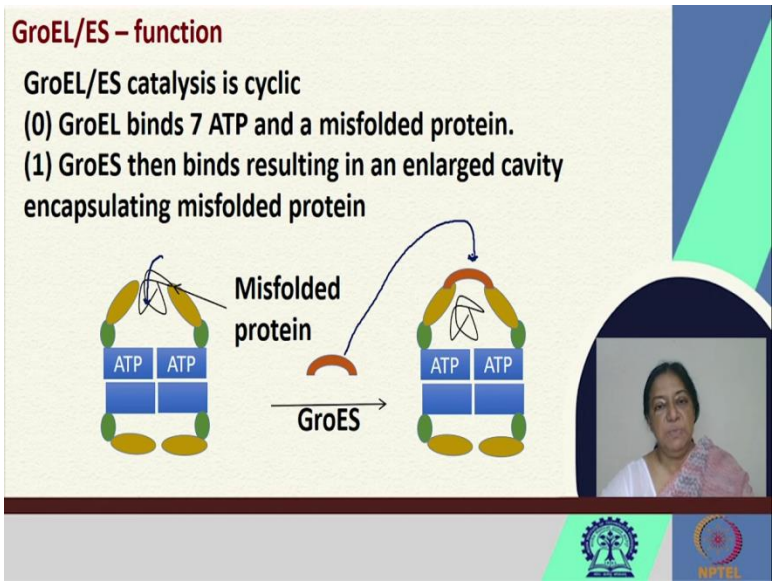

So the GroEL binds the 7 ATP and a misfolded protein; then the catalysis as we looked at is a cyclic process; the GroES then binds resulting in a larger cavity. We have this [refer to slide] come into the picture; this forms the cap on top here, we have the ATP bound 7, one ATP per protomass. We know it is a heptomeric capsule that is present there, two of them. So we have 7 ATP bound here.

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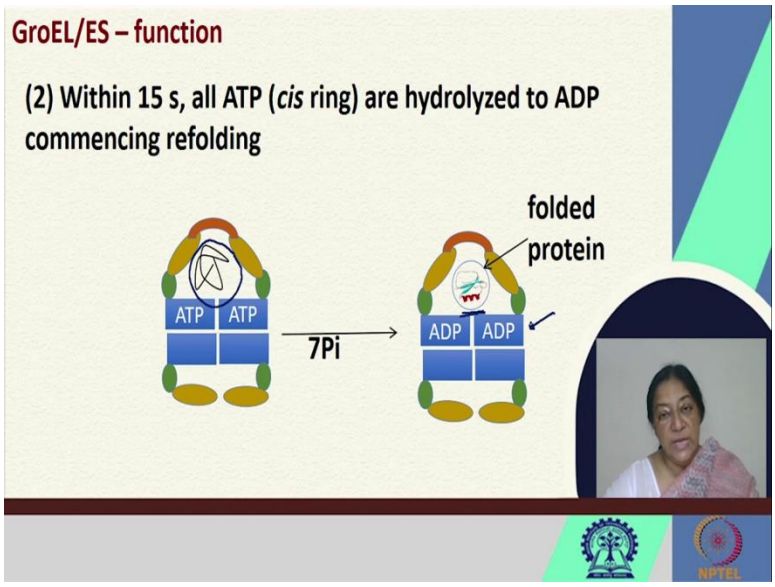

Then when we have the correct environment, when the ATP creates the environment that is hydrophilic in nature; we have the folded protein and the ADP form because the hydrolysis has created the hydrophilic environment.

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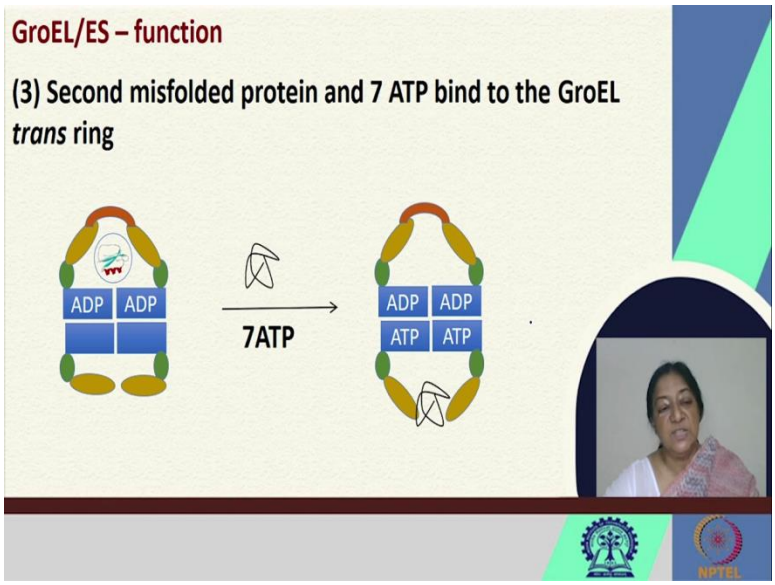

Then the cis ring are hydrolyzed to ATP. This allows the refolding to occur. Following this we have the second misfolded protein bind to the GroEL, that is the trans ring that is inactive at that time.

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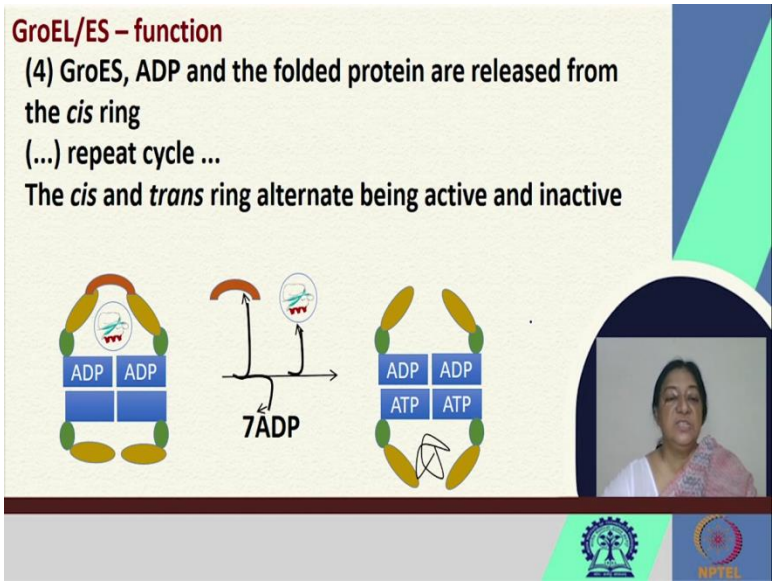

Then we have a specific formation when the protein is misfolded. So the GroES, ADP and the folded protein are released from the cis ring and this repeats the cycle between the cis and the trans ring; alternating between active and inactive. So one takes in the misfolded protein, ATP hydrolysis creates an environment for the folding to occur and then this is released.

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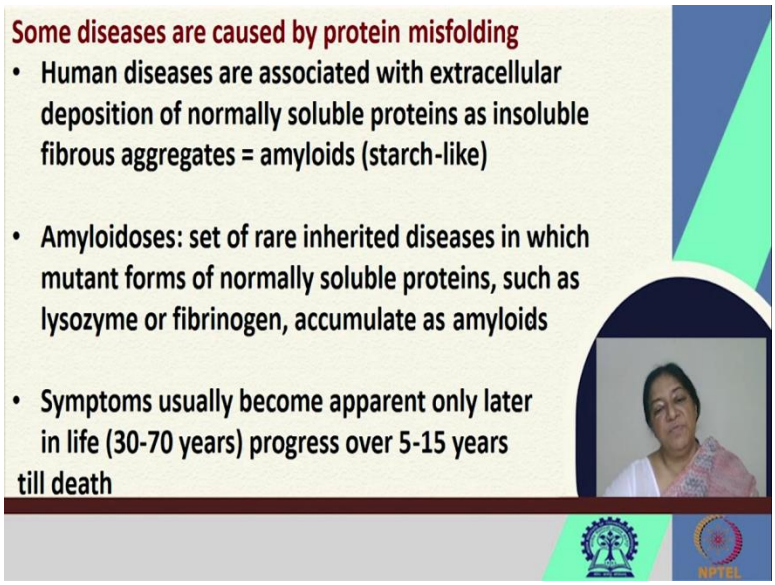

There are several diseases that are caused by protein misfolding. There are diseases that are associated with extracellular deposition of normally soluble proteins as insoluble aggregates, amyloids. Amyloids are formed in many neurodegenerative diseases, these are protein aggregation diseases.

Amyloidoses are a set of rare inherited diseases, in which a mutant form of normally soluble proteins for example lysozyme or fibrinogen, accumulate as amyloids. So there is a structural aspect that results in this aggregation that occurs. The symptoms usually become apparent later in life and they progress over 5 to 15 years.

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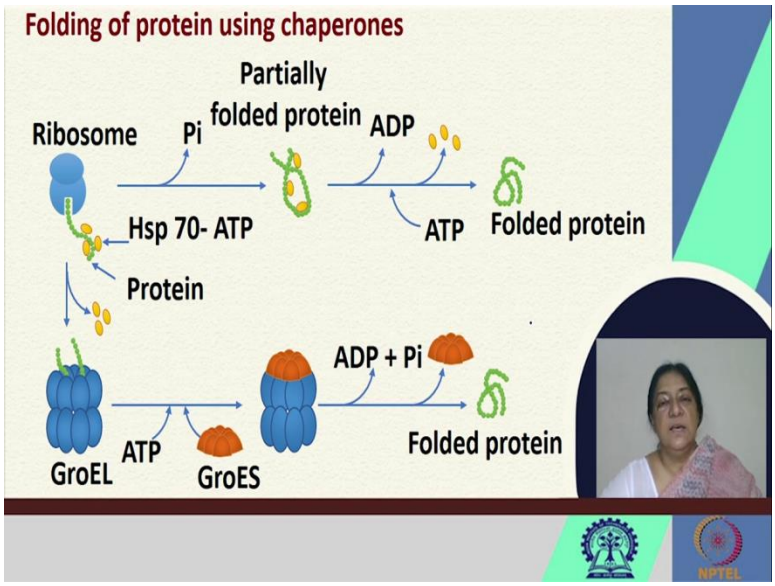

So if we look at the folding of the proteins using these chaperones, we have the ribosome that is creating or synthesizing the protein. The gradually partially folded protein is formed and we have

a proteasome complex that would take in any misfolded protein, that needs to have an environment to fold, but cannot fold correctly. It is then stamped with ubiquitin followed by which the proteasome results in its fragmentation or degradation.

In the partially folded protein what happens; there are hydrophobic amino acid residues that are on the surface and hydrophobic patches on the protein that are exposed due to the newly synthesized protein. Now to prevent any hydrophobic patches coming together, we know that a hydrophobic core is formed; but we also know that there is a specific structural integrity to the protein and it is not a random hydrophobic patch that is going to interact with each other.

So the hydrophobic patches in the partially folded protein, may interact in a manner that results in a misfolded protein. In that case what happens is, the chaperones come to the rescue and they bind to the hydrophobic patches in these proteins and create an environment that do not allow aggregates or misfolded proteins to form.

Through ATP hydrolysis they can create a folded protein. The GroEL/GroES complex works in a very complicated yet beautiful manner, in which it takes in a misfolded protein, creates a hydrophilic environment through ATP hydrolysis and allows a misfolded protein to fold correctly, creating our folded protein. This is the beauty of chaperone proteins, the heat shock proteins that respond to correct misfolded proteins and respond to heat shock and to stress.

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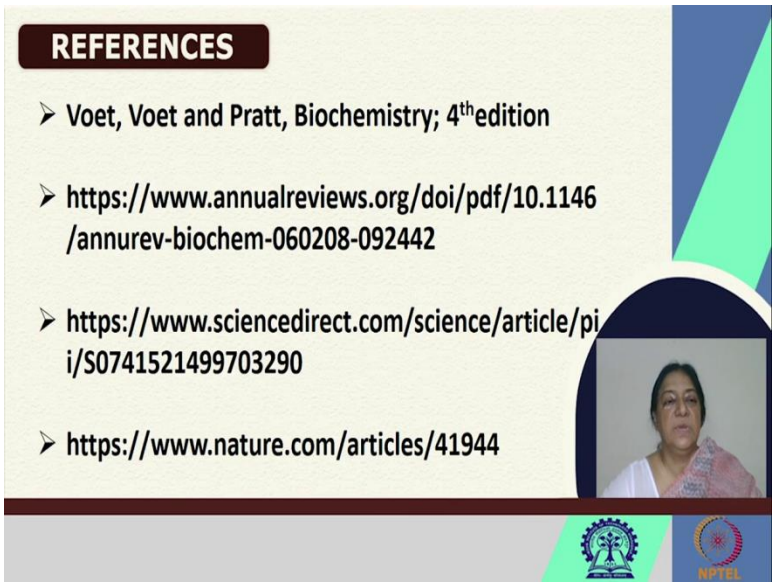

These [refer to slide] are the references.

Thank you.