Molecular Biology
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Module - 07
Translation
Lecture-32 Post-Translational Modifications

Hello everyone, this is Dr. Vishal Trivedi from Department of Bioscience and Bioengineering, IIT Guwahati and what we were discussing we were discussing about the different aspects of the molecular biology in this particular MOOC course. So, so far what we have discussed we have discussed about the cell biology, we have discussed about the different types of biomolecules, we have discussed about the role of these biomolecules into the cellular metabolisms and other kinds of important cellular functions such as cell division, cellular apoptosis, cell growth and the autophagy. In previous couple of modules we were discussing about the central dogma of molecular biology. So, we have discussed about the what is the requirement of the central dogma of molecular biology, why it is so important for the maintenance of the life on earth and what are the different processes which are being part of the central dogma of molecular biology. So, what you can see here is that the central dogma of molecular biology is consist of three important processes.

One is the duplication of the genomic content that is being done by a process known as replications followed by the for synthesis of the different types of RNA from the DNA and that process is being known as the transcription and then from the when the RNA is been synthesized it is going to be utilized by the ribosome and the protein synthesis machinery to synthesize the protein and this process is known as translation. So, in the current module we are discussing about the translations and so far what we have discussed we have discussed about the translation different types of steps in the translation in the prokaryotic system and as well as in the eukaryotic system and we have discussed about the genetic codes, how the people have discovered genetic code and what are the different types of genetic codes which are possible and then we have discussed about the initiation, elongation and terminations in the case of the prokaryotic as well as the eukaryotic system and what are the different significant differences between the translation in the prokaryote versus the translation in the eukaryotes. Now with this detailed discussion about the translation in the current lecture we are going to discuss about what happens once the protein is been synthesized and how the cellular system is changing this protein. So, that it becomes more useful for regulating the different types of events or how it is participating into the different types of processes.

So, what we have discussed we have discussed about the cell tone dogma and at the end

of the translations you are going to get the synthesis of the different types of proteins. Now once you have a protein right it is actually going to be present as a nascent protein or I will say it will not be a un mature proteins right. So, this protein has to be converted into a protein which is more of mature right. So, what is mean by the mature is that this protein may require the some kind of modifications which is not possible by the translational machinery to it. So, that it will be more useful and it will be more functional in terms of the doing the different types of task what is been given by the cellular system or what would be the requirement of the cellular system.

So, that is why I am writing this as protein as a immature it is not applicable to all the proteins it is possible that some of the protein which are been produced after translation could be in the final form and could be utilized as such. So, one of the classical examples what you have seen is the proteins are getting truncated right. So, one of the modification could be the truncation. Classical example is when you are converting a pro enzyme into active enzyme right. There are many examples such as pro-pepsinogen right sorry pepsinogen right.

This is a inactive form of the enzyme and when it is getting cleaved off it is forming a enzyme which is called as pepsin and that is the active enzyme. So, this is also happens after the synthesis of the pepsinogen. Now truncation is a very very important component then you also have the different types of the covalent or the non-covalent modifications mostly the covalent modifications such as the phosphorylation such as glycosylation such as ubiquitinylation or acetylation. So, all these are these different types of changes right and all these changes are bringing a very significant difference into that particular protein. For example, the phosphorylation it is going to impart a negative charge right and that negative charge is very important right because it may actually change the different types of scenarios and different types of interactions.

Similarly, the glycosylation that is actually going to impart a hydrophobic patch right. So, if you have a you know glycosylation it is actually going to reduce the solubility of that particular protein and because it is going to be less soluble it is actually going to increase the stability of that particular protein and we will discuss that in detail how it happens actually. Similarly ubiquitinylation that is also part of the protein degradation pathway so it is actually going to be a part of the protein degradation pathway so it is actually going to induce the protein degradation and acetylation is also going to induce the negative charge and it is actually going to add the or its actually going to add up the energy part right. So, it is going to add up the high energy bonds and that is how it is actually going to provide the energy into the system. Same is happening with the phosphorylation also that is also actually going to add up the energy into the system and that is how it is actually going to incorporate the high energy into the system.

Now let us first talk about the phosphorylation right. So, phosphorylation is one of the most important and the important modifications after the protein is been synthesized. So, post translational phosphorylations. Phosphorylation is an important post translational modification it is prevalent from the bacteria to higher eukaryotes sustainably as many as mainly two types. First it acts to functionally regulate the catalytic activity of protein by defining a rigid and permanent three dimensional structure.

Secondly temporarily phosphorylation proteins serve as an anchor for other protein substrate in signal transduction pathways. As such it act as a key player in the regulation of many cellular processes such as cell cycle, cell growth apoptosis and regulation of the cellular transduction. So, what it says is that if the protein is going to be get phosphorylated. So, it is going to be get phosphorylated with the help of the ADP and the protein is going to contain the going to take up the phosphate from the ADP. Then this protein is actually going to have or it is going to incorporate two different types of functionality functional you know functional things into the protein.

It is actually going to regulate its activity and you will see examples right. And the second part is it is actually going to add up into the interaction of the proteins during signal transduction. I am sure you might have seen the cellular signaling. So, you might have seen different types of signal transduction pathways. I think we have discussed about the apoptotic pathways intrinsic pathway and extrinsic pathway and how the cell signaling is occurring from the receptor pathway or the mitochondrial pathway and how the proteins are involved into this.

So, phosphorylation is also having the different similar kind of cascades and that is been maintained by the phosphate. So, some once you have the phosphorylation it is actually attract the other proteins and that is how you going to have a detailed signal transduction. So, for example, the insulin signaling and there are so many growth factor signaling there are so many signaling pathways which are very well decide and very well discussed. Similarly you have the regulation of enzymatic activity so, sometime the phosphorylation actually brings the higher activity sometime the phosphorylation means the lower activity. And you might have seen many of these examples when we were discussing about the glycolysis and Krebs cycle because you might have seen the many of the enzymes present in the glycolysis and Krebs cycle whose activities are been regulated similarly by the phosphorylations.

Now, let us see what is the mechanism of phosphorylations. So, in eukaryotic cell the phosphorylation is known to occur only at the side chain of the 3 amino acids serine, threonine and tyrosine ok. And what you see here is that there is a significant similarity

between the 3 amino acids and what is the similarity? Similarity is that they are all containing a hydroxyl group and which contains a lone pair of electrons. So, this is very very important feature of an of a amino acid because the amino acid should have a lone pair of electrons so, that it actually can participate into the phosphorylation reactions. And that is why you will see that in the eukaryotic system you have the serine, threonine and tyrosine.

But in the other system like for example, in the bacterial system you also have the aspartate which is getting phosphorylated you also have the histidine which is getting phosphorylated and so on. So, because the histidine is also having the nitrogen which is having the lone pair and the aspartate is having the this right aspartate is having this as a lone pair right. This is the terminal hydroxyl, a carboxylate group and you are actually going to have the lone pair on this. So, that is what it is actually going to do. So, in the histidine you are actually going to have the nitrogen, in the aspartate you are actually going to have the COOH group and this is actually going to participate into the phosphorylation reaction.

So, as I said in the lone pair of electron is going to be you know the requisition or going to be important for these kind of modifications. This is because these amino acids harbour a nucleophilic hydroxyl group. The terminal phosphate group on the universal phosphate donor adenosine ATP serves as the point of nucleophilic attack from the OH group which results in the transfer of phosphate group to the amino acid side chain. Magnesium ion act as a catalyst by chelating the gamma and beta phosphate group resulting into the lowering of the threshold for the phosphoryl transfer of the nucleophilic groups. So, that is what you are going to see right.

So, this is the example of the serine kinase. You can expect the similar kind of mechanisms even for the other modifications. So, what you see here is that this is the actually the protein where you have the serine and then serine has actually this lone pair of electrons. So, this lone pairs of electrons are actually going to act on to the on to this particular phosphate. So, what you see here is this is the ATP and in the ATP you have the three different types of phosphate.

You have the alpha phosphate, you have beta phosphate and you have gamma phosphate. So, it all the phosphorylation reactions actually works with the help of the gamma phosphate. So, in the gamma phosphate there will be a nucleophilic attack from the lone pair of electron what is present and as a result this particular group is actually going to get broken down from here and then this will go like this. So, it is actually going to get converted into ADP and this portion will actually go and attach here and that is how you are going to have this the phosphoserine and that phosphoserine is

actually going to be formed. Now, the similar kind of thing can happen even with the theronin or histidine and aspartate.

So, the enzymes name would be different in this case it is going to be called as serine kinase. If it is a aspartate then it is going to be called as aspartate kinase, if it is a histidine then it is going to be called as histidine kinase and so on. And majority as I said you know histidine kinase and aspartate kinase are more prevalent in the case of the prokaryotic system, but in the eukaryotic system you only have the serine, theronin and tyrosine. Now, these conformational changes can affect the protein in two different ways. So, the phosphorylation once you have a phosphorylation it is actually going to neutralize the or it is actually going to bring the additional negative charge.

What you see here is that right now it is actually uncharged it does not have any any charge this is the uncharged amino acid, but this one is negatively charged. So, it is actually bringing additional charge and because of this additional charge wherever the serine is present it either will make up the new interactions. So, it is actually going to contribute into the new interactions because negative is going to attract by the positive right. So, it is going to have the some kind of solvage interactions or hydrogen bonding interactions or van der waal interactions with the neighboring residues or it may actually break the interactions. Both there are both possibilities right it either can form the new interactions or it actually can break the pre existing interactions.

So, if it is going to bring the new interaction it is actually going to make the structure more compact and that is how or it may actually bring the conformational changes, but if it is breaking the interaction pre existing interactions then it is actually going to make the interaction make the structure more loose and majority of these interaction majority of these modifications always occurs in active site right. So, active site of an enzyme or sometime into the regulatory site also, but mostly if it is happen in the active site the active site could be more compact or active site could be more relaxed and both of these events are actually going to participate or going to modulate the catalytic activity of the enzyme. So, sometime they may not doing anything with the catalytic activity they may be actually bringing the additional you know surface right. So, the negative charge is there and it is actually going to bring the additional charge additional charge and that additional charge could be a would serve as a interaction site or docking site for new proteins right. This is what we said in the previous slide right that sometime it actually provides the docking site for the new interaction and that is how it is actually going to participate into the single transactions because until this phosphorylation does not occur this is not going to be recognized by the next proteins and once the next protein will go and bind this it is actually going to be get phosphorylated and so on and that is why there will be a cascade of the phosphorylation from one enzyme to another enzyme and the

third enzyme and so on and that is why it is actually going to make a single reductions.

So, once this happens it is actually going to bring the conformational changes and that may affect the protein in two different ways. Phosphorylation causing the conformational changes in the phosphorylated protein this conformational changes stimulate the catalytic activity of proteins. So, any protein can be activated or inactivated by the phosphorylation. So, this is what exactly happens when you have an inactive enzyme and if it is getting phosphorylated with the help of a kinase and that utilizes the ATP then it is actually going to be phosphorylated and that phosphorylated enzyme could be more active. Sometime it happens that and it may be other way around that you have active enzyme and then it becomes inactive enzyme, but that depends on the type of conformational changes it is bringing into the system and what could be how it is going to impact the structure of that enzyme.

Once you have the phosphorylated enzyme then there will be another set of enzyme which is called as the phosphatase enzyme and that phosphatase enzyme is going to bring the enzyme back into the unphosphorylated form and that is how it is actually going to shuttle between the two state one is active enzyme another one is the inactive enzyme and so on. So, phosphorylated proteins employ the neighboring proteins which have structurally conserved domain that distinguish and bind to the phosphor motifs these domains are specific for the diverse amino acids. Protein phosphorylation is a reversible post translational modification which is carried out by the kinases which phosphorylate and phosphatases which dephosphorylate to the substrate. These two type of enzyme make possible the dynamic nature of the phosphorylated proteins. So, that the balance concentration of the kinase and phosphatase is very important for the cell and it is also important for the catalytic efficiency of a particular phosphorylation site.

So, these two enzymes the kinase and the phosphatase are actually going to be always be present in a pair. So, you are going to have a tyrosine kinase you also going to have a tyrosine phosphatase. So, that when the tyrosine kinase is going to make the changes into any protein there will be a tyrosine phosphatase that is actually going to reverse these changes. So, that you are not going to have the only you know downstream signaling you also going to have the upstream signaling. So, that you are also going to nullify the effects otherwise you can imagine that if you have started a process it has to be shut down right and that shutting down is only by the these kind of pair of these enzymes.

For example, if the kinase is making the system on then the phosphatase is actually making a system off and that is what it is actually going to work in pairs. Now, the question comes what could be the ways in which you can be able to detect the phosphorylations. So, you can actually be able to use the activity which is called as

western blotting. So, you can actually be able to use the western blotting and you can use the these modification specific antibodies like for example, you can use the anti phosphoserine antibody although we have not discussed western blotting so far, but I think we are going to discuss. So, that time you will be able to understand full detail about how the people are doing the western blotting and how the people are using these antibodies and that anyway we are going to take up when we are going to talk about the molecular techniques in the subsequent modules.

So, you are going to use the anti phosphoserine as the primary antibody right. So, many of these terms many of these things you will understand once we are going to discuss about this. So, the first is you are actually going to do it with the western blotting and you are going to use the specific primary antibodies such as anti phosphoserine antibodies anti threonine phospho threonine antibodies or anti phosphotyrosine. So, these antibodies are being directed against the phosphorylated tyrosine. So, it is going to recognize only the phosphorylated tyrosine this is not going to recognize the unmodified tyrosine present in the proteins and that is how it is actually going to give you the signal.

Apart from that you can also use the specific proteins like anti phospho p 38 or those kind of proteins also those kind of antibodies also. Then the second is you can be able to change you can be able to use the spectroscopic method right. So, you can use you can be able to determine whether there is a modification of hopsville and you can actually be able to use the many type of dyes which are specific for the phosphorylations. So, you can use like you can use the malachite green. So, malachite green is specific for the phosphoproteins it does not react with the normal protein and that is how you can actually be able to use malachite green.

Number 3 you can also use the fluoromit fluorescence or fluorescence spectroscopy. So, or you can use the so here you can use the a probe which is been called as pro q. So, you can use a probe which is called as pro q pro q is a proprietary probe which is available from the molecular probes and when it reacts with the phosphoproteins it when it reacts with the phosphoprotein not the normal protein it actually going to give you the orange fluorescence. So, what you can do is you can just resolve the proteins on to a SDS page. So, it is actually going to give you the bands right and these bands you can actually be able to stain with pro q and when you do that it is actually going to give you the pattern right it is going to give you the pattern wherever you have the phosphorylation.

So, where your band is phosphorylated it is actually going to give you the signal into the orange fluorescence and that can be captured with the help of the scanners. So, you are actually going to have a specific scanners which can actually be able to capture the fluorescence at the particular wavelength. So, it is going to give you the image right and

that can be used very easily and very with a lot of you know there is no it is a user friendly techniques of doing that. Then the fourth is you can also use the NMR you can use the other kinds of analytical techniques. So, that I am not going to discuss in detail because we have discussed that in a one of our MOOCs course which is called as experimental biotechnology.

So, we have discussed in detail about how the phosphorylation of the protein can be detected and so on. Now phosphorylation is playing a very crucial role in many of the events and as I said you know when you have a protein which is getting phosphorylated and you are actually generating a phosphoprotein you are actually changing many things right you are changing the charge right. So, you are actually adding up the charge then you are also changing the energy level right or you are actually adding the high energy bonds into the system you are actually changing the size of the protein you are also changing the molecular weight because it could be attachment point for the other protein and that is how they may actually come and attach. Size means it is going to change the hydrodynamic surface area of the protein because the protein could be more compact or less you know it is going to be loose and depending upon that it is going to change the size right and it also going to change the activity of the enzyme. So, it is going to change the many things and phosphorylation is a very very very important post translational modifications.

Now let us move on to the next phosphorylation modification and the next modification is called as the glycosylation. So, glycosylation is the dire function of the biosynthetic secretory pathway in the endoplasmic reticulum and the Golgi apparatus. Approximately 50 percent protein characteristically expressed in a cell go through this alteration which involves the covalent addition of the sugar moieties to the specific amino acid. Mostly soluble and membrane non protein expressed in the endoplasmic reticulum undergoes glycosylation including all secretory protein surface receptors and ligand. Moreover some proteins are being transferred from the Golgi to the ecosystem for their glycosylations.

So, glycosylation is a very very important post translational modification because it actually been used as a system to for the vesicular trafficking. So, it is actually going to be used as a system to deliver a protein from the one compartment of the cell to the another compartment of the cell. So, you know that the protein are actually going to be synthesized onto the ribosome and these ribosomes are actually going to be attached onto the endoplasmic reticulum and that is why you are going to have two different types of endoplasmic reticulum. You are going to have the smooth endoplasmic reticulum and you are going to have the rough endoplasmic reticulum. So, in the rough endoplasmic reticulum the ribosomes are attached onto the ribosome and attached onto the

endoplasmic reticulum and when they synthesize the protein, the protein goes inside the endoplasmic reticulum and get folded.

And once it get folded, it is going to be packed into the vesicle and then it is actually going to be transported to the Golgi bodies. When it goes into the Golgi bodies, the Golgi bodies are adding the specific glycosylation pattern which involves the glucose, fructose, mannose, arabinose and all other kinds of drug, all other kinds of glucose and a combination of glucose. And that is how it is actually going to make the specific signal. It is going to make the specific tag and that specific tag is actually going to be recognized only by the specific receptors, what are present onto a particular organelle. For example, if the Golgi bodies has put the tag for the mitochondria, then that tag is and then it is actually going to be packed into a vesicle and then vesicle will go and interact with the endometacontria and that vesicle is actually going to be taken up by the mitochondria by the interaction of a tag and the receptor what is present onto the mitochondria.

And then at the end this protein is going to be delivered specifically to the mitochondria, not to the any other compartment, not to the glycosines or not to the plasma membrane or not to like nucleus or any other thing. So, this is a very, very important glycosylation. So, glycosylation is actually a very, very important modifications. Now, the glycosylation is being done by the different types of sugar. So, it is actually can be glucose, it can be fructose, it can be few course, it could be mannose, galactose, arabinose and so on.

Now, once you add a sugar, you are actually going to add the hydrophobic groups. Remember that the sugar is polar, but the sugar moieties are when they coming together, they are not very polar. So, they are actually going to bring the dual characters, they are actually going to bring the hydrophobic charges also and they are also going to bring the polar charges also because if you see size structure of the sugar, this is the sugar and what you see here is that it is going to have the OH, it is going to have H, but also going to have this ring. So, this ring is actually hydrophobic and these are actually the polar groups. So, it is actually going to bring the mixed environment into the interaction and that is how it is actually going to make the proteins, there are it is going to make the modifications.

Then the second is it is actually going to change the size because these are actually not going to get compact. When they are being present inside the protein structure, they are actually going to make the protein structure more loose and because of that it is actually going to impact the size of the protein. Then it also going to change the interaction of the protein with other proteins, with proteins or with substrate because you know if there is a

glucose molecule which is going to be attached, it is actually going to occupy that space and it is not may not provide the space for the substrate to interact. Similarly, it may actually allow the interaction of the some of the proteins. And glycosylation is also going to change as I said you know the surface chemistry or the charges of what are the present in.

So, that is what now the question comes how you can be able to study the glycosylation if the protein is getting glycosylated. Number one you can actually be able to use the western blotting with the help of the anti glycosylated antibodies and that anyway we are not going to discuss. Number two you can actually be able to use the SDS page. Remember that this method what we are discussing is more about the if you have the pure proteins. So, suppose you want to know whether the protein is getting glycosylated or not.

So, when you what you can do is you can add the protein you can add the glucose. So, what will happen is it is actually going to make the glycosylated protein. Now, whether this is forming or not that you can actually be able to know because it is it could be one single glucose or it could be a multiple glucose. So, when the glucose is going to bind it is actually going to change the size of the protein it is not going to change the molecular weight to that extent. So, it is not going to change the molecular weight it is actually going to change the size.

So, if the protein is very compact it may actually make the bigger balls. So, when you run it on the SDS page for example, so this is the protein and this is going to be the protein which is glycosylated or you have run the reaction for glycosylation. So, imagine that if you have a protein band here right then because the size is gone up right. So, it will behave like as a high molecular weight protein and that is how it is actually going to run like here.

Now, how you know that this is actually glycosylated. So, what you can do is you can actually be able to treat this with acid or you can treat it with the enzyme. There are specific enzymes like the glycosidase. So, if you treat it with the glycosidase right then what will happen is this is actually going to be get converted into proteins. So, if I have another lane right if I have another lane of saying that protein glycosylated minus plus enzyme or acid then what will happen is this band is again going to return back to the normal band. So, this is actually the protein band this is the glycosylated band and this is the unglycosylated band.

So, this will prove that the protein is glycosylated. If it is does not move if it is remain here then it is actually been a some kind of artifacts or it is not getting specifically been

glycosylated there are something happened funny and it cannot be explained by this. So, there are many other method you can actually have the some of the dyes periodic acid and all the so that anyway we are not going to discuss in detail about this. Now, the second another post translational modification is the ubiquitinylation. So, this is another post translational modification where ubiquitin which is a protein actually is added to the protein. Ubiquitin is the eukaryotic protein coded by the 4 different genes in the mammalian cells such as UBA 52, RPS 27A and UBB and the UBC.

Protein is made up of 76 amino acid and has a molecular weight of 8.5 KDA. It is characterized by the specific presence of C terminal tail and the 7 lysine residues. In ubiquitinylations basically the carboxylic acid side of the terminal glycine from the diglycine in the active activated ubiquitin forms an amide bond to the epsilon amino of the lysine in the modified protein. It marks the cellular protein for the process of degradation via the proteasome changes the protein localization prevent or promote the protein-protein interaction. So, what happened is that in the protein you have the different types of lysines.

So, when you are adding the ubiquitin and ubiquitin has a glycine. So, glycine on the C terminal and when you are going to do the ubiquitinylation reactions what will happen is that the protein is having a lysine and it is going to attach by glycine and the ubiquitin is going to be attached. So, this ubiquitin is going to be attached with the help of the terminal lysine what is present on the ubiquitin by binding to the lysine and that is how it is protein is actually going to be tagged for the cellular degradation. Now, how it happens that you are going to you know ubiquitinylate this protein not any other protein. So, there is a complete set of reaction what are going to happen when you are going to do ubiquitinylation of a particular protein and there is a balance and checks which actually allows and to the identifier that particular protein and then only it is actually going to be get ubiquitinylate.

So, it actually happens in the multiple steps. So, in the step one you are going to do the activation of ubiquitin. So, this is going to happen here. So, it occurs in a two step reaction process at first the ubiquitin interact with the ATP and forms a ubiquitinate adenylate intermediate. In the next step the ubiquitin is transferred to E1 active site enzyme containing the cysteine residue. This causes the formation of thioester linkage between the C terminal carboxyl group of the ubiquitin and the E1 cysteine cell filter groups.

So, this is what exactly happens. So, this is the step one where the this is the ubiquitin and it is actually going to be adenylate with ATP and that is how it is actually going to form the activated ubiquitin and that activated ubiquitin is going to bind the E1 ubiquitin

and it is actually going to you know form the thioester linkage. Then in the step two the step two there will be a transfer of ubiquitin E1 active site to the E2 active site via the transesterification reaction. So, that is what exactly happens then you are going to have the entry of the E2 ubiquitin and it is E2 enzyme and it is actually going to replace the E1 enzyme and it is going to form the E2 with the thioester linkage the ubiquitin is present. In the last step of the ubiquitin lylation cascade there is a formation of the isopeptide bond between the lysine of the target protein and the C terminal glycine of the ubiquitin via the activity of the one of the hundred of the E3 ubiquitin ligase. So, then you are going to have the E3 ubiquitin lysase and it is actually going to bind on one side E2 which actually has the ubiquitin and the target protein and the other side you are going to have the proteins which actually has the I mean lysine groups and then this is going to be transferred onto the amino group right of the lysine and that is why you are going to have the ubiquitin which is attached onto the target protein by the isopeptide bond.

So, this is actually a peptide bond but because you have a main chain peptide bond this will be called as the isopeptide bond okay. And you can have this cycle continue so from here it goes again here and then this cycle continues and then you are going to have the addition of multiple ubiquitin molecules. So, you can imagine that if you have a protein, protein may have the lysine and to this lysine the ubiquitin is going to be attached in this first cycle in the second cycle another ubiquitin is attached in the third cycle another ubiquitin is attached and so on. And remember that I told you right the ubiquitin also having the lysine. So, it is actually possible that sometime you may it may have the ubiquitin like this.

So, in some cases it happens that instead of adding the ubiquitin to the target protein it may actually add the ubiquitin to the existing ubiquitin because the ubiquitin has a 7 lysine residues the terminal C terminal amino acid. So, it is actually can attach 7 ubiquitin at once right and that is why the ubiquitin elation could be mono ubiquitin elations or it could be poly ubiquitin elations okay. And you can see that how it is actually going to change. So, you can have a mono ubiquitin elation. So, that only the one ubiquitin is going to attach onto the substrate protein or it could be a poly ubiquitin elations.

So, poly ubiquitin elation could be linear chain or it could be branch chains right. So, it could be branch change and the purpose of the ubiquitin elation is that it is actually going to mark the protein for the cellular degradation. So, it is going to deliver that protein into the proteasome systems. So, what happen is that when you are going to have a protein and when you are going to do the ubiquitin elations. So, if we say getting the ubiquitin elations it is going to be get converted into the protein ubiquitin okay.

So, I am just making taking a simple example of the mono ubiquitin elations okay. Now, ubiquitin is a 8.3 kDa protein okay. So, if the one protein is been attached it is actually going to change the molecular weight of the protein by 8.3 kDa okay. So, if it is a mono ubiquitin elation if it is a poly ubiquitin elation you can actually be able to you know multiply the same number and so when there will be a ubiquitin elation it is actually going to change the molecular weight of the target protein.

Number 2 it is actually going to change the size of the protein. Then number 3 it is actually going to change the surface interactions right. So, it is actually going to allow this protein to interact and because it is changing the molecular weight it is also going to impact the solubility of this particular protein because more and more protein you are adding it is becoming more and more heavy and if you know that heavy molecules are going to be less soluble compared to the light molecules right. Because the protein itself is soluble but when you are adding all these kind of thing it is going to be more soluble it is going to be less soluble. So, it is going to impact the solubility this means once it can impact the solubility it is actually going to make the particulate matter it is going to be form the particulate matter and these particulate matters are actually going to be a part of the proteasome degradation pathway. So, it is going to be attract by the proteasome system and that is how they will be delivered to the proteasome and they will be get degraded by the system.

So, it is going to be degraded. In some cases the monoubiquitinylation or polyubiquitinylation because it changes the size and the surface interactions it also brings the additional functionality into the system. Now how you can be able to study the ubiquitinylation? So, you can actually be able to have the as I said you know you are actually going to have the change in the molecular weight, you are going to have the change in the hydrodynamic surface area which means the size and it is also going to change the surface chemistry. So, first thing is you can actually be able to do the western blotting with the help of the specific antibody that we are not going to discuss number 2. You can also be able to check the molecular weight. So, you can actually be able to run the SDS page and SDS page is going to tell you because in the each ubiquitinylation round it is actually going to increase the size by 8.

3 kDa. So, you can imagine if you are going to have the addition of 5 ubiquitin then it is actually going to be approximately 40 kDa. There will be change in the 40 kDa and so on. So, if you run the system on the SDS page and suppose this is a protein and this is a protein ubiquitin, then it is actually going to change the pattern. So, it is going to change the pattern and remember that with the ubiquitinylation because you are going to get multiple species, you are going to have suppose this is a protein.

So, we are going to have a ubiquitin then you are going to have this. So, this is in the cycle 1. And again in the same protein, you are going to have ubiquitin then it is going to attach with another ubiquitin right, it is going to be another ubiquitin this is the cycle 2. So, in the cycle 2 you are going to have 2 ubiquitin then cycle 3 you are going to have 3, 4, 5, 6 like that. So, that is why you are not going to get single band you are going to get a band here then band here band here band here band here. So, it is actually going to show you a ladder kind of pattern or I will say it is actually going to show you the sphere.

So, if you are getting a smear then that means that it is actually going to be ubiquitinization. Now, how you are going to do the confirmatory experiment. So, what you can do is remember that the addition of the ubiquitin to the protein is been done by a bond which is called as the isopeptide bond right. See this isopeptide bond cannot be cleaved by the normal proteases ok, it is actually going to be cleaved by the cysteine proteases.

So, it is very sensitive for the cysteine proteases. So, what I can do is I can just take the PU and I will add the cysteine protease ok. So, if I add the cysteine protease this all ubiquitin is going to be removed and ultimately I am going to see a electrophoretic mobility of this protein to its original level. So, this is the protein, this is the protein plus ubiquitin and this is the ubiquitin we have lost the ubiquitin. So, all the ubiquitin you will see here actually you are going to see some of the ubiquitin smear on the bottom of the particular gel.

So, this is one of the method through which you can be able to detect the ubiquitin. Number 3 you can actually be able to do the gel filtration and as I said you know it is going to change the size of the protein. So, you can actually be able to use the gel filtration chromatography to know the size of the protein. We are not going to get into the detail of these because all these we have discussed in detail in my another MOOCs course which is called as the experimental viotechnology. So, if you are interested to know more about this you can be able to go through with this particular MOOCs course and it is actually going to tell you the complete detail about how the chromatography technique can be used for studying the ubiquitin dilations.

Now, let us move on to the next modification and the next modification is the methylation. This process refers to the addition of methyl group to the nitrogen or the oxygen to the amino acid side chain and methylation is irreversible whereas, o methylation is potentially reversible. So, methylation changes hydrophobicity of the amino acid and utilizes the negative charge when attached to the carboxylic acid. Many

methyl main methyl group contribution for such reaction is SAM or acetenyl methylene. This reaction is mediated by the enzyme which is called as methyl transferase and methylation process is involved in a epigenetic regulation as the histine methylation and demethylation. So, this is the methylation we have discussed in detail when we were talking about the chromatin romaned and genomic DNA and how the packaging of the genomic DNA occurs into the eukaryotic cell.

So, this is all about the post translational modifications and what we have discussed so far we have discussed about the central dogma of molecular biology where we have discussed about the replications transcription and translations. We have discussed we have tried to discuss in detail of this different processes into the prokaryotic as well as the eukaryotic system.