Bio-Physical Chemistry
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Lecture - 01
A Course on Bio-physical Chemistry

Hello everybody, welcome to this course on Biophysical Chemistry as you can see the title of the course on the slide. So, you know to tell you on the onset, this course involves a vast range of topics. So, it is very difficult for a single course to you know include each and every aspect which comes under this title of biophysical chemistry. But before we go more into what we are going to study in this course, let us look at a brief definition of what biophysical chemistry is.

Now, if you think about just the two words biophysical chemistry, well chemistry is chemistry. And biophysical if you try to divide it, you know break it up rather, so bio of biophysical comes from the word biology, and physical comes its physical like application of physical methods or physical principles.

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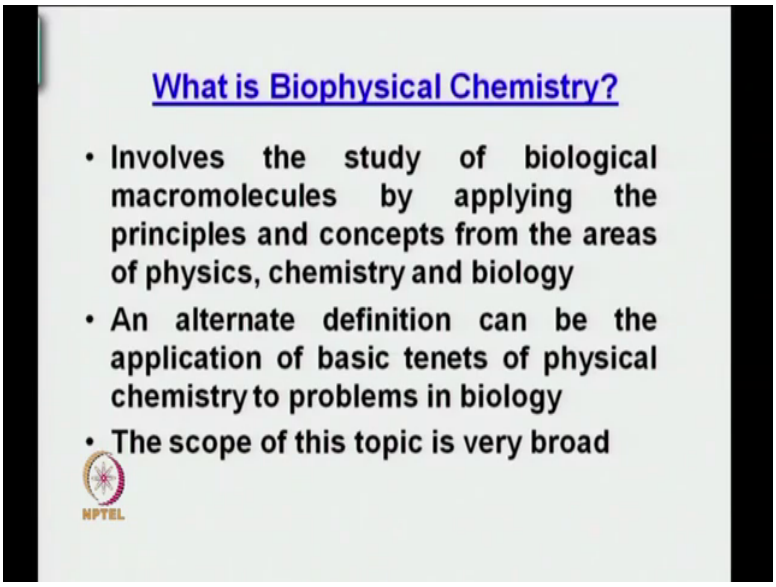

So, based on this or based on this understanding how can we define biophysical chemistry? So, one definition is it involves the study of biological macromolecules by applying the principles and concepts of physics, chemistry and biology.

So, you can understand just by this definition how wide the topics or how wide ranging the topics would be that can come under biophysical chemistry, so that means, you are applying principles of physics, principles of chemistry, and principles obviously of biology to solve biological problems. And how do we do that or in what respect do we do that is what the course or this course would try to tell you.

An alternate definition might be like this. So, an alternate definition can be the application of the basic tenets of physical chemistry to problems in biology. And as I said the scope of this topic is indeed very broad.

So, to try to tell you everything or whatever comes under biophysical chemistry in one course is next to impossible. Hence what I will do in the next slide is I will just give you a brief outline of the topics we are going to cover in this course spanning in about 42 to 45 lectures.

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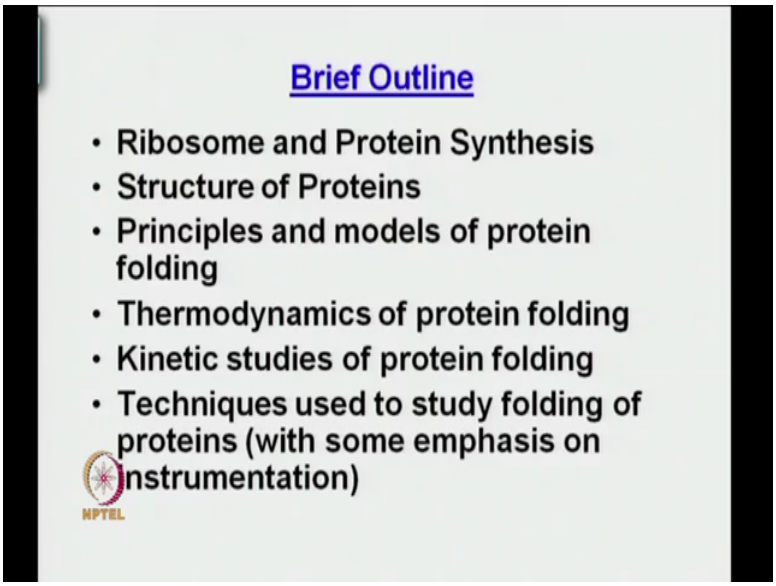

So, the brief outline the first thing which we are going to look at today is ribosome and protein synthesis. Now, please be advised that we are not going to go into too much detail about either the ribosome or either the actual synthesis of a protein. And we will come to know about it more when we actually start with the discussion.

So, it will mostly even for the ribosome protein synthesis, it will be kind of a schematic kind of an outline, so that you understand that even before the protein comes into existence, that means, even before protein folds or is released into the cytosol how does it come into existence.

The next would be definitely the structure of the proteins. Now, as you can see this course we are mainly talking about proteins, if you are not you know talking or focusing about nucleic acids right.

Then after having looked at the structures of proteins, different structures like you know protein generally have 4 levels of structures, primary which is a sequence of amino acids, secondary which are essentially the two most common ones are alpha helical proteins or beta sheet proteins, and combinations of these. Then the third level of structure would be the tertiary structure, and the fourth level would be the quaternary structure.

So, when we talk about structural proteins these are the different aspects we would be looking at. Not only that you would also look at different combinations and structures as I told you. And why those and why those combinations are necessary or why or what purpose that means what are the functions that these combinations serve I mean the combinations of structures.

The next we would cover is about principles and models of protein folding. Well, when a protein folds it has to be guided by some principles, and then if a protein is folding, how does it fold? Does it have a model, does it have a pathway does it follow? That it is it follows rather. And then we will talk about the thermodynamics of protein folding. So, this is relate very much related to the thermodynamics we talk about in chemistry.

After that having talked about kinetics, we have discussed about how fast does a protein fold, and when we are talking about fast we mean kinetic studies. And finally, we will be looking at some of the techniques that people have used over the years to provide in depth insights about how proteins fold starting from the thermodynamics, the kinetics, and the confirmations of

proteins under different circumstances like different external perturbations, say temperature, say chemical denaturation, say pH in this denaturation and so on right.

So, this is about the brief outline. So, you know as I was telling you before to that just by doing this, possibly we are not doing a justice to the course title which is biophysical chemistry. However, as we will proceed through the course what you will see is we develop enough basis of what this biophysical chemistry is all about, and the same things though in different contexts can be applied to other stuff which we are not going to focus in the course or covered in the course.

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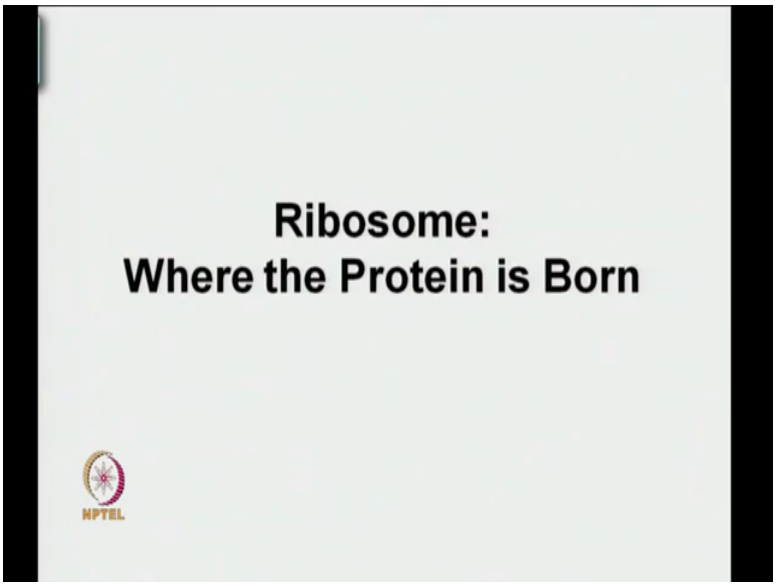

So, as I said this class we will talk about the ribosome that means where the protein is born or rather where the protein is synthesized. Now, you know you all of you know about the central

dogma of biology, again we will not go into that. Instead where will we start from, we will start from the aspect of from the moment that a protein starts getting synthesized.

See, for protein to come it into existence what has to happen is someone has to read the information that is being translated or being brought to us by the messenger RNA, it is picked up by somebody, and then according to that the protein according to that information which is transferred the protein is synthesized. Where is the protein synthesized or where does this information transfer taking place? It is taking place in the ribosome.

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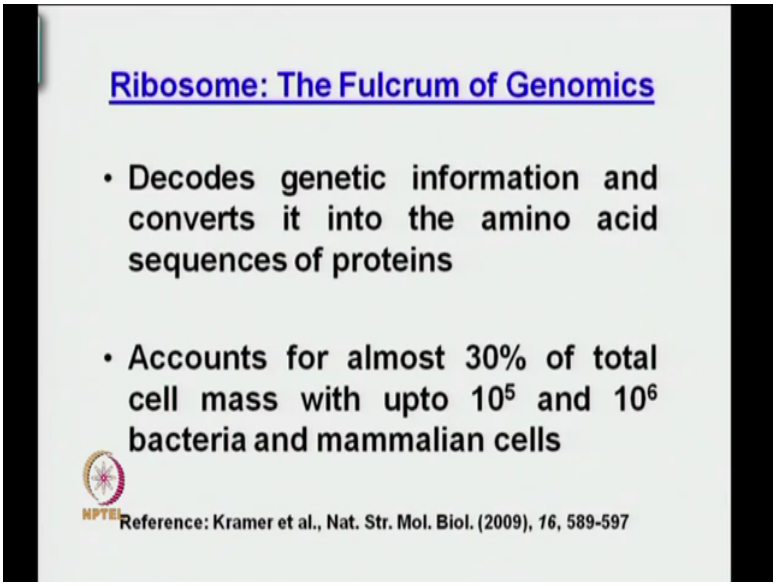

So, let us go ahead as the title says ribosome is indeed the fulcrum of genomics right. What does it do? As I just mentioned, it decodes the genetic information and converts it into the amino acid sequence of proteins right. We all know the primary structure of proteins; it is a sequence of amino acids.

It is a one which finally dictates what shape or what native structure the protein is going to acquire. The ribosomes they account for almost 30 percent of the total cell mass with up to 10 to the power 5 to 10 the power 6 bacteria and memorial cells.

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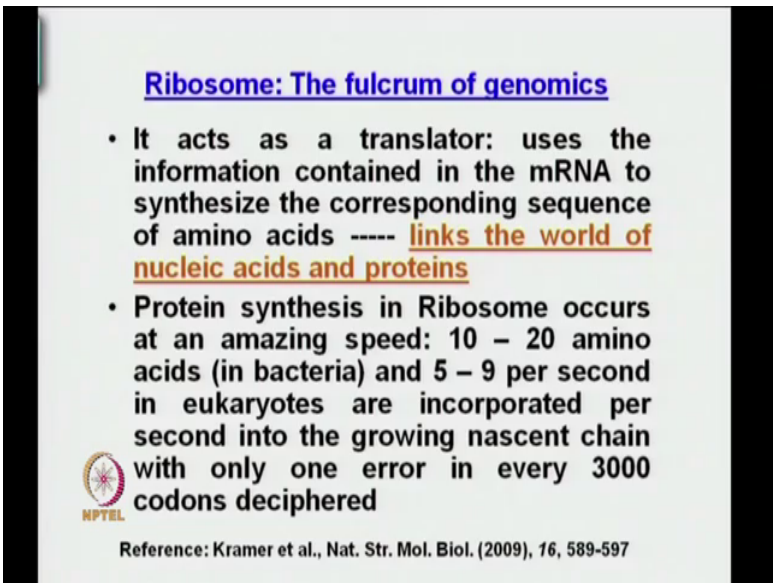

More what is the ribosome do? It acts as a translator. It uses the information contained in the mRNA to synthesize the corresponding sequence of amino acid. So, this links the world of nucleic acids and proteins, that means, you have a set of nucleic acids, it has information. What information does it have?

It has the information to tell you what protein is going to synthesize or be synthesized. And this is what the ribosome is doing. It is essentially acting as a bridge between the nucleic acids and the information content of the nucleic acids and the protein that is being synthesized.

How fast is this protein synthesis? And as it says the protein synthesis in ribosome occurs at an amazing speed, it is about 10 to the 10 to 20 amino acids in bacteria, and about 5 to 9 amino acids per second in eukaryotes. So, these amino acids are incorporated into the growing nascent chain, and you can see the fidelity that means with only one error in every 3000 codons right.

So, what it means is, if you go to a bacterial cell, then the number of amino acids per second being synthesized would be about 10 to 20; if it is an eukaryotes, it is about 5 to 9 amino acids per second.

And what do you mean by the nascent chain? The nascent chain is actually the chain of amino acids or that peptide chain which has started getting synthesized or started growing. Now, please again keep in mind that this nascent word we are going to use almost throughout today in many places.

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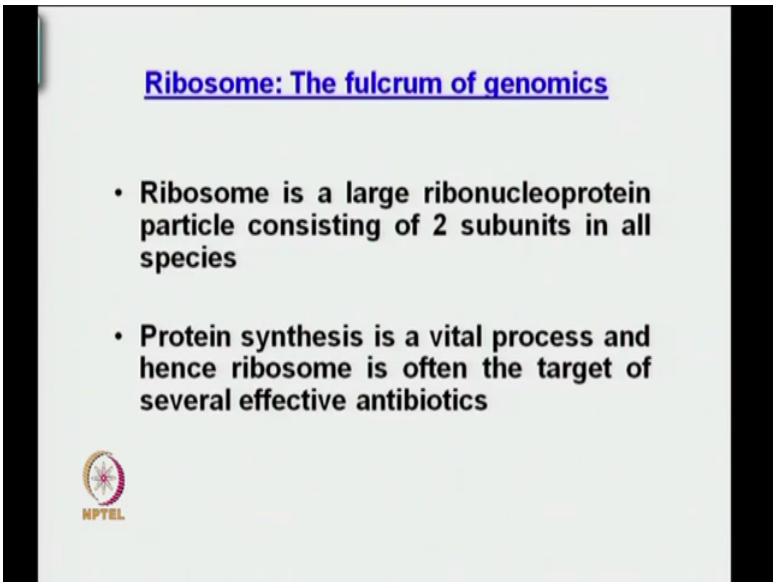

So, then what let us look at a schematic of the structure of the ribosome without going into too much of details. So, ribosome it is a large ribonucleoprotein consisting of particle rather consisting of 2 subunits in all species, whether it is bacteria or whether its eukaryotes.

Now, since protein synthesis is a vital process, now you can imagine if this protein synthesis is taking place in the ribosome then to stop the synthesis, this ribosome can often be targeted with antibiotics. So, that is why it says protein synthesis is a vital process, and its ribosome is often the target of several effective antibiotics right.

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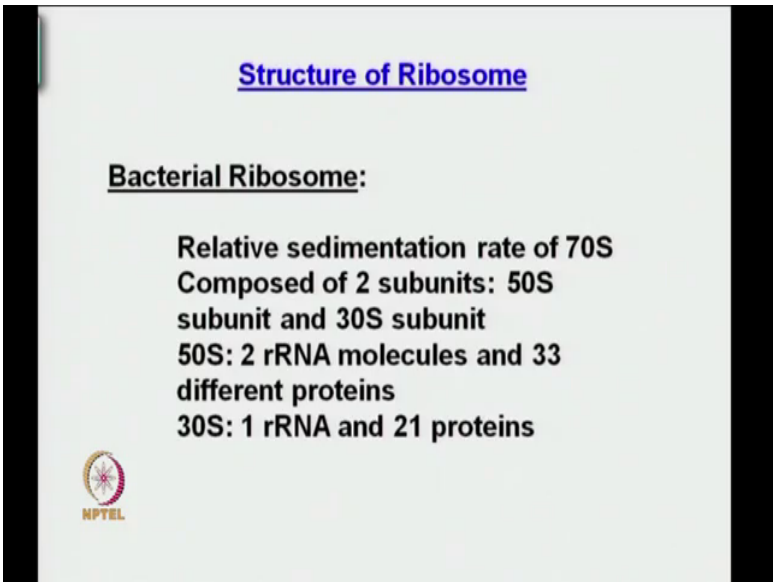

The structure of the ribosome well, as I said you have the bacterial ribosome and the eukaryotic ribosome. Whatever the bacterial ribosome the relative sedimentation rate is 70S, S stands for Svedberg unit. It is composed of 2 subunits. We will soon see a schematic of these sub units.

So, one subunit is having the 50S, and the other one is of 30S. Then the 50S subunit is composed of 2 rRNA molecules which is ribosomal RNA molecules and about 33 different proteins. And what about the 30S unit? There is 1 ribosomal RNA, and 21 proteins.

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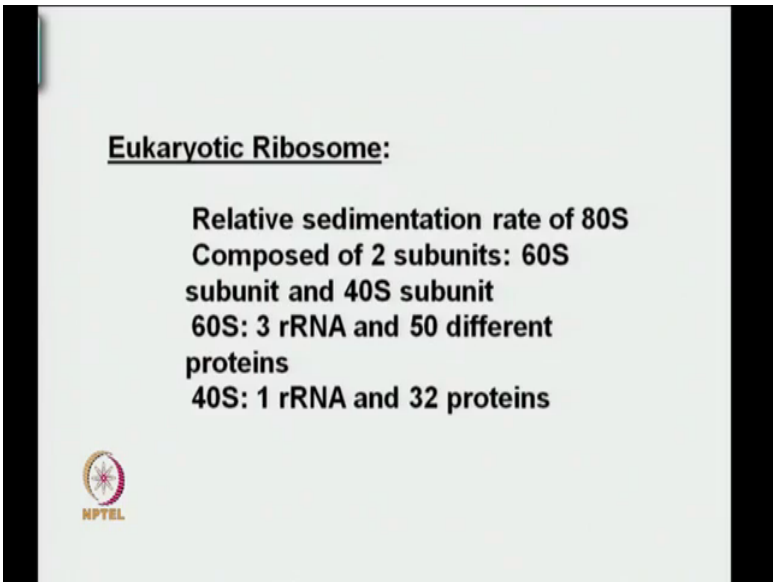

Now, what happens to the eukaryote ribosome, we can see its relative sedimentation rate is 80S; it is composed of 2 subunits. Now, one is of 60S which is the larger one, and the other one is a 40S being the smaller one. Similarly, like the other one the previous one the 60S has 3 rRNA molecules, and about 50 different proteins; the 40S has 1 RNA and 32 proteins.

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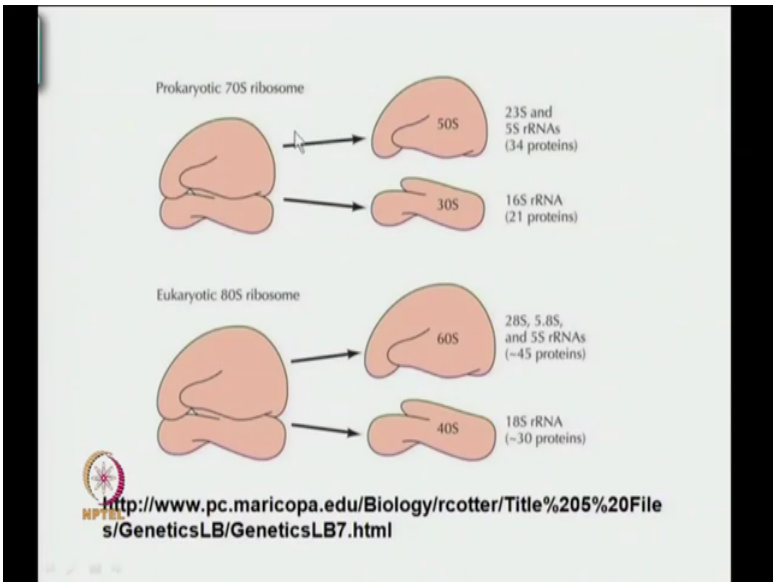

So, as I said this is a schematic ok. So, this is the prokaryotic and the bottom one is a eukaryotic. So, if you look, if you follow my arrow, you can see in the prokaryotic ribosome, the 70S one as I was telling you the bacterial one. So, there are these 2 units, the bigger one and the smaller one, and as it has been shown by two arrows. The bigger one again being the 50S and the smaller one being the 30S. And on the right hand side, you see what do you see other respective components of these units.

Similarly, if we look at the eukaryotic ribosome, it has the 60S subunit and the 40S subunit. And again in the text you see the composition of these two respective subunits. So, you know what does ribosome do? Is there a division of labour? So that means, among the smaller unit and the larger units or the sub units, what are these involved in? Is there a division of labour?

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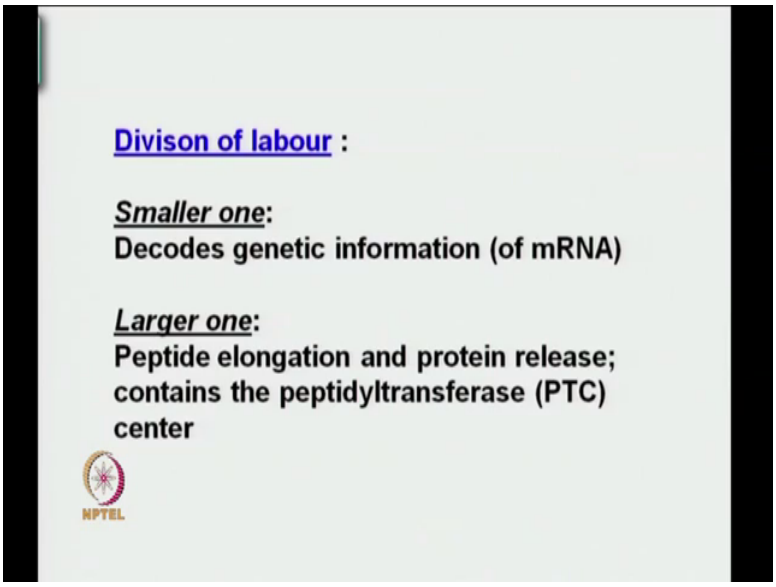

So, there is what is the smaller one do? The smaller one it decodes the genetic information which is carried by the messenger RNA or the mRNA. What is the larger one do? See if one unit is doing this, then the other unit better help us in synthesising the peptide.

So, that is what the larger one does. So, the peptide elongation and protein release is done by the larger one. And it contains a very important centre called the peptidyltransferase centre, where your peptide bond is actually being formed. Again we will not go into much details about this peptide bond formation. These I am sure you have ideas if you have done basic courses in biochemistry; if not, please look up.

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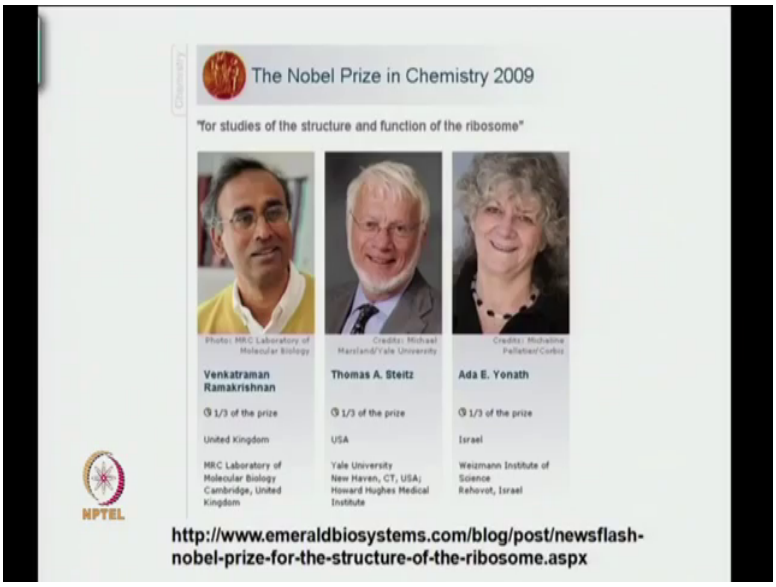

Now, to bring this whole discussion in to perspective, and this is one of the reasons why I thought that I would start this course focusing or spending some time on this topic. In 2009, remember the Nobel Prize, in 2009 in Chemistry was given to three very eminent scientists one is Venkatraman Ramakrishnan of Cambridge, the next one Thomas A. Steitz of Yale University, and Ada E. Yenath of Weizmann Institute of Science.

What were they given this Nobel Prize for? As you can see at the top, it is written all three of them shared the Nobel Prize for studies of the structure and function of the ribosome. So, now hopefully you can understand how important the study is or how important this aspect of protein synthesis is.

Now, as I said I will skip many things out here, but at least try to tell you some of the most important points, so some of the most relevant points. And what I mean by relevant? Relevant

in terms of the course or in terms of the topics, that we have to cover later in particular about how proteins fold or protein folding. So, as I said because the protein is being synthesized in the ribosome, now what will happen later?

Once the protein gets synthesized, that means, once you go on adding amino acids, the protein chain or the peptide chain will start elongating right. Now, see everything is happening in inside the ribosome.

But now once the protein synthesis is done, the protein has to come into the mainstream that is the cytosol. So, then it can go to its proper place, and exert its function or carry out its function that it is meant to do.

Which means or which tells you that there has to be an obvious opening in the ribosome itself through which the elongated nascent chain, that means, the elongated peptide chain of the peptide chain undergoing elongation because of subsequent addition of amino acids can come out. So, this is what the ribosomal tunnel does for you. So, that means, in the ribosome you have a tunnel a place through which the nascent chain can come out.

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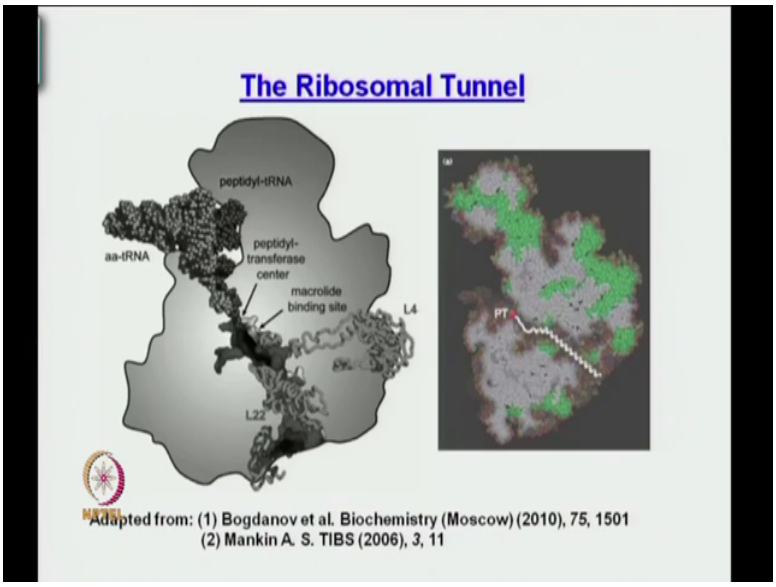

And this is what this figure shows you. So, let me if you follow the arrow, let me tell you the most important points out here. So, this peptidyl transferase center as I told you, it is the PTC; where the peptidyl is getting synthesized, so this is the peptidyl transferase center as this arrow says. Now, if you follow this arrow, this is how the peptide comes out; so this is the tunnel, this is the tunnel on the background here is the ribosome right.

And once the and at the end of the tunnel what you see is this is where the ribosome ends, and the peptide or the growing nascent chain is free to come out. Apart from that a couple of other things, this is a macrolide binding site, macrolide essentially a antibiotics, so they are based on these macrolide moieties or microlide structures. These antibiotics if you would administer antibiotic, it would typically bind at this place where the arrow has been shown.

And then you look at L 4 and L 22, these are two proteins amongst many other proteins which typically line the ribosomal tunnel. In other words, this L 4 and L 22 have been shown by people through many studies to interact with the growing or elongating peptide chain.

And to give you a clearer picture, if you go to the next figure the panel, you can see in white or the red is the peptidyl transferase center; the whole background is a ribosome and here you can see this white elongating peptide slowly coming out through the tunnel.

So, then what have you established, what we have said is that ok; I know that my peptidyl my peptide bond is getting synthesized here how, we are not going into that, but we know that this information is being carried it is been brought in by the messenger RNA.

Then at the peptidyl transferase center, your peptide bond is getting synthesized, your peptide elongates and it follows the exit point through the tunnel and comes out ok, so it comes out here ok.

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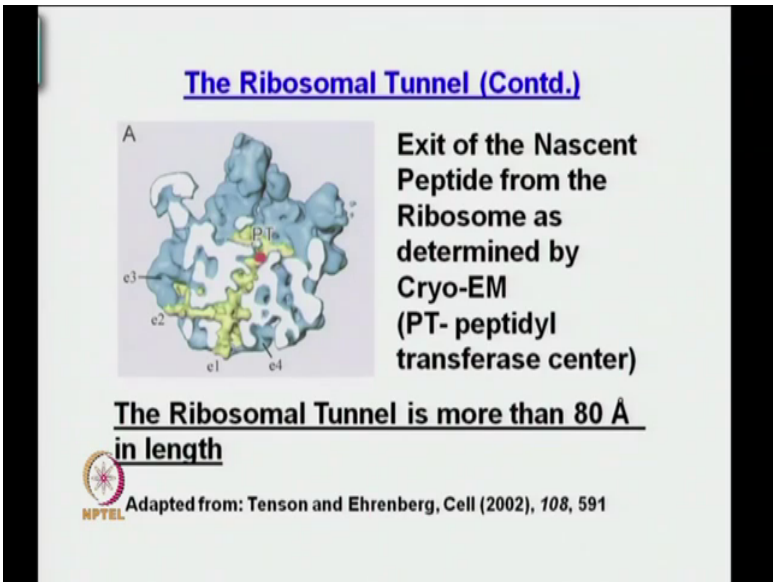

Now, continuing on with the ribosomal tunnel, if you see this panel, what it shows you is it is a Cryo-EM figure, and what is it show? It is exit of the nascent peptide from the ribosome as determined by Cryo-EM; again where PT or PTC stands for the peptidyl transferase center. Nobody will see out here is this red dot again being the PT, when we come down here these different labels e 1, e 2, e 3, e 4. Now, what is e stands for? e stands for the exit point or exit.

And if you are having e 1 to e 4, what it typically tells you is that instead of having one exits site, I can have multiple exit site that means, ribosome does have multiple exit sites. So, it might depend upon the protein that is being synthesized and depending upon what protein is being synthesized, it might take a different path that means a different exit path.

A little more information of the ribosomal tunnel and obviously, he would worry about a length of a tunnel; it cannot gone forever. The length of the tunnel is typically about 80 angstroms.

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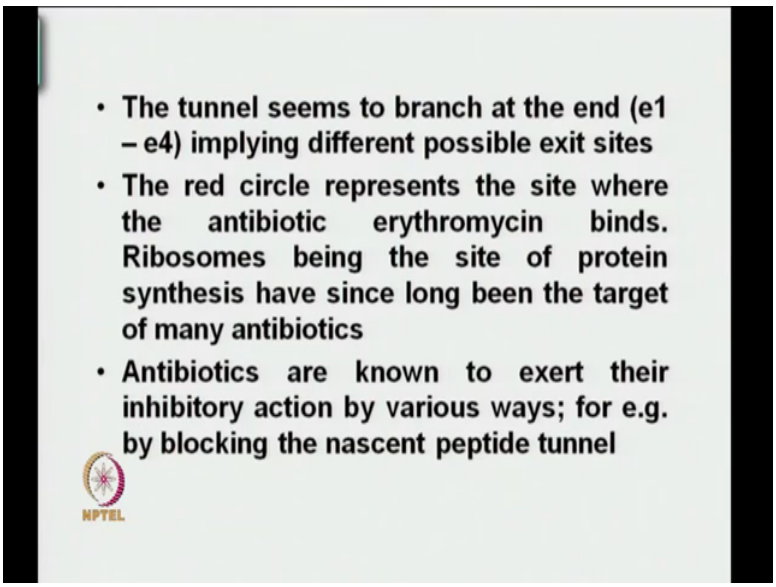

Now, as I said the tunnel seems to branch at the end implying different possible exit sites. The red circle represents the site where the antibiotic erythromycin binds. So, the ribosomes being the site of protein synthesis as I said have long been the target of many antibiotics.

And antibiotics are known to exert their inhibitory action by variety of ways; for example, one of the ways being by blocking the nascent peptide tunnel, so that means you are blocking the nascent peptide tunnel, what will happen? The peptide will not get synthesized ok.

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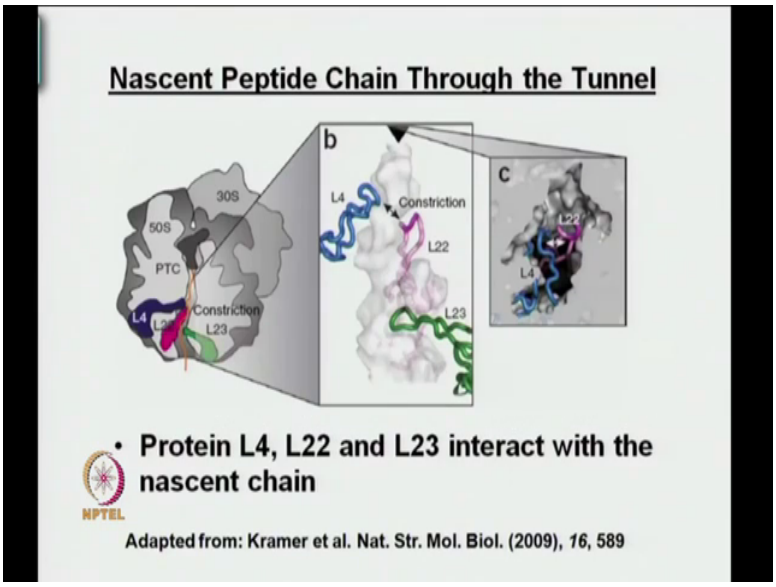

A little deeper into this tunnel, because see this is important, this is the place through which your peptide or elongating nascent chain is moving gradually. Now, if you look at the first figure focus attention on these two things; one is the L 4, and the other one is L 22, then there is another protein called L 23. The L 23 protein I will come to later, but just look about look at L 4 and L 22.

So, what happens is if you follow again my arrow, and if you follow the orange line which is coming out here which is coming through here, this orange line essentially is a growing peptide ok. So, consider that to be a growing peptide.

Now, if you can see at this place where you have the L 22 and the L 4 kind of a meeting right at these two places the blue and the magenta ones right. To the right of it is written

construction, what does it mean is that throughout your peptide or throughout your tunnel, it does not have a uniform width.

So, whenever you reach or as and when you reach this place that means, where you have the L 4 and L 22 points or the proteins merging, the tunnel is very constricted that means it is very narrow at that point. Now, if you blow up in this portion that is what you see in panel b, we can see this L 4 and we can L 22, and you see the construction is labelled here, that means that is the place which is possibly the narrowest point in your exit tunnel.

And this c goes more or it is like a more zoomed into version, I talks about or it tells you this L 4 and L 22 interactions, how they interact to maintain that construction of the tunnel.

So, the take home message from the slide is, when the protein is coming out or the where the peptide chain is getting elongated, it is coming through an exit tunnel, but at one point and typically at one point which is the construction point, it becomes the width of the tunnel really becomes very narrow.

So, in other words, what we can say is the protein L 4 and L 22 and I have not talked about L 23 though, but L 23 through the interact with the nascent chain. Now, you look at where L 23 is if this if you follow my arrow now, if this is where you can see this orange you know ribbon has come out, so this is where the peptide is slowly coming out of the ribosome assembly.

Now, L 23 is right at this exit point, and it can have certain interactions with the peptide chain that is coming out. But in what way, we will see very soon.

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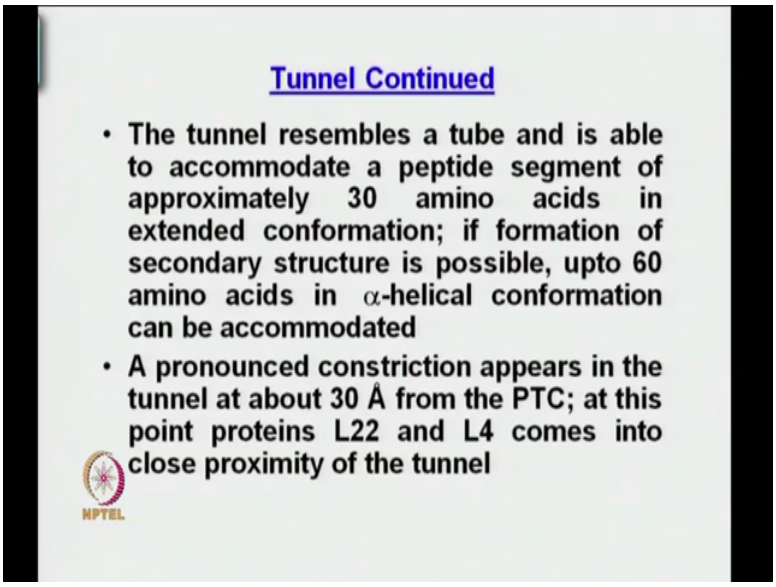

So, some more information of the tunnel the tunnel resembles a tube and is able to accommodate a peptide segment of approximately 30 amino acids in extended conformation. Now, if formation of secondary structure takes place, then up to 60 amino acids in alpha helical conformation can be accommodated.

Now, this is very significant, see one significance says that based on the length of the peptide tunnel, this is the stretch of amino acids you can accommodate. If the number of amino acid is 30, then essentially or rather is the peptide is unfolded, unstructured, then the number of amino acids that can be held inside the peptide tunnel without it being released to the cytosol or to the external medium is about 30.

However, if you gets that means if the protein starts getting structured, so now the protein is not that extended is slowly getting more compact, certain those considerations or under those conditions specially when its alpha helical, you can go to twice a number of amino acids.

Now, the importance being one was obviously it corresponds to the length of the tunnel, two it corresponds more importantly to the nature of the structure of the peptide; and this point hints at the following thing that some proteins even before they are release into the cytosol can actually start gaining some structure inside the ribosomal tunnel itself. And till date, there has been quite a few evidences towards pointing towards this particular feature.

Next as I said, a pronounced construction appears in the tunnel and about 30 angstroms from the peptidyl transferase center; and at this point proteins L 22 and L 4, they come into close proximity of the tunnel, ok.

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Now, before going to the next part what I will just do one is there is a small typo out here. Now, so what did we cover up till this far ok, the peptide is can synthesize and the peptide is coming out to the tunnel, and one feature of the tunnel we saw was there are few proteins with lines the tunnel, and at one point the tunnel is very narrow, right.

Now, the moment the tunnel ends there is the peptide or the elongating peptide chain immediately faced the cytosolic conditions, does it really happen like that, let us see. So, the next topic of a discussion is a trigger factor, now what you mean by the trigger factor of what does it do, let us proceed and see.

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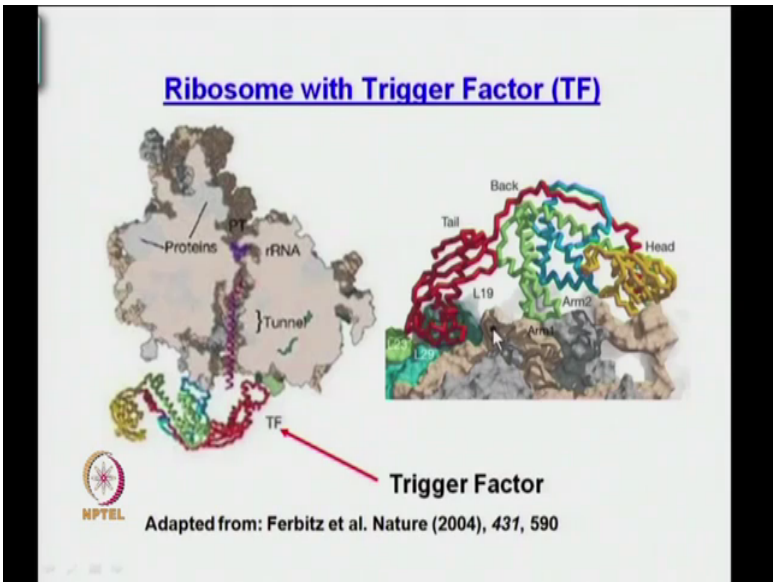

So, now you can see this is another schematic of the ribosome right. You can see this is the tunnel, this is the PT or the PTC, and you can see the silica like ribbon, this is your growing chain.

But there is one more addition to what we have seen before, this you can see here it is called TF in the short form of trigger factor, this corresponds to this protein assembly. You can see how this trigger factor is kind of cradling or protecting, this elongating nascent chain once it comes out of the ribosomal tunnel.

So, go back to the question we asked just a few minutes ago, does the protein go straight into the cytosol? The answer is no, there is this trigger factor; it is also known as a chaperone. We

will not talk about chaperones, but chaperones are those that help proteins to fold rather they prevent proteins for misfolding or aggregation ok.

They protect the proteins in a manner that they do not misfold or they do not self assemble into aggregates. So, hence this trigger factor is also known as a chaperone, because see this is what it does; it prevents the elongated peptide chain from getting exposed to other unwanted interactions in the cytosol right. So, this is the trigger factor.

Now, this is again a clearer picture of how the trigger factor looks. Now, look at this as I said L 23 is important, so this is your L 23 which is very close to the exit site. You see this black start, this is your exit site, this is the point through which the protein is coming out of the peptide chain is coming out; the nascent chain is coming out.

You can see this L 23 is a place where the trigger factor docks that means, is the connection between the ribosome and the trigger factor or the interaction between the ribosome and the trigger factor is mediated by this L 23 protein.

And you see how the trigger factor is kind of looking like an arch, its covering this exit site, it has essentially three different segments; one is the tail by which it docks on the ribosome, the other one is the back its the middle part, and the other one is the head.

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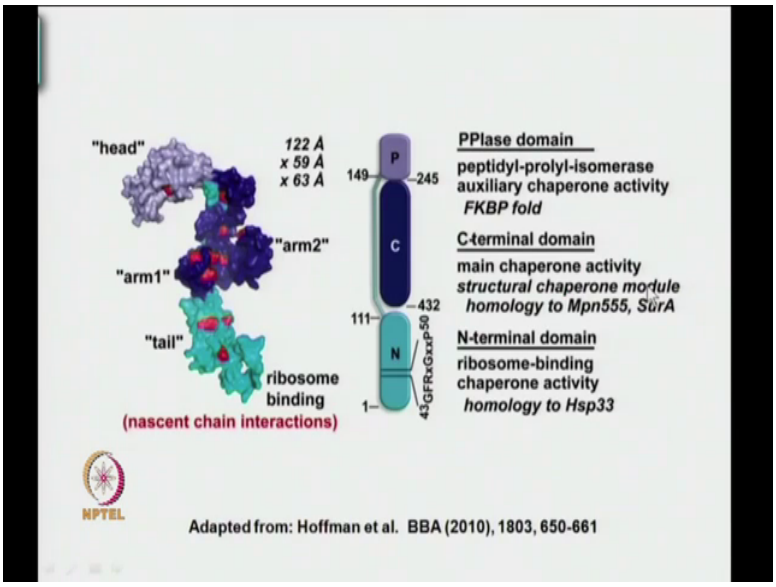

So, this is a more details about the structure of the ribosome and as I said, this is the head part, this portion is the middle part and this portion is the tail part. As you can see if you look at the tail part, this is where it is written ribosome binding, so this is the one which binds to the ribosome right.

So, this tail part is your N-terminal domain as it says its ribosome binding, it binds the ribosome and if you look at the sequence, if you look at the sequence, if you look at the sequence right.

So, this is a stretch of sequence which has or which this internal domain has and which is recognised by the L 23 protein of ribosome, so that this trigger factor can dock onto the ribosome through the L 23 protein.

Then you have this head which is called a PPIase domain or peptidyl-prolyl-isomerase domain, and one of the very important structural parts is the C-terminal domain, you can see this almost makes about makes up about half or the full protein or trigger factor.

So, this C-terminal domain is the one which exhibits the main chaperone activity I mean this is the one, which interacts with the protein in such a manner that it prevents it from misfolding or prevent and prevents it from going into any aggregated state.

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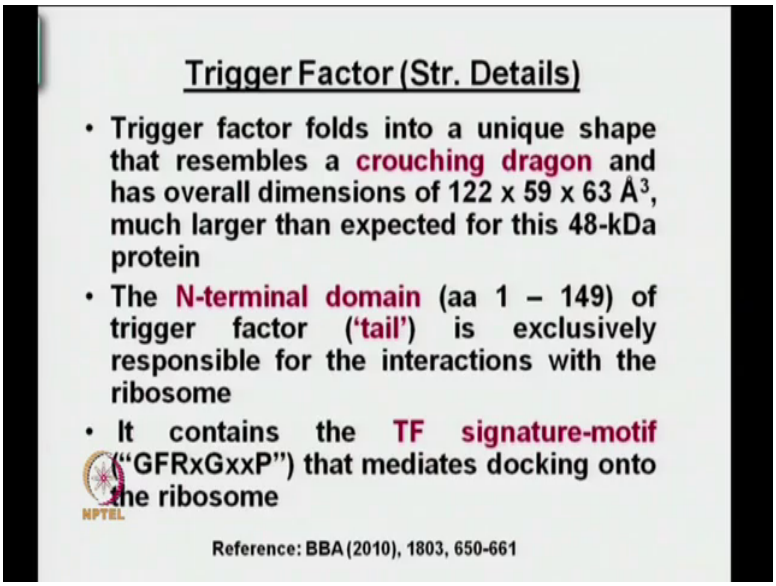

So, little more structural it was about the trigger factor. So, he said it falls into a unique shape that resembles a crouching dragon and has overall dimensions of this which is much larger than that expected from this 48 kilo Dalton protein. So, if you take this protein and if you

think of it as a sphere, because you do not know the structure say, then it would be much smaller.

But you can now figure out why the dimension is so long, because you can see the extended arch like structures; it is not spherical, it is an extended arch like structure that is what makes the dimensions so much more than expected.

So, the N-terminal domain the amino acids 1 to 149 of the trigger factor or in that domain it is the tail, and as it says, it is exclusively responsible for the interactions with the ribosome. And it contains the trigger factors signature-motif that I was just talking about that mediates docking onto the ribosome through this L 23 protein.

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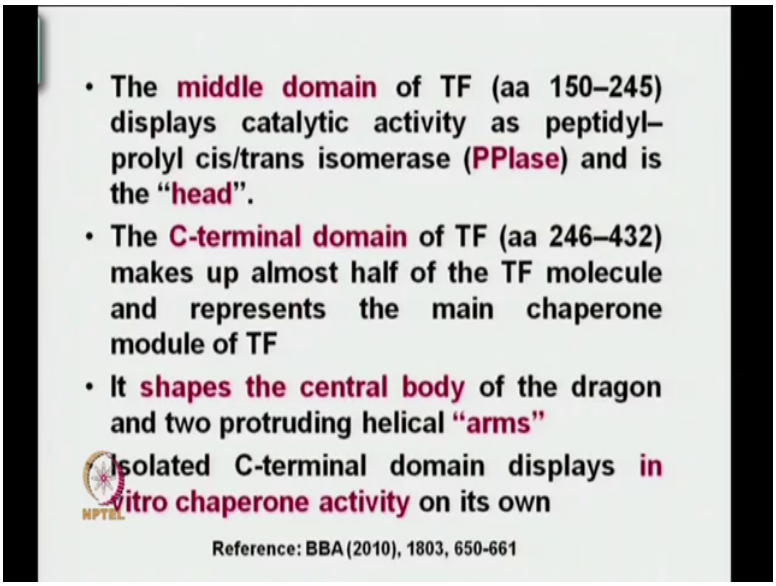

Next, the middle domain, it displays catalytic activity as the peptide the peptidyl-prolyl- cis, trans isomerase the PPIase and is referred to as the head of the domain. Now, next as I said one of the most important structural parts is a C-terminal domain, it has the one access from 246 to 432 makes up almost half of the TF molecule, and represents the mean chaperone module of TF the trigger factor.

One more important point is that if you just take the C-terminal domain in isolation, here if you just take the C-terminal domain in isolation, it itself will exhibit chaperone activity that means, just the C-terminal domain in isolation.

There is no internal domain, there is no PPIase domain; the C-terminal domain itself will be able to exhibit chaperone activity. And the third point which I just missed discussing, it shapes the central body of the dragon with and as you know two protruding helical arms. So, if you now go back, you will be able to look at these arms 1 and 2 right, ok.

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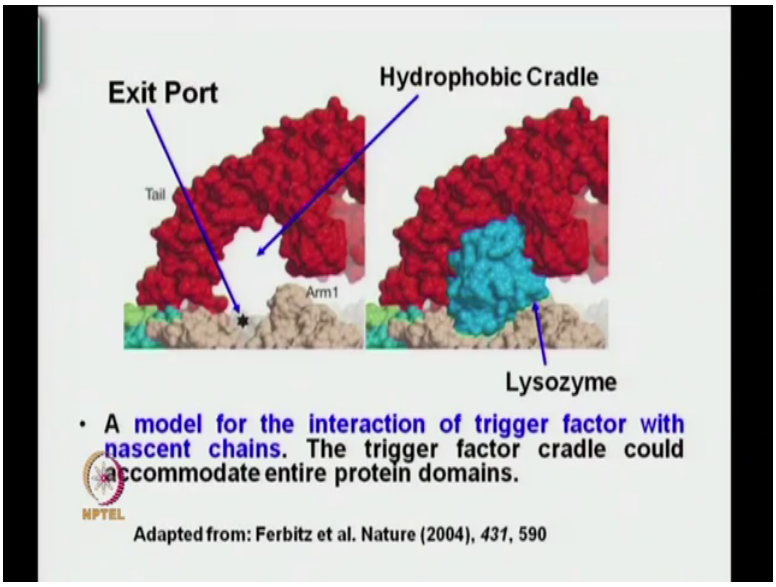

Now, what are we looking at here this is a model of a trigger factor interaction with nascent chains. So, as this is the trigger factor cradle could accommodate entire protein domains, because that means it is that big in size. So, here the black point or the black star is the exit port, this free space is referred to as your hydrophobic cradle that means, you have a lot of hydrophobic amino acids here.

And what you see here, in the second panel is a molecule of lysozyme of the protein lysozyme which is modelled onto this cradle that means, this protein fits in this cradle. Lysozyme thing is about 129 amino acids about 125 to 129 amino acids, and then it tells you that this cradle is that big that it can house a spherical protein of about that many amino acids; so it gives you an idea of the space that this cradle has.

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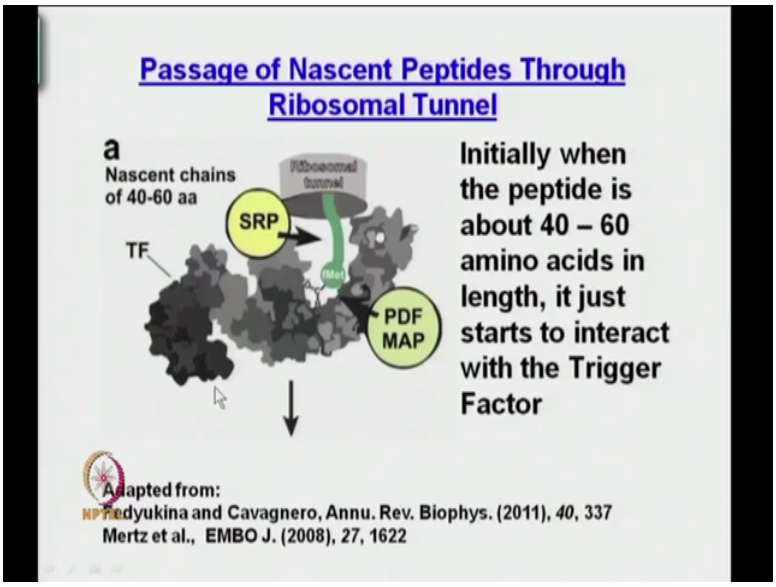

Now, looking at the passage of nascent peptides through the ribosomal tunnel, so what happens is say suppose you have started, so synthesize the protein you know which has a decent number of residues, say you know in 150, 160, maybe 200 whatever.

Now, this is the ribosomal tunnel you can see you can realise this is your trigger factor right, this is your tail this is the middle one where your C-terminal domain, this is your PPIase. Now, once a peptide or the nascent chain is coming out, here I can see two other things; this SRP stands for Signal Recognition Particle, the PDF stands for Peptide Deformylase and MAP stands for Methionine Amino Peptidase.

Now, this PDF what it does is it removes the formyle group, the MAP what it does is it removes the methionine group of the growing essentially, so that is why they are there, so that

means, at a certain point when this is coming out this PDF, MAP these are interacting with the growing chain that is what we need to know out here.

So, then what this figure is trying to tell you is that initially when the peptide is about 40 to 60 amino acids long or in length, it just starts to interact with the trigger factor ok. So, this is the start of or this is the initiation of interaction.

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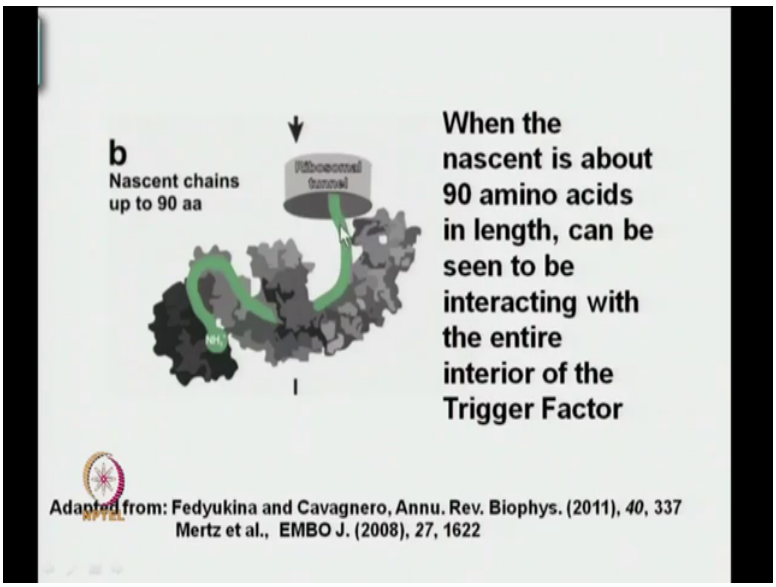

Now, what happens next is see the peptide is not growing in size right, because I said the peptides say about (Refer Time: 38:21) amino acids. So, the peptide goes on getting synthesized, the chain goes on elongating is that is why it is. Then when the nascent peptide is about 90 amino acids in length, you can see what has happened is.

So, this green one is the nascent peptide the same in the previous one, you can see what has happened is it now almost interacts with the whole of the trigger factor, so that means once you have the 90 amino acids you know, chain length peptide. So, these 90 amino acids can be seen to be interacting with the entire interior of the trigger factor ok, now think about this.

So, the full trigger factor is now involved in interacting with the amino acid chain, the growing amino acid chain. Now, what will happen is the protein goes on elongating and that is where it is supposed to right, it has many amino acids. So, after that what will happen is this.

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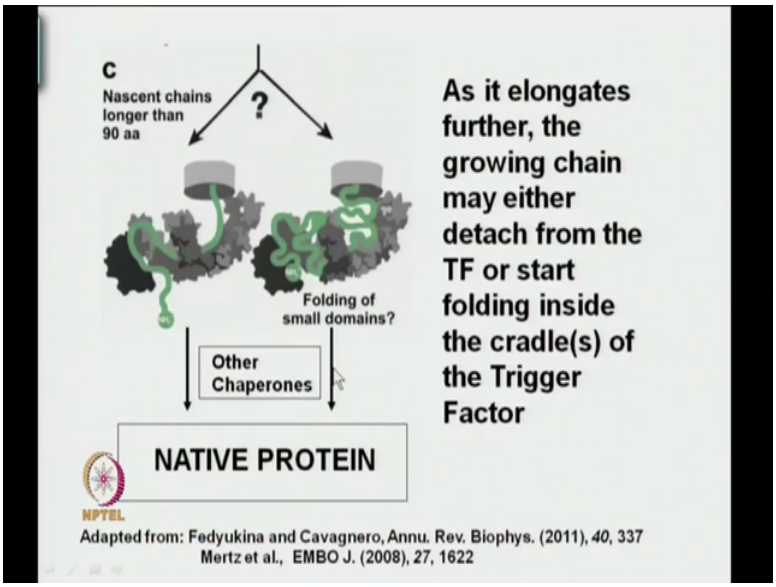

You can see this nascent chains what can happen there are two possibilities, so that is where this question mark; it will depend upon the specific protein, what path or what possibility would happen?

For example here, you would see is this green one just starts coming out that means, there is no other thing taking place the trigger factor realises that protein is elongated. So, it only engulfs that portion which it can cover through its entire stretch, the rest one which cannot be engulfed or cannot remain with the trigger factor is coming out and dangling into the cytosol say.

Now, what is the other option? The other option is as I was telling you, because these places these are not very compact that means, they have they are hollow sites that mean they the right space for the protein to fold. Then your protein instead of coming out, it can start gaining some structure inside the trigger factor itself, so these are the two possibilities are there.

Now, after that what will happen is as it says here, as it as it elongates further the growing chain may either detach from the trigger factor or start folding inside the cradles of the trigger factor, so that means its detaching its slowly coming out of the trigger factor and here it is remaining within the cradles of the trigger factor, it is not detaching.

So, what happens after that so trigger factor being a chaperone, there also many other chaperones; then with the help of the chaperones, these chaperones what they do is they make sure there were this protein or this protein they now go and finally, culminate at the native protein that is rights supposed to go that its endpoint its home, right.

So, before leaving this slide you know the message I want to want to give to you is at each and every level there is like a control factor, there is ribosome inside the of the peptide tunnel through which the peptide elongating chain is slowly coming out. But, once it comes out the ribosome does not feel that its function or it just once if it is over instead what it does is with the help of the L 23 protein, it helps the docking of this trigger factor chaperone.

Now, once the trigger factor chaperone docks this trigger factor chaperone helps the growing nascent peptide chain or the growing chain to fold or prevents it from other harmful interactions that might take it into a different pathway which will not culminate in the native floating.

Now, how can it do that one is it can engulf most of it by releasing a little part of it as a peptide goes into the cytosol or depending upon the protein and the interactions with the trigger factor, the protein can start falling into small domains ok; so evidences with regards to both are known.

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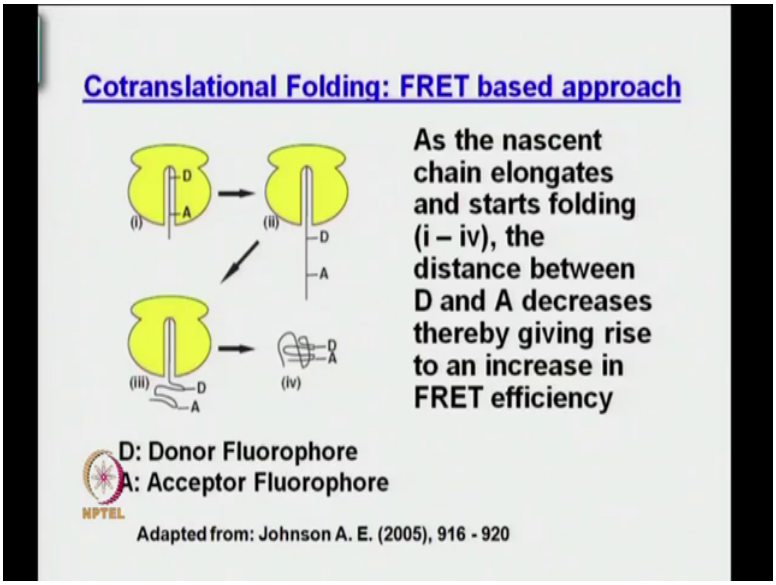

So, look at what this title says this title says cotranslational folding FRET based approach, again we will not go too much into details. FRET is a technique, it is based on fluorescence it is called Foster Resonance Energy Transfer or Fluorescence Resonance Energy Transfer this is an aspect we will come to later, almost at the last of the course when we talk about this technique.

Now, what does cotranslational folding mean? What it means is see folding is folding that means, the protein starts to fold cotranslational means as you translate that means, as you go

on synthesising the proteins that means, as you transfer the information from the mRNA to the information of what sequence of amino acids you would be having for the protein.

As a protein now get synthesized inside the ribosomal tunnel itself, it starts folding and that is what is referred to as cotranslational folding that means, folding happening simultaneously along with translation.

Now, there are ways of trying to look at it. So, one of the ways is using this FRET based approach. So, just to tell you now as it says it is you know resonance energy transfer or just focus on the words energy transfer, so what you can see out here is you have D it is the fluorophore, it is called donor fluorophore; A is an Acceptor fluorophore. Now, fluorophores are those molecules which are fluoresce which fluoresce that means, which emit light on excitation.

Now, what happens is this D will transfer energy to A and that is what energy transferase ok, and that is what this scheme or this technique is based on. Now, what do we see from 1 to 4 what we see here, so this yellow one is the ribosome, this stick or this line is your growing nascent chain.

You have what you have done is you have design experiments such that the moment the nascent chain peptide gets synthesized, one of the amino acids has a D attached to it, they will not go into details of how the system. And another amino acid has A attached to it, at some point along the chain. And you can see this D and A which are on certain amino acids, they are a little bit distant in sequence, ok.

Now, when you go to 2, what happens is this chain goes on elongating. Now, as I go to 3, what we now see is see from 1 to 2; the chain is elongated, but there is no structure developing that means, it is almost fully elongated like almost a linear one this is you know this is a hypothetical version, but what it tells you is that the peptide is not gaining any structure right now.

So, whatever the distance in one between D and A was you know it is almost the same in 2. Now, once you go to 3 and see what happens is you start developing some sort of structure in the moment that happens that D and A come close to each other. And finally, when the protein is released into the cytosol from the ribosome trigger factor combination, you can see what has happened is the end A and D are pretty close, ok.

So, if you look at the text to the right of your screen what it says is, as a nascent chain elongates and starts folding the distance between D and A decreases, thereby giving rise to an increase in FRET efficiency ok.

So, how are we following it what we are doing is? We are following the efficiency of energy transfer again do not worry about it, just think about the fact or just know this fact that this efficiency of energy transfer can be monitored using fluorescence ok; so that means, and this efficiency depends upon the distance between the two fluorophores the donor and the acceptor.

The farther they are that means, this is 1 and 2 the farther they are the lower is the energy transfer extent of energy transfer as they come closer the efficiency of energy transfer increases.

So, which means that if you have this design scheme, if you have this protocol, then if you would be looking at the distances that means, you would be looking at the energy transfer efficiency which is directly related to the distance between D and A; you would be able to figure out whether this core translational folding is actually happening or not.

In other words, if this is the one which is the native protein where this is pretty close right, and if this is the one where it is still attached to the ribosome, if and this one where there is no folding taking place. So, if this distance does not change in due course as it is on the ribosome that means, or when it is on the ribosome that means, no core transition folding is happening.

However, as long as the protein is on the ribosome, if you look at it if you look at the energy transform, if this energy transfer is changing, the only way this energy transfer efficiency can change is if the distance between A and D changes. And the only possible way this distance can change that means, the only possible way the distance can diminish that is the energy transfer efficiency can increase is when this peptide starts folding core translationally, ok.

And you can compare this one with the final native point or the final native protein. And because these are you know two amino acids at two specific positions, you can figure out how much of that core transitional folding at least with respect to those two amino acids have taken place when the peptide is in contact with the ribosome.

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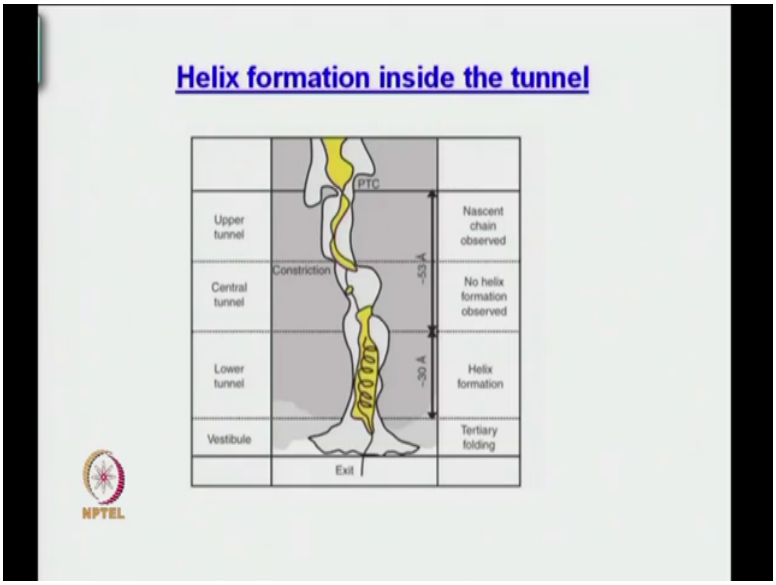

Now, there are other ways of doing it too ok. This is one of the ways it has been used. And as I said, we will not go into the full details of these, but at least we should be having an idea of

what takes place before a protein is released into the size of cytosol, so that it can fold into its native state. What are the different components they are need to be thought about that the protein has to go through in terms of interactions and other things before it is ready for folding.

And to end this discussion for today this is a picture of helix formation inside the tunnel. What you can see is again this is the peptidyl transferase center, the nascent chain is slowly elongating right, the nascent chain is slowly elongating. This is the upper part of the tunnel, this is the central part of the tunnel, and you can see this construction out here, this is the lower part of the tunnel.

And this is the vestibule in the sense this is the exit point, where it is the broadest where the nascent chain comes out. You can see where the helix formation is taking place; helix formation is taking place somewhere here, ok. This is with regards to the protein which is known to undergo helix that means, which has helical structure in its native state.

And then in this vestibule, people have talked about tertiary folding taking place, so that means, the primary is already there, the protein is getting synthesized. You have the secondary structure, this helical formation is taking place, alpha helix secondary structural element is forming.

And then at the tertiary folding, then even tertiary interactions can take place inside this tunnel. But you can see at this place no helix formation is observed that means, before this place, there is no helix formation taking place, ok.

So, this is what I want you to know about in terms of a protein getting synthesized and getting released into the cytosol, what it has to go through. So, in the next class, what we will do is, we will start looking at some basic structural features of proteins, you know different structural elements, you know that will take some time.

And in that what we will see is different combinations of structural elements, how they help as I was telling you at the right the beginning of the class, what are the functions, why do we

need them, do they have special designs, do they have special ways of packing against each other, because so many protein structures have been solved, the people have been able to come up with very general rules of packing of secondary structural elements in proteins.

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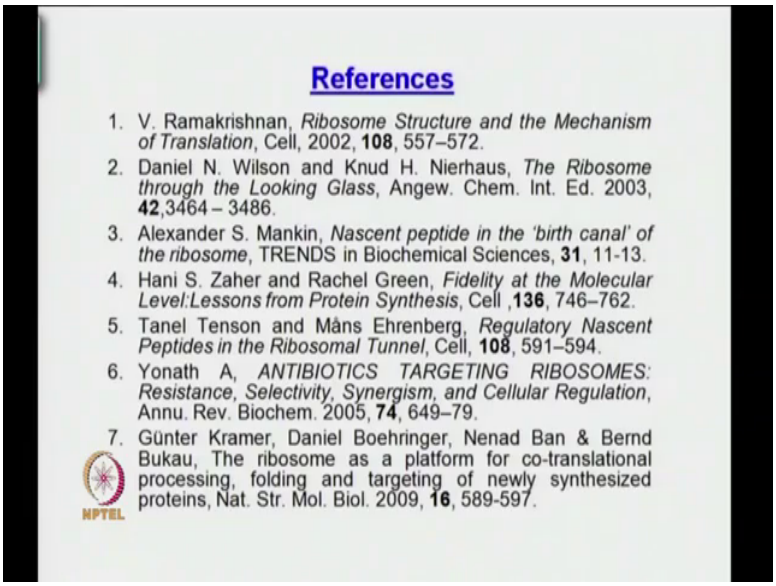

So, these are the some of the references that you might want to in, you definitely should go through which talks a lot about these respective interactions, it gives a lot more insights, and I have been able to give you today. Please go through this right. This will give you a real feeling what happens inside the ribosomal tunnel itself, and the trigger factor even before the protein is getting released into the cytosol, ok.

Thank you.