Molecular Biology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati
Module - 07
Translation
Lecture-30 Translation in Prokaryotic System

Hello everyone this is Dr. Vishal Tewedi from department of bioscience and bioengineering IIT, Guwahati and in the course molecular biology we are going to discuss about the different aspects of the cell biology we have discussed about the cell divisions different types of organelles what are present in the eukaryotic cell and then we also discuss about the different types of biomolecules. So, we discuss about the nucleic acid proteins enzymes and then subsequent to that we have discussed we are in last couple of modules we are discussing about the central dogma of molecular biology. So, within the central dogma of molecular biology so far what we have discussed we have discuss about the replications transcription. So, we have discuss about the replication different steps of replications in the prokaryotic system and the eukaryotic system and we have understood that how the machinery is actually unwinding the DNA how the DNA is being replicated and it is making the two copies of the DNA and what are the different types of events are happening within the prokaryotic system or the eukaryotic system. Similarly, we have also discuss about how the DNA is giving rise to the RNA through a process known as transcription and the transcription is also very different in the case of prokaryotic versus eukaryotic and in the prokaryotic system the transcription is been is much simpler compared to the eukaryotic system.

So, we have also discuss about the different types of differences between the prokaryotic system as well as the eukaryotic system. Now in today's lecture and in this particular module we are going to discuss about the translation. So, translation is the third step which is going to be where the RNA what is been produced within the transcription is going to be utilized for the production of the protein right and you know that the major reason why we are studying the central dogma of molecular biology because the central dogma of molecular biology is the basis of the life on the any kind of organisms or in the on the earth itself. Because all these events like the replication transcription or translations are you know tightly regulated and these events are tightly linked to each other.

So, if you want to you know make that two copies of a particular cell you are supposed to do a replication, you are supposed to do a transcription, you are supposed to do a you know the translation. Because until you were not going to do that replication the one DNA copy will not going to be you know make the two copies right. So, how you are

going to divide the genomic content between the two daughter cells. Then at the end at the same time when you are actually going to produce the next cell you are supposed to provide him the sufficient amount of the you know or different types of organelles you are supposed to provide him the different amount of other kinds of nutrients and other things. And for that you require the first the production of RNA through a process of transcription and then you are also going to have the another process of translation.

So, that you are actually going to synthesize the different amounts of proteins and enzymes and all other kinds of biomolecules. So, that you can be able to run the metabolism you can be able to govern the different types of events and so on. And you know that all these processes and all these events are very crucial for maintaining the life on the earth actually. So, that is why we are studying the central dogma of molecular biology. Now let us come back to the our topic.

So, in today's topic we are going to discuss about the translation. And when we say translation, translation means that you are actually going to synthesize proteins. And if you recall when we were discussing about the protein we said that the protein is made up of the amino acids. And if you want to synthesize the protein the amino acid has to be joined together. So, I hope that you remember what are the reactions are happening.

So, for example, you can imagine that if this is the amino acid 1 and if this is the amino acid 2 they are actually going to combine with each other with to through a peptide bond. So, this is a peptide bond what is present here. This is a peptide bond and then there will be a dehydration reactions. So, this is actually a standard way of synthesizing a protein with the help of the amino acids. But in the biological system you actually require the help from the different types of species or different types of system.

Because this under the in vitro system you can actually be able to choose the amino acid 1, you can be able to choose the amino acid 2 and that is how you are actually going to get a dipeptide. Where the first amino acid would be in the first place and second amino acid will be in the third place. You can even think about synthesizing the similar kind of protein with the help of the multiple amino acids. So, you can have amino acid 1, 2, 3, 4, 5, 6 I like that. But in biological system when you want to synthesize a protein the machinery supposed to you know select the amino acid 1, supposed to select the amino acid number 2 and so on.

So, how that works? So, that purpose the DNA is synthesizing the different types of RNA species. So, it is synthesizing the ribosomal RNA, it is synthesizing the transfer RNA and it is synthesizing the messenger RNA. All of these RNA species are being synthesized from the DNA with the process known as transcription that anyway we have

discussed in our previous class right or in the previous module. So, the why there is a reason why we are actually synthesizing the all these three different types of RNA species right. Because of the simple reason that we do not know the sequence of the amino acid in which we are supposed to attach right.

How in which amino acid will come first, then second, then third, then fourth like that. So, that sequence we do not know, but that sequence information is available on to the DNA right. Because DNA is actually the information system right. It is actually the hard disk or the brain of the particular cell. So, it actually knows which cell which protein which if you are synthesizing a protein X which amino acid will come first which amino acid will.

So, that information it is giving to the messenger RNA and that is why it is called as the messenger RNA right. So, it actually going to convey or it is actually going to take away the message from the DNA. And that message is nothing, but the different types of nucleotides right. So, it is actually going to have different types of nucleotides sorry. So, it is actually going to have the different types of nucleotides like A T A C G just like that ok.

So, this is actually going to be derived from the DNA. This process is also going to be done through a process of transcription. Now, once you have these then it is you are providing the information into the system, but who is actually going to read these information. So, that you can be able to know which amino acid. So, for example, this amino acid is actually going to read in pairs right and we are going to discuss in detail why it is been pair discussed in pair and this reading is actually going to be done by the tRNA.

So, tRNA is actually a amino acid where you have the two parts you are actually going to have we can imagine that it is actually having a two reading blocks on one or two other right. So, it is going to have the lower end which is actually going to sense the codes what are present on to the messenger RNA and on the top side it is actually going to have the amino acid what is present as the as like amino acid is attached covalently to this. So, this is actually a tRNA right. So, tRNA is actually going to have two parts and the lower end you are going that is actually going to help to read the codes what are mean what is been derived from the DNA right and the upper side it is actually going to have the corresponding amino acid right. For example, if this is for methionine then you are actually going to have tRNA which is going to be for methionine ok.

Same is true then this whole thing is and then they are actually going to do this condensation reactions right. So, that condensation reaction is actually going to be done

in the ribosomal RNA. So, ribosomal RNA is actually going to prepare more protein complexes and that is actually going to be called as ribosomes and ribosome is having a small subunit and it is going to have the large subunit and within these large and small subunit the tRNA is actually going to provide these amino acids and that is how these amino acids are actually going to combine with each other and through a condensation reactions and from the ribosomes you are actually going to have the synthesis of the proteins right. So, you can see that how complicated it is how systematic it is right and where the messenger RNA is actually you know conveying the or taking the message from the DNA tRNA is reading that message from the messenger RNA and as well as it is bringing the appropriate amino acids and then it is taking those amino acid into the ribosome which is actually a multimeric protein complexes involving the different types of ribosomal RNA and then it is actually going to do a condensation reaction just like this right. So, amino acid 1 and amino acid 2 is combining with each other to make a dipeptide.

Similarly this reaction can continue for several rounds and that is how you are going to have the synthesis of even a bigger protein like 300 amino acid, 400 amino acid, 500 amino acid protein right. So, this is just a brief overview of the importance of the different RNA species into the whole process of translations. Now if you want to understand the whole process of translation we are supposed to understand the many aspects related to messenger RNA, many aspects related to tRNA and many aspects related to ribosomes. So, what are the things we supposed to understand first? So, first thing what we have to understand is the structure of translational machinery which means we should understand the structure of the ribosomal RNA, we should understand the structure of the ribosomes right. So, then only you will be understand how the you know tRNA is getting into that ribosome how the tRNA is you know how the ribosome is catalyzing the condensation reactions and then you also should understand the structure of tRNA.

So, that it we will understand how it is binding to the amino acids, how it is recognizing the messenger at the course on the messenger RNA and then we also should understand the structure of the messenger RNA and very briefly we discuss about the messenger RNA structure. So, that you can be able to understand much quicker in the when we were going to discuss about this and then we also going to discuss about the genetic code remember that when we were talking about the messenger RNA the nucleotide sequence what is present on to the messenger RNA which is been derived from the DNA that is a you know genetic code which you are going to read and that is going to be read by the tRNA. Then we are going to discuss about the mechanism of translation. So, mechanism of translation involves the activation of amino acids, initiation, elongation and termination. This is a mechanism of translation could be you know could be for the

prokaryotic system or could be for the eukaryotic system.

So, remember that we are discussing these kind of mechanisms for both the systems. So, prokaryotic as well as the eukaryotic system. Although the basic steps remains the same that you are going to have the activation of amino acids. So, that amino acid going to attach to the tRNA and then you also going to have the different steps like initiation, elongation and termination all these are actually going to happen on to the ribosomes. And then so basic steps remains the same whether it is a prokaryotic system or eukaryotic system, but depending upon the complexity of the system depending upon the different types of requirements and different types of adaptations the prokaryotic system is adopting the different strategies to you know to perform these events or eukaryotic system is adopting the different.

Remember that the eukaryotic system is much more diversified much more you know wealthy in terms of the energy and in terms of the other kinds of resources and that is why it is actually doing the things in a very very different way compared to the eukaryotic system. So, let us start the first thing the first thing is the structure of the translational machinery. So, the in structure of the translational machinery where we are first going to talk about the messenger RNA. So, messenger RNA this all we have discussed before also. So, this is going to be a revision for you.

So, messenger RNA has a 3 prime 5 prime end has a 5 prime end 5 prime UTR ribosomal binding site coding sequences and 3 prime UTR. In eukaryotes there are additional structures such as 5 prime one in cap and poly A tail actually. So, technically the messenger RNA will have the 5 prime end 5 prime UTR these are going to provide the binding of the different types of factors then it is also going to have the ribosomal binding site then you are going to have the coding sequence this is the coding sequence which is going to be responsible for the synthesis of the proteins. So, it is actually and this is going to be a 5 prime UTR and the cap right. So, it is going to be a 5 prime cap and this remember that when we were talking about the post transcriptional modification we said that how the cap is been formed and how the poly A tail or the poly A dilation is happening.

So, messenger RNA has 3 reading frame out of which only one code for the desired protein. If it in the sequence of base there is no stop codon to interrupt the translation that met the entire peptide chain and that is called as the open reading frame. So, this is actually a coding sequence it is also going to be called as the open reading frame. Then let us talk about the this part right. So, it says that you are going to have the 3 reading frames.

So, when it says the 3 reading frame it actually is actually talking about the genetic codes right. And so, before we get into the discussion about the reading frames let us first discuss about the genetic code. So, genetic code so, messenger RNA is the random sequence of nucleotide differentiated by the base attached to them which are uracil, adenine, cytosine and guanine 3 nucleotide together code for a specific amino acid and these are called as the codons. So, you are actually going to have a codon where you are. So, it is actually going to be called as codon or I will say code right.

So, this code is made up of the 3 nucleotides right. So, for example, AUG ok and it always been 3. So, it has really going to be read as the 3 codon just like you know you are actually getting the OTP right. So, OTP is also 4 letter words right it is actually goes and give you the. So, when you do a you know banking transactions bank normally send you an OTP right.

That OTP is having 4 codes right 4 letter codes right. So, normally it is digits actually and when you enter that OTP the bank understand that it is actually the authentic person it is actually you who is doing the transactions. Similarly you are actually going to have the codons or the codes what is present on to the messenger RNA and all these codes are unique for their amino acids for example, the AUG is for methionine ok. So, similarly we have the codon for the other kinds of the amino acids. So, you see that so, codon has the 3 letters right first letter, second letter and third letter.

So, what you see here is the codon library. So, it is going to have the first letter, second letter and the third letter. So, if you go with the first, second and third what you see here is that you are going to have the multiple types of combinations and all these codons has to fulfill the criteria that it is actually going to be unique for that particular amino acid. It should be there should be no ambiguity right it should not be for other amino acids right. And the first question comes why there is a 3 nucleotide why not 4 just like in the OTP right you are getting the 4 amino acids.

So, you are getting the code which is of 4 digits right why not it should be 4 why not it should be 2 why not it should be 1 right. So, the people have done the experiments and people have mathematically also done the calculations and then they found that it is actually the 3 amino nucleotides which is actually been the optimal to provide the specificity and as well as the sufficient number. So, that you can be able to get the codon for all the 20 amino acids right. So, the genetic code is a triplet code right and it is called or scored it is known that we have only 4 type of nucleotide that make the whole genome. It is also known that the each codon consists of the 3 nucleotides which means that there are 4 to the power 3 that is the 64 possible amino acids.

However, since there are only 20 amino it is obvious that the more than one codon is for a single amino acid. So, these illustrated is called as the Bobel hypothesis. But there are this is this is just the mathematical way of explaining that why there is a 3 codon 3 nucleotides are present in the codon, but people have done the experiments. So, what we have done is they are actually taken the codon for example, you can take like this you can make a poly A amino acid poly A messenger RNA for example, right.

So, if I use this right ok. So, this one is having the 3 3 3 3 right. So, this is going to be 3 prime this is going to be 5 prime like. Now, if you translate if you translate this under the in vitro translation system you are going to get a protein which is of the 4 amino acids right. So, it will be 4 amino acids. So, this is going to be the first amino acid this is going to be the second amino acid this is going to be third amino acid.

So, you are going to get a polypeptide which has the 4 amino acids. So, it is going to be a 4 amino acid polypeptide. Now, imagine that I am going to make a mutation ok. So, I am going to erase this right. So, for example, I have I have prepared another messenger RNA and I have removed one of the amino acids.

So, I have removed one of the nucleotides. Now, what how many nucleotides I have? I have 1 2 3 4 5 6 7 8 9 and 11 earlier I was having the 12. Now, how many amino acids I am going to get? I am going to get the 3 amino acid right. Now, again if I remove one more suppose I remove one more then I am going to have the 10 even then I am getting the 3 if I remove one more then also I am getting the 3, but if I remove one more right I am going to get the 2 amino acids because then this one also is not going to code. So, same similar these kind of you know where people have synthesized the small RNA species in the you know the in vitro translations and so on and by doing so, they could they could be able to identify the code for the different amino acids. So, for example, if you do a you are what you are going to code is actually the what you are going to get the code for the for example, you are going to get a code for lysine ok.

So, this is the genetic code for the lysine. So, this means you are going to get lysine lysine lysine like that and that you can easily identify from the polypeptides right you can easily gets digest that polypeptide and it is actually going to tell you that this is actually the lysine. Same is true for other amino acids for example, I can make a messenger RNA with U U U right and I can say that oh it is actually coding for phenylalanine. Similarly, I can make the another amino another code like I can just make this kind of system and I can do a post you know in vitro transcriptions and then I will say ok U U A is coding for the lysine. But that is how they have actually come up with this kind of library which is called as the genetic code library ok or table ok genetic lab where this is the first letter this is second letter and this is third letter and you can easily

be able to know that ok if it is a U U U it is phenylalanine if it is C U U it is leucine and you see we have the 64 codons right. So, 64 codons for 20 amino acid that means, some of the amino acid will have more and more than one amino acids.

So, out of 64 the 3 amino acid 3 codon is for the stop codon and the 61 is for the amino acids right. So, what you see here is the 3 codons which are for the stop codon like the U A A U A G and U G A. So, these codons will not going to code for I mean any amino acids and that is why they are actually going to stop the synthesis of the translation this is very important to understand and it actually required when you are when we are going to discuss about the transformations. And so, this is this is very very you know detailed and remember that these codons are degenerate they are actually been present in all the organisms. So, same codon is being coded with in for a particular amino acid in all the organisms except there are few exceptions there are exceptions that some of the codons which are coding for methionine in the bacterial system, but they may they may be coding for something else in the eukaryotic system.

So, those kind of exceptions are there, but more or less more most majority of these codons are coding for the all coding for the same amino acid in all the organisms. So, these are the some of the properties of the genetic code. The genetic code is triplet and this means it is actually going to made up of the 3 nucleotides each codon coding sequence has a start and a stop codon to initiate and terminate the translation. Usually start codon is AUG right AUG which is for methionine right and which code for the methionine and stop codons are UA, UAG and UGA in some cases the starting codons are GUC or the UUC. So, these are the exceptions this are in the some of the bacterial system you are actually going to have the start codon as GUG or UUG.

We are going to discuss in detail why it is always methionine, but that you will understand once they are going to discuss about the initiation and other steps. The codon is the code is unambiguous which suggests that the code is for only one amino acid. That means, they are not going to get confused like. So, it is not like for methionine you have the same codon and the same codon is coding for the leucine also. Actually unambiguous it is only specific for that particular amino acids.

There is no gap and there is no comma in the codon. So, it is actually AUG there is no difference between these two rights there is no gap between or there is no comma actually. The code is degenerate this means that one amino acid has more than one codon for example, the phenylalanine is specific for two codons that is a UUU and UUC that this is the phenylalanine UUU and UUC. So, this is actually degenerate. So, you can actually have the flexibility of using a tRNA which contains the UUU or you can use the tRNA which is for a UUC. And it actually provides the flexibility into the system some

organism may use the UUU very extensively and some organism may use the UUC and that is why it may actually help the organism to adopt according to the according to the environment or according to the situation.

Only tryptophan and the methionine are coded by the single codon. Then the codon is non-overlapping for example, a code has AUG, CUG, GGU, GAU, UUU, GUA then codes will be AUG right. So, you go by 3 3 like. So, GU CUG, GGU and so on, but not like AUG, UGC, GCU and so on. So, this is what is actually going to say that there is only one reading frame which is possible right.

So, there are suppose I will explain you this in that. So, for example, you have AUG and CUG. So, I will just explain with two codon actually. So, what it says that you actually going to read this as first codon and you are going to read this as a second codon, but you are not going to read this as a overlap. For example, you cannot read like this you cannot read like you you can read like AUG and CUG, but you cannot read like this you cannot read like this because then it is actually going to be a problem right. So, if you are actually going to have the codons it can actually be able to and if you go by this you can be able to have the 6 different types of reading frames ok, but ideally we only have the one correct reading frame which actually provides the correct amino acids.

Genetic code is universal which suggests that the genetic code and its meaning is common for all life forms. However, there are some exceptions in this rule. For example, UGA is a stop codon, but it codes for tryptophan in the mycoplasma, spiroplasma and the mitochondria of several species. Similarly, CUG codes for the leucine in general, but in yeast mitochondria it codes for the threonine.

So, these are the some of the exceptions. Now, let us move on to the next structure and the next structure is the transfer RNA ok. So, the transfer RNA is a clover leaf structure in the 2 dimensional and L shaped structure in the 3 dimensional. Transfer RNA is a 73 to 94 ribose nucleotides in length. A transfer RNA molecule consists of 5 prime phosphate terminal and acceptor arm and ends in the CCA terminal at 3 prime end. D loops which often contains the dihydro uridine, anticodon loops and the t arm which has the t psi c where psi is a pseudo uridine.

CCA sequence is important as it is important for the recognition of tRNA and also site for attachment of the amino acid. So, this is the clover leaf model of the tRNA right. So, where you are going to have the 5 prime end and from the 5 prime end you are actually going to have the 5 different arms right. You are going to have the d arm, you are going to have the anticodon arms, you are going to have the di-cis group and you are going to have the CCA end.

And then you are also going to have the extra arm. This extra arm is being used for adjusting the extra nucleotides. So, it is actually used for adjusting the extra nucleotides. So, that you can always have the anticodon in a particular orientation, you also should have the CCA end in a particular orientations. These are the these 2 arms are actually containing the specific nucleotides for example, the d loop is having the dihydro uridine as a nucleotides. Similarly, t psi c has the pseudo uridine as one of the modified nucleotides what is present.

And then this is a very important codon because this is a anticodon loop which is actually going to recognize the codon onto the messenger RNA. So, it is actually going to recognize the codon what is present onto the messenger RNA. For example, if you have AUG, this AUG what is present onto the messenger RNA is going to be recognized by the anticodon what is present onto the tRNA. Similarly, this is the amino acid arm or CCA arm. So, this is the CCA arm where your amino acid is actually going to be attached and that is how this is actually going to participate into the condensation reaction.

So, this part is going to you know participate into the condensation reaction and this part is actually going to read the message onto the messenger RNA and that is how they are actually going to bring the correct tRNA into the actions. So, each tRNA is specific to the amino acid that it carries in the CCA arm. So, there are 30 to 45 different tRNAs in prokaryotes and the 50 different types in eukaryotes which suggest there is a more than one tRNA for a single amino. So, tRNA would be as per the genetic code. So, you are going to have the different types of anticodons for the different amino acids.

So, remember that we have the different types of genetic code for a specific amino acid or for a particular amino acid. Similarly, you are going to have the different types of tRNAs. So, you are going to have the 50 different types of tRNAs in the pro eukaryotic system and 30 to 45 different types of tRNAs in the prokaryotic system. For example, for glycine there are two tRNAs which are represented as tRNA gly 1 and is also called as tRNA gly 2 right.

So, these both are actually going to carry the glycine here. So, it is going to carry the glycine here, but the anticodon are probably different. Now, let us move on to the third machinery and the third machinery is called as the ribosome. So, structure of the translational machinery. So, ribosomes are the ribonucleotide proteins. So, these are the multimeric protein complexes where you are actually going to have the ribosomal RNA and the proteins.

Each ribosome is made up of the two subunits large subunit and the small subunit. In prokaryotes mitochondria and chloroplast of the prokaryotes chloroplast there is a 70S ribosome which is composed of 50S and 30S subunit. In equalize the 30S subunit consists of 16S ribosomal RNA, 21 ribosomal RNA and 50S ribosomal subunit and 50S subunit contains 23S ribosomal RNA, 5S ribosomal RNA and the other 31 different types of proteins. In eukaryote there is a 80S ribosome which is consists of 60S and the 40S ribosomal subunit. So, in eukaryote there is a 80S ribosome which is consists of 60S and 40S ribosomal subunit, 60S subunit consists of 20S RNA, the small ribosomal RNA and the 5.

8 ribosomal RNA and approximately 50 different types of proteins. The 40S ribosomal subunit consists of 18S ribosomal RNA and the 33 different types of proteins. So, this is what and it is actually going to have the 3 different types of activities is going to have the aminoacyl site, it is going to have the peptidyl site and it is going to have the exit sites. And one side it is going to have the messenger RNA binding site and on the other side it is actually going to have the tRNA docking site. So, the ribosomes are ribonucleoprotein that contain the RNA and protein and each ribosome is made up of the 2 subunits. So, it has actually going to so, 70S ribosome has 3 RNA tRNA binding sites has the P site, it has A site and has E site.

The P site is the peptidyl tRNA binding site, A site is the aminoacyl tRNA binding site and E site is the deacetylated tRNA site all the exit sites. So, it is going to have the 3 sites going to have the P site going to have the A site, it is going to have the E site. So, A site from where the RNA the tRNA is going to enter into the protein synthesis then it will enter it then it will move to the P site and it is actually going to catalyze the reaction of condensation and then from here it will either move to this site or it will actually move to this site depending upon what kind of codons are present at the bottom. If it is codon is next codon then it will enter here and then it will again take up the another amino acid, but if it is a stop codon then it will enter here and it will be get exited from this site ok. That anyway we are going to discuss in detail when we are going to discuss about the mechanism of the translation.

So, mechanism of translation so, it is going to have the 3 important events like the activation, initiation, elongation, terminations and we are also going to discuss about the post translational modifications. So, mechanism of translation so, first event is the activation of amino acids. During this process the amino acids are attached to the tRNA in the presence of the enzyme aminoacyl-tranvasanthase this enzyme activates amino acids by attaching covalently to the tRNA when the tRNA get charged it named it named as the amino T aminoacyl tRNA aminoacyl tRNA. During this process the amino acids are attached to the tRNA with a high energy bond so, that the it activates the amino

acids. So, you are going to have the amino acid you are going to have tRNA you are going to have ATP then you are going to have an enzyme which is called as aminoacyl-tranvasanthase and it is actually going to produce the aminoacyl tRNA and it is going to produce the AMP and VPI which is actually going to be produced from the ATP.

This is actually very high energy system where your amino acids are going to be attached on to this right and it is actually going to participate into the condensation reactions. In eukaryotes in eubacteria first amino acid is in the polypeptide chain is N-formylmethanine which is specific to 3 crores as AUG, GUG and UUG. Whenever these codes are present in the primary point they code for the N-formylmethanine but they are present in between the codon sequence they code for the methanine and valine respectively. How does this happen? This is because of the difference in the initiation tRNA and the one used in between the process of translation. Initiation tRNA has a unique feature that distinguish from the elongating tRNA in the bacteria.

So, you are going to have the amino acids tRNA and ATP and it is going to have synthesis of the amino acid tRNA. The only difference is that you are going to have the different initiation tRNA compared to the tRNA what is been going to be used for that during the elongation steps. Now let us talk about the mechanism. So, mechanism has the 3 steps is going to have the initiation, elongation and terminations. So, in the first step the small subunit of ribosome bind to the messenger RNA such as that the initiation codon lies in the P-side.

This gets possibly due to the activity of IF3 it basically prevents the untimely reassociation of the large and small subunit of the ribosomes. Moreover, it promises accuracy of initiation site selection. Ministries are there is a ribosomal binding site which consists of the shine-Turgano sequences and the initiation codon. This shine-Turgano sequence which is 5 prime A and this is located 10 base pair upstream of the initiation codon is complementary to the region near 3 prime and of the 16th ribosome a component of the small subunit of the ribosomes. So, this is the shine-Turgano sequence what is going to be present on to the initial on to the messenger RNA which is upstream to the start codon.

And then in the next step the initiation tRNA carrying the N-formylmethanine enters the P-side and binds to the messenger RNA via its anticodon loop. So, in the this is the initiation codon. So, it is actually going to have the binding and the assembly of the ribosomal machinery in such a way that this actually is going to bind to the P-side. And then once it binds to the P-side and then the IF2 is responsible for this activity. So, with the help of the initiation factor 2 it is actually going to allow the binding of the tRNA on to the P-side.

So, on the P-side the tRNA is actually going to recognize this initiation RNA and initiation t-methan-codon and that is how it is actually going to bind with the initiation amino acid. So, that is the 5-formylmethanine. It directs the initiation tRNA to its correct position in the initiation complex. It is also exhibit ribosome dependent GTPase activity.

Once the GTPase hydrolyze then the 50S ribosome and join to form the complete ribosomes. Finally, when the large subunit also join the complex it forms the complete P-side and the A-side. Second charge tRNA enters into the A-side this tRNA as per the rule has the anticodon corresponding to the codon into the messenger RNA. So, once the tRNA the initiation tRNA is going to be placed very nicely then the 50S ribosome subunit is going to enter. It is going to replace the initiation factor 1, 3 and 2 and it is going to hydrolyze the GTP as well. And then the large subunit will also going to bind in such a way that it is actually going to make the complete P-side, complete A-side and so on.

And then the P-side is actually going to take up the next tRNA that next tRNA would be the specific for the next codon. Now, let us move on to the elongation steps. So, it is a cyclic process elongation process starts from the formation of first peptide bond to addition of last amino acids. This amino acid added to the chain 1 at the time to nascent polypeptide chain. Addition of amino acid is very rapid process the peptide sequence is in order of codon and anticodon in the messenger RNA rate of elongation is nearly 15 amino acids per second.

So, there are some requirement regarding to the elongations that the messenger RNA and 70S ribosomes, aminoacyl tRNA and the elongation factors. So, you have the 3 different types of elongation factor elongation factor TU, elongation factor TS and elongation factor G. So, elongation factor TU it is a G protein which binds to the aminoacyl tRNA and direct it to the correct position and the ribosome A-side. It is main EFTS its main function is to regenerate the EF-TU and the hydrolysis of the GTP and then EFG it also has a G protein which mediate the translocations.

So, elongation is carried out by the ribosome in 3 steps. Remember that we have the 3 activities we have the P-side, we have A-side and we have E-side. And we already have the amino acid. So, in the initiation side we already have amino acid in the A-side in the P-side and it only moved. So, we actually have a tRNA which contains the another amino acid in the A-side.

So, first it is actually going to have the decoding. So, it is a codon directed binding during the process of ribosome select and bind to the incoming aminoacyl tRNA at a site

whose anticodon is complementary to the codon of the messenger RNA. Decoding region of 16 as ribosome confirm the proper base pairing between the codon and the anticodon. So, on the A-side there will be a codon and anticodon interactions. So, this is going to be messenger RNA what is running right and this is going to be anticodon what is present onto the tRNA and that is how this interaction will ensure that the correct amino acid and correct tRNA is actually going to bind into the A-side.

Then we are going to have the peptide bond formation. So, in this process the peptidyl group of P-side of tRNA is transferred onto the amino acid group in the peptide bond. So, this amino acid is actually going to be shifted onto this and that is how you are going to have a peptide bond which is going to be formed and this I mean this is going to be a free right. And then there will be a translocation. So, this there will be a translocation this is this whole complex is going to be translocated onto this side and that is how whatever is this dipeptide is going to be present onto the P-side.

And then again the third second nucleotide is going to be present here. So, in this case the tRNA of A-side is transferred onto a P-side to make a space for the next amino acid tRNA at A-side and the A-side of tRNA is shifted at the E-side and this shift is also coupled with the ribosome movement along with the messenger RNA. So, then so process of chain elongation in ribosomes. So, EF-Tu promote the entry of amino acid transfer A into the A-side of the ribosomes. First EF-Tu binds to the GTP and it activated the EF-Tu GTP complex which binds the tRNA when codon and anticodon base pairing stabilizes then hydrolysis of GTP occurs which converts into GDP and PI which helps in the binding of amino acid tRNA to A-side and after this EF-Tu is released. EF-Tu is catalyzed the release of the EF-Tu GTP from the ribosome and regenerate the EF-Tu GTP its main work is to recycle the EF-Tu.

So, this is what is actually going to happen in the elongation steps. And then you are going to have the peptidyl transferase. So, it is a peptide bond formation step which with which the amino acid of the peptide bonds are linked to the tRNA in A-side and a carboxyl end of the peptide chain uncoupled from the tRNA by in the P-side. This reaction is carried out by an enzyme which is called as peptidyl transferase. Peptidyl transferase is an enzyme which is associated with the 23S ribosomal RNA of 50S ribosomal subunit.

Peptide bond formation involves the O2N migration and the conversion of ester into the amide bond. So, this is what exactly going to happen in the P-side you are actually going to have the amino acid 1 and from here it is actually going to be shifted onto the amino group of the second amino acids and that is how you are going to have this dimer which actually going to have the peptide bond. So, this is actually going to be a peptide bond

what is going to be formed. So, three things are necessary for the translocations right deacetylated RNA moves from the P-side peptidyl transferase move from A to P-side and the ribosome should move on to the messenger RNA 1 codon so that the next codon can come at the A-side. Translocation steps carried out by the EFG factors during translation acceptor end of the both tRNA of A and P-side are interacting with the peptidyl transferase center of 23S ribosomal RNA of 50 subunit. In trans location A and P tRNA transfer to the P and E-side respectively as ribosome move 3 nucleotide along messenger RNA chain in 5 prime to 3 prime direction.

During trans location step the GTP is converted into GDP and the uncharged cRNA released from the P-side to E-side and newly formed a beta transfer from A to A-side. In longation process is nearly same in both the prokaryotes as well as the eukaryotes. This is what exactly going to happen this is the assembly of the system and this is going to be A-side, P-side and E-side. So, A-side is always going to welcome the new amino acid or new amino acid transferase. Whereas in this side you are going to have the peptide bond formations and once the peptide bond is been formed then it is actually going to be shifted on this side.

Whereas the old tRNA which does not contain amino acid is actually going to be shifted onto the E-side and from here it is actually going to be go into the it is actually going to be rejected and it is actually going to fall into the cytosol. Because so this tRNA is going to be rejected into the tRNA into the cytosol and then again the it this will going to participate into the another round of the the amino acetylations with the help of the amino acids and again it will be ready for supplying them in the amino acids into the A-side. And then if we have the terminations so termination of translation occur due to the stop codon there is a three stop codons UA, UAG and UGA. Out of these three when one of the stop codon appears in the A-side of the ribosome it causes the termination because there is no tRNA present corresponding to these codons.

So, tRNA is not binding codon and cause the terminations. During the termination process release factors are involved when the UA or UG is in A-side RF1 binds to the ribosome when UA or UGA is in the A-side RF2 binds to the ribosome. RF3 is a type of GTPase which maintain the function to catalyze the release process to GTP binding and hydrolysis. So, in the terminations release factor RF1 or release factor RF2 binds to the ribosome nearly at the A-side then the polypeptide chains are released from the ribosome by the peptidyl transferase complex. So, peptidyl transferase complex transfer the carboxyl terminal residue of the peptide chain from the tRNA of P-side to the water molecule.

Now the release factor RF and GTP released and its tRNA is also freed. Now the

ribosome assembly is unstable due to the presence of the initiation factor IF3 and IF1 and ribosome recycling factors as a result the ribosome 70 ribosome disrupt into the 30S and 50S ribosome and prepared for the initiation. So, this is exactly happened once you have the complete synthesis and you are actually going to reach to a stop codon then on the stop codon the release factors are actually going to participate and they are actually going to release the newly synthesized proteins from the newly synthesized protein from the ribosome. And once the protein is been released then the ribosomal large subunit and the small subunit is also going to assemble going to be disassembled and they are actually going to participate into the initiation with the another codons and another set of the translational machinery. So, this is what we have discussed so far we have discussed about the translational machinery we discuss about the structure of the tRNA we have discussed about the structures of the messenger RNA we discuss about the structure of the ribosomal RNA or ribosomes and then we also discuss about the different events. So, we discuss about the genetic codes we have discussed about the initiation, the elongation and terminations and we have also discuss about the different types of processes what is happening into the prokaryotic system.

In our subsequent lecture we are going to discuss about the eukaryotic translations and how it is different from the prokaryotic translations. So, with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss about the eukaryotic translation and how it is different from the prokaryotic system. Thank you. .