Molecular Biology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati
Module - 06
Transcription
Lecture-25 Transcription in Prokaryotic System

Hello everyone, this is Dr. Vishal Trivedi from the department of biosciences and bioengineering IIT Guwahati and what we were discussing we were discussing about the different aspects related to the central dogma of molecular biology. So in the previous module we have discussed about the replications and where we have discussed about the replication in the prokaryotes and replication in the eukaryotes. Apart from that we have also discussed about how the replication is playing a pivotal role in making the DNA damage and repairs and if you recall in the previous lecture we have also discussed about the what are the different other processes occurring in the central dogma of molecular biology. So what we have discussed that the central dogma of molecular biology is a very concentrated and very regulated process in which the DNA is going to be multiplied so that the DNA can be divided between the mother cell and the daughter cell and on the other hand the DNA is going to give rise to the RNA and that RNA is going to be responsible for the production of the proteins. So the production of or the synthesis of the RNA from the DNA is been catalyzed by the RNA polymerase and this process is known as transcription.

So in today's lecture we are going to discuss about the transcription and how the transcription is actually going to be done in the prokaryotic as well as the eukaryotic system. So in today's lecture we will discuss about the prokaryotic genes eukaryotic genes and how the prokaryotic transcription in the prokaryotes is been done versus the prokaryotic transcription in the eukaryotes. So when we say the transcription means that there will be a synthesis of the RNA from the DNA and this process is called as the transcription and this process is been catalyzed by a enzyme which is called as the RNA polymerase. And RNA polymerase is a very multimeric protein complex and it has the sub multiple subunits both in the prokaryote and as well as the eukaryotic cells and the RNA polymerase is a very very important enzyme for giving the DNA from the RNA.

Now before we getting into the details of the transcription it is important for us to understand the about the gene and how the gene is been different from the prokaryotes versus the eukaryotes. So what is transcription? So as we already discussed the transcription is the synthesis of the RNA from the DNA. So every cell contains three different types of RNA that is the transfer RNA or the tRNA, ribosomal RNA or the rRNA and the messenger RNA or the mRNA. Synthesis of RNA from the DNA template

with the help of the DNA dependent RNA polymerase is known as transcription. It occurs unidirectionally in which the chain is synthesized in the direction of 5 prime to 3 prime.

The segment which is transcribed from the DNA is known as the transcription unit. So the DNA the synthesis of the DNA to RNA is called as transcription and we have the three different types of RNAs. We have the ribosomal RNA, we have the ribosomal RNA, we have the tRNA and we have the messenger RNA. And if you recall when we were talking of when we were discussing about the biomolecules we have discussed about the structure of the messenger RNA and how you can be able to purify the messenger RNA from the cell with the help of the two different methods we have discussed about the trizole method and we have also discussed about the affinity purification as well. So the role of these RNA molecules are different right messenger RNA is actually being carried the message right.

So and it is required for providing the message in what sequence the amino acids are actually going to be attached. The tRNA is actually going to be carry the amino acid. You know that the proteins are made up of the amino acid that also we have discussed in the previous module. So it will actually going to carry the amino acid on one side and on the other side it also going to carry the information so that it will actually going to recognize carry the information to recognize the messenger RNA right. So it is actually going to carry it is going to recognize it is going to carry the amino acid and as well as it is actually going to recognize the messenger RNA and then the ribosomal RNA it is actually going to synthesize the protein.

So it is actually going to form the ribosomes and that is actually going to synthesize the protein by forming a peptide bond between the amino acid which is going to be carried by the transfer RNA. So basically all these RNA molecules are going to have one or other functions and they are actually going to be formed from the DNA which is by a process which is called as the transcription. So we have separate genes for synthesizing the ribosomal RNA, tRNA and messenger RNAs and the segment which is transcribed from the DNA is known as the transcriptional unit. So in a eukaryotes it is actually the monosystronic transcriptional unit which occurs to code for single polypeptide whereas in the prokaryote it is actually going to be polycystronic. It means it is actually going to transcribe and it is actually going to give you the more than one polypeptide.

So in a transcriptional unit what you have you have a promoter right this is the promoter then you are going to have the coding sequence and then you are also going to have the terminators and both are all these three segments have their specific role that promoter is actually going to provide a docking site for the RNA polymerase to initiate and in that

started whereas the RNA coding sequence is actually the coding sequence which actually is going to give you the RNA. It could be messenger RNA, it could be transfer RNA or it could be ribosomal RNA and once the synthesis is over then they are actually going to be a sequence which are actually going to bring the ending of these sequences. So promoter is actually going to be us going to help in the starting of the transcription replication transcription the coding sequence is actually going to allow the elongation and that is how the elongation will continue and the termination sequences are actually going to have the termination. So it is going to help in the termination and we are going to see the features of all of these components of the transcriptional units. So transcriptional unit as I said you know it is going to be having the promoters coding sequence and the terminators.

The promoter is going to be a starting point, it is going to be for elongation and this is for the termination. So what is the start point? It is the first base pair from where the transcription start and it is called as the start site. RNA polymerase from the moves from the start point along with the template synthesize the RNA up to the termination sequence and you have the upstream and as well as the downstream sequences. So upstream it is a non template nucleotide in the 5 prime end or the minus direction which is sequence before the start point. So this is actually the start point and before the start point you are going to have the promoter so that is going to be upstream sequences.

Then you also going to have a downstream sequences so it is a nucleotide in the 3 prime end or the plus direction so this is going to be the downstream directions and it is actually going to be a sequence after the start point. DNA is a double helix structure so during transcription only the one strand is transcribed so that the transcriptional sequence is identical with the one strand of the DNA known as the coding or the census strand and the other complementary strand is known as the template or the antisense strand. You know that transcription is going to occur in the direction of 5 prime to 3 prime this means it is actually going to read the information from the 3 prime to 5 prime strand. So it is actually going to read the sequence from the 3 prime to 5 prime strand and that is why so if you see that if the RNA polymerase is going to sit here and it is actually going to run in this direction on this strand then it is actually going to synthesize RNA which actually going to have the 5 prime on this side and 3 prime on this side and that is why this sequence is actually going to be a non-coding strand. This is going to be called as non-coding strand because this is actually going to be providing the template whereas this sequence is actually going to be a coding strand.

Before we discuss about the prokaryotic transcription and the eukaryotic transcription we will actually going to see the difference between the eukaryotic and the prokaryotic transcription. So what is the difference between the prokaryotic and the eukaryotic

transcription? Remember that the prokaryotic transcription is or prokaryotic genes are polycistronic whereas eukaryotic genes are monocistronic. So that is very very and that actually brings the difference in terms of their transcription. So the first difference is that the prokaryotic transcription or the prokaryotic genes are polycistronic which means they are actually going to code for many polypeptides whereas the eukaryotic gene is going to be monocistronic. So it is actually going to code for single polypeptide.

It means you are going to have the multiple genes present within the prokaryotic transcription unit whereas you are going to have the single gene. Since there is no nucleus right the prokaryotic transcription occurs within the cytoplasm right because the DNA is also present in the cytoplasm whereas the eukaryotic transcription occurs inside the nucleus and within the nucleus you are going to have the synthesis of the messenger RNA, tRNA and ribosomal RNA. Number 3 because the transcription is occurring in the two different compartments the transcription is not coupled with the translation in the case of the eukaryotic transcription because so it is not going to have the coupled transcription and the translation because the transcriptional unit and the translational units are present in the separate quarter right. So they are actually present in the separate compartment whereas in this case it is actually going to have the coupled transcription and the translation which means it is actually going to have the transcription as soon as the RNA comes out right it is actually going to be recognized by the translational unit and then the translation and the transcription will continue at the same time. Number 4 single type of RNA polymerase required for the synthesis of all type of RNAs.

So it is going to have the single RNA molecule RNA polymerase molecule which is actually going to be utilized for production for the synthesis of all type of RNA whether it is messenger RNA, tRNA and ribosomal RNA. Whereas in the case of eukaryotic system you are going to have the three different types of RNA polymerase which is required for the synthesis of the all different types of RNAs. Then number 5 you are going to have there is no need for any transcriptional factor for the initiation. So because why it is so because the RNA polymerase is competent enough to start the initiate the transcription whereas in the case of the eukaryotic transcription the eukaryotic transcription requires the transcriptional factor for the initiation. So actually the transcriptional factor are actually going to recognize the promoters and then only the RNA polymerase will come and bind.

So that anyway you will understand when we are going to discuss about the transcription in the eukaryotic as well as the prokaryotic units. The number 6 you are going to have the RNA polymerase are made up of T5 subunits whereas the RNA polymerase are made up of T10 to 15 subunits. So RNA polymerase is big and complex in the case of eukaryotic system whereas the RNA polymerase is small and simple in the

case of eukaryotic prokaryotic system. Now let us see what is the machinery of the prokaryotic system. So we will first discuss about the transcription in the prokaryotes and then we also going to discuss about the transcription in eukaryotes.

Many of these steps or the many of the basic steps are going to be remain same between the prokaryotic transcription and the eukaryotic transcription. So that we are not going to repeat when we are going to discuss about the transcription in the prokaryotes. So transcription in prokaryotes. RNA polymerase in prokaryotes a single type of RNA polymerase is present which is responsible for the synthesis of all types of RNA. U bacterial RNA polymerase is a holoenzyme and it is a multi subunit protein which contains the five different subunits alpha, alpha, beta, beta and sigma actually and alpha is the assembly of the core enzyme.

So you are going to have the alpha alpha as the assembly of the core enzyme. So alpha is actually going to be to form the core enzyme whereas the beta and beta prime are going to perform all the enzymatic and catalytic functions. So this is the beta and the beta prime which is actually going to perform the enzymatic and the catalytic activities and sigma is actually going to recognize the promoter sequences. So you are going to have the core enzyme which is going to be formed by the two alpha and beta and beta prime whereas the holoenzyme is going to contain the core enzyme plus the sigma factors. And these sigma factors are going to be different for the different types of genes.

So that is how you are actually having a very simple system where you are going to have the RNA polymerase made up of the five different types of subunits and all these five different subunits can be divided into two part. One is the core enzyme and the other is the sigma factor and within the core enzyme you are going to have the two alpha subunits and the beta and beta prime and this is being done only to conserve the energy because if you are synthesizing the RNA polymerase according to the different types of promoters or according to the different types of RNA species what you require then you are supposed to synthesize a large number of RNA polymerases and RNA polymerase is a big enzyme and a big protein. So to conserve the energy what bacteria has decided or the prokaryotic system has decided that I will actually going to have the core enzyme which will actually going to have all the activities so that it actually going to have the it will be able to read the DNA sequences it will be able to have the you know the synthesis activity and so on. But to recognize the genes to recognize the promoters we are actually going to have the sigma factors and that is how you can be able to have the single core enzyme associated with the multiple type of sigma factors and that is how you can actually be able to utilize the same for the multiple genes and that is how you can be able to conserve the energy. Now the second part is the promoter so prokaryotic promoters are simple compared to the eukaryotic promoters.

So they are simple so prokaryotic promoters typically consist of a 40 base pair region located near to the 5 prime end of the transcriptional start site promoter region consist of the two 6 pair consensus sequences called primbo box or the tata box and the minus 35 region. Primbo box is a 10 base pair upstream of start point and it is having a consensus sequence of TATAT whereas minus 35 region has the consensus sequence of TTGSEA. So this is actually going to be the promoter the typical promoter what is present in the prokaryotic system where you are going to have this is actually going to be called as the start site. So this is actually going to be if this is going to be the start site to the 5 prime end which means to the upstream of this you are going to have the minus 10 region and then you are going to have the minus 35 region and within the minus 35 region you are going to have the sequences which is going to be TTGSEA whereas in the minus 10 region you are going to have a sequence which is called as TATAT which means you are basically going to have a combination of these two and length or the distances between the two these two region is actually and the nucleotide what are present in these regions are actually going to decide whether the promoter is going to be strong promoter or it is actually going to be a weak promoter and depending upon that you are actually going to have the different types of you know the transcriptional activity of a particular gene is going to be different. This means the synthesis of the protein molecules are completely going to be governed by the strength of the promoter and that is how you can actually be able to modulate the expression as well as the production of a particular protein simply by modulating this because in a prokaryotic system remember that the transcription and the translation is going to be occurred simultaneously.

So as soon as the RNA species is going to be formed and it is going to be present in the cytosol or it is actually still be you know doing the transcription it will be available for the translational machine and it is going to start synthesis. So that is why you can actually have the better control over the protein production during the transcription itself and that anyway we are going to discuss and we are going to discuss about the control mechanism within the transcription and where we are going to discuss about the different types of operons. So here what we have is these are the three important or four important events what is going to occur in the transcription. So transcription in the prokaryote occurs in four stages one is number one is the template binding number two is the chain initiation number three is the chain elongation and a number four is termination. So the number one step is when the template is going to recognize this by the RNA polymerase and that is how it is actually going to initiate the synthesis of the RNA.

So the number one event is the binding of RNA polymerase to the template DNA and the chain initiation. So the DNA duplex should be opened so that the RNA pole can approach to the single standard DNA templates. Efficiency of the initiation is inversely

proportional to the melting temperature that is the TM and AT rich region has the lower TM because of the double bond hydrogen bonding and then the triple bond in GC region and thus it is more stable. Therefore the AT rich is good for melting of duplex and easy to create the open promoter complex than the GC rich region. So this region where the RNA polymerase is going to go and sit and then actually going to break the DNA or the unwind the DNA should be AT rich so that it should be easy for RNA polymerase to find the single standard DNA and that is how it is going to initiate.

So RNA polymerase has sigma factor so and you know that there are different types of sigma factors for the different types of genes. So you are going to RNA polymerase has sigma factor which recognize the promoter sequence at which the RNA polymerase holoenzyme binds and forms a complex which is known as the closed complex. In fact the sigma factor is released when the chain reaches nearly to the 10 base pair leaving the core enzyme for the elongation. So what happen is that suppose this is the promoter region right. If this is the promoter region, it is promoter region is actually going to be recognized by the sigma factor and as well as the sigma factor will go and bind then the RNA polymerase will come and it is actually going to bind the sigma factor and then this complex is going to unwind the DNA it is going to form the single standard DNA and that is how it is actually going to start the synthesis of the RNA.

So it is actually going to start running so RNA polymerase is actually going to start running in this direction and once the RNA polymerase start and it goes for another 10 nucleotides right. So if it goes for 10 nucleotides then the sigma factor will actually going to be dissociates right. So sigma factor is actually going to dissociate from the temp from the RNA polymerase and then it will actually be available for the next gene and that is how you see in this is event also the bacteria is trying to conserve the energy right. The same sigma factor suppose you have 10 different types of genes and you want to do the transcription right. So sigma factor will go and sit in the gene number 1 right and it will facilitate the process of RNA polymerase to come and start synthesis right.

So it is actually going to start the transcription right. So this is called gene number 1. Now as soon as this is done the sigma factor will come out from here and it will go to the gene number 2 right. Then from here as soon as it is done it would can go to the gene number 3 and so on right and that is how you see that you do not have to synthesize neither the RNA polymerase nor the sigma factor and you can be able to efficiently be able to synthesize the RNA from the DNA modules and that is how you can be able to have the efficient system and the you are at the other hand you are also going to have the conservation of the energy as well. So the first step is the binding of RNA polymerase to the template DNA and the initiation.

The second part is that the binding of RNA polymerase to the template DNA and chain initiation. So the DNA replays should be so this is what anyway we have discussed already. Then you are at this stage it is actually going to form the two different types of complexes. One is called as the open complexes the other is called as the closed complexes. So let us see what happened when it is going to form the open complex.

So open complex which is actually going to form when the sigma factor is going to bind the closed complex is converted into open complex by melting a short region of DNA that is the minus 10 base pair and the RNA polymerase bind at the promoter region and unwind and it covers minus 55 to plus 1. Remember plus 1 is the first nucleotide for the transcription. So total 50 base pairs 55 base pairs and start the initiation here of the one template strand available for the incomplete nucleotide for the base pairing and synthesis of RNA occurs. Minus 10 region of the template is essential for the recognition the promoter regions are double standard in closed complex and single standard in the open complexes. RNA polymerase has two binding sites for the nucleotide one is the initiation site and the other is the elongation site.

Elongation site binds to the first nucleotide within the open promoter complex at the plus 1 site which is usually a purine or the purine A or G. It means the first nucleotide would be either the ATP or the GTP. Elongation site binds with the second incoming nucleotide base pairing at the plus 2 positions. The two nucleotides are joined together and the first base is released from the initiation site and the initiation is complete. So this is what it is actually going to happen.

So first it is actually the sigma factor will go and recognize the promoter and then it is actually going to facilitate the binding of the RNA polymerase and then RNA polymerase when it binds to the promoter region is actually going to unbinds and it is actually going to uncover the 55 base pairs. So it is going to have the plus 1 site. So from minus 55 to plus 1 site it is actually going to unwind the DNA and then it is actually going to start the initiation which means at the first nucleotide it is actually going to add the nucleotide and it prefers that that particular nucleotide should be either A or the G. So that is why the first nucleotide would be either the ATP or the GTP. Then it is going to have the second nucleotide and there will be a bond which is actually going to be formed between the first nucleotide and the second nucleotide.

So it is going to have the bond which is going to be formed and that is how it is actually going to start the synthesis. So this is going to be the initiation step. After the initiation it is actually going to enter into the elongation step. So the chain elongation, chain elongation occurs in the 5 prime to 3 prime direction and RNA synthesis is carried out by the transcription bubble which forms due to the transient separation of double-

stranded DNA into the single-stranded RNA and the transcription takes place at a complex time. So once it is actually going to leave the promoter region it is actually going to enter into the elongation site and that is how the RNA polymerase will be keep moving and it is going to keep synthesizing the RNA.

Now after this it is actually going to reach to a region which is going to be a termination site and that is how it will enter into the termination region. So RNA chain synthesis occur basically at 5 prime to 3 prime ends direction by adding a nucleotide at the 3 prime end and the 3 prime end group of the last nucleotide is combined to the incoming of 5 prime gamma phosphate nucleotide. Alpha and beta phosphate groups are removed and only the gamma phosphate is used in the formation of phosphodiester bond. Likewise other nucleotide ada which are complementary to the template DNA and thus the RNA chain termination strand translocation occurs. In bacterial transcription rate is nearly 40 to 50 nucleotides per second at 37 degree Celsius which is nearly same as the translation in prokaryote which is 50 amino acids per second.

RNA polymerase points to the promoter and create a transcriptional bubble RNA polymerase moves along with the DNA RNA chain grows continuously the length of the transcriptional bubble is approximately 12 to 14 nucleotides. So this is this open area is going to be of 12 to 14 nucleotides and the length of the RNA DNA hybrid is about 8 to 9 base pairs. So within this there will be a region where the RNA and DNA will still have that double standard DNA double strands and it is going to have the RNA DNA hybrid. As the RNA polymerase moves the duplets reforms against the RNA hangs at the free nucleotide chain, free poly nucleotide chain the transcription bubble moves continuously by disrupting the DNA structure. Bioscope acids are added covalently to the 3 prime on the chain of the RNA beta and drama phosphates are removed from the incoming nucleotide and hadrosil is removed from the 3 prime carbon nucleotide present at the end of the chain.

So that is how it is actually going to occurs into the elongation phase. Now once it reaches to the termination side it is going to end up the into the terminations phase and that is how the termination or the transcription is going to occur. So chain terminations so when the RNA polymerase stop adding a nucleotide at the RNA chain it releases a complete product and the RNA chain get free from the termination sequence. During termination all the hydrogen bond breakdown which holds the DNA RNA hybrid together and when the RNA chain is separated from the DNA again from the duplet. So the nucleotide at which the enzyme stop adding a nucleotide is known as the chain termination side.

So at the chain termination side it is actually going to have the stop the progression of

the RNA polymerase that is the first event. Second there will be a disruption or the breaking of the hydrogen bond between the RNA DNA hybrid and after that the RNA is actually going to fall into the cytoplasm and along with the RNA RNA polymerase is also going to fall and that is how it is actually going to terminate the transcription. And once the termination is occurs the DNA which is duplex is going to be reformed. The there are two different types of mechanism which are being proposed for the termination one is called as intrinsic termination and the other is called as the row factor dependent terminations.

So let us discuss about the termination. So intrinsic termination intrinsic termination is being done by the sequence present within the termination side. So these sequences are unique sequences so they will actually going to have the one purpose that to stop the growth of RNA polymerase. If you stop the growth of the RNA polymerase RNA polymerase is a very big enzyme so the DNA RNA duplex what is being formed or the hybrid what is being formed is actually holding the RNA polymerase onto the template. So if there is a growth if there is a you stop the growth of the RNA polymerase then RNA polymerase cannot over be remain onto the template and that is how it is actually going to terminate the transcription. So how that this is occurs actually is that these intrinsic termination sites are actually going to have the sequences in such a way that it is actually going to form a loop like structure.

You see here you have the AATAGGGCAA like that and on this side also you are going to have the GGA GCCC. So if I show you this it is actually going to form a loop like structure like this it is going to form a loop like structure like this and this one also is actually going to form a loop like structure and because of that and you see that these are the high in GC content and high are AT content which is going to be followed on the other side. So because of that it is actually going to form the stem and the hairpin loop kind of structures and you know that once the hairpin like structure is going to be formed the RNA cannot actually have the possibility because these loops are actually going to have the strong GC content and because of that the RNA polymerase cannot break. So in this mechanism of termination the row factor is not required and the termination depends on the RNA product. It requires the GC rich hairpin, hairpin structure is followed by 7 U residues.

So RNA DNA hybrid requires the forces for holding the elongation complex together. Thus when the hybrid gets detached it collapse and the elongation complex which causes the termination. In this type of termination the depreciation of the polymerase occurs by destabilizing the attachment of the growing chain to the template. During this process the hairpin structure is formed by the transcription while complementary base pairing.

It includes the palindromic sequences. This stem loop structure include the GC rich region which is followed by the U rich region. So because of that it does not get the enough strength to hold the RNA and on the other hand the RNA polymerase will be going to stop by a strong GC rich region and because of these two events it is actually going to stop the transcriptional activity of the RNA polymerase. So the steps in the transcriptional termination is that the different steps are as follows. Here the two inverted repeat that the GC, GC, GC, GC are present in the DNA template which is transcribed. So nearly the six adenine residues follows the second inverted repeat that is the GC, CC, GC and number three is now inverted repeats are forming a hairpin structure which cause the RNA polymerase.

So this is what I was talking about. You are going to have the hairpin structure and it is been formed because you have a very high GC rich region. This is the high GC followed by the U region and U is actually going to have the low affinity. It is going to have the low affinity because the low U is actually going to have the higher affinity for the template and because of that this cannot withstand the RNA polymerase, cannot hold the RNA polymerase and on the other hand this will not allow the RNA polymerase to cross. So RNA polymerase if it is sitting here it cannot go on this side or it cannot actually break this particular bond. So due to the formation of the stem loop structure and the AU bond get breakdown leads the termination and the RNA molecule get separated.

This is what exactly it is actually going to happen. Now the second method is the row dependent termination. So row dependent termination this type of termination requires a row protein and the row is ATP dependent helicase that disrupt the RNA DNA hybrid. So row is actually having a protein which actually has a very high affinity for the RNA molecules. So it is an essential protein which causes the transcriptional termination. Row protein is a hexamer ATP dependent helicase and it actually sub its subunit contains the RNA binding and ATP hydrolysis domain.

These row proteins firstly bind to the sequence which is present at the upstream of termination site. Each sites are called root RUT sites. These sites are rich in the C residues. C residues row factor followed to the RNA polymerase until it do not catch the RNA polymerase. Row follows the RNA polymerase by its helicase activity which is driven by the ATP hydrolysis.

When the RNA polymerase reaches at the termination site the row protein feeds the structure of the polymerase and when the row factors collapse with the enzyme which causes the termination and the new chain get released. So this is what exactly happens. So row factor is actually binding the RNA polymerase and it is running along with RNA polymerase but when the RNA polymerase reaches at the termination site it speeds gets

slower down and that is how the RNA polymerase actually row actually proteins actually catches the RNA polymerase and that is how they are actually going to dislodge the RNA DNA hybrid and at the end the RNA is going to be RNA DNA hybrid RNA as well as row and RNA polymerase will fall into the heteroplasm and that is how it is actually going to terminate the polymerase and the transcription into the prokaryotes. So this is all about the transcription in prokaryotes. In our subsequent lecture we are going to discuss more about the transcription in eukaryotes.

So what we have discussed we have discussed about the transcriptional unit, we have discussed about what is the coding strand and what is the non-coding strands and we have also discussed about the transcriptional machinery in the prokaryotes where we have the sigma factor and the RNA polymerase and we have done with the previous few slides we have also discussed about the transcription in prokaryotes how the different events are occurring and how the termination is occurring. So with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss about the transcription in eukaryotes. Thank you.