Molecular Biology
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Module - 06
Transcription
Lecture-26 Transcription in Eukaryotic System

Hello everyone, this is Doctor Vishal Trivedi from department of Biosciences and Bioengineering IIT Guwahati. And in the course molecular biology we are discussing about the different aspects related to molecular biology and where we have discussed about the cellular structures. We have discussed about the prokaryotic structure and as well as the eukaryotic structures. Following that we have also discussed about the different types of biomolecules. So, we have discussed about the nucleic acids, we have discussed about the proteins and we have also discussed about the enzymes.

While we were discussing about the nucleic acid, we have discussed about the structure of DNA, we have discussed about how you can be able to isolate the DNA from the eukaryotic and as well as the prokaryotic cell and how you can be able to sequence the DNA and other kinds of related informations. While we were discussing about the RNA, we have discussed about the isolation of RNA. So, we have discussed about the different methods of RNA isolations. Following that we have also discussed about the central dogma of molecular biology where we have discussed about how the multiple processes are related to each other and that is how they are actually been responsible for governing the different events what is happening within the cell.

Now, following that we have also discussed the replications and in the current module we are discussing about the transcriptions. So, let us start discussing about this particular important aspects related to central dogma of molecular biology. Now, as far as central dogma of molecular biology is concerned, it is actually a regulated event where you have the multiple processes linked to each other and they are been responsible for production of the proteins and you know that the production of protein or the enzyme is been responsible for the phenotypic features what are going to be exhibited by the cell. In the previous lecture, we have discussed about the direct transcription in the prokaryotes. So, we have discussed about the transcriptional units, we have discussed about what is the structure of the transcriptional unit in the prokaryotic system and so on.

So, as far as the transcriptional unit is concerned, the transcriptional unit is in the prokaryotes is the polycistronic whereas, in the case of the eukaryotes it is actually the monocystronics. So, and every transcriptional unit is actually having the composition of that it is going to have the promoter, it is going to have the coding sequence and it is

going to have the terminations and all these transcriptional units are been present onto the DNA which is responsible for production of or the synthesis of the ribosomal RNA, tRNA and as well as the messenger RNA. You know that the ribosomal RNA is responsible for the formation of the ribosome and that is how they are actually going to be directly be involved into the protein synthesis. Whereas, the transfer RNA is actually going to be transfer the amino acid, you know that the protein is made up of the different types of amino acids, then messenger RNA is actually been responsible for the decoding the genetic information. Present on DNA right.

So, they these three RNA molecules are going to be produced from the DNA by a process which is called as transcription right and in the previous lecture we discuss about the transcription in prokaryotes. So, we have discussed about the different types of events, we have discussed about the initiation, elongation, and terminations. Now, as soon as we will talk about the transcription in eukaryotes, the transcription in eukaryote is going to be more complex because the transcription because the eukaryotic system there is a significant difference between the transcription in prokaryotes versus transcription in eukaryotes. One of the major differences is that in the in the case of prokaryotes, the RNA polymerase what it is actually going to utilized for what the synthesis of the RNA from the DNA is single or the same type. Whereas, in the case of the eukaryotes, it is going to be different for the different types of cells right.

The second point is that the transcription in the bacteria or in the prokaryotic system is going to occur in the cytoplasm. Whereas, in the case of the eukaryotes, it is actually going to occur inside the inside the nucleus. So, that is why the transcription is going to occur inside the nucleus, then it is actually going the transcripts what is going to be formed, it is going to be transported out of the nucleus and that is how it is actually going to be utilized for the translation. So, as soon as the transcription in eukaryote is concerned right. The transcription as I said you know in the case of prokaryotes, it is only utilizes the single type of RNA polymerase, which is been attached to the sigma factor and that is how it is actually going to make an hollow enzyme.

And that hollow enzyme is actually going to be utilized for the transcription of the different types of genes. Whereas, in the case of eukaryotes, you are going to have the different types of RNA polymerase. So, RNA polymerase is the enzyme which is responsible for the transcription. The RNA polymerase of the mitochondria and the chloroplasts are similar like bacteria, because you know that the mitochondria and chloroplasts are the prokaryotic in origin right. All eukaryotic RNA polymerase are multi subunit proteins, which contains three different type there are which there are three different types of RNA polymerase, which is responsible for the transcription in the eukaryotes.

You have the RNA polymerase 1, you have the RNA polymerase 2, and you have RNA polymerase 3. Now, RNA polymerase 1 is been utilized for the synthesis of the ribosomal RNA and it is sensitive it is resistance to the aminidine treatment right. So, it is not going to get affected and it mostly been found inside the nuclei ok. So, within the nucleus you have the region which is called as nuclei right. Then as far as the RNA polymerase 2 is concerned, RNA polymerase 2 is been utilized for the synthesis of the messenger RNA messenger RNA of the different genes right.

It is sensitive or the it is very sensitive for the aminidine treatment or the and it is been found into the nucleoplasm. So, it is not it is going to be present within the nucleus. Then we have the RNA polymerase 3, RNA polymerase 3 is required for the synthesis of the tRNA and it is intermediate between the between the RNA polymer 1 and RNA polymer 2 in terms of the sensitivity to the aminidine. So, it is less sensitive, but it is not that sensitive to the RNA pol 2, but it is less sensitive than. So, it is less sensitive than the RNA pol 2 and RNA pol 1 is anyway not sensitive at all and the RNA pol 3 is also been found within the nucleoplasm.

So, these are the 3 different types of RNA polymerases RNA pol 1, RNA pol 2 and RNA polymerase 3. Now, let us talk about the eukaryotic promoters right. So, each promoter contains some specific situations which get recognized by the transcription factor. Eukaryotic promoter has a longer region than the prokaryotic promoter because it contains all those sequences which are important regarding to initiation. It includes the core promoter elements at which the RNA polymerase get attached and form the initiation complex and also for the efficient transcription it requires an upstream promoter elements which are basically been G plus region and at which the transcription factors are bind.

So, just like the prokaryotic system in the eukaryotic system also you are going to have the initiation you are going to have for after that you are going to have the elongation and after that you are actually going to have the termination and in the initiation the promoter actually a region which is outside the coding region, but within the transcriptional unit is actually going to provide the docking site for the transcription factors. So, these are the some of the important event or important difference between the prokaryotic and the eukaryotic system. Remember that in the in the prokaryotic system the promoter was allowing the binding of the sigma factor whereas, in this case you are actually going to have the binding of the different types of transcription factor and although we are not covering the signal transaction in this particular course, but if you go through with any of the signaling event right for example, if the insulin is binding to the insulin receptor it is actually governing or it is actually activating the the important transcription factors then

these transcription when they go and bind to their respective promoters that is how they are actually going to recruit the RNA pol 2 for the synthesis of that particular gene and the gene product. And that is how the they are actually going to have the required enzyme and the required protein into the cytosol and that is how these are actually going to be responsible for the reduction in the glucose level. Same is true for other kinds of transcription other kinds of events for example, if we are getting the infection for example, there will be a COVID infection right then the cells are actually going to have the different types of signaling cascades and that is how it is actually going to activate the different set of transcription factors and these transcription factor will go and bind to the different set of genes the promoters of those genes and that is how they are actually going to have the different types of protein products.

And these protein products are going to be secreted out of the cell and these are mostly been called as cytokines and these cytokines are actually going to fight with the different types of infectious organisms same is true for the antibodies also right when there will be a clonal propagation then there will be a activation of the transcription factors and so on. So, transcription factor actually do play a very crucial role because they decide which promoter they are actually going to bind once the transcription factor goes and binds the promoter then the promoter is committed for the transcription because you have started you have in you have committed this particular promoter for the transcription. So, it is actually you have started the initiation and once the initiation done then the RNA polymerase will have no option but to sit on this and start the transcription right. So, this is been facilitated mostly by the transcription factor that you are actually going to have the transcription factor for the stress responses you have the transcription factors for the lowering the glucose level and so on. So, the set the battery of transcription factor going to decide which promoter or the promoters are going to be labeled for the transcriptional activity and once the promoter is the RNA polymerase is going to sit right mostly the RNA pol 2 is going to sit it is actually going to enter into the elongation phase then you are going to have the multiple cascade of reactions and that is how it is actually going to attach the nucleotides and that is how the elongation will go and then it is actually going to enter into the termination.

So, termination events are more or less the same as what we have discussed in the prokaryotic system as far as the mechanistic issues are concerned it is going to have the intrinsic termination or the row dependent terminations. So, these are the theme some of the events what we are going to discuss now. So, once the initiation is done then you are actually going to have so during the initiation during the initiation we are going to have a cascade of events. So, that the transcription factor will go and bind and then ultimate aim of the initiation when the initiation is going to happen that the RNA polymerase 2 will come and bind to the promoter and I am always saying RNA pol 2 because RNA pol 2 is

actually been responsible for the synthesis of the messenger RNA and we are mostly having the genome with the different types of genes where the gene product is going to be synthesized. So, mostly it is actually the RNA pol 2 which is going to be transcriptionally very active RNA pol 1 and 3 are only going to be utilized for the synthesis of tRNA or the ribosomal RNA.

So, their activity is going to be not that much compared to the RNA pol 2. So, the initiation so it in transcriptional initiation by the RNA pol 2 eukaryotic messenger RNA transcription required the initiation complex which consists of the general transcription factors and the mediators. So, you are these are the some of the transcription factors which are actually going to be present on to or which are actually going to have the active role into the initiation. So, what we have is the first is this right transcription factor 2D or it is also been called or it is it is been consist of the tata binding proteins as well as the TBP associated factors or tata binding protein associated factors, but the function of these TF 2 do TF 2D is that it is actually going to recognize to the core promoter or the tata box and it also going to recognize the core promoter which is non-tata box. So, it is going to recognize the tata box and it is going to be differentize the other transcriptional other other sequences within the promoter.

Then we have the TF 2 transcription factor 2A. So, it is actually going to stabilize the transcription the TBP and that transcription associated the factors binding then we have the TF 2B. So, it is going to help in RNA pol 2 and TF 2F recruitment and also helps in the start site selections. So, these are this is very important actually. So, this is the second factor then you are going to have the third factor then the fourth factor then you have the TF 2E.

So, it helps to RNA pol in the promoter binding then we have the TF 2E. So, TF 2E helps in the TF 2H recruitment modulation of the TF 2H and helicase activity ATPase activity and the kinase activity and then the last is the TF 2H. So, TF 2H is going to help in the promoter melting with the helicase activity and the promoter clearance by the phosphorylation activity. So, you will understand once we are going to discuss how the these transcription factors general transcription factors are going to be play a role in terms of initiating the initiation. So, these initiation is a very you know is a sequential steps and sequential in manner these transcription factor will come and bind to the promoter site and that is how they are actually going to recruit the RNA pol 2 and once the RNA pol 2 is been recruited then the transcription is going to be started.

So, transcription initiation these transcription factors are sequentially going to bind to the TATA box DNA to form a pre initiation complex. At last when the TF 2H get bind its phosphorylate the RNA polymerase to initiate the transcription in the presence of

ATP. So, you have this is a promoter right. So, you have the TATA box and you have the other region in the TATA box right. So, the first transcription factor what is going to bind is the D.

So, TF 2D right it will go and bind then it is actually going to the help or it is actually going to provide the docking site for the TF 2A to bind. So, the first is this is going to bind the second the TF 2A is going to bind and then that once these two are actually going to bind then it is actually going to allow the binding of the TF 2B and then followed by TF 2F E and H as soon as the TF 2E is actually going to provide the docking of the binding of the TF 2H and you know that the TF 2H is actually going to have the helicase activity and also going to have the kinase activity. So, it is actually going to bind or it is going to allow the binding of the RNA polymerase and it is actually going to phosphorylate the RNA pol 2 right you know that the DNA is negatively charged right. So, it is going to be negatively sorry sorry it is going to be negatively charged right. So, when the RNA polymerase is bind RNA polymerase is binding because RNA polymerase is recruiting or binding to this particular region because of the positively charged interaction.

So, there will be a positive charge on to the RNA pol 2 and that is how they are actually interacting with each other. Now, what will happen is once the TF 2H is going to be present what it is actually going to do is it is actually going to convert these positive charges by the phosphorylation. You know that if I have a protein and if I add the you know phosphorylation right. So, imagine that if A is a positive charge right if it is a positively charged protein right that is how it is binding to the DNA. So, that is why it is binding to a DNA.

So, this is not A this is RNA pol 2 and because this is positive and DNA is negatively charged right, but once I will actually going to have the TF 2H right TF 2H is actually going to have the kinase activity it is actually going to phosphorylate. So, what will happen is that it is actually going to generate this right and that is are that is actually it is going to impart the negative charge right. So, if it is imparting the negative charge then it is actually not going to destroy the affinity of the RNA pol 2 to the DNA, but it will actually going to allow the RNA polymerase to move up because it is initially it is binding and it is not moving because that interaction is very strong. Now, it has broken those interactions. So, that it actually can slide over this particular molecule right because the sliding of the RNA polymerase is very important and that is how this is the function of the TF 2H which is actually going to have the helicase activity.

So, it is going to break open the DNA and on the other hand it also going to have the phosphorylation activity. So, that it could be phosphorylate the RNA polymerase. So,

you can imagine that it is going to sit on the DNA and then it is going to slide along the DNA molecule and that is how it is actually going to start synthesizing the RNA molecule. So, it is going to start synthesizing the messenger RNA. So, this is once the initiation at the initiation the RNA all this happens then it will enter into the second phase that is the elongation.

So, elongation is very simple as like what we have discussed in the case of the prokaryotic system where the it is actually going to read the template and then depending upon the Watson-Crick base pairing requirements the A if it is A then it is going to put the T into the sorry U into the RNA into the RNA if it is G then it is going to provide the C remember that there is no T present in the into the RNA structure. So, if it is A then it is going to provide the U if it is G it is going to provide the C. So, this is actually going to be DNA in and this is going to be RNA that we have only discussed also if it is C then it is going to provide the G. So, these are these are going to be depending upon the Watson-Crick base pairing rule and so on. So, if that is the way it is actually going to keep adding and that is how the elongation will be keep happening.

So, from the 5 prime end it is going to start and then it is going to end on to the 3 prime end. Once the elongation is done then it the it will reach to a stage where it has to stop the synthesis and that is how it is going to enter into the third phase that is called as the trans terminations. So, transcriptional terminations so transcriptional terminations would be different in the case of the RNA pol 1 genes or RNA pol 2 genes and RNA pol 3 genes. So, RNA pol 2 transcription genes may continue to the hundreds or even thousands of nucleotides beyond the end of a coding sequence. Then the cleavage of the RNA strands occur by a complex which appear to be associated with the polymerase.

Cleavage of RNA is coupled with the termination process in occur at the same consensus sequence. The polyadenylation of the mature pol 2 messenger RNA occur at the 3 prime end which results in the poly A tail this process is followed by the cleavage and termination. Both process poly adenylation and termination occur at the both same consensus sequences and both of these processes are interdependent. So, termination is also been governed by the multiple factors and for different types of RNA polymerase you are going to have the different types of terminations. For example, for the RNA pol 1 you are going to have the row dependent terminations whereas, for the RNA pol 2 where you are going to have the most complex terminations that RNA pol 2 termination generally coupled with the RNA processing event in which the 3 prime end of the transcript undergoes the cleavage and poly adenylation.

Whereas, in the case of RNA pol 3 it is actually going to be the row independent terminations. Now, the transcription termination so, you can have the poly A dependent

termination. So, this type of terminations are basically coupled with the RNA maturation process in which the 3 prime end of the nascent RNA undergoes polyadenylation and cleavage and uses the 3 prime end processing reaction as carried out in 2 steps. So, transcription of a poly A followed by the cleavage or nascent and then the upstream product is polyadenylated and downstream product is degraded. Basically the 3 prime starts when the cis acting element in the poly A site of the nascent RNA transcript is recognized by the binding factors.

When these factors bind at 3 prime it form a strong complex result in a high shear force consequently processing down which cause the disruption of RNA pol 2 and DNA RNA. So, this is what exactly going to happen when there will be a transcription in terminations. So, this is going to be a poly A dependent terminations. So, this is all about the transcription in the prokaryotic and as well as the eukaryotic system. So, what we have discussed that from the DNA you are going to have the production of the ribosomal RNA you are going to have the production of t RNA and you are also going to have the synthesis of messenger RNA from in the case of prokaryotic system it is going to be the single RNA polymerase which is going to perform these transcriptional activity with the help of the different types of sigma factors whereas, in the case of the eukaryotic system it is going to be the different types of RNA polymerase which are going to be utilized.

So, you are going to have the RNA pol 1, pol 2 and pol 3 and all of these are actually going to go through with a very complex process of the transcriptional initiation where you are going to have the different types of the transcription factors and these transcription factors are going to be recruited on to the promoter site in a sequential manner and that is how they are actually going to form the pre initiation complex and once the pre initiation complex is going to be formed then it is going to allow the RNA pol 2 to enter into the elongation phase and once the elongation phase is over then it is actually going to enter into the termination phase and the termination is also different for the RNA pol 1 and pol 2 and pol 3 and they are actually going to have the different types of factors which are going to be utilized for the terminations and once the termination is over they are actually going to have the RNA transcripts and these RNA transcripts are further going to be utilized for the protein production or the other kinds of reactions like for example, in the case of RNA polymerase ribosomal RNA or tRNA they are going to be going to be processed for the attachment of the amino acids and other kinds of events whereas, in the case of messenger RNA it is actually going to be utilized for providing the information into the system so, that it is actually going to be utilized for the synthesis of proteins. So, with this brief discussion about the transcription in the prokaryotic and the eukaryotic system I would like to conclude my lecture here in our subsequent lecture we are going to discuss more aspects related to transcription. Thank you. Thank you.