Cell and Molecular Biology
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Central Dogma of Molecular Biology (Part 2)
Lecture - 39
Transcription (Part 2)

Hello, everyone. This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering at IIT Guwahati. So in the previous lecture, we discussed the differences between transcription in prokaryotes and eukaryotes. We have discussed the different contrasting features of transcription in prokaryotic and eukaryotic cells. How it is actually different is that the transcription varies in three major aspects between prokaryotic and eukaryotic cells.

First, the site of transcription in prokaryotes is within the cytosol, whereas in eukaryotes it is in the nucleus. And the second contrasting feature is that the transcription and the translation are both occurring simultaneously in the case of eukaryotes, whereas they happen differentially; the transcription occurs inside the nucleus, whereas the translation occurs inside the cytosol. So that also gives up regulatory events and allows you to control the transcription and translation processes separately in the case of eukaryotes. The third is that transcription does not require the transcription factor in the case of prokaryotes, while in the case of eukaryotes, it requires different types of transcription factors to recognize the promoter regions.

And that's how the transcription is going to be more tightly controlled in the case of eukaryotic cells. So in the previous lecture, we also discussed the different events and the structure of RNA polymerase in prokaryotic cells. And we have also discussed the different events, such as initiation, elongation, and termination. So, in today's lecture, we are going to start discussing transcription in eukaryotes. Subsequent to that, we are also going to discuss the post-transcriptional modifications.

Okay, so let's start discussing transcription in eukaryotes. So eukaryotic transcription is different from prokaryotic transcription. So eukaryotic transcription requires the transcriptional binding factors, enhancers, and RNA polymerase. Remember when we were talking about prokaryotic transcription? We said that prokaryotic transcription only requires RNA polymerase and that RNA polymerase is sufficient to recognize the promoters. It can also unwind the DNA, form the first closed complex, and then the open complex to initiate transcription.

It can then enter elongation and eventually reach termination. Whereas in the case of

eukaryotic transcription, you require the transcriptional binding factors or transcription factors. You require the enhancers, and you also require the RNA polymerase. So, what are the transcription factors? So, transcription factors are the proteins that are essential for transcription, but they are not part of RNA polymerase. These factors bind to the DNA template sequentially, and then RNA polymerase binds and forms the initiation complex.

The basal transcription factors create a structure at the promoter so that RNA polymerase can easily recognize it. In bacteria, a single RNA polymerase can transcribe all types of RNA, but in eukaryotes, different types of RNA polymerases are required for the different types of RNA structures. So you know that we have three different types of RNA molecules? We have the ribosomal RNA molecule, the messenger RNA molecule, and the transfer RNA (tRNA) molecule. So all three different types of RNA molecules are transcribed with the help of different types of RNA polymerases. Whereas, in the case of bacteria, all three species of RNA molecules are transcribed by a single RNA polymerase.

So that is also a very big difference between eukaryotic and prokaryotic transcription. So let's first discuss the machinery. So, in the case of eukaryotes, the machinery for eukaryotic transcription is the RNA polymerase. So RNA polymerase, the RNA polymerase of the mitochondria and chloroplasts of eukaryotic cells, is similar to that of bacteria. But all eukaryotic RNA polymerases are multisubunit proteins.

It contains three different types of RNA polymerase, which are responsible for transcription. So you can have three different types of RNA polymerases. You can have RNA polymerase I, RNA polymerase II, and RNA polymerase III. See RNA polymerase I is required for the synthesis of ribosomal RNA; it is resistant to amanitine, and it is also present inside the nucleus. Then we have RNA polymerase II.

RNA polymerase II is responsible for the synthesis of messenger RNA, is very sensitive to the amantadine treatment, and is present in the nucleoplasm. which means it is present inside the nucleus. And then we have RNA polymerase III. RNA polymerase III synthesizes transfer RNA, is less sensitive, and is present in the nucleoplasm. So you can see that it has three different types of RNA polymerases.

You have RNA polymerases I, II, and III. Their location, sensitivity to amanitine, and role in transcription are also very different in the case of eukaryotic transcription. Then, apart from that, you also have the eukaryotic promoters. So eukaryotic promoters exist; each promoter contains a specific sequence that is recognized by the transcription factors. So remember that in prokaryotic transcription, you have a similar kind of transcriptional arrangement, like the minus 10 box, the minus 35 box, the primase box, the TATA box,

and all those kinds of things.

But that kind of arrangement is not present in eukaryotic promoters. Eukaryotic promoters have specific sequences that are recognized by transcription factors, and they have a longer region than prokaryotic promoters because they contain all those sequences that are important for initiation. It includes the core promoter element at which RNA polymerase attaches and forms the initiation complex; for efficient transcription, it also requires the upstream promoter element, which is basically the GC-rich region where the transcription factors will bind. So transcriptionally, eukaryotic promoters are much bigger than prokaryotic ones because eukaryotic promoters have many regions. These regions are going to be responsible for binding RNA polymerase; on the other hand, they also require the binding of different types of transcription factors, and that's why transcription in eukaryotes is going to be tightly regulated compared to transcription in prokaryotes.

Then we have transcriptional initiation. So, transcriptional initiation is carried out by RNA polymerase II. So, this is what we are discussing regarding the transcription of messenger RNA. So, we are going to have different types of transcription factors. So, eukaryotic messenger RNA transcription requires the initiation complex, which can consist of general transcription factors and the mediator.

So, we have many types of general transcription factors. So, the general transcription factor we have is TF2D, and TF2D is actually going to be used to recognize the core promoter, such as the TATA box, or it will also recognize the core promoter, like the non-TATA boxes and other kinds of sequences. So, it can also have two different types of proteins: TATA binding proteins, which are called TATA binding proteins, or it can have TFEs, which are called TATA binding protein-associated factors. So, within the TF2D, you can have two different types of proteins. Then you can also have the transcription factor II A.

The function of transcription factor 2A is to stabilize the TATA binding proteins and the TATA binding protein-associated factors. Then it also requires the TF2B. TF2B is actually helping with RNA polymerase II and TF2F recruitment and also helps in the start site selection. So TF2B is very important because it actually provides the docking site for RNA polymerase and also for TF2F, and both are going to help in selecting the transcription start site.

Then we have the TF2F. TF2F is going to help RNA polymerase with promoter binding. Then we have the TF2E. TF2E is going to help in the TF2H recruitment and modulation of the TF2H helicases, ATPases, and kinase activity. Then it also required the TF2H. So, TF2H is going to help in promoter melting with its helicase activity and promoter

clearance by phosphorylation.

So, let us see how these transcription factors or the general transcription factor is going to participate in the transcription of eukaryotes. So, the transcriptional initiation by RNA polymerase II is going to be performed not only by one factor but by many factors. So, these transcription factors are going to sequentially bind to the TATA box DNA to form a pre-initiation complex. So what will happen is that you have that this is the promoter, right? So you have the Tata box, and then you have the other promoter regions. So the first transcription factor that is going to come and bind is TF2D, and when TF2D binds, it is actually going to provide the docking site for TF2A.

And then from the TF2A, it is actually going to bind to the TF2B, and the TF2B is going to provide the binding site for the TF2F, and it is also going to allow the binding of the RNA polymerase. RNA polymerase II is actually going to bind, and then our TFIIE is going to provide the binding site for TFIIH, and TFIIH is eventually going to allow the binding of the helicases. And that is how it is actually going to start unbinding the DNA, and that is how it is actually going to help the RNA polymerase initiate transcription. So at last, when the TF2H is about to bind, it phosphorylates RNA polymerase II to initiate transcription in the presence of ATP. So why is this phosphorylation important? Because RNA polymerase will bind to the DNA.

Because it's going to be positively charged and the DNA is going to be negatively charged, that's why in the last step, when TF2H binds, it is actually going to have intrinsic kinase activity, and that intrinsic kinase activity is going to phosphorylate RNA polymerase. So once the RNA polymerase is phosphorylated, it will have a lower affinity for the DNA, or that particular region of the DNA, and that's how it will actually start the transcription. The steps in elongation are going to be exactly identical to what we have discussed in prokaryotes, where it is first going to dock onto the transcriptional site one and then look for the A or G sequences, and then it will go to the transcriptional site number two, right? So, those events are the same, right? Then it will reach the transcriptional termination. So transcriptional terminations will be different in the case of different types of RNA polymerases. You can have RNA polymerase I, II, or III, and the transcription factors that are responsible for the termination of these transcriptions will be different.

So RNA polymerase II transcription may continue for hundreds or even thousands of nucleotides beyond the end of a coding sequence. Then the cleavage of the RNA strands occurs by a complex that appears to be associated with the polymerase. Cleavage of RNA is coupled with the termination process and occurs at the same consensus sequences. The polyadenylation of the mature messenger RNA occurs at the 3' end, which results in a

poly A tail; this process is followed by cleavage and termination. Both the processes of polyadenylation and termination occur at the same consensus sequence, and both of these processes are independent.

So, you see that if you have a polymerase H in a gene, it is going to be a row-dependent termination. If it is a Pol III gene, it is going to have row-independent terminations, and if it is a Pol II gene, it is going to be more complex, where you can have the Pol III terminations generally coupled with the RNA processing event in which the 3' end of the transcript undergoes cleavage and polyadenylation. This type of termination is basically coupled with the RNA maturation process in which the three-prime end of the nascent RNA undergoes polyadenylation and cleavage, and these three-prime end processing reactions are carried out in two steps. In step 1, the transcription of a poly A followed by the cleavage of the nascent transcripts occurs, and then the upstream product is polyadenylated while the downstream product is degraded. Basically, 3-by-1 processing starts when the cis-acting element in the poly(A) site of the nascent RNA transcript is recognized by the binding factors.

So you can see that when it reaches the stop codon or when it reaches the termination site, the RNA is going to be released from that site, and then it is actually going to undergo polyadenylation with the help of the polyadenylation machinery in step 2 when these factors bind at the 3' end. It forms a very complex structure that results in high shear forces; consequently, processing slows down, which causes the disruption of the Pol II and DNA-RNA hybrid complex, and ultimately, termination occurs. So you can see that when the polymerase chain reaction or the RNA polymerase reaches the termination site, it actually forms very complex structures. And because of this complex structure, it results in a high shear force, which means it becomes very difficult for RNA polymerase to move further up, and because of that, RNA polymerase, as well as the RNA-DNA duplex, is broken down, and that is how it ultimately ends up in the termination of transcription. So, this is all about transcription in eukaryotes.

What we have discussed is that transcription in eukaryotes is going to be very tightly regulated because it depends on the different types of transcription factors. You might have seen that we have different types of transcription factors, and the formation of the initiation complex is not possible until these transcription factors assemble onto the promoter site in a sequential manner. So, because of that, they are actually going to regulate the transcription within eukaryotes, and apart from that, the termination in eukaryotic transcription is also more complicated than the termination in the prokaryotic system. So, what we have discussed is transcription in eukaryotes, and we have also discussed transcription in prokaryotes. Now, once the transcription is done, it is actually going to form different types of RNA molecules.

Now, these RNA molecules, whether they are ribosomal RNA molecules, tRNA molecules, or messenger RNA molecules, are susceptible. For example, the messenger RNA molecule is a single-stranded RNA molecule. And these single standard RNA molecules are actually going to be susceptible to the different types of RNAs that are present in the cytosol because once these RNA molecules are synthesized in the nucleus, they are actually going to be transported outside into the cytosol. And once they are present in the cytosol, they are susceptible to different types of damage; for example, the messenger RNA is susceptible to the RNAs. The same is true for the tRNA, and the same is true for the ribosomal RNA.

Apart from that, some of these RNA molecules are going to be modified, aren't they? Some of these molecules require different types of modifications. So, this modification is required for two purposes. It is actually going to provide stability to the system. It's going to provide stability to the molecule so that messenger RNA, ribosomal RNA, or tRNA will be functional for a longer period of time within the cytosol. And also, it's actually going to be used; that modification is going to allow the function to be more optimal.

So it's going to optimize the function of that particular molecule. So once the modification is done, it's actually going to make the molecule more optimal at catalyzing the transcription. We are going to discuss post-transcriptional modification because RNA, whether it is ribosomal RNA, tRNA, or messenger RNA, is modified as it is formed. So that it actually achieves stability and also achieves optimal confirmation, enabling participation in downstream events like translations. So when we talk about post-translational modifications, we have to discuss the post-translational modifications of messenger RNA, ribosomal RNA, and tRNA.

So, for the post-translational modification of messenger RNA, what steps do you require? You require the three different types of post-translational modification; you have to add a cap to the 5' prime end. You also had to add polyethylene to the 3' prime end, and then you also required splicing out the introns. So let's first discuss how you can add the cap to the 5' end. So the addition of a cap to the 5' prime end is necessary.

So this is the cap structure. What you see here is that this is the 5' prime end of the messenger RNA, and the structure you see here is actually a cap structure. So this is called the cap, which is made up of a modified guanine molecule called 7-methylguanosine, and it is connected to the messenger RNA through the phosphodiester linkage. So, the capping in eukaryotic cells means that the messenger RNA is inherently unstable at the end, so we need to modify the end to protect it against ribonucleases; the messenger RNA is capped. So that it is protected from the ribonucleases and is important

in binding the messenger RNA to the ribosome for translation, it uses certain cap-binding protein complexes, right? So, the capping reaction starts soon after the transcription has started. As soon as the 20 to 30 nucleotides are formed, capping occurs, right? So, at the 5' end, the capping process occurs: a slightly modified guanine, like 7-methylguanosine, is attached to the transcript by a 5' to 5' linkage to the triphosphate of the first transcribed RNA.

Capping reactions include the condensation of GTP with the triphosphate at the 5' end, followed by the methylation of the guanosine at N7. Further methylation occurs at the 2-hydroxyl of the 2nd and 3rd nucleotides adjacent to the cap. So, this is what happens. You have the five primary nucleotides. So, you are going to have the phosphohydrolase that removes the gamma phosphate.

Then you are going to have modified guanine. So, guanine is also going to have. So, this is going to be a GTP where you are also going to have the gamma phosphate. There will be an enzyme; the enzyme-catalyzed reaction, because of guanyl transferase, will connect the guanine phosphate to the RNA molecules to form the cap. Then this cap is going to be methylated with the help of an enzyme called guanylyl 7-methyltransferase, and then this methyl group is going to be attached to the guanine molecules. And then you are going to have another round of methylation at the two prime positions, and that is going to be catalyzed by another enzyme, which is called 2'-O methyltransferase.

And that is how you are going to have the m7 guanine cap right, so this is going to be the cap that is going to be connected to the messenger RNA. And what is the function of this capping? The capping has two functions: first, it actually provides the binding site for the ribosome; second, it provides the binding site for the cap-binding proteins, and it has another function, which is to provide stability. The ribosome site is going to provide the binding site with the cap-binding proteins, and it is going to have another function, which is to provide stability. So this cap is not useless. If there is no cap present in the messenger RNA, that messenger RNA will not participate in the subsequent downstream translation events because it will not have efficient binding to the ribosomes.

Then the second is the addition of a poly A tail, and this event is called the tailing event. So the eukaryotic messenger RNA has a series of 18 residues ranging from 80 to 250 in number, forming a poly(A) tail at the three-prime end of the primary transcripts. This poly-A tail has several uses. For example, it can export mature messenger RNA from the nucleus. It increases the stability of the messenger RNA, and third, it serves as a recognition sequence for the binding of the translational factors during the initiation of translation.

The process requires the template-independent RNA polymerase activity, catalyzed by the enzyme known as poly(A) polymerase. So what happened is that when the RNA polymerase reaches the termination site, we have the binding of the CPFC, and these CPFCs are actually going to bind to this consensus sequence. And then we have the binding of the CSTF and CFs. To the polyadenylation sites, it is actually going to allow the binding of poly A polymerase, and once poly A polymerase binds to the messenger RNA, it is actually going to catalyze the synthesis of A RNA, which is going to be non-template dependent. So, it is going to be a non-template-dependent synthesis or addition of A residues onto the nascent RNA chains, and that's how it is actually going to synthesize and add varying amounts of A.

So this varying amount of A is actually going to determine the stability of this particular messenger RNA in the cytosol; it will also determine the age of this particular messenger RNA. So if you have 250, it's going to be more stable; if you have 80, it is going to be less stable. Apart from that, it also allows the export of messenger RNA out of the nucleus, increases the stability of messenger RNA, and serves as a recognition sequence for the binding of translational factors. So, that is why whether it is capping or tailing, both are actually responsible for the translation of particular messenger RNA into proteins.

Then we have the third event. The third event is called intron splicing. So an intron is a region. So what may happen is that in the eukaryotic genes, you have genes. So you have genes that are attached to one another.

And that's why you are actually forming genomic DNA. But in the genes, what you have is a region of the gene that does not code for any kind of protein, so these non-coding sequences are called introns, whereas these coding sequences are called exons. And these are the noncoding sequences. And in an event when you want to do the messenger RNA synthesis, all these coding sequences have to come together, which means if this is the gene I have to synthesize, when I synthesize the messenger RNA, I will synthesize the messenger RNA from that gene. But from the gene, you will have the coding regions.

You're going to have the non-coding regions. These non-coding regions, which are called introns, have to be removed. And this step is called intron splicing. So introns are the non-coding nucleotide sequences that are present in a gene and do not count for the protein and do not appear in the final messenger RNA molecule, which are removed by splicing. The protein-coding sequence of a gene, which is also called an exon, is interrupted by introns. So, in a particular gene, you have different regions; you can have different types of regions, including non-coding exons and coding introns.

So, these are the exons; these are the introns. And if you want to make the protein, then all these exons have to come together. So, all these exons have to come together, and that's how you can have the proteins from this particular gene. So the vast majority of eukaryotic genes are interrupted by non-coding introns, which need to be spliced out, meaning that you have to remove these introns from the messenger RNA. However, in vertebrates, one of the few exceptions is the stone protein-coding gene.

The occurrence of introns varies among eukaryotic species. Some yeast species lack introns, and many genes in eukaryotes carry dozens of them. Few bacterial and arterial genes also have introns. So introns can vary in length from 50 to 20,000 nucleotides. In higher animals such as humans, introns are more numerous than exons.

So there are four classes of introns. You can have group-one introns; you can have group-two introns. So both the self-splicing introns and the others do not involve any protein machinery. Then you can have the spliceosomal introns. They are not self-splicing, and you require splicing machinery.

Then you also have the introns that require ATP for splicing. So, there are four different groups of introns. One is an intron: group one and group two introns. These are the self-introns. Splicing introns means it doesn't require any protein machinery.

They can be spliced out on its own. Whereas you have also required the spliceosomal introns. They are not self-splicing, and they also require machinery. And then you also have the introns, which require ATP for splicing. So the splicing mechanisms of group 1 and group 2 introns involve similar steps of the true trans-esterification reaction. In which a ribose 2-prime or 3-prime hydroxyl group makes a nucleophilic attack on the phosphorus, and a new phosphorous bond is formed at the expense of the old.

Which means you can imagine that if this is group one and group two, then there will be a nucleophilic attack from the nucleotide present here onto the nucleotide, and that's how this particular region is actually going to be spliced out, forming a linkage. So, this means this OH is actually going to connect to this, which is the phosphate group present here, and that's how it is going to form a linkage. That is why this axon and that axon got connected, and this is the splice of the introns that are going to be removed. See the mechanism of this group transfer in the nucleophile that is used. The group uses the three-prime hydroxyl group of the guanine nucleotide as a nucleophile.

The group I introns are found in some nuclear, mitochondrial, and chloroplast genes that code for ribosomal RNA, messenger RNA, and tRNA. Whereas in group two, it will actually be performed by the lariat formations. And the lariat formation, whether it is a

group one or group two, has the same mechanism, except that you will have the A at the nucleotide, which will be utilized for the nucleophilic attacks. And then it's actually going to attack this, and then it's going to form a lariat; this lariat, and then this OH is going to attack this chain, and that is how it is going to form the axon. So, these two axons are going to be connected, and that is how it is actually going to release the intron in the form of a lariat.

Apart from this splicing mechanism, you can also have an alternative splicing method. So alternate splicing means you can actually have a distinction between the different types of splicing events, and that's how you can have alternate splicing in such a way that you can have different types of genes. So alternative splicing is a method substantially used for many mammalian genes that can result in multiple products that vary structurally and functionally from the same primary transcript. Sometimes alternative splicing is an upregulated phenomenon, while in others it is strictly regulated. One of the best examples of regulated alternate splicing occurs in the sex determination of Drosophila.

In Drosophila, three genes are involved in sex determination. Sex lethal genes, xa, xa1 transformer genes, tra genes, and doublesex genes are undergoing alternative splicing. So, for example, in alternative splicing, what will happen is that you are actually going to have—so this is the gene, right, for that particular protein, right? And it's going to produce the primary RNA. Now, in the primary RNA, you have exon 1, exon 2, exon 3, and exon 4. Now, if these four exons can actually undergo alternative splicing in two ways, in one way, it is going to take exons 1, 2, and 3. So if it takes the 1, 2, and 3, it is actually going to form protein A, which means it's going to have the decoding sequence that is present in exon 1, exon 2, and exon 3.

Now, in the second example, it can actually take exon 1, exon 3, and exon 4. So, if that happens, it is actually going to form protein B. So, you can have exon 1, exon 3, and exon 4, and that is why you see that the same gene has only one gene, and that gene is actually providing two different types of proteins: protein A or protein B, depending on whether it is using alternate splicing mechanism 1 or alternate splicing mechanism 2. That's why the number of proteins can be very high. And that's why it's actually going to help the organism not to increase its genome size. But at the same time, it can actually help in terms of producing different types of proteins.

You can even consider that it can actually go with one protein source. Go with the three and four, right? That is what we said, right? It can also be like this. One goes with two, then goes with four. So, alternative splicing is a very robust mechanism or tool that is present in eukaryotic cells and actually produces different types of products from a single gene. Due to alternative splicing, functional genes are produced in females and non-

functional genes are produced in males.

Alternate splicing occurs through two mechanisms. One, when two poly A or cleavage sites are available in the primary transcript. The cleavage occurs on one side, resulting in two different products; such a mechanism is followed by the variable domain of the immunoglobulin heavy chain, and its diversity is due to the mechanism of alternate splicing. Similarly, you can have alternative splicing with such mechanisms in the production of two different hormones: you can have the calcium-regulating hormone in the red thyroid and the calcitonin gene-regulated peptide in the red brain. The other mechanism involved more than three prime splicing sites for one five prime sliding site.

Hence, splicing occurs when taking either of these two results in different products. So we have different types of examples where you can actually use this alternative splicing. One of the classic examples is the IGG, right? So IGG, you know that IGG has two chains, right? One is the heavy chain, and the other is called the light chain, right? So then you also have the J chain, which is called a junction chain. So, IgG, you know that different types of antibodies can be produced according to the various types of organisms and all the different types of antigens. And all these antibodies have different types of structures and different sequences in their variable regions, right? So if you see a pro, the antibody structure is right; you have this antibody. This is the antibody structure, right? This is the heavy chain, right? This is the heavy chain, and this is the light chain, right? But this region is actually called the antigen-binding region.

And this region is very variable. So, from where does this variation come? You don't have that many genes. What happened is that these different combinations of the genes and exons, when they come together, are actually responsible for the formation of the different types of antibody molecules, and that is how they are responsible for providing the diversity within the antibody structures. So, because of alternative splicing, you can produce different types of antibody molecules. Similarly, you can have alternative splicing, which can also be responsible for the production of two different types of hormones. One is a calcium-regulating hormone, which is produced in the red thyroid, and the other hormone, which is calcitonin gene-related peptide, is found in the red base.

So from the same gene, you can have two different types of hormones. You can have the calcium-regulating hormones in the thyroid and the calcitonin gene-related peptide in the brain; hence, alternative splicing is a very robust tool that is present in the eukaryotic system and is also responsible for producing different types of proteins. Then we will talk about the processing of ribosomal RNA. So the processing of ribosomal RNA in eukaryotes involves 80S ribosomes, whereas prokaryotes have 70S ribosomes. Ribosomal RNAs are transcribed as longer precursor sequences, which are then modified

at specific bases and cleaved to give the mature product. In both bacteria and eukaryotes, RNA processing involves two basic steps: cleavage and base modification.

So, RNA processing in bacteria is different. So the RNA precursor in bacteria is a 30S ribosomal RNA, which is modified and cleaved to produce the 23S ribosomal RNA, 16S ribosomal RNA, 5S ribosomal RNA, and some tRNA segments that are also sometimes included. So what you have is the pre-ribosomal RNA in the bacteria, and it's going to be the 16S ribosomal RNA. That is going to be transcribed, and it is going to be cleaved to give you the 16S ribosomal RNA, 23S ribosomal RNA, and 5S ribosomal RNA. 30S pre-ribosomal transcript consists of a 16S ribosomal sequence followed by a spacer, which may include the tRNA sequence in some cases, and then there is a 23S ribosomal sequence followed by the 5S ribosomal sequences near the input 3' point event.

At times, there is one more tRNA sequence after the 5S ribosomal RNA sequence at the 3' end. There are seven different genes for ribosomal RNA in E. coli. They are essentially similar in the sequence of ribosomal segments but differ in the number and sequence of the tRNA segments. So, the maturation process involves the methylation of the 30S ribosomal precursor.

So, this is the 30S ribosomal precursor that you have. At the specific site occurring at the 2-prime hydroxyl group of the bases. Some bases, such as uridine, are modified to pseudouridine or dihydrouridine. Further cleavage processes are carried out using the enzymes RNase III, RNase P, and RNase E at sites 1, 2, and 3, respectively. Intermediate products are formed, namely the 17S tRNA. 25S and 5S are acted upon by certain nucleases to yield the final products of 16S tRNA, 23S, and 5S ribosomal RNA.

So this side, this is what you are going to have. You are going to have mature ribosomal RNA, which will be 16S, 23S, and 5S, and these will come assembled to give you the 70S ribosome. Similarly, you can have ribosomal processing in vertebrates and eukaryotes. So, in eukaryotes, the nucleolus is the center of ribosomal RNA processing. At 45, a 45 precursor is formed by RNA polymerase I and processed in the 90S preribosomal nuclear complex to produce the 18S, 28S, and 5.

8S ribosomal RNA. There is a tight coupling of RNA processing and ribosomal assembly. 5S ribosomal RNA is transcribed by RNA polymerase III from a separate gene. So, in this case, you can have the pre-ribosomal RNA. which is going to have the 18S, 5.8S, and 28S ribosomal RNA.

So it's going to form big transcripts, and then it is going to have a cleavage event. So, this cleavage event is going to give rise to the 18S, 5.8S, and 28S. And then you are also

going to have the 5S ribosomal RNA, which will be transcribed separately. And then the precursor RNA undergoes methylation at more than 100 bases from the 14,000 nucleotides at two-prime hydroxyl groups. Furthermore, there is a modification of bases such as uridine to pseudouridine, followed by a series of cleavage reactions.

In yeast, the entire process involves pre-ribosomal RNA 170, non-ribosomal proteins 70, SNRO RNAs, and the 78 ribosomal proteins. And this is what is going to happen. So, this pre-ribosomal RNA is going to form, then it is going to get cleaved, and it is going to form the 18S, 5.

8S, and 20S. And then there will be a separate synthesis of the 5-stable rimelay. And all of these are going to assemble to give you the 80S ribosomes. Then they can also have the RNA processing in the tRNA. So in the case of tRNA, you can have base modifications, and you can also have different types of modifications. So what is the processing in the tRNA in both eukaryotic and prokaryotic tRNA processing? It is transcribed as a long cursor; sometimes, single primary transcripts carry more than one tRNA segment, which are separated by cleavage. Processing of pre-tRNA involves cutting off the extra sequence by endonucleases such as RNase P at the 5' end and RNase D at the 3' end.

So in the tRNA molecules, what you have is the extra sequences. So the extra sequences at the 5 prime end are going to be cleaved by the RNase P, and the extra sequences at the 3 prime end are going to be cleaved by the RNase D. RNase P is a ribozyme with RNA exhibiting catalytic activity. After the removal of the sequences from the 3' end, the CCA sequence is added by an enzyme called tRNA nucleotidease transferase. So once the 3' end is cleaved by RNase D, the 3' end is going to be cleaved. Added like cca end is going to be added onto the 3' prime end with the help of an enzyme called tRNA nucleotidyl transferase.

Now this three-prime end is over. The enzyme binds to the CCA sequence at its active site, and a phosphodiester bond is formed with the three-prime end. Furthermore, there are base modifications occurring simultaneously, such as methylation, deamination, or reduction in the case where pseudouridine is removed and attached to the sugar through C5. So, after that, there will be a further cleavage, and then there will be a base modification so you can have the pseudouridine and all those kinds of things, and that's how ultimately you're going to have the final matured tRNA, which is going to be formed, and this tRNA is going to participate in the translation. So this is all about the transcriptions and what we have discussed so far. We have discussed the transcription in prokaryotes, and then we also discuss the different steps, such as the initiation of translation and the terminations.

So you can have the intrinsic terminations or the row-dependent terminations, and then we also discuss the transcription in eukaryotic cells or the eukaryotic system, where we have discussed the composition of RNA polymerase and different transcription factors that are responsible for the formation of the initiation complex. And then we also discuss the promoter sequences and all of the kinds of machinery that are required for transcription. And then we discuss the post-transcriptional modifications. Where we discuss the tailing as well as the capping in the case of the messenger RNA, then if we have discussed the splicing of group one, group two, and all other kinds of introns. And then we also discuss the relevance of alternate splicing and how alternative splicing gives rise to different types of proteins from a single gene.

We also discuss RNA processing in the case of ribosomal RNA as well as tRNA. So, with this, we would like to conclude our lecture here. In our subsequent lecture, we are going to discuss some more aspects related to the central dogma of molecular biology or the central dogma of life. So, with this, I would like to conclude my lecture here. Thank you.