

**Basics of Biology**  
**Professor Doctor Vishal Trivedi.**  
**Department of Biosciences and Bioengineering,**  
**Indian Institute of Technology, Guwahati**  
**Lecture - 28**  
**Transcription (Part-II)**

Hello everyone. This is Dr. Vishal Trivedi, Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing? We were discussing about the central dogma of life or the central dogma of molecular biology. And in this context, so far what we have discussed, we have discussed about the DNA dependent, DNA synthesis or the replications.

And we have understood the many aspects of the replications and how the replications can the steps, what are the present in the replications for the synthesis of the new DNA strand, can be modified or can be performed under the in vitro reactions, so that you can be able to amplify any given DNA strands and that is a very robust techniques which we have discussed in the previous lectures and the technique is called as the polymerase chain reactions.

Subsequent to that, we have also discussed about the DNA dependent, RNA Synthesis and that event is also been catalyzed by the RNA polymerases, and that process is called as the transcription. So, in the previous lecture, we discuss about the differences between the transcription in prokaryotes versus eukaryotes.

We have discussed about the different contrasting features of the transcription in the prokaryotic versus eukaryotic cells and how it is actually main different. So, the transcription is different in three major aspects between the prokaryotic vs eukaryotic cells. First is the sight of transcription, the sight of transcription in the case of prokaryotes is within the sight of all, where as in the eukaryotes it is the nucleus.

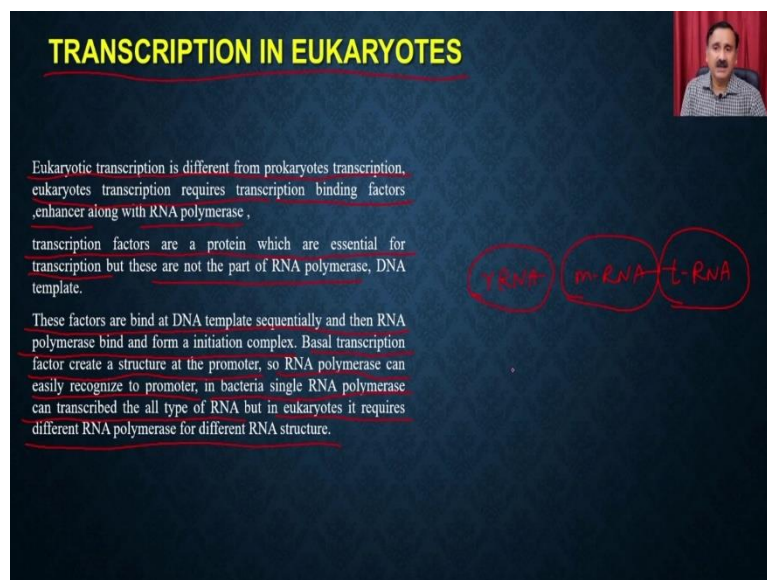
And the second contrasting feature is that the transcription and the translation both are occurring simultaneously, in the case of the prokaryotes, where as it is offering differentially, like the transcription is happening inside the nucleus, whereas the translation is happening inside cytosol, so that also gives up regulatory events and where you can actually be able to control the transcription and translation boards separately, in the case of eukaryotes.

The third is the, transcription is does not require the transcription factor in the case of the prokaryotes and in the case of eukaryotes, it require the different types of transcription factors

to recognise the promoter regions and that is how the action is going to be more technic control in the case of the eukaryotic cells.

So, in the previous lecture, we also discussed about the different events, the structure of the RNA polymerase in the case of prokaryotes cells and we also have discussed about the different events like the initiation, elongation and termination. So, in today's lecture, we are going to start discussing about the transcription in the eukaryotes and then subsequent to that, we are also going to discuss about the post of transcriptional modifications. So, let us start discussing about transcription in the prokaryotes and in the eukaryotes.

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**TRANSCRIPTION IN EUKARYOTES**

Eukaryotic transcription is different from prokaryotes transcription, eukaryotes transcription requires transcription binding factors, enhancer along with RNA polymerase.

transcription factors are a protein which are essential for transcription but these are not the part of RNA polymerase, DNA template.

These factors are bind at DNA template sequentially and then RNA polymerase bind and form a initiation complex. Basal transcription factor create a structure at the promoter, so RNA polymerase can easily recognize to promoter, in bacteria single RNA polymerase can transcribed the all type of RNA but in eukaryotes it requires different RNA polymerase for different RNA structure.

Diagram showing three types of RNA: rRNA, m-RNA, and t-RNA.

So, transcription in eukaryotes is different from the transcription in prokaryotes. It requires the entirely different machines. So, Eukaryotic transcription is different from the prokaryotic transcription, the eukaryotic transcription requires the transcriptional binding factors, enhancer and it also requires the RNA polymerase.

Remember when you are talking about the prokaryotic transcription, we said that the prokaryotic transcription only required the RNA polymerase and that are RNA polymerase is sufficient enough to recognise the promoters. It also can unbind the DNA and it also can form the first the close complex and then the open complex, to initiate the transcription and then it also can enter into the elongation and then eventually it can reach to the terminations.

Whereas, in the case of eukaryotic transcriptions, you require the transcriptional binding factors or the transcriptional factors, you require the enhancers and then you also require the

RNA polymerase. So, what are the transcriptional factors? So transcriptional factors are the proteins which are essential for the transcription, but these are not part of the RNA polymerase. These transcriptional factors are binding at the DNA template, sequentially and then the RNA polymerase binds and forms the initiation Complex.

The Basal transcription factors create a structure at the promoters. So, RNA polymerase can easily recognize to the promoter, in bacteria single RNA polymerase can transcribed the all type of RNA but in eukaryotes it requires different RNA polymerase for different RNA structure. So, you can, know that we are having the three different types of RNA molecules.

We have the ribosomal RNA molecules, we have the messenger RNA molecule and then we also have the transfer RNA molecule, so or tRNA molecule. So, all these three different types of RNA molecules are transcribed with the help of, the different types of RNA polymerase whereas, in the case of bacteria, all these three species of the RNA molecules are being transcribed by the single RNA polymerase. So, that is also a very big difference between the eukaryotic and the prokaryotic transcriptions.

(Refer Slide Time: 06:30)

**TRANSCRIPTION IN EUKARYOTES**

**RNA Polymerase:** The RNA polymerase of mitochondria and chloroplast are similar like bacteria. All eukaryotic RNA polymerases are multi subunit proteins which contain. Three different types of RNA polymerases are responsible for transcription.

Type of polymerase	Synthesis of RNA	Amantin sensitivity	Occurrence
1 RNA Pol I	synthesis of r RNA	resistant	Nucleoli
2 RNA pol II	synthesis of m-RNA	Very sensitive	Nucleoplasm → ↯
3 RNA pol III	Synthesis of t-RNA	Less sensitive	Nucleoplasm

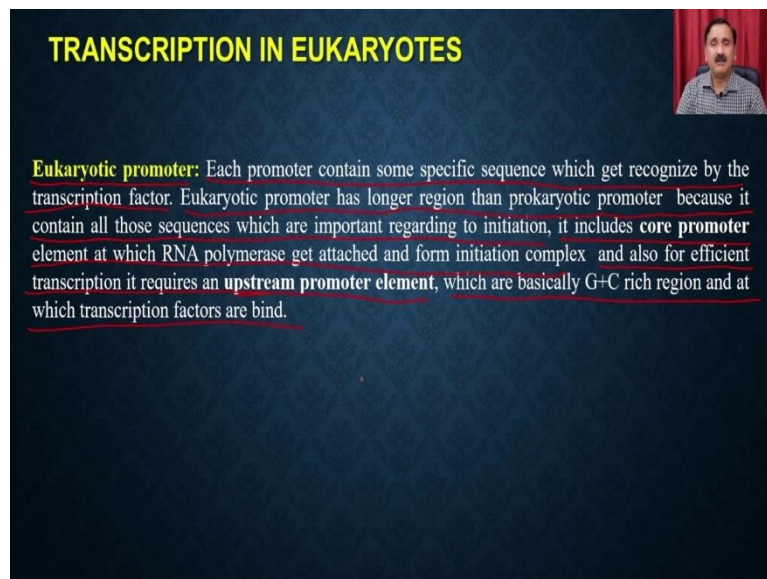
So, let us first discuss about the machinery. So, trans, machinery indicates of the eukaryotes, eukaryotic transcription is the transcript, the RNA polymerase. So, RNA polymerase, the RNA polymerase of the mitochondria and the chloroplast of the eukaryotic cells are similar to the bacteria. But all eukaryotic RNA polymerase are multi subunit protein which contains three different, multi-subunit proteins, it contains three different types of RNA polymerase, which is responsible for the transcriptions.

You can have the three different types of RNA polymerase you can have the RNA polymerase one, you can have the RNA polymerase two, you can have the RNA polymerase three. The RNA polymerase one is required for the synthesis of the ribosomal RNA and it is resistance for the eminent team, okay, and it is it is present inside the nuclei.

Then we have the RNA polymerase two, RNA polymerase two is responsible for the synthesis of the messenger RNA and it is very sensitive for the eminent treatment and it is present into the nucleoplasm, which means it is present inside the nucleus. And then we have the RNA polymerase three, RNA polymerase three is a synthesis of the transfer RNA and it is less sensitive and it is also present in the nucleoplasm.

So, you can see that it has the three different types of RNA polymerase, you have the RNA polymerase one, two and three, they are location, their sensitivity, to the eminent team and their role into the transcription is also very different in the case of eukaryotic transcriptions.

(Refer Slide Time: 08:11)



**TRANSCRIPTION IN EUKARYOTES**

**Eukaryotic promoter:** Each promoter contain some specific sequence which get recognize by the transcription factor. Eukaryotic promoter has longer region than prokaryotic promoter because it contain all those sequences which are important regarding to initiation, it includes **core promoter element** at which RNA polymerase get attached and form initiation complex and also for efficient transcription it requires an **upstream promoter element**, which are basically G+C rich region and at which transcription factors are bind.

Then apart from that, you also have the eukaryotic promoters, so, eukaryotic promoters, each promoter contains some specific sequence which get recognized by the transcription factors. So, remember that in the eukaryotic transcription, you have the similar kind of transcriptional arrangement like you have the minus 10 box, you have the minus 35 box and you, all this kind of rainbow box, TATA box and all this kind of thing.

But that kind of arrangement is not present in the eukaryotic promoter, eukaryotic promoter has the specific sequences, which are being recognized by the transcription factors and the

eukaryotic promoter has a longer region than the prokaryotic promoter, because it contains all those sequences which are important regarding to the initiations.

It includes the core promoter element at which the RNA polymerase get attached and formed the initiation complex and also for the efficient transcription, it requires the upstream promoter element, which are basically the GC rich region at which that transcription factors are going to bind. So, transcriptional the eukaryotic promoters are way bigger than the prokaryotic transcription.

Because the eukaryotic transcription, eukaryotic promoters are going to have the many regions and these mainly, these regions are going to be responsible for binding off the RNA polymerase and on the other hand it also requires the binding of the different types of transcription factors and that is why the transcription in the primer in the eukaryotes is going to be tightly regulated compared to the transcription in prokaryotes.

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General Transcription Factors (GTFs)	Function
<b>TFIID</b> i) <b>TBP (Tata binding protein)</b> ii) <b>TAFs (TBP Associated Factors)</b>	recognize to core promoter (TATA box) recognize to core promoter (Non TATA box)
<b>TFIIA</b>	Stabilized TBP and TAFs binding
<b>TFIIB</b>	It help in RNA polymerase II and TFIIF recruitment and also help in start site selection
<b>TFIIF</b>	Help to RNA Pol in promoter binding
<b>TFIIE</b>	Help in TFIIF recruitment, modulation of TFIIF, Helicase, ATPase, Kinase activities
<b>TFIIH</b>	Help in promoter melting with helicase activities promoter clearance by phosphorylation

Then we have the transcriptional initiation, for transcriptional initiation by the RNA polymerase two. So, this is we are discussing about the transcription of the messenger RNA. So, we are going to have the different types of transcription factors, so eukaryotic within the RNA transcription require the initiation complex, which can consist of the general transcription factors and the mediator.

So, we have the many types of general transcription factors. So, general transcription we have the TFIID and that TFIID is actually going to use to recognize the core promoter like the



TATA box are also going to recognize the core promoter like the Non TATA boxes like other kind of sequences. So, it can also have the two different types of proteins, it can have a tata binding protein, which is called as tata binding proteins or it can also have that TAFs which is called as Tata Binding Protein Associated Factors.

So, the, within that TFIID, you can have the two different types of proteins, then you can have the transcription factor two A data fusion factor to aid the function of the transcription factor to A that it is stabilizes the data binding proteins and that data binding protein associated factors. Then it also required that TFIIB, TFIIB is actually helping the RNA polymerase two and that TFIIF recruitment and also helps in the start site selections.

So, TFIIB is very important because it is actually going to provide the docking site for the RNA polymerase and it also provide the docking site for the TFIIF, and both are actually going to help in terms of the selecting the transcription start site. Then we have the TFIIF, TFIIF is going to help the RNA polymerase in promoter binding, then we have the TFIIE, TFIIE is going to help in that TFIIF recruitment.

And the modulation of the TFIIF helicases ATPase and kinase activity, then it also required the TFIIF, TFIIF is going to help in promoter melting with this helicase activity and the promoter clearance by the phosphorylations. So, let us see how these transcription factors or the general transcription factor are going to participate into the transcription of the eukaryotes.

(Refer Slide Time: 12:18)

## TRANSCRIPTION IN EUKARYOTES

**TRANSCRIPTION INITIATION BY RNA POLYMERASE II :**  
These transcription factors are sequentially bind to TATA box DNA to form a pre initiation complex.

At last when TFIIF get bind, It phosphorylates Pol II to initiate transcription in the presence of ATP.

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graph TD; TATA[TATA] --- INR[INR]; TFIID[TFIID] --> TFIIB[TFIIB]; TFIIB --> TFIIF[TFIIF]; TFIIF --> TFIIE[TFIIE]; TFIIE --> TFIIF; TFIIF --> Helicase[helicase]; TFIIF --> PolII[RNA Polymerase II]; Helicase --> Initiation[Initiation of Transcription]; PolII --> Initiation;
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So, the transcriptional initiation by the RNA polymerase is RNA polymerase two is going to be performed by the not only one factor, but by the many factors. So, these transcription factors are sequentially going to bind the TATA box DNA to form a pre initiation complex. So, what will happen is that you have that, this is the promoter, so you have the TATA box, and then you have the other promoter regions.

So the first transcription factor which is going to come and bind is TFIIB, and when the TFIIB is fine, it is actually going to provide the docking site for the TFIIA and then for the TFIIA, it is actually going to bind the TFIIB and the TFIIB is going to provide the binding site for the TFIIF and it is also going to allow the binding of the RNA polymerase, right.

So, that is RNA polymerase two is actually going to bind and then it is TFIIF is going to provide the binding site for the TFIIE and the TFIIE is eventually going to allow the binding of the TFIIH and the TFIIH is actually going to allow the recruitment of the helicases and that is how it is actually going to start unwinding the DNA, and that is when it is actually going to help the RNA polymerase to initiate the transcriptions.

So, at last, when the TFIIH is going to bind, it phosphorylates the RNA polymerase two to initiate the transcription in the presence of ATP. So, why this phosphorylation is important because the RNA polymerase will go and bind to the DNA? Because it is going to be positively charged and the DNA is going to be negatively charged.

And that is why, it is been in the last step in the TFIIH is actually when it binds, it actually going to have the intrinsic kinase activity and that intrinsic kinase activity is going to phosphorylate the RNA polymerase. So, once the RNA polymerase is going to be phosphorylated, it is going to have the lower affinity for the DNA or that particular region of the DNA and that is how it is actually going to start the transcriptions.

(Refer Slide Time: 14:38)

## TRANSCRIPTION IN EUKARYOTES

**TRANSCRIPTION Termination:**

RNA pol II transcription genes may continue up to hundreds or even thousands of nucleotides beyond the end of a coding sequence. Then the cleavage of RNA strand occurs by a complex which appears to associate with the polymerase. Cleavage of RNA is couple with termination process and it occurs at same consensus sequences. The polyadenylation of mature pol II mRNA occurs at the 3', which result in a poly(A) tail; this process is followed by cleavage and termination. Both processes polyadenylation and termination occurs at same consensus sequence, and both these processes are interdependent.

For Polymerase I gene	rho dependent termination
For polymerase III gene	rho independent termination
For polymerase II gene	It more complex, polIII termination generally coupled with the RNA processing event, in which 3' end of transcript undergoes cleavage and polyadenylation.

Then we have worked through, elongation is the steps in the elongation is going to be exactly the identical what we have discussed in prokaryotes, where the it is going to first dock onto the you know, transcriptional site one and then it is going to look for the A or gene sequences and then it will go for the transcriptional site number two, so that is that, those events are same. Then it will reach to the transcriptional terminations.

So, transcriptional terminations are going to be different in the case of different types of RNA polymerase, you can have the RNA polymerase one, two or three and the transcriptional factor which is responsible for the termination of these transcriptions are going to be different. So, RNA polymerase two transcription gene may and continue up to the 100s or even 1000s of nucleotide beyond the end of a coding sequence.

Then the cleavage of the RNA strands occurred 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 II messenger RNA occurs at the 3 prime, which results in a poly (A) tail, which process is followed by the cleavage and terminations. Both the process polyadenylation and termination occur at the same consensus sequence, and both these processes are independent.

So, you see that, if you have a polymerase, a chain gene, it is going to be rho dependent terminations. If it is pol three genes, it is going to be rho independent terminations. And if it is a pol II genes it is going to be more complex where you kind of have the pol 3



terminations, generally coupled with the RNA processing event in which the three prime end of the transcript undergoes cleavage and the polyadenylation.

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### TRANSCRIPTION IN EUKARYOTES

**TRANSCRIPTION Termination:**  
**Poly A dependent termination:** This type of termination are basically coupled with the RNA maturation process in which 3' end of nascent RNA undergo polyadenylation and cleavage, and these 3' end processing reaction are carried out in two steps:

Transcription of poly A followed by cleavage of nascent transcript, and then the upstream product is polyadenylated and downstream product is degraded. Basically 3' end processing starts when the cis acting element in the Poly A site of nascent RNA transcript is recognized by the binding factors.

when these factors bind at 3' end, it form a very complex structure which result in high shear forces consequently processing slow down which causes disruption of polymerase II and DNA-RNA hybrid complex; and ultimately termination occurs.

The diagram illustrates the molecular mechanism of poly A dependent transcription termination. It shows a DNA template with a 5' cap (m7G), a start codon (ATG), a stop codon (TGA), and a poly-A tail (AAAA(n)). RNA Polymerase II (RNA Pol II) is shown transcribing the DNA. The RNA transcript has a 5' cap (m7G) and a poly-A tail (AAAA(n)). The diagram shows the binding of factors Pcf11, Rtt103, P54/PSF, and Sen1 to the poly-A tail. The binding of these factors causes the RNA Pol II to stall at the stop codon, leading to the termination of transcription. The diagram also shows the binding of Rat1 and CPSF to the poly-A tail.

So, transcription, transcriptional terminations, so it can be a poly A dependent termination, which means it is going to form the polyadenylation at the end of the RNA, and that is when it is going to do the terminations. This type of terminations are basically coupled with the RNA maturing process in which the 3 prime end of the nascent RNA undergoes polyadenylation and cleavage and these 3 prime end processing reactions are carried out in two steps.

And that is step one, the transcription of a poly A followed by the cleavage of the nascent transcripts, and then the upstream product is polyadenylated and downstream product is degraded. Basically, 3 prime end processing starts when the CIS acting element in the poly A site of the nascent RNA transcript is recognized by the binding factor.

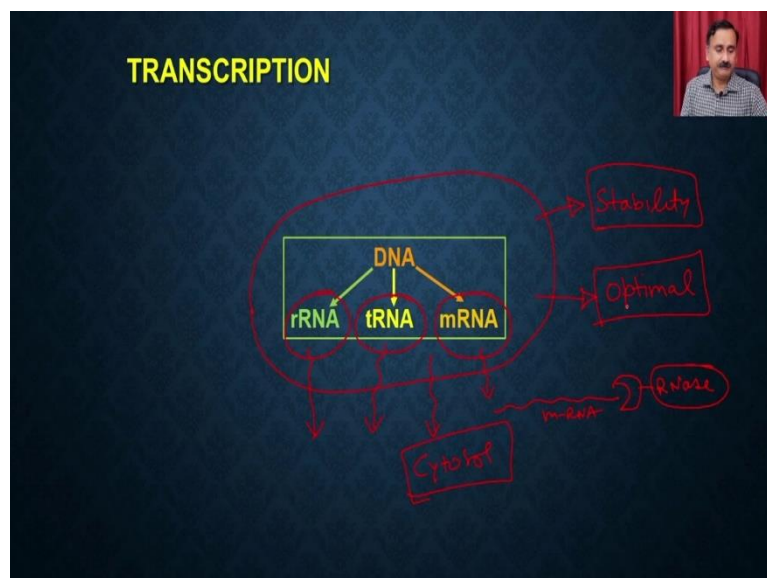
You can see that when it reaches to the stop codon or when it reaches to the termination site, the RNA is going to be released from that site and then it is actually going to do the polyadenylation with the help of the polyadenylation machinery. And the step two, when these factor binds at the 3 prime end, it forms a very complex structure which results in a high shear forces consequently processing slowdown which causes the disruption of the pol two and DNA RNA hybrid complex and ultimately the termination occurs.

So, you can see that when the polymerase chain reactions or RNA polymerase reaches to the termination site, it actually, it forms a very complex structures and because of this complex structure, it results in the high shear forces, which means it becomes very difficult for RNA polymerase to go further up, and because of that, the RNA polymerase as well as the RNA DNA duplex is getting broken down. And that is how the, ultimately it is end up in the termination of the transcriptions.

So, this is all about the transcription in the RNA polymerase in the eukaryotes, what we have discussed is that the transcription in the eukaryote is going to be very, 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, which and the formation of the initiation complex is not possible until these transcription factors or these factors are not going to assemble onto the promoter site in a sequential manner.

So, because of that, they are actually going to be regulated the transcription within the eukaryotes, and apart from that, the termination in the eukaryotic transcription is also been more complicated than in the termination in the prokaryotic system. So, what we have discussed? We have discussed about the transcription in the eukaryotes, and we have also discussed about transcription in the prokaryotes. Now, once the transcription is done, then it is actually going to form the different types of RNA molecules.

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Now, these RNA molecules, whether it is the ribosomal RNA molecules, whether it is the tRNA molecules or whether the messenger RNA molecules are susceptible. For example, the

messenger RNA molecule is going to be a single standard DNA, a single standard RNA molecules and these single stranded RNA molecules are actually going to be susceptible for the different types of RNase, which are present within the cytosol.

Because once these RNA molecules are going to be synthesized within the nucleus, they are actually going to be transported outside into the cytosol. And once they are present into the cytosol, they are susceptible for the different types of damages, like for example, the messenger RNA is going to be susceptible for the RNase, same is true for the tRNA, and same is true for the ribosomal RNA.

Apart from that some of these RNA molecules are going to be modified, some of these molecules require the different types of modification. So, this modification is required for the two purposes, one, it is actually going to provide the stability into the system, it is going to provide the stability into the molecule, so that the messenger RNA or the ribosomal RNA, or tRNA is going to be functional for a longer period of time within the cytosol.

And also, it is actually going to be used, that modification is going to allow the functioning be more optimal. So, it is going to make the optimal functioning of that particular molecule. So, once the modification is being done, it is actually going to make the molecule more optimally catalyzing the transcription.


So, we are going to discuss about the post transcriptional modification, because the RNA or the any RNA, whether it is ribosomal RNA, tRNA, or messenger RNA, when it is going to be formed, it is going to be modified, so that it is actually going to achieve the stability and it is also going to achieve the optimal conformation, so that it is going to participate into the downstream events like the translations.

(Refer Slide Time: 21:55)

## POST-TRANSCRIPTIONAL MODIFICATION

**m-RNA: Steps in RNA processing:**

- (1) Add a cap to the 5' end.
- (2) Add a poly-A tail to the 3' end.
- (3) splice out introns



So, when we are talking about the post translational modifications, we have to discuss about the post translational modifications of the messenger RNA. We have to discuss about the ribosomal RNA, and the tRNA. So, post translational modification for the messenger RNA. So, steps in the post translational modifications what you require?

You require the three different types of post translational modification. You have to add cap to the 5 prime end, you also had to add a poly-A tail to the 3 prime end and then you also require to splice out the introns. So, let us first start discussing about how you can be able to add the cap to the 5 prime end.


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## POST-TRANSCRIPTIONAL MODIFICATION

**m-RNA: Steps in RNA processing:**

- (1) Add a cap to the 5' end.

**Capping:** In eukaryotic cells, m RNA is inherently unstable at the ends. So needs to be modified the end to protect it against ribonucleases, mRNA is capped so that it is protected from ribonucleases as well as it is important in binding of m-RNA to ribosome for translation; it uses certain cap binding protein complexes. Capping reaction starts soon after transcription has started. As soon as 20-30 nucleotides are formed, capping occurs. At the 5' end capping process occurs, a slightly modified guanine (7-methyl G) is attached "backwards", by a 5' to 5' linkage, to the triphosphates of the first transcribed base. Capping reaction includes condensation of GTP with triphosphate at 5' end followed by methylation of guanine at N-7. Further methylation occurs at 2' hydroxyls of 2<sup>nd</sup> and 3<sup>rd</sup> nucleotide adjacent to the cap.



So, the adding a cap to the 5 prime end, so this is the cap structure, what you see here is that this is the 5 prime end of the messenger RNA and this is the structure what you see here is actually a cap structure. So, this is called, this cap is made up of a modified guanine molecule which is called as seven methyl guanosines. And it is connected to the messenger RNA through the phosphate diester linkage.

So, the capping is, in eukaryotic cell the messenger RNA is inherently unstable at the end. So, need to modify the end to protect it against the ribonucleases, this messenger RNA is capped so that it is protected from the ribonuclease as well as it is important in binding the messenger RNA to the ribosome for the translations. It uses a certain cap binding protein complexes. So, the capping reaction starts soon after the transcription has started.

As soon as the 20 to 30 nucleotides are formed, the capping occurs. So, at the 5 prime end, the capping process occurs a slightly modified guanine like the 7-methyl guanosine is attached to the backward by a 5 prime to 5 prime linkage to the triphosphate of the first transcribed base. Capping reactions includes the condensation of the GTP with the triphosphate at the 5 prime end followed by the methylation of the guanosine at the N-7.

Further methylation occurs at that two hydroxyl of the second and third degree are tight adjacent to the cap. So, this is what you happen. You have the 5 prime nucleotides, so you are going to have the phosphohydrolase that is going to remove the gamma phosphate. And then it is going to form a, then you are going to have the modified, the guanine.

So this is going to be a GTP, where you are going to also going to have the gamma phosphate and then they will be enzyme catalyzed reaction. So, because of the guanine transferase, this guanine is the guanine, the phosphate guanine is going to be connected to the RNA molecules to form the cap and then this cap is going to be methylated with the help of an enzyme which is called as the guanine, 7-methyl transferase.

And then this methyl is going to be connected, 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 the enzyme which is called as two O methyl transferase and that is how you are going to have the M7 guanine this cap. So, this is going to be the cap which is going to be connected on to the messenger RNA.

And this, what is the function of this capping? The capping is having that two function. One, it is actually going to provide the binding site for the ribosome, site is going to provide the binding site with the cap binding proteins and it is going to have the another function which is going to provide the stability.

So, this cap is not useless if there is no cap present in the messenger RNA, that messenger RNA is not going to participate into the subsequent downstream translation events, because it is not going to have the efficient binding to the transcription, to the ribosomes.

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**POST-TRANSCRIPTIONAL MODIFICATION**

**m-RNA: Steps in RNA processing:**

(1) Add a poly-A tail to the 3' end.

**Tailing:** Eukaryotic mRNA has series of adenosine residues ranging from 80 to 250 in number forming a poly (A) tail at 3' end of the primary transcript. This poly (A) tail has several uses- 1) it can export mature mRNA out of the nucleus. 2) It increases stability of mRNA. 3) It serves as recognition signal for binding of translational factors during ignition of translation. The process requires template-independent RNA polymerase called as poly (A) polymerase.

The diagram illustrates the steps of polyadenylation. It shows a primary transcript with a 5' cap (m7G) and a 3' poly(A) tail. The process involves the binding of CPSF to the poly(A) tail, followed by the binding of CstF and CF to the CA consensus sequence. Finally, poly(A) polymerase (PAP) adds a poly(A) tail to the 3' end of the transcript. A red arrow points to the PAP step with the handwritten note "Non-templated dependent".

Then the second is the addition of poly-A tail on, this event is called as the tailing event. So, the eukaryotic messenger RNA may have a series of adenosine residues ranging from the 80 to 250 in a number forming a poly-A tail at the 3 prime end of the primary transcripts. This poly-A tail has several uses, for example, it can export the mature messenger RNA out of the nucleus, it increases the stability of the messenger RNA.

And third, it serves as a recommendation sequence for the binding of the translational factor during the initiation of the translations. The process requires the template independent RNA polymerase activity called catalyzed by the enzyme which is called as poly (A) polymerase. So, what happened is that when the RNA polymerase reaches to the transcript, the termination site, then we have the binding of the CPSE and the CPSE 's are actually going to bind to this consensus sequence.



And then we have the binding of the Cstf and CF to the polyadenylation sites and then it is actually going to allow the binding of the poly (A) polymerase. And once the poly (A) polymerase is going to bind to the messenger RNA, then it is actually going to catalyze the synthesis of the A RNA and it is going to be non-template dependent. So, it is going to be a non-template dependent synthesis of or addition of a residues onto the nascent RNA chains.

And that is how it is actually going to synthesize and add the varying amount of A. So, this varying amount of A is actually going to decide the stability of this particular messenger RNA into the cytosol. It will also decide the age of this particular messenger RNA. So, if you have the 250, it is 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 the messenger RNA out of the nucleus, it increases the stability of the messenger RNA and it serves as a recognition signal for the binding of the translational factors. So, that is why the whether it is a capping or tailing, both are actually responsible for the translation of the particular messenger RNA into the proteins.

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**POST-TRANSCRIPTIONAL MODIFICATION**

**m-RNA: Steps in RNA processing:**

**INTRONS SPLICING:** Introns are noncoding nucleotide sequence within a gene that don't code for protein and don't appear in the final mRNA molecule and are removed by the splicing. Protein-coding sequence of a gene (known as exons), which are interrupted by introns. The vast majority of eukaryotic genes are interrupted with non-coding region (introns), which needs to be spliced out; however, histone protein coding genes in vertebrate is one among few exceptions. The occurrence of introns varies in eukaryotic species, some yeast species lack introns and many genes in eukaryotes carry a dozen of them. Few bacterial and Archeal genes also have introns. Introns can vary in length from 50 to 20000 nucleotides. In higher animals as humans, introns are more than exons.

There are four classes of introns:

Group I, (2) Group II; both are self-splicing introns and does not involve any protein enzymes.

(3) Spliceosomal introns- they are not self-splicing. →

(4) Introns that require ATP for splicing. →

The diagrams on the right illustrate the process: the top diagram shows a gene with alternating exons (shaded boxes) and introns (unshaded boxes). The middle diagram shows the resulting mRNA with introns removed and exons joined together. The bottom diagram shows a similar process with a different arrangement of exons and introns.

Then we have the third event, the third event is called as intron splicing. So, intron is a region of, so what will happen is in the eukaryotic genes, you have the non, you have the genes. So, you have a genes which are attached to each other and that is why you are actually forming the genomic DNA. But in the genes, what you have is, you have the sub region of the gene which is not coding for any kind of protein, so these non-coding sequences are called as introns whereas, these coding sequences are called as exons.

So, these coding sequences are the coding sequence and these are the non-coding these are non-coding sequence. And in an event, when you want to do the messenger RNA synthesis, all these coding sequences are had to be come together which means if this is the gene, I have to synthesize I have, so when I will synthesize the messenger RNA, I will synthesize the messenger RNA from the gene.

But from the gene, you are going to have the coding regions; you are going to have the non-coding regions. The non-coding regions which are called as introns have to be removed and this step is called as the intron splicing. So, introns are the non-coding nucleotide sequence which are present in a gene and that do not count for the protein and do not appear in the final messenger RNA molecule are removed by the splicing.

Protein coding sequence of a gene, which is also called as exon, which are interrupted by the intron. So, in a particular gene, you have the different regions. You can have the different types of regions, you can have the non-coding introns, you can have decoding introns. So, these are the exons, these are the introns and if you want to make the protein, then all these exon has to come together.

So, these all these exons have to come together and that is how you can be able to have the protein from this particular gene. So, the vast majority of the eukaryotic genes are interrupted with our non-coding region intron, which needs to be spliced out, which means you have to remove these introns from the finally, from the messenger RNA.

However, histone protein coding gene is in our debate is one among few exceptions. The occurrence of introns varies in the eukaryotic species, some Eastern species lacks introns and many genes in eukaryotic carry a dozen of them. Few bacterial and archaeal genes also have the introns. So, introns can vary in length from 50 to 20,000 nucleotides. In higher animals as humans, the introns are more than the exons.

So, there are four classes of the introns, you can have the group one introns, you can have group two introns. So, both are the self-splicing introns and does not involve any protein machinery. Then you can have the spliceosomal introns, they are not self-splicing, and you require a splicing machinery and then you require; then you also have the introns that require ATP for the splicing.

So, there are four different groups of introns, one is intron, the group one and group two introns. These are the self-splicing introns, which means they do not require any protein machinery, they can be just spliced out on their own. Whereas, you have also required the spliceosomal introns, they are not self-splicing, and they also require a machinery and then you also have the introns which requires the ATP for the splicing.

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**POST-TRANSCRIPTIONAL MODIFICATION**

**m-RNA: Steps in RNA processing:**

**Splicing mechanism of group I and Group II introns:** Splicing mechanism of both Group I and Group II involve similar steps of two **transesterification** reactions in which a ribose 2' or 3' hydroxyl group makes a nucleophilic attack on phosphorus and a new phosphodiester bond is formed at the expense of the old.

Mechanisms of these groups differ in nucleophile which is used. Group I uses 3' hydroxyl group of guanine nucleotide as nucleophile. Group I introns are found in some nuclear, mitochondrial, and chloroplast that code for rRNAs, mRNAs, and tRNAs.

The diagram illustrates two mechanisms of intron splicing. On the left, Group I splicing is shown in three steps: 1) A guanine nucleotide (G) at the 3' end of the intron has its 3' hydroxyl group (OH) positioned for a nucleophilic attack on the 5' phosphate group of the intron. 2) The reaction proceeds, forming a new phosphodiester bond between the 3' G and the 5' phosphate, while the 5' phosphate is released. 3) The final products are a spliced mRNA with a new 5'-3' phosphodiester linkage and a released intron containing a 5'-G phosphate group. On the right, Group II (Lariat Formation) is shown in three steps: 1) An adenine nucleotide (A) at the 2' position of the intron has its 2' hydroxyl group (OH) positioned for a nucleophilic attack on the 5' phosphate group. 2) The reaction proceeds, forming a new phosphodiester bond between the 2' A and the 5' phosphate, creating a lariat structure. 3) The final products are a spliced mRNA with a new 5'-3' phosphodiester linkage and a released intron lariat containing a 5'-A phosphate group.

So, the splicing mechanism of group one and group two introns are, the splicing mechanism of the board group one and group two intron in all, the similar step of the two-transesterification reaction in which our ribose, 2 prime or 3 prime hydroxyl groups makes a nucleophilic attack on the phosphorus and the new phosphodiester bond is formed at the expense of the old.

Which means, you can imagine that if this is a group one and group two, then there will be attack of the nucleophilic attack from the nucleotide, what is present here on to the nucleotide attack onto this and that, so, this particular region is actually going to spliced out and that is how it is actually going to form a linkage.

So, this means this OH is actually going to connect to this, which is the phosphate group what is present here and that is how it is actually going to form a phosphodiester linkage and that is why this exon and this is the exon, got connected and this is the splice, the introns which is going to be removed.

See, mechanism of these group transfer in nucleophile which is used, the group one uses the three prime hydroxyl group of the guanine nucleotide as a nucleophile. The group one introns, are found in some nuclear mitochondrial and chloroplasts that code for the ribosomal RNA, messenger RNA, and the tRNA, whereas in the group two, it is actually going to be formed by the Lariat formations.

And the Lariat formation also, whether it is a group one or group two, the mechanism is always the same, except that you are going to have the A at the nucleotide. What is going to be utilized for the nucleophilic attacks and then it is actually going to attack onto this and then there is going to form a Lariat, this Lariat and then this OH is going to attack onto the this chain at that point it is going to form the exon, so these two exams are going to be connected and that is how it is actually going to release the intron in the form of Lariat.

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**POST-TRANSCRIPTIONAL MODIFICATION**

**m-RNA: Steps in RNA processing:**

**Alternative splicing:** Alternative splicing is a method substantially used for many mammalian genes can result in multiple products that vary structurally and functionally from the same primary transcript. Sometimes, alternate splicing is unregulated phenomenon while in some it is strictly regulated. One of the best examples of regulated alternative splicing occurs in **sex determination in drosophila**. In drosophila three genes are involved in sex determination sex lethal gene (sxl), transformer gene (tra), double sex gene (dsx).

Due to alternative splicing, functional genes are produced in females and non-functional genes are produced in males. Alternative splicing occurs using two mechanisms - one when two poly (A) or cleavage sites are available in primary transcript. Cleavage occurs at either site resulting in two different products. Such mechanism is followed by **variable domains of immunoglobulins heavy chains** and their diversity is due to this mechanism of alternative splicing. Similarly alternative splicing with such mechanism results in **production of two different hormones**- calcium regulating hormone in rat thyroid and calcitonin-gene related peptide in rat brain. Other mechanism involves more than one 3' splice site for one 5' splice site. Hence splicing occurs by taking either of those 3' splice site resulting in different products.

Apart from this splicing mechanism, you can also have the alternate splicing mechanisms. So, alternative splicing means you can actually have the distinction between the different types of splicing event and that is why you can actually have the, alternative splicing in such a way that you can actually be able to have the different types of genes.

For example, and that is very common in the case of the, so alternate splicing is a method substantially use for many mammalian genes can result in the multiple product that very structurally and functionally from the same primary transcript. Sometimes, the alternate splicing is upregulated phenomena, while in some it is strictly regulated. One of the best examples of the regulated alternate splicing occurs in the sex determination of Drosophila.

In *Drosophila*, three genes are involved in sex determinations, sex lethal genes, or *sxl* one, transformer genes, *Tra* genes, and the double sex genes and these genes are getting the know, the going for the alternate splicing. So, for example, in the alternate splicing, what will happen is that, you are actually going to have the, so this is the gene, for that particular protein, right, and it is going to produce the primary RNA.

Now, in the primary RNA, you have the Exon 1, Exon 2, Exon 3 and Exon 4. Now, if these four exons, can actually be able to do the alternate splicing in two ways, in one way, it is going to take the one, two, and three. So, if it takes a one, two and three, it is actually going to form the protein A, which means it is going to have the coding sequence what is present in the Exon1, Exon2 and Exon3.

Now, in the second example, it can actually take the exon1, it can take the exon3 and it can take the exon4. So, if that happens, and then it is actually going to form the protein B, so you can have the exon1, exon3 and exon4 and that is why you see that the same gene, you have the only one gene and that gene is actually providing the two different types of protein.

The protein A or Protein B depending on whether it is going with the alternate splicing number one, or it is going with the alternate splicing mechanism to and these, that site, the number of proteins can be very, very high and that is why it is actually going to help in terms of the organism not to increase his genome size, but at the same time it actually can help in terms of you know, producing the different types of proteins.

You can even consider like it can actually go with like one, go with three and four, that is what we said. It can be even like this also, one going with two then going with the four. So, alternative splicing is a very robust mechanism or robust tool, what is present in the eukaryotic cell and that is actually going to give you the different types of products from the single gene. Due to the alternate splicing, the functional genes are produced in female and non-functional genes are produced in the male.

Alternative splicing occurs that by the two mechanisms, one we in, by the two mechanisms, one when two poly (A) or cleavage sites are available in the primary transcripts or the cleavage occur at the one side resulting in the two different products. Such mechanism is followed by the variable domain of the immunoglobulin heavy chain and their diversity is due to a mechanism of the alternate splicing.



Similarly, you can have the alternate splicing with such mechanism is the production of two different hormones, you can have the calcium regulating hormone in the red thyroids, and the calcitonin gene related peptide in the red brain. The other mechanism involved the more than 3 prime splicing sites for the one, 5 prime splicing site. Hence, splicing occur in the taking either of these two three results in the different products.

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**POST-TRANSCRIPTIONAL MODIFICATION**

**m-RNA: Steps in RNA processing:**

Such mechanism is followed by variable domains of immunoglobulins heavy chains and their diversity is due to this mechanism of alternative splicing.

Similarly alternative splicing with such mechanism results in production of two different hormones- calcium regulating hormone in rat thyroid and calcitonin-gene related peptide in rat brain. Other mechanism involves more than one 3' splice site for one 5' splice site. Hence splicing occurs by taking either of those 3' splice site resulting in different products.

The diagram shows a 'Gene' at the bottom with arrows pointing to 'IgG', 'heavy chain', 'light chain', and 'J-chain' components of an antibody structure.

So, here we have a different type of examples, where you can actually be able to use this alternative splicing. One of the classical example is the IgG. So, IgG, you know that IgG has the two chains. One is the heavy chain and the other one is called as the light chain. So, alternative splicing within these chains are, and then you also have the J chain, which is also called as junction chain.

So, IgG you know that the different types of antibodies can be produced according to the different types of organisms and all different types of antigens. And all these antibodies are having the different types of structures, different types of sequences at their variable region. So, if you see the antibody structure, you have this antibody, this is antibody structure, where this is the heavy chain.

This is the heavy chain and this is the light chain, but this region is actually called as the antigen binding region and this region is very much variable. So, from where this variation comes? You do not have those many numbers of genes, what happened is that, these different combination of the genes and different combination of the exons when they come together, they are actually been responsible for the formation of different types of antibody molecules.

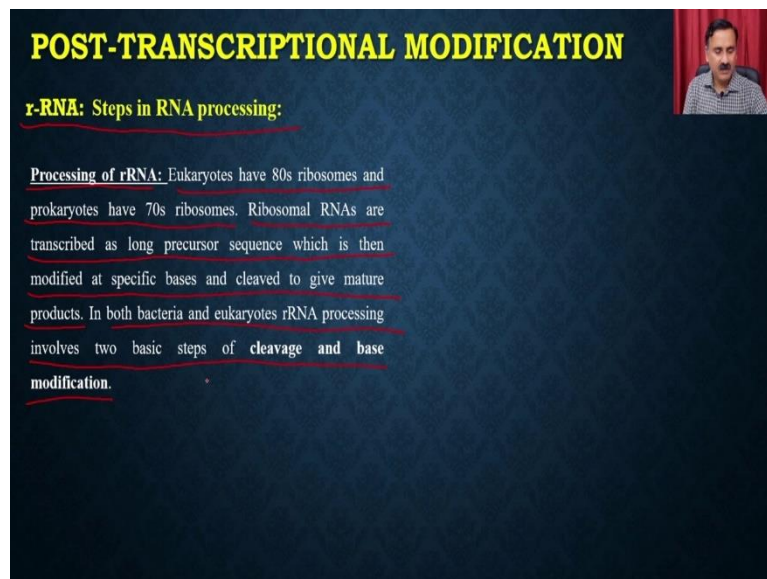


And that is how they are actually been responsible for providing the diversity within the antibody structures. So, because of the alternative splicing, you can be able to produce the different types of the antibody molecules. Similarly, you can have the alternative splicing, which can also be responsible for the production of two different types of hormones.

One is calcium regulating hormone, which is going to be produced at the rat thyroid and the other hormone which is going to be calcitonin-gene related peptide, which is going to be in the rat brain. So, from the same gene, you can have the two different types of hormones, you can have the calcium regulating hormones in the thyroids, and you can also have the calcitonin-gene related peptide in the case of brain.

So, hence the alternative splicing is a very easy, robust tool, what is present in the eukaryotic system and that is also going to responsible for producing the different types of proteins.

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**POST-TRANSCRIPTIONAL MODIFICATION**

**r-RNA: Steps in RNA processing:**

Processing of rRNA: Eukaryotes have 80s ribosomes and prokaryotes have 70s ribosomes. Ribosomal RNAs are transcribed as long precursor sequence which is then modified at specific bases and cleaved to give mature products. In both bacteria and eukaryotes rRNA processing involves two basic steps of cleavage and base modification.

Then, we will talk about the processing of the messenger ribosomal RNA. So, processing of ribosomal RNA, eukaryotes have the 80s ribosomes whereas, the prokaryotic have the 70s ribosomes. Ribosomal RNase are transcribed as a longer precursor sequence which is then modified at the specific basis and cleaved to keep you the mature product.

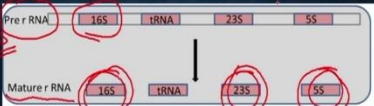
In both bacteria as well as the eukaryotes, RNA processing involved are two basic steps the cleavage and the base modifications.

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## POST-TRANSCRIPTIONAL MODIFICATION

**r-RNA: Steps in RNA processing:**

**rRNA processing in Bacteria:** rRNA precursor in bacteria is a 30s rRNA which modified and cleaved to give 23s rRNA, 16s rRNA, 5s rRNA and some t-RNA segments in between are also there sometimes. 30s pre-rRNA transcript consist of 16s rRNA sequence followed by spacer which may have t-RNA sequence in some cases and then there is 23s rRNA sequence followed by 5s rRNA sequence near to 3' end. At times there is one more t-RNA sequence after 5s rRNA sequence at 3' end. There are seven different genes for rRNA in E.coli, they are essentially similar in sequence of rRNA segments but differ with number and sequence of t-RNA segments. Maturation process involves methylation of 30s rRNA precursor at specific sites occurring at 2'hydroxyl groups of bases. Some bases such as uridine is modified to pseudouridine or dihydrouridine. Further cleavage process is carried out using enzymes RNase III, RNase P, and RNase E at sites 1, 2 and 3 respectively. Intermediate products are formed namely 17s, tRNA, 25S and 5S. These are acted on by certain nucleases to give final products of 16s, tRNA, 23s, 5s rRNA respectively.



The diagram illustrates the maturation of a 30S pre-rRNA transcript. The pre-rRNA transcript is shown as a single bar with segments labeled '16S', 'tRNA', '23S', and '5S'. Three cleavage sites are indicated by vertical lines labeled 1, 2, and 3. Below the transcript, the mature products are shown: '16S', 'tRNA', '23S', and '5S'. Red circles highlight the 16S, 23S, and 5S segments in both the pre-rRNA and mature products. A red arrow points from the pre-rRNA to the mature products.

So, RNA processing in the bacteria is different. So, RNA processing in bacteria it is it, so rRNA precursor in bacteria is a 30s ribosomal RNA which is modified and cleaved to give the 23 ribosomal RNA, 16s ribosomal RNA, 5s ribosomal RNA, and some t-RNA segment in between are also sometimes, so you what you have is you have the pre-ribosomal RNA in the bacteria and it is going to be a 16s ribosomal RNA.

That is going to be transcribed and that 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 16s ribosomal sequence followed by a spacer which may have the t-RNA sequence in some cases and then there is a 23 ribosomal sequence followed by the 5s ribosomal sequences near to 3 prime end.

At times there is a one more tRNA sequence after the fibres ribosomal RNA sequence at the 3 prime end. There are several, 7 different genes for the ribosomal RNA E.coli they are essentially similar in the sequence of ribosomal segments, but differ with 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, what you have; at the specific site occurring at the two prime hydroxyl group of the bases. Some bases such as uridine is modified to pseudouridine or dihydrouridine. Further cleavage process is carried out using the enzyme RNase III, RNase

P, and RNase E at the site one, two and three respectively. Intermediate products are formed namely 17s, tRNA, 25s and 5s.

These are acted on by certain nucleases to keep the final product of 16s, tRNA, 23s and the 5s ribosomal RNA. So, decide this is what you are going to have, you are going to have mature ribosomal RNA, which is going to be 16s, 23s and 5s and these will come assembled and give you the 70s ribosome.

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**POST-TRANSCRIPTIONAL MODIFICATION**

**r-RNA: Steps in RNA processing:**

**rRNA processing in Vertebrates (Eukaryotes):** In Eukaryotes nucleolus is the centre of processing ribosomal RNA. A 45s precursor is formed by RNA polymerase I and processed in 90s preribosomal nucleolar complex to give 18s, 28s, and 5.8s rRNA. There is tight coupling of RNA processing with ribosomal assembly. 5s rRNA is transcribed by RNA polymerase III from a separate gene. Precursor RNA undergoes methylation at more than 100 bases from 14000 nucleotides at 2' hydroxyl group. Furthermore there is modification of bases such as uridine to pseudouridine etc. followed by series of cleavage reaction. Cleavage and modifications are guided by snoRNAs (small nucleolar RNA). In yeast, entire processing involves pre-rRNA, 170 non-ribosomal protein, 70 snoRNA and 78 ribosomal proteins. snoRNA are supposed to be remnant of spliceosomes.

Pre r RNA	18S	5.8S	28S
Mature r RNA	18S	5.8S	28S

Similarly, you can have the ribosomal processing in the vertebrates or the eukaryotes. So, in the eukaryotes, the nucleolus is the centre of processing of ribosomal RNA, at 45s precursor is formed by the RNA polymerase one and processed in the 90s preribosomal nuclear complex to give the 18s, 28s and 5.8s ribosomal RNA.

There is a tight coupling of the RNA processing with the ribosomal assembly. 5s ribosomal RNA is transcribed by the RNA polymerase III from a separate gene. So, you, in this case, you can have the preribosomal RNA, which is going to have the 18s, 5.8s, 28s, so it is going to form a big transcript and then it is going to have the cleavage event. So, this cleavage event is going to give rise to the 18s, 5.8s and 28s and then you also going to have the 5s ribosomal RNA, which is going to be transcribed by separately.

And then the precursor RNA undergoes methylation at more than 100 bases from the 14,000 nucleotide at 2 prime hydroxyl groups. Furthermore, there is a modification of bases such as

uridine to pseudouridine, et cetera, followed by a series of cleavage reactions. So, cleavage and modifications are guided by the small nucleolar RNA.

In yeast, the entire processing involves preribosomal RNA, 170 non-ribosomal proteins, 70 snoRNA and the 78 ribosomal proteins, and this is what is going to happen. So, this preribosomal RNA is going to form, then it is going to form it is going to get the cleavage, and it is going to form the 18s, 5.8s, and 28s and then there will be a separate synthesis of the 5 subunits and all these are going to assemble to give you the 80S ribosomes.

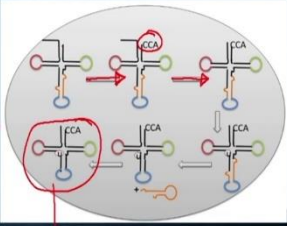
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**POST-TRANSCRIPTIONAL MODIFICATION**

**t-RNA: Steps in RNA processing:**

Processing of t-RNA: In both Eukaryotes and prokaryotes t-RNA processing occurs. It is transcribed as long precursor; sometimes single primary transcripts carry more than one t-RNA segments which are separated by cleavage.

Processing of pre tRNA involves cutting off of extra sequences by endonucleases such as RNase P at 5' end and RNase D at 3' end. RNase P is a ribozyme with RNA exhibiting catalytic activity. After removal of sequences from 3' end, CCA sequence is added via enzyme tRNA nucleotidyltransferase. This enzyme binds to CCA sequence at its active site and phosphodiester bond is formed with 3' end. Furthermore, there is base modification occurring simultaneously such as methylation, deamination or reduction; in case of pseudouridine, uracil is removed and reattached to sugar through C5.



Translated

Then they also can have the RNA processing in the tRNA. So, in the case of tRNA, you can have the base modifications and you can also have the different types of modifications. So, what is the processing in the tRNA? In both the eukaryotic and prokaryotic t-RNA processing occurs. It is transcribed as a long precursor; sometimes single primary transcripts carry more than one t-RNA segments which are separated by the cleavage.

Processing of pre tRNA involves the cutting off of extra sequences by the endonucleases such as RNase P at the 5 prime end and RNase D at the 3 prime end. So, in the tRNA molecules what you have, is you have the extra sequences. So, in the extra sequences at the 5 prime end is going to be cleaved by the RNase P and it is the extra sequences at the 3 prime end is going to be cleaved by the RNase D.

RNase P is a ribozyme with the RNA exhibiting catalytic activity. After removal of the sequences from the 3 prime end, the CCA sequence is added by an enzyme tRNA

nucleotidyltransferase. So, once the 3 prime end is going to be cleaved by the RNA is deep, then the 3 prime end is going to be added like CCA and is going to be added onto the A prime and with the help with the help of an enzyme which is called as tRNA nucleotidyltransferase.

Now, this is, this end is over, right, the 3 prime end is over. This enzyme binds to the CCA sequence at its active site and phosphodiester bond is formed with the 3 prime end. Furthermore, there is a base modification occurring simultaneously such as methylations, demanations or reduction, in case of the pseudouridine Uracil is removed and reattached 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, and that is how ultimately you are going to have the final mature tRNA which is going to be formed and this tRNA is going to transfer, participate into the translation. So, this is all about the transcriptions. And what we have discussed so far?

We have discussed about the transcription in prokaryotes and then we also discuss about the different steps like we have discussed about the initiation transcription and translation and we have also discussed about terminations. So, you can have the intrinsic terminations or rho dependent terminations, and then we also discuss about the transcription in the eukaryotic cells or eukaryotic system.

And where we have discussed about the composition of the RNA polymerase and different core, different transcription factors which are responsible for the formation of the initiation complex and then we also discuss about the promoter sequences, and all of the kinds of machinery, what is required for the transcription and then we discussed about the post transcriptional modifications, where we discuss about the tailing as well as the capping in the case of messenger RNA.

Then, we have discussed about the splicing of group one, group two and all other kinds of introns and then we also discuss about the relevance of the alternate splicing and how the alternative splicing is giving rise to the different types of proteins from the single gene. And then we also discuss about the RNA processing in the case of ribosomal RNA and as well as the 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.