RNA Biology
Prof. Rajesh Ramachandran
Department of Biological Sciences
Indian Institute of Science Education and Research, Mohali

Lecture - 13
RNA Transcription: Termination and RNA Modification

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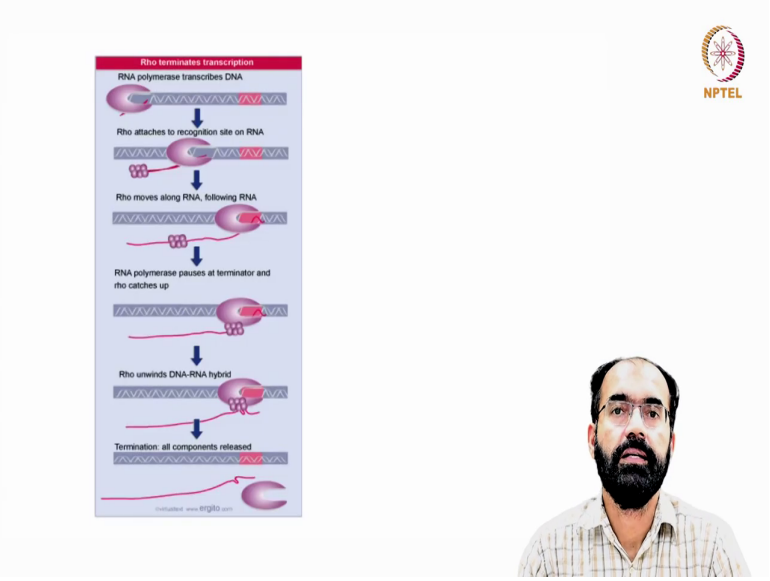

Hello everyone welcome back to another session of RNA Biology. So, we were on this slide in the previous class that is how two different mechanism of transcription termination takes place in bacteria rho independent and rho dependent. Rho dependent was the one which we were studying and as the name itself indicate rho independent do not require the protein rho rho factor or rho, rho and rho dependent require this protein that is named as rho rho factor.

And the concept also we have seen in one the rho independent mechanism you have to have a unique secondary structure that need to be formed by inverted repeats that is present in the RNA's end portion or where the termination is supposed to happen. Whereas, in the rho dependent situation the rho protein has to come and attach to the specific recognition site on the RNA and the RNA takes along the rho factor or the rho protein moves along with this RNA until the termination signal.

And the RNA polymerases poses at the terminator end that means, RNA polymerase also can have an identification where it is supposed to end because there are termination signals

present in the DNA itself. However, RNA polymerase is not in a position to detach or disassemble the complex it will stall and this stalling or this reduced rate of movement of the RNA polymerase can give hint to the rho protein.

So, that the RNA polymerase will pause at the terminator sequence region and rho will start into action and the rho unwinds the DNA RNA hybrid. What is DNA RNA hybrid? DNA which acting as a template and the RNA that is being formed, they pair together like a two stranded DNA itself, but in a normal DNA you have a sense strand and antisense strand both strands are DNA itself.

In RNA DNA hybrid the template strand is DNA and the other strand is RNA that is RNA DNA hybrid and the DNA RNA hybrid will be removed or detached and the termination takes place that all components are released to DNA, the newly formed RNA and the ribosomes will be released this is how the termination takes place.

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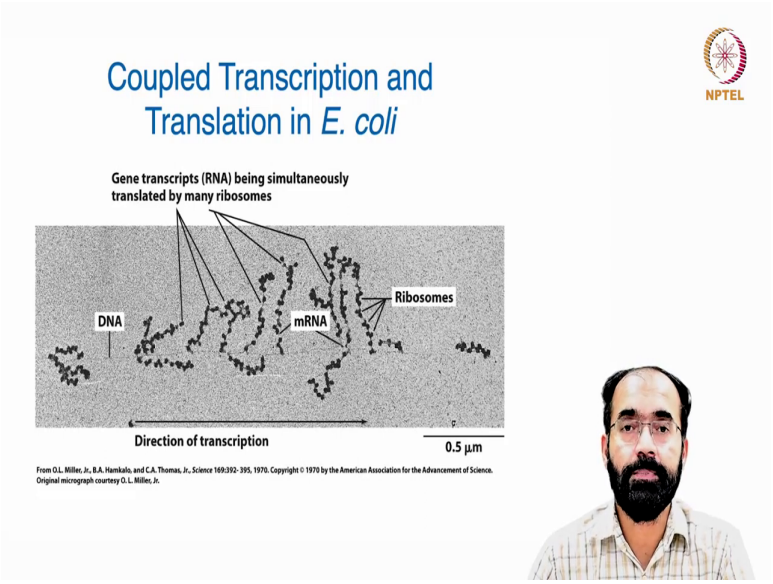

So, in the next picture you can see the coupled transcription and translation in E. coli. What is coupled transcription and translation? If the transcription and translation are occurring simultaneously, we call it as coupled transcription and translation. What does it mean?

Say if an RNA is 1000 bases long ok 1000 bases long can take a good amount of time to complete; however, if coupled transcription and translation is taking place as soon as the

RNA is formed say only 100 bases are formed then that part of the RNA itself can start the protein synthesis the translation will start.

Means, the entire RNA is 1000 base, but only 100 base is released from the RNA polymerase and the DNA combination. But that exposed part of the RNA can participate in the translation event and as the RNA keep growing the protein also keep increasing until the end point of the RNA or the stop codon of the RNA.

So, this is called coupled transcription and translation. So, this is possible in bacteria simply because there is no dedicated nuclease there is a chromosomal DNA single circular chromosomal DNA present in the bacteria which is attached on to the cell wall or the outer membrane of the bacteria that is the just like you are hanging a cloth on a stand or a fan hanging from the roof something like that.

The DNA is attached on to the cell membrane of the bacteria and then it is exposed directly to the cytoplasm which contains the translational machinery that is why coupled transcription and translation becomes possible let us see in detail. The gene transcript RNA that is being simultaneously translated by many ribosome that is another thing which you should understand if one ribosome binds onto one RNA.

It can give rise to one protein if one more ribosome binds it can give another protein if one more binds another protein. So, one RNA ribosome is not a huge structure, it is reasonably smaller in structure; that means, one RNA can simultaneously harbor 1000s of ribosomes. So, each ribosome basically is a pair a larger subunit and smaller subunit two subunits are there just like you know remember you are holding a pen in between your palms left palm and right palm and in between you have a pen you are holding it.

Here two subunits are holding the RNA in middle and each pair of ribosomes is capable of giving rise to one protein because this ribosome will travel from the first codon till the last codon. So, it can give rise to entire protein. So, as soon as one ribosome moved out of the first codon say it reached up to the third codon, then immediately another pair of ribosome sits.

So, now two pairs of ribosome one pair first pair and the second pair of ribosomes are binding and once they have moved out a third pair will join. So, just like a like a queue

normally to get into a train or flight you will stand in queue right. So, like that the ribosomes are standing in queue.

So, this is a real picture of the coupled transcription and translation in E. coli this strand DNA labeled is a thin strand if you look carefully you can see like a very small tiny line that you can see across this picture. And then you can see mRNA that has come out and remember bacteria do not require any RNA processing.

So, that is another advantage in eukaryotes even if there is no nucleus it is not possible why because majority of the eukaryotic gene do have introns that has to be removed and processed. So, since this RNA from bacteria do not have any such limitations the formed RNAs ready to function.

So, you have a DNA and you have got RNA that is formed and one more thing you should remember when there is a regulatory sequence on a particular gene upstream of a gene if there is one polymerase is bound on to that it will start walking does not mean that one more polymerase cannot bind. So, one polymerase bound and the RNA production started and another polymerase bound and again RNA polymerase function starts.

So, from one gene 1 RNA, 2 RNA, 3 RNA, 4 RNA like that it can keep on coming at different stages of completion none of them have completed this 1000 or 2000 whatever may be the length of the gene. So, at a different stages across this DNA you can see several RNA hanging and each RNA have got multiple ribosomes and each of this ribosome give rise to one protein sequence.

So, this way the coupled transcription and translation will enhance the rate of production of protein. So, within no time the protein level required for a cell can be achieved. So, it saves lot of time which is very important for bacteria because one bacteria's replication time for an E. coli at 37-degree Celsius replication time is roughly 20 minute. So, in 20 minute 1 bacteria has completed its life and so, this coupled transcription and translation becomes very handy.

So, what you see this dark color dot they are the ribosome and such multiple ribosome binding on to mRNA we refer to them as poly ribosome that is multiple ribosomes are binding on to 1 mRNA and this 0.5 micron is the scale. Remember 1000 micron 1000 microns is 1 millimeter.

So, half micron means you can imagine how small it will be and the direction of transcription is shown with this arrow head and so, maybe starting point will be somewhere here and you have got different stages of completion of RNA and protein synthesis exist.

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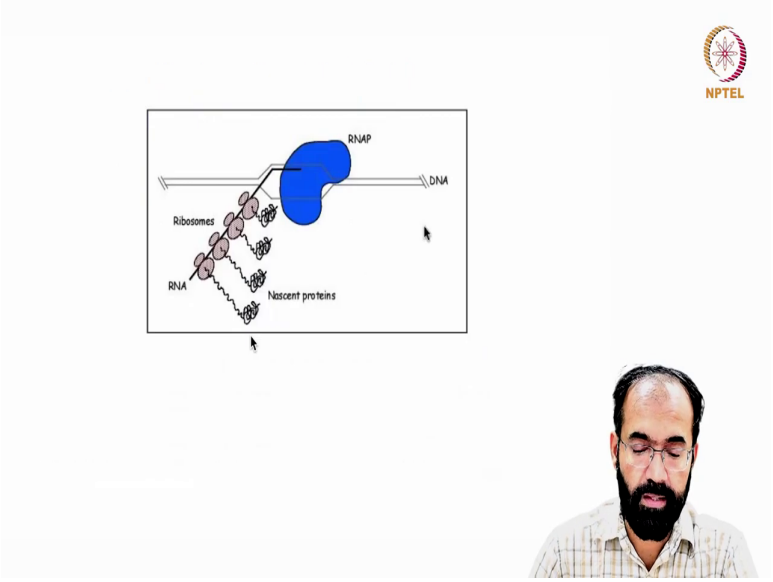

So, this is the cartoon of what we have already seen. So, this is the double stranded DNA and there is a replication or the transcription bubble, the DNA has kind of separated and the transcription bubble has moved from one end to the other as a result of which you have RNA that is coming out of this DNA or the transcription bubble and this RNA is now bound with 1 pair, 2 pair, 3 pair, 4 pair like that and each of them have got a varying length of nascent protein.

So, RNA transcription has not completed and the protein translation has not completed they are at different stages of completion.

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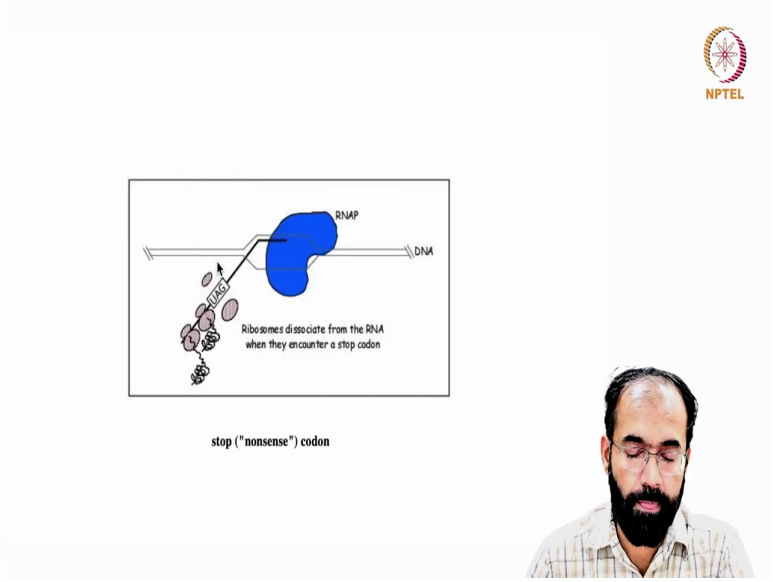

So, until this will continue until this top codon UAG; UAA, UAG, UGA these are the stop codon any one is enough not all the 3 is necessary in a given gene any one is enough as soon as the stop codon has reached the ribosomes will disassemble and the protein will come out of this translational machinery.

So, ribosomes dissociate from the RNA when they encounter a stop codon and the RNA has not completed its transcription yet, because it has to have the termination signals etcetera, but; however, that also will happen either in a rho dependent or rho independent manner depending upon which gene we are talking about and the RNA polymerase also will disassemble.

And remember once this RNA is released into the cytoplasm from the DNA, again it can participate in the translation. So, do not think that it can translate only when it is attached onto the; onto the DNA itself or the RNA polymerase now such rule is there. So, this will enhance. So, what is the benefit of coupled transcription and translation? It will enhance the rate of production of the protein.

And another advantage also is there if the RNA is exposed it is vulnerable for degradation. So, if the RNA is readily bound with ribosome, it will get protection from the various nucleases. So, this is the another advantage. So, enhance the speed of production of protein and also provide protection to the RNA from degrading nucleuses.

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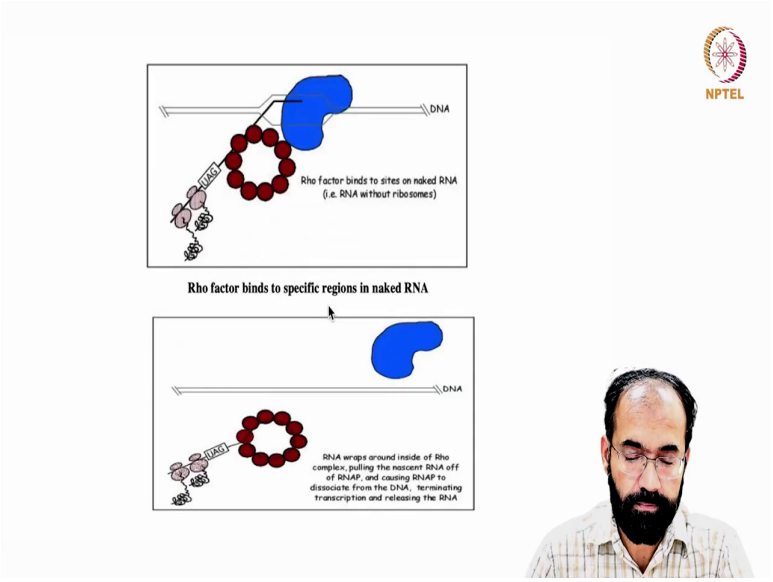

And if rho factor binds to the sites onto the naked DNA as soon as this is completed then it can enhance the rate of detachment. So, RNA wraps around inside of the rho complex pulling the nascent RNA off the RNA polymerase RNAP means, RNA polymerase and causing the RNA polymerase to dissociate from the DNA terminating the transcription and releasing the RNA.

Remember there are multiple ribosomes are standing in queue. So, first one only has completed and the second third fourth like that it will continue to complete and newer and newer ribosomes are adding from the 5 prime end.

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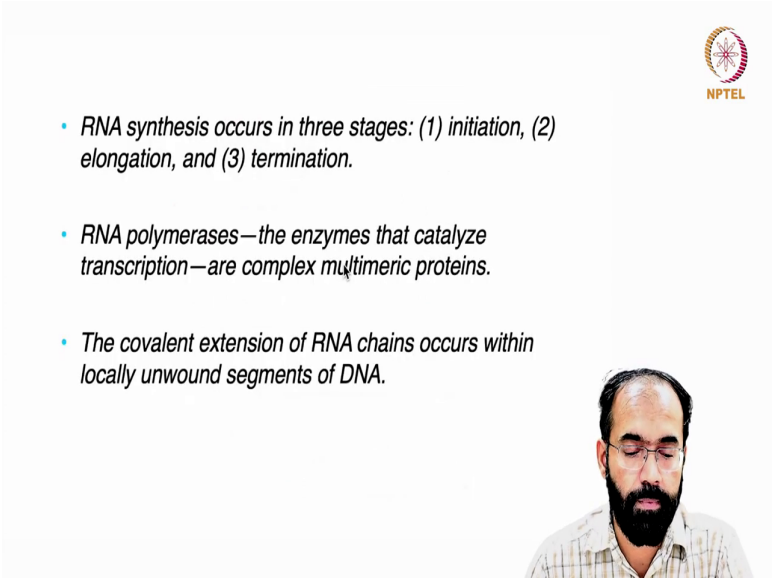

So, RNA synthesis in general occurs in 3 stages initiation, elongation and termination and the RNA polymerase the enzymes that catalyze the transcription are complex multimeric proteins we have seen it there is a tetrameric core and also sigma factor. So, that forms the holoenzyme. The covalent extension of RNA chains occurs within locally unwound segment of DNA. So, this is the recap of what we have seen so far.

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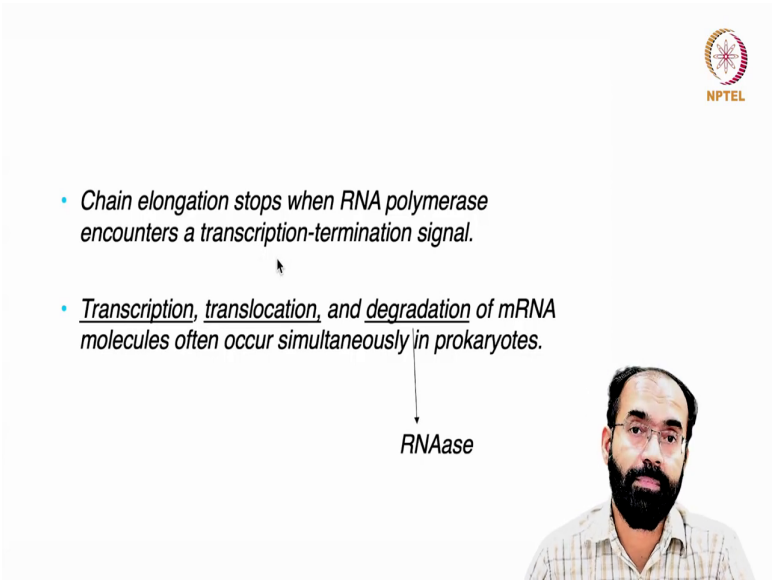

So, chain elongation stops when RNA polymerase encounters a transcription termination signal. The detachment or the dissociation is happening either in a rho dependent or rho

independent manner. So, transcription, translocation and degradation of mRNA molecules often occur simultaneously in prokaryotes; that means, the gene regulation happens at different levels.

However, the transcription and the translocation of these RNA into the ribosomal machinery and the translation even and also the degradation of the RNA happened almost instantaneously because the lifespan of bacteria is quite short. So, it has to complete the whole process in no time otherwise it will not be able to make a living and the degradation is often accomplished by dedicated protein enzymes such as RNAases.

Different types of RNAses are there, but RNAses in general are targeting the RNA molecules freely present in the bacteria same thing happens in eukaryotic cell also. So, if an RNA has to survive it has to fight against all the RNAases or any degrading factors in our body also our body fluids like your sweat saliva tears everywhere you have RNAse. So, if you are working with RNA you have to be very careful to prevent the degradation of your working material in your test tube.

So, because RNAse is there everywhere and it can cause its a protection you may wonder why RNAse need to be present because its a protection from various RNA viruses without which we will not be able to make a smooth living. So, thanks to our RNAases being produced even from our body fluids that will protect us from various infections.

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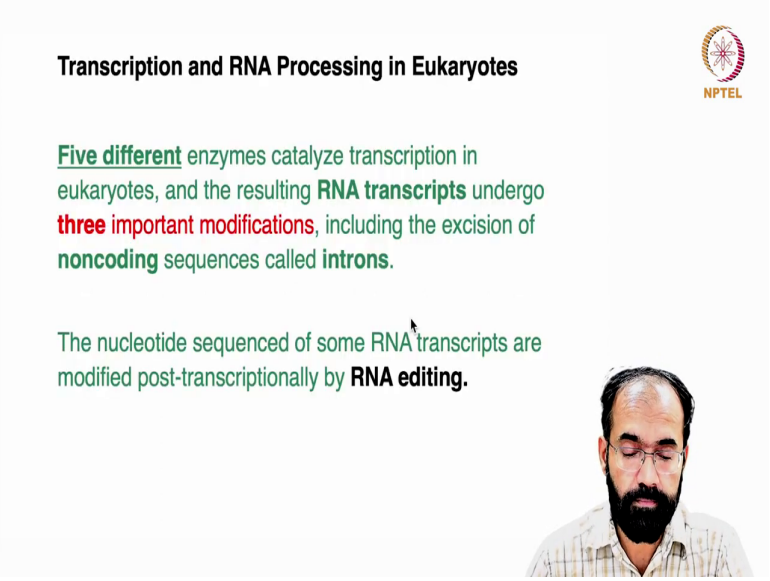

So, let us look into the transcription and RNA processing in eukaryotes how does this happen? There are five different enzymes that catalyze the transcription in eukaryotes and the resulting RNA transcripts undergo three major modification three important modification including the accession of the non coding sequence called introns.

So, this RNA production is governed or controlled by different types of polymerases. So, there are at least five different different RNA polymerases are there in eukaryotes. So, the nucleotide sequenced of some of the RNA transcripts are modified post transcriptionally by a process called RNA editing. We will see in detail about RNA editing time being you keep the idea clear in mind that RNA once it is formed need not necessarily have the same sequence.

So, RNA editing can be considered as some kind of mutation. Sometimes you may have seen people who do not like the shape of their nose or maybe part of their body they do plastic surgery and they increase the length of their nose or they decrease the length of their nose like that right that is the surgical intervention like that an RNA form also undergoes some changes say an A will change into C or A G will change into U.

So, like that RNA also edit not randomly anywhere at specific (Refer Time: 17:27) specific location and RNA editing is so, important that if some particular editing do not happen the animal will not survive. So, that much; that means, in the genome you have got a particular base and the RNA is formed corresponding to that, but you do not want that base to be there.

If you do not change that base the organism do not survive it will not develop it will not complete its embryonic development. So, RNA editing is as good as any other important process. So, we will see about RNA editing more in detail.

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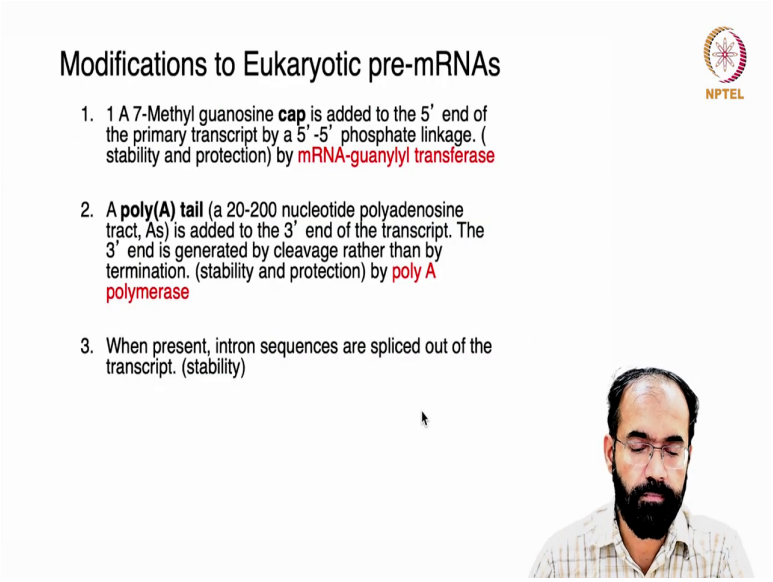

So, what are the modifications of eukaryotic pre mRNAs? So, one of the important one is A 7-Methyl guanosine cap that need to be there is a number 1 located here you can ignore it that is not the two ones have come. So, basically A 7-Methyl guanosine cap is added at the 5 prime end which is a post transcriptional modification at the 5 prime end of the primary transcript by a 5 prime 5 prime phosphate linkage.

So, normally we know the 5 prime is the end of a nucleotide that is the phosphate group is there and the 5 prime carbon of the ribose sugar is the one which is having the phosphate group and now the 3 prime carbon of the ribose sugar will have a hydroxyl group. So, the phosphor diester backbone takes place between the 3 prime OH and the 5 prime phosphate group like that it continues.

Now, here what happens? The 5 prime end of the nucleic acid or the RNA is now paired with another nucleotide whose 5 prime is being paired. So, there is a 5 prime 5 prime pairing takes place exposing the 3 prime. So, what will happen? Because of the 7 methyl guanosine cap the 5 prime end looks like a 3 prime end. So, this is the modification that happens and we will see more in detail about its relevance.

So, this enzyme responsible for that is guanylyl transferase mRNA-guanylyl transferase. Transferase in biology whenever you hear the word transferase means it is adding some transferring something kinase means it is adding phosphate. So, that is how the nomenclature is. So, guanylyl transferase means adding a guanine and that is having a unique its not any

other guanine it is having A 7-Methyl group its A 7-Methyl guanosine cap is there added on to the 5 prime end of a newly formed mRNA that is the 5 prime cap.

And then a poly A tail is added on to the 20 to 200 nucleotide long poly A tail. A stands for adenine poly A means multiple times A A A A A like that you keep counting how many times? It can be up to 200 nucleotide long poly adenosine track is added at the 3 prime end of the transcript.

So, the 3 prime end now generated by usually generated by the cleavage rather than termination. So, what happens whenever there is a transcription termination signal is there, the RNA polymerase continues beyond it for some more bases and then the rho factor or in eukaryote there is other termination signals will come into picture and it will fall off, but the release the release of the RNA has to be helped by certain nucleases.

So, soon after the poly A signal that is the termination signal the nucleases will cut it and as soon as the RNA is released an enzyme called poly A polymerase just like the guanylyl transferase poly A polymerase keep adding A and A and A in A template independent manner. So, far we know RNA transcription require a DNA template, but poly A polymerase its a polymerase means an enzyme that is able to polymerize something.

So, we say RNA polymerase means, it is able to add nucleotides sequentially making use of the template strand, but poly A polymerase will keep on adding A tail irrespective of any template or irrespective of any available template. So, poly A tail is added at the 3 prime end.

Now, the third important point we should understand if present not all eukaryotic genes have intron if present the intron sequences are spliced out the splicing of exon happened exon 1, exon 2, exon 3 exon 4 they are all joined together and the introns are removed out of the transcript and this will add to the stability.

Say all this process happens in the nucleus say if any of this process do not happen or one of the steps like capping failed or poly A tailing failed or the splicing failed then this RNA is marked for degradation we will study about different methods of degradation in the RNA destabilization topic.

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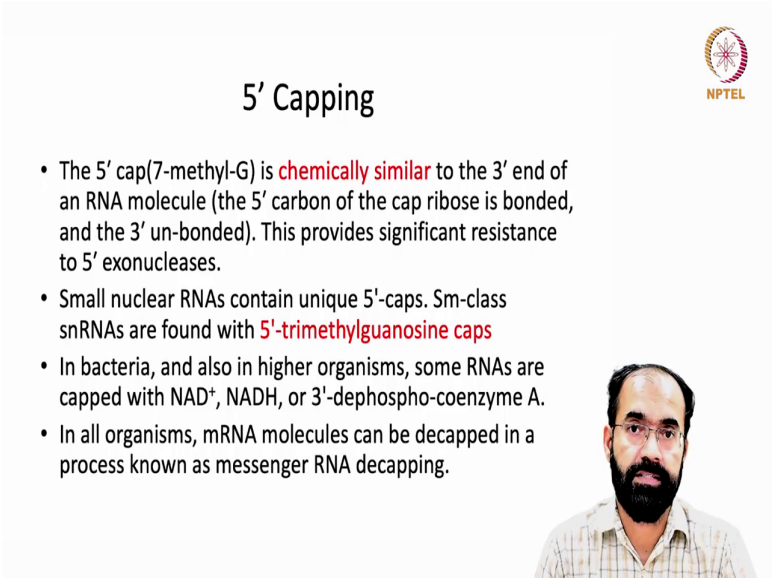

So, let us say more in detail about the 5 prime capping. So, the 5 prime cap which is nothing but 7-methyl-G is chemically similar to the 3 prime end of an RNA molecule like I told you because this nucleotides 5 prime is now bound with 5 prime of the RNA. So, it like you are joined instead of head to tail fashion you are having a head to head fashion.

So, that the tail is exposed and the 5 prime carbon of the ribose that is bonded with the 3 prime that remains unbonded. This provides significant resistance to exonucleases like RNAs are vulnerable for degradation endonucleases are there that cut in between and exonucleases are there that will chew from the edges.

There are 5 prime exonucleases and there are 3 prime exonucleases. So, you want protection from both. So, now, 5 prime exonuclease if it is supposed to come and bound for causing it degradation of an RNA. Then the enzyme exonuclease will get confused because the 5 prime end no more having a 5 prime phosphate group rather it is having a 3 prime OH group and this 5 prime exonucleus cannot degrade the 3 prime end.

Then it gets a protection like a decoy or a like a fooling system like if you any of you have watched some documentaries like there are several man eating tigars in Sundarban. So, lot of tribals go to the forest to collect honey as a part of their livelihood like many many humans make their living using resources from the forest and Sundarban is very rich. So, what they do?

They usually go in a straight line they do not go in group they go in a straight line if some 5 people are go in first fellow, second fellow, third fellow they go in like a straight line and the back most fellow will keep a mask a human face which is facing backward. So, the logic is simple animals normally do not attack from the front they always come from the back and, but since you are wearing a mask then that human face is always looking at the tiger although his real face is front.

So, like that you can see in nature some snakes are there, they will have tail look like its head. So, if any animal comes for attacking it will confused which is head it will not know. So, if it goes to the wrong head then this snake can attack the animal with its real head real mouth.

So, such a decoy or such a you know deceiving system is there even at molecular level. So, small nuclear RNAs contain unique 5 prime caps they are called sn class snRNAs they are found to with specific 5 prime cap that is 5 prime trimethylguanosine cap instead of 7-methyl guanosine cap as you find in mRNAs.

In bacteria and also in several higher order organism some RNAs are capped with NAD, NADH or also with 3 prime dephospho-coenzyme A. So, what we should understand the capping is a protection process just like you put you know shoe to horse horseshoe and also to bullocks you put to protect its hoofs from erosion. So, you put a metal shoes.

So, like that these molecules will deceive the exonucleases and provide stability to the RNA. In all organisms the mRNA molecules can be decapped in a process known as messenger RNA decapping and of course, there are dedicated decapping enzymes present to do this job. So, with this I will stop this topic today's this section and we will resume in another class.

Thank you.