RNA Biology
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Lecture - 17
RNA Processing and Life Cycle: Post Transcriptional Processing

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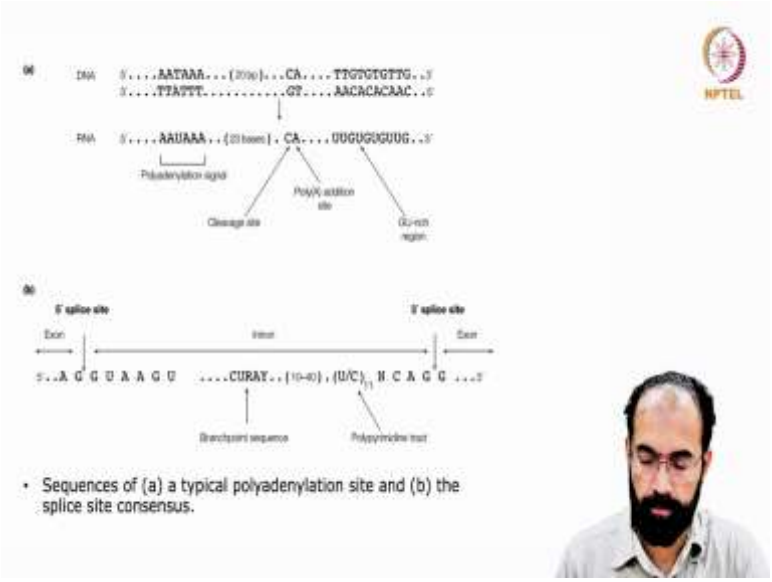

Hello, everyone. Welcome back to another session of RNA Biology and we were here in the last class. And, we were trying to understand the different signature sequence and the specific sequences necessary for the so called intron boundaries and how snRNAs recognize those boundaries, and their typical polypyrimidine track, and the branch point, and the 5 prime splice site are important because they are quite instrumental in this transesterification reaction.

Two transesterification reactions takes place. We will see them more in detail, but more or less it is identical to that of group I and group II introns performance more related to group II intron self splicing intron which we saw in the previous class. And, only thing here in the normal eukaryotic pre-mRNA splicing is it is assisted by spliceosomal machinery which comprise of snRNAs.

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Here you can see a cartoon of how the splicing is taking place, you can see here.

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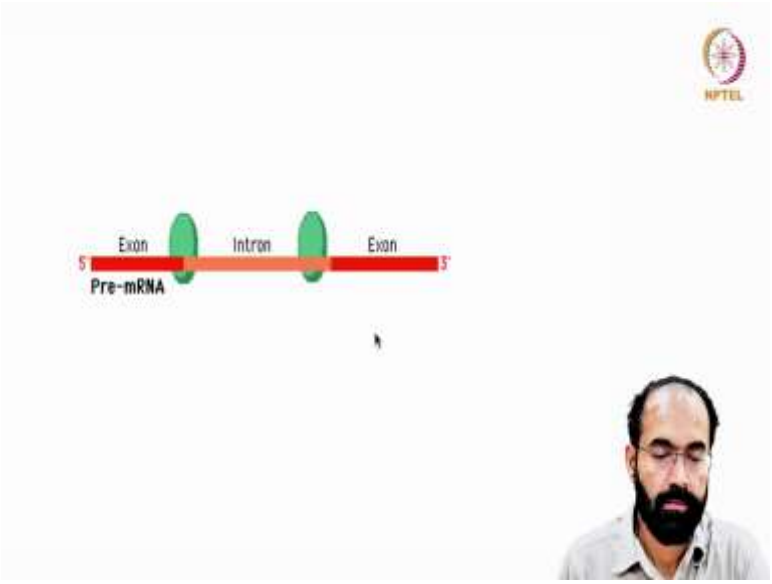

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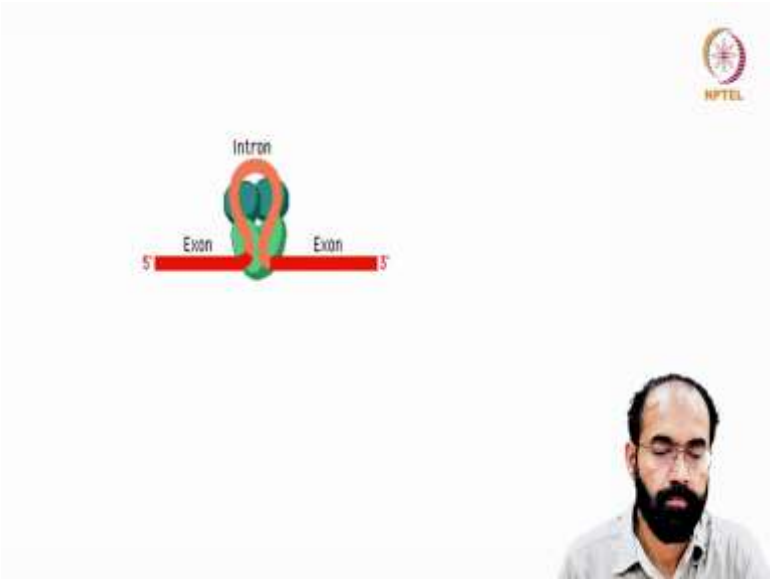

And, the splicing takes place with the help of snRNAs. Once again, I will play. Introns are removed and the RNA is formed. This is the basic principle how the RNA gets matured with the help of spliceosome.

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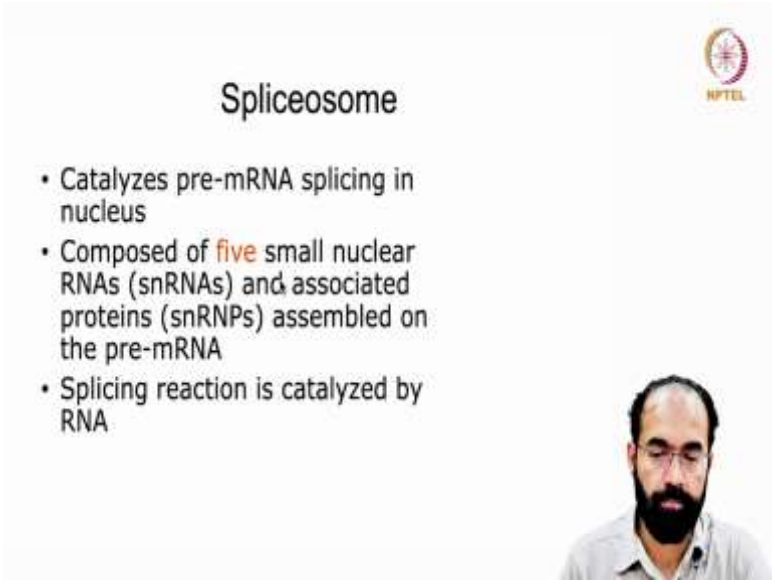

Now, the spliceosome in general, it has mainly components that is both RNA and protein in nature. It catalyzes the pre-mRNA splicing usually taking place in the nucleus. It composed of five small nuclear RNA. We call them as snRNA because it has role in spliceosome or the formation of spliceosome, and they have associated proteins. And, the snRNAs now are referred to as snRNPs.

And, they assembled on the pre-mRNA in a stepwise manner. Spliceosome is not present in a pre-made fashion; it is assembled similar to that of how RNA polymerase different subunits come one at a time. Same logic applies when you are talking about splicing also. Splicing reaction is catalyzed mainly by RNA because the proteins are mainly contributing to the stabilization of the spliceosomal machinery.

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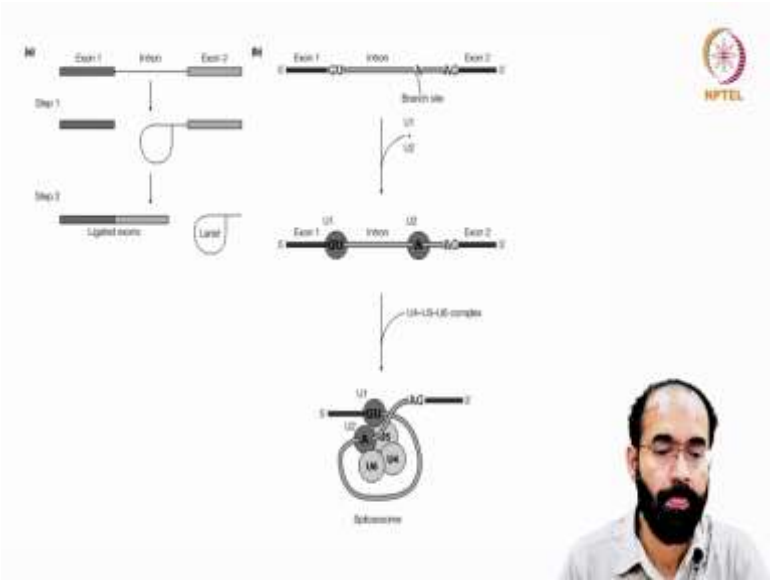

You can see here in this cartoon at left hand side you have an exon, intron and exon. At step 1 it has to form a lariat which is initiated by the branch point A and it forms a intron, intron pairing which then the second free exon, the 3 prime-end of the free exon participate in the second transesterification reaction onto the 5 prime-end of the second exon and it creates a fused exon or ligated exon and the intron lariat is lost.

But, to do that you need to have the boundary of exon - intron boundary should be made very clear, intron starts with GU end with AG and you need to have a branch point and between the branch point and AG that is the end of the intron you need to have a polypyrimidine track. If any of them are missing or any of them are mutated, you will have a splicing defect.

There are several disorders in humans that can come because of splicing defects. We will see them much later in the class. U1 and U2 sRNA they come and occupy the exon – intron boundary here and then the branch point and it also is important this pairing is also important for the proper folding of this intron because as I told you introns can be huge. They can be thousands of kilo bases; they are much much larger than the exons.

So, if the ends have to come closer the introns has to fold or the RNA that contains the intron sequence has to fold which is assisted by the spliceosome so that the transesterification reactions can happen smoothly.

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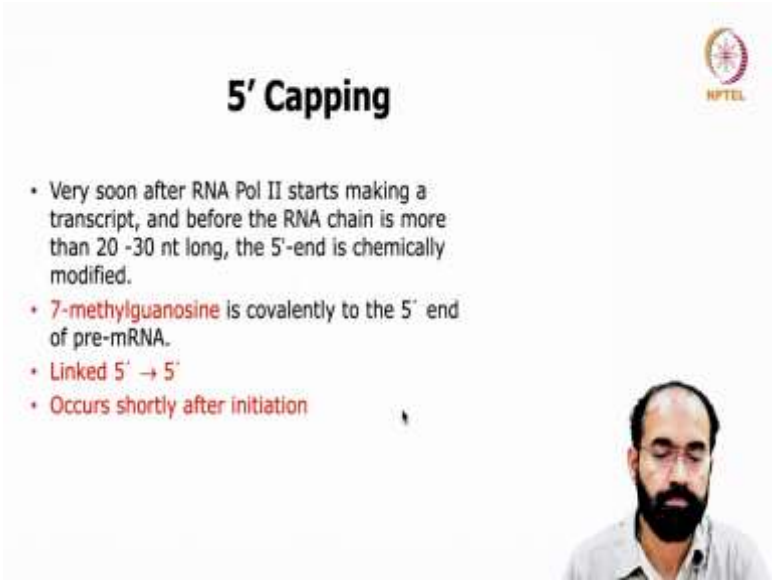

And, the 5 prime capping is like we discussed is very important. So far we know that it is helpful in protection from degradation etcetera, but it also have role in splicing. Soon after the RNA polymerase II starts making a transcript up to around 20–30 nucleotides the 5 prime-end is chemically modified which we know. And, it is the 7-methylguanosine cap onto the pre-mRNA and this is linked by 5 prime 5 prime fashion to mimic the end like a 3 prime and it happens shortly after initiation.

But, important things to notice if an RNA do not have the cap, then it will refuse to participate in the splicing function. So, cap is not only just for protection, but also for facilitating the splicing event.

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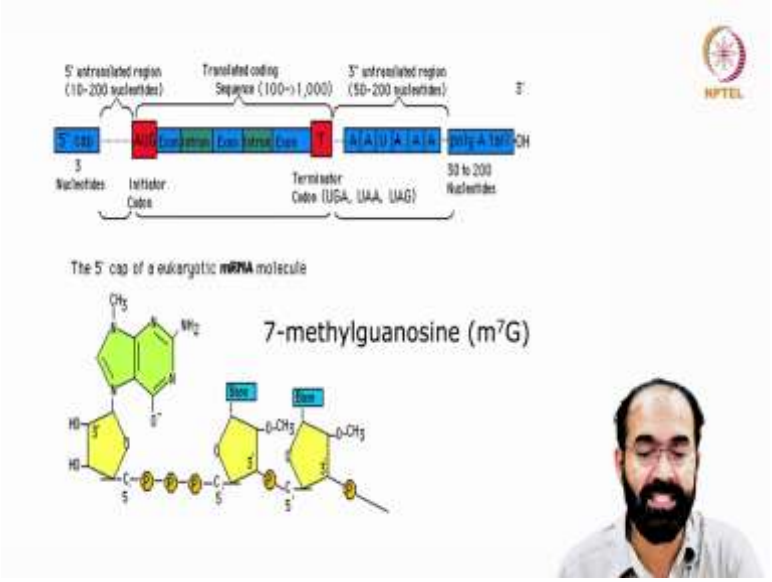

So, let us see this is a cartoon. You have a 5 prime cap and then the start codon AUG then you have exon, intron, exon, intron, exon and you also have terminator sequence and then you have polyadenylation signal and then you have a poly A tail. And, these sequences must be present in a typical mRNA gene or protein coding gene for them to act as a proper RNA.

And, the 7-methylguanosine cap is important and how it is paired you can see here in this cartoon.

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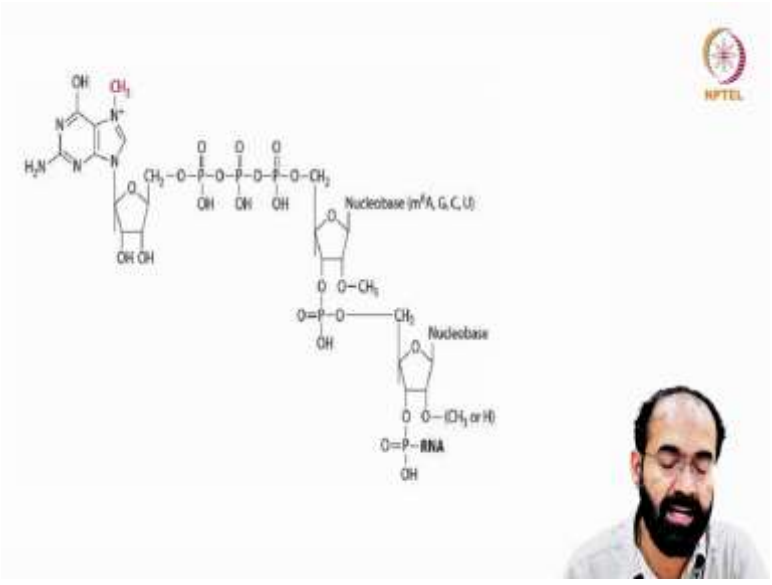

And, in the next slide also you can see how this pairing taking place you have this A base ribose sugar which has got a nitrogen nucleobase and then it is paired with another ribose sugar and again nucleobase is there this can be any base. It can be A, G, C or U, but this 7-methylguanosine cap has got a methyl group which also provides protection from the nucleases and also make them substrate less attractive for nucleases.

So, it is paired with phosphate group to phosphate group exposing the 3 prime OH 3 prime. This is 2 prime OH, this is the 3 prime OH of the ribose sugar. So, this 5 prime-end now resembles like a 3 prime end.

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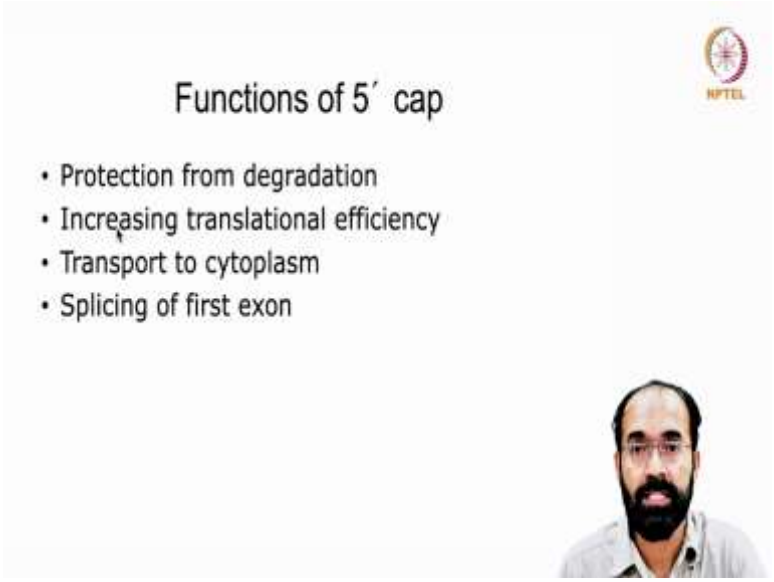

Let us see what are the functions of the 5 prime cap. Protection from degradation, we all know we discussed it and also increasing the translational efficiency. If an RNA having 5 prime cap 7-methylguanosine cap, it enhances the ability of this RNA to participate in protein translation. And it also facilitate in transporting to the cytoplasm. If this RNA is devoid of this cap it will not be pushed into the cytoplasm and because it will fail the quality control test in the nucleus.

And, splicing of the first exon; that means, exon 1 has to fuse into exon 2; that means, removal of the intron has to take place effectively and if the first exon is lacking because first exon is part of this extreme beginning that is the 5 prime-end of an RNA will never definitely will have the 5 prime end will have the cap and also the first exon. So, this first exons participation in splicing is assured by the presence of this cap.

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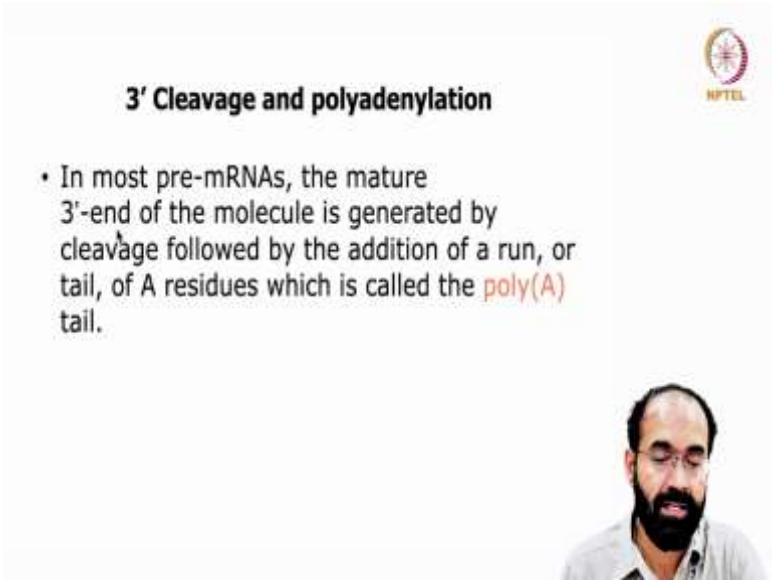

So, now comes the 3 prime-end end cleavage the and the polyadenylation. In most pre-mRNA's, the mature 3 prime-end of the molecule is generated by the cleavage followed by addition of a run or a tail; run means same sequence is being repeated and or a tail means again it will it does not have any complex structure it is A A A A it will continue it is called a poly A tail or a poly A track. That is added by a unique enzyme called poly A polymerase.

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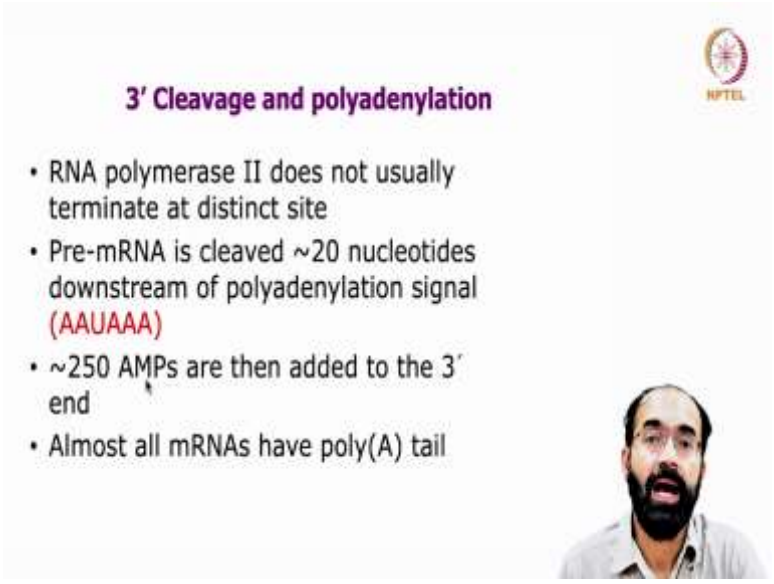

So, RNA polymerase II does not usually terminate at a distinct site. Normally RNA polymerase soon after the poly A signal will have a tendency because first of all the RNA

polymerase is working at a very high or faster rate. And, it will continue just like you are running and suddenly you have to stop, you cannot stop unless you are hitting on to a wall you will continue to go further.

So, you realize you can see that in many running race Olympic race etcetera, people put a ribbon. They do not put a metal rod in front when people are running and they will touch the ribbon or that line and then go. So, they will run few more base and then stop. RNA polymerase also is like that, it will cross the point and go some more distance and then it stops.

Pre-mRNAs that is cleaved approximately 20 nucleotide downstream of the polyadenylation signal. What is the polyadenylation signal? AAUAAA. Another polyadenylation signal possible is AUUAAA. No other sequence can act as a poly adenylation signal. This is different from poly A tail. So, do not get confused with the poly A tail. The poly A tail is a post transcriptional event it is not present in the DNA whereas, the poly A signal is definitely encoded in the DNA.

Sometimes the genes can have multiple polyadenylation signal and each of them will have role to play on the stability of this RNA. We will have such examples coming in the future classes. polyadenylation signal alternative utilization of polyadenylation signal for a given gene can ensure whether or not a given RNA is stable or how long this has to participate in translation and many more. We will see them one by one.

And, roughly around 250 adenosine monophosphate are present of course, it is started with ATP adenosine triphosphate, but now pyrophosphate is released and only one phosphate is present in the actual tail actual part of the nucleic acid. So, that is what we call it as AMP.

And, almost all RNAs have a poly A tail. Without a proper poly A tail RNA is vulnerable for degradation. And, it is also said that the poly A tail also gives an indication how many rounds an RNA mRNA has participated in protein translation. It is kind of a marking of the age of the mRNA.

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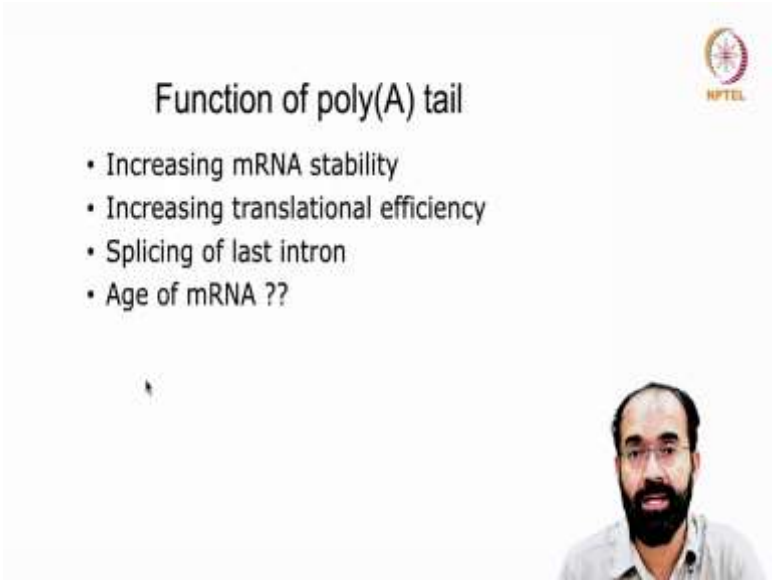

Let us see what are the functions of poly A tail. It increases RNA stability because 3 prime exonucleases are just waiting to cause the degradation of any given RNA because they are the scavengers. They are basically the cleaners of the system and our RNA a given RNA has to resist the degradation.

We just like defining lifespan. Lifespan of an organism is boundary between birth and death same logic applies to an RNA. Soon after an RNA is formed it is vulnerable for degradation. So, it has to resist. If it does not resist it cannot participate in protein translation or any other related function.

So, it also increases the translational efficiency. Those RNA, those mRNA that have got poly A tail is very much vulnerable for detection by the ribosomal machinery and it will enhance the rate of production of the protein. So, translational efficiency is increased which is the purpose of the mRNA itself.

And, this splicing of the last exon we saw 5 prime cap is helpful for the splicing of the first exon. Now, the last exon has to fuse with the previous exon say if a gene have got 10 exon we are talking about the 10th exon. So, 10th exon has to splice onto 9th exon. So, this will happen if the pre-mRNA that is formed already has the poly A tail.

So, remember soon after an RNA is formed it will have the 5 prime cap and then as soon as it is released it is cleaved by endonuclease, then it will have a poly A tail and without these two

features it will not go into the splicing reaction. And, poly A tail is also considered to be holding the information to give the system an idea that how old the RNA is. Sometimes an RNA may not degrade, but if it is too old it is vulnerable for mutations. It may have some errors. So, old RNA must be degraded.

So, who will tell age of an RNA? Poly A tail is supposed to give information about the age of an RNA. And of course, poly A tail is bound by specific proteins such as poly A binding protein and the absence of place for the poly A tail because they have got shortened also give indication to the various surveillance machinery in the cell whether this RNA has to be degraded or not.

In some cases, the shortened poly A tail will be extended by cytoplasmic poly A polymerases. It is not that only nucleus have got poly A polymerase cytoplasmic poly A polymerase also there, but it is not freely available for every RNA to access.

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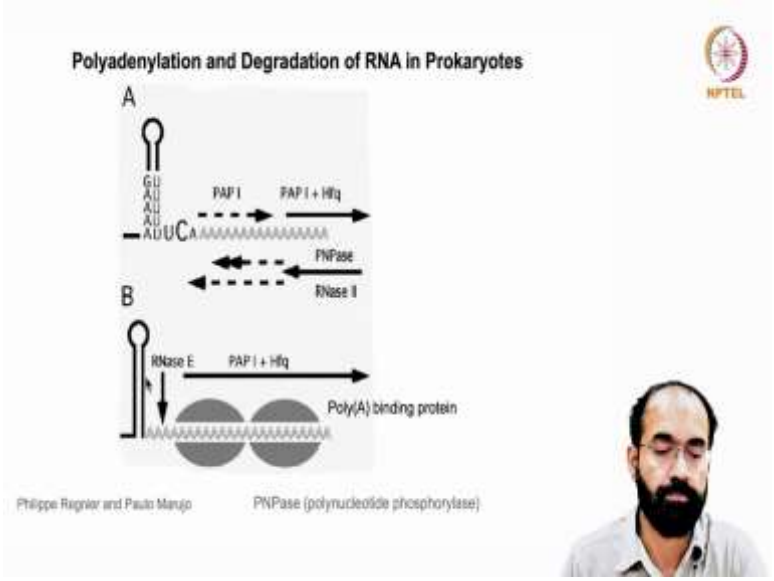

Now, let us see how poly adenylation how effectively it works in prokaryotes, it is quite interesting. You know two scenarios are there and in one scenario we know in bacteria also you have poly A polymerase I which is a weak adder of or a which weak enzyme that is adding the poly A signal or poly A tail to the RNA as you can see here dotted arrow.

And, but if it is added by host factor Q host factor Q protein PAP I plus Hfq, then the rate of production accelerates. Nonetheless it is vulnerable for degradation by PNPase and RNase II.

They will keep on decreasing the length. So, there is a you know tug of war between these 2 enzyme PAP I Hfq together will accelerate.

So, studies that show that Hfq is mutated or Hfq is degraded or not available then those bacteria have very short poly A tails. So, what it indicate? Although poly A polymerase is the functional enzyme the presence of Hfq. Sometimes it happens you may have seen it you want to go to a party, but you are lazy to go, you want a company.

If you have one company you will be quite interested to go. Alone, you will be bored. Although party enjoying dancing everything you have to do on your own, but company is not lifting you on that person shoulder. But, still having a company will elate or accelerate your mood to participate in a party. Same way many proteins are effective when another protein is available in collaboration.

And, we should also understand this PAP I Hfq can bind on to the poly A tail and it can influence the poly A binding protein to stay on to the poly A tail. If there is a gap where there is no poly A binding protein present, then what will happen? The exposed region are vulnerable for degradation by RNase E. RNase E when it cleaves between the RNA and the poly A tail part, then it can trigger a full fledged degradation of this RNA by various exonucleases.

So, we should understand the poly A polymerase interacts with other proteins to facilitate this whole process of poly adenylation.

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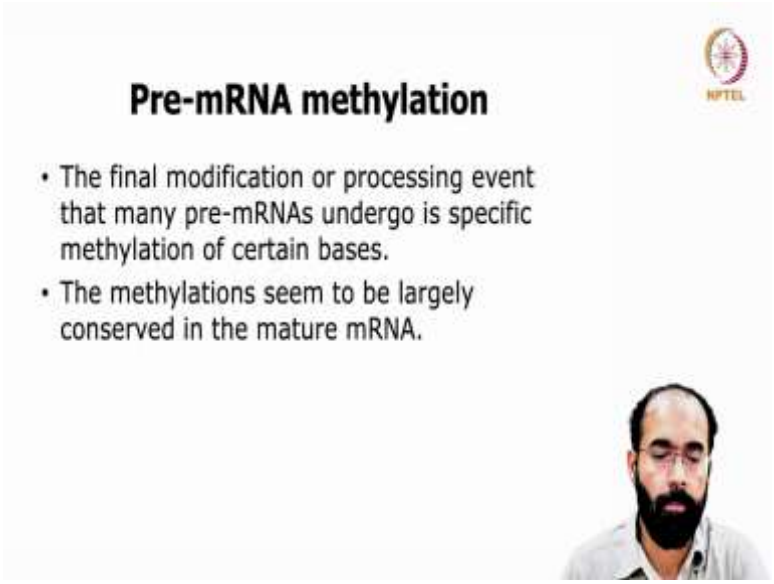

And, now let us see what is pre-mRNA methylation. The final modification or the processing event that may many pre-mRNA's have to undergo specific methylation at certain basis because the methylation make sure that this RNA is targeted for a particular location, RNA is targeted for a particular processing etcetera.

So, the methylation seems to be largely conserved in the mature mRNA; that means, when an mRNA has done it is capping, tailing, splicing etcetera then it undergoes modifications such as methylation. Majority of the methylations are making them resistant to degradation and also making them accessible for other modifying enzymes.

So, it is basically it provides a multiple just like you are a policeman wearing uniform. Uniform does protecting his body and also gives an identity to the layman that you know uniforms that particular uniform wearing person is a police officer and of course, badge, star and other features are also there.

So, this is something this modifications are somewhat like uniform or tagging with certain moieties, allow certain recognition and identification by other molecules present in the system.

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So, this is an article to read that is Comprehensive Analysis of mRNA Methylation that Reveals Enrichment in the 3-prime Untranslated Region and Near Stop Codons. So, those who are interested can read the article published in the journal Cell and it should be freely available to download and those who are interested can read it.

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Now, let us move on to alternative mRNA processing. What is alternative mRNA processing? The name itself says a lot. Alternative mRNA processing is, is there only one way? It is just like if someone is asking; someone is asking where is your house and you are

telling an address and then people are asking ok what are the different ways you can reach your house.

So, you might be normally going through the road that is a normal route which you take, but in some special circumstance special case say the road is broken and then you have no way of going. Then you will see oh there is a river side by beside my house that is I can get into the river with a boat and they can land beside my house and come through the back door.

Say, if river is having flood or there is no, there is lot of problem road is everything is flooded you want to get into your house then there is option is aerial route. You can come through helicopter. So, this is a simple example you can understand how do you handle a situation.

So, alternative mRNA processing is with a purpose in some case and in some case to add diversity. You will be able to add diversity from one source, just like you may have seen Swiss Army Knife, right. Swiss Army Knife look like a nail cutter, but it has got multiple viz.

They will have a scissors, it will have a can opener, it will have a knife, it will have a you know scissors many more things and even a screwdriver many things will be there in a Swiss Army Knife. So, it is quite handy one tool help you acting like a 10 tool. So, alternative mRNA processing also is something similar.

There are four different ways of alternative RNA processing exist one is alternative processing; another is alternative poly A site and another is alternative splicing and then RNA editing. So, these are all the ways in which from one source all I have is one say one gene is there.

So, how can I make diversity? Just like if you have just [FL] and rice, two carbohydrates you have got if you are smart, if you are not smart you will make only chapati and cook the rice and make it, but if you are smart you will come up with multiple dishes maybe you can fill the rice, cook the rice inside the chapati and make rolls and you say that you know this is a nice dish something like that.

Same way with one gene, one promoter you make use of the different features you have and make into something novel. That is what we will see them one by one.

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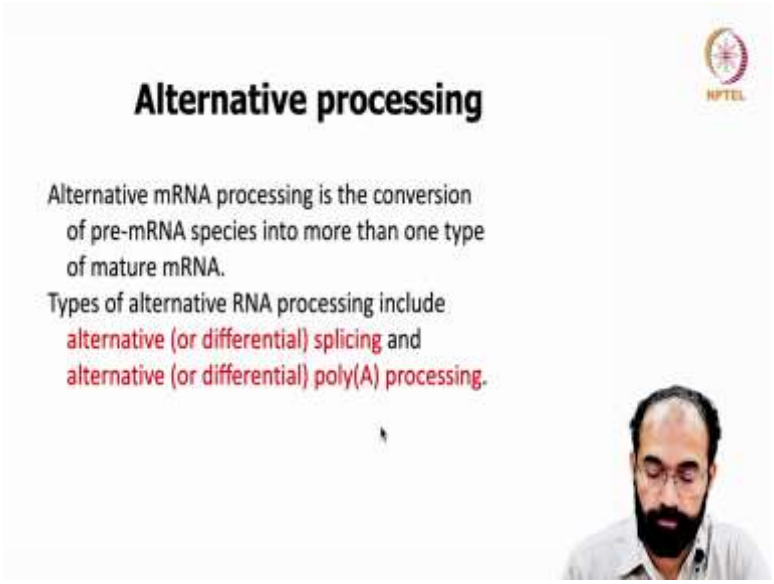

Alternative RNA processing is the conversion of the pre-mRNA species into more than one type of mature RNA. When you make more than one type of mature RNA it can technically give more than one type of protein, but the energy spent or the effort put is only for transcribing one gene, in a simplistic form we can say that.

So, types of alternative RNA processing include alternative or differential splicing and alternative or differential poly A processing because this require detailed and slow moving on to the topic. We will see them really slow so that you will catch it in the right spirit.

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So, one of this article which published in the journal genes and development ending the message only a signals then and now which is a interesting publication interesting article and those who are interested can read it is little old. I think it is published in 2011; it is quite interesting to read. You should try to read it and those who could not access it you can contact me and I will provide a ways of reading.

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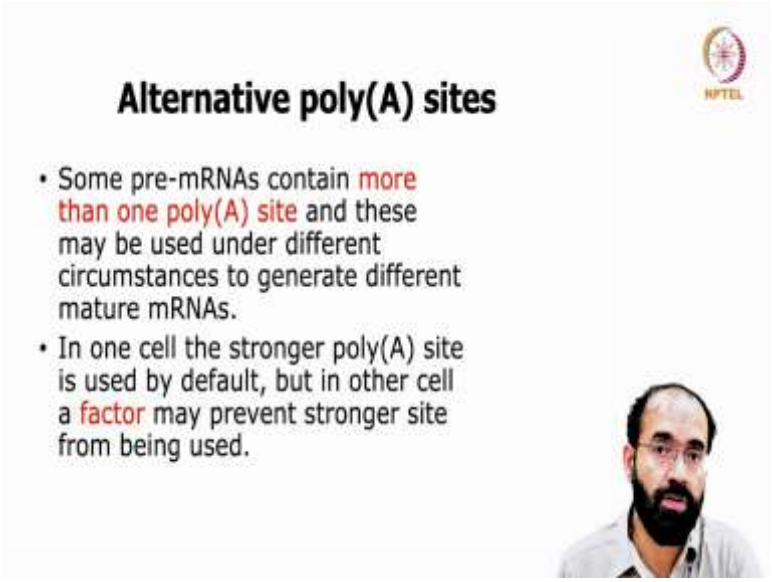

And, what are the alternative poly A sites? Because in the previous sections I already mentioned that poly A site can be many, not just one. Can be more than one some genes will have 3–4 poly A sites and which will use depends on which tissue you are talking about. One gene can be expressed in ten different tissues, but poly A site utilization need not be the same.

Some pre-mRNAs contain more than one poly A site and this may be used under different circumstances to generate different mature RNA. And, it is not influencing the coding, but different polyadenylation sites when it is present it can change the length of the untranslated region.

In one cell the stronger poly A site is used by default, and in another cell a factor means a protein factor may prevent the stronger site from being used. Stronger and weaker is a relative term; stronger is the one which is most preferred one. If I ask you to sing a song, you always sing one particular song I will say that ok this person have got a strong bias for this particular song.

That does not mean that you do not you know only one song, you may know another three more song as good as this song. But you do not sing it, you will sing only this song. So, that shows there is a strong link. So, that is what the idea you should get it when you say stronger poly A site there is no stronger or weaker, but whether that will be used or not depending upon the availability of certain protein factors.

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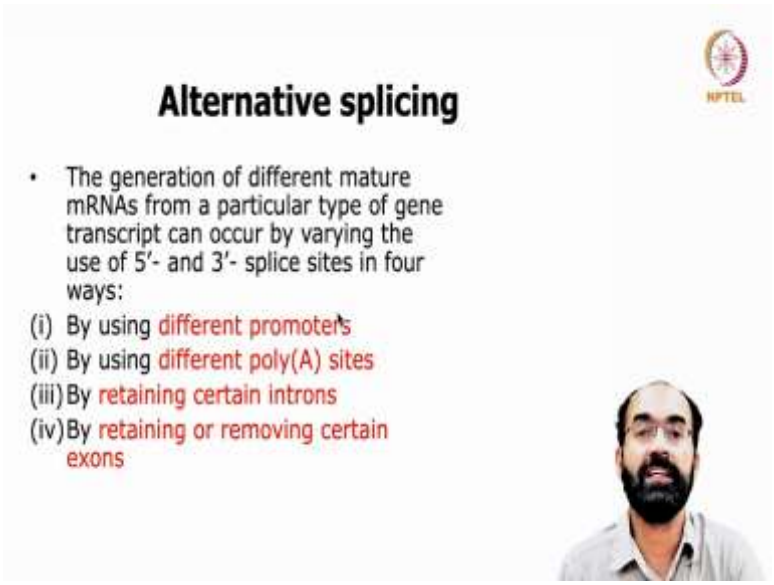

So, alternative splicing is basically the generation of different mature mRNAs from a particular type of gene transcript that can occur by varying the use of 5 prime and 3 prime splice sites in four different ways. That means we know every intron have got a 5 prime and a 3 prime-end every intron have.

Now, there are different ways in which you can utilize how to enhance the diversity, your goal is to enhance the diversity. If you are a classical singer if you have heard classical song, they use this [FL] different way [FL], they will keep changing and make different notation. Some people say [FL] like that you can keep changing. Like that introns also can bring in lot of variations to introduce complexity.

One way is by using different types of promoters; that means a stretch of upstream sequence in a given tissue it may or may not be acting as a promoter in specific region. So, you can have complex promoters depending upon which gene and which issue you are talking about.

Then using different poly A site. Then using retaining certain introns in the during the maturation process and, then lastly, by retaining or removing certain exons. We will see this more in detail in the subsequent classes time being we will end here and please go through the topic very carefully, so that you will be on the same page as I am and while listening the class itself it will be much easier for you to grasp.

Thank you.