RNA Biology
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Lecture - 36
Mechanisms of RNA Decay and Non Coding RNAs: mRNA Surveillance

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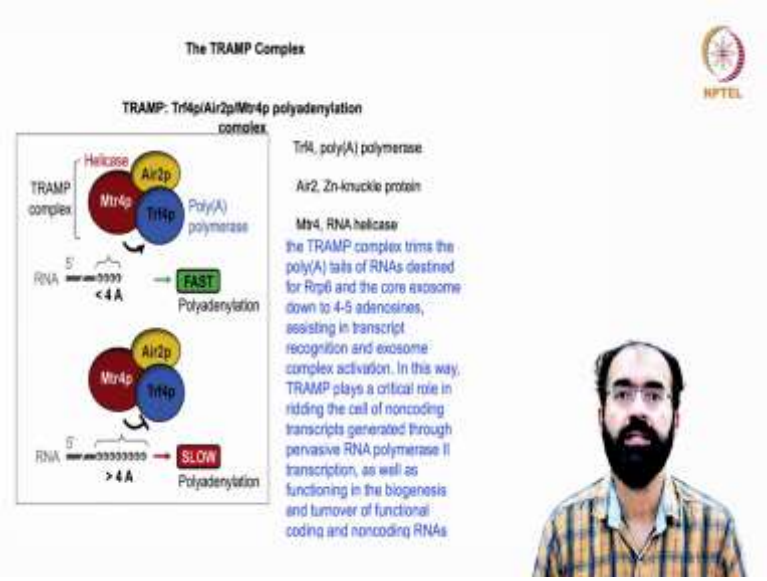

Hello everyone, welcome back to another session of RNA Biology. So, we were trying to understand the regulators of RNA degrading enzymes, that is exosome is one of the RNA degrading complex which targets the RNA from the 3 prime end to the 5 prime end. So, they are regulated by 2 complex, TRAMP complex and SKI complex and we were understanding the regulatory importance of TRAMP complex and this cartoon is basically indicating that.

So, before going into the details, we should also understand that RNA polymerase when it is transcribed, they always continue to transcribe even after the stop signal. So, we call it as transcriptional noise or pervasive transcription; that means, it is transcription goes like ripples. It is just like you are running a 100 meter race and in the finishing point I cannot expect you to stop within 1 millimeter.

You will continue to run based on how fast you are coming; you will continue to run at least some 15-20 meter. Same logic applies to the transcription stopping point, even after

the RNA is being released, mRNA is being released with the help of endonucleus from the complex, the transcriptional machinery continue, and this we call it as pervasive transcription or transcriptional noise.

So, how TRAMP complex, which works in the nucleus, how TRAMP complex comes into picture, we already have seen its a complex of Trf4p, Air2p and Mtr4p. So, in this Trf4 is basically doing the polyadenylation function, poly a polymerase. Whereas, Air2 is a zinc nickel protein and Mtr4p is a helicase. So, what we should understand, if a given RNA have got faster polyadenylation; that means, even if the length of the poly a tail is shorter, then it has to be handled in a different way.

Say for example, it has to be promoted; that means, the system understands this RNA is good and this RNA should be maintained. On the other hand, if the RNA polymerase is close to 4 4 to 5 the RNA polymerase mediated, transcription and the polyadenylation. If the 4 adenyl signal the poly a tail signal is in and around 4; however, the polyadenylation rate is pretty slow.

Let us imagine a scenario like this. Then what will happen, this helicase can Mtr4p can inhibit the poly a polymerase of unit that is Trf4p because Trf4p is a poly a polymerase also it can facilitate the polyadenylation. So, you do not want that adenylation because this polyadenylation is relatively slow that is an indicator, that this RNA is not a useful RNA, it is formed out of pervasive transcription.

So, let us see an example like, I already discussed that Trf4 is a poly a polymerase, Ar2 is a zinc nickel protein, Mtr4 is a RNA helicase. The TRAMP complex trims the poly a tails of RNAs destined for Rrp6 and other core exosome components and down to 4 to 5nucleotides that is adenosine nucleotides in length, assisting in transcript recognition and exosome complex activation.

If exosome complex is activated it is meant for degradation. In this way the TRAMP plays a critical role in reading the cell of its non-coding transcript generated through pervasive RNA polymerase to transcription. Pervasive transcription I already described I will not describe again. As well as functioning in the biogenesis and turnover of functional coding and non-coding RNAs.

Sometimes non coding RNAs require some kind of trimming. So, that time also this TRAMP complex should be able to direct the right path or right exosome complex to the non-coding RNA as part of its maturation. So, by and large the rate of adding of the a tail can be an indicative or indicative measurement to the cell of the quality of the RNA or the necessity of the RNA or even the nature of the RNA. So, that is how the TRAMP complex regulate the exosome function.

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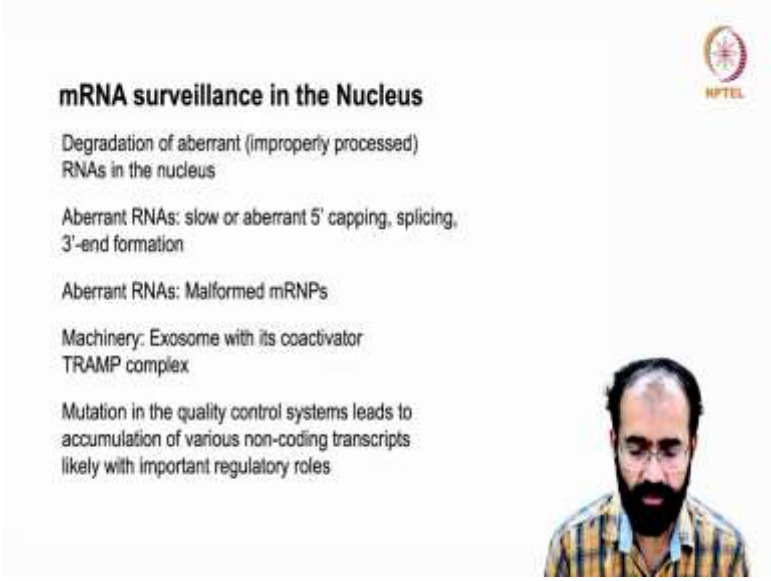

So, now let us see mRNA surveillance in the nucleus. So, degradation of aberrant or improperly processed RNA has to happen in the nucleus. So, no matter which RNA you are talking about. So, aberrant RNAs are showing slow or aberrant 5 prime capping or they can have aberrant splicing or they can have aberrant 3 prime information. So, when you say aberrant there is no definition of aberrant it can be a mixture of all or any one of them.

In general, if one of these either capping splicing or polyadenylation is improper, even it can be an RNA all the say 10 exosomes and 5, 9 introns are there, all of them are processed, but one intron is not spliced properly that is good enough that can be an indicator of an aberrant RNA.

So, aberrant RNAs often leads to the formation of malformed RNPs mRNPs; that means, they are not formed properly and they are having improper RMP structure, means it is

not welcome to process further it is not going to be pushed into the cytoplasm because it has to be handled or degraded in the nucleus itself.

So, the machinery used is exosome with its co-activator the TRAMP complex. So, they are the machinery that comes into picture and handles this aberrant RNPs. So, mutation in the quality control system often leads to accumulation of various non-coding transcripts likely with important regulatory roles.

Many a times what happens, these aberrant RNAs if they are accumulated in the nucleus sometimes these aberrant RNA will have some regulatory role such as, no more producing its own transcript sometimes it may have got other gene expression roles etcetera. But by and large these RNAs are degraded effectively they are not allowed to accumulate. If so, happens they are meant with a function they are meant for a given function. So, this also we have to keep in mind.

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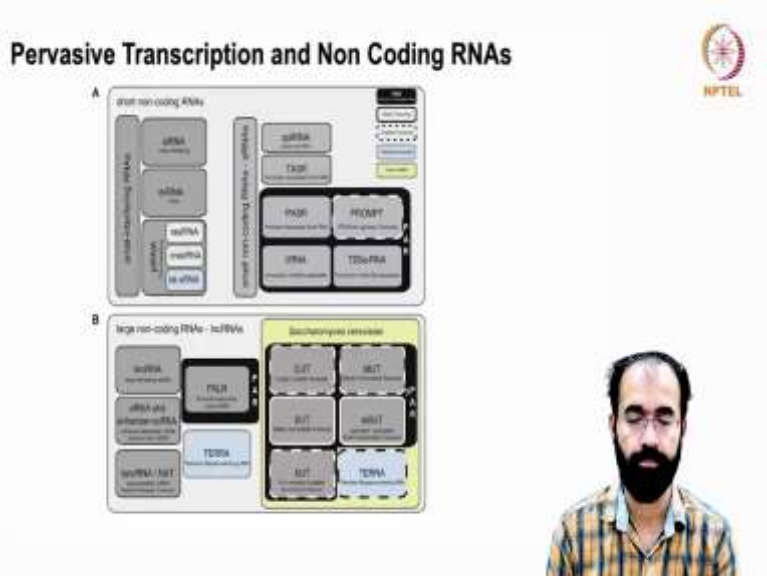

So, pervasive transcription and non-coding RNAs, let us see in this slide can be classified and this may look little complex, but it is one of the easier way of understanding, that how you can classify the non-coding RNAs like, broadly you can classify short non-coding RNA and large or long non-coding RNAs, in the top box you are dealing with short and the bottom box you are dealing with large.

So, short non-coding RNAs in general are small interfering RNA one group, which can be of siRNA and miRNA. siRNA means small interfering RNA, miRNA means micro RNA. And other examples include piRNA, piRNA and they are of rasiRNA, crasiRNA and telsRNA. So, these are some of the examples of pRNA. And small non-coding RNAs we often call them as snRNA and they are of few categories such as spliRNA, TASR RNA, PASR RNA, PROMPT RNA, tiRNA and TSSa-RNA.

These are some of the examples of small interfering RNA and small non-coding RNA. Same way large non-coding RNAs we have and we usually refer to them as lncRNA, Long Non-Coding that is LNC stands for. And they are of lincRNA, eRNA and enhancer non-coding RNA and fancRNA or NAT and their further groups are there PALR and TERRA.

So, these are all different types of the long non-coding RNA are plenty, there are more than 500 different types or maybe even some animals it is even high. Different number of non-coding around it can go in even in thousands, but the functioning of each them we do not understand fully, we know that there are plenty of non-coding RNA that exist and whether, what is the role they play lot of research is still going on in the field of non-coding RNA.

In example for example, saccharomyces cerevisiae yeast you have some examples of non-coding RNA CUT, MUT, SHUT, rsSUT and XUT and TERRA these are some examples extensively studied in saccharomyces cerevisiae. By showing this slide you get some understanding, some idea about the different types of non-coding RNA apart from the coding RNA such as, MRNAs. So, these non-coding RNAs are essential in several developmental pathways and even in homeostasis maintaining the steady state of a given cell.

So, we are not forgetting that ribosomal RNA and transfer RNA which is also non-coding RNA, but they are kind of housekeeping RNAs. So, these non-coding RNAs, long and short non-coding RNAs are important in a tissue specific manner. Some tissues have, some non-coding RNA, which is unique to that tissue and it is meant for regulating the gene expression in that tissue.

So, we can find plenty of examples of siRNA, miRNA and lncRNA that are influencing the gene expression events in a variety of cells. Some examples we will see as and when we go in different topics.

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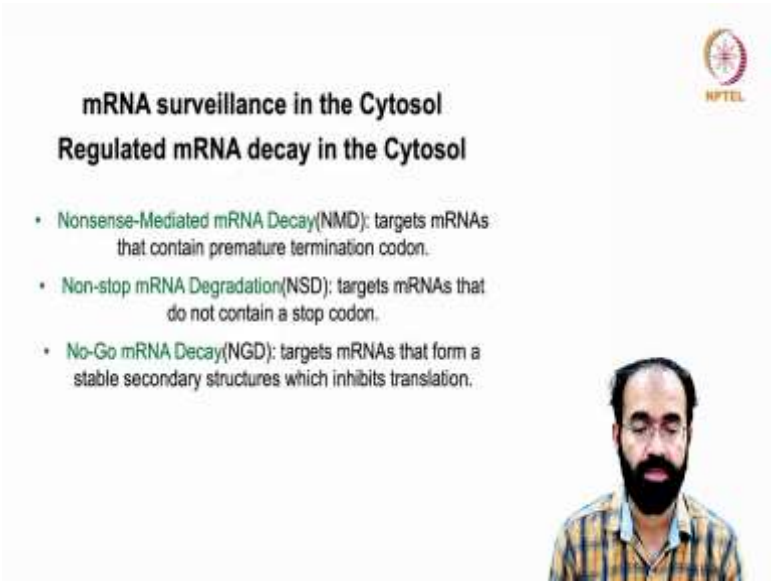

So, mRNA surveillance in the cytosol is regulated by we know it is regulated mainly by SKI complex and there are some typical examples we have that is the mRNA decay that happens and we can classify them. As Nonsense-Mediated mRNA Decay NMD and Non-Stop mRNA Degradation NSD, No-Go mRNA Decay NGD.

So, what is nonsense-mediated mRNA decay? It targets mRNAs that contain premature termination codon. You know UAA, UAG, UGA; these are the three stop codons of the 64 codons of which three of them can be anywhere. Of course, it has to be there towards the end of the coding sequence in any given mRNA, but it can form by RNA editing or simple mutation etcetera. It can come anywhere.

So, premature, the premature word itself indicates it is not present in a place where it is supposed to be there. Say for example, if a protein has got 100 amino acids. So, 100 codons should be perfect, 101 has to be a stop codon. This is what it infers. What if you are getting a stop codon at the place of 70? 71 amino acids onwards will be missing because 70th happened to be a stop codon.

So, this we call it as a premature stop codon. Stop codon came in a place where it is not supposed to be there. So, it is an indicator, it is a warning sign. So, cell is not having any use with that 70 amino acid long peptide. But do not forget we saw some examples of RNA editing, where the liver and intestine had apolipoprotein B with two different length.

That is ok, that is with a purpose. Of course, in evolution it would have evolved in such a way, so that it gained a function. We have seen several examples; we can also see several such examples, which is deliberately created, an alteration or an introduction of a premature stop codon is deliberately created and which is welcome for the functioning of that tissue.

But that is not the case in every premature stop codon. In such situation after the first round of translation this mRNA must be marked before degradation. If it does not do so the cell will keep accumulating more and more of this defective protein that can lead lot of complications to the survival of the cell itself. So, after first round of translation if there is a premature stop codon the sensing mechanism should mark this RNA for degradation.

So, that is what nonsense-mediated mRNA decay takes place, we will see them in detail. Then comes non-stop mRNA degradation; that means, targets mRNAs that do not contain a stop codon. Earlier one was, stop codon in an unwanted place. Now, we are talking about stop codon in a RNA is missing. So, that is called non-stop mRNA degradation. That means it is continuing all the way through the poly adenylation signal and it is now standing towards the tip of the RNA.

So, it is making lot of amino acid addition post the actual stop codon, where it is supposed to be there. So, this is another situation. And then comes No-Go mRNA decay that means targets those mRNAs that contain a stable secondary structure which inhibits translation. We all know RNA are capable of forming secondary structure. Sometimes the secondary structure can be found such a way that the ribosomes are no more able to go further.

It is just acting like a wall right in front of it. RNA has to be straight line, it has to be straight. So, that the smooth transition happens. It is just like you imagine a situation; you have taken a metal wire. Metal wire you know metal wire, electric copper wire or

aluminum wire it is a straight line you can put your hand and smoothly you can push till the end to end no problem.

What if in the half way through you made a bend and then you are trying to push your hand. So, it will come and stop where the bend is there. It does not allow your hand to smoothly go through this metallic wire. Same logic apply, same thing you can think about many things like you take a rope and you make a knot, a fat knot.

Then you are trying to people use it, if you are using rock climbing etcetera. You have a rope and in between you will put a knot in between. And so that you can happily put your leg or your toes in between the knot and you can climb. But if you want a smooth right, say you are smoothly gliding down a rope.

So, in between wherever there is a knot in the rope you will cannot smoothly go. You will be stuck here and there, right. So, that is called a No-Go. No-Go means, going becomes difficult, so that I s called No-Go mRNA Decay called NGD. We will see them in detail, so let us revisit one is nonsense mediated mRNA decay, that means stop codon came in an unwanted place premature stop codon.

Non-Stop mRNA degradation means absence of stop codon. RNA is continuing to translate even after the intended place where the stop codon is supposed to be there. And No-Go mRNA decay, RNA is perfectly fine, but some aberrant secondary structure is formed and this is preventing the ribosome from moving smoothly downstream. So, this is another situation. In all these three situations the RNA must be marked for degradation.

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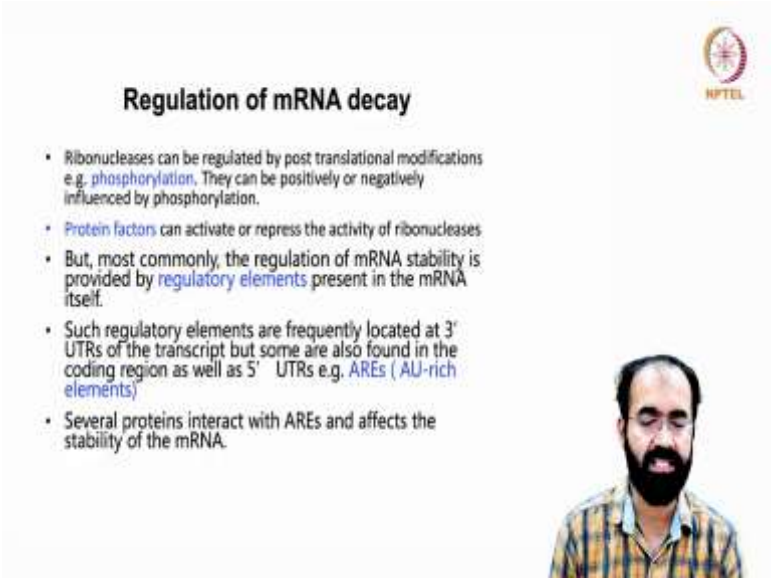

So, how the regulation of mRNA decay happens? Let us see some examples. Ribonucleases can be regulated by post translational modifications such as, phosphorylation. We have seen it like many proteins gets activated by phosphorylation, many times the activated protein gets deactivated by phosphorylation.

So, we cannot say that phosphorylation activate something. So, it depends which protein you are talking about. They can be positively or negatively influenced by phosphorylation, that is the exonucleases or ribonucleases. Protein factors can activate or repress the activity of ribonucleases, not just phosphorylation. Other proteins also can influence in a similar fashion. But most commonly the regulation of mRNA stability is provided by regulatory elements present in the mRNA itself.

So, mRNA is a kind of a self-sufficient system. So, the mRNA stability is kind of governed by the sequence and the secondary structure present within the mRNA itself. Such regulatory elements are frequently located at 3 prime UTRs of the transcript. But some are also found in the coding region as well as in the 5 prime untranslated region.

One example is AREs that is basically AU-rich elements that are present in the UTR. Many a times if they are present in the 3 prime untranslated region. They can influence the secondary structure and also the other proteins that is attached onto them that can influence the ability of this RNA to stay stable or unstable. So, several proteins they can

interact with the ARE that is AU-rich elements and effects the stability of the RNA. This is how they get influenced.

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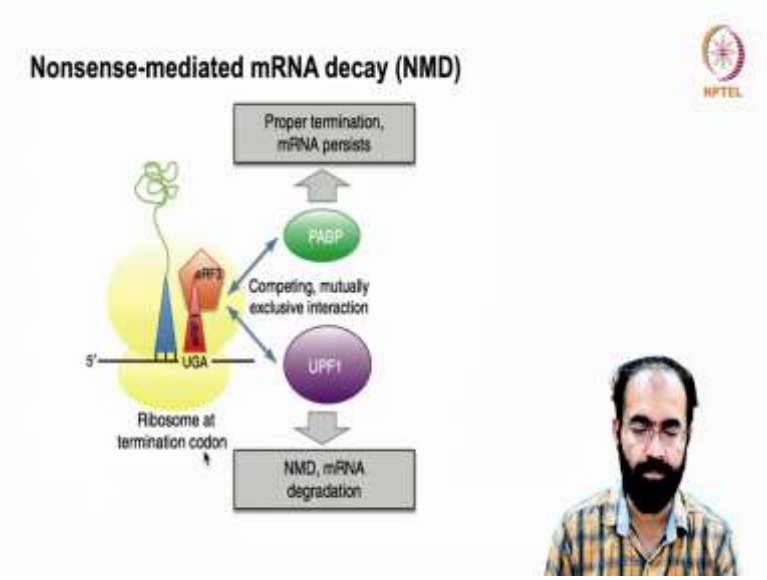

Let us see in detail with some pictorial description of the nonsense mediated mRNA decay. So, normally what happens, this yellow color two small flat balls are called ribosomes larger subunit smaller subunit. This line is the mRNA itself. So, each codons were there upstream and this is towards the last codon and this green color one is the newly formed protein peptide.

So, the last tRNA and the last amino acid is right here and it has completed. Because next one is a stop codon, UGA it will not attract a transfer RNA. Rather it will attract a eRF1, eRF3 complex. RF stands for release factor. So, release factor 1 and release factor 3 complex is supposed to enter into the ribosomal complex, where the stop codon is there.

And this is meant for disassembling the ribosome, peptide, tRNA and the mRNA this has to be disassembled. Now, let us see a situation. If the proper termination in an mRNA; that means, the stop codon is present in the place where it is supposed to be there, then a we all know what is PABP poly A binding protein.

Then this eRF3 can interact with the PABP and it says yes, it is perfect like you may see in movies you would have seen like if someone is you know; some character they will be

doing some you know some trafficking something, some smuggling or something like that, which is not which is illegal. So, they have their own codeword and to recognize is this person is the right to person, they will use some exchange some word this fellow will say something and that fellow will say in return.

If your; if it is a unknown person that person will not be able to react appropriately or if you see in normally you see in airport, if a visitor is coming they will stand with a placard Mr so and so or Mrs so and so. And you know your name, so looking at you go to that person and tell that oh ok you are waiting for me. But that person need not know you and of course, a smart person will confirm it with your ID card etcetera.

Otherwise, you can go to anyone, when you are coming from airport you can randomly go to any person and tell I am so and so. If they are not talented enough, then they will not confirm it with you. A smart person will confirm, show your ID card let me see. So, in your ID card they will know, that ok this is the person whom I am waiting for.

Such an interaction happens between PABP and eRF that is an indicator yes everything is fine PABP is supposed to be in the place where it is there and proper termination and the mRNA persists; that means, mRNA gets a life to live. It is not going to no one is going to trouble it.

Whereas, if the protein instead of PABP, it encountered another protein UPF1 instead of PABP, eRF3 recognized UPF1 which is supposed to be present in the exon-exon junction. We will see that more in detail the mechanism but remember eRF3 is looking for PABP; PABP is not there instead someone else is there, another protein is there UPF.

This indicates of course, disassembly everything will happen, but this indicates a sign this particular RNA should be degraded because the release factors could not encounter the PABP. So, it undergoes NMD degradation and so this is basically called nonsense mediated mRNA decay. So, this RNA is marked for that initially formed protein will be marked for degradation because now it countered a premature stop codon.

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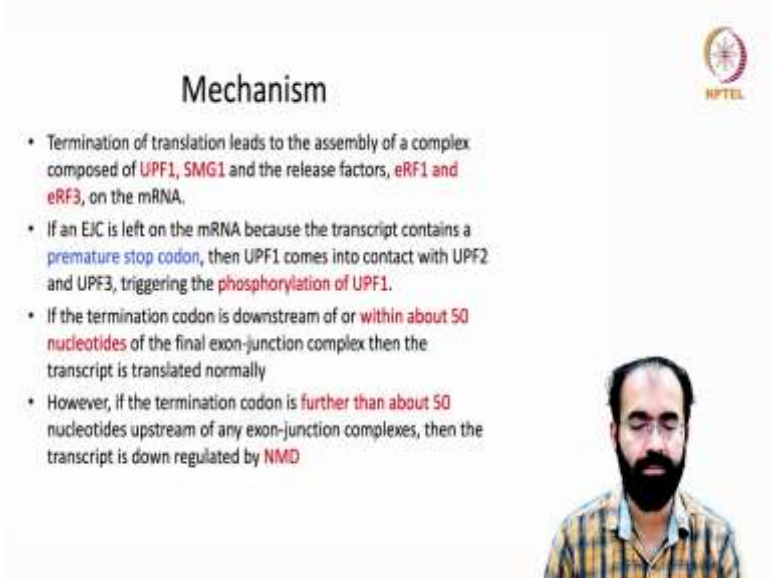

Let us see the mechanism. Termination of translation leads to assembly of a complex that is composed of UPF1, SMG1 and release factors, eRF1 and eRF3 on the mRNA usual case. If an exon junction complex called EJC exon junction means exon-exon junction. EJC is left on mRNA, which is naturally no mRNA usually is formed from one exon. Of course, exceptions are there, but usually at least two exons will be there.

So, if exon junction complex is left on the mRNA because the transcript contain the premature stop codon. Exon junction complex means exon-exon joining sequence plus a protein bound down to the exon junction complex. Then the UPF1 comes into contact with the UPF2 and UPF3 and this triggers the phosphorylation of UPF1.

If the termination codon is downstream or within about 50 nucleotides of the final exon junction complex; that means, exon-exon junction is there and you are within 50 nucleotides of that complex that junction, then the transcript is translated normally. However, if the termination codon is further than 50; that means, away from 50 nucleotides, the upstream of any exon junction complex then the transcript is down regulated by NMD.

So, what we should understand, this is not a full proof mechanism. Stop codon can come anywhere, but if the exon junction complex is intact, then chances of having a change or a RNA mediated change. If DNA has got a mutation naturally it will reflect no matter what, even if make 1 RNA, 2 RNA, 100 RNA 1000 RNA the mutation will persist. But if

the RNA has undergone some damage, some change then there is a good possibility, there is a good possibility that this RNA contains unwanted stuff.

So, what happens is, if the exon junction complex; that means, exon-exon fusion plus the protein associated with is staying intact, then chances of having a change or an editing within first 50 nucleotide is less. Even if it is there it will not be; that means, it will not be marked for degradation that does not mean that, that is welcome any change an unwanted change, unwanted stop codon is a problem.

But if there is a change that is a stop codon that occurred further to 50 base pair of this exon junction complex, then this will be RNA will be marked for degradation that is NMD mediated degradation we will happen. We will continue more in detail about this RNA decay in the cytoplasm in the next class.

Thank you.