RNA Biology
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Lecture - 37
Mechanism of RNA Decay and Non Coding RNAs: Mechanisms of RNA Decay

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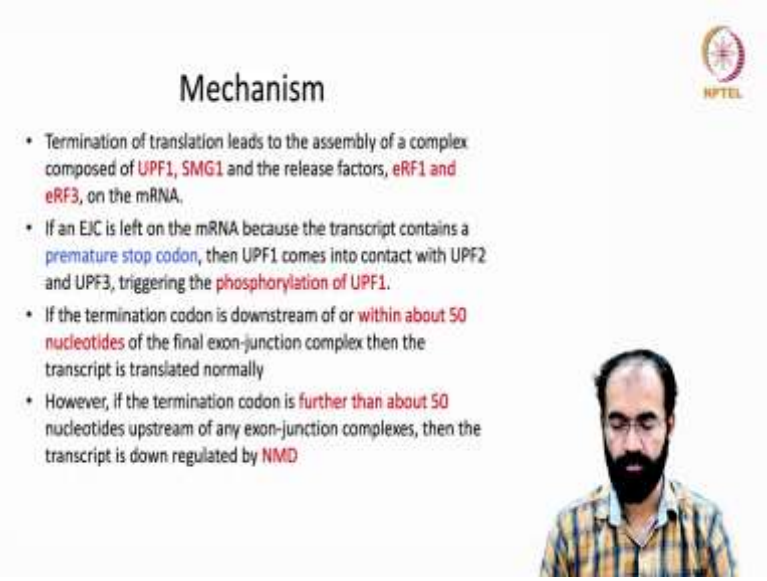

Hello everyone. Welcome back to another session of RNA Biology. So, we were trying to understand the Mechanism of RNA Decay that is occurring because of presence of a premature stop codon. So, premature stop codon usually leads to nonsense mediated decay.

And we also know it has something to do with exon junction complex. Exon junction complex is exon, exon fusion and some proteins associated with that. This is capable of interacting with the release factors, supposed to be coming into the ribosome complex when there is a STOP codon because no tRNA caters a stop codon.

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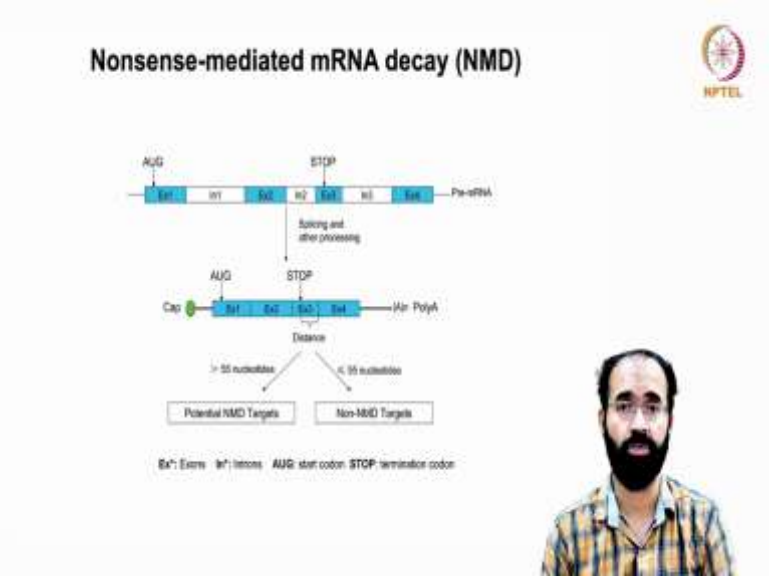

So, let us understand this more in detail. So, usually, you have a START codon which is inevitably AUG which brings in methionine, which is part of an exon 1 and you have intron exon 2, this and a STOP codon. It can be anywhere. Like, usually, sometimes you know there can be one full exon which is untranslated either in the 5-prime end or 3-prime end.

So, you have in this case, you have exon for which normally does not have any coding. So, it can be technically anywhere. So, but usually, it will go up to you know the last exon and usually the last exon contain the STOP codon. So, let us think about a scenario where you have the nonsense mediated decay. So, now the question is a STOP codon has arrived in a place where it is not supposed to be there. So, let us see how the system is dealing with it.

So, the RNA has got the cap, and also it has got this proper splicing that has happened, and also it has got a poly A tail. So, the RNAs quite good RNP has formed properly; despite having some problem in the in a premature STOP codon, but it formed the proper RNP and pushed into the cytoplasm.

Now, question is you know between exon, so this exon once it is fused with this exon you have got a exon exon junction, exon junction complex. So, this can have certain proteins, which stays until the first round of translation is done. Once the first round of translation is done, they are disassembled. So, you have protein here, you have protein

here, you have protein here and they are all moved by ribosomal machinery during protein synthesis.

However, here we have a STOP codon in a close vicinity to upstream part of a exon junction complex. Now, the question is the distance. If and the key here is ex stand for exon, in stand for intron, AUG is the start codon and STOP is the STOP codon, from this to better understanding of this whole cartoon.

If the distance that is distance of the STOP codon from this upstream, not upstream downstream exon junction complex; that means, the STOP codon must be upstream to a exon junction complex. If the distance this distance, that is STOP codon till this exon junction complex if it is more than 55 nucleotide scenario one another is less than 55 nucleotide which is another scenario.

So, this if so happens, if it is more than 55 nucleotides, then that kind of gives a warning signal to the system that despite having this exon junction complex in here, there was some alteration that happened. And that could most likely because of a post transcriptional modification, some kind of mutation or some editing occurred and unwanted editing. So, the system marks this as a unwanted editing.

This exon junction complex protein act like an umbrella so, you imagine a situation, you have an umbrella, a big umbrella. So, along with you if one friend is there less likely that person will get wet, right. If you have an umbrella, friend is there, and friend is still getting wet, that simply indicates that friend is not welcome to your umbrella. That person is coming with you, but not welcome in your umbrella, that person is getting wet in the rain.

Same way, if the distance is more, then some editing is occurred post transcriptionally. So, that is a potential target of NMD. Whereas, if it is close to it then chances are there it did not occur post transcriptionally. It is most likely because of a error in the genome or it is a needed or welcome or non-problematic, not a problematic. The system detects.

System cannot or the your surveillance system, cells a surveillance system cannot judge right now. It has only; it has to work on indications. So, if it is less than that is close vicinity of the exon junction complex, then what happens? Non, it is not a NMD target, that is non-NMD targets.

So, if it is a problematic situation, the cells or the mechanism or the homeotic surveillance should come with or the homeosis or homeotic mechanism should come with a novel strategy of handling this errors. But they will not be marked before NMD mediated. Means nonsense mediated decay will not come into picture. So, let us see how exon junction complex comes into picture.

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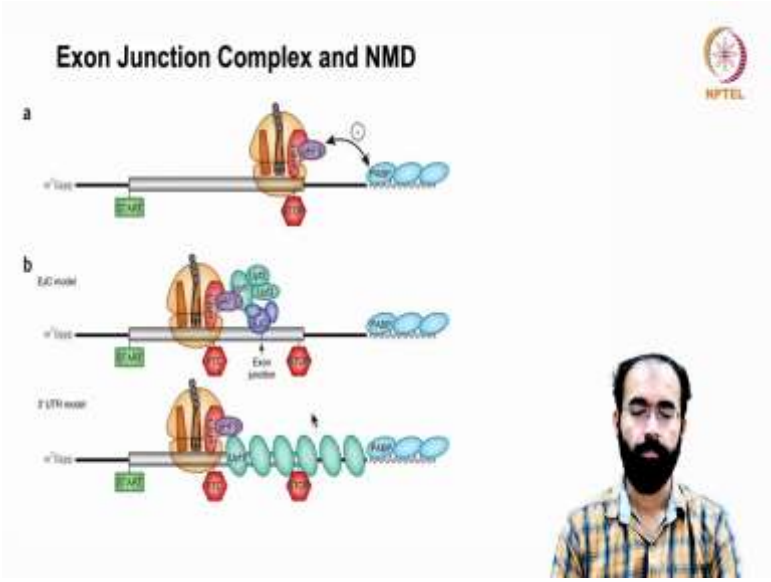

So, you have the cap, you have the start codon and you have the STOP codon and this is normal scenario, where the ribosome larger and smaller subunit came in, this is the peptide that is growing, and the release factors came. eRF1, eRF3 came and you have a UTR 3 prime untranslated region, then you have a poly A tail.

Poly A tail have got PABP, and PABP can happily interact with eRF3 which is a normal indication. Like, I told you in a previous example about you know communication between strangers, you use some code word etcetera. So, PABP and eRF3 interacts that is a successful communication.

So, during this process the protein is welcome, RNA is welcome and this RNA can participate in next rounds of translation. If EJC exist close to, this is an exon exon junction, this is an exon, this an exon. In RNA it is reflected in the form of a exon junction complex which contains plenty of protein. And this protein include Upf1, Upf2, Upf3.

And instead of interacting with PABP which is very much here, but very far, it is not able to this eRF cannot interact with PABP, its much downstream even after crossing this exon junction complex. Instead of PABP, it is now interacting with Upf, and this interaction happens, it is a warning bell, it is a problematic.

So, this is referred to a this is a STOP codon, this is premature STOP codon or premature termination codon. Actual STOP codon is here. Both are same, but it is in an unwanted place that is why we give it a name PTC, Premature Termination Codon. And this eRF3 in interacting with Upf1 is not a welcome sign for the RNA point of view.

In, and another situation this is exon junction model, another situation it is a 3 prime UTR model. It can happen that every protein may not have an exon junction complex. In that scenario what happens, this interaction with eRF3 and Upf1, this will invite a series of Upf1 binding onto rest of this RNA and that can mark for the degradation of this RNA.

So, two models are prevalent, that is exon junction model that is interacting with one of the protein. And another is 3 prime UTR model, where the Upf comes in contact with the eRF3 will trigger the binding of a series of Upf. And of course, their phosphorylation and other events can take place, and this can lead to degradation.

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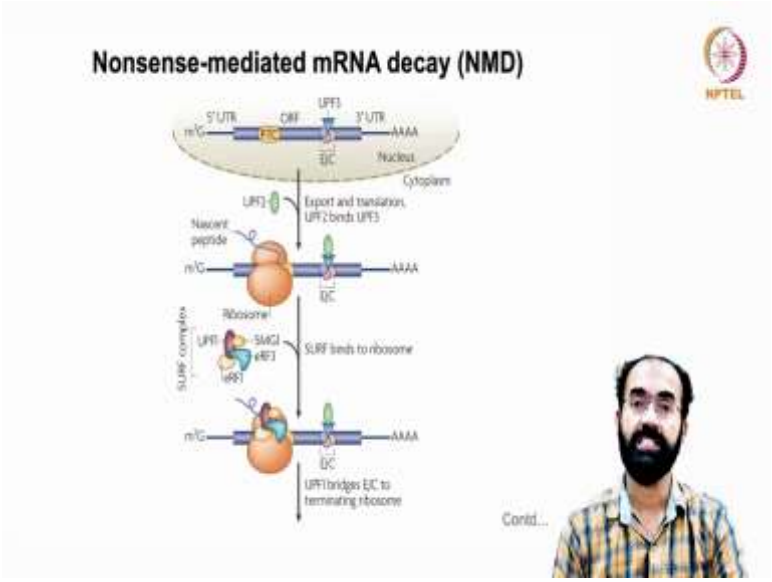

Let us understand the nonsense mediated decay in a different perspective. So, you have a cap, you have a premature termination codon in the ORF, and you have this exon junction complex, but this is a distant scenario, ok. So, you have, this is occurring in the nucleus and it pushed down to the cytoplasm. Because no surveillance system can catch a termination codon in a mRNA during processing in the nucleus.

So, the protein is being produced, a nascent peptide is being produced, and once the RNA reached cytoplasm, this UPF proteins incorporate in the exon junction complex they stay there. And a premature termination codon is existing there, until then a peptide is being produced. And then a new complex called surf complex comes into picture. So, surf complex that contain UPF1, SMG1, eRF3 and eRF1.

So, this complex joins it. This complex when it joins it can influence and interact with this stalled ribosomes. Ribosomes now have got eRF1 and eRF3, so they join and this whole complex that is called surf complex that will come in contact and this exon junction complex has got UPF already we know that. So, Upf1 bridges the exon junction complex to the terminating ribosome towards the end. And this will continue further.

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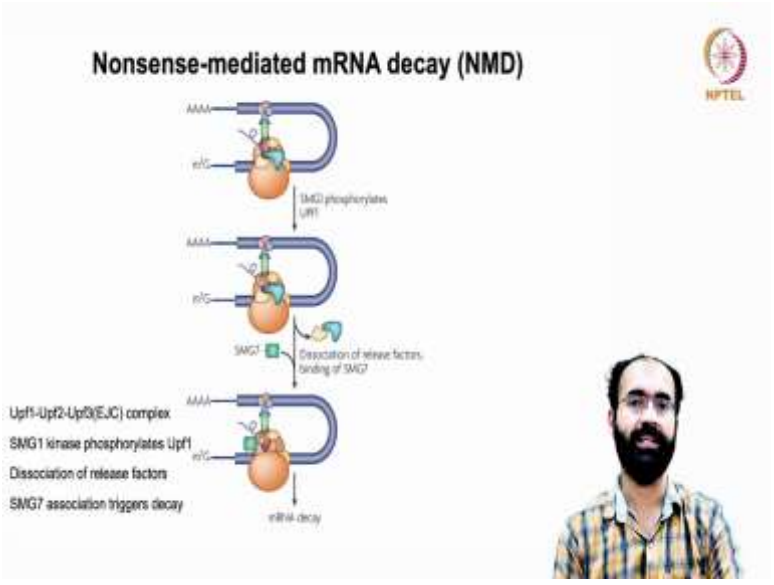

This interaction by forming a loop that will cause the SMG protein small mobility group protein that phosphorylates the UPF1, which is part of the exon junction complex and this leads to their mutual interaction and phosphorylation that will cause the dissociation of few components from this complex and SMG7 joins the complex.

So, the dissociation of this release factors that is eRF1 and eRF3 will take place, and joining of SMG7 takes place. And the Upf1, Upf2, Upf3 are present in the exon junction complex. SMG1 small mobility group one is a kinase that can phosphorylate the Upf1, and the dissociation of release factors is caused by the SMG7 association that can further trigger the decay.

So, once the SMG7 is joined, then this RNA supposed to be meant for decay. So, what we should understand? These steps of events happened mainly because of presence of a premature termination codon, which invited the surf complex to join. So, this is another mechanism of decay of RNA that contains the premature STOP codon.

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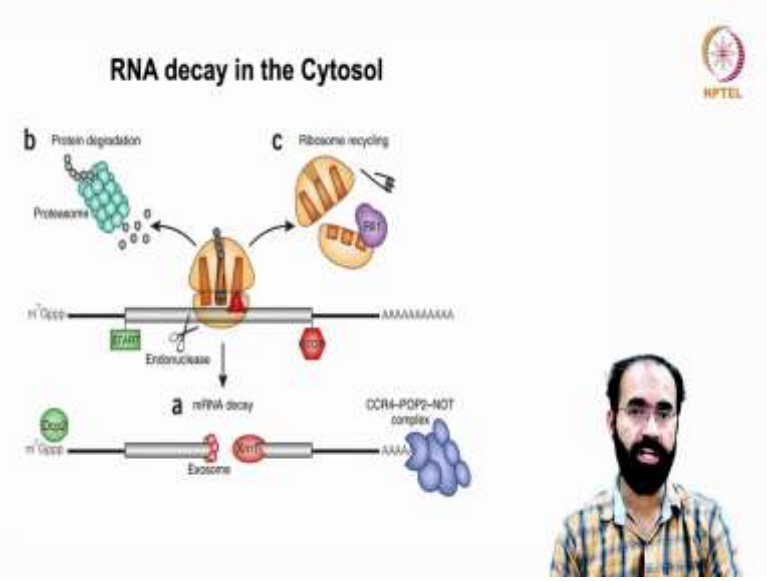

And one more thing I want to add, if it is less than 55 nucleotide, then this bending and looping and interaction is not possible. That is why it should be more than 55 nucleotides. So, that such an interaction and the bending of the RNA is possible because these proteins are bulky.

So, if RNA is too short, like if I give you a 1 meter long iron rod, you will be able to bend. If I give you iron rod which is of only 10 centimeter long and I am asking you to bend it. If it is a fat iron rod, you simply cannot bend it. If it is 1 meter long, you may struggle, you can still bend it so, because it has got this space to apply your force.

But short one means you cannot all you can hold it with your two hands and your hand is not powerful enough. If it is 1 meter long, you can put your leg and put your whole muscle power and try to bend it. Same logic applies, if it is shorter the bending and looping and this interaction become difficult.

Let us understand the RNA decay more in detail that is occurring in the cytosome. So, we know that if a premature termination codon is present, that protein also should be marked for degradation which is taken care of by the proteasomal machinery. So, proteosome are bodies or structures present in a cell that is meant for degrading the proteins, unwanted proteins.

So, this RNA starting from a specific start site and it came to a place where the termination codon or STOP codon is there which is a premature thing, and the ribosome has to be disassembled and recycled and a protein called Rli1 comes into picture. Once it is disassembled.

This RNA become an attractive substrate for endonucleases because no more protection from the ribosomes. So, it is vulnerable and exposed. And it will open up two new ends that is a, 3 prime end newly formed, 3 prime end and then newly formed 5 prime end because of this cleavage.

So, 5 prime end is mediated by Xrn 1 mediated 5 prime exonuclease activity and 3 prime end is taken care of by exosome mediated degradation. And of course, DCP decapping proteins comes into picture and CCR4 POP2 NOT complex also comes into picture for degrading this poly A tail. So, it acts after this endonuclease cut degradation happens from both ends, and after this endonuclease cut degradation happens from both ends of the other piece also.

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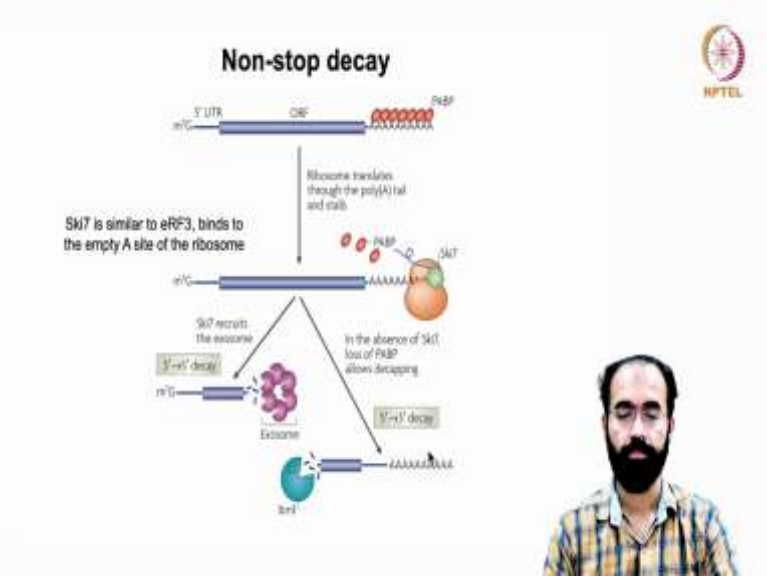

So, now comes the non-stop decay. So, non-stop decay, what it indicates? RNA is not having the STOP codon or ribosome is not stopping. That is called non-stop. You can interpret it in both way. Non-stop means STOP codon is not there or ribosome is not stopping because of absence of a STOP codon.

In either case in this scenario what happens, the 5 prime cap is there, UTR is there, ORF is there and the poly A tail is there, and PABP also is there, binding on to the poly A tail. So, ribosome translates through the poly A tail because no STOP codon, should have been somewhere here.

It is translating through the STOP codon and it ends there. It do not know what to do. So, the protein called Ski 7 is somewhat similar to eRF3. eRF3 you know, eRF1 and eRF3 comes in contact when there is a premature termination codon. Similar to that, Ski 7 comes in that place because here it cannot come; eRF1 and eRF3 cannot come because there is no STOP codon. It is just a stalling there. So, ribosome is stuck.

So, eRF3 and eRF7 cannot come and instead Ski 7 comes in there; binds to the empty A site of the ribosome. Empty A site means there is no more place available for the proper tRNA to come because of absence of a codon. It is just like you are walking on a on the on a roof. You can walk only up to the edge of the roof, and you can keep one more leg means you will fall down. So, that stage it is standing.

So, there in that empty space, the Ski 7 comes in. And remember the PABP is displaced by now because of the movement of the ribosomal machinery. Ski 7 can recruit the exosomes because Ski 7 is now in the extreme end of the 3 prime end of the RNA. It can cause the 3 prime to 5 prime decay by recruiting the exosomes. So, Ski 7 if it is bound onto a ribosome, attached to an RNA, that is a invitation or a dinner bell for the exosomes to come and start eating them up.

In the absence of Ski 7, sometimes it may not be there; the simple loss of PABP because the ribosome displaced it, allows the decapping. If PABP is missing in an mRNA that is a welcome sign or a welcome signal for decapping enzymes to start the decapping. Once the decapping, 7 methyl guanosine cap is removed by the DCP's then the Xrn 1 can start acting on them. So, they degrade by 5 prime to 3 prime decay.

So, to revisit this non-stop decay, we can see UTR is there, ORF is there, all we do not have is a STOP codon. Nowhere in this RNA there is no STOP codon. PABP also is perfectly there. So, this RNA is perfect in every sense except that there is no STOP codon.

So, ribosome will walk all the way through the poly A tail, it will translate through the poly A tail and you end up displacing the poly A binding protein. That can invite the decapping enzymes and that can cause the degradation by Xrn 1, 1 mechanism. Otherwise, if this Ski 7 came into the picture. Ski 7 occupied, and then Ski 7 itself can recruit the exosomes that will cause its degradation. So, this is the two mechanism or pathways through which the non-stop decay takes place.

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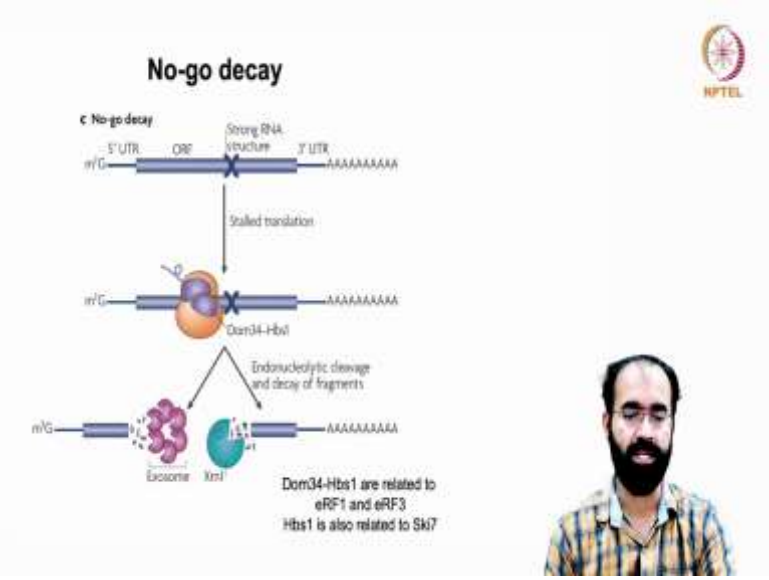

Now, let us think about the no-go decay. What is no-go decay? We have already discussed that no-go decay means ribosome is not going. It is just stalled. Credit goes to the secondary structure of the RNA. So, no-go decay, RNA is fine, it has got a cap, it has got a UTR, it has got an ORF, it has actual STOP codon, everything is there, it has poly A tail, everything is perfect, except that a strong RNA structure is formed right in the middle.

Of course, it can be because of some alteration. Any alteration need not necessarily bring a STOP codon. Some alterations may not bring even a change in the amino acid because we know codons are degenerate. 6 codons can bring in arginine. So, if one or two base change in this codon nothing will happen, arginine only will come in that place so, nothing wrong with the protein also. However, some scenario, the secondary structure can change. Even one base pair change can cause a havoc in the secondary structure.

So, some mutations need not necessarily bring in a change in the amino acid or change in the STOP codon. So, once this secondary structure has formed, strong secondary structure has formed, then the ribosome stalls during translation, it cannot go further. Then, there is a protein complex that is Dom34-Hbs1 heterodimer comes into picture in such scenario.

And this will cause Endonucleolytic cleavage and decay of the fragments can take place because this protein once comes into picture, it can create the breakage of the RNA. This

can now invite exosome mediated 3 prime decay and Xrn1 mediated 5 prime decay. Dom34-Hbs1 are related to eRF1 and eRF3 where the normal STOP codon is supposed to come. However, eRF1 and eRF3 will come only if the ribosome is encountering a STOP codon, but this is a related protein. So, Dom34-Hbs can come in even if there is no STOP codon.

All it wants is a slow. Just like if a train is going too slow or a bus is going too slow you can happily get into the bus and come out of the bus, if the bus is going only 5 kilometer per hour, just a little faster than you walk. Then, you do not need to worry. So, this slow going will become slow going ribosome become a target for, a target for the Dom34 and Hbs1.

Usually, they would not come. Same way eRF1 and eRF3 can come only with the RNA is our RNA ribosome complex as stalled. If tRNA is coming frequent interval, then protein translation will takes place smoothly. No problem at all. But if there is a reduction in the speed, then eRF1 and eRF3 comes provided there is a STOP codon. But if there is a reduction in speed, but no STOP codon is there, then Dom34-Hbs1 comes into picture because they are related to eRF1 and eRF3.

Hbs1 is also related to Ski 7 because Ski 7 we have seen it, they will attract the exosomes. So, here the degradation the breaking of the RNA happens because of this complex and Ski 7 used to attract the exosome. Similarly, the Hbs1 can attract the exosome and it can trigger a exosome or Xrn1 both will trigger from both ends of the RNA.

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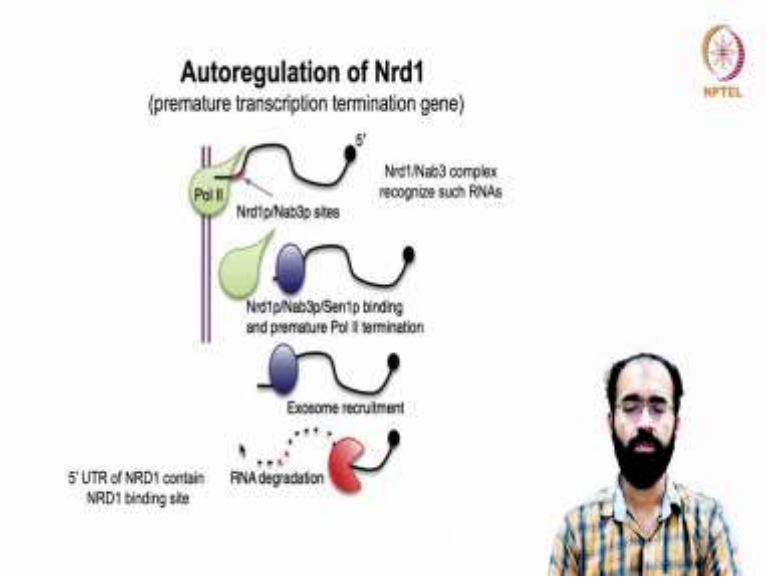

Now, let us see some examples of autoregulation. Autoregulation is a wave RNA degradation, based on demand. We have seen some such examples before also. Concept is simple. If a protein is present more than required, then the protein should have evolved a strategy to degrade its own RNA, so that the production of that protein will be reduced. Transcription is happening normally. The production of that protein is decreased.

And it will go to such an extent; it is no more able to STOP its production. Because there is no, it is just like you have got 1 lakh rupee, you keep on spending, spending, spending and your rate of spending will be high when you have 1 lakh rupee and once that is come down to 100 rupee, your rate of spending will decrease.

And now you became penniless. You have 0 rupee with you. Then, there is a chance that; now you cannot go become minus; you will slowly start earning. Same logic applies that RNA the protein level is high the RNA will degrade because this protein is contributing to its degradation.

And once the level of the RNA has gone down then protein also automatically will go down because protein also got a half-life right, then no more the protein is available to degrade its own RNA. Then, the newly formed RNA will start translating and it will start doing its functions kind of oscillation.

So, you can see an example the Pol 2 transcribing, this example of this Nrd1 and which is basically encountering a situation where you have premature transcription termination gene. Nrd1 and Nabp has got multiple sites here and Nrd1, Nab3 complex recognized such RNAs.

And what happens? Nrd1p and Nabp and Sen1p has got a binding site and the premature Pol 2 termination site. Whenever you have got Nrd1, Nabp binding site Nab3p binding site, it will once it is exposed, they can come and bind and this binding can trigger the exosomes to create their degradation.

So, the 5 prime untranslated region of Nrd1 contained the Nrd1 binding site. So, this can trigger the auto regulation. That means, the Nrd1p, Nab3p, and Sen1p they form a complex and bind to the premature polymerase two termination. That means, you are talking about a situation where the termination is premature. That means, it is occurring in a situation where the RNA is incomplete.

If the RNA is incomplete, the binding of these factors, soon after its transcription because the binding site is now exposed, that is Nrd1 Nab3 binding sites are recognized and that can cause the degradation of such RNA. We will see more in detail about the Autoregulation of similar examples in the next class.

Thank you.