RNA Biology
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Lecture - 34
Mechanisms of RNA Decay and Non Coding RNAs: Decay Pathways

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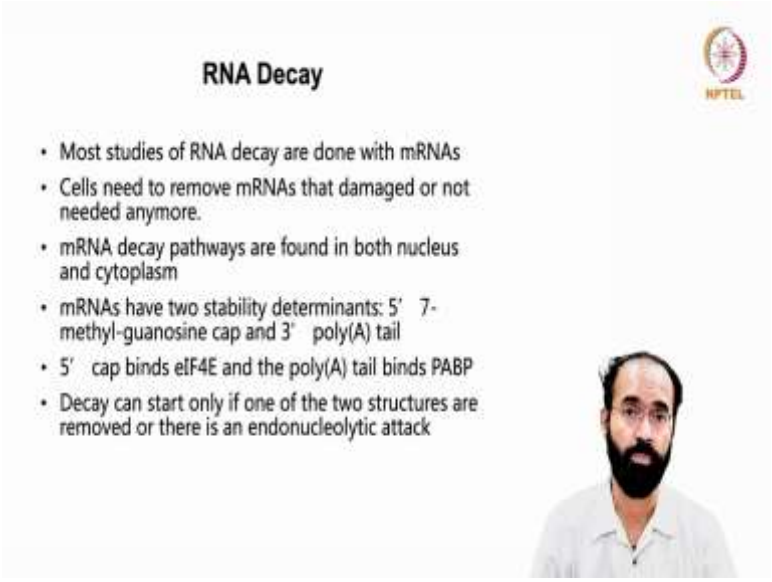

Hello everyone. Welcome back to another session of RNA Biology and we were here in the previous class that we were trying to understand the importance of RNA decay because RNA decay is as important as the RNA production itself and it has to be monitored stringently.

We also should understand one more thing. If an RNA important RNA is being formed in the cell and it is marked for degradation then the system suffers, the cell suffers. So, that is another thing that RNA degradation is important, but premature degradation or unwanted degradation also should be prevented. But these things keep happening in a cell. So, the adequate monitoring system, adequate surveillance system is important for maintaining the healthy quantity and quality of the RNA population inside a cell.

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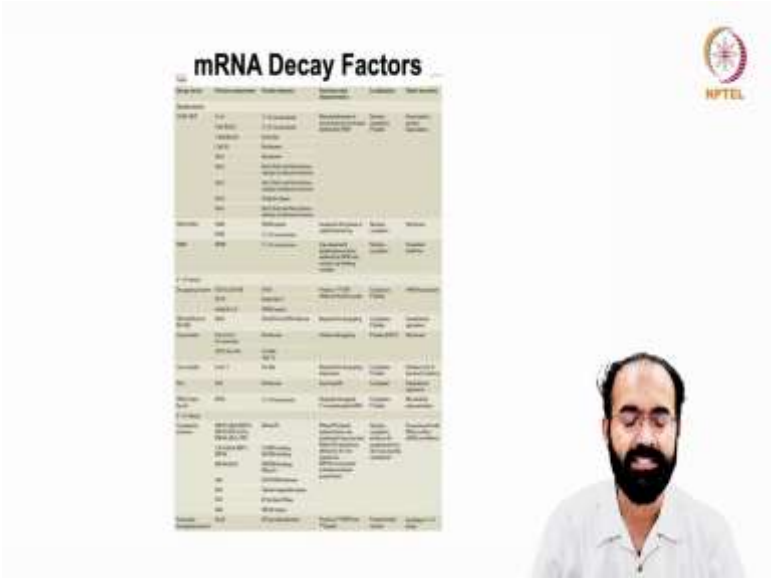

Let us see RNA decay factor. This is a complex slide. Do not worry about it because our purpose is not to go into detail. Of course, you can see these factors in detail, but the important thing is to sensitize you that the RNA decay factors, the mRNA decay factors are not just 1 or not 2 or not 3 or not 4. They are so many are there.

They are in hundreds and a lot of them are listed here and understand many more are not discovered yet because we understand some mRNA population and their degradation, but we do not even know how many mRNA population we have in a human body. That itself we say enigma. We do not know how many genes constituted human being. We do not even know an arbitrary number. We have only a guessed number that we say around 1 lakh genes, mRNAs are needed that is anyone's guess. It is a; it is a ballpark figure.

So, it will take lot and lot on years of research to understand the entire mRNA decay machinery, but whatever we have listed in this table we will be trying to address the important members of this list and we also try to understand what is the mechanism and there are different categorization also exist for the mRNA decay pathway.

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So, one category is deadenylation dependent mRNA decay and name itself says that poly a tail is there which is made of adenine and adenine tail if it is removed or degraded or lysed then we call it a deadenylation. So, the removal of poly a tail from the mRNA is called deadenylation.

So, deadenylation can trigger an RNA decay so, that is deadenylation dependent. And another example is deadenylation independent; that means, the RNA can still have the poly a tail intact, but still it can be marked for degradation. So, we call it as deadenylation independent. Then comes the third category endoribonuclease mediated mRNA decay.

Like we saw nucleases can act right in the middle of the RNA and it can cause creation of two ends, 5-prime end and 3-prime end which is not protected. Usually the 5-prime end supposed to be protected by 7 methyl cap, but you created by cutting right in the middle of the RNA. So, that can have vulnerability to exonucleases and also 3-prime end new 3-prime end is created because of the endonucleolytic cleavage and that is not polyadenylated. So, that is also vulnerable.

So, deadenylation dependent mRNA decay, bulk of mRNA's undergo decay by this pathway. A majority bulk means a good number of the mRNA population undergoes degradation by this pathway. The 5-prime, 3-prime exonuclease pathway kicks in on top of that and the exosome mediated 3-prime, 5-prime exonuclease pathway also kicks in.

So, once a deadenylation has happened it is vulnerable to both kinds of exonuclease activity 5-prime to 3-prime exonuclease and 3-prime to 5-prime exonuclease.

Of course, you may wonder, oh cap is there, what happened to cap? Of course the cap will be removed by decapping enzymes. So, if it is just like, if you see a national geographic channel, if you are watching you will see in a herd, normally you see if there are a herd of buffalo which is 100 or 200 buffalos are there, if your lions are attacking them, they will be just a charging one to them, they will just go after them.

In this process they will create a panic and you may wonder why they have to do this futile exercise. They are searching for the weak. When they chase a herd, the weak one will linger, weak one will struggle and they will fall back or they will start showing signs of weakness.

Same logic applies; if an mRNAs cap is removed then it is a free for all. Other nucleus will jump in, remove the other parts like it can, if the cap is removed or if the poly a tail is removed then immediately the decapping enzymes will come. Just like how lions will go after a weak animal. Same logic applies when it comes to enzymatic degradation of the RNA.

And the deadenylation independent means poly a tail is intact, but still some specific decapping enzymes come into picture and it can cause the degradation of degradation of the RNA population. And even if both these ends are intact, endonucleus can come right in the middle and cause the degradation.

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So, let us see deadenylation dependent mRNA decay, how does it work? Shortening of poly a tail by deadenylation enzymes like I told you, many enzymes are capable of causing removal of the poly a tail and we call it as deadenylation. And if the poly a tail has fallen below certain threshold, the PABP is no more able to bind. PABP need a certain stretch of poly a tail in order to bind.

If the deadenylation happened, then PABP will not find the target attractive and it becomes a naked RNA. And this can cause deadenylation enzyme acting on it and it will reduce. It will not cause the degradation; it will simply get rid off the poly a tail. Just like poly a polymerase was adding the a tail, the deadenylating enzymes will remove the poly a tail sequentially. And this step is reversible.

Why you can have cytoplasmic poly a polymerase? Just like you fall sick, does not mean that it is a death sentence for you. You are sick now, you are not well, you will go to hospital, you will get a saline drip or maybe medicines and you will back up and running. So, same way, sometimes deadenylation happen and if poly a polymerase recognizes in time and it realize this an important.

And then the poly a polymerase will keep adding the poly a tail onto the reduced tail bearing mRNA and it will now become longer and it will back to normal. So, just like how you go to hospital and get recovered from your ailment. Followed by either removal of a 5 prime cap by the decapping machinery and 5-prime 3-prime decay or 3-prime 5-

prime decay; this reversible process did not happen say deadenylation happened, repair did not happen. Just like you are sick, you did not take medicine, did not go to hospital. Situation can worsen and it can be life-threatening. So, we have to be careful enough to go to hospital in time.

Same way, if an RNA is problematic, then you have to take care of the system effectively. That is why people who get some fever, some ailment like typhoid or maybe chicken pox, etcetera. After that they realize, after that they are losing lots of their hair. Their you know their skin has wrinkles, you know they are not well, they are weak, etcetera.

Why? Because the body is using a lot of its resources in its immunity or fighting the virus so, the normal procedure like you know growing hair, which is I am not saying hair is an unimportant part, but hair is a outcome of the biology of your cell, your epithelial cell in certain part of your body. Of course, it has got its own role, like protecting from cold; you know your head has to be kept warm, especially in cold conditions. So, hair does this insulation work function. Many things we can discuss on and on about it.

But when you are sick body is not able to handle those so-called unimportant RNA population, then there is no repair happens. There is no, those RNA responsible for hair growth may not get this helping hand of you know extra poly a poly. There is hardly any poly a polymerase available because they are running short.

So, they will not get extended and some proteins will go missing and that will result in hair loss. This happens all of you would have seen even pregnant mothers after their delivery, once you know their body is demanding a lot in the you know milk production etcetera.

So, a mother's body is literally spending lot of energy in the uplifting or upbringing of the newborn baby. So, her body is suffering from nutrition. So, they also will face hair loss. I gave an example like this because this does not mean that your brain function can be compromised or your you know digestion or some of your heart beating etcetera can be compromised. So, they are vital functions. They will be retained.

So, so-called unimportant functions will be like maintaining a strong say you had a strong muscle and you have to daily do exercise and eat good protein rich food etcetera

to maintain it. But if your body is little bit suffering or body is wanting urgently energy for some other source, then your muscles will start becoming loosen muscles. You will you will have you know loose skin, saggy skin etcetera.

Because body is not spending enough energy to retain the production of certain proteins required in those tissues by you know facilitating the rejuvenation of some defective RNA or a prematurely dying RNA. They are not repaired effectively because body do not have energy. It has been utilized for something else.

So, we have to understand deadenylation dependent mRNA decay although it is reversible, it need not be reversible. If it is not reversible the polyadenylation or poly a tail is not reversed, then it will be marked by removal of the 5 prime cap and this itself can trigger the 5 prime 3-prime exonuclease. And the absence of poly a tail will trigger a 3-prime to 5-prime exonuclease activity.

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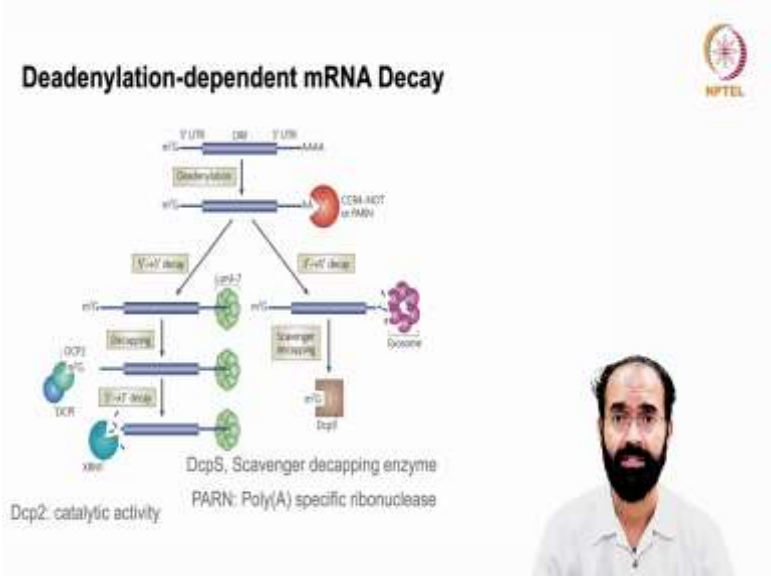

So, deadenylation dependent mRNA decay, we can see something like this. You have a mRNA proper ORF, 5-prime untranslated region and 7-methyl guanosine cap and you have a 3-prime UTR and the poly a tail. This since it is a cartoon, do not think that poly a tail is only 4-ase. For convenience, 4-ase are shown here.

If the deadenylation happened by whom by an enzyme called CCR4, not or also called as PARN. So, these are enzyme PARN, poly a specific ribonuclease that is the short form

and CCR4 not is the name of another enzyme that is capable of taking down the poly a tail or reducing the length.

So, if this happened, then it can follow 2-trac. One is 5-prime 3-prime exonuclease activity that is starting from the 5-prime end or 3-prime 5-prime exonuclease activity. How does it happen? Once this poly a tail is shortened, then this can be bound by Lsm1 to 7. It is a ring protein, Lsm-1, Lsm-2, Lsm-3 like that, Lsm1 to 7 proteins form a ring around the UTR untranslated region and this can trigger the decapping.

Once this Lsm1 to 7 protein, just like I told you, I heard of buffalo, one buffalo is limbing. That is the dinner bell for the lion, which is chasing it. So, it can easily catch, oh if I perceive little bit, this buffalo is my food. So, this Lsm1 to 7 is just like a signature for the decapping process. This will trigger the decapping and decapping is done by enzyme DCP2 and DCP1.

DCP1 and DCP2 are 2 enzymes complex that will cause the 7 methyl guanosine to be lost. Now, this will invite a 5-prime, 3-prime exonuclease such as XRN1. These names of these enzymes are very important. If you do not understand the names of these enzymes, you will not understand the decay of the RNA. So, XRN1 is a 5-prime, 3-prime exonuclease whereas, exosome, we saw in one of the earlier class also about exosome.

But say for example, Lsm1 to 7 did not bind for whatsoever reason depending upon the UTR structure, secondary structure. Let us not go into the details what made Lsm1 to bind onto this dehydenylated and translated region of an mRNA. That is a different topic altogether. But understand, not every species of RNA which is devoid of poly a tail will attract Lsm1 to 7 some population attract Lsm1 to 7 which will cause the decapping eventually.

But whereas, if Lsm1 to 7 did not bind, does that mean the RNA will be safe? The answer is no because the poly a adenylation is important. So, what will happen if the poly a tail is reduced and cap is intact, but this in turn will attract the exosome. Exosomes are a group of 3-prime to 5-prime exonucleases and that can eventually cause a scavenger decapping also. That means enzymes such as DcpS, DcpS is a S stands for Scavenger. Here we saw DCP-1 and DCP-2.

So, exosome itself is capable of doing the 3-prime to 5-prime exonuclease, but this will be further aggrieved just like you know putting salt in the wound. So, it will further exacerbate the situation by introduction of DcpS which is a scavenging enzyme scavenger decapping that will start the 5-prime. Then it is ok for XRN1 also because once the decapping has happened or the 7-methyl guanosine is removed then XRN1 can start acting on it.

So, whichever situation whether it is via DCP-1-2 complex or via DcpS the decapping effect has happened it is an attractive substrate for XRN1 exonuclease, 5-prime, 3-prime, exonuclease activity whereas, if exosome directly coming into picture it will degrade by itself. It do not need the help of any other protein because exosome itself is a exonuclease. It will cause the 3-prime to 5-prime exonuclease activity.

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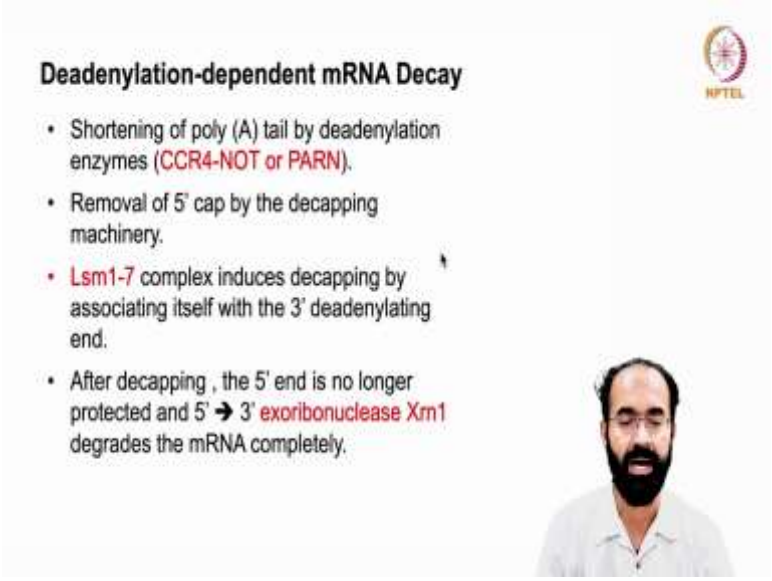

Let us see how this process are explained inward. So, this is we are talking deadenylation dependent mRNA decay. So, shortening of poly a tail by deadenylation enzymes such as CCR-4- NOT or PARN. So, poly a specific ribonuclease that is PARN and removal of 5-prime cap by the decapping machinery DCP-1-2 complex and then Lsm-1 to 7 complex induces decapping by associating itself with the 3-prime deadenylating end. So, this is another requirement for the Lsm1 to 7 mediated decapping.

Although they do not cause the decapping, but their presence will attract the DCP1 and 2 to the 5-prime end of those RNA. After decapping the 5-prime end no longer protected

and 5-prime 3-prime exonuclease, exoribonuclease, XRN1 and that degradace degrades the mRNA completely from 5-prime end of the mRNA.

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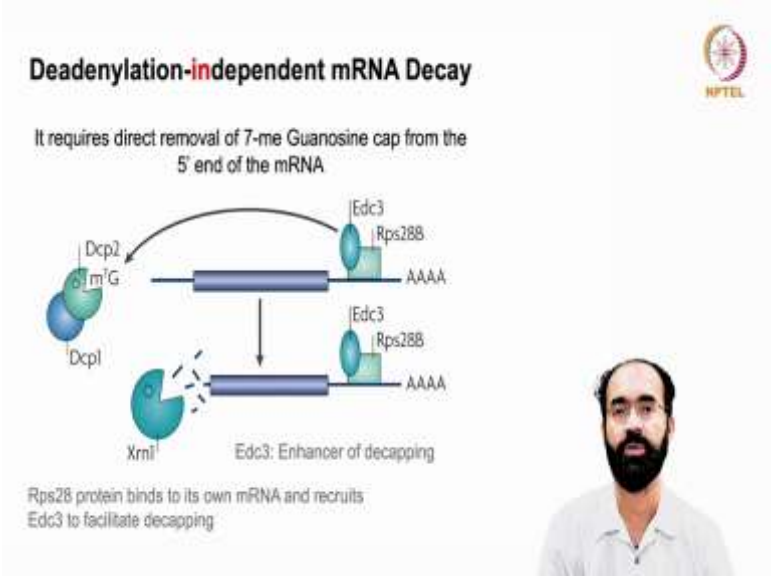

Now, let us see deadenylation independent mRNA decay. In this how does it happen? So, it requires direct removal of 7-methyl guanosine cap from the 5-prime end of the mRNA means it will not bother whether 3-prime end is intact, 3-prime end is useful, functional, nothing, it will not bother. Here also the decapping enzyme is Dcp1 and Dcp2 same as where you saw Lsm1 to 7 was binding onto a deadenylated poly a tail.

However here the Dcp1 and 2 is not looking for Lsm1 to 7 because poly a tail is intact, but here what happens although an RNA that has got poly a tail intact the binding of 2 proteins that is Rps28B and Edc3 these complex or this is a heterodimer. If they are bound between the coding region and the polyadenylate poly a tail or polyadenylated region this we call it as 3-prime untranslated region.

If Edc3 and Rps28B heterodimer binds onto this UTR that can attract just like how Lsm1 to 7 attracted Dcp1, Dcp2 complex. Similarly, this can attract this molecule and this in turn will remove the 7 methyl guanosine cap which makes the 5-prime end vulnerable to Xrn1 exonuclease.

So, this is basically called the Edc3 protein's name is enhancer of decapping; that means, Edc3 when it is found it will bring it will attract the Dcp1, Dcp2 complex and the

moment Dcp1 Dcp2 complex that has come to the 5-prime end of an RNA the cap is lost and once cap is lost Xrn1 will find it an attractive substrate to cause the degradation.

Rps28 protein binds to its own mRNA and recruits Edc3 to facilitate decapping this is a kind of auto regulation. You should understand a given cell say for example, you think of a situation Rps28 protein is present plenty more than actually say if only 100 molecule are required this cell has got 10,000

Then what will happen every RNA will not be able to function because every RNA has a proper poly a tail proper cap, but what will happen Rps28 will go and bind down to their 3-prime UTR attract decapping enzyme and degrade it. So, it is almost like you are like you are drawing a line on a board you are moving from left to right using your right hand and your left hand with the duster you are erasing it will look so, counter intuitive and basically a useless job.

So, you do not want that to happen. So, who will control it this has to be controlled by Rps28B itself. So, Rps28B if protein is their plenty what it will do it will go and bind on to its own RNA UTR and then it will invite Edc3 and cause its own degradation. So, once Rps28 protein is causing or Rps28 protein is causing the degradation of its own RNA then the protein level will decline.

And protein level will decline to some extent then other RNAs will get a chance to survive and function and once the RNA is no more the Rps28 level is declining to a low level then what will happen it will not be able to degrade its own RNA, but RNA is being constantly produced. Then the Rps28 protein will start increasing its level because say any given cell is producing 100 Rps28 RNA let us assume, then each RNA can produce multiple copies of this protein.

Then if that much number is there that will cause the degradation of lot of RNA then this 100 Rps28 RNA itself is superfluous it is much more then it will cause this protein will cause its own degradation. So, the 100 number can come down to 3 or 2 you have cell has only 2 Rps28 RNA. So, the protein will automatically will come down. And once it comes down then other RNA will be able to perform.

But the other RNA when it is not able to or it is not requiring to do its job or its purpose is done you need Rps28 then what will happen the Rps28 RNA is constantly being

produced then the degradation is now prevented by Rps28 level is very low. Because it already degraded its own you know RNA population, but now since the production of Rps28 is going on constantly it will build up eventually.

So, you can think of like somewhat like an oscillation like a wave it goes up then down again up just like something like you can see sometimes if you watch share market fluctuation you can say it goes up and then comes down again go up. It is not exactly like a wave, but trend somewhat if market is going up now it can come down if it comes down it will go up similar something similar. So, this oscillation has to happen in Rps28 level to provide ample opportunity for other RNA to function.

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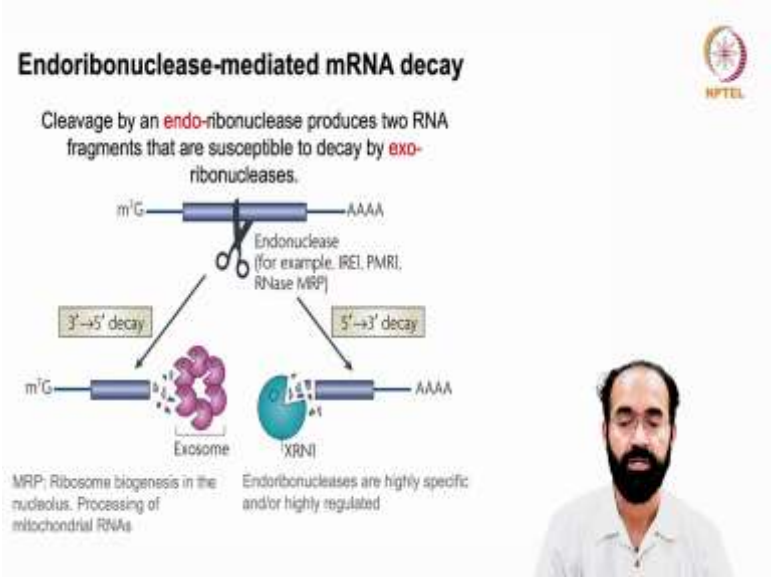

Now, let us see endoribonuclease mediated mRNA decay word itself says enough because endoribonuclease do not bother 5-prime end or 3-prime end whether it is protected or not protected. It can simply act right in the middle create new two ends which is 5-prime and 3-prime ends and exosome and the XRN1 can happily eat chew them up. So, that is exactly what happens.

Cleavage by an endoribonuclease produces 2 mRNA fragments that are susceptible to decay by exonuclease that this 5-prime end is protected this 3-prime end is protected, but when you cut it you are exposing creating a new 3-prime end and you are creating a new 5-prime end. So, the 3-prime end will be chopped up by exosome the 5-prime end will be chopped up by XRN1 simple.

So, what are the enzymes doing this IRE1, PMR1 and RNAse MRP these enzymes can come and act right in the middle of some RNA. And remember RNA always fight against it by forming polyribosome complex like every RNAse jump act with multiple ribosomes then the ribonucleus are not welcome there because there is no gap no place. Some of them will form specific bodies they will form like some you know round shaped structure and protect themselves from the degradation.

But once this endonuclease have acted upon them the fate is sealed. So, MRP is name of a ribosome biogenesis that is important in the nuclease its contributing because processing of mitochondrial RNA also is controlled via this exonuclease endonucleases. And endoribonuclease are highly specific and are highly regulated because we will see them this degrading enzymes have to be controlled very effectively and very specifically.

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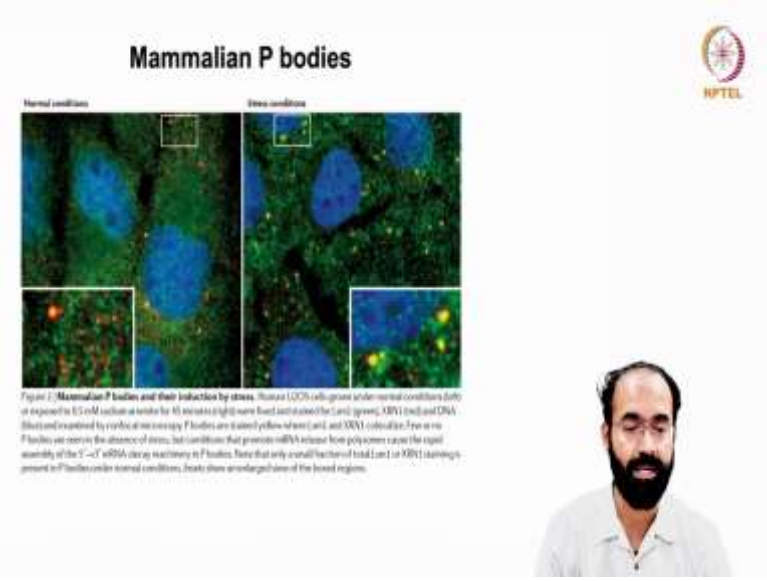

Let us see one real-time picture of mammalian P bodies what is this mammalian P bodies they are structures where the RNA degradation can happen effectively and they are they are induced when stress is there like stress can come to anyone, like if you are stressed you are having tension you do not sleep effectively your body is suffering actually. So, this you do not eat food all are stress.

What happens? Whenever there is a stress you can see you can prove it. So, in this study this is culture cell culture this blue color is the DAPI staining of the nucleus and the green color one is the cytoplasm green and red dots you can see. What is green? Green

one is Lsm1 we have seen it Lsm1 binds down to the 3 prime UTR after the decapping and XRN1 is an exonuclease.

So, in a normal cell you can see Lsm1 green which is separate and XRN1 is also separate means they are not bound or they are not in the close vicinity; that means, they are there ready for action, but not acting right now, but when there is a stress, you see lot of yellow parts are there yellow dots are there.

So, green and red combined together when they come. So, close vicinity it will look like yellow in color. So, after stress you can see stressed condition there is plenty of yellow bodies; that means, Lsm1 now attracted XRN molecule XRN1 exonuclease and caused decapping. Of course, DCP1 and 2 complex would have come and it caused the degradation. We will see more details about this RNA Decay Pathway in the next class.

Thank you.