RNA Biology
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Lecture - 38
Mechanisms of RNA Decay and Non Coding RNAs: Autoregulation of RNAs

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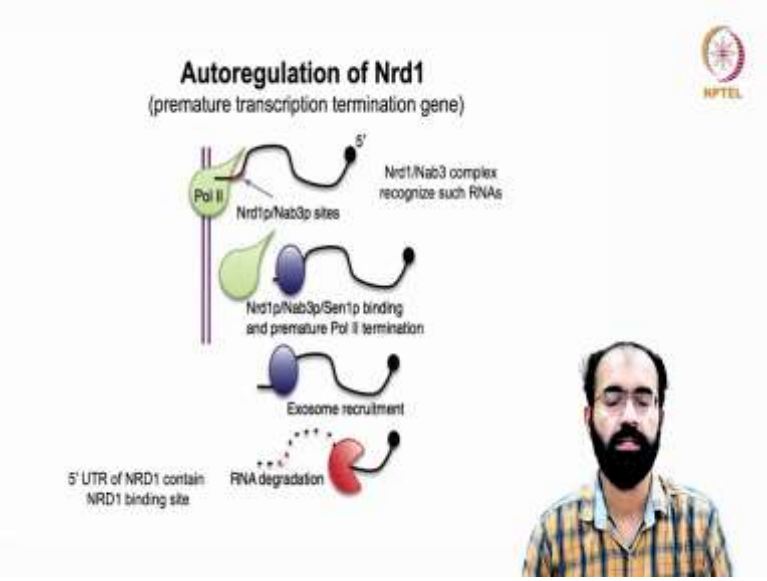

Hello everyone, welcome back to another session of RNA Biology and we were looking at the Autoregulation of certain RNAs. How are they binding on to the sequence of their own RNA and how is influencing the stability etcetera.

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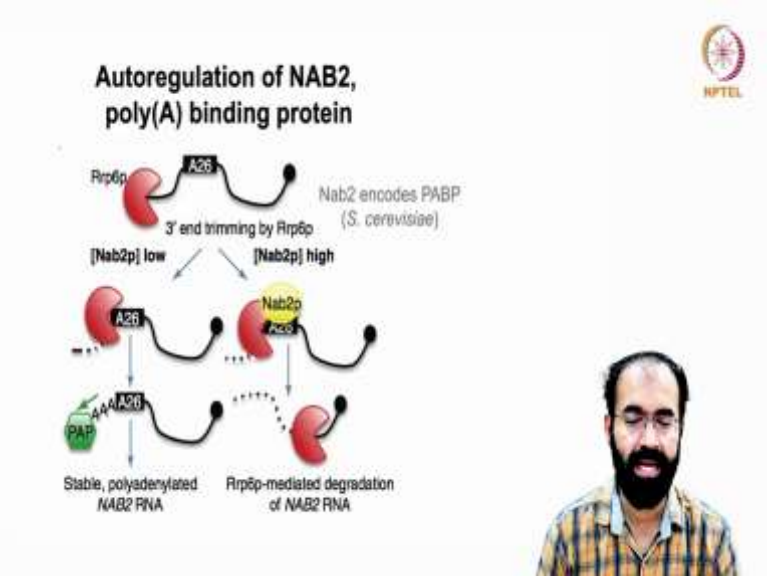

So, let us see another example, the autoregulation of Nab2 and the poly A binding protein. So, let us see how is it working? So, we can see the Nab2 is a homologue of poly A binding protein in Saccharomyces cerevisiae. So, we know its function, we will not go into the detail because poly A binding protein the name itself says, where it is supposed to bind.

So, the stretch of sequence that is present in the RNA, especially a region called A26 that is a stretch of A s up to 26. And it can be influenced further by trimming enzymes; such as, Rrp6p and that will influence the length of the poly A tail. And this is a cap what you are seeing and this is basically the trimming by Rrp6p. And it will reach up to a adequate length say A26; that means, 26 adenines.

Now, you have a scenario where Nab2p that is a protein, Nab2p is low and Nab2p is high. Remember Nab2 is a PABP in Saccharomyces cerevisiae. Now, if Nab2, Nab2 is low, then it will follow a given pathway, Nab2 is high it will follow a given pathway. We kind of discussed in the previous class that many autoregulations are demand based autoregulation; that means, if there is more product you do not want more further more product.

So, you want to reduce it. So, one way of reducing it is getting rid of the RNA encoding that gene. So, that no more protein is formed. So, when Nab2 is high then it can bind onto A26 region and this binding can stimulate the Rrp6p that is a exonuclease and it

will further chew up beyond this A26 region and it will completely degrade the RNA when Nab2p is high, now if Nab2p is low, trimming is happening trimming is happening by Rrp6p and it will come up to A26 region.

But remember Nab2p is low; it is not available for binding because unless the level of Nab2p is high it is not going to bind onto A26. So, what happens? No more binding is happening and once it reached up to A26 then it can be a signal for the poly A polymerase. That means addition of poly A tail can continue and this will make a stable polyadenylated Nab2 RNA.

So, this trimming will continue again you know if Rrp6p is not going to keep quiet, it will come and trim again come up to A26. But whether or not Nab2p is binding that will decide the eventual degradation. So, if this RNA is stable, it can participate in the protein translation and it can continue to produce the Nab protein which eventually bind onto the A26 region and cause the degradation by this exonuclease. So, this is the autoregulation.

So, when more Nab is there less RNA will be stable or RNA will be unstable, Nab2 RNA will be unstable and if Nab2p protein is not available because of its low abundance then A26 is free. So, no more Nab2p is binding onto that A26 region and the poly A polymerase will continue its extension.

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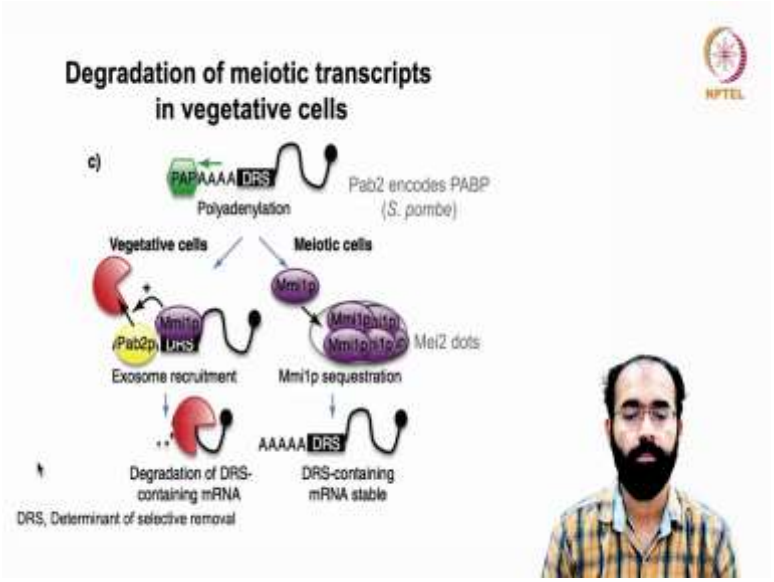

Now, let us see another example of degradation of the meiotic transcripts. So, this is something interesting. So, all of you know what is meiosis? In yeast, Saccharomyces cerevisiae it can undergo the degradation of the degradation of certain RNA based on whether the cell is in the mitotic stage or meiotic stage. So, let us see an example in how a vegetative cells of Saccharomyces cerevisiae behave.

Because vegetative cells are not meiotic, so in a vegetative cell you do not need meiosis. So, all of you may have heard about cell division. We have two kinds of cell division are there; mitosis and meiosis. When you have a cell death or when you have a wound in your hand, you want to repair that wound then the cells undergo mitosis in our body and even yeast when yeast has to increase its cell number, it will undergo mitosis.

So, in mitosis we also have like mitosis entire body, entire human body the cells are dividing by mitosis, no doubt about that. Even during development, the cells are dividing and dividing and dividing. And this undergoes a bulk production of cells and you can use for wound healing like there is a production of blood cells in your bone marrow that is also mitosis. Your hair has fallen or your skin is eroded, your intestinal epithelium is eroded, replacement is by mitosis.

And so, a two-end cell or a deployed cell makes another deployed cell. But when you are talking about meiosis, there is something called reduction division takes place. Reduction division means say we have 46 chromosomes our diploid number is 23 pairs, 46 chromosomes. During meiosis, where gametes are formed, this happens in gonads that is testis and ovary where the gametes are being formed.

There has to be a reduction division. That means the 46 chromosome now reduced to 23 chromosome or the pairing is disrupted. Not randomly some 23 chromosomes are removed; it is exactly like 23 pairs is no more 23 pairs. It is 23 singletons, so that is what is present in the gametes. And the remaining 23 will come, during fertilization. So, this is the basis of sexual reproduction. All of you would have studied this in some of your earlier class.

So, in yeast also, it has to undergo something similar. It has to maintain the decorum of the cell. A vegetative cell should remain vegetative, a meiotic cell should remain meiosis. So, this yeast, it is a single cellular organism. It does not have the so called,

what you call; it does not have the so called tissue organization like, you know, gonads are separate, liver is separate, kidney is separate, no such things.

So, a given cell switches from mitotic to meiotic, meiotic to mitotic. So, if this happens, it may not be equivalent. I will give after giving an example of this, I will also explain you how something similar degradation happens in a eukaryote also, we will see that example. So, for convenience, let us go through the degradation of meiotic transcript; that means, transcript give rise to proteins and their proteins, those proteins are helpful in maintaining the meiotic status of the cell.

So, a meiotic transcript in a vegetative cell, which is mitotic by the way, vegetative cell is unwanted. So, I told you that the cells switch between mitotic and meiotic phases. So, a vegetative cell has to deal with the meiotic transcript, you have to get rid of them. How does yeast is doing that? So normally, a RNA, say, Pab2, which encodes 4 poly a binding protein, we have seen it.

And we also know that each RNA will have a cap, and also it has got a sequence called DRS. What is DRS stands for? Determinant of Selective Removal and this sequence is vulnerable for binding of certain proteins, and it has a poly a tail and this is taken care by poly a polymerase. So, this continues normally, a given transcript, which is meiotic specific.

And based on the nature of the cell, it has to change. So, let us see, how does it deal with it? In vegetative cells say, given transcript, what will happen is this DRS is bound by Mmi1p that is the protein Mmi1p protein. If it is bound, then what will happen? It can create an exosome recruitment usually, if it can cause a exosome recruitment, then that will eventually lead to the degradation.

We know exosomes are meant for 3 prime to 5 prime exonuclease activity. So, if DRS is bound by Mmi1p and the Pab2p can get influenced by this exosome, exosome will get recruited, and you will end up getting; you will end up getting a degraded RNA. So, degradation of DRS-containing, DRS is a sequence that is determinant of selective removal.

DRS containing RNA will be removed under the influence of Mmi1p and Pabp, because there is a poly a tail here, this is a 5 prime end and it is a 3 prime end and they will

recruit the exosome and can cause the degradation. On the other hand, what will happen if Pab, if what will happen, if the cell has changed its identity to meiotic cell; that means, cell decided no more vegetative phase, let us go into meiosis.

Under what circumstance a yeast cell will decide etcetera, we will not go into the detail. But in a simplistic form we can understand in the reproductive phase, it will undergo the meiotic switch in a vegetative phase, when there is enough nutrition, enough space, enough reagent then it will continue to increase in number.

When there is a reduction in nutrition, when there is adverse condition, it will switch on to the meiotic phase, where it has to preserve the genome or the identity of the organism, it will wait for a convenient situation. So, in meiotic cells what happens? Mmi1p is present in abundance, then what will happen; same Mmi1p which was able to recruit the exosome is not recruiting that. What it is doing?

It is forming a cluster, it is forming Mmi1p complex by binding onto the RNA, same DRS containing RNA and it formed a cluster and this Mmi1p is now removed onto a Mei dots and these Mei dots are kind of acting like a scavenger. So, because of this removal, so some small amount it can bind, if it is small amount, it can bind and cause the degradation.

But meiotic cells this Mei dots Mei2 dots, this will sequester the Mmi1p proteins and they are not available for binding, because such a dots are not being formed in the vegetative cells. So, Mmi1p proteins are sequestered and they are not available for binding onto DRS as it was available in the case of vegetative cells.

But we should understand Mmi1p if it is present in small quantity, even in meiotic cell, it can bind and it can do the same job of degrading it, but the credit goes to this Mei2 dots where the Mmi1p are sequester like a sponge, they are no more available. It is just like you know when there is a fire accident, the one of the earlier way of extinguishing the fire is to open a CO2 cylinder, carbon dioxide cylinder and you blow there.

So, carbon dioxide once you push in, it will cut off the oxygen supply. So, if oxygen is not there, fire cannot kick start. So, normally if a firewood or plastic or something is getting fire, you can simply pour water. So, once it is wet, it cannot catch fire. But it would not work if it is a petroleum hydrocarbon, is because petroleum hydrocarbon do

not mix with water, it will come on top of water and it will catch fire. So, fire will go up and up.

So, such situation, you have to cut off the communication of this compound with oxygen, because without oxygen, you cannot catch fire. So, you use CO2, something similar strategies being used, Mmi1p is no more available to bind down to the DRS. Because of this, the DRS-containing RNA are stable, available and they will proceed with translation. So, interesting thing to note is that DRS-containing RNA are used and they are useful in the maintenance of meiotic status.

So, what we should understand, DRS-containing RNA, if they are degraded from the vegetative phase, there is no loss occurring to vegetative cell. So, if DRS-containing RNA are stabilized in meiotic cell, those RNA are producing proteins required for the meiosis of the organism. So, in both case, it is a win win situation. So, the vegetative cell finds this DRS containing RNA as a nuisance, whereas, meiotic cell finds this DRS-containing RNA as a blessing.

So, this is what you should keep in mind. So, the idea of Mmi protein being present freely or being present but not available freely decides, whether a bunch of DRS containing RNA should be expressed or should not be expressed. So, I was telling that similar situation happens, so I will give an example of tissue regeneration for you to have an idea.

Normally, when a tissue is damaged, there are different ways of fixing that. One way is to induce stem cell, which normally many lower vertebrates do it. So, they induce stem cell by expressing the pluripotency factors. So, pluripotency factors are you know, sox two c-Myc, Lin28, Klf4, Oct-4 like that. Total 6 are there any 4 can act as pluripotency.

We will not go into the details of that. There are two groups of factors, Thomson factors and Yamanaka factors. But the important thing, how Lin28, one of the pluripotency factors are influencing the expression of other pluripotency factors is something interesting. We should know that the induction of stem cells happen via the expression of this pluripotency factors.

So, this RNA are expressed and the RNA should be translated. But there is a catch, every say neuronal tissue, many differentiated tissue say for example, neuronal tissue, they will

have a high level of let-7 microRNA. MicroRNA are present abundantly. So, this let-7 microRNA, they will prevent the translatability of a bunch of mRNAs that are required for the mitosis.

That means, a differentiated tissue such as neuronal tissue do not want any proliferation to occur. They want their proliferation to be kept in check. So, this pluripotency factors say for example, Oct4, Lin28, c-Myc these kind of genes when they are induced. Immediately, cell can kick into a proliferative phase. But the let-7 microRNA will go and bind on to their UTR and prevent their translatability.

Even if these RNA are produced like MYC or Lin or many such genes are their pro-mitotic genes, if they are expressed, the let-7 microRNA prevents the translatability and the stability of this mRNA. So, what you have to do? First if the reintroduction of stem cell or the reprogramming of stem cell has to happen, the first thing is you should get rid of the let-7 microRNA.

If let-7 microRNA is there, all the other efforts to induce pluripotency genes will go down the drain. It is now of no use. So, one of the important genes that is required is the Lin28. Lin28 is an RNA binding protein that can create the degradation of the let-7 and it can also block the production of let-7. So, we have seen it the earlier the microRNA production that DGCR8 and Drosha works in the nuclease and the dicer works in the cytoplasm for the production of the microRNA in general.

So, the Lin28 prevents Drosha from producing the microRNA and Lin28 can recognize the mature let-7 microRNA, cause its degradation. So, what actually for a stem cells to form is get rid of the lead-7 microRNA and Lin28 does that. So, Lin28 is produced in an injured cell and I am talking all these things based on the regeneration of the zebra fish retina.

So, I am telling lessons from that. So, this Lin28 degrades and gets rid of the let-7 microRNA. And the let-7 microRNA when it is absent the other pluripotency factors and pro-mitotic genes can happily get translated and now this stem cells can form. So, something similar you have seen in the case of this Mmi1p in the shifting of vegetative and the meiotic phases.

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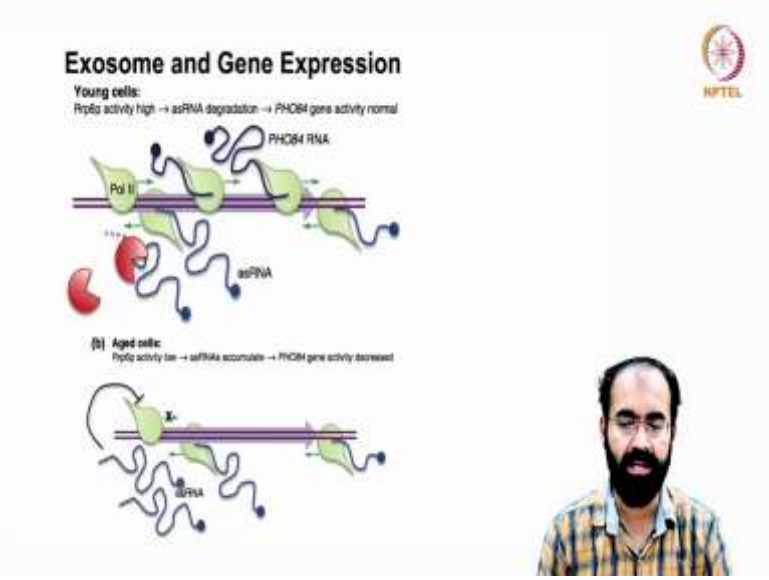

Now, let us see another example that is exosome and gene expression. How exosomes will influence the gene expression? Let us see example. Here we are comparing young cells versus aged cell. In young cell what happens? Rrp6p, we have seen Rrp6p which is basically a exosome here and Rrp6p activity are high is pretty high in young cell.

So, what will happen? It can do a antisense approach. How does it do? So, it can cause the degradation of a antisense RNA and because of this a gene called 484 gene activity remains normal. So, here what we should understand? You have a gene that is 484 that has got two strands that is transcribed. One is making 484 sense strand and you have another strand making the antisense strand of 484.

Remember many of this kind of way of regulation happens in several mRNA and several non-coding RNA. This antisense RNA is not coding for a gene not coding for a protein rather this antisense RNA to this 484 gene is meant only to regulate the expression of this 484. This is what you should keep in mind. So, this antisense RNA can form and this Pol II can produce 484 RNA.

Now, when the Rrp6p which is an exosome when the activity is high the antisense RNA can be degraded and because of this the 484 RNA can be formed properly without any problem. Because there is no one to pair with the 484 RNA and it will be happily translated and the 484 protein is formed.

Now, think about a situation where the cell is aged you are having an aged cell. So, where Rrp6p activity is low which is an exosome an exonuclease. Rrp6p activity is low and antisense RNA is stable now because Rrp6p activity is low and no one is there to degrade the antisense RNA. Because of this the 484 gene activities decreased how because antisense RNA will pair it will form a dimer or duplex with 484 RNA. When 484 and antisense of 484 gene pairs then it will invite a degradation.

So, double stranded RNA are not welcomed, double stranded RNA will be immediately attractive RNA interference and it will cause the degradation, in any system you do not want a forward and reverse stand pairing together it will invite double stranded RNA will be a warning sign for the degradation mechanism.

So, in aged cell Rrp6p activity is low and that will allow stable antisense RNA and 484 is no more able to produce because it pairs with the antisense RNA and it is getting degraded. So, the change happened mainly because of whether a cell is young versus whether a cell is old. So, the age of the cell decided whether the Rrp6p is activity strong activity or weak activity.

So, young cells it is strong and that is getting rid of the antisense RNA hence the 484 can happily translated. Whereas, the activity of Rrp6p is low and the antisense RNA remained stable and that could cause the degradation of the that could cause the degradation of the 484 RNA. And of course, antisense RNA will be degraded because of this pairing, but antisense RNA is not coding for anything, hence no definitive action or definitive effect on the cell.

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Now, we have seen with these examples, the distinction between RNA quality control system and gene regulation is not very clear. Lot of things are clear, but the question remains how can the entire list of entire group or entire bunch of RNA can be regulated this way. That is why I told you in the beginning of this RNA decay pathway or RNA decay studies, it is still incomplete.

First of all, we do not know how many mRNAs are there even in the most important organisms such as human it is only a guessed amount. So, hence unless you know your enemy, you cannot say whether you won over your enemy. Same way unless you know how many species of RNA is there, no point in discussing the stability of that unknown RNA. All we know it is there, it is quite elusive the RNA different types of RNA.

Therefore, the RNA decay pathways are not there just for the removal of the junk alone, because RNA decay pathway often introduces lots of tiny fragments. Each of these fragments were going to have a biological function like classic example is micro RNA. Nobody knew micro RNA can create, so much of effect on the survival maintenance homeostasis of a given cell.

Now, we realize that micro RNA can do lot more than it was thought to be. Initially, it was thought to be a post degraded fragments and nobody thought that micro RNA are produced with a purpose and they have a role to play. So, RNA biology is quite baffling topic and it will continue to baffle us. So, we should try to explore each and every

information we get it in a live cell perspective, so that we can assume, novel and novel pathways that might work for the survival and the propagation of a species.

Thank you.