RNA Biology
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Lecture - 35
Mechanisms of RNA Decay and Non Coding RNAs: The Exosomes

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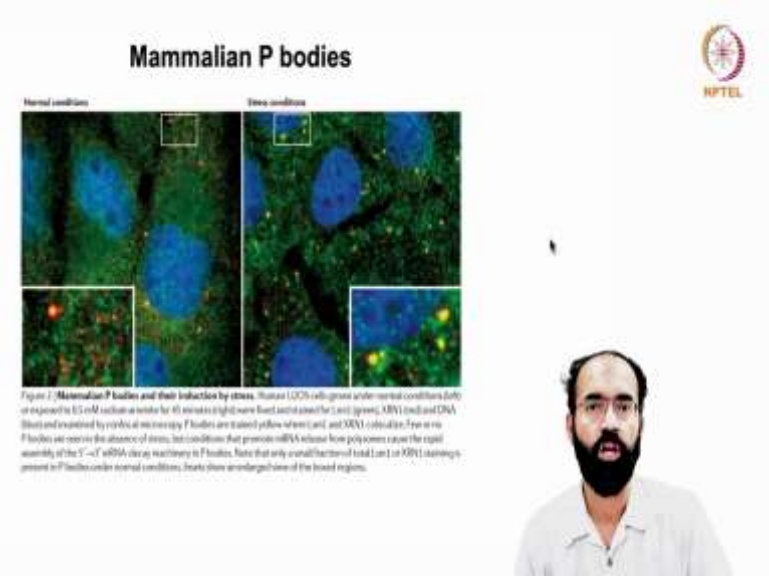

Hello everyone, welcome back to another session of RNA Biology. So, we were here in the previous a class that this is an example, real time example of a Mammalian P Bodies, where you are having the congregation or association of an inviter and inviter is Lsm1 and the actual player is XRN1. So, what we can see is the green colored labelled Lsm1 is uniformly distributed in the normal conditions. And also, the red colored one also distributed uniformly in normal conditions.

So, what it indicates, a normal cell have got both Lsm1 and XRN1 adequately and distributed evenly in the cytoplasm. But they are not coming together. It is just like you have fire everywhere, you have petrol everywhere. As long as the fire does not come close to the petrol, you are safe, there is no problem. Fire means do not think literal fire, the fire-litting match box or something, something like that you should understand.

The moment they come together; you will see the fire. Petrol will be in one color, match box will be in another color, but if they come together the fire will be in another color. That is what the red color is XRN1, the green color is Lsm1.

Of course, they do not have color, ok. So, they are made by immunofluorescence that is using antibody. I will not go into the detail because it is a cell biological technique where you detect proteins of your interest using antibodies, labelled antibodies against them. And they go and bind and you use a primary, and then secondary, and then you read them.

So, those who are interested can read about immunofluorescence in you know cell biology studies. So, like that you are giving, you can give any color of your choice. In this particular experiment they have done that.

And then in stress condition, you can give stress in multiple way, you can give nutritional stress, you can give you know oxygen stress you know or temperature stress, you can give inner tissue culture cell, you can give stress in multiple ways. In any case, they are giving stress.

And like in the case of human like I told you, if you do not sleep, do not eat food, you are exerting yourself, you are thinking a lot and getting tensed or you fall sick because of some infection and many things, everything is stress. Or you took even some medicines, high dose of antibiotic, everything is stress on your body.

But does not mean that all the stress will lead to you know RNA degradation. But majority of the stress once it goes beyond certain limit. So, even running on the ground is also stress. That does not mean that your RNA will start degrading. That is also stress, but you will recoup back.

But if you are running only, you are running whole day which you cannot, that is a different thing, but if you are running then your body will fall apart. You simply cannot, your body want you to run, and then you know recover, so that it will build etcetera. Just like going to gym also, you leave whole day in gym, you know your muscles will fall apart.

So, you have to have a break time where body will recover and rejuvenate back. But when you have an infection, there is no, bacteria will not say, your virus will not say ok, 10 to 2 o'clock, I am taking a break, so you will let your body build up. No, it does not happen. It is constantly using your resources. So, that is a perennial stress, throughout stress.

Until your immune system comes into action or if it is a bacteria, your doctor gave you an antibiotic and you took correct dose of antibiotic, the correct nature of type of antibiotic against that bacteria, then you have a helping hand. So, body will get help to fight this stress.

If so happens, if the stress is unchallenged, means it is not being, there is no, relenting happens onto the stress, then relentless stress can lead to RNA damage. That is what they are showing. The green and the red came together. Lsm1 and XRN1 came together; and that means, nothing but the degradation of the RNA.

So, that is what they are shown here that when very few cells are there, even in normal condition, very few cells are there having a low cell like you can see here, one yellow, one yellow here, but that is ok. That is the normal degradation because even in normal cell need to have RNA degradation, right?

But you see the number of yellow spots here, a lot are there yellow spots and the number of yellow spots are high. That means Lsm and XRN1 came together, until they can do spectral overlap because RNA molecules are very small. Once they come closer, that will give a yellow color signal because green and yellow both are coming simultaneously from one spot.

So, that is what you should infer that because of stress, you can end up getting too much of this stress granules there. You have congregation of Lsm1 and XRN1. Like this, many proteins can be evaluated. That is how you discover like whether what is the role of individual proteins and you knock down one of them.

Let us say total different line of experiment, which take much more time to explain how you go ahead and address to find the function of a gene, etcetera. Now, let us see what are the different molecules that is contributing to the degradation.

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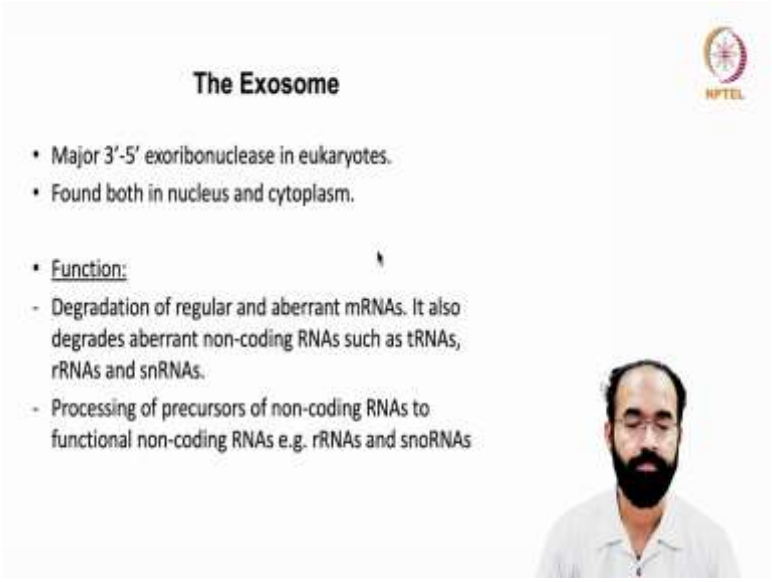

One example is exosomes. And we know exosomes, they are major 3 prime to 5 prime exoribonuclease in the eukaryotes. Major means we have seen the major spliceosome minus spliceosome. Major means that is prevalent one. So, here exosome mediated 3 prime to 5 prime exonuclease activity is the prevalent one and it is found both in nucleus and in the cytoplasm. And because you need to degrade the RNA by exonucleolytic way, both in nucleus and the cytoplasm.

To function, so the degradation of regular and aberrant mRNA. Regular mRNA once the job is done. Aberrant mRNA, it should prevent its the RNA from doing its job because it is aberrant, it has to be degraded instantaneously. So, it is not biased towards a particular species of RNA. Normal, after doing its job has to be degraded and abnormal has to be degraded immediately.

So, it also degrades aberrant non-coding RNAs such as transfer RNAs, ribosomal RNA and SNRNAs, spliceosome RNA. So, many of these not; it is not handling just a RNA. It can handle other non-coding RNAs also. Non-coding means it is not coding for any protein. That is the meaning of non-coding.

So, processing of precursors of non-coding RNAs to functional non-coding RNAs that is ribosomal RNA and snoRNA. They require some trimming, some maturation etcetera. So, do not think exosomes are meant only for degradation purpose. Of course, they are doing degradation, but degradation need not necessarily the entire RNAs itself. A small

portion of the RNA can be removed, so that it undergoes the processing, it undergoes the maturation.

Like if you buy a full coconut, you do not eat the shell of it, right, you eat only the inside part of it. So, you break it, use the inside part whatever you require, remaining will be removed. So, we say I processed the coconut, and I used it for making some food or dishes whatever.

So, same way precursor RNAs are not functional yet. They have some unwanted portions. So, that has to be chopped off by these exonuclease coming under the exosome family. So, they are important in the maturation of ribosomal RNA and small nucleolar RNAs.

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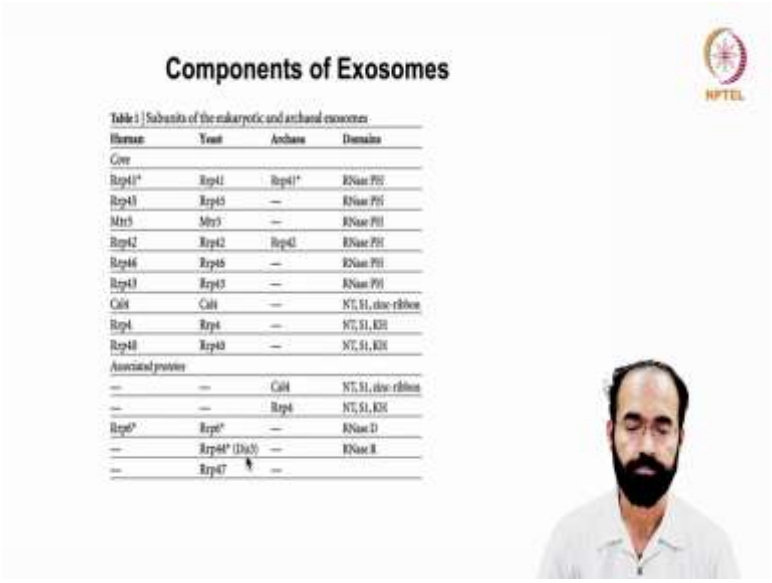

Let us see the components of exosome. You are having examples from human, yeast, archaea and also, we have a column that says what is the domain, what is the functional domain of this.

So, the subunits of eukaryotic and archaeal exosome. Archaea means very primitive prokaryote. So, yeast is an intermediate. It is a; it is a unicellular eukaryote. Whereas, human means you know it is a highly developed a complex eukaryote. And it has got a core portion that is, the names are not important like Rrp41, Rrp45, Mtr3, Rrp42 etcetera etcetera.

In yeast you have a similar name, Rrp41, here 45, Mtr3 and sometimes the name can vary, like by and large the name is retained here. And archaea here it is Rrp41 is Rrp41, Rrp42 is 42, but you do not have a representative of Rrp45 in archaea. So, and also you can see some of the associated protein because exosomes also require some helper protein associated molecule. You can see in archaea, you have CSL 4, Rrp4 like that and in yeast you have some helper proteins such as Rrp6 etcetera. In human also have Rrp6.

So, yeast and archaea require more of helper, but in human not too much of helper molecules are needed. So, and they also have specific domains. Almost all of them they have got RNase PH domain. So, RNase domain that also contain a PH domain that is proline, histidine, amino acid containing domains.

And also, they have some zinc-ribbon domain and KH domain, K stands for lysine and H stands for histidine. Some of them have got a KH domain, so on and so forth. So, we should understand that the components of exosome, it is a multi-protein complex.

Exosome is not just one protein. It is a multi-protein complex, and it come together, they assemble together in the degradation process of a decap or in the not decap a de-adenylated because recapping is normally attracting XRN 1 not the exosomes. Exosomes are normally targeting the 3 prime to 5 prime exonuclease activity.

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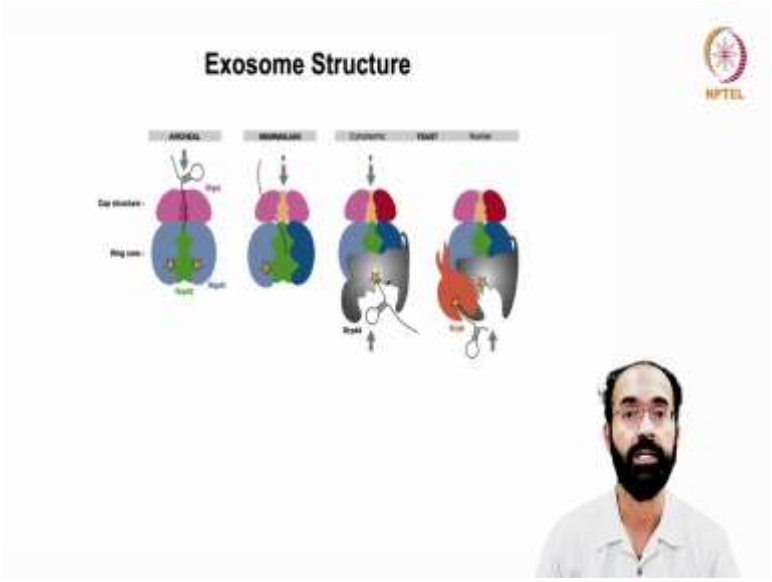

Let us see the exosome structure. So, archaeal exosome, mammalian exosome and you also have cytoplasmic exosome and nuclear exosome from yeast. So, you have archaeal, mammalian and yeast.

By and large they have a similar structure. You have a ring core, and also a cap structure, and this is the RNA which is having a loop and this is the place where it is supposed to act. So, this is normally seen in the archaea. And in mammal, you have more or less the similar structure this is the RNA loop, and also, yeast have got a cytoplasmic and nuclear.

Structure remains more or less the same the number of subunits changes. It has got a RNA detection or RNA interaction domain. The names of these proteins are not that important. We should understand it matches somewhat like that of a ribosome structure, but it starts its attack from one end. So, one end of the RNA, the exosomes will start its nucleolytic mechanism or the cleavage of nucleotides one by one, one by one, one by one, and it will clear the whole structure.

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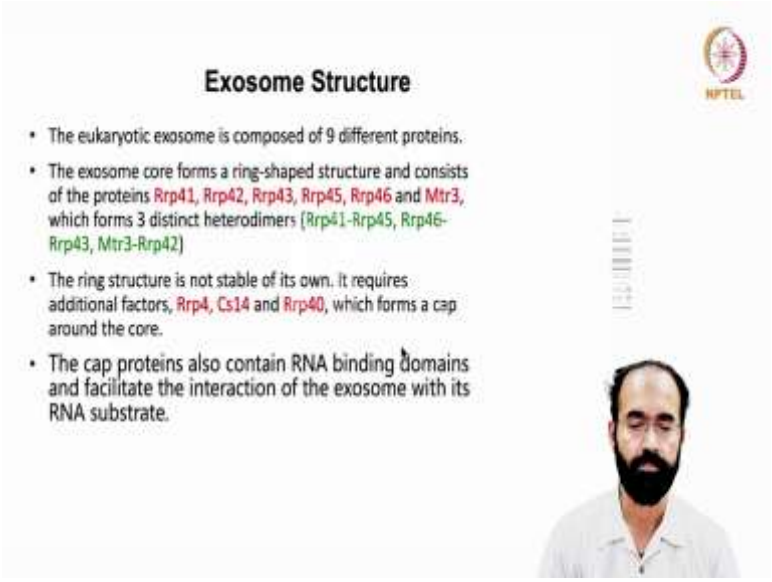

So, coming back to exosome structure, you can see the eukaryotic exosome is composed of 9 different proteins. The exosome core forms a ring shaped structure and it consists of proteins such as Rrp41, Rrp42, Rrp43, Rrp45, Rrp46 and Mtr3 and which forms 3 distinct heterodimers.

What are those heterodimers? Rrp41 and 45 a pair, Rrp46 and 43 another pair, and Mtr3 and Rrp42 another pair. So, 3 heterodimers form total 6 proteins.

The ring structure is not stable on its own, it requires further proteins such as Rrp4, Cs14 and Rrp40 which forms a cap around the core, so that it does not move apart. We just like you are making a hut, you made 10 pillars in a circular fashion.

There is a good possibility that 10 pillars may fall apart. So, if you put a roof on top of that, so all the pillars will stay united. All the pillars is strong on its own, but they are not connected to each other in a common structure. So, that common structure is roof. If you make a roof, then it will prevent the pillar from falling apart.

So, all the roof is setting sitting on top of this pillars, but the roof also provides a structural support to the pillars, preventing it from falling apart. Same logic applies. So, the unstable ring is now stabilized by a cap structure formed by Rrp4, Cs14 and Rrp40.

So, the cap proteins also contain RNA binding domains and facilitate the interaction of exosome with its RNA substrate. So, what you should understand? Just like you can say roof, if you made a hut, you are inside, but the outside of the roof is interacting with interacting with say atmosphere, like rain, sun or sunlight etcetera.

So, when they are interacting with each other, the molecule that is the RNA that has to come from outside. So, the cap structure interacts with the RNA and facilitate just like how rain and water is having a gap of hut in between. The pillar and the rain is prevented in between here. The roof is attracting, the cap is attracting the RNA.

So, cap proteins also contain RNA binding domains just like or act like a glue or a if you apply a glue onto a paper and throw some glitters on it, wherever there was glue it will stick, right. So, they can cap proteins can bind RNA and facilitate the interaction of exosome which is made of a temporary ring made of heterodimer 3, heterodimer total 6 protein. So, that is a member of the exosome with its RNA substrate.

So, cap not only stabilize the structure, but also brings you know RNA substrate onto the target.

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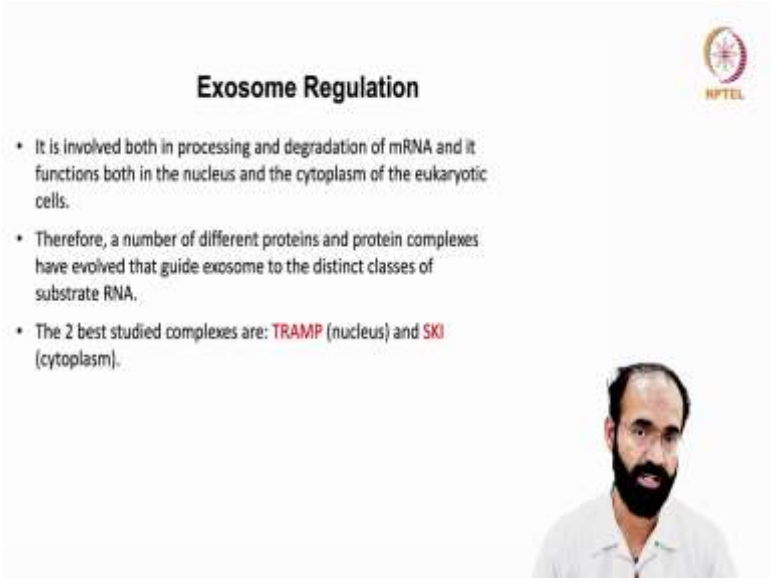

So, let us see how exosomes are regulated. Now, you say exosome, we know what is the circumstance in which exosomes bind onto the RNA substrate. Deadenylated RNA or endo nucleolytic cleavage by a endonuclease on the RNA will attract exosomes, but exosome cannot be left uncontrolled, right that also has to be controlled. So, it is involved both in processing and degradation.

Exosome is important you can call it as a necessary evil. Like, you can say in physics people say friction is a necessary evil, without friction you cannot walk on the road. But friction also causes lot of wear and tear.

Your chappal is eroding because of friction. So, we should understand lack of friction you cannot walk, you cannot walk on top of an ice, surface of a ice bar. If you try to walk, you will slip because there is no friction. It is good for your chappal, but it is not good for you.

So, the shoes or any of your footwear is eroding because of friction, like many mechanical parts, but it is necessary, but it is evil. So, exosome also can be called as a necessary evil necessary for the degradation of unwanted RNA and necessary for the maturation of some premature RNA, some pre-RNA everything is important. But it can be evil also. If it is degrading all RNA, then all those efforts of splicing RNA production everything will go wasted. So, it has to be controlled.

So, it is involved both in processing and degradation of RNA, and it functions both in the nucleus and cytoplasm of eukaryotic cell. But it comes with a price unless it is controlled.

Therefore, a number of different proteins and protein complexes have evolved that can guide exosome the distinct classes of substrate RNA, where to act the actual place should be shown to them, otherwise they will act randomly. The two best studied examples are the TRAMP complex in the nucleus and the SKI complex in the cytoplasm. Two complexes regulate the exosome function. TRAMP in the nucleus. SKI in the cytoplasm.

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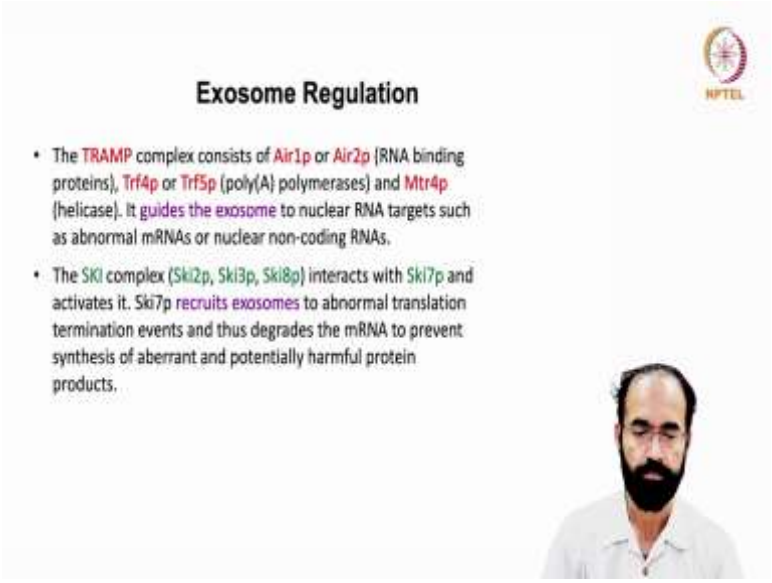

Let us see how is it regulated. The TRAMP complex consists of Air1p, Air2p, they are RNA bending proteins and Trf4p and Trf5p they are poly A polymerases. Remember, poly A polymerase can add the poly A tail. And Mtr4p helicase which is helicase is involved in changing the structure, secondary structure of the RNA.

So, we have Air1p, Air2p which are RNA binding protein, it can recognize RNA target. Trf4p, Trf5p which is poly A polymerase that can add poly A tail to a shortened poly A tail bearing RNA and Mtr4p which is a helicase.

So, it can guide the exosome to nuclear RNA targets, such as abnormal mRNA or nuclear non-coding RNA. So, those RNA which fails the quality control test has to be recognized by this TRAMP complex components that is made of total 5 proteins, Air1p,

Air2p, Trf4p, Trf5p and Mtr4p. That will guide the exosome. That this RNA is problematic why do not you degrade it. So, that is a message it is going to give to the exosome.

This SKI complex on the other hand, it handles the cytoplasmic affairs. So, Ski2p, Ski3p, Ski8p that interacts with Ski7p and activates it. Ski7p recruits the exosome to abnormal translation termination events because cytoplasm mainly handles only the quality control connected with protein translation because cytoplasm no splicing is happening. Cytoplasm there is no transcription is happening. So, you cannot handle that.

So, once the RNA has come into the cytoplasm, it has passed all those quality control tests in the nucleus. But in cytoplasm this RNA may misbehave in terms of protein translation.

So, SKI complex consisting of Ski2p, Ski3p, Ski8p that interacts with Ski7p and activates it. Ski7p is capable of recruiting the exosome to the abnormal translation termination sites. Thus, degrades the mRNA to prevent the synthesis of aberrant potentially harmful protein product.

If a protein product like an RNA has got a premature stop codon, then if that keep producing this protein which is of no use, it will can cause some inclusion bodies, it will not fold properly and that can damage the cells. Like, you know example of Alzheimer's disease because of a improper folding of the alpha sign nuclei, many proteins are there, like tau proteins many are there. They will form unfolded junk in your brain neurons and eventually it will make holes.

So, your brain instead of looking like a butter, now it is looking like butter eaten by ants, it will have holes, that is called spongy. You may have heard about this, bovine spongiform encephalopathy. So, it will look like holes. What is this holes? Neurons are lost or neurons are not working. So, the connections are lost, that is why you end up getting memory problems, that communication is not there.

All of a sudden, if you remember what you were which school you studied in your second grade, that is a memory. Who was your class teacher, it is a memory. Or what is your best childhood friend, that is a memory. So, this information are not being able to retrieve because of the holes created.

So, misfolded or unwanted junk protein can give lots of trouble. Sometimes, this unwanted protein like we discussed in one of the classes about p53, it is the guardian of the cell. Because of this mutation, it is not able to do the job. p53 is no more p53, it is only a ghost of p53, only a relic of p53. It is not doing the job, then cell will suffer because p53 was the guardian of the cell.

So, you do not want such situation to happen, then the system has to come up with the strategy to degrade those mRNA, ok. The p53 had problem, so let me degrade this mRNA. However, this will not be helpful if the genome itself is mutated because if a fresh RNA comes, again it will have the mutation.

Just like we discussed the sequel cell anaemia case. Glutamic acid is changed into alanine, and it causes one amino acid change causes a sequel cell shaped RBC which can cause you know, it can clog your coronary artery and can cause heart attack at low oxygen temperature.

So, but of course, it can be degraded. But the newly formed RNA also have this problem that is why we call it as genetic mutation, genomic mutation, problem is at the source. So, controlling the RNA will not help.

Sometimes you know tiny mutations because glutamic acid to alanine is not a stop codon, it is creating a protein having a different shape. But if that shape was so abnormal, then the RBC would have been dead. Now, RBC is not dying, but it is forming a very funny shape sequel cell shape. That is what is there.

So, do not think that only one mutation ever occurred in the history of RBC. There are plenty of such mutations are there, but they will be removed. That is why with they are not staying. That RBC will be killed or that RNA will be killed like that.

But some will escape, like p53, some p53 mutation can lead to cancer. If the surveillance was working perfectly fine, now cancer should have happened. So, although all this exosome and other surveillance system and the quality control system exist, there are problems that will come out of this problem. We saw the splicing defect disorders and many ribo RNA transporting disorders etcetera. But problems can come somewhere or the other.

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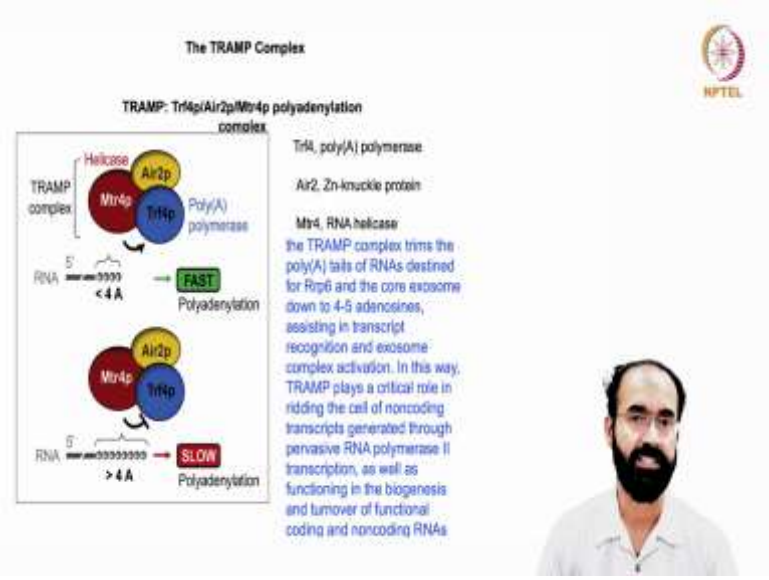

So, the TRAMP complex, if you are looking closely, the TRAMP complex which is in the nucleus consists of Trf4p, Air2p, Mtr4p and polyadenylation complex, and this is the TRAMP complex. And you have these proteins acting together, and they also have got elements for poly A polymerase because it allows a chance, second chance for this RNA to repair. That is why this poly A polymerase etcetera has to be present in the TRAMP complex.

If it fails so, then it will guide the exosome to start showing up this RNA. So, the RNA when the length of poly A tail is less than 4, that is why a the process called polyadenylation, then it will be marked for degradation and if this start rate of de-adenylation is very fast. Then, it will be recruited by the TRAMP complex, and it will mark for a repair option, can we repair it.

But if the polyadenylation is slow very slow, the de-adenylation is very slow, what happens, the Mtr4p will inhibit one of the components of its own member and that will trigger a different mechanism. So, the rate of de-adenylation although they it will be triggered when the length is close to 4, one is less than 4 and another is close to 4, but it is more than 4. So, both the situation, it can trigger a different mechanism of controlling over the exosomes.

We will study more in detail about this mechanism of TRAMP complex action in the next class.

Thank you.