RNA Biology
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Lecture - 33
SnRNA, rRNA, miRNA, siRNA Processing, Export and Function: RNA Quality Control

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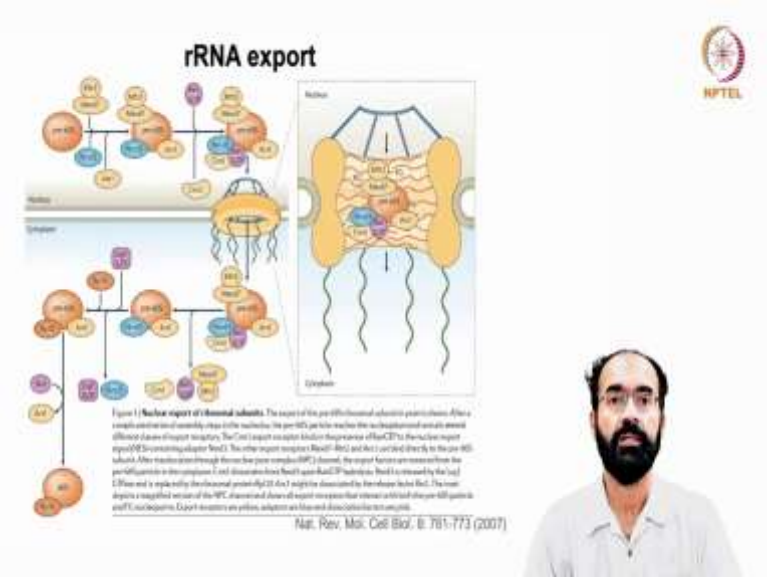

Hello everyone, welcome back to another session of RNA Biology. And this was the last slide where we stopped in the previous class. And this is a cartoon of the ribosomal RNA export through the nuclear power complex and we discussed in detail about the way in which the ribosomal RNA and the ribosomal RNP, in fact passed through this nuclear power complex with the help of FG containing protein which is lining the lumen of the nuclear power complex.

And those who are interested can read this review, which is published in Nature Reviews in Molecular Biology a few years ago which is a quite exclusive review, which has also linked to several latest publications in the field.

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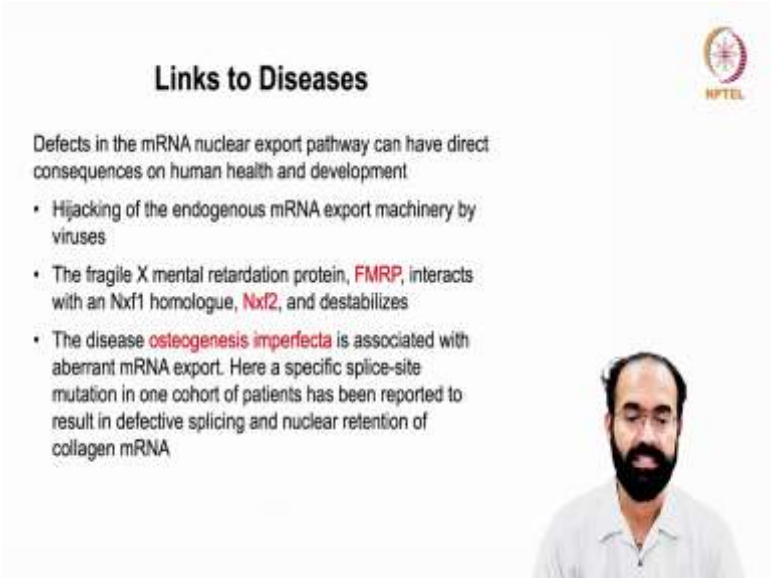

Now, let us see what are the major disorders or diseases that is linked to the RNA movement to and from the nucleus. So, what are the possible defects and troubles? We also saw some splicing defects and the diseases related to that. So, defects in the mRNA nuclear export pathway can have direct consequences on human health and development.

Health means day to day life, development means embryonic development. It can even cause a defective embryonic development etcetera. So, one has to be or one system or one organism system has to be perfect in order to have a normal development and normal way of living.

Let us see some example. Hijacking the endogenous mRNA export machinery by viruses can often have a deleterious effect. You may realize, if you have an infection say virus infection of course, your body responds normally the cell mediated response comes into picture which takes around 6 days to reach to its perfection.

So, when a when you have a viral infection, let it be any infection whether it is a chickenpox, whether it is COVID or any other doctors normally say viral you know infection or viral fever etcetera. Before you get that real fever, fever is your body's response right the high temperature etcetera.

You will have one or two days of uneasiness that malice, basically you will feel not have feeling to have food like, you have some people have body ache or not we normally say

that I do not have good mood I am not in good mood. So, this mainly because the virus which entered your body is hijacking the machinery what you have.

It is entering, it is increasing in number because initially our immune system may not be capable enough or surveilling the immune system may not be powerful enough to detect the single virus or two virus etcetera although ideally it should detect, but it can take time. So, in this meanwhile the virus will enter the cells and hijack your machinery for its own benefit or its own utilization or propagation or survival.

So, this can have too much low energy production in your body. The ATP production other normal metabolic reactions will be compromised. And that is the reason for that the, so called feeling of not so good feeling. And before you get the onset of fever comes when your body detected there is some problem and body tries to kill it by elevating the body temperature.

So, this is one situation, where the viruses when they hijack the transport machinery and of course, virus will hijack pretty much every machinery in your cells. But transport machinery also virus will hijack which can give lot of trouble. And another example Fragile X Mental Retardation Protein called FMRP and which interacts with a homologue of Nfx, Nxf1g that is called Nxf2 and destabilize it.

And this kind of situation the you know FMRP is important in the movement of RNA across the nucleus we have seen it some examples earlier. So, this FMRP may not be effectively available for the transportation functions that is, another example. Another example is the disease called osteogenesis imperfecta.

So, osteogenesis imperfecta is characterized by fragile bones. So, normally the density of the bone becomes lesser and lesser, which makes you vulnerable for bone or fracture, bone fracture etcetera. And this osteogenesis imperfecta is associated with aberrant mRNA export.

So, here a specific splice site mutation in one of the cohort of patients has been reported to result in defective splicing and nuclear attention of collagen mRNA. We know collagen mainly, different types of collagens are there mainly 4 collagens are important. Collagen 1, 2, 3 and 4 although. The family of genes are around 20 different genes are there approximately.

So, collagen is important for maintaining the flexibility of the tissue, it is there in you know wherever you have tissues that is we. So, called the what you call structural cells which we called they do not have any specialized function those cells. Like fibroblast cells which is kind of covering the gap or filling the gap between the differentiated tissues say, when you have a wound in your hand or leg. So, that gap is filled by fibroblast.

So, many times your collagen is there pretty much everywhere. It basically make sure the smooth transition, smooth movement of cells among themselves and also the you know your ear your, ear pinna, nose so these are all very flexible you know that. This is mainly because we have got cartilaginous tissue. So, which is calcium derived, but it is not very strong, it is not very dense as bone your bone wont bend like this, right.

So, like that many places we have tip of your nose here, behind here it is solid, but little bit down it moves your nose the there will be slight flexibility is there your ear moves around. So, there here and there some places you have got cartilaginous tissue. Again the credit goes to the collagen the distribution of collagen, like you are able to move your hands like some part of your body, you are able to turn and twist etcetra. The credit goes to the collagen.

If you do not have a adequate amount of collagen, your bone formation the maintenance of your bony structure etcetera everything can get compromised. Remember your bone keep decalcifying and continue to calcify, so it does not happen like in around 7 years, approximately 7 years your entire skeleton is replaced. So, if a person is 21 years old, he is on the third version of his skeleton. So, you can keep multiplying.

If a person is 70 years old, he is on the 7th version; 10th version of his skeleton. So, your skeleton on an average stays only around 7 years. So, it does not happen one day morning the entire skeleton is replaced it continues to replace. So, so why I am saying is the bone you may have a dense bone right now. But that does not mean that it will continue like that forever.

So, the food you take the rate at which the bone density is maintained or the rate at which the calcium deposition happens in your bony, bony parts decides how strong the bone you have etcetera. So, one such disease that comes osteogenesis imperfecta is due to a splicing defect and the nuclear retention of the collagen mRNA happens. Because if

there is a splicing defect, collagen does not come to the cytoplasm, collagen RNA does not come to the cytoplasm because it is retained because of a splicing defect.

So, lot of patients it is reported that the collagen RNA is not coming to the cytoplasm. Hence the collagen protein is not being formed effectively and collagen is secreted out of the cell. And it functions outside the cell like I told you it acts like a glue, it lot of collagen and their mutation are very important like if certain mutations or even isoforms of collagen allow people body very flexible, some people body will be very flexible compared to the other.

So, collagen being a structural protein can tolerate to some extent some mutations because it is not as important as a functional protein. I am not saying that mutations in structural protein are unimportant not like that. They are also important to maintain their amino acid sequence, but sometimes some structural proteins tolerate variations and some such changes from the general population allow much flexible body.

Body is very flexible you can bend it you can twist it whatever way you want you can do it. The credit goes to the collagen deposit between the joints. So, bone you cannot bend it, whoever it is the body cannot bone cannot bend it. But still the bone will remain tough credit goes to the nature of the collagen. So, if collagen is not being formed, adequately then you can end up getting situation like osteogenesis imperfecta where you are more vulnerable for bone defects or bone damage or bone breakage etcetera.

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Those who are interested can read some of these references. Exporting RNA from nucleus to cytoplasm which is a nature review, molecular cell biology, came few years ago. And mRNA nuclear export at a glance is another review published in Journal of Cell Science in 2009. So, those who are interested can read it because it will make your impression about the subject much better.

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Now, we will move on to another topic mRNA Decay. We know that any mRNA that is formed from the cell has to have a lifespan. You do not want an RNA to stay forever, no matter how precious the RNA is no matter how tough to make this RNA like we have seen dystrophin it goes in mega base at size and that produces around 17 KB size RNA and so many exomes are there.

So, that glycine energy consumption everything is too much is there. That does not mean that an RNA should stay forever that can give you lot of problem, say for example, you think about an RNA is important during the development of an organism. And once that development is done you do not want it, say you go to a restaurant and have food. Will you carry the plate with you? No, you will you carry your hand just like you know I love this food, so in between I can you know have a taste of it.

So, will you carry normally? No, however tasty, however great. The ambiance and place you will wash your hand and you will come out as you entered inside. Of course, with belly full, but that is not obvious you went for that purpose same way, when an RNA is

being formed it has to perform the task of it say whether it is a structural RNA or an mRNA which is supposed to produce proteins. Once the job is done, the RNA should be cleared. Otherwise what will happen? It will continue to do the same job and that will be overdoing it.

And say a proliferation an RNA or a protein which is facilitating cell proliferation. Cell proliferation means multiplications say you got a wound, your hand got cut with a blade and there is an open wound. Now after maybe a 10 days or 1 week that wound will be closed. And who closed it by cell proliferation, new cells are formed they are filling the gap just like your house, wall is broken you cannot cover it with your hanging a calendar or putting an almirah in front of that. You have to bring bricks cement and seal it.

And so that the wall is restored back our body also does the same thing produce cells fill, the gap and make the damaged part look normal. Now, if the genes responsible for the cell proliferation or the multiplication of cell, is not stopping once the job is done. It is still on, so cells are continuing to divide. Now its dividing and dividing and dividing.

So, what the situation is called? It is called cancer. It is a problematic situation, you do not want that. So, why a normal wound when you heal it, you do not end up getting cancer because the RNA or the gene regulation are stringently controlled. So, they are controlled in two ways, one way is stopping the production of that RNA one way of regulation.

What is the other way of regulation? Degrade the existing RNA. So, if it stays back, it can give rise to lots of trouble, lots of problems like you may have seen in many situations like if there is a communication between some high level officials. You do not want you can be tracked by enemy countries.

So, what they do is it is a prime minister or president making a communication to a high level official. They immediately damage that phone contact, whether it is a mobile phone or whatever they just to make that particular call. Then that card or connection everything is disrupted. They want to make one more call they call with a new number, they call it communicated and again they disrupt it.

Because they do not want their positions to be revealed to anyone. So, they immediately; if it stays back its possible that some it can give rise to lot of troubles. So, they do not

want that. RNA also like that, when you have a job is done you have to degrade it. So, we have to understand that RNAs degradation is much more important than even its production. If it is not degraded in time effectively, then that can lead to lots and lots of problems.

So, this has to be stringent also you do not want like morning 10 o'clock in a cell it turned on ok RNA degradation pathway let it be on. And all the RNA in the cell is now damaged, that way it cannot happen. It has to happen to those, if there are in a cell there are 100 different types of mRNA are there in a given time. You do not want all the 100 to be degraded, you want just it 10 to be degraded.

So, remaining 90 should stay back. So, RNA destabilization is a vast topic. Lots of people dedicate their whole career in understanding the RNA destabilization, RNAs you know degradation it is not an easy topic. RNA we all know that it is a highly vulnerable molecule to degrade RNA you do not need to do anything it is like your sweat, your tears, your saliva everything has got RNAs.

So, RNA is basically fighting against its degradation. However, when it comes to selective degradation. If you fail to do so, then there can be lots of trouble to the cell, including developing complicated conditions such as cancer. So, RNA degradation is a or RNA decay is an important phase or important signs of RNA biology or important part of RNA biology.

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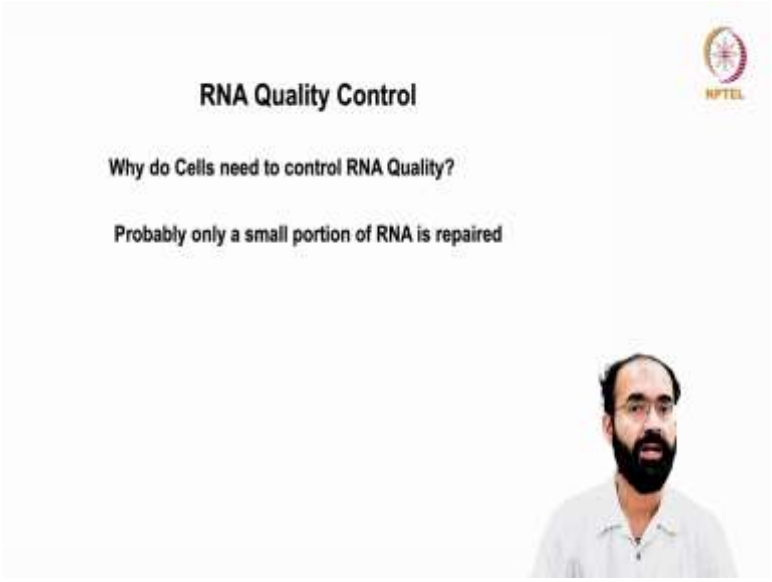

So, RNA usually starts its quality controlled right from its production. We all know in any company, when they are producing a product, if there is any damage then they will not, they simply will not proceed to the next stage because if. So, first of all companies reputation will go, second that product may not be if optimally usable.

So, any defect if it is there you will simply you know discard it. You may some of you may have used many centrifuge machines right, like some class of centrifuges are called ultra centrifuge, which can run rpm of 1 lakh 30,000, 1 lakh 50,000 those kind of RPM. So, the machine will cost say if it is costing around 1 crores of rupee.

The major part of that goes into the rotor because rotor is not even the mortar, you may wonder oh mortar mortar you can make it, however powerful you want you can even people make it you know aeroplane also you make the mortar. But the rotor which you are making has to be the most sensitive part in a centrifuge. Especially in an ultra centrifuge, so it is made of special alloy and they have to cast it multiple times and screening for the defect.

So, it is said, that if you make around 12 rotors of the same dimension, same size only one can be marketed. The remaining 11 has to be discarded because they will not pass the quality control. That is why the rotors are one of the costliest of the ultra centrifuge part, where they can go in lakhs.

Like 12 lakh, 50 lakh, 20 lakh like that. It goes lot of and when you look you will say it is a metallic part made of some alloy how does that matter no. It is discarded lot of them are discarded same way RNA is maintained very stringently by quality control at every step.

So, we can ask the question. Why do cells need to control RNA quality and I do not think I need to discuss this because by now you know how important the RNA is, how important a given sequence of RNA is. Only a small portion of RNA is repaired, if an RNA has got a defect, if it is not passing the quality control, it can be repaired to some extent by using you know RNA modifying enzymes etcetera etcetera.

But RNA editing also can be called as a kind of repair because a given RNA is problematic it is not going to be useful. So, hence it has to be repaired it has to be fixed. So, only a small portion of RNA is repaired, that means, any RNA that do not pass the

quality control must be marked before degradation. So, it starts from its production that is right from the nucleus.

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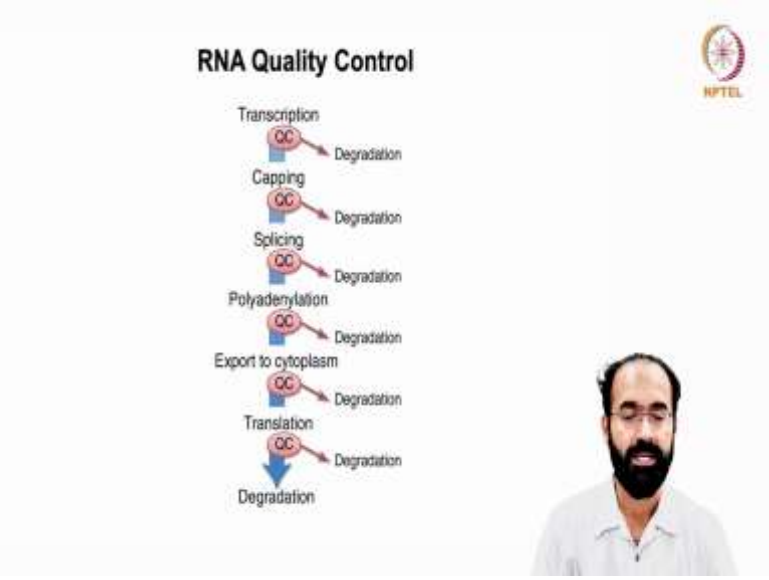

Now let us see RNA quality control what are the steps, what are the stages it is happened. Transcription, QC stands for Quality Control and it can cause degradation or it can be marked before degradation right from the transcription. Capping if capping has an issue 7 methyl guanosine cap, if any problem cap does not work effectively. It has to be marked for degradation.

Splicing if there is any defect should be marked for degradation. Polyadenylation if there is any problem with the polyadenylation, due to whatsoever reason it should be marked for degradation. Export to the cytoplasm and RNA it is capped, spliced, polyadenylated, but somehow it is not effectively forming the RNP.

Hence it is failing to get exported it is lingering standing here should be marked for degradation and translation an RNA which is translating in the cytoplasm, mRNA translating in the cytoplasm sometimes it does one round properly, then it detects something is not right. The protein that is found is not right it will be marked for degradation.

So, every stage it has to be marked for quality control, mediated, degradation. Then comes if it is utilized properly, RNA it was perfect passed quality control in every stage

and it did its job still that does not mean that RNA is eternal that also should be marked for degradation because after some rounds of say protein translation or maybe some rounds of its actual function. The RNA lose its integrity in terms of its secondary structure and ability to form RNP. It was a perfect RNA just like you can see your mobile phone, it was perfect when you purchased.

After few years why it goes slow? Although you did not do anything because the parts inside the electronic parts inside are not able to conduct electricity or electrons as it was used to do it. It is still working, but in a very slow and defective manner, any instrument for that my including you, you may not have that much energy as you used to have in your 5 years or 6 years old that time you had full of energy.

As you grow old, you have you are energetic, you are muscular, but it will slowly come down. Same logic applies to RNA also. Although it does not have any defect, it is used a lot then also it should be marked for degradation.

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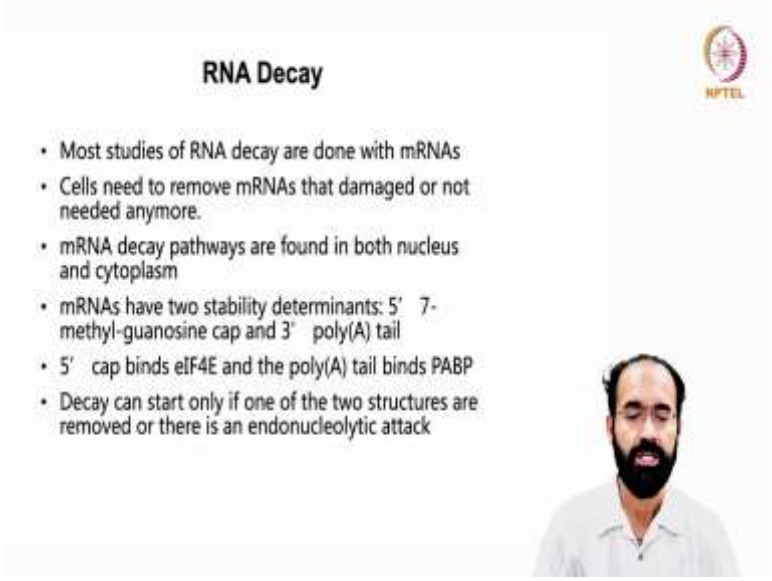

So, RNA decay in most studies of RNA decay are done with mRNA with a purpose. Because mRNAs are diverse and mRNAs are unique because a given cell a given mRNA may have a longer lifespan, but that may not be so for tRNA ribosomal RNA etcetera. Because they are mundane RNAs like they do not have any; what you call?

They do not have any special identity to them, although they are important, although they are essential, but they have a mundane job or routine job. Whereas, mRNAs that is not the case a given mRNA expressed in a cell can completely transform the outfit of that cell.

Means a kidney cell you can convert into liver, purely by expressing the mRNA, you may have heard about Yamanaka factor. Yamanaka who converted, the fibroblast cell like we were discussing earlier today, fibroblast cell into stem cell so just express few RNA few genes you express and he expressed just 4 genes. 4 genes you express we call them as Yamanaka factors and we also call them pluripotency factors.

Just express 4 of them, 4 RNA. It fibroblast which was doing no special job now became stem cell, where you can convert them into any tissues. Stem cells can be converted into any tissue in your body. So, they became pluripotent stem cell. So, RNA as small as 4 can convert huge change in the outfit of the cell that is why a lot of decay studies have done in mRNA. Cells need to remove mRNA that damaged or do not need any more.

So, do not think when an RNA is degraded it is a damaged. No it is no more needed anymore, just like I gave the example of you know once a communication is done between two high power officials they damage the phone or break the phone because they do not want any tracking same way.

So, mRNA decay pathways are found in both nucleus and cytoplasm. Because both places there is RNA and quality control happens both in nucleus and cytoplasm based on which stage of RNA production is going on. So, mRNA's have two stability determinants, that is 5 prime that is the 7 methyl guanosine and at 3 prime the poly a tail. So, these are all the two boundary yardsticks.

So, if RNA lacking any of this should be marked before degradation. Because both are needed and the 5 prime cap binds to elF4E which we saw, that CBP will be removed as it comes into the cytoplasm from the nucleus the mRNAs the 7 methyl cap bound CBP will be removed and elF4E binds onto it. And the poly a tail binds to the poly a tail binding protein PABP.

So, poly a binding protein is very important to have identity to the poly a tail of an RNA. So, elF4E binds to the cap, in this I am talking about in the cytoplasm. And PABP poly a

binding protein bind to the poly a tail and decay can start only if one of the two structures are removed or there is an endonucleolytic attack.

So, if elF4E is bound onto the 5 prime end PABP bind down to the 3 prime end everything is fine with the RNA. But they are still vulnerable for endonucleolytic attack where you are getting cut by an enzyme right in the middle of the RNA, which exposes two tails one 5 prime end this exposed, a 3 prime end this exposed new because of the cut, that can be utilized by exonucleases and can cause degradation.

So, it has to fight against the endonucleolytic cleavage by enzymes also. So, we will see more in detail about the RNA decay pathway in the next class.

Thank you.