RNA Biology
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Lecture - 29
SnRNA, rRNA, miRNA, siRNA Processing, Export and Function: RNA Helicases

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Hello everyone, welcome back to another session of RNA biology. So, we were here in the previous class, we were talking about what are the mutations and what are the splicing defects that can lead to various human diseases. And so, from these examples we should infer that RNA splicing is a very crucial step in the gene expression event. If RNA splicing have got any defect whether it is in the splicing machinery or a given candidate splicing, the outcome can be quite devastating.

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So, we will move on to a new topic that is RNA helicases. So, we have seen helicases enzyme and their this terminology we have used it before when you are discussing about the transcription, RNA transcription. Because they are important in changing the structure of a nucleic acid, a given RNA secondary structure. So, we will see which all the steps, at what are the conditions in which RNA helicases become important.

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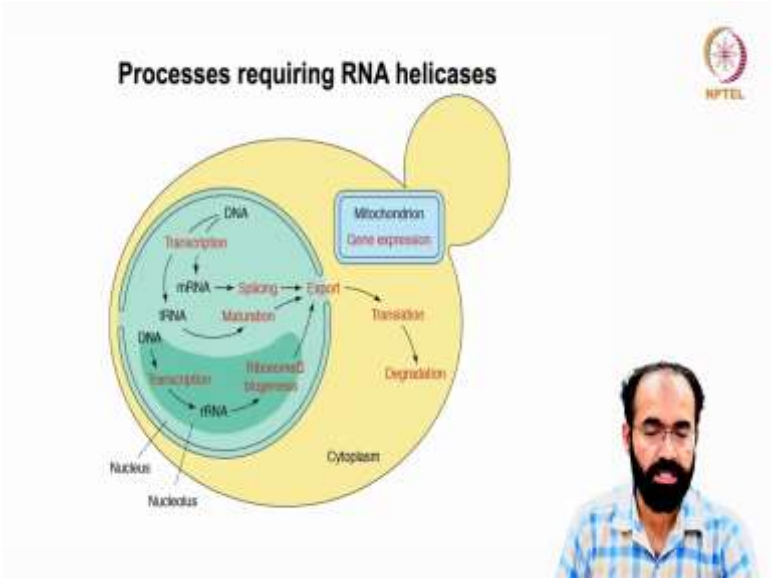

So, there are plenty of processes that require RNA helicases. In a nutshell, we can say pretty much every function in the gene expression or gene related functions require RNA

helicases, at some time point or the other. Like, we can see here, here you have a DNA and it is transcribing transcription and into mRNA, tRNA and mRNA undergoes splicing, tRNA undergoes maturation and it gets exported and translation and degradation.

And each of them which is shown red in color, they all require helicases action in some way or the other. And you can also see mitochondrial DNA, because mitochondria is a extracellular or extracellular organism that parasitized into a eukaryotic cell, into a cell which eventually became eukaryote in during millions of years in the evolutionary timescale or history.

So, this DNA when gets transcribed mitochondrial DNA and also ribosomes are need to be synthesized and RNA export into the cytoplasm etcetera, you require RNA helicases. So, by and large every stage in the gene expression event require RNA helicases.

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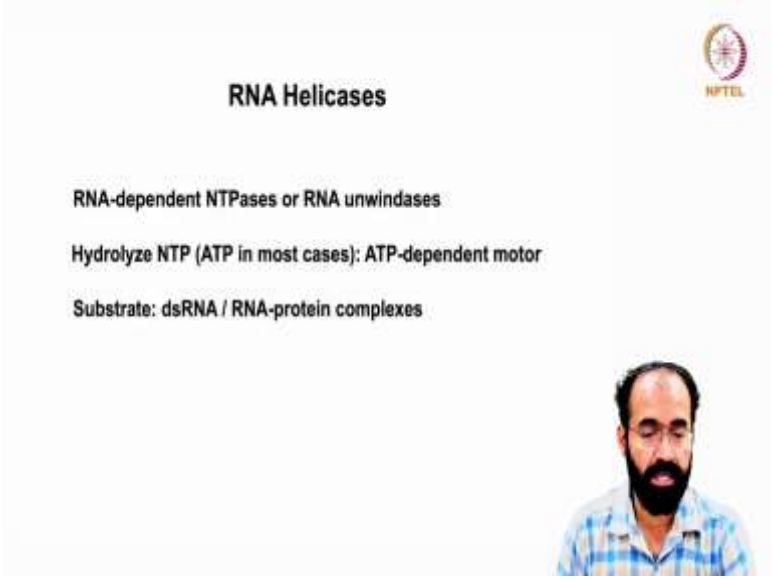

Now, let us see some examples. RNA dependent NTPases or RNA unwindases. So, unwind itself, the word unwinding itself you get an idea that RNA need to have change in its secondary structure. Sometimes this change in secondary structure is important for them to get another secondary structure or get interaction with another RNA. So, for many things certain sequences in the RNA which is buried inside the RNA secondary structure need to be exposed that function is done by the helicases.

And they are needed for the hydrolysing the NTP and in most cases it is the ATP, and this can be called as a ATP dependent motor. And many a times the substrate tweaking and double standard RNA into RNA-protein complexes etcetera, they require RNA helicases. Let us see the family of RNA helicases.

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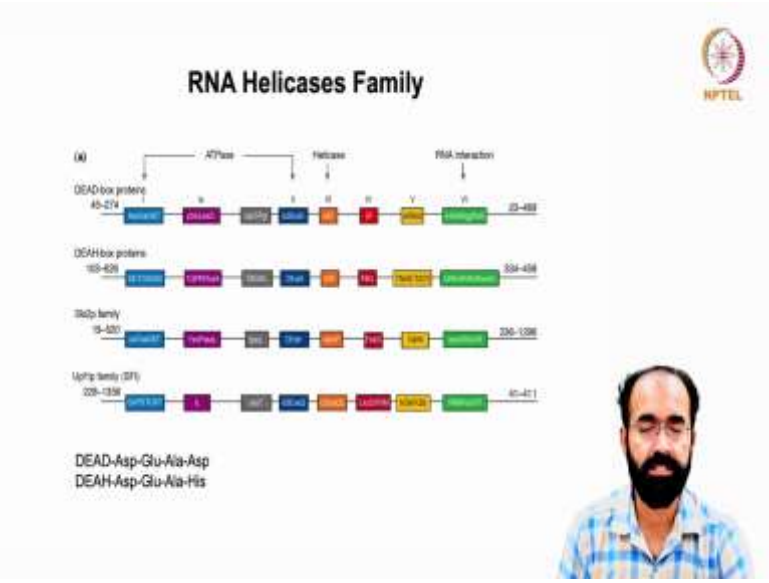

So, this slide indicates the RNA helicase family. And many a times they have a ATP utilizing domain, we call it as ATPase domain. And then, you have a typical helicase domain and then they have a RNA interaction domain. So, if you are talking about an RNA helicase, it need to recognize the RNA, it need to have the helicase activity and also it should be able to make use of the energy that is ATP.

So, some of these boxes which are highlighted in different color and you can see AxxGxGKT. So, these are all single letter code. x stands for any amino acids can come there. So, this is a box and this is called dead box protein because they contain the dead domain. That means they are the single letter code for some amino acid. You can see here DEA and D.

So, what we should understand that many of these proteins which have got helicase activity they have certain common features, but they are not identical, remember that. Because some of these boxes are meant for attracting other proteins for further modification of the enzyme.

So, normally, how these enzymes work is that they need to recognize an RNA substrate and they should be able to cause a structural change means a secondary structural change. And then they should be able to utilize energy and then this RNA will be vulnerable for a next step of reaction. That means existing RNA need to be made ready for some other reaction.

Just like if a human baby is born, you send them to school and they get trained in many things, so that they can grow up in their career etcetera. So, this is what RNA helicases will do. An existing RNA need to be trained in order to perform certain task. So, this DEAD or DEAH, they stands for aspartic acid, glutamic acid, alanine and aspartic acid. So, that is DEAD. And same way here aspartic acid, glutamic acid, alanine and histidine. So, that is what you see in multiple places. That is not important.

What is important is the function what they are able to do because they have different boxes, do not think that the protein will have different boxes. It is for our convenience we are just categorizing them, because these boxes can be seen in some other protein. They need not be helicase at all. They can be seen in some other protein also.

So, many such boxes are common because they have some unique features. Such family proteins have some unique features which they use for their you know functioning and interaction with a specific protein etcetera. So, these domains are important, but by and large the helicase activity depends on whether it can utilize ATP by ATPase domain and helicase and also their ability to interact with the RNA, RNA interaction domain.

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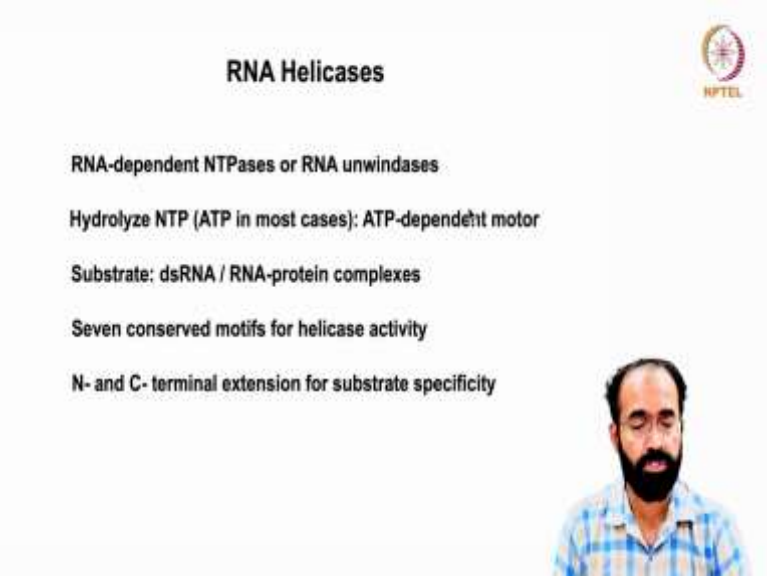

So, helicases are in general are RNA dependent, NTPases or RNA unwindases and they can hydrolyze the NTPs, usually it is the ATP and that will perform ATP dependent motor. And they use the substrate as double stranded RNA or RNA-protein complex that is RNPs. And they usually have seven conserved motif for helicase their RNA helicase activity, and they also have N and C terminal extension for substrate specificity.

So, it need to retain the substrate specificity and it should be able to utilize the ATP and then it should have the helicase activity without which that protein will not be qualified as a helicase.

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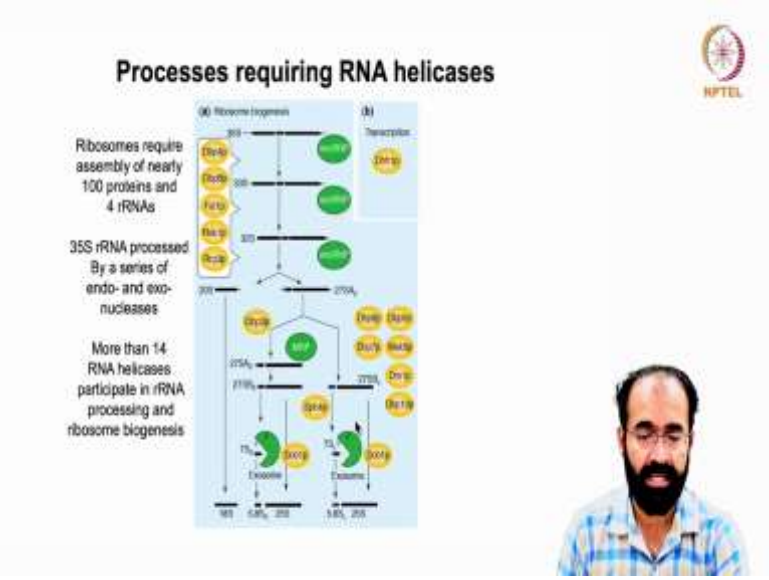

Now, let us see what are the processes that require RNA helicases. And this are numbered like a, b like that you can see and you can see the example. Ribosome require assembly of nearly 100 proteins and for ribosomal RNAs. You can see here starting from 35, 33, 32 S different svedberg unit RNAs are there, and they also have to interact with SNO snoRNP means small nucleolar RNP, means RNA plus protein that is snoRNP is there in the snoRNP form.

And the helicases that perform this assembly are Dbp4p, Dbp8p, Fal1p, Rok1p and Rrp3p. So, these are all different enzymes. These are all helicases. So, they contribute for the assembly. And also, that is not it, there are further Dbp3p and plenty of other whichever you see in yellow color box, they are all helicases.

So, 35S ribosomal RNA processed by a series of endo and exo-nucleases. And more than 14 RNA helicases participate in the ribosomal RNA processing and ribosome biogenesis. And one helicase that is important in the transcription are Dhh1p. The names of these helicases are not important. We are showing these helicases for you to have an idea that the RNA helicases are very important for each and every function that is occurring related to gene expression.

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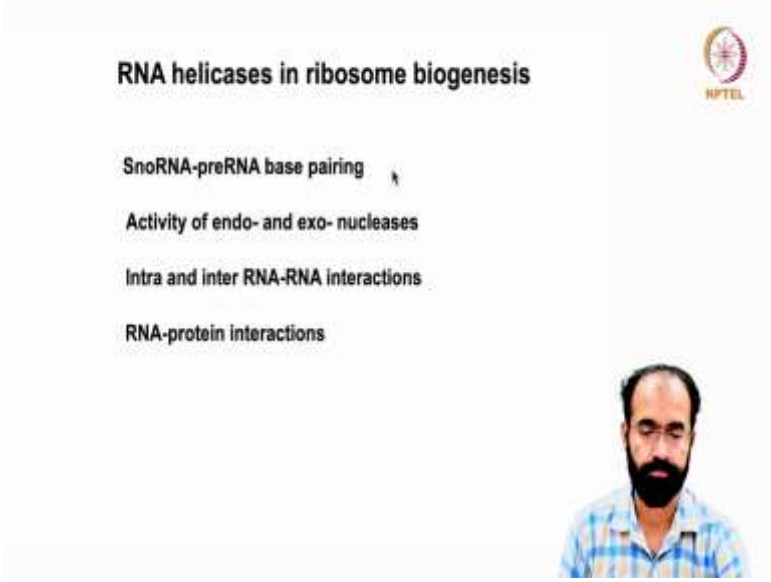

And snoRNA and preRNA base pairing is the important process which is governed by RNA helicases during ribosome biogenesis. And the activity of endo and exo-nucleases because many times the ribosomal RNA requires some trimming, maturation, cleaving etcetera. And also intra and inter RNA-RNA interactions also important, and also RNA-protein interactions. These are governed during the ribosome biogenesis, mainly done by the RNA helicases enzymes.

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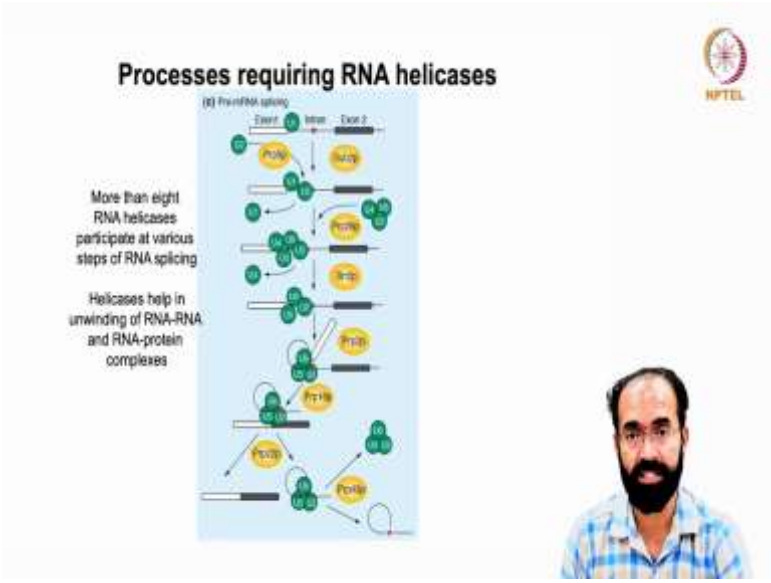

And let us say some more process, with regard to you saw a and b, with regard to ribosomal RNA. And now you are seeing c, pre-mRNA splicing. We know what is RNA splicing we will not go into the detail. But more than 8 RNA helicases participate at various steps of RNA splicing.

And helicases help in unwinding of RNA-RNA and also RNA-protein complexes. Means they can tweak or change the existing structure of an RNA, means a secondary structure, not the primary structure primary structure is the sequence of the RNA itself, the secondary structure. And also structure of an RNP for good reason because it can proceed further.

Like, we will not go through each of this RNP's each of these helicases whichever shown yellow in color, they are all helicases that participate at a different stages of the RNA splicing.

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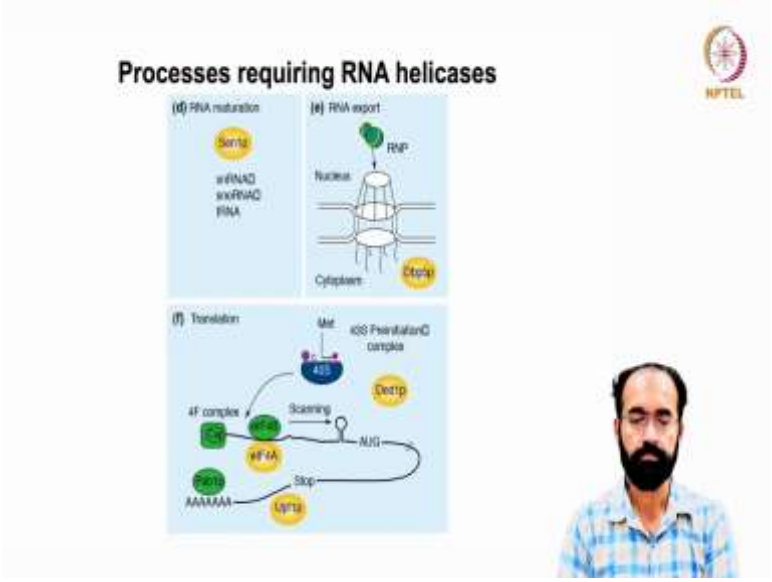

And let us see step d, RNA maturation. So, snRNA snoRNA and tRNA maturation is governed by Sen1p. And there is another step 2, that is RNA export that is governed by Dbp5p, one of the important cytoplasmic RNA helicase that contribute to the movement of RNP across the nuclear pore complex.

And then comes f, that is the translation this during the production of proteins from the mRNA in the cytoplasm. That means, you know elF4A which is important for the

assembly of the ribosomes to start with for the process of initiation of transcription and also the 43S preinitiation complexes controlled via Ded1p, another helicase.

So, and even the stopping of the protein translation require Upf1p, another helicase. So, helicases are important in pretty much every step of RNA biology.

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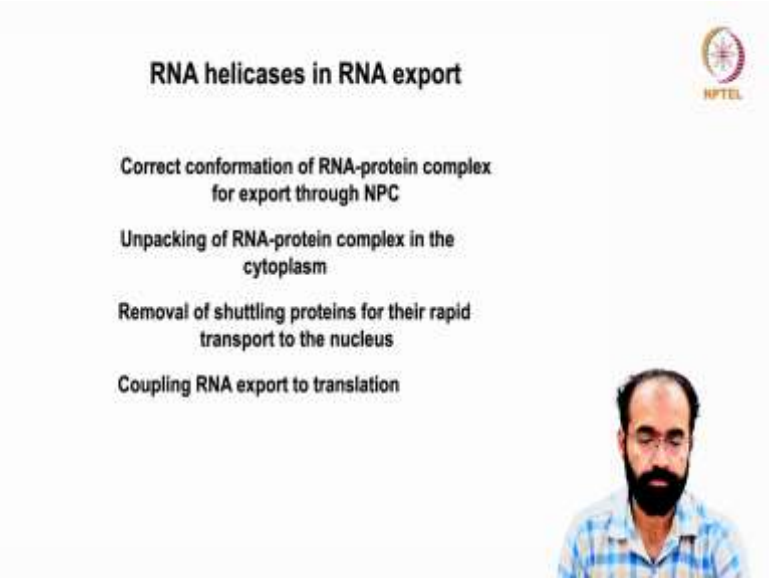

And let us say how RNA helicases contribute to the RNA export that is movement of RNA from the nucleus to the cytoplasm. The correct confirmation of RNA-protein complex for export through NPC. What is NPC? Nuclear Pore Complex. So, helicases need to come into the picture.

Unpacking of RNA-protein complex in the cytoplasm also require RNA helicases. Removal of shuttling proteins for their rapid transport to the nucleus. Many proteins interact with the RNA transiently for the purpose of movement across the nuclear pore complex. So, they need to be unpacked and categorically removed. So, that is done by the RNA helicases. Coupling RNA export to translation. This is another function done by the RNA helicases.

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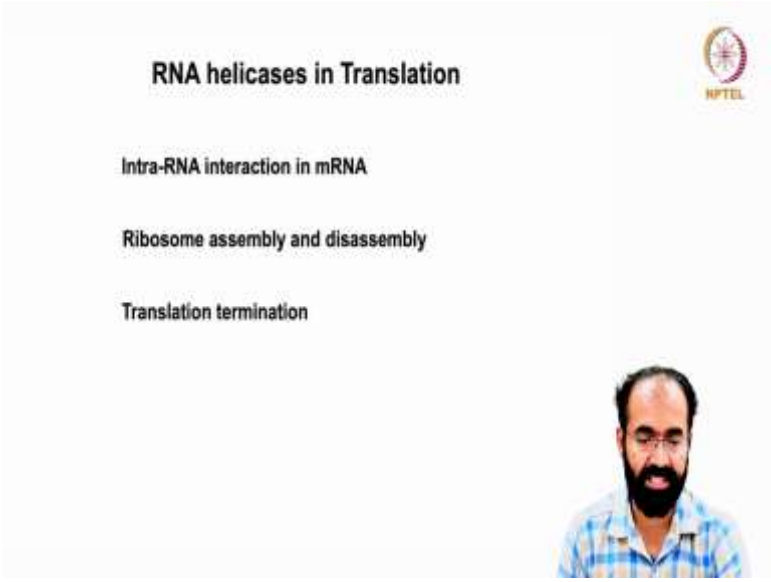

Now, let us see what are the major steps or roles played by RNA helicases in protein translation. Intra-RNA interaction in mRNA; that means, many times the mRNA has to interact with ribosomal RNA present in the ribosome which is facilitated by specific helicases. Ribosome assembly and disassembly, assembly to start the translation and disassembly once the translation is stopped. And the translation termination all this require RNA helicases.

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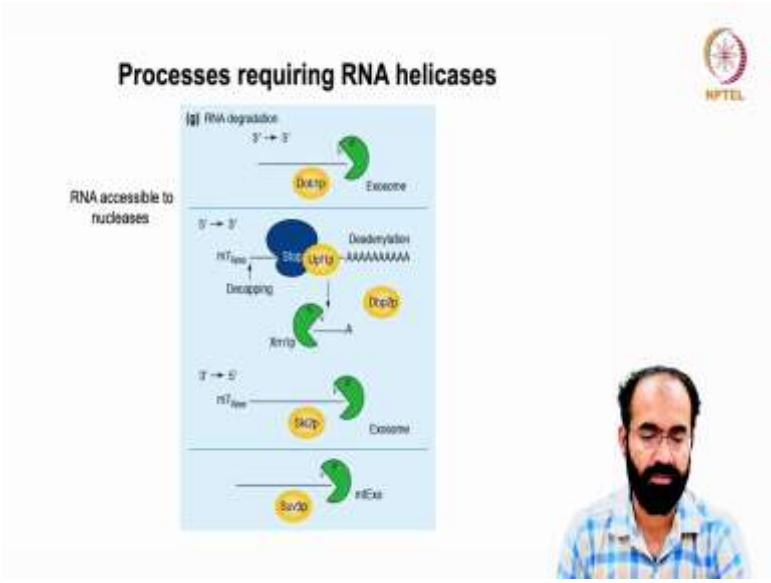

Let us see some more example where RNA helicases are needed, that is the step g, that is RNA degradation. RNAs can be degraded in multiple ways, whether it is endo-nucleolytic cleavage or exo-nucleolytic cleavage. Endo-nucleolytic cleavage means RNA will be cut inside that is in the center or in the middle wherever there is no tip or the edge of the RNA is there internal cut that is called endonuclease.

Exo-nuclease start either from the 5-prime end of the RNA or 3-prime end of the RNA. You can see here one of the helicase dob1p which is important for the assembly of the exosome. Exosome is a protein complex that cause the degradation of the RNA via exo-nucleolytic method that is cleaving from the 3-prime end, not from the 5-prime end. This cleaving from the 3-prime end.

We will see more in detail about the RNA degradation, but time being we are just little bit touching upon the enzymes in a RNA helicase point of view. We will see later what are the enzymes, how the decapping happens, etcetera as a part of RNA degradation. So, some RNA when the exo-nuclease has to act from the 5-prime end, then decapping is required because if the cap is there, the 7-metheleguanosin cap is there, it will not allow any exonuclease to act.

So, the cap has to be removed. And decapping is needed, and you need to have requirement of a Upf1p. And remember, this will be done for those RNA which is utilized; that means, it has encountered a stop codon, the ribosome blue color ribosome is situated here. Once it encountered the Upf1p will come and it will disassemble the ribosome and the RNA. Then, that will be an invitation signal for the decapping process.

And of course, just like the decapping, deadenylation is another step that has to happen and which is governed by Dbp2p another helicase. And there is another enzyme just like exosome which is starting from the 3-prime end that is the exo-nuclease. There is another exo-nuclease which is working from the 5-prime end that is Xrn1p.

And this is mainly happen or mainly function if the decapping has taken place effectively. And then 3-prime to 5-prime direction when you want to regulate the exosome. Exosome if it is on, the RNA will be degraded from the 3-prime end. But again this has to be regulated. It cannot go at a tremendous pace. So, that is regulated by Ski2p.

And there is another exo-nuclease that is mtExo which is similar to that of the exosome this mtExo is regulated or it is influenced by Suv3p another RNA helicase. So, we are highlighting the importance of RNA helicases. So, whether it is RNA's biogenesis, its maturation, its export, its translation or its degradation, it require RNA helicases at different stages of the RNA biology.

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So, this is an article un unwinding RNA in saccharomyces cerevisiae and DEAD-box protein and related families. So, it is a review article. Those who are interested can read. It is quite interesting article to read. It came in transient biochemical sciences tips.

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Now, we will understand RNA export. We know RNA is produced in the nucleus and it need to be mobilized into the cytoplasm for its, sometimes for maturation, sometimes for its functioning.

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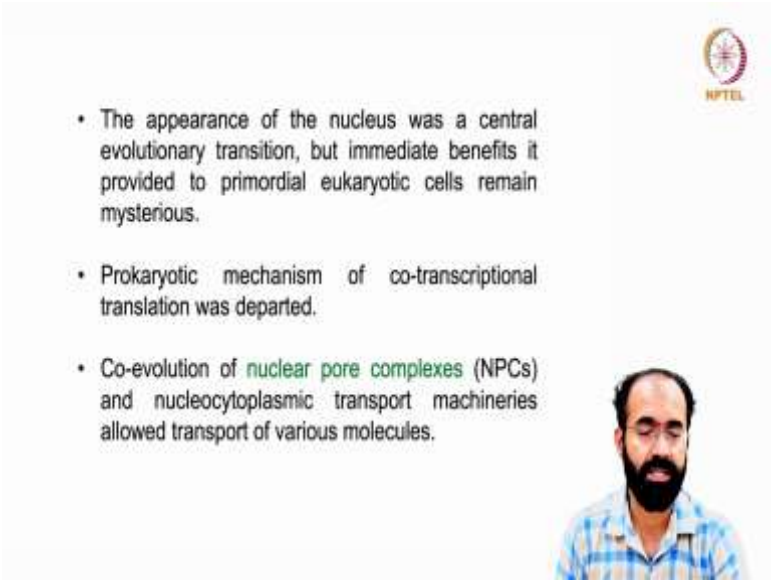

The appearance of nucleus was a central evolutionary transition, but immediate benefits it provided to the primordial eukaryotic cells remain quite mysterious till today. Prokaryotic mechanism of co-transcriptional translation was departed. That means, it has

changed because there is a nucleus, then there is a entry exit into and out of the nucleus need to be monitored, and it slows down.

Then, this having the nucleus should have some advantage also, without that the propagation of that feature in the evolutionary timescale will not be favoured. So, some advantage would have been there if you have nucleus in the so called primitive organism. So, co-evolution of nuclear pore complex that is NPCs and nucleocytoplasmic transport machineries allowed transport of various molecules. We should understand, what is co-evolution.

Co-evolution means if you; I can give a simple example. You do not know to write unless you have a pen with you, right. You cannot write on a paper with your nail or a; of course, you can make some dents on the paper or you can use your nail to carbon a wall etcetera, but you cannot write meaningfully properly.

So, as long as you are available to use a pen, there is always a need, there is always a demand for different types of pen. So, same way with the mobile phone, if there is a user you are very much dependent on mobile phone, then there is always a chance that much newer, much efficient mobile phones keep evolving keep coming.

If there is no demand, there is less likely, there will be too much of demand of another product which you are depending on. So, this is called co-evolution. If there is a demand and if there is a supply and if that organism have got an advantage, then both are getting benefited then that will be favoured. So, that is called co-evolution.

So, as the nuclear pore complex as the nucleus is maintained, and the nuclear pore complex is maintained, the associated molecule that is helping the transport in and out also facilitated. It is just like if a city is big or if a railway station is big, there is always a good chance that there will be helping staff.

If the railway station is very small, you would not get too much of helping staff, you have to help yourself if you want to carry your cargo, transport anything. Because it is not useful for a small railway station to have too much of supporting staff. So, that will not evolve. So, this idea you should keep in mind.

For a prokaryote having a nucleus or having some of this related structure was cumbersome and it was counter-intuitive and counterproductive. Whereas, for an eukaryote having a nucleus and having associated structure was beneficial and it was selected and it was proceeding further.

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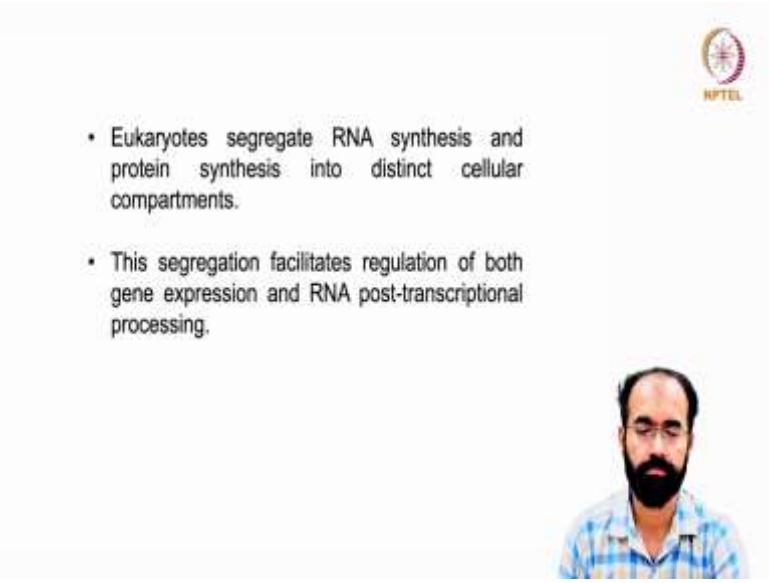

So, eukaryotes segregate RNA synthesis and protein synthesis into distinct cellular compartments because they segregate both of them separately for the way in which they handle the gene expression events. This segregation facilitates regulation of both gene expression and RNA post-transcriptional processing.

Both are needed. Having a feature does not ensure anything. Having a feature and retaining that feature across the evolutionary time scale simply means that was needed, that was utilized, and that was advantageous. So, in eukaryotes what you see having nucleus and having nuclear pore complex and also proteins helping in the shuttling of the RNA all were facilitated and this comes with a benefit over the so called prokaryotes. So, that is why it was retained even today in the advanced eukaryotes.

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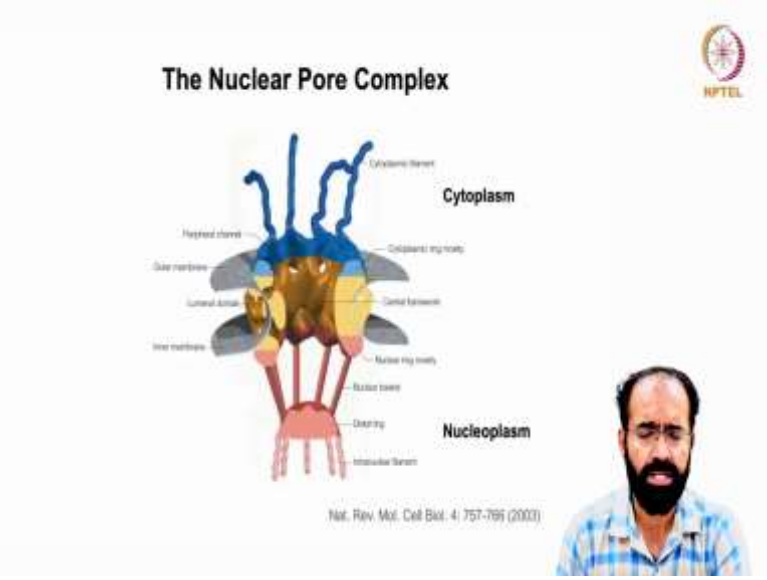

So, let us see what is nuclear pore complex. In the simplistic form this is what you can depict. You have a cytoplasmic side, and you have a nuclear side that is the nucleoplasm. What you are seeing here, it has got an outer membrane and that inner membrane. It is a double membrane structure.

And you also see a bunch of protein, some are hanging their projections into the cytoplasm and some of them are hanging into the nucleus. And these are hanging with a purpose because the RNPs that has to transport out of the nucleus or back into the nucleus they make use of this rope.

Imagine you want to climb a floor and there is no ladder, what if there is a rope hanging from the roof. What you will do? You will catch hold of that rope, and if you are strong enough or you are muscular enough you will hang on to it and climb onto that roof. This is exactly what the RNAs will do.

On the other hand, you want to jump from the roof, if you directly jump there is a good chance you will get damaged. What if there is a rope hanging? You will slowly come down, and you will be able to move yourself into the that compartment with minimum damage because RNPs also can get damaged very badly.

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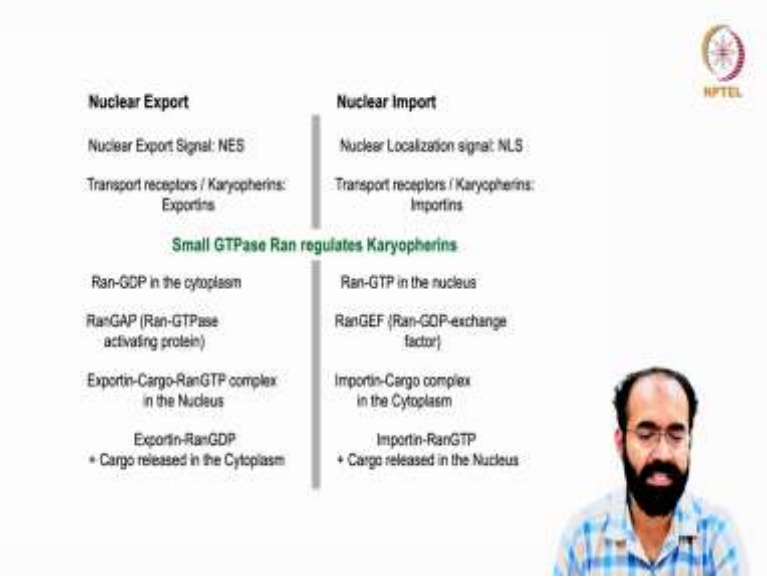

So, let us see nuclear export and nuclear import how does it work. Nuclear export that the molecule should have nuclear export signal and nuclear import that means, bringing by export means moving from the nucleus to cytoplasm. Nuclear import means bringing the cytoplasmic RNA back into the nucleus that is import. So, nuclear export signal is written as NES and nuclear import signal is NLS. And the transport receptors are mainly karyopherins and exportins.

Whereas, transport receptors are karyopherins and importins. And they you both utilize small GTPase Ran which regulates the karyopherins. And we will see how the Ran is cycled more effectively. And the Ran-GDP stays in the cytoplasm, Ran-GTP is in the nucleus. And Ran-GAP and Ran-GTPase activating protein is present in the nuclear export function, whereas, Ran-GEF Ran-GDP exchange factor it is the full name is present in the nuclear import.

And Exportin-Cargo-Ran-GTP complex is in the nucleus and Importin-Cargo complex in the cytoplasm. And Exportin-Ran-GDP plus cargo is released in the cytoplasm. Importin-Ran-GTP plus cargo released in the nucleus.

We will revisit this nuclear export and import more in detail with picture in the next class.

Thank you.