RNA Biology
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Lecture - 32
SnRNA, rRNA, miRNA, siRNA Processing, Export and Function: RNA Export Mechanisms

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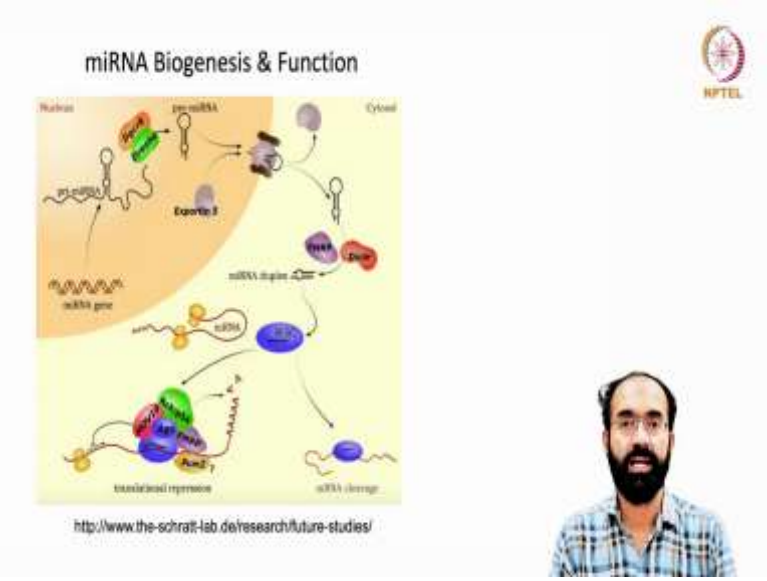

Hello everyone, welcome back to another session of RNA Biology. And we were here in the previous class that the micro RNA biogenesis and their function. And we know the micro RNA has got two major phases, one is the production in the nucleus that is in the form of primary micro RNA which is matured through the pre micro RNA by the action of Drosha and DGCR8 and goes into the cytoplasm which is further acted upon by Dicer and FMRP and which is can act into onto mRNAs and prevent the translatability or affect the stability of this mRNA species.

In a simplistic form we can say microRNAs are meant for preventing the availability or the formation of protein from an mRNA.

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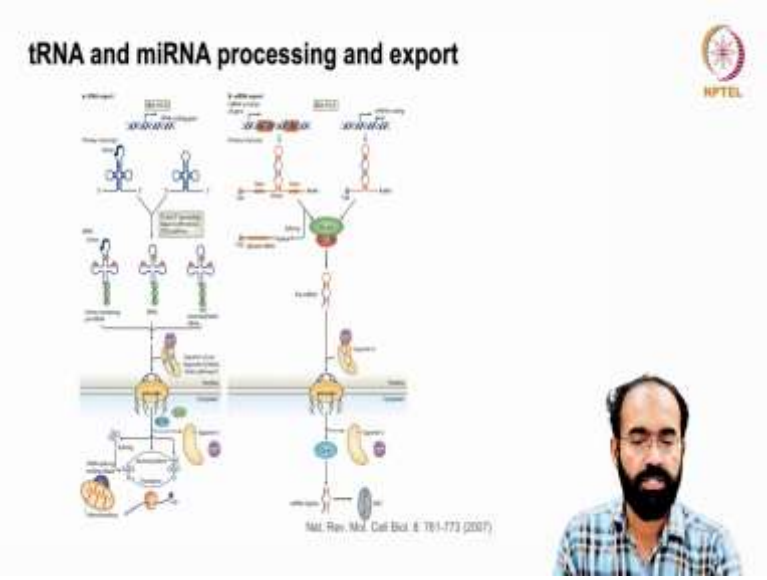

So, the transfer RNA and microRNA processing and export can be seen in this cartoon. You can see, the entire side section is the nucleus and the bottom below this line is the cytoplasm which is clearly mentioned here. And transfer RNA we saw they are three species of transfer RNA incomplete and complete and amino acylated, they pass through the nuclear core complex and function in the cytoplasm.

Whereas mRNA that can be exported through unique set of proteins and the microRNAs are present either in the form embedded inside the intron of mRNA or there can be dedicated microRNA that can be produced by polymerase 3. And polymerase 2 and polymerase 3 participate in the production of microRNA intermediates, which is processed by Drosha and DGCR8 and they become pre microRNA which in turn is exported via exportin 5.

And eventually it will find Dicer in the cytoplasm and the exportin is released and this microRNA can function onto target via the RNA induced silencing complex called RISC.

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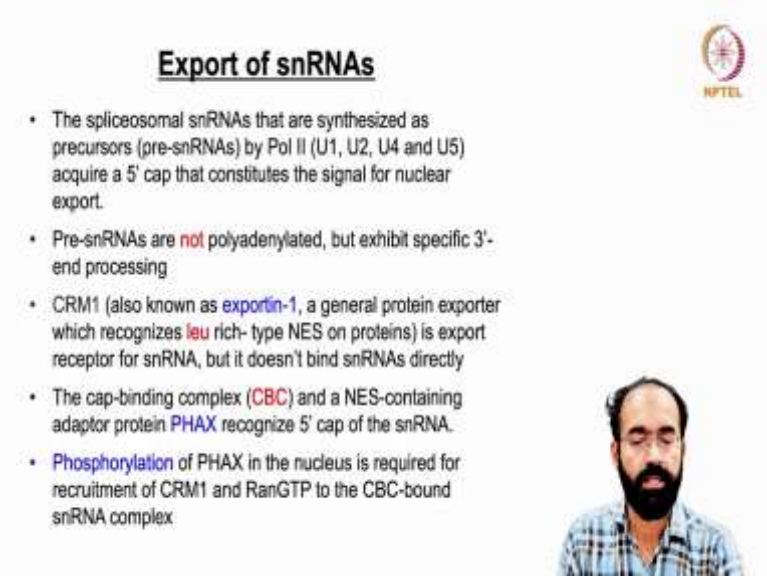

Let us now understand the export of snRNAs. What are snRNAs? snRNAs are spliceosomal snRNAs that are synthesized as precursors and we call them as pre snRNAs by polymerase 2 the transcription is via RNA polymerase 2. And we know they are U1, U2, U4, U5 and they require they acquire a 5 prime cap that constitutes the signal for nuclear export.

So, the pre snRNAs are not polyadenylated, but exhibit specific 3 prime end processing. Of course, every snRNA need to be protected from the nucleus and their way of functioning is not via poly adenylation. So, there is a protein called CRM1 it has another name exportin-1. We have seen exportin-t earlier and we also have seen exportin-5 here we are talking about exportin-1.

And this protein has another name CRM1, which is a general protein exporter which recognize leucin-rich type NES on proteins associated with this snRNA. And the CRM1 protein is export receptor for snRNA, but it does not bind snRNA directly. So, it binds the snRNA via another group of proteins.

So, the Cap Binding Bomplex CBC and the NES containing adapter protein PHAX recognize the 5 prime cap of the snRNA. So, these 2 molecule the cap binding complex and the NES containing adapter protein PHAX, they recognize the 5 prime cap of this snRNA.

Though the phosphorylation of PHAX, we know phosphorylation change the structural property and functional property of a target protein. Phosphorylation of PHAX in the nucleus is required for the recruitment of CRM1, we saw CRM1 is similar to exportin this (Refer Time: 05:24) family protein and the exportin-1 is the another name.

So, it is helpful the phosphorylation of PHAX is helpful for the recruitment of CRM1 and the RanGTPase. We know RanGTPase cycle by now and to the CBC bound snRNA complex. So, the snRNA when it is bound with the CBC can be pushed into the cytoplasm.

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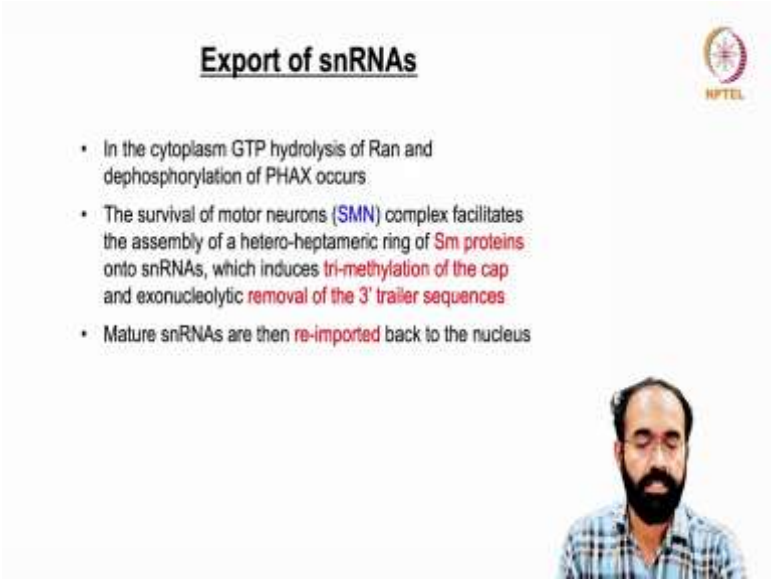

So, let us see, in detail about the export of snRNA. In the cytoplasm the GTP hydrolysis of Ran and the dephosphorylation of the PHAX occurs, because PHAX protein is phosphorylated in the nucleus. And another protein called survival of motor neuron SMN complex, that can facilitate the assembly of hetero-heptameric ring of SN protein.

We saw in the earlier class how the Sm rings are assembled during the maturation of snRNA. On to this snRNA which induces the tri-methylation, it comes from the nucleus monomethylated and now it induces the tri-methylation of the cap pro; cap nucleotide the cap guanosine, monomethylated now become tri-methylated.

And exonucleolytic removal of the 3 prime trailer sequence, there is no polyadenylation, but there is exonucleolytic removal of the 3 prime few bases from the snRNA that takes

place in the cytoplasm. The mature snRNAs are then re-imported, back to the nucleus once these features are done the tri-methylation, trimming of the three prime end and if such maturation even has taken place in the cytoplasm.

These mature SNRNAs are re-imported or re-localized back to the nucleus because they have to function in the nucleus.

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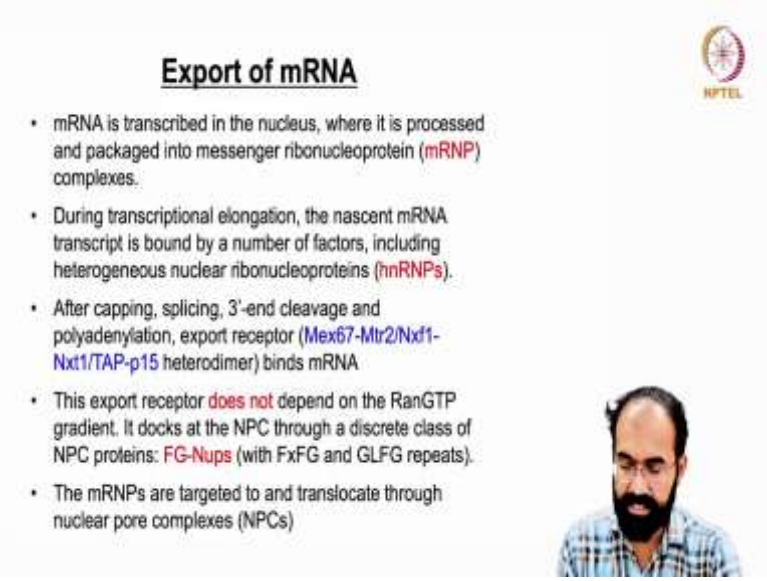

So, now let us understand the export of mRNAs we know mRNA's do not use the Ran-GTP at all, they do not require the Ran-GTPasm cycle. So, mRNA is transcribed in the nucleus, where it is processed and packaged into messenger ribonucleoprotein and we call it as MRNP complexes.

So, during transcriptional elongation the nascent mRNA transcript is bound by a number of factors including heterogeneous nuclear ribonucleoprotein and we call it as hnRNPs. And majority of these proteins are meant for substrate recognition by downstream enzymes and also for protection. In bacteria you know coupled transcription and translation the association of ribosome prevents the degradation of this mRNA.

But whereas, such a thing does not happen in the nucleus. So, hnRNP become handy for the survival of this RNAs. After capping, splicing and 3 prime end cleavage and poly adenylation onto this mRNA export receptor, which is formed of a multi-protein complex.

What are those protein? Mex67, Mtr2, Nxf1, Nxt1, TAP-p15; TAP-p 15 heterodimer. So, TAPp15 is a heterodimer and they bind to the RNA. So, the export receptor is not single form like exportin-t or exportin-1 not like that because mRNA require a group of protein which act as the exportin because of the unique secondary structure and unique molecular weight higher molecular weight of the mRNA.

This export receptor does not depend on the Ran-GTP gradient, which we already have seen before. It docks at the nuclear pore complex through a discrete class of NPC protein and we call it as FG-Nups or FG-nups. Because they have FxFG; that means, x stands for any amino acid, F stands for phenylalanine and x stands for any amino acid and Fx. And then another repeat GLFG that is glycine, leucine and then phenylalanine glycine repeats.

So, this FG-Nups have Fx, FG repeat and GLFG repeats. So, the mRNPs are targeted to and translocate through the nuclear pore complex. So, all molecules all RNA molecule cargo, they go in and out of the nucleus through the nuclear pore complex. However the protein associated with individual cargo and the proteins of the NPC Nuclear Pore Complex they interact can vary. So, depending upon which species you are handling, the nuclear pore complex interaction can vary.

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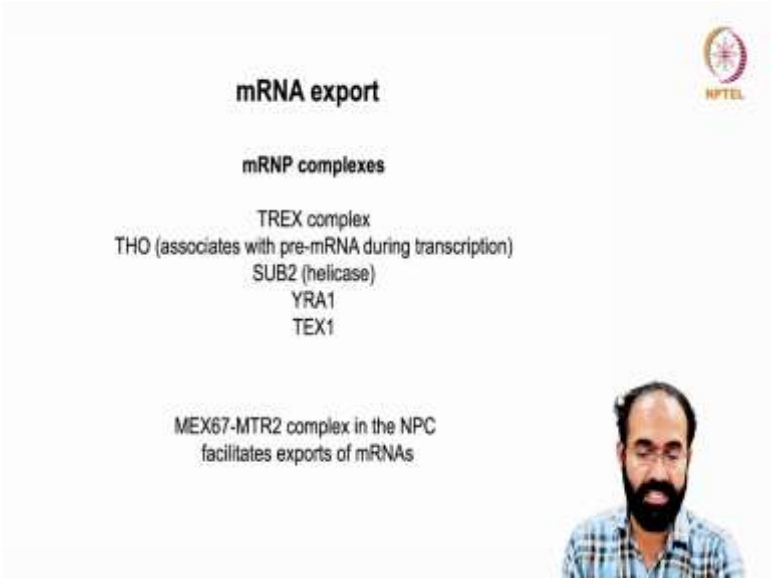

So, coming back to mRNA export. So, mRNP complex are important because that will protect the RNA and also provide stability and this complex involve TREX complex and

THO associates with pre mRNA during the transcription and another protein SUB2 which is a helicase and YRA1 and TEX1.

So, TREX is a protein, THO is a protein, SUB2 is a protein, YRA1 is a protein, TEX1 is a protein. And another complex in the nuclear pore complex is MEX67 and MTR2 complex in the nuclear pore complex is important in the movement of the cargo, that facilitate the export of mRNAs.

So, mRNA export from the nucleus to the cytoplasm is a complex process and it involves TREX, THO, SUB2, YRA1, TEX1 and also MEX67, MTR2 complex in the NPC. So, these are associated with the RNA and these are present in the NPC Nuclear Pore Complex.

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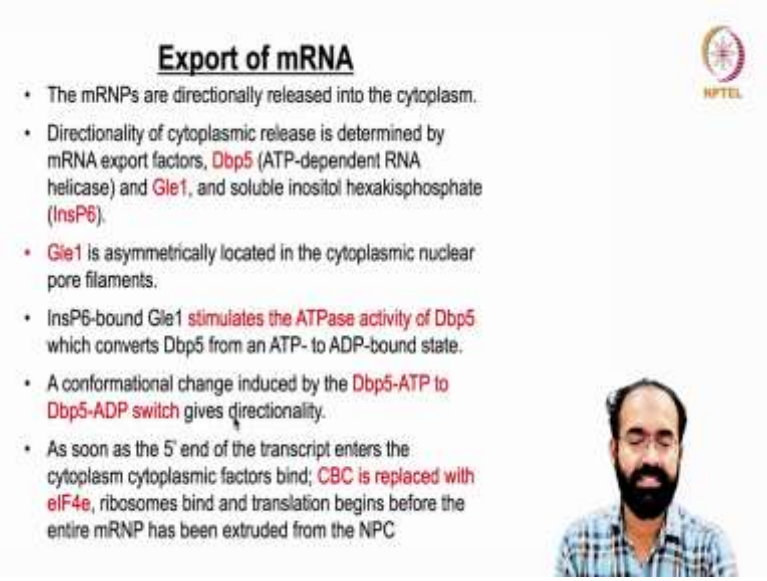

Let us see stepwise the export of mRNA. The mRNPs are directionally released into the cytoplasm; that means, it does not come from the cytoplasm to the nucleus. It is always nucleus to the cytoplasm. Directionality of cytoplasmic release is determined by mRNA export factors such as Dbp5 which is an ATP dependent RNA helicase.

You know importance of helicase because they have to maintain proper structure. If structure tweaking of secondary structure tweaking of an mRNA is needed, helicase has to come into action. And another protein DBP5 which is a helicase and Gle1, which is a

soluble inositol hexabisphosphate that is also known as InsP6. And. So, three proteins Dbp5, gle1 and a soluble inositol hexabis/by phosphate known as INSP6.

Three proteins Dbp5, gle1 and InsP6 that provides the directionality via the export factors. And gle1 is asymmetrically located in the cytoplasmic nuclear pore filaments. That means it look does not look like a mirror image, they are distributed asymmetrically. And InsP6 bound, gle1 stimulates the ATPase activity of Dbp5, which converts Dbp5 from an ATP to ADP bound state.

Now, you remember, the role of Ran-GTPase and the GAP protein, GEF protein like that. Which were contributing to the utilization of GTP energy? Same way the InsP6 bound gle1 stimulates the means acting like a GAP protein. Stimulates the ATPase activity of Dbp5. So, Dbp5 is not using GTP, but it is using ATP for the energy purpose. Which converts the Dbp5 from an ATP to ADP bound state. So, the energy can be utilized for the transport purpose.

A conformational change induced by Dbp5-ATP to Dbp5-ADP switch gives directionality. Many a times you may have seen it, like in many places where you go, where you want to control, where you want to control the movement of people, you will see some doors, some gates which open only one direction, it cannot open other way round. You can move from one place to another because this door cannot open opposite.

So, you just to push the door, it like many a times in laboratory. See you can see in dark rooms, when you are entering into dark room, you enter, then you will enter into a small chamber and you twist the door, then the outside door will be closed and it will open into inside. So, it is like a circle door. So, they are kind of unidirectional.

So, many such doors you can see it. So, here this conformational change of Dbp5-ATP to Dbp5-ADP bound which gives a conformation change. So, that it cannot go other way around. The RNA cannot come from the cytoplasm to the nucleus, but it can go from nucleus to the cytoplasm. This is what the directionality mean, which is structure induced directionality.

So, remember Dbp5-ATP and Dbp5-ADP bound state decides, which direction the RNA should go. So, Dbp5-ATP bound is in the nuclease, Dbp5-ADP is in the cytoplasmic side. So, this direction structural change allow the cargo to move into the cytoplasm. As

soon as the 5 prime end of the transcript enter the cytoplasm, cytoplasmic factors bind that is CBC is now replaced with elF4e. And the ribosomes bind and the translation begins before the entire mnRNP has been extruded from the nuclear pore complex.

Because once an RNA is pushed into the cytoplasm, it is vulnerable for degradation, even if it is bound with many proteins because many nucleases are there. So, as soon as it is entered, the CBC is now replaced by with elF4e. And elF4e; CBC stands for Cap Binding Complex and elF4e and the ribosomes come together and they will continue to start the translation process.

Ribosomes assemble one after the other and the translation begins. So, we should understand that similar to what you saw in bacteria, coupled transcription and translation, here as soon as an RNA comes out of the nucleus, it is situation is similar to the coupled transcription and translation. Just that transcription is already completed.

But it is something similar because where the release is from the DNA; here the release is from the nuclear pore complex that is the only difference.

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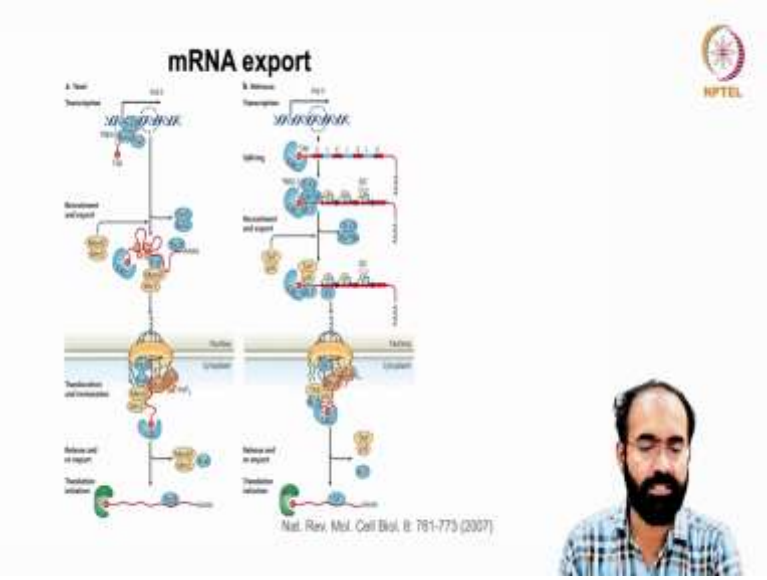

So, if you see the mRNA export, both in yeast and metazoan, they have common features. Except that in metazoan, there will be plenty of more factors coming into picture. So, in this cartoon, you can see the recruitment and export of a bunch of proteins is very; like you can see here, CBC here, here also you can see CBC, which is cap

binding complex or the 7 methyl guanosine cap is protected by the CBC proteins. And they are released out like you can see here, it is a release is happening.

And the CBC is immediately recognized and it will start the translation process and the translation initiation takes place once elF4e is bound because CBC is now replaced by elF4e. And same way you can see in metazoan also the same principle takes place and it is important to note that some of this fused exon, here you had exon, intron, exon, intron is there.

Once exon is fused, you have some EJC Exon Junction Complex. And exon junction complex are kept on the RNA on exon, exon boundary with a purpose. Because we will see them, you keep this in mind because they become handy in nonsense mediated decay or the RNA decay.

But time being you remember exon junction complex contains protein that stay bound onto the RNA until their first translation is completed. After their first round of translation that will be lost and that is why the premature stop codon has to be sensed and detected.

So, compared to yeast, metazoans also have more or less the same type of you know nuclear pore complex and their function and CBC is there, elF4A is replaced and you know and also this cargo is detached, here also you can see the cargo is released and detached and this process continues smoothly.

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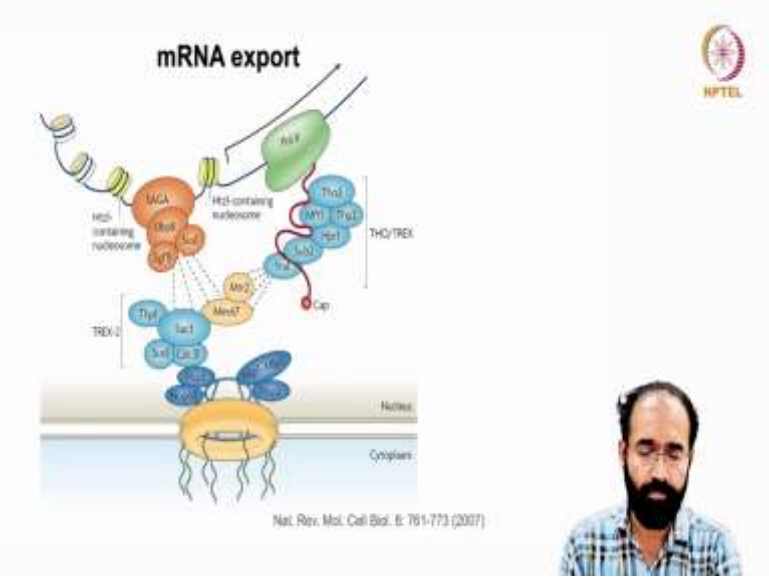

And if you look closely the mRNA export through the nuclear pore complex, this is the chromatin what you are seeing and the chromatin contains a lot of proteins and the RNA polymerase two moves across and the RNA is produced and the capping has taken place and a bunch of protein that is part of this THO, TREX complex you saw earlier.

They have assembled onto it and both this THO and TREX complex proteins are important to be recognized and some of them are recruited like Mtr2, Mex67 they also join the group. And they are important for the recognition by the nuclear pore complex protein. You can see here this yra1 interacts with Mtr2, MTR with Mex67 and also a bunch of other protein like SAC3, TSP1 like you see here, THO TREX complex, here you see TREX2 complex. TREX2 complex is present in the nuclear pore complex.

So, this is the nuclear pore complex and these proteins are projecting into the nucleoplasm and these protein complex interact with these members and slowly passing it through the nuclear pore. And this cap and CBC is not shown here and CBC also will be occupied here and that will be exposed and that is replaced by Elf4A once it reached into the cytoplasm.

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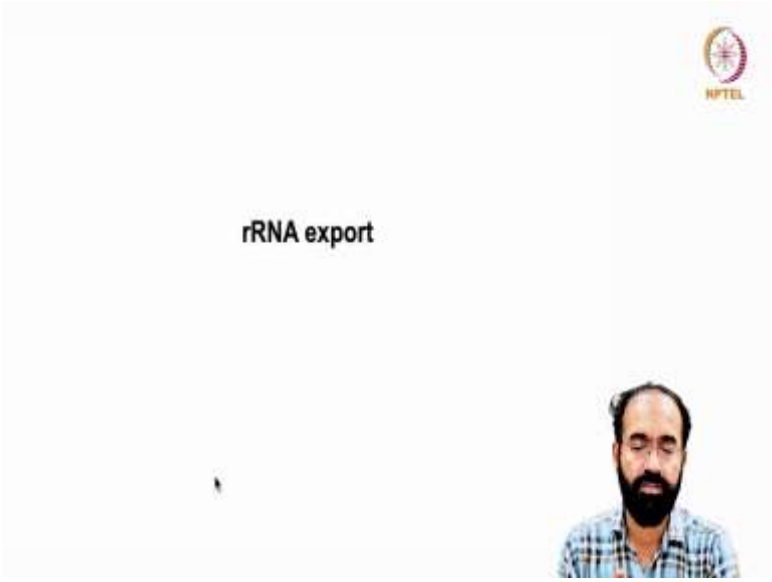

Now, we will see ribosomal RNA export, how ribosomal RNA is getting exported from the nucleus to the cytoplasm.

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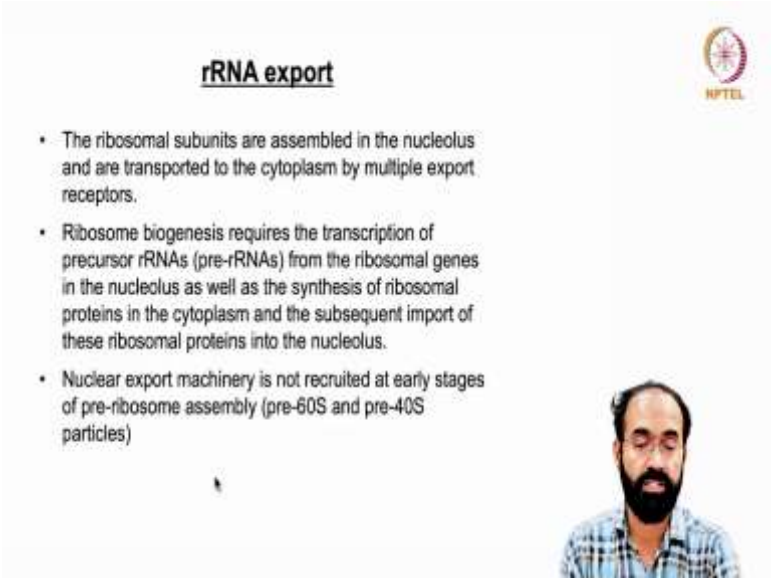

The ribosomal subunits are assembled in the nucleus and are transported to the cytoplasm by multiple export receptor. Because ribosomal RNA is not one species just like, mRNA there are plenty of different type and size and length. And secondary structure ribosomal RNAs are there and ribosome biogenesis requires the transcription of precursor ribosomal RNA we call it as pre-rRNA.

And from the ribosomal genes in the nucleolus that is looking like a heavily pigmented part inside the nucleus. So, nucleolus is a part inside the nucleus where the ribosomal RNA biogenesis is taking place. As well as the synthesis of ribosomal proteins in the cytoplasm and subsequent import of this ribosomal proteins into the nucleolus.

Many a times these proteins are produced which are supposed to interact with the ribosomal RNA they are produced in the cytoplasm they are imported back into the nucleus. So, that this freshly made ribosomal RNA can assemble with them and that is what happens in the nucleolus.

So, the nuclear export machinery is not recruited at early stages of pre-ribosome assembly. That is pre-60S and pre-40S in the case of eukaryote or metazoans. So, this particle, so the machinery is not recruited, but it will happen eventually.

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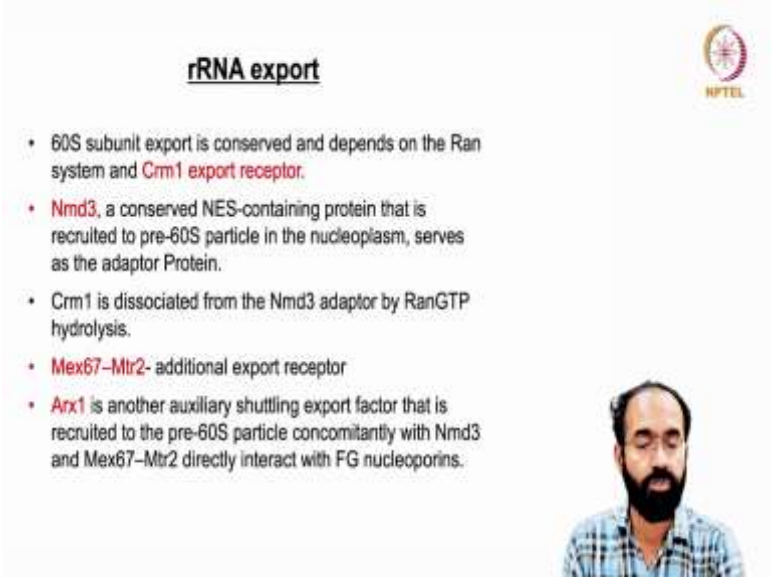

So, 60S subunit export is conserved and it depends on the RanGTPase, Ran system that is RanGTPs, RanGDP cycle. And it also make use of ACrm1 export receptor. Another protein Nmd3, which is a conserved nuclear export containing protein like I told you in the previous class, nuclear export signal need not necessarily be present in the RNA primary or secondary structure.

It can be present on specific sequences of the protein associated with them. That is recruited to the pre-60S particle in the nucleoplasm and serves as the adapter protein. So,

Crm1 is dissociated from the Nmd3 adapter by Ran-GTP hydrolysis. And then two more proteins that is Mex67 and Mtr2 additional export receptor also joins this complex.

And finally, there is an Arx1 which is the axillary protein export factor that is recruited to the pre-60S particle concomitantly means, subsequently with Nmd3 and Mex67 Mtr2 dimer. And they directly interact with the FG nucleoporin. So, it is a systematic step where Crm exporter receptor Nmd3, Mex67-Mtr2 heterodimer and Arx protein.

So, they come together and they assemble for the 60S ribosomal RNA complex formation in the nucleus.

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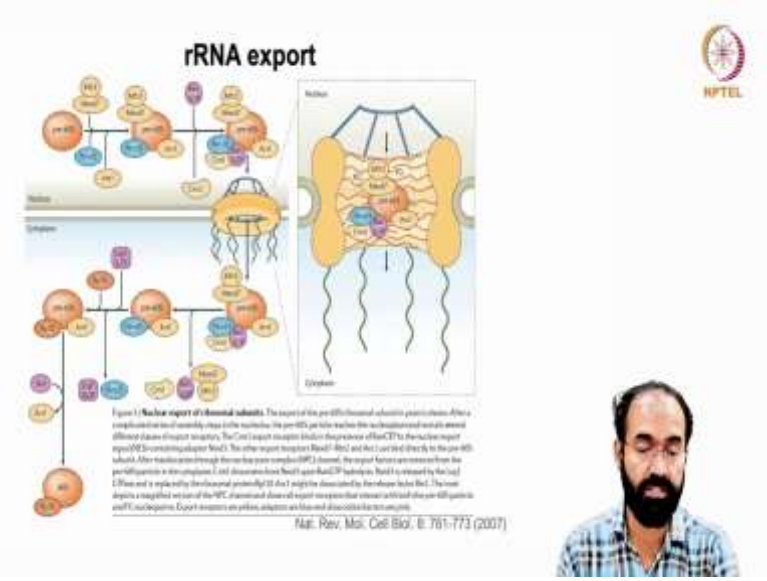

So, ribosomal RNA export if you see closely, this is the nucleoside this is the cytoplasmic side and this is the nuclear pore complex. You can see pre-60S they assemble, Mtr2 and Mex67 and NMD3 and also ARX1 they join together to form a complex and the Ran-GTP joins and Crm1 also joins and you end up getting a huge complex and this Ran-GTP undergoes the GTPase in the cytoplasm.

Once it comes out of the once it comes out of the nucleus in the cytoplasm and you end up disassembling of these proteins and you end up making a 60S ribosomal protein complex, that is ribosomal RNA plus permanently associated protein which is present in the 60S ribosomal structure or subunit.

And you can see more in detail about this in this review. And you can also see this is the enlarger structure where you have the nuclear pore complex and you can also see how individual proteins like you can see here NMD3, Crm1 how they interact with FG proteins present in the lumen of this nuclear pore complex.

So, that their movement is slow and steady, rather than just falling into a well or falling from the roof of a building like that it should not happen that fast because it will disassemble. It has to be smooth that is why the FG proteins becomes handy for this transport. So, we will study more in detail about various diseases that is associated with you know export of the RNA in the next class.

Thank you.