RNA Biology
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Lecture - 31
snRNA, rRNA, miRNA, siRNA Processing, Export and Function Nucleoporins and miRNAs

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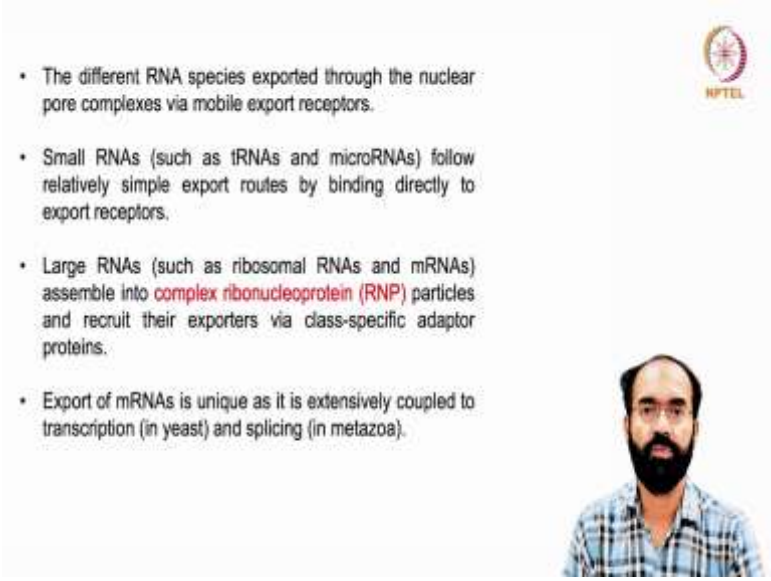

Hello everyone. Welcome back to another session of RNA biology and we were here in the previous class and we were discussing about the nuclear export and nuclear import of various RNA cargo into and out of the nucleus. So, the mRNA's they follow a different strategy, different tract unlike rest of the RNA which depends on the Ran GTP cycle.

So, we saw in detail the Ran has Ran GTP and Ran GDP have got gradient across the nucleus with a purpose that it will be helpful in mobilizing various helpful or supportive proteins for the movement of RNA cargo in and out of the nucleus. Now, let us see a diagrammatic representation of the nucleoporins in the RNA export.

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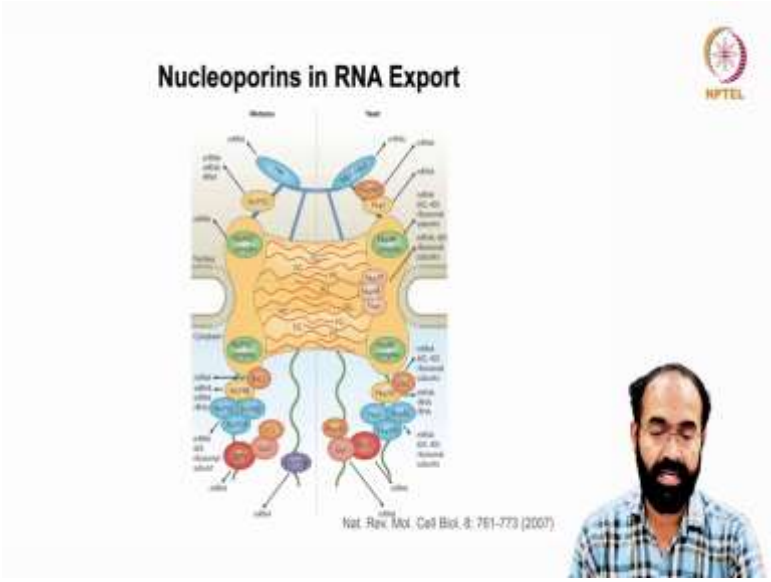

This slide will look very complex and quite cumbersome, but understand this many number of proteins are present in the nuclear pore complex and there is no easier way we can simplify it if we want to know the strength and depth of these proteins. We should understand there are mainly three different categories of proteins in the nuclear pore complex.

The ones which provide structural support of the nuclear pore complex which do not play any role in the transport of the cargo that is the RNA cargo, but they just hold the nuclear pore complex into the membrane. So, nuclear membrane is a bi-wall structure, two wall structure is there and they are present and remember it is a three dimensional structure, what you are saying is a cross section and it is like a pipe it is just like a cylinder.

Now, you have some proteins hanging into the hanging into the cytoplasm and there are some factors hanging into the nucleus and left hand side you can see typical metazoan that is all multicellular organism in right hand side you are seeing yeast specific proteins; that means, you have NUP 84 complex whereas, NUP 107 complex in the metazoan.

So, this is the main difference. So, functionally they are similar, but name wise or amino acid sequence wise they are different. So, same way for rest of the protein so the entire half you can see and you can also see many FG proteins hanging into the lumen and you

saw in the previous class that the FG proteins are the pockets we gave the example of rock climbing etcetera the pockets in which the cargo literally interacts with them.

And so, you can also see many associated protein along with the structural protein that is present for interacting with the cargo or the proteins associated with the cargo that is here cargo we are referring to the RNA species. So, this is the nucleoporins in the RNA export mainly in the metazoan and also in the yeast.

Now, let us see in detail with proper description how each of these proteins are important in the transport of the cargo.

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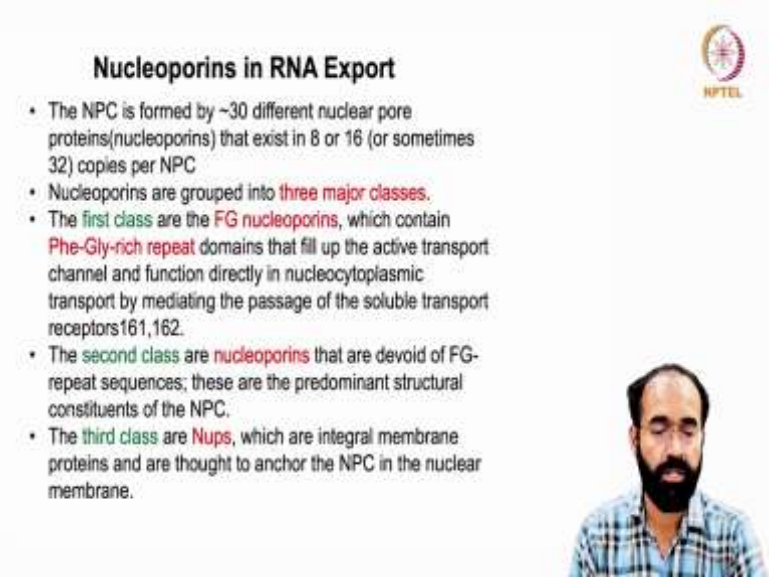

So, nucleoporins in RNA export. So, nuclear pore complex NPC is formed by approximately 30 different nuclear pore proteins and we call them as nucleoporins that exist in 8 or 16 or sometimes even 32 copies per nuclear pore complex, note the point that they are in pairs there will not be 9 or there will not be 17. So, they are 8, 16 and 32. So, they are kind of occupy the diagonals quite effectively.

So, depending upon which cell type or which cells nucleus you are talking about accordingly like some tissues there will be active transcription going on like say liver or sometimes neuronal tissues etcetera. So, they need to have more effective more efficient migration.

So, they may have more proteins associated it is like the less transcription is occurring like in some of the epithelial cell or some of the lining of the intestine those kind of cells they do not have any dedicated function they are playing more of a structural role. So, the gene expression events may not be that robust as you see in the case of some of the glands or some nervous tissue like that.

So, nucleoporins are grouped into three major classes. The first class are the FG nucleoporins, that is those nucleoporins that contain the FG repeat phenylalanine glycine repeat which contain this phenylalanine glycine rich repeat domains that fill up the active transport channel and function directly in the nucleocytoplasmic transport by mediating the passage of the soluble transport receptors 161 and 162. So, these are the FG nucleoporins.

What is the another class, they are the first class of nucleoporins. The second class of nucleoporins that are devoid of the FG repeat sequence; they do not contain the FG repeat these are the predominant structural constituents of the NPC; that means, it provides structural support, the nuclear pore complex should be of a particular shape and they need to have this structural support.

The third class are the Nups, Nups which are integral membrane proteins that are thought to anchor the nuclear pore complex to the nuclear membrane because they are also structural in category, but they have nothing to do with the nuclear pore complex function.

They are holding just they are acting like anchors or hooks just like if you are hanging a fan in the ceiling you need to have a metallic hook and metallic hook has nothing to do with the functioning of the fan except that the fan is in place hanging from the roof because of this hook. So, something like that this is the third class of protein.

So, let us see the first class are the FG nucleoporins and the second class are the nucleoporins porins that do not have the FG repeat and the third class are the Nups they are basically holding the nuclear pore complex onto the nuclear membrane.

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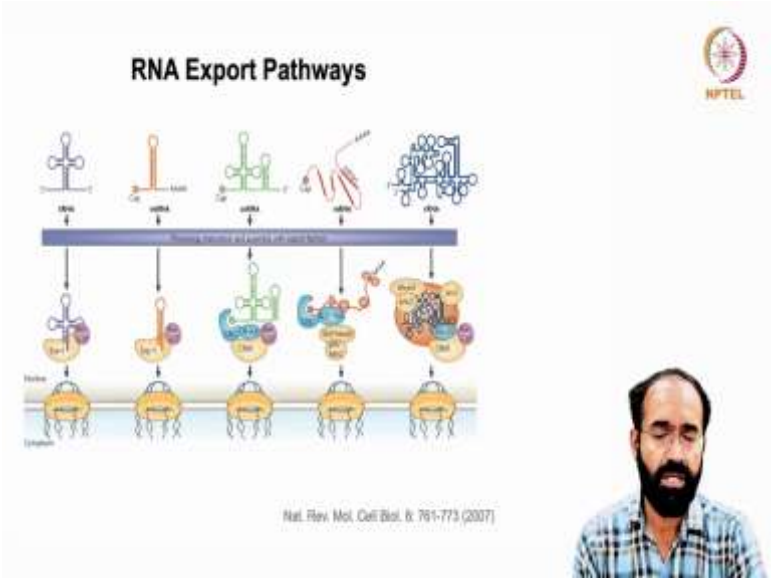

So, the RNA export pathways, you can see them in this cartoon that the RNA category include transfer RNA micro RNA, snRNA, mRNA and ribosomal RNA. And the movement of all this RNA category like tRNA require Ran GTP cycle, mi RNA require Ran GTP cycle, snRNA require Ran GTP cycle and mRNA do not require Ran GTP cycle ribosomal RNA require Ran GTP cycle.

You can also see the associated protein like here exportin- t whereas, here it is exportin-5 and here you can see CRM1 and like that each of these molecules have a different set of associated proteins and this associated proteins help in two ways. One thing is recognizing the nature of the cargo whether it is a tRNA, whether it is an snRNA, whether it is a micro RNA this recognition is done by these proteins.

And another way is that they have to have a specific downstream event; that means, presence of presence of a given protein associated with the cargo can decide what should be the destination of this cargo. It is not that they are just associated how effectively this should be transported, how what is the rate of transport everything can be decided by the protein associated with that.

So, we should understand this cargo protein complex depending upon which species being transported they will have a dedicated exporting associated with that and this is the nuclear pore complex and the what you see downwards is the cytoplasm. So, based on the nature of the RNP that is formed or assembled accordingly it will pass through the

nuclear pore complex and nuclear pore complex proteins also interact with specific proteins associated with this RNP.

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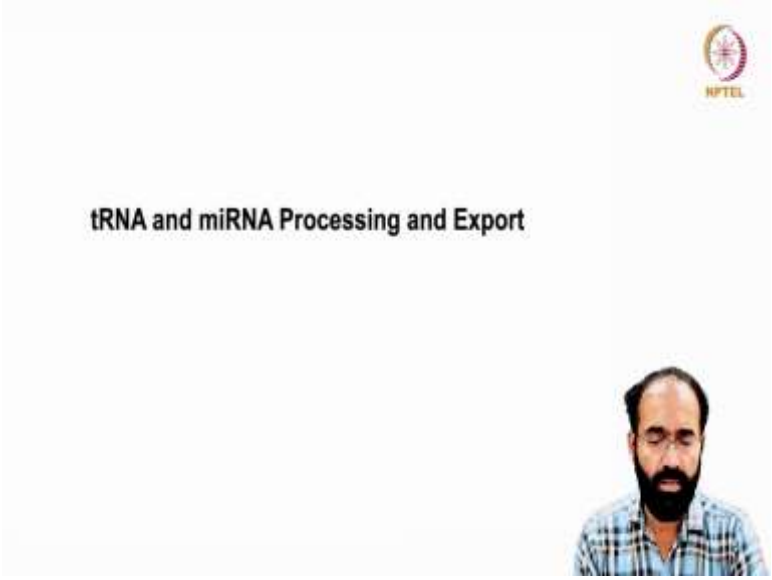

So, let us see individually how different RNA species are transported or do they have anything in common or are they unique. Let us see transfer RNA and micro RNA processing and their export through the nuclear pore complex.

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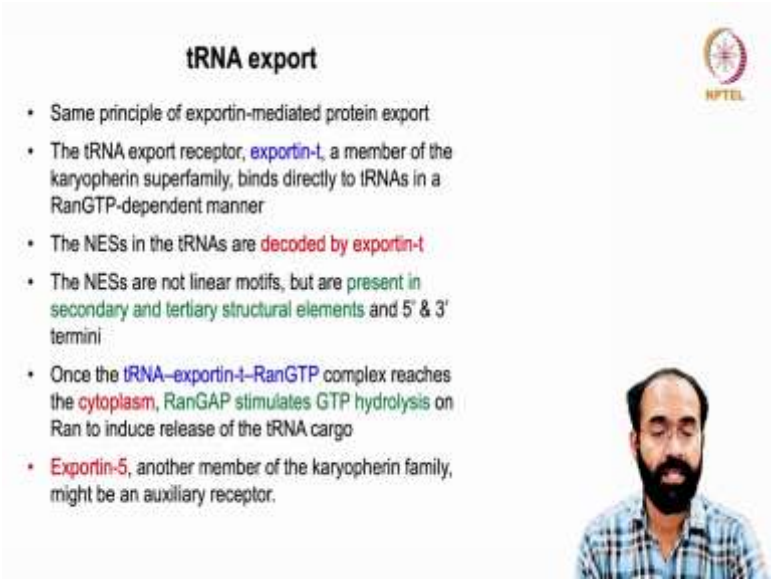

Let us see tRNA export at first. It has the same principle of exportin-mediated protein export what we saw earlier that the tRNA export receptor that is exportin-t it is a member

of the karyopherin super family, it binds directly to the tRNA in a Ran GTP dependent manner. Ran GTP we will not go into the details, we know that Ran GTP it provides the phosphate to the karyopherin for the release of the cargo in the cytoplasm. Otherwise, in the cytoplasm also it will hold the hold the cargo and we do not want that.

So, the nuclear export signals in the tRNAs are decoded by exportin-t. So, this decoding is very important because without the exportin-t the tRNAs nuclear export signals cannot be read. So, exportin- t's role is to identify the right cargo. So, the NES nuclear export signals are not just a linear motif not that you know UUUCA UU like that you saw poly a signal which is AAUAAA.

So, it is not like that there that is a linear motif, but here it is not like that, but they are present in the form of secondary and tertiary structural elements at the 5 prime and 3 prime termini. So, this is what you should understand that they have a unique dedicated secondary structure that is obvious in the 5 prime and 3 prime terminus of this tRNA molecule.

Once the tRNA exporting and t-Ran GTP complex reaches the cytoplasm, the Ran GAP you know that GTPase activating protein. Ran GAP stimulates the GTP hydrolysis on the Ran to induce the release of the tRNA cargo. Otherwise, export in will not release the cargo it will hold on to the cargo. So, the moment the Ran GTPase is acted upon or activated by Ran GAP then you will have the release of the cargo.

Another export in exportin- 5 is a member of the karyopherin family and it can act as a auxiliary receptor; that means, it is kind of a supportive in function. Sometimes in some tissue if the exportin-t is not available or abandoned then or exportin-t is not functional adequate enough then the auxiliary receptor it is just like an assistant it is like a personal assistant you have personal assistant cannot replace you, but personal assistant can enhance your efficiency. So, this is exportin-5 can act as a auxiliary receptor.

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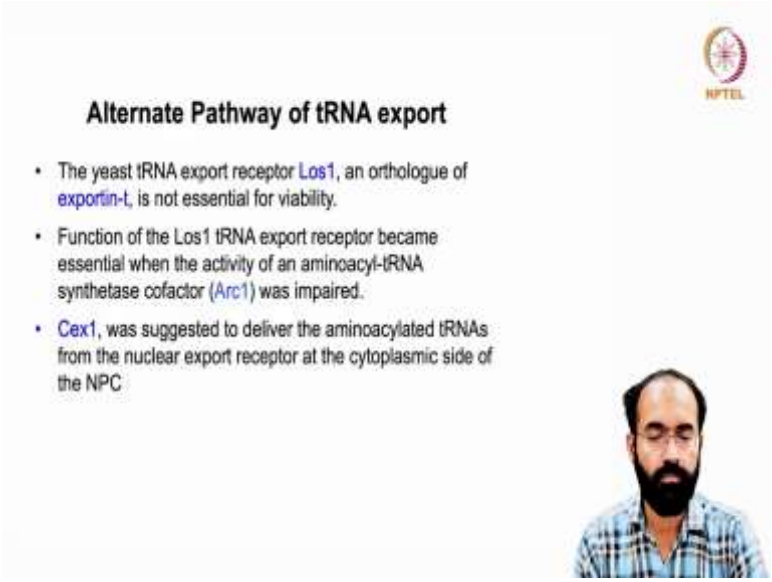

And alternate pathway of tRNA export, let us see is this the only way what you saw in the Ran GTPase exportin-t mediated is there any other method and in yeast the tRNA export receptor los 1 is an orthologue of exportin-t. Exportin- t is not there in the yeast whereas, you have another protein called Los 1.

And, but interestingly it is not essential for viability, it may sound little surprise because you cannot assume that the tRNA need not be transporting to 1 from the nucleus in the case of yeast that is not the case because Los 1 is the functional counterpart of exportin- t seen in metazoan.

So, how does this happen? The function of Los 1 tRNA export receptor became essential when the activity of an amino acyl tRNA synthetase cofactor known as Arc 1 was impaired. So, what it indicates los 1 is doing the job of exportin- t, but if Los 1 is missing then it is not affected. So, we should assume the tRNA transport is not affected. So, we should assume there is some assistant someone else is doing the function of the Los 1 and we could guess it who could be that be.

So, if Los 1 is defective along with the Arc 1 which is a aminoacyl tRNA synthetase cofactor then Los 1 absence of Los 1 become important. So, another protein that is Cex 1 was suggested to deliver the amino acylated tRNAs from the nuclear export receptor at the cytoplasmic side of the nuclear pore complex.

So, another protein Cex 1 was able to facilitate the movement of the or the maturation of the tRNA even in the absence of Los 1. So, Arc 1 if it is missing along with the Los 1 it becomes problematic because another protein Cex 1 was acting as a mediator for the interaction of the tRNA with the amino acyl tRNA synthetase.

So, we should understand systems or the organisms they evolve with diverse strategy they can have unique strategies through which they evolve and this we also you also may remember that we talked about the evolution by gene duplication. So, this can be one example like Los 1 gene is important it is necessary it is doing its job everything is working fine, but there is also a second in charge or second person in charge which is there a second molecule in charge is there.

So, Arc 1 and Cex 1 can work together and they can complement the absence of Los 1 when it comes to the transport. So, naturally what happens in evolution the Los 1 can acquire some novel functions eventually. So, whether it acquire or not is a question of time, but it is possible because Los 1 is kind of free even if it is present it is kind of free from doing it is this job along it can do another job.

So, this is an example which we can think about that Arc 1 and Cex 1 they can kind of complement the absence of Los 1, but if Arc 1 also is missing then it is a serious issue.

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So, you can read this review that is exporting RNA from the nucleus to the cytoplasm which came nature reviews molecular cell biology few years ago. And it is quite interesting and I have used some of the images from this review and it is an excellent review. So, Student should go through it for a better understanding of the subject.

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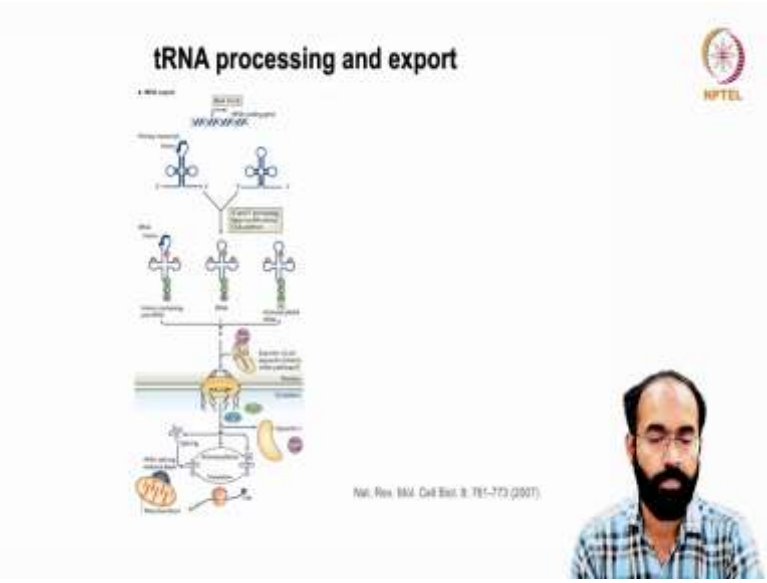

So, tRNA processing and export like you can see in this picture a tRNA normally is transcribed with RNA polymerase 3 and it has it has to undergo the maturation process and once it is undergoes maturation then this 5 prime and 3 prime processing and the CCA addition of the tail everything has to happen.

Now, in the nucleus you can have three species one is tRNA with intron that is immature and it can have the CCA maturation already and or properly processed tRNA and you can also have amino acylated tRNA. So, all these three has to find its way out it is holding the tRNA along with the intron probably because it is defective it has to be marked for degradation and some of them the aminoacylation will happen in the cytoplasm not in the nucleus for some the aminoacylation can happen in the nucleus itself.

So, for all this category you need to have exportin-t and exportin-s and also other pathway proteins they have to come together and act along with the nuclear 4 complex proteins and they move into the cytoplasm. And in the cytoplasm it will disassemble because the Ran GTPase cycle come into picture and the Ran GAP will help in the GTP

hydrolysis and the cargo will be released and the cycle will continue the amino acylation can happen to those tRNA which do not have the amino acid attached the amino acylated tRNA can directly participate in the protein synthesis.

Remember there are 20 different amino acids and you also have 30 different tRNA species which has to be catering the 61 different codons of the genetic code.

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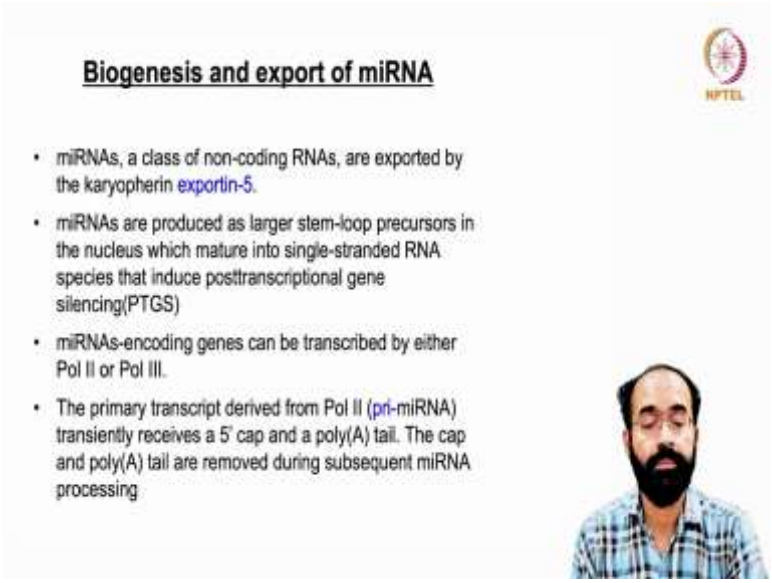

And let us see how the biogenesis and export of micro RNAs happen micro RNAs are group of non-coding RNAs similar to ribosomal RNA transfer RNA, but their role is post transcriptional gene silencing. That means they are performing the gene regulation which is on the mRNA after the transcription. So, miRNAs are a class of non-coding RNA they are exported by the karyopherin exportin- 5 we saw for tRNA it was exportin- t.

So, mi RNAs are produced as larger stem low precursors in the nucleus which mature into single stranded RNA species that induce post transcriptional gene silencing PTGS transcriptional gene silencing means a gene regulation occurring at the transcriptional level; that means, whether or not a RNA is produced or not whereas, post transcriptional gene silencing means it has to be regulated after the fact or after the production of the of the mRNA that is happening in the cytoplasm.

Micro RNAs encoding genes can be transcribed by either Pol 2 or Pol 3. So, they can be depending upon like we will see them in detail because micro RNA can be embedded

inside the intron of an mRNA. So, in that situation the micro RNA transcription is regulated by polymerase 2 RNA polymerase 2 whereas, some micro RNA can have their own promoter their own genes. So, that transcription is polymerase 3.

So, the primary transcript derived from polymerase 2 we refer to them as pri-miRNA that is primary micro RNA and it transiently receives a 5 prime cap and a poly a tail because that is embedded inside the intron of some other gene. The cap and the poly a tail are removed during subsequent micro RNA processing.

Many a times this micro RNA if they are transcribed they even can have their own 5 prime and cap and the 3 prime tail which will be removed because eventually the micro RNA are just 22 nucleotide in length and they are functioning in the cytoplasm onto the mRNA target to prevent their translation.

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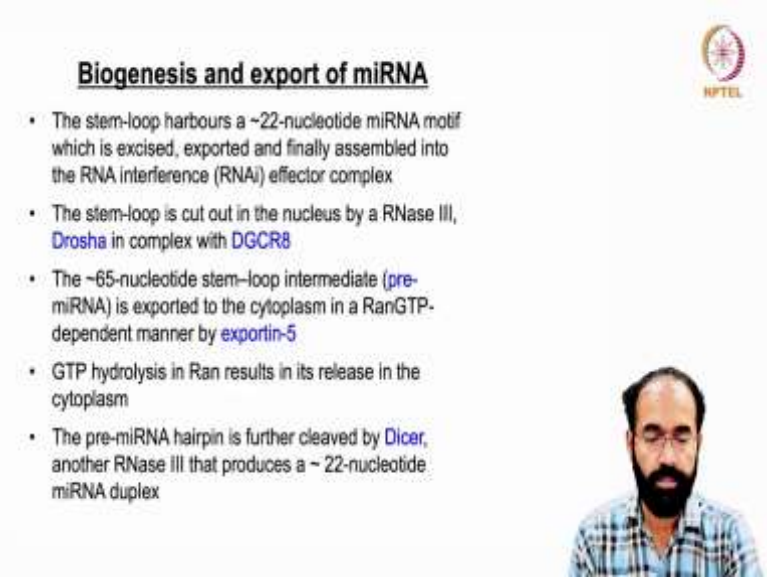

So, let us see the biogenesis and export of micro RNA in detail. The stem-loop structure that is present when it is formed it harbours a 22 nucleotide micro RNA motif. Remember the stem-loop structure of the micro RNA can be around 150, 160 nucleotide. So, somewhere in this 150, 160 nucleotide you have a complementary means both strands are pairing each other.

That is why disabled form a stem in the first place. Loop region does not have any pairing. The stem region have got complementary sequence that is why it is forming a

stem and it has around 22 nucleotide long micro RNA motif which is excised and exported and finally, assembled into the RNA interference effector complex. So, we call it as RNA induced to silencing complex or it is called RISC.

The stem-loop is cut out in the nucleus by an RNase 3 enzyme and we call it as Drosha in the complex with another protein DGCR8. So, remember the stem-loop structure micro RNA is acted upon by RNases Drosha along with the help of a protein DGCR8. So, stem-loop can be of around 65 nucleotide in length.

The 65 nucleotide stem loop intermediate we call it as pre micro RNA is exported. Earlier it was pre micro RNA and now after cut by the RNase 3 drosha and DGCR8 we call this product as pre micro RNA which is now around 65 nucleotide stem loop intermediate is exported to the cytoplasm in a Ran GTP dependent manner by exportin-5.

So, exportin- 5 is contributing to the transport and the GTP hydrolysis in Ran that results in the release of the cargo that is the 65 nucleotide stem loop intermediate micro RNA into the cytoplasm. So, the pre micro RNA hairpin is further cleaved by Dicer. So, this action happens in the cytoplasm. So, another nuclease called Dicer comes into picture and this produces. So, this is also an RNase 3 family protein which produces a 22 nucleotide micro RNA duplex.

So, this is precise no matter which micro RNA you are talking about it is 22 nucleotide in length which is in complementary sequence it is pair. And one of the strand is the one which participate in the silencing event both strands do not participate. So, one of the strands of this 22 nucleotide participate in the silencing event.

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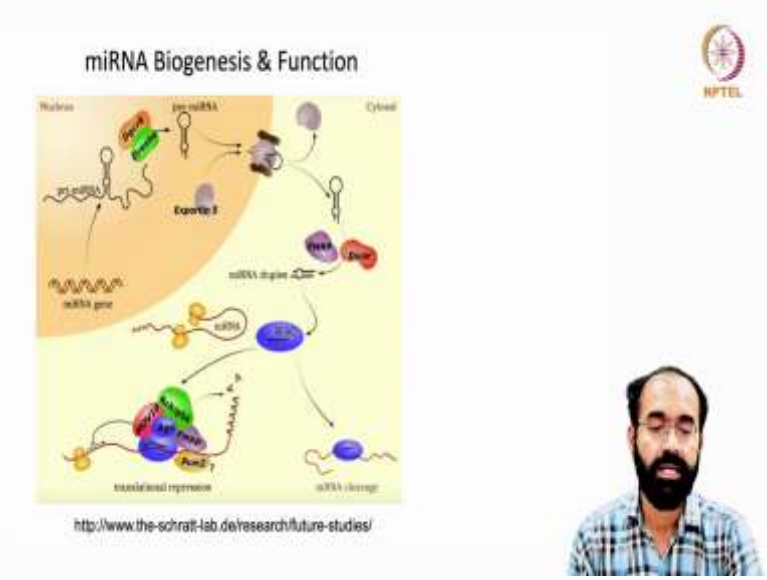

Now, in this micro RNA biogenesis you can see in this cartoon you have this nucleus and you have this primary micro RNA which has formed the loop and this is from the DNA the primary micro RNA has formed and this is the stem loop structure which is acted upon by Drosha nuclease and DGCR8 and you end up getting a pre micro RNA and this pre micro RNA is now with the help of exportin through the nuclear pore complex it is released into the cytoplasm.

So, in the cytoplasm the exportin is released back because of the Ran GTPase cycle and this pre micro RNAs now acted upon by Dicer along with another protein FMRP and you end up getting a micro RNA duplex which is to 22 nucleotides in length. And this can assemble into RNA induced silencing complex RISC complex onto mRNA species and this complex involves a bunch of protein, one of the major protein is argonaut protein which binds onto the mRNA and causes the degradation.

And it can cause translational repression that is preventing the mRNA from protein being translated or mRNA cleavage, the mRNA can be marked for degradation both way it functions. So, we can see more in detail about the micro RNA and its function in the next class.

Thank you.