RNA Biology
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Lecture - 30
SnRNA, rRNA, miRNA, siRNA Processing, Export and Function: Nucleo Cytoplasmic Transport

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Hello everyone, welcome back to another session of RNA Biology course and we were here in the previous class and we were kind of discussing the portions and we did not discuss adequately enough to we just had a flavor of this nuclear export and nuclear import signals and what is the mechanism that is happening and we will see them in detail today.

Nuclear export and nuclear import by now you would already know because export means taking something from here to somewhere else and import means bringing in something from somewhere else to here. So, when a gene point of view or gene expression point of view the here is the nucleus, ok. So, you always refer within terms of nucleus.

So, when you say nuclear export which basically mean removing or taking something from the nucleus because that is the origin that is the origin of a gene origin of a gene expression event. So, from there to somewhere that is called export and from somewhere

back to here that is to nucleus is the import. So, anything which has to move out of the nucleus need to have the nuclear export signal like you need passport to move out of the country.

So, if you do not have passport you simply could not have moved out of the country. So, hence now question of coming back to the country without a passport because in the first place you will not move out of a country in a normal circumstance without a passport. So, you need to have nuclear export signal.

Especially, for a RNA which is specifically produced in the nucleus. Things are not so for protein because no protein is produced in the nucleus every protein is produced in the cytoplasm and they get located into the nucleus for a protein point of view. But whereas, RNA no RNA is being produced in the cytoplasm every RNA is produced in the nucleus, but in cytoplasm the RNA undergoes maturation, degradation etcetera.

And degradation can happen in the nucleus also we will see them what are the circumstance such things happen. So, let us have clarity on the export and import concept. So, export means moving something here we are referring to RNA from the nucleus to the cytoplasm and they must have nuclear export signal. Nuclear export signal can be its not that you know just like if someone ask you how passport of a country look like you can say it is a small booklet which is of so and so dimension you can say.

So, export signal are not so there is nothing so specific about export signal it varies from RNA to RNA. It is just like if I give you a pen to hold you use your hand, but you use a specific set of muscles especially the fingers to hold the pen if you are writing it. But if I ask you do not write just to hold the pen you will use a different set of you will tightly hold in your palm.

On the other hand if I give you a thermo flask or a glass of water to hold you will use finger, but finger alone may not be sufficient enough depending upon the strength of that material. If I give you a laptop to hold definitely you will not choose to hold just with the finger alone, you will use much more quantity and space in your body and much more quantity of muscles and occupy lot of space in your body.

And if I give you a gas cylinder to hold you will use maybe your shoulder or use your both hands etcetera. Why I gave this example is based on the size of the RNA every

RNA is not of uniform size based on the size of the RNA they have to be held together by various factors in the nuclear pore complex accordingly the nuclear export signal also will vary.

So, same idea you should have, there is nothing specific that this is this sticker you put it on the RNA that indicates it is an export signal. No, every RNA will have a different set of export signal. All we need to understand if an RNA has to move from the nucleus to cytoplasm through the nuclear pore complex it must have to have the nuclear export signal.

And mainly the transport receptors especially the karyopherins and exportins are the readers of this nuclear export signal in the nuclear export. And we should understand also that GTPase GTP is something similar to ATP which yields energy when the phosphate normally TP means tri-phosphate means it has got three phosphate group alpha, beta, gamma.

So, usually the third one which is cleaved and you end up getting a GDP and phosphate this breakage of bond releases energy which is utilized for various biological function. So, we can use ATP also because when you consume you know food your body breaks it down by glycolysis and krebs cycle and produce lots of ATP and it produces small quantity of GTPs also. We will not go into the kreb cycle details etcetera right now, but understand ATP and GTP are the energy sources for our body.

And GTP has to be utilized by GTPase like if a organism have to eat food it need mouth no point in having nose or no point in having hands etcetera you need mouth in order to eat food. Same way if you want utilized GTP that need to have an enzyme that can utilize the GTP that is Ran one of the GTPase do not think that Ran is the only GTP there are plenty of GTP utilizing enzymes we call them as GTPase and this Ran GTPase can regulate the karyopherins .

So, whether or not this karyopherins have the energy provided by the Ran-GTPase it will decide to function in a given manner; that means karyopherins are important in nuclear export and they are important in nuclear import also. Just like you have a door in your house you will not use for getting into the house you use the same door for getting out of the house also.

So, both situation, you use the door you open and close. So, if you are entering the house you open the door and then enter the house and then close the door. Again when you want to come out of the house you again open the door and close the door. So, same logic apply when it comes to karyopherins.

And the Ran when it is utilizing a GTP it will hold the GDP in within itself and that is called Ran-GDP. Because the phosphate is utilized it has been already been delivered to the karyopherin. And so there in this process the Ran-GDP is holding back into the cytoplasm. And then there is one more protein comes into the picture that is RanGAP, what does it mean? Ran GTPase activating protein.

So, what it indicates? Ran is a GTP's but it is lazy. The moment it sees a GTP it will not jump and start action it need a stimulator, it need a catalyst and that is called RanGAP. So, that is GTPase activating protein. So, if you are sleeping and I kept to breakfast beside your bed. So, I cannot assume you will have the breakfast, right. So, I have to shake you and wake you up and that is the role of this RanGAP. So, that you can see the breakfast and you eat it.

So, then comes the next step in export nuclear export is the Exportin-cargo-Ran-GTP complex in the nucleus. So, Ran is in the nucleus, GTP is in the nucleus, cargo which is the RNAs in the nucleus and exportin also it is in the nucleus. So, what it forms? Exportin-cargo-Ran-GTP complex stays in the nucleus before exporting the cargo into the cytoplasm.

So, exportin Ran-GTP plus cargo released in the cytoplasm. So, during this complex formation what happens? The Ran-GTP utilize the GTP for energy and as a result Ran is no more having the GTP it is now having the GDP the phosphate is taken by this complex.

And then the cargo because of this process the cargo will be released into the cytoplasm. If the Ran did not give the phosphate to the karyopherin then the karyopherin will hold, the karyopherin will hold the cargo the moment it give the phosphate group to the karyopherin then it will release the cargo, what is the cargo? RNA is the cargo.

So, it will release into the it is just like if a child is holding something important your mobile phone, child is holding your mobile phone he is not giving he or she is not giving

back then what you will do? You will give an eclair or a chocolate to the child and the child will no more interested in the mobile, but go after the chocolate and you get your phone back.

So, this is how many a times the enzymes work. So, it gave the phosphate group and changed. And now do not think that you know karyopherins have its like a child because this phosphorylation changes the conformation. Because of the change in conformation of this protein it no more hold the cargo and the Ran-GDP is available for cycling.

Now, let us see what happens in the nuclear import. And nuclear import we know it need to have the nuclear localization signal and many a times this nuclear localization signal is associated with the protein, protein of the RNP you know RNA never stays as empty or naked RNA it always associated with proteins. And many a times when they have to come back the nuclear localization signal has to be the protein associated with that.

One example we saw one of the 5 prime you know mono methyl guanosine has to become tri methyl guanosine in order to come back into the nucleus. So, in the previous class we have seen it. So, they are also kind of a nuclear localization signal. Of course, that has to be recognized by specific proteins in the nuclear pore complex and also specific proteins associated with this RNP is important.

And the transport receptors are mainly karyopherins and importins whereas, in the case of export it was karyopherins and exportins and these are all categories of protein it is like when you say animal what you understand you do not think it is dog, you do not think it is cat, you do not think it is rat, when you say animals it can be anything. So, it is like a generic name.

So, importins are group of proteins. And the Ran-GTP usually stays in the nucleus before the movement of the cargo from the nucleus to the cytoplasm. So, for this further the nuclear import you need to have just like the RanGAP was helpful for activating the GTPase there is another protein in the cytoplasm in the nuclear import that is RanGEF what does it indicate? Ran-GDP exchange factor.

So, when Ran is associated with the GDP it need to need the support from an exchange factor somewhat similar to the activating protein like GTPase activating protein gap. Like exchange factor is important for exchanging the GDP with a fresh GTP. So,

important cargo complex stays in the cytoplasm. Remember earlier it was exportin in the nuclear export now it is importin cargo, what is the cargo? The RNA which is present in the cytoplasm right now is the cargo.

And importin Ran-GTP plus cargo gets released in the nucleus. Now, we are talking about a situation where the RNA that arrived in the cytoplasm like snRNA we have seen it snRNA comes from the nucleus to the cytoplasm in the form of snRNP and it undergoes further processing so that this snRNP can go back to the nucleus because splicing happens in the nucleus.

So, it need to be available in the nucleus. So, such a scenario the RNA cargo must go back into the nucleus. And so what happens? Importin Ran-GTP plus cargo, cargo gets released with the influence of this RanGEF because it exchange the Ran-GDP with a GTP and so important Ran-GTP stays together and cargo is released in the nucleus which is available for its function. The same thing we can see in a pictorial manner in this slide.

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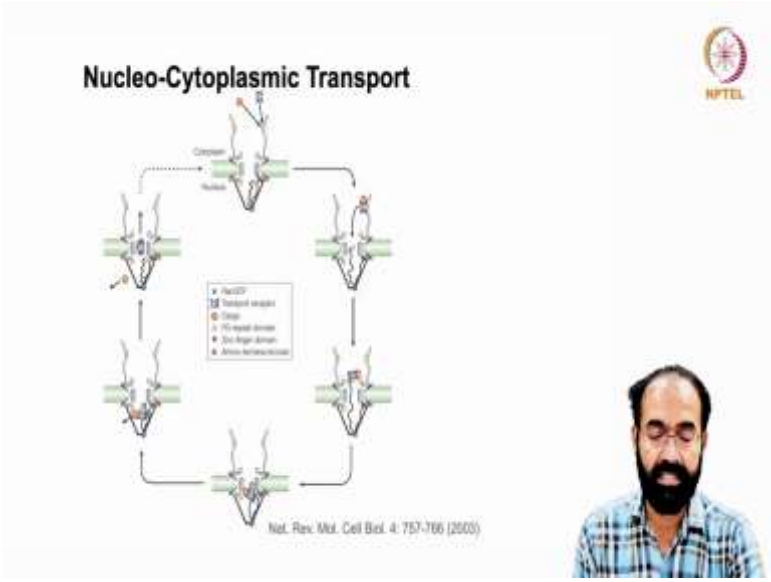

So, this is nucleocytoplasmic transport and what happens? See one side that is the entire circle what you can see here in the cycle the whole thing the box etcetera are the part of the nucleus or this is the nuclear pore complex. So, inside this is nuclear side, nuclear side, nuclear side, this is nuclear side, nucleus side and nuclear side rest of them entire thing projecting outward is the cytoplasm.

So, now let us see how things are, you can start from anywhere for convenience you can start from any particular location and you need to understand this key here that is this particular blue color shape is Ran-GTP and this is transport receptor, the big purple color and the orange color one is cargo and the small yellow circle is the FG repeat protein FG stands for phenylalanine glycine repeat containing protein and this blue circle is the zinc finger protein and this reddish circle is amino terminal domain of the protein.

So, this is for our easier understanding this key is given. So, you can see here this is the nuclear pore complex wherever you see this yellow color small circles shows FG containing FG repeat containing proteins which are important for the recognition of the cargo.

Because they have it is just like if you are doing rock climbing you need to have some specific holds to keep your shoes, you do not have place for keeping your entire foot, you have some small small crevices will be there in the actual rock or even you can see in some malls you will be having something mimicking the rock climbing kids will be playing.

So, there also you can see on the wall there will be tiny crevices. So, this FG domains are something like that where you specifically interact with the cargo. They are kind of like hooks you can call it as hooks and this tiny portions are meant for that. That is what you can see they are attaching there, what are that? Transport receptor and the cargo they are binding here in one of the FG location.

Then what happens? They will get you know swivelled into the lumen or into the cavity or the opening of the gate. And there as soon as it is swivelled it will be recognized by another FG protein does not belong to this, but some other protein. Say you have a long thread hanging and that FG protein it is interacting and from there it is now further swiveling inside and which is recognized by another FG protein that is located inside this is the cytoplasmic side inside and then the cargo is released.

So, it is going on like a cycle and you can see Ran-GTP is effectively utilized in this cycling and in the next slide we can see little bit clearly.

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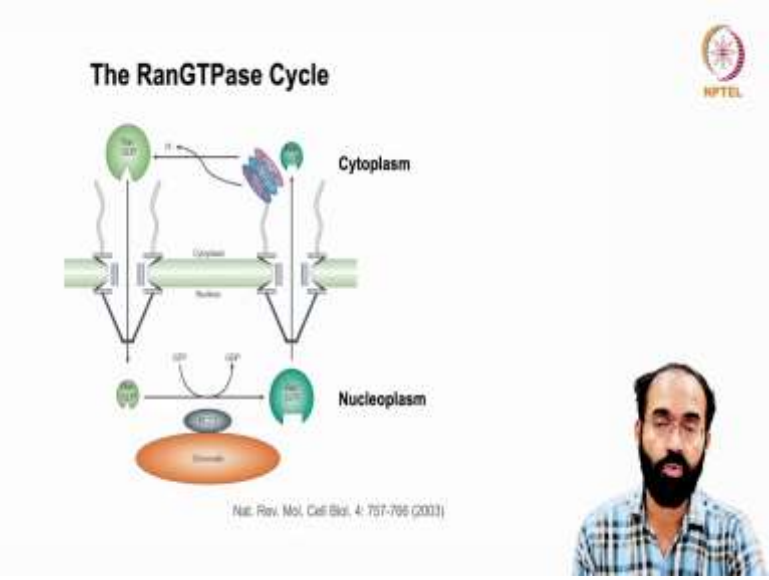

How the RanGTPase cycle functions? So, this is the cytoplasmic side, this is the nucleoside. So, the entire thing is the nucleus and we call it as nucleoplasm and what is outside the nucleus we call it as cytoplasm. Now let us see how the cycle is happening. So, cycle like you can see it is going in a anti-clockwise side.

You can start from anywhere because it is a cycle. So, cycle does not have a beginning or ending you can start from anywhere. So, let us see for convenience starting from Ran-GDP, you may wonder why this Ran-GDP is bigger here and Ran-GDP is smaller here. Not that you have a bigger molecular or a smaller molecular it is for convenience it is given the Ran-GDP concentration is high in the cytoplasm whereas, Ran-GDP concentration is low in the nucleus.

Same way Ran-GTP is bigger and Ran-GTP is smaller in the cytoplasm, the bigger Ran-GTP in the nucleoplasm indicates Ran-GTP concentration is very high because Ran-GTP has got function. So, it has to be there it has function in the nucleus. And the Ran-GTP concentration in the cytoplasm will be low.

And this increasing and decreasing levels also is important just like how a river is flowing river will flow or how you are getting water in your pipe because the water is in the overhead tank. So, overhead tank water has always a tendency to come down if there is an opening and your tap is the opening and credit goes to the gravity.

Same way molecules move from their greater concentration region to the lesser concentrated region if there is given a chance. And the gradient is maintained based on their utilization if there is utilization is there you are eating lunch or breakfast after some time nothing will be there in your stomach, why? It is utilized by your body it is digested and passed down to the next it is a intestine from the stomach went to intestine, small intestine, large intestine like that.

So, there is a gradient developed; that means, as soon as a heavy lunch or heavy breakfast or heavy dinner you cannot eat further food because there is no demand, there is no gradient possibilities are there. Outside table plenty food is there, but inside there is no place. So, once it is moved after couple of hours you come to the same table or after half a day you come to the same table you will continue to eat because there is a demand. So, this logic you should have whenever you talk about the gradient.

So, what happens when Ran-GDP is here it will have it more in the cytoplasm it will have a tendency to move into the nucleoplasm, in the nucleoplasm what happen? The Ran-GDP catch hold of GTP and it will get converted into Ran-GTP. So, again the GTP donates its phosphate group to the GDP and become GTP and you have a higher concentration of GTP.

And this GTP will be utilized for the nuclear export, we saw it in the previous slide here see you have Ran GTP-exportin-cargo complex that is in the nucleus. So, that will effectively will be formed and that will be pushed along with the cargo into the cytoplasm. So, this Ran-GTP further will be converted into Ran-GDP with the help of a bunch of exchange factors and again the Ran-GDP concentration will be maintained.

And because Ran-GDP concentration is high in the cytoplasm it will get a easy access or easy demand into the nucleus. Because nucleus has got a low demand of Ran-GDP and it is maintained low because this Ran-GDP is converted into Ran-GTP. So, this Ran GTP cycle or Ran-GTPase cycle is one of the central topic of nuclear export and import. If there is no Ran-GTP cycle going on there is no way the RNA cargo can be pushed into and out of the cytoplasm.

So, this idea should be very clear and thorough in your mind, this Ran-GDP higher in the cytoplasm lower in the nucleus, Ran-GTP higher in the nucleus low in the cytoplasm this continues and it is maintained so with a purpose.

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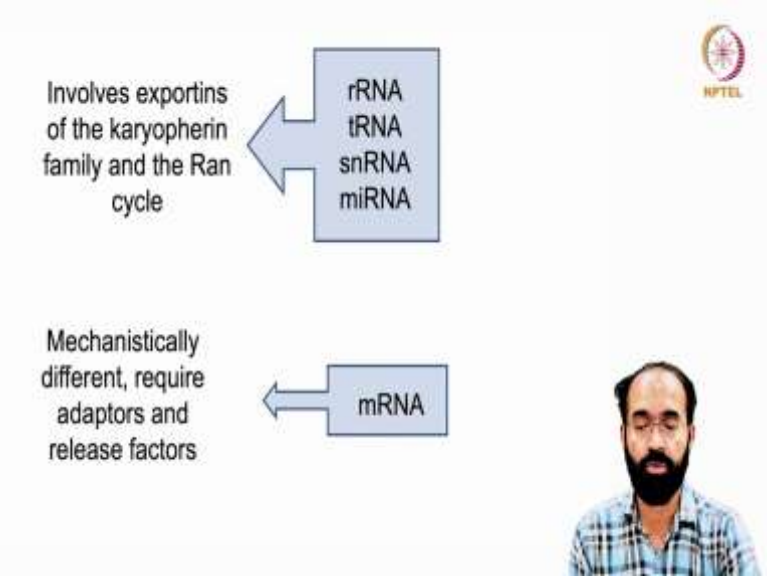

So, now let us see what are the RNA species which are exported into and out of the nucleus and what are the RNA species, whether they are similar and dissimilar compared or can be group them. Say just like if you can think like in many places, many cities you would have seen there are some pavements, there will be some barricades would be given where the animals four legged animals like cow, buffalo or bulls cannot enter, but you can enter happily.

Because they have a bigger body, if they come through that gap they will see another wall hitting their body. So, they cannot go. But what you do? As soon as you enter you turn left or turn right and you can go because your body is small, but they cannot go. So, what they are doing?

They are making a barricade not for humans, but for animals, you have seen in the many cities you will see because their shop they do not want any cow or buffalo entering into their shops or something. So, they make this, but they want humans to come. So, they do this kind of effective way of barricading those who have not seen whenever you visit a city which has this such structures you can see it.

So, our ribosomal RNA, transfer RNA, snRNA, micro RNA they follow somewhat the similar track and that involves exportin of the karyopherin family and the Ran cycle. Whereas, mRNA like that is a different species like I was talking about humans and the

four legged you know animals. So, mRNA they are mechanistically different and they require adapters and release factors.

So, what you understand from here? You can also imply one more thing here because mRNA once it is made processed properly there is no need that mRNA should come back, they are huge in size they are only exported, mRNA's never imported back into the nucleus remaining every RNA and to some extent it is true with other RNA species such as ribosomal RNA you do not want ribosomal RNA back.

All want and also transfer RNA back all you want is snRNA and to some extent some non-coding RNA because they are important in gene regulation, they are welcome back into the nucleus. So, they are categorized like ribosomal RNA, tRNA, snRNA and micro RNA they follow one common cycle which is involving the Ran-GTPase cycle and it involves the exportins of the karyopherin family.

And whereas, mRNA which is meant for protein translation they are huge in size like we have seen the muscular dystrophin gene is around 17 kilobases. So, you can imagine how big that mRNA will be. So, they follow a different tactic or different strategy.

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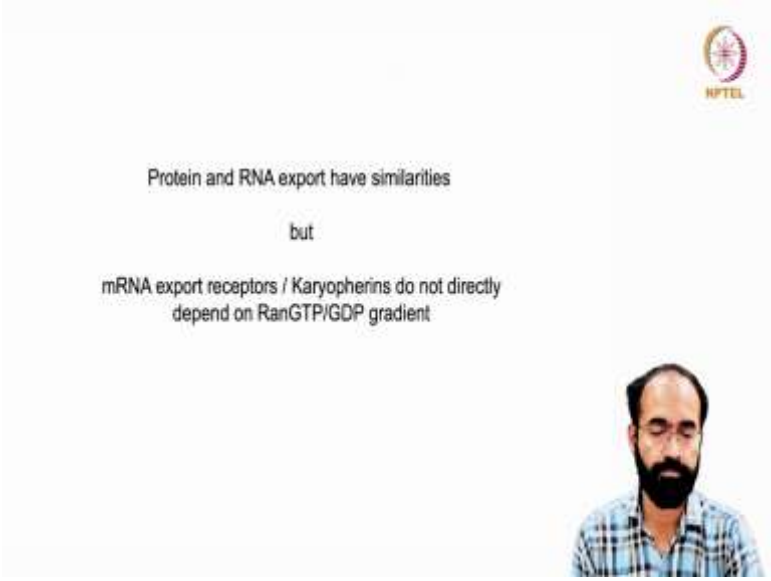

Protein and RNA export have similarities, but mRNA which export the receptors and they also have specific karyopherin do not directly depend on the Ran-GTP, Ran-GDP gradient. So, we should understand the Ran-GTP, Ran-GDP gradients are mainly

governed via they are mainly governed for the purpose of transport of ribosomal RNA, transfer RNA, snRNA and micro RNA. Whereas, mRNA do not depend on this Ran-GTP, GDP gradient.

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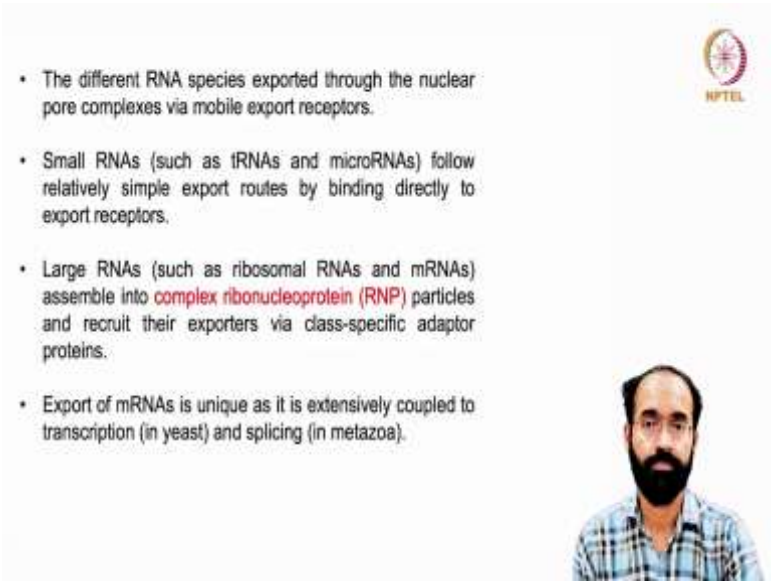

So, the different RNA species exported through the nuclear core complex via mobile export receptors. When you say receptor we should understand receptors can be temporarily fixed on a place or they can be mobile receptors also many such examples are there. So, receptor means it can be of different two mainly two different types.

So, small RNAs such as transfer RNA and micro RNA's they follow relatively simple export routes by binding directly to the export receptors. And large RNA's such as ribosomal RNA and mRNA's they assemble to complex ribonucleoproteins particles to recruit their exporters via classic specific adaptor protein that is case by case, they have unique dedicated protein species both in the nucleus and also interaction, interacting proteins in the nuclear pore complex.

And the export of mRNA's is quite unique and it is extensively coupled to transcription especially studied in yeast and splicing. So, the transcription the rate of transcription, the moment a transcription is going on and the moment a splicing is going on both are decided and detrimental for the moment of RNA from the nucleus to the cytoplasm and they do not depend on the Ran-GTP cycle.

So, we will see more in detail about this RNA and their dynamics in the next class with more diagrammatic representation.

Thank you.