RNA Biology
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Lecture - 08
RNA as Enzymes: Structure and Functions

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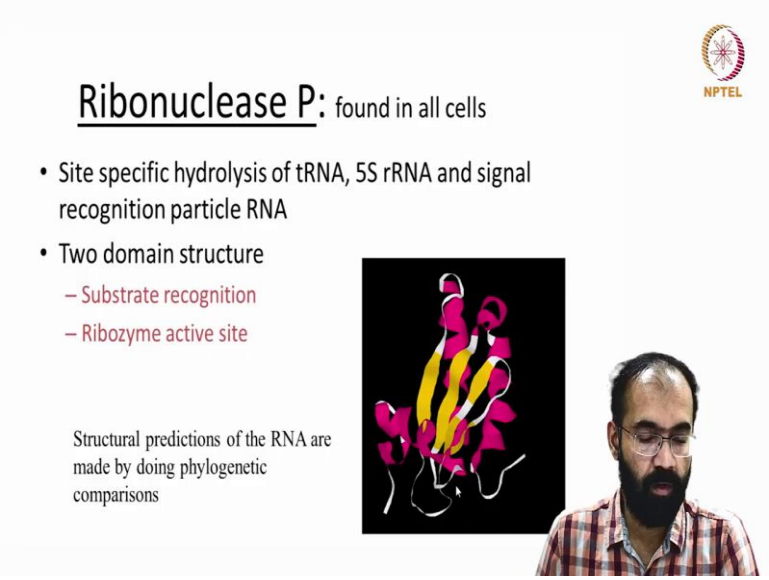

Welcome back to another session of RNA Biology and we were studying about Ribozymes and this was the slide which we left in the previous class. And it is talking about the Ribonuclease P which is found in all cells which require the tRNA, 5S RNA etcetera and also the signal recognition particle. So, what is the role of ribonuclease P is basically allow the maturation of this premature tRNA, premature 5S rRNA and also premature signal recognition particle which has to undergo maturation process.

And this contains two domain structure. That is substrate recognition region also ribozymes active site. And the substrate is nothing, but the premature tRNA or premature 5S RNA etcetera. So, this has to be acted upon by ribonuclease P for the RNA molecule to undergo maturation. So, this is something which is very important for us to understand about the ribonuclease P. And the structural predictions of the RNA are made by doing phylogenetic comparisons.

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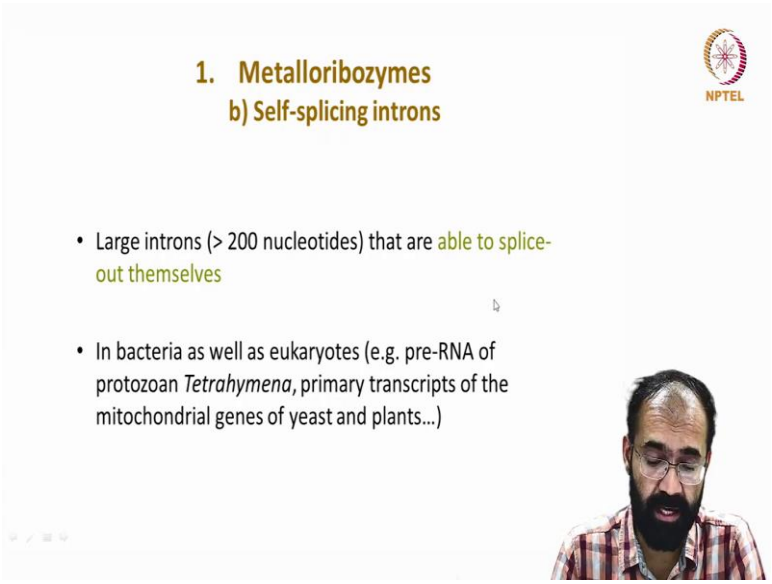

Now, we are going on to the second group that falls under the metalloribozyme category. The first category was ribonuclease P and the second one is self splicing introns. So, normally the self splicing intron which is a metalloribozyme they need to have this need to have more than 200 nucleotide as the intron. Intron like we already mentioned in the previous class that is the intervening sequences present in a RNA which is not useful for coding purpose or not useful in the mature RNA.

So, introns have got their own importance and significance etcetera which we will discuss as the classes progress. But time being you understand that when an RNA is being formed it has regions that are need to be present in the mature form and regions that need not be present in the mature form.

So, those regions which are need not be present in the mature form are called as introns. And for self splicing intron you need to have them roughly 200 or more in nucleotides in length. And then they will be able to self splice or splice out themselves without any intervention.

In bacteria as well as in eukaryotes the pre mRNA or pre-RNA of the protozoan tetrahymena the primary transcripts of the mitochondrial genes of yeast and plants all undergoes a self splicing process and which is governed or which is conducted by the self splicing introns. One more thing that this intron itself is the ribozyme, ok.

So, when you talk about self splicing ribozyme or self splicing intron no one no other molecule come from outside it is basically the same intron fold in such a way that it can get itself out of the RNA structure. So, that is called self splicing introns or self splicing ribozymes.

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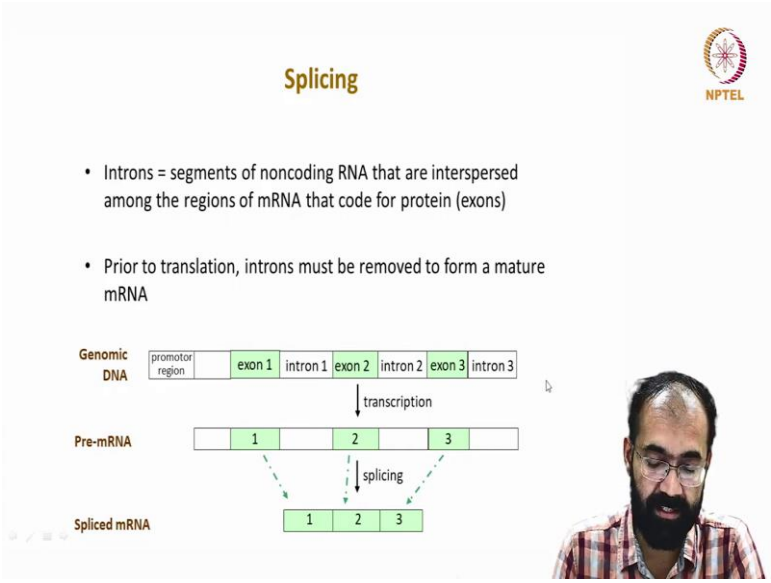

Let us quickly look int into what is splicing or how splicing is important. So, introns by definition are segments of non-coding RNA that are interspersed among the regions of mRNA that code for protein which we usually refer to as exons. So, if a region in the mRNA that is capable of coding for amino acid, we call it as exon and region which you do not code, we call it as intron. And normally the introns are located in between various exon.

So, prior to translations introns must be removed to form a mature mRNA. let us look into this cartoon genomic DNA it has got a promoter region and some untranslated region and then exon 1, exon 2, exon 3 and in between exon 1 and exon 2 you have intron one in between exon 2 and exon 3 you have intron 1 and then after exon 3 you have intron 3. So, while transcription all this exon and intron are present in the RNA and we call it as pre-mRNA that is it is premature and then it undergoes.

So, called splicing where exon 1, exon, 2 exon 3 are spliced together to form a mature mRNA as you can see here in the bottom whereas, the intron 1, intron 2 and intron 3 are removed they are no more part of the mature mRNA. So, this is the basic principle of

splicing. And now we were talking about the self splicing introns. So, say if the intron one was self-splicing do not think that intron 1 always will be self splicing let us think about a situation where a given intron is self splicing intron then it can form a structure unique.

So, that the exon 1 and exon 2 can come together and the intron will just disappear from the pre mRNA. So, that is what self-splicing means.

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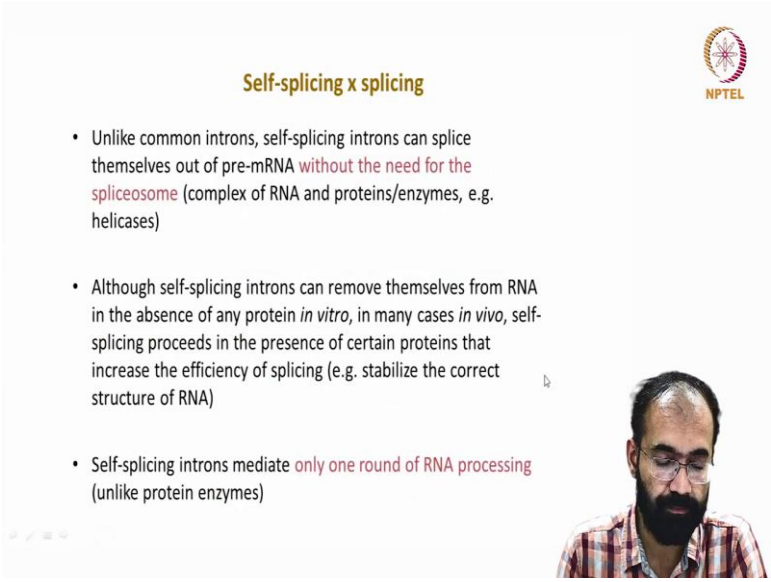

So, what is the difference or similarity between self-splicing and the actual splicing? Unlike common introns self-splicing introns can splice themselves out of the pre-mRNA without the need of spliceosome. Remember the normal splicing require an extensive splicing machinery which we call it as spliceosome machinery.

Which is not required for the splicing of self-splicing introns. And it is basically complex of spliceosome is a complex of RNA and protein and enzymes such as helicases they are present in these spliceosome machinery. Although, self-splicing introns can remove themselves from the RNA in the absence of any protein in vitro in many cases in vivo in reality inside a cell. In vitro basically mean a lab condition in a test tube in vivo means within the organism.

So, inside the real scenario. So, that is in vivo. So, in many cases in vivo self-splicing proceeds in the presence of certain proteins that increase the efficiency of splicing. So,

you can understand if a kid or a school boy is there with his friends he will be playing and can be naughty.

But in presence of his teacher or presence of parent he will behave quite sober and quite decent although teacher is not saying anything although parents are not saying anything they are not actively controlling him. Mere presence is changing the way they behave something similar to that in vivo presence of certain proteins in the vicinity can enhance the efficiency of splicing.

So, it could be because of stabilizing the structure of the RNA these proteins can allow a proper conformation of the RNA that could contribute to the splicing efficient splicing of the self-splicing intron. So, the self-splicing introns mediate only one round of RNA processing unlike the protein enzyme. If you look into any protein enzyme it can do several round say you have got all of you know in your saliva you have got the enzyme salivary amylase.

So, salivary amylase act on to starch and give rise to glucose. That is why when you chew chapati or rice for some time after some time it will become sweet. Chapati is not sweet to start with, but you keep chewing it will become sweeter because the salivary amylase acted on it.

As the time passes the same salivary amylase can act more and more starch molecule and convert them into glucose, but that do not happen in the case of self-splicing intron. Simply because the function it is doing is self-splicing once it is spliced out of this pre-mRNA.

It cannot do that function again like if you pass the intermediate exam, you cannot pass it again you already passed you can study for graduation something like that; however, this removed portion of the intron can participate other biological reaction not the splicing related, but that is also been shown.

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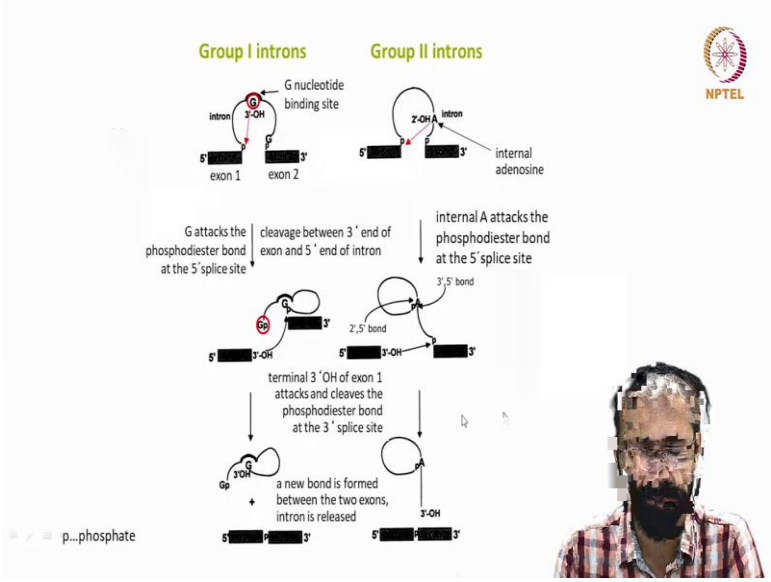

So, let us see how group 1 and group 2 introns are functioning. Group 1 intron what happens it recruits a guanosine into one of the branches of the intron. So, this line what you are seeing is an intron or the black box and this black box are exon 1 and exon 2. So, this recruited a extra guanosine and this guanosine have got two free hydroxy group one is in the 2 prime and another is in the 3 prime carbon.

Both of them have got a hydroxy group in this case the 3 prime OH remember it is not part of this intron it is a recruited guanosine from the outside. So, this 3 prime OH participate in a nucleophilic attack on to the 5 prime end of splicing.

That is towards the end of first exon. It participate in a nucleophilic attack. As a result, the exon 1 and the intron 1 junction is broken it is no more connected here. And then what happens the exon 1 now have got a free OH earlier it was not there 3 prime OH was part of this double helic part of this RNA long chain that is part of the phosphodiester backbone, but once this nucleophilic attack happened you will have free OH that is from the first exon, exon 1. This 3 prime OH does a second nucleophilic attack on to the 5 prime end of the intron.

That is in the 5 prime end of five sorry, 3 prime end of the intron and the 5 prime end of the exon 2. So, the second nucleophilic attack happens from the exon 1 on to the 5 prime end of the second exon. And as a result, this intron will be released from that 3 prime end also 5 prime end was released because of the nucleophilic attack from the G recruited G

and the second end of the intron is released because of the 3 prime OH from the exon 1. And as a result, the intron is released completely and you have exon 1 and exon 2 fused together. And coming to group 2 introns it is also very much similar to that of group 1 intron.

Except that the group two introns have got a endogenous A instead of G it has got an endogenous A which is not externally recruited it is present in the intron sequence itself and we call it as an internal adenosine. And this internal adenosines 3 prime is already part of this phosphodiester backbone it is not available for any reaction whereas, the 2 prime OH is free. So, this 2 prime carbons hydroxyl group participate a similar nucleophilic attack on to the 5 prime end of this intron releasing the 5 prime end of the intron from the exon.

And now freeing the 3 prime OH of the exon 1. And this 3 prime OH undergoes second nucleophilic attack on to the 5 prime end of exon number 2 fusing or splicing the exon 1 and exon 2 and you end up getting a intron lariat that will be released out. So, group 1 and group 2 introns does similar job only difference is group 1 require a external guanosine.

And whose 3 prime OH participates in the trans esterification or nucleophilic attack reaction. Whereas, in group 2 intron it is an internal adenosine that participate and whose 2 prime OH is participating in the nucleophilic attack and the transesterification reactions.

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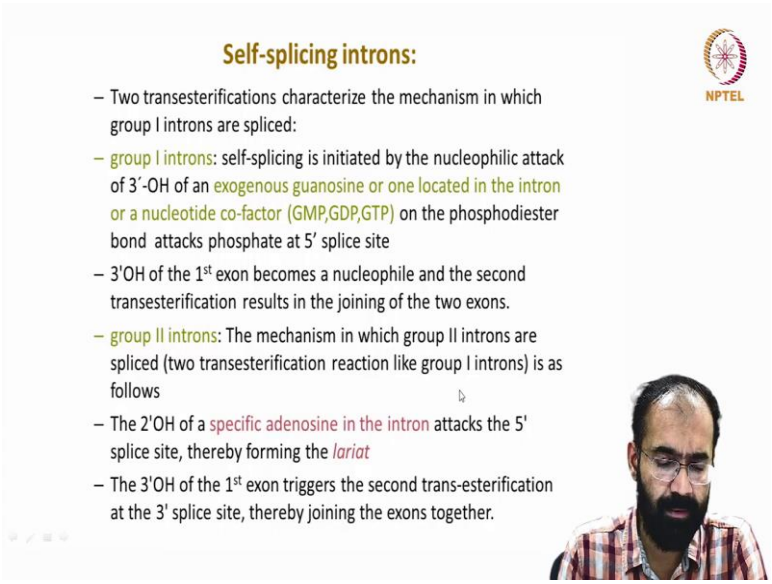

Same thing is whatever we discussed is listed in the in text I will not go through the details because this you can read it because whatever we discussed is typed down for easy understanding for the audience.

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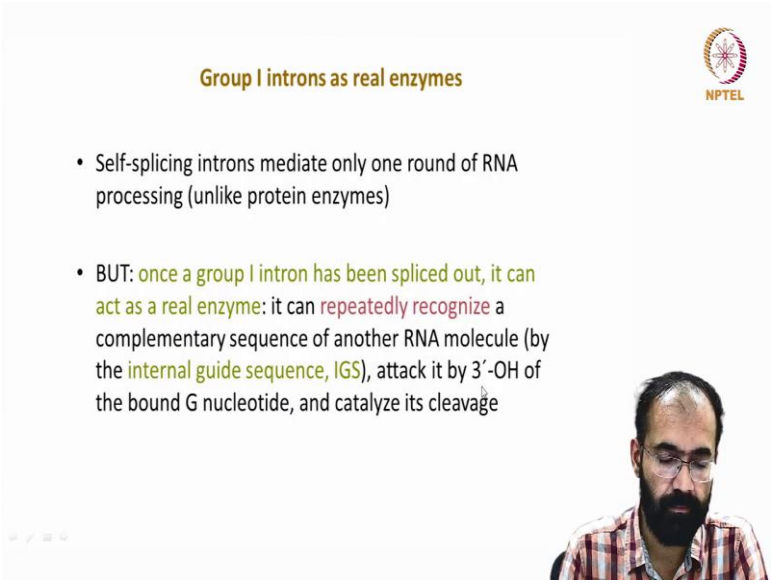

So, group 1 introns are acting as the real enzymes, self-splicing introns mediate only one round of RNA processing unlike the protein enzyme which we already discussed. But once the group 1 intron has been spliced out it can act as a real enzyme other than the splicing function.

It can repeatedly recognize a complementary sequence of another RNA molecule by a region called internal guide sequence or IGS that can attack it by 3 prime OH of the bound G nucleotide. Remember this spliced out group 1 intron is holding a externally recruited guanosine whose 3 prime OH is always available for attack nucleophilic attack and it can catalyze the degradation of various target RNA. Let us see how it is doing in detail.

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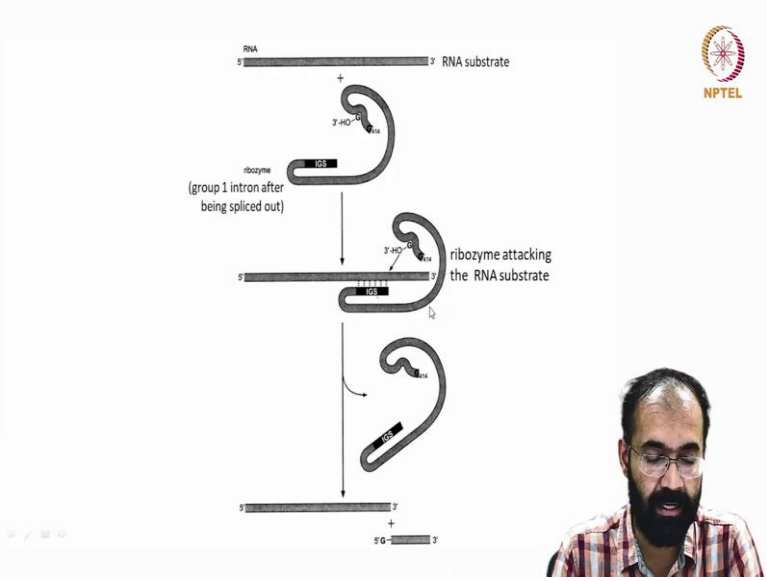

See this is a target RNA and we call it as a RNA substrate what is in the top most panel. And now this is a spliced-out group one intron it has got a external G attached on to it whose 3 prime OH is also available for the biological function. Now it has a unique sequence called IGS internal guide sequence which can pair on to the RNA substrate at a selective region.

Once this pairing happened what will happen this guanosine can do same nucleophilic attack on to the specific regions of this RNA similar to that what it did on to the 3 prime end of the exon number one or whichever sequence which is available in the vicinity in this case.

So, as a result what happen the RNA substrate now gets cleaved. Because this 3 prime OH of the external guanosine can attack on to the substrate RNA and breaking this RNA into two pieces. Now this group one intron ribozyme will be released and you end up

getting two pieces of the substrate RNA. And this is something very interesting because the IGS sequence can be tweaked.

So, you can design the IGS sequence as you wish. If you have an RNA which you wanted to disturb or disrupt, say a person is having an HIV RNA in his body. So, you can design a IGS containing ribozyme and deliver into that patient. So, that the IGS will selectively recognize the HIV RNA and it can cleave it. So, this has got medical relevance this is how you can make use of a ribozyme for clinical intervention.

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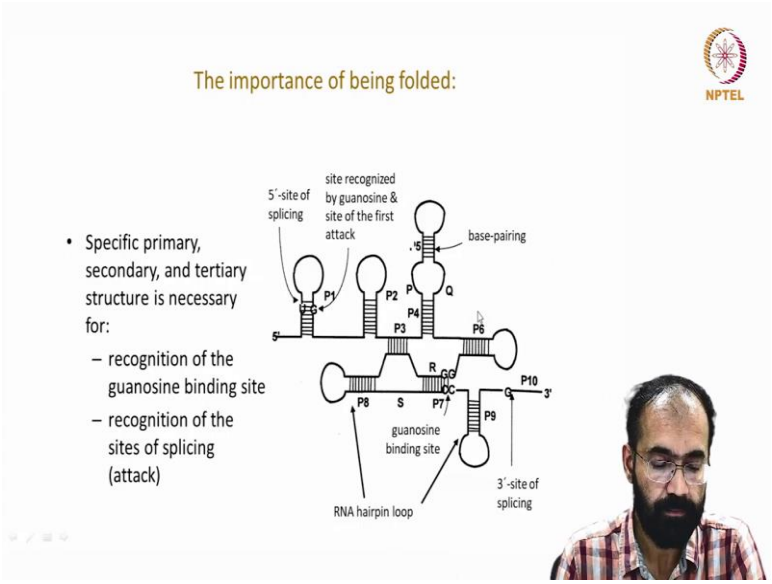

So, if you look the importance of the G recruitment. The extensive secondary structure you can see here the intronic sequence self-splicing intron 1 starts from here you can see a stem loop and again a long line again stem loop and it is continuing and remember it is pairing with a long distance after.

So, it is going up again forming two loops and two stems again continuing and then after long distance it is again pairing. So, it has got different arms P1, P2, P3, P4 different arms are there which is very important for stabilizing the structure of this ribozyme. And then it is this place where the guanosine is recruited.

And this specific location allows the movement of this guanosine because guanosine got recruited does not mean that it can perform the task it is the structure all these loops are important for the proper structure to be formed in the three-dimensional structure. Then

this 3 prime OH of the G can participate in the trans esterification reaction nucleophilic nucleophilic attack can happen.

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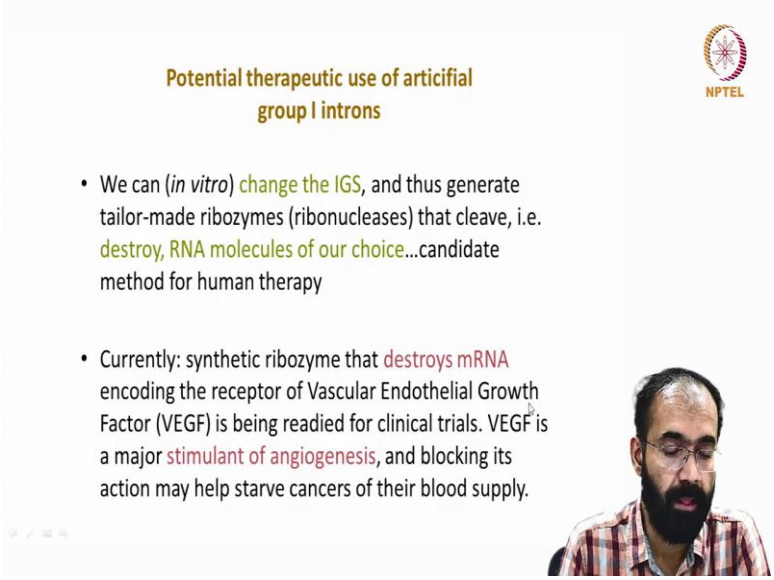

So, some potential therapeutic use of the artificially made group 1 introns are listed here. We can in vitro change the IGS internal guide sequence and thus generate tailor made ribozymes that is ribonucleases that can cleave that is destroy mRNA molecules of our choice like I told you about the HIV RNA.

And it is a candidate method for human therapy. Currently one such achievement has happened is synthetic ribozyme that destroys mRNA encoding the receptor for vascular endothelial growth factor in short form it is called as VEGF. VEGF is an important molecule; VEGF is a ligand or a growth factor binds on to the receptor and creates the blood vasculature.

You know when cancer is developing it need faster supply of blood. So, when you have a tumour then inside of the tumour need adequate oxygen adequate glucose nutrition etcetera, but when you are rapidly dividing blood vessels need not form. So, these blood vessels require or the formation of blood vessels require VEGF.

So, if you target VEGF or VEGF receptor then you can prevent the growth of cancer. So, what is done is that you can create a VEGF receptor targeting ribozyme and it is a major stimulant for angiogenesis. The formation of blood vessels is called angiogenesis. During

our development it is very important when an organism is developed from the zygote it is very important, but in case of cancer angiogenesis is not a friendly thing.

Because the blood vessels now supply nutrition for a non-useful or a destructful cause. So, you do not want such damage happening and the blocking of its action or the VEGF receptor if you block it by degrading its RNA it can starve the cancer cells off their blood supply and can cause the retardation or the degradation of the tumour bulk or tumour size. So, these are the some of the clinical approach that is done using artificially created IGS sequence onto the ribozyme. Now coming to the group 2.

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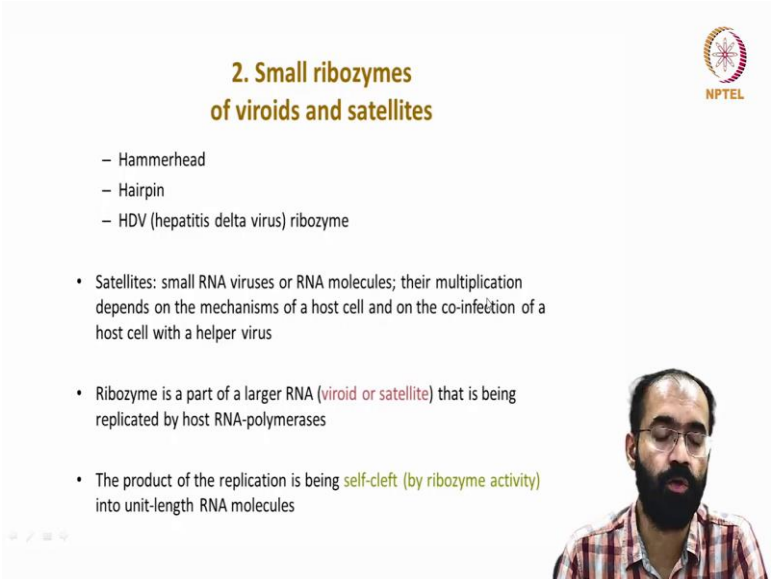

So, we have seen metalloribozymes two of the ribonuclease P and the self-splicing intron and then comes the group 2 that is small ribozyme. Ribozymes basically these ones are normally the viroids and the satellites. So, they are of three major type one is hammer head ribozyme, hairpin ribozyme and hepatitis delta virus ribozyme. So, these are all the major groups of ribozyme that come under small ribozyme because they are shorter in structure. What are satellites? Satellites are small RNA viruses or RNA molecules.

And their multiplication depends on the mechanism of a host cell and on the co infection of a host cell with a helper virus. Normally, satellites are harmless sequence they do not do anything much, but they will wait for another inflection to occur then it becomes a dangerous situation it can enhance its effective way of attacking a host we will see the example.

How hepatitis delta virus and hepatitis B virus is HDV and HBV is interacting. And ribozyme is a part of larger RNA and we call it as viroid or satellite. Why it is called viroid? Because it is harmful sequence; however, it is not having a existence of its own it require another pathogen virus to enter into the host.

So, ribozyme is a part of larger RNA that is being replicated by host RNA polymerases. So, the product of the replication is being self-cleft that is by the ribozyme activity. Say you have a sequence that is being produced by a rolling circle mechanism it need to be broken at frequent intervals.

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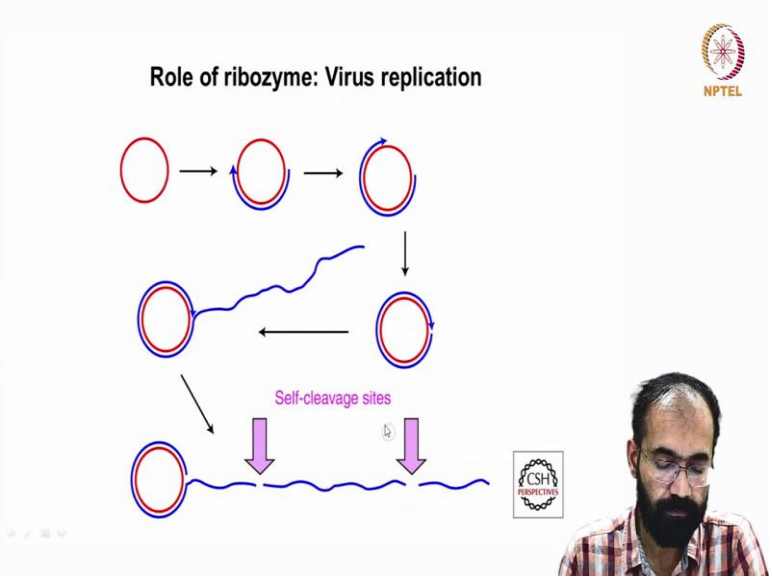

So, that it will have the meaning that is what is been shown here. You have a RNA sequence during virus replication and this blue line is what a newly formed strand and it is continuing and finishing one circle. And now this strand is being replaced by the newly forming strand. So, it will replace this strand which is now fallen out of this rolling circle. Now this strand need to be cut at frequent interval without which it would not be able to make a meaningful.

And these pieces now it will circle and give rise to the old individual copy. And this cleaving and frequent intervals is happening because of a ribozyme action encoded in this structure. Like you may have seen a lizard shedding it straight, if a wall is at is intimidated we call it as autotomy. So, it is breaking without any external influence. Of

course, its mechanism is not there was I think few months ago there was a elegant paper published in the journal science about it how the protein structure contribute.

It is like almost like a ball and socket joint you just detach it just falls off and the lizard tail is more made of multiple such segments and you can break wherever it want. Lizard can break wherever it want by simply by unlocking it. So, lock gets unlocked and it falls off. Same way that is a self-action same way these RNA also broken at frequent intervals.

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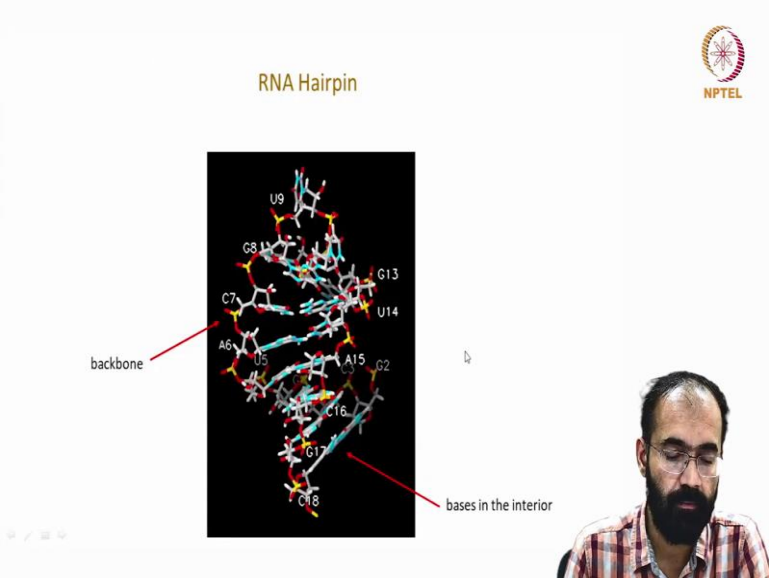

So, this is a molecular structure of the RNA hair pin and it has a backbone and also it has got unique bases in the interior. And both are important for structure and function point of view. Because the hairpin when you are talking it has to form in a unique fashion not any random fashion without a proper structure it will not be able to perform the task no matter which is the task you are talking about whether it is a cleaving at frequent intervals or this assisting a specific region to be expelled from an RNA everything is important for the everything depends on the structure of the RNA hairpin.

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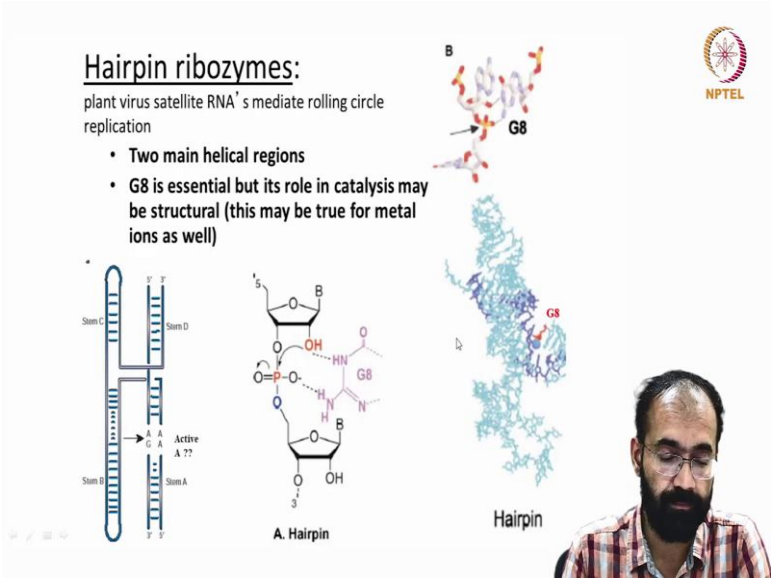

If you look further into the hairpin ribozyme many plant virus and satellite RNA mediate the rolling circle replication. There has got two main helical region and one of them is called G8. G8 the guanosine 8 is essential, but its role in catalysis may be purely structural. This may be true for various metal ions which we discussed like many a times magnesium and manganese such divalent cations kind of stabilize the ribozyme structure. Same way G8 in the hairpin ribozyme stabilize.

The various stems like you can see stem A, stem B, stem C and stem D. But some regions specific bases allow the stability structural stability to be ribozyme like you can see here a region called G8 and which allow the hairpin to be stabilized such a way that the ribozyme is able to perform the task.

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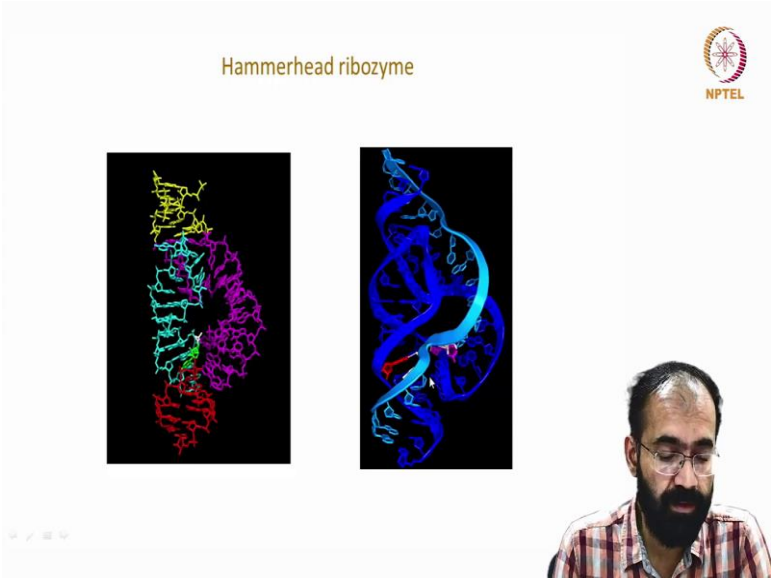

So, we will stop here and in the next class we will continue with the hammer head ribozyme and we will learn more about the ribozyme and its functions.

Thank you.