RNA Biology
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Lecture - 09
RNA as Enzymes: The Present and Future

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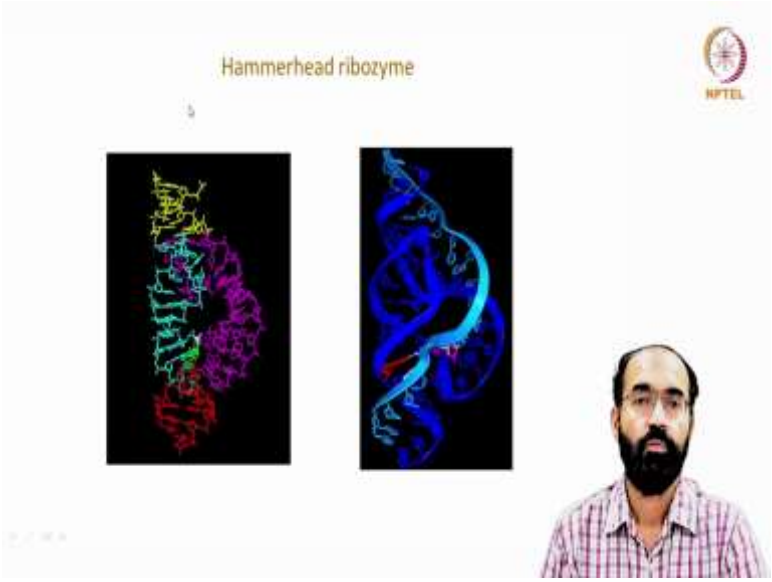

Welcome back for another class on ribozymes and we left here in the last class that is structure of hammerhead ribozyme. As you can see from this picture itself that it resembles the head of hammerhead shark hence the name hammerhead ribozyme is given. So, what I would say is that, these kind of molecules which are named after different shapes are quite handy for the functioning of various biological functioning of the cell and we will see them more in detail.

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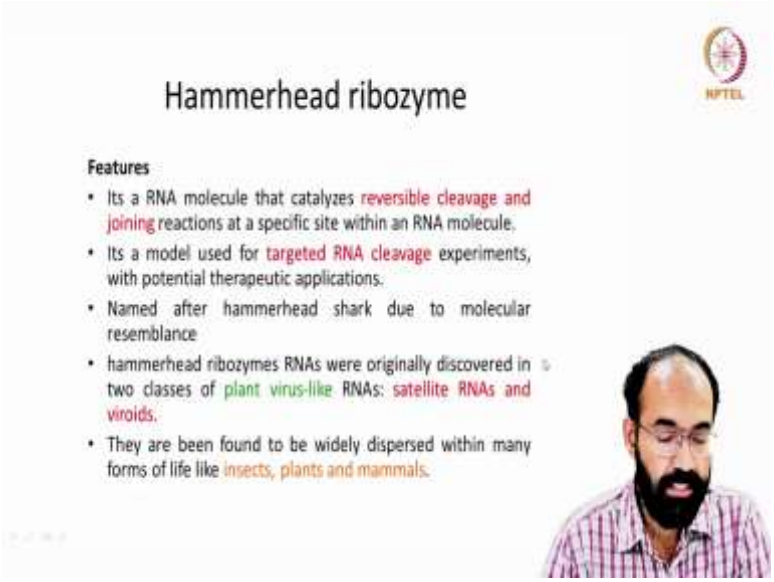

So, some features of the hammerhead ribozyme. It is an RNA molecule that catalyses reversible cleavage and joining reactions at specific site within an RNA molecule. So, the major function is reversible cleavage and joining; that means, it causes the cleavage of nucleic acid in a reversible manner and it is a model used for targeted RNA cleavage experiments and with potential therapeutic application.

That is why you will find lots of research work going on with hammerhead ribozyme because you can literally engineer them. And you can use them for targeting some viral RNA etcetera, which is infected into a host and as I already told you named after the shape resemblance to the head of a hammerhead shark and at molecular level it resembles.

So, and hammerhead ribozymes RNAs are originally discovered in two types of plant virus like RNA like the satellite RNA and viroids. And they also have been found to be widely dispersed within many forms of life forms such as insects, plants and mammals. So, this is not restricted to this is not restricted to just plants they are seen in other animals also.

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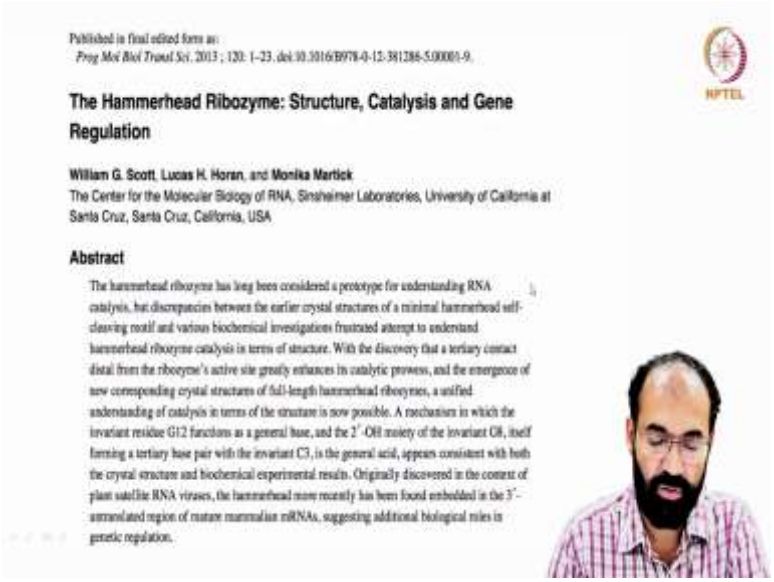

So, you can see more in detail about the hammerhead ribozyme structure and its catalysis and gene regulation in this article those who are interested can read more in detail about this hammerhead ribozyme.

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And hammerhead and hairpin ribozymes can be found in several satellite RNAs associated with plant viruses. Majority of the plant viruses are RNA viruses one example is tobacco ringspot virus they do have the hammerhead ribozyme and it is also has been

known to be embedded in the UTRs UTR stands for Untranslated Region UTRs of several mammalian RNAs.

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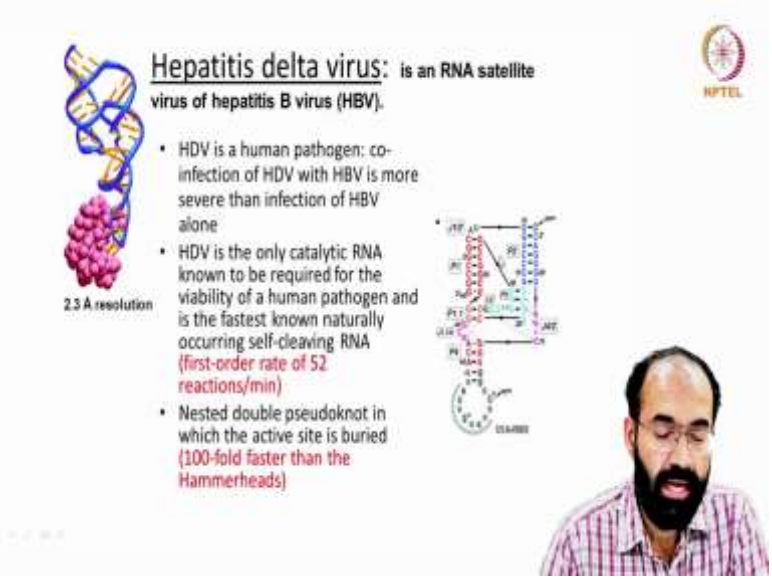

And now moving on to another ribozyme that is hepatitis delta virus in short form it is called HDV. It is a satellite RNA of the hepatitis B virus or HBV hepatitis B virus is a very important and quite problematic viral infection that infects the liver and it is as important as HIV in terms of its spread ability through blood.

So, it is more infective than even HIV like you can spread its quite contagious, but of course, it passes through body fluid especially blood. So, HDV is a human pathogen co infection of HDV with HBV. Remember hepatitis delta virus along with hepatitis B virus is more severe than the infection of HBV alone.

Or in other words if a person has got HDV alone it is less likely to be detrimental or crucial for the life of an organism whereas, if that person encounters an HBV hepatitis B virus infection then things can go really wrong. HDV is the only catalytic RNA known to be required for the viability of a human pathogen what is that pathogen? HBV and is the fastest known naturally occurring self-cleaving RNA and its rate is around 52 reactions per minute.

It is one of the fastest replication rate and nested doubles pseudoknot knockout that is the structure what it follows for the breaking of the nucleic acid in which the reactive site is

remaining buried. So, this nested double pseudoknot is the structural feature what we reflect as you can see here in the right hand side in this picture.

And this structure allows this faster rate of reaction roughly around 100-fold faster than hammerhead ribozymes, hammerhead ribozymes also reasonably faster, but HDV ribozyme is much much faster the structural feature called nested double pseudoknot is quite capable of imparting this fast reaction.

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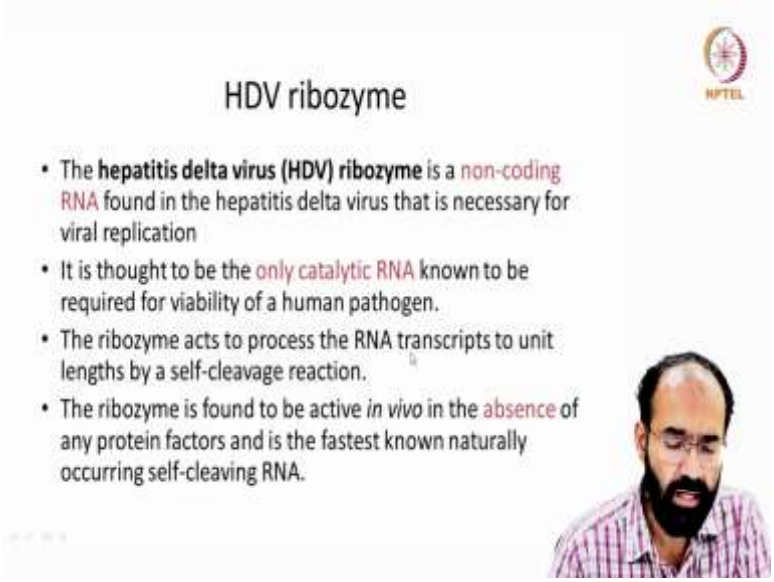

For moving on to more details about HDV, the hepatitis delta virus is a non-coding RNA found in the hepatitis delta virus that is necessary for the virus replication and it is thought to be the only catalytic RNA known to be required for the viability of human pathogen. So, this catalytic RNA part is very very important for the viability of the human pathogen such as HBV.

The ribozyme acts to the process the RNA transcript to unit length by self-cleavage reaction; that means, as we saw in the previous class, it is almost like a circle like a bangle and a copy has to be formed from the existing single stranded RNA template. And now it continues in a rolling circle fashion and this ribozyme is found to be active in vivo in the absence of any protein factors and is the fastest known naturally occurring self-cleaving RNA.

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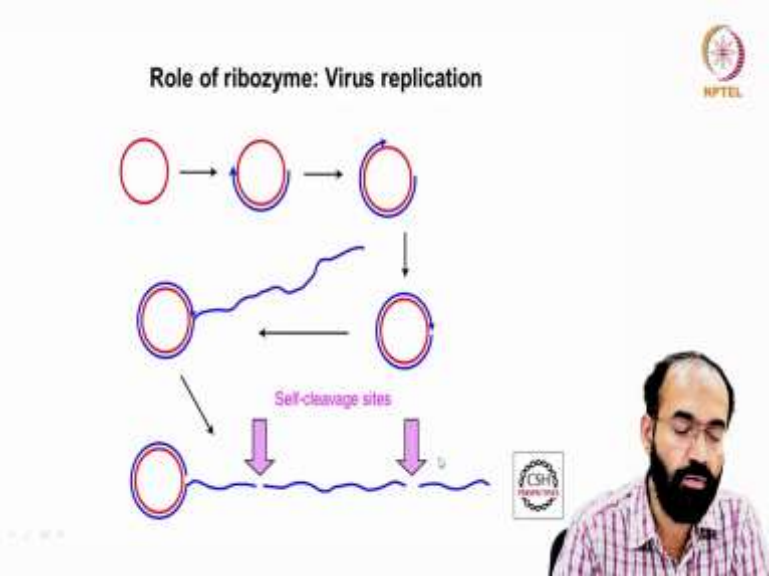

So, this is how it occurs this red colored circle is the entire ribozyme in the circular form and now the blue arrow is the copy that is the red one is the template and blue is the copy and it completes one circle and then it start replacing this already formed old newly formed strand and it comes out of the circle and this is called a rolling circle replication. As it continues it need to have a feature to cut at uniform distance and this will now circled together and form a independent bangle like structure as you see here.

So, this cutting at specific low size specific distance happens because of the structural features embedded within just like group 1 and group 2 introns we saw the structural feature is lying within the sequence itself no one comes from outside no protein comes from outside. So, that is what you should keep in mind that ribozymes act on their own.

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Now, moving on to riboswitches. Riboswitches are elements of bacterial mRNA that control gene expression via binding of small molecules such as coenzyme, amino acid, nucleobases. So, they are sequences present in the bacterial mRNA they are embedded inside, but they can control the expressivity of this mRNA.

One example is GlmS ribozyme that is located in the 5 prime untranslated region of an mRNA that mRNA codes for glucosamine-6-phosphate abbreviated as GlcN6P synthetase. So, glucosamine 6 phosphate synthetase is the enzyme whose 5 prime untranslated region has this GlmS ribozyme in the presence of GlcN6P product that is the product of this mRNA it cleaves its own mRNA which downregulates the production of the synthetase.

That means if the product is present then it will recognize this 5 prime untranslated region ribozyme sequence when the product is there that ribozyme will recognize the product once it is recognized it will cause the degradation of the mRNA so, that no more new product is formed.

So, riboswitches may have functioned as the metabolite sensors in primitive organism; that means, demand and supply it is like if you are eating food and if your plate has got enough food no point in keep on delivering food because then it will start spilling out you will not be able to eat.

So, if those plates are empty I should be able to or anyone else should be able to serve food. So, this is the logic if the product is there mRNA should not keep producing more and more product. So, the mRNA should be degraded and when the product is less then the mRNA should not be degraded. Remember mRNA is constantly being produced from the organism in those cases where the mRNA is regulated.

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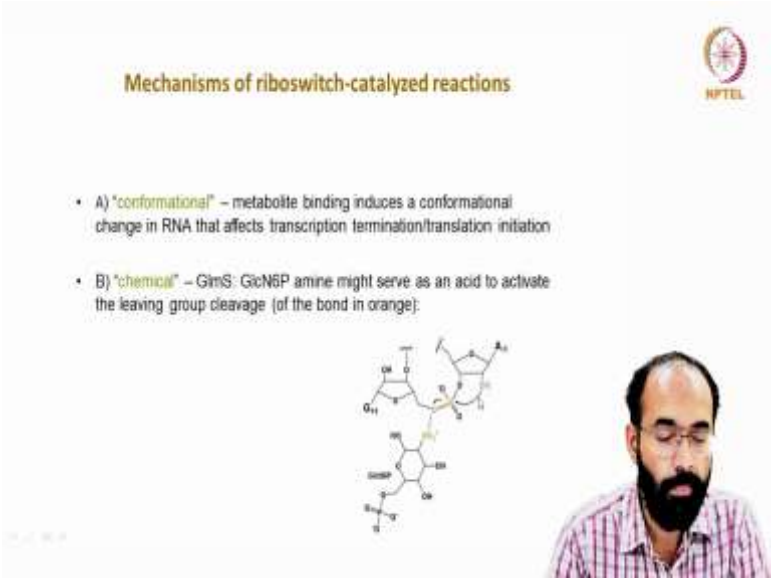

So, let us look into the mechanisms of riboswitch catalyzed reaction. First one is a conformational regulation. So, the metabolic binding induces; that means, in the previous case a 5 prime untranslated region has the ribozyme sequence and the product goes and binds on to it and so, the metabolite binding induces a conformational change in the RNA that affects the transcription termination or translation initiation.

So, in some case it is the conformational change induced in the mRNA because of the metabolite binding on to it. Second case is a chemical in this case GlmS what happens? GlcN6P amine might serve as an acid to activate the leaving group or after the cleavage. So, this change happens because once the degradation of the RNA happens the leaving out product should be stabilized without which the system the bacterial system can suffer.

So, that is a chemical means of stabilizing the leaving group as you can see here the bond which is cleaved is given in orange in color. So, once the product this is the GlcN6P

which comes and binds on to the target where the ribozyme sequence is there and it can cause the cleavage and this product stabilizes the leaving group.

So, that there will not be any unwanted effect and there is no repairing of this RNA and returning back of this mRNA should not happen. So, that is the idea. So, remember riboswitch can catalyze in two different way one is conformational and another is purely chemical means.

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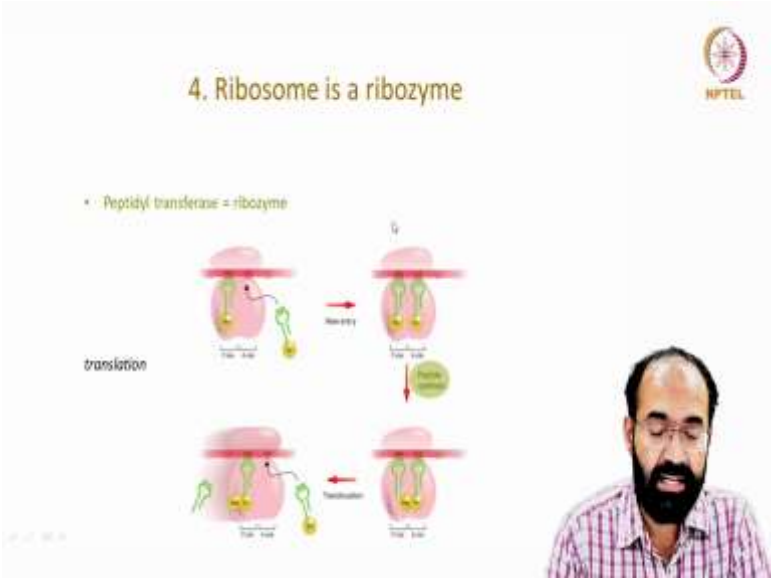

So, ribosome is another example of a ribozyme which we have seen in the earlier classes also, but in this context we have to quickly touch upon the ribosome also. So, the function of the ribosome is peptidyl transferase; that means, it is creating a peptide bond and that is done by the ribozyme action of the ribosomal RNA.

So, what you can see here? During translation there has to have like I told you there are two sites one is an A site acceptor site P-site peptidyl site or peptidyl transferase site. So, two sites are there in the ribosome and it has got two units one is a large subunit and a small subunit and the middle what you are seeing is the mRNA.

What you see this green color small structure is a tRNA and this yellow ball is the amino acid. So, in the acceptor site this tRNA amino acid complex comes in through the acceptor site and we call it as aminoacyl tRNA which is an amino acid containing tRNA which comes inside and this always the translation is started by AUG, AUG is the first

base which brings in the methionine which is already here through the acceptor site that has already come.

Now, the second one UCA. So, a serine is coming in UCA which enters in here then what happens? It moves inside and binds on to the codon and the tRNA has got a sequence called anticodon and it binds in here and now this peptide synthesis has to happen between the methionine and the serine Met and Ser methionine its an amino acid serine is another amino acid it has to form the peptide bond, which is done by the ribozyme action.

Then what happens there will be translocation; that means, this methionine is now jumping onto the serine by peptide bond formation and the methionine containing transfer RNA is now expelled out and now it move it is present in the P-site now it is ready for the another amino acid containing tRNA to enter into the A site.

So, what happened? Peptide bond is formed and then also it caused a jump in; that means, the serine containing tRNA is no more sitting in the A site, but it jumped on to the P-site and making the A site empty so, that newer tRNA containing amino acid can come inside and the peptide synthesis can continue and eventually you have a long protein.

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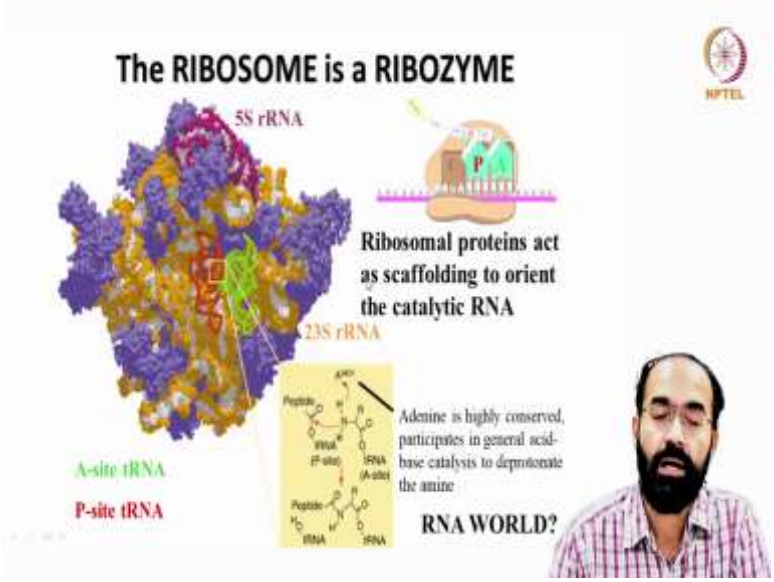

So, this is the structure of the ribosome this also we saw in the earlier class that mainly it has got a A site and it has got a P-site which is revealed from the structural crystallography studies.

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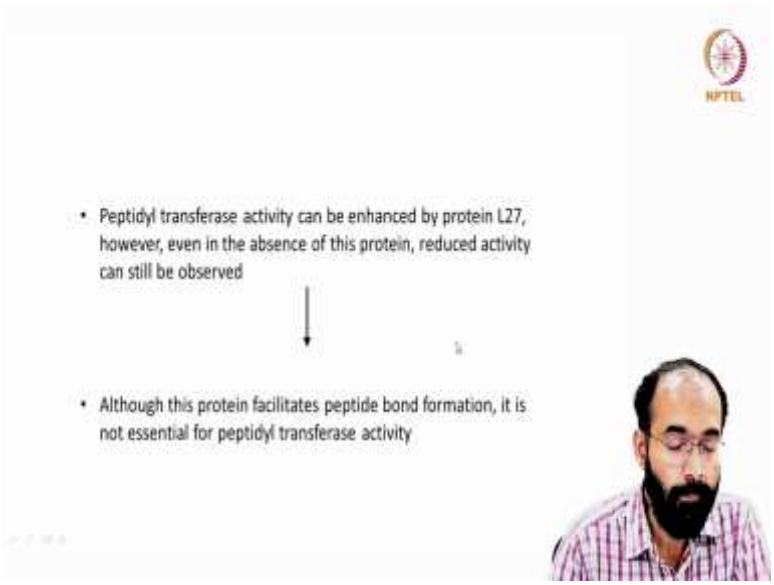

So, the peptidyl transferase activity can be enhanced by a protein called L 27. L 27 stands for a ribosomal protein, which do not participate in the peptide bond formation, but rather it is necessary for enhancing the activity; however, even in the absence of this protein which protein. L 27 protein reduced activity can still be observed. Although this protein facilitate peptide bond formation it is not essential for the peptidyl transferase activity of the ribosome because which indeed is carried out by an RNA molecule.

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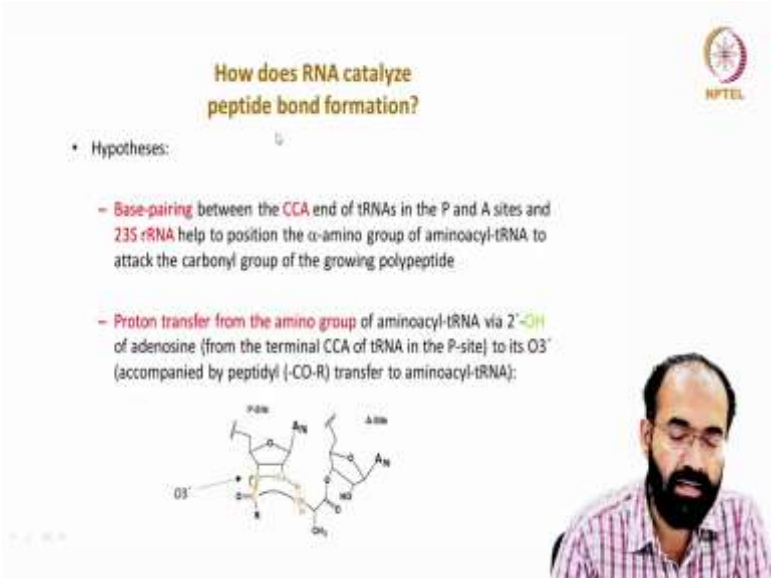

So, how does the RNA catalyst peptide bond formation? Base pairing between the CCA, CCA remember it is a tip of the transfer RNA which is formed by the post transcriptional modification. So, the base pairing between the CCA end of the tRNA in the P and A site remember P is the peptidyl site and A is the acceptor site and the 23S ribosomal RNA which is part of a ribosome itself help in the position of the alpha amino group of the aminoacyl tRNA to attack the carbonyl group of the growing polypeptide.

So, this is the chemistry that happens during the peptide bond formation. Now, the proton transfer from the amino group of the aminoacyl tRNA via the 2 prime OH. Remember 2 prime carbons hydroxyl group is highly reactive it is helpful in this splicing and various other biochemical reactions and this 2 prime OH of the adenosine.

That is that adenosine is belonging to the CCA from the terminal CCA C and C starts for two cytosine residue and then you have got A adenine residue and this adenine residues 2 prime OH is participating and that of this tRNA is helpful in the P-site that is present attached on to the P site.

And what happens? This 2 prime OH to be O 3 prime of that is of the accompanied by the peptidyl group of or in other words peptidyl group is written as CO dash R. R is basically a common group CO is the peptide bond peptidyl or a carbonyl bond we call it as that and transfer to the aminoacyl tRNA. So, this proton transfer from the amino group

of the aminoacyl tRNA via the 2 prime OH of the adenosine to its oxygen at the 3 prime of the CO-R group is what is going to contribute this peptidyl peptide bond formation.

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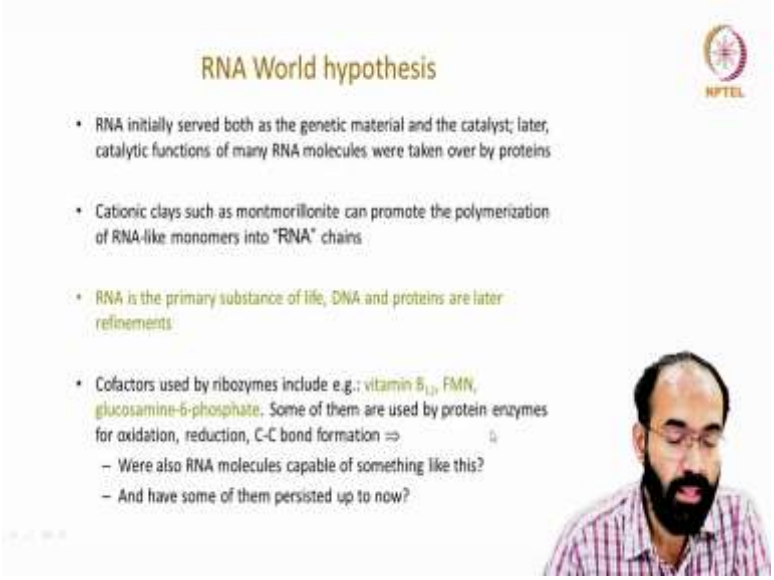

Now, coming back to RNA world hypothesis. RNA initially served both as a genetic material and catalyst. Later the catalytic function of many RNA molecules were taken over by proteins as a convenience because proteins can adopt much diverse structure and it also have about 20 amino acids to play with unlike the ribosome.

So, hence protein became quite handy. So, RNA world was there that time itself the proteins were handy by certain RNA molecules were capable of doing this job. So, various cationic clays such as montmorillonite clay can promote the polymerization of RNA like monomers into RNA chains.

So, this is what would have happened in the RNA world. So, RNA is the primary substance of life and dDNA and proteins are later refinements for better performance of this RNAs function or RNAs properties. So, cofactors used in ribozymes include vitamin B12, FMN, glucosamine 6 phosphate etcetera.

Some of them are used by the protein enzymes for oxidation reduction and carbon-carbon bond formation even today. So, what we should understand? Lot of cofactors which RNA enzymes made use of are also been made use of by the protein enzyme and their role more or less will remain the same as well.

So, we were they were also had had several RNA molecules that is capable of something like this and the answer is yes there are plenty of such RNA molecules had such cofactor utilization and can some of them can persist even today and the answer is yes a lot of them persist even today and they perform the task more or less similar fashion.

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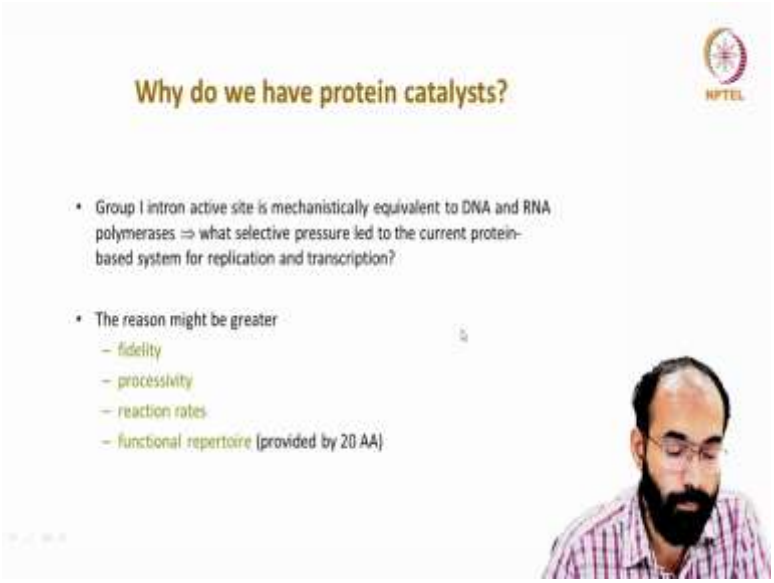

Why do we then have protein catalyst? We need to have an explanation for that. We have seen group one introns active site is mechanistically equivalent to the DNA and RNA polymerases which we see in protein inside DNA and RNA polymerases are nothing, but protein enzyme and the group one introns active site resembles a lot to that of mechanistically equivalent to that of DNA and RNA polymerases.

What selective pressure led to the current protein based system for replication and transcription? Because in the modern world no DNA replication happen without a protein no RNA replication happen without a protein; that means, the job which was done by the ribozyme is now almost completely taken over by the protein enzyme is there any reason behind it?

And the reasons might be much much greater than what we can assume one could be fidelity. Fidelity how accurately a job can be done and then comes the processivity how accurately the processing of the job can be done and then reaction rate how effectively a RNA can be produced and then functional repertoire; that means, the interaction can be

much much divergent; that means, RNA has only four bases A C U and G whereas, proteins 20 amino acids are there.

And each of them when you say basic amino acid not that having a constant basicity, it can have less basic, more basic extremely high basic like that or if you say if you say acidic also it is not just in a fixed manner. So, this varies quite a lot and this allows the fidelity, processivity, reaction rate and functional repertoire is possible that could be one of the reason why the RNA world slowly switched into protein world.

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Now, let us quickly look into the future of the ribozymes can we adopt newer and newer function to the ribosome zymes? The In Vitro molecular evolution of RNA is very much possible with the help of ribozyme and we can also cause high throughput screening; that means, you can design ribozyme and you can do a screening and based on which you can create ribozyme based therapies are very much possible.

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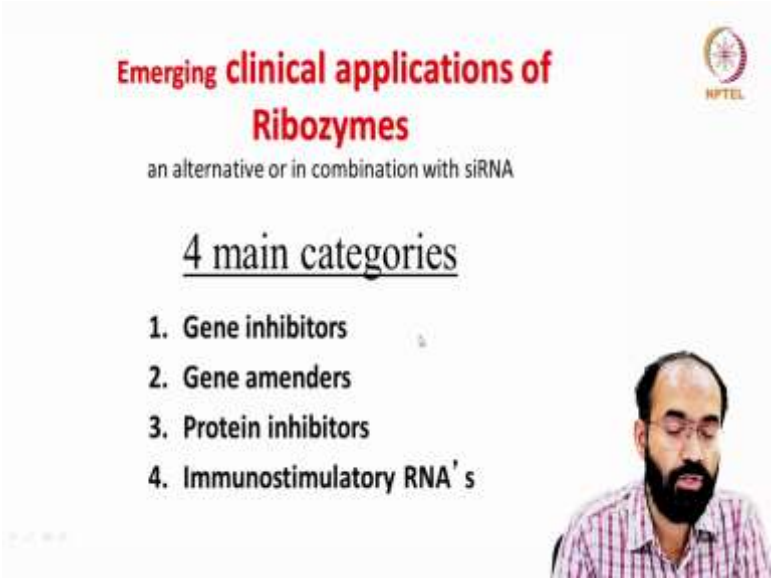

So, let us quickly see some of the emerging clinical applications of ribozyme and it is an alternative or a combination with siRNA. siRNA is small interfering RNA we will see more in detail how they are targeting an RNA because we have not discussed about it, but time being you understand siRNA can also negatively regulate gene expression; that means, siRNA can inhibit an RNAs expressivity we will see more in detail when we study siRNA.

So, time being we are addressing only the ribozyme. So, four main categories that can be made use of ribozyme in clinical application 1 as gene inhibitors, 2 as gene amenders, 3 as protein inhibitors, 4 as immunostimulatory RNAs. So, these are all the four categories in which ribozymes can be made use of gene inhibitors means, disrupt the function of a gene.

Gene amenders means, you tweak it like you do not want that much of expression of a gene reduce it like you know you are increasing or decreasing the volume of a phone or a audio device and protein inhibitors; that means, certain protein alone can be inhibited you do not want to degrade it, but you can inhibit it. And then immunostimulatory RNA and certain ribosomes can be used to induce immunogenicity in the host so, that it can provide protection against certain protein four nRNAs and proteins.

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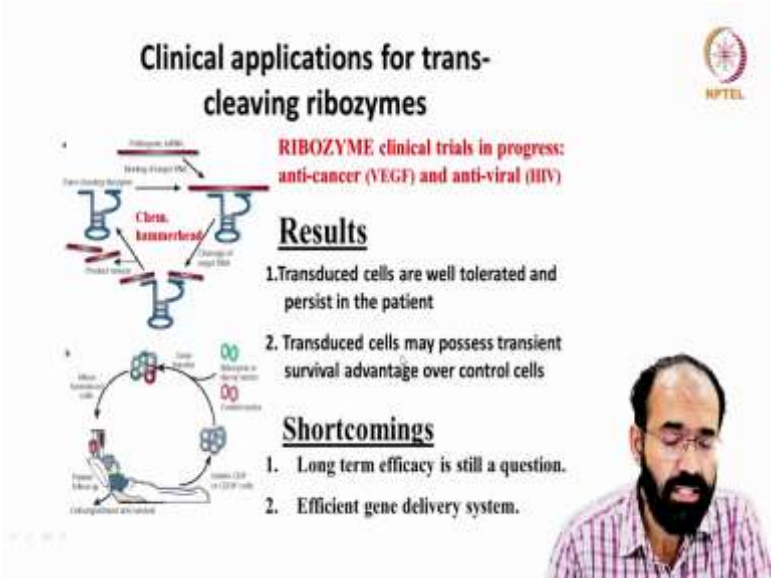

So, some of the clinical applications of trans cleaving ribozyme let us see. Ribozymes clinical trials were in progress it is going on one of the anti-cancer approaches VEGF we have also seen about the degradation of VEGF in the earlier class and also anti HIV or antiviral treatment against the HIV virus which causes the AIDS.

VEGF I told you that it can cause cancer, when there is too much of VEGF and its receptor is present inside the tumor the musculature can form very effectively and cancer survives and you do not want that happen. So, what happens here is if you have a pathogenic RNA and you design a ribozyme that is a trans cleaving ribozyme it will go and bind as you can see here at specific (Refer Time: 24:05) and it causes a break in.

So, this is a chemically produced hammerhead ribozyme it can cause a cleavage on to the target RNA. Then another example how do we produce how you deliver it? So, you have a patient and you collect the cells from the patient that is CD4 or CD34 cells you collect from the bloodstream and you introduce this ribozyme in a vector into the cells and deliver back into the same patient so, that in the bloodstream the person has got this ribozyme and it can cause a protective effect against VEGF or against HIV.

So, the results are the transduced cells last you can see here transduced cells into the CD4 and CD34 cells are well tolerated and persist in the patient. And 2nd result is transduced cells may possess transient survival advantage over controlled cells both are good and the shortcoming the long term efficacy is still a question.

So, every time you may have to keep doing this, but it is a life saver approach and the efficient gene delivery system also is a question can we deliver very effectively. So, these are all the things to be addressed when you want to come into the effective therapeutic intervention using ribozyme.

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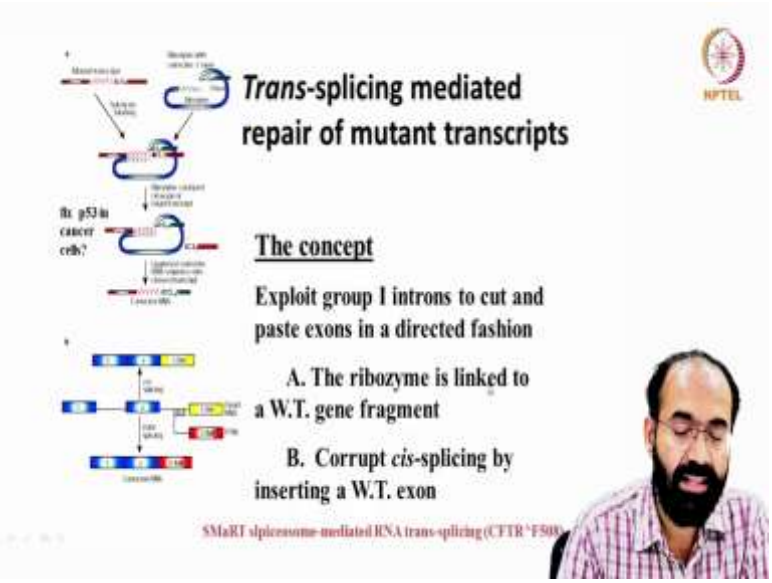

Another example is employing trans splicing mediated repair of mutant transcripts. So, here what happens? You have a mutant transcript; that means, like many such examples are there Duchenne muscular dystrophy you know that mutation causes very severe case of organisms muscles and the person does not survive and it is very much affected human males.

So, let us think such an example a mutated transcript and you want to repair it. So, you can make use of a ribozyme and that is having a corrected exon that the mutated part is now repaired and ribozyme acid and this ribozyme can cause selective cleavage on to the place where, there is a damage and it can cause a splicing because many ribozyme have got a ligase activity also.

So, it cuts and then ligates and this ligation happens with the fixed correct or the normal exon which is carried by the ribozyme. One way many cancers are caused because of the mutation in the gene called p53 if you correct the p53 gene the mutated p53 you may get a cure to the cancer because p53 is a guardian of the cell and this way this trans splicing approach can help a lot in fixing in vivo.

And like you can see here if you have a exon exon and exon mutated one you corrected it fixed it here and you end up getting a corrected RNA which can function quite effectively. So, the concept is exploit the group one introns to cut and paste exons in a directed fashion what we saw a group one introns are functioning.

The ribozyme is linked to a wild type gene fragment and the corrupt means damaged cis-splicing by inserting a wild type exon is very much possible one such example is smart spliiceosome mediated RNA trans splicing. So, this is a way in which you end up fixing damaged exons.

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So, those who are interested can read about evolutionary insights into RNA trans splicing in vertebrates and spliceosome mediated trans splicing these are all interesting article to read.

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So, in clinical trial ribozymes and lot of pharmaceuticals are there ribozyme and pharmaceutical incorporated and angiozyme is a product, which is used to target VEGF and heptazyme is a product that is targeting against Hepatitis C virus infection. So, these things are quite handy for the repair of the.

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And in clinical trial anti HIV therapy also is going on with bone marrow sample and to treat stem cell with retroviral vectors and it encodes gene for anti-HIV ribozyme and re

implant the treated cells. So, these are all the way in which you carry out ribozyme mediated treatment.

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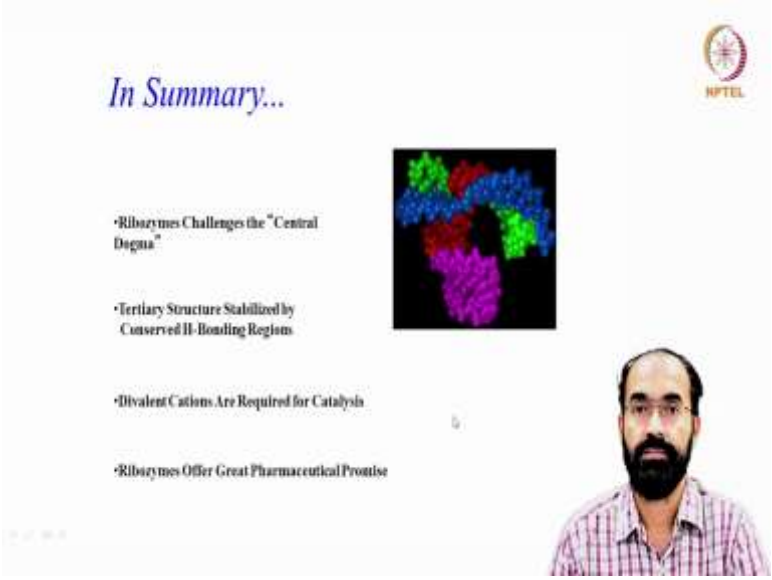

To summarize ribozyme challenges the central dogma of biology, tertiary structure stabilized by conserved H bonding regions also possible and divalent cations are required for the catalysis of the ribozyme and ribozyme offer great pharmaceutical promise in general and with this I end the ribozyme part and you will see a new topic in the coming classes.

Thank you.