**RNA Biology**

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**Lecture - 07**
**RNA as Enzymes: The Ribozymes**

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Welcome back to a new session of RNA Biology. So, now, we will start learning about Ribozymes. We have heard this name before that they are the RNA enzymes; that means, RNA that is capable of performing the task of an enzyme protein enzyme, which has the huge potential to maintain itself. And of course, there are different functions that an RNA enzyme can perform.

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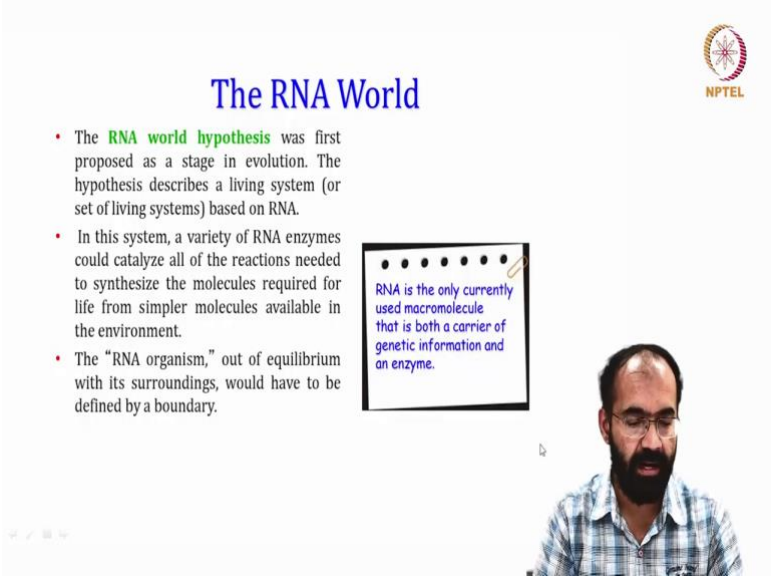


So, coming back to the RNA world itself when you look closely into the RNA world, we know ribozymes are the integral components of the RNA world and the RNA world hypothesis itself is standing because of them. Now, as I told you earlier that the RNA world hypothesis have a lot of plus points and also it is holding back because of lots of minus points and we will address them one by one.

And can ribozymes hold the key to answer or can ribozymes effectively come forward and defend for the RNA world hypothesis? So, it was proposed at a very early stage in the evolution and this hypothesis describes that in living systems the ribozymes could have been an integral part at least for several billions of years of evolution.

So, we should understand a different species of RNA enzymes they would catalyze almost all possible biological reactions of those days for that times living organisms requirement. And it has to be formed from the simpler molecule that is available and we coin the term RNA organism.

RNA organism is that organism, which depends on ribozymes to perform the required functions what that organism needed in order to survive. And it is very much in equilibrium with its surrounding and it has to have a defined boundary like we have seen that it has come from the environment what it is living. So, RNA is the only currently used macro molecule that is both a carrier of genetic information and an enzyme neither protein nor carbohydrate, nor lipids can claim this job.

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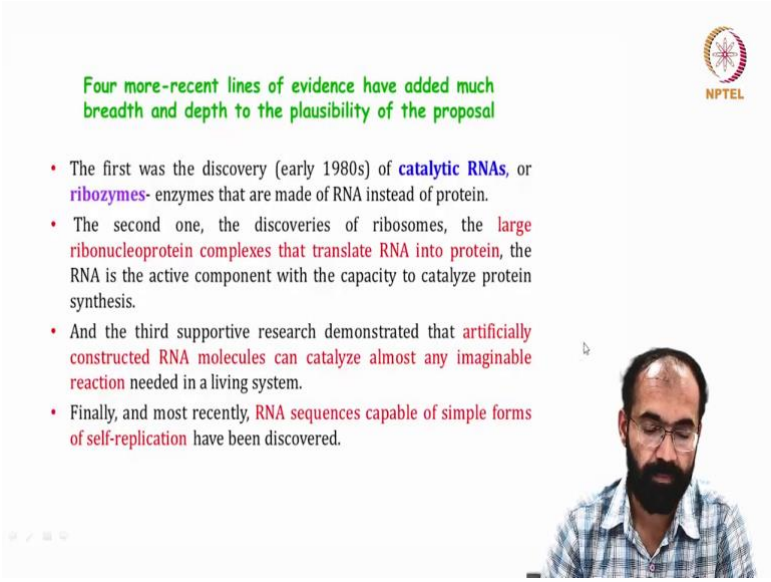


So, four major evidences we have that added lot of breadth and depth to the plausibility of the proposal. Let us see what are they one by one. The first was the discovery in 1980s the catalytic RNA or the ribozyme the so-called ribozymes that are made of exclusively RNA and no proteins involved.

The second one is the discoveries of the ribosomes itself that is an essential organelle for the protein biosynthesis. Large ribonucleoprotein complexes that translate the RNA, mRNA into protein, the RNA is the active component and with the capacity to catalyze the peptide bond formation in the protein biosynthesis.

And the third supportive research demonstrated that artificially constructed RNA molecule in the laboratory condition that can catalyze almost any imaginable biological reaction that is needed for a living organism because that is very much possible. Because to perform a task you need to have a unique three-dimensional structure which is possible to be created with RNA. Of course, lot of programming and designing has to go into that, but; however, it is possible to do that.

Finally, and most recently we know that the RNA sequences capable of simple forms of self-replication have been discovered; that means, RNA do not depend on any other molecule to make a copy of itself even in modern world. What does it say? It simply says RNA world hypothesis is definitely valid and the evidence has to come mainly from the ribozymes.

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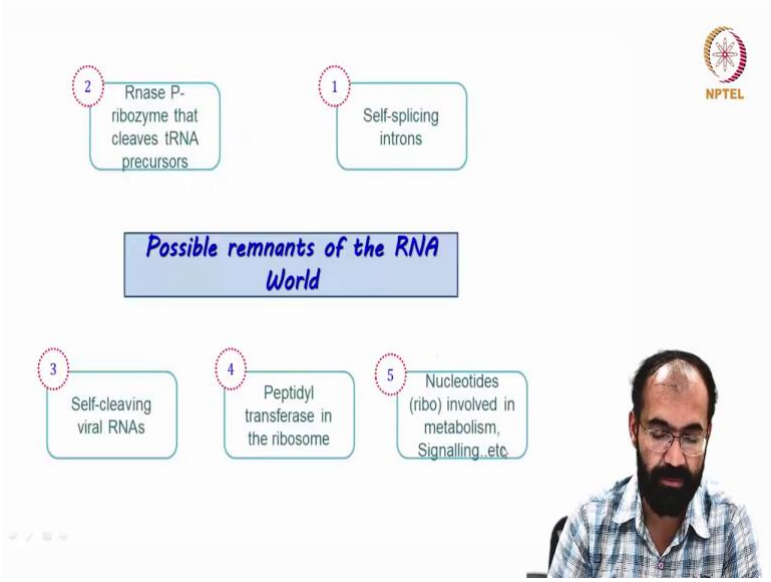


So, let us see some possible remnants of the RNA world. Can we prove it the RNA world with the help of examples of ribozyme? One is self splicing introns. There are many genes which has got the non-coding or unimportant part rather than using the word non-coding it would be convenient to use that unimportant or not required part in the RNA that has to be removed they have to be spliced out.

And there are many self splicing introns are there in many mRNA's or pre mRNA's which has to be spliced and become mRNAs or a mature RNA instead of using the word mRNA one could always use a pre-RNA to a proper RNA by self splicing introns. Then examples you have is Rnase P that is a ribozyme that cleaves transfer RNA precursors. We will see more in detail about Rnase P which is a important ribozyme.

And then we have number 3 that is self-cleaving viral RNA. Many examples are there hepatitis delta virus is a good example we will see them more in detail. Then comes the peptidyl transferase activity in the ribosomes which is a ribozyme action and then nucleotides that is the ribonucleotides involved in the metabolism signaling like we saw in the very beginning that many vitamins or energy yielding ATP, GTP, etcetera they all components of an RNA molecule.

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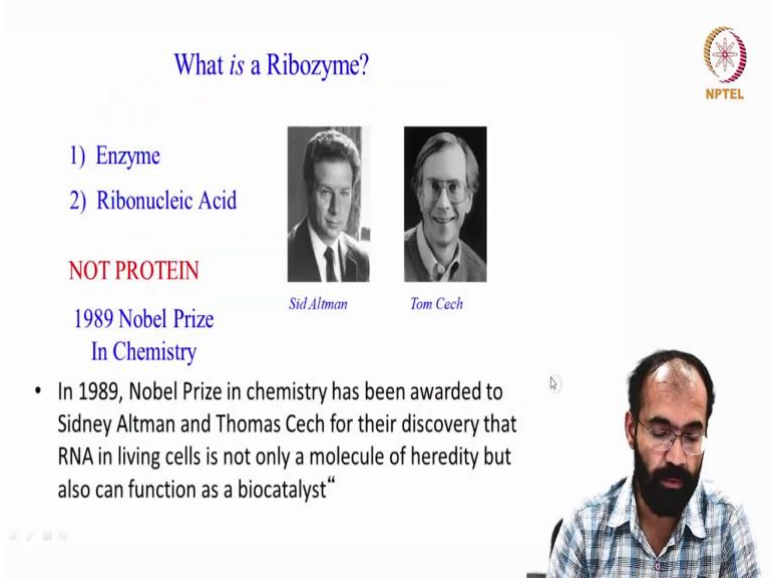


So, let us ask what is a ribozyme? We know the answer we will not go into the detail. This basically an enzyme and also a nucleic acid, but this is definitely not a protein. So, in 1989, Sid Altman and Thomas Cech independently discovered they were awarded Nobel Prize later on in Chemistry. And it is which is been found that you can have RNA molecule that is capable of performing catalytic role which is present in some prokaryotic or protozoan very primitive organisms very abundantly and quite efficiently.

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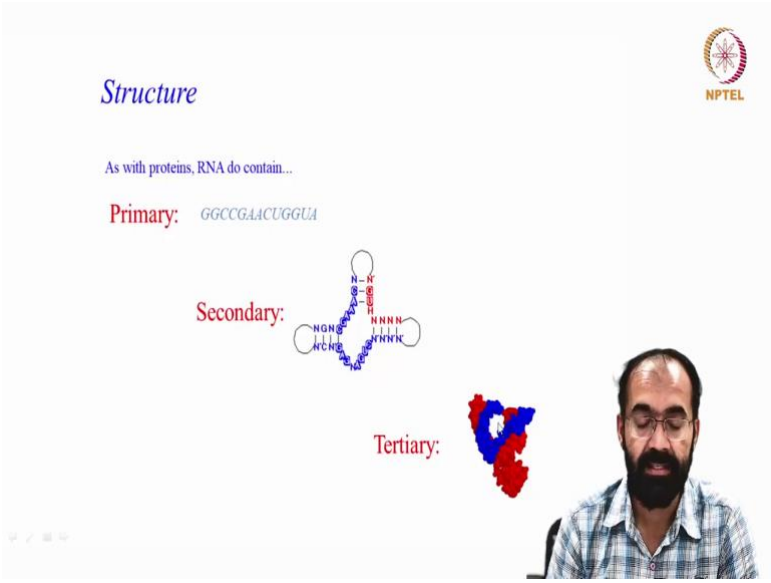

We will see more in detail about the examples in subsequent classes. So, if you look into the structure, as with proteins which has got a three-dimensional structure ribozyme also need to have a three-dimensional structure. And it has got a primary sequence, and it has got a secondary sequence, and it also has got a tertiary sequence. Remember the tertiary sequence is the final structure which is performing the biological function.

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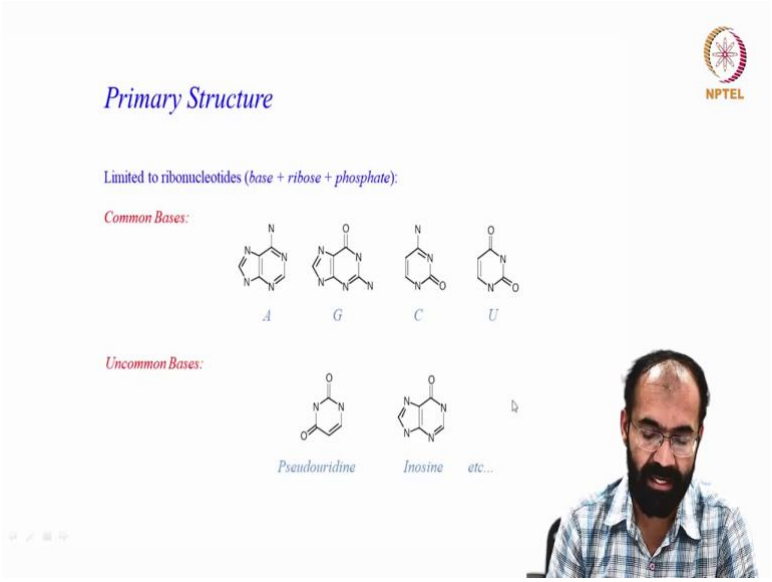


Now, if you look more in detail, we can see that the ribonucleotides which is basically nitrogen, base, ribose and the phosphate. They are essential part they are important in the carrying out of the biological activity of a ribozyme that adenine, guanine, cytosine and uracil are the four major nucleotides that is present in the ribozymes.

And the uncommon bases also there. Sometimes as a result of introduction of some of the uncommon bases the RNA the ribozyme in question gets some extra ability to prevent degradation; that means, it can be resistant nucleus etcetera. One such example is pseudo uridine which gets incorporated post transcriptionally. Once an RNA's being formed it will be edited with a uncommon base pseudo uridine then inosine and there are many more examples which we will see them one by one as and when we go.

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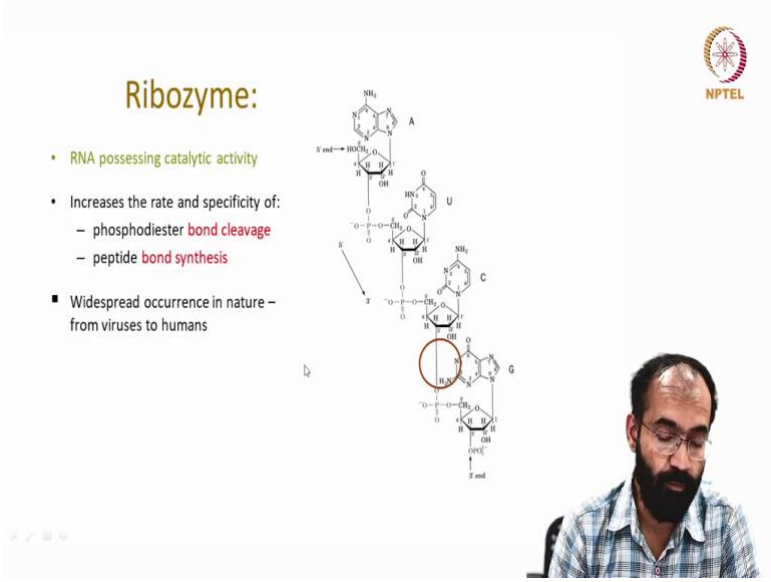


So, ribozyme as you can see it is an RNA processing, catalytic activity an enzyme which has got a processivity ability is there associated with the ribozyme. It increases the rate and specificity based on the phosphodiester, bond cleavage and peptide bond synthesis. Lot of functions can be assigned to a ribozyme, but the rate and the specificity depends a lot on the phosphodiester bond cleavage and also on the peptide bond synthesis. And the widespread occurrence of ribozymes in nature can be seen from viruses to humans.

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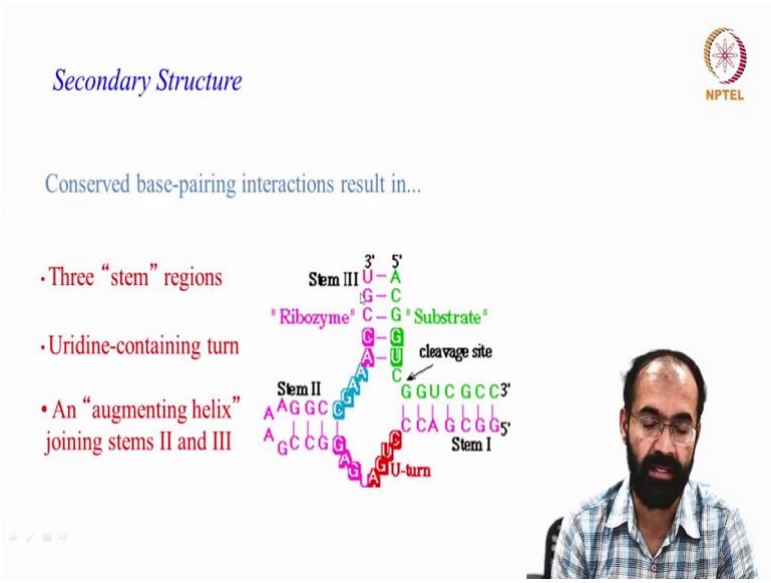

But remember the structure is very simple, but they adopt a unique structure to perform the task of a ribozyme. So, we should also see very conserved base pairing interactions in various domains or various sequences within the ribozyme. And if you can see closely as you can see in this picture you have multiple stems stem I, stem II and stem III regions are there. This green color sequence is the substrate; that means a RNA stretch which is acted upon by a so called ribozyme.

So, it pairs and this area where the arrow mark is put that is the place where the cleavage has to happen. And these three stems stabilize the structure without the stem I, stem II and stem III the ribozyme structure will not be strong or structure will not be stable enough to perform.

The substrate action or the action on the substrate to cause its cleavage, then there is also a specific uridine containing turn which is basically acts like a hinge like you know why hinge is important for door. There is no hinge you cannot open and close the door effectively. So, the uridine rich term is very important in acting like a hinge for the enzyme.

So, and then also there is a new term called augmenting helix. That is basically for joining stem II and III. You have stem II and stem II. Once they come together what happens? Usually, this area will have a big stretch and this will get cleaved and this will be detached because stem II and stem III is now holding over the other. As a result, this will detach. So, that is how the ribozyme act on to a substrate.

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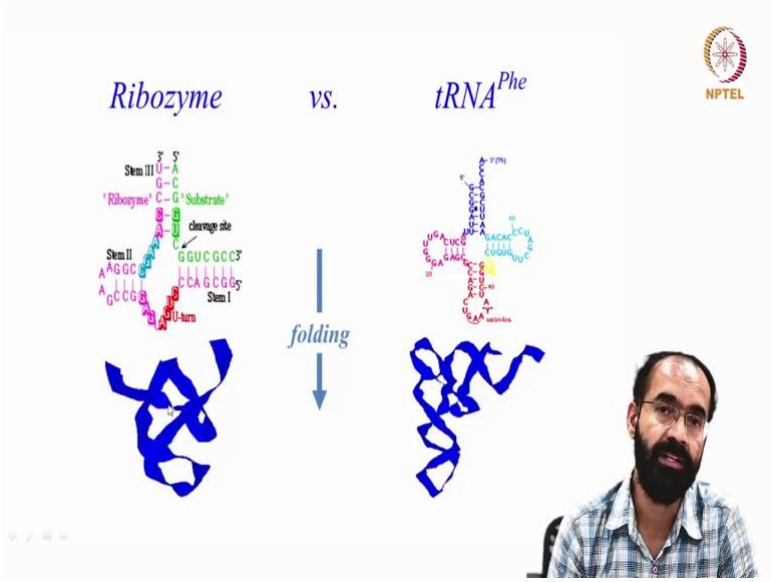


If you compare, we saw the previous structure of a ribozyme bound on to a substrate. Now let us compare the ribozyme structure to that of a tRNA. Just out of curiosity it does not have any biological major biological role. And you will see a huge similarity because transfer RNA also have got multiple regions we will see transfer RNA structure more in detail, but you can see ribozyme and transfer RNA have got lot in common with regard to its structure.

So, what it says that the structural features of a given RNA molecule can be copied over and over if it is found to be quite useful. Like we will not go into the more details of this right now. Because when time or the topic is appropriate, we will get into those details. And the transfer RNA's maturation once it is properly folded again that also mimics the mature transfer RNA or properly folded transfer RNA also mimics a functional ribozyme in its final structure.

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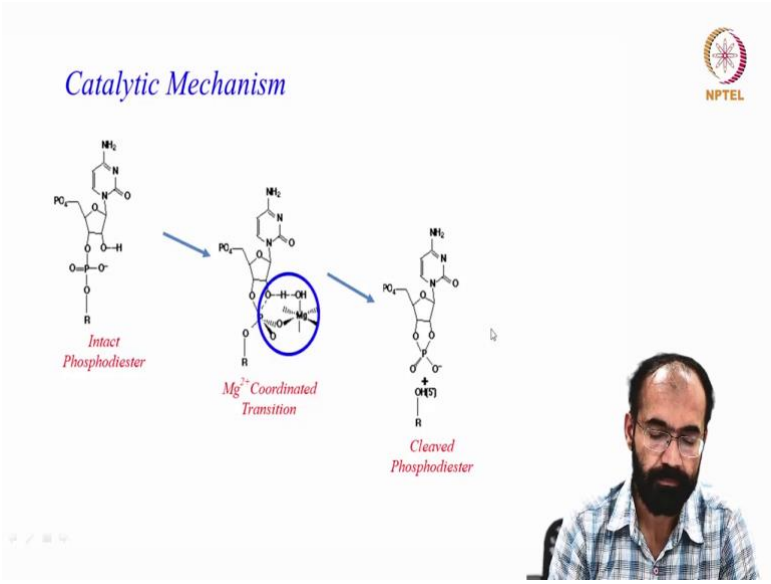


So, now coming back to the tertiary structure should see the catalytic mechanism depends a lot on the tertiary structure. And as you can here there is an intact phosphodiester backbone that is present in to start with. And this will further mature into interaction with certain metal ions such as magnesium ions.

There are many divalent cations that comes into picture or comes handy when it comes to providing stability. Remember in the prebiotic world there are no proteins only ions are there and also nucleic acid that are randomly formed are there. Now, we should know

whether these molecules are providing any structural stability and magnesium is one of them that provide structural stability and more and more enzymatic activity to the ribozyme.

And as the magnesium ions comes in contact it can influence the phosphodiester backbone and it can create a cleaved phosphodiester backbone because of the enzymatic activity of the whole ribozyme. Remember this structure is not a whole ribozyme, but it is a part of a ribozyme just a relevant portion is been highlighted.

But to cleave you need to have metal ions such as magnesium also come into picture. Remember this is meant exclusively for structural stability magnesium ion do not participate in the cleaving of the phosphodiester backbone.

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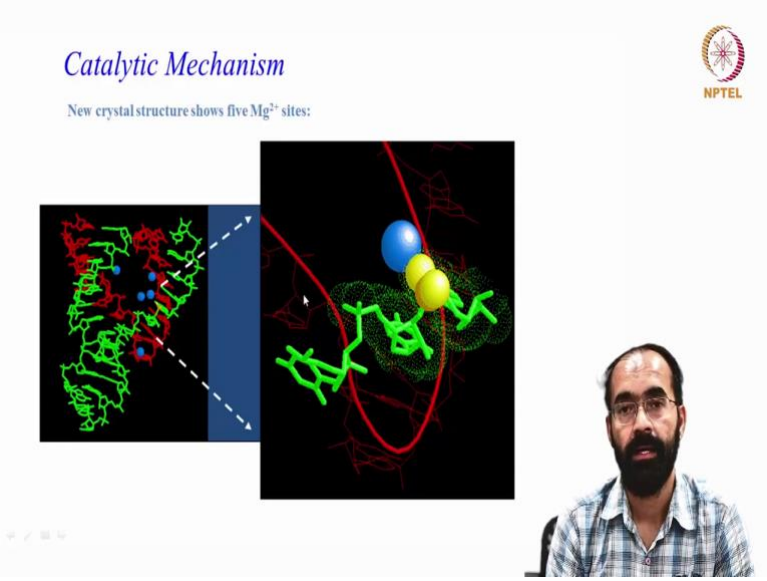


If you look further into the catalytic mechanism at its crystal structure level you can see this blue color dots are nothing but the magnesium ions. And there are around five magnesium ions that come into picture come handy for the stabilization of the ribozyme structure. And this is in enlarged picture you can see how ribozymes act and how the metal ions come together to cause a bending or a breaking of the phosphodiester bond.

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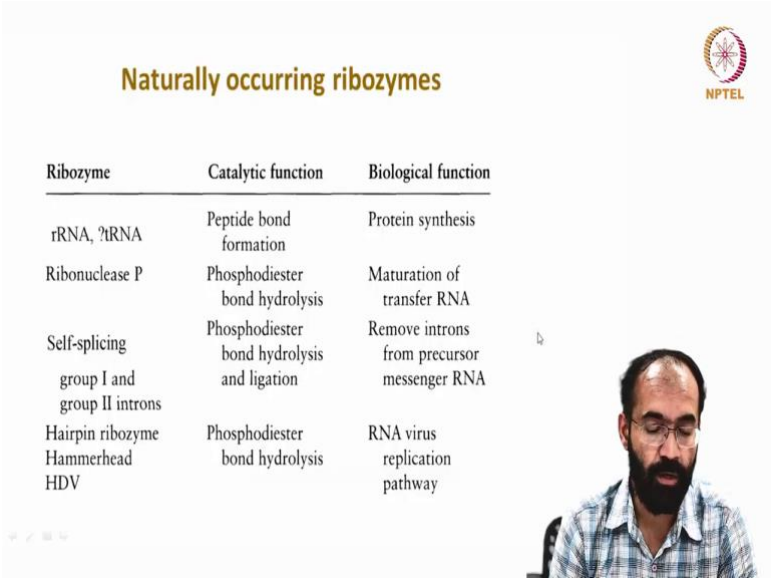


Now, let us see do we have some examples of naturally occurring ribozyme as an example. So, there are several naturally occurring ribozymes exist and we will see them one by one. And ribozymes can have a proper name such as ribosomal RNA, transfer RNA, Ribonuclease P, self splicing group 1, group 2, etcetera.

Let us see ribozyme their catalytic function and the biological function. So, ribosomal RNA and transfer RNA they are mainly involved in the peptide bond formation and the biological function is the protein synthesis. Ribonuclease P which is basically meant for phosphodiester backbone hydrolysis and it is important for the maturation of the transfer RNA.

Then self splicing ribozymes are there they are of two types group one and group two we will see them more in detail how do they perform, but they are meant mainly for cleaving of the phosphodiester backbone as a part of maturation of an RNA that is splicing event takes place in this self-splicing ribozyme.

That is the removal of the introns and from the precursor messenger RNA. Then examples involve hairpin ribozyme, hammer head ribozyme, hepatitis delta virus ribozyme they all important for the phosphodiester bond hydrolysis. And they are important in RNA virus and various replication pathway of their genome.

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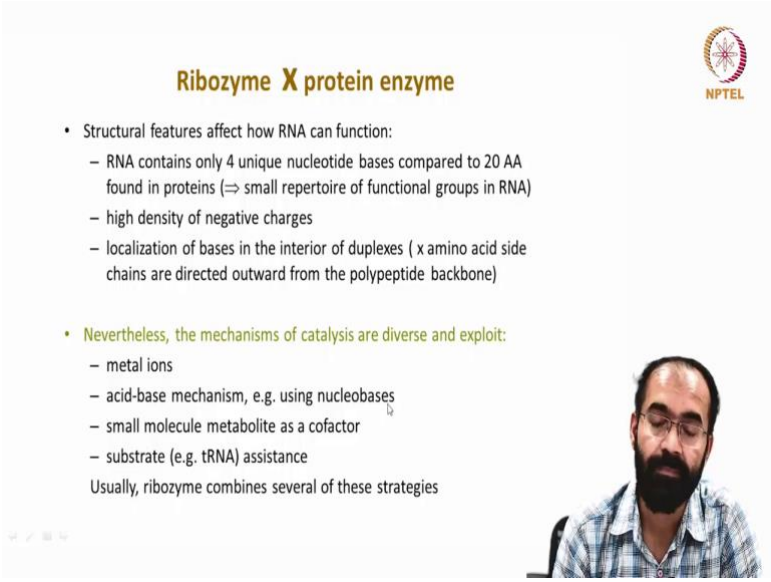


If you compare ribozyme with their protein enzyme you can always see a lot are in common; the structural features that affect how RNA can function. That RNA as we know has only 4 unique nucleotides. Means it has to have the permutation of all these 4 nucleotides, it cannot have diversity as you can see in the case of proteins, why? Because proteins have 20 of such amino acids are there based on which it can create lots of permutations.

And a small repertoire of the functional groups are present in the ribozyme. So, the complexity in structure of ribozymes are imparted by the secondary and tertiary structure that is formed. And the catalytic role is played by very few one or two nucleotide sequence present in the core of the ribozyme. And it also has high density of negative charges.

This is important point to remember because high density of negative charges makes the ribozyme able to act only on those substrates which has got positive charges. Because if a substrate has got high negative charge, then it becomes very difficult for them to interact. So, these are all some of the points to remember.

Localization of bases in the interior of the duplexes are also unique compared to the amino acid side chains that are directed outward in the case of a protein enzyme. So, these are all some points which we should compare and contrast with ribozyme with that

of a protein. Nevertheless, despite all this problem the mechanism of catalysis are quite unique and they are diverse and they exploit metal ions and acid base mechanism.

Example using the nuclear bases also small molecule metabolic acting as a cofactor and also substrates such as tRNA come handy for assistance purpose. Usually, ribozymes are capable of combining all these strategies means using a metal ion, acid base mechanism, small molecule co-factors and also unique substrates assistance such as tRNA it makes use of all these features to carry out a given biological function.

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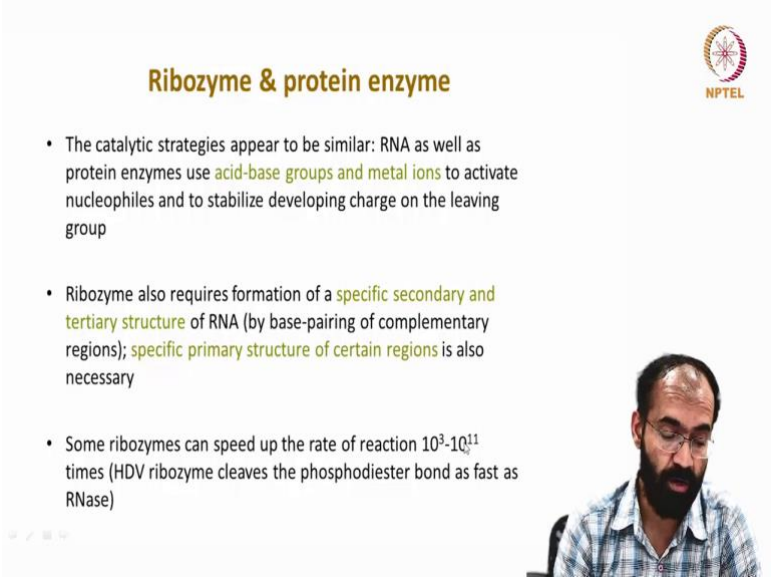


If you compare further to that of protein enzyme the catalytic strategies appear quite similar RNA as well as the protein enzymes use the so-called acid base interaction or if the enzyme has got an acid catalytically active domain or a region it will interact with the basic domain of the substrate. So, that the interaction becomes stronger. So, this is a common feature.

And also, utilization of metal ions both ribozymes as well as protein enzyme make use of metal ions to activate various nucleophiles and also to stabilize the developing charge onto the leaving group. Many a times as a part of an enzymatic activity you end up creating products. And products if they are highly reactive it is bad for the cell the system and also to the enzyme itself.

So, you have to nullify it just like you can understand if you study the history of match boxes like people match box earlier times the match boxes are so sensitive if you put a match box in your pocket if you walk around, it will catch fire and you will get you know a fire accident.

But now match boxes are made such that you have to take the match tick and rub on to the correct spot where you are supposed to you cannot rub anywhere then only it will catch fire which is outside. So, inside match box there are match sticks and outside you have to rub. Same logic applies when it comes to a product that is being formed from an enzymatic reaction that product is dangerous to the cell or to the enzyme itself then it is counterproductive.

So, this metal ions make sure that the newly formed product is passive, it is no harmful that is why many a times they will remain as sodium salt or potassium salt or they will remain associated with these ions to make them less reactive. So, metal ions become handy for that also. So, ribozyme also requires the formation of specific secondary and tertiary structure of an RNA by simple by base pair to its complementary regions.

So, it also has to have specific primary structures in certain regions and it is very very important not only for stabilizing the structure of the ribozyme, but also for pairing this particular primary sequence to the substrate. This will become very evident when you study the RNA splicing, we will see how spliceosomal RNA is binding on to a pre mRNA.

So, that time you will remember how important this pairing is for the splicing purpose. We will see that in the splicing section. And some ribozymes can speed up the rate of reaction 10 raised to 3 to 10 raised to 11 times more powerful. Some examples include HDV ribozyme hepatitis delta virus ribozyme that cleaves the phosphodiester bond as fast as RNase.

RNase is a protein enzyme one of the very powerful very stable enzyme. That if you heat it up to around 200 degrees Celsius it is stable. So, such protein enzymes are very effective and very efficient they are competent enough to these HDV ribozymes are competent enough to accelerate the phosphodiester cleavage as fast as a RNA's which is also causing the same thing cleaving of the phosphodiester backbone.

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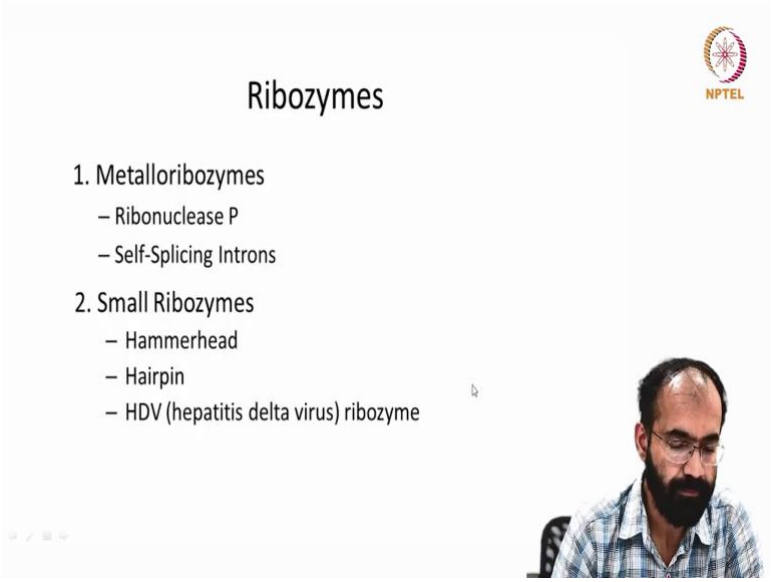


So, ribozymes can be classified broadly into two that is metalloribozymes and small ribozyme. And metalloribozymes include two major groups ribonuclease P, self splicing introns these come under metalloribozyme. As the name itself indicates you can get a clue metalloribozymes require metal ions for their biological action. Whereas, small ribozymes it contains hammerhead ribozyme, hairpin ribozyme and HDV hepatitis delta virus ribozyme. So, these are pretty small, but quite efficient in their rate of reaction.

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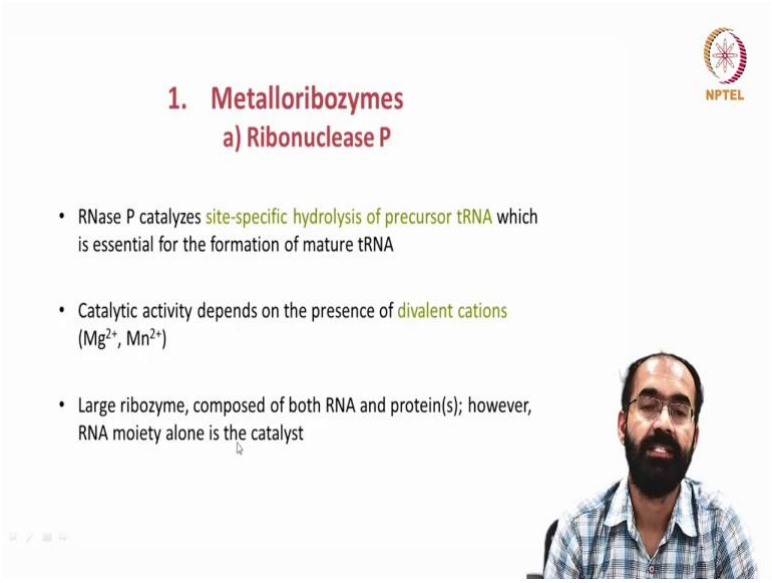

Now, if you look further into metalloribozyme. Let us see the example of ribonuclease P or RNase P. RNase P catalyzes site specific hydrolysis of precursor tRNA. tRNA means transfer RNA important in the protein synthesis which is essential for the formation of the mature tRNA, tRNA's are formed immature or precursor form which has to undergo the cleavage at specific locations. So, that it can mature into a functional tRNA which is done by RNase P ribozyme.

And then catalytic activity of this enzyme ribonuclease P depends on the presence of divalent cations such as magnesium and manganese divalent cations which are important without which this RNase P is not biologically active. And it is a large ribozyme it composed of RNA as well as proteins. However, the RNA moiety is acting as the catalyst similar to that what we saw in the case of ribosome that is why ribonuclease P is coming under metalloribozyme.

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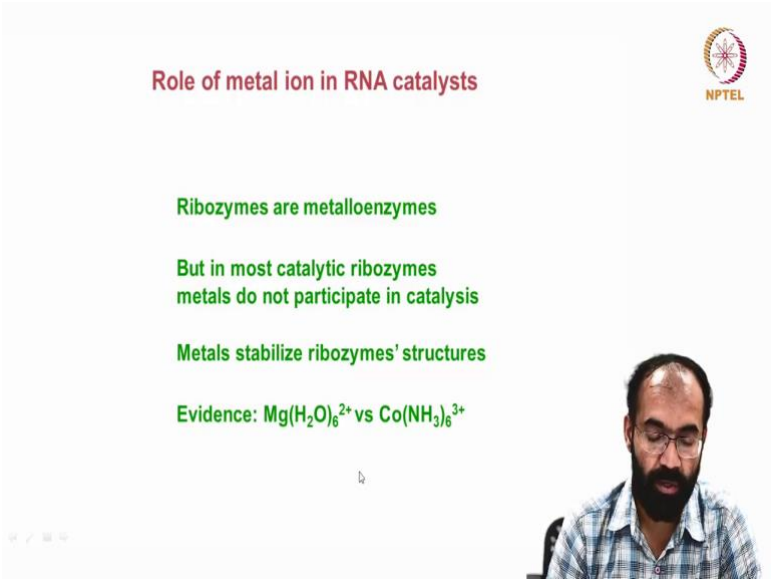


And let us see what is the role of metal ions in RNA catalysis. Ribozymes are metalloenzymes, but in most catalytic ribozyme's metals do not participate in catalysis, it participates only for stabilizing the ribozyme. Metals stabilize ribozyme structures without which the catalytic activity is not possible. An examples include magnesium compounds and also cobalt compounds which participate of which magnesium and cobalt is going to be part of the integral component of the ribozyme.

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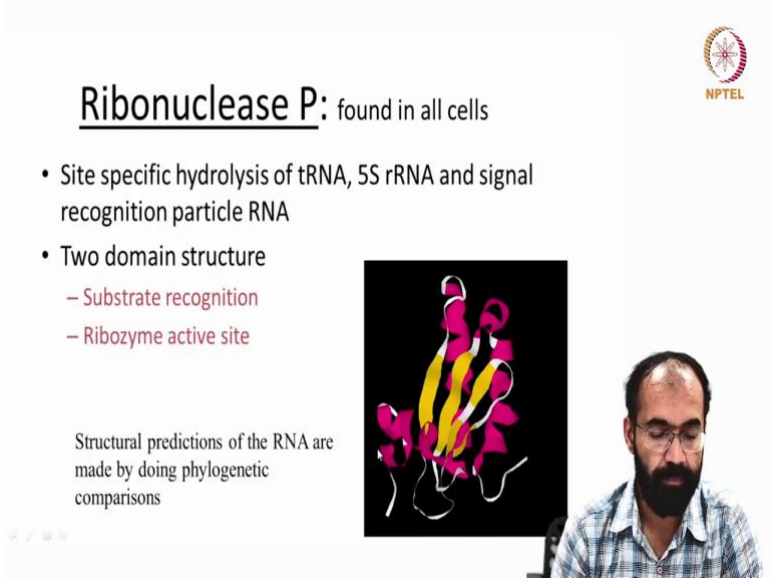


And if you look into the structure of the ribonuclease P it is found in all cells because every cell need tRNA and it also have got site specific hydrolysis of tRNA, 5S ribosomal RNA and signal recognition particle in the RNA. We will see 5S RNA and SRP RNA much later. And it has got two domain structure.

One is for substrate recognition and ribozymes active site that is it has to recognize the immature tRNA and also a region to perform the cleavage of the unwanted part from the tRNA. And this is a picture that shows the structural prediction of this ribonuclease P and we will see more in detail about the ribozymes in the next class.

Thank you.