RNA Biology
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Lecture - 05
Introduction to RNA Biology and RNA World-RNA Self Replication

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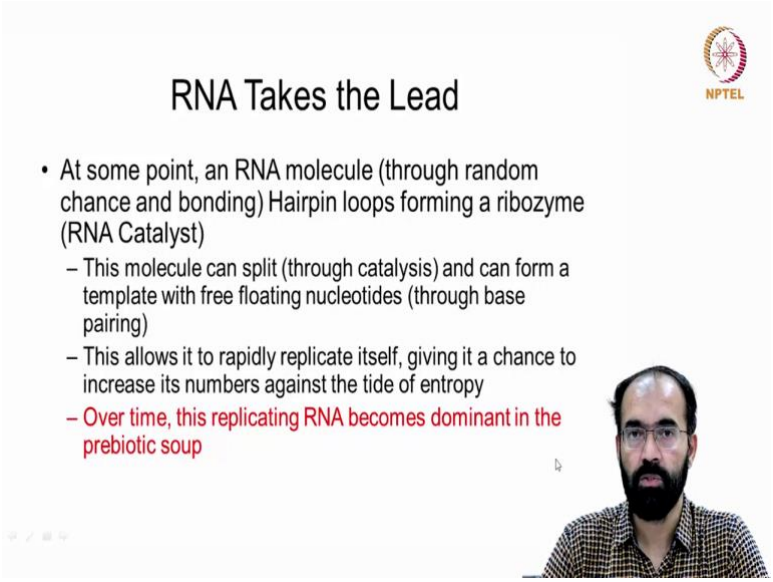

Welcome back to another session of RNA Biology. So, we left here in the last class that how RNA takes the lead in the prebiotic world. So, it is a question of ability or not having the ability to make a copy of itself decides which species molecule of RNA dominates in the prebiotic world.

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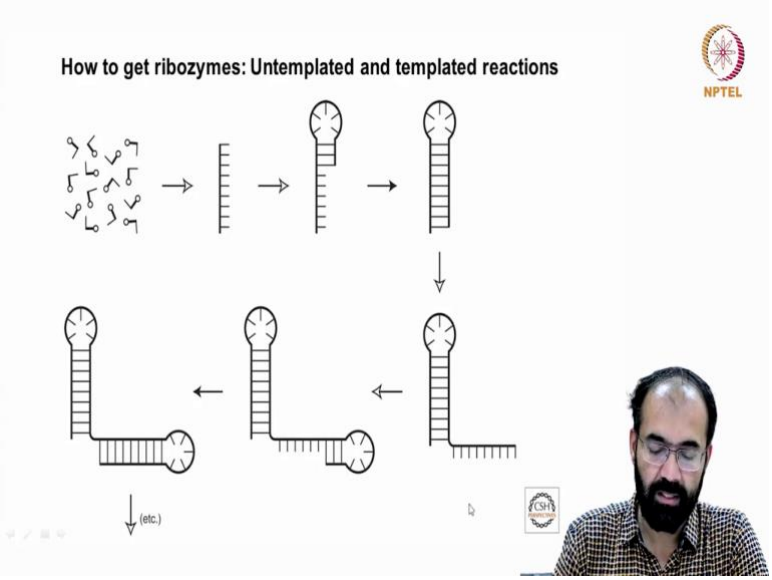

Now, you can see this in this picture you have several monomers of the RNA in the left-hand side and they randomly assembled with the help of montmorillonite clay catalyst etcetera, it made a strand. And it can be any sequence, not that it has to be you name it any random sequence using these 4 nucleotides it can form any random sequence, but one of them happened have the ability to form a pairing.

Now, it is a question of time whether it can make a template copy like you can see a loop and then a stem and this stem is growing in all directions possible.

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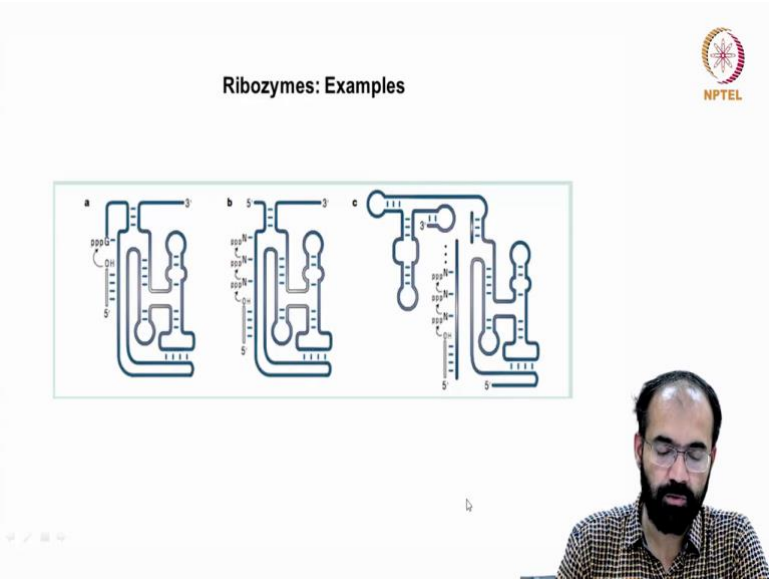

And, this will continue to make a very complex structure as you can see in this picture that any long possible stem loop again stem again loop structure is possible and this continue to grow. Remember during this process it is adopting newer and newer secondary tertiary structures.

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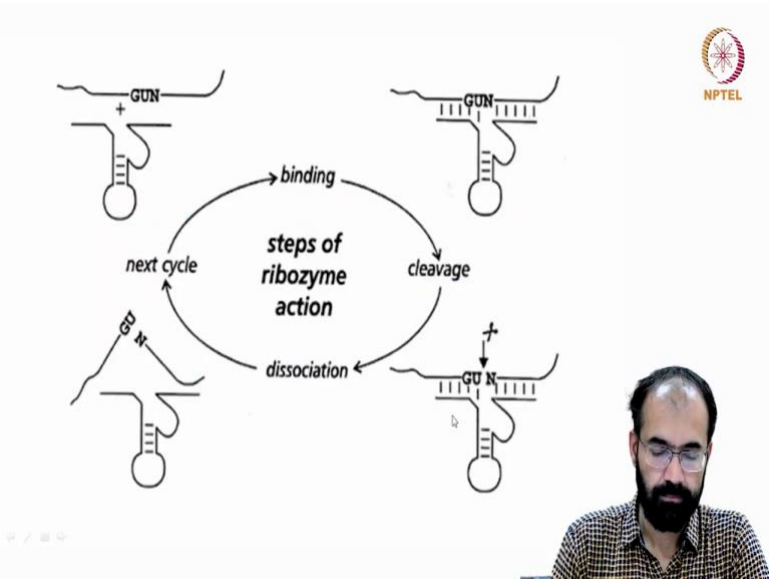

Now, let us see do we have some evidence for an RNA to cannibalize another RNA molecule and extract energy from that and we usually call such functional RNA molecule as the ribozyme. Let us quickly see a typical ribozyme action as a catalytic molecule. You can see here in the left-hand side top that there is a stretch of RNA in that one sequence is written clearly. It is a full stretch of RNA sequence, but G U and N, G is for Guanosine, U for Uracil and N for any Nucleotide, it can be A, C, G or U itself.

This can be paired with another molecule. This which is having already a stem loop structure, this we can call it as a ribozyme for convenience. Now, it undergoes so called pairing at specific regions on this you know target or a substrate molecule. So, we can for convenience we can call this GUN bearing RNA as a substrate whereas, the stem loop bearing molecule as a enzyme or a ribozyme or a functional RNA molecule.

Now, it underwent a pairing at specific sequence now it behaving it is behaving like a DNA molecule. And, now we are more interested in this GUN region. What happens after binding initially after binding it performs a cleavage reaction; that means, there is a

symbol of scissors is given, it cuts between the U and the N. And, what you end up getting is getting a cleaved a substrate and this substrate will broker and gets released.

But, during this process it can absorb the energy and catch hold of another molecule and again continue this process like a cyclic fashion. And, this is basically making use of energy available from pre-existing RNA molecule to perform a task. So, we can in the simplistic form we can call this ribozyme as a nuclease.

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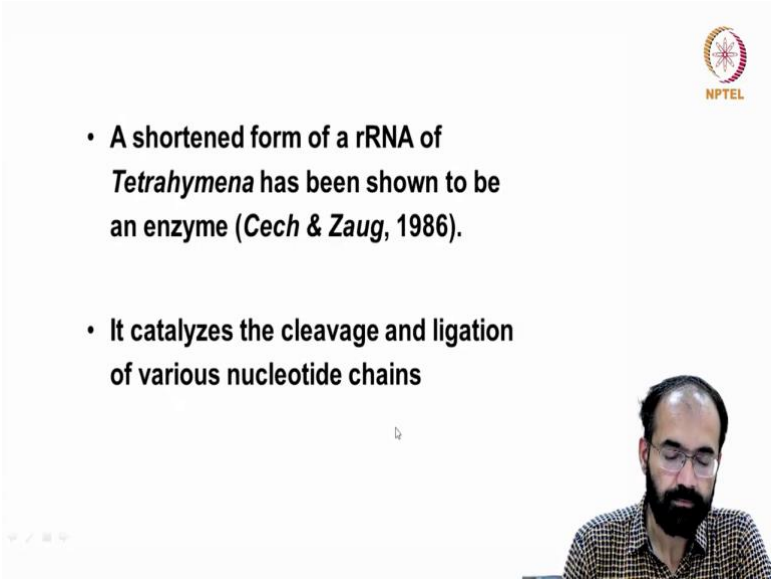

Now, we have similar examples in some small protozoa called Tetrahymena. A shortened form of ribosomal RNA of Tetrahymena as shown to act as an enzyme. This was discovered in 1986 by Cech and Zaug and they have eventually discovered the properties of a ribozyme using Tetrahymena as a model. It could catalyze the cleavage and also the ligation.

So, this molecule not only cleaves a template, but it can also attach a nucleotide to another molecule of various nucleotide chains. We can see them more closely how does it do.

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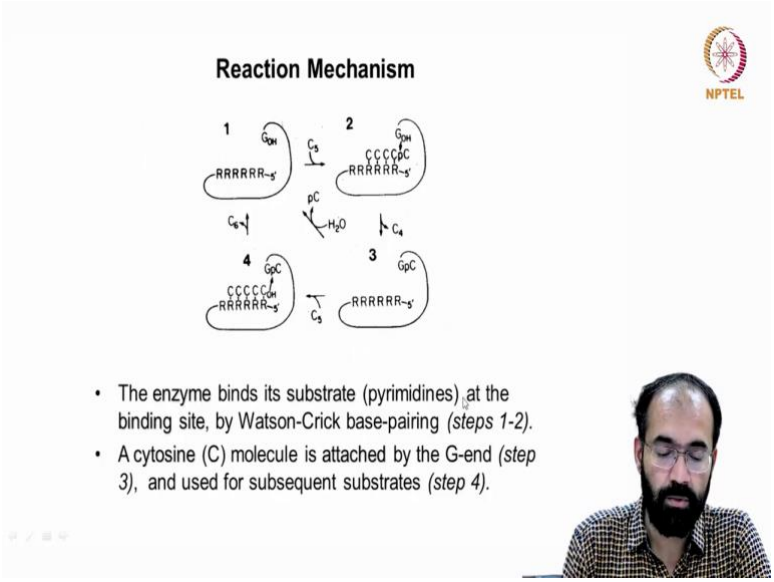

You can see this reaction mechanism in this picture. So, this RNA have got a purine rich stretch. You can see R R R R R stands for purine. Purine means what? Adenosine and guanosine, whereas, uracil and cytosine are pyrimidines. Now, one end of this RNA molecule have got a G and its O H is highlighted. Every base has got an O H, but this Gs O H is highlighted for convenience, because it has a function. It catch hold of a stretch of 5 cytosine nucleotide.

So, there is a RNA molecule which is only 5 nucleotide in length, that is written as C and C and C and C and C. So, what happens in such situation it can catch hold of this 1, 2, 3, 4, 5 C bearing molecule and this hydroxyl group. And, remember this guanosine 2 prime hydroxyl group like I told you in the previous class that the 2 prime hydroxyl group is very reactive.

The 2 prime hydroxyl group cleave the phosphodiester backbone of this 5 C or 5 cytosine nucleotide or a stretch of nucleotide cleaves this phosphodiester backbone. And, it release a C 4, it started with a C 5, 5 cytosine; now it release the C 4 and this detached single cytosine is now attached onto the G. So, they end G now became G phosphodiester backbone and C.

And, now what it will do? It can catch hold of another template which is again C 5 and it can transfer this carrying C, the G pairing C that C can be attached by this newly recruited C 5. So, you end up getting a C 6. So, what you should understand? This

ribozyme action involves the release of 1 C from a C 5 and holding temporarily and then catching hold of another C 5 and attaching the whole C 5 onto the existing C as a result you end up producing a C 6.

So, you saw both now here, one is cleavage and another is ligation. So, it removed 1 C from 1 template and its hold catch hold of another template, it made C 5 into C 6. So, this is a simplistic form of nuclease as well as ligase activity.

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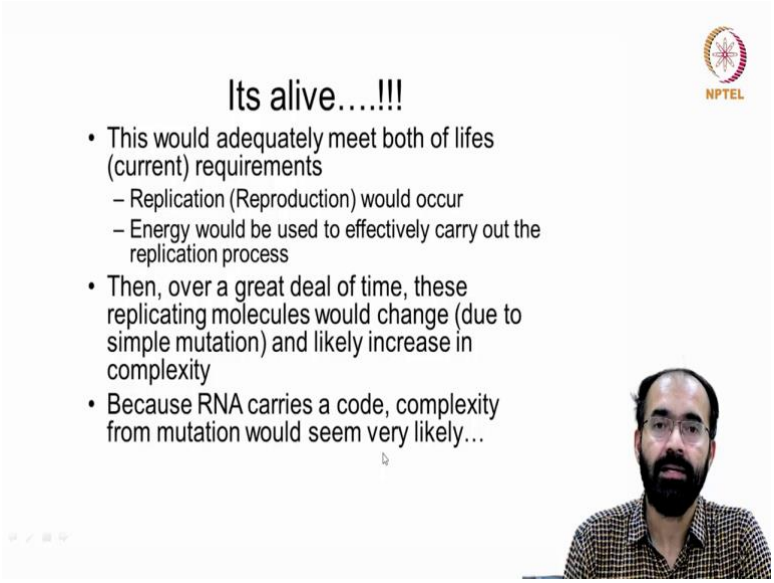

So, we should understand it is a living action, it is always alive when you talk about the catalytic activity of a RNA enzyme. So, this would adequately meet both the life's or both the explanations on the requirements. The replication which is nothing but reproduction of itself would occur and the energy, remember we defined the life in the beginning; that means, all major things it require is ability to make a copy of itself and ability to utilize the energy.

So, the RNA can technically make a copy of itself and it can also utilize the energy either by chemical means or by the physical means which allows the replication to happen smoothly. Then, over a great amount of time these replicating molecules would then change due to simple mutation because although the changes or the making a copy of itself using a template strand it is always error prone.

And, remember even in modern day we talked about RNA dependent RNA polymerase right which is protein enzyme which is present in the viruses which do not have the proofreading activity, that is why people who get flu, keep getting flu after every 6 months or they can get frequently because the flu virus which infected you has undergone change.

Because, it is having an enzyme RNA dependent RNA polymerase which do not have the proofreading activity, unlike DNA dependent RNA polymerase which has the proofreading activity. So, what we should understand even when a ribozyme is making a copy of itself it is not full proof, it has the ability to incorporate wrong bases; that means, it is incorporating a base which should not have been there.

Say for example, if A is there it should have incorporated a U in the complementary strand. If it there is a G is there it should have incorporated C not any other base. But, by mistake the C can be anything else then that is called a mutation and this mutation increases the possibility of changing structure.

And, because the RNA also carries a code and this code can evolve, this code can change and this complexity from the mutation is very much likely and this can add a lot of changes, lot of variations in the existing molecule. So, although a template is being copied into a complementary strand, that complementary strand is not 100 percent the same. And, this will allow either to acquires a new character or simply lose the character and perish from the system.

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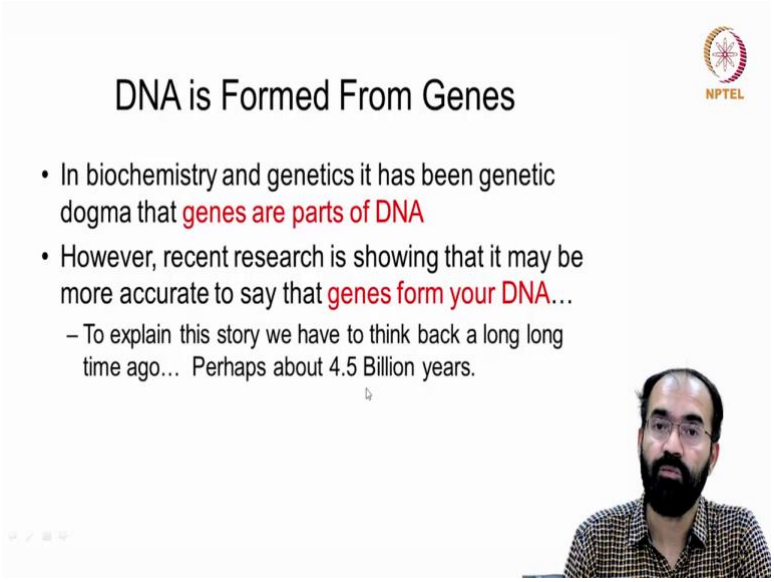

Now, let us think at what point or what time this there was a decision to convert the RNA to into DNA. We should understand normally we study in classes that genes are nothing but DNA and the genes are encoded in the form of DNA. Now, we should understand DNA is formed from the genes. It may sound little bit you know inconvenient to understand.

Here, what is gene? Gene means a property; gene means a quality. Like when you say this person have got so and so gene and this organism have got so and so gene, it is always associated with a property. But here RNA has a property, what is that property? It is able to make a copy of itself and it is able to do catalysis and this one is now retained in the form of a gene and now we can say this property formed first.

And, then it is registered just like you earned lot of money, lot of gold and you put in locker. So, we cannot say that locker discovered gold. We will say the gold discovered or the presence of gold or some precious item made the people to discover locker. Just like you know in like in colloquial form term we will say that which came first, chicken came or egg came first. So, this is always a big question.

Similarly, in this case we always say the property which is encoded in the RNA now stored in the form of DNA. So, the DNA is formed from genes. Genes here what we are referring to as a given property or a given quality. In the biochemistry and genetics, it is always been the genetic dogma that genes are part of the DNA.

However, recent research is showing that it may be more accurate to say that the genes form your DNA. Genes here what we are referring to as a property. To explain this story we have to think, your time scale is long because you have to think 4.5 billion years that is the age of the earth itself. It may sound little impractical, but the time span what has gone through for carrying out this large-scale experiment is sufficient enough to carry out all these biological reactions.

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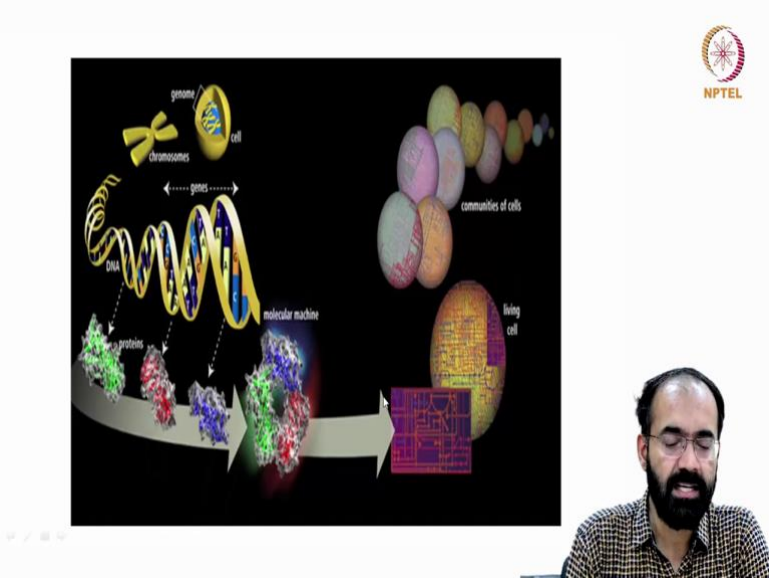

So, this is a cartoon which shows it is a single cell as a nuclease and it has a genome in the form of chromosomes and then the chromosomes when you loosen it, it becomes the double stranded DNA. And, this DNA interact with various proteins and eventually can give rise to RNA transcriptional machinery and these genes what it is being formed from the DNA is allowing to behave a given behavioral property to a cell and these cells become different communities of the cell.

So, every organism started from a single cell deployed cell that we call it a zygote, but no organism remain like a zygote. Although, every cell have got the information of the entire organism, a given set of genes are expressed and that allow a group of cells to differentiate from the existing group of cells and that allows tissues and organs to be formed during development, that is how complex organisms arose later on.

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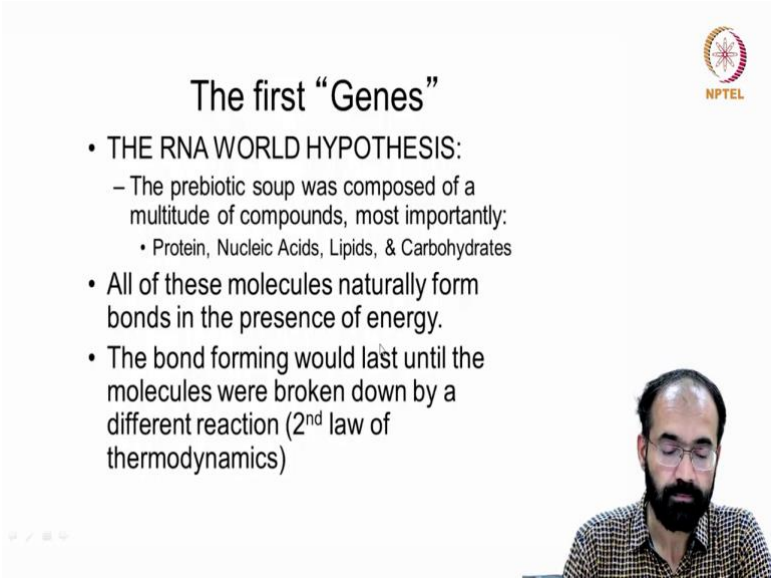

So, the first genes if you think about learning the light or the putting the information, we got it from the RNA world hypothesis. The prebiotic soup that was composed of multiple components not just RNA and this include protein, nucleic acid, lipids and carbohydrates. All were there, but none of these molecules were able to sustain itself. Although, they are present their collaboration or their congregation enhanced their ability to survive.

Although, this molecule protein is stable, nucleic acid is stable, lipid is stable, carbohydrate is stable there are all there, but stable is not its a relative term. But, when they come together, they always had a chance of having a better survival or better meaning for their survival. All of these molecules naturally form bonds in presence of energy, let it be chemical or physical.

The bond forming would last until the molecules were broken down by a different reaction that is basically the 2nd law of thermodynamics. So, it is like if someone ask how long a organism can live? As long as it is alive or as long as someone did not kill it or as long as someone kill it, there is a end point for the organism. Same law applies to the molecule also, a molecule is stable until when it is degraded.

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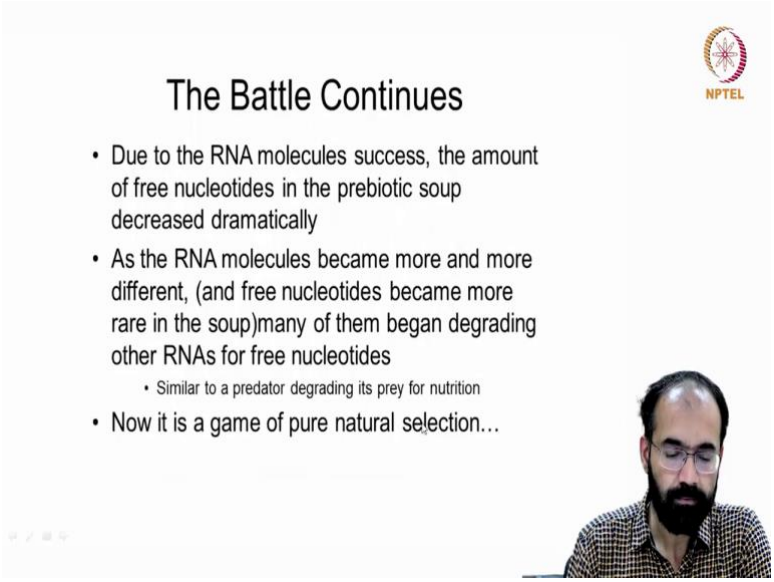

So, this battle continues for longer and longer time. Due to the RNA molecule success, the amount of free nucleotides in the prebiotic soup became lesser and lesser and lesser because there is always a demand. Like you can think about in the old planet earth there was lot of gold and people kept on mining and mining and mining and all golds are either in pure gold blocks and you know in the form of ornaments.

Now, the density of golds have decreased and decreased and decreased, but of course, it is still there of course, people are keep on mining. Same logic applies, the more and more nucleotides are being utilized to form the RNA; the nucleotides became a scarcity. As the RNA molecule became more and more different and free nucleotides have become more rare in the soup, many of them began degrading other RNAs to get the free nucleotides; is just like I have enough food to eat, I do not bother others.

But I do not get enough food to eat, I was catch hold of another organism and eat whether it is a plant or an animal. If I have enough food I do not bother. Same logic applies when it comes to RNA molecule. So, those ribozymes which is now capable of making a copy of itself should also have the ability to cleave other incapable RNA molecule that is a some kind of predation.

So, similar to a predator degrading its prey for nutrition, now it is a game of pure natural selection who wins. Like if I am 50 kilo body weight, if a 100-kilo body weight fellow comes, I will lose. If a 20-kilo body weight fellow comes I win. So, the size of the body

decides whether I will be a winner or loser. Same logic applies how effectively a given RNA was able to make a copy of itself and it can degrade other RNA molecule, no matter what is that other RNA molecule. So, it can grow.

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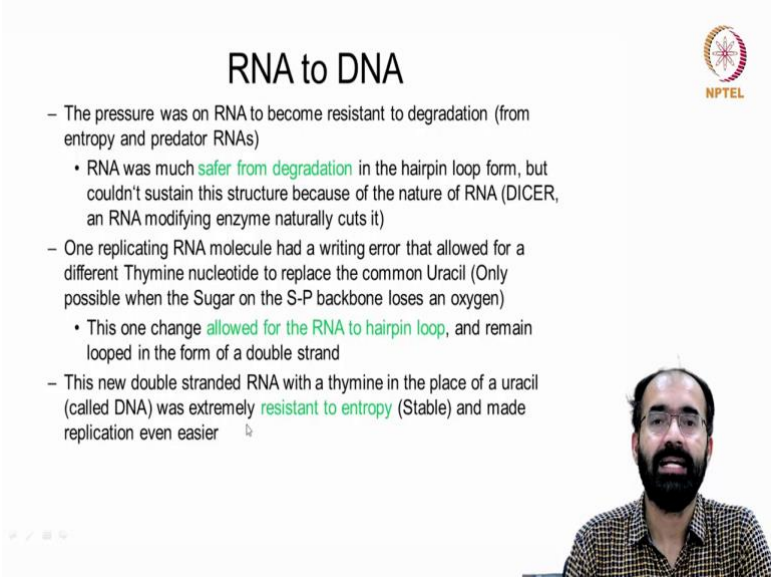

So, now RNA eventually turns into DNA with circumstantial pressure. The pressure was on the RNA to become more resistant to degradation, but we should understand RNA has got its own innate property. And, this degradation it has to resist from the entropy and also from the predator RNA molecule, another RNA molecule that is trying to attack.

So, RNA molecules at least a group of RNA molecules became helpless, they want to survive somehow. So, RNAs was much safer from degradation in its hairpin loop form because it is quite it forming a secondary structure which protects vulnerable areas and sequences from the exposure to the external environment.

But it could not sustain the structure because of the nature of RNA itself because if an RNA has to perform its task just like in winter season you are wearing so many jackets and jackets and jackets, in winter season you are feeling very cold, but if you want to take bath you can take bath with jackets on right. So, same way in the prebiotic world you have plenty of this RNA molecule that are protected from its secondary structure.

However, when there is a need for replicating it has to unwind it which makes it vulnerable. Like you can see a typical enzyme, it is a protein enzyme called dicer, it is an

RNA modifying enzyme that has an affinity specifically for given secondary structure RNA; that means, it do not depend on an RNA based on its sequence, but a secondary structure makes a template. Dicer will catch hold of an RNA template provided if that RNA is in a given secondary structure or a stem loop structure.

So, one replicating RNA molecule had a writing error, why I said about the dicer is because there can be another ribozyme that can detect a substrate RNA; although it is protected in its own secondary structure. It is in its secondary structure become attractive for another RNA to take it as a substrate. So, that is no longer a valid option of saving itself.

So, one replicating RNA molecule had a writing error that allowed writing error means copying error while making a copy of itself; that means, error in the complementary strand allowed for a different thymine nucleotide to replace the common uracil. Remember, RNA always have got uracil, but now all of a sudden this uracil, the place of uracil a mimicking molecule appeared that is thymine.

Only possible this is possible only when the sugar on the S-P backbone that loses 1 oxygen, that is two conditions happen. Thymine is welcome in the place of uracil provided it do not have the oxygen group in the 2-prime carbon of its ribose sugar. So, this was a condition which allowed a better stability to this RNA molecule. This one change allowed the RNA to form a unique hairpin loop and remain looped that is very important, remain looped in the form of a double bond.

So, that it gets an extra stability. So, this new double stranded RNA with a thymine in the place of uracil, now we can technically call it as a DNA was extremely resistant to entropy that is very stable. Resistance to entropy means is increasing the stability and made the replication even easier, because if a molecule is stable then it can undergo replication much much easy.

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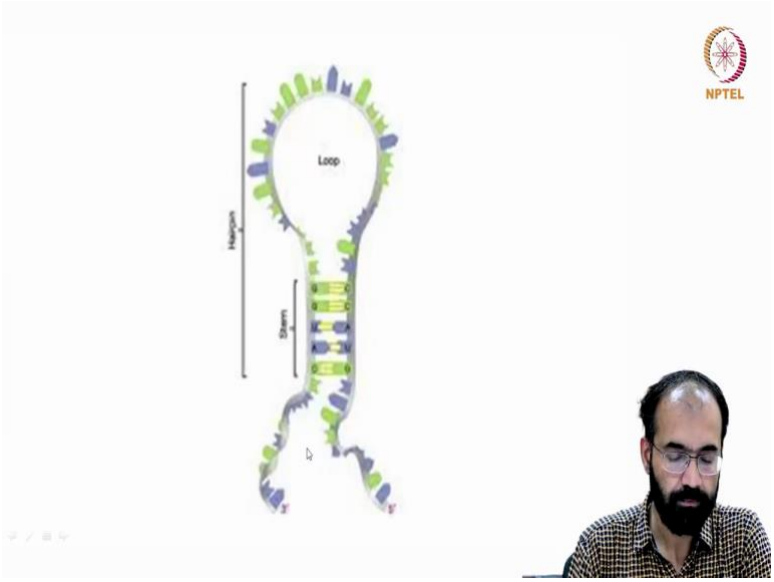

So, this is what a crude structure of stem loop, RNA molecule and this change in the place where A has to pair U, it can if it is changed into a T then it has a higher chance of survival.

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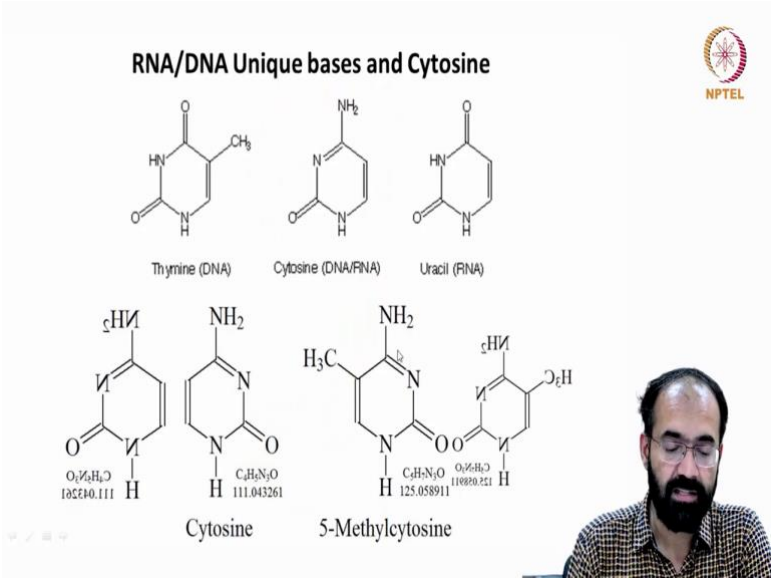

Now, let us look into the molecular structure of thymine, cytosine and uracil. They are pretty similar, you have a thymine here, you have a cytosine here, you have a uracil here. If you look closely, you can see uracil and thymine are very much similar except for a methyl group, otherwise they are basically the same. Same way you can see cytosine and

methyl cytosine. Like in DNA many attempts like I told you the cytosine can undergo further change that is we called it as a methylated cytosine.

So, that is another change which DNA acquires to protect its some information which we will not go into the detail. But why these mirror images are kept for this cytosine is to have similarity and complementarity that it is similar to that what you see here, you have a methyl group here which is very much similar to the methylated cytosine what you are seeing here.

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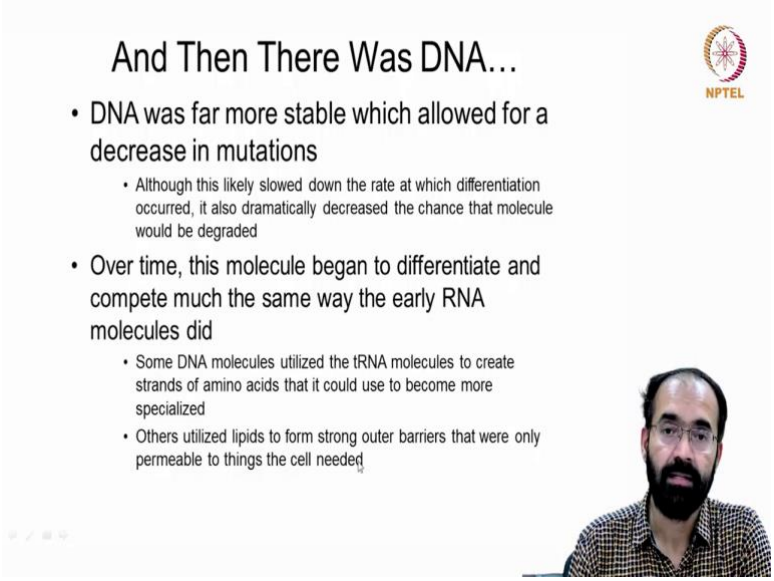

And, then there was the production of the DNA. DNA was far more stable which allowed a drastic reduction in the mutation. So, what you understand from here is that if there is a good quality or a good information that is present, that is preserved for a long time because it is stable and it is resist mutation. Although, this likely showed down the rate at which differentiation occurred, it also dramatically decreased the chance that the molecule will be degraded.

Because, any random change can make the molecule vulnerable for degradation also of course, it can evolve that is one thing. It is like if you are staying in your house, you are not leaving your house you will not get a chance to see the world, you will not get a chance to grow. But, leaving the safety of house makes you vulnerable for predators and you know various other intruding factors.

So, it is a trade between these two. Over the time, this molecule began to differentiate and compete much the same way early RNA molecule did; that means, you have a DNA now the RNA got converted into DNA, it is very stable. And, then it has no ability to evolve then although with less efficiency or less frequency if there is a change and if that change allow some extra features to be present then it becomes very effective way of evolving for the DNA in the DNA form itself.

Some DNA molecule utilized another small RNA called tRNA molecule, transfer RNA we will see them more in detail in the subsequent classes. But, remember it is one of the species of RNA called transfer RNA molecule to create strands of amino acids, amino acids are building blocks of protein that it could be used to become more specialized.

That means, now you have got a DNA strand which can utilize transfer RNA and this transfer RNA carries amino acid and you can assemble them and give rise to a protein molecule. So, now it is almost like I am inviting a friend to my home and who is a very efficient singer. So, if I invite him for the party, if he sings, I am saving money for a musician whom I should have invited.

Same way this DNA molecule can make use of the tRNA and assembled a protein then this protein can perform a task. And, now this DNA is not 1, it is 2. It is able to perform two tasks: one is storing the information stably in the form of DNA, then it can produce a protein that can perform a task. So, this gets a advantage.

Others utilized lipids to form strong outer barriers that were only allow them to store, that allow permeable to things that they self needed. So, some DNA made use of the tRNA and allowed assembly of the amino acid whereas, the other one made use of the lipids and now it is a question of time who is winning this battle.

And, we will continue about this in the next class.