RNA Biology
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Lecture - 03
Introduction to RNA Biology and RNA World-Origin of Monomers

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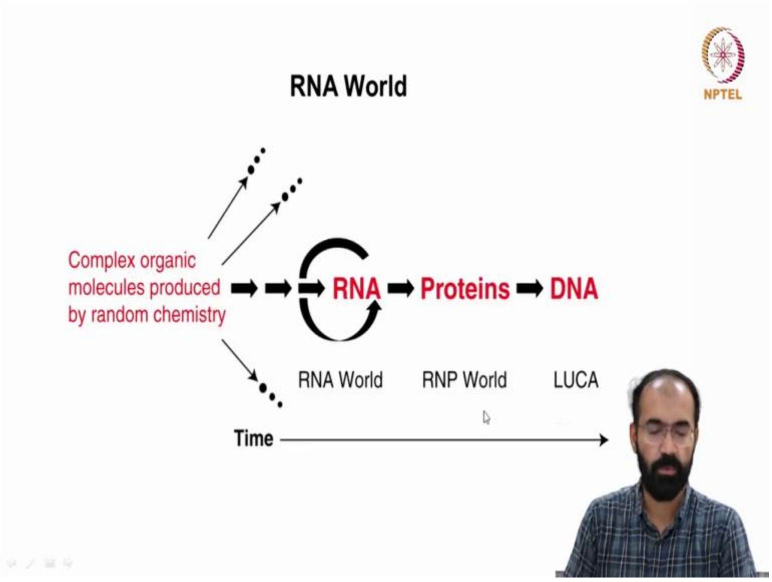

Welcome back! To continuation of another class of RNA Biology. So, we were here in the last class that we reached up to LUCA that is Last Unique Common Ancestor. And, this LUCA has acted as the fundamental life form which eventually evolved into unicellular and multicellular organism.

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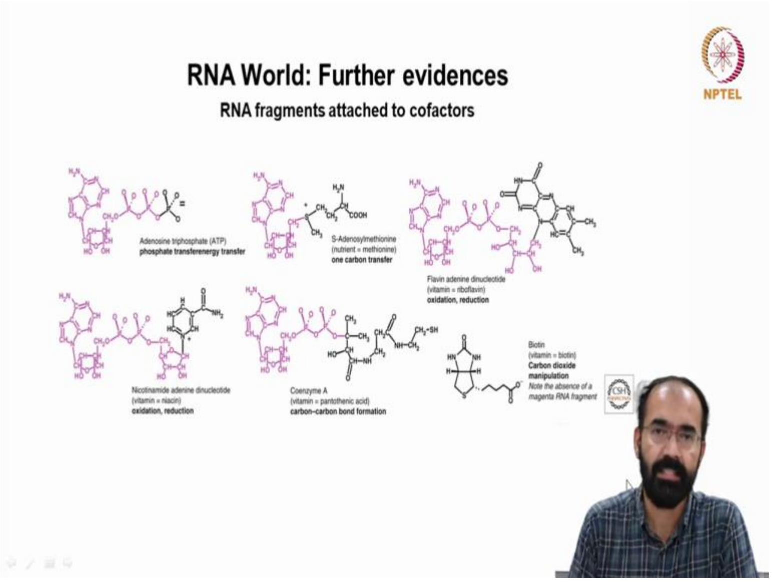

Now, let us think do we have any evidences for the RNA world? We have been saying about RNA world a lot, but do we have any evidence in the modern world. So, do we have any relics? We have already mentioned about two of them. One is existence of ribozymes and also the presence of ribosomes. More than that do we have any real RNA nuclei left out in the living organisms of the modern world and some of them are listed here.

The classic example is the ATP. All of you would have heard about ATP that is adenosine triphosphate, which is the major source of energy for almost all biological reaction. And, and I am not forgetting about GTP because many signaling events rely on the GTP which is guanosine triphosphate, ATP stands for adenosine triphosphate.

This molecule have got an adenosine nuclei followed by 3 phosphate groups. So, this third phosphate is the one which is used for energy yielding reactions and this is nothing but a monomer of RNA or DNA. So, this is ATP is a monomer of RNA not DNA because, we will come to the difference of the ribose sugar much later. And, then another example is S adenosylmethionine and then comes flavin adenine dinucleotide.

Some of them we classify them as vitamins, vitamins are essential components of several protein enzymes in the living system. And, then you can have niacinamide which is we also call it as niacin of which is coming under the B family of vitamins. Then, you have the classic coenzyme A and then we have got the biotin. So, these are all molecules where we call them as vitamins etcetera, but they are all part of the RNA world which staying back even today in the modern life forms including humans.

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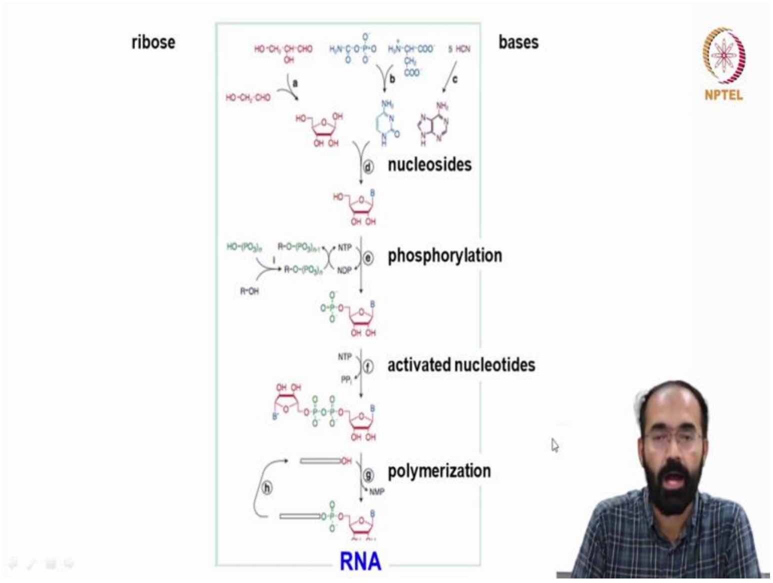

Now, let us think how a RNA monomer is formed. We know the ribose and the base plus the phosphate adds to a nucleotide. So, how does this happen? The nitrogenous base can be formed from HCN or hydrocyanic acid and also the phosphate the phosphate can come from the rock weathering and ribose can be ribose sugar can be formed by the foremost reaction.

And, you can also have purines and pyrimidines that is formed from the inorganic material and this can eventually combine and we call them as nucleosides. And, this eventually can combine with phosphate that is formed from the rock weathering and we call it as phosphorylation. In biology, any molecule that is added with a phosphate group we usually call them as phosphorylation.

So, the nucleosides which is nothing but nitrogen base plus sugar that is the pentose sugar when added with phosphate group, we call that phosphorylated nucleosides that is also called as activated nucleotides which can undergo the so-called polymerization reaction. Polymerization happens between 2 nucleotides and which direction we can discuss later. But, this assembly of this monomer the so-called nucleotides can give rise to a extended molecule called RNA.

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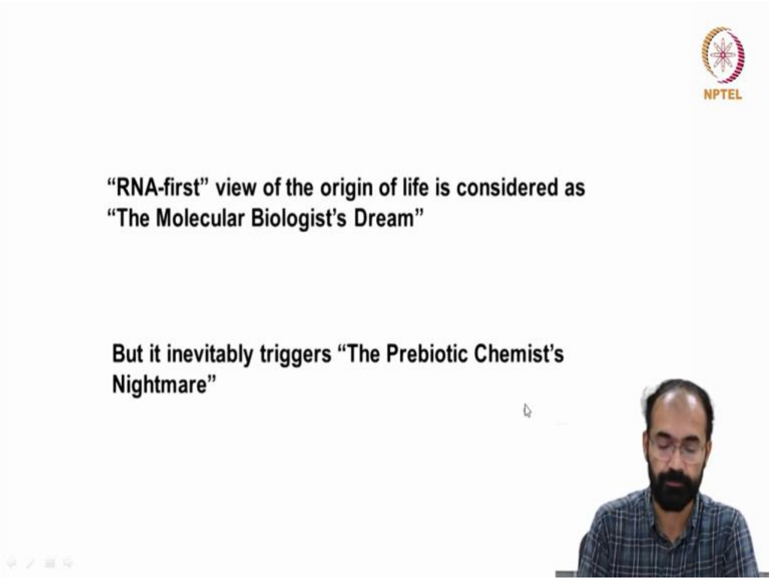

Now, RNA first is a view of the RNA world hypothesis and it is basically is considered as a molecular biologist's dream. Because, it is very easy to explain how the life or the molecular structure or the cell biology of a living organism evolved over the years, but it also triggers a prebiotic chemist nightmare.

So, we can understand that RNA first molecule concept is a molecular biologist dream because it is much easy to explain whereas, when you talk about the chemistry it is also a nightmare because of the conditions in which the prebiotic world existed.

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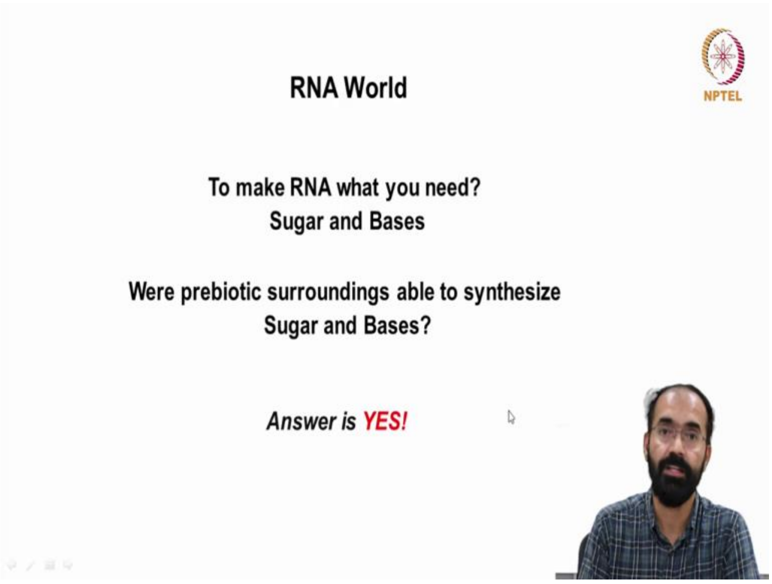

So, let us look back into the RNA world again. To make an RNA what you actually need? We know that it needs sugar and bases. But, when the prebiotic surroundings are able to synthesize sugar or base in the proportion and in the quantity, were they able to produce? This is a big question, because although you can produce 1 molecule or 2 molecule that is not going to trigger the production of a the so, called RNA molecule capable of giving rise to life forms.

So, what we should understand that this should have been a feasible reaction and a preferred reaction and also a sustainable reaction. And, the answer is yes; despite all the odds the conditions of the prebiotic world was very much in favor of not only production of sugars and bases, but they also get selectively stabilized and preferred. Let us see whether how can we go about this further.

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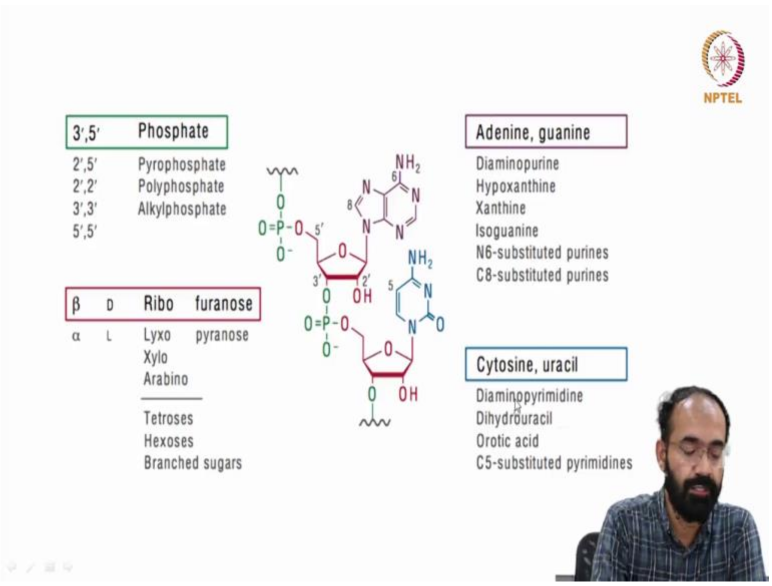

If you look into this left panel top, few molecules are kept in box that 3 prime, 5 prime phosphate and you also have 2 prime, 5 prime pyrophosphate, 2 prime, 2 prime polyphosphate, 3 prime, 3 prime alkyl phosphate and so on and so forth. And, if you see left hand side down you also have beta D ribo furanose and you have got alpha L lyxo pyranose. What this D stands for? Dextro rotatory that is the chemistry, this optical activity of organic molecules and L stands for levo rotatory.

Those who are studied chemistry will know that optical activity is a property of various chemical molecules which has the ability to rotate the plane of polarization of a plane polarized light while in solution. I will not go into the detail because it is part of chemistry. But, remember all amino acids in a living system are L forms whereas, the carbohydrates are D forms in all living organism. Exceptions are there, but in by and large that is how it is preferred.

So, you are talking about different types of sugars in the left-hand panel down here and at right side you can see adenine and guanine. We know they are purines and you also have diamino purine, hypoxanthine, xanthine, isoguanine etcetera etcetera. And, remember of all these purines that are formed adenine and guanine are only circled and you can guess why it is so.

And, in bottom you have a list of pyrimidines that is cytosine and uracil are given in circle whereas, you also have diaminopyrimidine, dihydrouracil, orotic acid etcetera. And so, what this shows that in prebiotic world due to the lot of physical and thermal activity a lot of molecules could have been formed and existed.

However, not all of them are recruited into the RNA structure. Some of them are recruited, what could be that reason? And, we should not forget that this hypoxanthine and xanthine these are all pigmentation molecules even in flowers, even today. Because, a plant produce this molecule by chemical reactions to give coloration to various you know parts of its flower etcetera.

So, these molecules are still there, but they are not part of the RNA molecule. So, like you can see here in this slide whichever mentioned in box, they all have given rise to becoming eventually part of the RNA.

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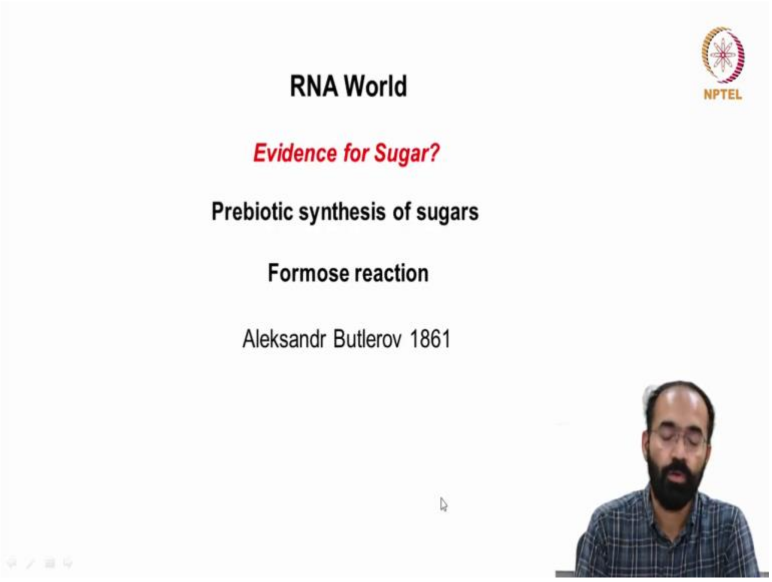

So, looking further into the RNA world, do we have evidence for sugar and is it possible that prebiotically sugars can be synthesized? And, the answer is yes. And, the reaction is called Formose reaction and it was discovered by Aleksandr Butlerov in 1861, that Formose reaction is pretty simple.

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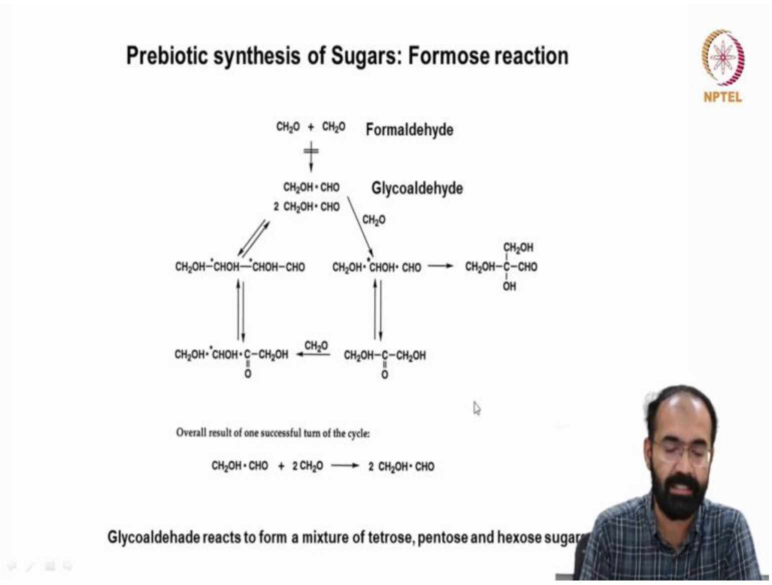

It is although it is a lengthy process, the starting material is formaldehyde. What is the chemical formula? CH2O and formaldehyde can combine and formaldehyde can be spontaneously formed in the prebiotic world and they combine and give rise to glycoaldehyde. And, they again combine with formaldehyde and give rise to intermediate compound.

And, remember they are reversible reaction; that means, reaction can go in either way. Eventually, you can end up getting glycoaldehyde that can react with a bunch of tetrose, pentose and hexose sugars. And, you end up getting a diverse sugar molecule and remember the ribose sugar is the one which is preferred and recruited into the RNA molecule. Reasons can be many and which we will discuss one by one.

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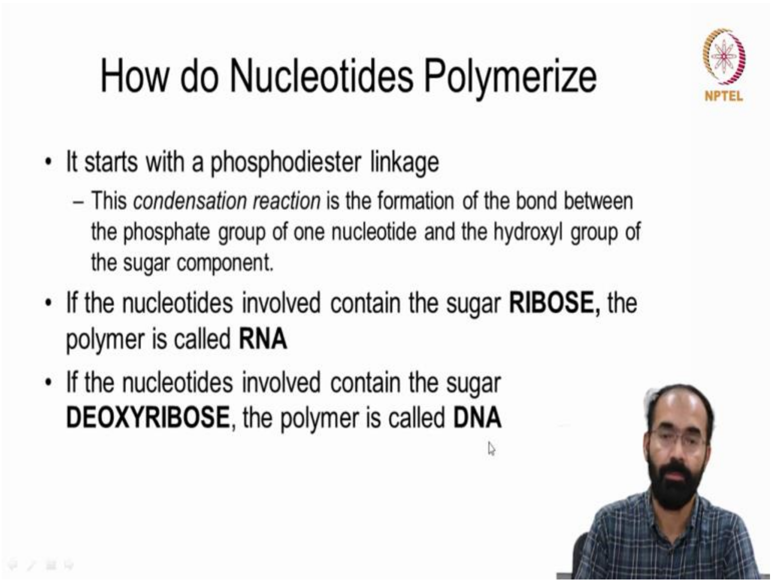

So, let us quickly think how do the nucleotides polymerize. Polymerization we know that the assembly of the monomers which is the nucleotides and once they polymerize, the end product has to be a polymer called RNA. So, it starts with a phosphodiester linkage. What is a phosphodiester linkage? Phosphodiester linkage basically is formed between a sugar and phosphate group which continues over downstream chain. It is formed by a condensation reaction.

A condensation reaction is what? 1 hydrogen molecule and a hydroxyl molecule react together and 1 water molecule is formed; H plus OH. So, one molecules H and another molecules OH, together participate in release of an H2O, 1 water molecule will be released.

And, here in this formation the phosphate group and the sugar participate the pentose sugar, ribose sugar participates in this condensation reaction and it continues. And, this sugar like I told you in the previous class it has got 5 carbons; carbon number 1, carbon number 2, carbon number 3, carbon number 4, carbon number 5.

So, the phosphate reacts with the carbon number 5 and the subsequent downstream the 3 prime carbon participate in the downstream and the 5 prime end reacts with the phosphate group, like that the chain continues. And so, we should understand the bond formed between the phosphate group of one nucleotide and the hydroxyl group of the sugar component and you end up getting a release of water molecule, H2O will be released.

And, this is called the fundamental backbone or the basic backbone of a RNA molecule. So, the nucleotides involved contain the ribose and this phosphate reacting with ribose which already containing a nitrogen base which we call it as nucleoside. And, this polymer now we call it as nucleotide polymer or RNA or ribo nucleic acid.

If the nucleotides involved that contains the sugar deoxyribose, then the polymer is called DNA. So, like I told you the ribose sugar have got 5 carbon atom and the 2nd and 3rd carbon is very important. In DNA, the 2nd carbon do not have an oxygen whereas, the third-one have got a hydroxyl group, 2nd carbon has a OH group in the case of RNA.

But, in the case of DNA the 2nd carbon has got only the H, oxygen is missing that is why we use the word deoxy whereas, the 3 prime of both DNA and the RNA; the 3 prime carbon has got OH group which participate in the polymerization reaction. So, the 3 prime and the 5 prime is important molecule, 3 prime and 5 prime carbon of the pentose sugar or the ribose sugar is very important for the polymerization reaction.

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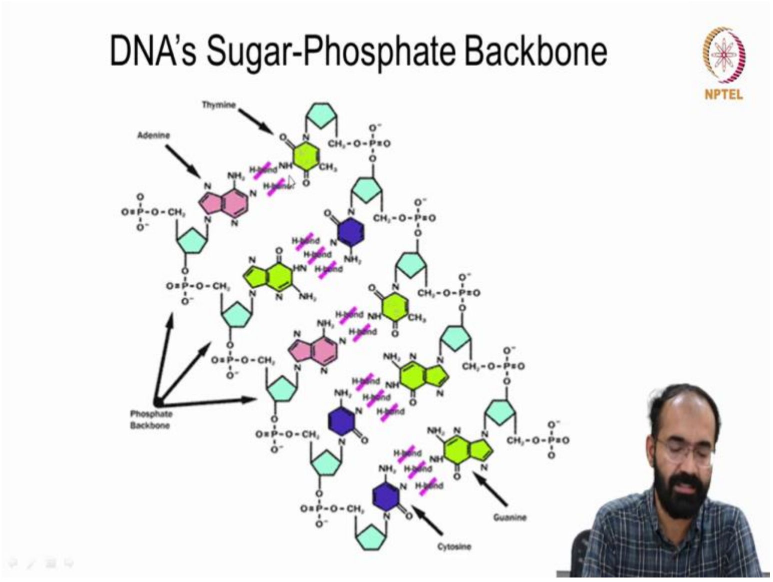

So, this is what you can see the DNA or the RNA that has got a typical sugar phosphate backbone as you can see here and the nitrogen space is capable of pairing with another nitrogen-base pair of another RNA or another DNA and this adenine nitrogen base pairs with the thymine and whereas, the guanine nitrogen base pairs with the cytosin. So, adenine pairing with thymine or a adenine pairing with uracil.

Remember, RNA prefers uracil over thymine, although they are molecularly very close by, we will see them more in detail. A always pairs with T or U in a double bond whereas, G pairs with C. This is the short form. A is the short form of adenine; T is the short form of thymine or U for uracil and C for cytosine and G for guanosine.

So, G to C pairing is always a triple bond, A to T or A to C is always a double bond as you can see here. Remember, this is shown with respect to DNA, but RNA also have the ability to form such pairing.

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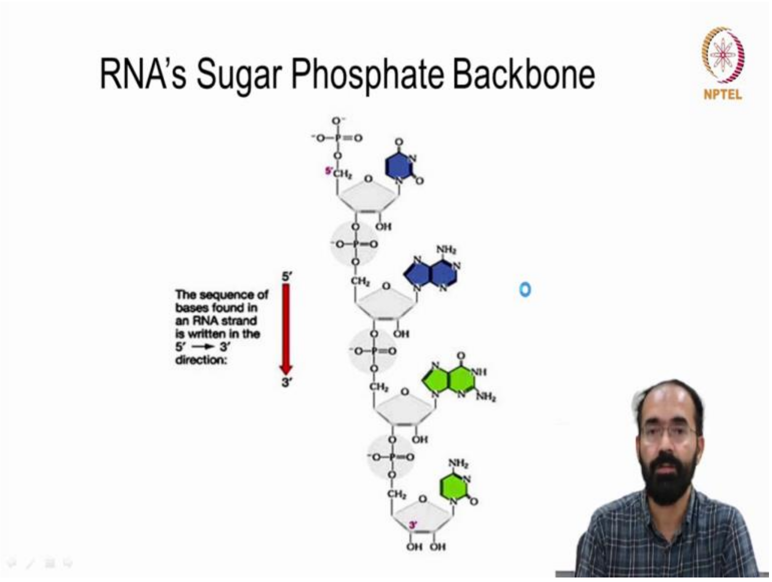

So, the RNAs sugar phosphate backbone is very much similar to that of the DNA, but only difference is the sugar has got OH group in the 2 prime, rest everything remains the same. And, there is also a directionality, when you talk about the RNA or DNA for a given strand we always refer from 5 prime to 3 prime end, that is the 5 prime carbon to the 3 prime carbon.

And, say if I want to say left side and right side, if I am giving you a RNA which is like a ribbon; it has got which is lying in front of it has got a left hand and a right end. So, the left hand will always will be 5 prime and the right end always will be 3 prime. We will come to more discussion about this much later, time being you just need to know this much.

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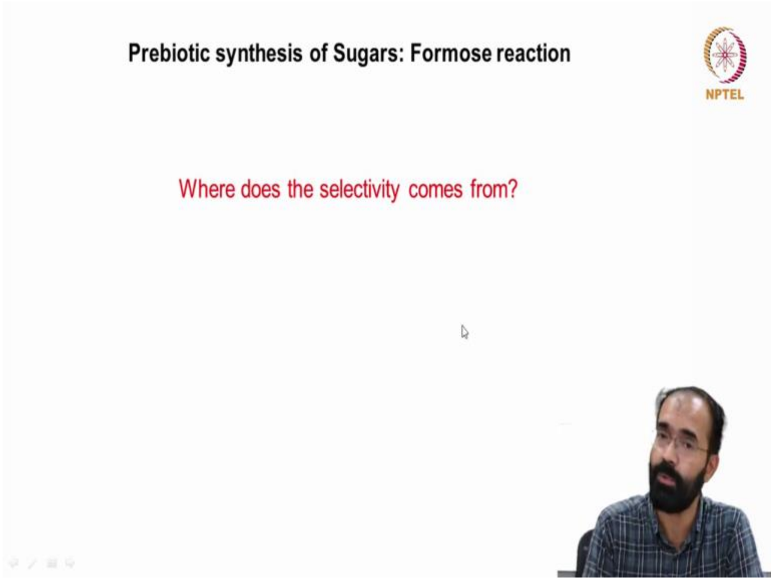

So, if you think about the prebiotic synthesis of sugars by Formose reaction, we have to understand lot of such molecules are formed by the Formose reaction. Because, formose reaction do not favor a pentose sugar ribose, it can favor lot of molecules and we have seen many of them are bi direction.

So, what is so much great about this pentose sugar ribose? Where does the selectivity come from? Who is selecting? Is there any someone sitting and selecting ok, this molecule is important for RNA? That is something which we have to bother.

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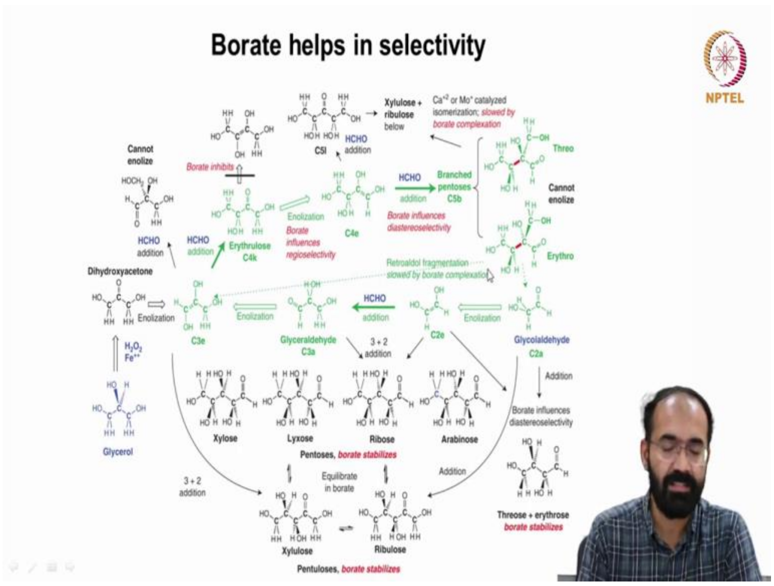

And this is a complex slide, but do not worry about the molecular structure in detail. What we should understand is there is a mineral that is called borate mineral that is very important in preferring selectivity; that means, if you see certain molecules borate influences diastereoselective whether D is to be preferred over L or a pentose sugar is to be preferred over hexose sugar or any other more complex structure is all decided by the borate mineral, that is present.

It can borate can also influence regioselectivity and borate can inhibit the propagation to a particular form. Say a pentose sugar should not become hexose sugar, that can be inhibited and borate can also stabilize some specific type of pentose sugar and borate along with various other molecules can facilitate the selective accumulation of a given type of sugar.

This does not mean that borate will prevent the formation of other sugar molecule, but presence of borate minerals can enhance the availability of a given type of sugar. It is like if you see a given fruit item is available in a fruit market, in mango season if you go you will see every vendor will have only mango.

So, if you go for buying fruit, you there is a good chance that you end up returning home with mangoes. Same way if you have only a given type of pentose sugar then there is a good chance that pentose sugar will be incorporated in meaningful or without any meaning into some or the other molecules. And, credit goes to the so-called selectivity provided by the borate mineral.

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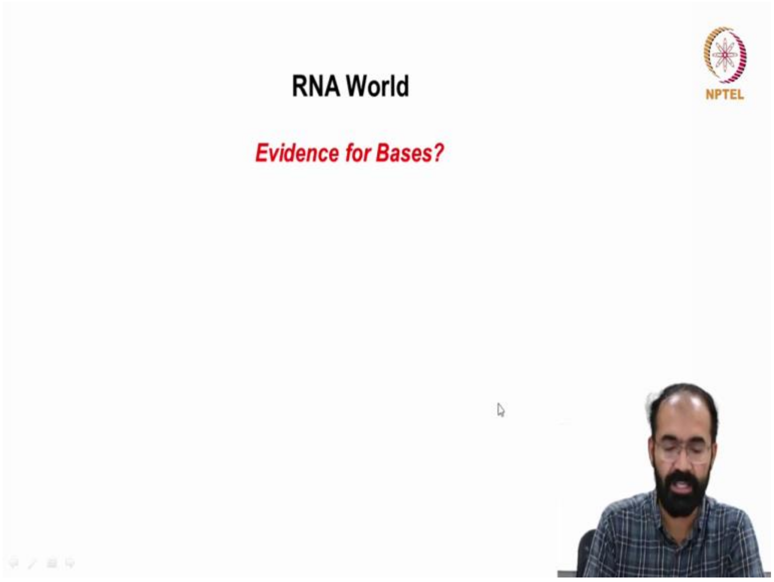

And, let us think in RNA world do we have evidence for bases that are being formed?

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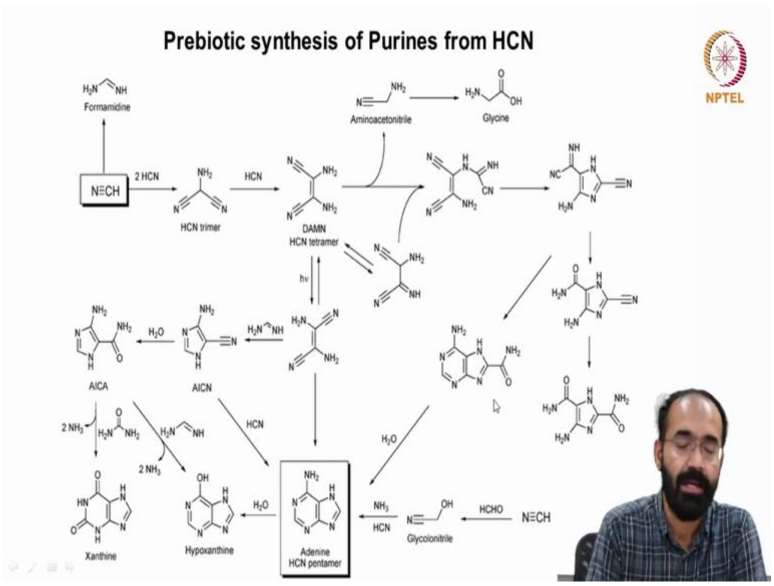

And, you can see hydrogen cyanide that 2 hydrogen cyanide can form a dimer or hydrocyanic acid and it can keep on combining and it can give rise to a sequential reaction event. And in the end of the day, you end up getting adenine which is nothing but a pentamer of hydrogen cyanide.

Hydrogen cyanide or hydrocyanic acid which is also present in lot of tuber plants like such as tapioca. It has got like that is why if you are cooking some of this underground tuber plant, you put them in water for a long time to get rid of hydrocyanic acid or hydrogen cyanide. Hydrogen cyanide remember at high concentration can be very toxic. So, otherwise people end up getting headache etcetera.

So, hydrogen cyanide is a very easily available molecule. All it has hydrogen, carbon and nitrogen. So, this is abundantly present in the prebiotic world. So, as you can see here xanthine can be formed, hypoxanthine can be formed and also adenine can be formed. Similarly, other purine that is the guanine also can be easily formed by similar chemistry. So, purines can be formed as a polymer of hydrogen cyanide.

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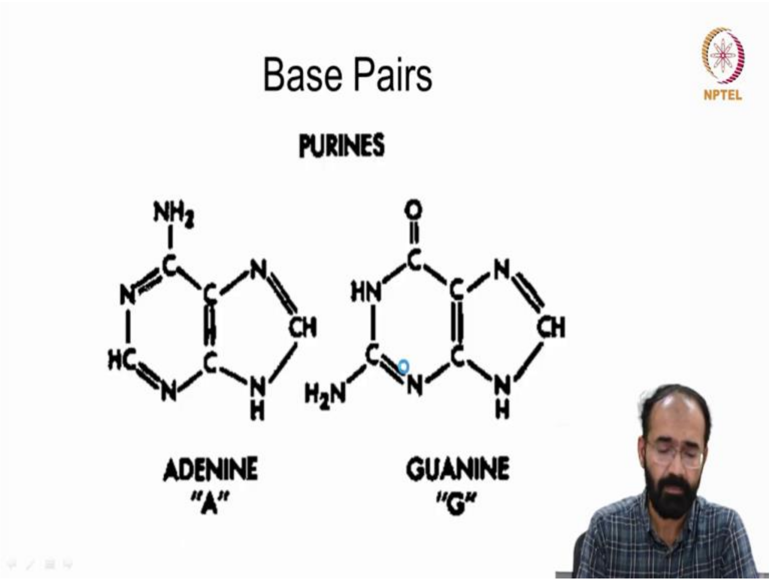

And, let us look into the structure of the purines. Adenine and guanine, they are very much similar in structure except that an amino group is in the adenine and whereas, the amino group is changed in this position in the case of guanine and the place where the amino group is there in the adenine now you have got a oxygen. Whereas, rest of the chains and parts are not very drastically different; they are more or less the same and they are coming under the category called purines.

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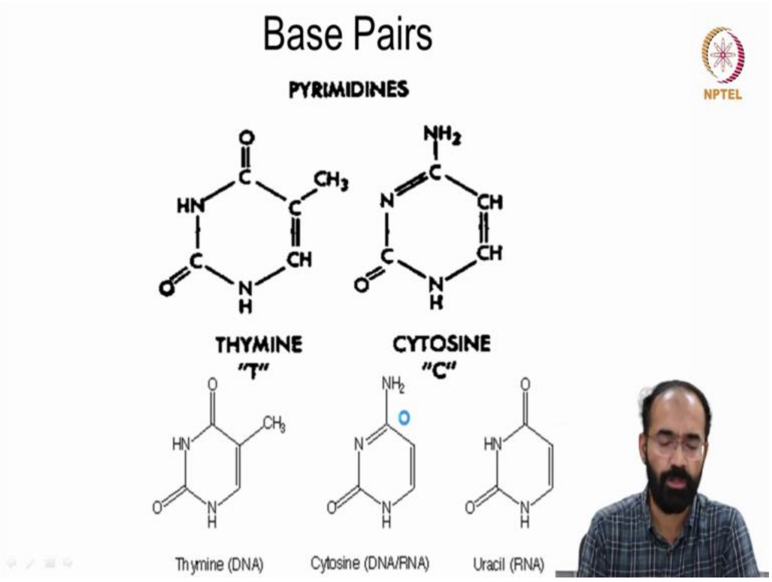

Now, let us look into pyrimidines. Pyrimidines are of two types: thymine and cytosine in the DNA whereas, uracil and cytosine is in the RNA. If you look closely into the structure, they are also very much similar that the thymine has got a methyl group whereas, cytosine has got a amino group.

If you look closely thymine and cytosine and then I have mentioned here about the uracil. Uracil and thymine are not present together, in DNA you have got thymine whereas, RNA you have got uracil. both cannot exist in one molecule. Either any one of them only it can exist in the entire species of RNA species or DNA species molecules and cytosine is somewhere in between.

Interestingly, what you have to see is uracil and thymine are one and the same except for one methyl group. We can also call uracil as methylated uracil that is thymine. So, if you take uracil and add a methyl group it becomes thymine and we should also understand in like in higher order epigenetics you also study the cytosine also can undergo similar methylation and we call it as methylated cytosine.

So, understand the uracil has undergone methylation to become thymine which eventually become part of the DNA, otherwise there is no difference between the uracil and thymine.

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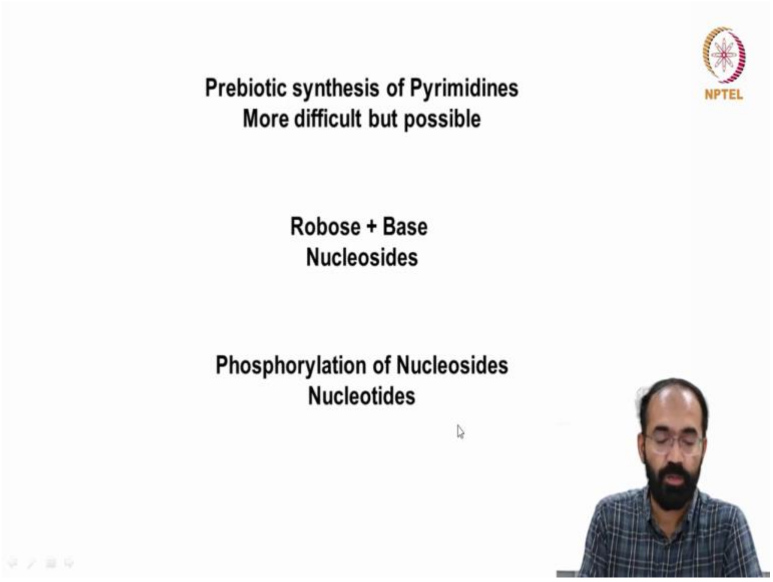

Now, if you think about the prebiotic synthesis of pyrimidines, it is little difficult than purine, but it is definitely possible. And, the ribose and the base can combine to give rest to nucleoside and the phosphorylation of this nucleosides give rise to nucleotides. So, what are we proving here? We are proving that we can easily have all the monomers of the RNA and which can assemble together and become eventually an RNA molecule.

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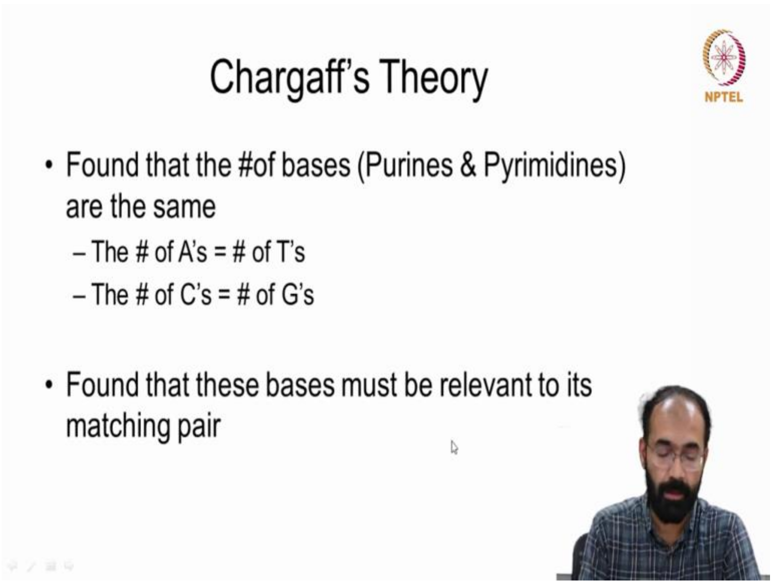

So, now let us quickly go into Chargaff's theory. Chargaff is a scientist who discovered by chemical means that the number of adenines is equal to number of thymine on a DNA and the number of cytosine is equal to number of guanine. This kind of give clue probably A have something to do with the T or C have something to do with the G.

And, this gave the hypothesis that they might be pairing, this was very useful for the discovery of structure of the DNA. And, he found that the bases must be relevant to its matching pair else it is impossible to have a equivalents number between A's and T's and G's and C's.

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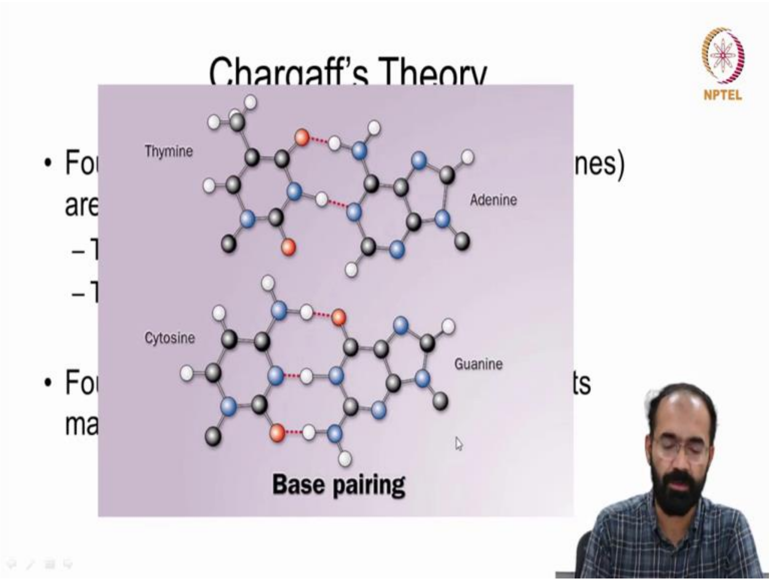

This is what you can see from the picture. Thymine always pair with adenine, cytosine always pair with guanine. And, remember in the case of RNA, thymine need not be thymine it is the uracil, but the pairing rest of them remains the same.

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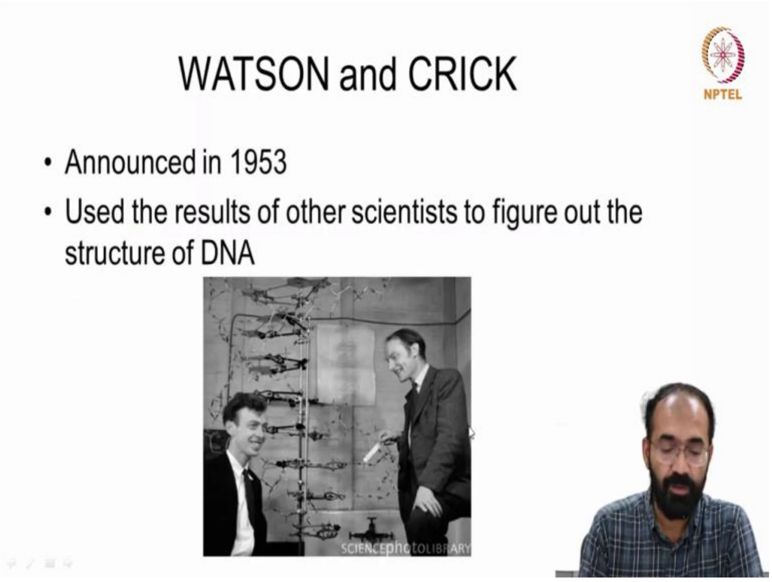

And, in 1953 Watson and Crick discovered the structure of the DNA and this emphasized and this established this pairing concept and this is very important. You may wonder why talking about DNA in an RNA biology class, because the discovery of DNA has proven the ability of this nucleotides or the nucleic acids to polymerize and also pair because DNA is a pair of two strands and they form a DNA double helix.

And, they depended on the results extra crystallography results from Maurice Wilkins, Rosalind Franklin etcetera and they discovered the structure of the DNA. So, this is a picture of Watson and Crick.

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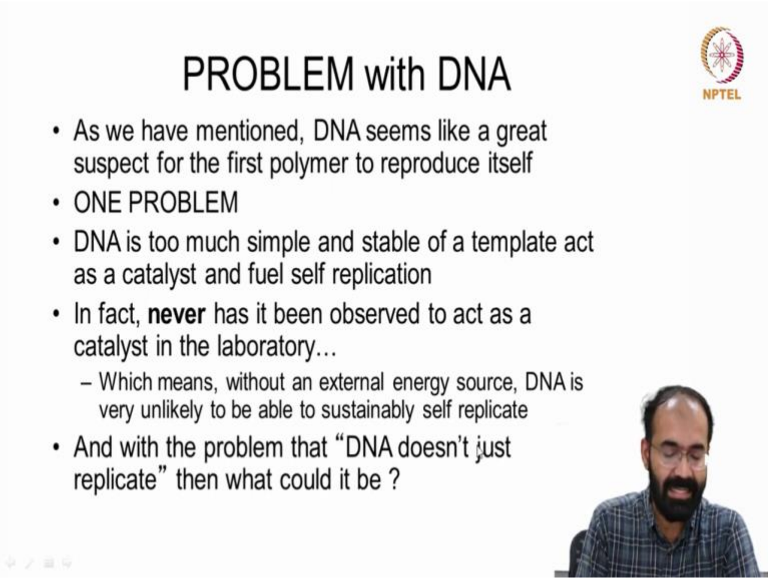

And, let us think what is wrong? If DNA has got lots of such features, pairing, structure etcetera etcetera, why not think DNA is the starting material, why RNA? What is so unique about it? As we have already discussed, the DNA seems like a great suspect of being the first polymer to reproduce itself. But, is it genuinely true? Because, it poses a big problem; what is that problem?

Problem is DNA is too simple and extremely stable to act as a template and to act as a catalyst and to fuel itself to form a new copy. Like we already discussed that if any molecule, any life form has to have any success; it should be able to make a copy of itself. If it did not make a copy of itself, it will disappear from the face of earth. So, DNA is very stable, but is it active? No. Can it be more dynamic? The answer is no.

Then, how can it perform a function? And, the answer shows that it simply may not be a good candidate to act as a early biological molecule and especially when it comes to self-replication. So in fact, never ever DNA has been shown to act as a catalyst in a laboratory. Like various approaches have been taken to prove whether DNA can act as a catalyst.

No, DNA never could perform any biological reaction on its own, remember on its own. Here, we are talking about without any help from other outside which means without any external energy source DNA is very unlikely to be able to sustainably make a survival on its own or in this case it is self-replication.

And, the problem with DNA that DNA does not just replicate, then what it could be doing? We will see that more in detail that why DNA is important and what is the contribution of DNA, what could it be so special about the DNA. And, why the so-called RNA world eventually turned into DNA and what is the benefit it was getting. Those things need to be discussed more in detail and we will continue with that in the next class.

Thank you.