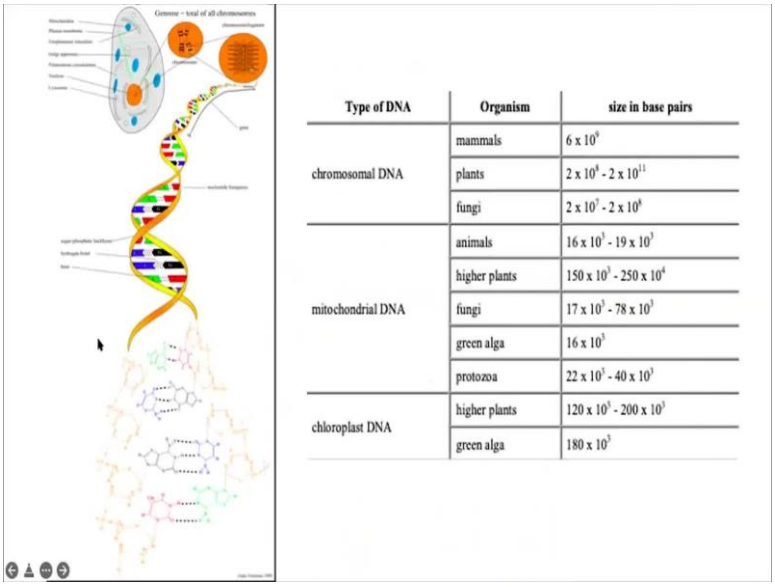
Introduction to Cell Biology
Professor Girish Ratnaparkhi
Professor Nagraj Balasubramanian
Department of Biology
Indian Institutes of Science Education and Research Puneoo=====
An Overview of Central Dogma of Molecular Biology - Part 2

(Refer Slide Time: 0:17)



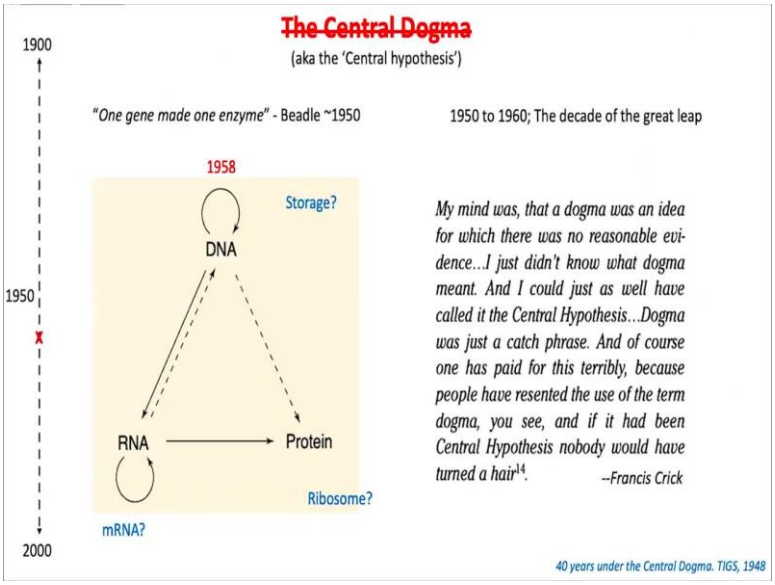
Type of DNA	Organism	size in base pairs
chromosomal DNA	mammals	6×10^9
	plants	$2 \times 10^8 - 2 \times 10^{11}$
	fungi	$2 \times 10^7 - 2 \times 10^8$
mitochondrial DNA	animals	$16 \times 10^3 - 19 \times 10^3$
	higher plants	$150 \times 10^3 - 250 \times 10^4$
	fungi	$17 \times 10^3 - 78 \times 10^3$
	green alga	16×10^3
	protozoa	$22 \times 10^3 - 40 \times 10^3$
chloroplast DNA	higher plants	$120 \times 10^3 - 200 \times 10^3$
	green alga	180×10^3

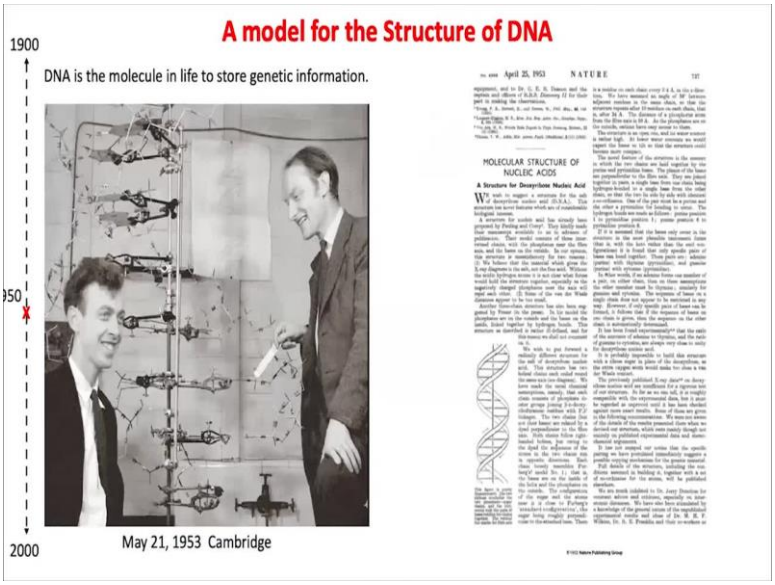
Student: Sir what is base pair?

Professor: What is base pairs? If you look over here, each one of these is a nucleotide over here and when two nucleotides of opposite strands connect to each other through hydrogen bonds, we call this a base pair. So for example, A and T are for A from one strand is hydrogen bonded to the T on the other strand, and together A and T make up a base pair, because DNA pretty much is always in a double standard form.

Student: Okay sir, thank you.

(Refer Slide Time: 0:52)





Student: Sir, why do we believe that protein synthesis does not directly come from DNA, why is it not feasible or why do we believe this?

Professor: So this is all a hypothesis. In 1958 these were the proposals, but these proposals were completely hypothetical, these were models the same way DNA as shown over here is a model, models are what you think is happening, but somebody has to prove it, it took 10 years to actually collect crystal structures of DNA to prove that Watson and Crick were actually right. And that was done by crystallographers.

Similarly, there is something happening inside our cells, we can propose what is happening using the information we have gathered from literature by researchers who are actually doing

the experimental work, but what is happening is what is happening, you cannot change that somehow evolution has reached a stage where certain biochemical reactions are happening, and other reactions are not happening.

So information is flowing from DNA to RNA to protein, information in our cells is not flowing from DNA to protein. Now, maybe there is a universe out there in a planet where information is flowing from DNA to protein. But to the best of our knowledge in the last 70 years of research, information is not flowing directly from DNA to protein, because there are not any molecular machines, which are inside the cell which can do this job.

For example, if I asked you, can you make a molecular machine which can take a protein sequence, which is a series of amino acids and make RNA, it turns out that there is no such machine inside the cell, cell can make DNA from DNA, the DNA dependent DNA polymerase, cell can make RNA from DNA, which is DNA dependent RNA polymerase, cell can make RNA from RNA, RNA dependent RNA polymerase.

So, these machines, these enzymes are present inside the cell. And other enzymes are not present inside the cell and why is it, it has something to do with fitness and evolution.

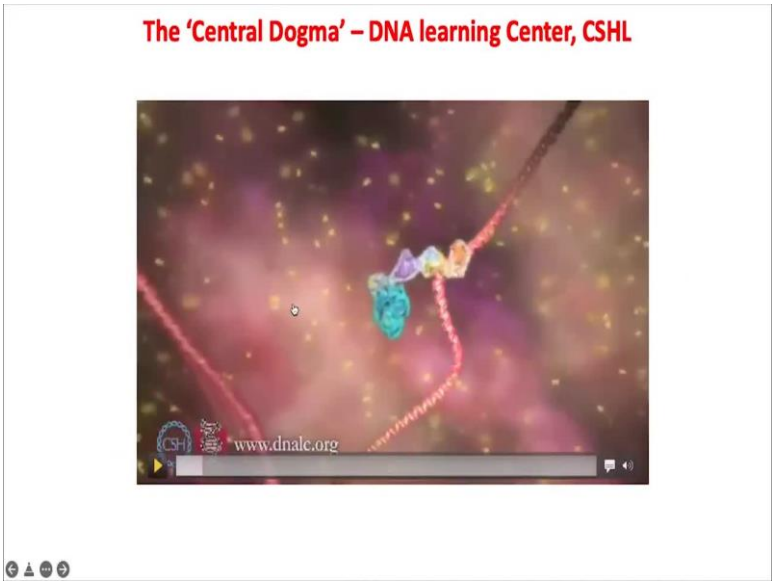
Student: Okay sir.

Professor: Any other question?

Student: Sir, please can you repeat the X-ray crystallography and fiber diffraction method.

Professor: I will actually go back to that in the next class in a little bit more detail. But what you should know very simply is that we are talking of molecules which are extremely small, you cannot look at them through a microscope.

(Refer Slide Time: 3:28)



Their size scale is to the 10s of angstroms, which is pretty much what this this movie is trying to show. Okay, they are very, very small and how do we take pictures of these very small proteins and DNA and the way we do it is we purify them, so that there is only one species for example, you can purify DNA, or you can purify this transcription factor, or you can purify RNA. So when you purify them in let us say milligram amounts, and you put them in certain salts and buffers, certain special salts and buffers, they tend to crystallize.

And when they crystallize the crystalline lattice is such, it is structured in such a way that if you point X rays at a crystalline lattice, you get diffraction patterns, the X rays bounce through the crystalline lattice and give you a sort of a, I will show you pictures of what a X ray diffraction image looks like. These images can then be interpreted to build the molecular structure of the protein or the DNA or the RNA you want to solve.

So, that is how crystallography is done. For fiber diffraction, when you cannot form a crystal, the molecule refuses to form a crystal, it is not easy to do. You can take fibers and you can point which are basically wet fibers, these have a lot of water in them, you can point X rays that are in a certain direction, which is basically along the axis of the fiber and you can get diffraction patterns.

And these diffraction patterns basically will allow you to interpret the possible spacing between and the possible length scales of the bonds inside the actual structure. So, you can use X ray crystallography as a tool to get diffraction patterns and the diffraction patterns

through a process which is complicated allows you to solve the molecular structure of the molecule which are looking at, maybe DNA RNA or protein.

Student: Thank you sir.

Professor: Any question?

(Refer Slide Time: 5:33)

Student: Sir, could you please give a brief description of what a thymonucleate is?

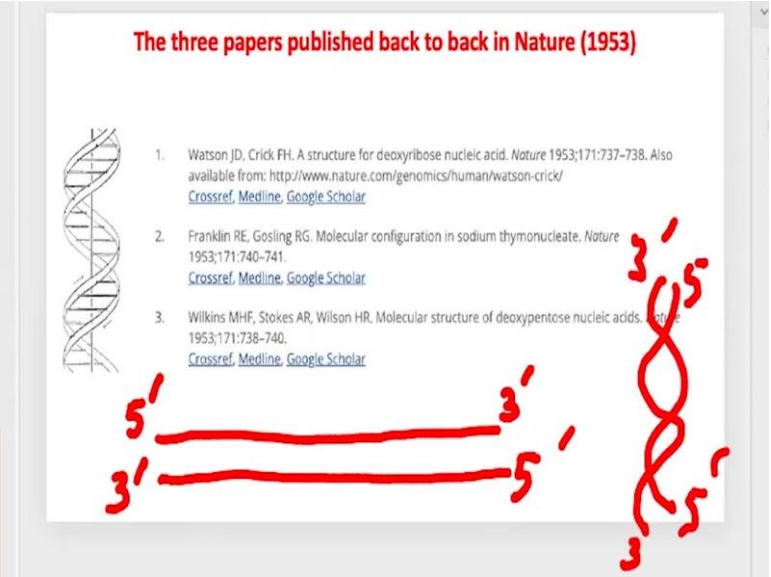
Professor: So, these are basically, these are terminologies which are not used, basically, it is a sodium salt of DNA.

Student: Okay, sir.

Professor: So, these are terminologies which modern biology does not use. It is a sodium salt of DNA.

Student: I do not understand the meaning of double-stranded DNA running parallel to each other.

(Refer Slide Time: 6:15)

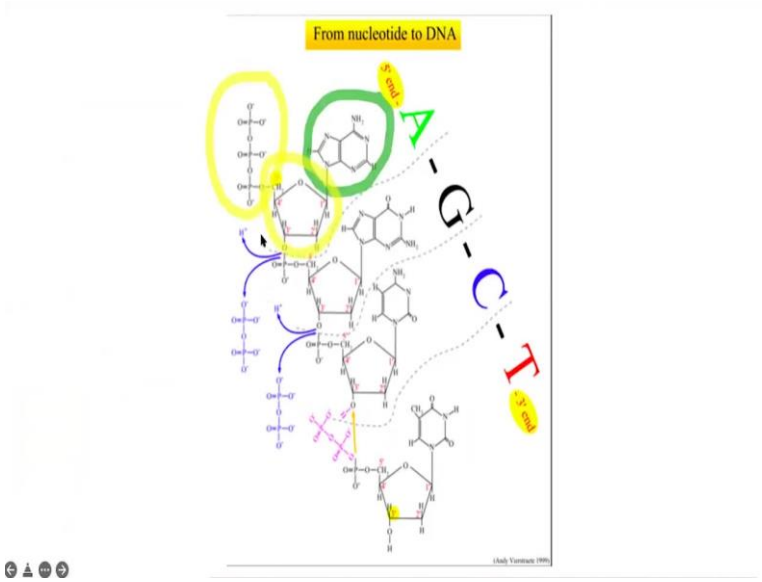


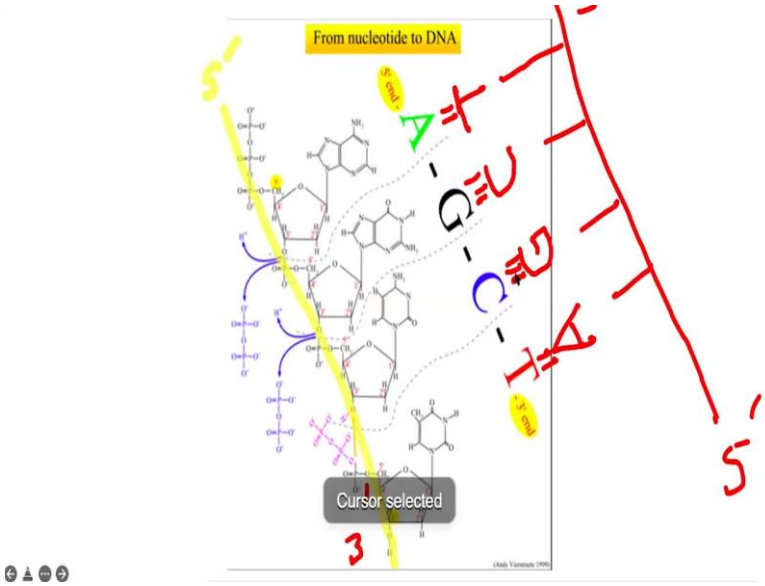
Professor: I am doing a very simple thing over here, but it is for those of you who do not seem to be familiar with DNA, basically, you have a strand of DNA, this is a very simplified way of making a DNA. And in order to make a double strand, you have a second strand of DNA. Now, we use terminology and I will explain this terminology that a strand of DNA has polarity, it starts with a 5 prime phosphate and ends with a 3 prime of the phosphate backbone.

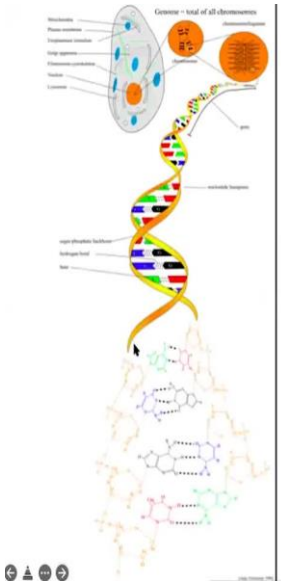
And the second strand which is forming this double helix is anti-parallel, it runs in the opposite direction. So these 2 strands basically form the double helix with 5 prime, 3 prime and 3 prime 5 prime. Now what is this 3 prime 5 prime is something I will explain to you.

Student: Sir in one of them you wrote 3 prime 5 prime where in the second one it is 3 prime and 5 prime.

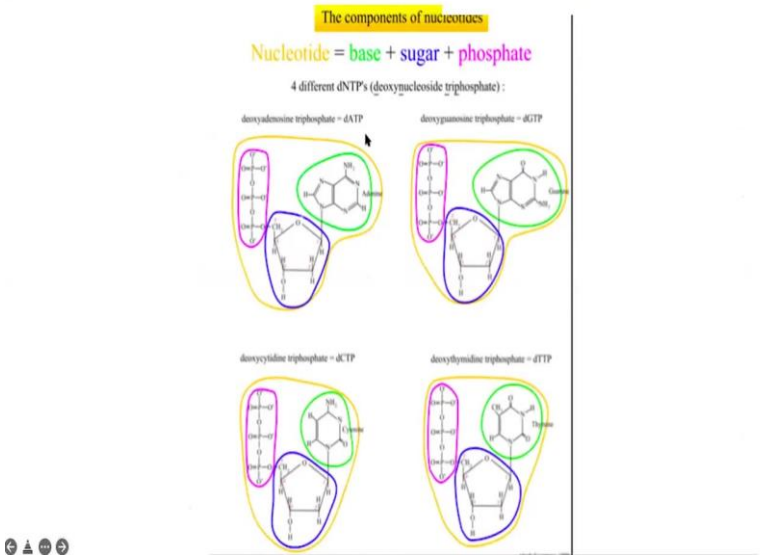
(Refer Slide Time: 7:15)







Type of DNA	Organism	size in base pairs
chromosomal DNA	mammals	6×10^9
	plants	$2 \times 10^8 - 2 \times 10^{11}$
	fungi	$2 \times 10^7 - 2 \times 10^8$
mitochondrial DNA	animals	$16 \times 10^3 - 19 \times 10^3$
	higher plants	$150 \times 10^3 - 250 \times 10^4$
	fungi	$17 \times 10^3 - 78 \times 10^3$
	green alga	16×10^3
	protozoa	$22 \times 10^3 - 40 \times 10^3$
chloroplast DNA	higher plants	$120 \times 10^3 - 200 \times 10^3$
	green alga	180×10^3



Professor: So, basically, these are 2 strands which are anti-parallel. Now, let me try and explain them to you over here. Now, these are the 4 nucleotides of DNA, here is the nucleotide. So, this is A-Adenine, Guanine, Cytosine, Thymine. Now, the orientation of the strand is 5 prime to 3 prime, because, so, now, let me just start see if you can understand this. So, this is the nucleotide, this is the sugar and this is the phosphate backbone still remained yellow, that is fine.

So, now, what is happening is if you look carefully, you will realize that the backbone of a single strand of DNA is basically this phosphate backbone, do you see a backbone over here and the nomenclature is such that this is the 5 prime end of the phosphate and the 3 prime end of this nucleotide will attach to the 5 prime end of the next nucleotide to the next nucleotide to the next nucleotide and so on and so forth.

So, you are connecting these 4 nucleotides A G C T, through the phosphodiester backbone. So, this is the phosphate this is the backbone which is connecting A G C T together. Now, what happens when you connect these nucleotides together using phosphodiester bonds, these 4 nucleotides are projected out from the strand and opposite to these nucleotides, when you make a double strand.

Let us see if I can do that. So, there will be a second double strand over here. And basically there will be a projection of T over here, C over here, G over here and A over here and this is in the orientation of 5 prime to 3 prime and this will be in the orientation 5 prime to 3 prime and these 2 strands of DNA the one on the left and the one on the right will come opposite to each other and they will form hydrogen bonds and the hydrogen bonds basically GC is a triple hydrogen bond and AT is a double hydrogen bond.

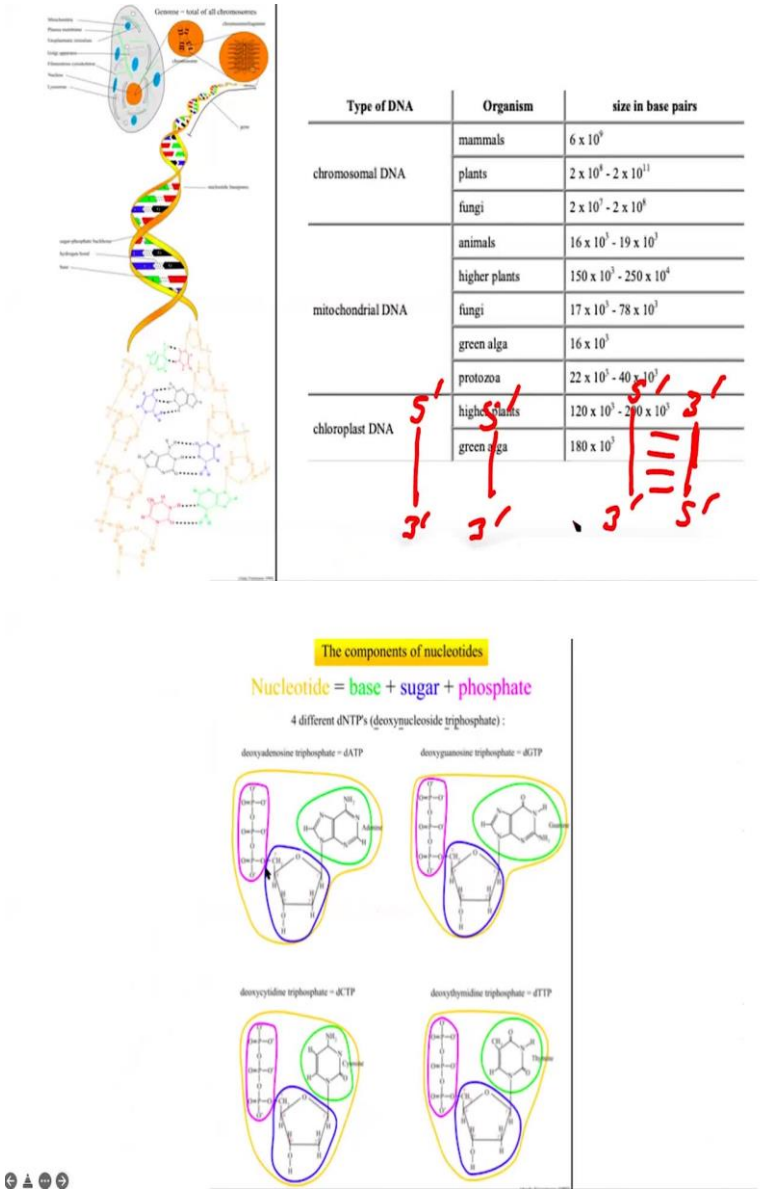
This is something you will you will see carefully when you look at the structure of DNA. So, these 2 strands are going to come together because of hydrogen bonds, which are forming between AT GC for both these 2 anti-parallel strands of DNA, and which is what is shown over here. Here are the 2 strands of DNA over here, and they are coming together using these hydrogen bonds. So the dotted lines over here are the hydrogen bonds.

And for each nucleotide, as I have told you earlier, this is the structure, there is a base, so need to form the hydrogen bond in green, there is the sugar, which acts as a spacer between the phosphate and the nucleotide. And it is this purple part, the phosphate groups, which are going to bond with each other forming the phosphodiester backbone. Is this clear enough? I can repeat this in the next class.

Student: You said that the 3 prime and 5 prime have to do with something with polarity?

Professor: It is directionality, the one strand is going 5 prime, see both strands are going 5 prime to 3 prime.

(Refer Slide Time: 11:03)



Type of DNA	Organism	size in base pairs
chromosomal DNA	mammals	6×10^9
	plants	$2 \times 10^8 - 2 \times 10^{11}$
	fungi	$2 \times 10^7 - 2 \times 10^8$
mitochondrial DNA	animals	$16 \times 10^3 - 19 \times 10^3$
	higher plants	$150 \times 10^3 - 250 \times 10^4$
	fungi	$17 \times 10^3 - 78 \times 10^3$
	green alga	16×10^3
	protozoa	$22 \times 10^3 - 40 \times 10^3$
chloroplast DNA	higher plants	$120 \times 10^3 - 200 \times 10^3$
	green alga	180×10^3

So let me draw it again both strands are basically 5 prime to 3 prime. But they cannot make hydrogen bonds, they cannot form a direct double helix. Unless one strand is 5 prime to 3 prime and the other strand flips and makes 3 prime to 5 prime. And it is only then that these 2 strands can come together to form a double helix. Is that clear?

Student: Yes, sir. So the 5 and 3 are the charges on the phosphate?

Professor: On the phosphate. It is it is nomenclature. It is the attachment of the phosphate to the sugar. And I think I need to define the nomenclature for you, which I will do in the next class. So for example, here is the 5 prime this is this phosphate group that I attached to the 5

prime of the sugar and when it forms a bond, it will be the 3 prime of the of the sugar. I will go through this nomenclature in the next class.

Student: Thank you, sir.

Professor: So I have to end here. I will come back to DNA in the next class.