Introduction to Cell Biology
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Central Dogma: Replication of DNA Part 1

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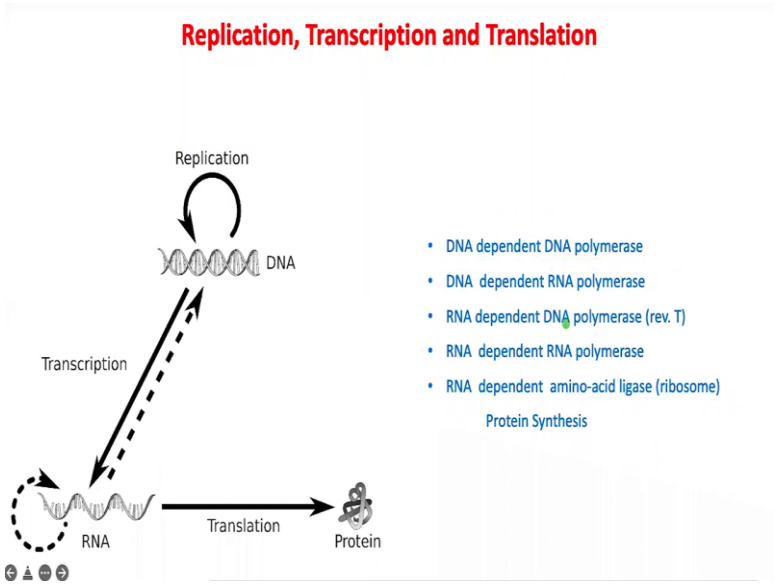

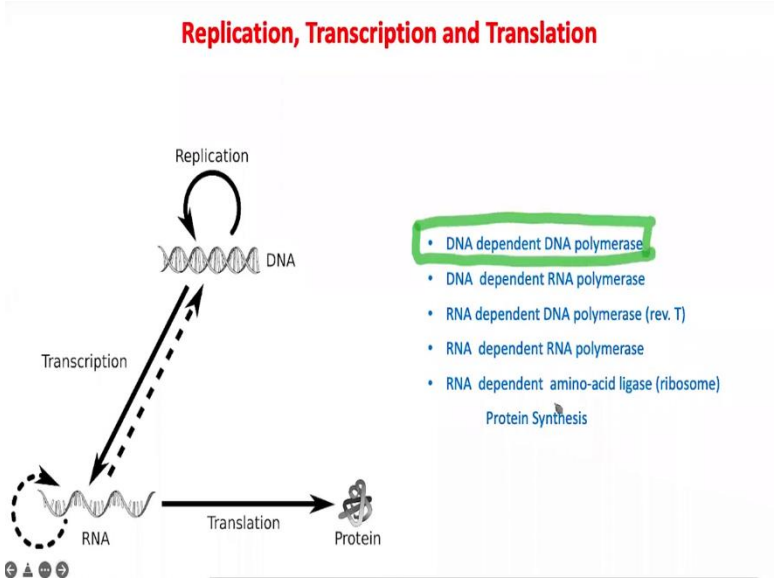

As I said, put the central dogma, the flow of information as the centerpiece of the way I am teaching this semester. And what I will be doing in today's class an tomorrow's class is focusing on replication and transcription. And I will wrap up translation in the next week. And then I will move on to the other parts, which I am supposed to teach you.

So let us look at translation, let us look at replication first. And then we will look at transcription. Remember, each time when I am talking like, these are all protein machines,

with the exception of a ribosome, which is RNA machine itself, RNA protein machines, they all do chemical functions, very, very critical chemical functions.

There are not always a single molecule, there are many DNA dependent DNA polymerases that is something you should understand, especially when we go from prokaryotes to eukaryotes, you will realize there is a world of DNA polymerases out there a world of RNA polymerases out there, and so on and so forth.

These are all molecular machines. And we are going to see one of these molecular machines in action today, which is basically this machine, the DNA dependent DNA polymerase, and supporting it will be other machines, which we will not spend too much time on, and you will learn more about them in future molecular biology process.

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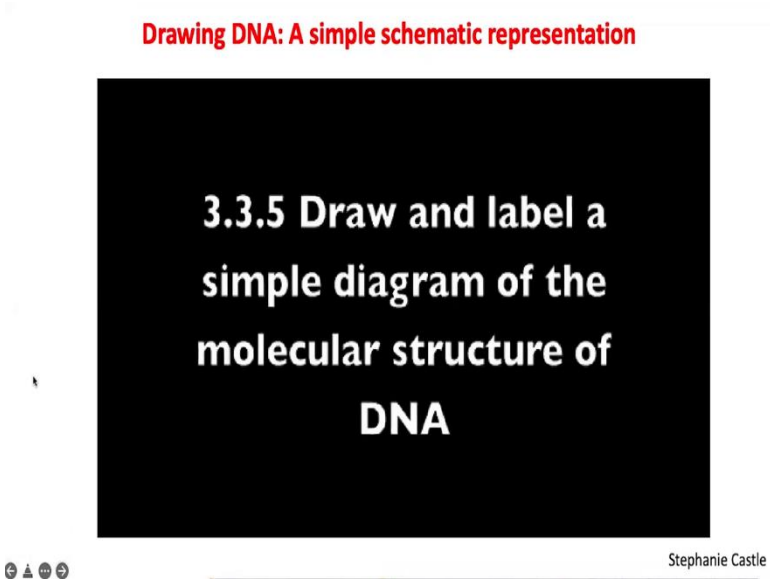

So let us start with the building block. Because the building block is really very, very important. And the reason I am also showing you here.

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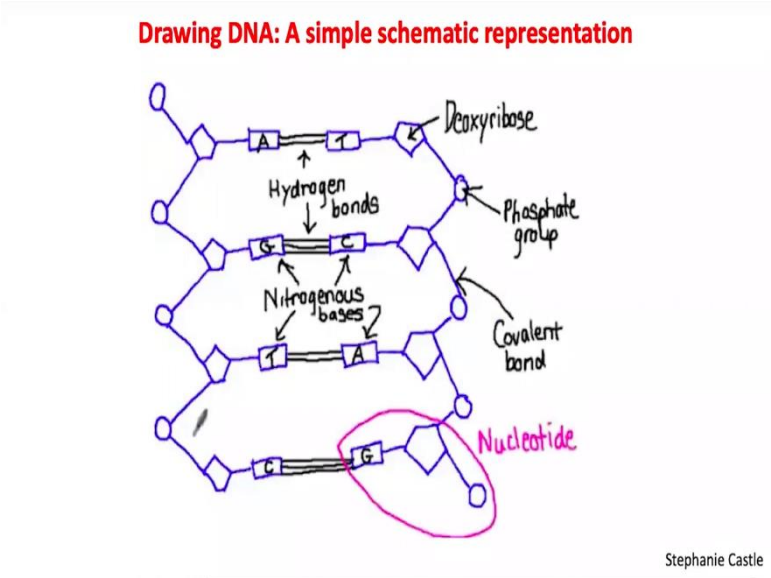

Speaker: So here I am going to demonstrate drawing the simple structure of DNA. And now you do not need to be an artist to do this. So what the only cares about is that you get the relative positions of the phosphate group the deoxyribose sugar, both of those known as the sugar phosphate backbone, and the nitrogenous bases relatively, they need to be in the correct positions.

Now, as I draw the second strand here, please note that it is anti-parallel, my deoxyribose pentose sugar is effectively like pointing down, this is somewhat difficult to draw on a computer screen for you, what you might want to do is actually turn the page upside down, so you are drawing the same shape, it is just in the opposite direction.

Now once you have drawn it, you want to make sure that you label everything so that is the hydrogen bonds, the covalent bonds, the nitrogenous bases, deoxyribose, the phosphate group, and go ahead and draw a line around a nucleotide as well.

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Professor: So this reminds you of what I have shown you earlier. Let us go to replication.

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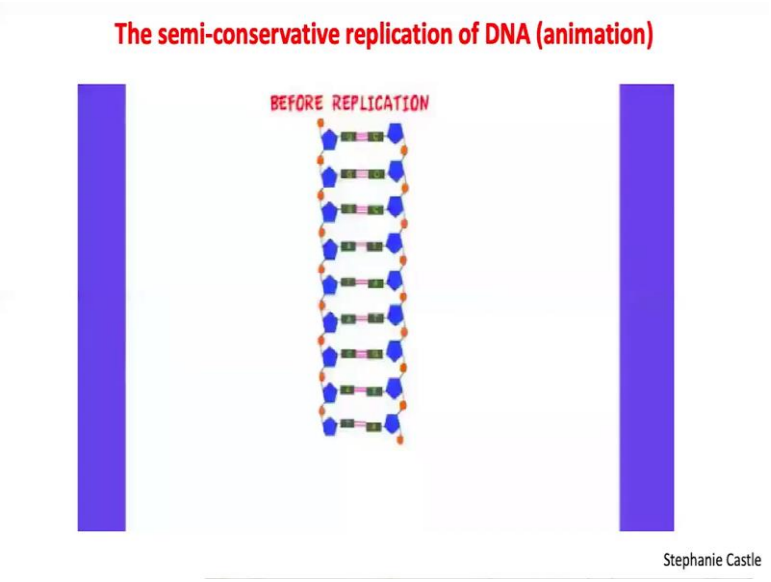
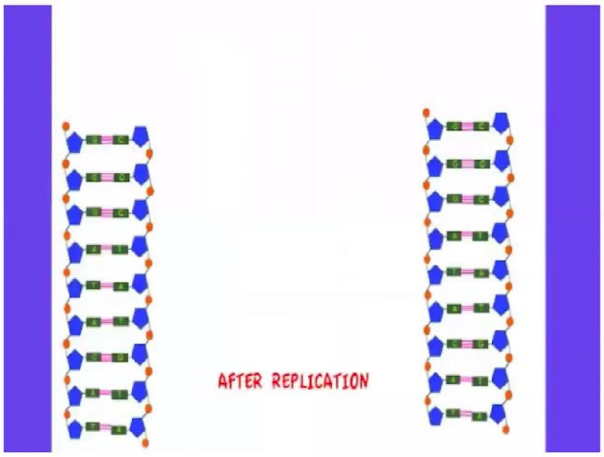


The semi-conservative replication of DNA (animation)



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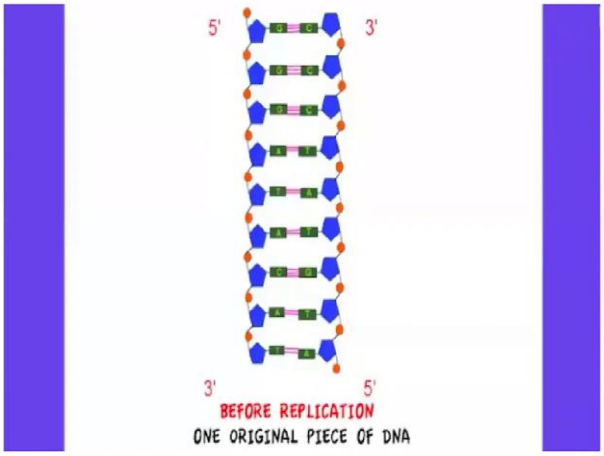

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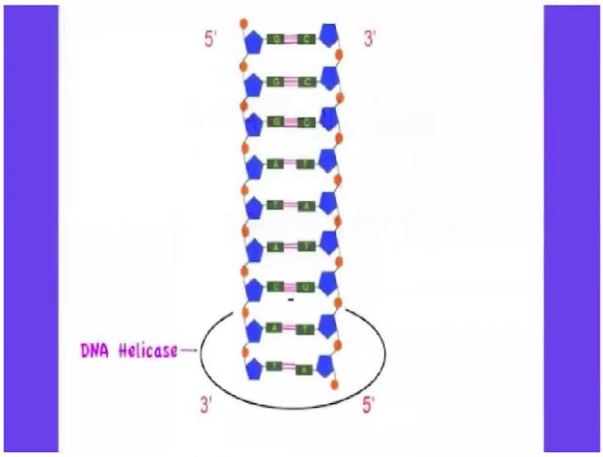

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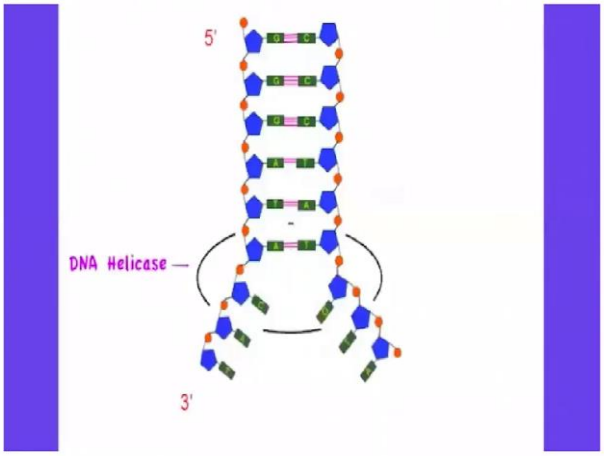

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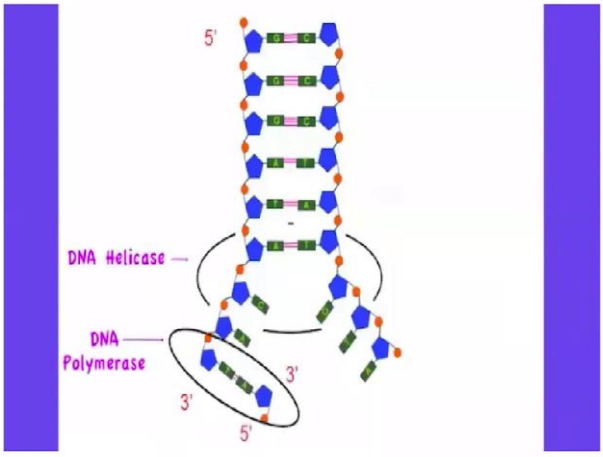

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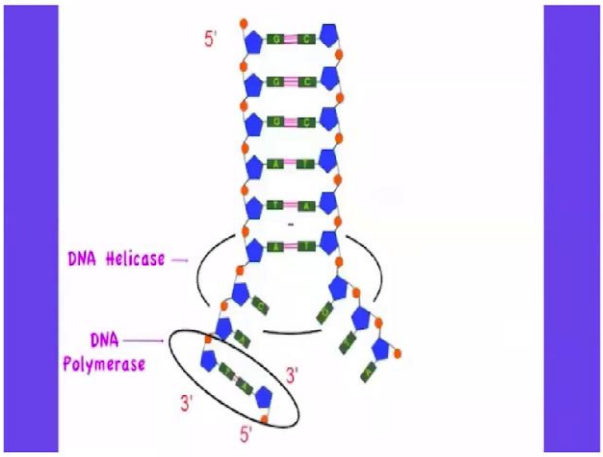

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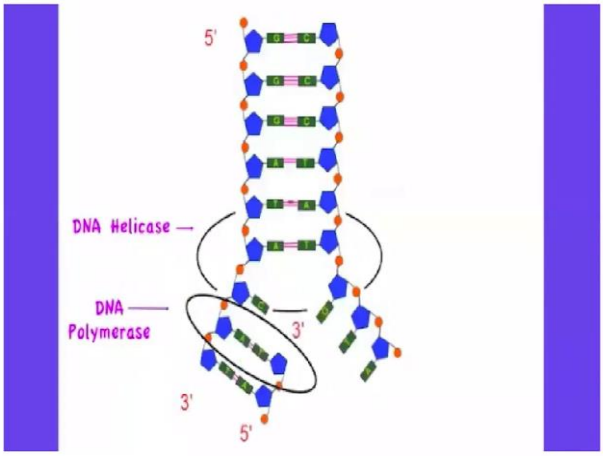

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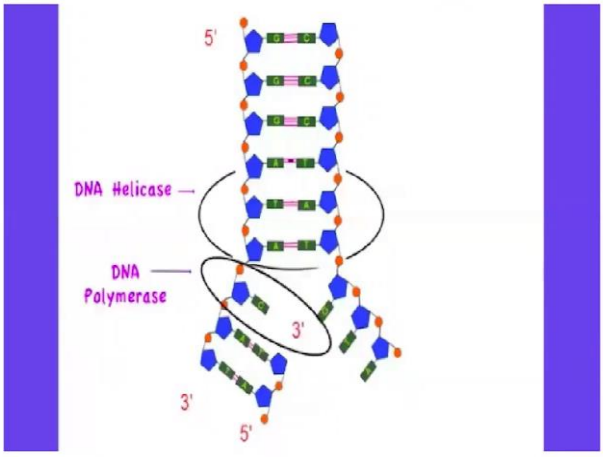

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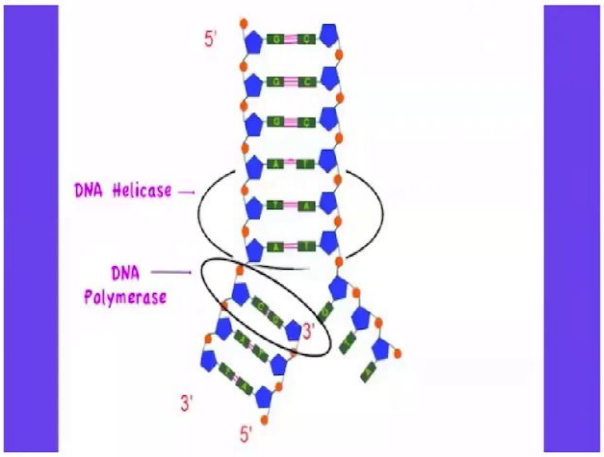

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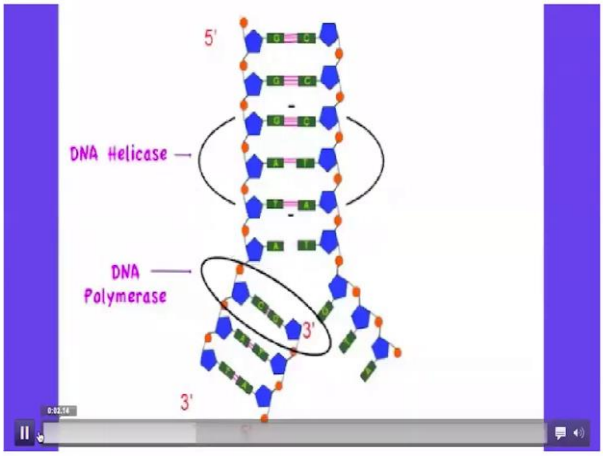

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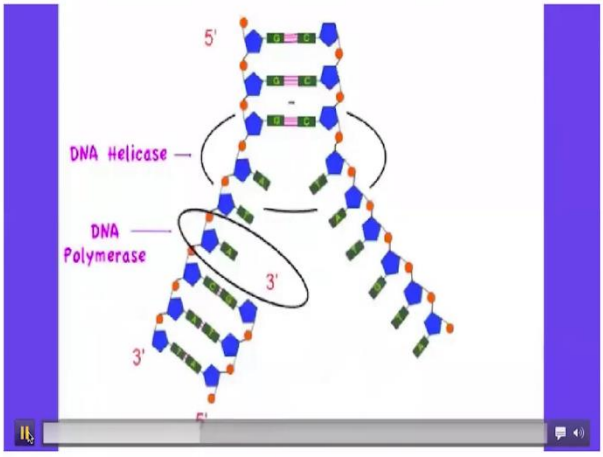

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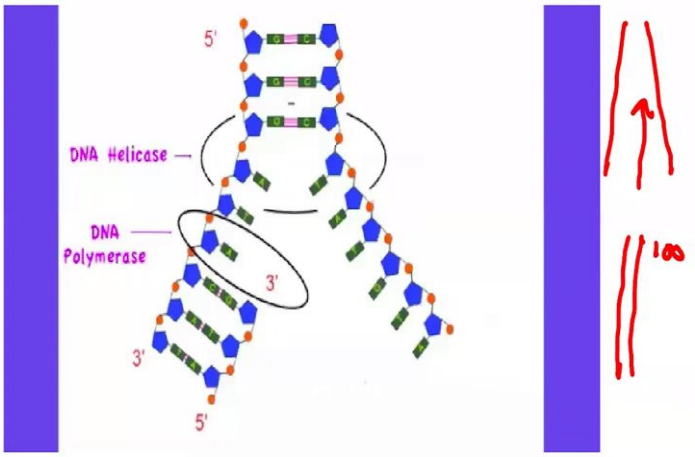

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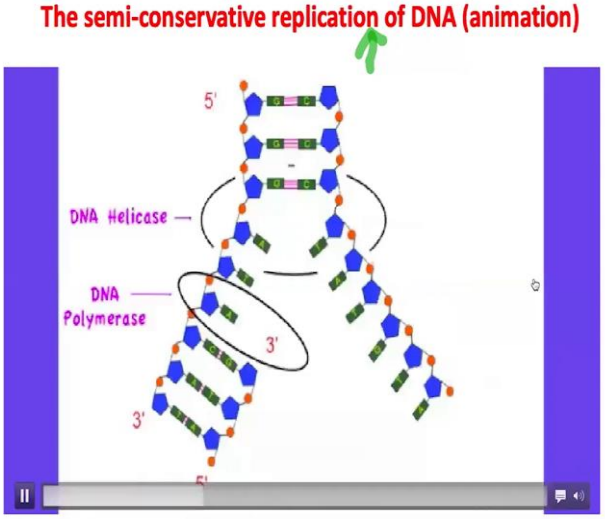

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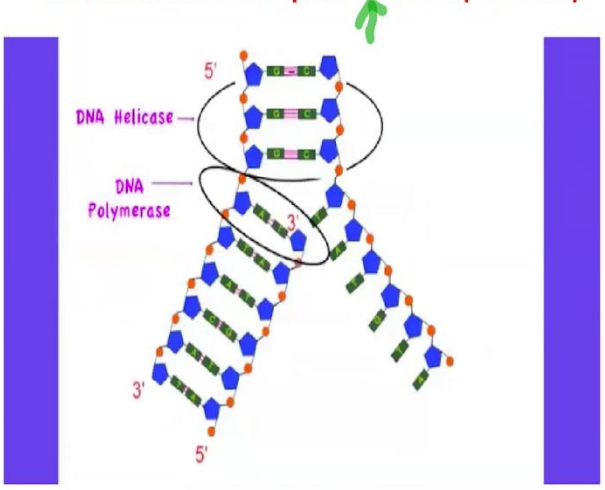

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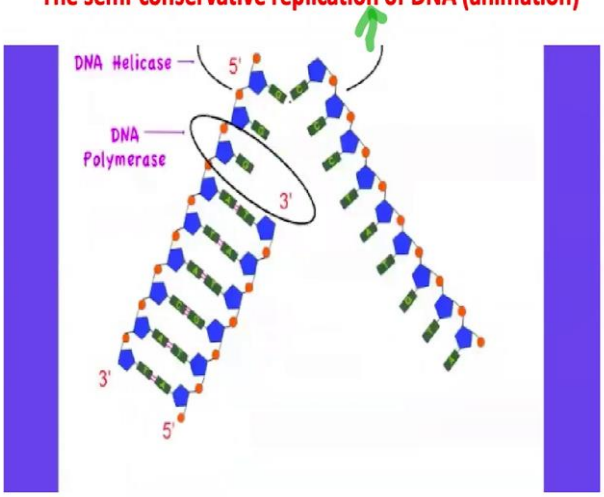

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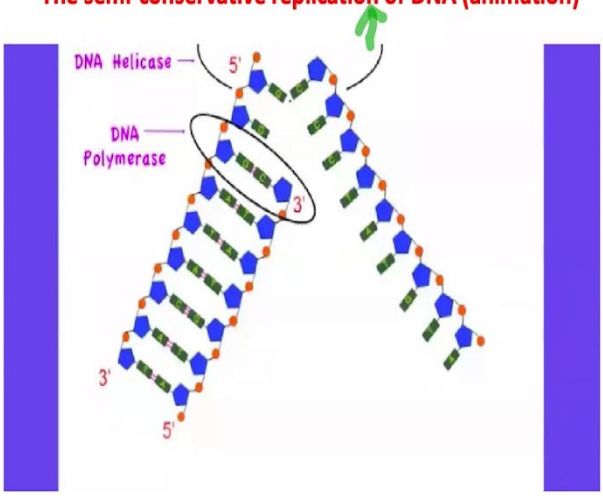

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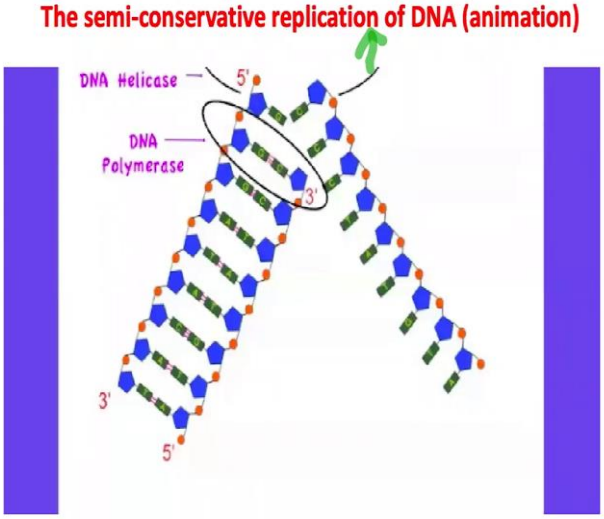

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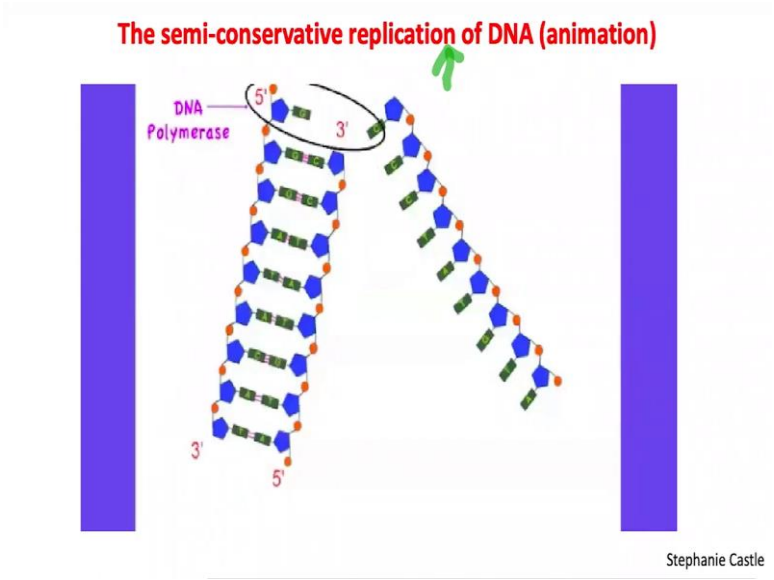

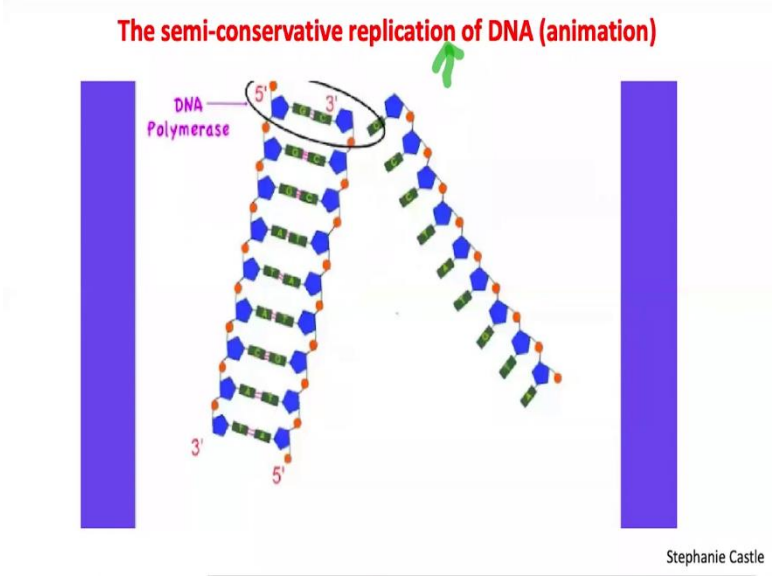

Speaker: DNA replication is the process by which one DNA molecule is duplicated to make two identical DNA molecules so that the next generation of cells can contain the same genetic information. To understand how DNA replication takes place, we first need to remind ourselves of the structure of DNA with regards to the prime ends as shown here. DNA is double stranded and notice that the right hand strand is anti-parallel to the left hand strand.

Each of these strands undergoes DNA replication in a slightly different fashion. So I will deal with each one of them individually. And then at the end, I will show you how it takes place together. So we will first deal with what we call the Leading Strand, the leading strand is the strand on the left hand side, which goes from 3 prime at the bottom to 5 prime at the top.

Professor: I am going to stop here and ask those of you who have not done biology, to start worrying about nomenclature, I know nomenclature is a little difficult, but the way I am taking you through DNA replication and all other processes in a stepwise fashion. As long as you take the trouble of noting down and revising things, you will be able to pick up all the nomenclature.

Speaker: First of all, the enzyme DNA helicase unwinds and separates the strands by breaking the hydrogen bonds between the complementary base pairs. Next, the enzyme DNA polymerase adds complementary DNA nucleotides. Notice that the newly synthesized strand complementary to the original strand is anti-parallel.

Professor: So to go through this again, before the movie completes, what DNA helicase is doing, it is called helicase for a reason, it is unwinding the helix. And if these are two strands of DNA, it is basically acting to break these two strands of DNA. Now, let us assume that the DNA strands are 100 bases long.

So you have 100 such bases which have to be broken, but DNA replication also takes place in viral genomes in bacterial genomes, and also in human genomes. And all of you know that they range somewhere around 10 raised to 4 base pairs to 10, raised to 9 base pairs. So that is a huge set of stretch of DNA, which has to be unbound, and which has to be remade using DNA polymerase.

And this is pretty much what I want you to imagine, as you go along and ask questions related to this very complex, it is a lot of work to make so many, so many base pairs. So helicase is doing its job, it is opening up the DNA polymerase on the leading strand is trying to basically copy the leading strand.

And polymerase has this ability to attach nucleotides only on the 3 prime side, and this will create a problem and you will understand what is the problem?

Student: Sir what is DNA polymerase and DNA helicase? Like they are machines, or they are some kind of molecules or what?

Professor: They are proteins, and I am calling them machines, because they do a lot of work. It is like having a vacuum cleaner or mixie. They do functional work, but they are made up of proteins which are pulled.

Students: So means they are enzymes and they are doing work.

Professor: Exactly.

Student: But how exactly does that a helicase unwinds the DNA?

Professor: So these are the, so what you have just asked me is about what we define in, research and in biological research as an open question? So you will find very fascinating details of how these molecules were discovered. And it struck scientists who discovered them, this the same kind of questions we are asking, you are asking me, how is it doing it?

What is, what are the steps? What is the energy source? And the most obvious question which will strike many of you? If you start opening up a helix? Does it not cause strain, for example, on the side, which is further away? As you keep on opening up a helix? Are not you increasing strain on the rest of the DNA, which is which has yet to be opened? And how do you break a bond? How does the polymerase move on this DNA?

Does it grip the DNA? All these questions have been asked in the last 20, 30 years. And they are answers for most of them. And what has happened is now at least till the time, I was a master student, which is in the 90s. I had to visualize these things by myself because there were no animations at that time.

Today, by the end of this talk, hopefully, the animations will give you a better molecular picture. So what we have today is we have structures of DNA polymerase, we have structures of DNA helicase, we have structures of all of these with bits and pieces of DNA. So we actually can see at atomic resolution, mostly using x ray crystallography, static pictures of what, what we are seeing.

Now, what unfortunately, we still do not have his dynamic pictures. And for dynamic pictures, we have to literally make animations correct to molecular level detail, and then try and understand what is going on.

Speaker: DNA polymerase is only able to add a nucleotide onto the 3 prime end of the previous nucleotide.

As DNA helicase continues to unwind, the DNA polymerase continues to build the newly synthesized strand in the 5 prime to 3 prime direction, the same direction as the movement of

the replication fork. As a result of this, we say that DNA replication on the leading strand takes place continuously.

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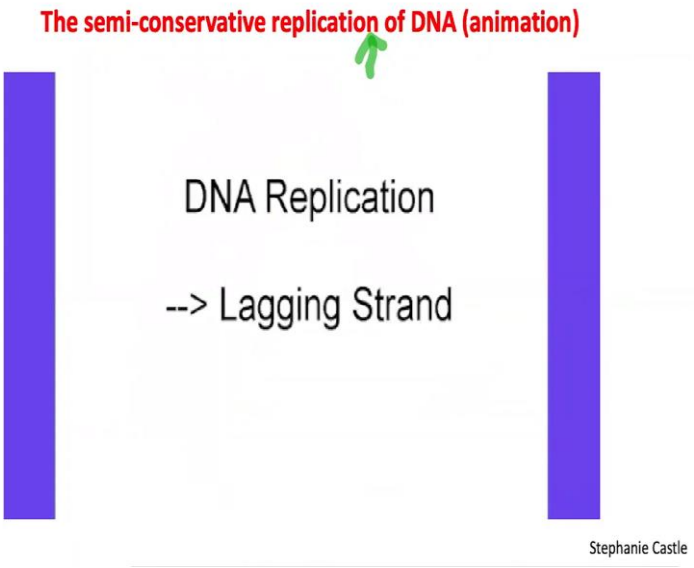

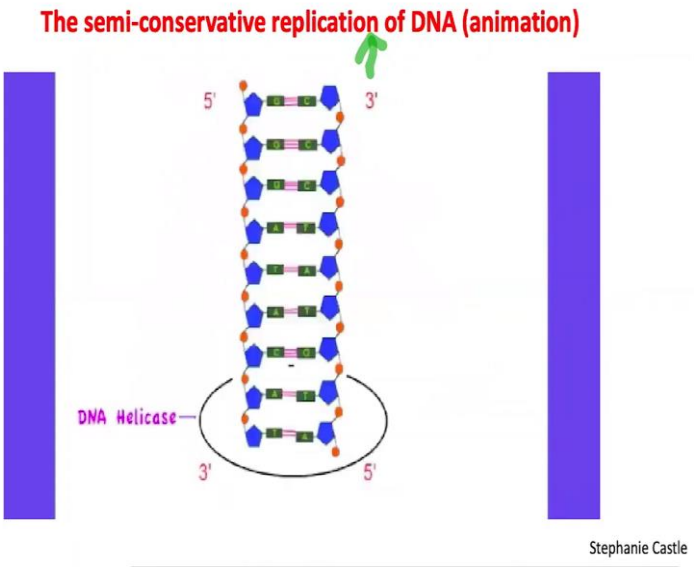

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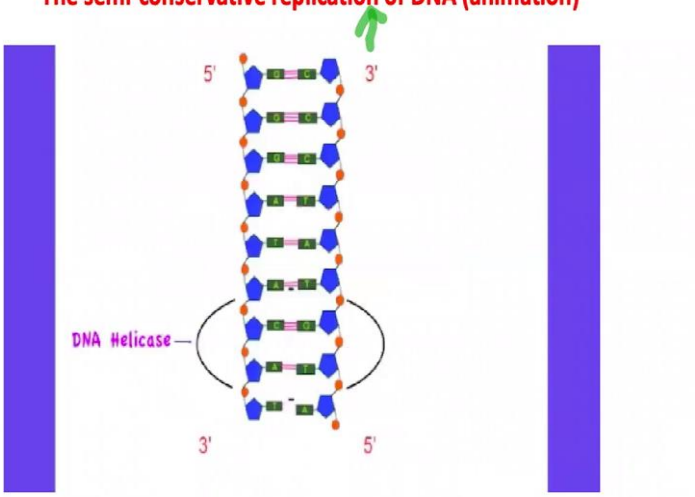

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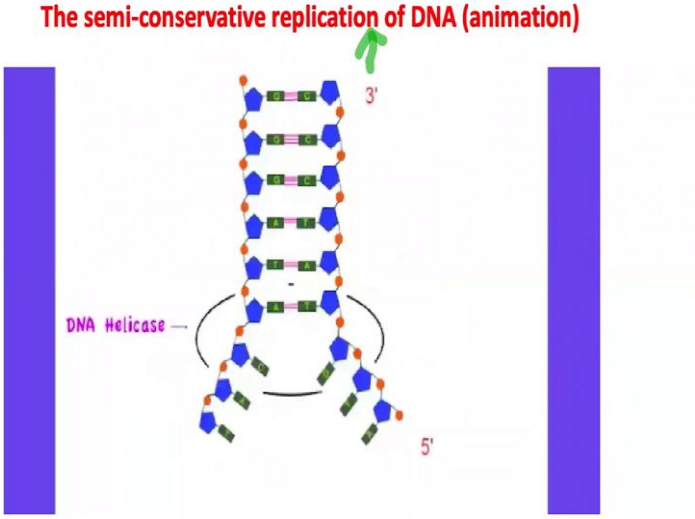

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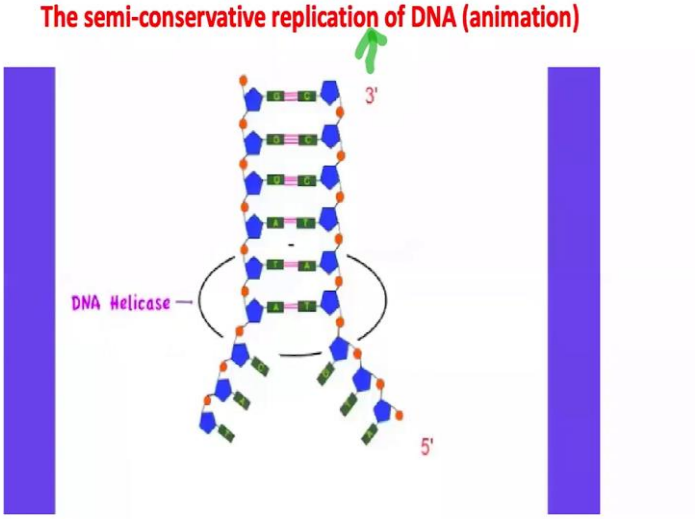

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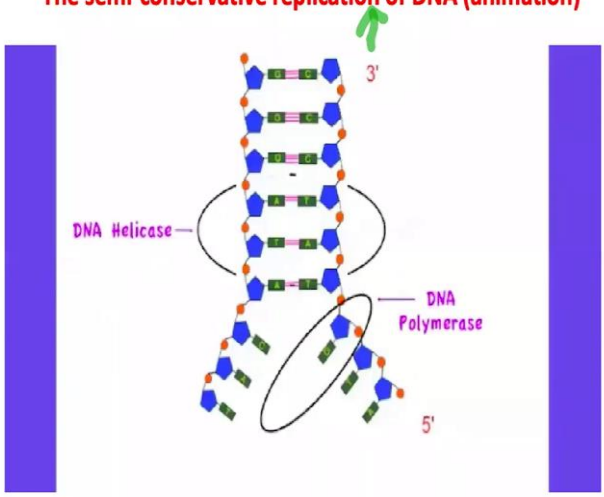

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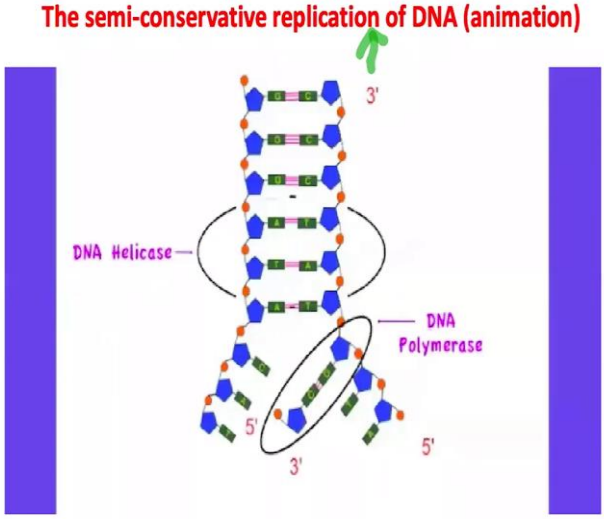

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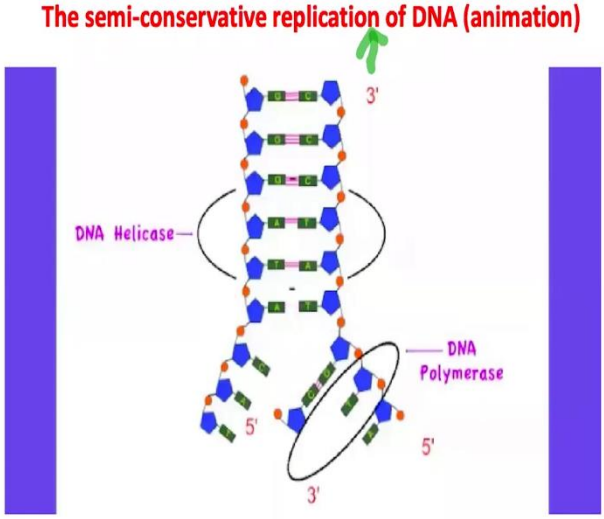

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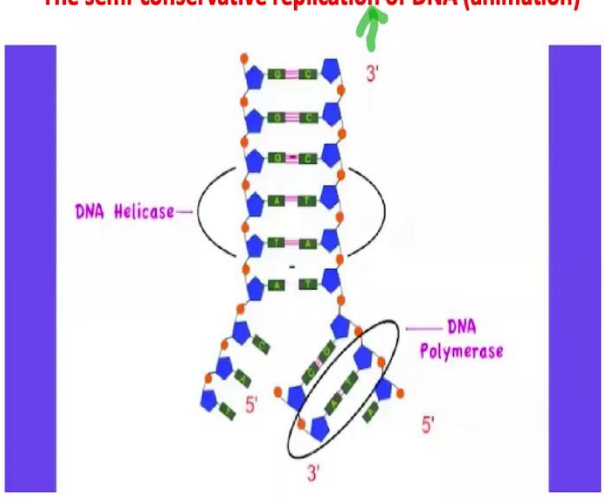

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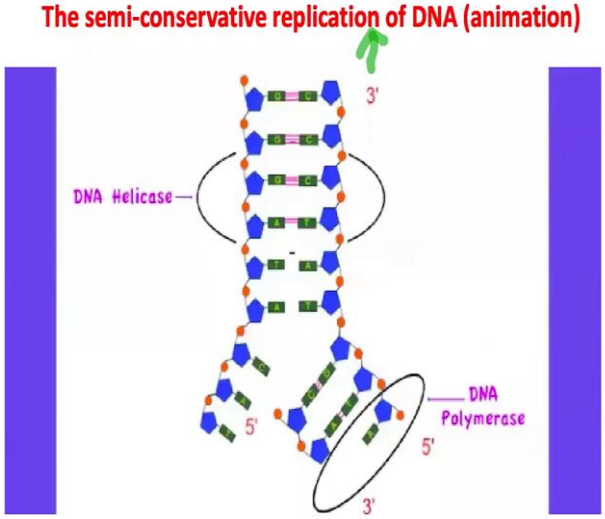

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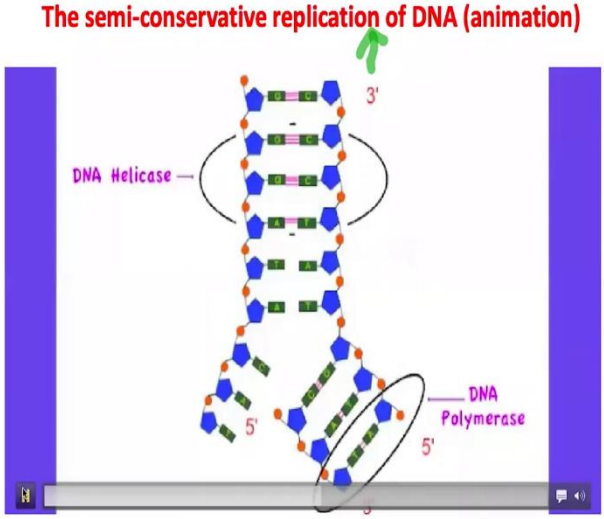

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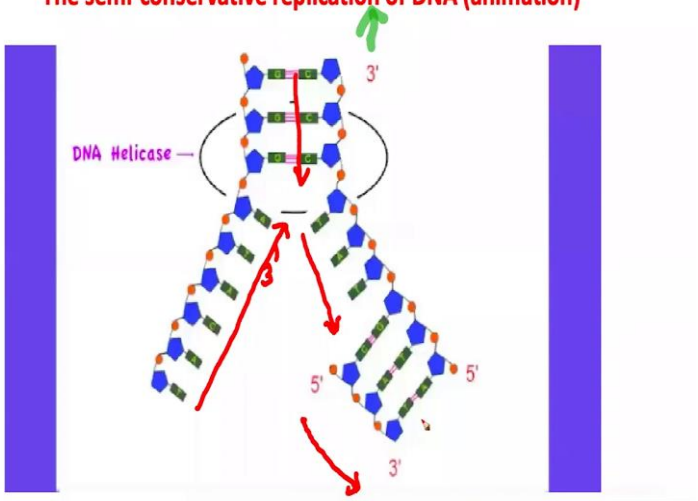

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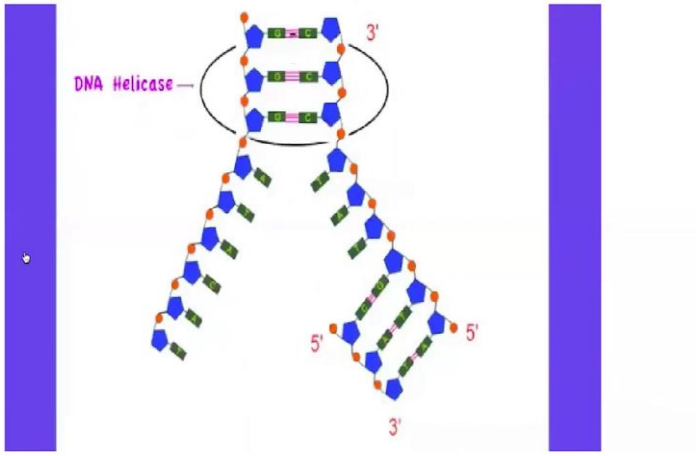

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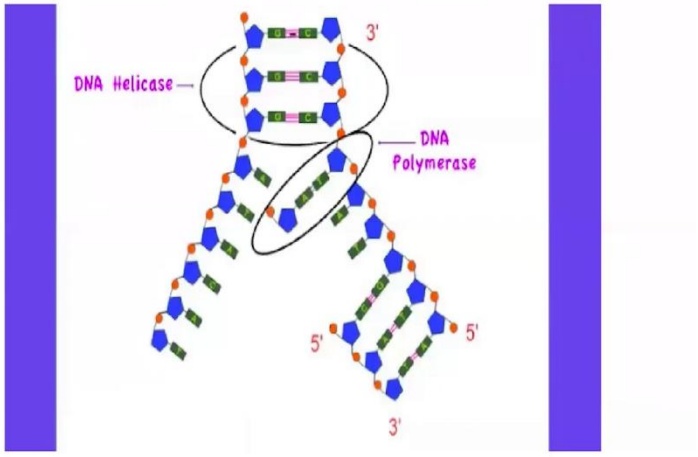

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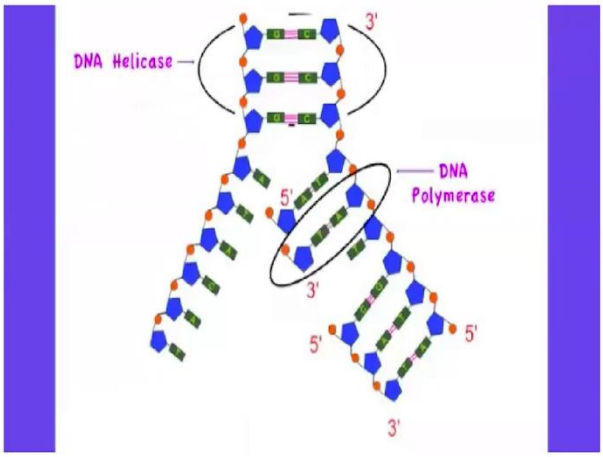

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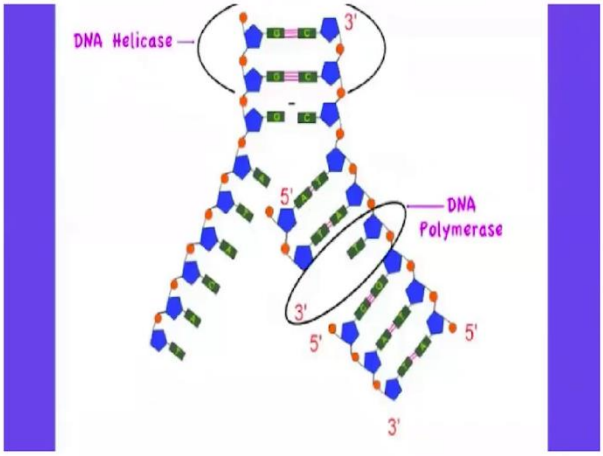

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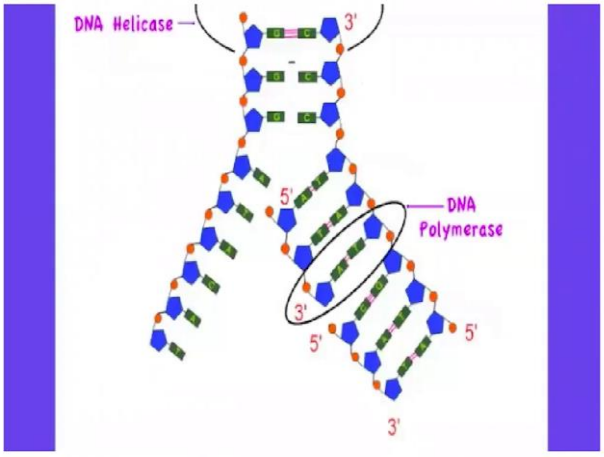

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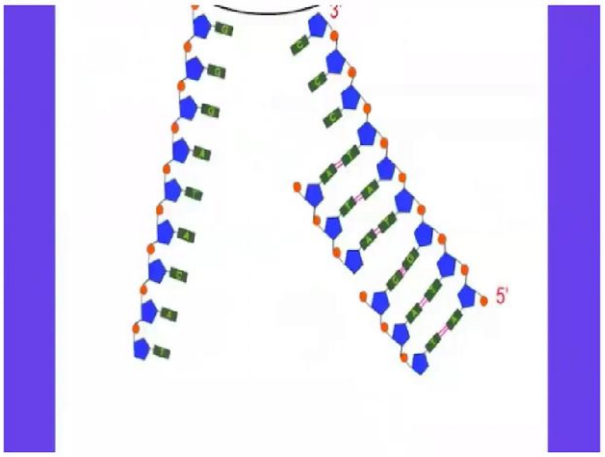

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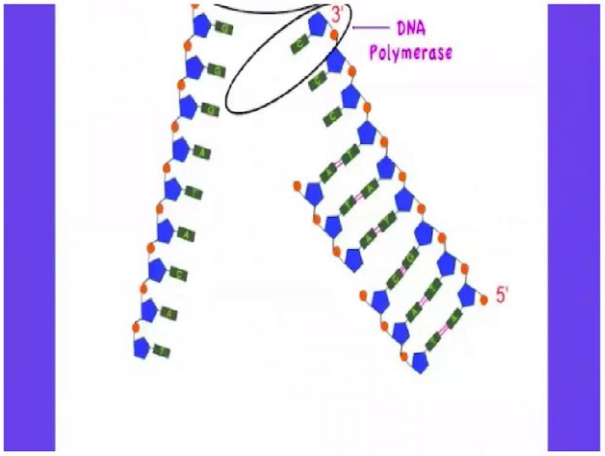

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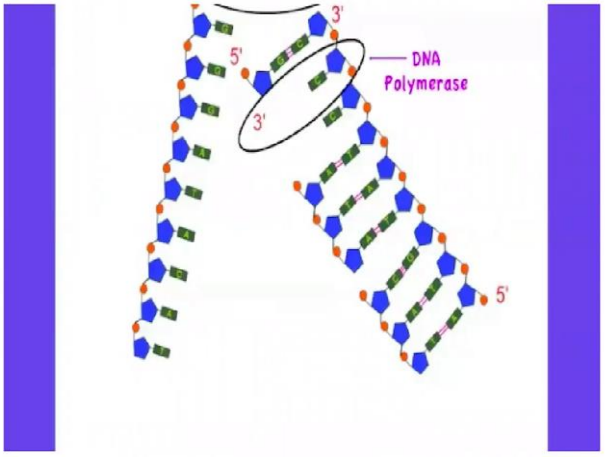
The semi-conservative replication of DNA (animation)



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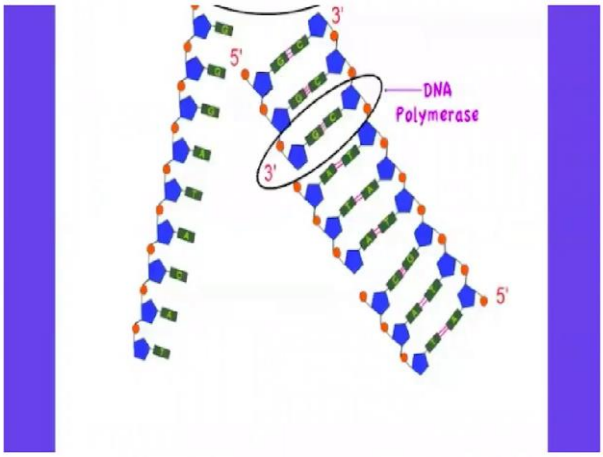

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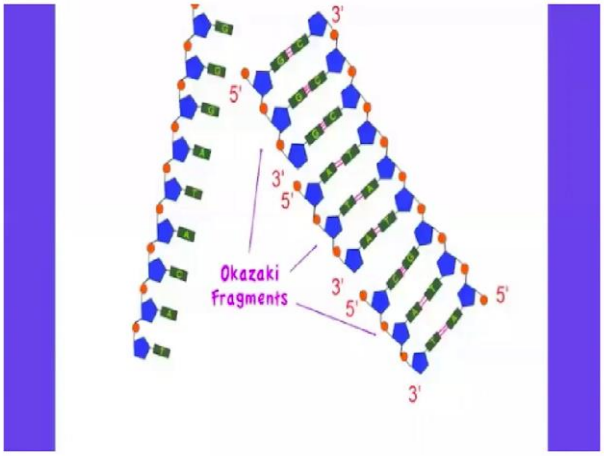

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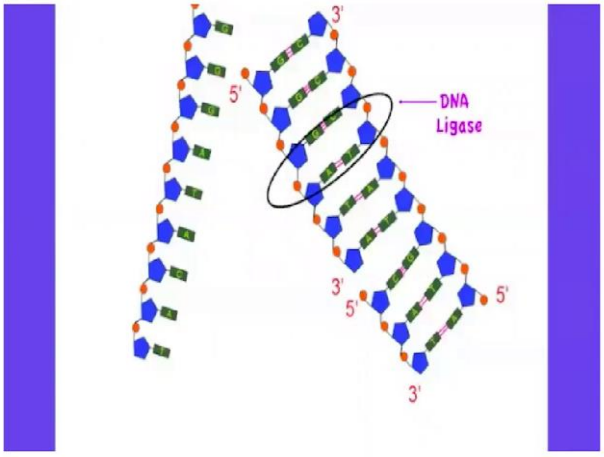

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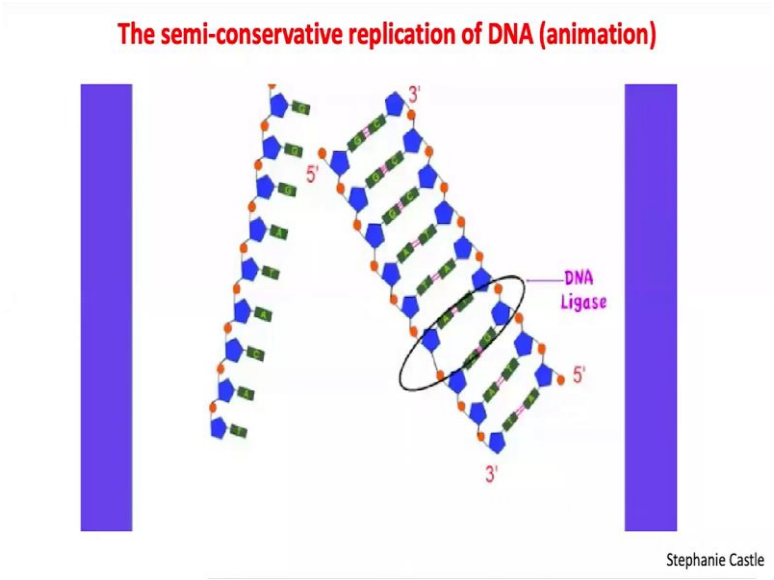

Now let us look at the other side of the original DNA strand. We call this the lagging strand. In the same way as before DNA helicase unwinds and separates the two strands breaking the hydrogen bonds. DNA polymerase also, as before, adds complementary DNA nucleotides. However, because of the anti-parallel nature of the original DNA strand, DNA polymerase has to work in the opposite direction on the lagging strand.

Professor: So let me explain this a little bit more clearly. As the helicase is opening things up. Initially it has opened, let us say 3 or 4 base pairs. Now in this direction, things are fairly simple. As the helix keeps on opening, the polymerase can extend to 3 prime and it keeps on adding nucleotides, so it is a continuous stretch.

Initially, only 3 base pairs were open. And the polymerase a second polymerase makes a little patch of DNA because it can only extend to 3 prime. By the time it is finished doing its job. 3 more base pairs base pairs have opened up. So it does the job again, 3 more. After that you will realize 3 more open up it will does, it will do its job again and put 3 more.

The number 3 is arbitrary. It is it can be 5 base pairs can be 10 base pairs, but it can only extend in the 3 prime direction DNA polymerase. Therefore, it is forced to do things in a discontinuous manner, 3, 5, 10 base pairs at a time.

The net result is unlike the copying of the leading strand, which is on the left hand side over here, which is continuous, the making of DNA, the making a copy of DNA during DNA replication in all biology is discontinuous. And that is what I want you to take as a take home thing. And let us see what this movie continues to say.

Speaker: Recall that we just mentioned that DNA polymerase was only able to add a nucleotide to the 3 prime end of the previous nucleotide. So what is different here with the lagging strand is that each time the DNA helicase moves up, the DNA polymerase is operating in the opposite direction.

This results in the completion of the lagging strand in shorter fragments. The lagging strand is therefore being completed discontinuously as compared to the leading strand that was completed continuously. The fragments created are known as Okazaki fragments. In order to complete replication on this strand, the Okazaki fragments need to be joined together, and this is done by DNA ligase.

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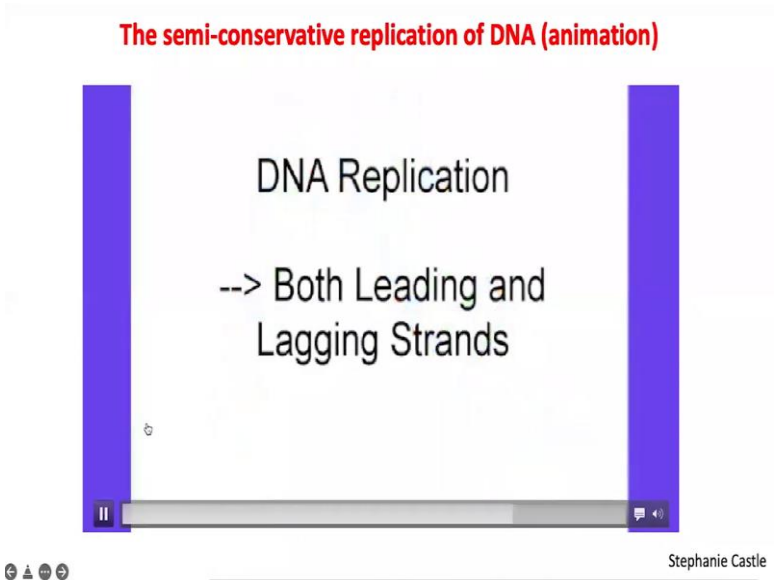
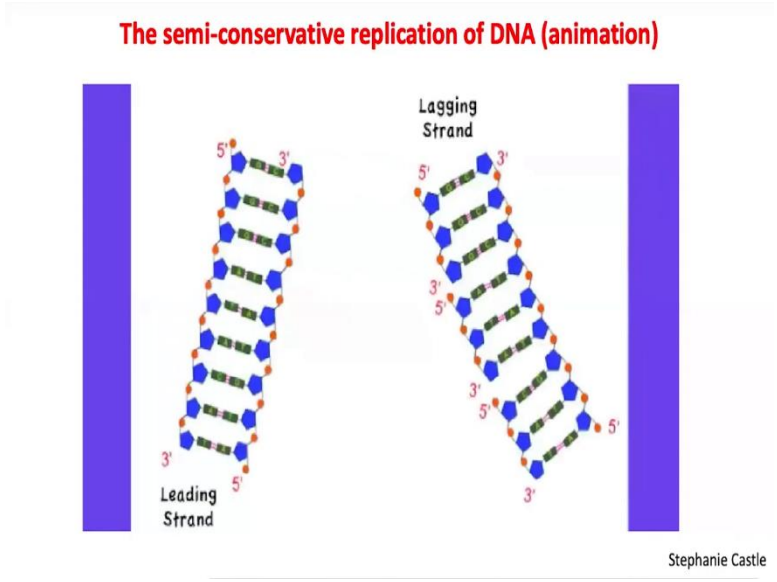
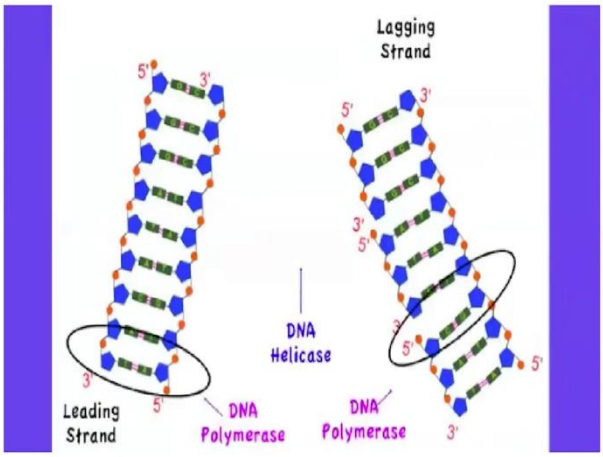




The semi-conservative replication of DNA (animation)



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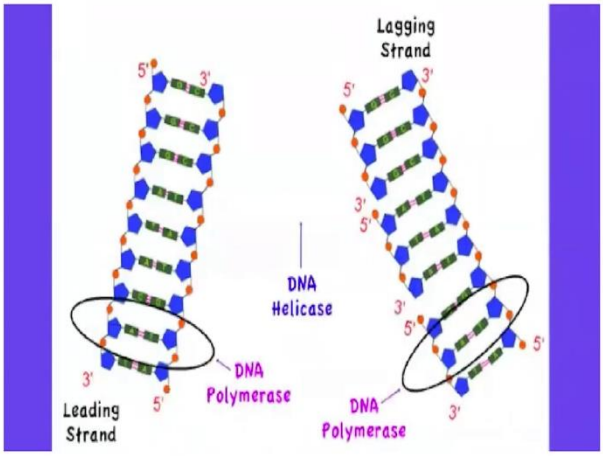

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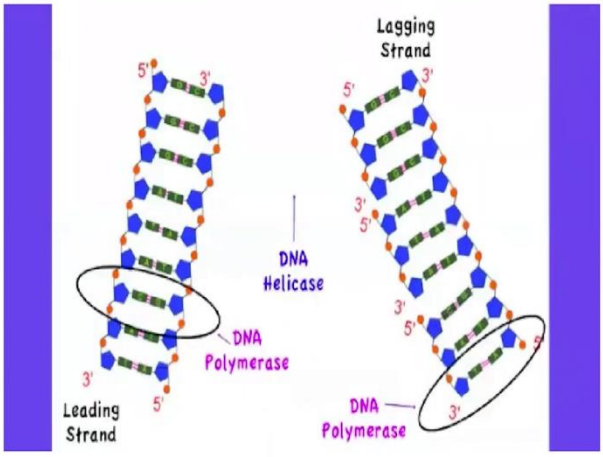

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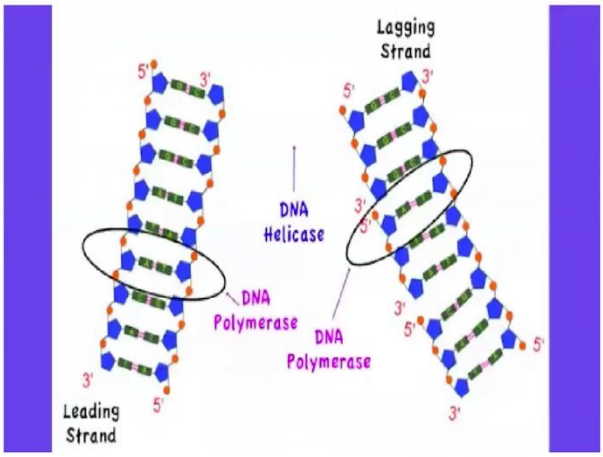

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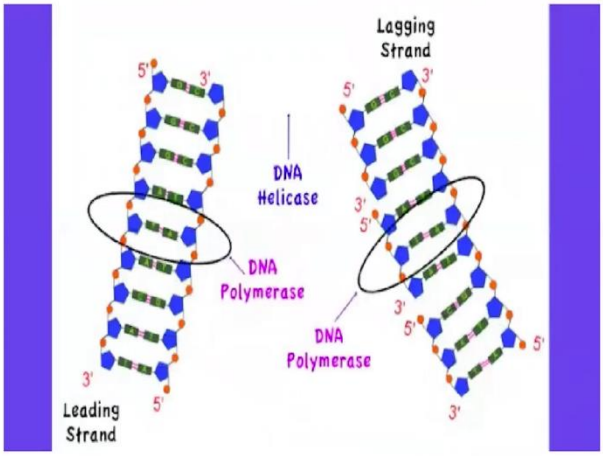

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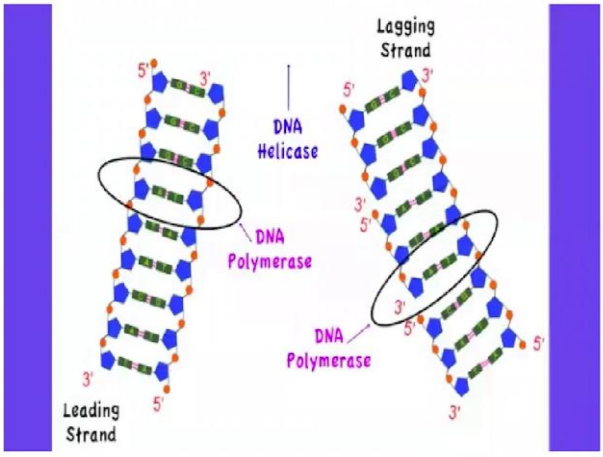

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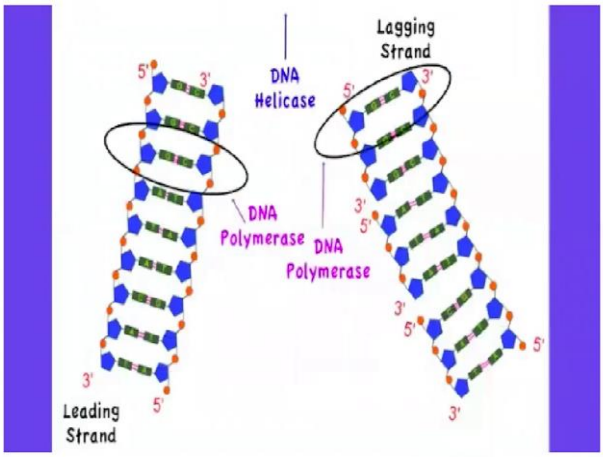
The semi-conservative replication of DNA (animation)



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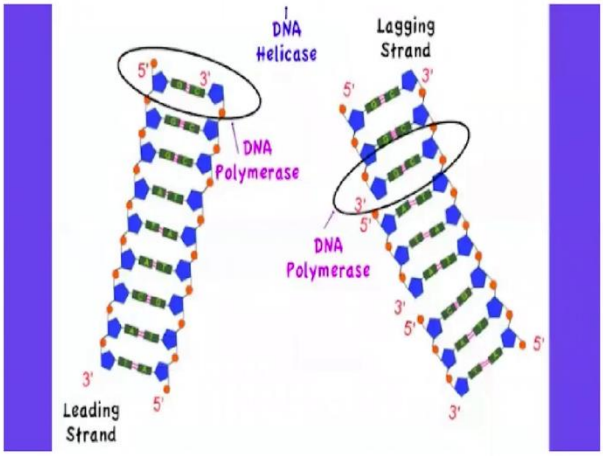
The semi-conservative replication of DNA (animation)



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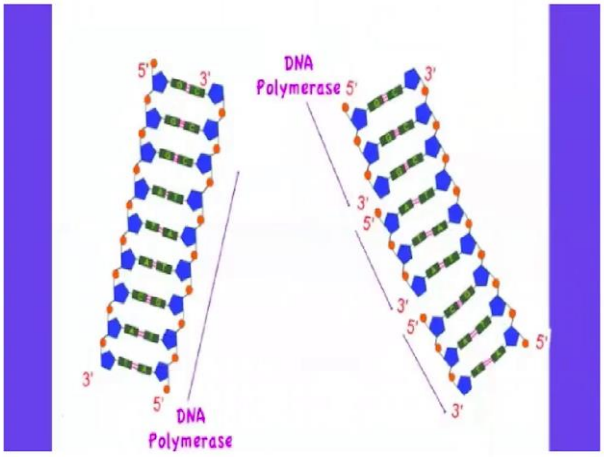

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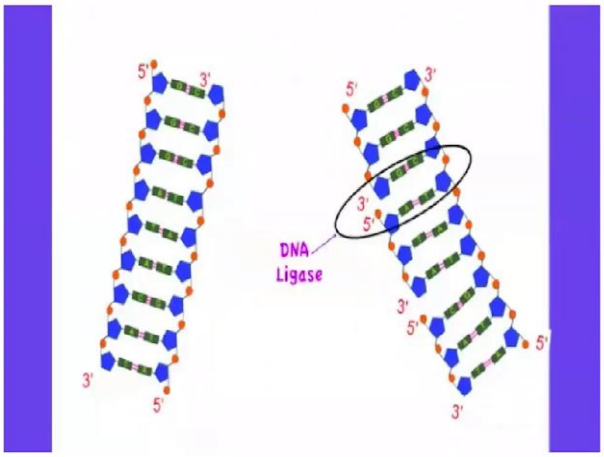
The semi-conservative replication of DNA (animation)



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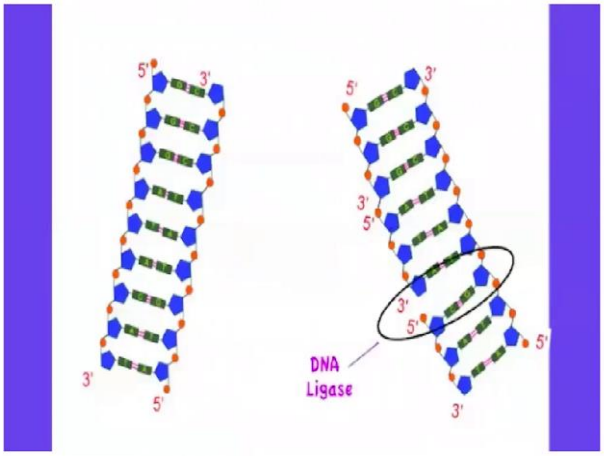

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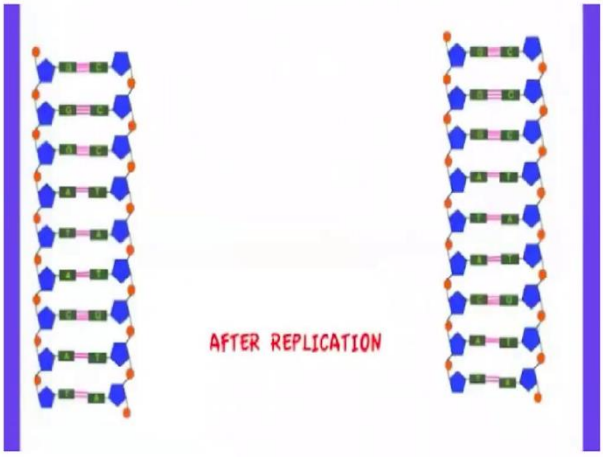

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So to put it all together here, you can visualize the action of the enzymes on the leading and lagging strand and how they compare. Although in this animation, I do show the nucleotides already there. Recall that it is the DNA polymerase that adds those complementary nucleotides. To review, notice that on the leading strand, the DNA polymerase works in the same direction as the DNA helicase.

Moving up the strand from the 3 to the 5 prime direction on the original strand. In contrast, on the lagging strand, DNA polymerase and helicase work in opposite directions. And this leads to the creation of Okazaki fragments, which must be joined by DNA ligase. Finally, two identical daughter molecules of DNA are created which are identical to the original piece of DNA. These daughter molecules then rewind to form a double helix.

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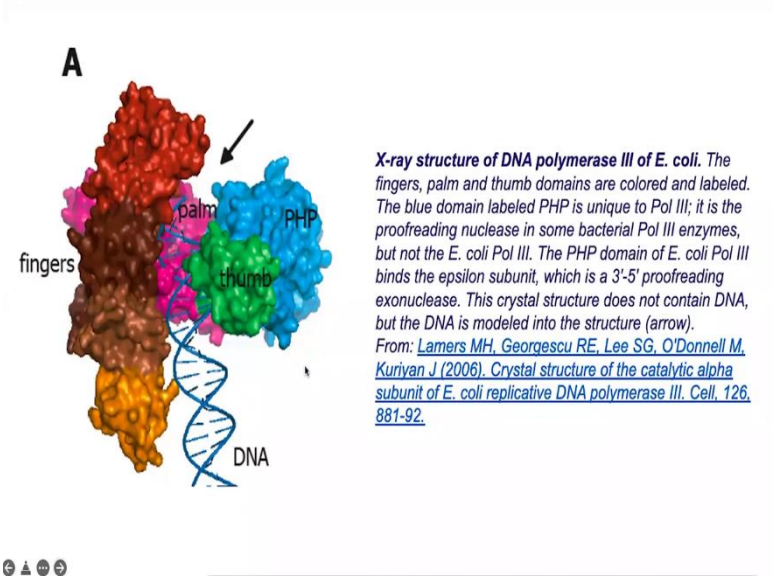

Professor: So let me show you another couple of animations. Now, this is actual structure at atomic resolution of DNA polymerase. It is one of the many polymerases, which we have purified crystallized and solved structures of, when I say we, I do not mean me, I mean scientists in general, in order to help people to visualize what this is doing, we look at DNA polymerase with a hand, and you have to put your hand on the screen, well not on the screen next to the screen.

And if you for example, I am using my right hand, and I am bending my fingers, and I am bending my thumb. And in the palm of my hand, I can hold a rod or a pen. And that is basically what the DNA is. And DNA polymerase moves on this DNA with the DNA moving through the hole which is there, which is created by the fingers thumb and the palm.

And very obviously, somewhere in the palm and thumb is the actual set of amino acid residues, which are adding nucleotides, which are consulting the strand which is already there, and adding complementary nucleotides in a continuous manner. So this is the best way I can, at this point, give you a visualization of how DNA polymerase looks like. It is a protein machine, it is made completely of, of proteins, and it does its job.

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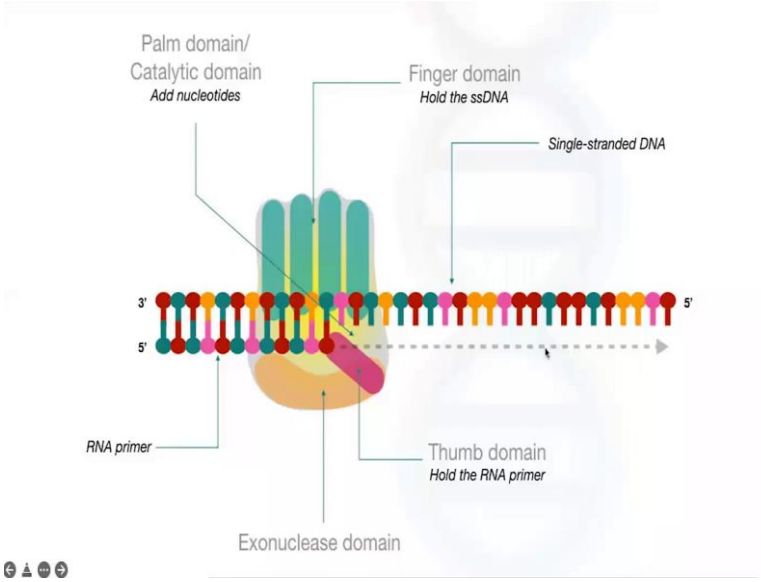

So again, pictorial view, here is a, let us say, call it a leading strand, which is in the 3 prime to 5 prime orientations. polymerase sits on the single stranded DNA. I will explain what RNA primer is. This is an interesting concept, but basically, it is holding the single stranded DNA on the palm of its hand.

And in a stepwise manner, it is adding nucleotides, which are copies or complement of the 3 prime, 5 prime strand and polymerase can only extend in the 3 prime direction which is shown as a gray dotted line over here. So now let us see a couple of movies and then we will end this class.

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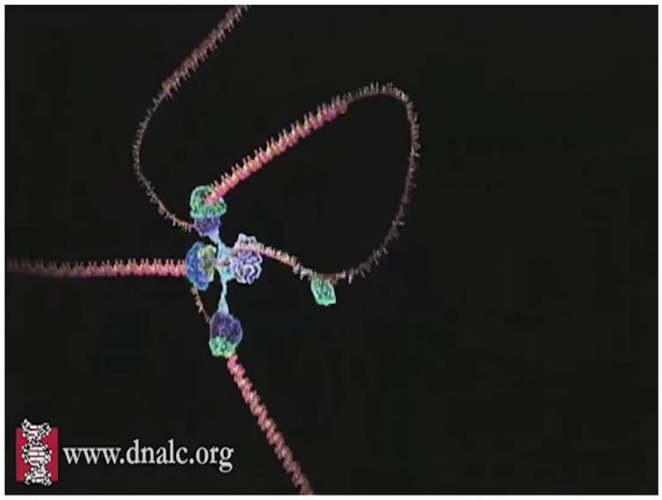

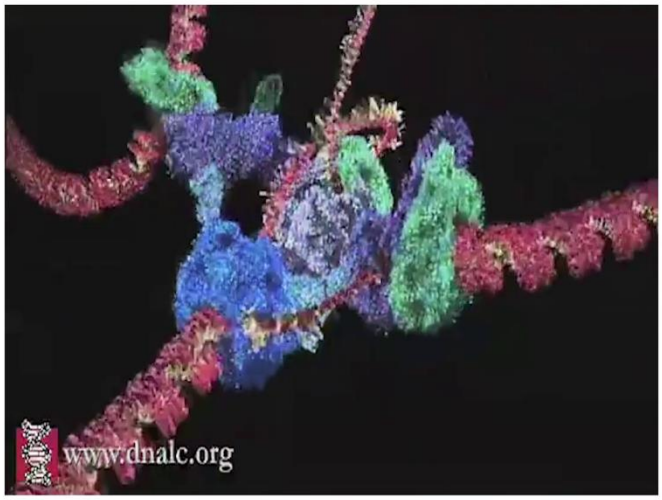

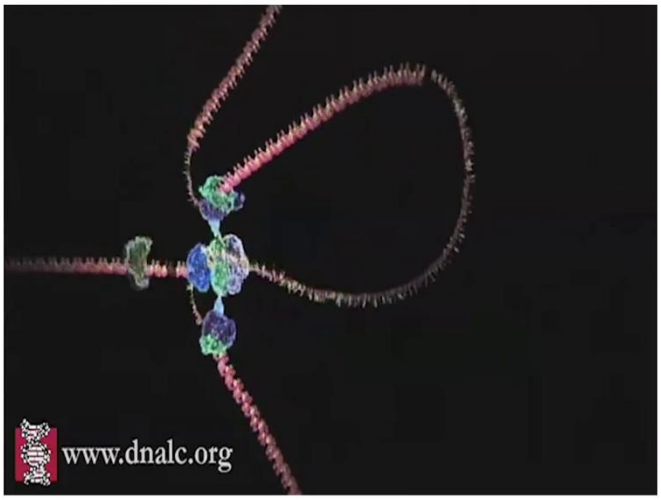
www.dnalc.org


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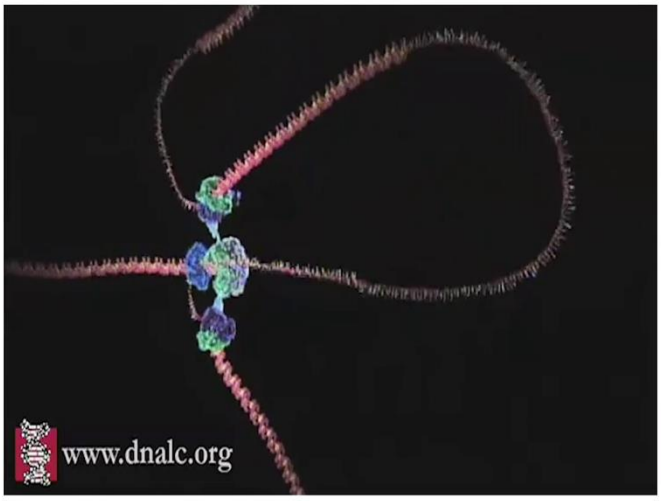
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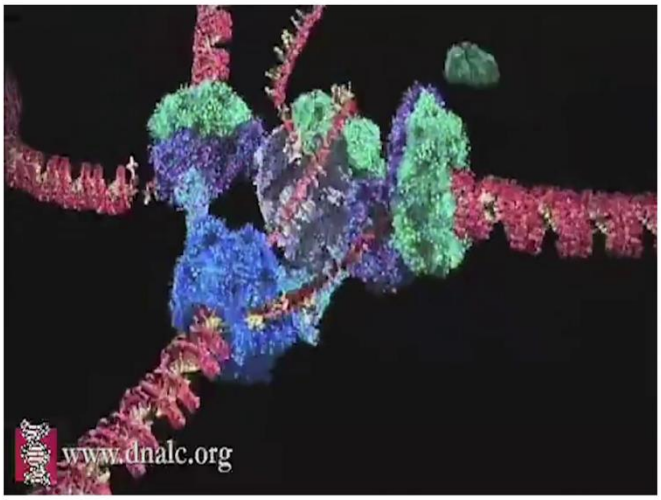
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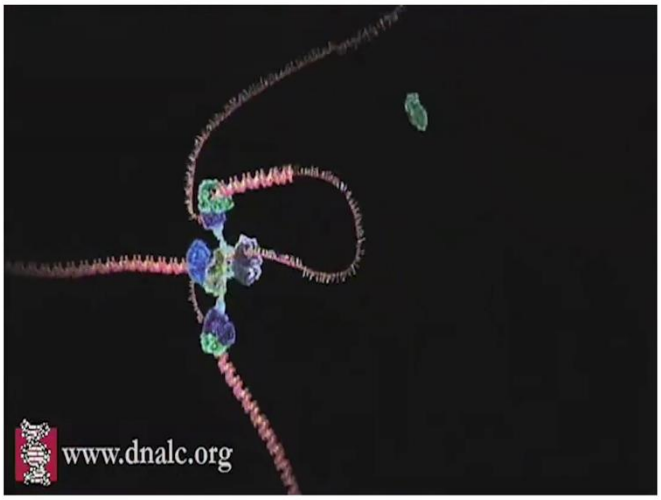
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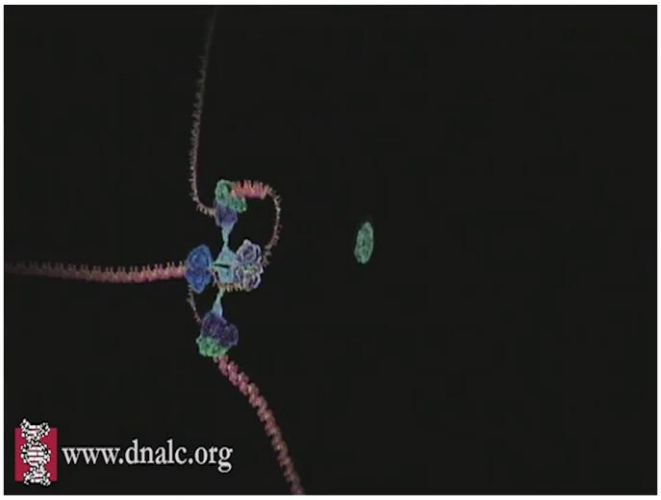
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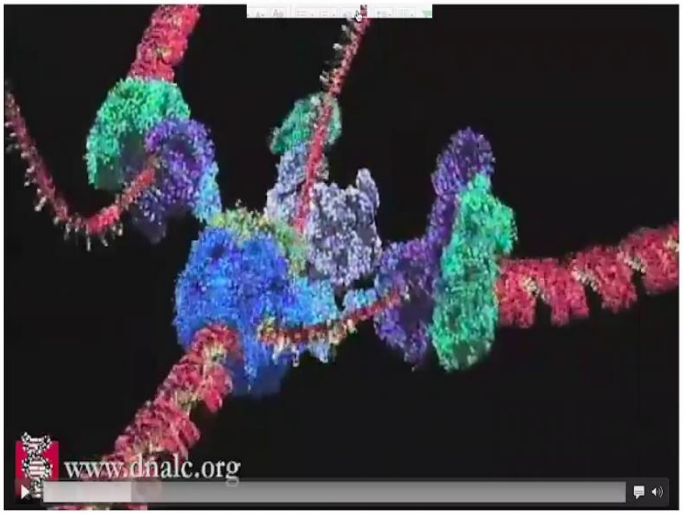
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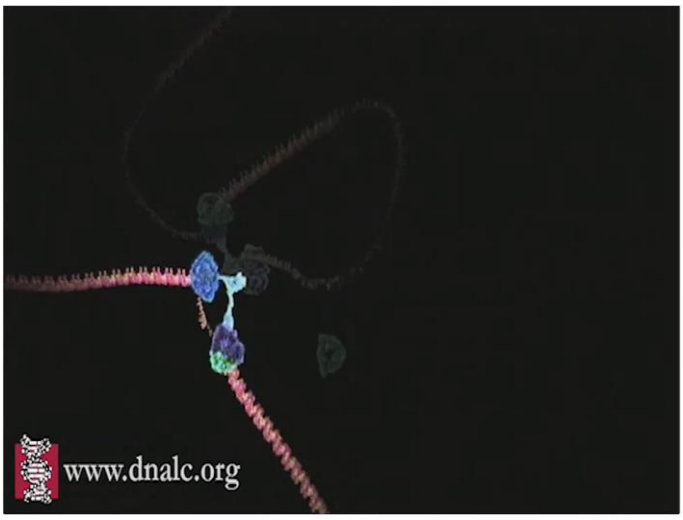
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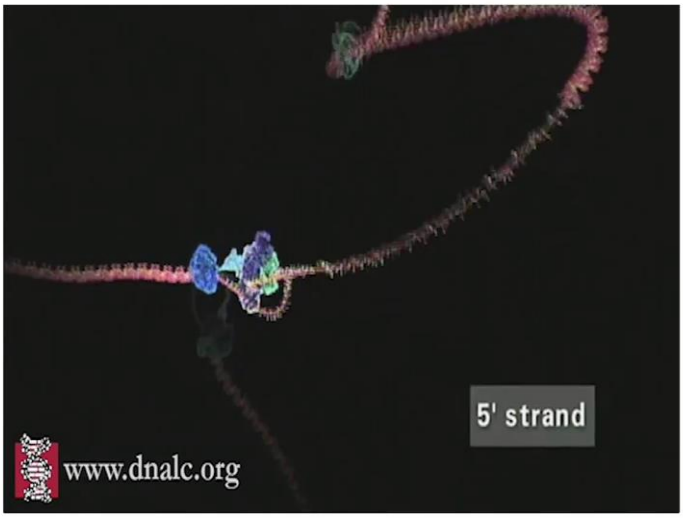
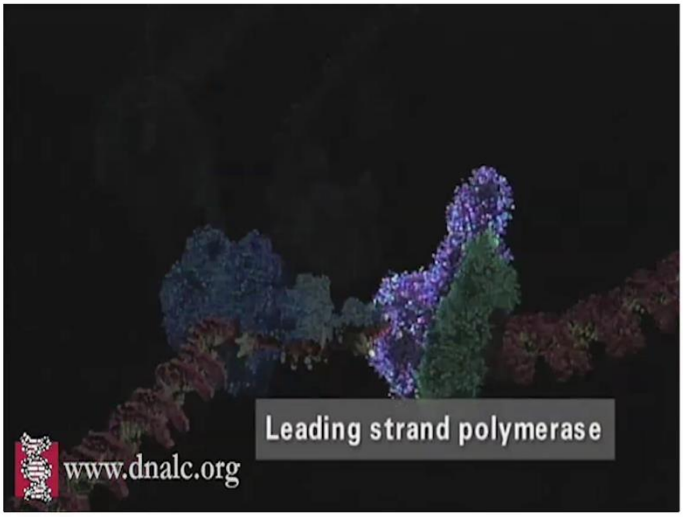

5' strand
www.dnalc.org


Leading strand polymerase
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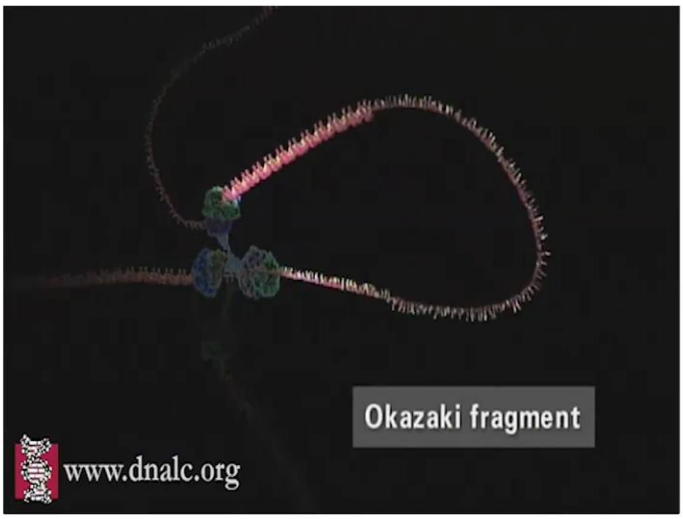
Okazaki fragment
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Speaker: Using computer animation based on molecular research we are now able to see how DNA is actually copied in living cells. You are looking at an assembly line of amazing miniature biochemical machines that are pulling apart the DNA double helix and cranking out a copy of each strand. The DNA to be copied enters the production line from bottom left. The whirling blue molecular machine is called helicase.

It spins the DNA as fast as a jet engine as it unwinds the double helix into two strands. One strand is copied continuously and can be seen spooling off to the right. Things are not so simple for the other strand, because it must be copied backwards. It is drawn out repeatedly in loops and copied one section at a time. The end result is two new DNA molecules.

Professor: I am going to play this once more and I am going to play the same movie in another form after this. So, this is the double helix which is going to be copied, on the bottom over here is the leading stand simple to do and lagging strand becomes complicated. And each of these fragments is Okazaki fragment.

Speaker: The DNA to be copied entrust the production line from bottom left. Whorlling blue molecular machine is called helicase. It spins the DNA as fast as a jet engine as it unwinds the double helix into two strands. One strand is copied continuously and can be seen spooling off to the right. Things are not so simple for the other strand, because it must be copied backwards. It is drawn out repeatedly in loops and copied one section at a time. The end result is two new DNA molecules.

Professor: So now I am going to show you the same movie, which is now an advanced version of the movie which you saw.

Speaker: During DNA replication, both strands of the double helix act as templates for the formation of new DNA molecules. Copying occurs at a localized region called the replication fork, which is a y-shaped structure where new DNA strands are synthesized by a multi enzyme complex.

Professor: So this is an replication fork where the ligase is opening things. On the right hand strand is on the right hand side is the simple polymerase, which is making a second copy of the leading strand. It is the lagging strand where things become more complicated. You have to do things in bursts simply because the DNA is not in the in the in the orientation, which is suitable for DNA polymerase.

Now you can ask another open question. Why did not whoever made biological machines, why did not they have DNA polymerases which could copy both in the 3 prime and the 5 prime orientation? And that remains an open question we have never found a polymerase, which can copy in both directions because if that was so we would not have Okazaki fragments.

Speaker: Here the DNA to be copied enters the complex from the left. One new strand is leaving at the top of frame and the other new strand is leaving at the bottom. The first step in DNA replication is the separation of the two strands by an enzyme called helicase. This spins the incoming DNA to unravel it at 10,000 rpm in the case of bacterial systems.

The separated strands are called 3 prime and 5 prime distinguished by the direction in which their component nucleotides join up. The 3 prime DNA strand also known as the leading strand is diverted to a DNA polymerase and is used as a continuous template for the synthesis of the first daughter DNA helix.

The other half of the DNA double helix, known as the lagging strand has the opposite 3 prime to 5 prime orientation, and consequently requires a more complicated copying mechanism. As it emerges from the helicase, the lagging strand is organized into sections called Okazaki fragments. These are then presented to a second DNA polymerase enzyme in the preferred 5 prime to 3 prime orientation.

These sections are then effectively synthesized backwards. When the copying is complete, the finished section is released and the next loop is drawn back for replication. Intricate as this mechanism appears, numerous components have been deliberately left out to avoid complete confusion. The exposed strands of single DNA are covered by protective binding proteins. And in some systems, multiple Okazaki fragments may be present.

Professor: So I will stop here.