Organic Chemistry In Biology And Drug Development
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Lecture - 28
Central Dogma: DNA Replication, Transcription and Translation

The topic of last lecture was oligonucleotide synthesis.

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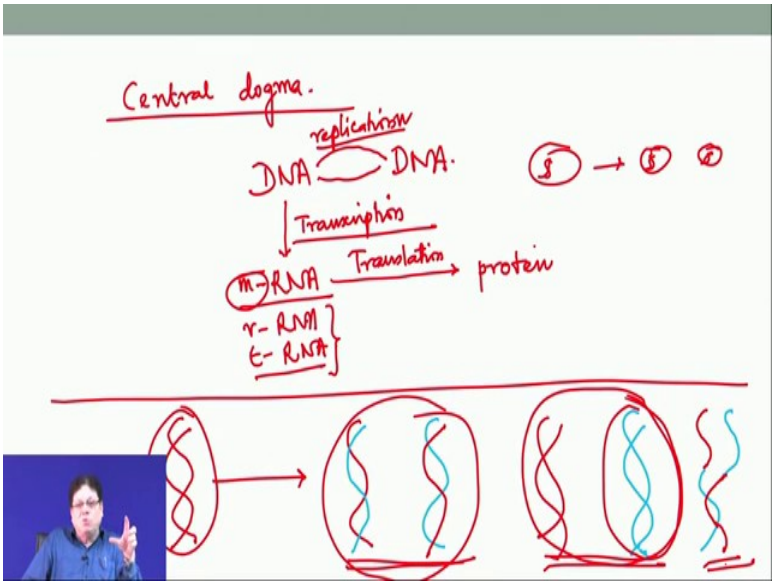

Now will see how this central dogma of biology involves different processes, different chemistry for the flow of information from DNA to the protein. What is central dogma in biology? It has to do certain processes and the first process is the replication, i.e making the copy of the existing DNA in the cell. So, one cell is converted to two cells, both having the pieces of the DNA.

After DNA replication, DNA has to be transcribed into RNA. There are several classes of RNA- m RNA *i.e* messenger RNA, t RNA transfer RNA, r-RNA *i.e*. Ribosomal RNA. Conversion of DNA to RNA is called transcription- it could be ribosomal RNA/ or it could be t-RNA the transfer RNAs and that is what is called transcription.

Then messenger RNA with the help r RNA and the t-RNA forms the protein and this process is called translation. Possibly you have read this in the biology books at the

lower level, but we will talk about the chemistry involved in these three processes -one is replication, another is transcription and translation. At first, I will discuss replication. DNAs are replicated to form new two double stranded DNA.

One possibility is that the new strand is forming double strand with the old strand. It could be that the old strands remain coupled with each other hand the new ones are forming double strand with each other. Third possibility is that a portion of the strands consists of part of the old and the new strand.

So, basically three possibilities exist. At first, we have to sort out the pathway by which DNA is being copied. What is called conservative replication? If it goes through conservative pathway the old strands remain with each other and the new strands are the daughter strands remain with each other. In case of semi conservative pathway, the old and the new combine with each other to form a double helix. For dispersing pathway, each strand is composed of old and the new one.

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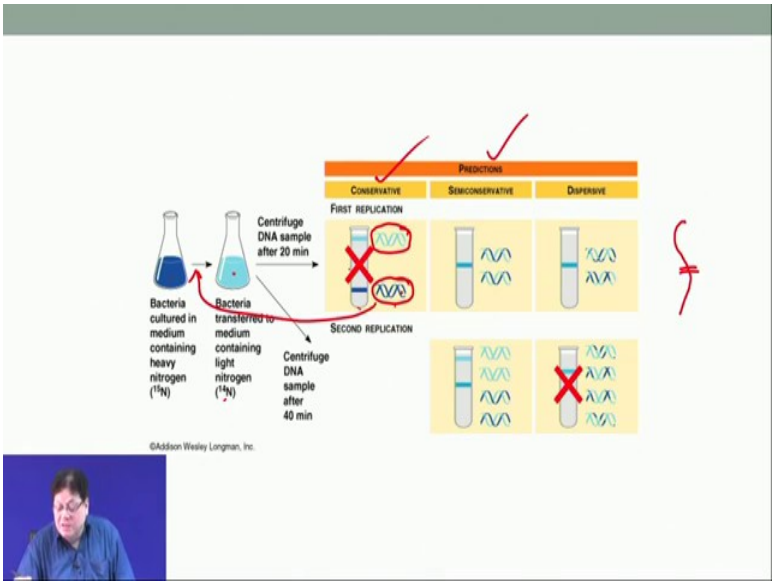

If your initial DNA is red colored one and the and the new DNA is when it is made that new DNAs it is colored like that ok.

So, what will happen in conservative replication?

Old DNA are together to form the double helix and new DNA strands are also forming double helix with each other. DNA contains a lot of nitrogens in the bases and each base

contains more than one nitrogen. If bacteria are allowed to grow in ammonium chloride medium then nitrogen comes from ammonium chloride. So, if you use ammonium chloride containing only ^{15}N level (heavy isotope of nitrogen) the DNA that will be formed here only consists of ^{15}N initially. Now this bacteria are transferred to normal ammonium chloride medium containing ^{14}N i.e lighter isotope of nitrogen and allowed to grow one cycle. What will happen?

In conservative replication, you will have a heavy DNA consists of only ^{15}N and a light DNA consists of only ^{14}N. There will be significant molecular weight difference between this two DNA double helix. If you do centrifugation the heavier DNA will collect at the lower side of the centrifugation tube and the lighter one should be at the higher level than the heavier one. If it follows conservative pathway this should be observed. But actually it is not seen.

Now consider all the three possibilities. If it is semi conservative then what will happen? After the first cycle (i.e when the 2 strands become 4 strands), it is expected that we will have two DNA strands each having one ^{14}N strand and one ^{15}N strand. It will be intermediate between this fully ^{14}N and fully ^{15}N and we expect a band in between these two. So, there should be only one band after first cycle if it is semi conservative.

If it is dispersive 50 percent of this will be coming from the old strand and 50 percent from the new strand. So, the molecular weight of these DNA will be similar because this is 50 50. So, there will be no difference between you have 4 strands and out of which 50 percent is the light blue. Here also you will have 4 four strands and out of that 50 percent is the light blue. So, their molecular weight will be same. After the first cycle, you cannot distinguish between the dispersive and the semi conservative modes.

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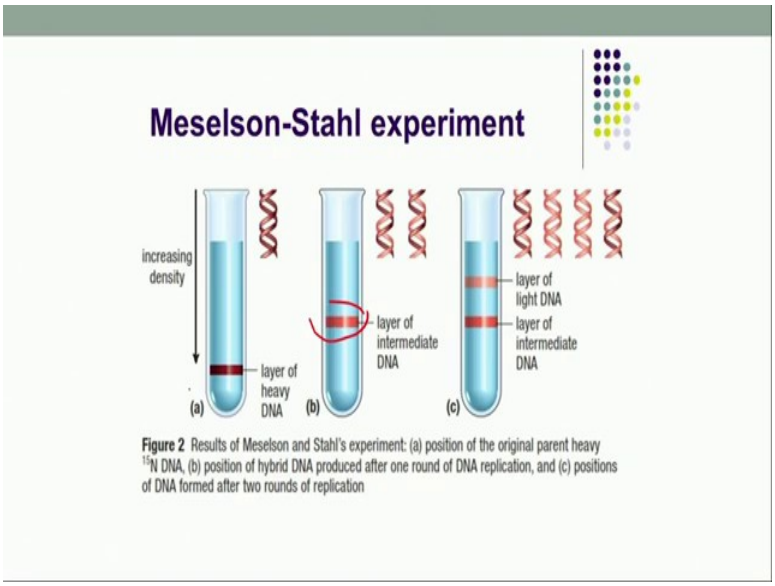

But it can distinguish the conservative ones. The famous experiment was done by Meselson Stahl. After the first cycle, it was found that there is only one band and it is higher than the first band which was made from only ^{15}N leveled DNAs. You have to go for the second cycle in order to distinguish between the dispersive and the semi conservative.

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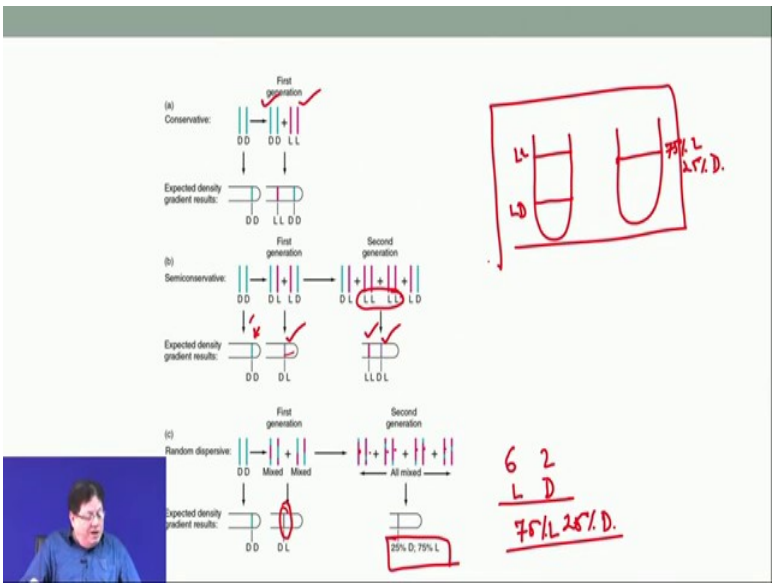

There is another schematic diagram where the old strands are mentioned by D and the new strand is mentioned by L. For conservative pathway, there should be two types of

DNA-DD and LL. So, you should see two bands and that is written here. After first cycle, the initial band consisting only DD should have two bands.

For the semi conservative, if you have this DD you should see a DL at little higher level than DD. Because it will have lower molecular weight than DD. In case of dispersive pathway, 50 percent is covered by the new DNA strands and the rest 50 percent is the old after first generation.

When you go to the second generation of now semi conservative pathway, you will have LL and DL.

If it is like dispersive then what will happen? Here after the second generation, there are 8 strands. Out of this eight, 6 strands actually comes from the newly generated strands and two are the old strands. So, the molecular weight of this DNA is basically 25 percent.

That will be 75 percent of L and 25 percent of D. It will be little bit on the lower side of the LL, but it will be in between LL and DL. For semiconservative pathway, after second generation this is pure LL, this is LD and this is 75 percent L and 25 percent D. So, Meselson Stahl came to the conclusion that it is actually semi conservative.

So, DNA replication is semi conservative.

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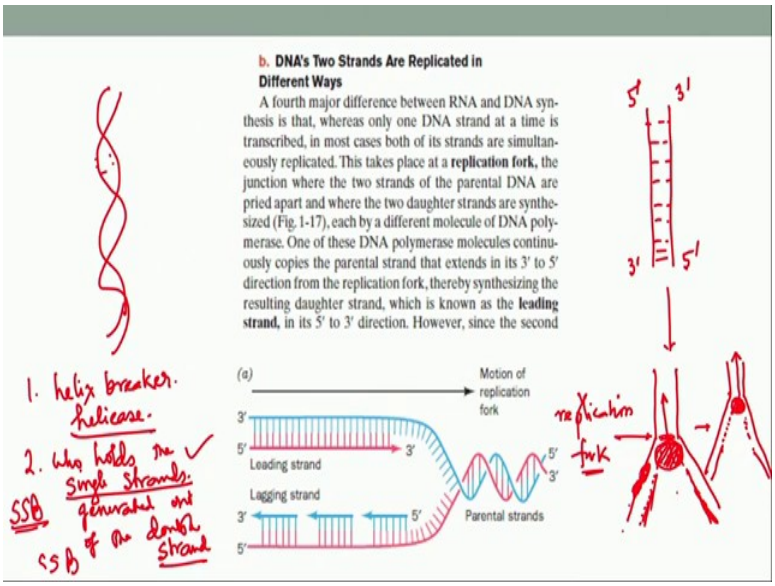

As DNA replication is semi conservative, these two strands need some separation to start replication. The old strand will act as the template for making new strand. Inevitably the question will arise that there is a stability of this double helix. So, you have to unwind the double helix. Remember one strand goes from 5 prime to 3 prime, another strand goes from 3 prime to 5 prime.

So, the first step that is needed is the DNA needs to be unwind. That means, double helix have to be converted into single helix single strand and then new strands can be synthesized on this. Why is it semi conservative? You have to separate the new strands from the old strands. As the synthesis is progressing you make these new strands. That new strand will be already tied up to the old strand. Now who actually does that?

Who disrupts this helix? There must be something which is this double helix breaker. You have to break the double helix at a particular point and then that needs to be progressed further. Once this part is over the synthesis has to progress. Helix breaker which is an enzyme breaks the hydrogen bonds between two strands and slowly proceeds ahead. As it proceeds new strands of DNA are synthesized and this point is called the replication fork.

An enzyme called helicase is acting as helix breaker. After breaking the helix, these two strands again want to really go back to the double strand by forming the same Watson Crick Base pairs. So, something also needs to be there that will hold these two separated strands. Because this has to be separated for some time till the synthesis of the other new strands are complete otherwise they will come back and will rejoin or reanneal again. So, the next question is who holds the single strands generated out of the double strand?

SSB proteins i.e Single Strand Binding proteins stabilize these single strands. So, these strands do not fall back and anneal with itself or hybridized with the other one which is separated.

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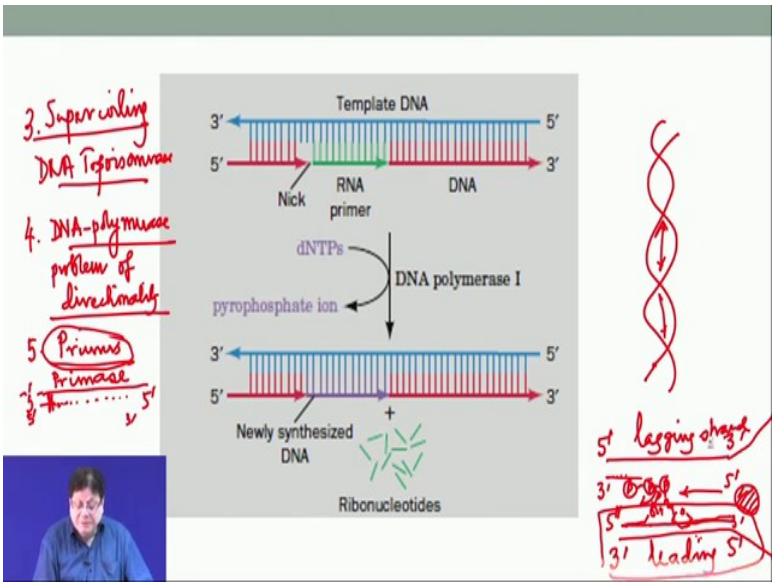

If you try to unwind the double helix the gaps between the major and minor grooves will decrease. This will create super coiling ahead of the helices. At some point of time, it will be very difficult to open up the portion of the DNA which you want to replicate. Why there is supercoiling? Whenever a helix is taken out, you will always see that there will be supercoiling in the front. That means, a force which does not want to allow further unwinding of the DNA. Now this super coiling can be prevented by an enzyme called DNA topoisomerase. When there is super coiling the best way to reduce that strain due to super coiling is to nick or cut the chain at certain point. The chain which is going below the in order to avoid or reduce the super coiling that will turn around and go to the to the top.

As the helicase progresses you need to cut or nick the DNA at difference points to reduce the super coiling.

This topoisomerase will cut DNA. When copying of the DNA is done, that cuts can be sealed again. So, topoisomerase takes care of the super coiling. Number 4 is that in case of DNA when you have a DNA strand like this 5 prime to say 3 prime direction. The other strand also has to be copied but that is a little bit complicated. We will go to that aspect later on. The enzyme called DNA polymerase adds the oligonucleotide one after another. Remember DNA polymerase works by from 5 prime to 3 prime direction.

3 prime OH is attacking the 5 prime triphosphate.

There is a problem of copying this or making the complementary DNA for the original DNA which runs from 5 prime to 3 prime direction. There is no such problem for the other strand. For the other strand, the DNA synthesis can start from here because now this is the 5 prime and going to the 3 prime. What happens to the complementary strand? It has got a 3 prime OH and there is another oligonucleotide which will have a 5 prime triphosphate. So, this OH will attack the phosphate, that goes out and that forms the phosphodiester linkage. So, one synthesis is not a problem because there is no the direction of the movement of the helicase or the replication fork. In this case, as the helicase moves, the replication also moves to the right side. The synthesis of the complimentary DNA strands which runs from 3 prime to 5 prime direction is not a problem. Because the synthesis of the complementary DNA strand moves towards 5 prime -3 prime directions.

Also in complementary strand, DNA polymerase does the synthesis of oligonucleotide. There is a problem of directionality. Our system the biological system has sorted out this problem. DNA synthesis cannot take place on a strand where there is no portion of any double strand. So, you cannot start the DNA synthesis. If you can remember the Sangers method there is something is called a primer. DNA polymerase can work towards 5 prime-3 prime direction in presence of a slight piece of double stranded DNA i.e primer. Tiny portion of the double strand could be a DNA RNA double strand or it could be a DNA double strand.

In this case the primers are RNA primers and these RNA primers are synthesized. There must be some enzyme which will synthesize this primer. So, these primers are synthesized by an enzyme called primase. Both the strands are now called template strands because the strands are separated and they act as a template to synthesize the new complementary strands. In one case, there is no problem because the direction of synthesis matches with the progress of the replication fork or the helicase.

Let us consider this strand where there is no problem because here continuous DNA synthesis takes place. By the way this is called the leading strand and this is what is called the lagging strand. This is called lagging strand because the DNA synthesis cannot be continuous. So, they are made in pieces. However, in the leading strand DNA synthesis is continuous because in that case the 5 prime to 3 prime DNA synthesis is matching with the progression of the replication fork and the helicase.

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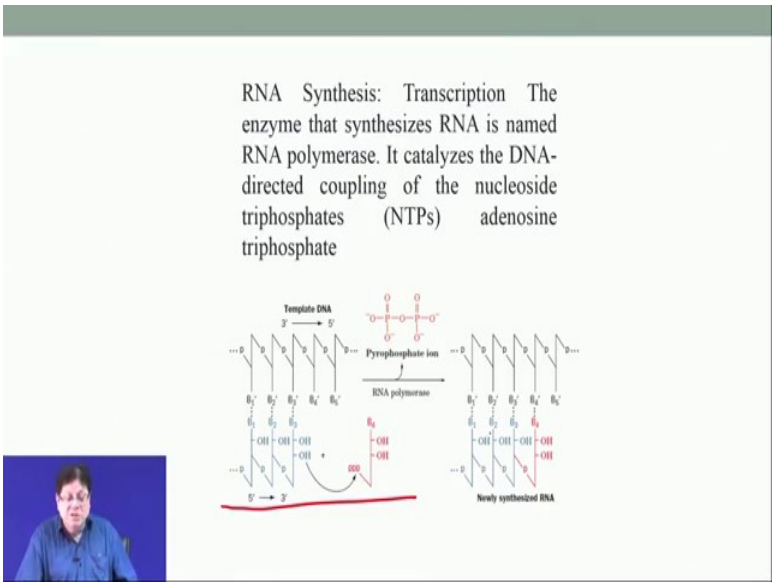

Now this is the reaction where the 3 prime is attacking the 5 prime phosphate and then that increases the chain.

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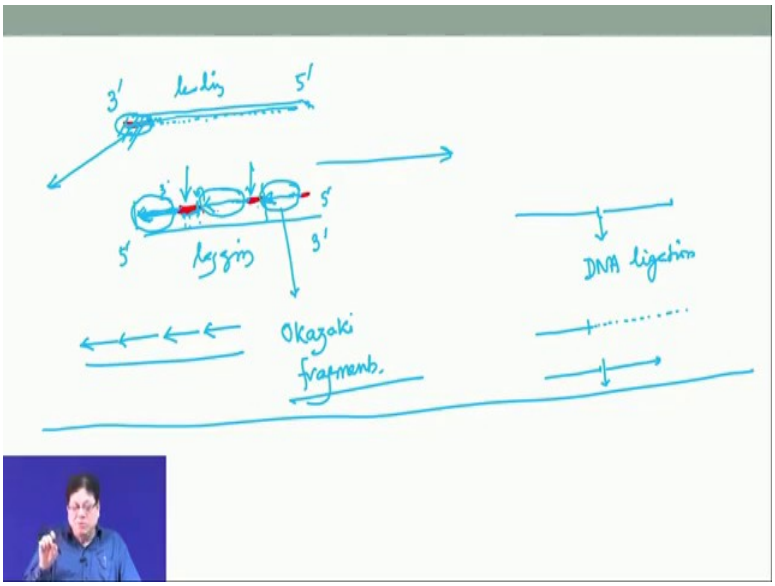

This is your leading strand that runs from 5 prime to 3 prime direction and here is the lagging strand which runs from 5 prime to 3 prime in this direction. There is this RNA primer that has to be synthesized by primase and then the DNA polymerase comes and adds the nucleotide according to the sequence in the leading strand. In case of the lagging strand, the synthesis has to go towards this direction i.e 5 prime to 3 prime. At first, RNA primer is made and then The DNA polymerase strats the synthesis. Remember this is going in the opposite direction of the replication fork.

The origin of the replication fork is at the point where it started, but then this is continuously moving. This is done along with this RNA primer. So DNA polymerase now jumps back here to make this strand from the 5 prime to 3 prime direction because it is lagging behind. So, it has to go back and then again start synthesizing the piece of DNA. But every time when it starts it needs a piece of double strand here.

Only one RNA primer serves for the leading strand, but you need several RNA primers for the lagging strand. The RNA primers are by the way joined here join to the new piece of DNA. Every time it comes to the starting point of the RNA. So, it again goes back and makes the RNA. The RNA primer is again made and then the DNA synthesis takes place up to this point. At the end of the game, you have the DNA which are made in pieces or fragments. So, this is called the lagging strand because here the DNA synthesis is not

completed. In leading strand, DNA synthesis is almost complete except the first primer that has to be removed later on.

But here the DNA is synthesis takes place in fragments. This fragments are called Okazaki fragments. So, once the synthesis is all done then what will happen? For the leading strand, you have to take this RNA primer out and this should be replaced by a DNA. This portion should only contain DNA. So, this RNA has to be chopped out making it free and then you add the piece of DNA to ligate this position.

So, you get the complete daughter strand which is complementary to the leading strand. For the lagging strand, there are more work to do. This has to be chopped off because here are the DNA. So, that will be chopped off and that will be replaced by DNA. But the problem is that there is no connectivity between this part of the DNA and this new DNA will replace the RNA. So, you have to do ligation.

DNA ligase comes and adds these DNA pieces. You need joining of these two ends and that is done by DNA polymerase. DNA polymerase can only do addition from one end and then add all the time. But if there are two DNA fragments facing each other then there is a nick in between. The nick can only be repaired by DNA ligase.

So, basically what we have learned? We have learned that first of all DNA replication is semi conservative. As DNA replication is semi conservative in nature, the two old strands have to be separated. For separation, you need an enzyme called helicase.

You need SSB protein i.e single stranded binding proteins which will stabilize the two isolated strands. Then what happens? Primers are synthesized. Primers are made of RNA. The RNA primers bind to the old DNA strand. The DNA synthesis starts because DNA polymerase cannot work on it. RNA primers have to be synthesized and then the synthesis starts. Who does the synthesis? That is the DNA polymerase.

There are different categories of DNA polymerase. DNAs polymerase 3 does the synthesis by putting the oligonucleotides one after another depending on the sequence of the leading strand. Now there is this leading strand. There is no problem because it runs the synthesis runs from the 5 prime to 3 prime. It runs towards the direction of the movement of the helicase or movement of your replication fork. The lagging strand runs

from 5 prime to three prime. So, the complementary strand should run from 5 prime to 3 prime in a direction opposite to the movement of the replication fork or the helicase.

In that case, the synthesis has to be taken in fragments, the RNA primer is synthesized and then the DNA synthesis starts by the DNA polymerase. But as it goes to the origin of the replication, it has to go back and then again synthesize the RNA primer to start the synthesis of the DNA. When it comes to the almost end it stops. Here again goes back and then synthesize RNA primers by primase.

Now RNA primer and this DNA are joined. This DNA at this point is not joined to the RNA because the synthesis started from this and ended here. So, there is a gap i.e nick between these portions. Now what will happen? DNA polymerase 1 takes care of this replacing the RNA primers by DNA.

But there is still nicking. DNA piece is not connected to the new the DNA piece which is replacing the primer. As here it runs in the opposite direction there will be a disconnection between these fragments. These are called Okazaki fragments. So, after this the DNA ligase comes and joins these nicking points. So, that is the whole story about the replication of DNA. So, you see it is a very complicated process. Many enzymes are involved here- helicase i.e a helix breaker, topoisomerase that takes care of supercoiling.

This primase makes the primers. There are two types of DNA polymerase. One is DNA polymerase 3 which does the synthesis. DNA polymerase 1 takes care of the replacement of the RNA primers and replaced by the DNA. Finally, DNA ligase joins the Okazaki fragments to make the complete piece of the complementary strand. So, that is the story behind your replication DNA replication.

Thank you.