Biology for Engineers and Other Non-Biologists
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Week- 04
Lecture - 20
DNA Replication

Hello and welcome back to these series of videos on biology for engineers and other non-biologists. Today we are going to talk about a very important process, the process of DNA replication. Now, before I get into the nitty-gritties of exactly how DNA copies itself, it is important to know and refresh our memory about the DNA structure. Now we all know that DNA is a double standard molecule, right, and it is made up of smaller units which are called as the nucleotides. Now each nucleotide itself is made up of a phosphate sugar backbone. So it has a phosphate, a pentose sugar and the nitrogenous base.

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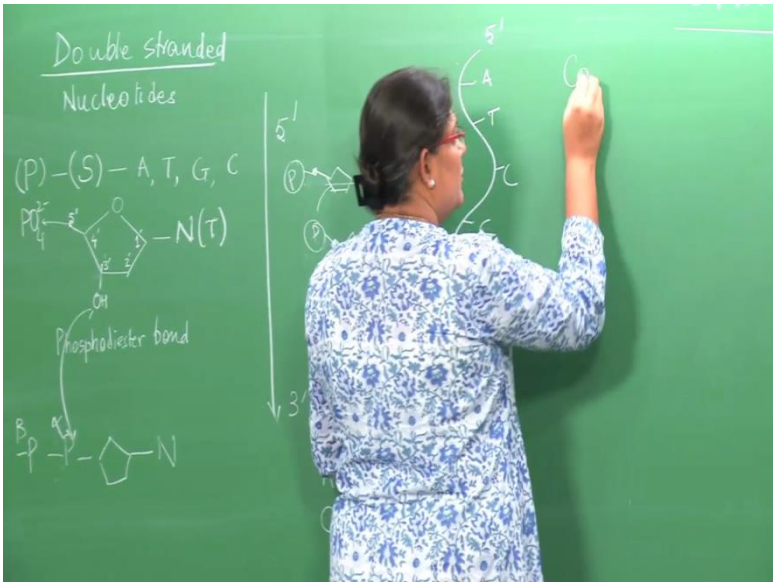

Now this nitrogenous base could be either an adenine or a thymine or a guanine or a cytosine. So each nucleotide basically has a nitrogenous base and this nitrogenous base is attached to a pentose sugar, right? And the (f) fifth carbon of the pentose sugar in turn is attached to the phosphate group, okay? Now we know that each nucleotide, so let us say this is nucleotide 1; now this nucleotide 1 in its pentose sugar has the first, the second, the third, the fourth and the fifth carbon, right?

Now we know that every time this nucleotide has to interact with the next incoming nucleotide, it is the hydroxyl group present in the third carbon of the pentose sugar which is

deoxyribose in this case, has to interact (it) this hydroxyl group at the (third) 3 prime carbon, interacts and forms of phosphodiester bond, right, with the next incoming nucleotide.

So if you have let us say a (next s) let us say this nucleotide was anything; let us say it was a T, okay? And then it has to form phosphodiester bond with the next incoming nucleotide; this 3 prime hydroxyl in the pentose sugar will form the phosphodiester bond with the next incoming nucleotide which again will have its own pentose sugar, will have its own nitrogenous base and will have alpha, beta and gamma phosphates. So this 3 prime hydroxyl is then going to form the phosphodiester bond, will have a nucleophilic attack on this (phospho) on the first alpha phosphate and hence you get the first phosphodiester bond formed.

So what do you get? You get the first nucleotide, with its phosphate, with its sugar and with its nitrogenous base let us say N1, and this forms, this third carbon forms again a bond with the next incoming nucleotide, let us say N2. Now what you notice is that it is always the 5 prime, the phosphate which is attached to the 5 prime carbon, so you will have another carbon here and then this, right?

Similarly in this case this will be the fifth carbon attached to the pentose ring. So you always find at, this is the third carbon of the sugar which is forming the phosphodiester bond with the phosphate which is attached to the fifth carbon. That is why you find that the Strand or the polymerisation, building up of more and more nucleotides for DNA happens from a 5 prime to a 3 prime direction.

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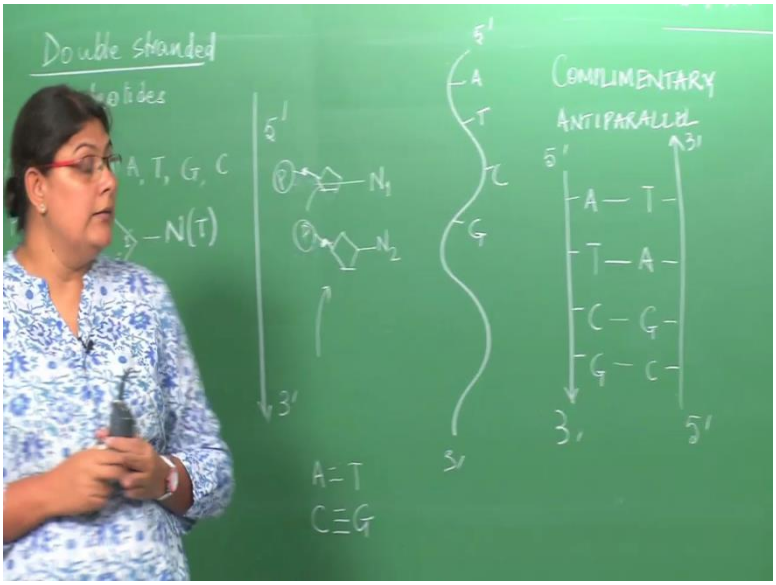

So before I get into DNA replication, there are few things which is very important to know. The first one, as I told you that DNA is a double stranded structure, it is a polymer of nucleotides, each incoming nucleotide attaches to the previous nucleotide through a phosphodiester bond and this incoming always happens at the 3 prime end of the given Strand. So this is one thing that I want you to remember and I will come back to this when we are talking about the mechanism of DNA replication.

The other (th) thing which you have to remember is that when you look at a DNA double helix; let us say this is one strand which is going 5 prime to 3 prime, the other strand is going to be complementary to it. Now what do you mean by complimentary, we have already studied that always the adenine base will form hydrogen bond with the thymine group while the cytosines will always form hydrogen bonds with the guanine. So every time in this strand, 5 prime to 3 prime, you let say have an adenine, a thymine, a cytosine and a guanine, what will happen is the second strand will be complementary to it.

So the DNA structure is also, the 2 strands are complimentary, right? So if this is the first strand with a certain sequence going 5 prime to 3 prime, the second strand will be complimentary to it but will also be antiparallel, right? So let us, for simplicity sake, let us say this is one strand which is going 5 prime to 3 prime with this sequence, A, T, C and G, then the (s) complementary strand at this position will have a C, at this position will have a G, at this position will have an A and here it will have a T.

And the sugar phosphate bone which holds it together will be going in the opposite direction, 5 prime to 3 prime, right? So this is, these are 2 major features I want you to keep in mind, one that the 2 strands of DNA are complimentary, so in a sense if you know the sequence of one strand, that one strand knows and has information (of) as to what all would be the sequence of nucleotides in the second strand.

So that information is kind of coded already in the DNA, thanks to the ability of base pairing, okay? The second thing is that the 2 strands of DNA are antiparallel. And how do you decide the directionality as I just mentioned through the formation of phosphodiester bonds that it is in the direction of 5 prime to 3 prime because every incoming nucleotide will form a phosphodiester bond with the 3 prime hydroxyl of the previously sitting nucleotide. So the polymerisation, adding in of successive nucleotides is happening in a 5 prime to 3 prime direction.

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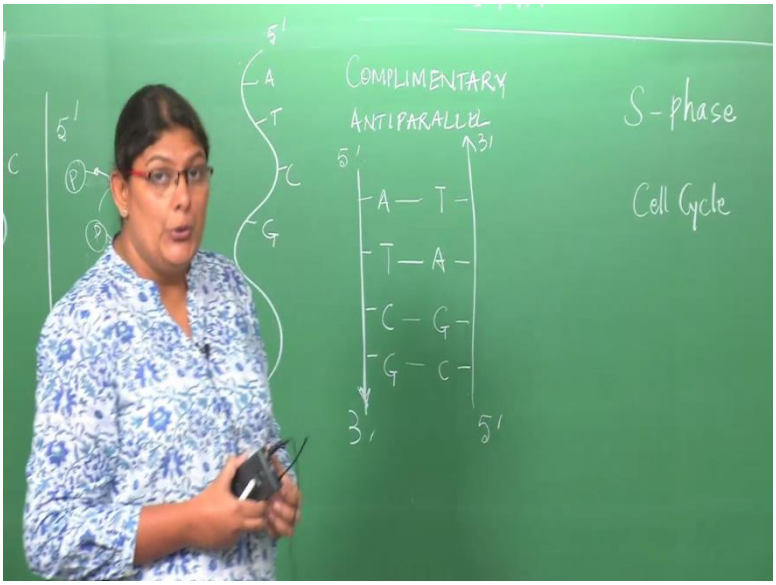

Now this is some of the important basics about DNA structure and the relevance of this will become more apparent when we start looking at exactly how DNA replication happens. Now, you may ask me what is DNA replication. Well, DNA replication is nothing but a process in which a DNA will copy itself and as we had seen in cell cycle this process wherein a DNA copies itself happens in case of eukaryotic cells during the stage of S-phase in cell cycle.

Remember I told you that in cell cycle a cell prepares itself to pass on the information to the daughter cells and the way it does that is that it first duplicates its DNA before actually dividing and undergoing the process of cell division. So that is what we are talking today, we

are talking today exactly how a DNA is able to copy itself and pass on the same information to the daughter DNA molecule.

So let us look at what happens during DNA replication. Now, I will, want you to remember again I am (a) reiterating 2 things, antiparallel strands, complementarity and formation of phosphodiester bonds from 5 prime to 3 prime, or (in) the incoming of the newer nucleotide and formation of a 5 (p) phosphodiester bonds and hence are directionality in 5 prime to 3 prime.

Now, let us look at the DNA replication itself. Now DNA as I had mentioned earlier also, does not exist as a linear molecule in our cells, it kind of remains in a condensed form because of its interaction with histones. And around the time when the cell is ready, sorry, chromatin, right; around the time that the cell is getting ready to replicate, it loosens up and it exists as a chromatin, okay? Now it is important to note that DNA is a helix, right? It is an alpha helix, it is a twisted helical structure and; so let me draw DNA molecule.

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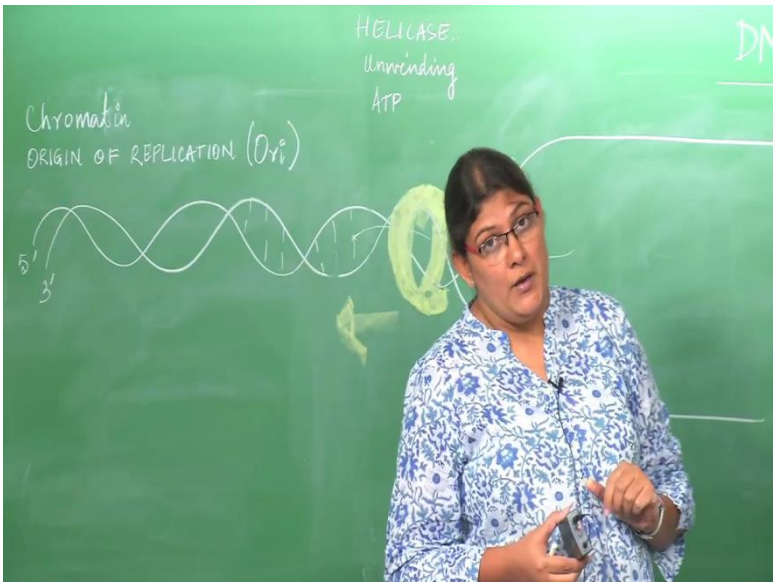

Let us say this is a DNA molecule, this is the first strand going 5 prime to let us say 3 prime, okay? And then you have a second strand which is complementary to do it, where this becomes the 5 prime end and this becomes the 3 prime end. Now it is important if the DNA has to replicate, it has to first unwind, right? And this unwinding has its consequences and (wh) imagine it like this, you have a nylon rope and you are trying to pull apart the 2 strands of a nylon rope. Now what will happen is, every time you are trying to pull apart the 2 strands of the nylon rope, they will try to recoil back. Now that is a tendency, right?

Now the same thing, and same problem one encounters when there is going to be the separation of the 2 strands and how is it that the cell makes sure that once the strands have segregated and separated, they are prevented from recoiling back till the process of DNA replication is over. Now that is one thing to ponder.

So let us look at how DNA replication happens. Now, the human DNA is told you is really big. Now what I am going to (t) talk today about DNA replication is going to hold true, (I) what I am going to cover is just the basic features of DNA replication and just give you the common features. There are slightly different in prokaryotes than in eukaryotes, we will talk about that later, but right now we are just looking at exactly how DNA copies itself.

Now DNA has the signature sequences on its arrangement of nucleotides, which act as recognition for a point where the replication has to start. Now these regions where the DNA replication starts are called as origin of replication, okay? In bacteria it is called as the Ori sequence. So there are specific regions on the DNA double strand which will direct the entire machinery of the DNA replication to that telling them, that this is where the DNA replication should start. So that region where the replication actually starts is called as the origin of replication.

Now let us come back to this DNA strand. So we have these 2 strands and the unwinding has to happen, right, so this strand has to segregate. So it is like you imagine that this is your double helix DNA and then it has to open up. So it is like you have a zip and then you have to unzip this entire molecule of DNA. And then this is the next strand, okay? Now this opening of the 2 helices, somewhere here will be the origin of replication and this is where the replication has to start, the first thing that has to happen is the 2 strands have to unwind. And this unwinding happens with the help of a particular enzyme, let me draw this here, so it is like a clamp like enzyme okay?

And this enzyme is called as Helicase. So what this enzyme really does is nothing but, since the 2 strands are held together the complementary strands are held together through hydrogen bonds, it basically breaks these hydrogen bonds, so it starts moving inwards, so it would have first bound here, right; starts moving inwards and it starts breaking the hydrogen bonds and as it moves in this direction it keeps unwinding, keeps unzipping. So it is like the clamp on the zipper which is just unzipping the 2 strands which are held together through hydrogen bonds.

So the helicase is the first enzyme which comes in and it causes the unwinding of the 2 DNA strands. Obviously this process is not going to happen spontaneously, it does require some sort of energy and this energy comes from ATP. I mentioned to you during cell cycle that before a cell commits itself to cell division and cell replication it ensures that sufficient energy is there and now you can appreciate why because this process of unwinding is a pretty (int) energy intensive process and it does require expenditure of ATP, so helicase is the first enzyme which will come and open up the 2 strands of DNA.

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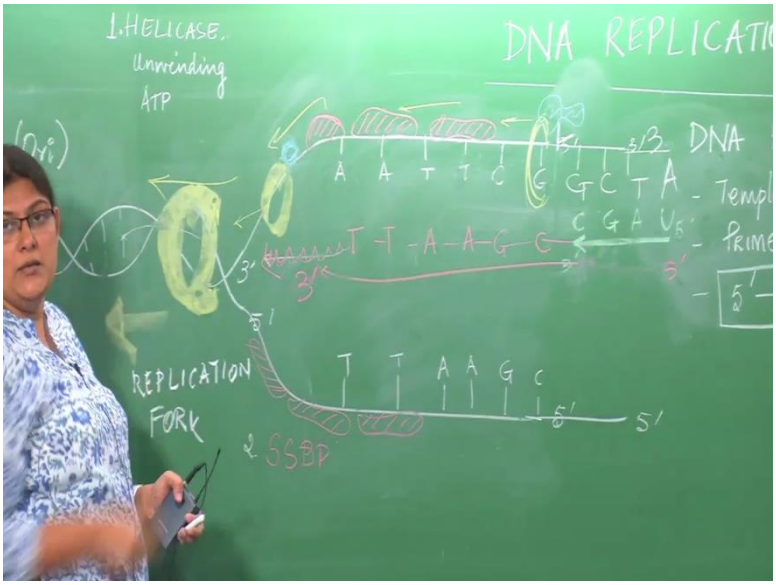

Now let us look at what will happen. Now (as) as I mentioned the (t) strands will try to come back together and that should not happen because the DNA has still not finished its job of replicating it and the way DNA prevents this is by a clause of another proteins which as soon as the unwinding has happened bind to these strands, okay? These proteins will bind on either of the 2 strands, both the 2 strands actually, okay, and it will prevent it not only from mining to each other; if the DNA (tras) the separated strand tries to recoil with itself, it will prevent that also.

Now these proteins which prevent that are called as single strand binding proteins, okay? So this SSBPs will now keep the separated strands separated. Okay, that is fine. But how does a new DNA come into existence? Now that happens because, so (we) let me (f) before I get there let me again tell you the (f) there is origin of replication that the process of replication will start; you have the first enzyme which comes in and which helps you in unwinding the DNA which is the helicase. Once the DNA has been unwound the 2 separated strands remain

separated thanks to the second class of proteins which are called as single strand binding proteins.

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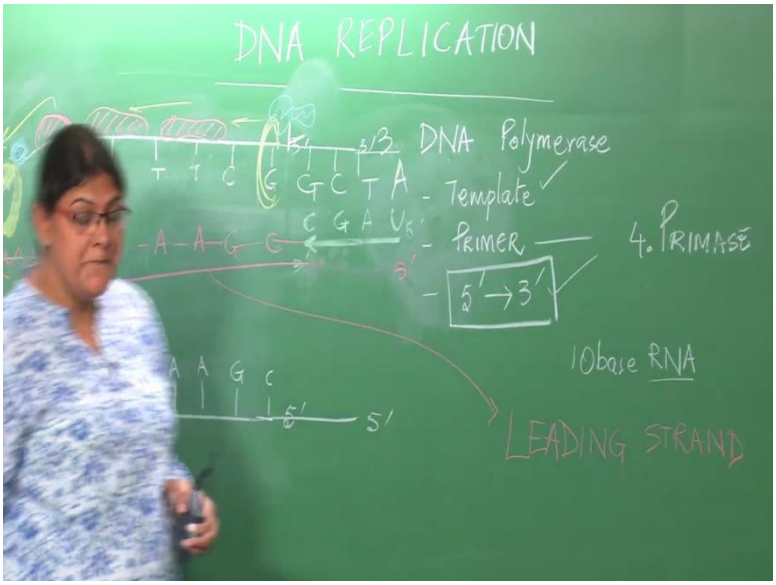

Now once that has happened the (polom) there has to be a (go) enzyme which will then come and, you know, meticulously will start bringing in 1 nucleotide at a time and make a second strand. So let us take this separated strand and let us (lo) let us give it some sequence. Let us say it has a sequence of an A, another A, a T, another T, say a C and a G, okay? Now since this strand was complementary to this strand, it should exactly have the complementary information, which is it should have had a C here, a G here, right, and A here, another A here, a T here and a T here, okay?

So, now this separated strand has to give a new copy, it cannot go back and reanneal with its partner which was there earlier, it has to give rise to new strand. Now this polymerisation, this bringing in of new (tucloni) (2 neu) new nucleotides as per the sequences just dictated by this strand is done by an enzyme called as DNA polymerase, all right? (Now ther) there aredifferent types of DNA polymerases both in prokaryotes and eukaryotes, I am not getting into details of those, I want to keep this clause very simple.

So these DNA polymerases will bring in 1 nucleotide and attach it to the next nucleotide in the 3 prime end. So what happens is this DNA polymerase needs 2 things, first and the foremost it needs something to guide it, something to tell it that after this nucleotide (the) this nucleotide has to come. So it needs what we call as a template, right? So this separated strand acts like a template for DNA polymerase, the first requirement is a template.

The second requirement is the more bigger of a deal because it is this stringency of DNA polymerase which will create some problems for us and we will see how. The DNA polymerase de novo cannot start making, so if the new strand which has to come here has to get synthesised in 5 prime to 3 prime direction. Remember there is a formation of phosphodiester bond and this happens because the incoming nucleotide, (w) and, will, which is, has a phosphate at its fifth carbon of the sugar has to form phosphodiester bond with an existing nucleotide having a 3 prime hydroxyl.

So it has to make a chain in this 5 prime to 3 prime direction. The problem is, polymerase on its own cannot start making a new strand. It needs, one it needs a template to tell how to put in and in what sequence to put in the nucleotides, the second is it requires a primer, all right? So it needs something to go and tell it that (s) from there you need to start adding the nucleotides. So (the) it has 2 have some sort of an indicator which will tell us that from here you need to start adding nucleotides in a certain direction.

And DNA polymerase always adds nucleotides in a 5 prime to 3 a prime direction, okay? So basically this is a requirement of a DNA polymerase. So when we have separated the 2 strands, the separated strand acts as a template, so this requirement has been taken care of, it will polymerase only in the direction of 5 prime to 3 prime; that also we know white happens. But how will it decide where to start adding? Now this requirement of where to start adding these nucleotides one at a time, is brought about by another enzyme which is called as the primase, okay.

Now primase is a very (n) interesting enzyme, so let me just rub this off again, okay? Now primase is a very interesting enzyme. It kinds of tags along every time with a helicase, okay? And as the unwinding happens, this (the the) let us say, earlier the helicase was sitting here and then started moving in this direction, reached here, now it has continued to move like that. Now, as it started unwinding the helicase, the (primer) Peggy bagged primase kind of piggybanks itself along with the helicase, and as and when there is unwinding happening, it can immediately form a primer.

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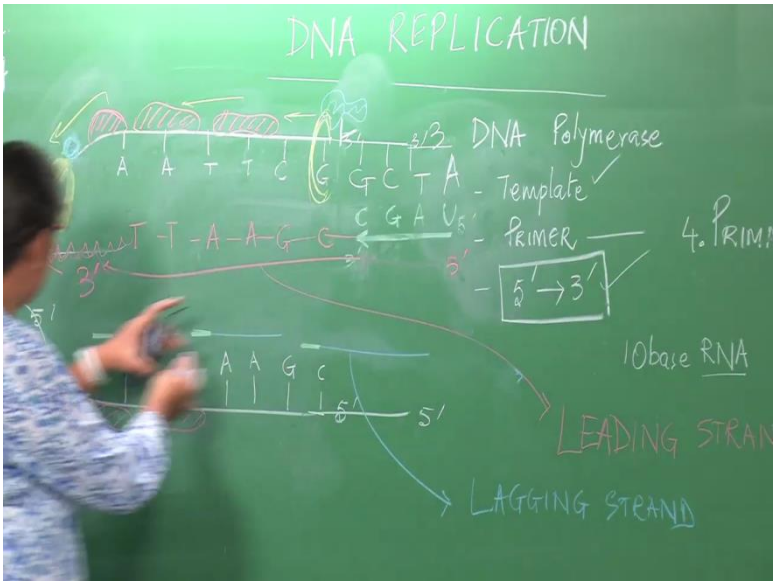

Now primer is nothing but about 10 bases long RNA sequence, so this is like an RNA polymerase, all right? So what it does is that if you have this as a sequence, it will form, let me continue this, this is 3 prime, this continues as 5 prime. There are let us say another (set) set of bases here, G, C, T, so on, okay? So what it does is, reads this, immediately synthesises a small RNA molecule, okay? T will always pair with an A, right, C will pair with a G, G will pair with a C and if it is an RNA molecule and let us say there was an A here; now RNA will not give thymine it will give a uracil. So it synthesises this small stretch of RNA as a starting point.

So this primer now acts as the base on which the DNA polymerase can start adding the nucleotides, okay? Now this is formed by the enzyme primase and once the (R pr) RNA primer is there DNA polymerase, so this is 5 prime, so the 3 prime hydroxyl here is free, right, of the pentose sugar. So this will now form of phosphodiester bond with a new nucleotide which is coming in and what will decide which nucleotide will come in; that is decided by the sequences on the template.

So this is how the polymerase will start, so let us give another colour, so let us say it starts acting so it will put a C here, we will put a G here, it is an A here and A again. Now this is DNA, this is not RNA, right? So this will put thymine here and a thymine here. And this process will keep on continuing. So what happens here is all that DNA polymerase needs is somewhere to start. And it is the primer which gives the direction that start adding nucleotides from here and that is exactly what happens. Once the primer is there in vicinity

the DNA polymerase hops along and moves, and it started to polymerise in the direction of 5 prime to 3 prime, okay?

Now, what you notice here is 2 things, one; as soon as the 2 strands have opened and they have got separated you have a formation of a structure which has taken place and this structure thanks to the uncoiling by the helicase is called as the replication fork. So this is a replication fork. Now in that replication fork for one of the strands, the 2 strands remain separated thanks to the single strand binding proteins, once they remain separated for one of the strands, what happens is you start having a quick polymerisation. As soon as the primer has been put in, thanks to the primase you find that the DNA polymerase continuously starts adding the nucleotides because DNA polymerase works only in the direction of a 5 prime to 3 prime direction.

Now this newly synthesised strand which is pink in colour, this one, right, till here it is a RNA and then it continues as a pink strand; it keeps on continuously moving, so as the helicase keeps on unwinding this particular strand is continuously getting synthesised because DNA polymerase works in the 5 prime to 3 prime direction, and one primer is enough to keep the extension going. This strand which is continuously synthesised is called as the leading strand, okay?

Everything is fine so far so good. But then the problem comes for this one. Now here the 5 prime to 3 prime direction is going this way, right, this strand has a 5 prime end here and a 3 prime end here. And if the RNA (pol) sorry, if the DNA polymerase has to work, the DNA polymerase will work in the complimentary fashion 5 prime to 3 prime, okay? But there is another problem it (h) encounters because the helicase is moving faster, keeps on unwinding and as it keeps on unwinding the Peggy bagged primase keeps on adding primers, all right? So in this strand what has happened is for this particular complementary strand the DNA polymerase cannot, at a stretch, synthesised the newly nucleotides; it has to keep on adding these things in stretches.

So let us say, the DNA polymerase started polymerising this thing, started adding the bases in the 5 prime to 3 prime direction, by the time it finished it, this region further uncoiled and when a new primer was formed, so the DNA polymer sends to come back and jump and then synthesise again. Then this uncoiled further, again there was a primer formed here and then it again had to (unc) extend it in a 5 prime to 3 prime direction. So what you find is that when you have this (so) short stretches synthesised, the second strand is not getting synthesised in a

continuous manner. Though it is getting synthesised in a 5 prime to 3 prime direction it is not happening in a continuous manner. And this, (um) what happens for this one is that you end up having small small stretches of new strands synthesised.

Every time you have a RNA primer,RNA primer and a RNA primer. So this strand is a little is lower than the first strand and it is called as the lagging strand. And this process keeps on continuing, okay? And then once this has been formed, what will now have is let us say, this is how I am just redrawing this with much simpler form, so you have this DNA molecule, right, and then you had this one as the (f) 3 prime end, right, this point give you the 5 prime and then this was a 3 prime and then this was the 5 prime. So what is happening now is that you are getting a new molecule synthesised, sorry, with the leading strand which is moving towards the replication fork and a lagging strand which is getting synthesised in bits and pieces.

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Now each of these tiny pieces of the (lag) lagging strand are called as the Okazaki fragments, okay? Now, so what has happened, this one molecule is now giving rise to 2 molecules and these Okazaki fragments have to be later rejoined; not only are they supposed to be rejoined, whatever RNA which was sitting, right, there were primer which were sitting here, there was one primer which were sitting here, right, you have primers here, primers here, primer here. All these primers have to be removed and have to be replaced with a DNA sequence, because in the end it is about copying the DNA and not DNA (hi)RNA molecule.

So what happens is the primers get removed after the Okazaki fragments have been formed by another type of DNA polymerase. That is why I told you there are different kinds of DNA polymerases; I am not going into all of them, this is a different DNA polymerase. This DNA polymerase will re-synthesise the missing pieces which were (or) originally occupied by the RNA and then for the lagging strand once these have been synthesised, these pieces have to be stitched together, right, because it has to be a continuous stretch and that stitching happens by the one of the another crucial enzymes called as DNA ligase.

So what do you notice here is, you started with a double helix, the helix got uncoiled, thanks to the helicase and the 2 separated strands were maintained in a separate position because of the single strand binding proteins. Not only that, as soon as the uncoiling happened you had the primase come in; it started putting in small stretches of RNA which you call as the primer. You need this primer because DNA polymerase has to then build upon it, (also) RNA primer kind of acts as a foundation for this DNA polymerase to then keep on adding the nucleotides in a 5 prime to 3 prime direction.

Of the 2 separated strands, one strand just grows continuously so that is called as the leading strand but since here the uncoiling is happening at this end and the polymerase only works in 5 prime to 3 prime, for the other strand the polymerase adds these stretches in small pieces, which is what you call as the Okazaki fragments. The Okazaki fragments are finally stitched together as a single strand by the DNA ligase while the RNA sequences are removed by another DNA polymerase. So this is how the DNA replication happens and then finally once it has copied itself the DNA polymerase will reach a point (aranear) the way you have origin of replication, you will have another signature sequence sitting on the DNA which is called as the terminator, a terminator sequence in case of prokaryotes, in case of eukaryotes you have regions which are called as telomere.

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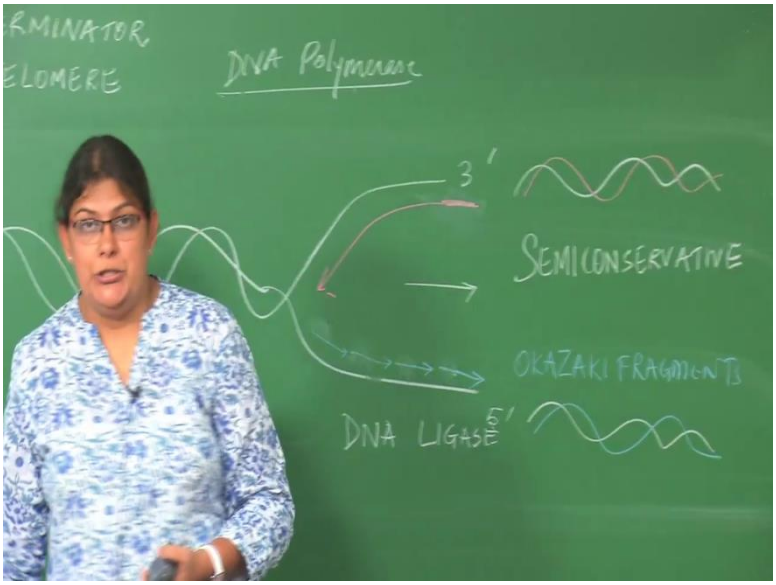

So this is how DNA replication takes place. Now you notice at the end of this entire process, once this DNA replication has happened what you end up getting our 2 daughter DNA molecules, this one formed because of a leading strand, right, and this one formed because of the lagging strand. By what you notice is in each new daughter DNA molecule one strand is the parental strand which came from the original DNA while one strand is the newly synthesised strand. The same thing happens in this molecule, one strand is from the parental DNA, the other strand is the newly synthesised strand.

So (the) such mode of DNA replication, where it still has the original parental strand, one of it and one of this is newly synthesised because of complementarity is called as semi-conservative mode of replication. So with that we have covered how DNA replicates itself. I just, again I just want, before I wind up, I just want to highlight few points.

The 2 strands of DNA are antiparallel, the base pairing provides the complementarity between the 2 strands and then you have a whole (array) array of enzymes which are required for uncoiling of the DNA, for polymerisation of DNA. The DNA polymerisation always happens in the direction of 5 prime to 3 prime. And that is simply because of the chemistry by which the sugar phosphate bone in DNA is actually formed.

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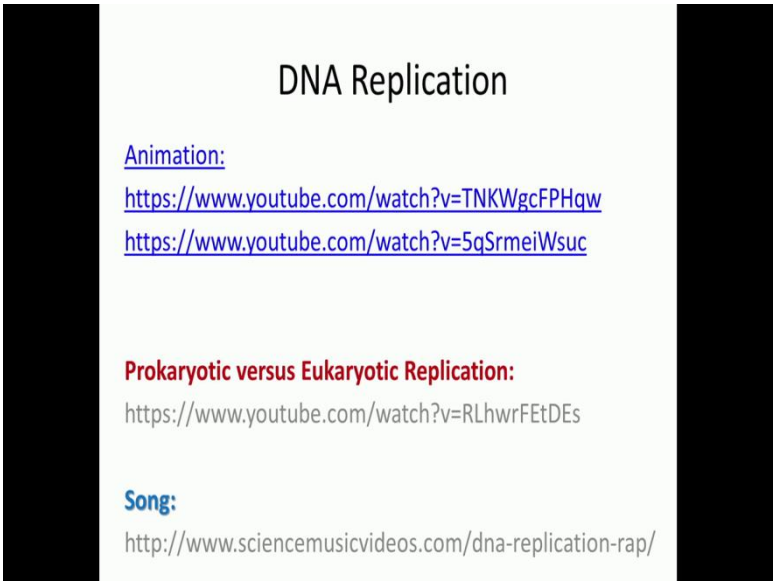

Now, I would like you to, if you have time, to go through some of these fun videos and the first one is, there are 2 videos which very briefly in animation actually show to you exactly how this process of replication takes place, what has taken me about 30 minutes to explain you can see it in about 5 to 10 minutes video. And this is a very interesting video.

There is another video which also will tell you if you are interested to know, what is the difference between DNA replication and prokaryotes versus eukaryotes. And the last is a fun rap song again by Glenn Wolkenfeld which will just help you kind of quickly grasp the various quick players of DNA replication. Thank you and I will see you later. Bye-bye.