Basics of Biology
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Lecture 25
Replication

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing? We were discussing about the different properties of a living organism. And in that context, So, far what we have discussed, we have discussed about the classification of the different organisms and then we subsequently understand how these organisms are evolved over the course of a time.

And then we have alSo, discussed about the structure of the prokaryotic as well as the eukaryotic cells. And in the previous module, we have discussed about the different types of biomolecules. So, we discussed about the nucleic acid, we discussed about the carbohydrates, we discussed about the proteins. And then we alSo, discussed about the lipids.

Apart from understanding the structure and functions of these biomolecules, we have alSo, understood the role of these biomolecules into the cellular activities. Now, once we have understood the biomolecules and their structure and functions, it is important for us to understand the, how these biomolecules are playing the role in different types of activities and that is what we are going to discuss in the current module.

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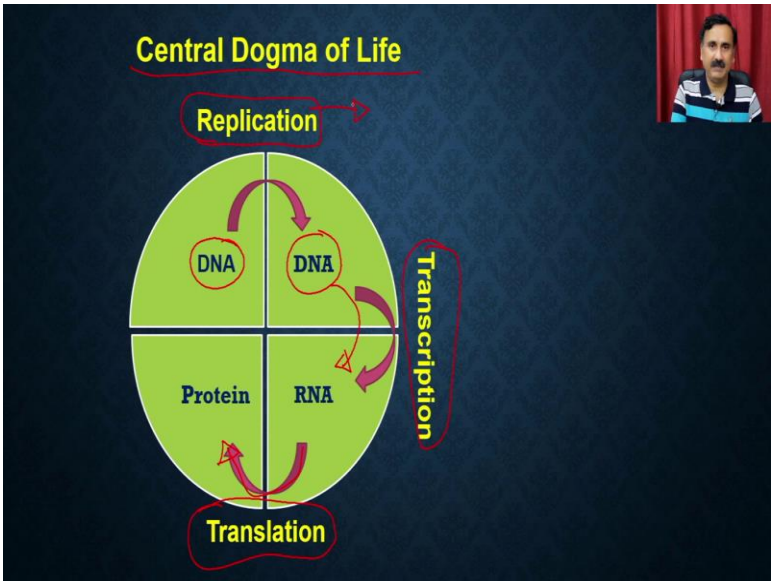

So, what is the central dogma of life is that you are going to have the DNA dependent DNA synthesis, which is called as the Replication. So, we are going to understand about the replication. Then we can alSo, discuss about the DNA dependent RNA synthesis, which is called as the Transcription. And then we alSo, can have the RNA dependent protein synthesis and which is alSo, called as the Translation. So, let us discuss the first process and the first process is called as the replications.

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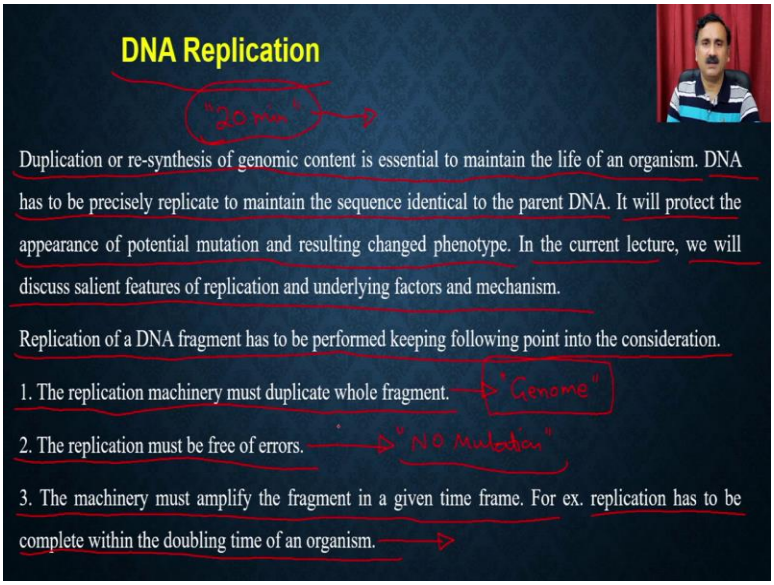

Now, Replication or the DNA replication is the duplications or the re-synthesis of the genomic content and it is essential to maintain the life and organism. DNA has to be precisely replicate to maintain the sequence identical to the parent DNA. It will protect the appearance of the potential mutation and resulting changed phenotype.

In the current lecture, we will discuss salient feature of the replication and underlining factors as well as the mechanism. Replication of a DNA fragment has to be performed keeping the following point into the consideration. What is the point? The replication machinery must be duplicate the whole fragment, which means it is actually going to replicate the whole genome of that particular organism.

It is not like you can do the partial replications. You cannot do the partial replications. The replication must be free of errors, which means there should be no mutations. So, there should be no mutation. That is the ideal conditions. Although, we know that there could be a mutation when the DNA is replicating from one copy to another copy.

And the machinery must be amplify the fragment in a given time frame. For example, the replicons has to be complete within the doubling time of an organism, which means there is a time restriction for the machinery to replicate the DNA. For example, the bacteria is actually, or the E.Coli is actually going to duplicate into 20 minutes, it is going to make the one bacteria into two bacteria.

This means the replication has to complete within this timeframe. Now, before getting into the details of the DNA replication, it is important to recall the, what are the properties of a particular DNA. Remember when we were talking about nucleic acid, we were discussing about the different types of properties. So, what are the different properties? Just, we can, very quickly summarize or review what we have discussed.

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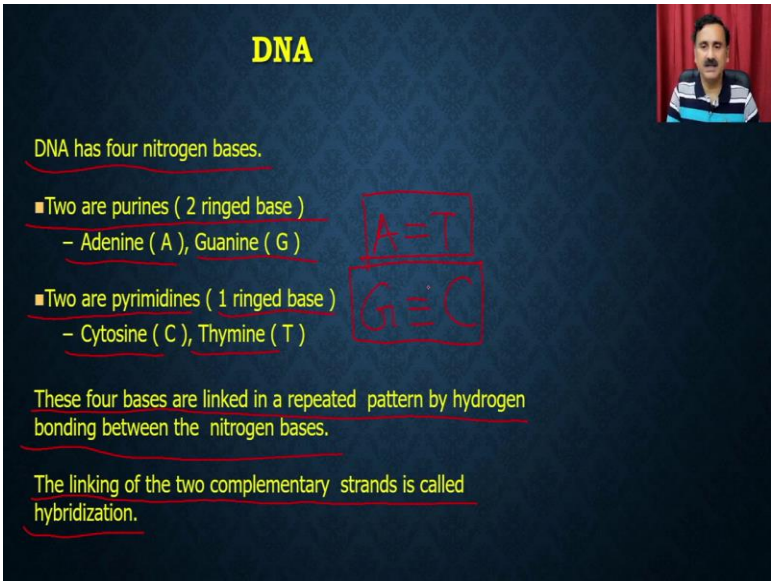

So, DNA has the four nitrogenous bases. We can have To have the purine bases, where you have the Adenine and Guanine. Then you can have the two of the pyrimidine bases which are the one-ringed bases, these are called as the Cytosine and Thymine. And these four bases are linked in a repeated pattern by the hydrogen bonding between the nitrogenous bases, you remember that.

The A is always making a double bond, double hydrogen bonding with the T, and G is always making a triple bond with the C. So, the linking of the two of the complementary strands is called as the hybridizations, which means A is always going to make with a pair with T and G is always going to make with a C. And this is important because that is how the DNA is actually going to maintain the complementarity in their sequences.

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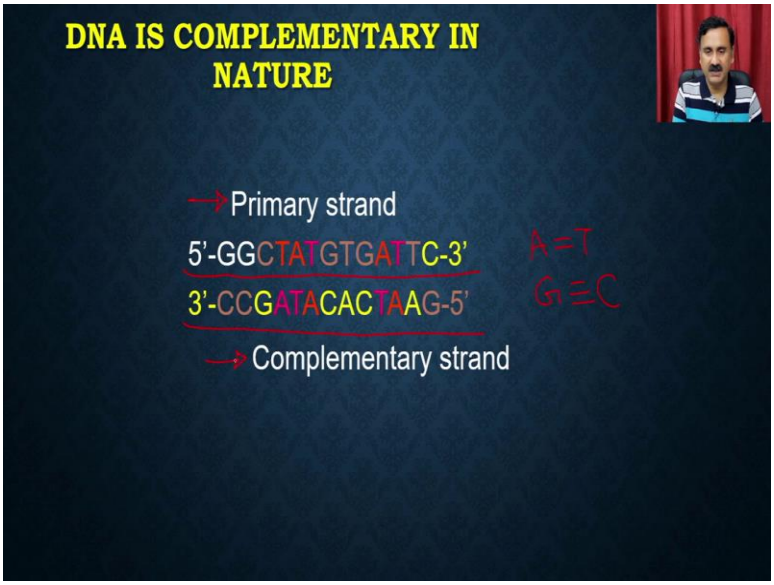

So, what is mean by the complementarity? Complementarity means if that you are going to have the primary sequence. So, imagine that this is the first strand synthesis, and then this is going to be complementary because the A is actually going to make a pair with G and T, and the G is always going to make a pair with C.

So, wherever you have the G, it is going to be C, and wherever you have the A it is actually going to be T. And that is why the sequence on the primary, first strand is going to be the, and on the other hand, it is going to be complementary strand. And that is why the first strand is called as a primary strand, and the second strand is called as a complementary strand.

So, before getting into the detailed process of the DNA replication, let us first discuss about de mush machinery what is responsible for the replications.

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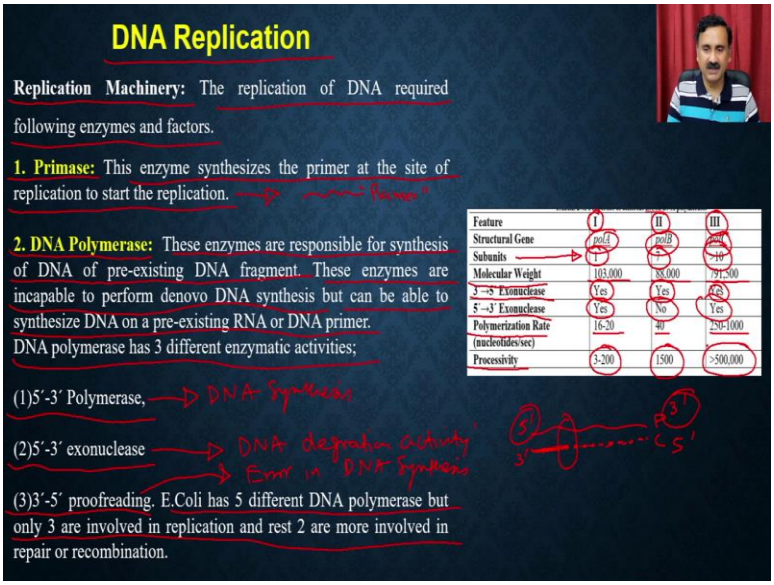

So, we have the DNA replication machinery. So, what is there in the replication machinery? The replication of the DNA required the following enzyme as well as the factors. The first enzyme what is required is called as Primase. The enzyme synthesizes the primer at the side of replication to start the replication.

So, this is going to be the first enzyme which is going to synthesize the small stretch of RNA, and that small stretch of RNA is actually going to function as the primer. Then we have the DNA polymerase. These enzymes are responsible for the synthesis of the DNA of pre-existing fragments. So, DNA polymerase is the main enzyme which is alSo, called as the DNA dependent DNA polymerase.

And these enzymes are incapable to perform the denovo synthesis but can be able to synthesize the DNA on a pre-existing RNA or the DNA primer. What it means is that DNA polymerase cannot synthesize the denovo synthesis. They actually can add the nucleotide on the existing DNA fragment, which means if you have a DNA fragment the DNA polymerase will not be able to add the nucleotides on to the other strand.

But if you have a small strand like this, then it actually can actually add the nucleotide on this according to the nucleotide what is present on to the primary strand. So, this is going to be complementary strand, this is going to be the primary strand. And that is why this

also, require the action of the Primase because the Primase is actually going to synthesize this RNA primer.

And that is how we are, this can be utilized by the DNA polymerase to synthesize the second strand. DNA polymerase can have the three different types of enzymatic activity. It can have the 5 prime to 3 prime polymerase activity, which means it actually going to do a DNA synthesis into the direction of 5 prime to 3 prime.

Remember that when we were talking about the DNA, we were talking about that it has, it has a 5 prime phosphate and the 3 prime hydroxyl group, and it is going to be complementary. So, it is going to be like this. Which means, on the 3 prime, you are going to have the 5 prime, and that is how you are going to add the 5 prime end. So, this is going to be DNA synthesis.

Then it also, has the 5 prime to 3 prime exonuclease activity, which means that is going to degrade the DNA, So, DNA degradation activity. And that activity is also, nucleotide specific. And then you also, have the 3 prime to 5 prime proofreading activity. So, E.Coli have five different polymerases but only three are involved in the replication, and two are involved into the repair or to the recombination.

So, 3 prime to 5 prime proofreading activities, that it is actually going to check the errors into the DNA synthesis. So, now, you can have the three different types of RNA, DNA polymerases, what is present into the organisms. And the DNA polymerases are called as DNA pol 1, DNA pol 2 and DNA pol 3, and are going to be synthesized by a gene which is called as polA, polB, polC.

Then it alSo, has the different amount of subunits. So, in a DNA pol 1 you have the single subunit, whereas, in the DNA pol 2 you can have the seven subunit, and in DNA polymerase 3, you can have the more than 10 subunits. The molecular weight is alSo, different. So, you can have the 100 kDa, 88 kDa and the 790 kDa.

Then the 3 prime to 5 prime exonuclease activity, So, that is presenting the 1 and, So, there are, that is present in all the three types, 1, 2 and 3. Then you can have the 5 prime to 3 prime exonuclease activity. So, that is present in 1 and 3, but it is absent in the DNA

polymerase 2. Then you can have the polymerization rate which means it is going to be 5 prime to 3 prime polymerization activities.

And that is 16 to 20 nucleotides per second in the case of DNA polymerase 1, whereas it is going to be 40 nucleotides and, per second and it is going to be 250 to 1000 nucleotides per second, which is for the DNA polymerase 3. Then the processivity, processivity is going to be 3 to 200 in the case of DNA polymerase 1, 1,500 in the case of the DNA polymerase 2, and it is going to be more than 500,000 in the case of DNA polymerase 3.

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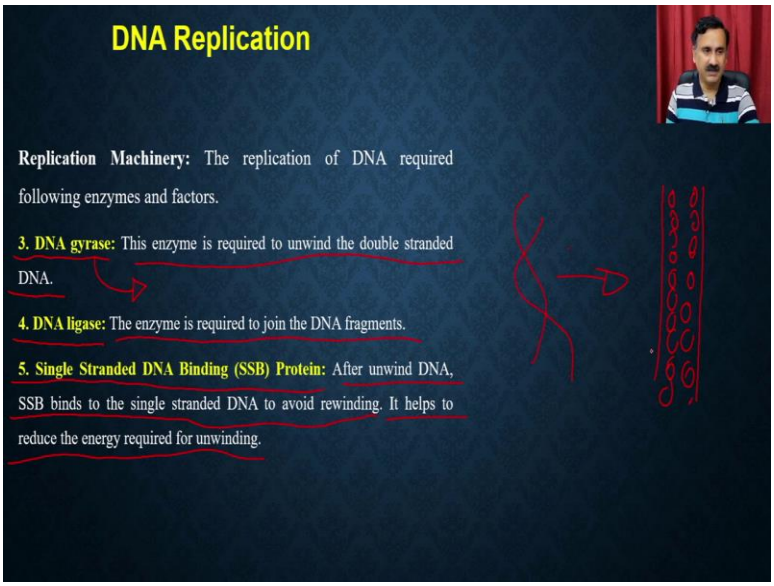

Apart from the DNA polymerase and the primase, you can also, have the DNA gyrase. So, this enzyme is required to unwind the double stranded DNA. Remember that when we were talking about the plasmid isolation, we said that you can actually be able to use the DNA gyrase to convert the different form of the enzyme. And then you also, require the DNA ligase. So, this enzyme is required for joining the DNA fragments.

And then you alSo, require a single stranded DNA binding protein. So, after the unbinding of the DNA by the DNA gyrase, the SSB is going to bind to the single stranded DNA to avoid the rewinding. It helps to reduce the energy required for the unbinding, which means, once you have the double stranded DNA, and if you, DNA gyrase is going to bind, it is actually going to make the single stranded.

Like, it is going to remove the unbinding. But once it removed then binding, since the DNA has the endogenous ability to rotate and it gives you the double stranded DNA, it actually, the single stranded binding protein is actually going to bind to the all the nucleotides. And that is how it is actually going to allow the DNA to be remain as the as the unwinded.

And because of that, it actually can be utilized by the different components of the replication machinery, like for example, the primase or the DNA polymerase and therefore, it is actually going to facilitate the DNA synthesis.

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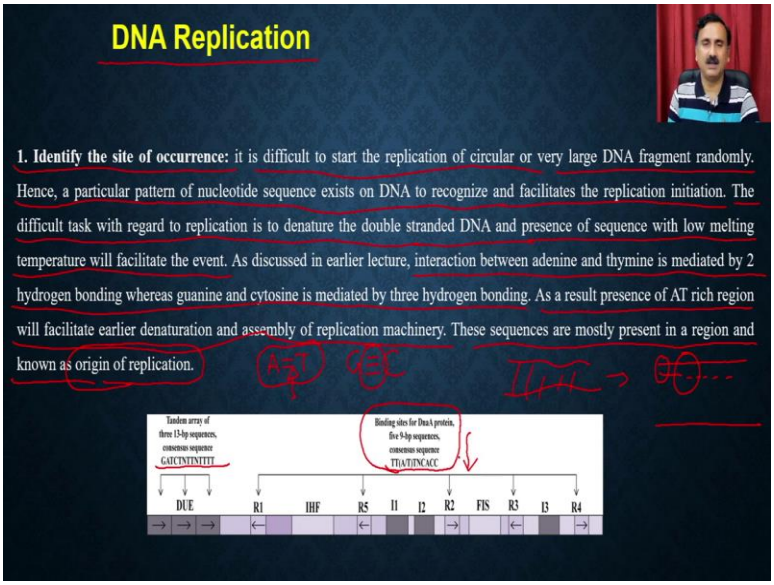

Now, let us discuss about the processes. So, the processes is, first you have to identify the site of occurrence, like the where the replication has to start. So, it is difficult to start the replication of circular or very large DNA fragment randomly. You cannot start the work randomly. When you go for a site, for example, if I have to start making a building, I have to go for a site, I have to first inspect where the, where I could have the front door, where I could have the back door where I could have toilet, those kind of different things.

So, the, when the when the machinery will go to the site, it actually cannot start randomly. It has to first decide from which side I should start making the road, which side I should start making the different types of things. So, hence, a particular pattern or

nucleotide sequence existed on DNA to recognize and facilitate the replication initiations. The difficult task with regard to replication is to denature the double stranded DNA, and the presence of sequence with low melting temperature will facilitate the event.

So, what is the first, what is the most difficult part into DNA replication is that conversion of a double stranded DNA into a single stranded DNA. Once you do that, then there is, the other tasks are very easy, because then you can actually have the primes, which is sitting and then synthesizing the primers, and then see subsequently you can have the RNA polymerase and that alSo, going to add the nucleotides.

So, interaction between the adenine thymine is mediated by the two hydrogen bond whereas the guanine and cytosine is mediated by the three hydrogen bonds. So, where you can actually do the melting, where you can actually do the denaturation easy, because the A is making to best pair with T, and G is actually making the three hydrogen bonding with the C. So, this is actually going to be stronger.

So, you can actually be able to look for the sequence where you can have the more amount of A and T. And that is actually going to say that y, this is the site from where you can actually be able to open the DNA very easily. So, as a result, the presence of at AT region will facilitate the earlier denaturation and assembly of replication machinery. These sequences are mostly present in a region and known as the origin of replication.

So, the region were you going to have the AT rich sequences that is going to be called as the original replication. So, you see this, this is a origin of replication of an E.Coli. And what you see here is the different types of components, like you can have the 13 base pair tandem repeats, and you can alSo, have the, this sequence.

So, you see here, it alSo, has a very high quantity of the AT rich sequences. And these are actually going to be the site where you can actually start the replications. Once you have identified the site of occurrence, then all other machinery is actually going to installed onto this particular sequence and then it is actually going to start the replications.

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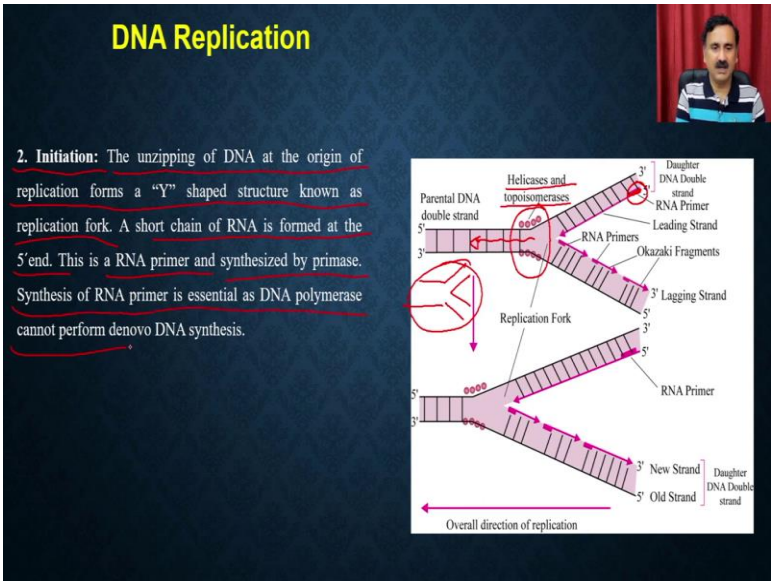

Then we can have the first event, and that is called as the Initiation. So, what you have here is, this is the initiation. So, the unzipping of the DNA at the origin of replication forms a Y-shaped structure known as a replication fork. So, once the, you have the, once you have the, identifying the site, then you are actually going to unzip the DNA.

And once you unzip the DNA, you are actually going to form the Y-shape DNA. And this Y-shaped DNA is called as the replication fork. And then what will happen, the first is that RNA primase, or the primase is actually going to sit onto this small stretch of DNA, which is going to be open and then it is actually going to synthesize the RNA primer.

Once the RNA primer is synthesized, then on this, you are actually going to fit the DNA polymerase and it is going to start doing the synthesis. You see as the fork is opening, fork is opening by the molecules like the helicases as well as the topoiSo,merases. So, topoiSo,merases keep moving in the forward direction, and keep opening the DNA.

And that is how this Y-shaped replication fork is going to be more and more available for the machinery to replicate. A short stretch of DNA is formed at the 5 prime end. This is the RNA primer and it is synthesized by the primase. Synthesis of RNA primer is essential as the DNA polymerase cannot perform the denovo DNA synthesis.

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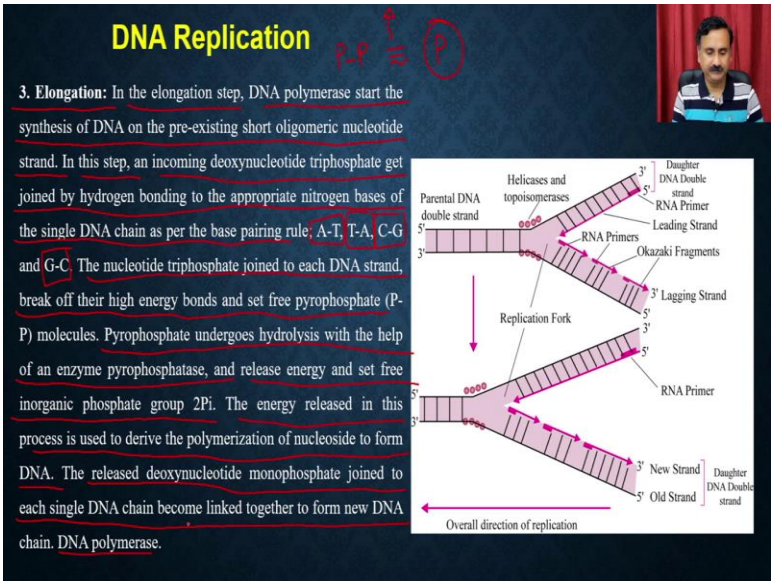

Now, we can have the elongation. So, elongation is, is nothing but the addition of the nucleotides on to the different incoming new 3 prime ends. So, in the elongation step, the DNA polymerase has start the synthesis of the DNA onto the pre-existing short oligomeric nucleotide strands.

In this step, an incoming deoxynucleotide to get joined by the hydrogen bonding to the appropriate nitrogen base of the single stranded chain as per the base pairing rule, which means A is making a pair with T, T is making a pair with A, C is making a pair with G, and G is making a pair with C. The nucleotide triphosphate joint to each DNA strand break off their high energy bond and set the free pyrophosphate.

So, it is actually going to set the free pyrophosphate which is actually going to digest and it is actually going to generate the free phosphate and in this process, actually going to have the energy regulations. So, the pyrophosphate undergoes hydrolysis with the help of an enzyme which is called as the pyrophosphatase and it releases the energy and set the inorganic phosphate to 2Pi.

The energy released in this process is used to derive the polymerization of the nucleotides to form the DNA. The released deoxynucleotide monophosphate joint to each single stranded DNA become linked to form the new DNA chains.

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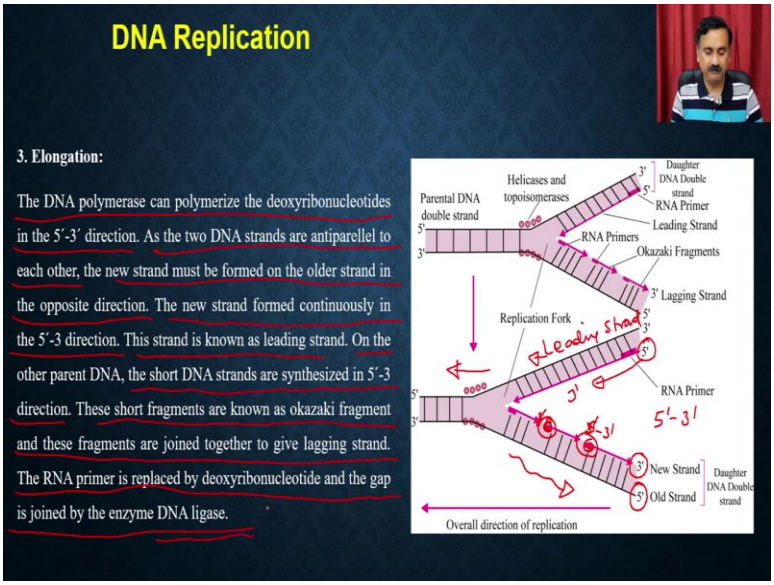

Then we have the DNA polymerase. DNA polymerase can polymerize the deoxynucleotides in the direction of 5 prime to 3 prime, which means it actually can synthesize on to the 5 prime to 3 prime. As the data strands are anti-parallel to each other, the new strand must be formed onto the older strand in the opposite directions. The new strand forms continuously into the 5 prime to 3 prime directions.

So, this is the strand which actually is going to form as a continuously, it is going to run along with the replication fork where, So, this is strand is called as the leading strand. So, this strand is called as a leading strand, because it is actually going to synthesize along with the opening of the replication fork. So, the strand which going to be go for synthesis in the same direction as the replication fork, that is called as the leading strand.

Whereas on the other parent, or the other strand, like on this strand, it is actually going to wait because the DNA synthesis is always been in the 5 prime to 3 prime directions, and you know that you have a 5 prime direction. So, it is going to be a 3 prime here. So, this means it has to wait for the DNA to go for So,me unzipping, and then the primase is actually going to sit and synthesize the RNA primer.

And then you are actually providing a 3 prime site. And that is how it is actually going to utilize and go for a synthesis of 5 prime to 3 prime. This means on this strand, on the

other strand, the short DNA strands are synthesized into the 5 prime to 3 prime. And these short stretches are known as the okazaki fragment and these fragments are joined to give, and these join together to give the lagging strand.

This means this is going to be the leading strand, and on this one, this strand has to wait for the DNA to be unwind for So,me amount of time. So, this is going to unwind, it is going to, RNA primase is going to sit, and going to synthesize the primer. Then this small stretch of DNA is going to be synthesized.

Then it has to wait for So,me more time, then this amount of DNA is going to be open and then again or a primate will sit, and then it is going to be synthesized. So, these individual fragments are called as the okazaki fragment, and since this strand is running in the opposite direction to the direction of the fork, this strand is alSo, called as a lagging strand.

The RNA primer is replaced by de deoxynucleotide and the gap is joined by the enzyme which is called as the DNA ligase. So, eventually what will happen, these RNA primers are going to be removed, and then this is going to be filled by the deoxynucleotides and the gap is going to be joined by an enzyme which is called as the DNA ligase.

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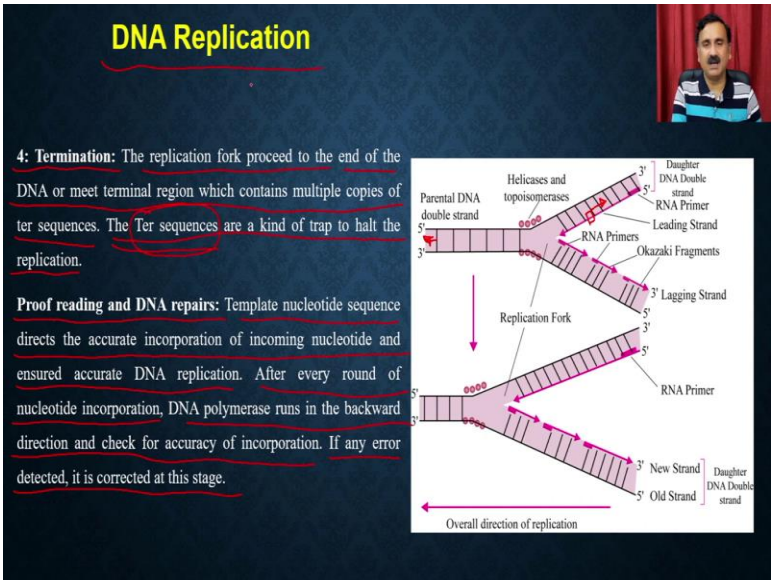

Now once the elongation is over, then it was going to have the termination. So, the replication, once the replication fork is going to reach at the end of the DNA, or meet the terminal region which contain the multiple copy of the Ter sequences. The Ter sequence are going to trap the halts of the replication, which means once the replication fork is going to be reached to the end of the tube or end of the DNA, that it is actually going to have the multiple Ter sequences or the termination sequences.

And these termination sequences are actually going to stop the moment on the replication fork. And that is how it is actually going to have the terminations. Apart from this particular type of activity, you can alSo, have the proofreading as well as the DNA repair activity. So, the template nucleotide sequence directs the accurate incorporation of the incoming template and ensure the accurate DNA replication.

After every round of nucleotides incorporations, the DNA polymerase runs in the backward direction and check for the accuracy of the incorporation. If any errors is directed, it is corrected at this stage. So, what will happen is, when the DNA is synthesizing, it is actually synthesizing in this direction but the DNA polymerase is alSo, moving into the reverse direction and keep checking whether the nucleotide what he has added is as per the template DNA or not.

If it is not as per the template DNA, then it is actually going to remove those nucleotides by utilizing its exonuclease activity and then it actually going to do the synthesis again. So, that is why the DNA proofreading and the DNA repair activity is important for the successful synthesis of the DNA, and making a copy which is almost identical to the host DNA.

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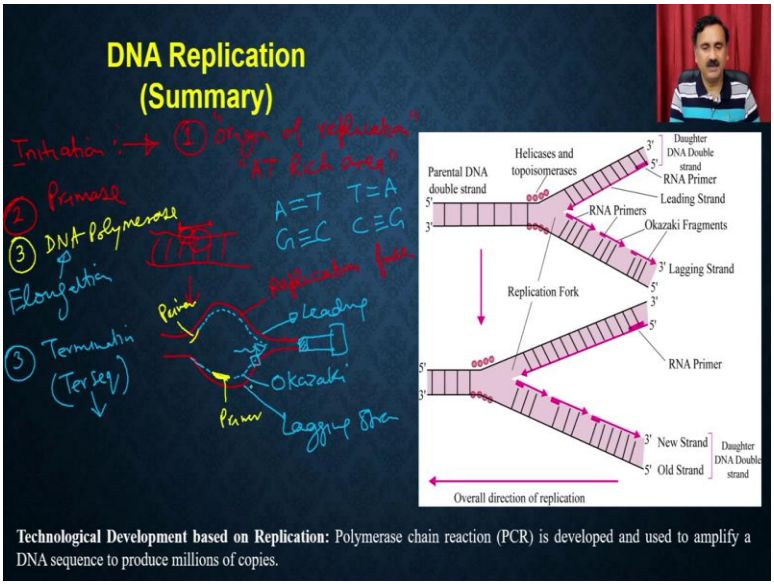

So, now, what we have discussed, we have discussed about the different types of steps. So, what we have is we have the three events, we can have the initiation. So, in the initiation, what will happen is that, the first is that it is actually going to recognize the origin of replication. So, over origin of replication it is going to synthesize, or recognize the origin of replication. And origin of replication is actually going to have AT rich area.

So, at the AT rich area, the helicases and the topoiSo,merases are going to go and sit. So, they will actually going to have the double stranded DNA and imagine that if this is the region where you have actually the AT rich area, So, on this area, the helicases and the topoiSo,merases will go and sit, and then they are actually going to open this strand. They are going to open the middle area.

And that is how this actually is going to form the replication fork. So,, once the replication fork is formed, So, it is going to be formed. So, then it is actually going to start the synthesis. So, it is actually going to allow, So, in the initiation, first is this, the second the, the Primus is going to sit, and then it is actually going to synthesize a small stretch of RNA. So, it is going to synthesize the small stretch of RNA.

So, it is going to synthesize the small stretch of RNA. Now remember that this strand is actually a leading, lagging strand, So, on this side. And then it is actually going to have

the third event, third event is where you are actually going to have the DNA polymerase. And DNA polymerase is actually going to have the synthesis of the DNA. So, DNA polymerase is going to sit and it is actually going to start the synthesis of the DNA.

So, it is going to start synthesis of the new strand. So, it is going to start synthesis of the new strand. And it is actually going to see what is the nucleotide present. So, if the A is present, it is actually going to have the T, and if it is, G is presented then it is going to have the C, and if the T is present then it is alSo, going to have the A, and if the C is present, then it is alSo, going to have the G.

And utilizing that, it actually is going to reach to the end. So, then the DNA replication is going to start. And for this strand, it has to open for So,me time, and then from this side, the DNA polymerase, the primase is are actually going to sit. So, primase is actually going to sit, and start going to synthesize these small stretch of RNA. So, it is going to start the synthesis of primer.

So, this is actually a primer, which is going to be synthesized in both the cases. And then the DNA polymerase is going to go and sit. So, DNA polymerase is going to go and sit and start the synthesis of the DNA in this reverse direction. And that is why it is actually going to have the multiple such fragments.

And these fragments are called as the okazaki fragments. And that is why this is going to be the leading strand, and this is going to be the lagging strand. Now once they reach to the end of the tube, So, after this, this is going to be the second step, it is going to be elongation. So, you are going to have the elongation. And the elongation is where the DNA polymerase is going to synthesize the DNA.

And the third is, you are going to have the termination. So, once this DNA is actually going to reach to the end strip, it is actually going to have the Ter sequences. So, these Ter sequences are actually going to be rich in the GC sequences. And because of that, it is actually going to stop the moment of the forks, because see, the fork is moving in this direction. It is actually going to stop the ter, stop the replication.

So, that is why the, this strand is going to be going, synthesize up to this and this one alSo, is going to be synthesized in reverse directions. So, this is all about the central dogma of life, and what we have discussed? We have discussed about the many events what is happening inside the cell and what is responsible and what are the different types of molimolecules are going to be produced.

And what we have understood is that the protein is actually been responsible for making the different types of activities within the cell. And the protein synthesis is always been governed by the sequence dependent synthesis of the RNA. And the sequence dependent RNA synthesis is being done by the DNA.

And this, all these events are actually been coming together under the central dogma of life, or the central dogma of molecular biology as it is been stated by the Francis. What we have discussed, we have discussed about the replications, like the DNA dependent DNA synthesis, which has been done by the DNA polymerase, and we have discussed about the different events where we have discussed about the origin of replications, we have discussed about the initiation, elongation and terminations.

So, with this, I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss So,me more aspects related to the central dogma of life, where we are going to discuss about the Transcriptions. So, with this, I would like to conclude my lecture here. Thank you.