Module 10 / DNA Synthesis

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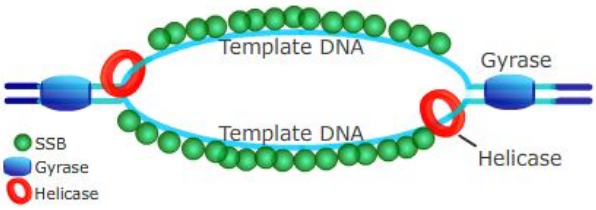
Describe the formation of the Open Complex at the origin of replication.	Explain how the appropriate substrate for DNA polymerase at the replication fork is established.	Distinguish the difference between continuous and discontinuous DNA synthesis.
Expound on why Okazaki fragments are formed on the lagging strand.	Describe the process of connecting the Okazaki fragments into a continuous strand of DNA.	

Initiation of synthesis from double stranded DNA

Having explored the requirements for DNA polymerase, it is clear that the prokaryotic and eukaryotic genomes fail the first requirement of a single stranded sequence of DNA. Therefore to initiate DNA replication it is necessary to create the conditions that will allow DNA polymerase to carry out DNA synthesis.

1. First the DNA must be opened by breaking the hydrogen bonds between the base pairs and unwinding the DNA. This is initiated by a protein called the **initiator protein or DnaA** that recognizes a specific DNA sequence called the **origin of replication**, binds to the sequence and opens up the sequence to create a replication bubble (**open complex**). An enzyme called a **helicase** binds to the open complex and extends the melted region of the double stranded DNA to further open the replication bubble. There now exists a single stranded segment of DNA exposed on both chains of the DNA double helix. A small protein called **SSB** (Single Stranded Binding protein) coats the open complex.

The Open Complex



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Without SSB present, what would you expect to happen to the open complex?

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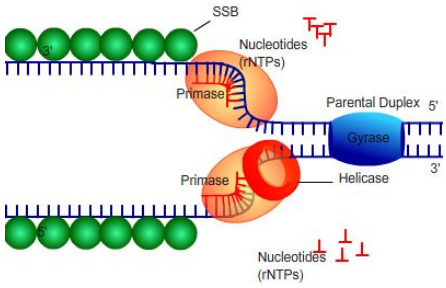
2. With formation of an open complex, one of the requirements for DNA polymerase is satisfied: single stranded DNA is generated. At either end of the open complex where the single stranded and double stranded DNA meet, a structure referred to as the **replication fork** exists. From this point we will focus on the process of replication at one of the replication forks. Remember that the replication will be occurring simultaneously from both forks in opposite directions. To allow the binding of DNA polymerase, a second requirement must be met for the structure of the substrate. An enzyme called **Primase** synthesizes a short (approximately 10 nucleotides), complementary RNA primer on each strand of the DNA in a 5' to 3' unidirectional fashion. This complex of DNA and protein at the replication fork is referred to as the **replisome**.

Replisome at one fork



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Based on the illustration of the replisome given above with the RNA primers shown in red, once the primase has left, where do you expect DNA polymerase to start its action?

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3. The substrate requirements for DNA polymerase have been satisfied and synthesis begins. Helicase continues to melt the DNA in front of DNA polymerase.

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What do you notice about the direction of synthesis on each strand at the replication fork? If you allow the synthesis to proceed for the addition of 10 nucleotides, draw the structure of the replication fork that you expect at that time.

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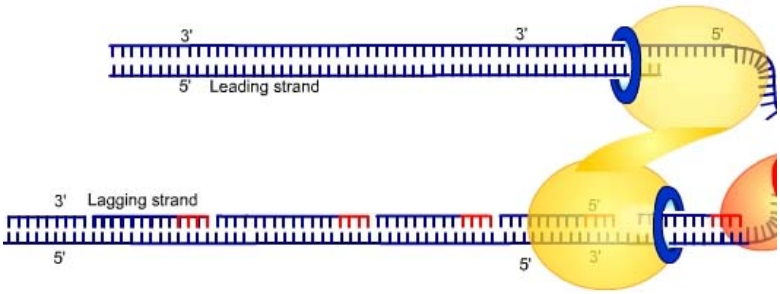
4. As single stranded DNA is generated between the 'back' of the RNA primer on one strand and the opening replication fork, a new segment of RNA primer is laid down near the opening fork and DNA polymerase synthesizes a new complementary strand of DNA from the primer toward 5' end of the existing primer and stops. This segment of DNA that is not connected to the next segment is referred to as an **Okazaki Fragment**. Synthesis on this **lagging strand** is said to be **discontinuous** since it is generated in uniform lengths of DNA starting from one primer and stopping before the next RNA primer.
5. Synthesis on the opposite strand is a continuous uninterrupted process starting from the initial primer and continuing as long as the DNA continues to open up at the replication fork. This strand on which **continuous synthesis** takes place is referred to as the **Leading Strand**.



Okazaki fragments on the lagging strand

The following animation depicts the complete process of DNA replication. In the animation you should be able to identify each of the stages of synthesis on each of the strands. At the end of the synthesis you will see that the leading strand has a continuous double helix generated while the lagging strand has a discontinuous set of Okazaki fragments that must be connected before the synthesis is complete.

To accomplish this mending of the DNA, a separate DNA polymerase binds to the 'nick' left in the DNA between the 3' end of the newly synthesized DNA and the 5' end of the RNA primer. The substrate requirements for DNA polymerase are met even though there is no apparent single stranded DNA present. The DNA polymerase removes the RNA primer and a few nucleotides of DNA in front of it while simultaneously synthesizing DNA on the 3' end of the existing DNA. This process is referred to as **nick translation**.

When only DNA is present, the DNA polymerase releases from the DNA leaving a nick in the DNA between two DNA segments. DNA polymerase does not have a function to join the two ends of the DNA together and

thus a separate enzyme, **DNA Ligase**, recognizes the nick in the DNA and links the 3' end of the one

thus a separate enzyme, **DNA Ligase**, recognizes the nick in the DNA and links the 3' end of the one segment to the 5' end of the next segment.

This process of DNA replication continues until the entire sequence of DNA is synthesized. The process is fundamentally the same in prokaryotes and eukaryotes.

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A closed circular prokaryotic chromosomes replicates from a single origin of replication. If the rate of DNA synthesis by DNA polymerase is 500 bp/sec and the chromosome contains a million bp, how long will it take to replicate the chromosome?

- 1 hour
- 33 minutes and 20 sec
- 16 minutes and 40 sec
- 8 minutes and 10 sec

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