Module 10 / DNA Replication

62

Describe the results of semiconservative replication.	Explain the substrate requirements for DNA synthesis by DNA polymerase.	Characterize the direction of DNA synthesis.

DNA replication is the process by which DNA is copied resulting in a faithful replicate of the original double stranded DNA. Key to the inheritance of the code for materials produced and used in a cell, DNA replication must be faithful thus not allowing or minimizing errors during the process and it must generate new DNA that can be transmitted to the next generation. Because DNA replication generates a copy of the original, it is referred to as **DNA dependent DNA synthesis**. It is dependent on DNA because it must have a template to use in making the copy and the result is a new strand of double stranded (duplex) DNA.

Semi-conservative Replication

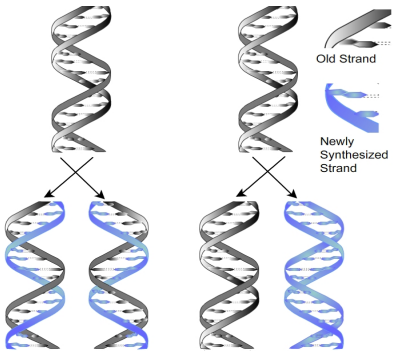
The process of DNA replication requires that the original DNA act as a **template** for the newly formed DNA. How the new DNA acts as a template could occur in multiple ways. As depicted below the double stranded DNA (anti-parallel strands indicated by the grey and opposite polarity blue strand) could be completely copied leaving the original strands of DNA intact (grey) and yielding a newly synthesized double strand DNA containing two new strands (blue). This is referred to as conservative replication. The second model of DNA replication starts with the same double stranded DNA but the products each contain one of the original strands and a complementary newly synthesized strand. This is referred to as **semi-conservative replication**. Biological organisms have been shown to replicate their DNA by semi-conservative synthesis.

The “Learn More…” that follows shows the Meselson-Stahl experiment that was performed to prove that semi-conservative replication is the mechanism within biological systems.

MANY STUDENTS WONDER...

The Meselson-Stahl Experiment

Semi-conservative vs Conservative replication models



DNA synthesis

DNA synthesis in biological systems is a very directed process controlled by specific enzymes that both dictate where synthesis begins and the exact structural conditions required to undertake DNA synthesis. Knowledge of these conditions and the process involved in DNA synthesis has become the basis for understanding other biological processes involving DNA and in the design of a set of tools essential to the advancement of the study of modern molecular biology.

DNA polymerases (setting the rules)

DNA polymerase is an enzyme that has very specific substrate requirements and thus controls where the synthesis of DNA can take place. An understanding of the basic mechanism of this enzyme will make the understanding of how DNA replication works become clear.

1. As with any enzyme, DNA polymerase has a very specific set of requirements for recognition of its substrate. For DNA polymerase the substrate is **single stranded DNA** not double stranded DNA. Thus DNA polymerase is not able to bind to or recognize an intact genome but rather it uses each single strand of DNA as a **template**.
2. While DNA polymerase requires single stranded DNA as a template, it does not have the ability to start anywhere on the strand and initiate de novo synthesis. The active site of the polymerase requires a segment of DNA or RNA bound in a complimentary manner to the single stranded template as an initiation point for the synthesis reaction. This segment of DNA or RNA is generally referred to as the **primer**.
3. If you examine the structure of either DNA or RNA there are two places that a new deoxy-nucleotide can be added to extend the length of the complementary strand.

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What are the possible attachment sites for a new nucleotide to the ends of an existing polynucleotide? Why doesn't it matter if the primer for DNA synthesis is either a segment of RNA or DNA?

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While DNA synthesis could be from either end of the primer, during replication using the enzyme DNA polymerase the synthesis is restricted by the enzyme to growth in only one direction. Deoxy-nucleotides are only added to the 3' end of the primer and thus **synthesis is unidirection from 5' to 3'**.

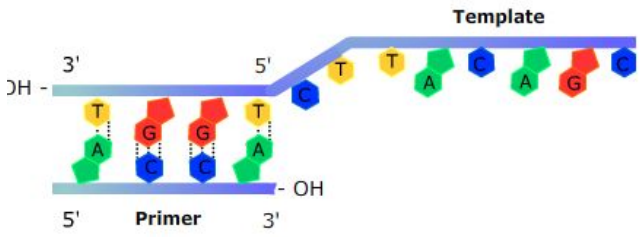
4. For synthesis to proceed, the enzyme imposes a third restriction on the substrate. Not only does it

4. For synthesis to proceed, the enzyme imposes a third restriction on the substrate. Not only does it require a DNA template and a primer, but the primer must have a **free, 3' hydroxyl group** for the addition of the next deoxy-nucleotide.
5. And finally, all four deoxy-nucleotide triphosphates must be present to completely synthesis the complementary strand of DNA. For DNA synthesis (replication) this means dATP, dTTP, dCTP, and dGTP must be present. Collectively these are referred to as dNTPs (**d**eoxy-**N**ucleotide **T**ri**P**hosphates).

More than one DNA polymerase enzyme is known and each carries out a specific function depending on the situation. For example, DNA polymerase III is the enzyme used in replicating a genome while DNA polymerase I is used in DNA repair. Even with clear differences as to when each of the DNA polymerases is used during the replication of DNA, the basic substrate requirements are the same.

The following diagram depicts the structural requirements for the operation of DNA polymerase enzymes.

Perfect Substrate for DNA Polymerase



Synthesis (the making of new DNA)

Once the appropriate structural requirements for the synthesis of DNA are met, DNA synthesis occurs continuously in a unidirection process from 5' to 3' along the template. The following animation demonstrates the building of a complementary strand using the components required by DNA polymerase. This process can take place wherever the initial conditions for operation of DNA polymerase exist.

DNA Polymerase binds to DNA at the 3' end of the primer and builds a complementary sequence.

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DNA Replication