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**ASSIGNMENT QUESTIONS**

- **Highlight the steps of DNA replication**
- **Outline the functions of DNA Replication enzymes.**

**ANSWERS**

- Deoxyribonucleic acid, commonly known as DNA, is a nucleic acid that has three main components: a deoxyribose sugar, a phosphate, and a nitrogenous base.
- Since DNA contains the genetic material for an organism, it is important that it be copied when a cell divides into daughter cells. The process that copies DNA is called replication.
- Replication involves the production of identical helices of DNA from one double-stranded molecule of DNA.
- Enzymes are vital to DNA replication since they catalyze very important steps in the process.
- The overall DNA replication process is extremely important for both cell growth and reproduction in organisms. It is also vital in the cell repair process.

**DNA Structure**

DNA or deoxyribonucleic acid is a type of molecule known as a nucleic acid. It consists of a 5-carbon deoxyribose sugar, a phosphate, and a nitrogenous base. Double-stranded DNA consists of two spiral nucleic acid chains that are twisted into a double helix shape. This twisting allows DNA to be more compact. In order to fit within the nucleus, DNA is packed into tightly coiled structures called chromatin. Chromatin condenses to form chromosomes during cell division. Prior to DNA replication, the chromatin loosens giving cell replication machinery access to the DNA strands.

**Steps of DNA Replication**

**Step 1: Replication Fork Formation**

Before DNA can be replicated, the double stranded molecule must be “unzipped” into two single strands. DNA has four bases called adenine (A), thymine (T), cytosine (C) and guanine (G) that form pairs between the two strands. Adenine only pairs with thymine and cytosine only binds with guanine. In order to unwind DNA, these interactions between base pairs must be broken. This is performed by an enzyme known as DNA helicase. DNA helicase disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the replication fork. This area will be the template for replication to begin.

DNA is directional in both strands, signified by a 5' and 3' end. This notation signifies which side group is attached the DNA backbone. The 5' end has a phosphate (P) group attached, while the 3' end has a hydroxyl (OH) group attached. This directionality is important for replication as it only progresses in the 5' to 3' direction. However, the replication fork is bi-directional; one strand is oriented in the 3' to 5' direction (leading strand) while the other is oriented 5' to 3' (lagging strand). The two sides are therefore replicated with two different processes to accommodate the directional difference.

**Replication Begins**

**Step 2: Primer Binding**

The leading strand is the simplest to replicate. Once the DNA strands have been separated, a short piece of RNA called a primer binds to the 3' end of the strand. The primer always binds as the starting point for replication. Primers are generated by the enzyme DNA primase.

**Step 3: Elongation**

Enzymes known as DNA polymerases are responsible creating the new strand by a process called elongation. There are five different known types of DNA polymerases in bacteria and human cells. In bacteria such as E. coli, polymerase III is the main replication enzyme, while polymerase I, II, IV and V are responsible for error checking and repair. DNA polymerase III binds to the strand at the site of the primer and begins adding new base pairs complementary to the strand during replication. In eukaryotic cells, polymerases alpha, delta, and epsilon are the primary polymerases involved in DNA replication. Because replication proceeds in the 5' to 3' direction on the leading strand, the newly formed strand is continuous.

The lagging strand begins replication by binding with multiple primers. Each primer is only several bases apart. DNA polymerase then adds pieces of DNA, called Okazaki fragments, to the strand between primers. This process of replication is discontinuous as the newly created fragments are disjointed.

**Step 4: Termination**

Once both the continuous and discontinuous strands are formed, an enzyme called exonucleaseremoves all RNA primers from the original strands. These primers are then replaced with appropriate bases. Another exonuclease “proofreads” the newly formed DNA to check, remove and replace any errors. Another enzyme called DNA ligase joins Okazaki fragments together forming a single unified strand. The ends of the linear DNA present a problem as DNA polymerase can only add nucleotides in the 5’ to 3’ direction. The ends of the parent strands consist of repeated DNA sequences called telomeres. Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing. A special type of DNA polymerase enzyme called telomerase catalyzes the synthesis of telomere sequences at the ends of the DNA. Once completed, the parent strand and its complementary DNA strand coils into the familiar double helix shape. In the end, replication produces two DNA molecules, each with one strand from the parent molecule and one new strand.

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Functions of DNA replication enzymes

DNA replication would not occur without enzymes that catalyze various steps in the process. Enzymes that participate in the eukaryotic DNA replication process include:

- DNA helicase - unwinds and separates double stranded DNA as it moves along the DNA. It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.
- DNA primase - a type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as templates for the starting point of DNA replication.
- DNA polymerases - synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.
- Topoisomerase or DNA Gyrase - unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.
- Exonucleases - group of enzymes that remove nucleotide bases from the end of a DNA chain.
- DNA ligase - joins DNA fragments together by forming phosphodiester bonds between nucleotides.