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QUESTION 1-Highlight the Steps on DNA Replication

ANSWER

Why Replicate DNA?

DNA is the genetic material that defines every cell. Before a cell duplicates and is divided into new daughter cells through either mitosis or meiosis, biomolecules and organelles must be copied to be distributed among the cells. DNA, found within the nucleus, must be replicated in order to ensure that each new cell receives the correct number of chromosomes. The process of DNA duplication is called **DNA replication**. Replication follows several steps that involve multiple proteins called replication enzymes and RNA. In eukaryotic cells, such as animal cells and plant cells, DNA replication occurs in the S phase of interphase during the cell cycle. The process of DNA replication is vital for cell growth, repair, and reproduction in organisms.

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.

Step 2: Initiation

Replication begins at a location on the double helix known as "oriC" to which certain initiator proteins bind and trigger unwinding. Enzymes known as helicases unwind the double helix by breaking the hydrogen bonds between complementary base pairs, while other proteins keep the single strands from rejoining. The "topoisomerase" proteins surround the unzipping strands and relax the twisting that might damage the unwinding DNA. The cell prepares for the next step, elongation, by creating short sequences of RNA called primers that provide a starting point of elongation.

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 exonuclease removes 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.

QUESTION 2- Outline The Functions Of DNA Replication Enzymes.

Enzyme	Function in DNA replication
DNA Helicase	Also known as helix destabilizing enzyme. Unwinds the DNA double helix at the Replication
DNA Polymerase	Builds a new duplex DNA strand by adding nucleotides in the 5' to 3 direction. Also performs proof-reading and error correction.
DNA clamp	A protein which prevents DNA polymerase Ill from dissociating from the DNA parent strand
Single-strand Binding (SSB) proteins	Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus maintaining the strand separation.
Topoisomerase	Relaxes the DNA from its super-coiled nature.
DNA Gyrase	Relieves strain of unwinding by DNA helicase; this is a specific type of topoisomerase
DNA Ligase	Re-anneals the semi-conservative strand and joins Okazaki Fragments of the lagging strand

Primase	Provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand.
Telomerase	Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes