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QUESTION: 1.HIGHLIGHT THE STEPS OF DNA REPLICATION

There are three main steps to DNA replication: initiation, elongation, and termination.

Initiation:

DNA synthesis is initiated at particular points within the DNA strand known as 'origins', which are specific coding regions. These origins are targeted by initiator proteins, which go on to recruit more proteins that help aid the replication process, forming a replication complex around the DNA origin.

Within the replication complex is the enzyme DNA Helicase, which unwinds the double helix and exposes each of the two strands, so that they can be used as a template for replication. It does this by hydrolysing the ATP used to form the bonds between the nucleobases, therefore breaking the bond between the two strands. DNA can only be extended via the addition of a free nucleotide triphosphate to the 3'- end of a chain. As the double helix runs antiparallel, but DNA replication only occurs in one direction.

DNA Primase is another enzyme that is important in DNA replication. It synthesises a small RNA primer, which acts as a 'kick-starter' for DNA Polymerase. DNA Polymerase is the enzyme that is ultimately responsible for the creation and expansion of the new strands of DNA.

Elongation:

Once the DNA Polymerase has attached to the original, unzipped two strands of DNA, it is able to start synthesising the new DNA to match the templates. This enzyme is only able to extend the primer by adding free nucleotides to the 3'-end of the strand, causing difficulty as one of the template strands has a 5'-end from which it needs to extend from.

One of the templates is read in a 3' to 5' direction, which means that the new strand will be formed in a 5' to 3' direction. This newly formed strand is referred to as the Leading Strand. Along this strand, DNA Primase only needs to synthesise an RNA primer once, at the beginning, to help initiate DNA Polymerase to continue extending the new DNA strand. This is because DNA Polymerase is able to extend the new DNA strand normally, by adding new nucleotides to the 3' end of the new strand.

However, the other template strand is antiparallel, and is therefore read in a 5' to 3' direction, meaning the new DNA strand being formed will run in a 3' to 5' direction. To counteract this, DNA Primase synthesises a new RNA primer approximately every 200 nucleotides, to prime DNA synthesis to continue extending from the 5' end of the new strand. To allow for the continued creation of RNA primers, the new synthesis is delayed and is such called the Lagging Strand.

The leading strand is one complete strand, while the lagging strand is not. It is instead made out of multiple 'mini-strands', known as Okazaki fragments. These fragments occur due to the fact that new primers are having to be synthesised, therefore causing multiple strands to be created, as opposed to the one initial primer that is used with the leading strand.

Termination:

The process of expanding the new DNA strands continues until there is either no more DNA template left to replicate (i.e. at the end of the chromosome), or two replication forks meet and subsequently terminate. The meeting of two replication forks is not regulated and happens randomly along the course of the chromosome.

Once DNA synthesis has finished, it is important that the newly synthesised strands are bound and stabilized. With regards to the lagging strand, two enzymes are needed to achieve this; RNAase H removes the RNA primer that is at the beginning of each Okazaki fragment, and DNA Ligase joins two fragments together creating one complete strand.

Now with two new strands being finally finished, the DNA has been successfully replicated, and will just need other intrinsic cell systems to 'proof-read' the new DNA to check for any errors in replication, and for the new single strands to be stabilized.

2. OUTLINE THE FUNCTIONS OF DNA REPLICATION ENZYMES.

Functions of DNA Replication Enzymes

Replication in eukaryotes starts at multiple origins of replication. A primer is required to initiate synthesis, which is then extended by DNA polymerase as it adds nucleotides one by one to the growing chain. The leading strand is synthesized continuously, whereas the lagging strand is synthesized in short stretches called Okazaki fragments. The RNA primers are replaced with DNA nucleotides; the DNA remains one continuous strand by linking the DNA fragments with DNA ligase. Below is a summary table of the major enzymes addressed in this reading, listed in rough order of activity during replication.

Important Enzymes in DNA Replication

Enzyme	Function
Topoisomerase	Relaxes the super-coiled DNA
DNA helicase	Unwinds the double helix at the replication fork
Primase	Provides the starting point for DNA polymerase to begin synthesis of the new strand
DNA polymerase	Synthesizes the new DNA strand; also proofreads and corrects some errors
DNA ligase	Re-joins the two DNA strands into a double helix and joins Okazaki fragments of the lagging strand