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1) Highlight the steps of DNA replication.

DNA replication can be thought of in three stages; **Initiation, Elongation,**

Termination

1. 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.

There are multiple origin sites, and when replication of DNA begins, these sites are referred to as **Replication Forks**.

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, it means growth of the two new strands is very different (and will be covered in Elongation).

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.

2.Elongation

Once the DNA Polymerase has attached to the original, unzipped two strands of DNA (i.e. the **template** strands), 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 (as the two strands are antiparallel to each other). 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 (how DNA Polymerase usually works).

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. This is an issue as DNA Polymerase doesn't extend in this 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 of **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.

3.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.

Topoisomerase} Relaxes super coiled DNA.

DNA helicase} Unwinds the double helix at the replication fork.

DNA polymerase} Builds a new duplex DNA strand by adding nucleotides in the 5' to 3' direction. Also performs proof-reading and error correction. There exist many different types of DNA polymerase, each of which perform different functions in different types of cells.

(RNA polymerase) primase} Provides a starting RNA short primers for DNA polymerase to begin synthesis of the new DNA strand.

DNA clamp} A protein which prevents elongating DNA polymerase from dissociating from the DNA parent strand.

Single-Strand Binding (SSB) proteins} Bind to ssDNA and prevent the DNA double helix from reannealing after DNA helicase unwinds it, this maintaining the strand separation and facilitating the synthesis of the nascent strand.

DNA ligase} Re-anneals the s and joins okazaki fragments of the lagging strand.

2.outline the function of dna replication enzyme

Important Enzymes in DNA Replication

Enzyme Function

Topoisomerase	Relaxes the super-coiled DNA
DNA helicase	Unwinds the double helix at the replication
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