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Question: highlight the steps of DNA replication

Steps of DNA replication are:

The process of replication can be divided into three stages:

1. Initiation

2. Elongation

3. Termination.

1. Initiation:

- Initiation of DNA replication involves unwinding (separation) of two complementary DNA strand sand formation of replicating fork.
- Unwinding occurs at a single, specific site at a particular DNA sequence on circular DNA of prokaryotes. The site is called the origin of replication, where active synthesis occurs. This region is called replicating fork.
- Replication of double stranded DNA is bidirectional.
- In eukaryotes replication begins at multiple sites composed almost exclusively of A-T base pairs along the DNA helix and is referred to as a consensus sequence.

Steps involved in initiation

1. First DNA a protein recognizes and binds to the “ori” of the DNA and successively denatures the DNA.

2. DNA B protein (helicase) then binds to this region and unwinds the parental DNA, and

form a “V” where active synthesis occurs. This region is called the replicating fork.

3. The stress produced due to unwinding by helicase is released by topoisomerases by cutting either one or both DNA strands.

4. The Single stranded binding (SSB) protein stabilizes the separated strands and prevents theirreassociation.5.To initiate the DNA synthesis by DNA polymeraseIII, it requires RNA primer. The RNA primers are short pieces of RNA (some 5–50 nucleotides in length) formed by the enzyme primase (RNA polymerase) using DNA as a template.

2. Elongation

• Once RNA primer has been synthesized at each of the replicating forks, a DNA polymerase III initiates the synthesis of new DNA strand by adding deoxyribonucleotide to the 3' end of the RNA primer. Thus, DNA polymerase III can synthesize a new chain only in the 5' to 3' direction. Both the DNA strands are synthesized simultaneously but in opposite direction one is in direction towards the replication fork, the other in a direction away from the replication fork.

• The DNA chain which runs in the 3' →5' direction is copied by polymerase III as a continuous strand, requiring one primer. This new strand is known as the leading strand.

• The DNA chain which runs in the 5’ →3' direction is copied by polymerase III as a discontinuous manner because synthesis can only proceed in the5' to 3' direction. This new strand is known as the lagging strand. This requires numerous RNA primers. As the replication fork moves, RNA primers are synthesized at specified intervals. These RNA primers are extended by DNA polymerase III into short pieces of DNA called Okakazi fragments.

• The completion of lagging strand synthesis, the RNA primers are removed from fragments by DNA polymerase I. DNA polymerase I also fills the gaps that are produced by removal of the primer leaving only a nick. It cannot join two polynucleotide chains together, an additional enzyme DNA ligase is required to perform this function. This enzyme catalyzes the formation of a phosphodiester bond to seal the Okazaki fragments.

3. Termination:

Termination sequences, e.g. “ter”, direct termination of replication. A specific protein ter binding protein, binds these sequences and prevents the helicase (DNA B protein) from further unwinding of DNA and facilitates the termination of replication.

Proofreading

• DNA is copied by DNA polymerase with high fidelity (accuracy). Incorrect nucleotides are incorporated with a frequency of one in 10^{8}–10^{12} bases, which could lead to mutation. But the error ratio during replication is kept at a very low level by specific process. This process is known as proofreading.

• Mismatches, occur more frequently but do not lead to stable incorporations because the all three DNA polymerases have 3' to 5' exonuclease activity (proofreading activity).

• DNA polymerase I and II are known to excise mismatched nucleotides before the introduction of the next nucleotide.

Question 2: outline the function of DNA replication enzymes

1. 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.
2. DNA primase--- a type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as template for the starting point of DNA replication.
3. DNA polymerases--- synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.
4. Topoisomerase or DNA Gyrase--- unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.
5. Exonucleases--- group of enzymes that removes nucleotide bases from the end of a DNA chain.
6. DNA ligases--- joins DNA fragments together by forming phosphodiester bonds between nucleotides.