Mechanism of DNA Replication:2

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The separation of DNA 2 chains is termed as unzipping**.** It takes place due to the breaking of H-bonds.It requires helicase ,Mg^{2+} .
In E. coli DNA replication has been investigated most extensively. It was thought that in eukaryotes probably similar mechanism operates. However, it has been found that in E. coli replication always starts at a very unique site called the origin or Ori –site.

Through this replicating point DNA thread moves and replication is accomplished. In eukaryotes enzyme moves along the DNA thread. It has earlier been described that E. coli possesses three types of DNA polymerases; each reads DNA template in 3'→ 5' direction and catalyses the synthesis of DNA in 5'→ 3' direction.

The polymerases read deoxyribonucleotide triphosphates (dATP, dGTP, dCTP, dTTP) as substrate and a DNA template.

To the 3′ end of growing point, the nucleotides are added after interaction of 3′-OH end of deoxyribose with alpha (first) phosphate group of substrate releasing pyrophosphate as below**:**

Before the replication begins, DNA double helix must be unwinded to give rise to single strand. The unwinding process is facilitated by helicases.

Overall DNA replication is accomplished in the following stages :

(i) Unwinding of Double Helix:

Helicases are responsible for unwinding of double helix. They use energy from ATP to unwind short stretches of helix just ahead the

replication fork. After separation of strand it is very necessary to keep them single stranded through single stranded DNA binding proteins (SSB).

It may bind as a binding sites of 8-10 nucleotides . However there is possibility of leading tension and formation of super coils in helix.

The relieving of tension and promotion of unwinding process are done by the enzyme topoisomerases which transiently break one of two strands in such a way that it remains unchanged. It ties or unties a knot in DNA strand. DNA gyrase is one of the E. coli topoisomerases that removes super coiling of DNA during replication. Thus there is formation of a ssDNA template.

(ii) DNA Replication:

DNA replication is accomplished in several steps. The first step is the RNA-primer synthesis on DNA template near origin of replication. Synthesis of RNA primer is very necessary because during DNA replication there is chance of more error in initial laying down of first few nucleotides to pre-existing DNA template. DNA Pol I and Pol II cannot synthesize DNA without an RNA primer; therefore a special RNA polymerase called primase synthesizes an about 10 nucleotide long short primer.Therefore, DNA Pol III holoenzyme starts synthesis of DNA in 5'→ 3′ direction at the end of RNA primer .

The second step is chain elongation. A new DNA strand starts synthesizing by addition of deoxyribonucieoside triphosphates to the 3′ end of last nucleotide of RNA primer. DNA synthesis occurs in 5'→ 3′ direction catalysed by the replisome. The replisome has two DNA Pol III holoenzyme complexes. It is a very large complex containing DNA Pol III and several proteins.

The γ and β-subunits bind the holoenzyme to the DNA template and primer. The a-subunit synthesizes the DNA. One polymerase continuously copies the leading strand (i.e. a strand growing in the direction of replication fork and showing continuous replication).

The lagging strand (i.e. a strand growing in opposite direction of replication fork and showing discontinuous replication of strand) loops around replisome continuously. There is formation of Y shaped replicating fork at the point where two strands are separated.

On leading strand DNA synthesis occurs continuously because there is always a free 3′-OH at the replication fork to which a new nucleotide is added. But on the opposite strand called lagging strand, DNA synthesis occurs discontinuously because there is no 3′ -OH at the replication fork to which a new nucleotide can link. On this strand there is free 3′ -OH at the opposite end away from growing point. Therefore, on lagging strand a small (11 bases long) RNA primer must be synthesised by primase to provide free 3′ -OH group.

Finally, about 1000-2000 nucleotides long fragment in bacteria and about 100 nucleotides long fragment in eukaryotic cells are synthesized. These fragments are called the Okazaki fragments after the name of a Japanese discoverer, R. Okazaki.

(iii) Removal of RNA Primer and Completion of DNA Strand:

When the Okazaki fragments are formed; most of lagging strands become duplicated. The RNA primer is removed by DNA Pol I or RNAase . DNA polymerase I synthesizes a short segment of complementary DNA to seal the gap. Possibly Pol I remove the primer nucleotide at a time and replace it with suitable complementary deoxyribonucleotide .

(iv) Joining of Fragments:

At the end, the fragments are joined by DNA ligase that forms a phosphodiester bond between 3′-OH end of growing strand and 5’ end of an Okazaki fragment . Reaction of DNA ligases . In mutants defective ligase is produced; therefore, joining of Okazaki fragments is greatly improved.

E. coli DNA ligase derives energy from NAD. It is first adenylated by AMP moiety of NAD releasing the nicotinamide mononucleotide (NMN). The adenylated ligase reacts with ssDNA having a nick and forms phosphodiester bond.

The complete reaction is as below:
E. coli ligase + NAD → ligase – AMP + NMN

Ligase – AMP + DNA (with break) → phosphodiester + ligase + AMP

AMP + NMN → NAD

Obviously, DNA replication is a very complex process. If any error is made during replication, it leads to mutation. E. coli makes error about 10^{-6} per gene per generation. The DNA Pol I and Pol III act as proof reader of the newly formed DNA.
These move along new DNA synthesized.