DNA Replication:

Before each cell division, new copies must be made of each of the many molecules that form the cell, including the duplication of all DNA molecules. DNA replication is the name given to this duplication process, which enables an organism's genetic information (genes)to be passed to the two daughter cells created when a cell divides.

DNA replication is fundamental process occurring in all living organism to copy their DNA. The process is called replication in sense that each strand of ds DNA serve as template for reproduction of complementary strand.

What is replication of DNA?

1. DNA replication is the process by which DNA makes a copy of itself during cell division .
2. This process takes us from one starting molecule to two daughter molecules , with each newly formed double helix containing one new and one old strand .
3. DNA found within the nucleus , must be replicated in order to ensure that each new cell receives he correct number of chromosomes. The process of duplication is called DNA replication .
4. Fundamental process occurring in all cells for copying DNA to transfer the genetic information to daughter cells .

Models for DNA replication:

1) Semiconservative model:

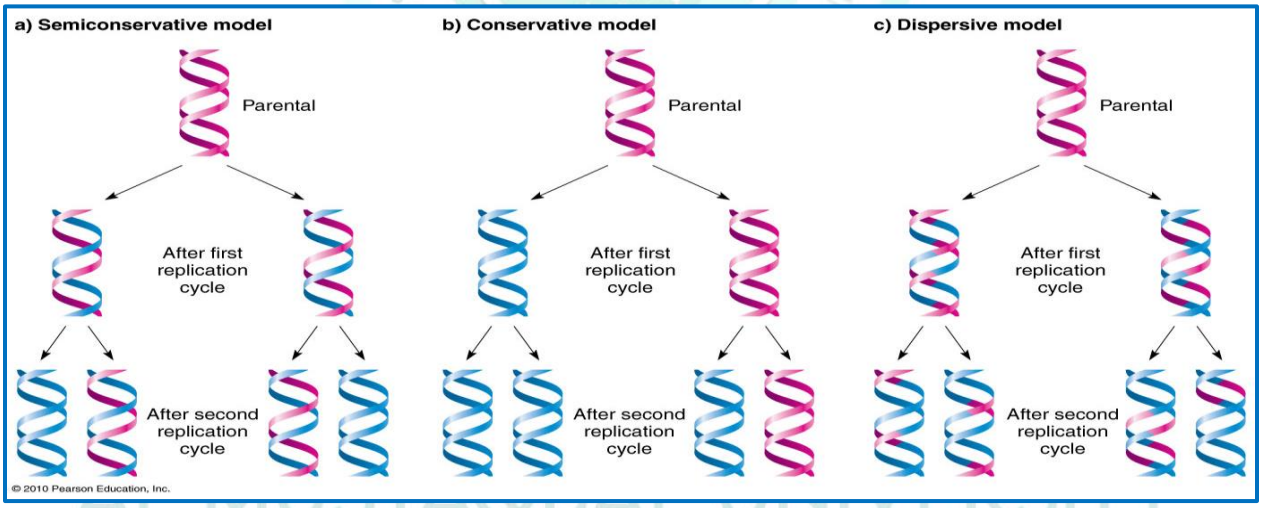
The semi-conservative method suggests that each of the two parental DNA strands act as a template for new DNA to be synthesized; after replication, each double-stranded DNA includes one parental or "old" strand and one "new" strand.

2) Conservative model:

After DNA replication, the parental DNA remains together, and the newly formed daughter strands are together.

3) Dispersive model:

Both new copies of DNA have double- stranded segments of parental DNA and newly synthesized DNA interspersed.



Meselson and Stahl resolved the issue in 1958 when they reported results of a now famous experiment. which showed that DNA replication is semi-conservative, where each strand is used as a template for the creation of the new strand.

General feature of DNA replication

- DNA replication is semi conservative
- It is bidirectional process
- It proceed from a specific point called origin
- It proceed in 5'-3' direction
- It occur with high degree of fidelity
- It is a multi-enzymatic process.

DNA replication occurs by three steps

1. Initiation:

2. Elongation:(Leading strand synthesis and Lagging strand synthesis)

3. Termination.

1- Initiation

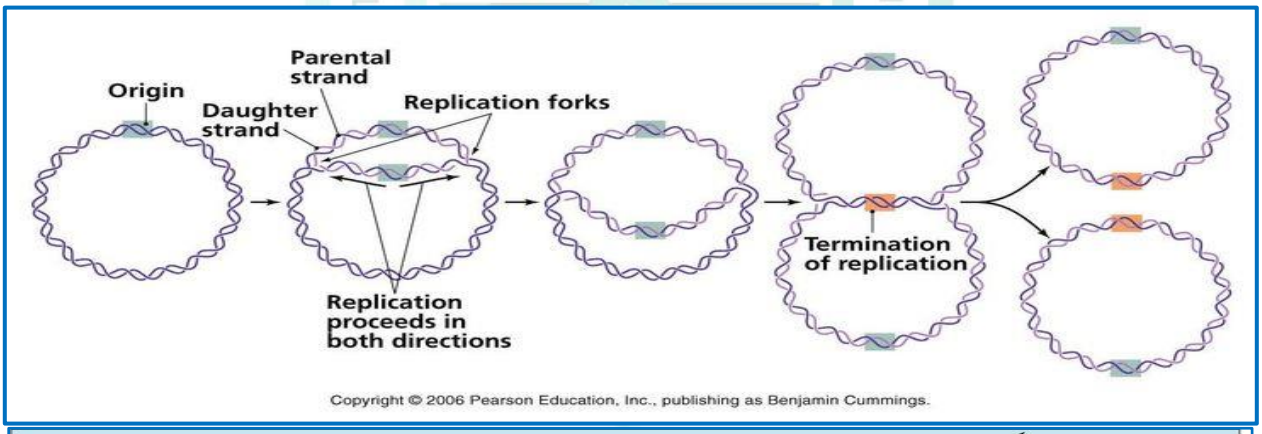
The replication of both prokaryotic and eukaryotic DNAs starts at a unique sequence called the origin of replication, which serves as a specific binding site for proteins that initiate the replication process.

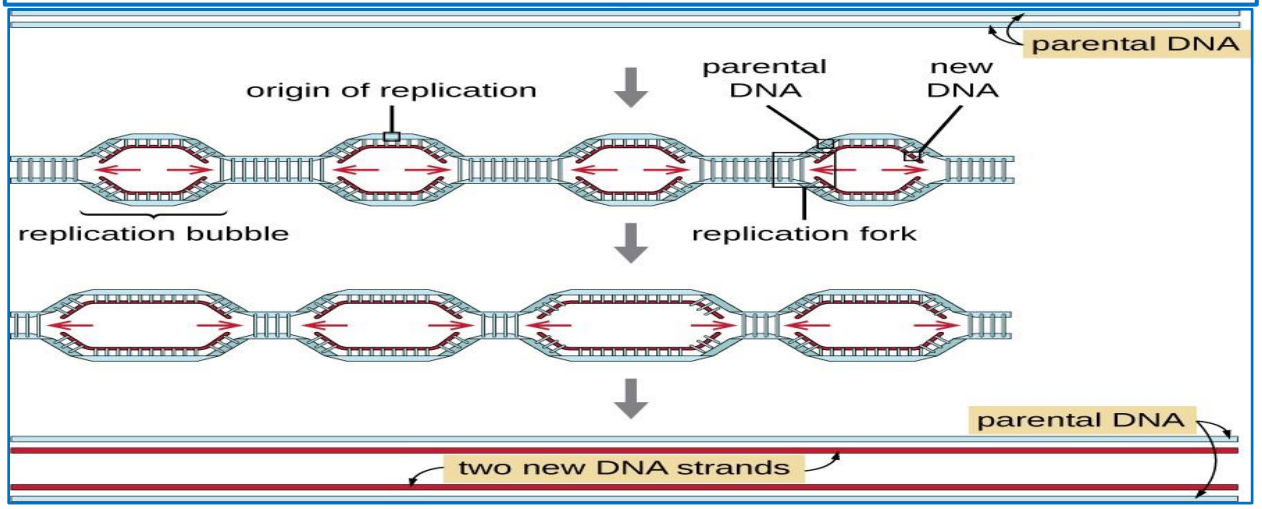
In *E. coli*, which has a single origin of replication on its one chromosome (as do most prokaryotes), it is approximately 245 base pairs long and is rich in AT sequence (rich in adenine and thymine bases), because A-T base pairs have two hydrogen bonds (rather than the three bond in a C-G pair) and easy to break in this site.

The origin of replication (oriC) is recognized by certain proteins that bind to this site called Initiator proteins. These proteins (DnaA in prokaryotes, origin recognition complex in yeast) binds specifically to the AT-rich replicator sequence

oriC to form a specific DnaA-oriC complex. An enzyme called helicase unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process.

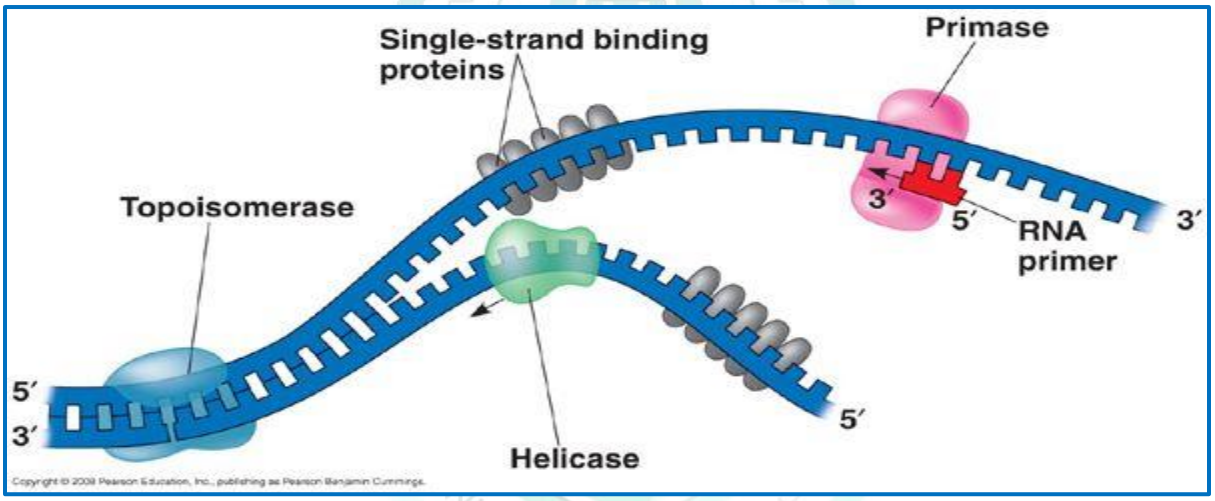
As the DNA opens up, Y-shaped structures called replication forks are formed. Two replication forks are formed at the origin of replication and these get extended bi- directionally as replication proceeds. (Two replication forks begin at a single replication origin in bacteria and proceed in opposite directions which look like a bubble , moving away from the origin till reaching the opposite direction in one point called Ter Terminus (Teri).





The mechanism of eukaryotic DNA replication is similar to that of prokaryotic DNA replication but it is more complex. There are multiple origins of replication on the eukaryotic chromosome so multiple replication bubbles will form.

Single-strand binding proteins (SSBPs) bind to the single strands of DNA near the replication fork to prevent the ssDNA strands from winding back into a double helix,thus maintaining the strand separation.

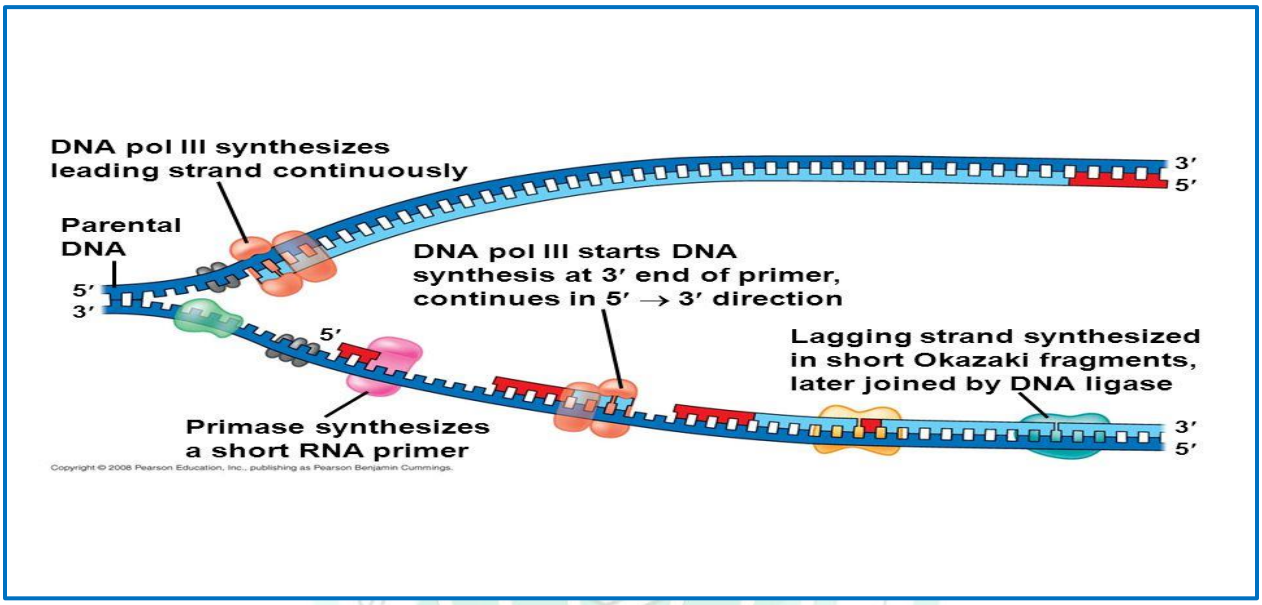


One of Key molecule in DNA replication is the enzyme DNA polymerase is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be only extended in this direction).It also requires a free 3'-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3'-OH group is not available.

The problem is solved with the help of an RNA sequence that provides the free 3′-OH end. RNA primase, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA template .

Because this sequence primes the DNA synthesis, it is appropriately called the primer. DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand. (example: A in the template strand is complement to T in new growing strand , and G in the template strand is complement to

C in new growing strand strand)… The primer is RNA rather than DNA because DNA polymerases cannot start chains de novo.



2- Elongation step

DNA double helix is anti-parallel; that is, one strand is in the 5' to 3' direction and the other is oriented in the 3' to 5' direction. Both strands of parental DNA serve as templates for the synthesis of new DNA. A new DNA strand is always synthesized in a 5’ to 3’ direction. Thus, the replication of both the strands goes in two in different ways .

One strand, which is complementary to the 3' to 5' parental DNA strand, is synthesized continuously in 5----3 direction towards the replication fork because the DNA polymerase III can add nucleotides in this direction. This continuously synthesized strand is known as the leading strand.

All known DNA polymerases synthesize DNA in the 5′→3′ direction but not in the 3′ → 5′ direction.

The other strand, complementary to the 5' to 3' parental DNA, is extended away from the replication fork discontinuously; in small fragments known as Okazaki fragments, each requiring a primer to start the synthesis (this strand needs a new primer for each of the short Okazaki fragments) . Okazaki fragments are then synthesized via extension of these RNA primers by DNA polymerase.

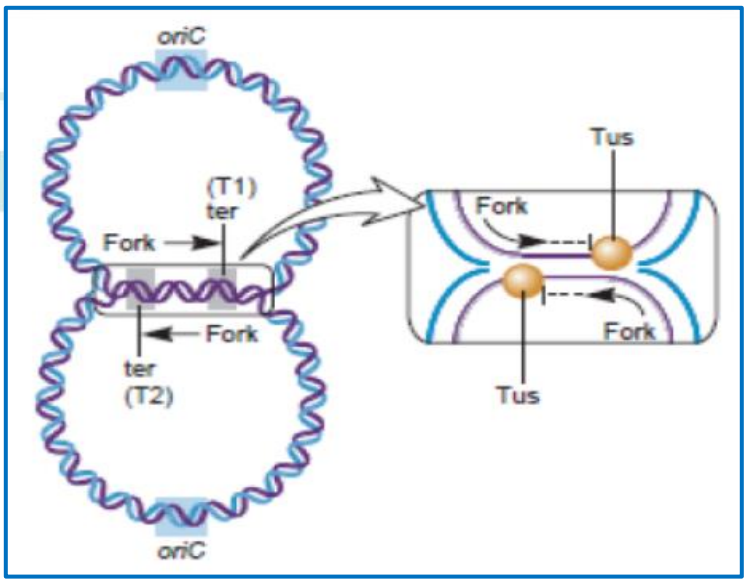
Okazaki fragments are named after the Japanese scientist Reiji Okazaki (1968) who first discovered them. The strand with the Okazaki fragments is known as the lagging strand.

The primers are removed by the exonuclease activity of DNA polymerase I in prokaryotes, and the gaps are filled in by deoxyribonucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme DNA ligase.

In eukaryote, The enzyme ribonuclease H (RNase H), instead of a DNA polymerase I, removes the RNA primer, which is then replaced with DNA nucleotides. The gaps that remain are sealed by DNA Ligase.

3-Termination

Termination requires that the progress of the DNA replication fork must stop or be blocked. Because bacteria have circular chromosomes, termination of replication occurs when the two replication forks meet each other on the opposite end of the parental chromosome.



- Removes the primer (RNA fragments), by 5'-3' exonuclease activity of polymerase I, and replaces the RNA nucleotides with DNA nucleotides. and fill the gaps.

- When this is complete, a single nick on the leading strand and several nicks on the lagging strand can be found. Ligase works to fill these nicks in, thus completing the newly replicated DNA molecule .

- Topoisomerase IV will : separate the two complete daughter chromosome in to two chromosome.

-To form a continuous lagging strand of DNA, the RNA primers must eventually be removed from the Okazaki fragments and replaced with DNA.

Termination in Eukaryotic cell

Eukaryote cell initiate DNA replication at multiple points in the chromosome, so replication forks meet and terminate at many points in the chromosome.

Primer removal at the end of the chromosome leaves a gap that can't be filled in (there is no DNA polymerase coming along to fill in that piece. (Remember that DNA synthesis can ONLY occur 5'-3'). So on every round of replication, a little piece is lost from the end of the chromosome.

Enzymes involve in DNA Replication

1.DNA helicase :- unwind and separates double stranded 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 . RNA molecule acts as templates for the starting point of DNA replication.

3. DNA polymerases :- Synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands .

4. DNA Gyrase or (Topoisomerase) :- unwind and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled .

5. DNA ligase :- joins DNA fragments together by forming phosphodiester bonds between nucleotides .

6. Single strand binding proteins :- Keep the DNA single stranded after it has been melted by helicase .

7. RNA primer:- RNA primer composed of multiple bases that attached to the template strands to initiate the DNA replication .

8. Telomerase:- Finishes off the ends of DNA strands .

9. Exonucleases:- Group of enzymes that remove nucleotide bases from the end of a DNA chain.