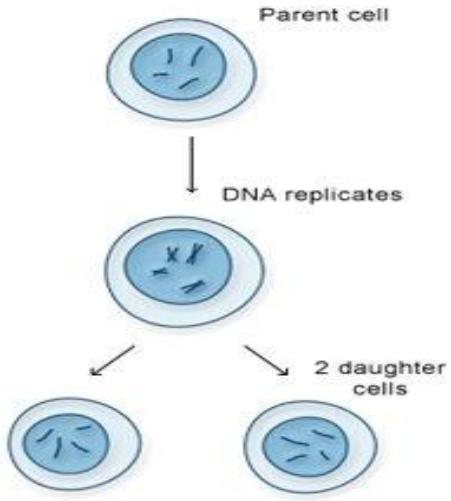
DNA REPLICATION

DNA replication is a biological process that occurs in all living organisms and copies their exact DNA. It is the basis for biological inheritance.

Biological significance Of DNA Replication

- extreme accuracy of DNA replication is necessary in order to preserve the integrity of the genome in successive generations
- In eukaryotes, replication only occurs during the S phase of the cell cycle.
- Replication rate in eukaryotes is slower resulting in a higher fidelity/accuracy of replication in eukaryotes



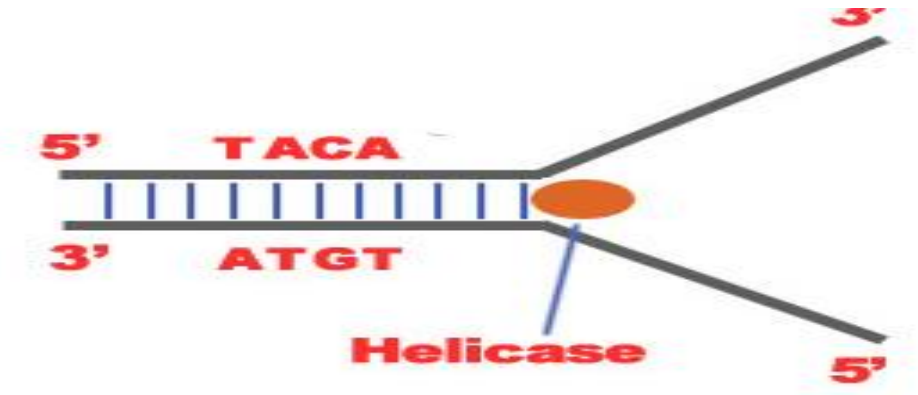
The first major step for the DNA Replication to take place is the breaking of hydrogen bonds between bases of the two antiparallel strands. The unwinding of the two strands is the starting point. The splitting happens in places of the chains which are rich in A-T. That is because there are only two bonds between Adenine and Thymine (there are three hydrogen bonds between Cytosine and Guanine).

The nucleotides of DNA synthesis are very important in three ways.

They function as:

1. The building blocks of DNA (and RNA)
2. The information stored in DNA (more precisely, the sequence of nucleotides serves this function)
3. The energy source (in the triphosphate forms) for synthesis

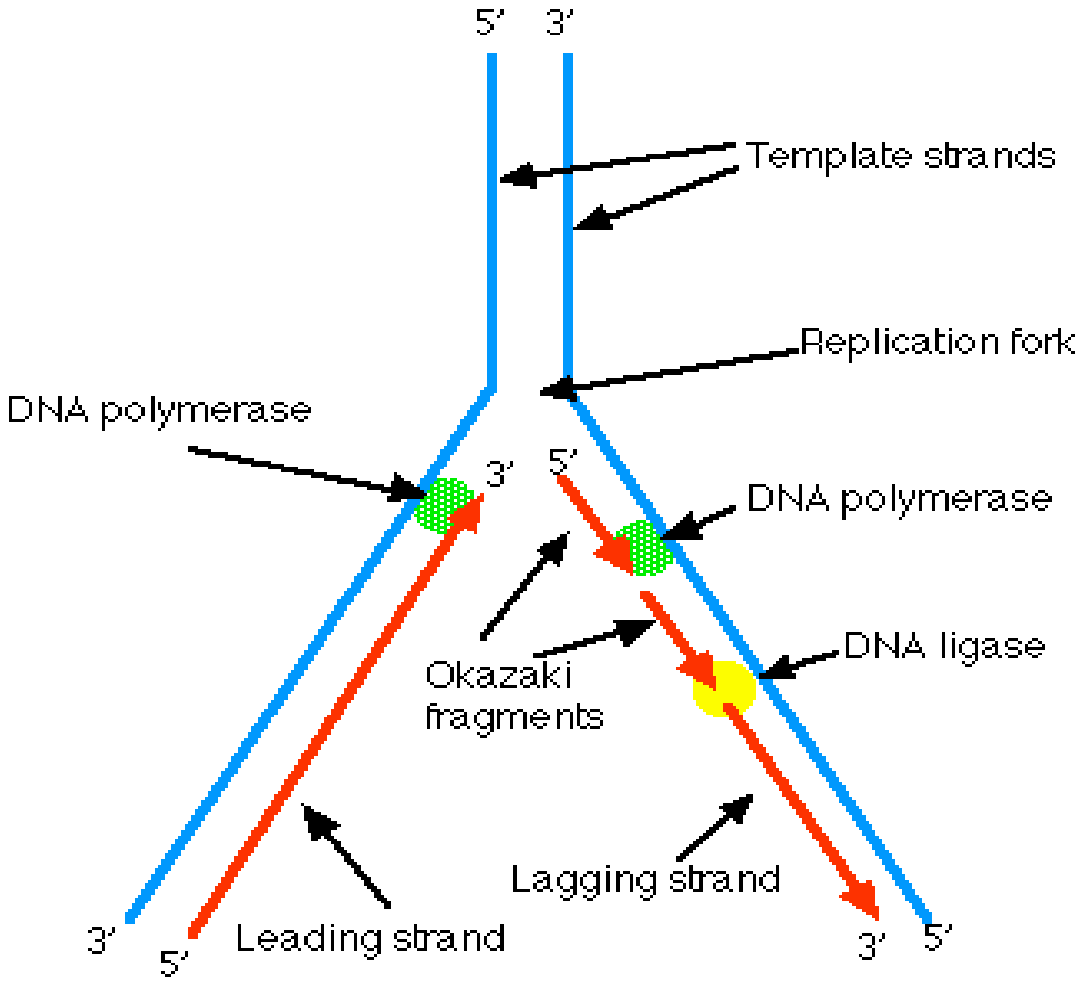
Helicase: Is the enzyme that splits the two strands. The structure that is created is known as "Replication Fork".



In order for DNA replication to begin, the double stranded DNA helix must first be opened. The sites where this process first occurs are called replication origins. Helicase unwinds the two single strands.

Replication Fork

The replication fork is a structure that forms within the nucleus during DNA replication. It is created by helicases, which break the hydrogen bonds holding the two DNA strands together. The resulting structure has two branching, each one made up of a single strand of DNA. These two strands serve as the template for the leading and lagging strands, which will be created as DNA polymerase matches complementary nucleotides to the templates; the templates may be properly referred to as the leading strand template and the lagging strand template.



Single-Strand Binding Proteins

Single-Strand DNA Binding Proteins, SSB for short, work to bind individuals strands in a DNA double stranded helix and aid the helicases in opening it up into single strands. They are particularly useful in **stabilizing** the unwound single-stranded formation.

Basic rules of replication

A. Semi-conservative

B. Starts at the ‘origin’

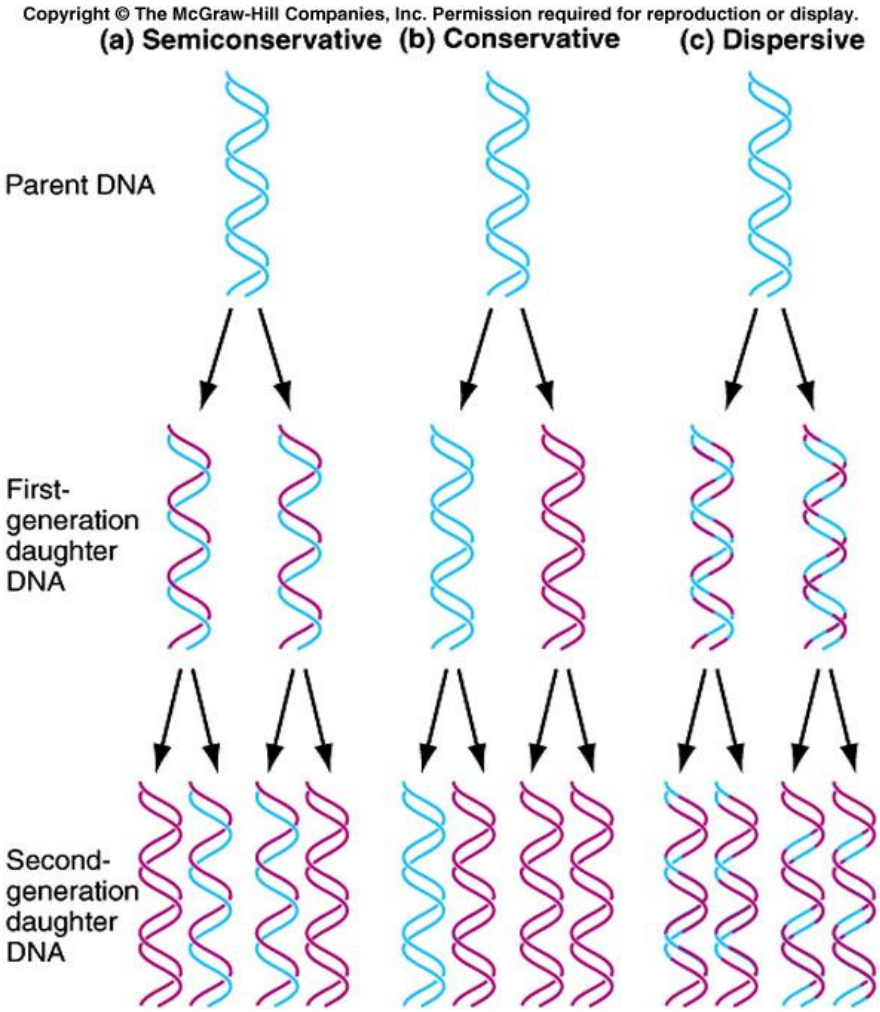
C. Synthesis always in the 5-3' direction

D. Can be uni or bidirectional

E. Semi-discontinuous

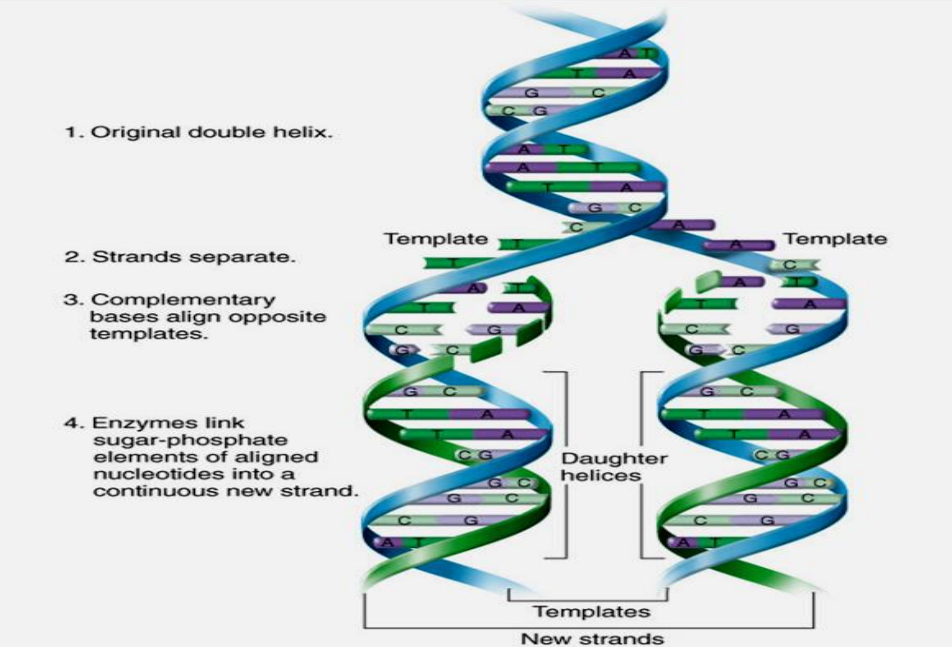
F. RNA primers required

DNA replication 3 possible models



Semi-conservative replication:

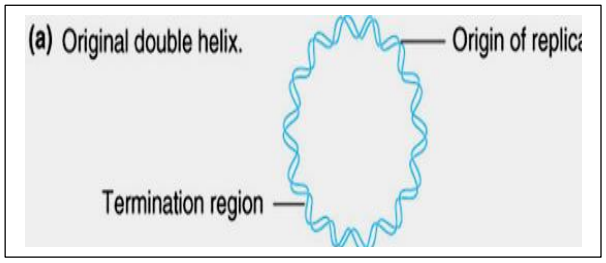
One strand of duplex passed on unchanged to each of the daughter cells. This 'conserved' strand acts as a template for the synthesis of a new, complementary strand by the enzyme DNA polymerase. Each new double helix is consisted of one old and one new chain. This is what we call **semiconservative replication**.



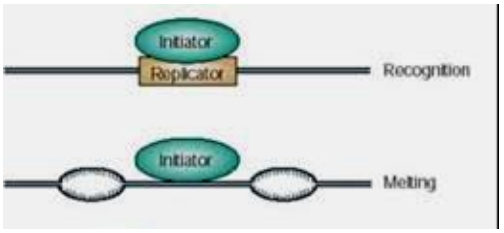
Starts at origin

Prokaryotes – single origin site E.g *E.coli* - *oriC*

Eukaryotes – multiple sites of origin (replicator)

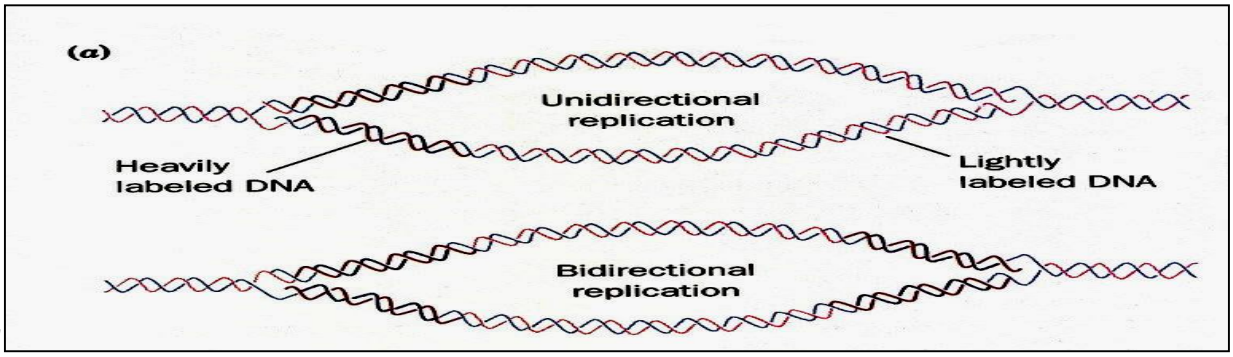


Prokaryotes



Eukaryotes

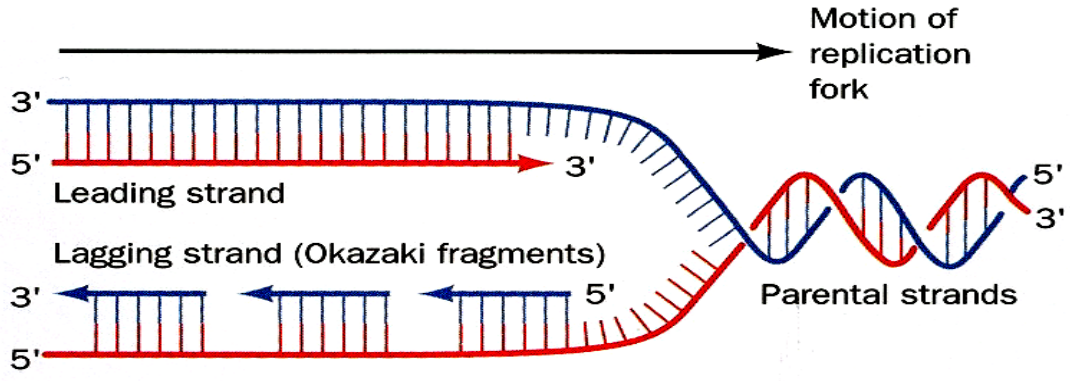
Uni or bidirectional



Semi-discontinuous replication

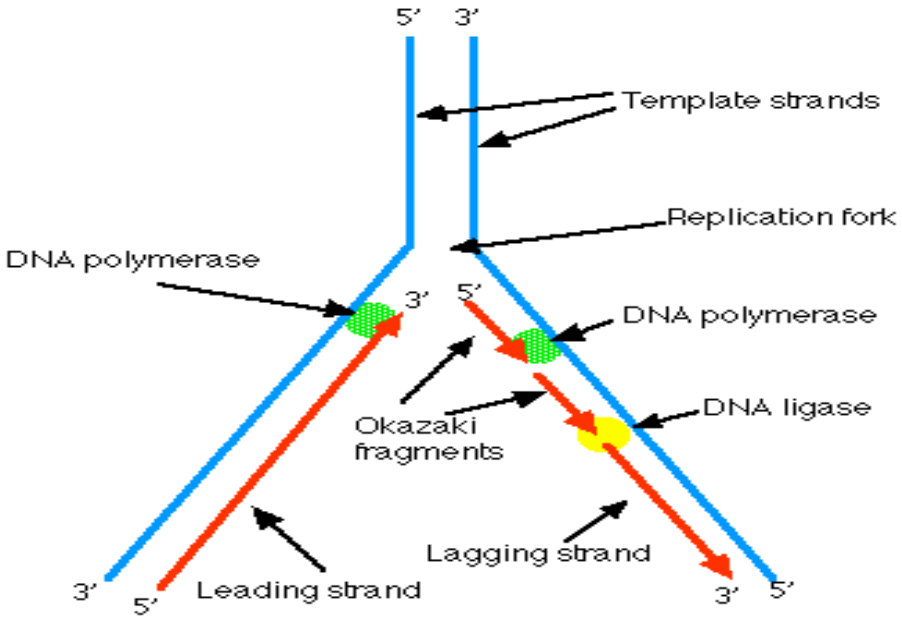
Anti parallel strands replicated simultaneously

- Leading strand synthesis **continuously** in 5'– 3'
- Lagging strand synthesis in **fragments** in 5'-3'

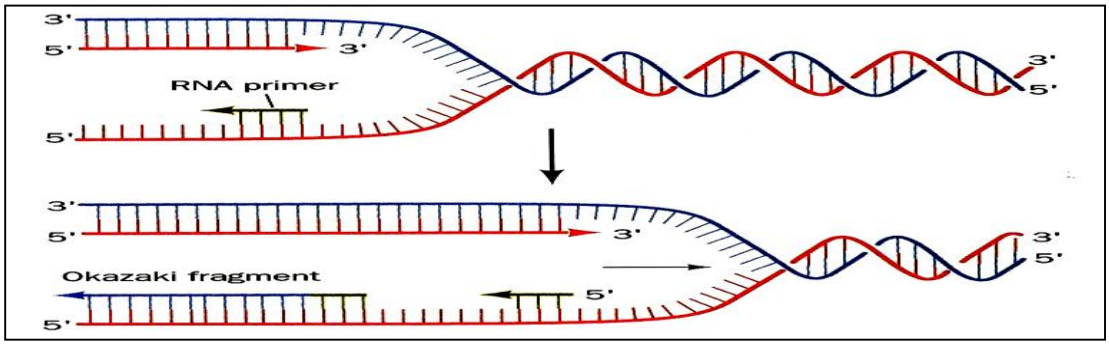


The elongation process in DNA replication, both daughter strands are synthesized in their 5'-3'direction. The 3'-5' proceeding daughter strand -that uses a 5'-3' template- is called leading strand because DNA Polymerase III can "read" the template and continuously adds nucleotides (complementary to the nucleotides of the template, for example Adenine opposite to Thymine etc).

The gap between two RNA primers is called "**Okazaki Fragments**".



RNA primers required



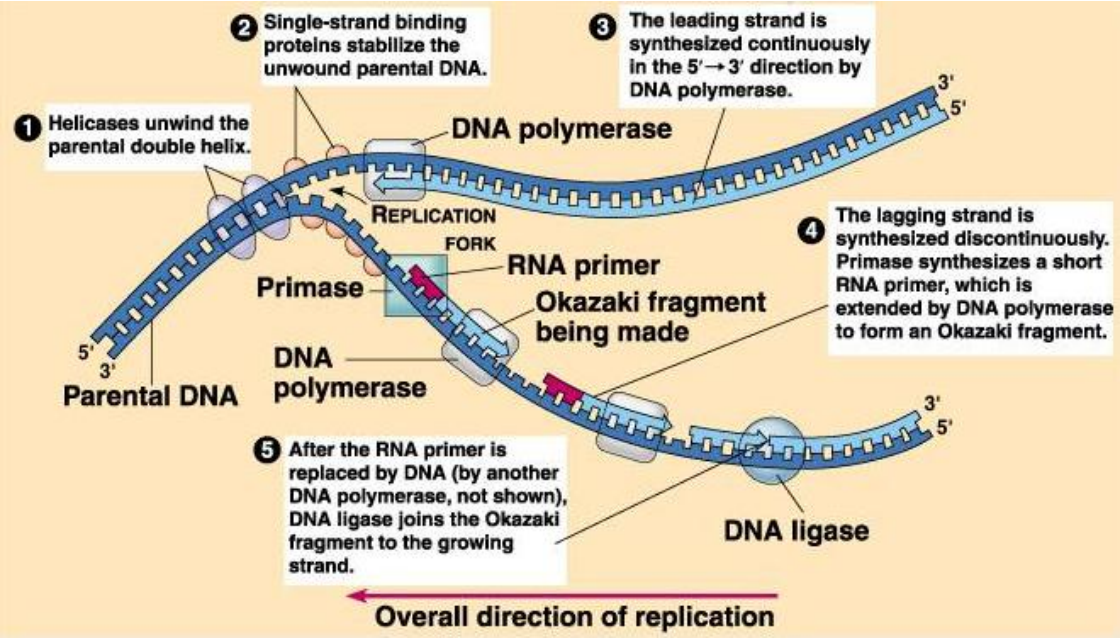
RNA Primase: One of the most important steps of DNA Replication is the binding of **RNA Primase** in the initiation point of the 3'-5' parent chain. **RNA Primase** can attract RNA nucleotides which bind to the DNA nucleotides of the 3'-5' strand due to the hydrogen bonds between the bases. RNA nucleotides are the primers (starters) for the binding of DNA nucleotides. The elongation process in DNA replication, both daughter strands are synthesized in their 5'-3'direction. The 3'-5' proceeding daughter strand -that uses a 5'-3' template- is called leading

strand because DNA Polymerase III can "read" the template and continuously adds nucleotides (complementary to the nucleotides of the template, for example Adenine opposite to Thymine etc).

The total mechanism requires a cycle of repeating steps that include:

1) Creation of RNA Primers (Primase)
2) Synthesizing a short segment of DNA between the primers (Polymerase III)
3) Replacing the RNA primer with DNA (Polymerase I) and finally The binding of these pieces (Ligase)

The last step of DNA Replication is the Termination. This process happens when the DNA Polymerase reaches to an end of the strands. We can easily understand that in the last section of the lagging strand, when the RNA primer is removed, it is not possible for the DNA Polymerase to seal the gap (because there is no primer). The DNA Replication is not completed before a mechanism of repair fixes possible errors caused during the replication. Enzymes like nucleases remove the wrong nucleotides and the DNA Polymerase fills the gaps.


2 Single-strand binding proteins stabilize the unwound parental DNA.
3 The leading strand is synthesized continuously in the 5′→3′ direction by DNA polymerase.
1 Helicases unwind the parental double helix.
DNA polymerase
3'
5'
REPLICATION FORK
4 The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
RNA primer
Primase
Okazaki fragment being made
5'
3'
Parental DNA
DNA polymerase
3'
5'
5 After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.
DNA ligase
Overall direction of replication