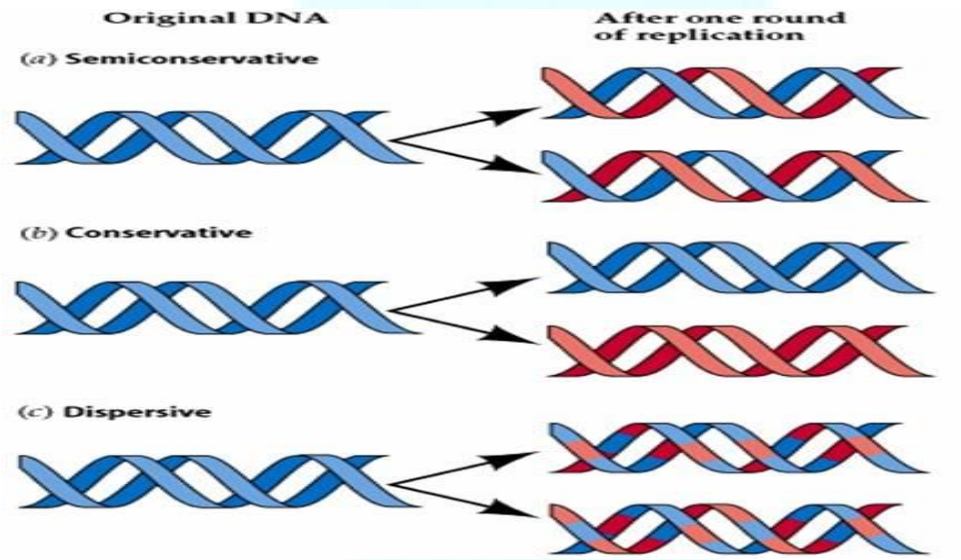
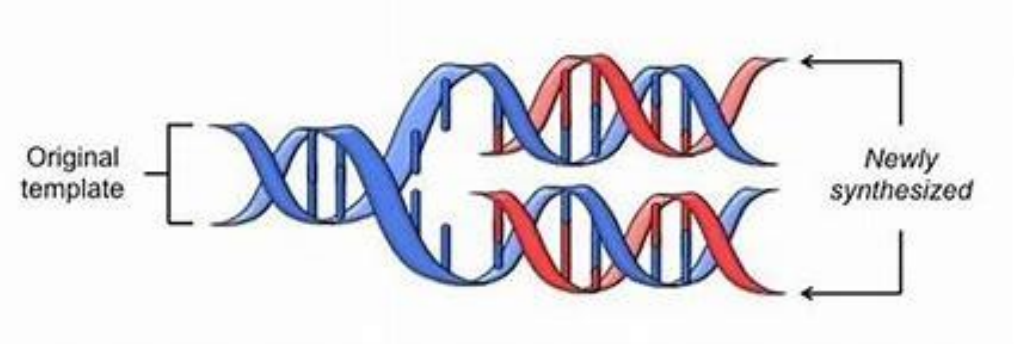
DNA REPLICATION

- The transfer of genetic information from cell to cell and generation to generation necessitates the faithful replication of the DNA molecule.
- Three mechanisms were suggested to explain the process of replication of DNA.
    1. Semi consevative replication
    2. Conservative replication
    3. Dispersive replication.

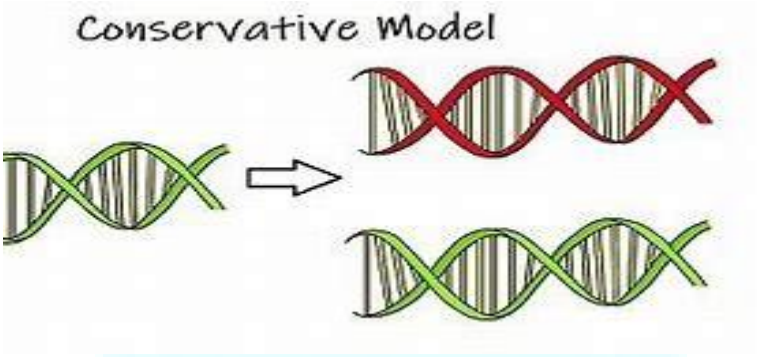


- **Semi conservative replication**



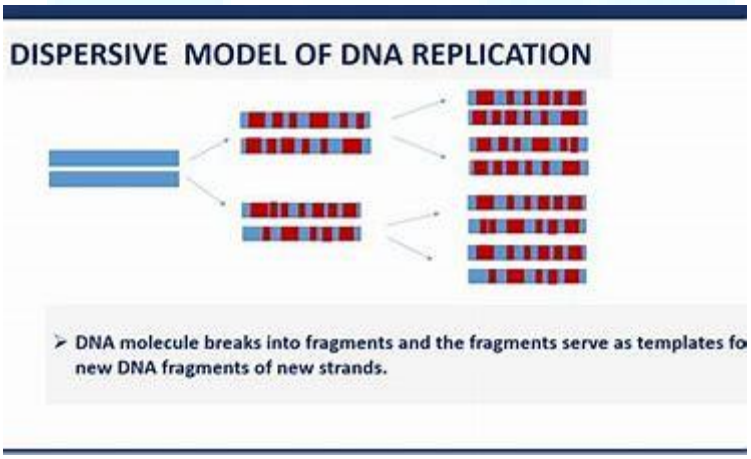
- It was proposed by Watson and Crick
- Each single strand of the double helix acts as a template for its complement and the newly formed helices will have one old strand conserved and a newly synthesized strand.

- **Conservative Replication**



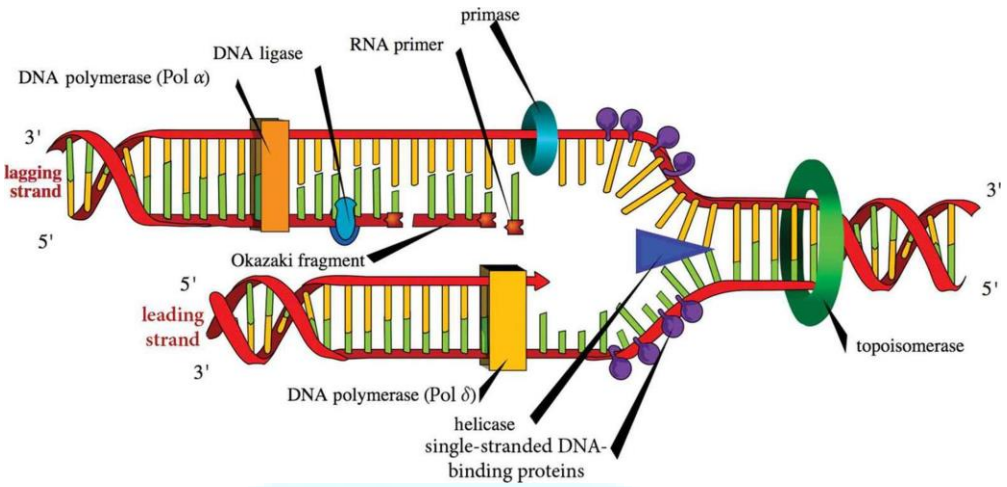
- According to this, two new strands are synthesized from the parent DNA which directly functions as the template without undergoing unwinding and strand separation.

- **Dispersive replication**



- It is to take place by the break down of the double helical strands along their length into small pieces.
- After each strand has synthesized its complementary strand they would be randomly reunited to form a patchwork single string of dispersed old and new pieces.

- **Enzymes involved in DNA replication**



1. Nucleases
2. Polymerases
3. Ligases
4. Topoisomerases

Nucleases

- It hydrolyze a polynucleotide chain to component nucleotides by breaking the 5' – 3' phosphodiester linkages between them.
- There are two kinds of nucleases are present – the endonucleases and exonucleases.
- The exonucleases begin their action from the free end of a polynucleotide.
- Depending on its specificity an exonuclease may begin its activity from the free 3' OH of the polynucleotide and proceed towards the 5' end or it may proceed on the reverse direction.
- The endonucleases preferentially break the phosphate bonds occurring in the interior of a polynucleotide chain.

- If the polynucleotide chain is a single stranded linear structure it will be cut into two by the action of this enzyme.
- In the case of a double stranded helix, only a nick will be created forming two free ends susceptible to the action of exonucleases.

Polymerases

- These are enzymes responsible for the formation of a polynucleotide chain complementary to the template strand.
- They catalyze the addition of individual nucleotides to the growing primer in a 5′ – 3′ direction.
- At least 5 different DNA polymerases have been identified in E.coli.
- DNA polymerase I is a single polypeptide with a molecular weight of 109,000 Daltons.
- This enzyme is also known as the Kornberg enzyme.
- In addition to the polymerase activity, it has two other enzymatic activities. – a 5′ – 3′ exonuclease activity and a 3′ – 5′ exonuclease activity.
- It has been shown eventually that this enzyme is involved in the repairing activities only and is not associated with the replication of the DNA.
- Later two other DNA polymerases were also discovered.
  Of these the DNA polymerase II is a repairing enzyme, just like the polymerase I.
- The principal replicating enzyme, DNA polymerase III is a complex enzyme composed of different subunits.
- It has got a 5′ – 3′ polymerase activity and a 3′ – 5′ exonuclease activity but no 5′ – 3′ exonuclease activity.
- DNA polymerase III is a complex multimeric enzyme with a molecular mass of 900,000 Dalton in its complex or holoenzyme form.
- The minimal core that has the catalytic activity contains three subunits: α,ε and θ.
- The α subunit possesses the polymerizing activity as well as the 5′ – 3′ exonuclease activity.

- The ε subunit possesses the 3′ – 5′ exonuclease activity.
- One or more of the remaining polypeptides bind an ATP molecule needed DNA polymerase III to commence synthesis at the end of an RNA primer.
- The addition of the τ subunit results in the dimerization of the catalytic core and increased activity.
- The catalytic core synthesizes rather short DNA strands because of its tendency to fall off the DNA template.
- In order to synthesize the long DNA molecules present in chromosomes, this frequent dissociation of the polymerase from the template must be eliminated.
- The β subunit of the DNA polymerase III forms a dimeric clamp that keeps the polymerase from falling off the template DNA.
- The β dimeric forms a ring that encircles the replicating DNA molecule and allows DNA polymerase III to slide along the DNA while tethered to it.
- The fact that the ploymerase enzymes could function as an exonuclease also enable them to perform the 'proof reading', giving the DNA replication a much higher fidelity.

Ligases

- The function of the ligases involves the joining of the DNA fragments into single linear strand.

Topoisomerases

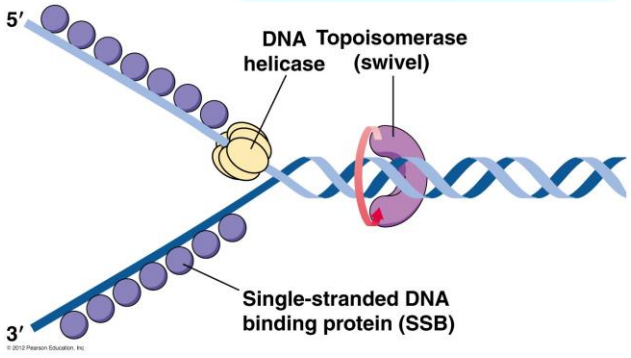
- Semi conservative replication requires that two strands of a parental molecule be separated during the synthesis of new complementary strands.
- So replication requiring an unwinding mechanism.
- Each turn of DNA is about 10 nucleotides long, a DNA molecule must be rotated 360^0 once for each 10 replicated base pairs.

- In E.coli DNA replicates at a rate of about 30,000 nucleotides per minute.
- Thus a replicating DNA molecule must spin 300 revolutions per minute to facilitate the unwinding of the parental DNA strands
- This furious process of unwinding raises the possibility of positive super coils developing ahead of the replication fork.
- Such super coils are relaxed by the DNA topoisomerases.
- The topoisomerases catalyze transient breaks in DNA molecules but use covalent linkages to hold on to the cleaved molecules.
- These transient breaks provide an axis of rotation that allows the segments of DNA on opposite sides of the break to spin independently.
- Thus during DNA replication, only a short segment of DNA in front of the replication fork need to spin – the segment up to the closest transient nick by topoisomerase.

Mechanism of DNA Replication

- During DNA replication, two processes are in motion simultaneously.
- One is the unwinding of the parental strands to provide single stranded templates and the other is the template – guided synthesis of new strands.
- The role of the template DNA is important in DNA synthesis as it serves as master copy upon which the synthesis of the new DNA occurs.
- The replication begins at a specific initiation site in a double helix at which a nick is produced in one of the two strands of the double helix.
- The nick is produced by an endonuclease enzyme enables the cut stands to unwind there by forming two single stranded templates.
- For the occurrence of the unwinding, both the hydrogen bond and the hydrophobic interactions of the DNA have to be eliminated.
- Two strands of double helix at the replication site do not spontaneously come apart frequently enough permit the rapid rates of DNA polymerization.

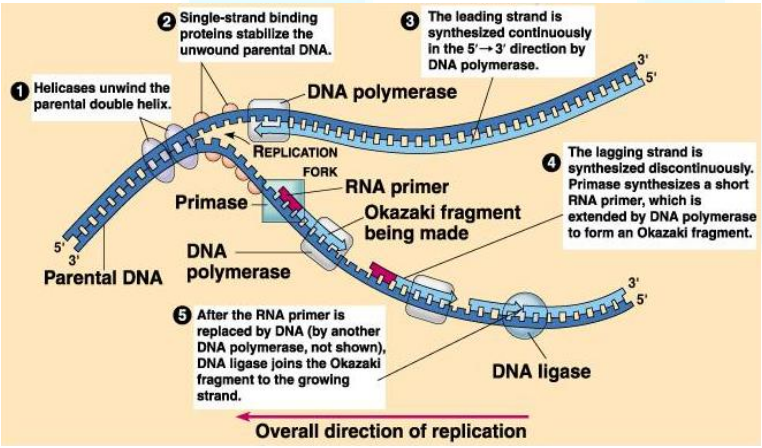
- To facilitate strand separation the cells contain a specific class of proteins – the Helicases – which bind to ATP and to the strands of the DNA.
- They use energy derived from the breakdown of ATP to ADP to move progressively along and increase the rate of DNA strand separation.
- Two different helicases, helicase II and helicase III and the rep proteins are involved in strand binding.
- The rep proteins bind to the leading strand and the helicases bind to the lagging.
- The conversion of bound ATP to ADP must lead to conformational changes that propel the helicases unidirectionally along the DNA strands ahead of the replication fork.
- The single strands thus formed become covered by large amounts of single stranded DNA binding proteins (SSBs).
- The binding of one SSB tetramer promotes the binding of another one to an adjacent section of a single stranded, DNA, a process called cooperative binding.
- Single stranded DNA that is covered by SSBs has a semi rigid extended from without bends of kinks.
- This rigid configuration is essential for the functioning of DNA as a template upon which complementary DNA strands can be initiated and elongated.



- One important aspects about the DNA polymerases is that such enzymes could add on nucleotides to pre existing polynucleotide chains only.

- Short stretches of RNA have been found attached to the 5′ ends of many newly synthesized DNA chains suggesting that these RNA fragments act as the initiating points.
- This starting points for DNA replication is recognized by specific RNA polymerases.
- The primers on the lagging strand are made by a special class of enzymes called primases which recognize specific DNA sequences along the single stranded chains.
- The E.coli primase is a single polypeptide of 60 K.Daltons.
- By itself it is relatively inactive and functions only when complexed with six or seven other polypeptides including the DNA helicase. Such a complex is called a primosome.
- Removal of the RNA primers occurs later through the action of other enzymes like DNA polymerase I.
- RNA primers are used to start virtually all DNA chains probably has its origin in the need to prevent a lethal number of mistakes near the starting points of DNA chain.
- The laying down of the first few nucleotides along a DNA template may be inherently much less accurate than when nucleotides add on to long sections of an already formed double helix.
- So there may be great selective advantages for an initiation mechanism that allows easy discrimination and subsequent elimination of starting nucleotide sequences.
- The opposing chain directions(5′ – 3′ and 3′ – 5′) of the two strands of a double helix mean that the two daughter strands being synthesized in each replication fork must also run in opposite directions.
- But the DNA polymerases are strictly direction oriented and can synthesize DNA only in the 5′ – 3′ direction.

- The resolution of this paradox occurred with the demonstration that the synthesis of the other strand is discontinuous.
- At the molecular level the synthesis of the complementary strands of DNA is occurring in opposite physical directions.
- The synthesis of the strand being extended in the overall 5′ – 3′ direction, called the leading strand and it is continuous.
- The strand being extended in overall 3′ – 5′ direction, called the lagging strand grows by the synthesis of short fragments(synthesized 5′ – 3′) and subsequent covalent joining.
- The short fragments of DNA synthesized on the discontinuous strands are called Okazaki fragments after Reiji and Tuneko Okazaki who discovered them in the late 1960's.
- Okazaki fragments of E.coli are found to be 1000 to 2000 nucleotides long where as in eukaryotes they are found to be only 100 to 200 nucleotides long.



- Most aspects of DNA replication are basically the same in both prokaryotes and eukaryotes.
- RNA primers and Okazaki fragments are shorter in eukaryotes than in prokaryotes.

- DNA synthesis takes place only within a small portion of the cell cycle in eukaryotes where as it is a continuous process in prokaryotes.
- The long DNA molecules present in the eukaryotic cells would take a much longer period for replication and hence contain multiple origins of replication.
- Rather than using two catalytic complexes of the same DNA polymerase to replicate the leading and lagging strands of a replication fork, eukaryotes use two different polymerases.