DNA REPLICATION

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DNA REPLICATION IS SEMI CONSERVATIVE

One of the most important properties of DNA is that it can make exact copy of itself. This process is called replication. The two strands of a DNA double helix are united by hydrogen bonds between the purine and pyramidine base pairs. When hydrogen bonds break the two strands separate and unwind. The free nucleotides present in the nucleus pairs with the nucleotides of the two separated strands by means of hydrogen bonds. In this way a new strand is formed around each old strand. The result of replication is the formation of two double helices each identical to the original double helix and this occurs during interphase.

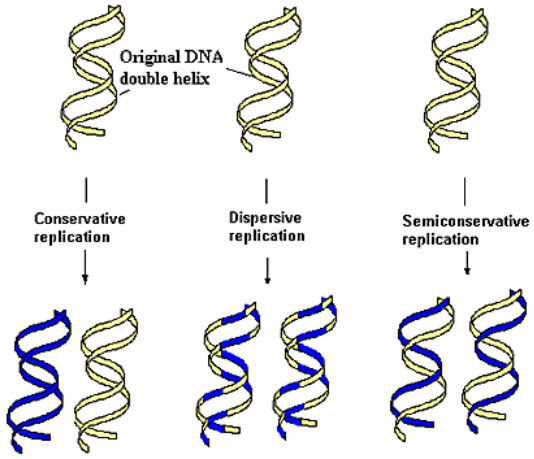
Delbruck suggested that the Watson and Crick model of DNA could theoretically replicate by three modes

1. Conservative
2. Semiconservative
3. Dispersive

According to conservative mode of the two double helix formed, one would be entirely of old material and the other entirely of new material. Thus the old parent double helix would be unchanged.

According to semi conservative mode proposed by Watson and crick, each strand of the double helices formed would have one old and one new strand.

According to dispersive method of replication the DNA double helix would break at several points forming many pieces. Each piece would replicate and then the pieces would reconnect at random. Thus the two double helix formed would have a patch work of old and new pieces.



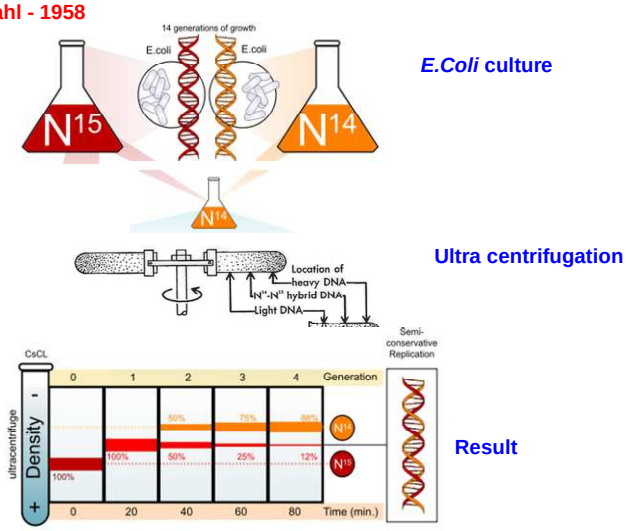
Possible Models of DNA Replication

Meselson and Stahl in 1958 conclusively demonstrated the semi conservative nature of replication. *E. coli* was grown in a medium containing a nitrogen (N) source with an atomic mass of 15 instead of the usual mass of 14. The *E. coli* was grown for several generations until all of the nitrogen in the DNA was N^{15} and the presence of the isotope of high atomic mass had made the DNA of the bacteria significantly denser than normal. The N^{15} medium then was replaced with usual medium containing N^{14} and the *E. coli* was grown for several generations. During the growth of the cells in the N^{14} medium, cells were harvested and their DNA extracted and centrifuged in cesium chloride/ sucrose gradient to provide a density gradient that separated DNA's of different densities.

1. After the first generation in the N^{14} medium all the DNA was the same hybrid density *i.e.* of medium density between N^{14} and N^{15} DNA.
2. After the second generation, 50% was hybrid density DNA and 50% was N^{14} DNA.
3. After the third generation, 25% was hybrid density DNA and 75% was N^{14} DNA.

If the DNA replicated conservatively one would expect to find two layers one of N^{14} and the other of N^{15} in the first generation and similarly for subsequent generations. With dispersive replication tubes of all generation would be expected to show a single layer $N^{14}N^{15}$, since the DNA would contain both new and old material mixed up. In semi conservative replication, the first generation would be expected to show a hybrid $N^{14}N^{15}$ layer. With each generation after the second the N^{14} layer would show a greater accumulation of material. Actual observations correspond to this expectation. This shows that replication of DNA is of semi conservative manner proposed by Watson and Crick, *i.e.* the double strands formed are identical to the parental strands.

Meselson and Stahl - 1958



E.Coli culture

Ultra centrifugation

Result

MAJOR ENZYMES INVOLVED IN DNA REPLICATION

1. **DNA POLYMERASE 1:** Arthur Kornberg discovered this enzyme in the extracts of *E. coli*. This enzyme is able to synthesize DNA from four precursors *i.e.*, dNTPs'- dATP, dGTP, dCTP, dTTP as long as a DNA molecule to be copied (template) is provided. Some years later it was found that polymerase I, though playing as essential role in the replication process is not the major polymerase in *E. coli*. It is believed to take part in the repair of DNA. Polymerase I is a single polypepetide chain with a molecular weight of 1, 09, 000. About 400 molecules of polymerase I are present in *E. coli*. The activities of polymerase I are
2. **5'→3' polymerization:** The synthesis of a new DNA chain (polymer) from nucleotides (monomers) is called polymerization. Polymerase I polymerizes nucleotides at the rate of about 1,000-1,200 molecules per min at 37^{o}C in *E. coli.*
3. **3'→5' Exonuclease activity:** Polymerase I catalyses the breakdown of one of the DNA strands into its nucleotides in the 3'→5' direction (opposite to polymerization direction). It functions as a proof reader and edits mismatched nucleotides at the primer terminus before proceeding with re-synthesis of the strand. Errors made during polymerization are corrected by polymerase I. It thus acts in repair synthesis. Since base pairing is checked twice the replication of DNA is accurate.
4. **5'→3' Exonuclease activity:** Polymerase I can also remove nucleotides in the 5'→3' direction. This 5'→3' exonuclease activity has an important role in the removal of thymine

dimmers. Since such dimer cannot fit into the double helix it blocks replication unless removed. This activity is also responsible for removing the RNA segment and then filling in the gap by deoxyribonucleotides. As polymerase I move ahead it cuts off ribonucleotides in front and adds deoxyribonucleotides behind.

5. **DNA POLYMERASE II:** It is a single polypeptide chain with molecular weight of 90,000. There are about 40 molecules present in *E. coli*. Polymerase II shows 5'→3' polymerization activity and also contains an associated nuclease digesting in the 3'→5' direction. About 50 nucleotides are polymerized per minute in *E. coli*. Not more than 50 nucleotides have been recorded till date. The 3'→5' exonuclease activity of polymerase II indicates that it may have an editing role in repair replication of UV induced DNA damage. Also polymerase II can elongate OKAZAKI fragments in the absence of polymerase I.
6. **DNA POLYMERASE III:** *E. coli* polymerase III is a very complex enzyme. In its most active form it is associated with nine other proteins. The smallest aggregate having the function of polymerase is called core enzyme. Polymerase III has 5'→3' polymerase activity and 3'→5' exonuclease activity. Polymerase III can polymerize about 15,000-60,000 nucleotides per minute at 37ºC.
7. **DNA LIGASE:** Seals OKAZAKI fragment in the lagging strand of DNA replication. *E. coli* DNA ligase can join a 3'OH to a 5' P as long as both groups are termini of adjacent base-paired deoxynucleotides. However, this enzyme cannot bridge the gap.
8. **PRIMASE:** Also known as dna G product. DNA polymerase cannot lay down the first nucleotide to initiate chain growth but requires a RNA primer. This RNA primer is synthesized by copying a particular base sequence from one DNA strand and differs from typical RNA molecule in that after its synthesis , the primer remain hydrogen bonded to the DNA template.
9. **SLIDING CLAMPS**: Allows DNA polymerase to remain attached to their DNA stretches of DNA efficiently without falling off the template DNA.
10. **Dna A-** It is a origin binding protein, binds to origin of replication just before initiation of replication.
11. **Dna B-** Also known as helicase. Helicase unwinds DNA strands using ATP energy and moves processivelly (*i.e.*, does not leave the DNA until replication is finished). It encircles the DNA strand in the 5'—3' direction along DNA.
12. **Dna C-** It forms a complex with dna B (helicase) to load and function on DNA template.

13. **SINGLE STRAND BINDING PROTEIN**: It does not itself unwind the DNA but binds to and stabilizes unwound single stranded DNA.
14. **DNA Gyrase:** Also known as **TOPOISOMERASE II**. It relieves torsional stress during replication *i.e.*, double stranded DNA is unwound by dna B/ helicase so that each strand can be replicated into new daughter double stranded molecules. However, the unwinding activity of helicases at one position along a DNA molecule causes the downstream portion of the same DNA molecule to become over wound. This over winding is relieved by an enzyme called Gyrase. Gyrase uses ATP energy to introduce negative super coiling into the DNA to ovoid over winding.

MECHANISM OF REPLICATION IN PROKARYOTES

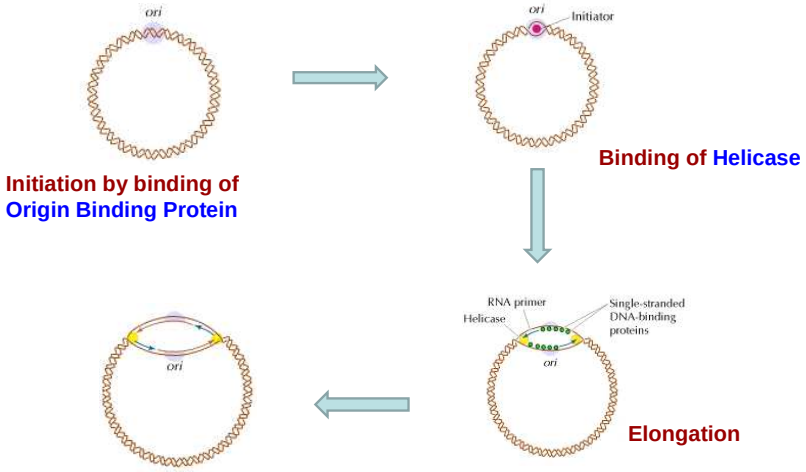
E. coli is taken as a representative example for study of prokaryotic DNA replication.

The replication is initiated at a specific point on DNA template which has a specific sequence known as origin. Origin binding protein (dna A) binds to this point which is recognized by the dna C which in turn brings dna B (Helicase) to this site. Initially it binds to single strand opposite to the origin binding protein but later occupies the center of the double strand in order to break the bonds between the two anti parallel polynucleotides and also relaxing the helical nature of DNA. The dna B protein is also known as helicase. It opens up the double stranded DNA into single strand in an action of Zip. Thus forming a 'Y' shaped structure wherein the junction of two single stranded DNA strand is known as replication fork. Single strand binding proteins bind to the single stranded DNA to avoid re-annealing and avoiding degradation. As the replication fork moves down the DNA double helix, the parental strand wind imposing a torsional strain which is relaxed by topoisomerase also known as DNA gyrase. DNA gyrase works by nicking and closing the DNA strand under stress.

Initiation of DNA replication requires an RNA primer. Primer RNA is a short stretch of polynucleotide (60 bases) with 5' PO4 and 3'OH end. The primer is synthesized next to origin of replication by enzyme primase. Replication is always in 5'→3' direction. In one strand the synthesis starts on 3' end strand and in opposite strand the direction starts in opposite direction to the first template but always in 5'—3' direction. DNA polymerase III synthesizes DNA from 3'OH of RNA primer. The new strand in which the replication takes place uninterruptedly is called leading strand. The new strand in which there is disrupted or discontinuous synthesis is called lagging strand. The new strands of DNA synthesized on lagging strand are called OKAZAKI fragments. The primer is removed by the exonuclease action of 5'-3' action of DNA polymerase I. Also wherever proofreading

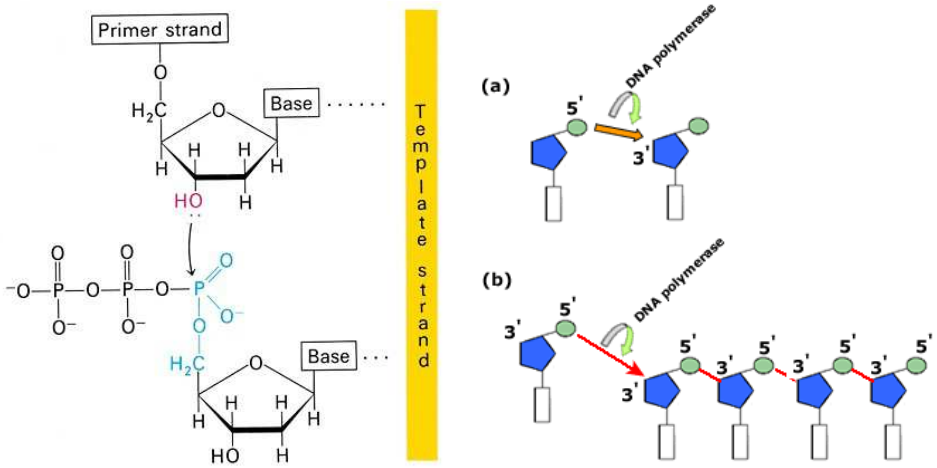
is required the polymerases function and a true copy of parent is formed at the end of replication. When two adjacent nucleotide meet with 3'OH and 5'PO4 DNA ligase ligates the adjoining nucleotide. Topoisomerase relaxes the torsional stress during the entire period of replication.

Mechanism of Replication in Prokayrotes



In a unidirectional replicating molecule, replication terminates at the origin. In a bidirectionally replicating molecule, there are two possible modes of termination. 1. There is a defined termination sequence or 2. Two growing points collide and termination occurs wherever the collision point happens to be. In both cases, termination might occur exactly halfway around at the circle (at the antipode of the origin). Both termination modes have been observed.

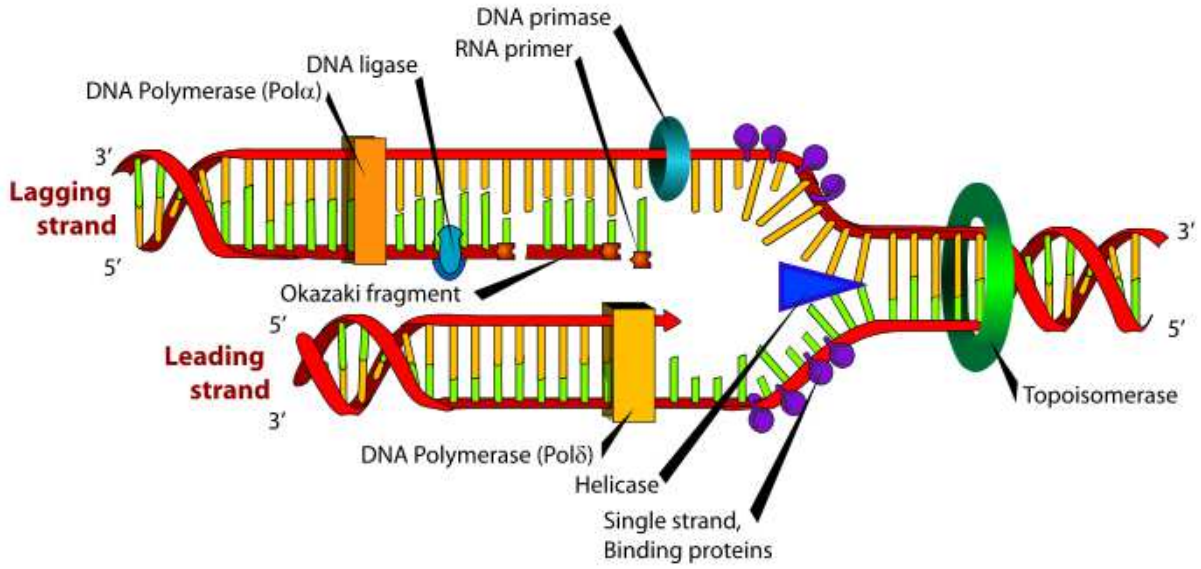
Mechanism of polymerization



3’ ← ← ← ← ← ← ← ← ← ← ← 5’

Chain Growth from 5’ to 3’

Replication Fork



REPLICOSOME: The unit consisting of enzymes, template strand and newly synthesized daughter strand at the replication fork is called replicosome / replisome.

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