DNA METABOLISM

The DNA metabolism includes two processes namely DNA replication and DNA repair. The DNA replication refers to *enzymatic biosynthesis of true copy of a DNA molecule.* The DNA replication is semi-conservative in nature that is *each DNA strand serves as template for the synthesis of a new strand producing two new daughter molecules of DNA each with one new strand and one old strand.* The semi-conservative nature of DNA replication was demonstrated by Meselson-Stahl Experiment (1957) as described below:

Meselson and Stahl grew *E. coli* cells for many generations in a medium in which the sole nitrogen source (NH_4Cl) contained 15N, the "heavy" isotope of nitrogen, instead of the normal, more abundant "light" isotope, ^{14}N. The DNA isolated from these cells had a density about 1% greater than that of normal [^{14}N]DNA (Fig. 1.a). Although this is only a small difference, a mixture of heavy [^{15}N]DNA and light [^{14}N]DNA can be separated by centrifugation to equilibrium in a cesium chloride density gradient. The E. coli cells grown in the ^{15}N medium were transferred to a fresh medium containing only the 14N isotope, where they were allowed to grow until the cell population had just doubled. The DNA isolated from these first-generation cells formed a single band in the CsCl gradient at a position indicating that the doublehelical DNA molecules of the daughter cells were hybrids containing one new ^{14}N strand and one parent ^{15}N strand (Fig. 1.b).

This result argued against conservative replication, an alternative hypothesis in which one progeny DNA molecule would consist of two newly synthesized DNA strands and the other would contain the two parent strands; this would not yield hybrid DNA molecules in the Meselson-Stahl experiment. The semiconservative replication hypothesis was further supported in the next step of the experiment (Fig. 1.c). Cells were again allowed to double in number in the ^{14}N medium. The isolated DNA product of this second cycle of replication exhibited two bands in the density gradient, one with a density equal to that of light DNA and the other with the density of the hybrid DNA observed after the first cell doubling.

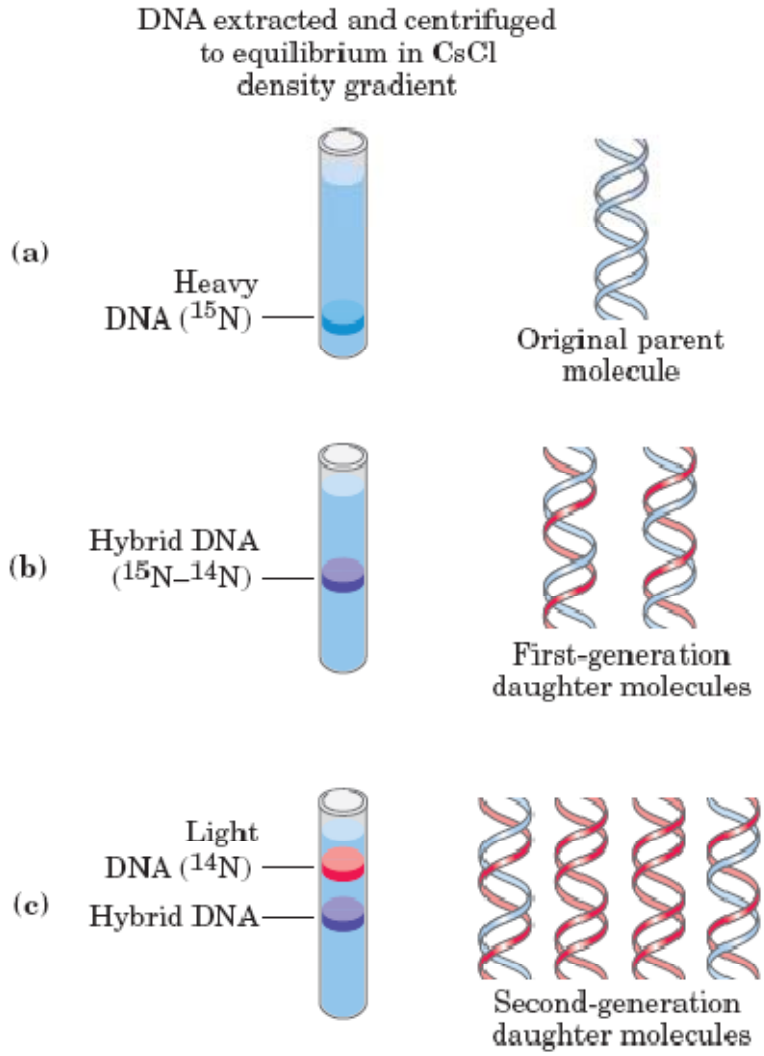


Fig.1. Schematic diagram of Meselson-Stahl Experiment

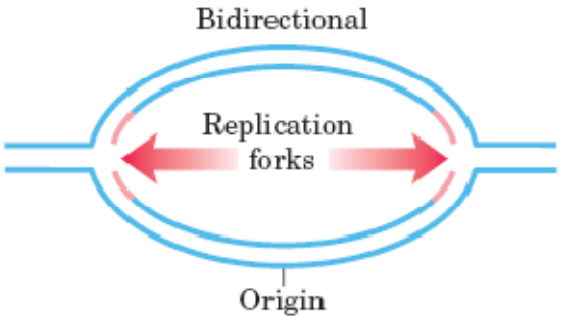
The general reaction of DNA replication is

$$\underset{\text{DNA}}{(\text{dNMP})_n} + \text{dNTP} \longrightarrow \underset{\text{Lengthened DNA}}{(\text{dNMP})_{n+1}} + \text{PP}_\text{i}$$

The basic rules of DNA replication:

1. The nucleotide monomers are added one by one to the end of a growing strand by an enzyme called DNA polymerase.
2. Sequence of nucleotide in new strand is complementary to the nucleotide sequence of parental strand (Old Strand).
3. The old strand or the parental strand acts as template strand for the synthesis of new strand.
4. The synthesis of new strand proceeds from 5`→3` direction. That is every new incoming deoxyribonucleotide is added at 3` position of the growing new strand.
5. The site of DNA in which the synthesis of new strand begins is called origin of replication. The bacterial DNA has single origin of replication while the mammalian DNA has multiple origin of replication.

6. The DNA replication is *SEMI-DISCONTINOUS*. As the DNA replication always proceeds in 5`→3` direction and both the strands of DNA are antiparallel. The strand synthesis that takes place in the direction of movement of DNA polymerase is called continuous strand or leading strand. While the other strand that is oriented in reverse direction of movement of enzyme is called lagging or discontinuous strand.
7. DNA replication proceeds bidirectionally.



REPLICATION IN PROKARYOTES

Enzymes and proteins required for initiation of DNA replication

1. dnaA protein: Recognizes the origin of replication.
2. Helicase (dnaB): this enzyme unwinds DNA at the origin of replication.
3. Primase: this enzyme is a kind of RNA polymerase that synthesizes the RNA primer.
4. DNA gyrase (DNA topoisomerase II): this enzyme relieves the torsional stress generated by DNA unwinding.
5. DNA polymerase III: this enzymes carries out the polymerization of new strand over the parental template strand during the DNA replication.

PROPERTIES OF DNA POLYMERASE

1. They require template for polymerization of new strand. The enzyme always move from 5`→3`direction of new strand.
2. They always require a primer. They cannot initiate the reaction of polymerization. But can add nucleotide to a pre-existing short strand which is called primer. The primer is an ologonucleotide of RNA rather than DNA synthesized by an enzyme called primase which is a type of RNA polymerase.
3. Proof reading: the DNA polymerases have the capability of correcting error of adding wrong nucleotide during replication. This activity is reverse of polymerization. It is refered to as 3`→5` exonuclease activity.
4. 5`→3`exonuclease activity: this activity is present only in DNA polymerase I. Through this activity, the DNA polymerase I removes the incorrect nucleotide at the end of replication. It simply slides on the new strand and checks for the wrong nucleotides and when found removes it and put correct ones. It removes the RNA primers.
5. Polymerization rate (nucleotides per second): the Polymerization rate of DNA polymerase III is 1000 nucleotides per second and of DNA polymerase I is 20 nucleotides per second.
6. Processivity: it refers to number of nucleotides added by the DNA polymerase before it gets dissociated from the DNA strand. The Processivity of DNA polymerase III is 500,000 nucleotides and of DNA polymerase I is 200.

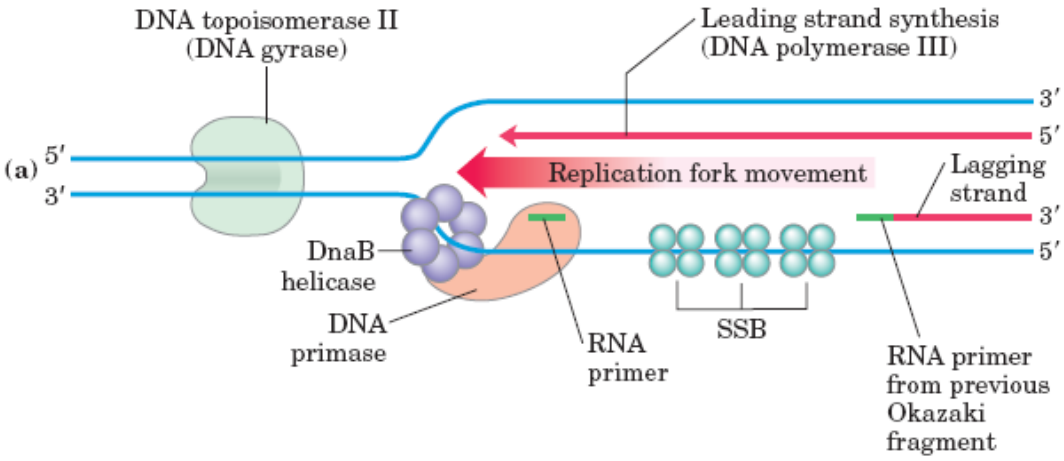
There are 3 phases of DNA replication:

1. Initiation
2. Elongation
3. Termination

INITIATION OF PROKARYOTIC DNA REPLICATION:

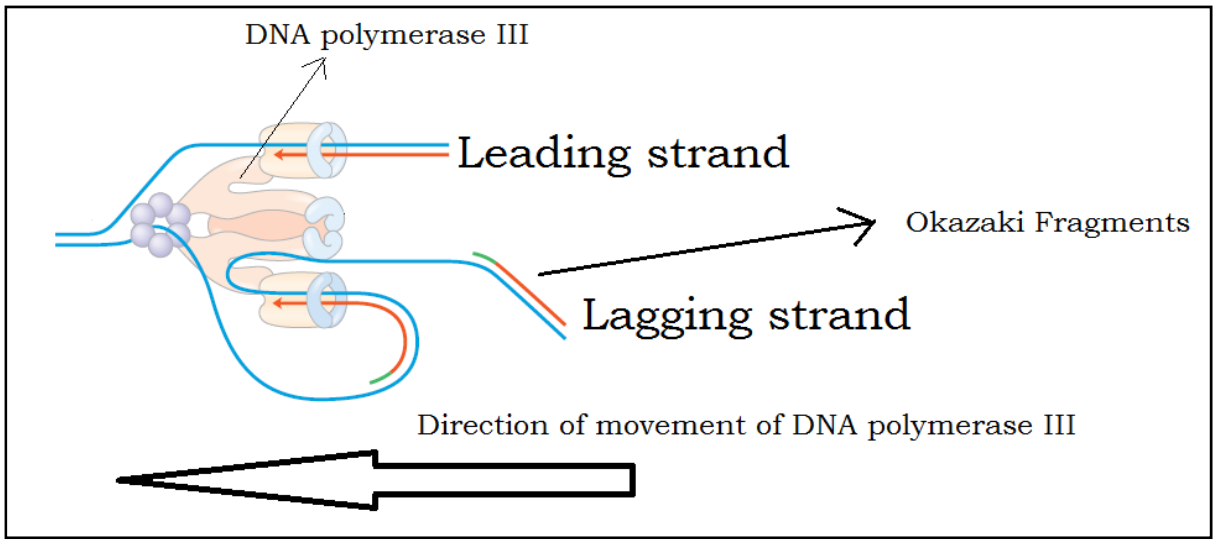
It occurs in following steps:

1. dnaA protein binds the origin of replication.
2. The enzyme DNA helicase (dnaB protein) opens the double helix by separating the two strands.
3. Single strand binding protein (ssb proteins) binds the separated strands (they prevent the rewinding of the strands).
4. DNA gyrase (topoisomerase II) binds the DNA ahead of replication fork to relieve the torsional stress owing to the opening of DNA double helix by the enzyme helicase.
5. The enzyme primase (dnaG protein) synthesizes the short oligonucleotide RNA primer on the parental old strand (Template strand).
6. The DNA polymerase III extends the primer at 3` end to form the new strand by adding deoxy-ribonucleotides.

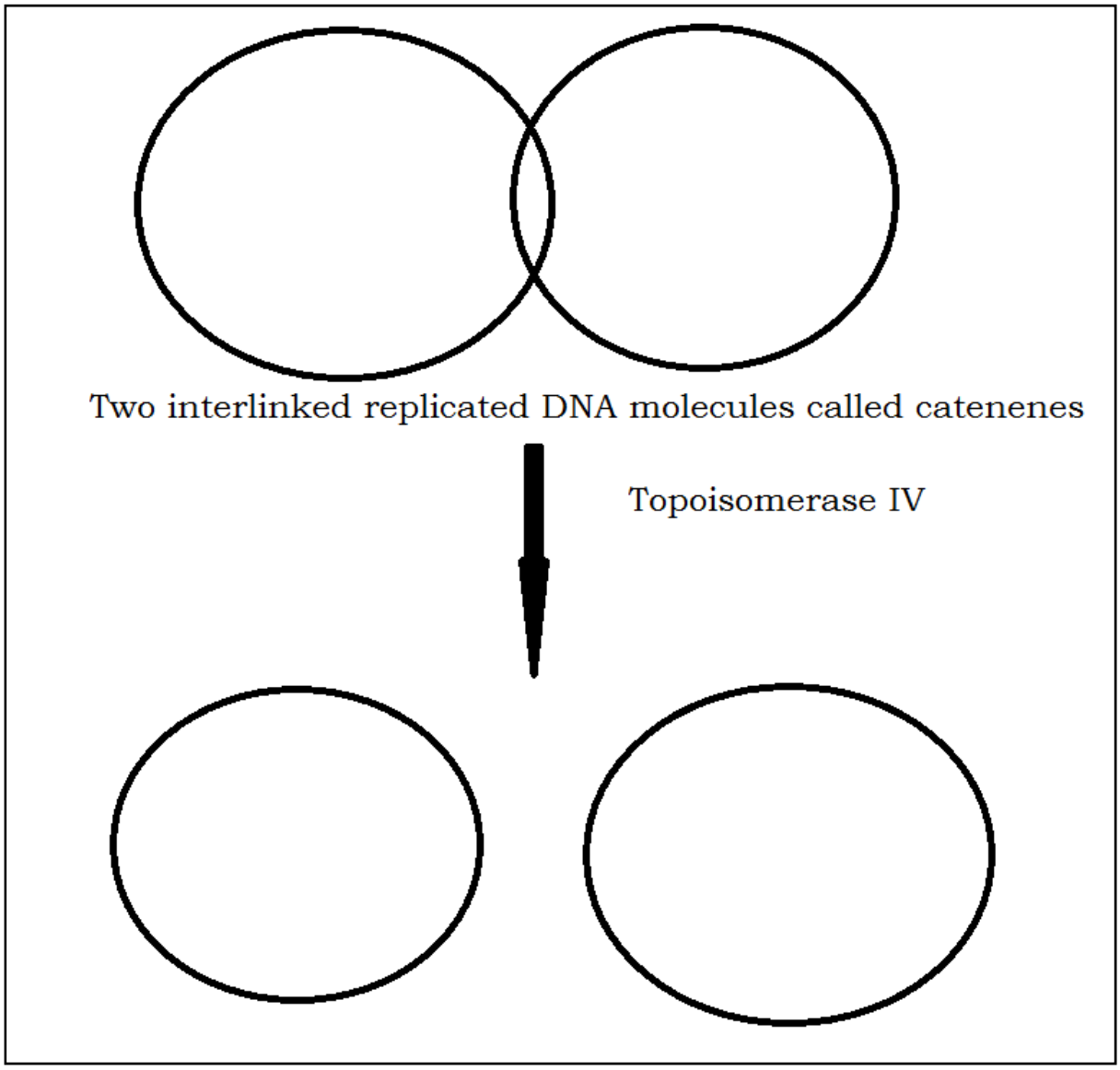


ELONGATINON:

This phase includes two distinct processes namely leading strand synthesis and lagging strand synthesis. The leading strand synthesis occurs towards the direction of movement of enzyme or towards, the direction of replication fork. It begins with synthesis of 10-60 nucleotides RNA primer by enzyme primase. But in case of lagging strand synthesis, the direction of synthesis is opposite to the direction of movement of enzyme. So, in order to turn both the directions (direction of movement of enzyme and direction of lagging strand synthesis) in the same direction, the lagging strand is turned to opposite direction towards the direction of movement of enzyme by looping the lagging strand. After the synthesis of lagging strand within the loop, the loop get straightened and again a new loop is formed for lagging strand synthesis. The primer is synthesized every-time with the formation of new loop. Because of this the lagging strand is also called as discontinuous strand. While in case of leading strand, the primer is synthesized only once hence the strand is continuous (hence called continuous). *The discontinuous fragments of DNA of lagging strand are referred to as Okazaki fragments* (Reiji Okazaki, 1960s).



TERMINATION: The replication fork when enters into a region of DNA called *ter* sequence it gets arrested. This region of DNA is bound by another protein called Tus protein. This protein aids in arresting the replication fork. As in case of majority of prokaryotes the DNA is circular, the replicated DNA remains as interlinked DNA molecules called *catenanes*. The interlinking of replicated DNA molecules is broken by the enzyme topoisomerase IV.


Two interlinked replicated DNA molecules called catenenes
Topoisomerase IV