REPLICATION OF DNA

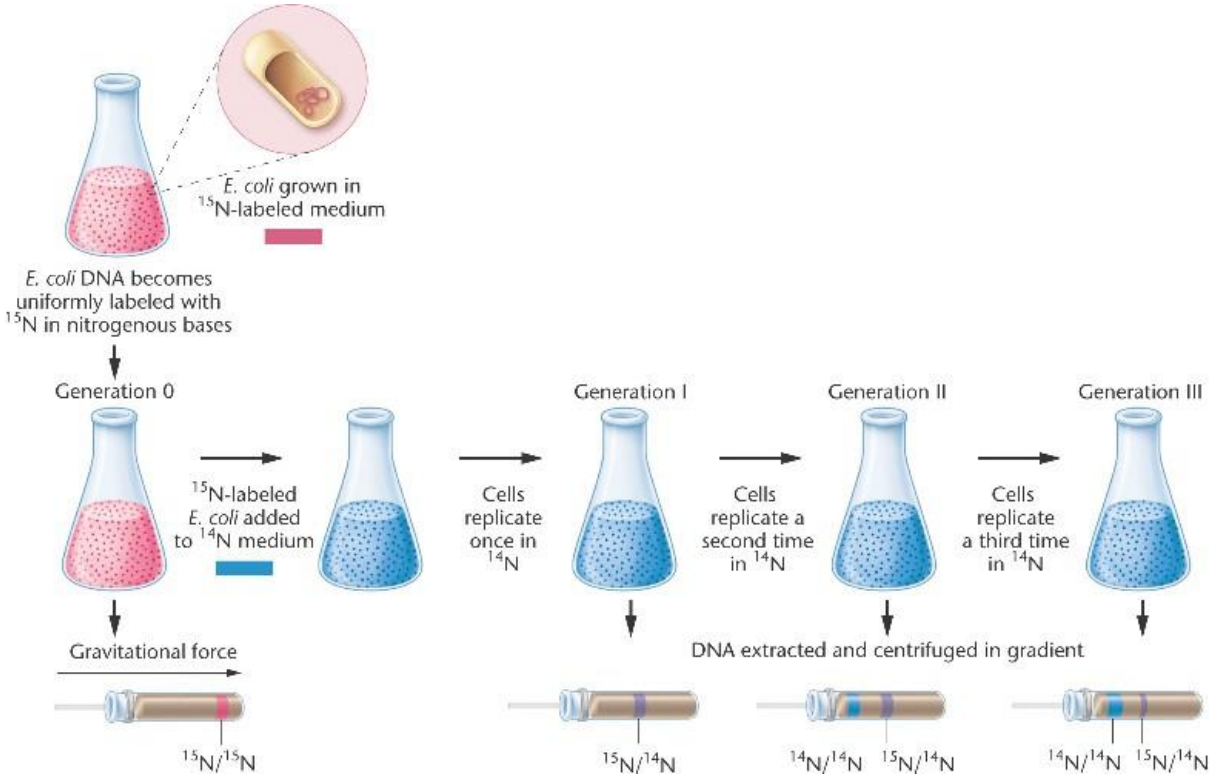
Replication is the process of synthesis of daughter nucleic acid molecules identical to the parental nucleic acid (Principles of Biochemistry: 4th edition).

BASIC RULES OF DNA REPLICATION

DNA replication is semi conservative

Each DNA strand serves as a template for a synthesis of a new strand. As a result, two new DNA molecules are formed. Each DNA molecule has one new strand and one old strand and hence, the mode of replication is called Semi-conservative in nature.

Hypothesis of semi-conservative replication was first proposed by Watson and Crick and this hypothesis was proved by Mathew Meselson and Franklin Stahl.



The Meselson and Stahl Experiment The Expected results of two generations of semi-conservative replication in the Meselson-Stahl Experiment

Replication begins at an Origin and typically continues bidirectionally

Experiments by John Cairns with the help of autoradiography using DNA of E. coli indicate that replication is a highly co-ordinated process in which the parent strands are simultaneously unwound and replicated.

Denaturation mapping was used by Ross Inman and colleagues for determination of origin of replication. This technique revealed that the replication loops always initiate at a unique point called Origin. It also confirmed the earlier observation made by Cairns that replication is usually bidirectional.

For circular DNA molecules, the two replication forks meet at a point on the side of the circle opposite to the origin. Specific Origins of replication have since been identified and characterized in bacteria and other lower eukaryotes.

DNA synthesis progress in a 5' to 3' direction and is semi discontinuous

3'OH is the point of elongation of DNA molecules; hence, synthesis of new strand is always from 5' to 3' direction. Reiji Okazaki et.al. discovered that one of the daughter strands is synthesized in short sections. These short segments are termed Okazaki fragments. It was concluded from his work that one daughter strand is synthesized continuously while the other one in discontinuous fashion. The strand in which synthesis occurs continuously in the direction of movement of replication fork is called Leading strand. The strand in which synthesis of new strand is discontinuous in the opposite direction of movement of replication fork is called lagging strand.

Enzymes required for DNA replication

DNA Helicases

DNA Helicases help in separation of two strands of parental DNA. These enzymes bind to the single stranded DNA and move along the single stranded DNA. These enzymes thus help in unwinding double stranded DNA.

Single Stranded Binding Proteins

Single stranded DNA Binding Proteins bind to the separated single stranded DNA. It helps in stabilization of single stranded DNA after their separation from its complementary strand. Hence, the strand is in elongated state for being used as template for synthesis of daughter strand. Topoisomerases

It removes super coiling of double stranded DNA as it unwound at the replication fork.

Primase

It is a special type of RNA polymerase that makes 5 to 10 nucleotide long primers on single stranded DNA template.

DNA polymerases

DNA polymerases catalyze the synthesis of DNA strand. There are different types of polymerase having diverse function ranging from catalyzing biosynthesis of new DNA molecules to degradation of nucleic acid residues.

RNAse H

This enzyme removes RNA primers. RNAse H removes all the ribonucleotides except the one that is bound to the deoxyribonucleotide molecule directly.

DNA ligase

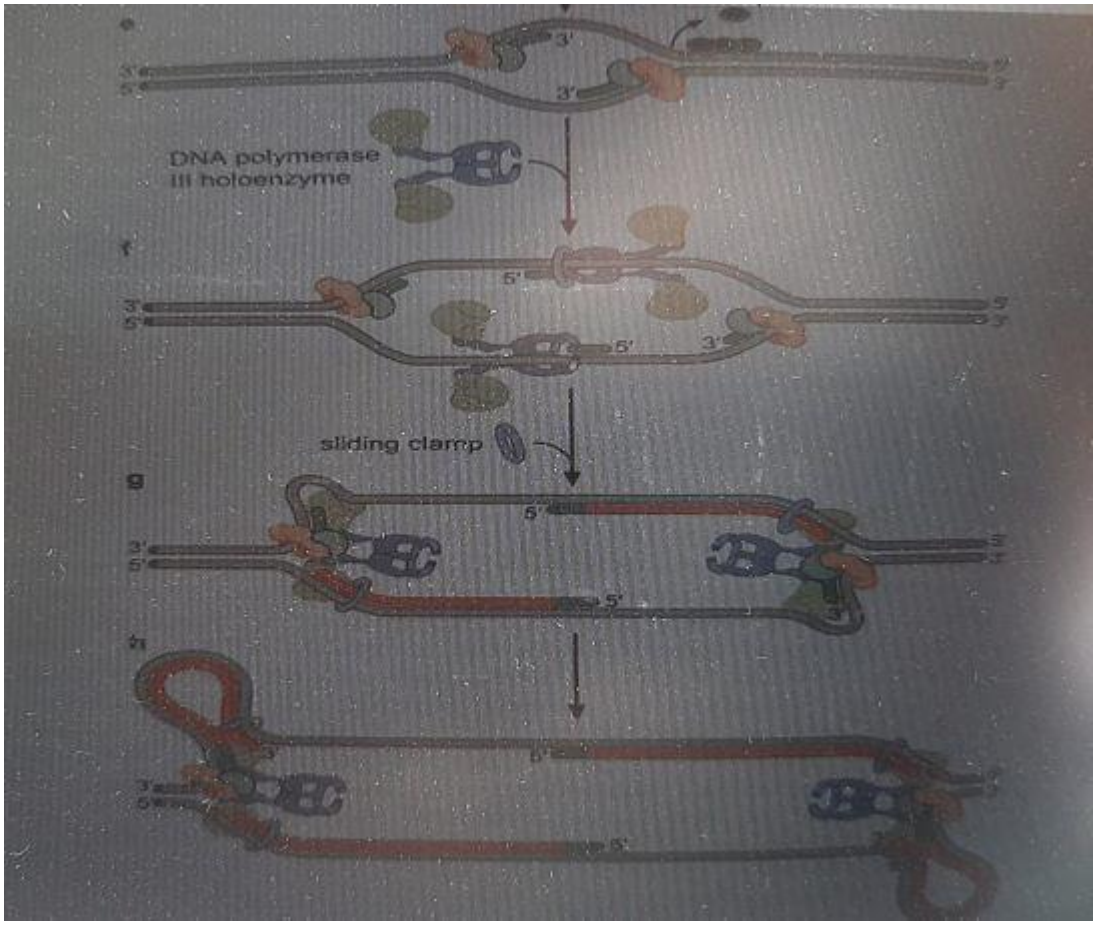
It repairs the "nick" present in DNA strand. ("Nick" is a break or absence of any bond in the polynucleotide backbone between the 3'OH and 5'phosphate)

Replication in prokaryotes (E. coli)

INITIATION

Replication occurs from Replication origin. In E.Coli replication origin is called “ori C” which is 245 base pairs long sequence. Different proteins that are involved in initiation of replication is given in tabular form (From Principles of Biochemistry; 4th edition)

Protein	Number of Subunits	Function
DnaA protein	1	Recognizes “oriC sequence; opens duplex at specific sites in origin
DnaB protein (Helicase)	6 (Identical subunits)	Unwinds DNA
DnaC protein	1	Required for DnaB binding at origin
HU	2	Histone-like protein; Dna binding protein; stimulates initiation
DnaG protein (Primase)	1	Synthesizes RNA primers
Single stranded Binding protein (SSB)	4 (Identical subunits)	Binds single stranded DNA
RNA polymerase	5	Facilitates DnaA activity
DNA topoisomerase II (DNA gyrase)	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	1	Methylates (5’) GATC sequence at “oriC”



Description of above given Figure (Figure is from Molecular Biology of the Gene; 5th edition)

a. DnaA-ATP complex binds at the 9 mer repeat sequence present within the "oriC" region.

b. This binding facilitates strand separation within 13 mer repeat sequence.

c. DnaB and DnaC associate with the origin bounded by DnaA

d. DnaC catalyzes the opening of DnaB protein ring and its placement around single stranded DNA at the origin. DnaC dissociates itself from DnaB and activate DnaB.

e. Each DnaB recruit a primase to synthesize RNA primer on each template. Movement of DnaB also removes bounded DnaA.

ELONGATION OF DNA REPLICATION

Elongation occurs both at the leading (3' end at the replication fork) and lagging (5' end at the replication fork) strand simultaneously though the process of replication at these strands differ slightly.

Deoxyribonucleotides are added to the primer located at the replication origin with the help of DNA polymerase III.

In the leading strand, addition of deoxyribonucleotides proceeds continuously, keeping pace with the unwinding of DNA at the replication fork.

In the lagging strand, addition of deoxyribonucleotides is discontinuous. Small daughter strands are formed which are known as Okazaki fragments.

Both leading and lagging strands are formed by the single DNA polymerase III dimer holoenzyme. Enzyme complex responsible for synthesis of daughter DNA strand at the replication fork is called Replisome

Synthesis at lagging strand occurs by looping the DNA. Synthesis of Okazaki fragments is supported by Primosome (Association of DnaB and DnaG)

At the lagging strand, DnaB unwinds the DNA at the replication fork. DnaG sporadically associate with DnaB to synthesize RNA primer. Sliding clamp of DNA polymerase III holoenzyme assemble on RNA primer for synthesis of Okazaki fragment in 5'to 3' direction.

Replication arrests after synthesis of Okazaki fragment and sliding clamp dissociate itself from DNA polymerase III core. This process is repeated for the synthesis of next Okazaki fragment. In this way, complementary daughter DNA strand is formed for lagging strand. DNA polymerase I excise the primers of both leading and lagging strand and fill the gap by adding deoxyribonucleotides.

DNA ligase finally joins the nicks.

Table showing proteins at the replication fork (From Principles of Biochemistry, 4th edition)

Protein	Number of subunits	Function
SSB	4	Binds to single stranded DNA
DnaB protein (Helicase)	6	DNA unwinding, primosome constituent
DnaG protein (Primase)	1	RNA primer synthesis, primosome constituent
DNA polymerase III	17	New strand elongation
DNA polymerase I	1	Fills the gap, excision of primers
DNA ligase	1	Ligation
DNA topoisomerase II (DNA gyrase)	4	Supercoiling removal

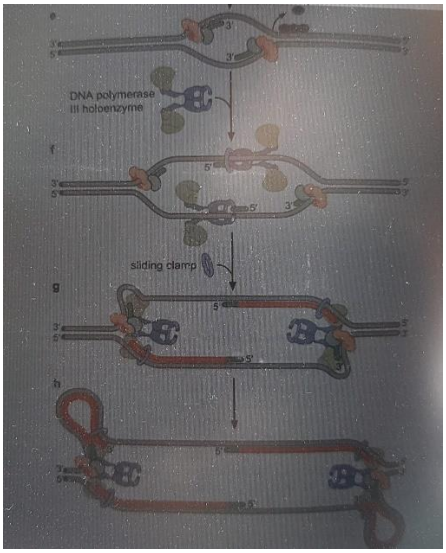


Figure showing elongation of DNA replication (From Molecular Biology of the Gene; 5th edition

TERMINATION OF DNA REPLICATION

Ter region present in the parental DNA of E.coli block the movement of replication fork.

Ter sequence binds with Tus (Terminus utilization substance) forming Tus-Ter complex.

When any of the two replication fork encounters the Tus-Ter complex first replication comes to an end.

Single Tus-Ter complex act per replication cycle.

In this way, two topologically interlocked circular chromosomes are formed and known as catenanes.

Topoisomerase IV separates the catenanes.

Thus, two circular chromosomes are synthesized during S phase of cell division.

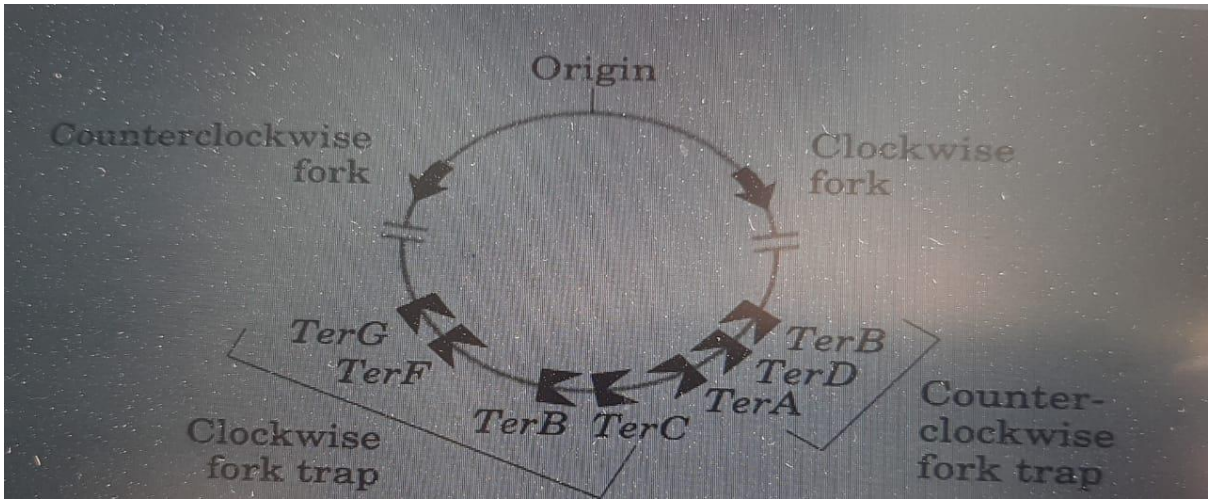


Figure showing The Ter sequence position on the chromosome of E.coli in two clusters with opposite orientations (Figure from Principles of Biochemistry, 4th edition)

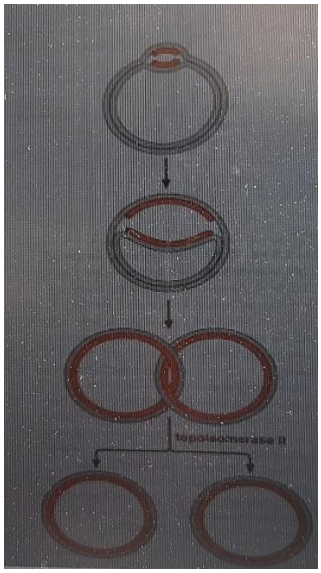


Figure showing Topoisomerase II action for decatenation of replicated circular DNA in E. coli