2.4 PROCESS OF DNA REPLICATION IN PROKARYOTES (6)

The process of DNA replication is very well studied in prokaryotes because of the smaller size of genome. Bacteria *E. coli* has 4.6 million base pairs in a circular double-stranded chromosome and it takes only about 42 minutes to replicate. This replication begins from a single origin of replication and proceeds along the circle in both the directions. The rate of fork movement is about 1000bp per second per fork. The process is quite rapid and occurs without many mistakes.

The DNA replication produces two indentical copies of daughter DNA molecules using the parental DNA strand as template strand. In this chapter we earlier discussed that each molecule has one strand from parent DNA and the other strand is daughter strand. This mode of replication is called as **'semi-conservative'** mode of replication.

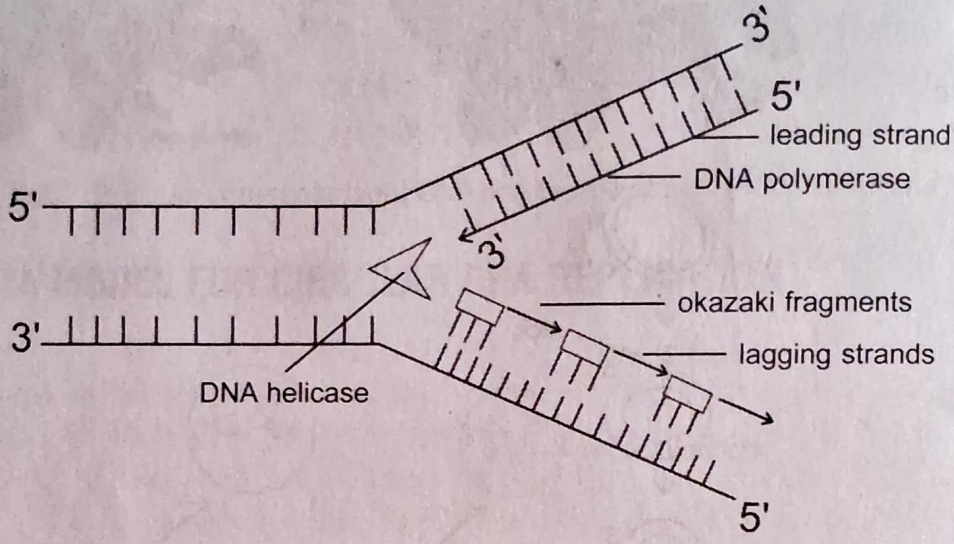

Fig. 2.8. *DNA replication in prokaryotes.*

The DNA replication in prokaryotes are divided into three stages: initiation, elongation and termination.

(i) **Initiation:** The initiation of replication starts at ORI C (origin of replication) and has 24 bp DNA. It contain 9 bp repeats called 9 mer and contains these 13 bp **repeates**. The 13 bp sequences are AT-rich sequences. The 13 mer region is called as DUE region. DNA unwinding region while 9 mer regions are called as DAR i.e. Dna Assembly Region. These two sites are the binding sites for Dna A protein that initiates replication. Another AT-rich sequence is present adjacent to ori C site that facilitates local melting of the duplex exposing single strands to DNA replication machinery.

Dna A protein which is either ATP or ADP bound protein. It binds to the four 9 mers ori-C site forming an "initial complex" that contains 10-20 protein subunits. The binding of Dna A protein to ori C facilitates the denaturation of AT-rich sequences present at three 13 mer region. This process of melting requires an energy which is supplied by ATP molecules associated with Dna A protein and ultimately yields an "open-complex". One

open–complex is formed creating single stranded strand then single stranded binding protein (ssb) binds to each strand which prevents reannealing of both the strands.

There are H-bonds between the nitrogenous bases of the strand. In order to separate DNA molecule completely the H-bonds between the bases has to be broken. These H-bonds can be broken by Dna B protein which is a helicase enzyme and a hexamer of identical sub units. Dna B protein is associated with Dna A protein which is a helicase loader and acts as a clamp around each of the two single strands in the open complex. This dna C protein escorts the Dna B to the Dna A proteins, yielding the pre- priming complex. Once the complex is formed the Dna B protein breaks the H-bonds between the bases and two complete separated DNA strands in is formed in the form of replication bubble.

After this, Dna G protein which is a primase is required to prime the reaction. Primase is a special RNA polymerase that is the product of the Dna G gene. It is a 60 KDa single polypeptide which synthesize short stretches of RNA that are used as primers for DNA synthesis. The primase protein binds to the respective strand and lays down the primer in the form of small piece of RNA stretch of about 11-12 bases.

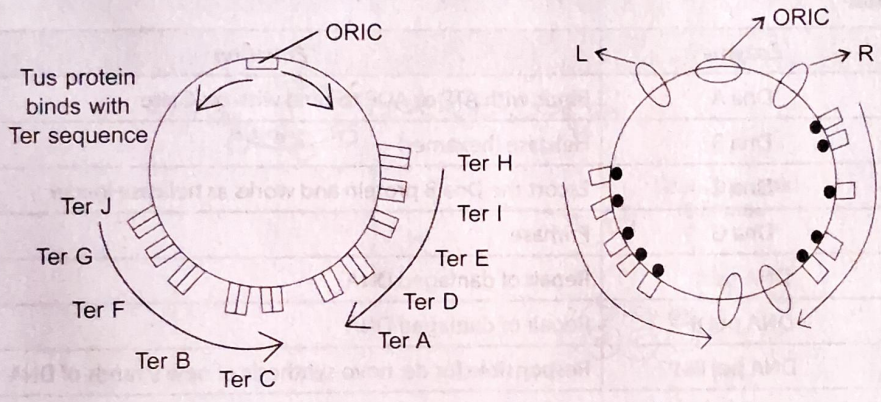

Fig. 2.9. *DNA replication in prokaryotes-process of termination.*

- The role of DNA polymerase is to synthesize a new DNA on a template DNA strand. DNA polymerase is of three types. DNA pol I, DNA pol II, DNA pol III. DNA pol I performs repair of damaged DNA and fill the gaps between okazaki fragments. DNA pol II also plays a role in the repair of damaged DNA while DNA pol III is responsible for denovo synthesis of new strands of DNA. The structure of DNA pol III has complex and consists of a 'palm' which is the site of polymerase function DNA polymerase III is the main enzyme is replication of prokaryotes which is complex and made up of 10 different polypeptides.

The core polymerase consists of three sub units:

α-active site for nucleotide adotition

ε – 3′ to 5′ exonuclease to remove incorrectly added nucleotide

θ-function is not known

(*ii*) **Elongation:** After the addition of nucleotides by DNA pol III new strand is formed on both the template strand. During the elongation process another molecular binds to the DNA molecule called as DNA gyrase. It is a topoisomerase enzyme. It relieves stress caused by supercoiling. The DNA pol III adds nucleotides on 3′ OH end only and the synthesize of new strand always occurs in 5′ to 3′ direction. The strand which synthesize

continuously is known as **leading strand**. The other strand where discontinuous synthe occurs is called as **lagging strand**. On the lagging strands the DNA are synthesized short stretch of DNA is called as okazaki fragments. The leading strand can be elonga by a single primer only whereas the lagging strand needs a new primer for each of short okazaki fragments.

(iii) **Termination:** RNA primers are removed by primase enzyme and gaps are repaired DNA pol I. The gaps between the DNA fragments are sealed by DNA ligase. At the e the duplicate chromosomes from one another are formed. The replication in circul chromosomes initiates at a origin called as Ori C site. The formation of replication fo occurs and both forks move in the opposite direction until reached to termination or 'Te sites. There are 10 different Ter sequences starting from Ter J to Ter C in one direction a from Ter H to Ter A in other direction. Five ter sequences are Ter J, Ter G, Ter F, Ter B a Ter C present on one direction whereas on the right side other five Ter sequences are T H, Ter I, Ter E, Ter D, and Ter A.

TABLE 2.2 *Enzymes involved in DNA replication in prokaryotes.*

S. No.	Enzyme	Function
1	Dna A	Binds with ATP or ADP to bind with ori C site
2	Dna B	Helicase (hexamer)
3	Dna C	Escort the Dna B protein and works as helicase loader
4	Dna G	Primase
5	DNA pol I	Repair of damaged DNA
6	DNA pol II	Repair of damaged DNA
7	DNA pol III	Responsible for de novo synthesis of new strands of DNA
8	SSB	Binds to SS DNA
9	Ligase	Seals the gap

Five Ter sequences which are present on the left side have permissive faces outside to th left and five Ter sequences on the right have permissive faces facing to the right. Thes 'Ter' sites sequences are recognized and bound by termination utilization substance Tu protein monomers. The Ter-tus complex is asymmetrical and has one face that blocks th progression of replisome which is called as 'non-permissive' face and a second face tha allows replication to continue is known as 'permissive' face.

There are two replication fork on the left and on the right because this is a bidirectiona DNA replication. The left fork continues replication and passes through the 5 Ter – Tu complex. and is stopped only by Ter A sequence because the fork at Ter A sequence face the non-permissive face of Ter A sequence, in the same way right fork which is denoted a R fork continues replication and pass through 5 Ter-Tus complex and is stopped only by Te C sequence because the fork faces the non- permissive face. As the two protein dislodges the replisome replicates the two strands creating the two complete daughter copies.

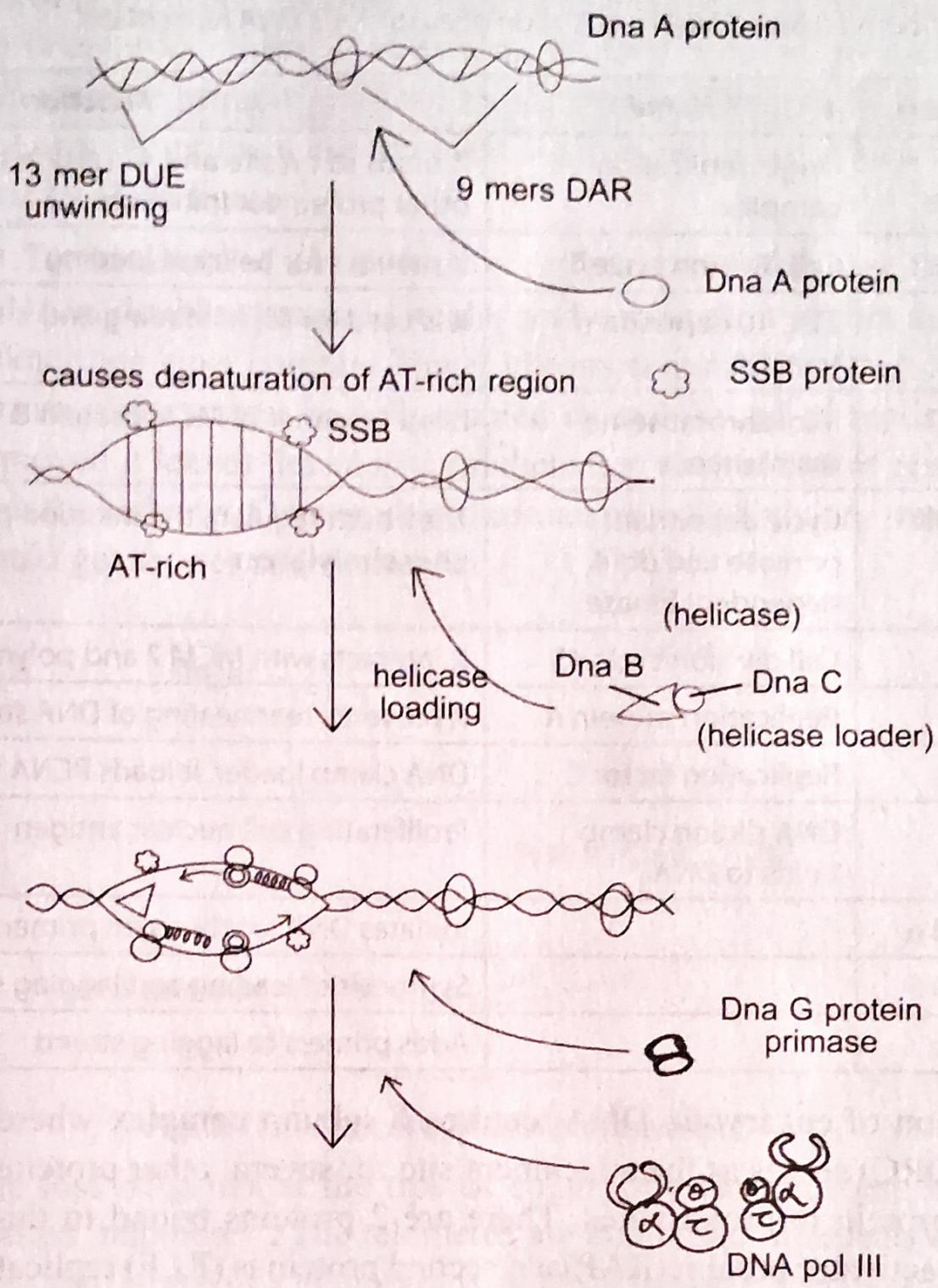

Fig. 2.10. *DNA replication initiation in prokaryotes.*