PROKARYOTIC REPLICATION

-Ms. Sanchari Sarkar
Department of Microbiology
Shivaji Science College, Nagpur

➢ The Replication Fork :

▪ Both Strands of DNA Are Synthesized Together at the Replication Fork

• In the cell, both strands of the DNA duplex are replicated at the same time.
• This requires separation of the two strands of the double helix to create two template DNAs.
• The junction between the newly separated template strands and the unreplicated duplex DNA is known as the replication fork.

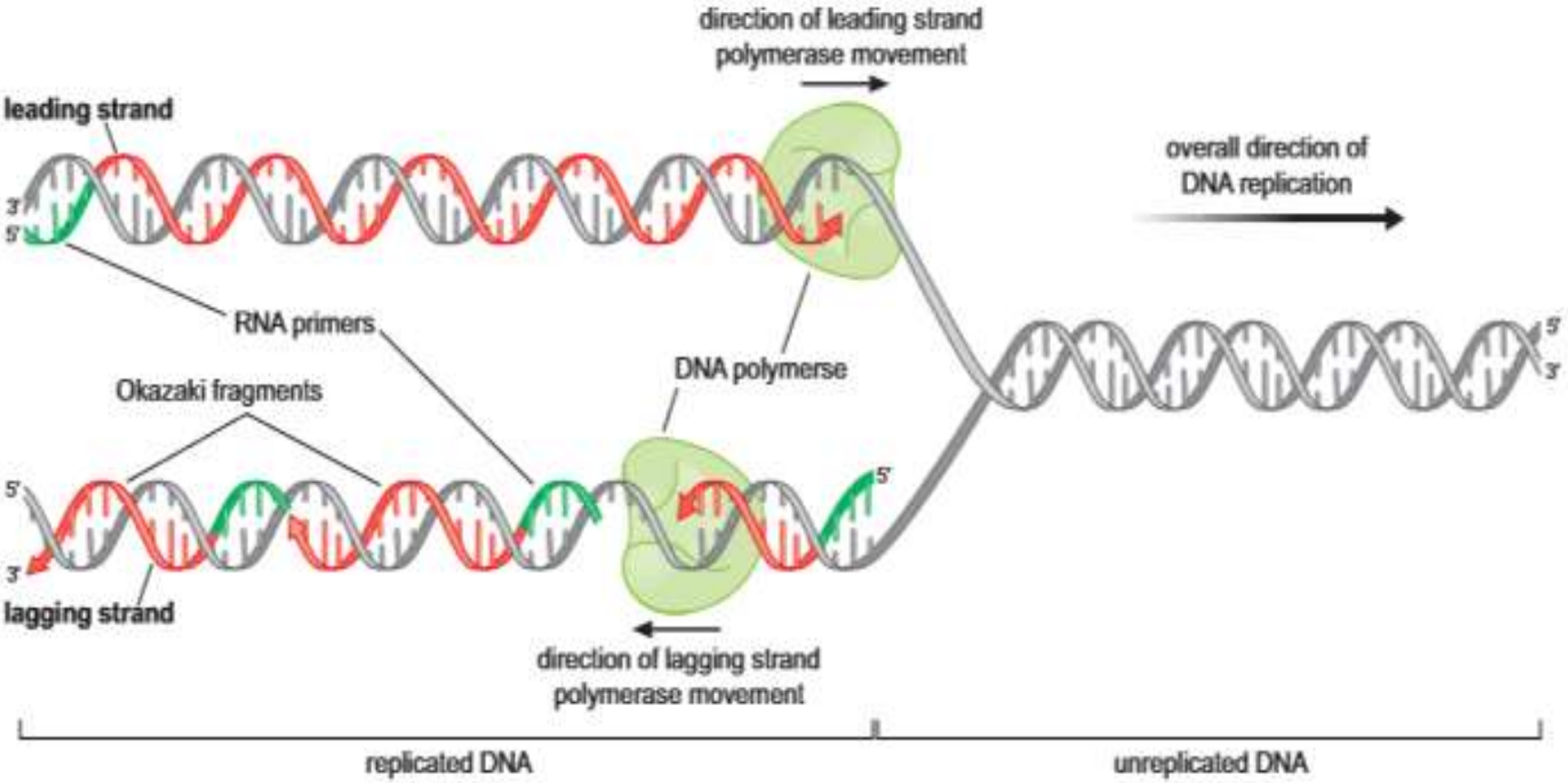


FIGURE 9-12 Replication fork. (Red) Newly synthesized DNA; (green) RNA primers. The Okazaki fragments shown are artificially short for illustrative purposes. In the cell, Okazaki fragments can vary between 100 and 2000 bases depending on the organism.

• The replication fork moves continuously toward the duplex region of unreplicated DNA, leaving in its wake two ss DNA templates that each direct the synthesis of a complementary DNA strand.
• The antiparallel nature of DNA creates a complication for the simultaneous replication of the two exposed templates at the replication fork.
• Because DNA is synthesized only by elongating a 3′ end, only one of the two exposed templates can be replicated continuously as the replication fork moves.

• On this template strand, the polymerase simply "chases" the moving replication fork.
• The newly synthesized DNA strand directed by this template is known as the leading strand.
• Synthesis of the new DNA strand directed by the other ssDNA template is more complicated.
• This template directs the DNA polymerase to move in the opposite direction of the replication fork.
• The new DNA strand directed by this template is known as the lagging strand. As shown in figure, this strand of DNA must be synthesized in a discontinuous fashion.

• The resulting short fragments of new DNA formed on the lagging strand are called Okazaki fragments and vary in length from 1000 to 2000 nucleotides in bacteria and from 100 to 400 nucleotides in eukaryotes.

• Shortly after being synthesized, Okazaki fragments are covalently joined together to generate a continuous, intact strand of new DNA.

• Okazaki fragments are therefore transient intermediates in DNA replication.

➢ Replicon and origin of replication

• DNA replication does not start at random locations but at particular sites, called the origins of DNA replication.

• A unit of DNA in which replication starts from an origin and proceeds bidirectionally or unidirectionally to terminus site is called a replicon, a unit of DNA replication.

• Replicon can be linear or circular. Prokaryotic replicons are usually circular.

❖ Replication Initiation

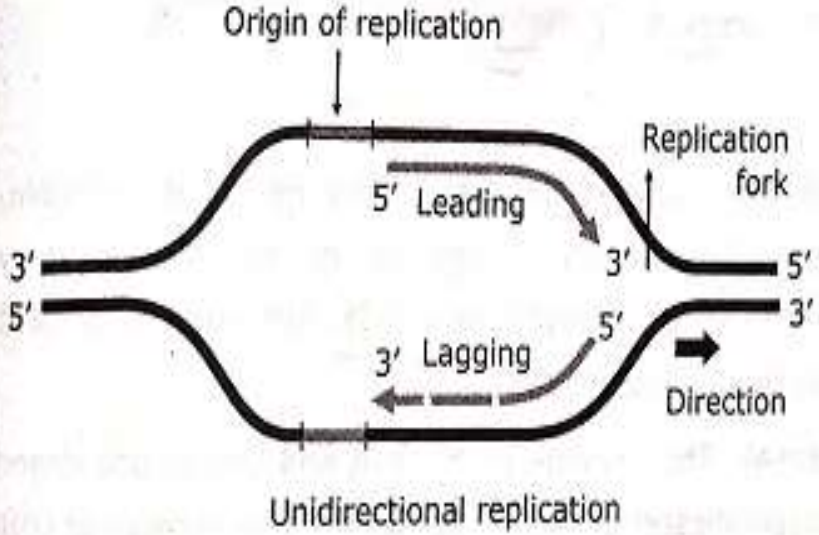
▪ Origin of replication : the *OriC* system

• Replication of chromosomal DNA initiates bidirectionally at a unique sequence called oriC.

• The minimal oriC sequence for replication is 245bp.

• It contains two short repeat motifs

a) 9 bp sites: The nine nucleotide repeat, five copies of which are dispersed throughout oriC, is the binding site for a protein called DnaA.



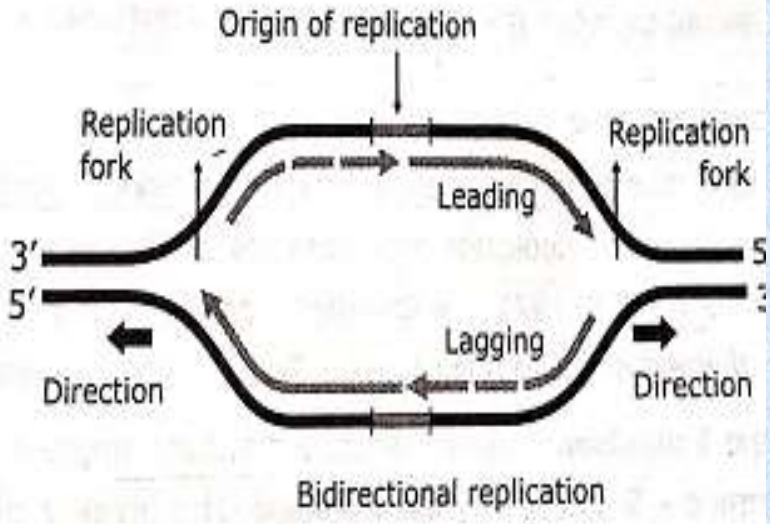


Figure 1.75 Unidirectional and bidirectional replication. The point at which replication is occurring is called the replication fork (sometimes also known as the growing point). A replication fork moves sequentially along the DNA, from its starting point at the origin. In bidirectional replication, two replication forks are formed; they proceed away from the origin in opposite directions.

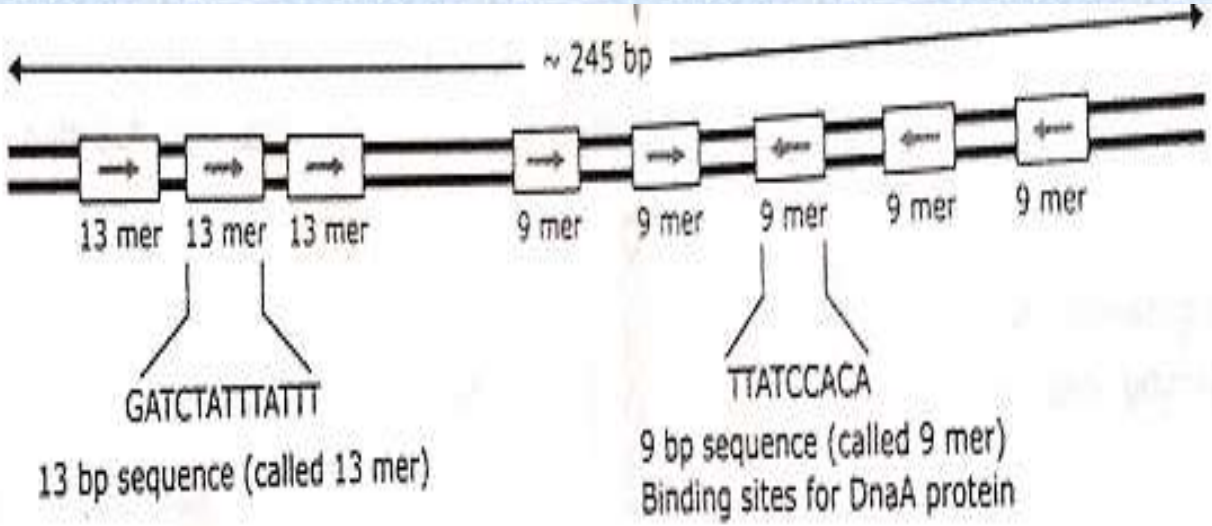


Figure 1.73 *E. coli* origin of replication, oriC. oriC contains repetitive 9-bp and A.T rich 13-bp sequences referred to as 9-mers and 13-mers, respectively. Multiple copies of DnaA protein bind to the 9-mer and the 'melt' the 13-mer segments.

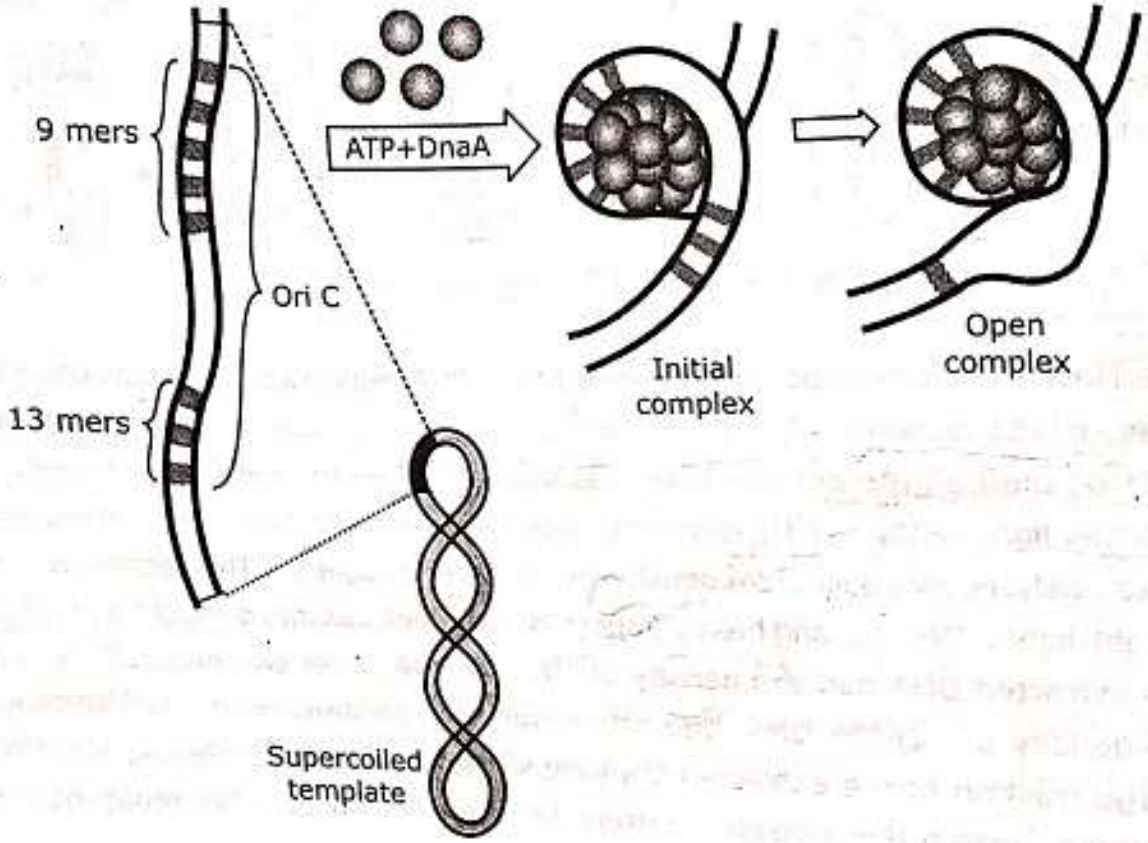
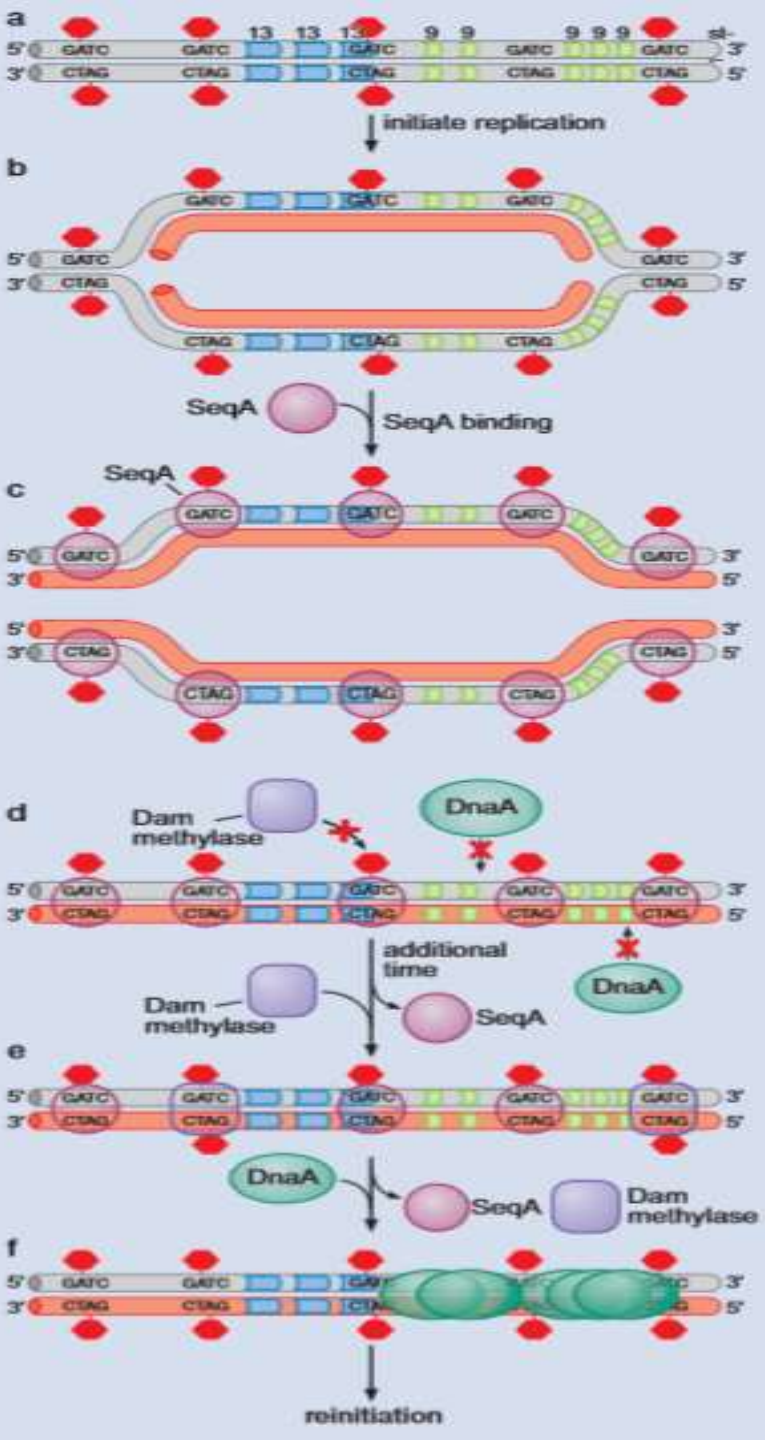


Figure 1.74 Initiation at oriC occurs after DnaA protein binds the five 9 mers. The 13 mer region is then denatured, and this open complex serves as a replication start site. Adapted and redrawn from D. Bramhill and A. Kornberg. Cell, 1988,54; 915-918.

b) 13 bp sites: The result of DnaA binding is that the double helix opens up (melts) within the tandem array of three AT-rich, 13- nucleotide repeats located at one end of the oriC sequence.

c) Recognition sequences for Dam methylase: oriC contains 11 copies of 5′-GATC-3′ repeats that are methylated on adenine on both strands. Only fully methylated origins can initiate replication; hemimethylated daughter origins cannot be used again until they have been restored to the fully methylated states.


a
13 13 13 9 9 9 9 9
5' GATC GATC GATC GATC GATC 3'
3' CTAG CTAG CTAG CTAG CTAG 5'
initiate replication
b
SeqA
SeqA binding
c
SeqA
d
Dam methylase
DnaA
additional time
DnaA
Dam methylase
SeqA
e
DnaA
SeqA
Dam methylase
f
reinitiation

❖ Mechanism of replication initiation:

During initiation, the enzymes that synthesize DNA at the replication fork are assembled on template DNA at the origin of replication. In many replicons, specific initiator proteins bind the origin sequence to begin assembly.

i) Initial complex: The first step in initiation from oriC is the binding of the Dna A protein to the 5 copies of 9 mer sequences. Dna A is weak ATPase that binds ATP and slowly hydrolyzes it to form DnaA-ADP.

ii) Open complex: Dna A-ATP promotes the opening of the DNA duplex in the region of the 13-mers. This converts the initial complex into an open complex.

iii) Pre-priming complex : After the opening of the origin of replication, the DnaB protein, escorted by DnaC protein is transferred to the exposed ssDNA.

iv) Primosome and primer formation:

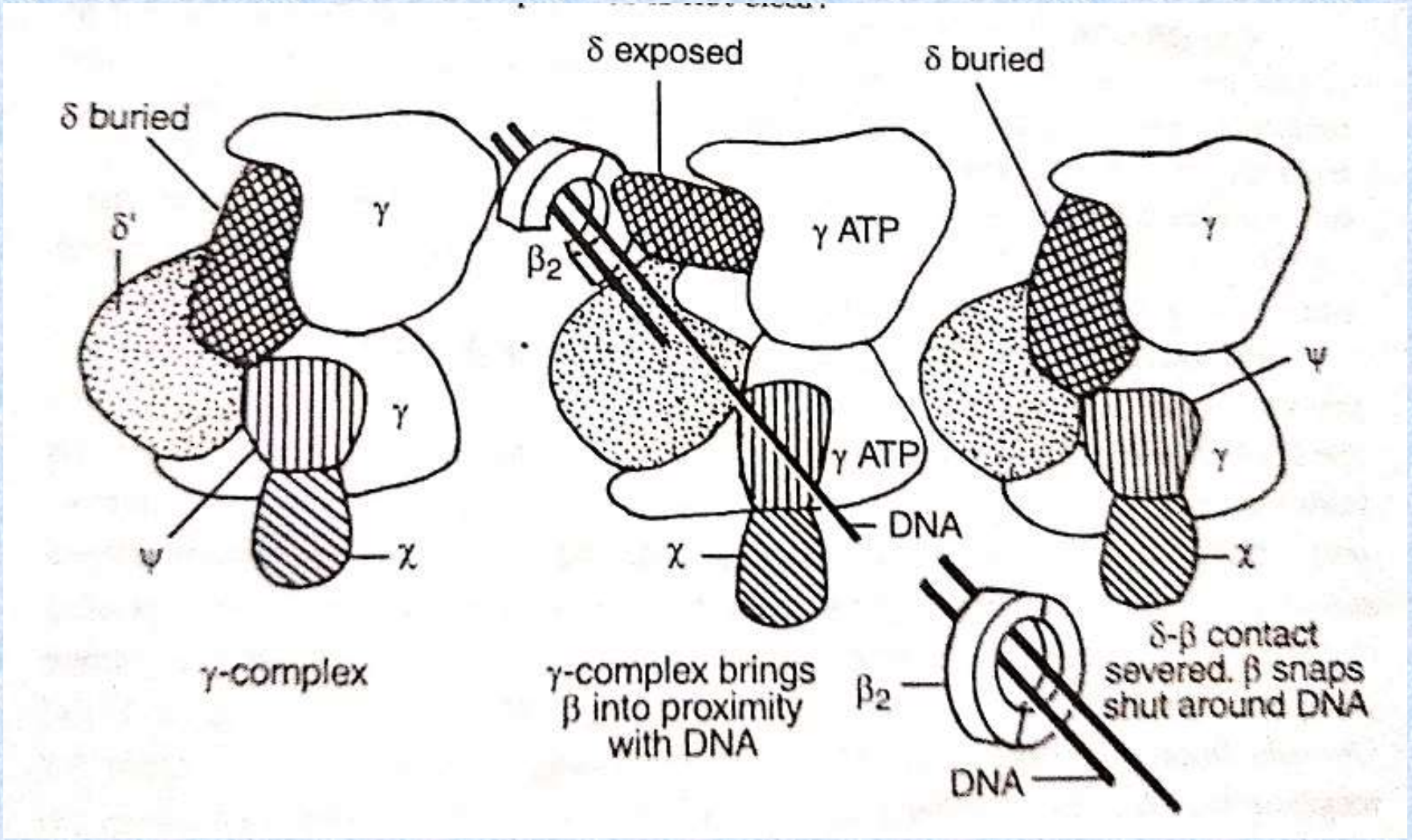
DnaB + DnaG $\longrightarrow$ primosome

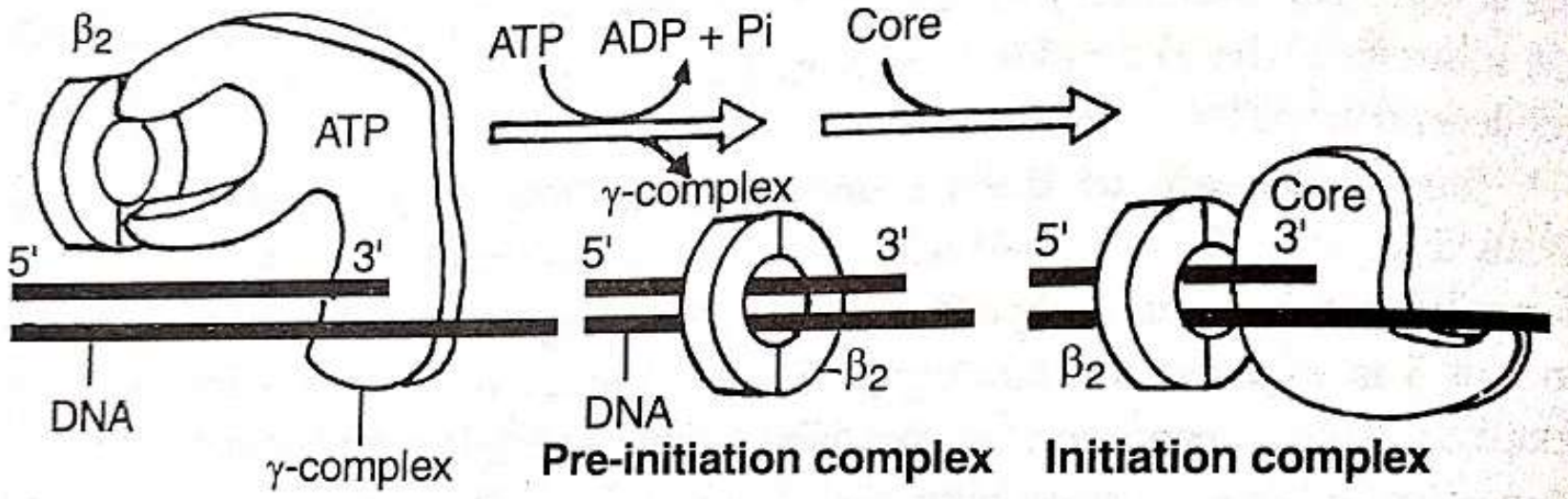
❖ Replication Elongation:

i) Replisome: DNA synthesis is carried out by a multienzyme complex called the replisome that assembles at the prokaryote replication fork. It is not a independent unit, but assembles from its components when replication begins.

a) Dna B
b) Dna G
c) DNA polymerase III holoenzyme
d) DNA gyrase

ii) Single strand DNA binding proteins (SSB)


δ buried
δ'
γ
γ
ψ
χ
γ-complex
δ exposed
β2
γ ATP
γ ATP
DNA
χ
γ-complex brings
β into proximity
with DNA
δ buried
γ
ψ
γ
χ
β2
DNA
δ-β contact
severed. β snaps
shut around DNA



γ–complex recognizes primed DNA template

ATP hydrolysis
β-assembled onto DNA
γ-complex dissociates

Core assembles with β-clamp to form processive polymerase

Fig. 14.32. Two stages in the assembly of a processive DNA polymerase.

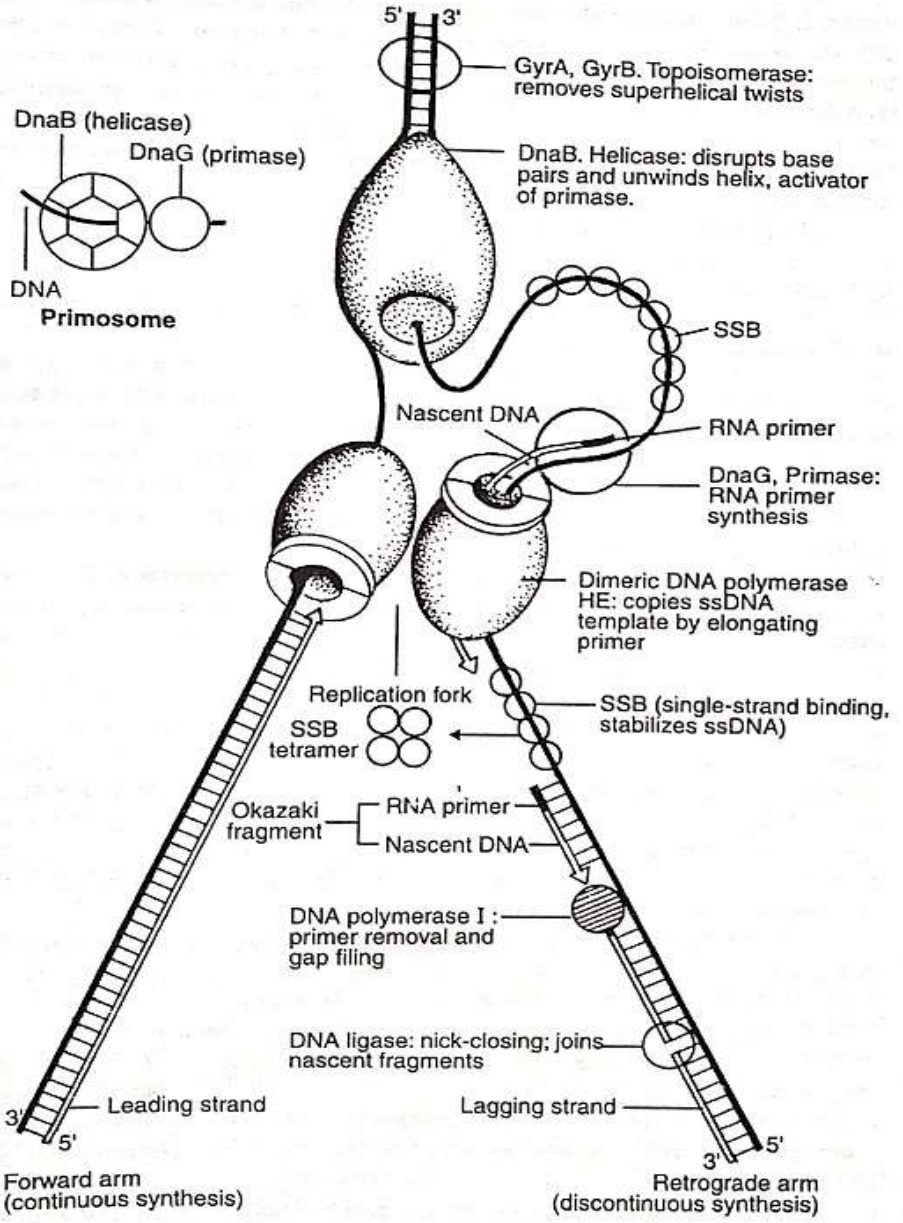
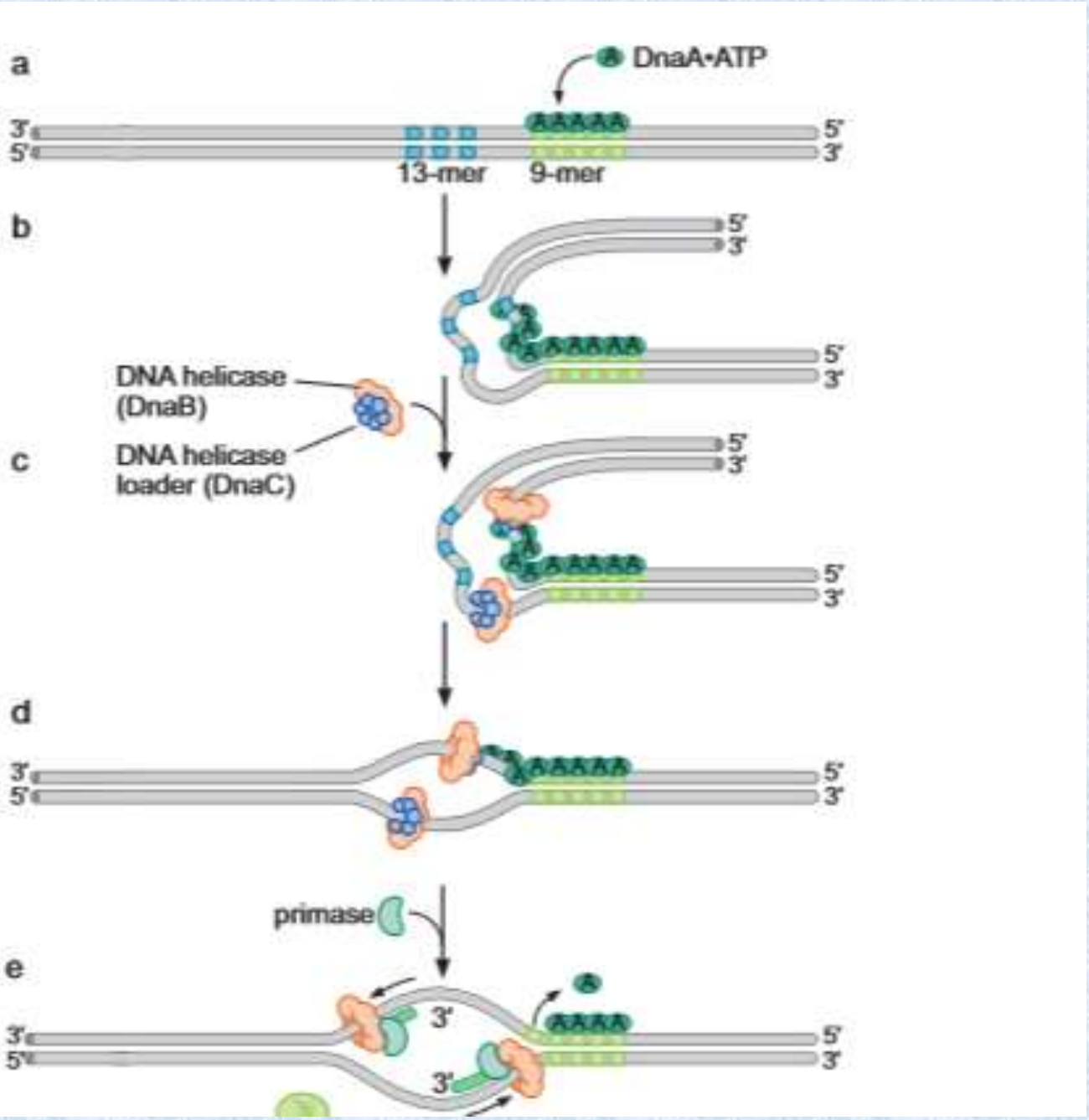
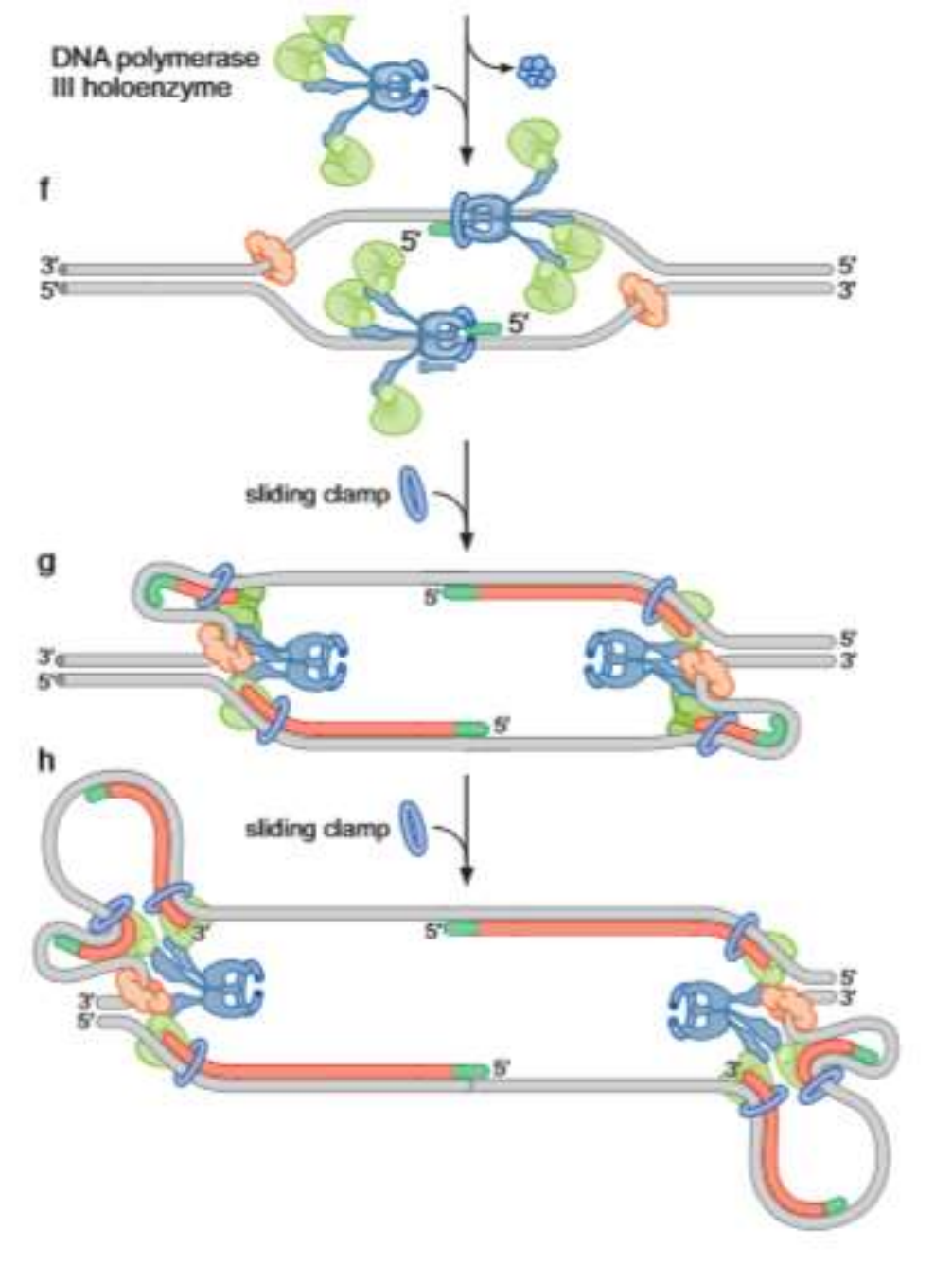


Fig. 14.33. Enzymes involved in the replication of *E.coli* DNA.


a
DnaA•ATP
3'
5'
5'
3'
13-mer
9-mer
b
5'
3'
5'
3'
DNA helicase
(DnaB)
c
DNA helicase
loader (DnaC)
5'
3'
5'
3'
d
3'
5'
5'
3'
primase
e
3'
5'
3'
3'
5'
3'


DNA polymerase
III holoenzyme
f
3'
5'
5'
3'
5'
5'
sliding clamp
g
5'
3'
5'
5'
3'
5'
h
sliding clamp
3'
5'
5'
3'
3'
5'
5'
3'