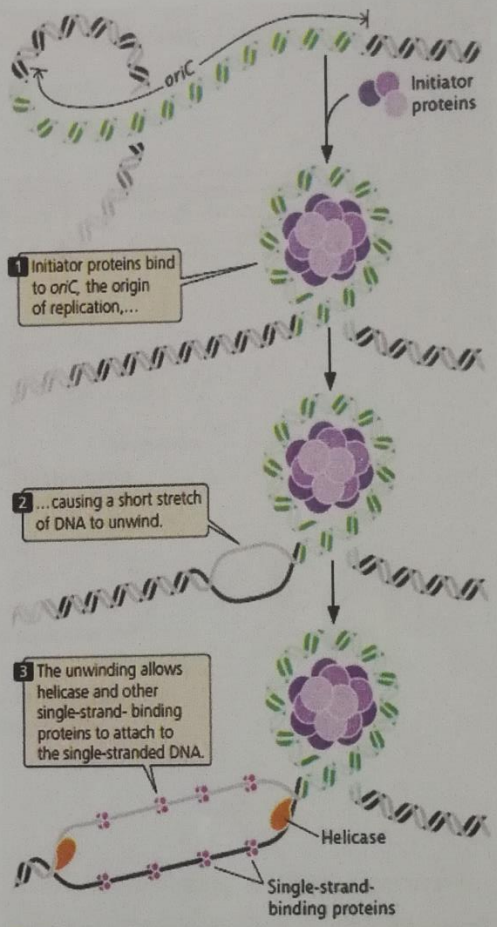
Bacterial DNA Replication

1- Initiation

The circular chromosome of *E. coli* has a single replication origin (*oriC*). The minimal sequence required for *oriC* to function consists of 245 bp that contain several critical sites. **Initiator proteins** bind to *oriC* and cause a short section of DNA to unwind. This unwinding allows **helicase** and other **single-strand-binding proteins** to attach to the polynucleotide strand.

2- Unwinding

Helicases bind to the lagging-strand template at each replication fork to break the hydrogen bonds that exist between the bases of the two nucleotide strands of a DNA molecule and move in the 5 3 direction along this strand, thus also moving the replication fork.



12.11 *E. coli* DNA replication begins when initiator proteins bind to *oriC*, the origin of replication, causing a short stretch of DNA to unwind.

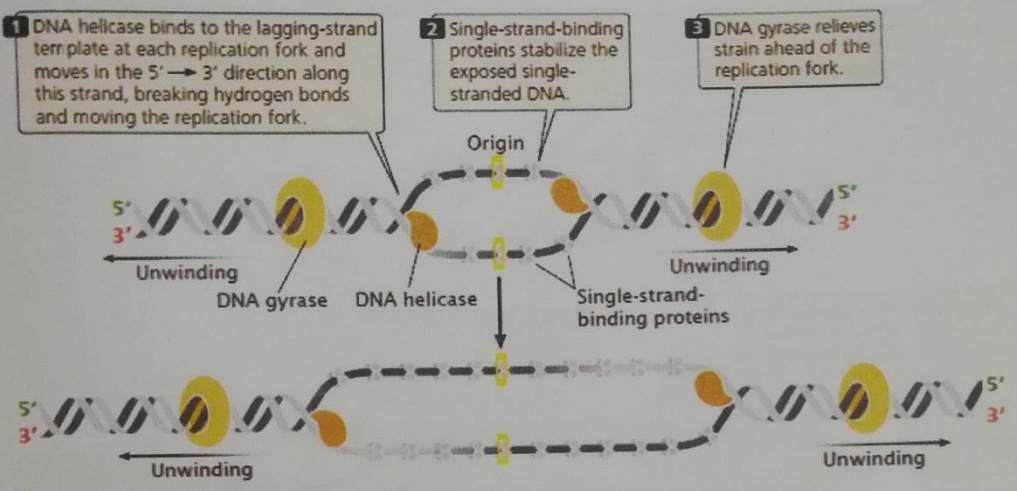
*** **Note**: *the single stranded nucleotide chains have a tendency to form hydrogen bonds and re-anneal (stick back together)*. To prevent re-anneal occur, single-strand-binding (SSB) proteins attach tightly to the exposed single-stranded DNA cover from 35 to 65 nucleotides.

- DNA gyrase reduces torsional strain (torque) that builds up ahead of the replication fork as a result of unwinding. It reduces torque by making a double-stranded break in one segment of the DNA helix, passing another segment of the helix through the break, and then resealing the broken ends of the DNA. This action removes a twist in the DNA and reduces the supercoiling.

3- Primers

An enzyme called **primase** synthesizes short stretches of nucleotides **(primers of RNA)** to get DNA replication started (about 10–12 nucleotides long), which provides a 3- OH group to which DNA polymerase can attach DNA nucleotides.

- On the leading strand, where DNA synthesis is continuous, a primer is required only at the 5_ end of the newly synthesized strand.
- On the lagging strand, where replication is discontinuous, a new primer must be generated at the beginning of each Okazaki fragment.



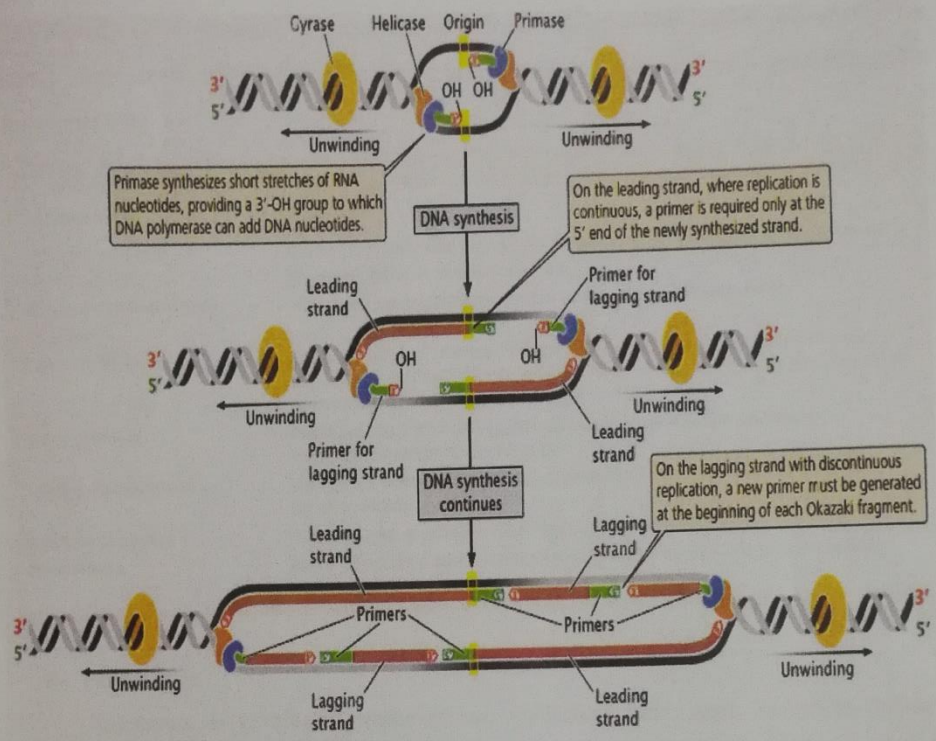
12.12 DNA helicase unwinds DNA by binding to the lagging-strand template at each replication fork and moving in the 5′→3′ direction along the strand.

4- **Elongation**

DNA polymerases elongate the polynucleotide strand by catalyzing DNA polymerization. *E. coli*, which has at least five different DNA polymerases. DNA polymerase III synthesizes nucleotide strands by adding new nucleotides to the 3 end of growing DNA molecules. This enzyme has two enzymatic activities.

- Its 5 3 polymerase activity allows it to add new nucleotides in the 5 3 direction.
- Its 3 5 exonuclease activity allows it to remove nucleotides in the 3 5 direction, enabling it to correct errors.

DNA polymerase I also possess 5 3 exonuclease activity, which is used to remove the primers laid down by primase and to replace them with DNA nucleotides by moving in a 5 3 direction. DNA polymerases IV and V function in DNA repair.



12.13 Primase synthesizes short stretches of RNA nucleotides, providing a 3′-OH group to which DNA polymerase can add DNA nucleotides.

Table 12.3 Characteristics of DNA Polymerases in *E. coli*

DNA Polymerase	5′→3′ Polymerization	3′→5′ Exonuclease	5′→3′ Exonuclease	Function
I	Yes	Yes	Yes	Removes and replaces primers
II	Yes	Yes	No	DNA repair; restarts replication after damaged DNA halts synthesis
III	Yes	Yes	No	Elongates DNA
IV	Yes	No	No	DNA repair
V	Yes	No	No	DNA repair; translesion DNA synthesis

5- DNA ligase

After polymerase I have replaced the last nucleotide of the RNA primer with a DNA nucleotide, a nick remains in the sugar phosphate backbone of the new DNA strand. The 3-OH group of the last nucleotide to have been added by DNA polymerase I is not attached to the 5- phosphate group of

the first nucleotide added by DNA polymerase II. This nick is sealed by the enzyme **DNA ligase,** which catalyzes the formation of a phosphodiester bond without adding another nucleotide to the strand. Some of the major enzymes and proteins required for replication are summarized in the following table.

Table 12.4 Components required for replication in bacterial cells

Component	Function
Initiator protein	Binds to origin and separates strands of DNA to initiate replication
DNA helicase	Unwinds DNA at replication fork
Single-strand-binding proteins	Attach to single-stranded DNA and prevent reannealing
DNA gyrase	Moves ahead of the replication fork, making and resealing breaks in the double-helical DNA to release torque that builds up as a result of unwinding at the replication fork
DNA primase	Synthesizes short RNA primers to provide a 3′-OH group for attachment of DNA nucleotides
DNA polymerase III	Elongates a new nucleotide strand from the 3′-OH group provided by the primer
DNA polymerase I	Removes RNA primers and replaces them with DNA
DNA ligase	Joins Okazaki fragments by sealing nicks in the sugar–phosphate backbone of newly synthesized DNA

6- Termination

Replication is terminated whenever two replication forks meet. In others, specific termination sequences block further replication. A termination protein, called ***Tus*** in *E. coli,* binds to these sequences. Tus blocks the movement of helicase, thus stalling the replication fork and preventing further DNA replication.