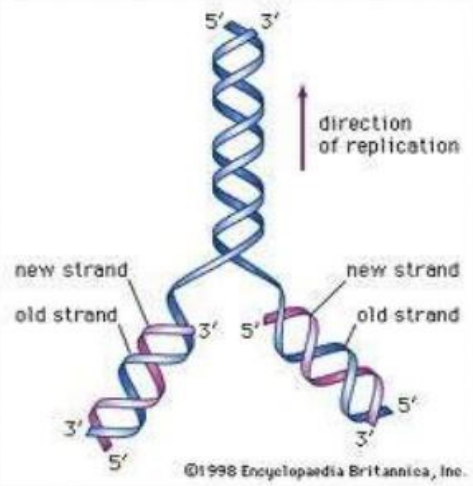
General Features of DNA replication

- DNA replication begins with the ***unwinding*** of two anti-parallel complementary strands, resulting in the formation of two single strands.
- This unwinding produces the two ***replication forks***
- The replication proceeds in **5'-> 3' direction** and is ***semi-discontinuous***
- DNA replication is ***semi-conservative***
- DNA replication begins at the ***origin of replication (ori)***
- DNA is synthesized by the enzyme ***DNA polymerases***
- Of the two strands, one strand is synthesized **continuously** (5' to 3')in the direction of movement of the replication fork called ***leading strand***; while the other strand is synthesized **discontinuously** away from the movement of replication fork in short segments called the ***lagging strand***
- DNA replication is ***bidirectional*** from the origin of replication

DNA Replication



Replication is Semi-conservative

INTRODUCTION

- **Deoxyribonucleic acid** (**DNA**) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses.
- Within cells, DNA is organized into long structures called **chromosomes**
- During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes
- **DNA replication** is the process of producing two identical copies from one origin
- This biological process occurs in all living organisms and is the basis for biological inheritance

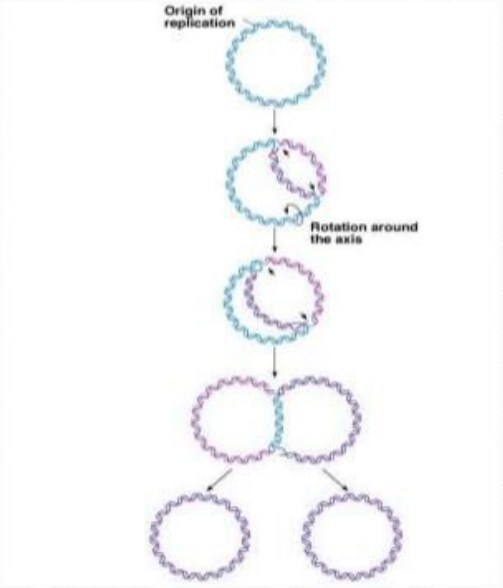
DNA replication in prokaryotes

The bacteria multiply by a process of binary fission; before this occurs, each cell must duplicate its genetic information so that each daughter cell has a copy. By a process called DNA replication

- DNA replication:

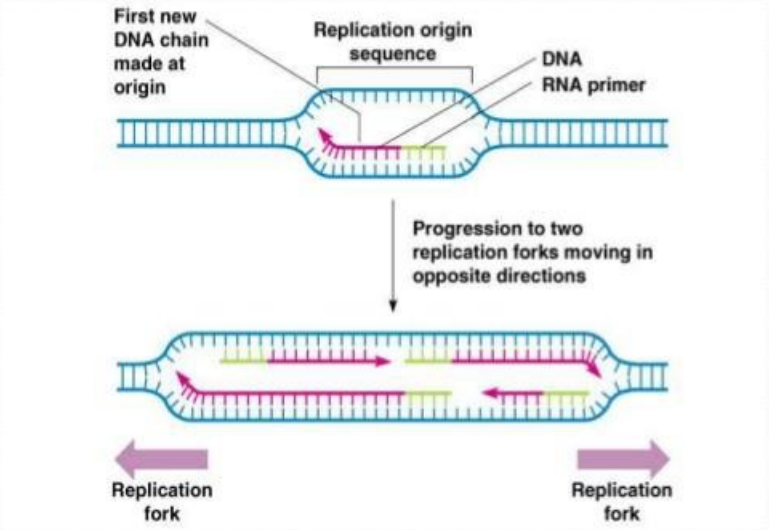
is the process of producing two identical copies from one origin This biological process occurs in all living organisms and is the basis for biological inheritance.

Bidirectional replication of circular DNA molecules



Prokaryotic DNA Replication

- The chromosome of a prokaryote is a circular molecule of DNA.
- Replication begins at one **origin of replication** and proceeds in both directions around the chromosome.



The Mechanism of DNA Replication (Prokaryotes)

By
Dr. Swasti Srivastava
Department of Biotechnology
CSJM University, India

The Mechanism of DNA Replication

- Tightly controlled process:
– occurs at specific times during the cell cycle.
- Requires:
– a set of proteins and enzymes,
– and requires energy in the form of ATP.
- Two basic steps:
– Initiation
– Elongation.
- Two basic components:
– template
– primer.

The Mechanism of DNA Replication (Prokaryotic)

- DNA polymerase – the enzyme that extends the primer;
- Pol III - produces new stands of complementary DNA;
- Pol I - fills in gaps between newly synthesized Okazaki segments
- additional enzymes/proteins –

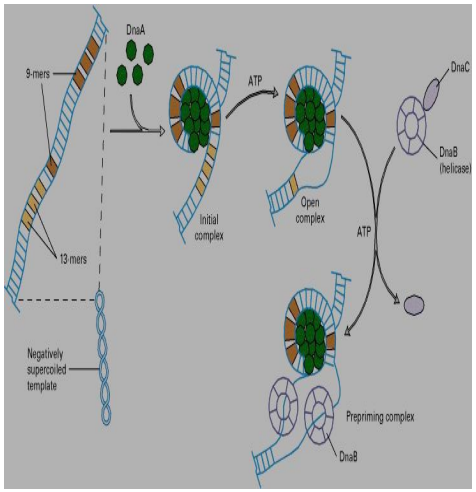
i) DNA helicase – unwinds double helix
ii) Single-stranded binding proteins – keep helix open
iii) Primase – creates RNA primers to initiate synthesis
iv) Ligase – welds together Okazaki fragments

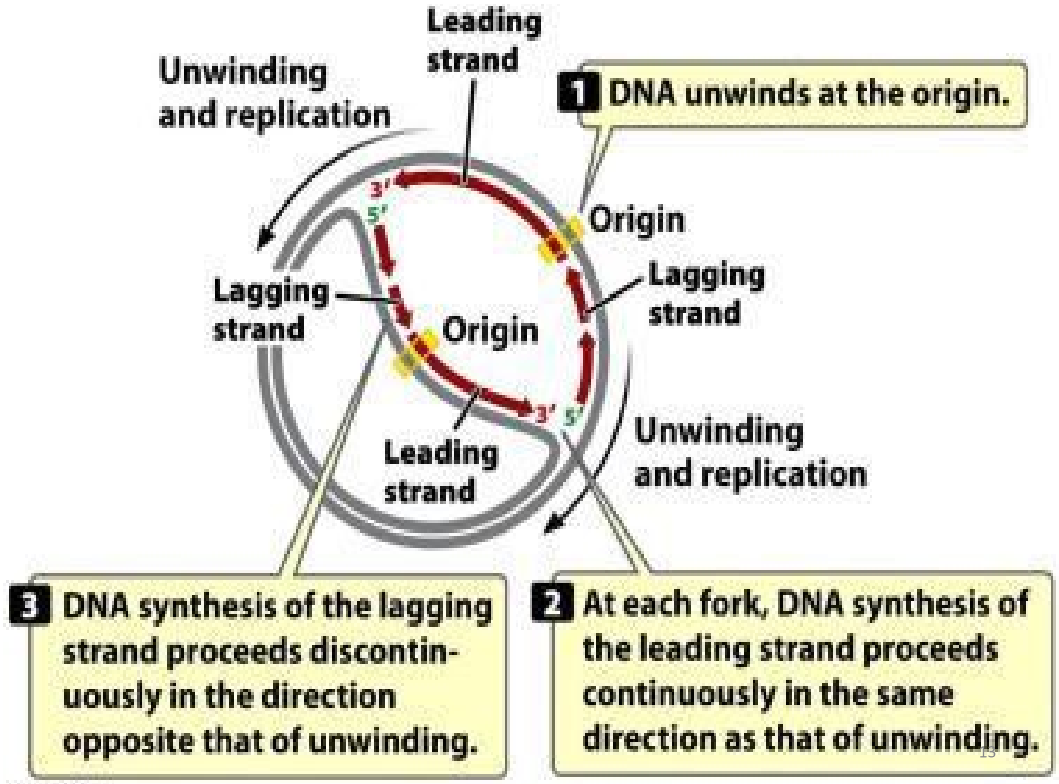
DNA Replication in Prokaryote (*E.coli*)

- The genome of *E.coli* is replicated bi-directionally from a single origin, oriC . *E. coli* replication is circular with no free ends. Replication of DNA in E. coli is also known as theta replication and it occurs in three steps:
  1) ***Initiation***
  2) ***Elongation***
  3) ***Termination***

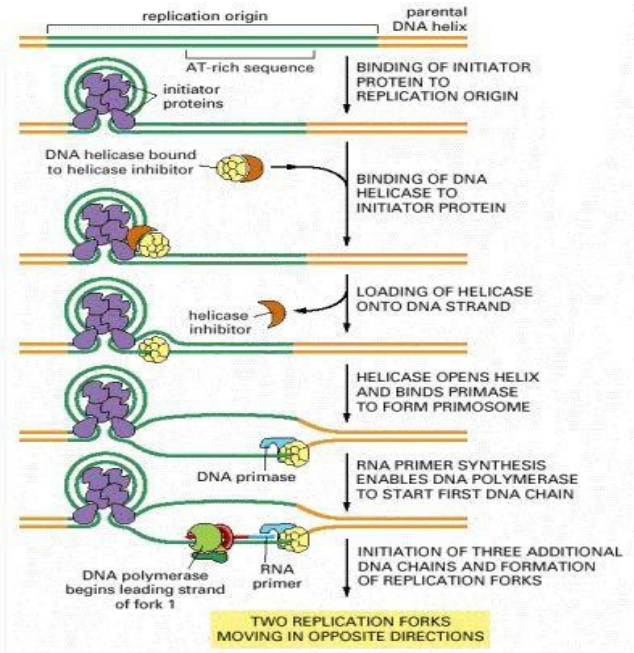
Model of initiation of replication at *E. coli oriC*

The 9-mers and 13-mers are the repetitive sequences shown in Figure 12-5. Multiple copies of DnaA protein bind to the 9-mers at the origin and then "melt" (separate the strands of) the 13-mer segments. The sole function of DnaC is to deliver DnaB, which is composed of six identical subunits, to the template. One DnaB hexamer clamps around each single strand of DNA at *oriC,* forming the prepriming complex. DnaB is a helicase, and the two molecules then proceed to unwind the DNA in opposite directions away from the origin. [Adapted from C. Bramhill and A. Kornberg, 1988, *Cell* **52**:743, and S. West, 1996, *Cell* **86**:177.]





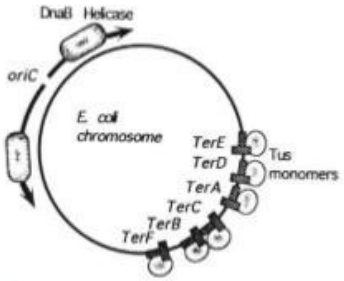
Replication Initiation

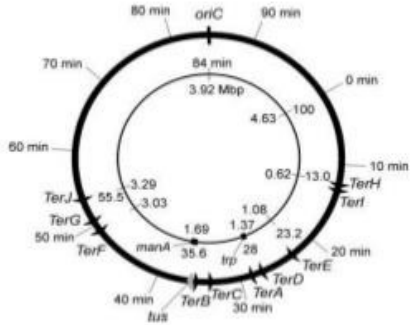


Initiation

- In *E. coli* , initiation at origin (oriC) requires several protein factors. These are:
  - >**DnaA protein** – recognizes oriC sequence, opens duplex at specific sites in origin
  - >**DnaB protein** (helicase) – unwinds DNA
  - >**DnaC protein** – required for DnaB binding at origin
  - >**Primase (DnaG protein)** – synthesizes Rna primers
  - >**SSB** – bind to ss DNA
  - >**DNA gyrase(DNA topoisomeraseII)** – relieves topological stress by DNA unwinding
  - >**Dam methylase** – methylates (5')GATC sequences at oriC

Termination:-



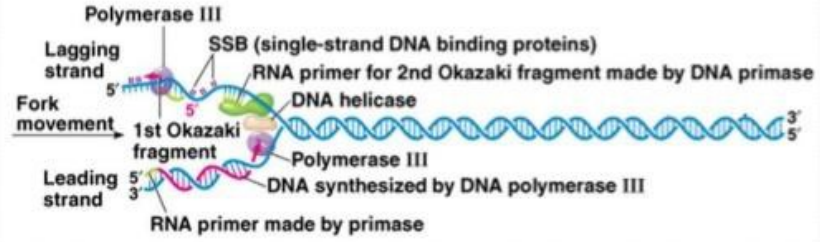


- In prokaryotes DNA replication terminates when replication fork reach specific 'termination site'.
- The arrest of DNA replication in Escherichia coli is triggered by the encounter of a replisome with a Tus protein-Ter DNA complex.
- A replication fork can pass through a Tus-Ter complex when traveling in one direction but not the other, and the chromosomal Ter sites are oriented so replication forks can enter, but not exit, the terminus region.
- The Tus-Ter complex acts by blocking the action of the replicative DnaB helicase, but details of the mechanism are uncertain
- The two replication fork meet each other on the opposite end of the parental circular DNA

Elongation

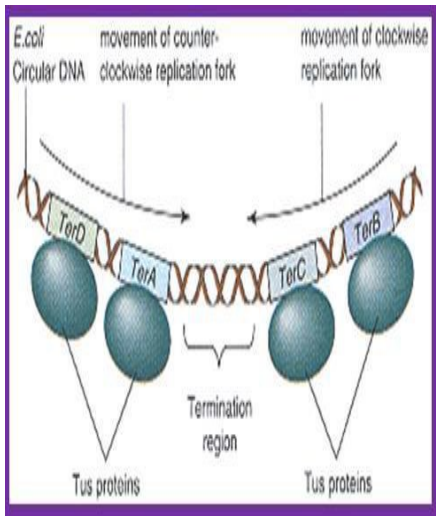
- Primase binds to the first priming sequence on the leading strand template and synthesizes a short RNA primer that is complementary to the DNA template.

DNA Polymerase III uses the primer to initiate DNA synthesis by adding deoxyribonucleotides to its 3' end. The leading strand requires only one priming event, because DNA synthesis is continuous thereafter, in the 5' ⟶ 3'direction.

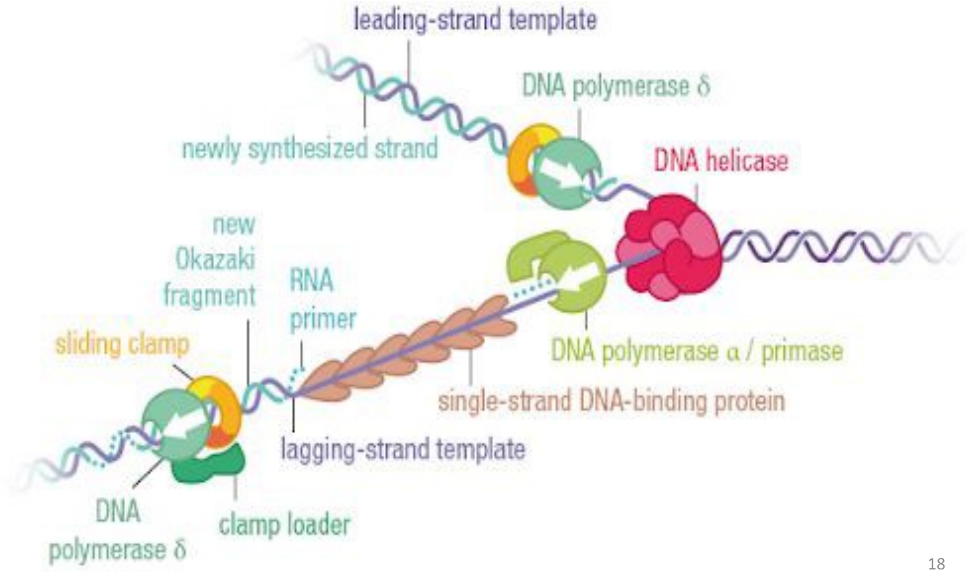


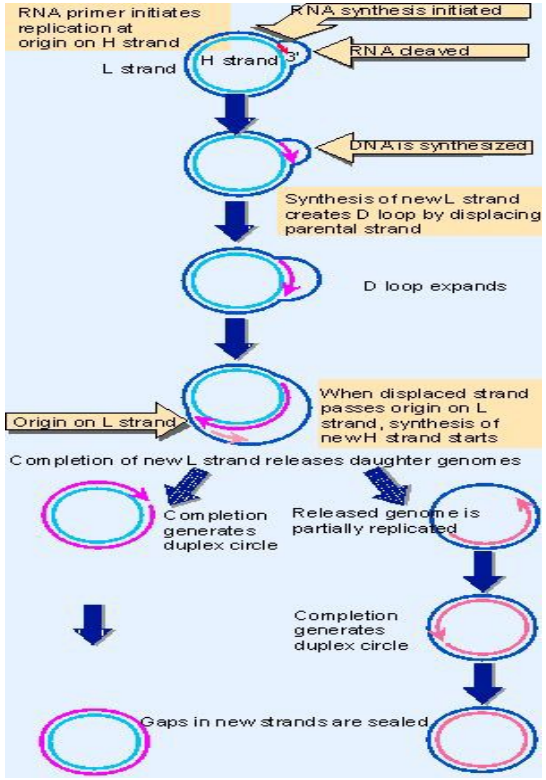
Termination

- The replication of *E. coli* is bidirectional from one origin, and the two replication forks must meet at one point called ter at 32.
- All the primers will be removed, and all the fragments will be connected by DNA-pol I and ligase.

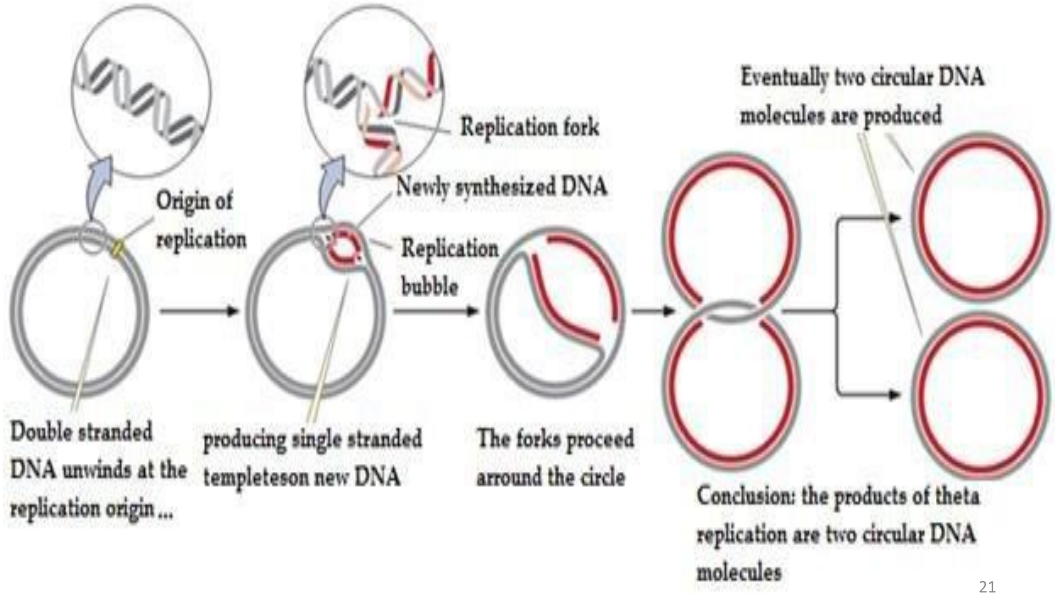


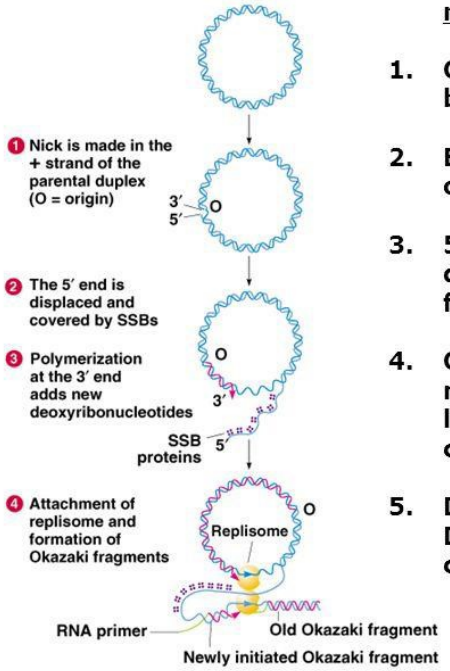
Elongation





Theta (θ) Model of DNA Replication





Rolling circle model of DNA replication (3.11):

1. **Common in several bacteriophages including λ.**
2. **Begins with a nick at the origin of replication.**
3. **5′ end of the molecule is displaced and acts as primer for DNA synthesis.**
4. **Can result in a DNA molecule many multiples of the genome length (and make multiple copies quickly).**
5. **During viral assembly the DNA is cut into individual viral chromosomes.**