DNA REPLICATION IN PROKARYOTES

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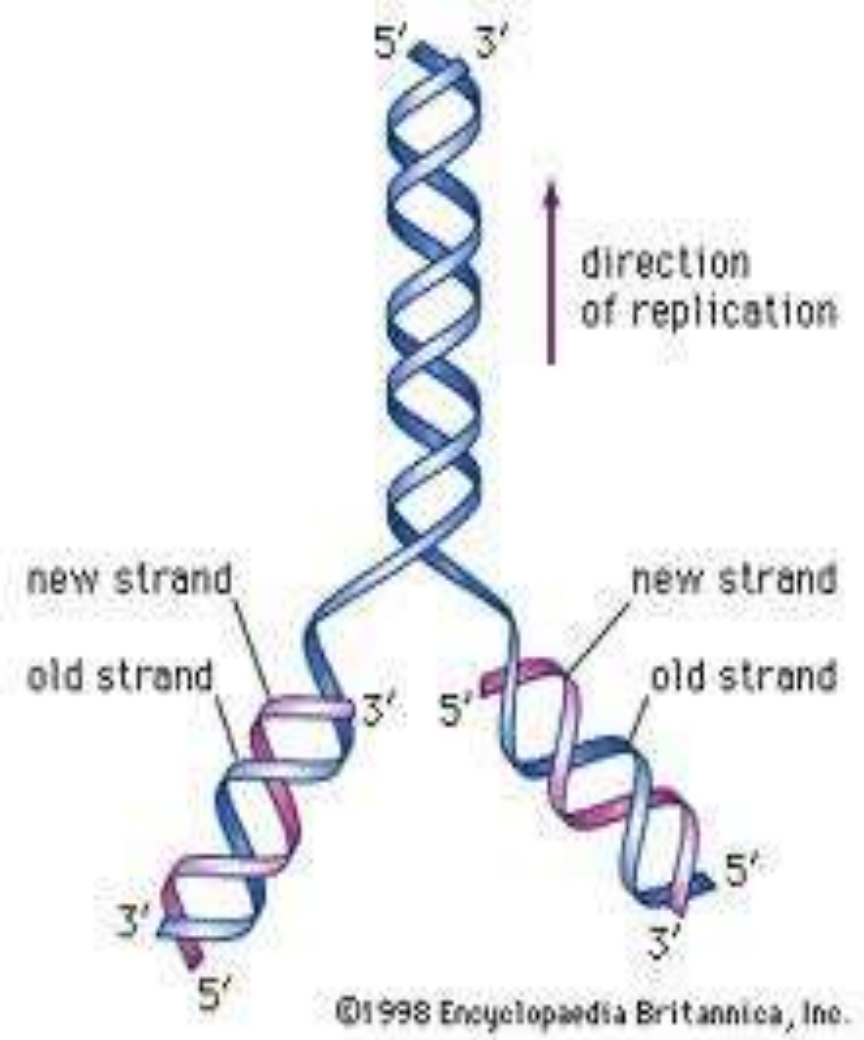
INTRODUCTION

- **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


5′
3′
direction
of replication
new strand
old strand
3′
5′
new strand
old strand
5′
3′
3′
5′
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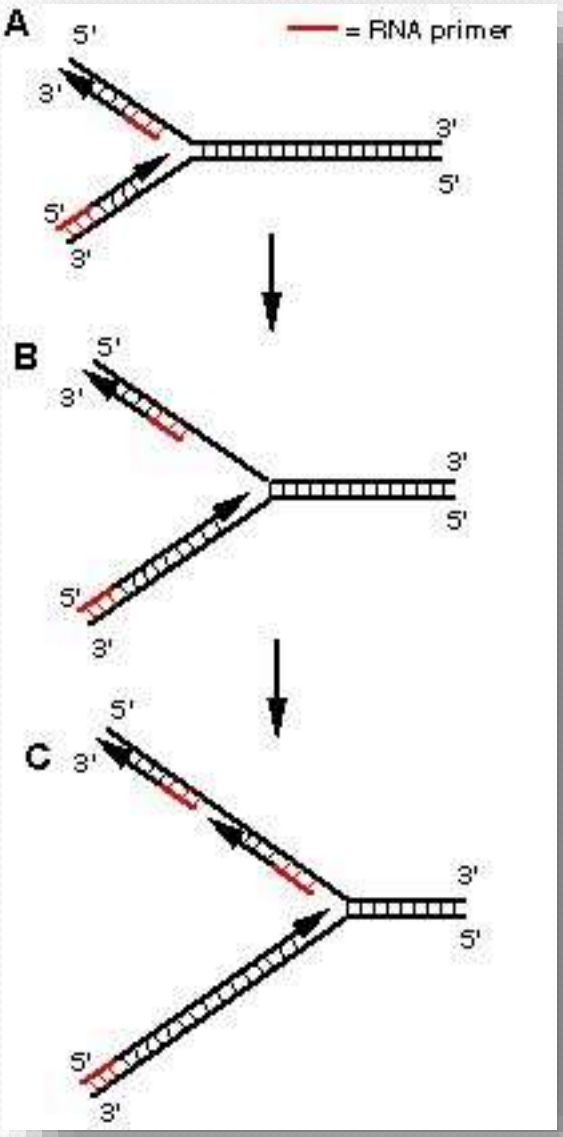
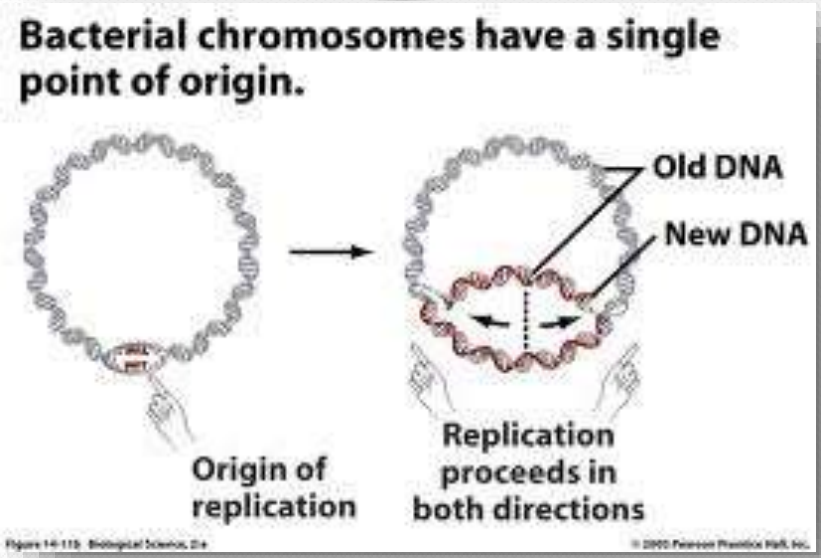
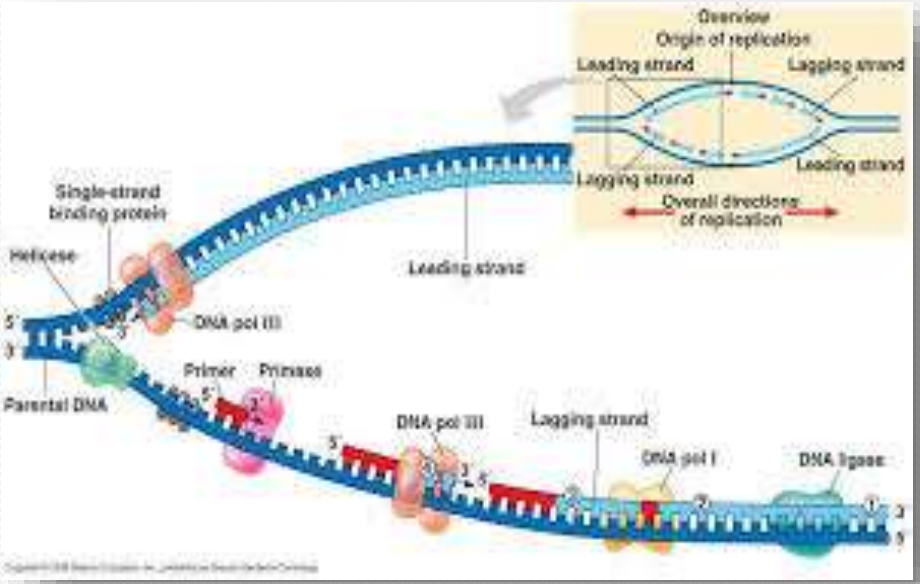
General Features of DNA replication

- 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


A
= RNA primer
5'
3'
3'
5'
5'
3'
B
5'
3'
3'
5'
5'
3'
C
5'
3'
3'
5'
5'
3'


Bacterial chromosomes have a single point of origin.
Old DNA
New DNA
Origin of replication
Replication proceeds in both directions


Overview
Origin of replication
Leading strand
Lagging strand
Lagging strand
Leading strand
Overall directions of replication
Single-strand binding protein
Helicase
Leading strand
DNA pol III
Primer
Primase
Parental DNA
DNA pol III
Lagging strand
DNA pol I
DNA ligase

DNA is Synthesized By DNA Polymerase

DNA polymerase is an enzyme that carries out the synthesis of a new strand on the template strand. DNA polymerase is found in both prokaryotes and eukaryotes. There are several types of DNA polymerase. Only few participates in polymerization of the new strand, while the other take part in proofreading activites. Some DNA polymerase catalayze the synthesis of a DNA strand complementary to the RNA. These DNA polymerase are termed as ***RNA-dependent DNA polymerase.***

DNA polymerase I was first enzyme to be isolated,which has ***5'-> 3' exonuclease activity***. In *E. coli* ***DNA polymerase III*** is the *principle replication enzyme*. In eukaryotes ***DNA polymerase*** δ is responsible for leading strand synthesis

✓The main enzymatic activity of DNA polymerases is the 5'—>3' synthetic activity.

✓However, DNA polymerases possess two additional activities of importance for both replication and repair

✓These additional activities include a 5'—>3' exonuclease function and a 3'—>5' exonuclease function

✓The 5'—>3' exonuclease activity allows the removal of ribonucleotides of the RNA primer, utilized to initiate DNA synthesis, along with their simultaneous replacement with deoxyribonucleotides by the 5'—>3' polymerase activity

✓The 5'—>3' exonuclease activity is also utilized during the repair of damaged DNA.

✓The 3'—>5' exonuclease function is utilized during replication to allow DNA polymerase to remove mismatched bases and is referred to as the **proof-reading** activity of DNA polymerase.

DNA polymerase in *E.coli*

- There are atleast five DNA polymerases associated with *E.coli* DNA replication
- These are
  - **DNA polymerase I** – it is the first DNA pol to be isolated and purified. The enzyme is encoded by polA gene. The polymerase has 5'->3' exonuclease activity
  - **DNA polymerase II** – the enzyme is encoded by polB gene. It has no 5'->3' exonuclease activity, rather it has 3'-5' exonuclease activity(proofreading activity)
  - **DNA polymerase III –** this is the principle replication enzyme,encoded by the gene polC. It has 5'->3' proofreading activities. It lacks 3'->5' exonuclease activity. It has a very high proccessivity
  - **DNA polymerase IV and DNA polymerase V** are involved in an unusual form of DNA repair

DNA Polymerase III

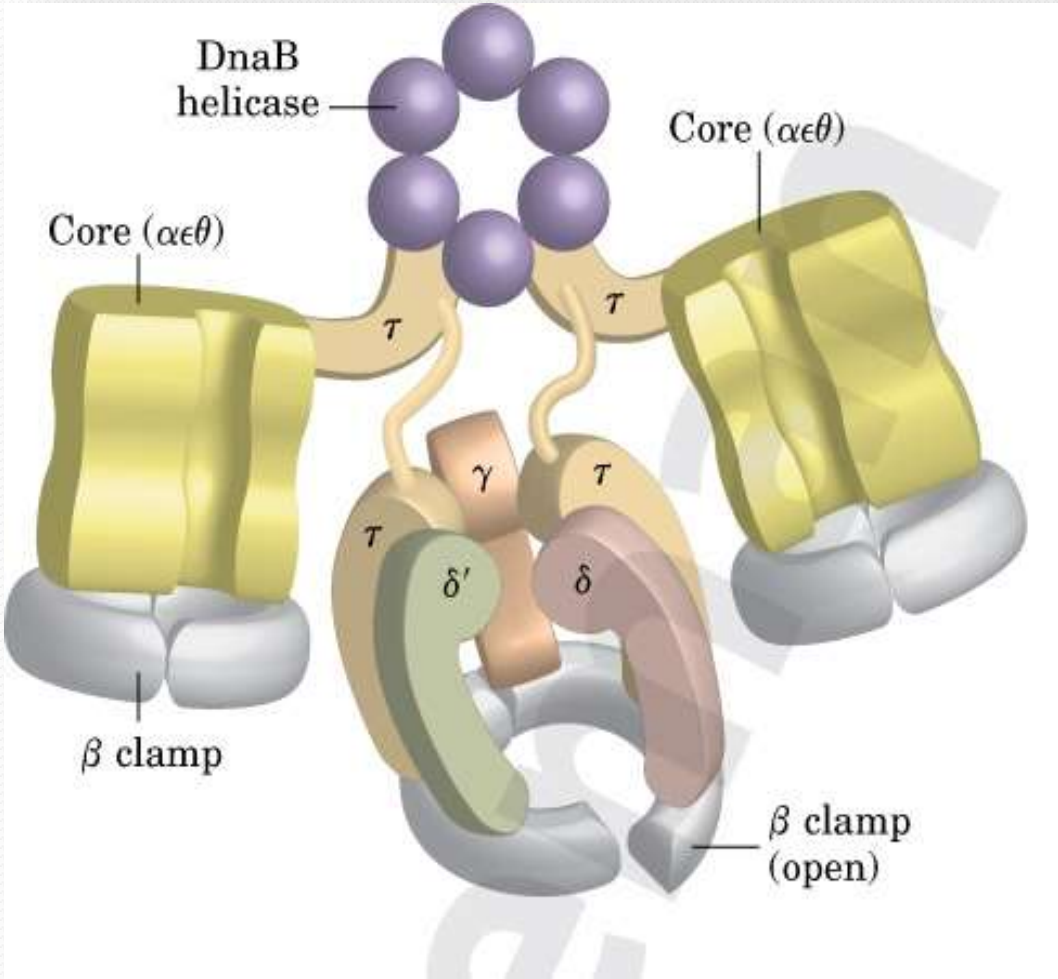
- **DNA polymerase III holoenzyme** is the primary enzyme complex involved in prokaryotic DNA replication
- DNA polymerase III synthesizes base pairs at a rate of around ***1000 nucleotides per second.***
- As replication progresses and the replisome moves forward, DNA polymerase III arrives at the RNA primer and begins replicating the DNA, adding onto the 3'OH of the primer
- DNA polymerase III will then synthesize a continuous or discontinuous strand of DNA
- DNA polymerase III has a ***high processivity*** and therefore, synthesizes DNA very quickly.
- This high processivity is due in part to ***the β-clamps*** that "hold" onto the DNA strands.

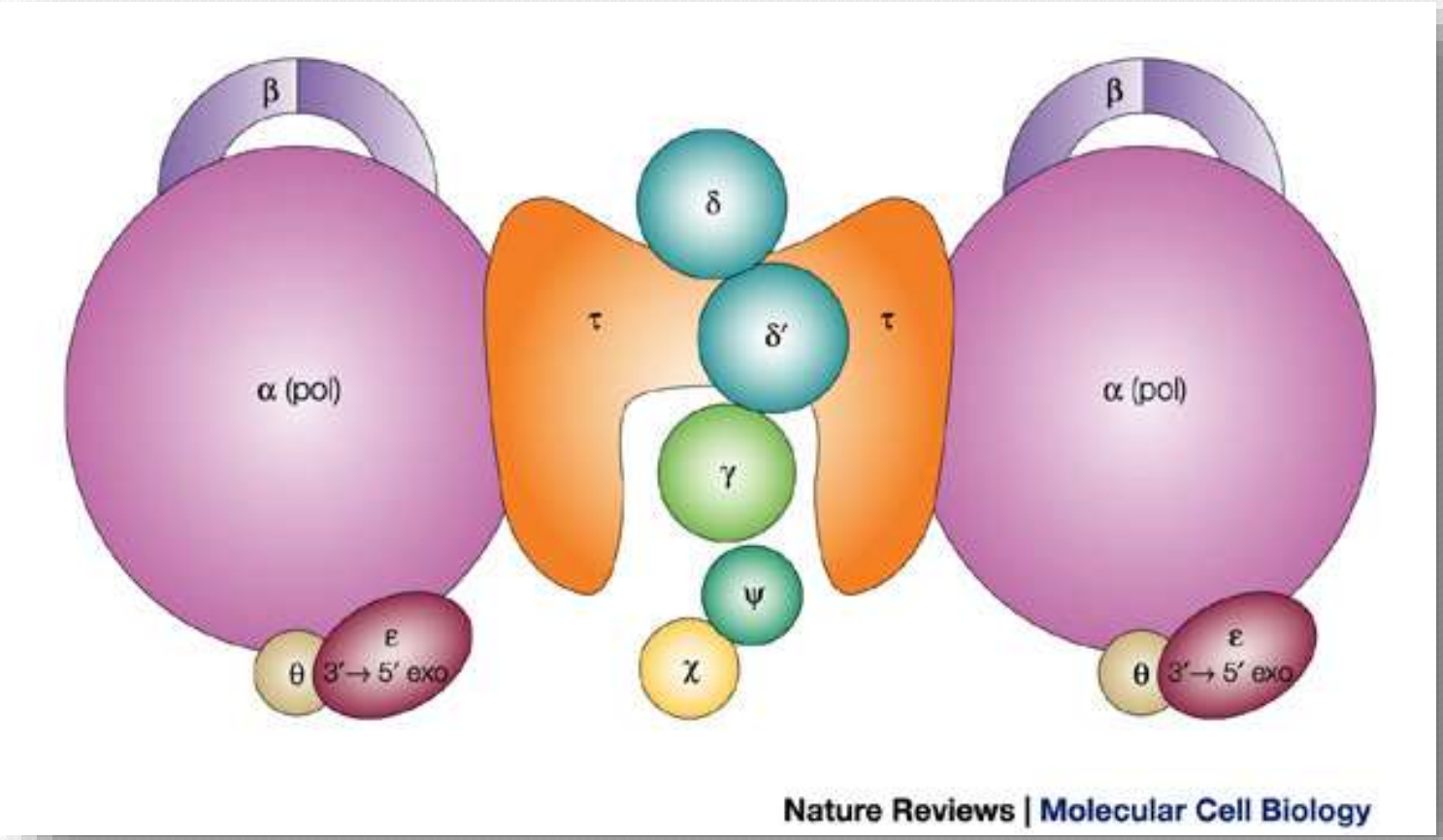
❖It consists of three assemblies: the pol III core, the beta sliding clamp proccessivity factor and the clamp-loading complex

❖The holoenzyme contains two cores, one for each strand, the lagging and leading

❖The beta sliding clamp proccessivity factor is also present in duplicate, one for each core, to create a clamp that encloses DNA allowing for high proccessivity

❖The Pol III catalytic core has three tightly associated subunits: alpha, epsilon and theta. The alpha subunit is responsible for the DNA polymerase activity, while the epsilon subunit is the 3'-5' proofreading exonuclease




β
β
δ
τ
δ′
τ
α (pol)
α (pol)
γ
ψ
χ
ε
θ
3′→5′ exo
ε
θ
3′→5′ exo
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Replication Requires Many Enzymes and Protein Factors

- ***DNA helicase*** - unwinds the ds DNA
- ***Topoisomerase*** – relieves the topological stress produced in the helical stuctures of DNA during unwinding
- ***Single stranded DNA binding proteins*** – stabilizes the single strands of DNA after unwinding
- ***RNA primer*** – synthesized by primase (a specific RNA polymerase)
- ***DNA ligase*** – catalyses the formation of a phosphodiester linkage.

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***

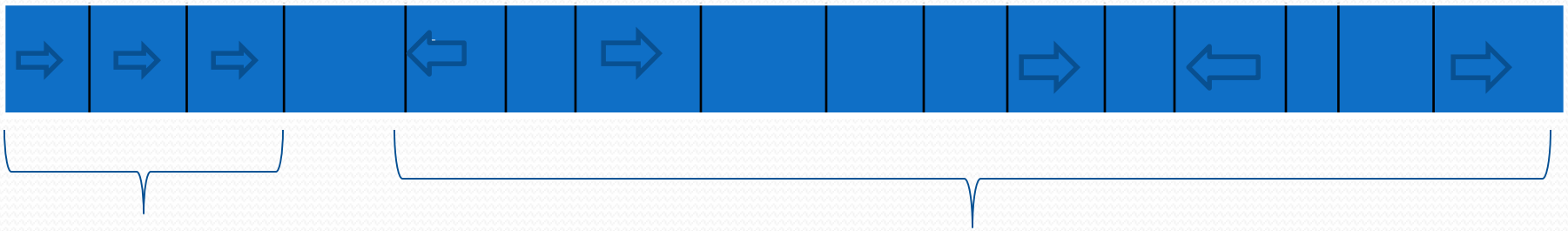
Initiation

- 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

Sequence of events during Initiation

- The *E. coli* oriC , consists of **245bp of DNA** , which are highly conserved. It consists of **two short sequences:**

  one is of *five repeats of 9bp sequence sites*, remains dispersed , where DnaA protein binds and the other is *three repeats of 13bp sequence* , which is continuous and a region rich in A=T base pairs, called DNA unwinding elements (DUE) .
- The binding of DnaA to the 9bp requires ATP, which facilitates the initial strand separation of *E. coli* DNA duplex, which leads to the denaturation in A=T rich DUE region.
- DnaC protein binds to the DnaB protein, which results in the opening up of DnaB ring. The two DnaB ring is loaded on the denatured DUE , one on each strand
- DnaC is released and two DnaB remains bound to the two ss DNA
- ***DnaB helicase*** is then loaded on to two ss strands , which then travels along the ss DNA in the 5'->3' direction unwinding the DNA strand, creating two replication forks.
- Many SSB then binds to ss DNA to stabilize the strands separation
- Unwinding produces stress which is removed by ***DNA gyrase***
- Finally the oriC DNA is methylated by the ***Dam methylase*** , which methylates the adenine at N^6 within the palindromic sequence (5') GATC.



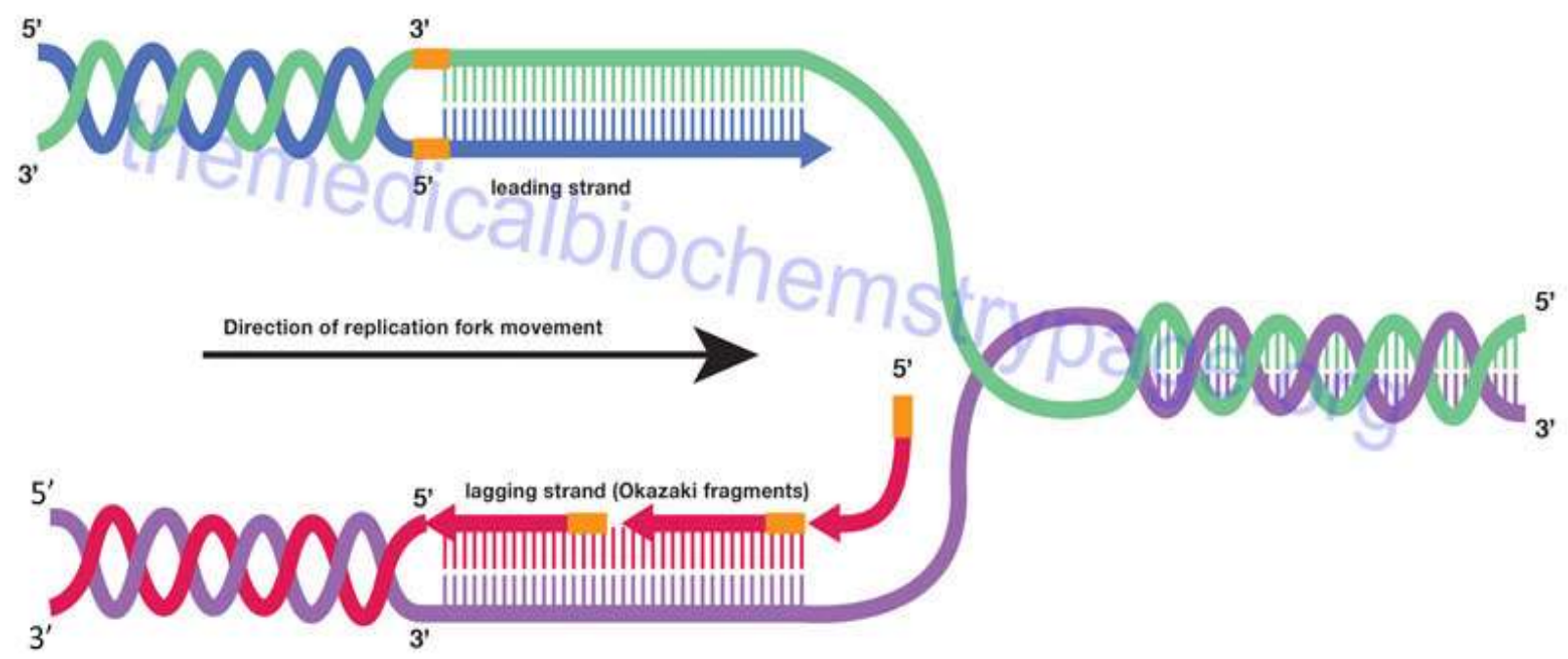
THREE REPEATS OF 13 BP SEQUENCES

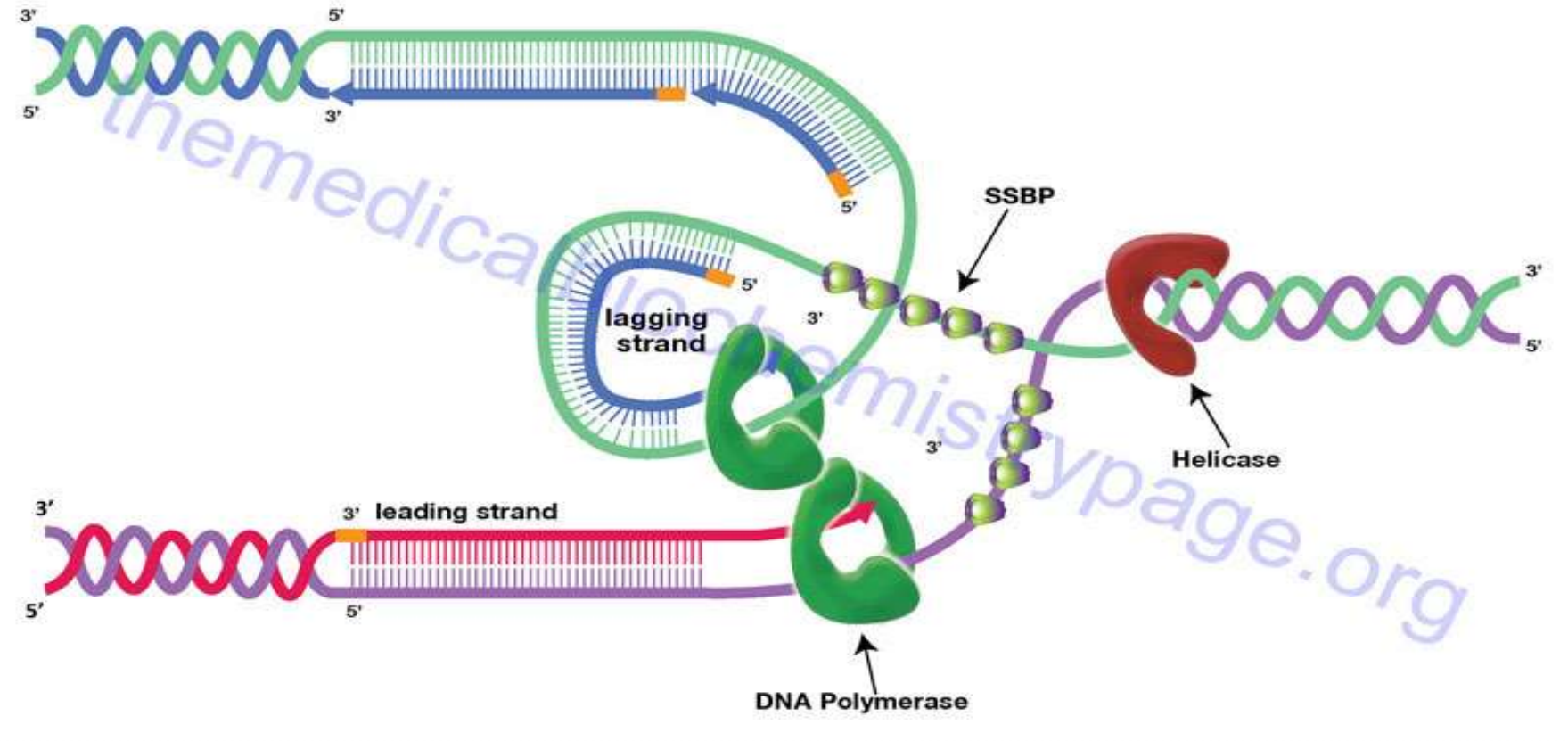
BINDING SITES OF DnaA PROTEIN , FIVE 9-BP SEQUENCES

FIG: ARRANGEMENT OF SEQUENCES IN THE *E. coli* REPLICATION ORIGIN. oriC

ELONGATION

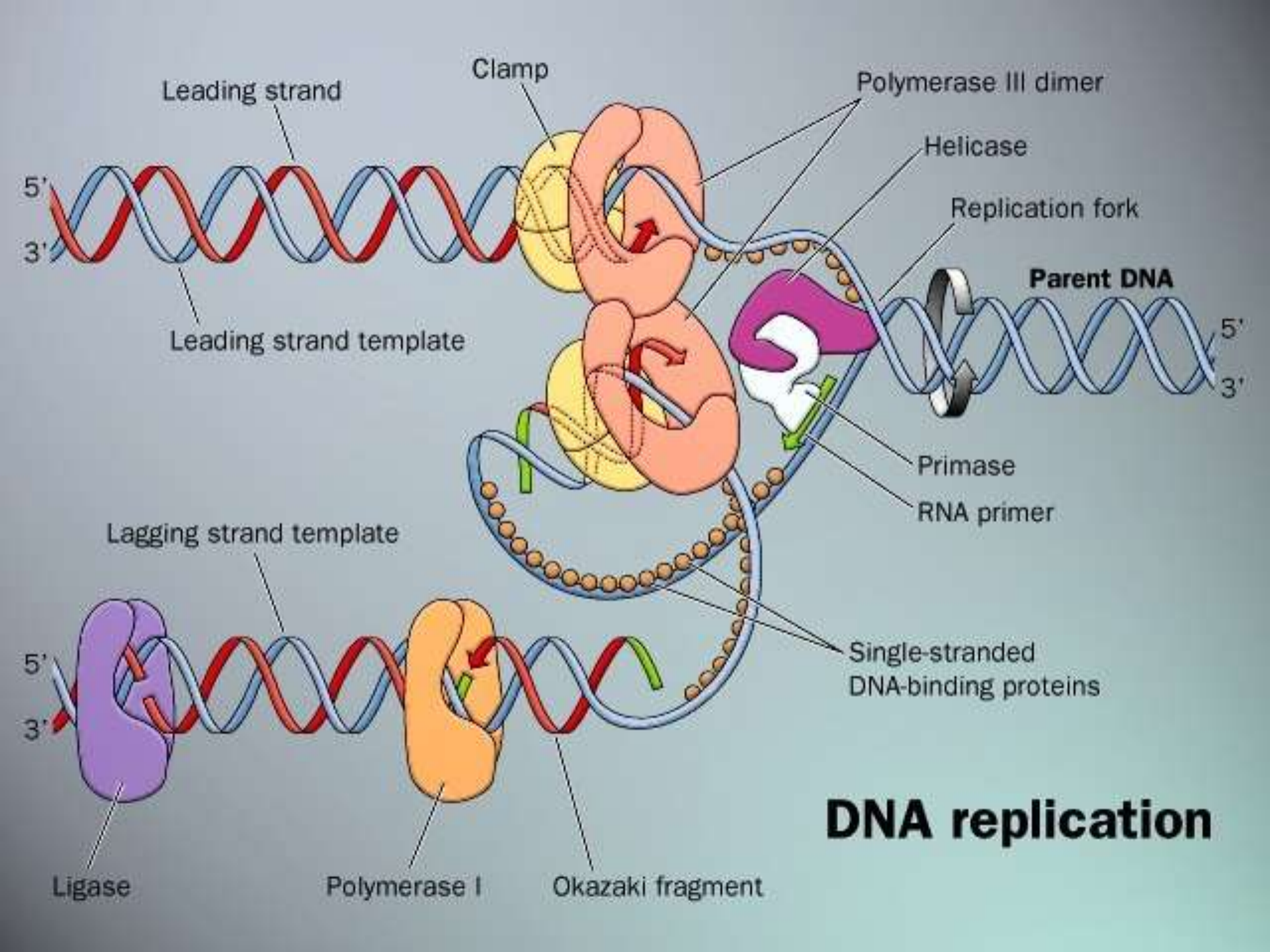
- In this phase, the synthesis of two new daughter strand takes place complementary to the template strand
- *DNA polymerase III* is the enzyme that synthesizes the daughter strands
- At this point*, a primer* is needed so that DNA polymerase III can begin to act.
- A primer is a short segment of RNA, that provides *3'-OH group* to which a nucleotide can be added
- This phase is marked by the synthesis of *leading strand and lagging strand*
- Leading strand is synthesized continuously in *5' to 3'* direction along the direction of the movement of replication fork
- Lagging strand synthesis occurs discontinuously *by loop formation* in short segments called *Okazaki fragments*.
- The lagging strand is *looped* so that DNA synthesis proceeds steadily on both the leading and lagging strand templates at the same time.
- The synthesis of Okazaki fragments on the lagging strand requires *DnaB helicase and DnaG primase* that constitute a functional unit within the replication complex, the *primosome.*
- Of the two core subunits of DNA polymerase III , one of the core subunit cycles from one Okazaki fragment to the next on the looped lagging strand.
- *DnaB helicase* first unwinds the replication fork
- *DNA primase* then associates with DnaB helicase, which synthesizes a short RNA primer
- The clamp loading complex of DNA pol III loads a *β-sliding clamp* to the primer
- The primer is then extended by the DNA pol III, which completes the synthesis of one Okazaki fragment
- When synthesis of an Okazaki fragment has been completed, replication halts, and the core subunits of DNA polymerase III dissociate from their sliding clamp (and from the completed Okazaki fragment) and associate with the new clamp This initiates synthesis of a new Okazaki fragment. Once an Okazaki fragment has been completed, its RNA primer is removed and replaced with DNA by DNA polymerase I, and the remaining nick is sealed by DNA ligase .
- DNA ligase catalyzes the formation of phosphodiester bond


5'
3'
3'
5'
leading strand
Direction of replication fork movement
5'
5'
3'
5'
5'
lagging strand (Okazaki fragments)
3'
3'


3'
5'
5'
3'
5'
SSBP
5'
lagging
strand
3'
3'
5'
3'
Helicase
3'
3'
leading strand
5'
5'
DNA Polymerase

TERMINATION

- Replication of bacterial genome proceeds bi-directionally which terminates at a position diametrically opposite to the origin of replication.
- Replication terminates at the terminus region containing multiple copies of a 20bp sequence called ***Ter(terminus)*** sequences
- Ter sequence works as the binding site for protein ***Tus (terminus utilization substance)*** which stops the DnaB helicase, resulting in termination of DNA replication
- The completed chromosomes then partitioned into two daughter cells during cell division


Leading strand
Clamp
Polymerase III dimer
Helicase
Replication fork
5'
3'
Parent DNA
Leading strand template
5'
3'
Primase
RNA primer
Lagging strand template
Single-stranded
DNA-binding proteins
5'
3'
Ligase
Polymerase I
Okazaki fragment
DNA replication

CONCLUSION

- DNA replication is semi-conservative, with each existing strand serving as a template for the synthesis of a new strand.
- Replication begins at, and proceeds bi-directionally from the point of origin.
- On one strand (the leading strand), synthesis is continuous; on the other strand (the lagging strand) synthesis is discontinuous, in short fragments called Okazaki fragments,which are subsequently ligated by DNA ligase
- DNA polymerase III is the main replication enzyme, DNA polymerase I is responsible for special functions during replication and repair
- Ter sequence works as the binding site for protein Tus (terminus utilization substance) which stops the DnaB helicase, resulting in termination of DNA replication

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