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DNA Replication In Prokaryotes

BY

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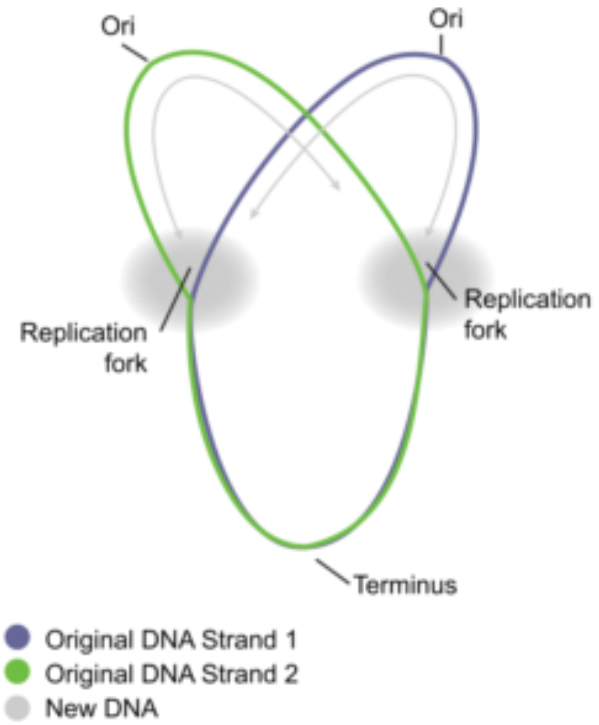
ZOOLOGY: SEM- V, PAPER- C11T: MOLECULAR BIOLOGY, UNIT 2: DNA REPLICATION

There are specific nucleotide sequences called **origins of replication** where replication begins. *E. coli* has a single origin of replication on its one chromosome, as do most prokaryotes as show in the figure below:



DNA replication in prokaryotes, which have one circular chromosome.

- DNA replication has been very well studied in prokaryotes primarily because of the small size of the genome and because of the large variety of mutants that are available.

- E. coli has 4.6 million base pairs in a single circular chromosome and all of it gets replicated in approximately 42 minutes, starting from a single site along the chromosome and proceeding around the circle in both directions.

- This means that approximately 1000 nucleotides are added per second. Thus, the process is quite rapid and occurs without many mistakes.

- DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process.

- One of the key players is the enzyme DNA polymerase, which adds nucleotides one by one to the growing DNA chain that are complementary to the template strand.

- The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them, similar to ATP which has three phosphate groups attached.

- When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain.

- In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair.

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Phases of DNA Replication in Prokaryotes

The three main phases of DNA replication in prokaryotes are:

1. Initiation

2. Elongation

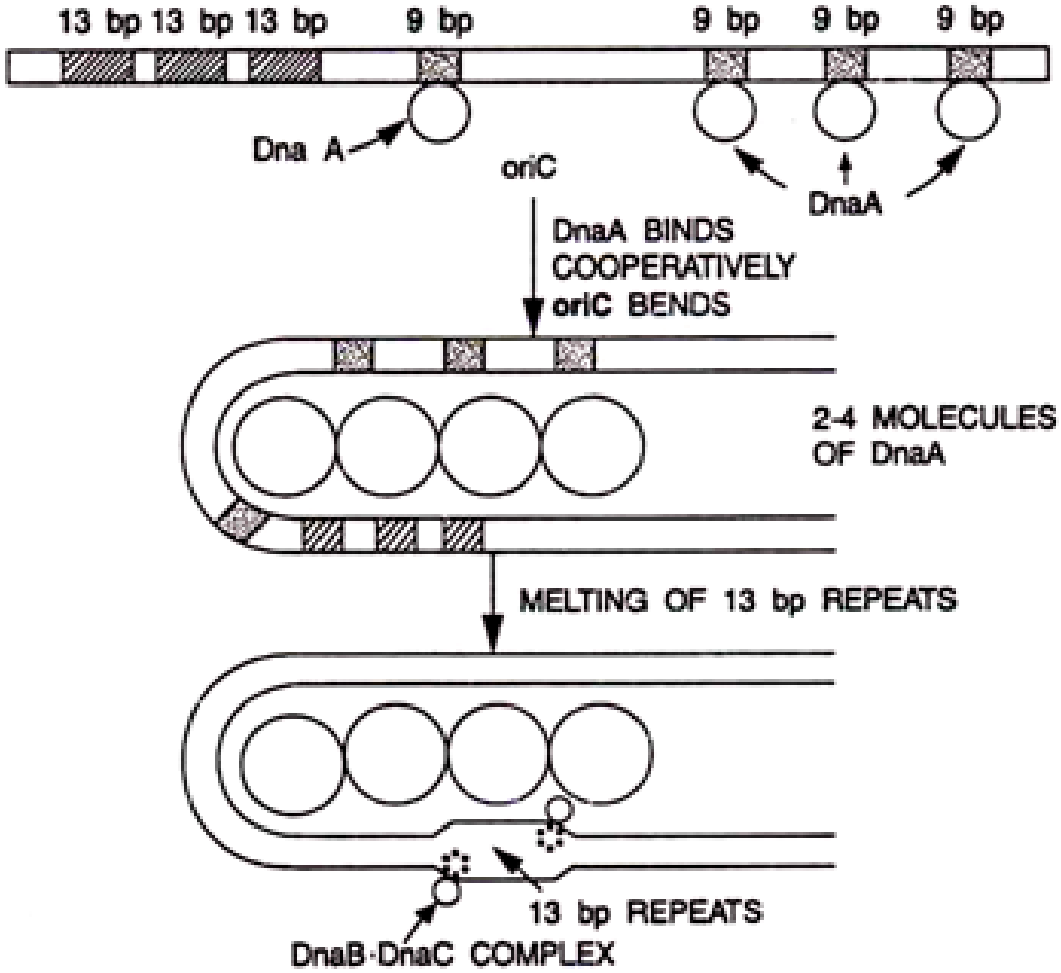
3. Termination

Phase: 1. Replication Initiation:

1. Recognition of origin,
2. The origin of replication is approximately 245 base pairs long and is rich in AT sequences.
3. This sequence of base pairs is recognized by certain proteins that bind to this site.
4. DNA melting, i.e., separation of the two strands in the origin region,
5. Stabilization of the single strands,
6. Assembly of primosome at the two forks so produced, and finally, and
7. Start of synthesis of the two daughter strands.

- Replication initiation in E. coli requires 6 proteins, viz., DnaA, DnaB, DnaC, HU, gyrase and SSBP (single strand binding proteins).
- First, 2-4 molecules of DnaA bind oriC; this results in the folding of the origin Oric DNA around DnaA aggregate.
- As a result, DnaA now induces melting at OricC.
- Now an aggregate having 6 molecules each of DnaB and DnaC binds to each of the three separate single-stranded regions produced by DnaA.
- The aggregate eventually displaces DnaA, and DnaC loads the DnaB hexamer at the two forks produced by melting.

- An enzyme called helicase unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs.
- ATP hydrolysis is required for this process because it requires energy.
- DnaB functions as helicase and begins to unwind the DNA.
- Gyrase facilitates unwinding by helicase as it provides a swivel. SSBP bind to the single-stranded regions so produced and stabilize them.
- Initiation of replication generally requires ~ 60 bp of unwound DNA, and the process consumes ATP.
- One DnaB hexamer binds to each of the two forks produced by unwinding at the origin.



In *E. coli*, replication initiation begins with binding of DnaA to *oriC*, which induces melting. DnaB (⁘) then attaches to the potential forks and begins unwinding.

- As the DNA opens up, Y-shaped structures called replication forks are formed.
- Once a replication fork is generated, primosome assembles at the origin, and initiates primer synthesis; this is called **priming**.
- Priming occurs only once and at the origin for the replication of the leading strand.
- But for replication of the lagging strand, priming occurs repeatedly at intervals of 1000 to 2000 bases.
- Priming reaction at OriC is rather simple the primosome consists of a single protein, DnaG.

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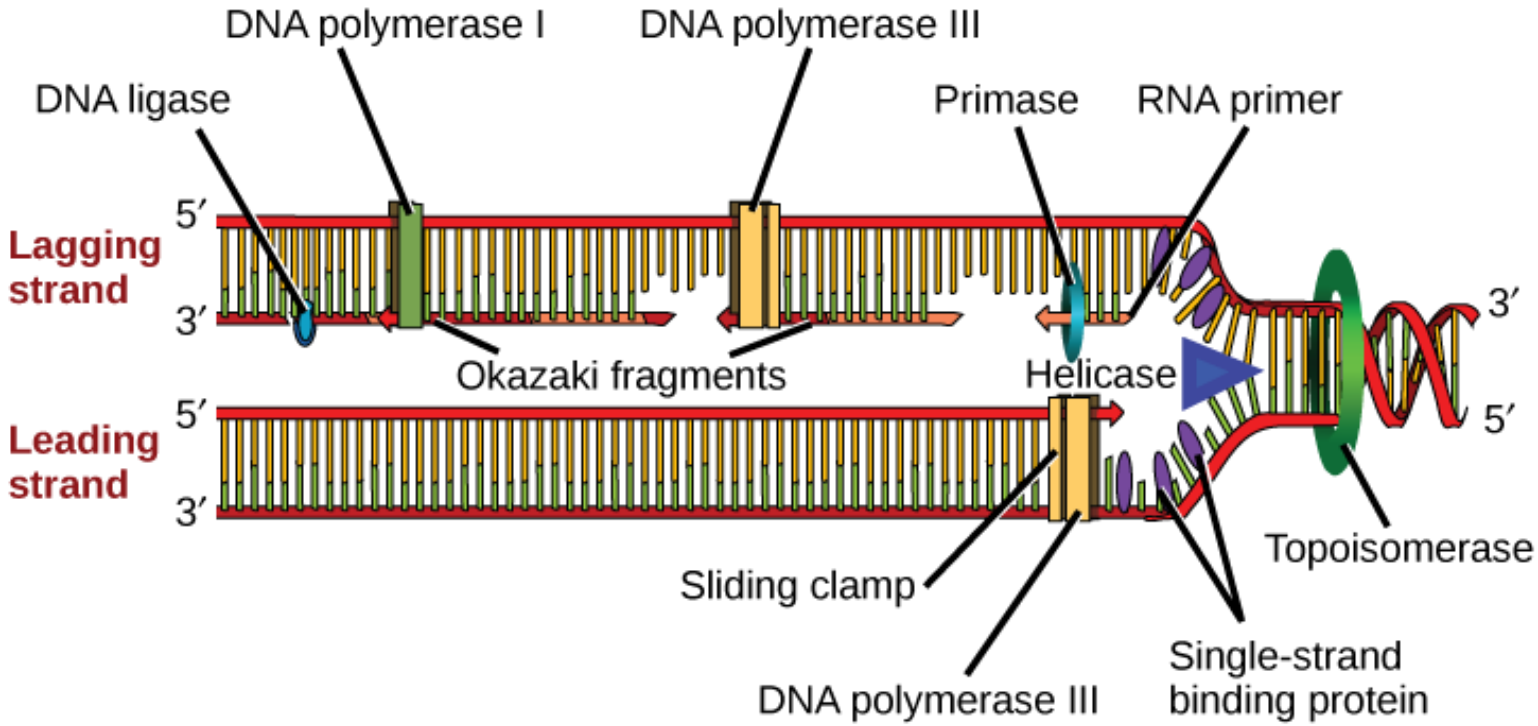


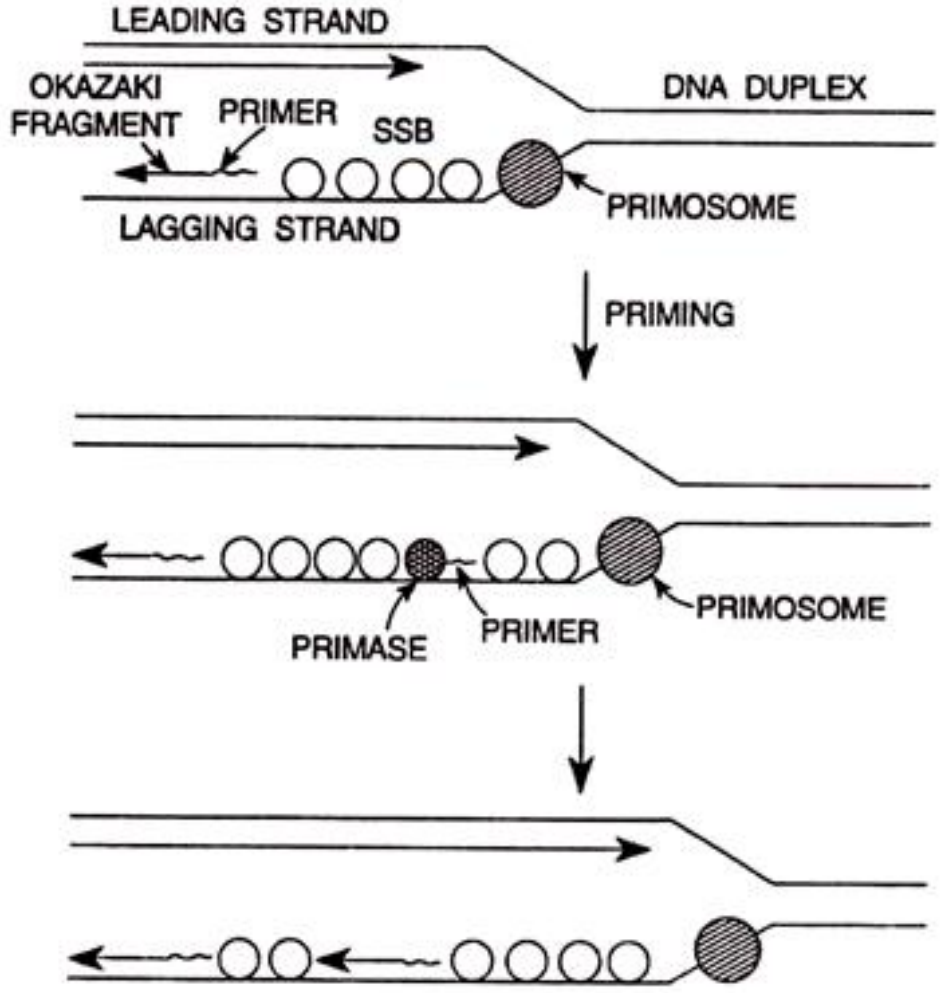
Replication fork is formed when helicase separates the DNA strands at the origin of replication. The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this supercoiling. Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming. Primase synthesizes an RNA primer. DNA polymerase III uses this primer to synthesize the daughter DNA strand. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. DNA polymerase I replaces the RNA primer with DNA. DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule.

- DnaG needs to be activated by DnaB.
- DnaB also serves as helicase, while DnaG carries out primer synthesis; primers of 15-50 bases are normally synthesized.
- The replication fork proceeds in the 5'→ 3′ direction in relation to the lagging strand.
- Single-strand binding proteins (SSBPs) coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.
- The replication fork advances and generates a single-stranded region of the lagging strand bound to SSBP ahead of the primosome.

- The primosome moves along this single-stranded region.
- When the primosome reaches a site at which priming can occur, it synthesizes an RNA primer.
- This primer sponsors synthesis of a new Okazaki fragment.

Energy from ATP is required during:

- Melting of DNA by DnaA,
- Release of DnaB at the forks by DnaC,
- Helicase action of DnaB,
- Swivel action of DNA gyrase,
- Activation of primase DnaG by DnaB, and
- Activation of DNA polymerase III to begin replication.



A simplified schematic representation of the events involved in priming during replication of the lagging strand. Prisomosome may consist of simply DnaG at *oriC* or DnaG plus 5 other proteins in case of ϕX type replicons.

Phase: 2. Primer Elongation (DNA Replication):

- Once the primer has been synthesized, DNA synthesis is taken up by replisome, which is a complex of proteins.
- In *E. coli*, DNA replication activity is provided by DNA polymerase III component of replisome.
- DNA polymerase III, also known as DNA pol III, adds nucleotides one by one to the growing DNA chain.
- The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them.

- ATP structurally is an adenine nucleotide which has three phosphate groups attached; breaking off the third phosphate releases energy.
- In addition to ATP, there are also TTP, CTP, and GTP.
- Each of these is made up of the corresponding nucleotide with three phosphates attached.
- When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the existing chain.
- In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. DNA pol III is the enzyme required for

DNA synthesis; DNA pol I is used later in the process and DNA pol II is used primarily required for repair.

- Each *E. coli* cell has ~ 10 molecules of DNA polymerase III; most of these molecules are associated with replication forks.
- The complete enzyme, holoenzyme, molecule has the following subunits; α_2, θ_2, ε_2 Υ, χ, ψ, δ, δ', τ_2, β_4.

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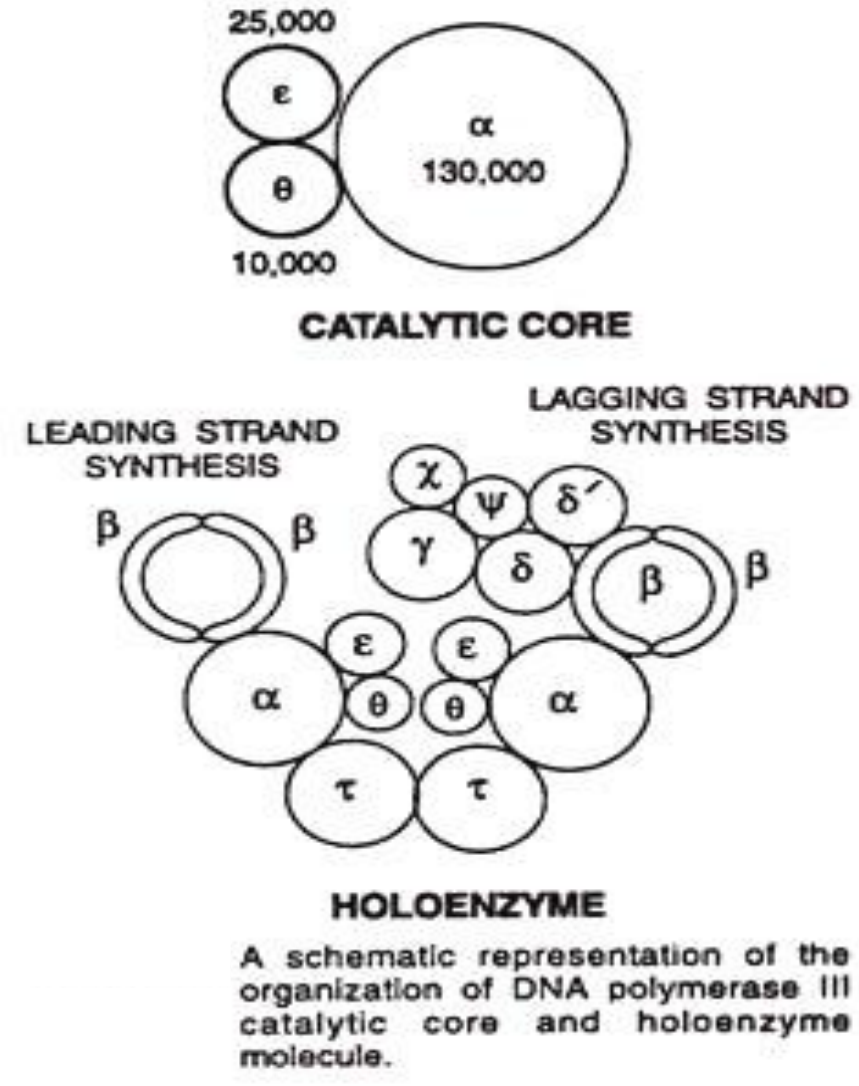


A schematic representation of the organization of DNA polymerase III catalytic core and holoenzyme molecule.

The enzyme is assembled at the replication fork as follows:

1. First, the γ- δ complex (subunits γ δ δ' X ψ) or 'clamp loader' and a pair of β subunit (the 'clamp') recognize the primed-template and bind to it.
2. They now attach to a catalytic core (∝ θ ε subunits).
3. Subunit τ now joins the complex. It brings two more β subunits and another catalytic core to the complex. This generates a DNA polymerase III holoenzyme.

➢ According to one model, a single holo-enzyme molecule functions at one replication fork.

- Each holoenzyme molecule has 2 catalytic cores; one catalytic core catalyzes the replication of leading strand, while the other catalyzes that of the lagging strand.
- In the case of leading strand, the catalytic core extends the primer one nucleotide at a time.
- DnaB progressively unwinds the duplex and the replication fork moves along.
- Replication of the lagging strand will begin sometime later.

- When DnaB associated with the advancing fork reaches a site suitable for priming, it activates DnaG to synthesize a primer in the normal 5′ → 3′ direction, i.e., moving from the fork toward the origin.
- When the primer become 10-14 bases long, the other catalytic core begins to elongate this primer in the 5'→ 3′ direction.
- The lagging strand, is in effect, pulled up by the replisome in the process of replication; it therefore, forms a progressively larger loop between the fork and the replisome.

- When the replisome reaches the 5′-end of the primer of the previous Okazaki fragment, it stops replication and dissociates from the lagging strand.
- Meanwhile DnaB continues to move forward with the replication fork.
- When it reaches the appropriate site, it again induces primer synthesis by DnaG and the events described above take place again.
- In eukaryotes, two different enzymes are used to replicate the leading and the lagging strands.

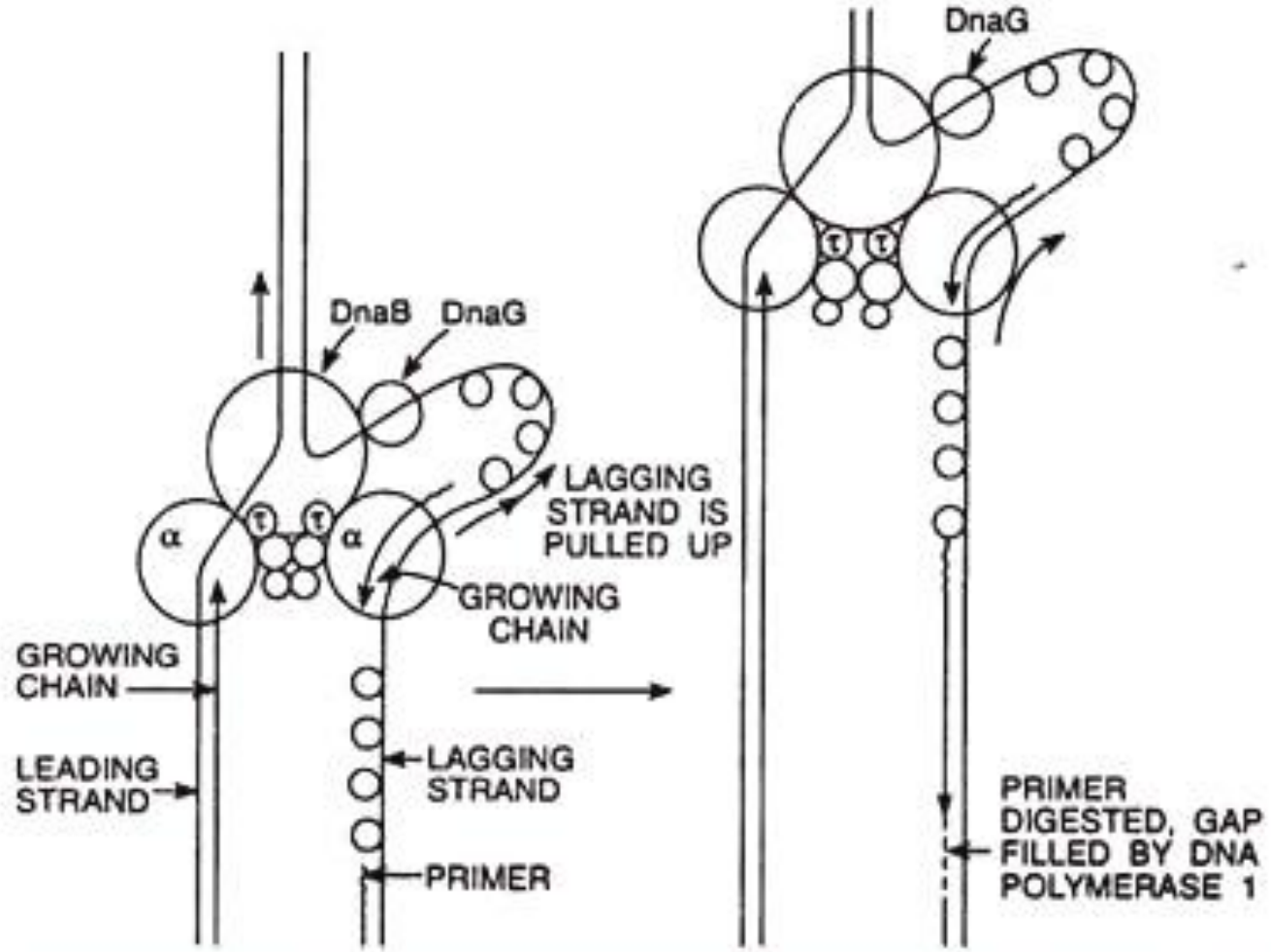


FIG. 28.13. Coordinated synthesis of leading and lagging strands by the same holoennzyme molecule of DNA polymerase III.

- Leading strand is replicated by DNA polymerase δ, while replication of the lagging strand is due to DNA polymerase ε.
- Primase activity is due to DNA polymerase α, which primes both the leading and the lagging strands.
- It also begins to synthesize DNA using this primer, but is soon replaced by DNA polymerase δ (in the case of leading strand) and ε (in the case of lagging strand).
- DNA polymerase is able to add nucleotides only in the 5′ to 3′ direction (a new DNA strand can be only extended in this direction). It requires a free 3′-OH group (located on the sugar) to which it can

add the next nucleotide by forming a phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide.

- This essentially means that it cannot add nucleotides if a free 3′-OH group is not available.
- So this problem is solved with the help of a primer that provides the free 3′-OH end.
- Another enzyme, RNA primase, synthesizes and RNA primer that is about five to ten nucleotides long and complementary to the DNA. RNA primase does not require a free 3′-OH group.

- Because this sequence primes the DNA synthesis, it is appropriately called the primer.
- DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand.
- The replication fork moves at the rate of 1000 nucleotides per second.
- DNA polymerase can only extend in the 5′ to 3′ direction, which poses a slight problem at the replication fork.

- As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5′ to 3′ direction and the other is oriented in the 3′ to 5′ direction.
- One strand, which is complementary to the 3′ to 5′ parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction.
- This continuously synthesized strand is known as the leading strand.
- The other strand, complementary to the 5′ to 3′ parental DNA, is extended away from the replication fork, in small fragments known as Okazaki fragments, each requiring a primer to start the synthesis.

- Okazaki fragments are named after the Japanese scientist who first discovered them. The strand with the Okazaki fragments is known as the lagging strand.
- The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments.
- The overall direction of the lagging strand will be 3′ to 5′, and that of the leading strand 5′ to 3′.
- A protein called the "**sliding clamp**" holds the DNA polymerase in place as it continues to add nucleotides.

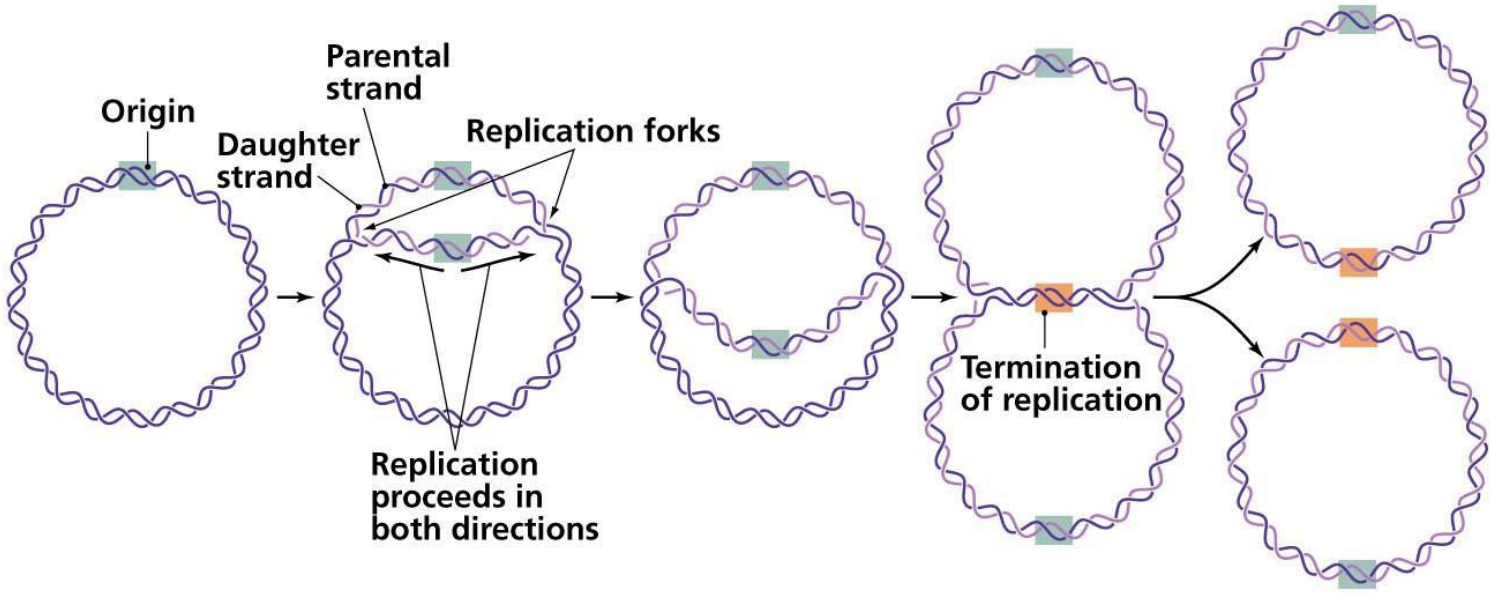
- The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place.
- Topoisomerase prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it.
- As synthesis proceeds, the RNA primers are replaced by DNA pol I, which breaks down the RNA and fills the gaps with DNA nucleotides.
- The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme DNA ligase that catalyzes the formation of

phosphodiester linkage between the 3′-OH end of one nucleotide and the 5′ phosphate end of the other fragment.

Phase: 3. Termination of DNA Replication:

- In *E. coli*, termination is signalled by specific sequences called ter (Terminal Sequence) elements, which serve as a binding site for protein Tus.
- Tus (Terminus Utilization Sequence) protein binds to ter element and stops DnaB (helicase) from unwinding DNA.
- This stops the movement of the replication fork.

- The leading strand is replicated up to the ter element, while the lagging strand replication is stopped 50-100 bp before the ter element.
- It is significant that Tus protein is able to stop fork movement in only one direction.



Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division. The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication.
2. Helicase opens up the DNA-forming replication forks; these are extended in both directions.
3. Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
4. Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling (over-winding).
5. Primase synthesizes RNA primers complementary to the DNA strand.

6. DNA polymerase III starts adding nucleotides to the 3′-OH (sugar) end of the primer.
7. Elongation of both the lagging and the leading strand continues.
8. RNA primers are removed and gaps are filled with DNA by DNA pol I.
9. The gaps between the DNA fragments are sealed by DNA ligase.

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The enzymes involved in prokaryotic DNA replication and the functions of each are given in the table below:

Prokaryotic DNA Replication: Enzymes and Their Function	
Enzyme/protein	**Specific Function**
DNA pol I	Removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5'-3' direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments to

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Prokaryotic DNA Replication: Enzymes and Their Function

Enzyme/protein	Specific Function
	create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Topoisomerase	Helps relieve the strain on DNA when unwinding by causing breaks, and then resealing the DNA
Single-strand binding proteins (SSB)	Binds to single-stranded DNA to prevent DNA from rewinding back.
HU Protein	HU is a small (10 kDa) bacterial histone-like

Prokaryotic DNA Replication: Enzymes and Their Function	
Enzyme/protein	**Specific Function**
	protein that resembles the eukaryotic Histone H_2B. HU acts similarly to a histone by inducing negative supercoiling into circular DNA with the assistance of topoisomerase. The protein has been implicated in DNA replication, recombination, and repair.

Significance of DNA Replication:

1. DNA replication is the process by which an organism duplicates its DNA into another copy that is passed on to daughter cells.

2. Replication occurs before a cell divides to ensure that both cells receive an exact copy of the parent's genetic material.
3. DNA replication uses a semi-conservative method that results in a double-stranded DNA with one parental strand and a new daughter strand.
4. Prokaryotic DNA replication is often studied in the model organism *E. coli*, but all other prokaryotes show many similarities.
5. DNA Replication is bi-directional and originates at a single origin of replication (OriC).
6. It takes place in the cell cytoplasm.
7. Its synthesis occurs only in the 5′to 3′direction.

8. It occurs with high degree of fidelity.
9. It is a multi-enzymatic process.
10. Individual strands of DNA are manufactured in different directions, producing a leading and a lagging strand.
11. Lagging strands are created by the production of small DNA fragments called Okazaki fragments that are eventually joined together.

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