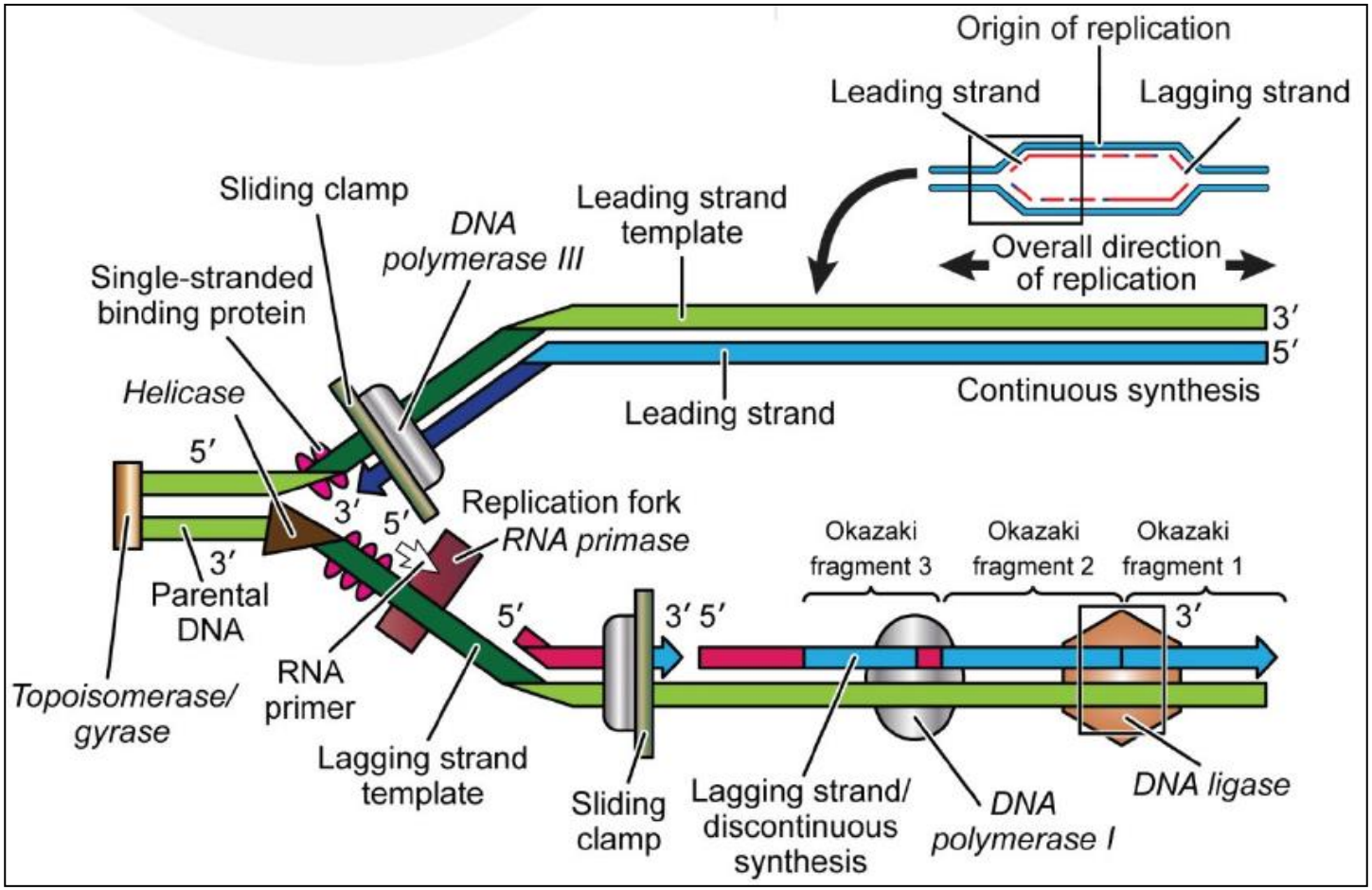
DNA REPLICATION IN PROKARYOTES AND EUKARYOTES



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- DNA carries all of the genetic information that is transmitted to the daughter cells after division. The knowledge of the structure of DNA enabled scientists to work out the detailed mechanisms of processes such as DNA replication and recombination.
- During replication, DNA makes a copy of itself by copying the genetic information. DNA replication is also referred as duplication.

- Before each division, duplication of DNA molecules takes place by replication resulting in formation of copies of molecules. DNA replication is the process by which DNA makes a copy of itself during cell division.
- In prokaryotes, a single, double-stranded DNA molecule is present in the form of a loop or circle. In contrast there are several double-stranded linear DNA molecules present in the genome of eukaryotes.

Role of different enzymes used in DNA replication

Enzyme	Function in DNA Replication
DNA Helicase	Also known as helix destabilizing enzyme. Unwinds the DNA double helix at the Replication Fork.
DNA Polymerase	Synthesizes new duplex DNA strand by adding nucleotides in the 5´ to 3´ direction. Also performs proof-reading and error correction.
Primase	Provides a starting point of RNA (or DNA) for *DNA polymerase* to begin synthesis of the new DNA strand.
DNA Gyrase	A kind of topoisomerase that removes DNA supercoils ahead of replication fork.
Topoisomerase	Relaxes the DNA from its super-coiled nature.
Single-Strand Binding (SSB) Proteins	Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus maintaining the strand separation, and facilitating the synthesis of the nascent strand.
DNA clamp	A protein which prevents *DNA polymerases* from dissociating from the DNA parent strand during the process of replication.
DNA Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Telomerase	Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes.

Various steps involved in DNA replication in prokaryotes can be summarized as follows:

1. The double helix structure of the DNA molecule is unzipped. This process is carried out with the help of an enzyme *helicase* that breaks the hydrogen bonding between the complementary bases.
2. The separation of DNA strands into two single strands creates a 'Y' shaped replication 'fork'. Two single strands act as templates for making the new strands of DNA.
3. One of the strands is oriented in the 3′→5′ direction (towards the replication fork). This is referred as the leading strand. The other strand is oriented in the 5′→3′ direction (away from the replication fork). This is called as lagging strand. Because of the difference in orientation, the two strands replicate differently.
4. A fragment of RNA called Primer comes along and binds to the leading strand. The primer starts the DNA synthesis. *DNA polymerase* binds to the leading strand and moves ahead adding new complementary nucleotide bases to the DNA strand in the 5′ to 3′ direction (Fig. 11.15). This type of replication is called continuous.
5. Numerous RNA primers (synthesized by enzyme primase) bind at various points to the lagging strand. Fragments of DNA, called Okazaki fragments, are then added to the lagging strand also in the 5′→3′ direction. This type of replication is called discontinuous as the fragments will need to be joined up later (Fig. 11.15).

6. The bases are matched up (A with T, C with G) with the help of enzyme called exonuclease that removes the primer(s). The gaps are filled by complementary nucleotides. The new strand is proofread to make sure there are no mistakes in the new DNA sequence.

7. Enzyme called *DNA ligase* seals up the sequence of DNA into two continuous double strands. Hence after DNA replication, DNA molecule formed consists of one new and one old chain of nucleotides. Hence the DNA replication is described as semi-conservative.

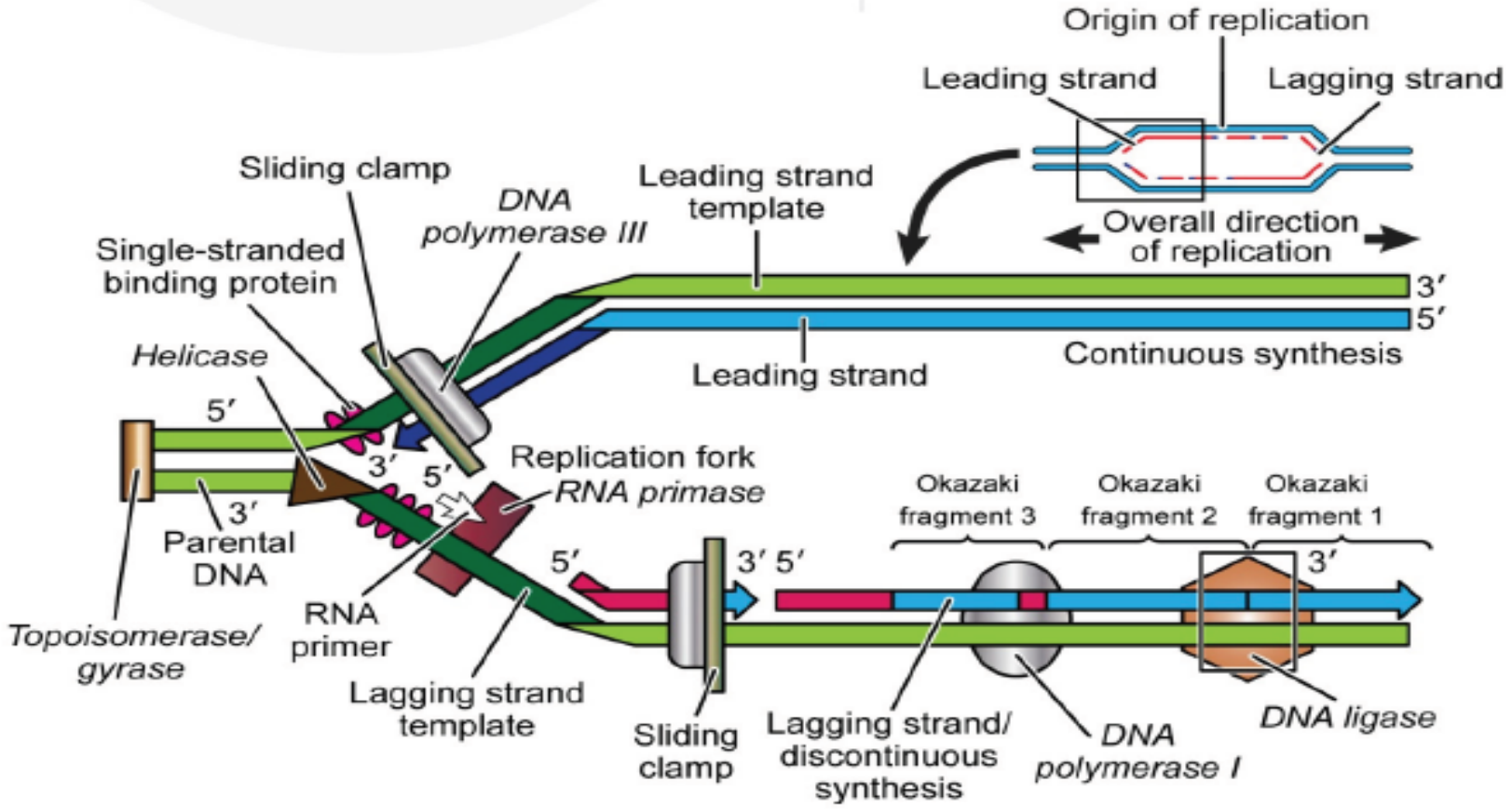

Fig. 11.15: An overview of DNA replication in prokaryotes.

- **In prokaryotes such as *E. coli* DNA replication occurs in a semi conservative manner. This refers to a replication process in which two strands separate from one another, maintain their integrity and each strand synthesizes a new complementary strand from the pool of nucleotides.**

The replication of DNA begins with unwinding of the double helix at sites called origin of replication. At these sites, the hydrogen bonds between the bases are broken and paired bases separate. The sites where the pair of replicated segments come together and join the non-replicated DNA are called as **replication fork**. In bacterial chromosome, DNA replication always begins at specific sites called the **origin**. Each origin controls the replication of a unit of DNA called a replicon. The bacteria have a single specific origin of replication

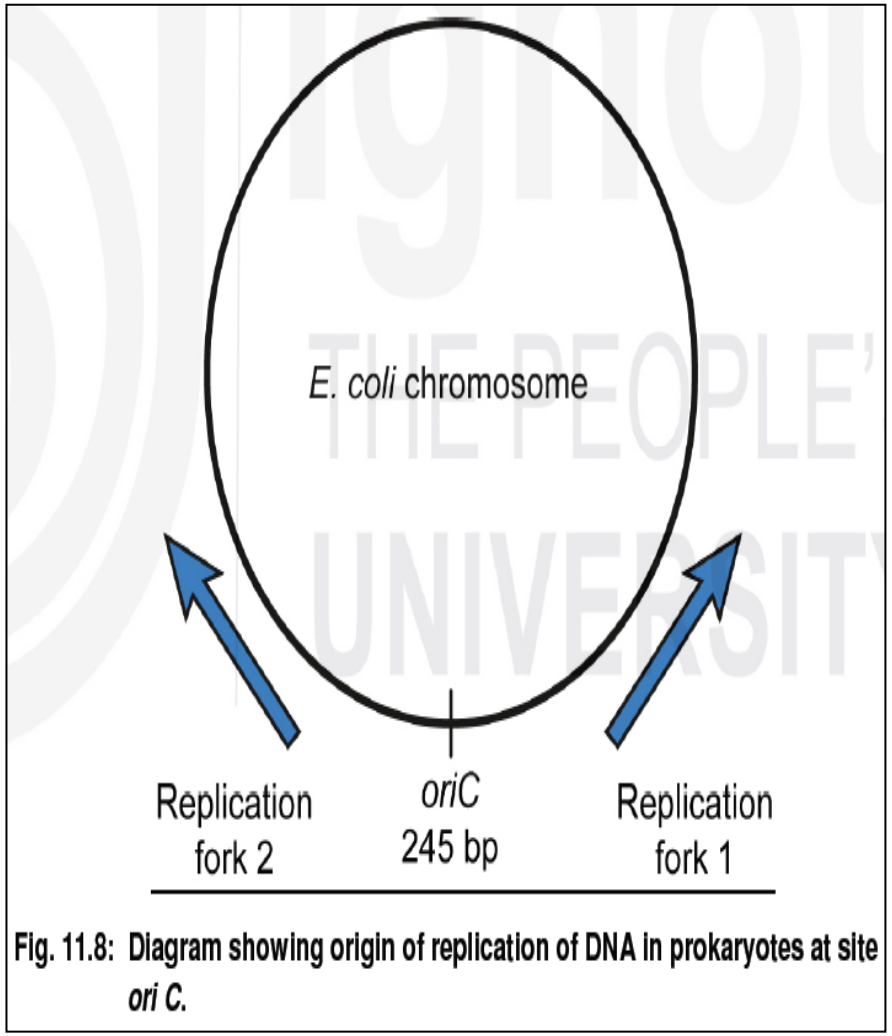

Fig. 11.8: Diagram showing origin of replication of DNA in prokaryotes at site *ori C*.

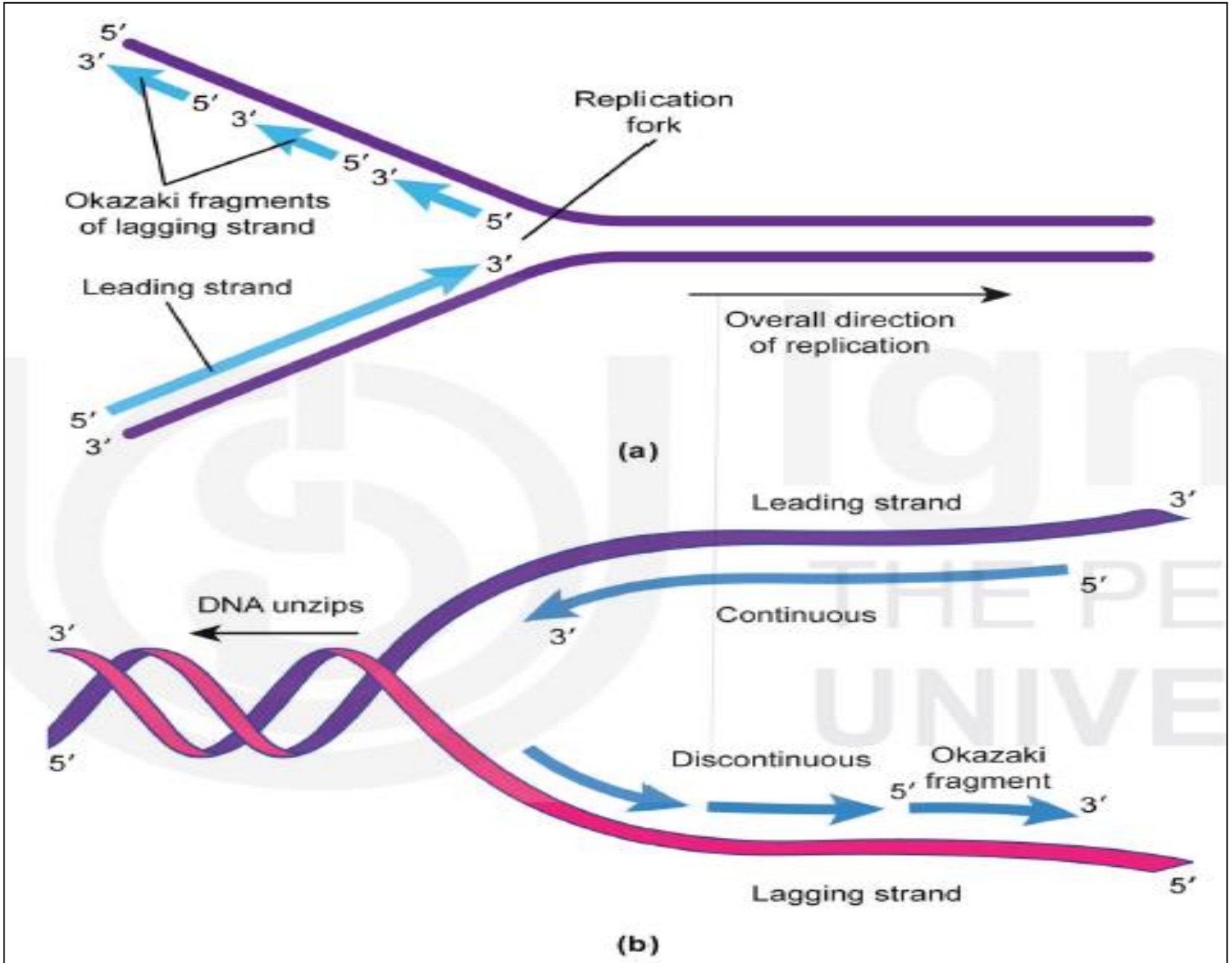

Fig. 11.10: Diagrammatic representation of the replication fork formed during DNA replication.

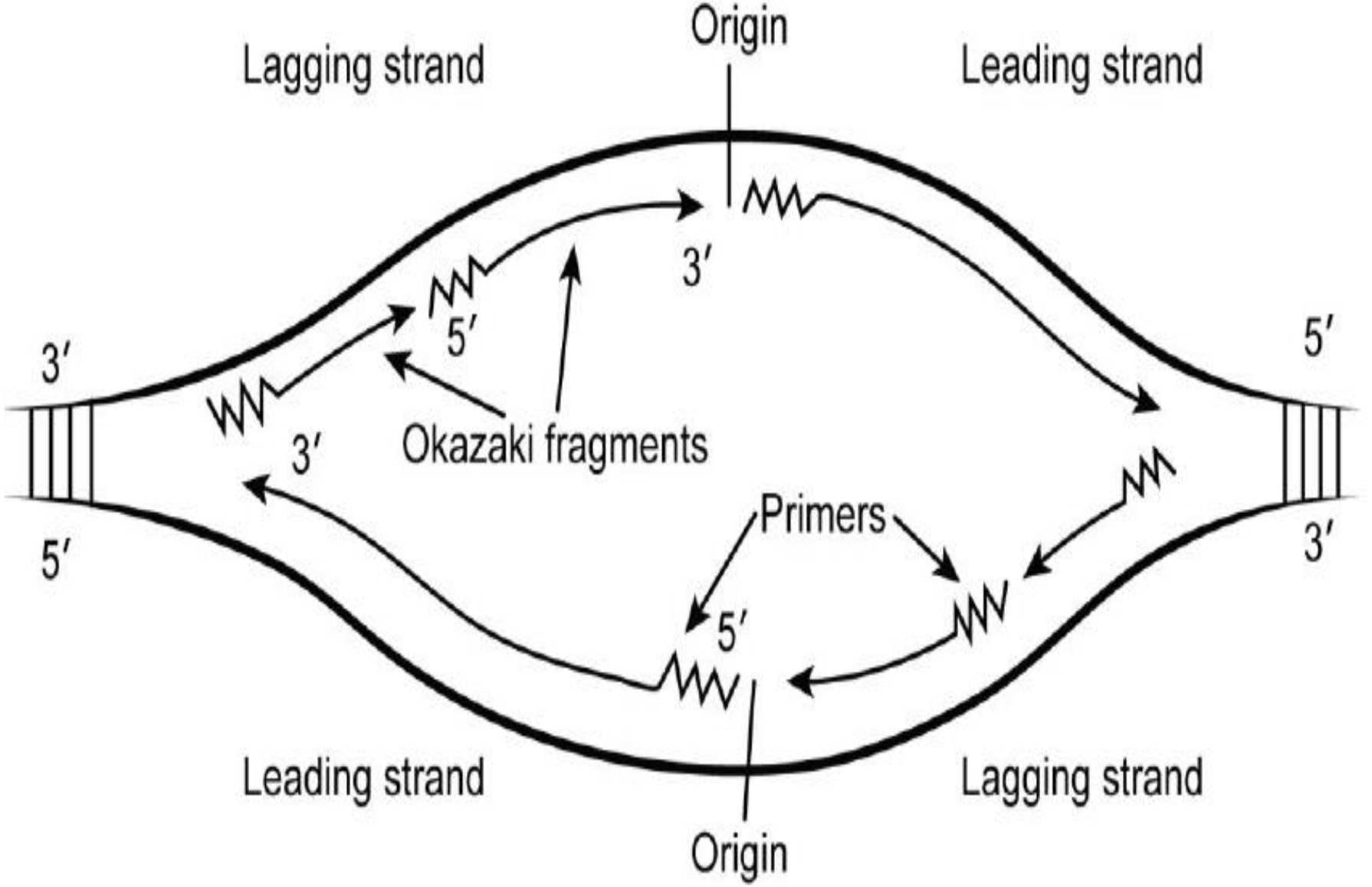

Fig. 11.11: Mechanism of DNA replication in prokaryotes.

DNA REPLICATION IN EUKARYOTES

The DNA replication in eukaryotes occurs in a semi conservative manner. In a semi conservative mode, two complementary polynucleotide chains are synthesized on a template strand. The parental DNA strand undergoes cleavage and the two new double helices formed that carry old fragments and the newly synthesized DNA (Fig.11.16). Since the parental strands are dispersed into two newly synthesized helices it is called dispersive replication.

Though most aspects of DNA replication are similar in prokaryotes and eukaryotes but still some prominent differences are noted between them. Some of the major differences have been listed below:

- The eukaryotic cells have larger genomes and complex chromosomal structures.
- DNA synthesis takes place in a small portion of the cell cycle in eukaryotes. Replication occurs in the S phase of the cell cycle.

- DNA synthesis is not continuous as in prokaryotes.
- The large DNA molecules in eukaryotes take more time to replicate of each chromosome contained a single origin.
- The eukaryotic chromosomes contain multiple origins of replication. DNA replication is initiated at multiple origins in a coordinated manner.
- Two or more *polymerases* are used for replication of leading and lagging strands at replication fork.

The process of DNA replication in eukaryotes 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 polymerase I*.
9. The gaps between the DNA fragments are sealed by *DNA ligase*.

During replication some problems are faced by eukaryotes. Some of these have been listed below:

- The chromosomes are linear in eukaryotes and they possess more amount of genetic material. A typical animal cell has 50 times more DNA than the bacterium. e.g. *E. coli* has 4.6 million base pairs, humans have 3000 million base pairs.
- Eukaryotes show high level of packaging in the form of nucleosomes (DNA wound around histones).
- *DNA polymerases* found in eukaryotes work slowly. More enzymes are required to speed up the process of replication.
- The replication initiates at multiple sites scattered some 30 to 300 kb apart.

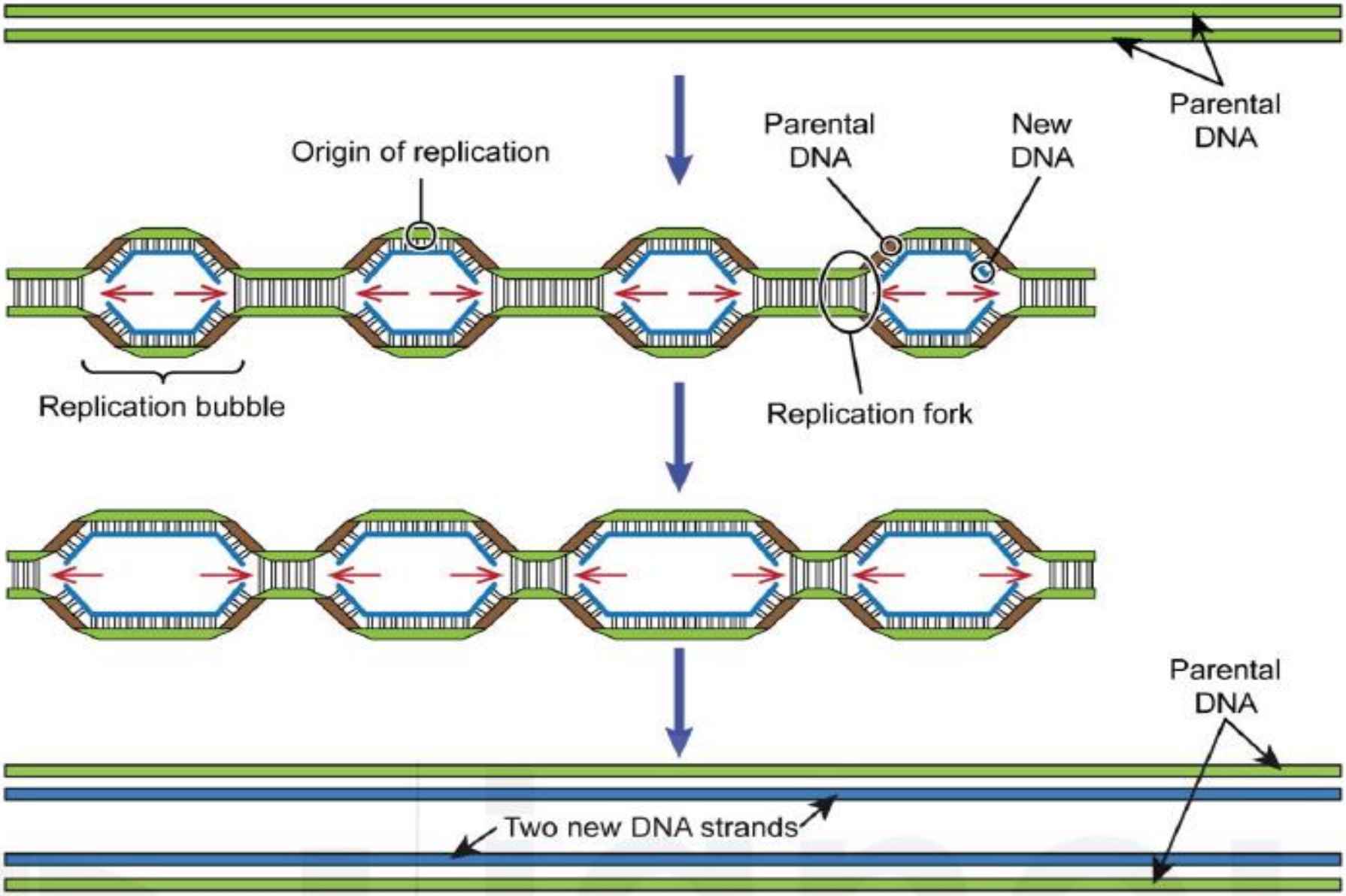

Fig.11.16: Diagrammatic representation of various origin of replication in eukaryotes.

Comparative account of DNA replication in prokaryotes and eukaryotes

DNA Replication in Prokaryotic Cells	DNA Replication in Eukaryotic Cells
Occurs in the cytoplasm	Occurs in the nucleus
There is a single origin of replication for each DNA molecule.	There are multiple origins of replication
Replication occurs at one point in each DNA molecule.	Replication at several points simultaneously in each chromosome.
Only one replication fork is formed in one DNA molecule.	Numerous replication bubbles are formed in one DNA molecule.
Both initiation and elongation is carried out by *DNA polymerase III*	Initiation is carried out by *DNA polymerase α* while elongation occurs with the help of *DNA polymerase δ* and ε.
DNA gyrase is required	*DNA gyrase* is not required.
Replication is very fast i.e. about 2000 base pairs/nucleotides are added per second.	Replication is slow i.e. about 100 nucleotides are added per second.
The Okazaki fragments are very long (1000-2000 nucleotides long)	The Okazaki fragments are short (100-200 nucleotides long)
RNA primer is removed by *DNA polymerase I*.	RNA primer is removed by *DNA polymerase β*.
DNA is circular, hence telomeres are not replicated	Telomeres present at the end of DNA are replicated

In prokaryotes, *Polymerase I, II, III* play a major role in DNA replication while in eukaryotes it is *polymerase* **α, β, γ, δ, ε** which play a crucial role in DNA replication and repair (Table 11.2).

Table 11.2: Role of different *DNA polymerases* found in eukaryotes

DNA polymerases	Subunits	3´ to 5´ *exonuclease* activity	Function
Alpha α	**4**	**No**	RNA/DNA primers, initiation of DNA synthesis
Delta δ	**4**	**Yes**	Lagging strand synthesis, DNA repair, proof reading
Epsilon ε	**4**	**Yes**	Leading strand synthesis, proof reading
Gamma γ	**2**	**Yes**	Mitochondrial DNA replication and repair/ editing
Beta β	**1**	**No**	Base-excision DNA repair
Eta η, zeta ζ, kappa κ, iota ι	**1,2,1,1**	**No**	Damaged/distorted (translesion; TLS) DNA synthesis
Theta θ, lambda λ, mu μ	**1,1,1**	**No**	DNA repair
Nu υ	**1**	**No**	Unknown
REV1	**1**	**No**	DNA repair

A SUMMARY OF DNA REPLICATION