Semi-Conservative, Bidirectional & Semi-Discontinuous Replication

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Watson and Crick proposed the double helical structure of DNA with complementary base pairing. The structure of DNA suggested the possible mechanism for replication of DNA molecules. Three models of DNA replication were suggested namely conservative, dispersive and semi-conservative (Fig. 11.1). In **conservative** type of replication, double stranded molecule is conserved as such and a new copy of DNA is synthesized from the old molecule. In **dispersive** mode of replication, the old molecule disintegrates and two molecules are synthesized, while in **semiconservative** type of replication, the two strands separate from each other, maintain their integrity and synthesize its complementary strand from the pool of nucleotides (Fig. 11.1).

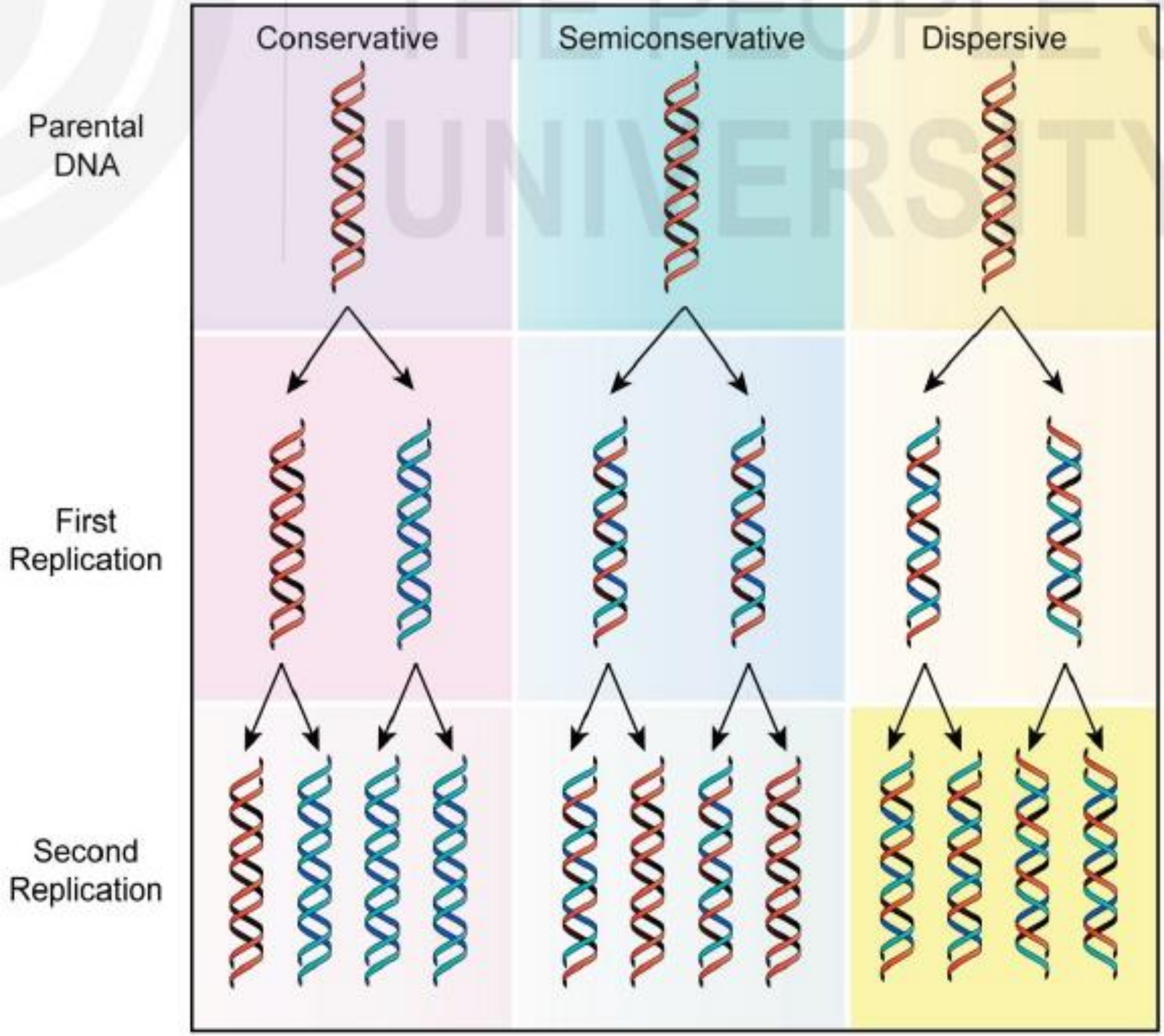


Fig.11.1: Diagram showing different modes of DNA replication.

Semi-Conservative Replication

Watson and Crick proposed that two complementary strands of the DNA double helix unwind and separate. Each strand guides the synthesis of a new complimentary strand. The sequence of bases in each parental strand is used as a template. The template guides the sequence of bases in the newly synthesized strand. This mechanism of DNA replication is called semi conservative replication. In this type of replication, half of the parental molecule is conserved. In 1958, Matthew Meselson and Franklin Stahl demonstrated that chromosome of *E. coli* replicate in a semiconservative manner.

Meselson-Stahl Experiment

Meselson and Stahl proved that DNA replication was semiconservative using nitrogen which is a major constituent of DNA. They grew *E. coli* in a medium containing $^{15}NH_4Cl$. The medium contained a heavy isotope of nitrogen, ^{15}N (^{14}N is the naturally abundant isotope). After growing the bacteria on ^{15}N containing medium for several generations, they found that DNA of cells had a high density (heavier). Meselson and Stahl transferred the bacteria grown on a ^{15}N medium to a medium containing only ^{14}N. The new DNA, replicated in the ^{14}N medium, showed density intermediate between those cells grown independently on light (^{14}N) and heavy (^{15}N) medium. Since the replication was semiconservative, double stranded DNA was synthesized in which one strand had ^{15}N DNA and the other strand was having ^{14}N DNA. If replication had been conservative, two strands have either heavy (^{15}N) or light (^{14}N) DNA (Fig.11.2). If the method of replication had been dispersive, various multiple-banded patterns would have appeared, depending on the degree of dispersiveness.

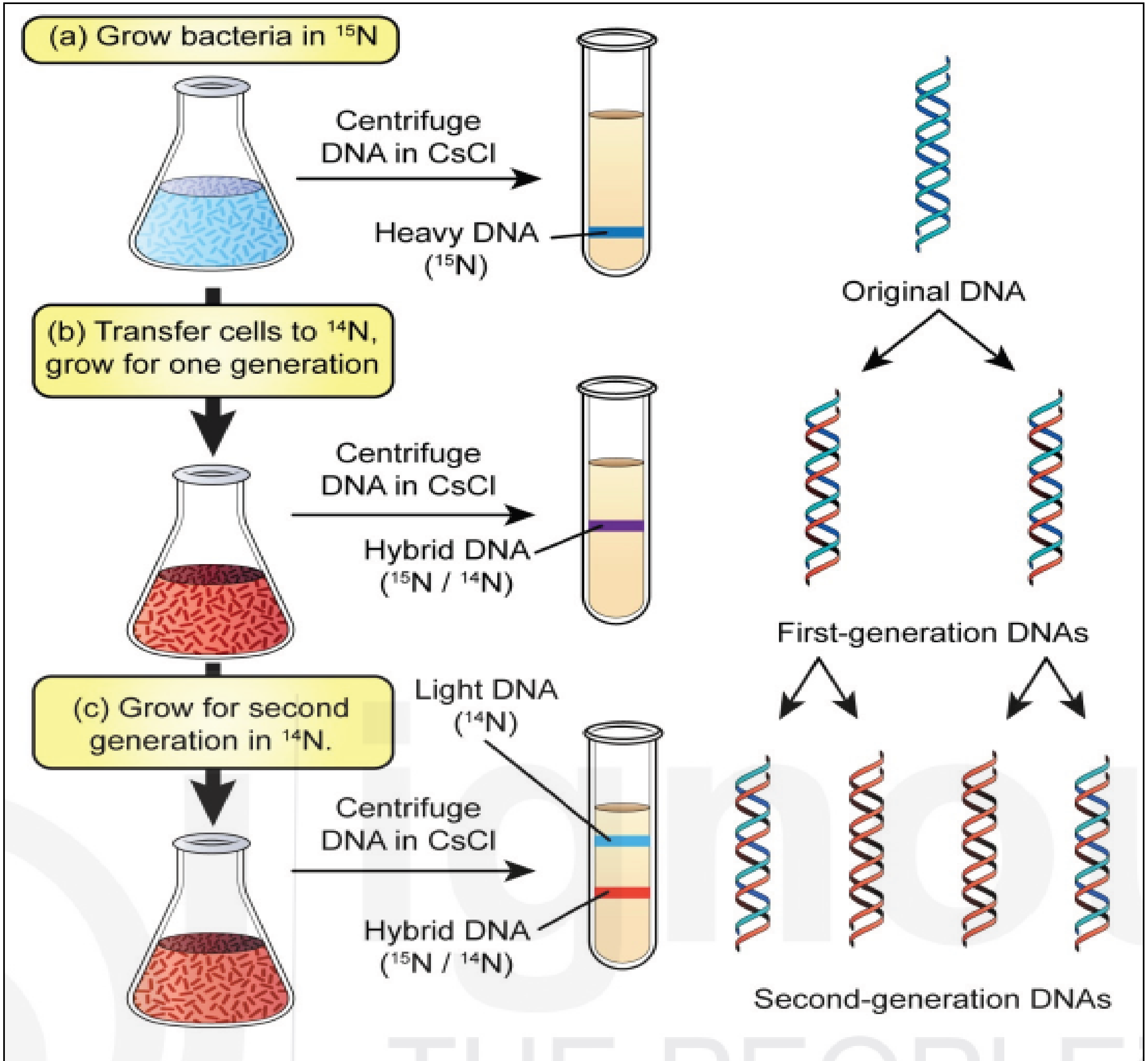


Fig. 11.2: Meselson-Stahl experiment.

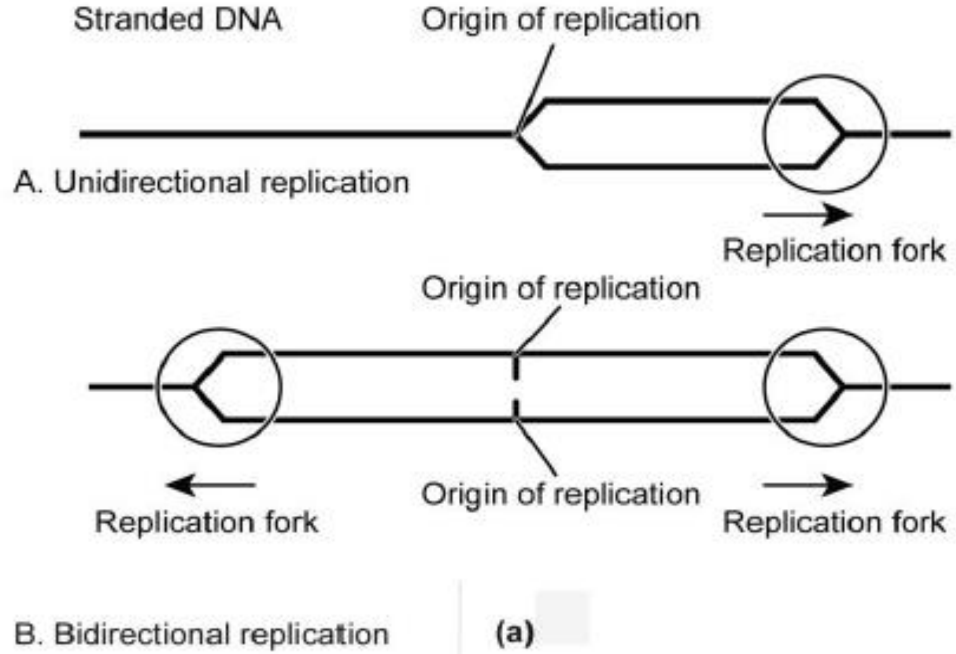
The density of the strands was determined using a technique known as **density-gradient centrifugation.** In this technique, a cesium chloride (CsCl) solution is spun in an ultracentrifuge at high speed for several hours. Eventually a density gradient is established in the tube with an increasing concentration of CsCl from top to bottom. DNA formed a band in the tube at the point where its density is the same as that of the CsCl. If DNA with different densities are added, then several bands are formed.

Meselson and Stahl took cells growing in the medium containing ^{15}N for several generations, washed them to remove the medium containing ^{15}N and transferred them to medium containing ^{14}N. The cells were allowed to grow in the presence of ^{14}N for varying periods of time. The DNA was extracted and analyzed using CsCl density gradient. The result indicated semi conservative mode of DNA replication. The DNA isolated from cells had a density half way between the densities of heavy and light DNA. The intermediate density is called as hybrid density. After two generations of growth in the medium containing ^{14}N, half of the DNA showed hybrid density (^{15}N-^{14}N) and the other half showed light density (^{14}N). The results proved the semiconservative replication in DNA.

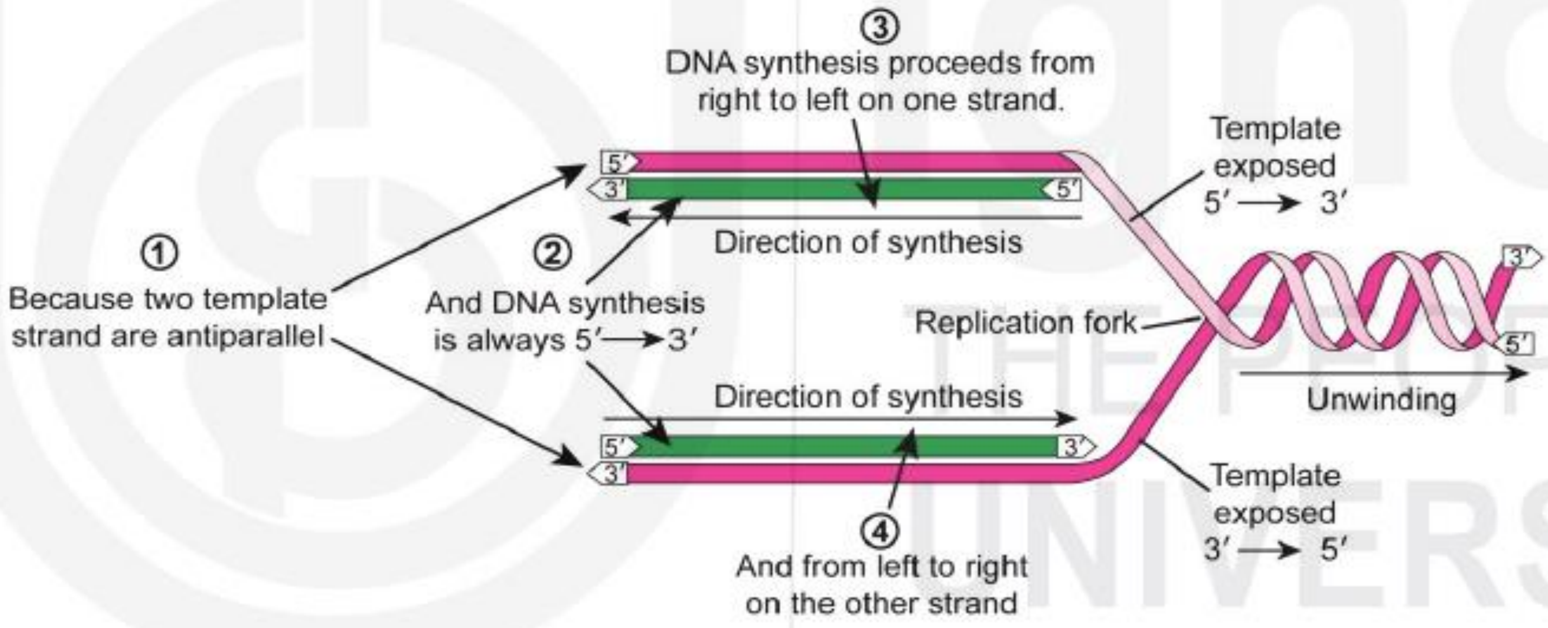
Bidirectional Replication

The replication in DNA can be **unidirectional** or **bidirectional. This depends** upon whether the replication from the point of origin proceeds only in one direction or proceeds in both the directions. Replication eye may appear in both the types unless the replication starts from one of the two ends of a linear DNA molecule. In **unidirectional replication,** one of the two ends of the replication eye will be **stationary** and the other end will move with replication.

On the other hand, in **bidirectional replication,** both ends will be moving and none will be stationary (Fig. 11.5). Distinction between unidirectional and bidirectional replication can be made by the study of autoradiographs If radioactively labelled nucleotides are used during DNA synthesis.


Stranded DNA
Origin of replication
A. Unidirectional replication
Replication fork
Origin of replication
Origin of replication
Replication fork
Replication fork
B. Bidirectional replication

(a)


③
DNA synthesis proceeds from
right to left on one strand.
Template
exposed
5′ → 3′
5′
3′
5′
Direction of synthesis
①
Because two template
strand are antiparallel
②
And DNA synthesis
is always 5′→3′
3′
Replication fork
5′
Unwinding
Direction of synthesis
5′
3′
3′
Template
exposed
3′ → 5′
④
And from left to right
on the other strand

(b)

Semi-discontinuous replication

The newly synthesized strand is synthesized only in 5′→3′ direction. One strand i.e. the leading strand is synthesized continuously because its synthesis continues as the fork advances, while the other strand i.e. the lagging strand is synthesized discontinuously because the initiation of each fragment waits for the parental strand to separate and expose the additional template (Fig. 11.12).

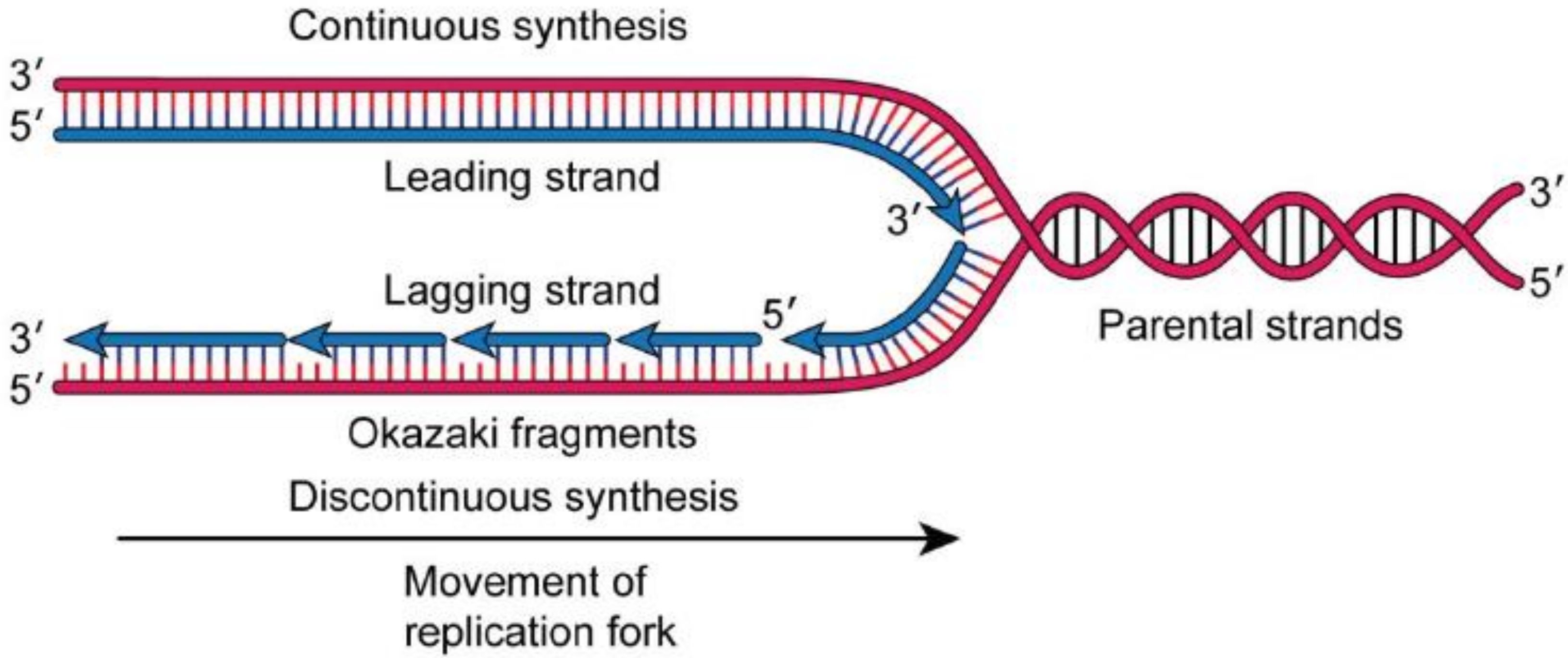


Fig. 11.12: Diagrammatic representation of Continuous and discontinuous type of DNA replication.

Since one strand is synthesized continuously and other discontinuously, the replication is called as semidiscontinuous. The leadings strand shows continuous replication by adding nucleotides to the growing end while the lagging strand shows discontinuous replication by adding short nucleotides segments to the growing end (Fig.11.13).

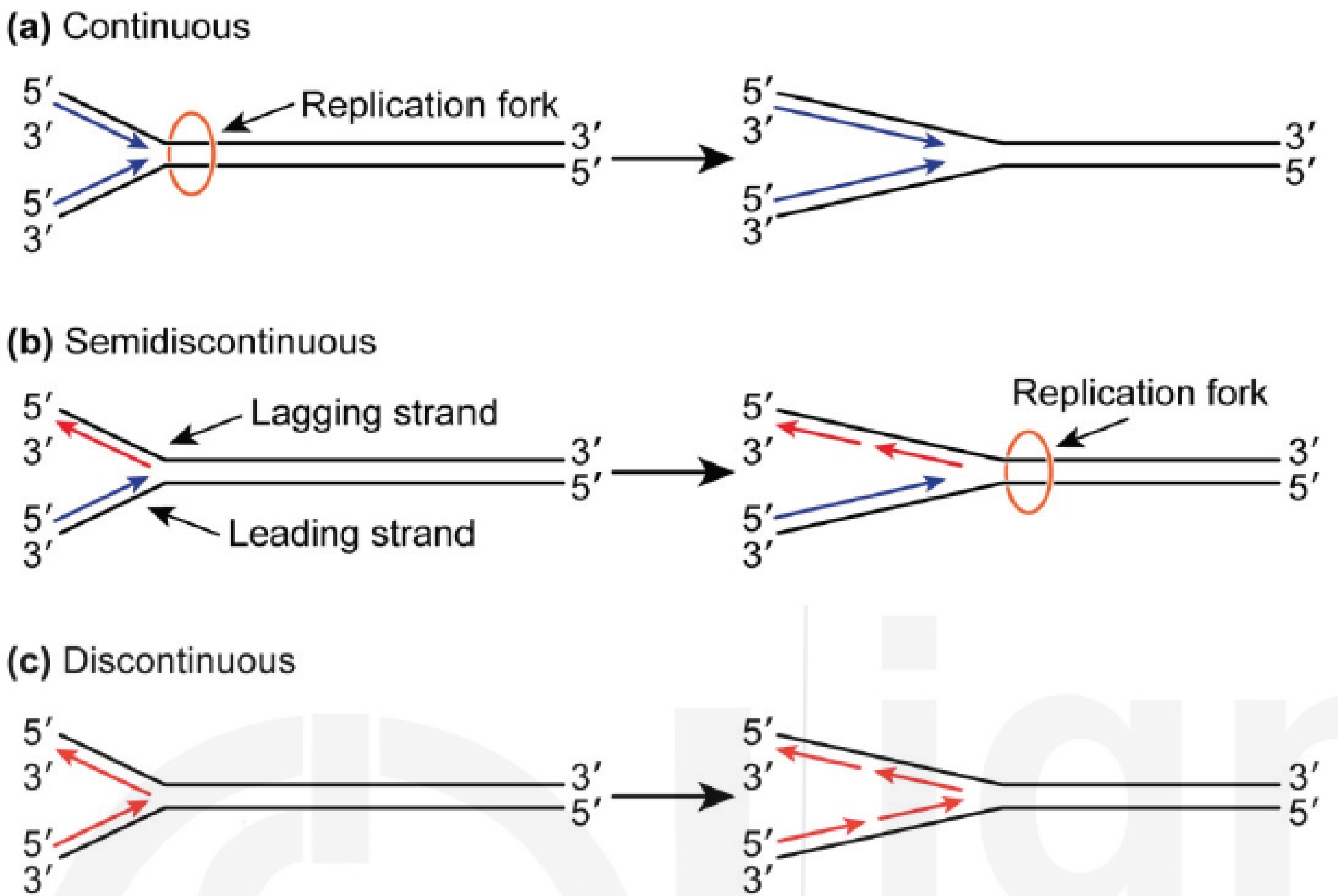


Fig. 11.13: Comparison of different types of DNA replication.