DNA Replication

Learning Outcomes:

1. Describe the structural features of DNA that enable it to be replicated.

2. Analyze the experiment of Meselson and Stahl and explain how the results were consistent with the semiconservative model of DNA replication.

- Before cell division occurs, the genetic material must be copied by process, known as **DNA replication,** the original DNA strands are used as templates for the synthesis of new DNA strands.
- DNA replication depends on the complementarity of DNA strands, based on the AT/GC rule.
- During replication process, the two complementary strands of DNA unwind and serve as **template strands, or parental strands**, for the synthesis of two **new strands of DNA or daughter strands.** DNA is replicated in such a way that both copies retain the same genetic information the same base sequence as in the original molecule.

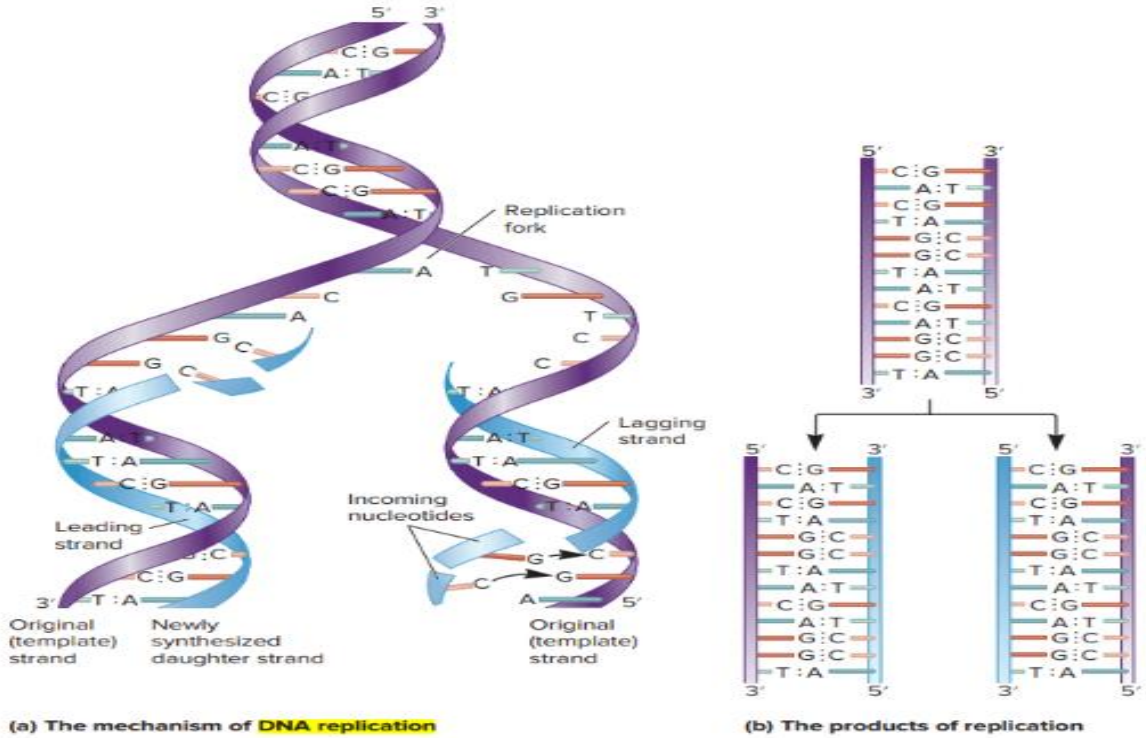


Figure 1: The structural basis for DNA replication. (a) The mechanism of DNA replication. (b) DNA replication produces two copies of DNA with the same sequence as the original DNA molecule (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

- Scientists in the late 1950s considered three different mechanisms to explain the net result of DNA replication.

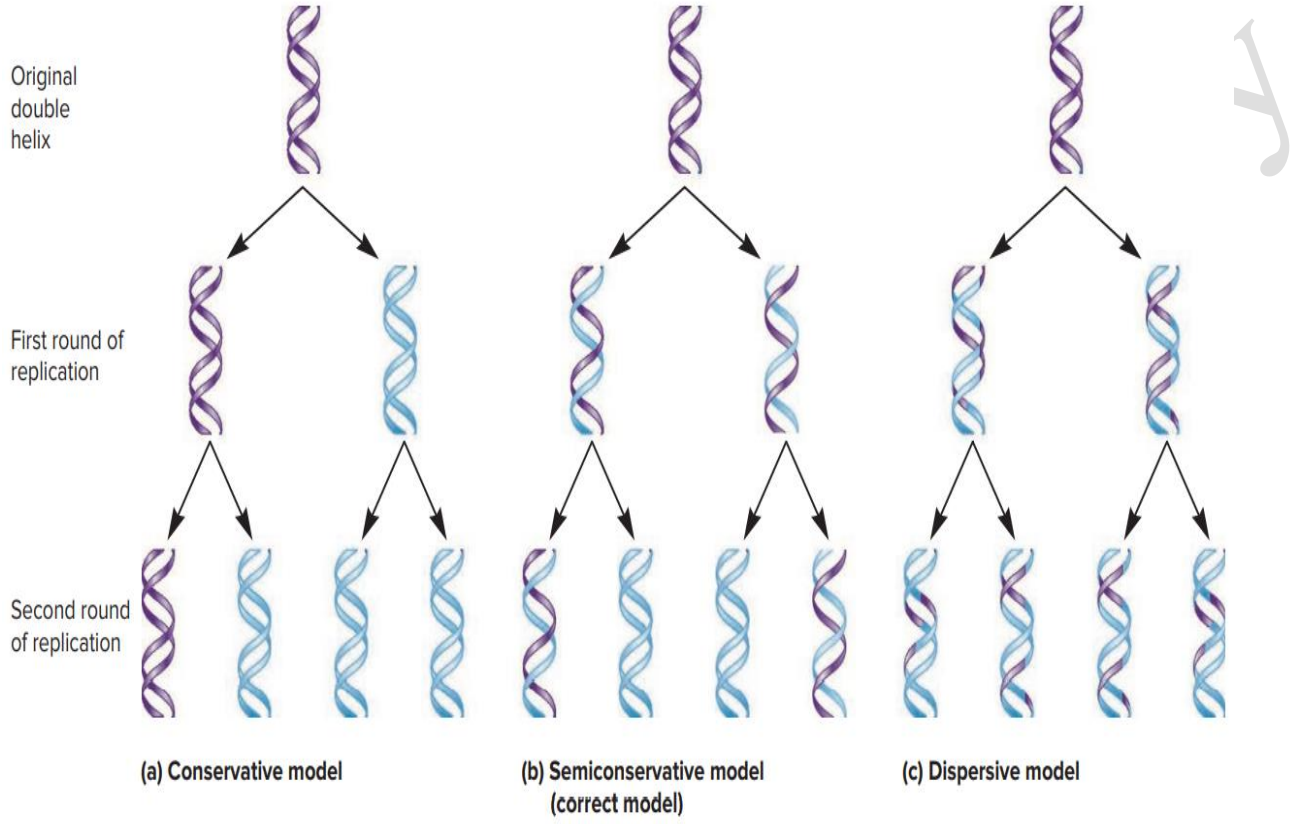


Figure 2: DNA replication models. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US)

1- The first is referred to as a **conservative model.**

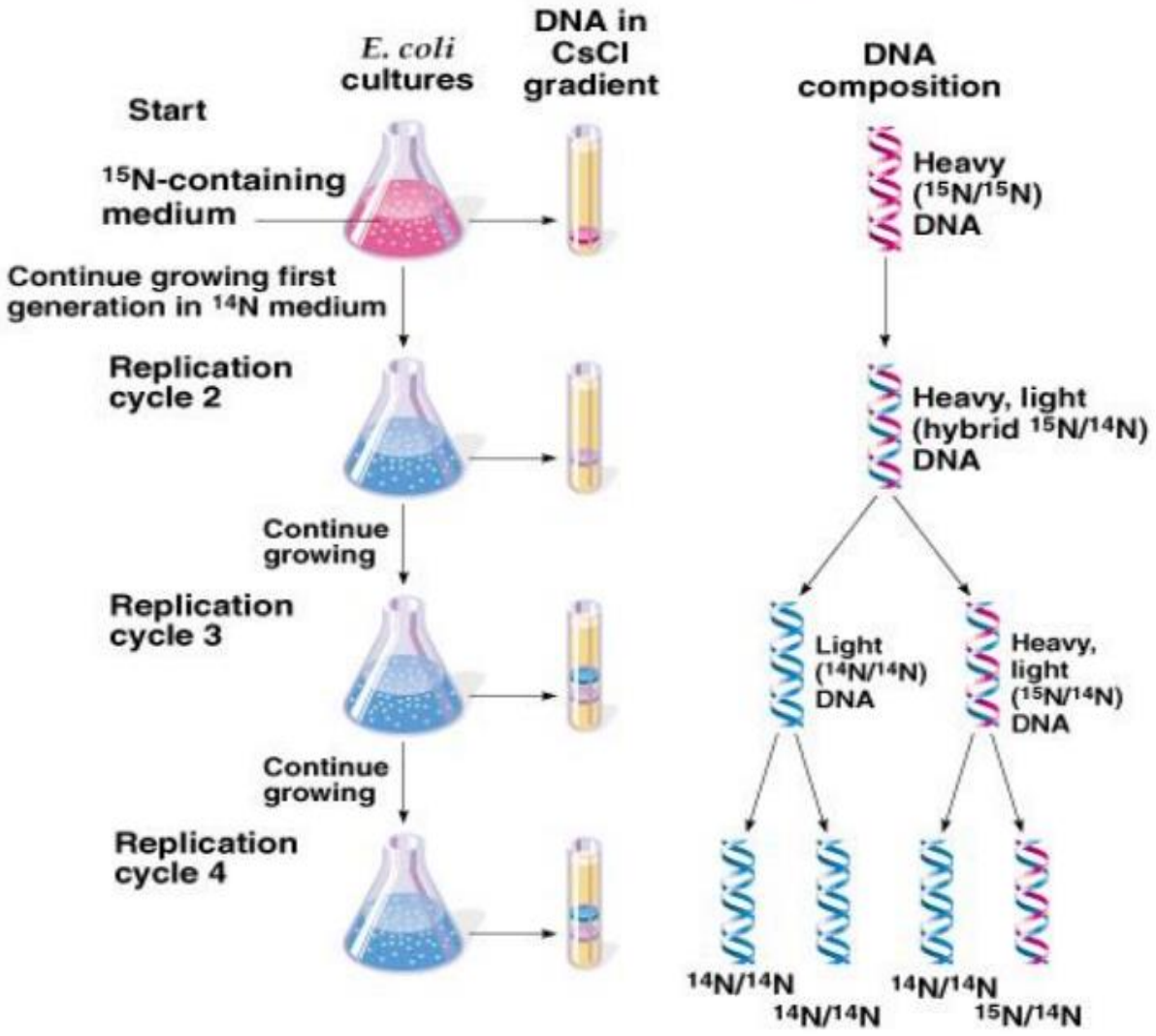
According to this hypothesis, both parental strands of DNA remain together following DNA replication. In this model, the original arrangement of parental strands is completely conserved, while the two newly made daughter strands also remain together following replication.

2- The second mechanism is called **a semiconservative** model. In this model, the double-stranded DNA is half conserved following the replication process. In other words, the newly made double-stranded DNA contains one parental strand and one daughter strand.

3- The third mechanism, called the **dispersive** model, proposes that segments of parental DNA and newly made DNA are interspersed in both strands following the replication process.

Only the **semiconservative** model is actually correct. In 1958, Matthew Meselson and Franklin Stahl devised a method to experimentally distinguish newly made daughter strands from the original parental strands.

Starting material: A strain of *E. coli* that has been grown for many generations in the presence of ^{15}N as a NH_4Cl in the culture medium. All of the bases in the DNA are labeled with ^{15}N. Then, researchers shifted the cells to medium containing normal 14N, and took samples at time points. DNA was extracted from each sample and analyzed in CsCl density gradients. DNA containing ^{15}N is denser (because it is heavier) than DNA with normal ^{14}N, and so can be separated by CsCl density gradient centrifugation.



Figure(3): DNA replication models. (Russell, P. , I Genetics, a molecular approach , 3rd edition , 2009, edited by Yue-Wen Wang. Pearsons,USA).

Replication in Bacteria

- Replication of bacterial chromosomal is stated from single site known as the **origin of replication (oriC)**. The synthesis of new daughter strands is initiated within the origin and proceeds in both directions, or **bidirectionally**. This means that two replication forks move in opposite directions outward from the origin.
- DNA replication begins with the binding of **DnaA** proteins to AT rich sequences within the origin of replication as well as other protein and the results is the separation of the two strands.

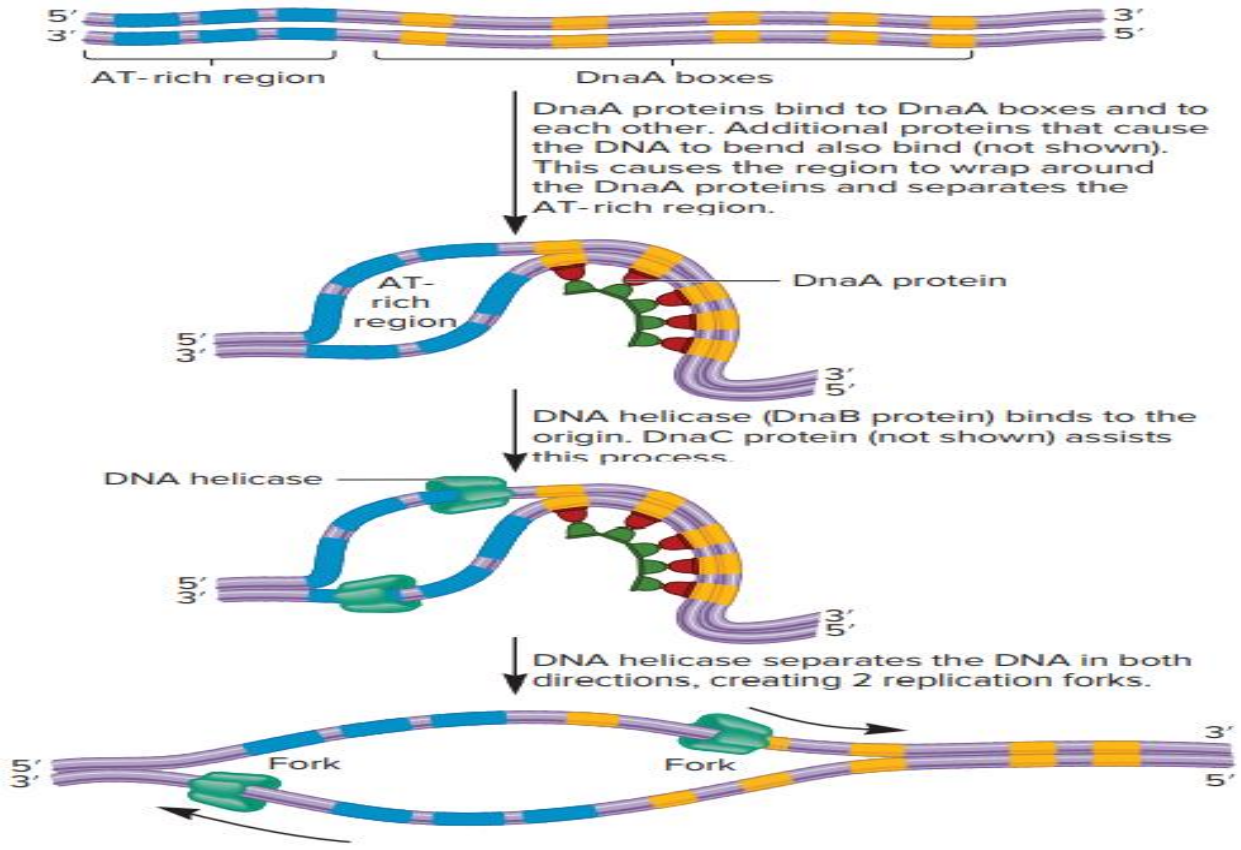


Figure 4: The events that occur at oriC to initiate the DNA replication process. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

- the enzyme **DNA helicase** is recruited to the replication fork. DNA helicase breaks the hydrogen bonds between the two strands, thereby generating two single strands. In *E. coli*, DNA helicases bind to single-stranded DNA and travel along the DNA in a 5′ to 3′ direction to keep the replication fork moving. also, this action generates positive supercoiling ahead of each replication fork. an enzyme known as **topoisomerase II, also called DNA gyrase,** travels in front of DNA helicase and solving the positive supercoiling.
- To prevents the DNA strands from coming back together, single-strand binding proteins is binding to the separated strands.

- Then short strands of RNA (rather than DNA), about 10-12 ribonucleotides, called **RNA primers** are synthesized in complementary manner to DNA. These strands of RNA are synthesized by the linkage of ribonucleotides by an enzyme known **as primase**. These short RNA strands start, or **prime**, the process of DNA replication. In the **leading strand**, a single primer is made at the origin of replication. In the **lagging strand**, multiple primers are made.
- DNA helicase and primase are physically bound to each other to form a complex known as a **primosome.**
- An enzyme **DNA polymerase** is responsible for synthesizing the DNA along the leading and lagging strands. This enzyme catalyzes the formation of covalent bonds between adjacent nucleotides and thereby makes the new daughter strands. It has specific characteristic that its unable to synthesize DNA denovo and only add nucleotides in the direction 5′ to 3′ by linking the phosphate group at the 5′ of the new nucleotide to the free OH at the 3′ of the pre-existing nucleotide.
- The synthesis of RNA primers by primase allows DNA polymerase III to begin the synthesis of complementary daughter strands of DNA.

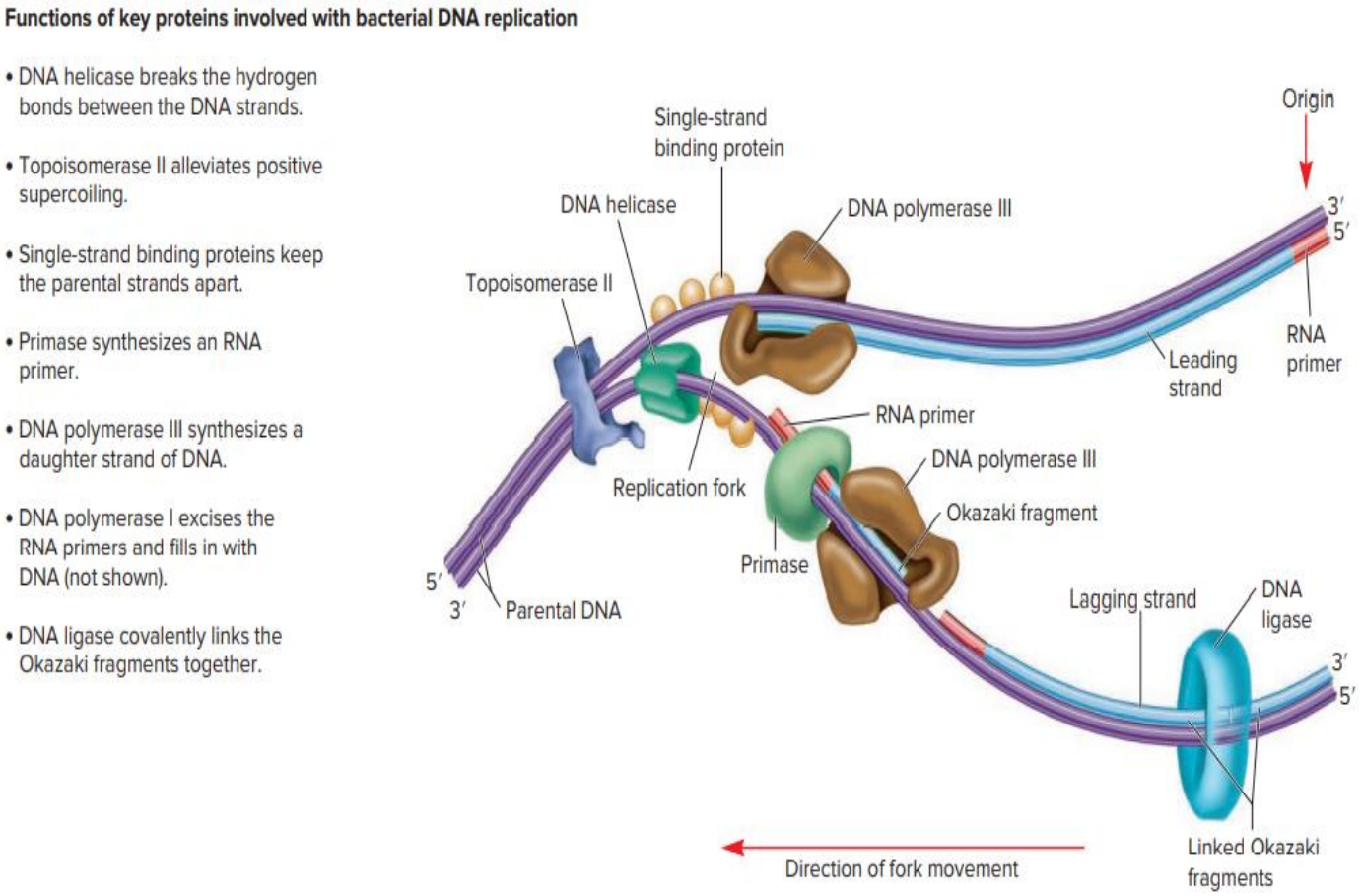


Figure 5: The proteins and enzymes involved in bacterial DNA replication. (Russell, P, I Genetics, a molecular approach , 3rd edition , 2009, edited by Yue-Wen Wang. Pearsons,USA)

- **In the leading strand**, one RNA primer is made at the origin, and then DNA polymerase III attaches nucleotides in a 5′ to 3′ direction as it slides toward the opening of the replication fork. The synthesis of the leading strand is **continuous.**

- **In the lagging strand**, the synthesis of DNA also proceeds in a 5′ to 3′ manner, **but it does so in the direction away from the replication fork**. In the lagging strand, **RNA primers repeatedly initiate the synthesis of short segments of DNA**; the synthesis is discontinuous. The length of these fragments in bacteria is typically 1000–2000 nucleotides. In eukaryotes, the fragments are shorter: 100–200 nucleotides**. Each fragment contains a short RNA primer at the 5′ end**, which is made by primase. The remainder of the fragment is a strand of DNA made by DNA polymerase. The DNA fragments made in this manner are known **as Okazaki fragments.**

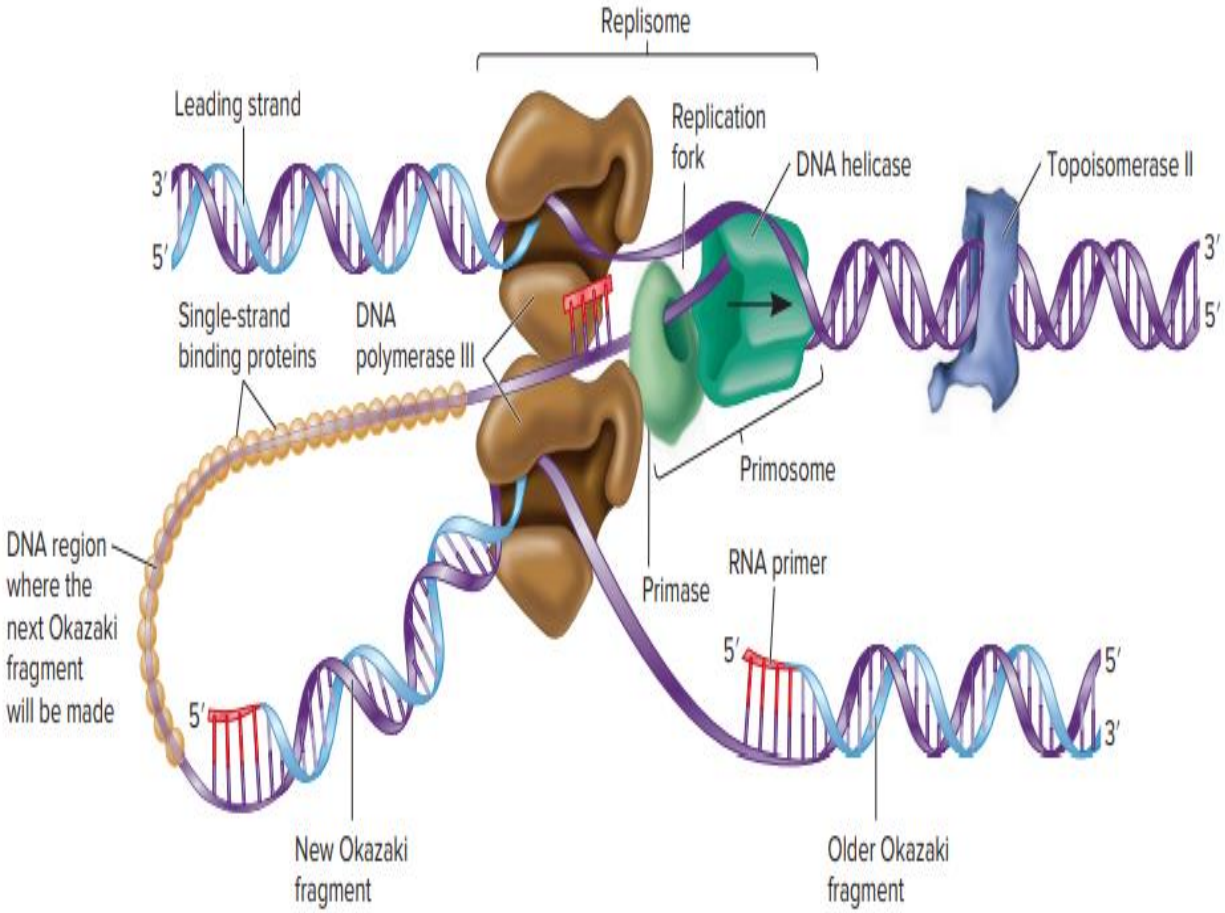


Figure 5: The prosses of replication. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

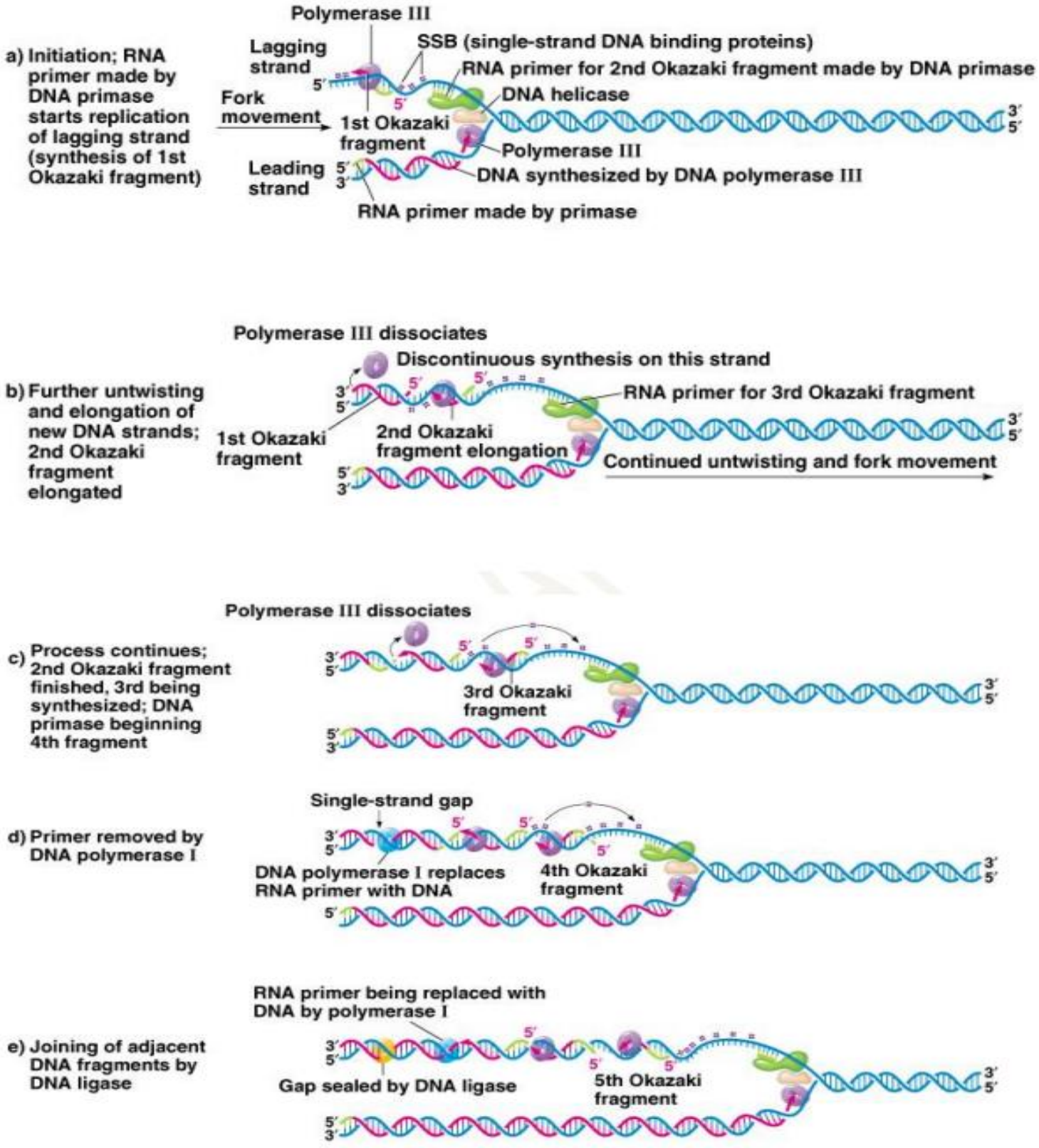


Figure 6: The replication forks and Okazaki fragments. (Russell, P , I Genetics, a molecular approach , 3rd edition , 2009, edited by Yue-Wen Wang. Pearsons,USA)

- In *E. coli*, the RNA primers are removed by the action of DNA polymerase I. This **3´→5´ exonuclease activity or "proofreading" function.** Then DNA polymerase I then synthesizes DNA to fill in this region.
- DNA ligase is an enzyme catalyzes a covalent bond between adjacent Okazaki fragments to complete the replication process in the lagging strand
- The primosome is physically associated with two DNA polymerase holoenzymes to form a **replisome.**
- When the new stands synthesis is completed, On the opposite side of the *E. coli* chromosome from oriC is a pair of **termination sequences**, known as **ter** sequences. A protein known as the **termination utilization substance (Tus)** binds to the **ter sequences** and stops the movement of the replication forks.
- In bacteria DNA polymerases and are designated polymerase I, II, III, IV, and V. DNA polymerases I and III are involved in normal DNA replication, whereas DNA polymerases II, IV, and V play a role in DNA repair and the replication of damaged DNA. DNA polymerase III is responsible for most of the DNA replication.

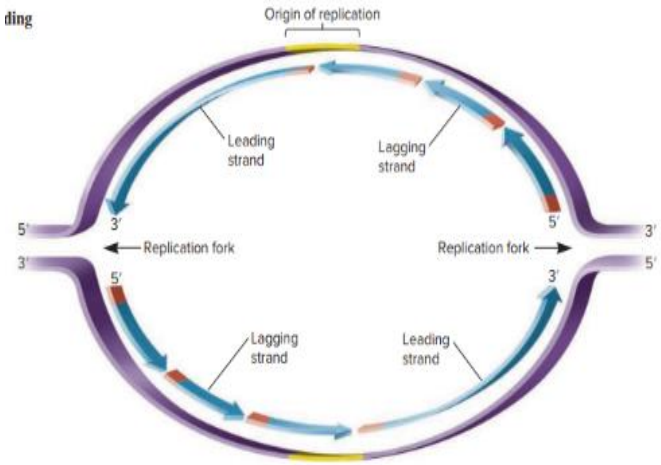


Figure7: The synthesis of leading and lagging strands outward of single origin of replication. (Brooker, 2018).

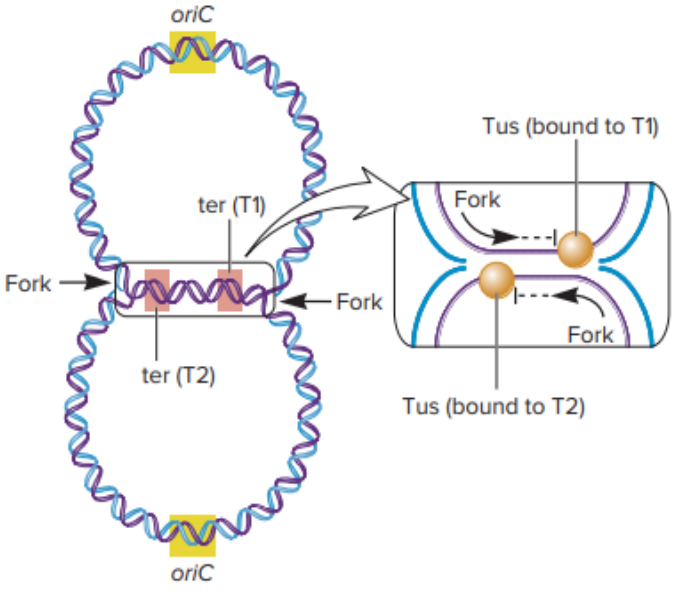


Figure8: Replication termination. (Brooker, 2018).

Table 1: Proteins and enzymes involved in DNA replication. (Brooker, 2018).

Proteins Involved in *E. coli* DNA Replication	
Common Name	**Function**
DnaA proteins	Bind to DnaA box sequences within the origin to initiate DNA replication
DnaC proteins	Aid DnaA in the recruitment of DNA helicase to the origin
DNA helicase (DnaB)	Separates double-stranded DNA
Topoisomerase II (DNA gyrase)	Removes positive supercoiling ahead of the replication fork
Single-strand binding proteins	Bind to single-stranded DNA and prevent it from re-forming a double-stranded structure
Primase	Synthesizes short RNA primers
DNA polymerase III	Synthesizes DNA in the leading and lagging strands
DNA polymerase I	Removes RNA primers, fills in gaps with DNA
DNA ligase	Covalently attaches adjacent Okazaki fragments
Tus	Binds to ter sequences and prevents the advancement of the replication fork

Eukaryotic DNA replication

The replication in eukaryotes showed extensive similarities in the general features of DNA replication with prokaryotes, but its more complex in eukaryotes. The eukaryotes cells have large, linear chromosomes, the chromatin is tightly packed within nucleosomes. Therefore, multiple origins of replication are needed to complete the whole genomic DNA.

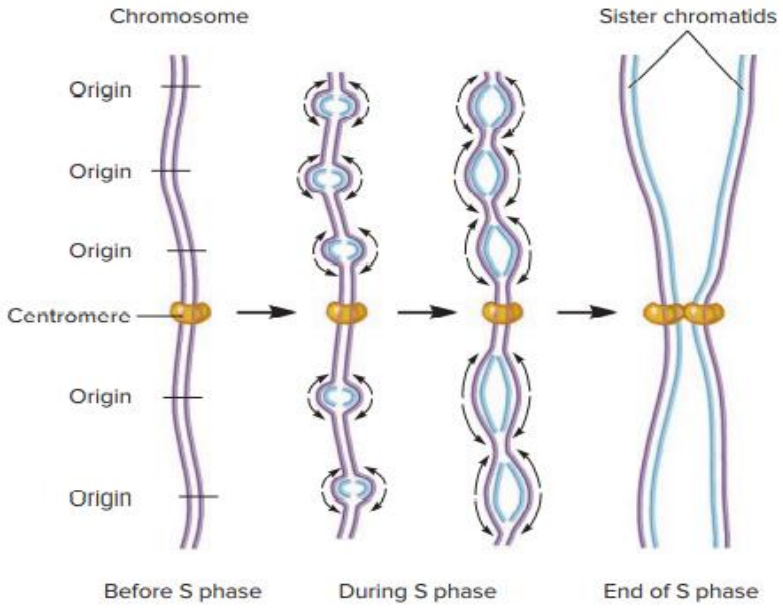


Figure 9: The multiple origins of replication in Eukaryotic chromosome. (Brooker, 2018).

- DNA replication in eukaryotes requires the assembly of a **prereplication complex (preRC)** during the G1 phase of the cell cycle, the **prereplication complex (preRC**) is formed from a group of six proteins called **the origin recognition complex (ORC**). The binding of **MCM helicase** to the leading strands completes a process called **DNA replication licensing**.
- Eukaryotes have many types of DNA polymerases. For example, mammalian cells have well over a dozen different DNA polymerases. Four of these, designated α (alpha), ε (epsilon), δ (delta), and γ (gamma), have the primary function of replicating DNA. DNA **polymerase γ** functions in the mitochondria **to replicate mitochondrial DNA**, whereas **α, ε, and δ are involved with DNA replication in the cell nucleus**. DNA polymerase α is the only eukaryotic polymerase that associates with primase. The functional role of the **DNA polymerase α/primase complex is to synthesize a short RNA-DNA primer** of approximately 10 RNA nucleotides followed by 20–30 DNA nucleotides.

Replicating the Ends of Chromosomes:

1. When the ends of chromosomes are replicated and the primers are removed from the 5' ends, there is no adjacent DNA strand to serve as a primer, and so a single-stranded region is left at the 5' end of the new strand. If the gap is not addressed, chromosomes would become shorter with each round of replication.
2. Most eukaryotic chromosomes have short, species-specific sequences tandemly repeated at their telomeres. Blackburn and Greider have shown that chromosome lengths are maintained by telomerase, which adds telomere repeats without using the cell's regular replication machinery.
3. In the ciliate Tetrahymena, the telomere repeat sequence is 5 TTGGGG3" .

A- Telomerase, an enzyme containing both protein and RNA, binds to the terminal telomere repeat when it is single stranded, synthesizing a 3-nt sequence, TTG.

B- The 3" end of the telomerase RNA contains the sequence AAC, which binds the TTG positioning telomerase to complete its synthesis of the TTGGGG telomere repeat.

C- Additional rounds of telomerase activity lengthen the chromosome by adding telomere repeats.

4- After telomerase adds telomere sequences, chromosomal replication proceeds in the usual way. Any shortening of the chromosome ends is compensated by the addition of the telomere repeats, If the sequence of the telomerase RNA is mutated, telomeres will correspond to the

mutant sequence, rather than the organism's normal telomere sequence. Using an RNA template to make DNA, telomerase functions as a reverse transcriptase called TERT (telomerase reverse transcriptase).

5-Telomere length may vary, but organisms and cell types have characteristic telomere lengths. Mutants affecting telomere length have been identified, and data indicate that telomere length is genetically controlled. Shortening of telomeres eventually leads to cell death, and this may be a factor in the regulation of normal cell death.

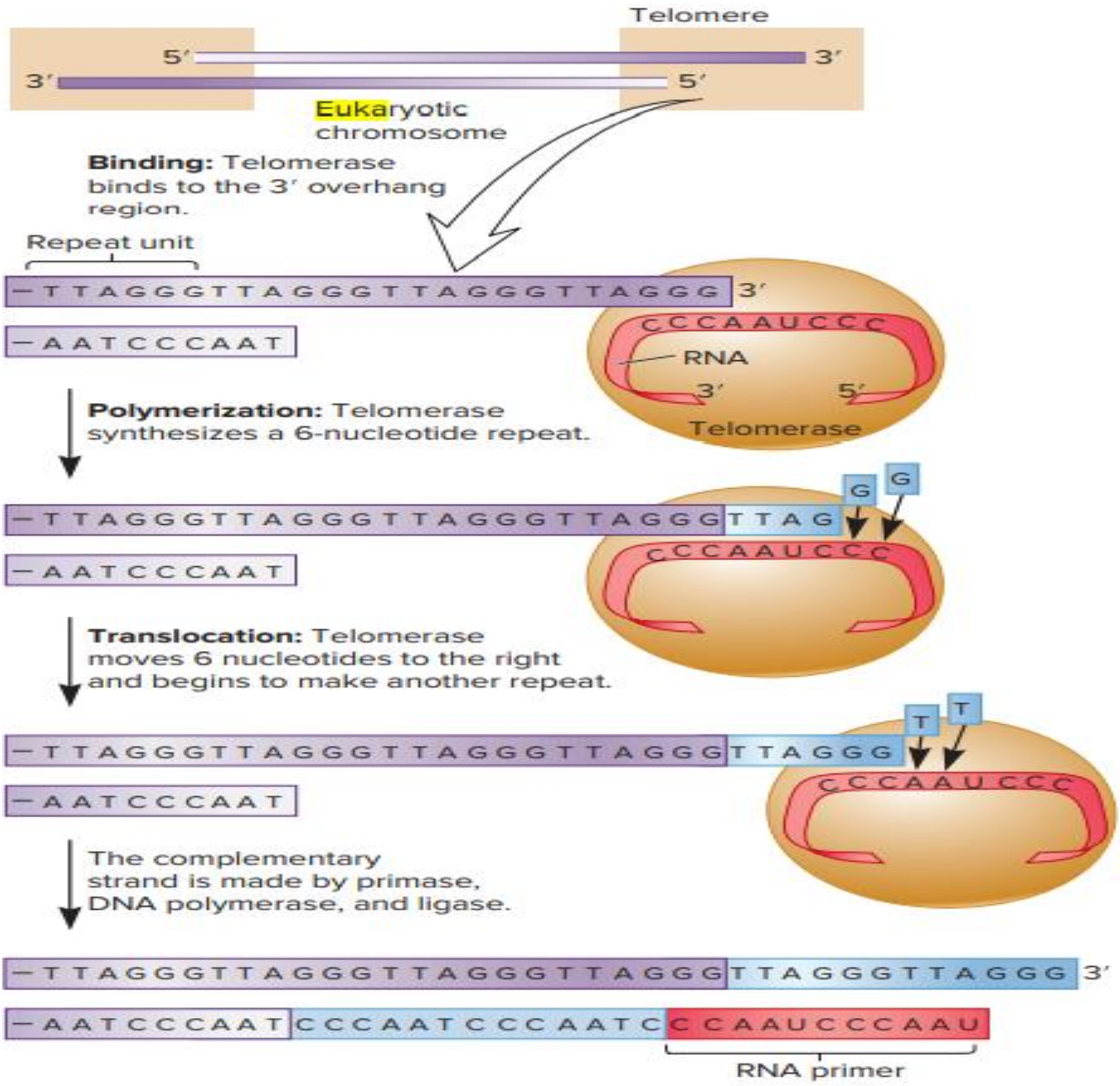


Figure10: the replication of the end of the chromosome/ telomer (Brooker, 20218).