# DNA REPLICATION

The DNA double helix is composed of two DNA strands, and the individual building blocks of each strand are nucleotides. The nucleotides contain one of four bases: adenine, thymine, guanine, or cytosine. The double-stranded structure is held together by base stacking and by hydrogen bonding between the bases in opposite strands. A critical feature of the double-helix structure is that adenine hydrogen bonds with thymine, and guanine hydrogen bonds with cytosine. This rule, known as the AT/GC rule or Chargaff's rule, is the basis for the complementarity of the base sequences in double-stranded DNA. The strands within a double helix have an antiparallel alignment. This directionality is determined by the orientation of sugar molecules within the sugar–phosphate backbone. If one strand is running in the 5' to 3' direction, the complementary strand is running in the 3' to 5' direction.

During the replication process, the two complementary strands of DNA come apart and serve as template strands, or parental strands, for the synthesis of two new strands of DNA. After the double helix has separated, individual nucleotides have access to the template strands. Hydrogen bonding between individual nucleotides and the template strands must obey the AT/GC rule. To complete the replication process, a covalent bond is formed between the phosphate of one nucleotide and the sugar of the previous nucleotide. The two newly made strands are referred to as the daughter strands.

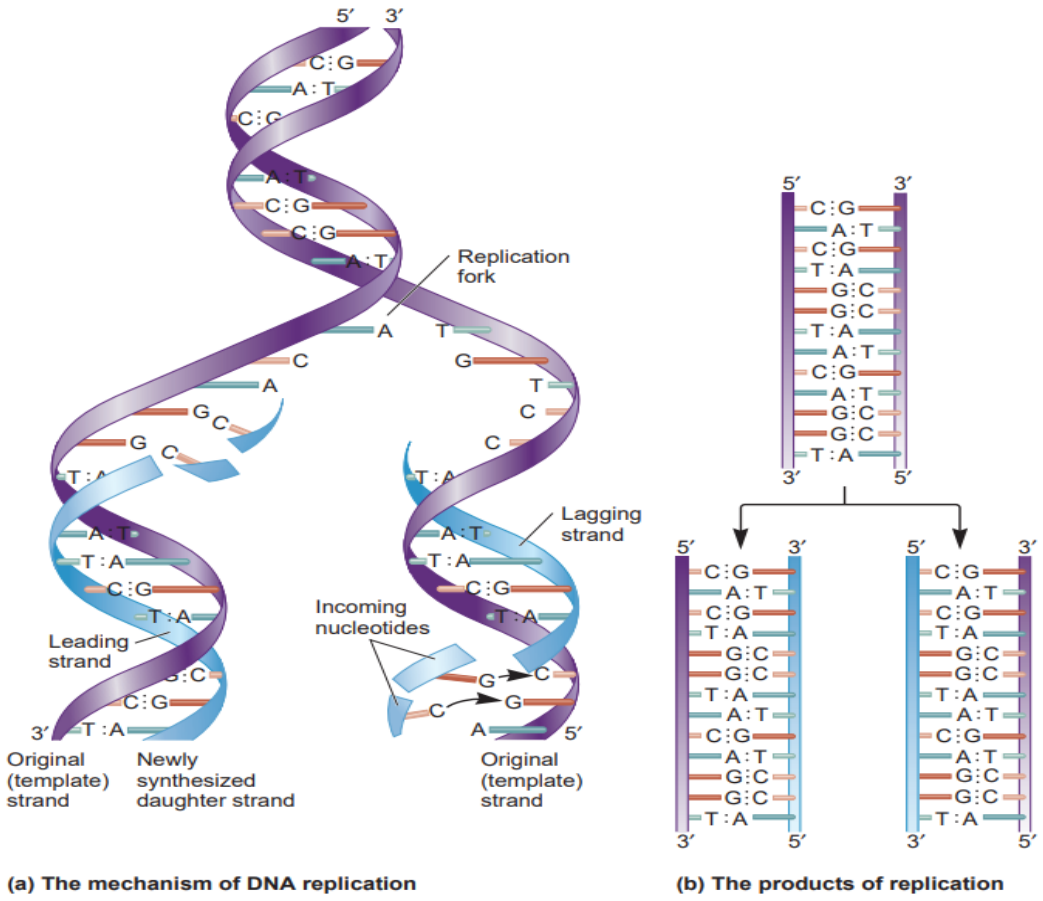



**FIGURE 11.1** **The structural basis for DNA replication.** **(a)** The mechanism of DNA replication as originally proposed by Watson and Crick. As we will see, the synthesis of one newly made strand (the leading strand) occurs in the direction toward the replication fork, whereas the synthesis of the other newly made strand (the lagging strand) occurs in small segments away from the replication fork. **(b)** DNA replication produces two copies of DNA with the same sequence as the original DNA molecule.

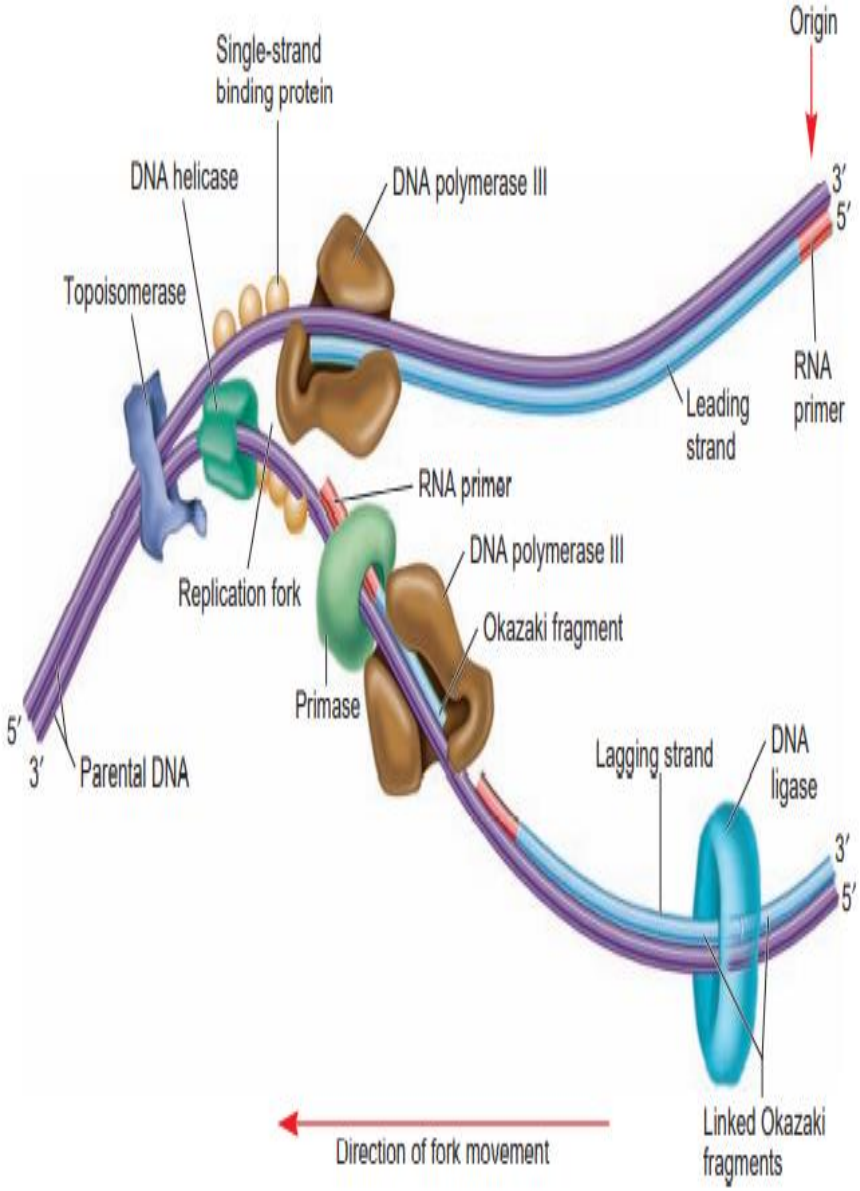
# ENZYMES INVOLVED IN DNA REPLICATION

To act as a template for DNA replication, the strands of a double helix must separate. As mentioned previously, the function of DNA helicase is to break the hydrogen bonds between base pairs and thereby unwind the strands; this action generates positive supercoiling ahead of each replication fork. An enzyme known as a topoisomerase (type II), also called DNA gyrase, travels in front of DNA helicase and alleviates positive supercoiling. DNA replication requires single-strand binding

proteins that bind to the strands of parental DNA and prevent them from re-forming a double helix. The next event in DNA replication involves the synthesis of short strands of RNA (rather than DNA) called RNA primers. These strands of RNA are synthesized by the linkage of ribonucleotides via an enzyme known as DNA primase, or simply primase. This enzyme synthesizes short strands of RNA, typically 10 to 12 nucleotides in length. 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. A type of enzyme known as DNA polymerase is responsible for synthesizing the DNA of the leading and lagging strands. This enzyme catalyzes the formation of covalent bonds between adjacent nucleotides and thereby makes the new daughter strands. In E. coli, five distinct proteins function as DNA polymerases and are designated polymerase I, II, III, IV, and V. DNA polymerases I and III are involved in normal DNA replication, while 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.

**Functions of key proteins involved with DNA replication**

- DNA helicase breaks the hydrogen bonds between the DNA strands.
- Topoisomerase alleviates positive supercoiling.
- Single-strand binding proteins keep the parental strands apart.
- Primase synthesizes an RNA primer.
- DNA polymerase III synthesizes a daughter strand of DNA.
- DNA polymerase I excises the RNA primers and fills in with DNA (not shown).
- DNA ligase covalently links the Okazaki fragments together.




ONLINE ANIMATION

**FIGURE 11.7** The proteins involved with DNA replication.

Note: The drawing of DNA polymerase III depicts the catalytic subunit that synthesizes DNA.