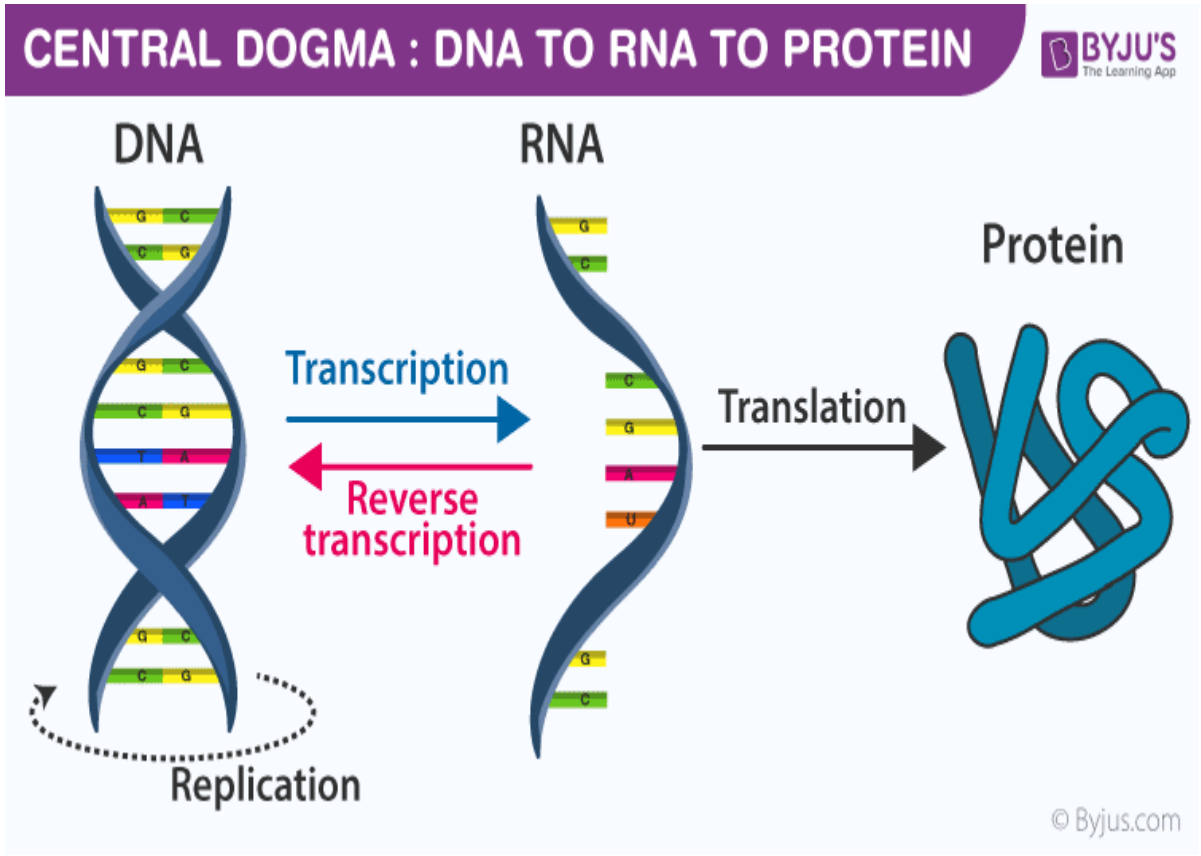
FDE 330
FOOD BIOTECHNOLOGY

DNA Replication



Central Dogma

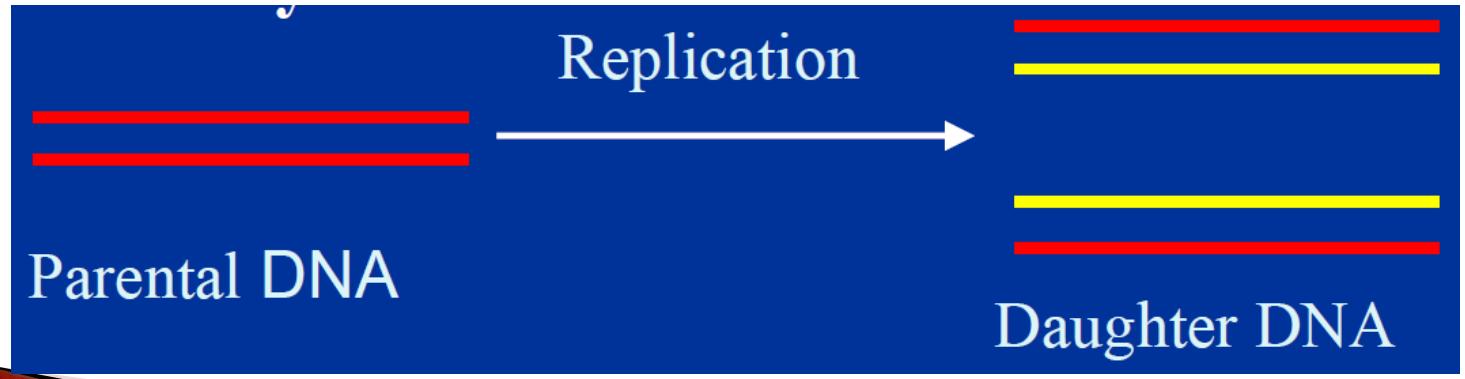
DNA Replication-Introduction

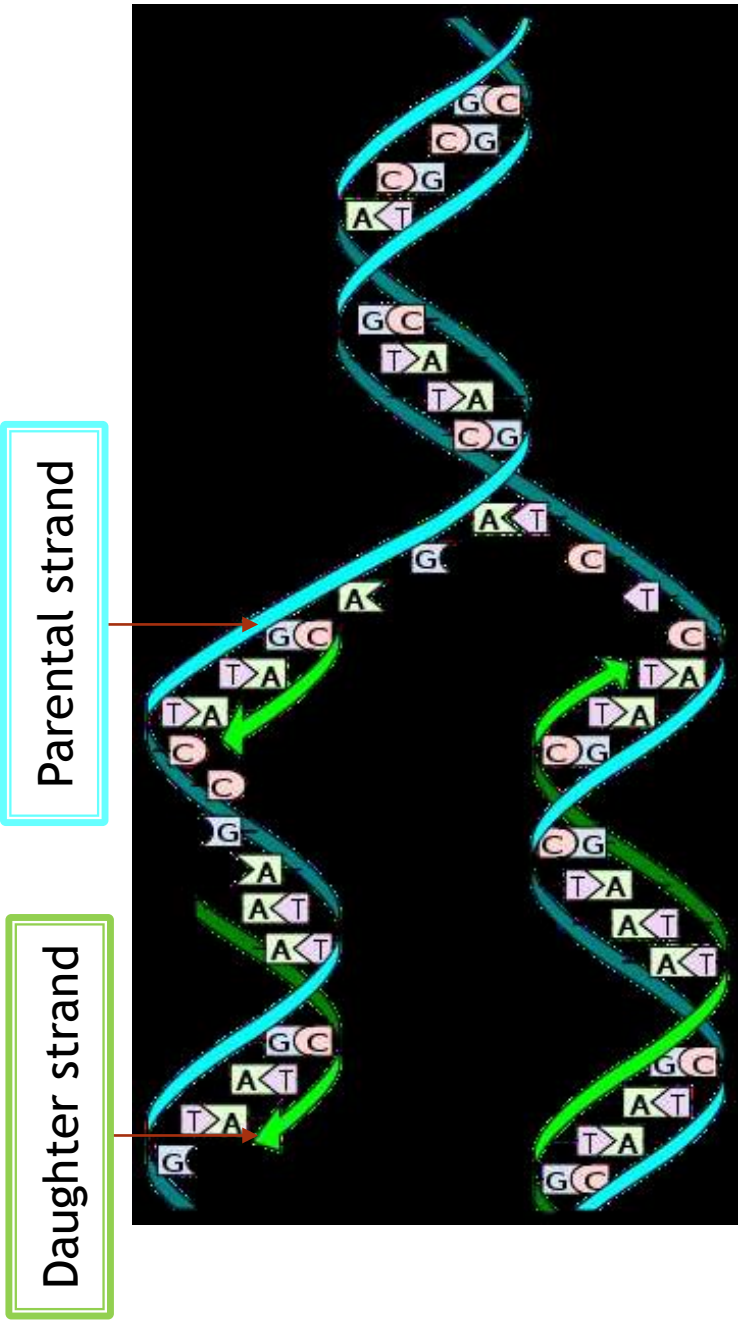
- **DNA Replication** is the biological process of producing two same copies of DNA from one original DNA molecule.
- **DNA replication** occurs in all living organisms and copies their exact DNA.
- It is the basis for biological inheritance.
- **Replication** is the process of synthesis of daughter DNA from parental DNA by the enzyme DNA Polymerase.
- **DNA replication** uses a semi-conservative method that results in a double-stranded DNA with one parental strand and a new daughter strand.

DNA Replication-Introduction

- A reaction in which daughter DNAs are synthesized using the parental DNAs as the template.

- Transferring the genetic information to the descendant generation <u>with a high fidelity</u>.

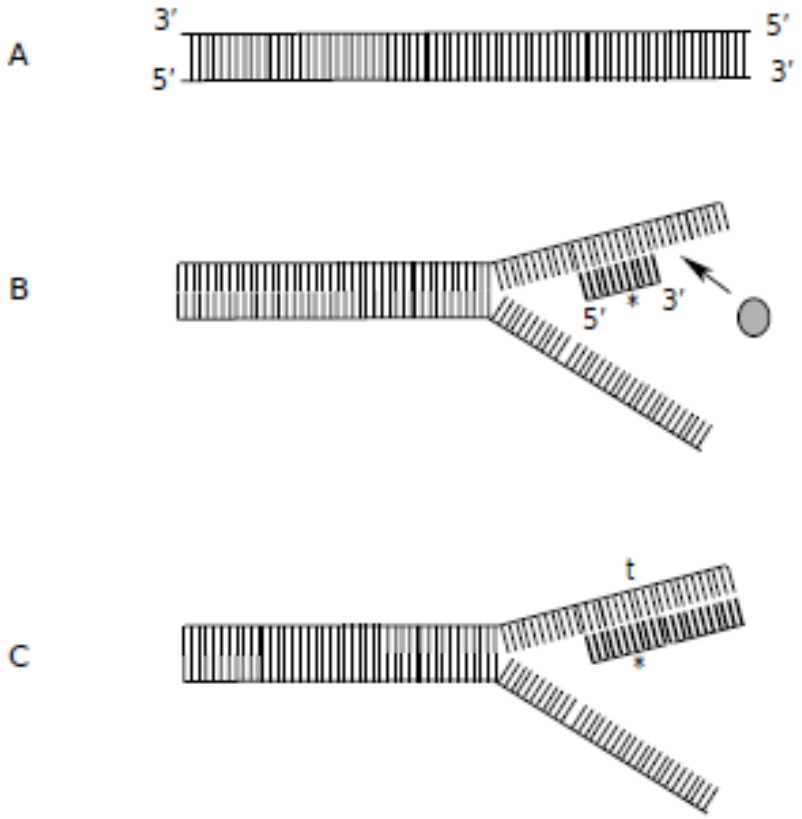




DNA Replication-Introduction

- Each cell must replicate its DNA before division.
- When a cell divides, it must ensure that both daughter cells have identical genetic structure; in other words, each cell must have the same sequence of DNA. This is achieved through **DNA replication**.
- In both bacteria and eukaryotes, this is accomplished through a suite of proteins and enzymes that separate the DNA into single strands, allowing DNA polymerase to use each strand as a template to build a new complementary strand.
- The following characteristics of DNA replication are particularly important; they are crucial to various techniques of molecular biology, including PCR:
  - DNA polymerase cannot start to build a new strand of DNA unless it has **an RNA primer** that is complementary to a sequence on the template strand. DNA polymerase is able to extend a complementary DNA strand from this primer and later replaces the RNA primer with DNA. DNA primers are often used to control the starting point of DNA replication.
  - The new DNA strand is always synthesized **from the 5′ to the 3′ end**.
- DNA replication in cells also requires a specific DNA sequence that acts as an "origin of replication" (*ori*) on the chromosome (eukaryotic cells often have multiple sites of origin on each chromosome).

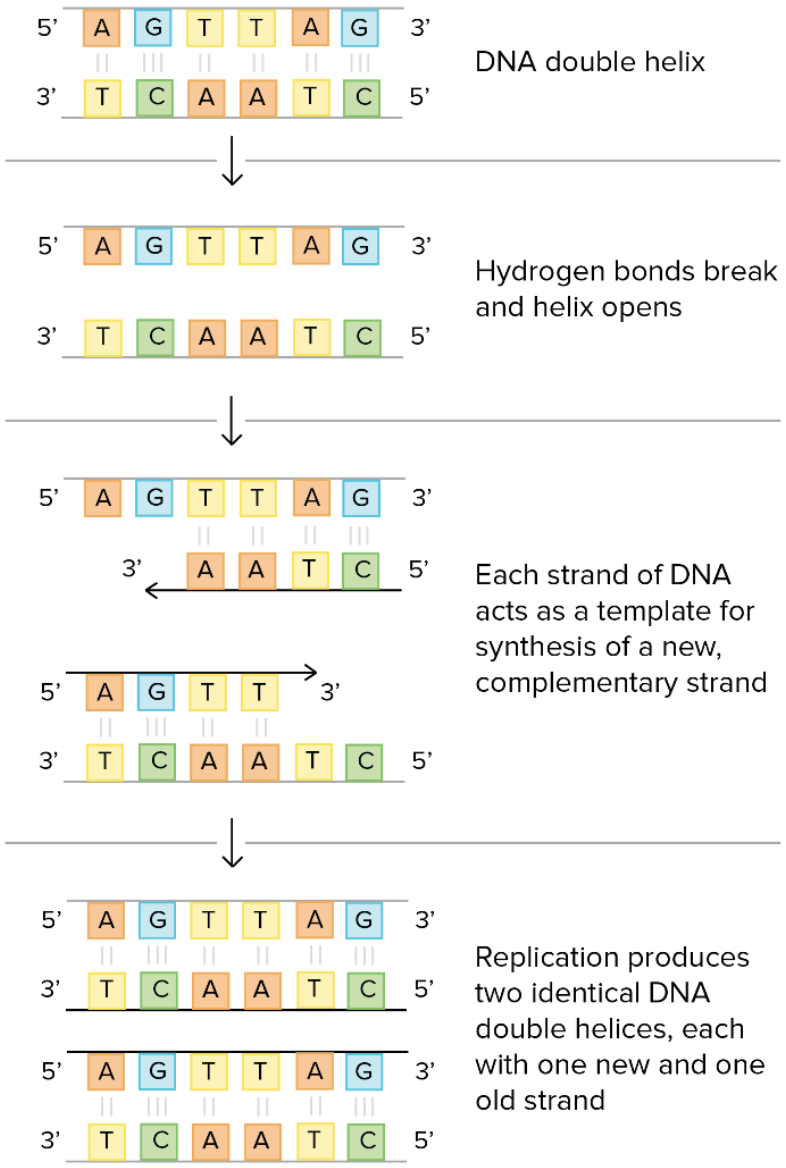
Replication of DNA

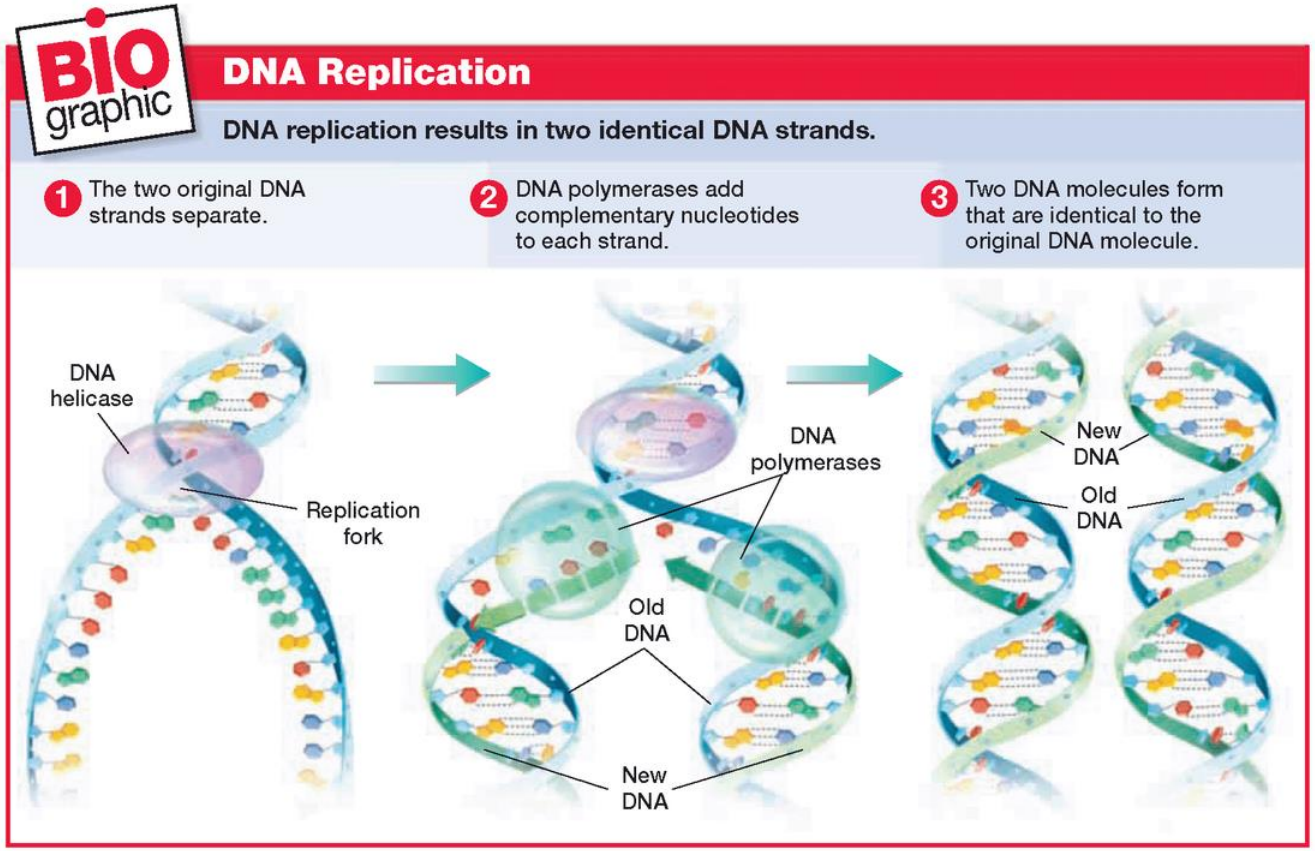


- The double-stranded DNA (**A**) separates into single strands.
- A primer (*) anneals to one of the strands (**B**), and DNA polymerase (filled circle) extends the new strand (**C**), starting with the primer, and adding bases complementary to the template strand (t).
- The other original strand would also act as a template.

Key points

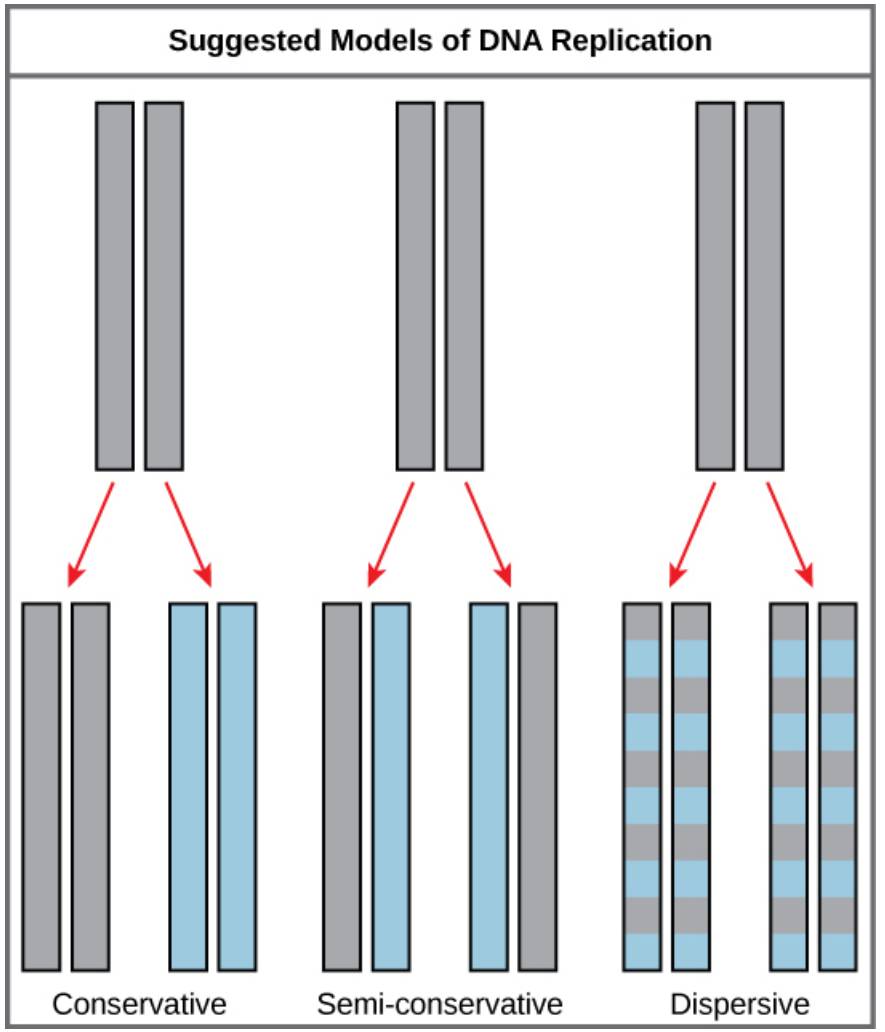
- DNA replication is **semiconservative**. Each strand in the double helix acts as a template for synthesis of a new, complementary strand.
- New DNA is made by enzymes called **DNA polymerases**, which require a template and a **primer** (starter) and synthesize DNA in the 5' to 3' direction.
- During DNA replication, one new strand (the **leading strand**) is made as a continuous piece. The other (the **lagging strand**) is made in small pieces.
- DNA replication requires other enzymes in addition to DNA polymerase, including **DNA primase**, **DNA helicase**, **DNA ligase**, and **topoisomerase**.


5’ A G T T A G 3’
3’ T C A A T C 5’
DNA double helix
Hydrogen bonds break and helix opens
Each strand of DNA acts as a template for synthesis of a new, complementary strand
Replication produces two identical DNA double helices, each with one new and one old strand


BIO graphic
DNA Replication
DNA replication results in two identical DNA strands.
1 The two original DNA strands separate.
2 DNA polymerases add complementary nucleotides to each strand.
3 Two DNA molecules form that are identical to the original DNA molecule.
DNA helicase
Replication fork
DNA polymerases
Old DNA
New DNA
New DNA
Old DNA

Three Possible Replication Patterns

1. Semiconservative Replication
2. Conservative Replication
3. Dispersive Replication



Suggested Models of DNA Replication: The three suggested models of DNA replication. Grey indicates the original parental DNA strands or segments and blue indicates newly-synthesized daughter DNA strands or segments.

Characteristics of Replication

1. Semi-conservative replication
2. Bidirectional replication
3. Semi-discontinous replication
4. High fidelity

DNA Replication Follows a Set of Fundamental Rules

Early research on bacterial DNA replication and its enzymes helped to establish several basic properties that have proven applicable to DNA synthesis in every organism.

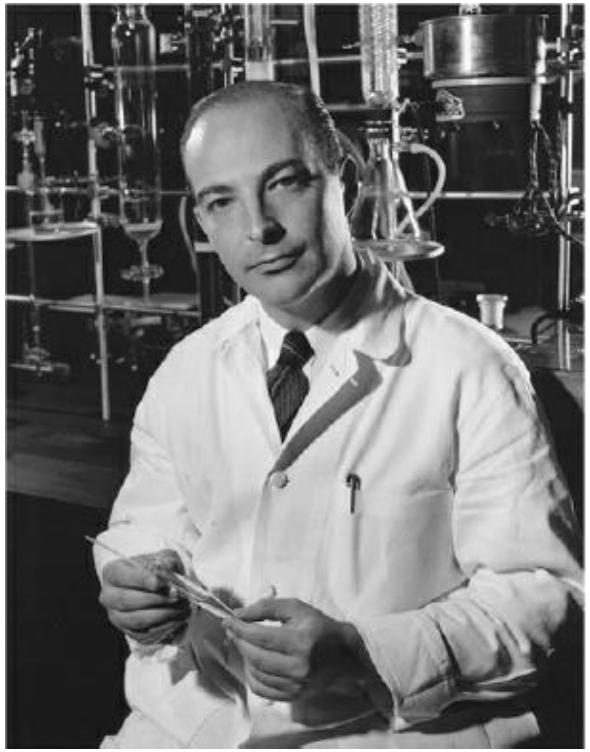
- **DNA Replication is semiconservative.**
- **Replication begins at an origin and usually proceeds bidirectionally.**
- **DNA synthesis proceeds in a 5'→3' direction and is semidiscontinuous.**

DNA Is Degraded by Nucleases

- **Nucleases** degrade nucleic acids.
  - Specifically, DNases degrade only DNA; RNases degrade only RNA.
- Every cell contains several different nucleases, belonging to two broad classes: **exonucleases** and **endonucleases**.
- **Exonucleases** degrade nucleic acids from one end of the molecule. Many operate in only the 5′→3′ or the 3′→5′ direction, removing nucleotides only from the 5′ or the 3′ end, respectively, of one strand of a double-stranded nucleic acid or of a single stranded DNA.
- **Endonucleases** can begin to degrade at specific internal sites in a nucleic acid strand or molecule, reducing it to smaller and smaller fragments.
- **Exonucleases** cleave bonds that remove nucleotides from the **ends** of DNA.
- **Endonucleases** cleave bonds **within** a DNA sequence.
- A few exonucleases and endonucleases degrade only single stranded DNA.
- There are a few important classes of endonucleases that cleave only at specific nucleotide sequences (such as the restriction endonucleases that are so important in biotechnology.

DNA Is Synthesized by DNA Polymerases

- The first DNA polymerase (DNA polymerase I) was discovered in *E. coli* in 1958 by Arthur Kornberg who received Nobel Prize in physiology & medicine in 1959.
- DNA Polymerase is considered as Kornberg Enzyme.
- Much later, investigators found that *E. coli* contains at least four other distinct DNA polymerases.

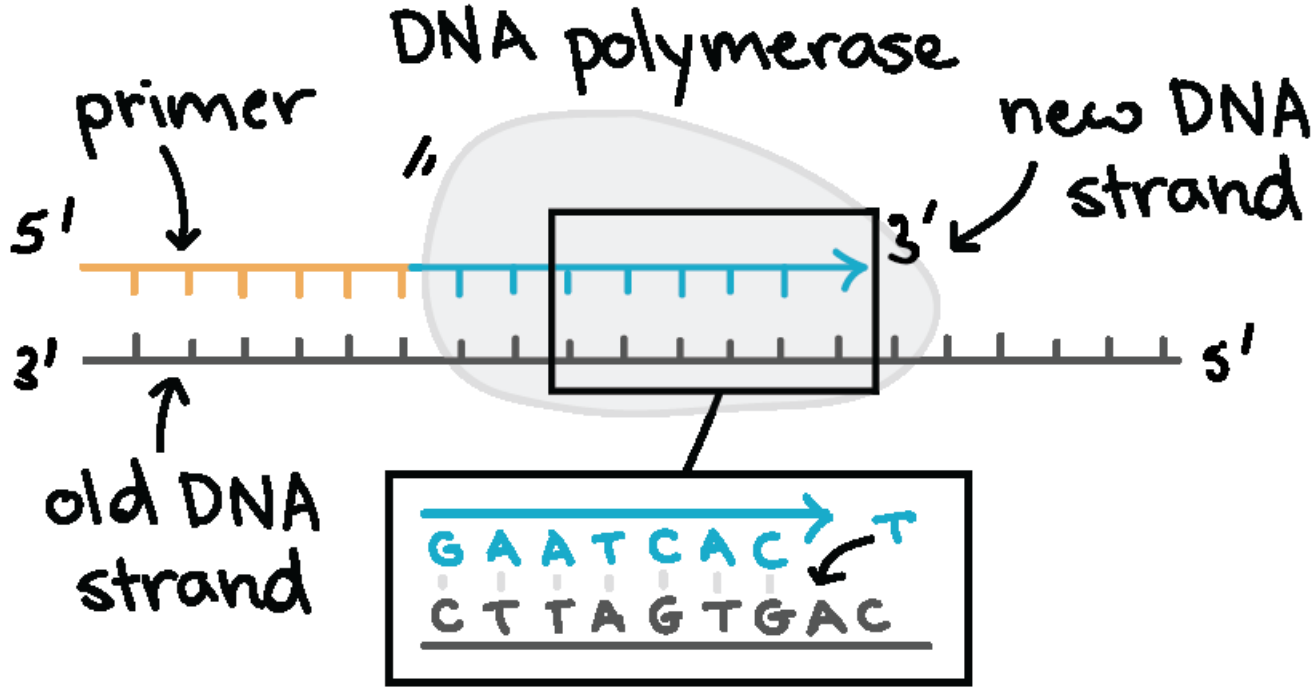


Arthur Kornberg, 1918–2007

DNA Polymerase

- One of the key molecules in DNA replication is the enzyme **DNA polymerase**.

- DNA polymerases are responsible for synthesizing DNA: they add nucleotides one by one to the growing DNA chain, incorporating only those that are complementary to the template.



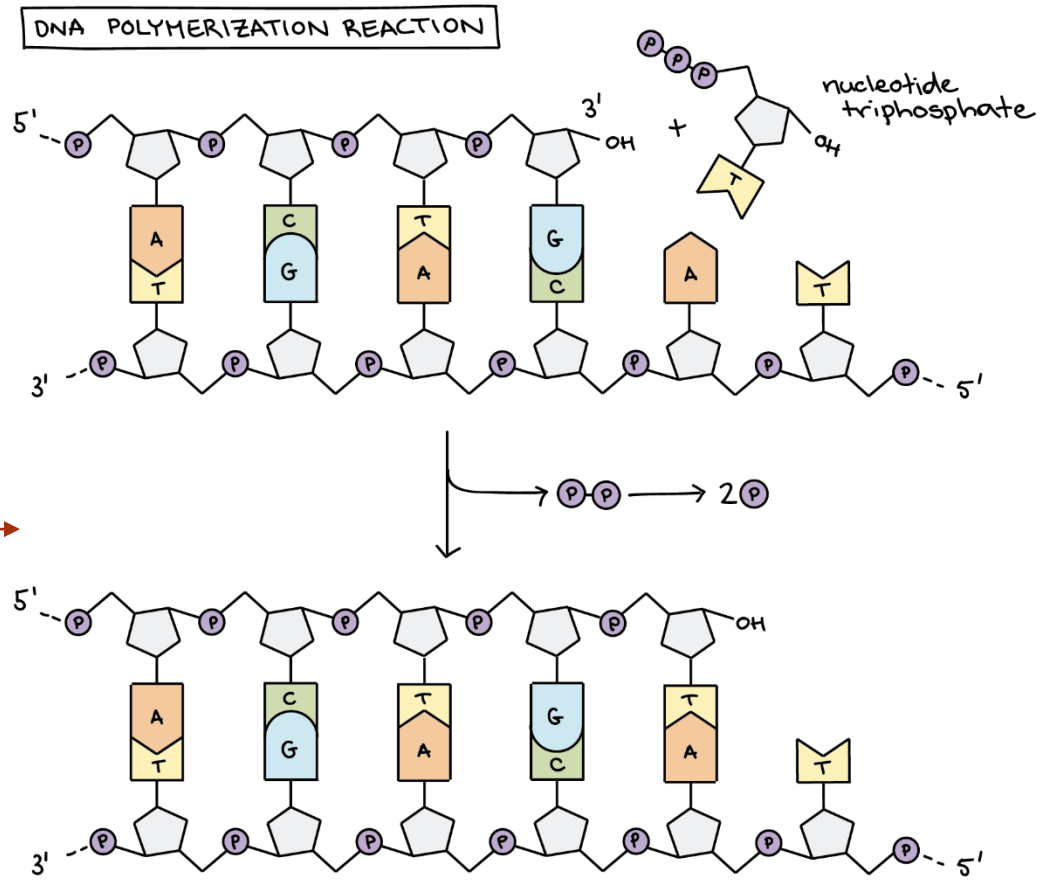
DNA Polymerase

Some key features of DNA polymerases:

- They always need **a template**.
- They can only add nucleotides to the 3' end of a DNA strand.
- They can't start making a DNA chain from scratch, but require a pre-existing chain or short stretch of nucleotides called **a primer**.
- They **proofread**, or check their work, removing the vast majority of "wrong" nucleotides that are accidentally added to the chain.

The addition of nucleotides requires energy. This energy comes from the nucleotides themselves, which have three phosphates attached to them (much like the energy-carrying molecule ATP). When the bond between phosphates is broken, the energy released is used to form a bond between the incoming nucleotide and the growing chain.

In prokaryotes such as *E. coli*, there are two main DNA polymerases involved in DNA replication: DNA pol III (the major DNA-maker), and DNA pol I.



Two Central Requirements for DNA Polymerase

A template & A primer

- **First**, all DNA polymerases require **a template**. The polymerization reaction is guided by a template DNA strand according to the base-pairing rules predicted by Watson and Crick: where a guanine is present in the template, a cytosine deoxynucleotide is added to the new strand, and so on. This was a particularly important discovery, not only because it provided a chemical basis for accurate semiconservative DNA replication but also because it represented the first example of the use of a template to guide a biosynthetic reaction.

- **Second**, the polymerases require **a primer**. A primer is a strand segment (complementary to the template) with a free 3′-hydroxyl group to which a nucleotide can be added; the free 3′ end of the primer is called the primer terminus. In other words, part of the new strand must already be in place: all DNA polymerases can add nucleotides only to a preexisting strand. Many primers are oligonucleotides of RNA rather than DNA, and specialized enzymes synthesize primers when and where they are required.

DNA Polymerase

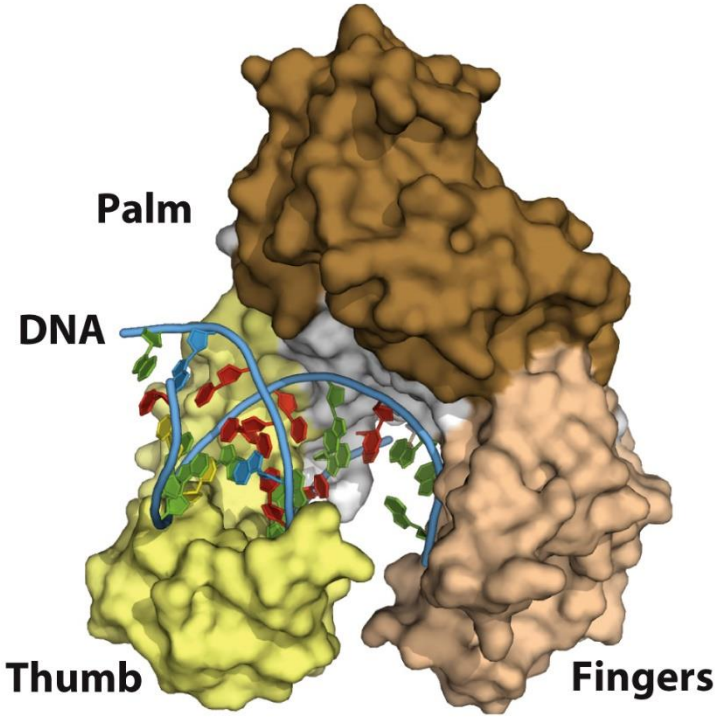


Figure 25-5c
Lehninger Principles of Biochemistry, Seventh Edition

- The core of most DNA polymerases is shaped like a human hand that wraps around the active site.
- The structure shown here is DNA polymerase I of *Thermus aquaticus*, bound to DNA.

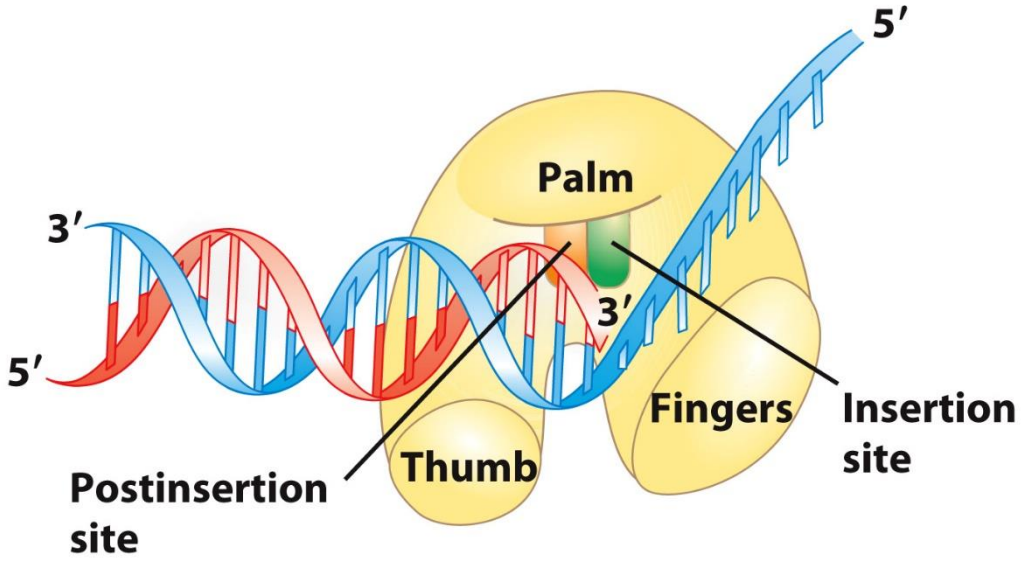


Figure 25-5d
Lehninger Principles of Biochemistry, Seventh Edition

- A cartoon interpretation of the polymerase structure shows the insertion and postinsertion parts of the active site.
- The insertion site is where the nucleotide addition occurs, and the postinsertion site is the site to which the newly formed base pair translocates.

DNA Replication Requires Many Enzymes and Protein Factors

- Replication in *E. coli* requires not just a single DNA polymerase but 20 or more different enzymes and proteins, each performing a specific task. The entire complex has been termed the **DNA replicase system** or **replisome**.
- Access to the DNA strands that are to act as templates requires separation of the two parent strands. This is generally accomplished by **helicases**, enzymes that move along the DNA and separate the strands, using chemical energy from ATP.
- Strand separation creates topological stress in the helical DNA structure, which is relieved by the action of **topoisomerases**.
- The separated strands are stabilized by **DNA-binding proteins**.
- Before DNA polymerases can begin synthesizing DNA, primers must be present on the template—generally, short segments of RNA synthesized by enzymes known as **primases**.
- Ultimately, the RNA primers are removed and replaced by DNA; in *E. coli*, this is one of the many functions of DNA polymerase I.
- A specialized nuclease that degrades RNA in RNA-DNA hybrids, called RNase H1, also removes some RNA primers.
- After an RNA primer is removed and the gap is filled in with DNA, a nick remains in the DNA backbone in the form of a broken phosphodiester bond. These nicks are sealed by **DNA ligases**.

DNA Replication Stages

1. Initiation
2. Elongation
3. Termination

Steps in DNA Replication

Replication occurs in three major steps:

1. **Initiation**
   - During initiation, proteins bind to the origin of replication while helicase unwinds the DNA helix and two replication forks are formed at the origin of replication.

2. **Elongation**
   - During elongation, a primer sequence is added with complementary RNA nucleotides, which are then replaced by DNA nucleotides.

   - During elongation the leading strand is made continuously, while the lagging strand is made in pieces called Okazaki fragments.

3. **Termination**
   - During termination, primers are removed and replaced with new DNA nucleotides and the backbone is sealed by DNA ligase.

Okazaki Fragments:

- Many DNA fragments are synthesized sequentially on the DNA template strand having the 5´- end. These DNA fragments are called Okazaki fragments. They are **1000 - 2000 nt** long in prokaryotes and **100-150 nt** long in eukaryotes.

- The daughter strand consisting of **Okazaki fragments** is called the **lagging strand.**

DNA Replication in Eukaryotes

- The basics of DNA replication are similar between bacteria and eukaryotes such as humans, but there are also some differences:
  - Eukaryotes usually have multiple linear chromosomes, each with multiple origins of replication. Humans can have up to 100,000 origins of replication.
  - Most of the *E. coli* enzymes have counterparts in eukaryotic DNA replication, but a single enzyme in *E. coli* may be represented by multiple enzymes in eukaryotes. For instance, there are five human DNA polymerases with important roles in replication.
  - Most eukaryotic chromosomes are linear. Because of the way the lagging strand is made, some DNA is lost from the ends of linear chromosomes (the telomeres) in each round of replication.

SUMMARY: DNA Replication

■ Replication of DNA occurs with very high fidelity and at a designated time in the cell cycle. Replication is semiconservative, each strand acting as template for a new daughter strand. It is carried out in three identifiable phases: initiation, elongation, and termination. The process starts at a single origin in bacteria and usually proceeds bidirectionally.

■ DNA is synthesized in the 5′→3′ direction by DNA polymerases. At the replication fork, the leading strand is synthesized continuously in the same direction as replication fork movement; the lagging strand is synthesized discontinuously as Okazaki fragments, which are subsequently ligated.

■ The fidelity of DNA replication is maintained by (1) base selection by the polymerase, (2) a 3′→5′ proofreading exonuclease activity that is part of most DNA polymerases, and (3) specific repair systems for mismatches left behind after replication.

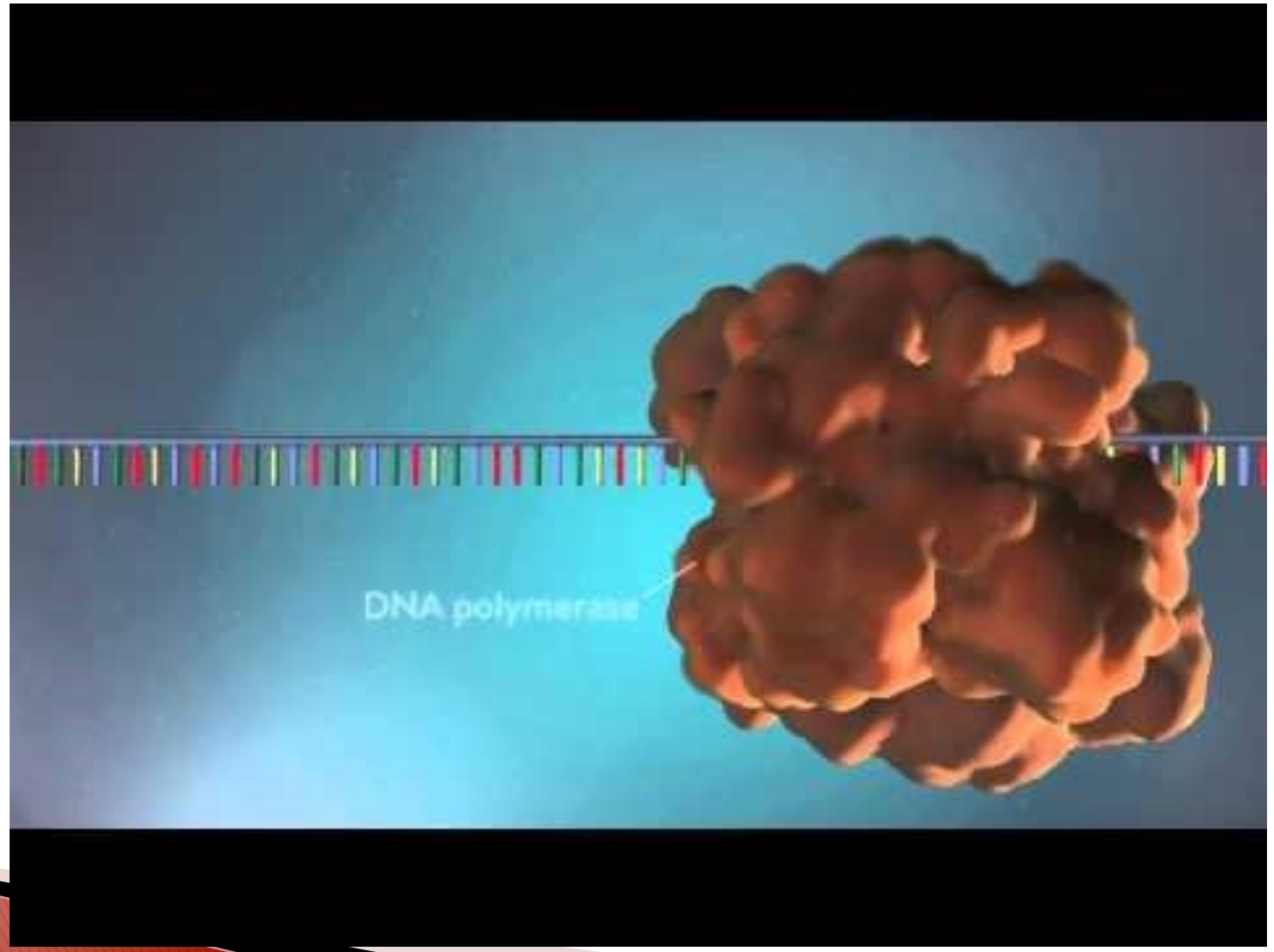
■ Most cells have several DNA polymerases. In *E. coli*, DNA polymerase III is the primary replication enzyme. DNA polymerase I is responsible for special functions during replication, recombination, and repair.

■ The separate initiation, elongation, and termination phases of DNA replication involve an array of enzymes and protein factors, many belonging to the AAA+ ATPase family.

■ The major replicative DNA polymerases in eukaryotes are DNA polymerases ε and δ. DNA polymerase α functions to synthesize primers.

Access link:

https://www.youtube.com/watch?v=TNKWgcFPHqw



Accesss Link: https://www.youtube.com/watch?v=EYGrElVyHnU

Topoisomerase 1 and 2