



# CHAPTER 16

## THE MOLECULE BASIS OF INHERITANCE

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### Section B: DNA Replication and Repair

1. During DNA replication, base pairing enables existing DNA strands to serve as templates for new complimentary strands
2. A large team of enzymes and other proteins carries out DNA replication
3. Enzymes proofread DNA during its replication and repair damage to existing DNA
4. The ends of DNA molecules are replicated by a special mechanism

# Introduction

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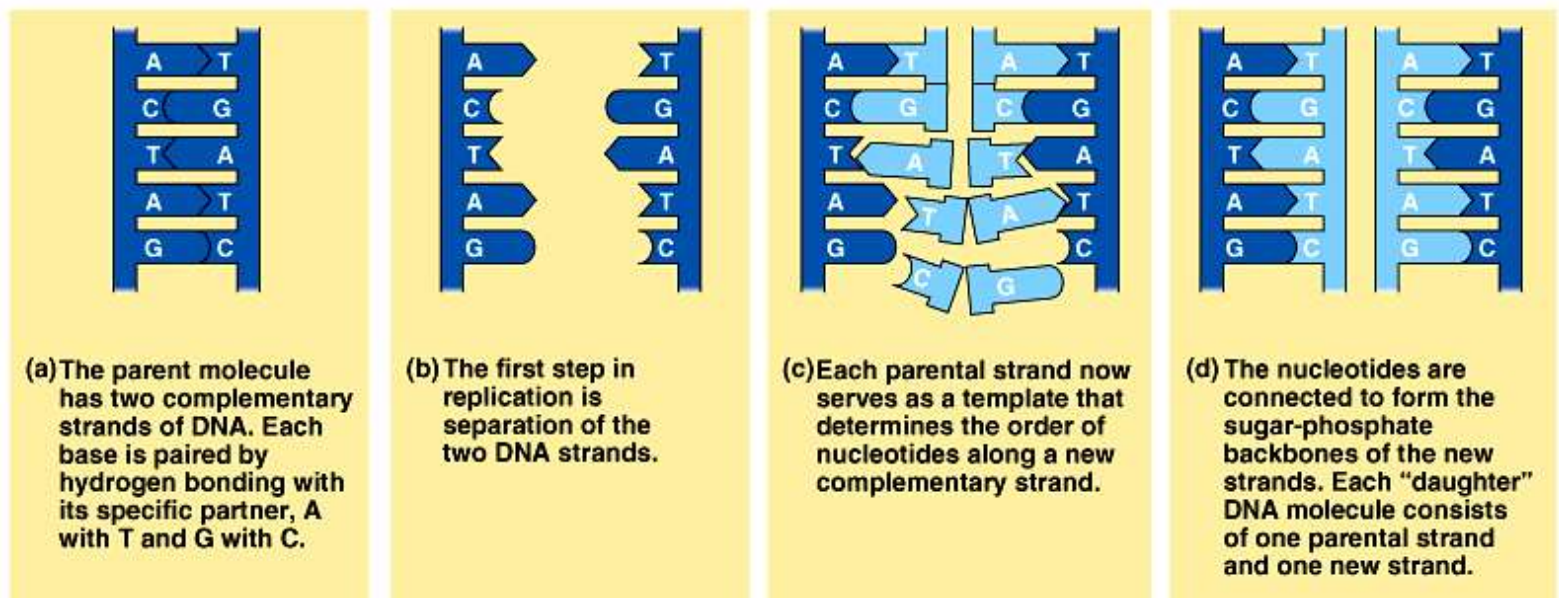
- The specific pairing of nitrogenous bases in DNA was the flash of inspiration that led Watson and Crick to the correct double helix.
- The possible mechanism for the next step, the accurate replication of DNA, was clear to Watson and Crick from their double helix model.

# 1. During DNA replication, base pairing enables existing DNA strands to serve as templates for new complimentary strands

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- In a second paper Watson and Crick published their hypothesis for how DNA replicates.
  - Essentially, because each strand is complementary to each other, each can form a template when separated.
  - The order of bases on one strand can be used to add in complementary bases and therefore duplicate the pairs of bases exactly.

- When a cell copies a DNA molecule, each strand serves as a template for ordering nucleotides into a new complementary strand.
  - One at a time, nucleotides line up along the template strand according to the base-pairing rules.
  - The nucleotides are linked to form new strands.



**Fig. 16.7**

- Watson and Crick's model, **semiconservative replication**, predicts that when a double helix replicates each of the daughter molecules will have one old strand and one newly made strand.
- Other competing models, the conservative model and the dispersive model, were also proposed.

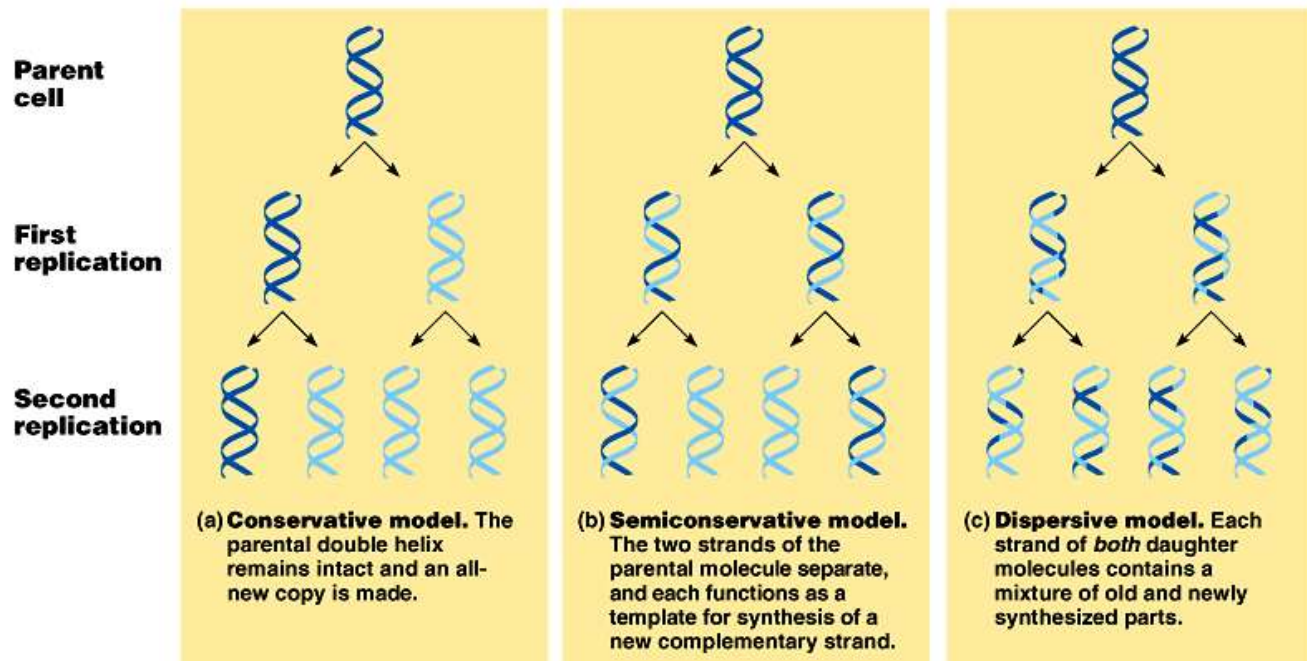


Fig. 16.8

- Experiments in the late 1950s by Matthew Meselson and Franklin Stahl supported the semiconservative model, proposed by Watson and Crick, over the other two models.
  - In their experiments, they labeled the nucleotides of the old strands with a heavy isotope of nitrogen ( $^{15}\text{N}$ ) while any new nucleotides would be indicated by a lighter isotope ( $^{14}\text{N}$ ).
  - Replicated strands could be separated by density in a centrifuge.
  - Each model: the semi-conservative model, the conservative model, and the dispersive model, made specific predictions on the density of replicated DNA strands.

- The first replication in the  $^{14}\text{N}$  medium produced a band of hybrid ( $^{15}\text{N}$ - $^{14}\text{N}$ ) DNA, eliminating the conservative model.
- A second replication produced both light and hybrid DNA, eliminating the dispersive model and supporting the semiconservative model.

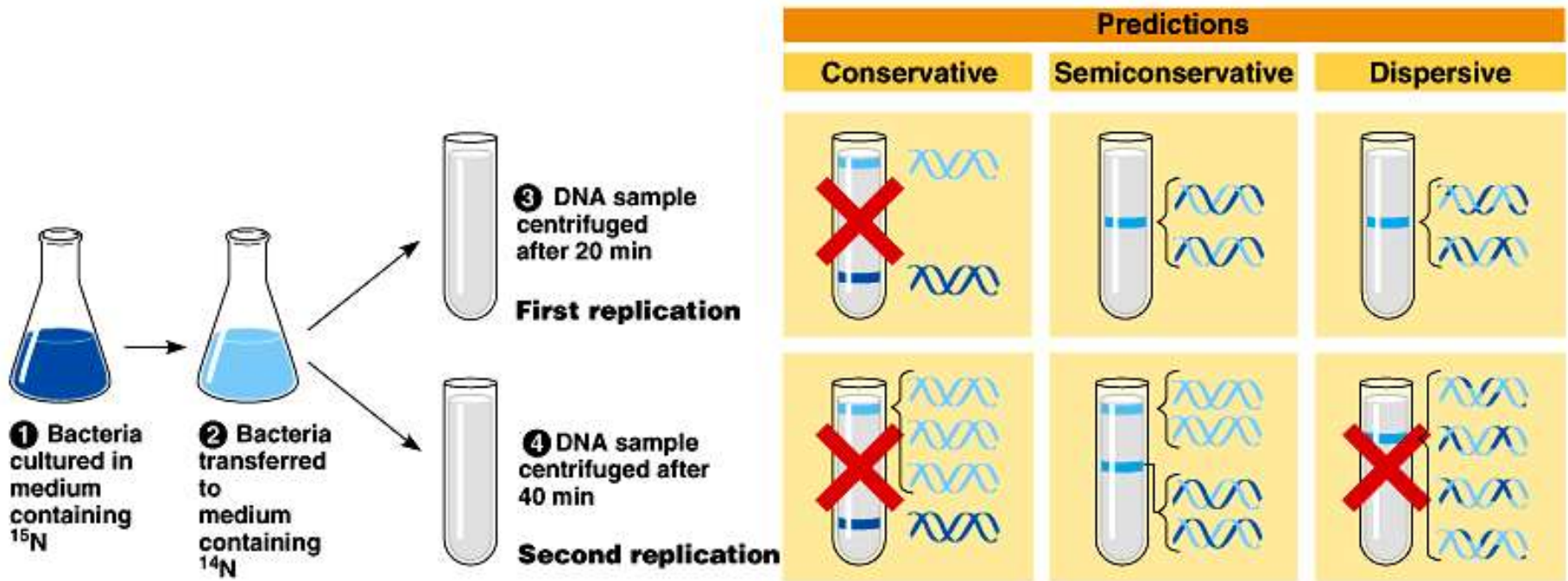


Fig. 16.9



## 2. A large team of enzymes and other proteins carries out DNA replication

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- It takes *E. coli* less than an hour to copy each of the 5 million base pairs in its single chromosome and divide to form two identical daughter cells.
- A human cell can copy its 6 billion base pairs and divide into daughter cells in only a few hours.
- This process is remarkably accurate, with only one error per billion nucleotides.
- More than a dozen enzymes and other proteins participate in DNA replication.



- The replication of a DNA molecule begins at special sites, **origins of replication**.
- In bacteria, this is a single specific sequence of nucleotides that is recognized by the replication enzymes.
  - These enzymes separate the strands, forming a replication “bubble”.
  - Replication proceeds in both directions until the entire molecule is copied.

- In eukaryotes, there may be hundreds or thousands of origin sites per chromosome.
  - At the origin sites, the DNA strands separate forming a replication “bubble” with **replication forks** at each end.
  - The replication bubbles elongate as the DNA is replicated and eventually fuse.

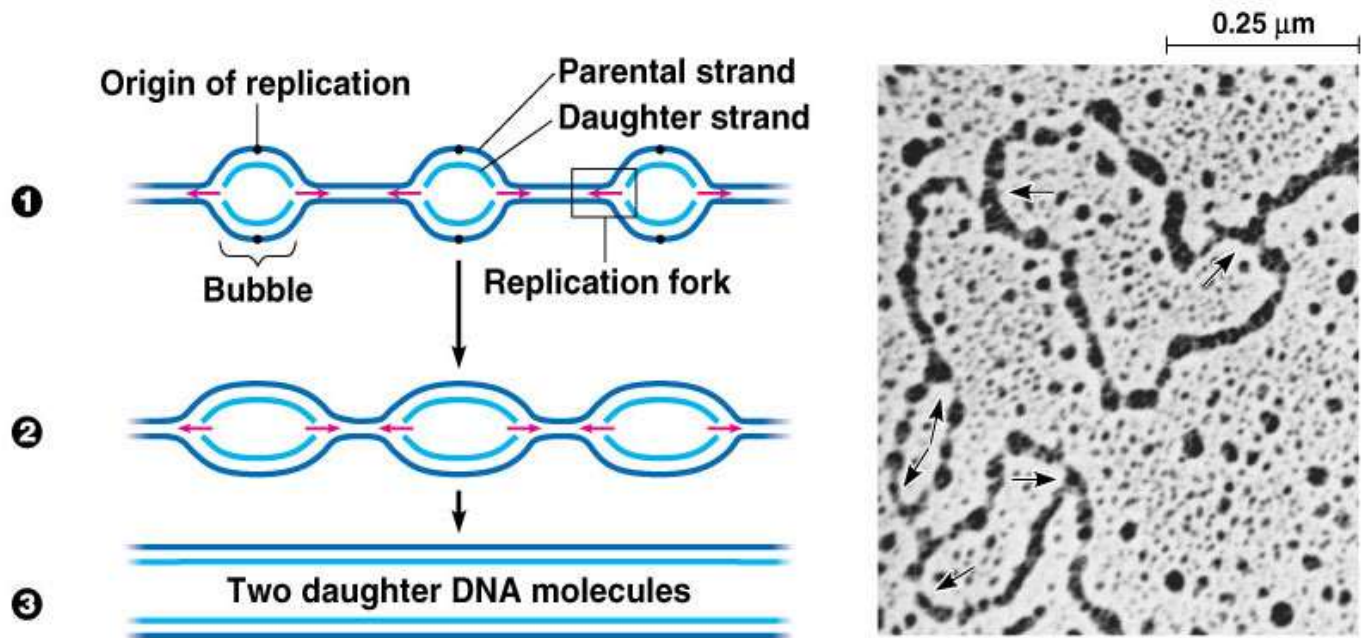


Fig. 16.10

- **DNA polymerases** catalyze the elongation of new DNA at a replication fork.
- As nucleotides align with complementary bases along the template strand, they are added to the growing end of the new strand by the polymerase.
  - The rate of elongation is about 500 nucleotides per second in bacteria and 50 per second in human cells. The raw nucleotides are nucleoside triphosphates.
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  - Each has a nitrogen base, deoxyribose, and a triphosphate tail.

- As each nucleotide is added, the last two phosphate groups are hydrolyzed to form pyrophosphate.
- The exergonic hydrolysis of pyrophosphate to two inorganic phosphate molecules drives the polymerization of the nucleotide to the new strand.

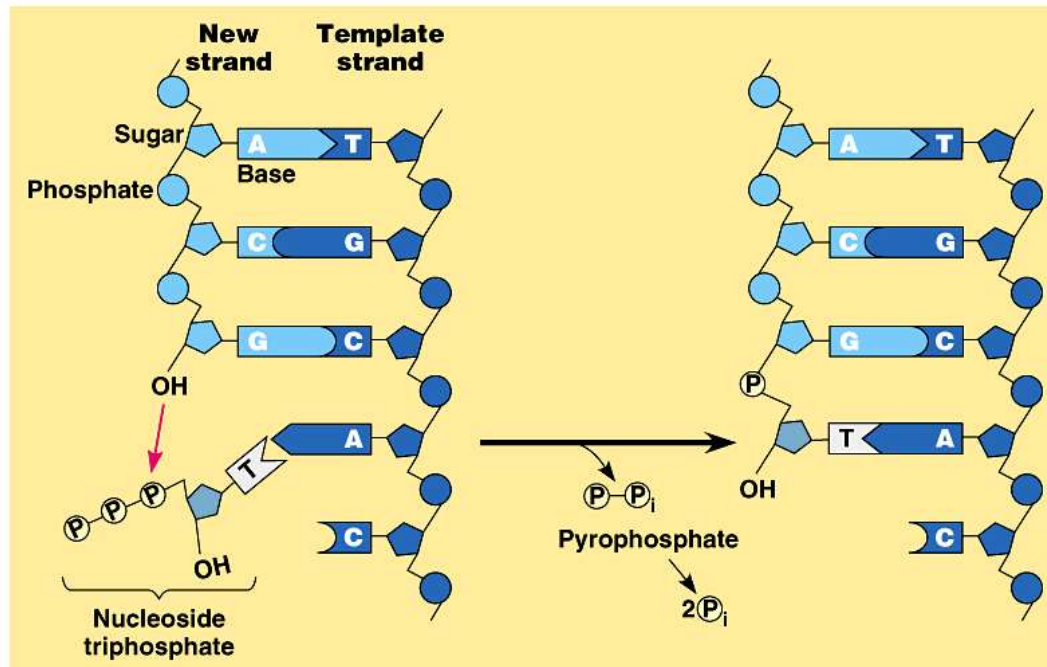


Fig. 16.11

- The strands in the double helix are *antiparallel*.
- The sugar-phosphate backbones run in opposite directions.

- Each DNA strand has a 3' end with a free hydroxyl group attached to deoxyribose and a 5' end with a free phosphate group attached to deoxyribose.
- The 5' → 3' direction of one strand runs counter to the 3' → 5' direction of the other strand.

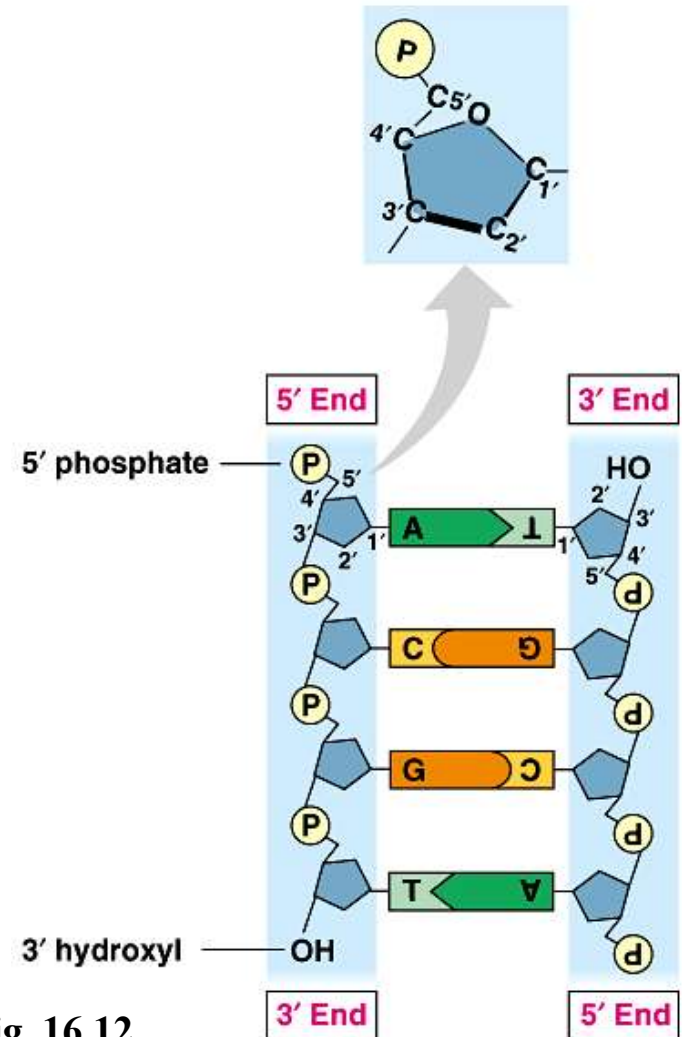


Fig. 16.12

- DNA polymerases can only add nucleotides to the free 3' end of a growing DNA strand.
- A new DNA strand can only elongate in the 5' → 3' direction.
- This creates a problem at the replication fork because one parental strand is oriented 3' → 5' into the fork, while the other antiparallel parental strand is oriented 5' → 3' into the fork.
- At the replication fork, one parental strand (3' → 5' into the fork), the **leading strand**, can be used by polymerases as a template for a continuous complementary strand.

- The other parental strand (5' → 3' into the fork), the **lagging strand**, is copied away from the fork in short segments (Okazaki fragments).
- Okazaki fragments, each about 100-200 nucleotides, are joined by **DNA ligase** to form the sugar-phosphate backbone of a single DNA strand.

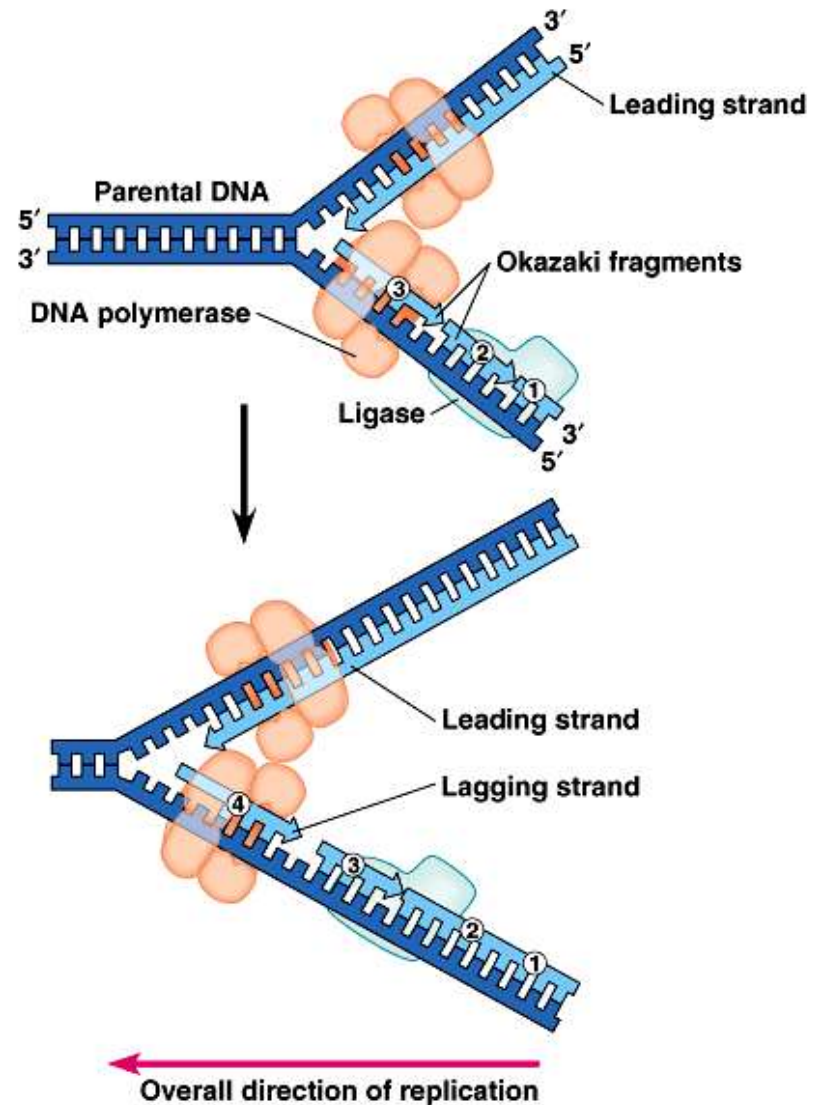


Fig. 16.13



- DNA polymerases cannot *initiate* synthesis of a polynucleotide because they can only add nucleotides to the end of an existing chain that is base-paired with the template strand.
- To start a new chain requires a **primer**, a short segment of RNA.
  - The primer is about 10 nucleotides long in eukaryotes.
- **Primase**, an RNA polymerase, links ribonucleotides that are complementary to the DNA template into the primer.
  - RNA polymerases can start an RNA chain from a single template strand.

- After formation of the primer, DNA polymerases can add deoxyribonucleotides to the 3' end of the ribonucleotide chain.
- Another DNA polymerase later replaces the primer ribonucleotides with deoxyribonucleotides complementary to the template.

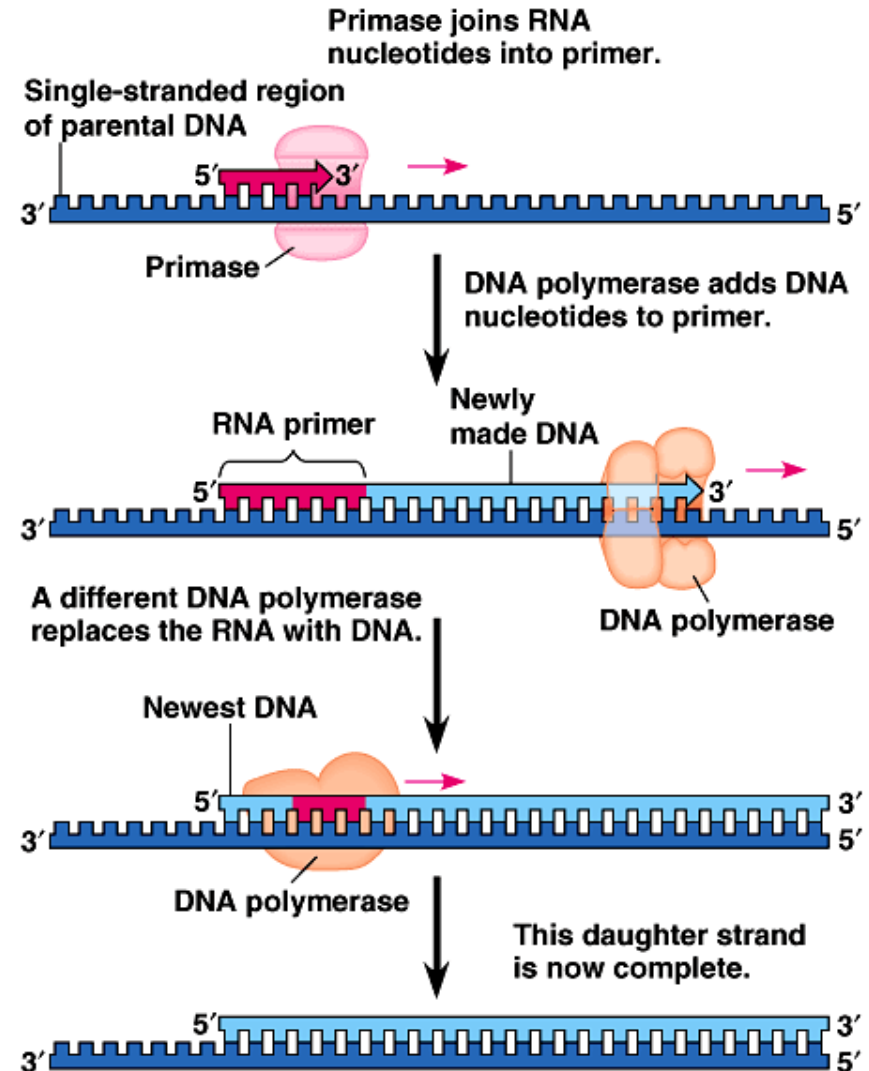


Fig. 16.14

- Returning to the original problem at the replication fork, the leading strand requires the formation of only a single primer as the replication fork continues to separate.
- The lagging strand requires formation of a new primer as the replication fork progresses.
- After the primer is formed, DNA polymerase can add new nucleotides away from the fork until it runs into the previous Okazaki fragment.
- The primers are converted to DNA before DNA ligase joins the fragments together.

- In addition to primase, DNA polymerases, and DNA ligases, several other proteins have prominent roles in DNA synthesis.
- A **helicase** untwists and separates the template DNA strands at the replication fork.
- **Single-strand binding proteins** keep the unpaired template strands apart during replication.

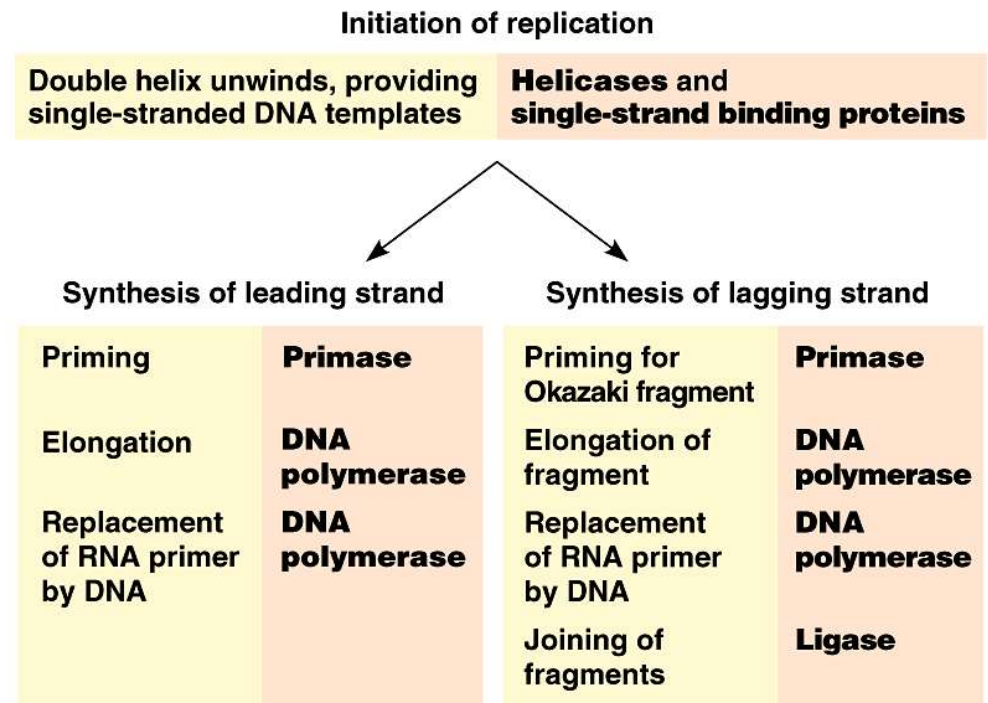


Fig. 16.15

- To summarize, at the replication fork, the leading strand is copied continuously into the fork from a single primer.
- The lagging strand is copied away from the fork in short segments, each requiring a new primer.

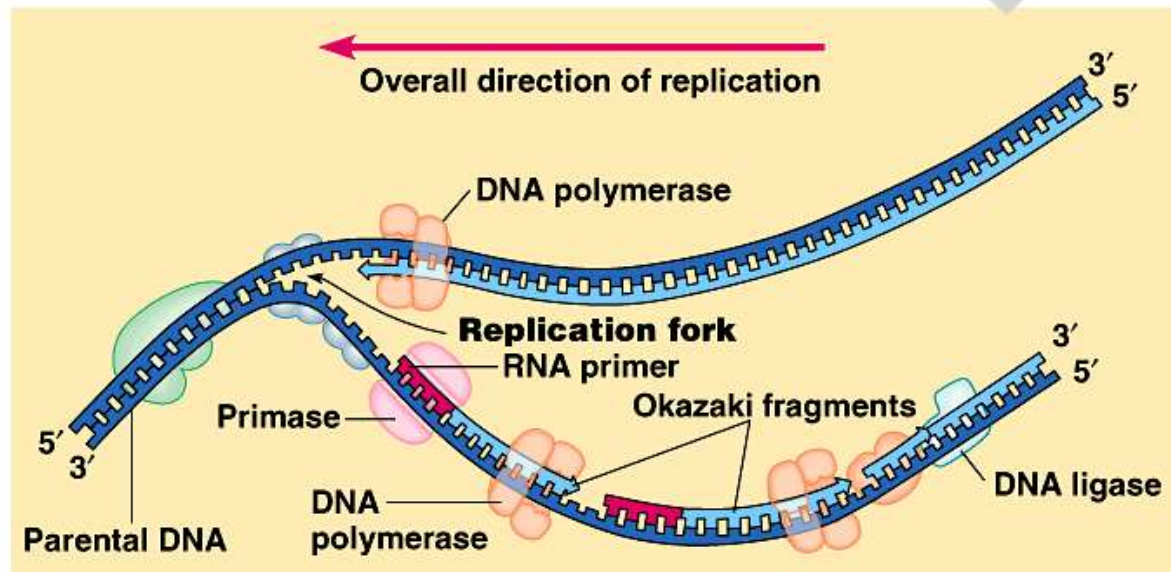
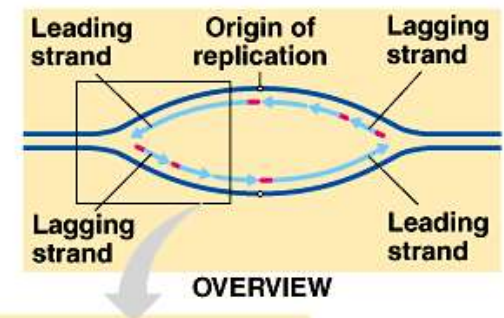


Fig. 16.16

- It is conventional and convenient to think of the DNA polymerase molecules moving along a stationary DNA template.
- In reality, the various proteins involved in DNA replication form a single large complex that may be anchored to the nuclear matrix.
- The DNA polymerase molecules “reel in” the parental DNA and “extrude” newly made daughter DNA molecules.

### 3. Enzymes proofread DNA during its replication and repair damage in existing DNA

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- Mistakes during the initial pairing of template nucleotides and complementary nucleotides occurs at a rate of one error per 10,000 base pairs.
- DNA polymerase proofreads each new nucleotide against the template nucleotide as soon as it is added.
- If there is an incorrect pairing, the enzyme removes the wrong nucleotide and then resumes synthesis.
- The final error rate is only one per billion nucleotides.



- DNA molecules are constantly subject to potentially harmful chemical and physical agents.
  - Reactive chemicals, radioactive emissions, X-rays, and ultraviolet light can change nucleotides in ways that can affect encoded genetic information.
  - DNA bases often undergo spontaneous chemical changes under normal cellular conditions.
- Mismatched nucleotides that are missed by DNA polymerase or mutations that occur after DNA synthesis is completed can often be repaired.
  - Each cell continually monitors and repairs its genetic material, with over 130 repair enzymes identified in humans.

- In **mismatch repair**, special enzymes fix incorrectly paired nucleotides.
  - A hereditary defect in one of these enzymes is associated with a form of colon cancer.
- In **nucleotide excision repair**, a **nuclease** cuts out a segment of a damaged strand.
  - The gap is filled in by DNA polymerase and ligase.

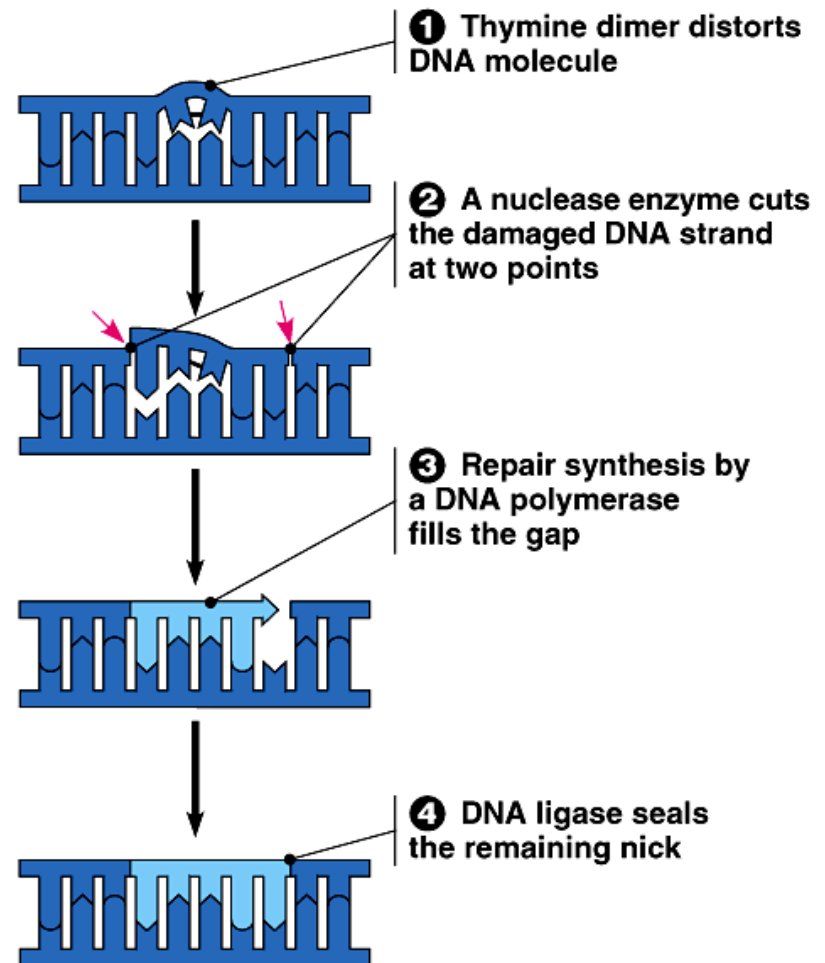


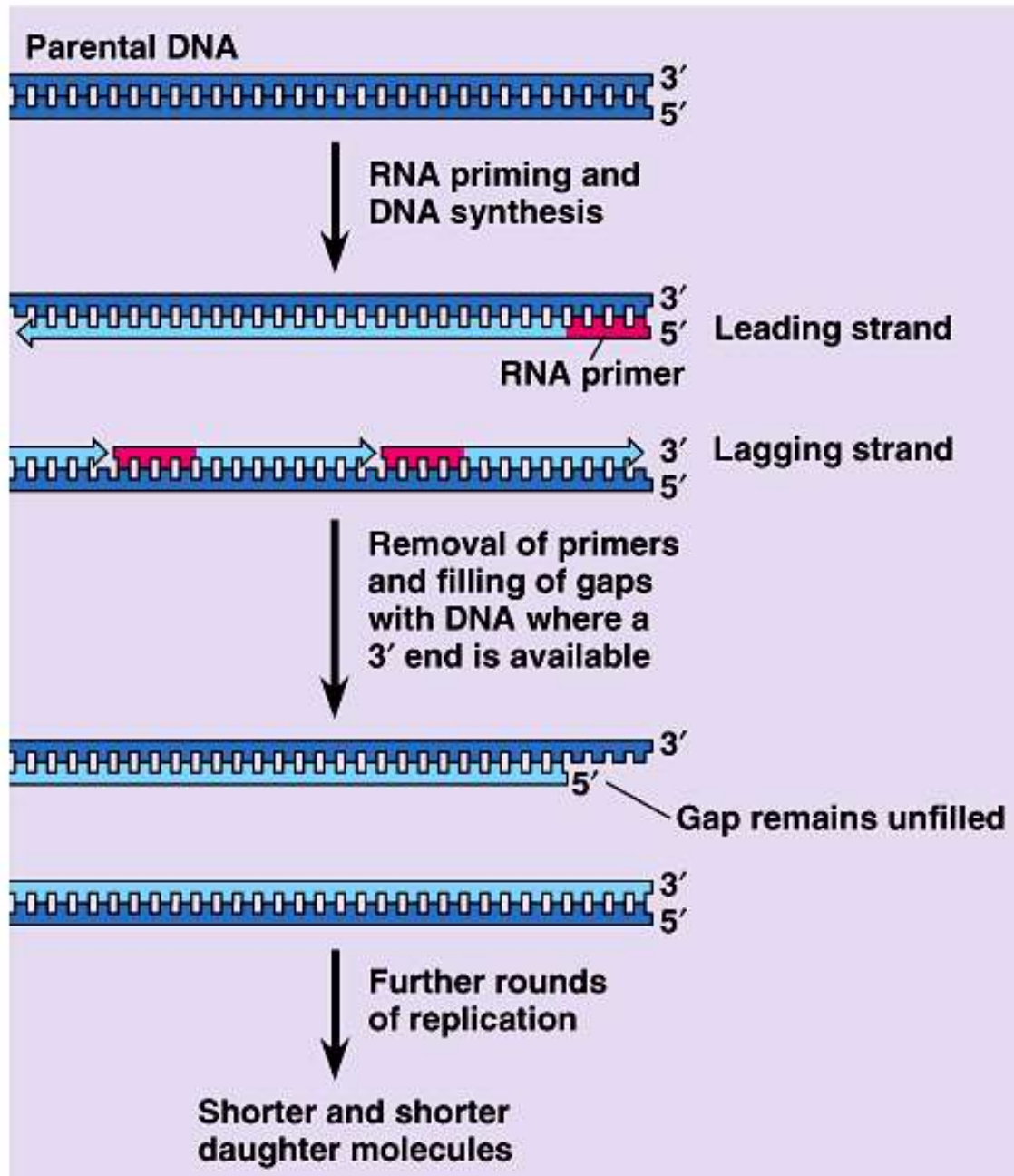
Fig. 16.17

- The importance of proper function of repair enzymes is clear from the inherited disorder xeroderma pigmentosum.
  - These individuals are hypersensitive to sunlight.
  - In particular, ultraviolet light can produce thymine dimers between adjacent thymine nucleotides.
  - This buckles the DNA double helix and interferes with DNA replication.
  - In individuals with this disorder, mutations in their skin cells are left uncorrected and cause skin cancer.

## 4. The ends of DNA molecules are replicated by a special mechanism

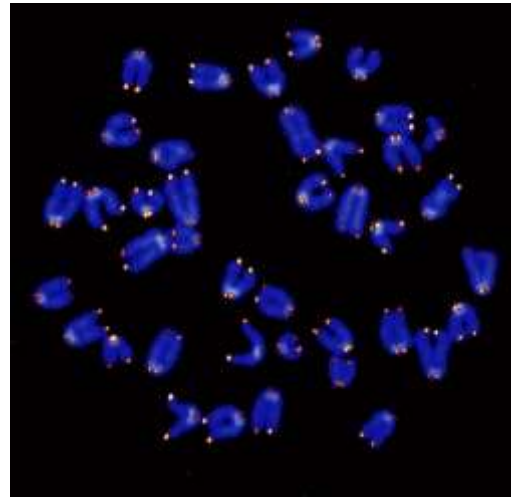
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- Limitations in the DNA polymerase create problems for the linear DNA of eukaryotic chromosomes.
- The usual replication machinery provides no way to complete the 5' ends of daughter DNA strands.
  - Repeated rounds of replication produce shorter and shorter DNA molecules.



**Fig. 16.18**

- The ends of eukaryotic chromosomal DNA molecules, the **telomeres**, have special nucleotide sequences.
  - In human telomeres, this sequence is typically TTAGGG, repeated between 100 and 1,000 times.
- Telomeres protect genes from being eroded through multiple rounds of DNA replication.



**Fig. 16.19a**

- Eukaryotic cells have evolved a mechanism to restore shortened telomeres.
- **Telomerase** uses a short molecule of RNA as a template to extend the 3' end of the telomere.
  - There is now room for primase and DNA polymerase to extend the 5' end.
  - It does not repair the 3'-end "overhang," but it does lengthen the telomere.

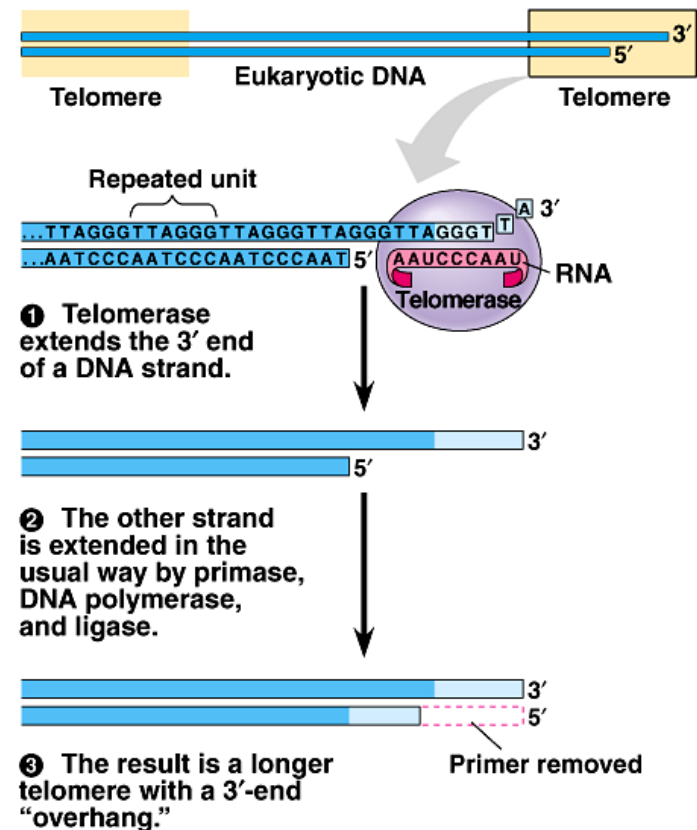


Fig. 16.19b



- Telomerase is *not* present in most cells of multicellular organisms.
- Therefore, the DNA of dividing somatic cells and cultured cells does tend to become shorter.
- Thus, telomere length may be a limiting factor in the life span of certain tissues and the organism.
- Telomerase is present in germ-line cells, ensuring that zygotes have long telomeres.
- Active telomerase is also found in cancerous somatic cells.
  - This overcomes the progressive shortening that would eventually lead to self-destruction of the cancer.