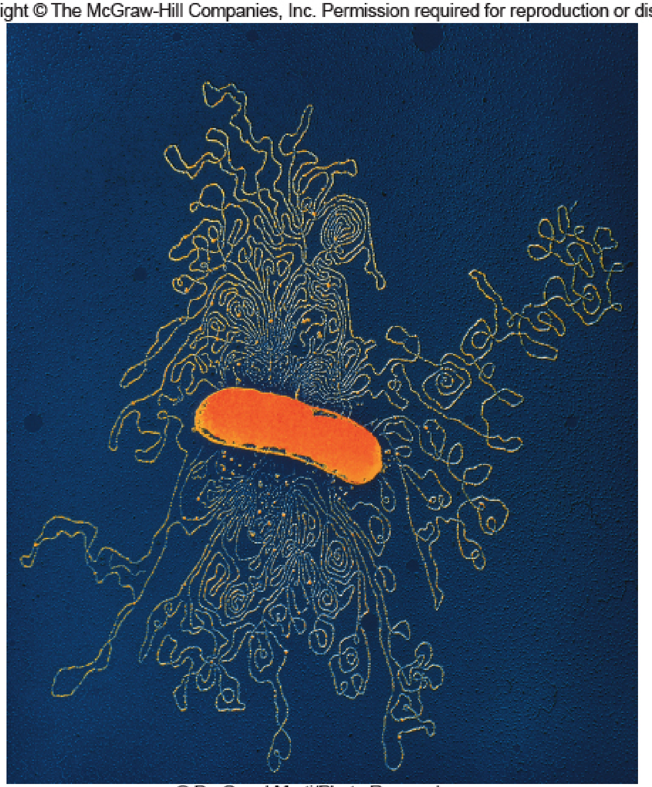
DNA Structure & Replication (Outline)

- Historical perspective (DNA as the genetic material):
  - Genetic transformation
  - DNA as the transforming agent
  - DNA is the genetic material in bacterial viruses (phage)
- Historical perspective (Structure of DNA):
  - Identifying ribose and deoxy ribose
  - Equal parts of nucleotide parts
  - The base-pairing rule
  - DNA structure: double stranded anti-parallel strands
  - DNA structure: helix
- Basis for polarity of SS DNA and anti-parallel complementary strands of DNA
- Models of DNA replication
- Mechanism of DNA replication: steps and molecular machinery
- Fidelity of DNA replication

Genetic Material

"A genetic material must carry out two jobs: duplicate itself and control the development of the rest of the cell in a specific way."

- Francis Crick, 1953



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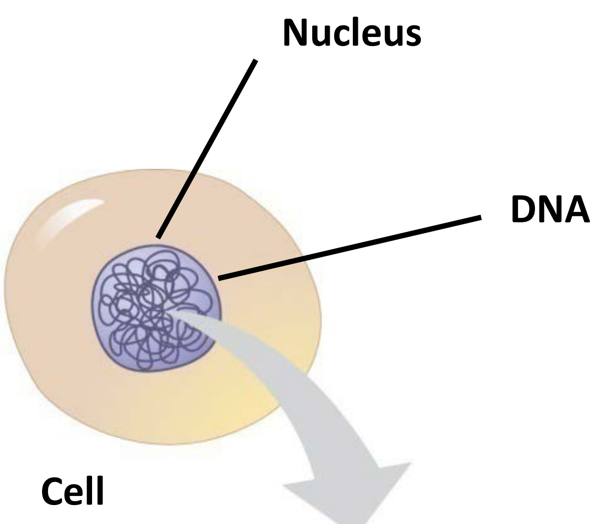


The Road to the Double Helix

Table 9.1 **The Road to the Double Helix**

Investigator	Contribution	Timeline
Friedrich Miescher	Isolated nuclein in white blood cell nuclei	1869
Frederick Griffith	Transferred killing ability between types of bacteria	1928
Oswald Avery, Colin MacLeod, and Maclyn McCarty	Discovered that DNA transmits killing ability in bacteria	1940s
Alfred Hershey and Martha Chase	Determined that the part of a virus that infects and replicates is its nucleic acid and not its protein	1950
Phoebus Levene, Erwin Chargaff, Maurice Wilkins, and Rosalind Franklin	Discovered DNA components, proportions, and positions	1909–early 1950s
James Watson and Francis Crick	Elucidated DNA's three-dimensional structure	1953
James Watson	Had his genome sequenced	2008

History leading to establishing DNA as the genetic material

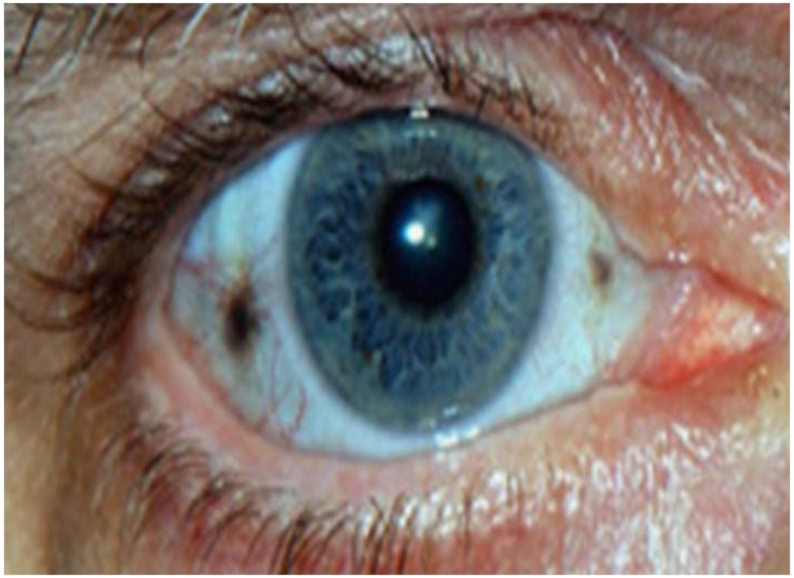
Friedrich Miescher, 1871

- Swiss physician and biochemist
- Isolated white blood cell nuclei from pus
    - Acid substance with nitrogen and phosphorus
    - *"Nuclein" later changed into nucleic acid*

History of DNA

Archibald Garrod, 1902

- English physician
- Linked inheritance of "inborn errors of metabolism" with the lack of particular enzymes
- First described the disease alkaptonuria



History of DNA

Frederick Griffith, 1928

- English microbiologist
- Established the concept of **transformation**:

a change in genotype (*genetic makeup)* by a foreign substance that changes the phenotype (*observed properties or trait*)

Frederick Griffith

Worked with *Diplococcus pneumonia,* which exists in two types

- Type S (Smooth) = Produces capsule
- Type R (Rough) = No capsule
- Capsule associated with virulence (causing disease)

Discovery of Bacterial Transformation

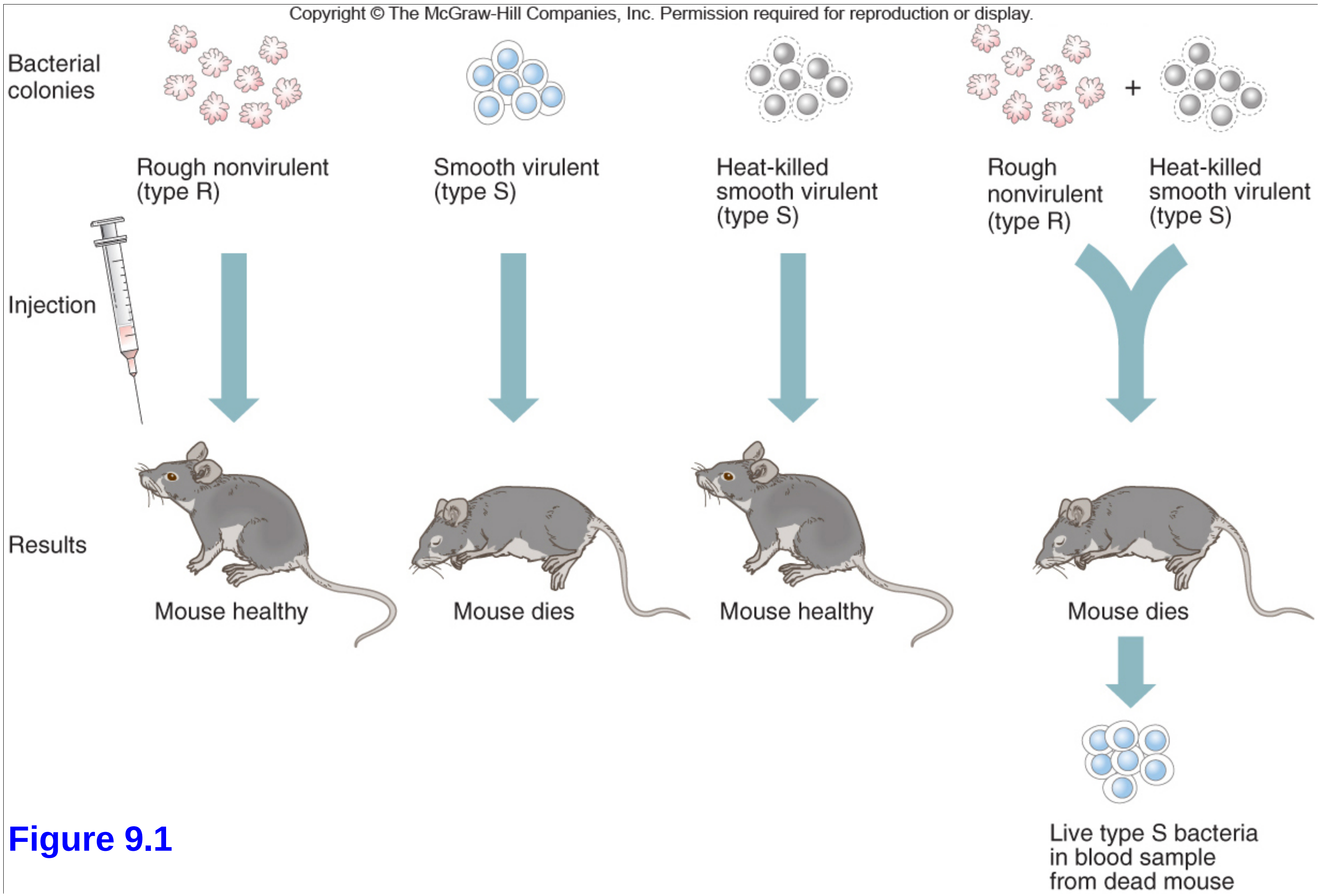


Figure 9.1

History of DNA

Avery, MacLeod, and McCarty, 1944

- American physicians
- DNA is the **transforming material**

  (Can convert Type R bacteria into S)

The Transforming Principle

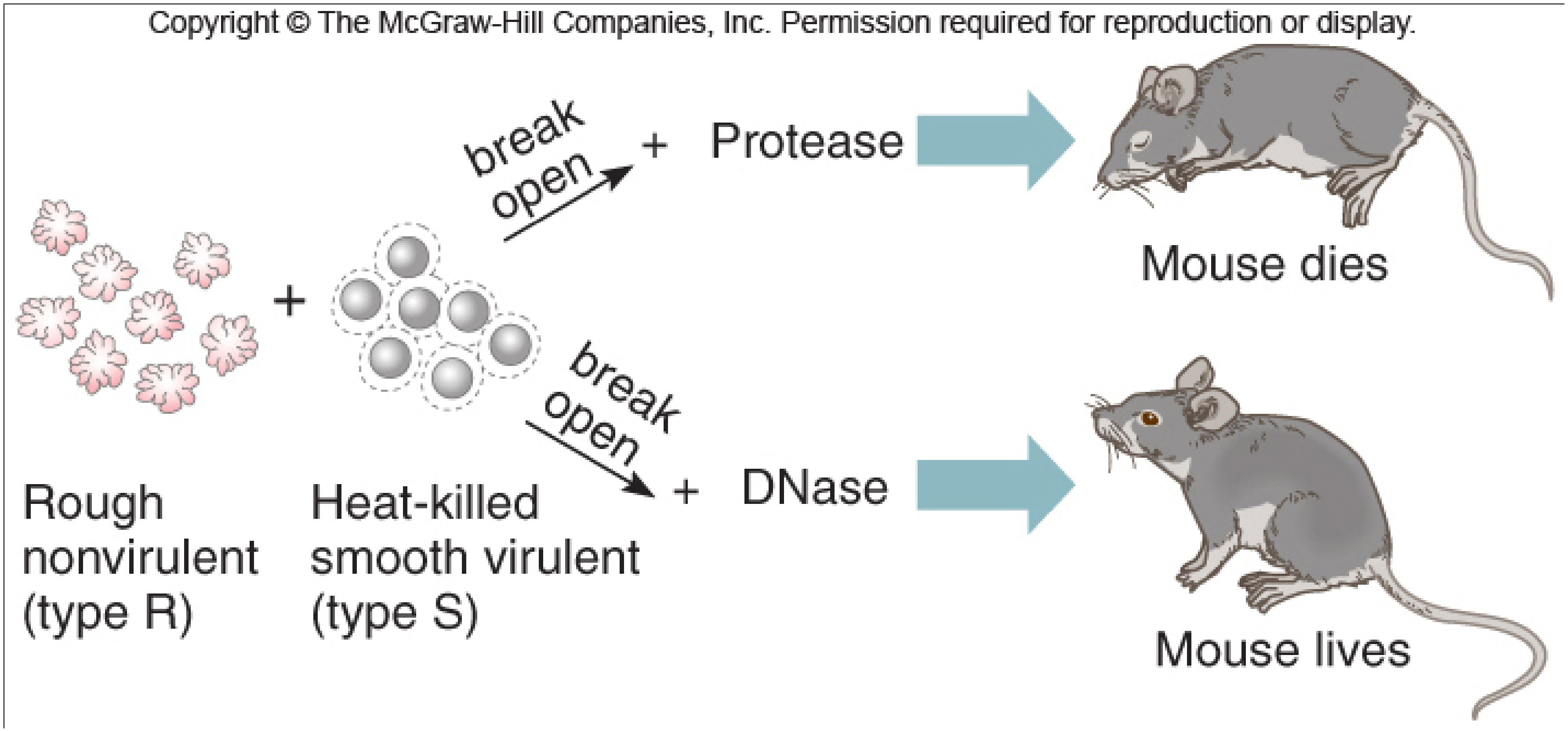


Figure 9.2

History of DNA

Alfred Hershey and Martha Chase, 1953

- American microbiologists
- Viruses can infect *E. coli* bacteria
- A virus in not a cell, it has protein “head” and DNA core
- Can replicate only using host living cells as host
- DNA is the genetic material of these viruses

Discovering the Structure of DNA

Phoebus Levine

- Russian-American biochemist
- Identified the 5-carbon sugars ribose in 1909 and deoxyribose in 1929
- Discovered that the three parts of a nucleotide are found in equal proportions
    - **Sugar**
    - **Phosphate**
    - **Nitrogen Base**

Discovering the Structure of DNA

Erwin Chargaff, 1951

- Austrian-American biochemist

- Analyzed base composition of DNA from various species and observed regular relationships:
  - Adenine + Guanine = Thymine + Cytosine
  - A = T and C = G

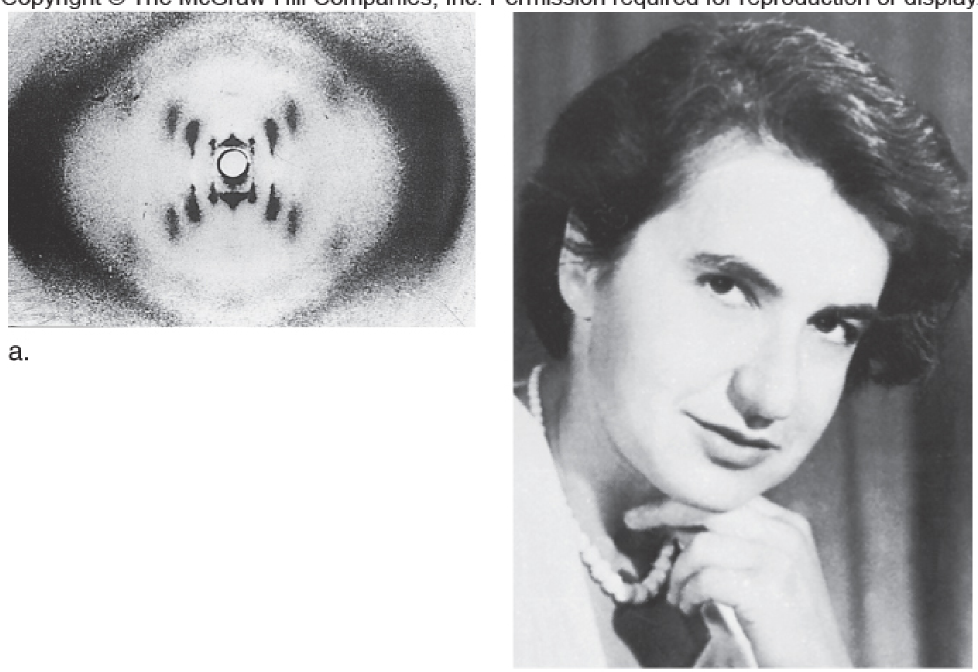
Discovering the Structure of DNA

Rosalind Franklin and Maurice Wilkins, 1952

- English scientists
- Used a technique called X-ray diffraction
- It took Franklin 100 hours to obtain “photo 51”

Discovering the Structure of DNA

Franklin reasoned that the DNA is a helix with symmetrically organized subunits



a.

b. Rosalind Franklin 1920–1958

a: © Science Source/Photo Researchers; b: From The Double Helix by James D. Watson, 1968, Atheneum Press, NY. Courtesy Cold Spring Harbor Laboratory Archives

Figure 9.4

Discovering the Structure of DNA

James Watson and Francis Crick

- Did not perform any experiments

- Used results of others and cardboard cutouts to build a model of the structure of DNA

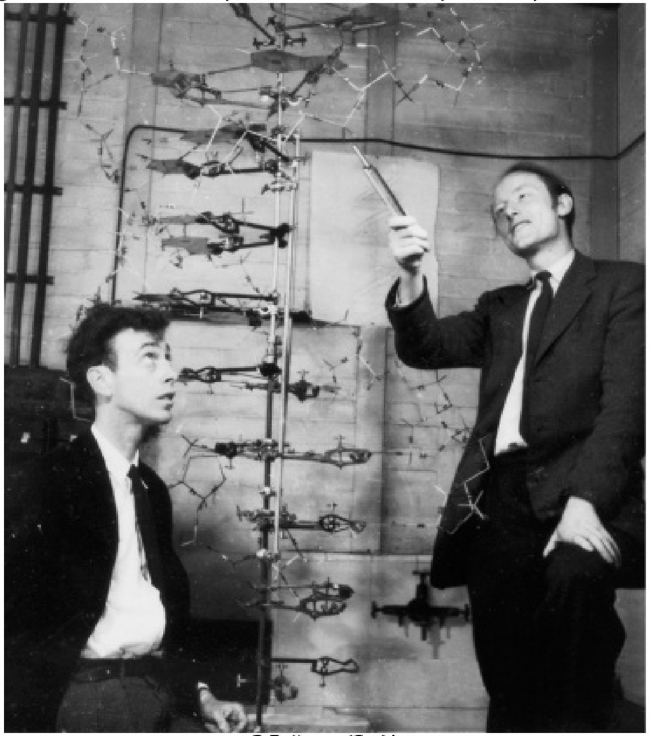


Figure 9.5

DNA Structure

A single building block is a **nucleotide**

Each nucleotide is composed of:

- A deoxyribose sugar
- A phosphate group
- A nitrogenous base; one of four types
    - Adenine (A), Guanine (G) = Purines
    - Cytosine (C), Thymine (T) = Pyrimidines

DNA Structure

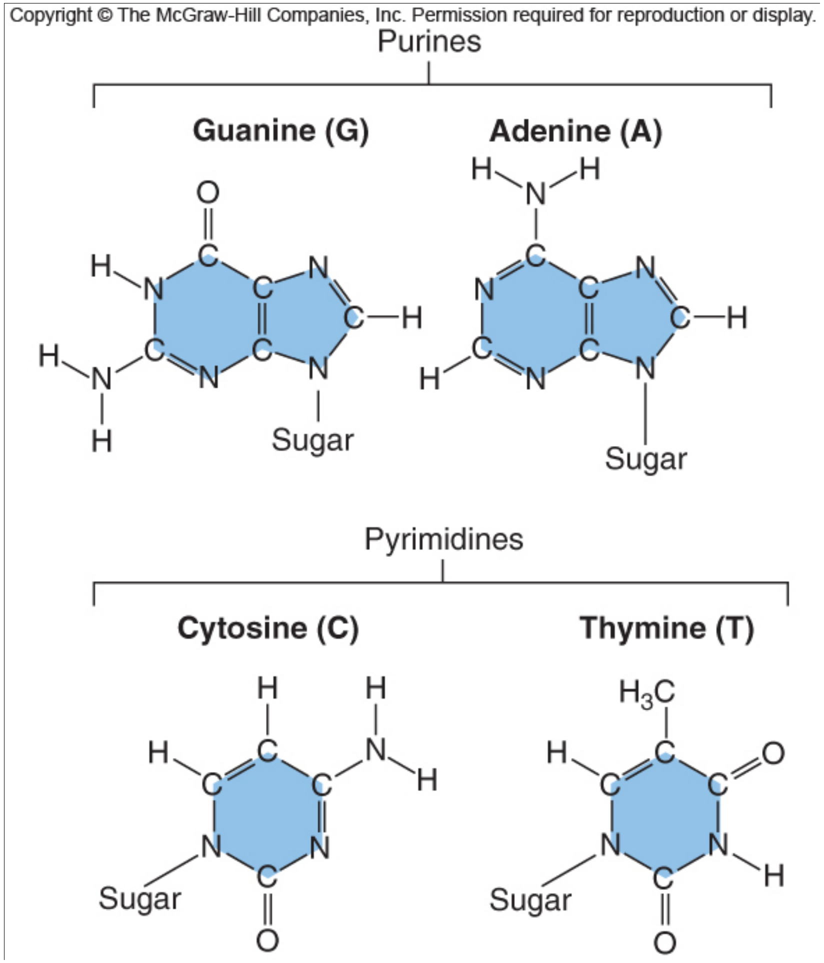


Figure 9.6

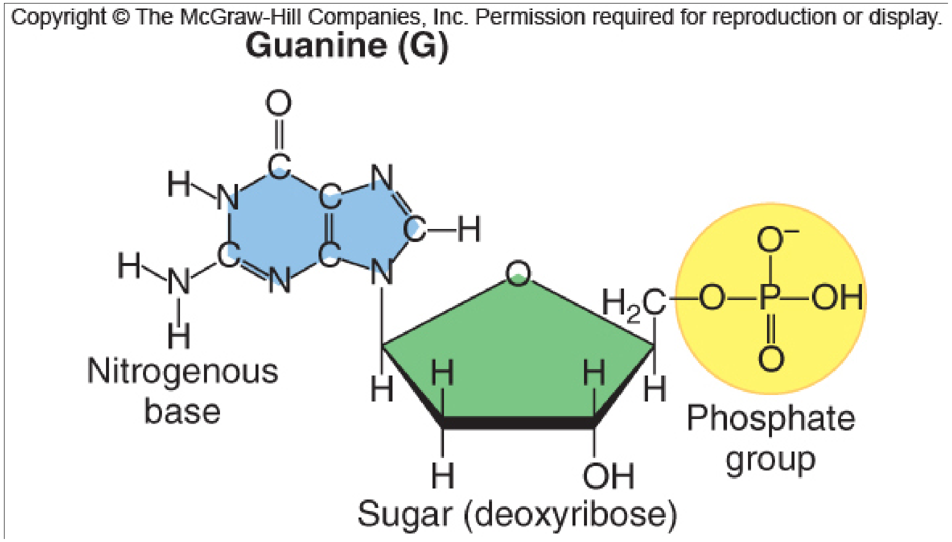
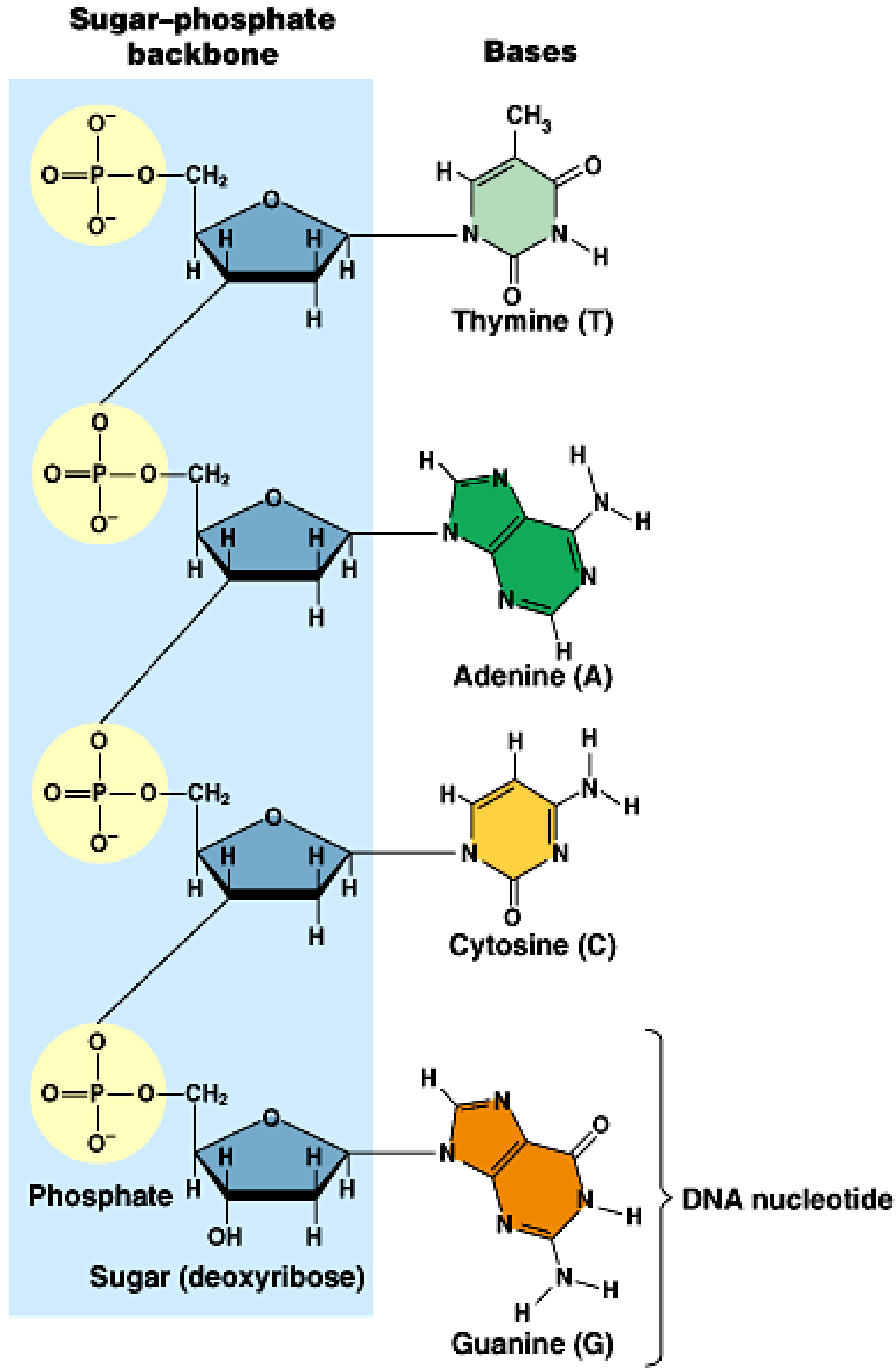


Figure 9.7



Sugar–phosphate backbone
Bases
Thymine (T)
Adenine (A)
Cytosine (C)
Guanine (G)
Phosphate
Sugar (deoxyribose)
DNA nucleotide

DNA Structure

Nucleotides join via a bond between the 5'-phosphate of one and the 3' hydroxyl of another

- This creates a continuous sugar-phosphate backbone

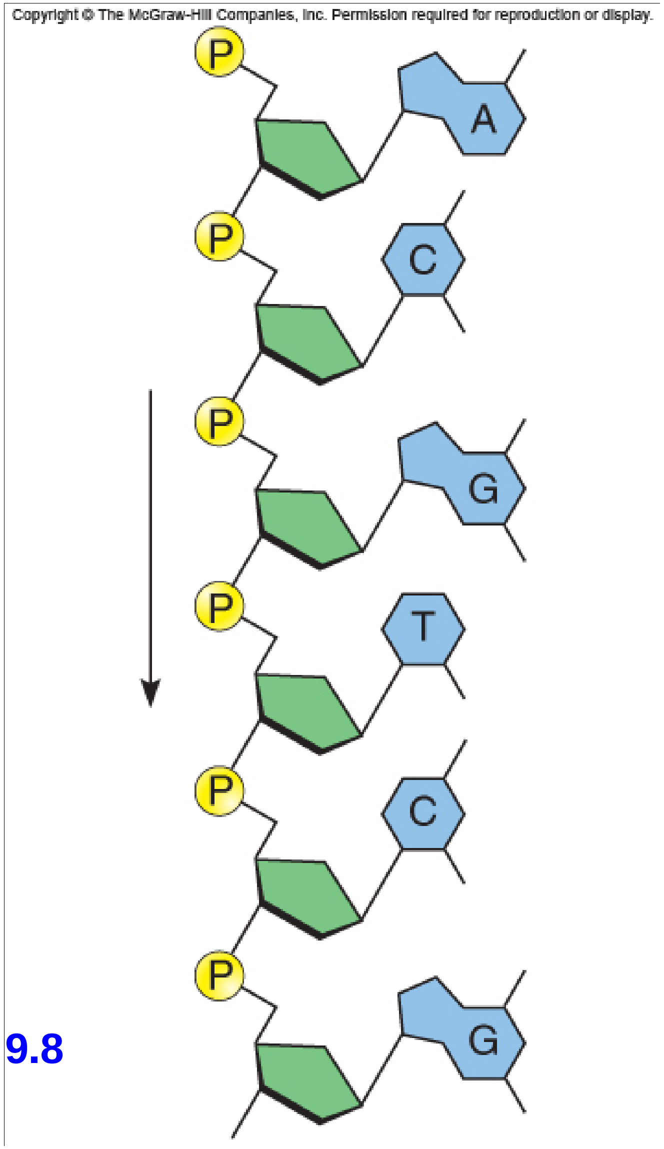
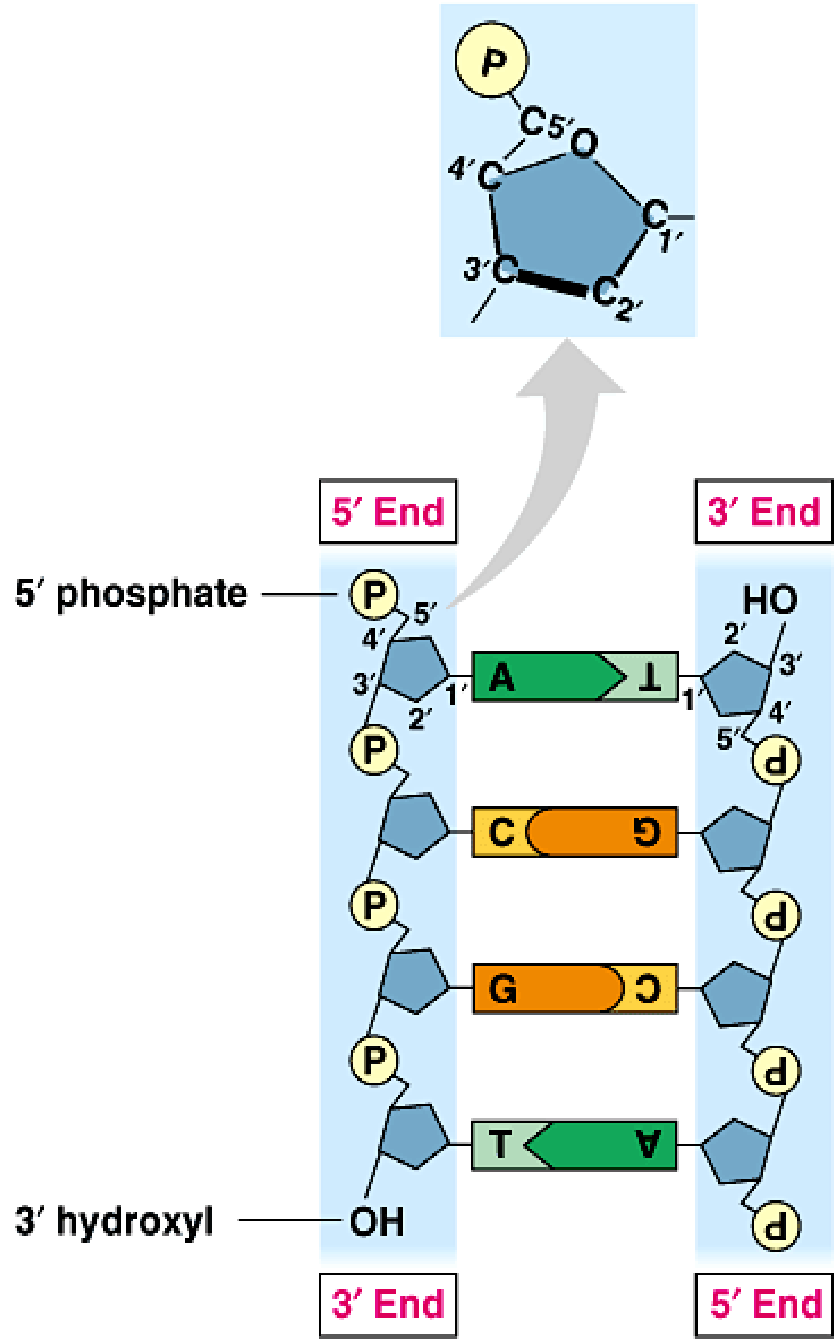


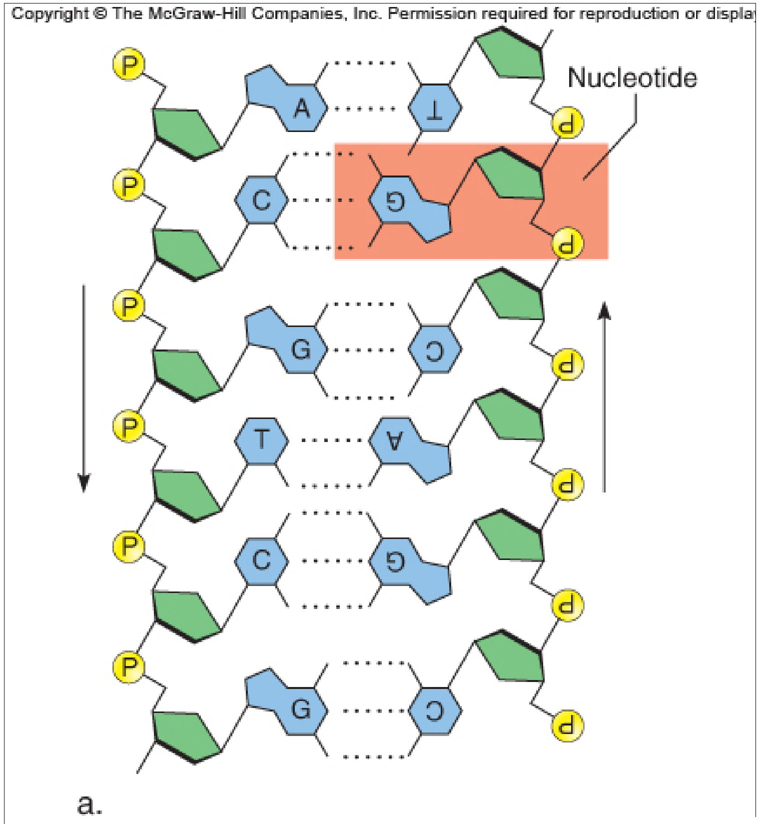
Figure 9.8

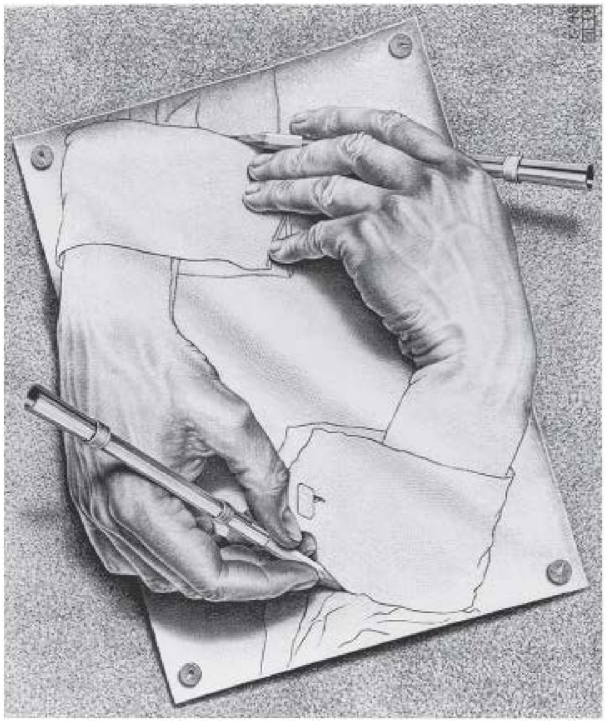
Polarity and anti-parallel nature of the two DNA strands (5’ and 3’ ends)



Two polynucleotide chains align forming a **double helix**

- The opposing orientation (head-to-toe) is called **antiparallelism**



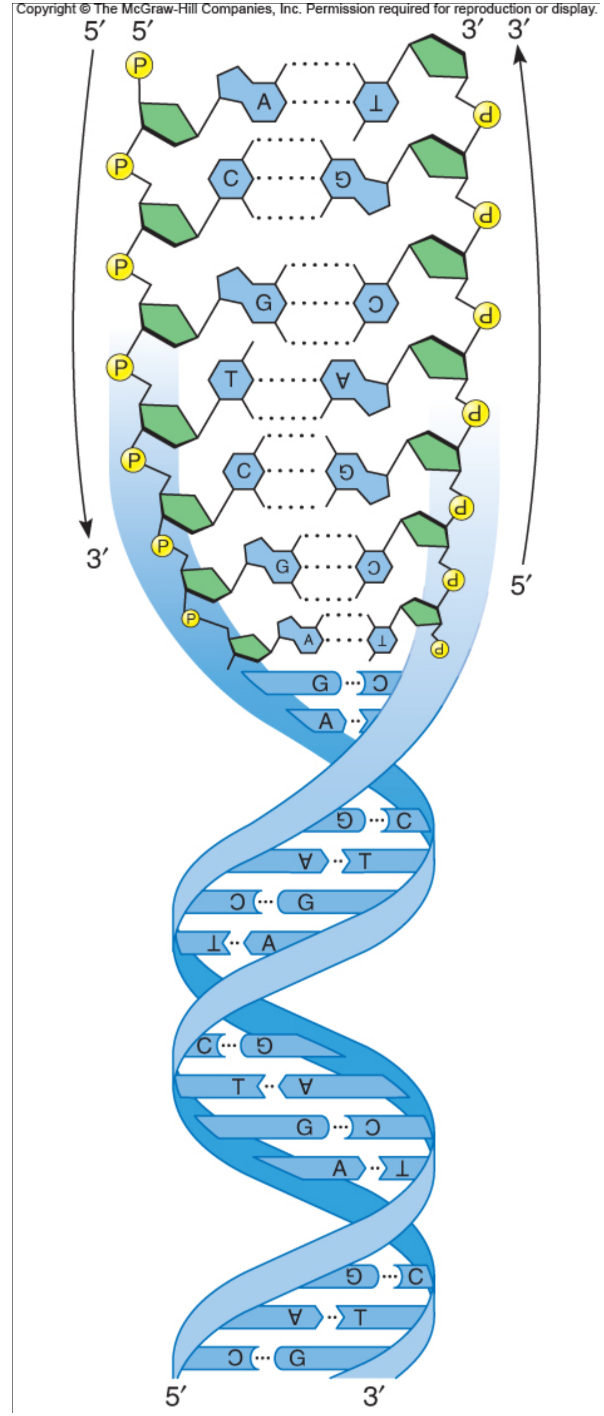


b. © M.C. Escher's "Drawing Hands" © 2007 The M.C. Escher Company-Holland. All rights reserved. www.mcescher.com

Figure 9.9

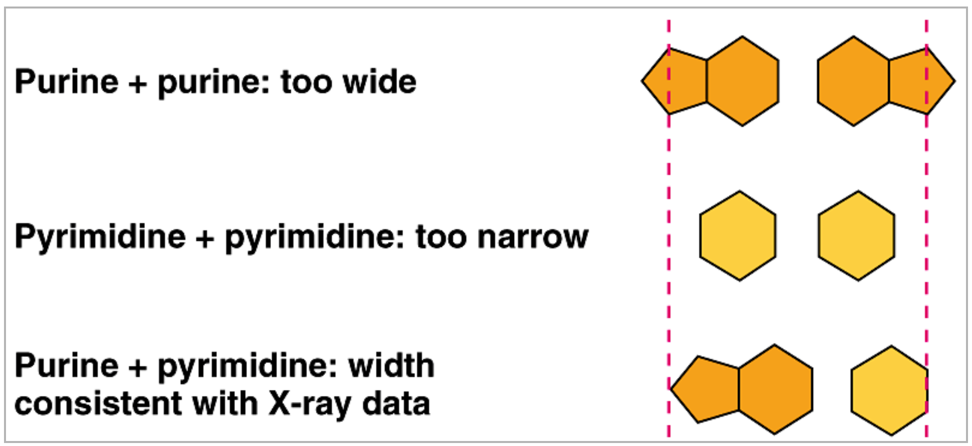
One strand of the double-helix runs in a 5’ to 3’ direction, and the other strand runs in a 3’ to 5’ direction

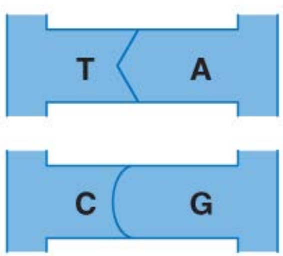
Figure 9.11

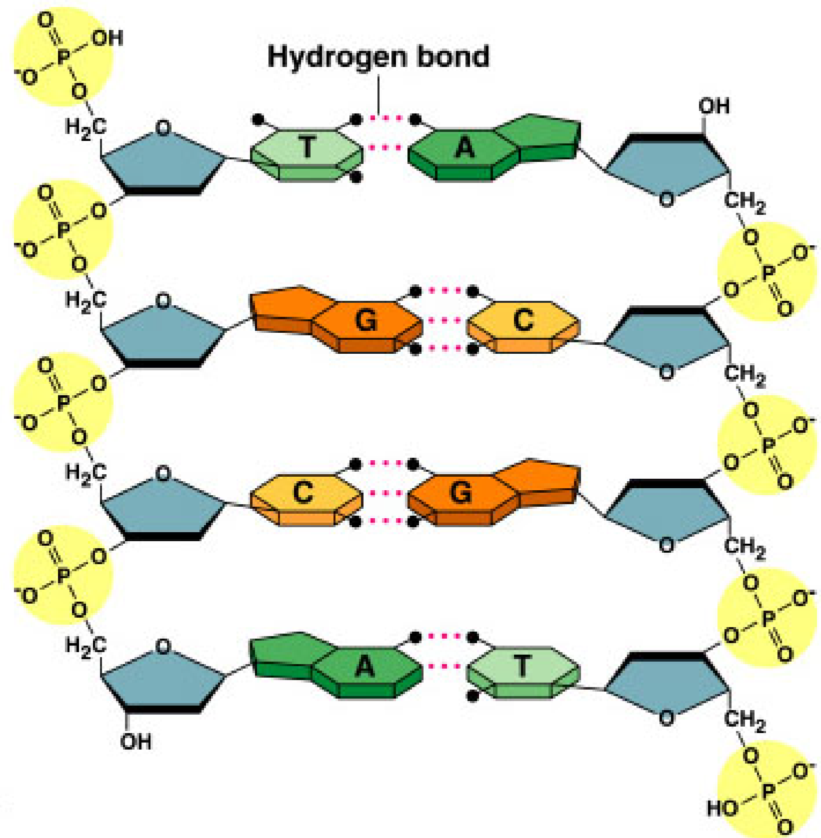


DNA Structure

The key to the constant width of the double helix is the specific pairing of its complementary bases via hydrogen bonds







(b) Partial chemical structure

DNA is Highly Condensed

The DNA coils around proteins called **histones**, forming a bead-on-a-string-like structure

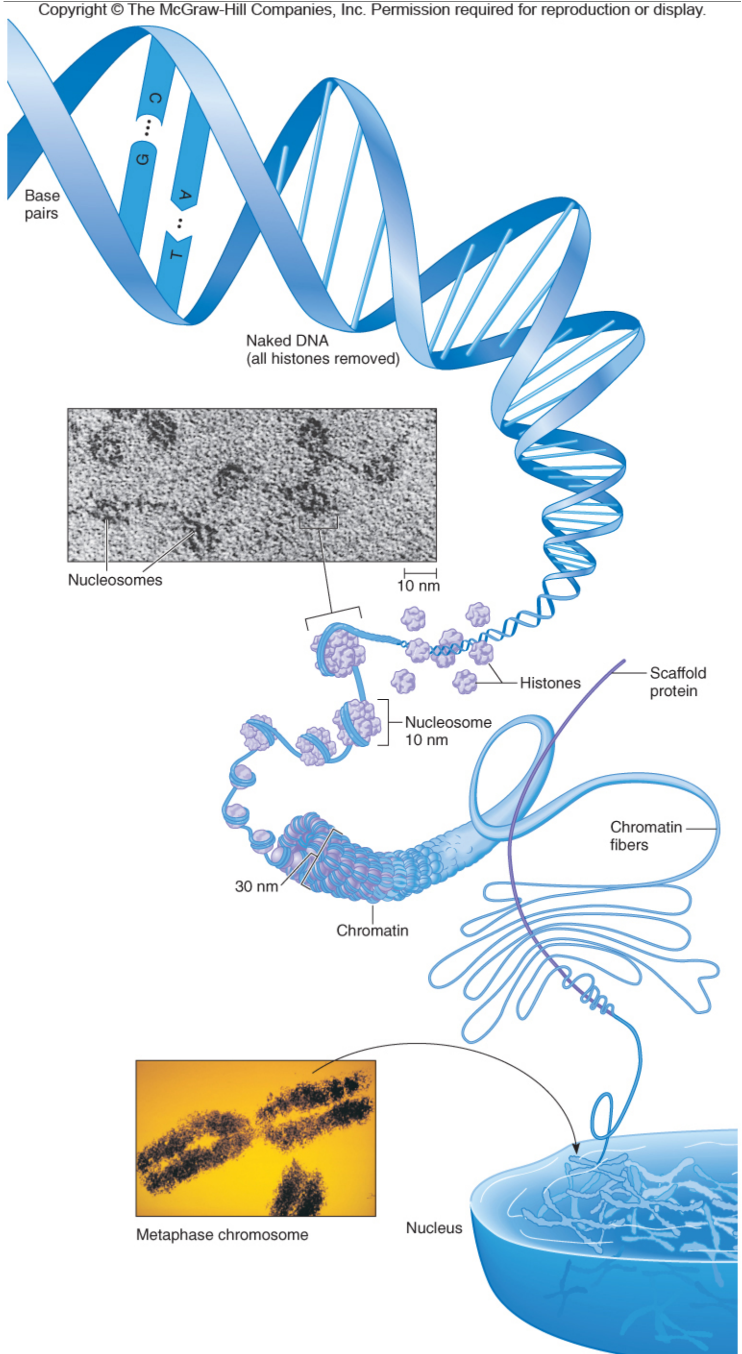
The bead part is called the **nucleosome**

The nucleosome in turn winds tighter forming **chromatin**

Chromatin fibers attach in loops to **scaffold proteins**

http://www.biostudio.com/demo_freeman_dna_coiling.htm

Figure 9.13


C
G
A
T
Base pairs
Naked DNA (all histones removed)
Nucleosomes
10 nm
Histones
Scaffold protein
Nucleosome 10 nm
Chromatin fibers
30 nm
Chromatin
Metaphase chromosome
Nucleus

Molecular Definition of a Gene

A **gene** is a segment of DNA that directs the formation of RNA to produce protein

The protein (or functional RNA) creates the phenotype

Information is conveyed by the sequence of the nucleotides

DNA Replication

At first, researchers suggested that DNA might replicate in any of 3 possible ways

Model of DNA Replication	Organization of DNA Strand
1. Conservative	old/old + new/new
2. Semiconservative	old/new + new/old
3. Dispersive	mixed old & new

DNA Replication

Matthew Meselson and Franklin Stahl, 1957

- Grew *E. coli* on media containing ^{15}N for several generations

    DNA with ^{15}N is heavy

- Moved bacteria to media containing ^{14}N
- Then traced replicating DNA
- Determined that DNA replication is semi-conservative

Meselson-Stahl Experiment

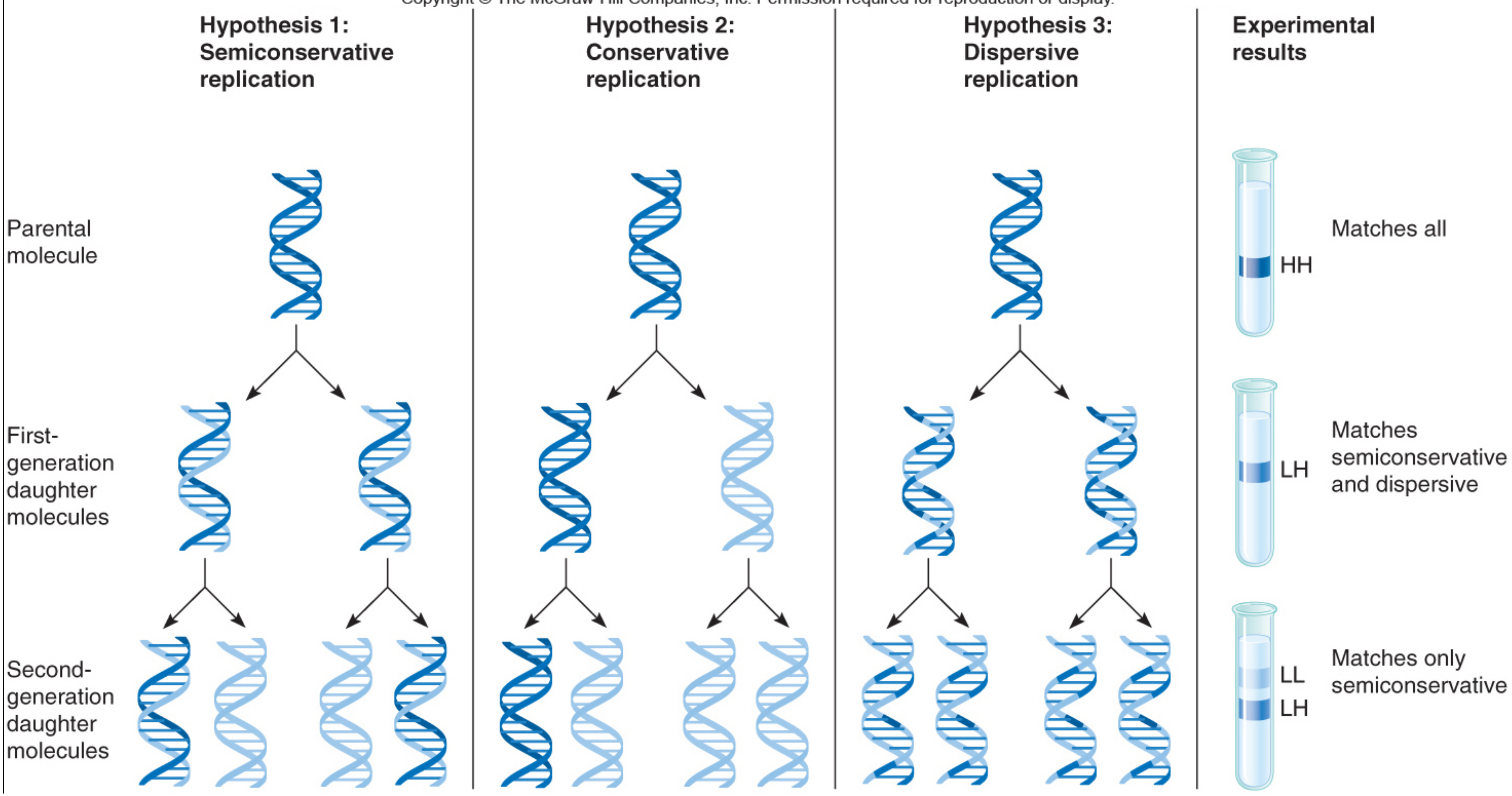


Figure 9.14

Overview of DNA Replication

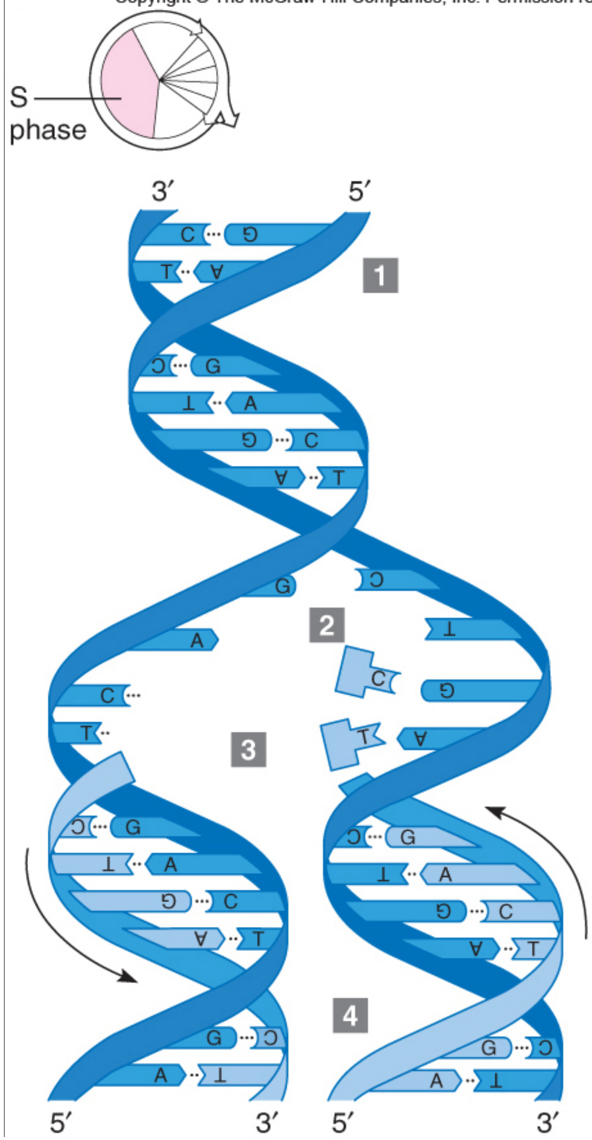
DNA replication occurs during the S phase of the cell cycle, prior to cell division

Human DNA replicates about 50 bases/sec

A human chromosome replicates simultaneously at hundred points along its length

A site where DNA is locally opened is called a **replication fork**

Overview of DNA Replication



1 Parent DNA molecule.

2 Parental strands unwind and separate at several points.

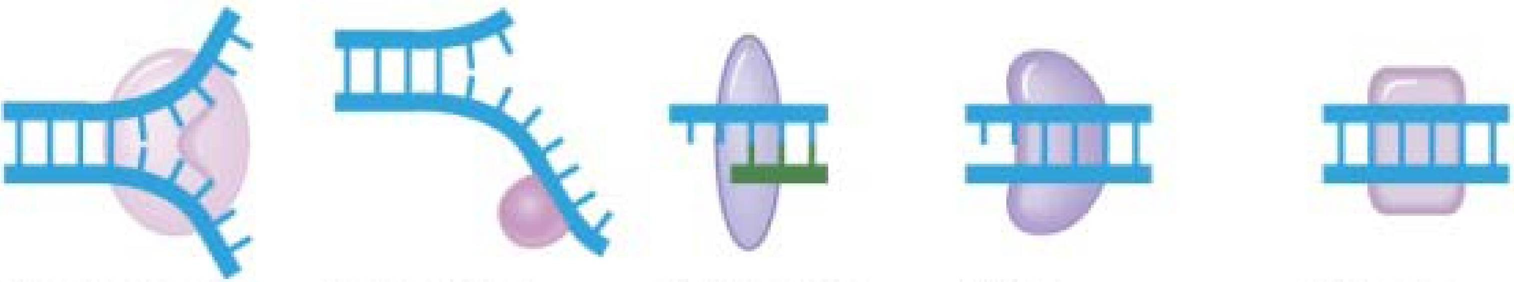
3 Each parental strand provides a template for DNA polymerase to bind complementary bases, A with T and G with C.

4 Sugar-phosphate backbones of daughter strands close.

Figure 9.15

Enzymes in DNA Replication

Enzymes in DNA replication



Helicase unwinds parental double helix.

Binding proteins stabilize separate strands.

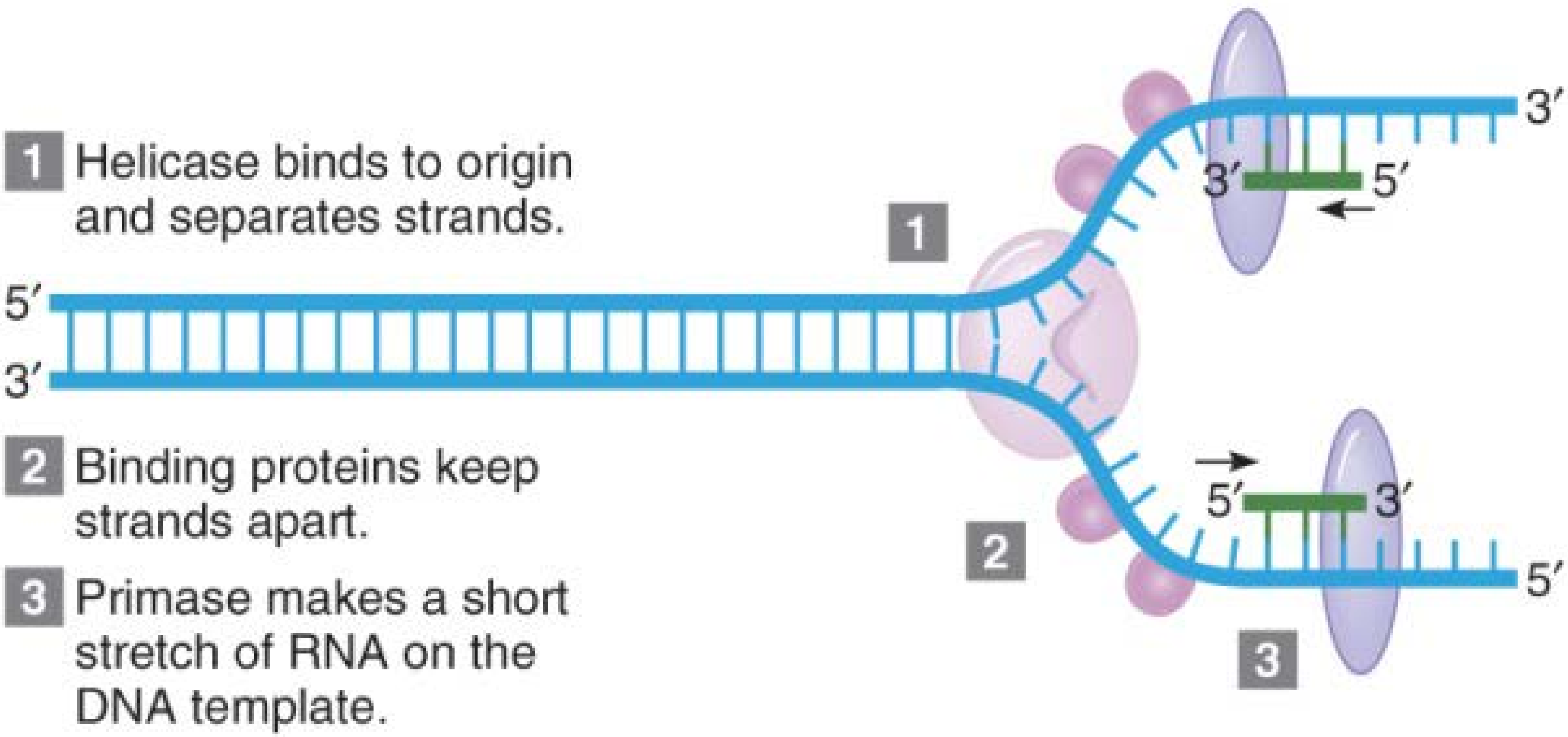
Primase adds short primer to template strand.

DNA polymerase binds nucleotides to form new strands.

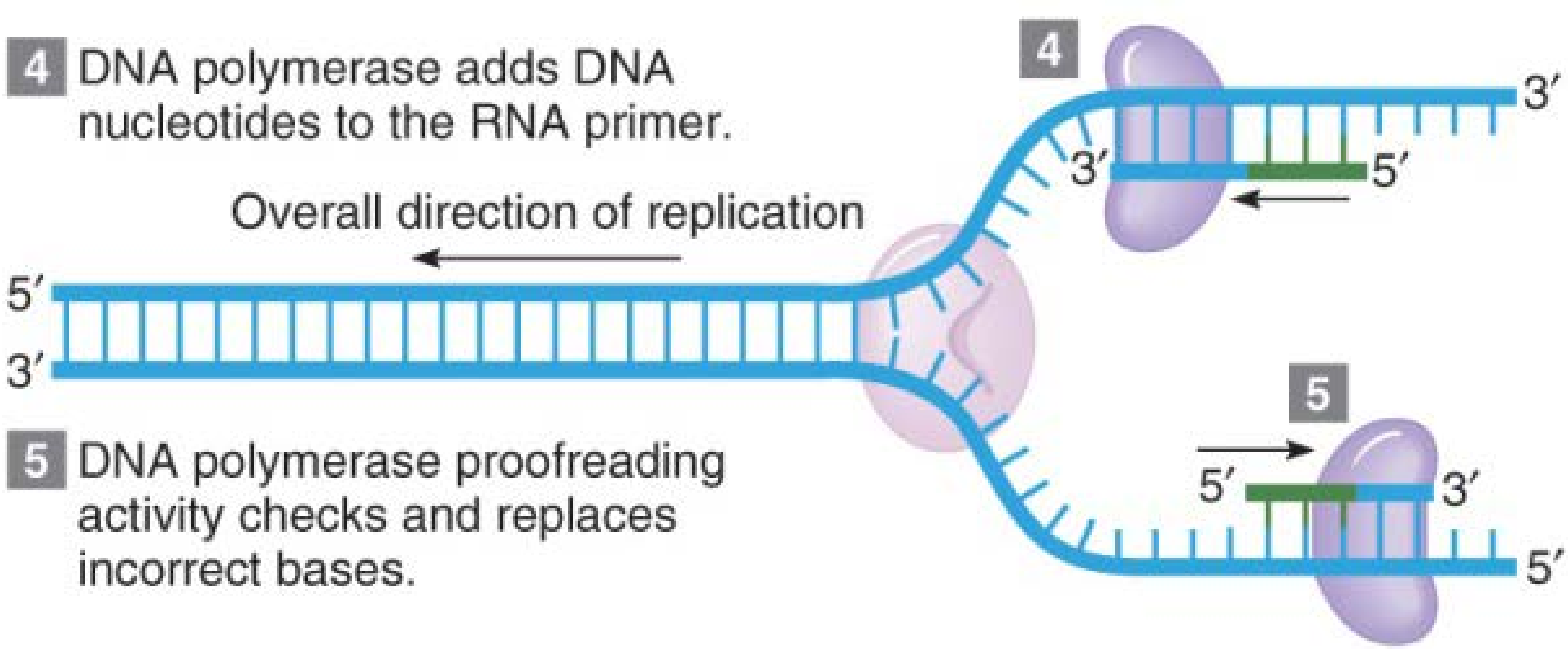
Ligase joins Okazaki fragments and seals other nicks in sugar-phosphate backbone.

Figure 9.16

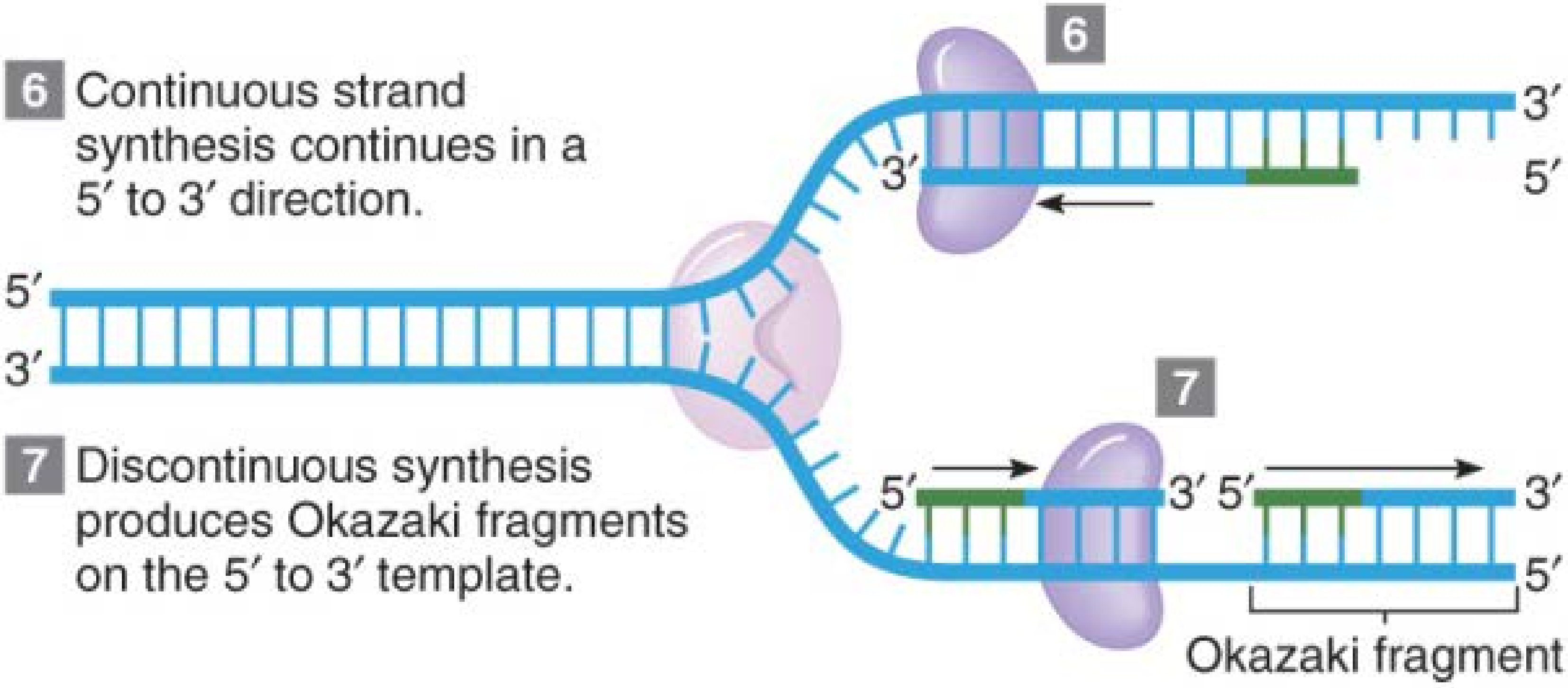
Activities at the Replication Fork



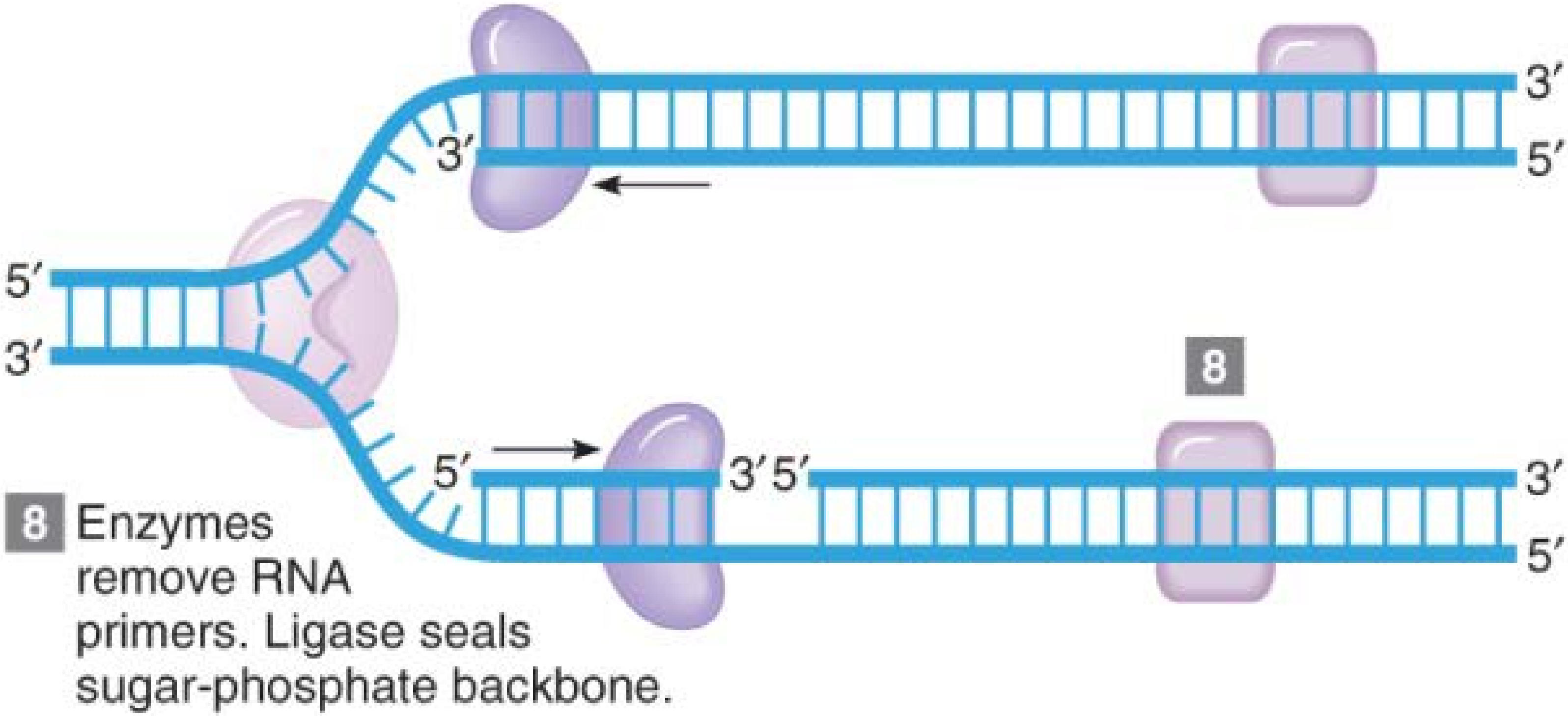
Activities at the Replication Fork



Activities at the Replication Fork



Activities at the Replication Fork



Fidelity of DNA replication & maintaining DNA integrity

Maintained by:

1. Proof-reading function of DNA polymerase
2. DNA repair systems

http://www.hhmi.org/biointeractive/media/mismatch_repair-lg.mov

DNA damage and repair in general

http://www.youtube.com/watch?v=y16w-CGAa0Y&feature=related

http://www.youtube.com/watch?v=nPS2jBq1k48

Genetic Integrity and Diversity

Need for maintaining genetic integrity is balanced by having enough genetic variability for natural selection to act on

Few errors of DNA replication are not corrected!