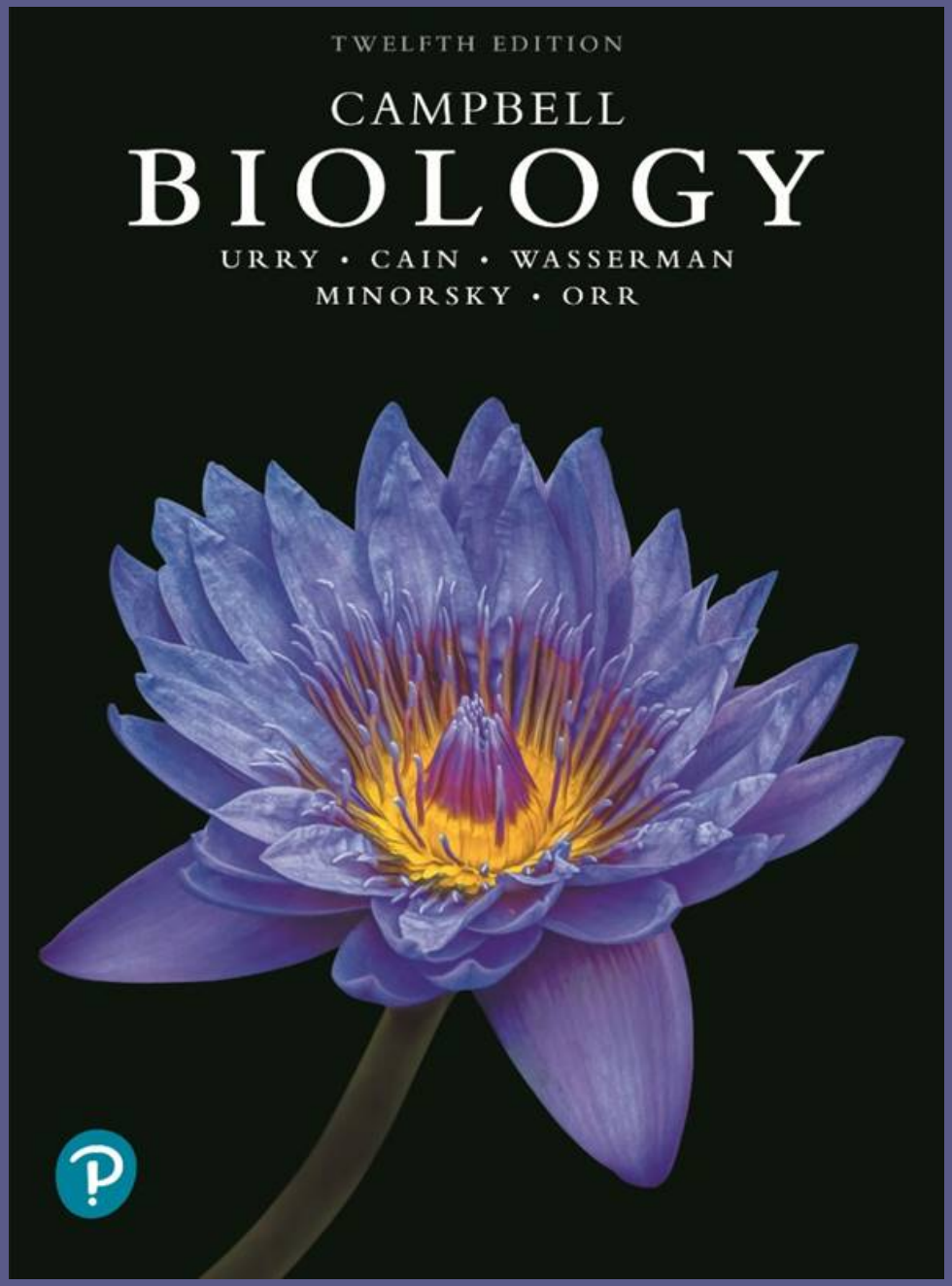


Chapter 16

The Molecular Basis of Inheritance

Lecture Presentations by
Nicole Tunbridge and
Kathleen Fitzpatrick

Figure 16.1a

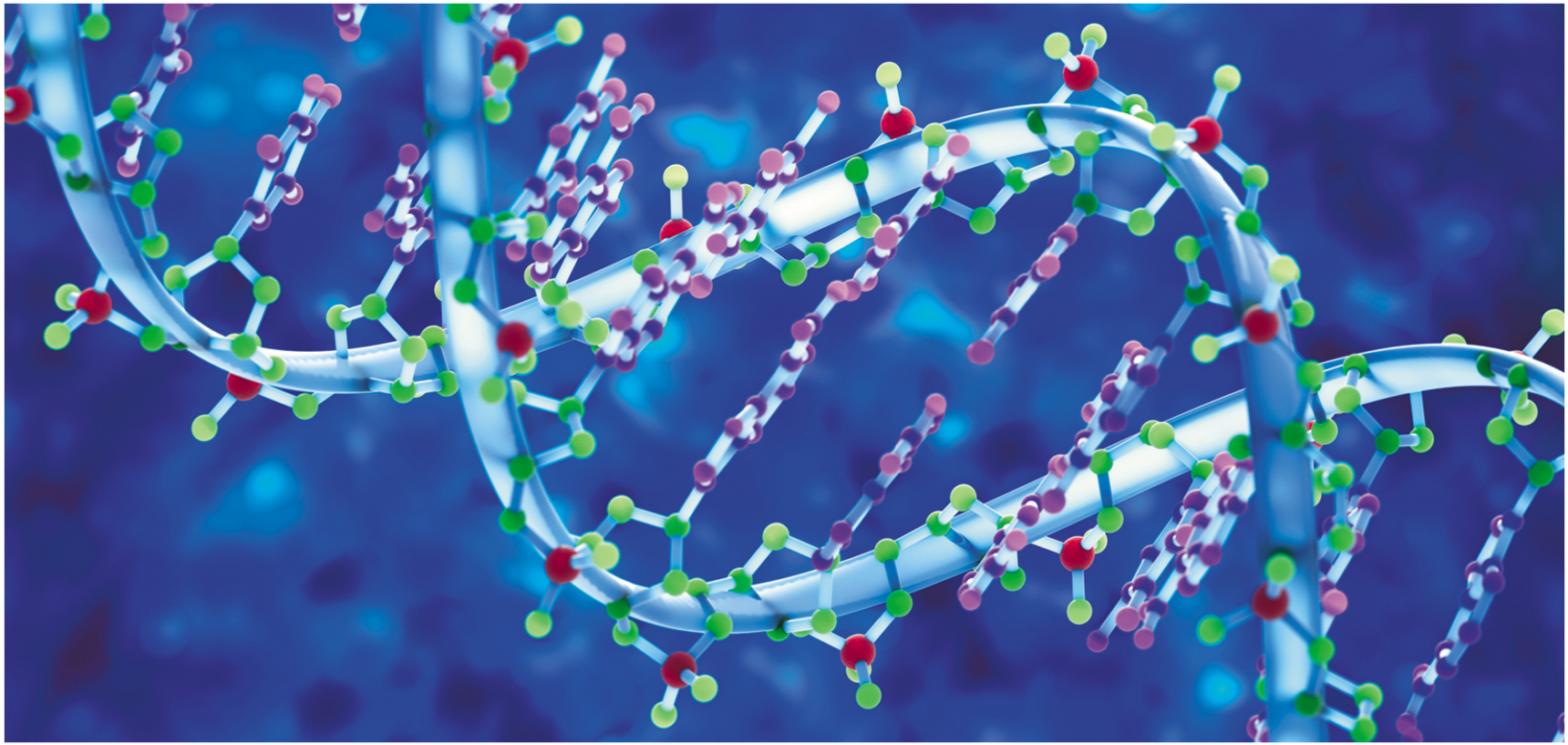
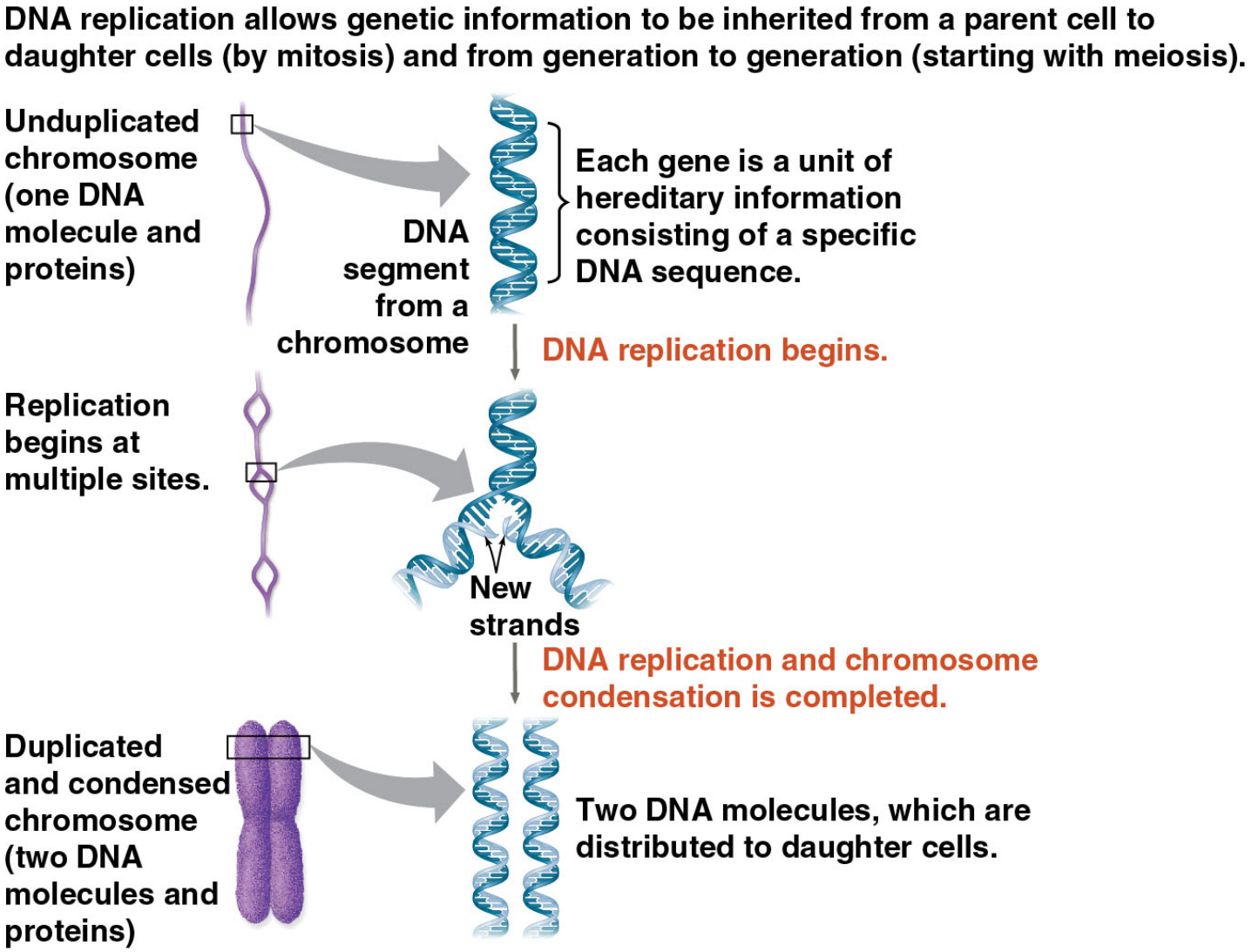


Figure 16.1b


DNA replication allows genetic information to be inherited from a parent cell to daughter cells (by mitosis) and from generation to generation (starting with meiosis).
Unduplicated chromosome (one DNA molecule and proteins)
DNA segment from a chromosome
Each gene is a unit of hereditary information consisting of a specific DNA sequence.
DNA replication begins.
Replication begins at multiple sites.
New strands
DNA replication and chromosome condensation is completed.
Duplicated and condensed chromosome (two DNA molecules and proteins)
Two DNA molecules, which are distributed to daughter cells.

CONCEPT 16.1: DNA is the genetic material

- Early in the 20th century, the identification of the molecules of inheritance posed a major challenge to biologists

The Search for the Genetic Material: *Scientific Inquiry*

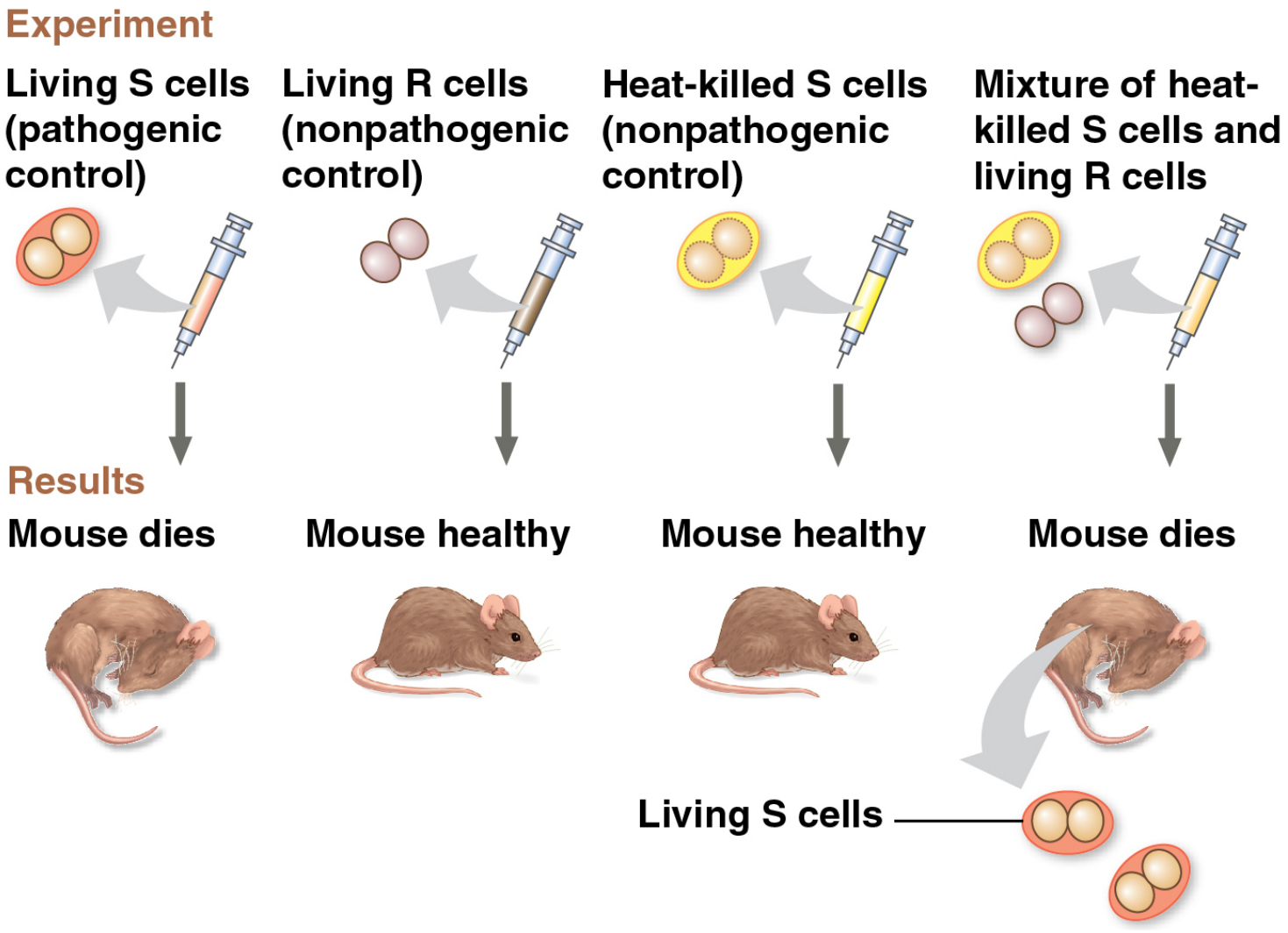
- When T. H. Morgan's group showed that genes are located on chromosomes, the two components of chromosomes—DNA and protein—became candidates for the genetic material
- The role of DNA in heredity was first discovered by studying bacteria and the viruses that infect them

Evidence That DNA Can Transform Bacteria

- The discovery of the genetic role of DNA began with research by Frederick Griffith in 1928
- Griffith worked with two strains of a bacterium, one pathogenic and one harmless

- When he mixed heat-killed remains of the pathogenic strain with living cells of the harmless strain, some living cells became pathogenic
- He called this phenomenon **transformation**, now defined as a change in genotype and phenotype due to assimilation of foreign DNA

Figure 16.2

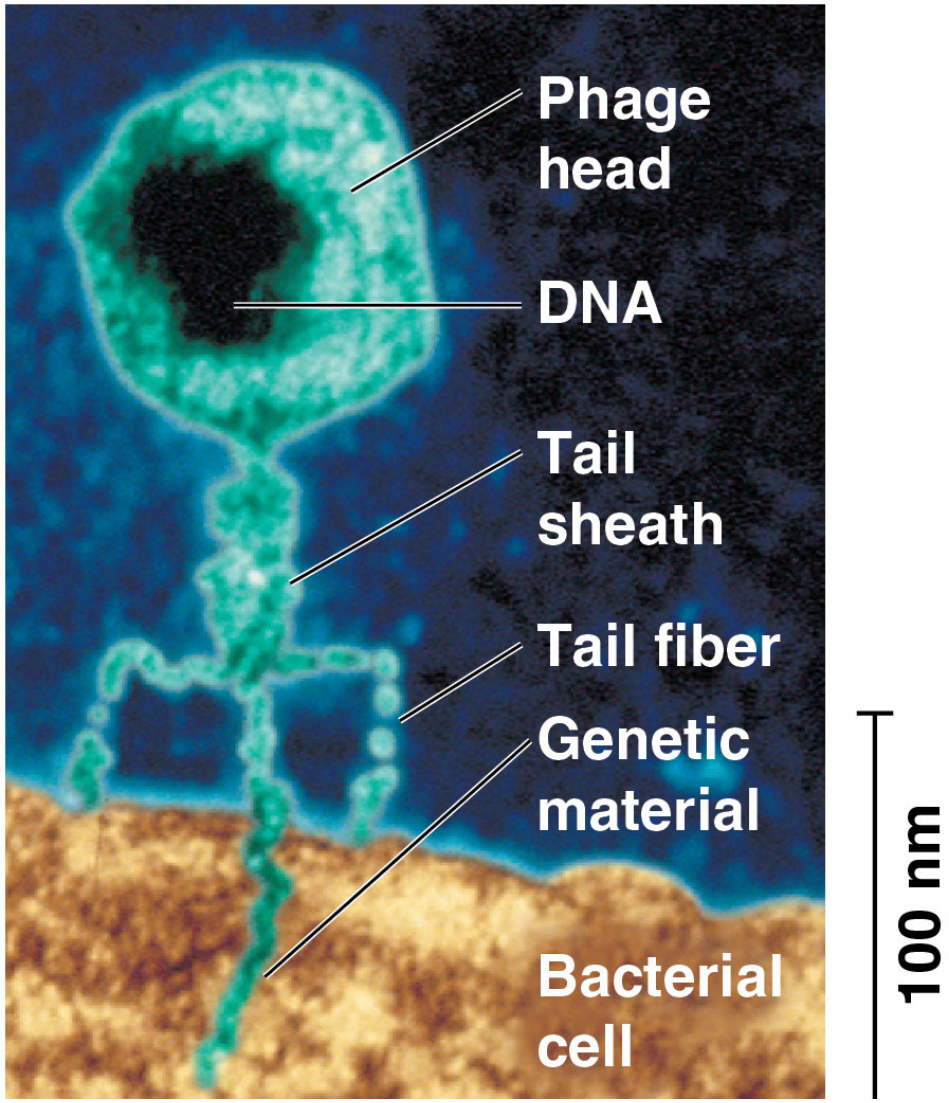

Experiment
Living S cells (pathogenic control)
Living R cells (nonpathogenic control)
Heat-killed S cells (nonpathogenic control)
Mixture of heat-killed S cells and living R cells
Results
Mouse dies
Mouse healthy
Mouse healthy
Mouse dies
Living S cells

- Later work by Oswald Avery, Maclyn McCarty, and Colin MacLeod identified the transforming substance as DNA
- Many biologists remained skeptical, mainly because little was known about DNA

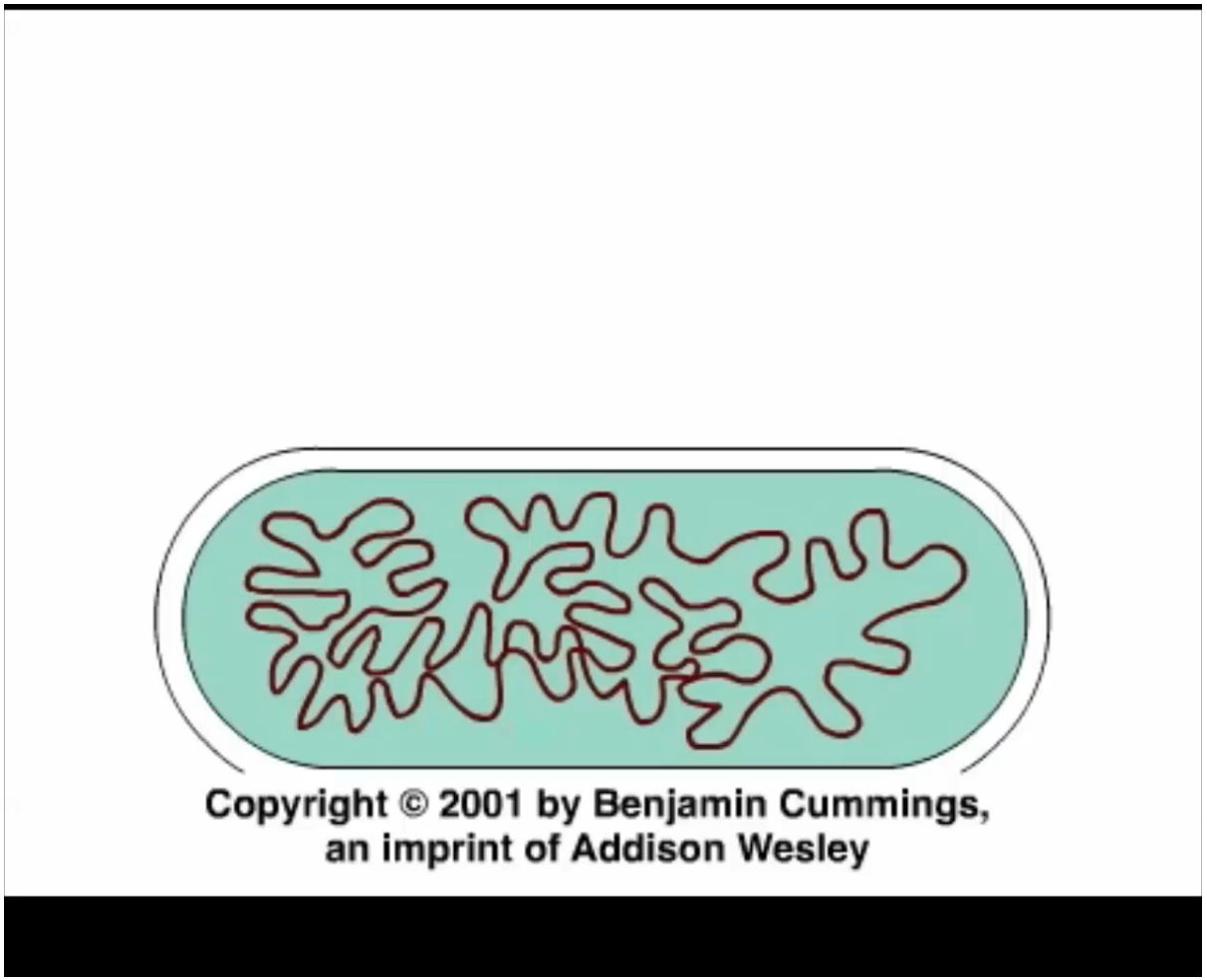
Evidence That Viral DNA Can Program Cells

- More evidence for DNA as the genetic material came from studies of viruses that infect bacteria
- Such viruses are called **bacteriophages** (or **phages**)
- A **virus** is DNA (sometimes RNA) enclosed by a protective coat, often simply protein
- Phages have been widely used as tools by researchers in molecular genetics

Figure 16.3

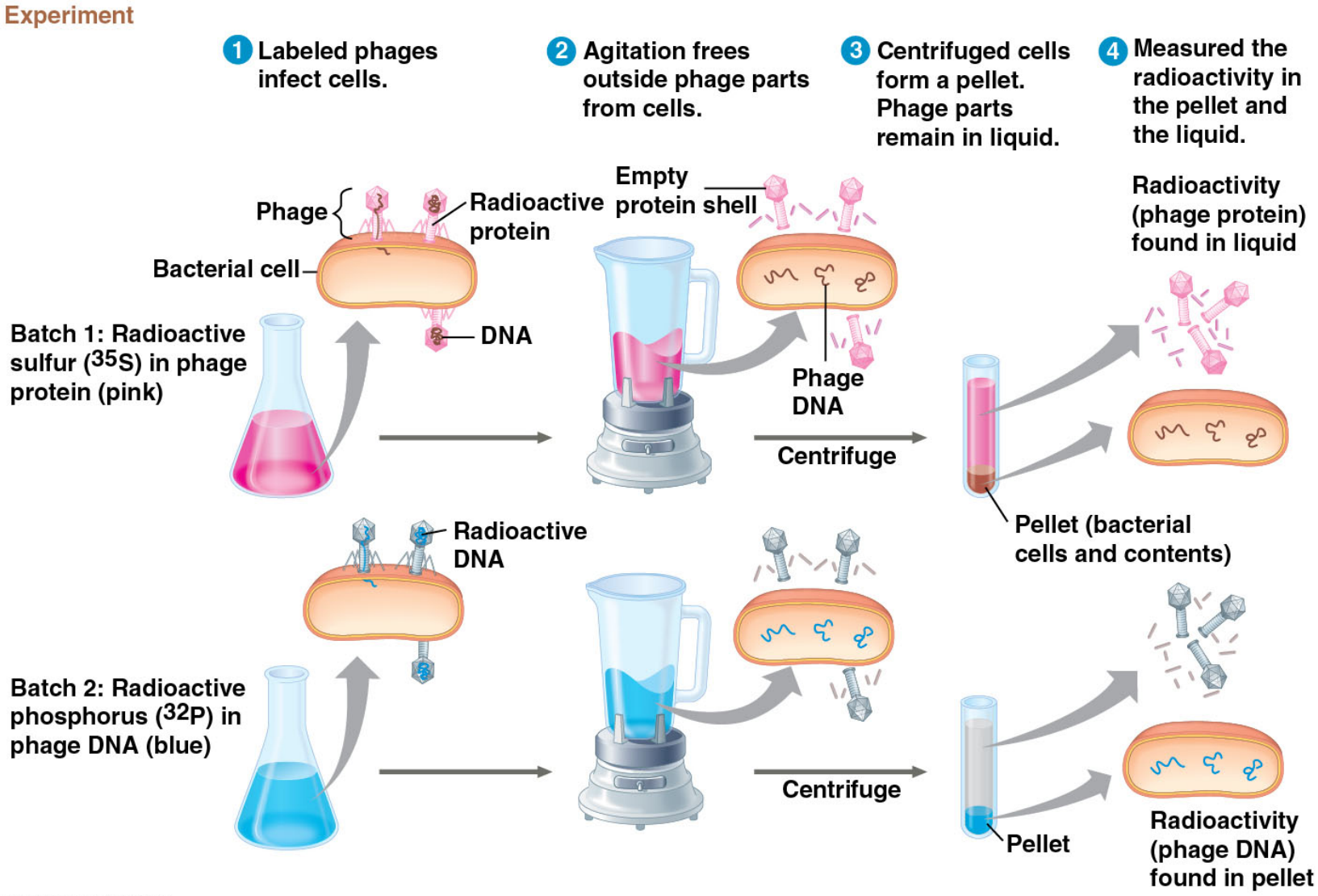

Phage head
DNA
Tail sheath
Tail fiber
Genetic material
Bacterial cell
100 nm

Animation: Phage T2 Reproductive Cycle



- In 1952, Alfred Hershey and Martha Chase showed that DNA is the genetic material of a phage known as T2
- They designed an experiment showing that only one of the two components of T2 (DNA or protein) enters an *E. coli* cell during infection
- They concluded that the injected DNA of the phage provides the genetic information

Figure 16.4


Experiment
1 Labeled phages infect cells.
2 Agitation frees outside phage parts from cells.
3 Centrifuged cells form a pellet. Phage parts remain in liquid.
4 Measured the radioactivity in the pellet and the liquid.
Phage
Radioactive protein
Bacterial cell
DNA
Batch 1: Radioactive sulfur (^{35}S) in phage protein (pink)
Empty protein shell
Phage DNA
Centrifuge
Radioactivity (phage protein) found in liquid
Pellet (bacterial cells and contents)
Radioactive DNA
Batch 2: Radioactive phosphorus (^{32}P) in phage DNA (blue)
Centrifuge
Pellet
Radioactivity (phage DNA) found in pellet

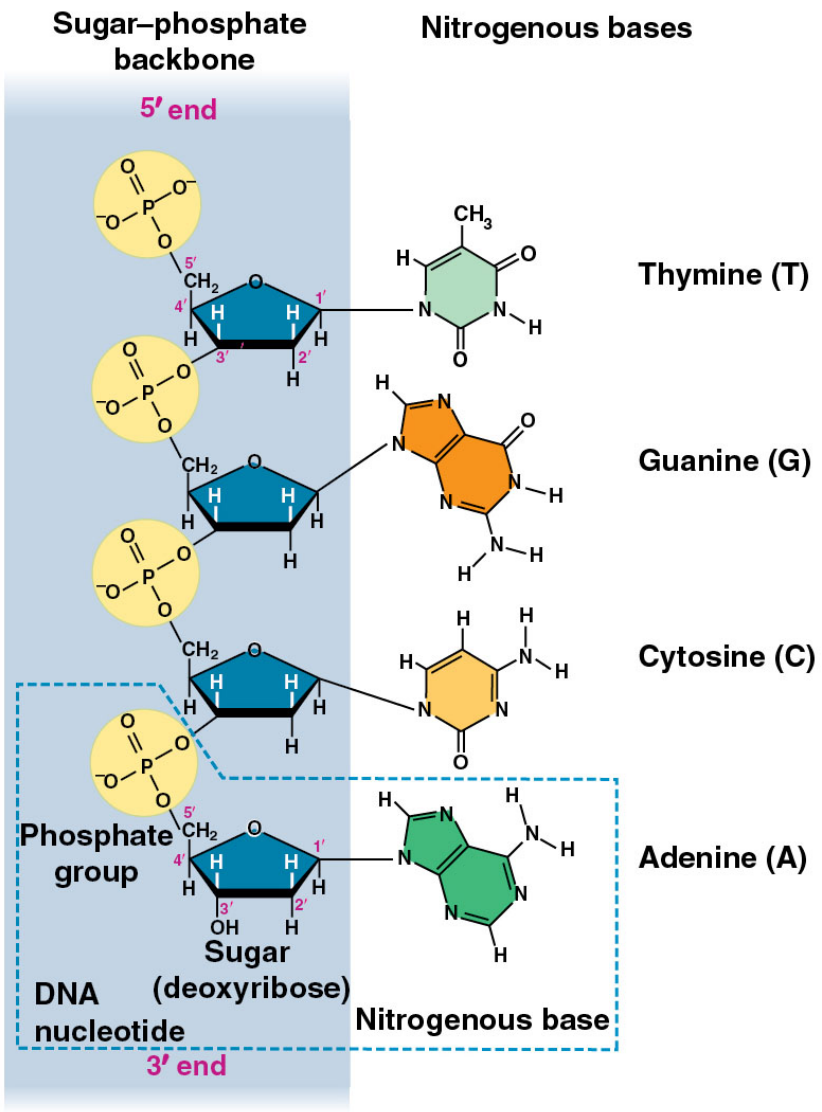
Animation: The Hershey-Chase Experiment

Additional Evidence That DNA Is the Genetic Material

- DNA is a polymer of nucleotides, each consisting of a nitrogenous base, a sugar, and a phosphate group
- The nitrogenous bases can be adenine (A), thymine (T), guanine (G), or cytosine (C)
- In 1950, Erwin Chargaff reported that DNA composition varies from one species to the next
- This evidence of molecular diversity among organisms made DNA a more credible candidate for the genetic material

- Two findings became known as Chargaff's rules
  - The base composition of DNA varies between species
  - In any species the number of A and T bases is equal and the number of G and C bases is equal
- The basis for these rules was not understood until the discovery of the double helix

Figure 16.5

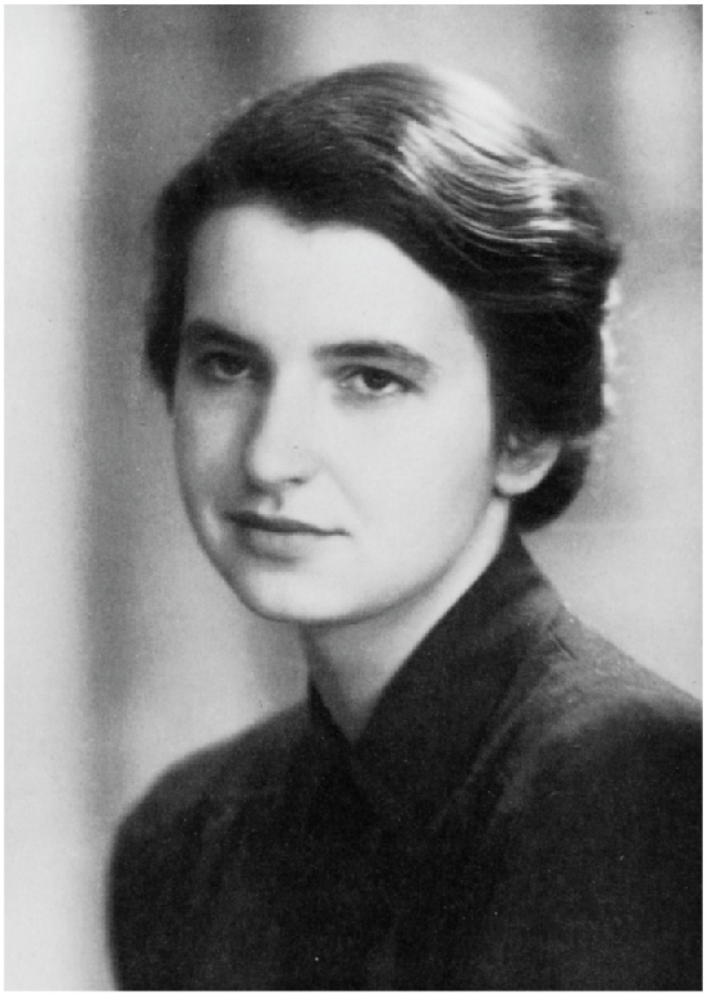

Sugar–phosphate backbone
Nitrogenous bases
5′ end
Thymine (T)
Guanine (G)
Cytosine (C)
Adenine (A)
Phosphate group
Sugar (deoxyribose)
DNA nucleotide
Nitrogenous base
3′ end

Animation: DNA and RNA Structure

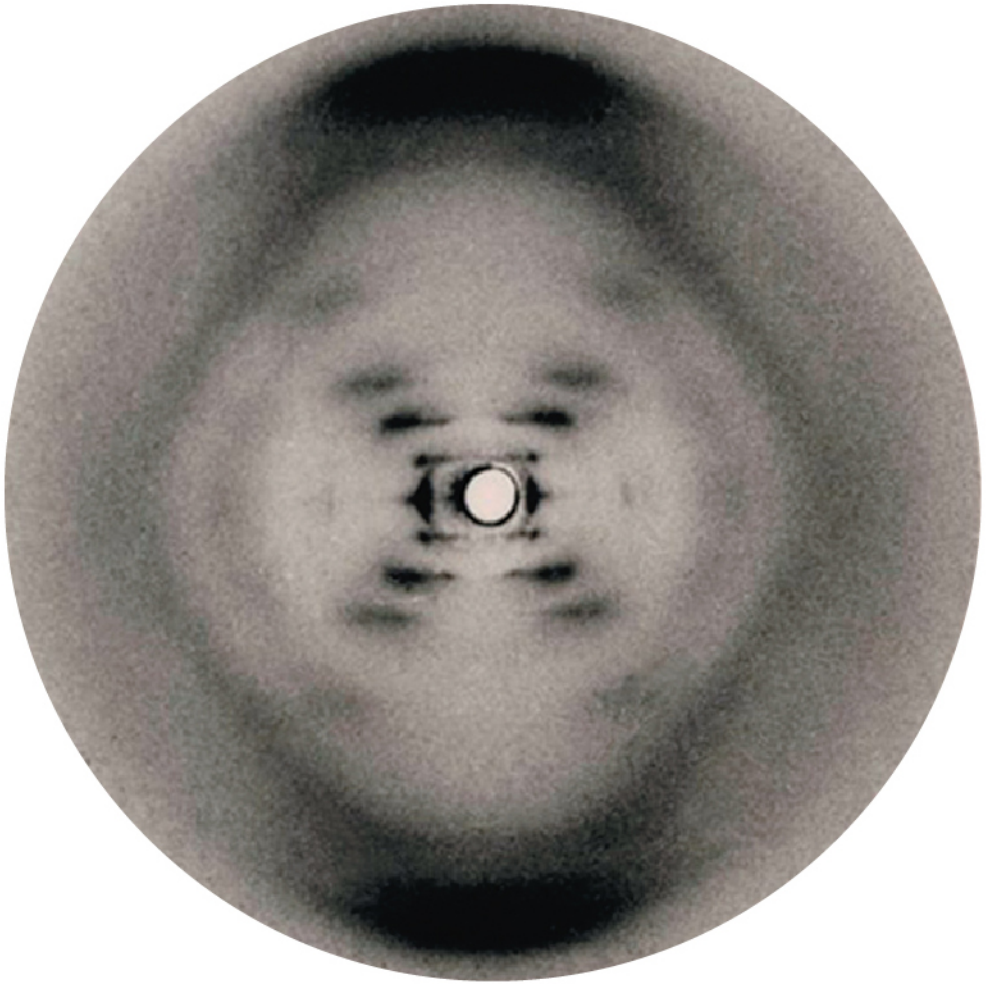
Building a Structural Model of DNA

- After DNA was accepted as the genetic material, the challenge was to determine how its structure accounts for its role in inheritance
- Maurice Wilkins and Rosalind Franklin used a technique called X-ray crystallography to study molecular structure
- Franklin produced a picture of the DNA molecule using this technique

Figure 16.6



(a) Rosalind Franklin

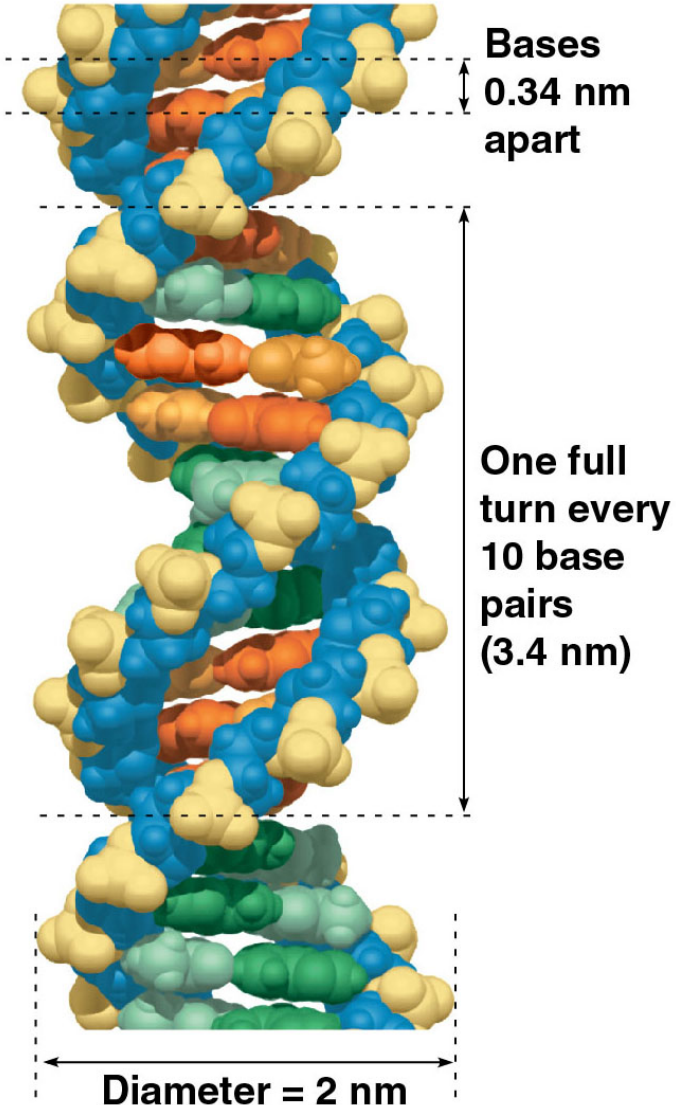


(b) Franklin's X-ray diffraction photograph of DNA

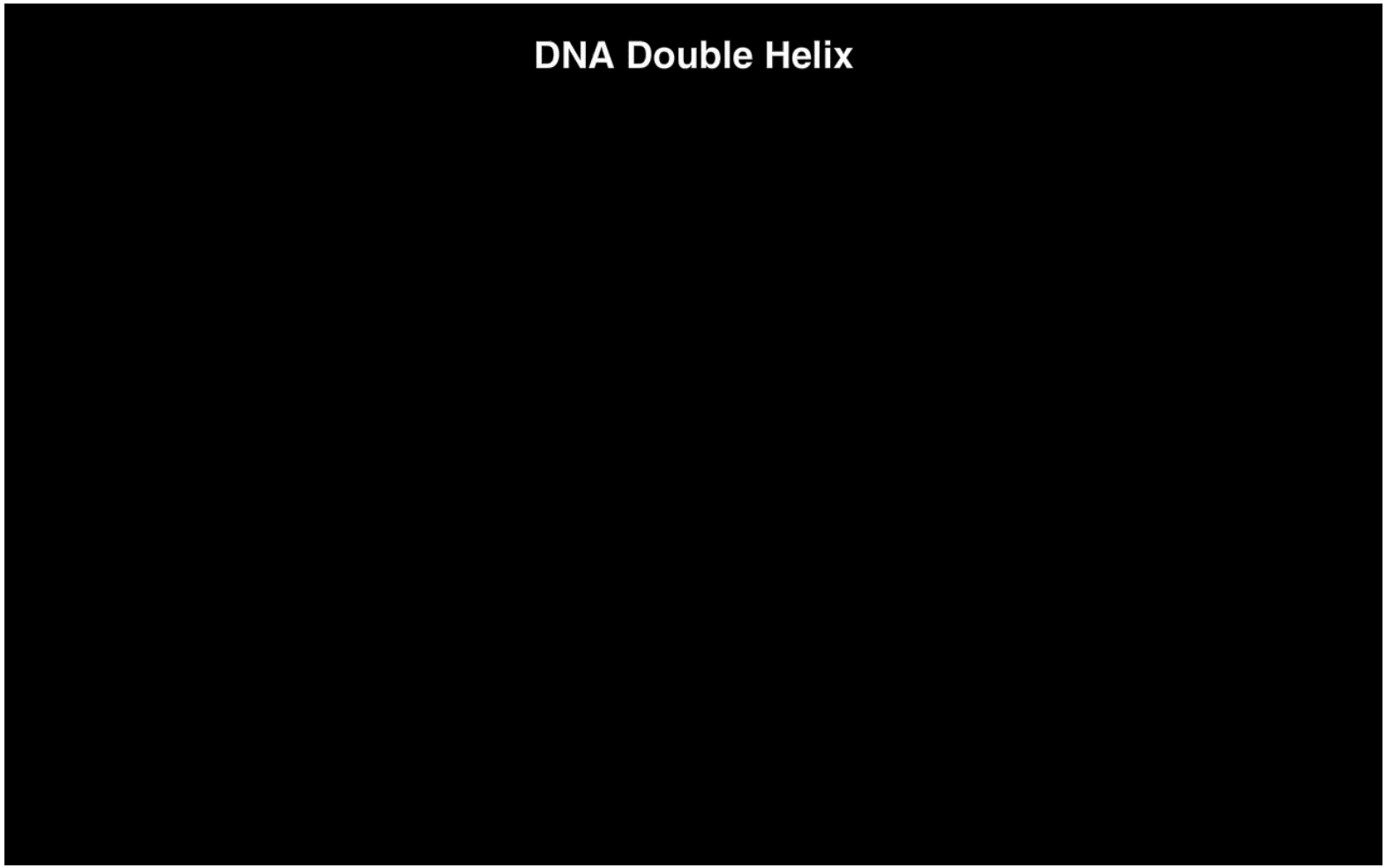
- Franklin's X-ray crystallographic images of DNA allowed James Watson to deduce that DNA was helical
- The X-ray images also enabled Watson to deduce the width of the helix and the spacing of the nitrogenous bases
- The pattern in the photo suggested that the DNA molecule was made up of two strands, forming a **double helix**

- Watson and Crick built models of a double helix to conform to the X-rays and chemistry of DNA
- Franklin had concluded that there were two outer sugar-phosphate backbones, with the nitrogenous bases paired in the molecule's interior
- Watson built a model in which the backbones were **antiparallel** (their subunits run in opposite directions)

Figure 16.7

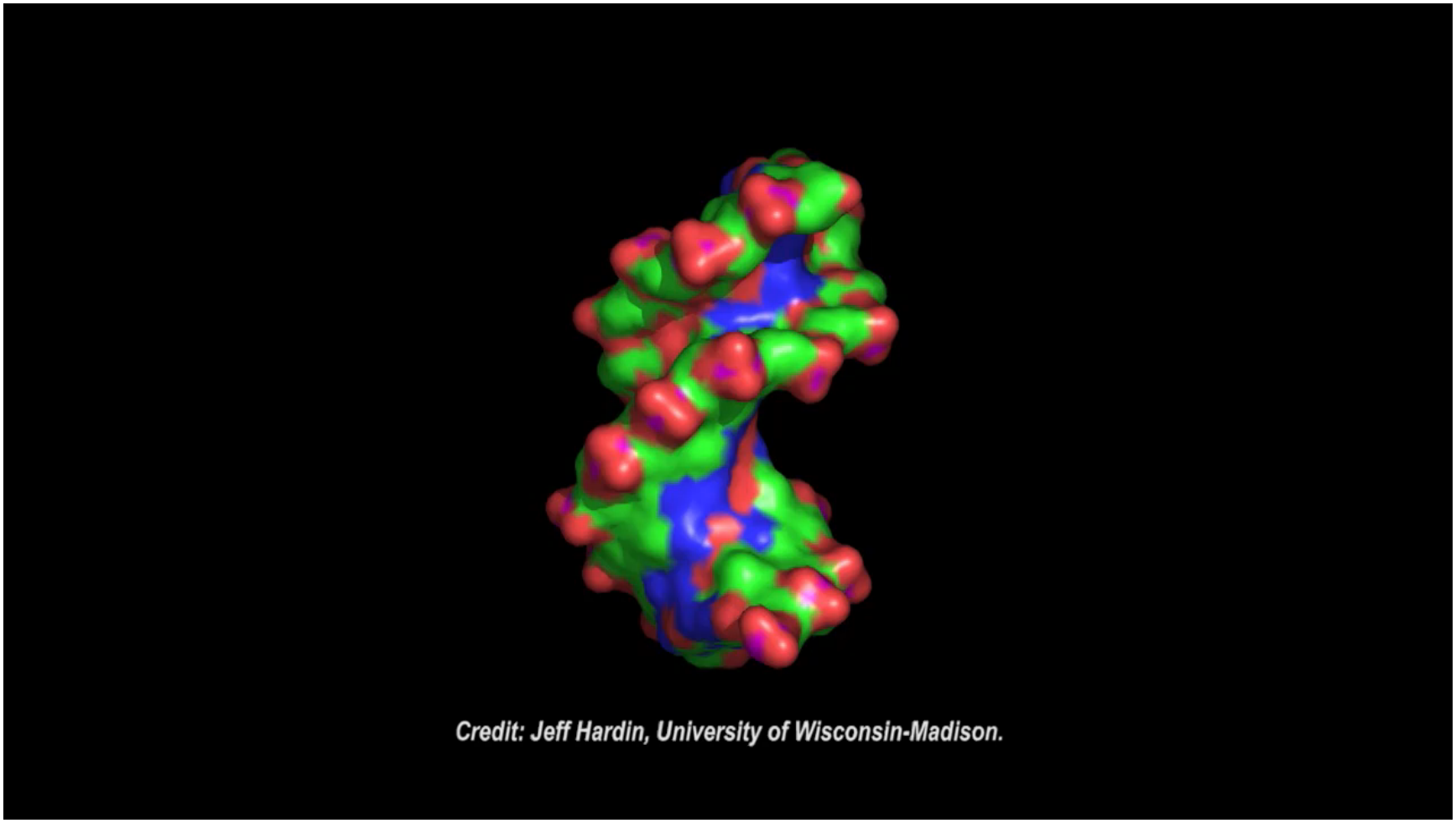

Bases
0.34 nm
apart
One full
turn every
10 base
pairs
(3.4 nm)
Diameter = 2 nm

Animation: DNA Double Helix



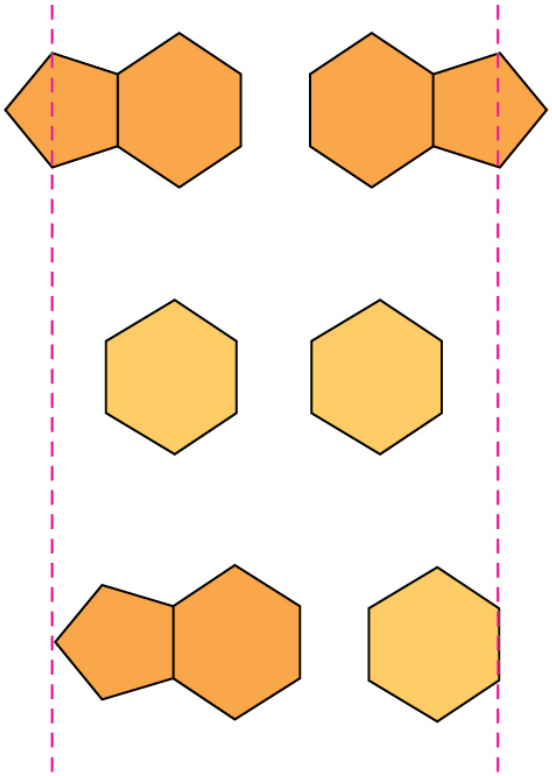
Video: Stick Model of DNA

Video: Surface Model of DNA



- At first, Watson and Crick thought the bases paired like with like (A with A, and so on), but such pairings did not result in a uniform width
- Instead, pairing a purine (A or G) with a pyrimidine (C or T) resulted in a uniform width consistent with the X-ray data

Figure 16.8



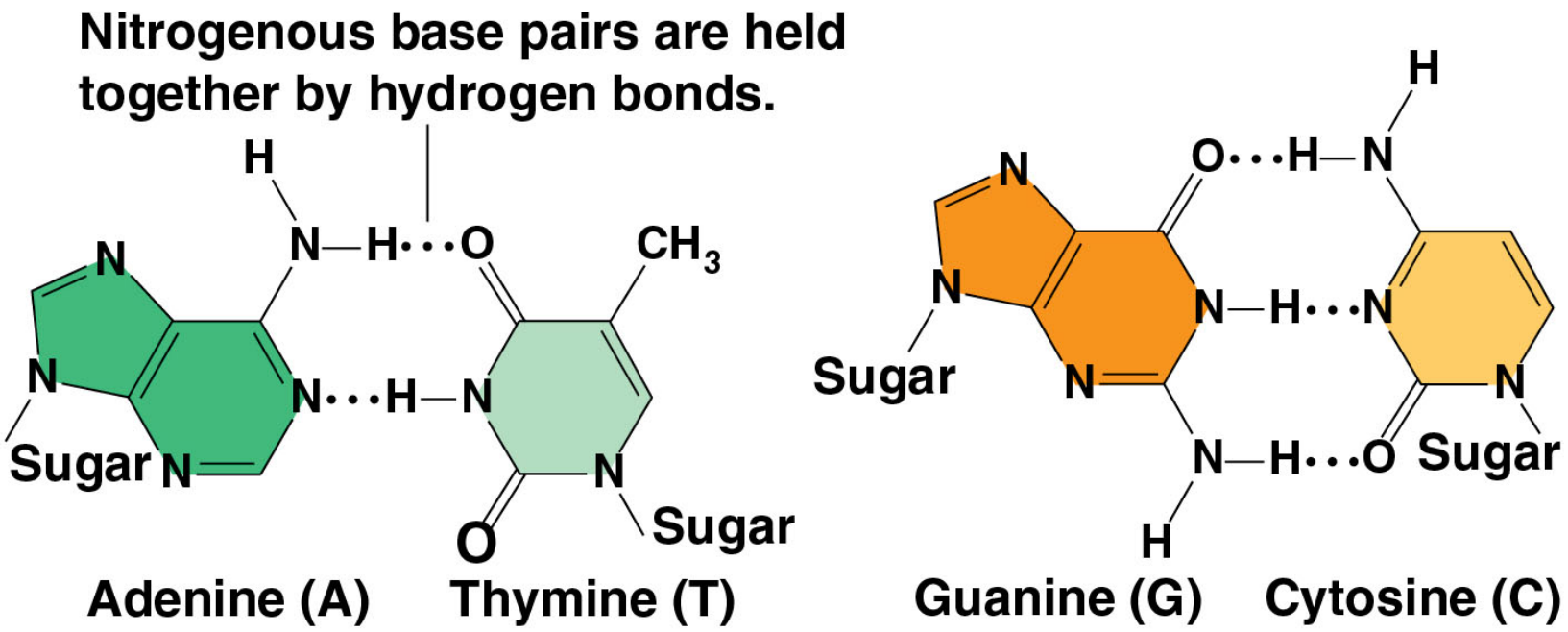
Purine + purine: too wide

Pyrimidine + pyrimidine: too narrow

Purine + pyrimidine: width consistent with X-ray data

- Watson and Crick reasoned that the pairing was more specific, dictated by the base structures
- They determined that adenine (A) paired only with thymine (T), and guanine (G) paired only with cytosine (C)
- The Watson-Crick model explains Chargaff's rules: in any organism the amount of A = T, and the amount of G = C

Figure 16.9


Nitrogenous base pairs are held together by hydrogen bonds.
H
N—H···O
CH3
N
N
N···H—N
Sugar
N
N
O
Sugar
Adenine (A)
Thymine (T)
H
O···H—N
N
N
N—H···N
Sugar
N
N
N
N—H···O
Sugar
H
Guanine (G)
Cytosine (C)

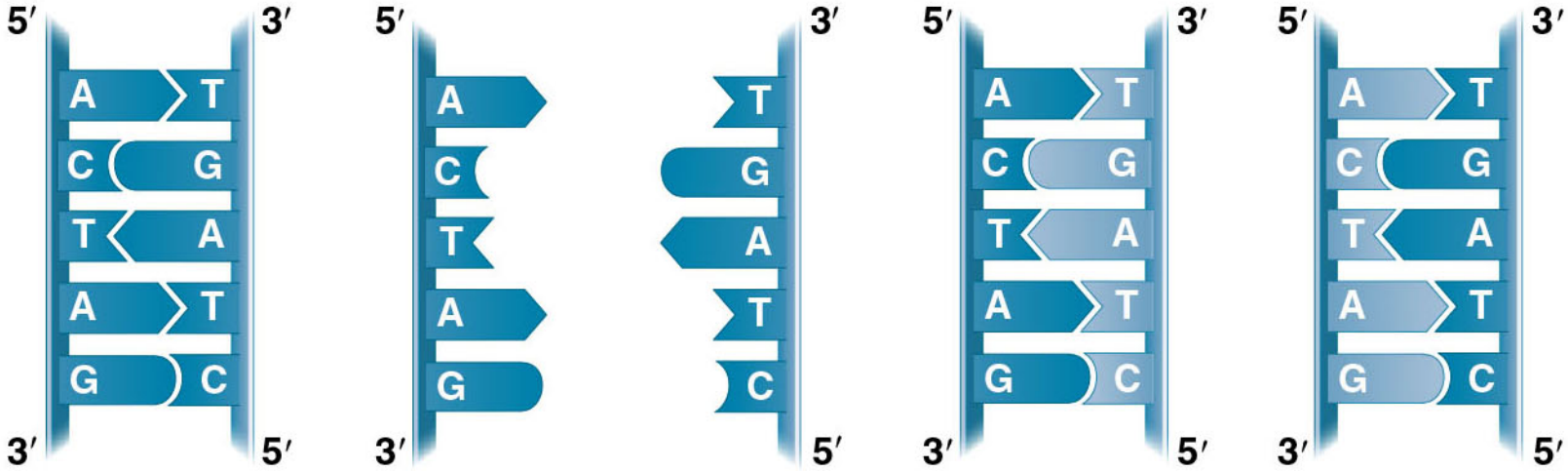
CONCEPT 16.2: Many proteins work together in DNA replication and repair

- Resemblance of offspring to parents relies on accurate replication of DNA prior to meiosis and its transmission to the next generation
- Replication prior to mitosis ensures the faithful transmission of genetic information from a parent cell to the two daughter cells
- Watson and Crick noted that the specific base pairing suggested a possible copying mechanism for genetic material
- The copying of DNA is called **DNA replication**

The Basic Principle: Base Pairing to a Template Strand

- Since the two strands of DNA are complementary, each strand acts as a template for building a new strand in replication
- This yields two exact replicas of the “parental” molecule

Figure 16.10



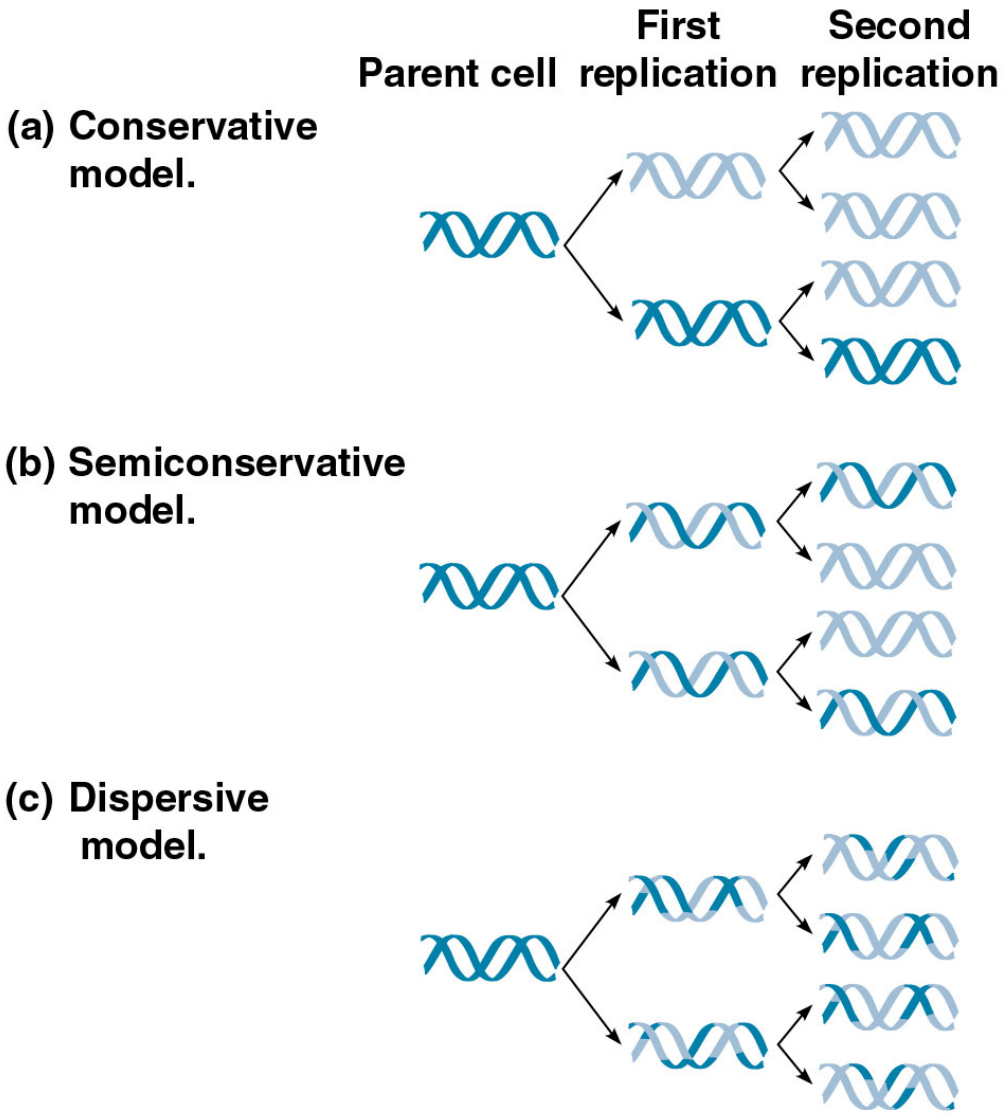
(a) Parental molecule

(b) Separation of parental strands into templates

(c) Nucleotides complementary to the parental (dark blue) strand are connected to form the sugar-phosphate backbones of the new "daughter" (light blue) strands.

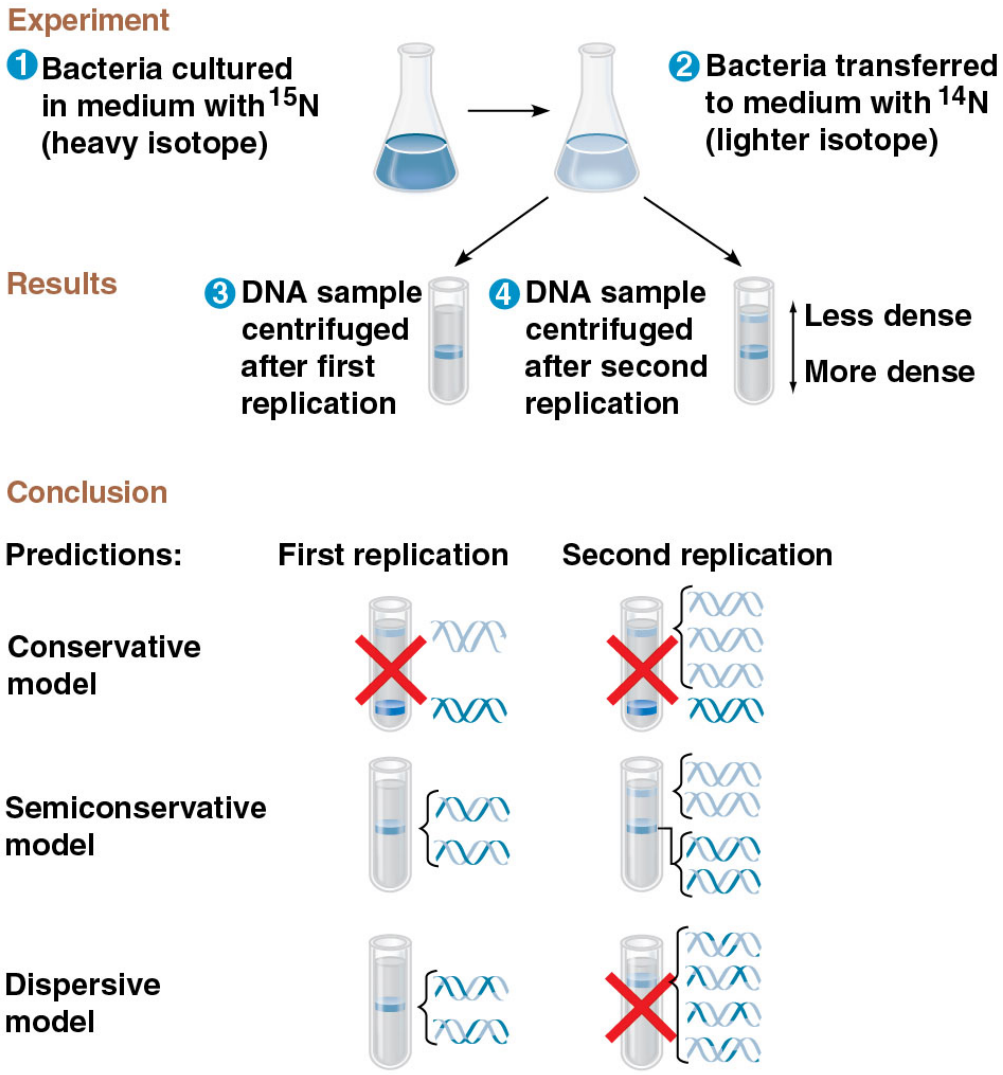
- Watson and Crick's **semiconservative model** of replication predicts that when a double helix replicates, each daughter molecule will have one old strand (derived or "conserved" from the parent molecule) and one newly made strand
- Competing models were the conservative model (the two parent strands rejoin) and the dispersive model (each strand is a mix of old and new)

Figure 16.11


Parent cell
First replication
Second replication
(a) Conservative model.
(b) Semiconservative model.
(c) Dispersive model.

- Experiments by Matthew Meselson and Franklin Stahl supported the semiconservative model

Figure 16.12


Experiment
1 Bacteria cultured in medium with ^{15}N (heavy isotope)
2 Bacteria transferred to medium with ^{14}N (lighter isotope)
Results
3 DNA sample centrifuged after first replication
4 DNA sample centrifuged after second replication
Less dense
More dense
Conclusion
Predictions:
First replication
Second replication
Conservative model
Semiconservative model
Dispersive model

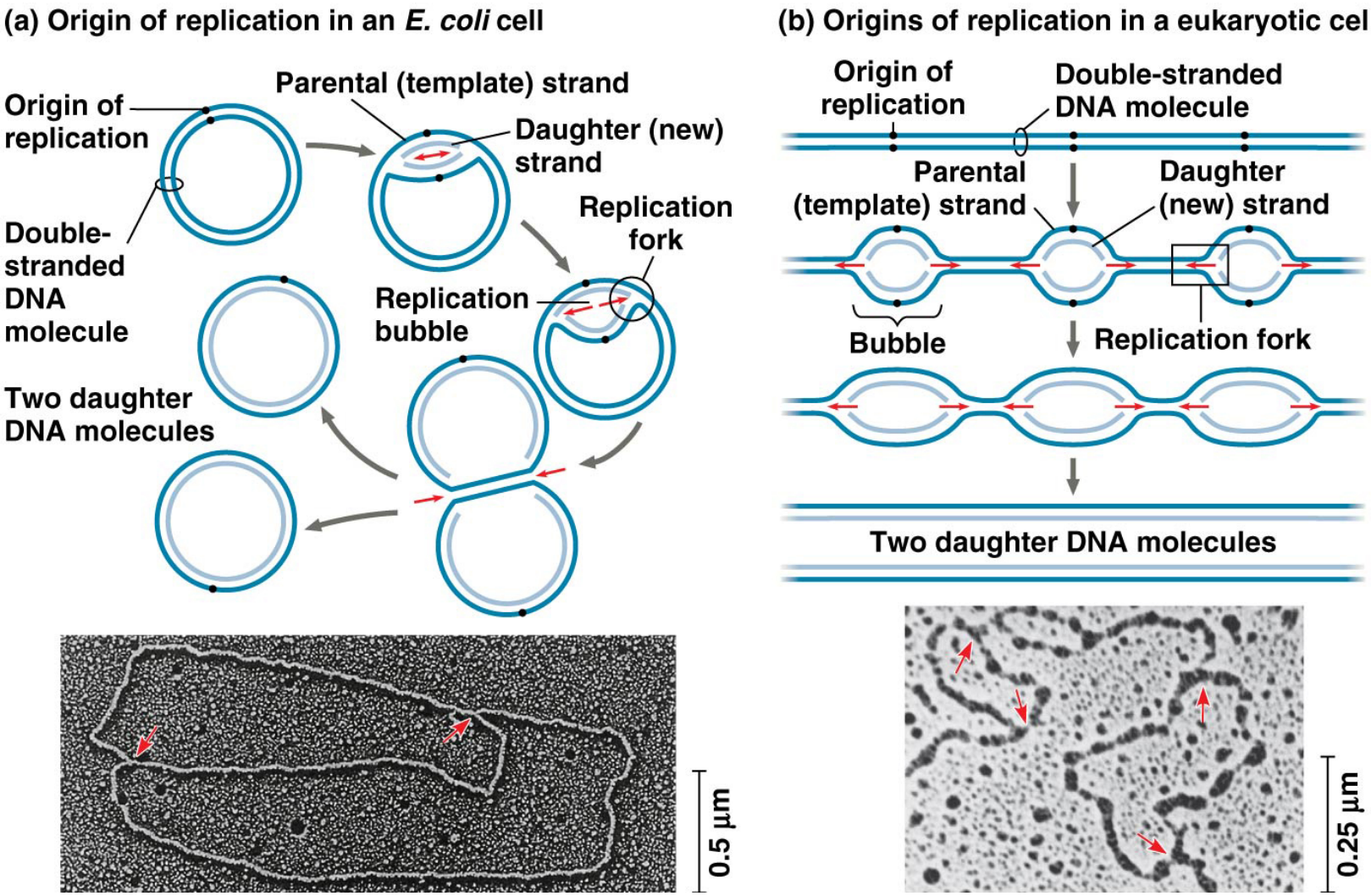
DNA Replication: *A Closer Look*

- The copying of DNA is remarkable in its speed and accuracy
- More than a dozen enzymes and other proteins participate in DNA replication
- Replication in bacteria is best understood, but evidence suggests that the replication process in eukaryotes and prokaryotes is fundamentally similar

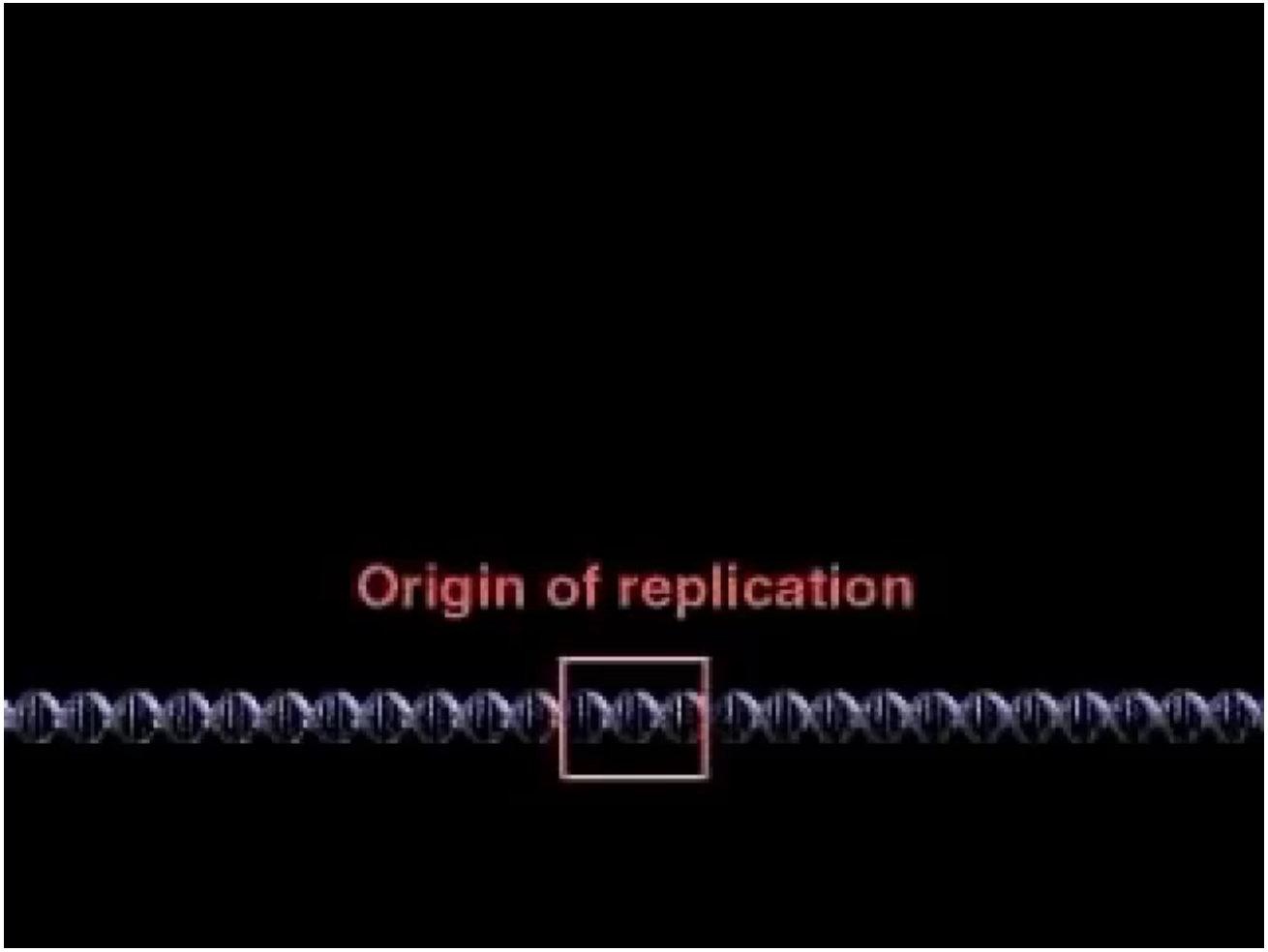
Getting Started

- Replication begins at particular sites called **origins of replication**, where the two DNA strands are separated, opening up a replication "bubble"
- A eukaryotic chromosome may have hundreds or even thousands of origins of replication
- Replication proceeds in both directions from each origin, until the entire molecule is copied

Figure 16.13

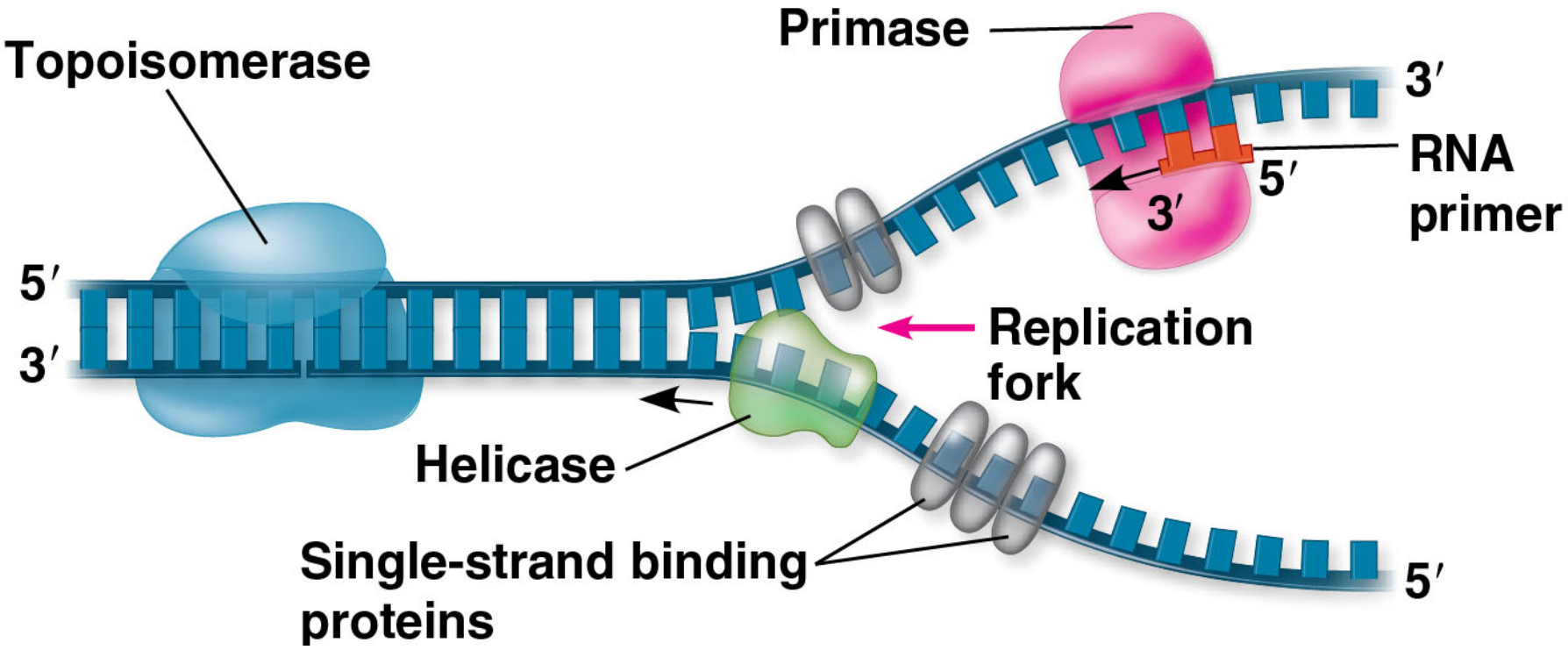

(a) Origin of replication in an *E. coli* cell
Origin of replication
Parental (template) strand
Daughter (new) strand
Replication fork
Double-stranded DNA molecule
Replication bubble
Two daughter DNA molecules
0.5 µm
(b) Origins of replication in a eukaryotic cell
Origin of replication
Double-stranded DNA molecule
Parental (template) strand
Daughter (new) strand
Bubble
Replication fork
Two daughter DNA molecules
0.25 µm

Animation: Origins of Replication



- At the end of each replication bubble is a **replication fork**, a Y-shaped region where parental DNA strands are being unwound
- **Helicases** are enzymes that untwist the double helix at the replication forks
- **Single-strand binding proteins** bind to and stabilize single-stranded DNA
- **Topoisomerase** relieves the strain of twisting of the double helix by breaking, swiveling, and rejoining DNA strands

Figure 16.14


Topoisomerase
Primase
3′
RNA primer
5′
3′
5′
3′
Replication fork
Helicase
Single-strand binding proteins
5′

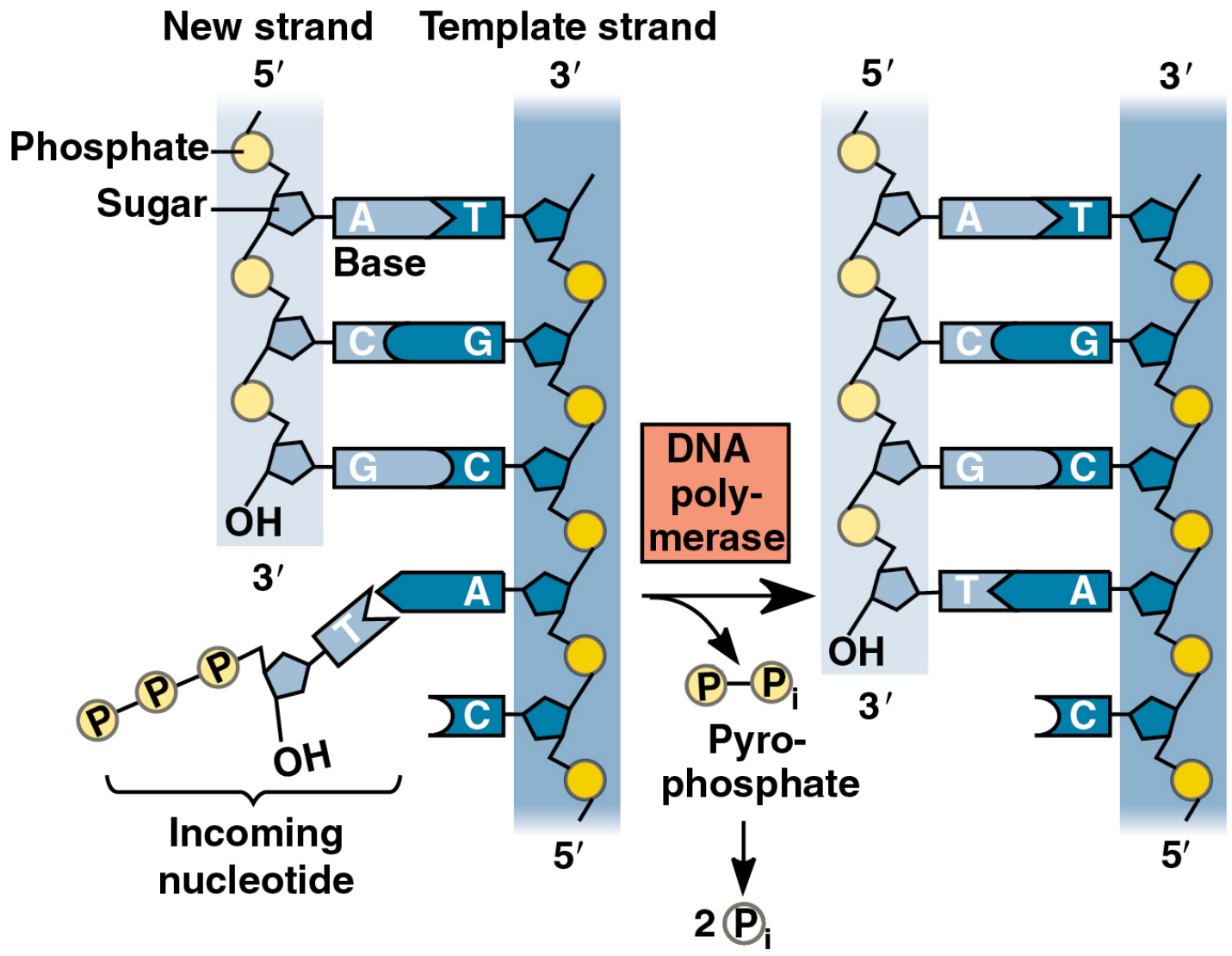
Synthesizing a New DNA Strand

- DNA polymerases require a primer to which they can add nucleotides
- The initial nucleotide chain is a short RNA **primer**
- This is synthesized by the enzyme **primase**
- The completed primer is five to ten nucleotides long
- The new DNA strand will start from the 3′ end of the RNA primer

- Enzymes called **DNA polymerases** catalyze the synthesis of new DNA at a replication fork
- Most DNA polymerases require a primer and a DNA template strand
- The rate of elongation is about 500 nucleotides per second in bacteria and 50 per second in human cells

- Each nucleotide that is added to a growing DNA strand is a nucleoside triphosphate
- dATP supplies adenine to DNA and is similar to the ATP of energy metabolism
- The difference is in their sugars: dATP has deoxyribose while ATP has ribose
- As each monomer joins the DNA strand, via a dehydration reaction, it loses two phosphate groups as a molecule of pyrophosphate

Figure 16.15

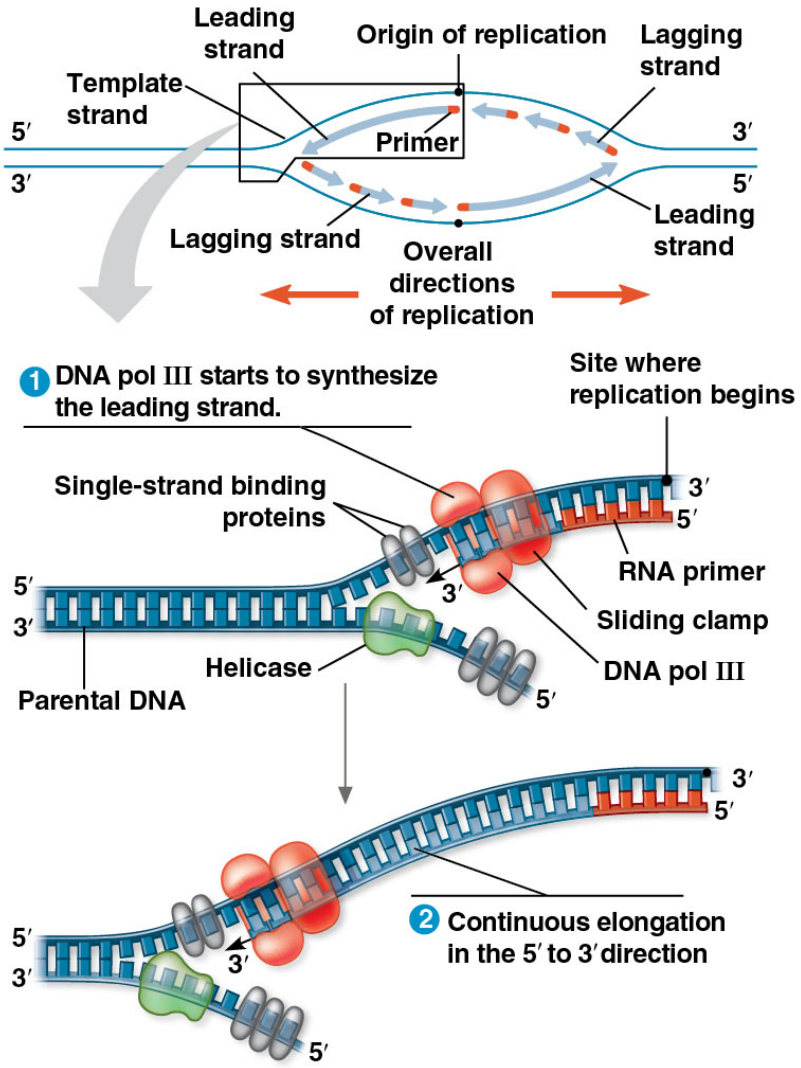

New strand
Template strand
5′
3′
Phosphate
Sugar
Base
A
T
C
G
G
C
OH
3′
A
T
P
P
P
OH
C
Incoming nucleotide
5′
DNA poly-merase
P
Pi
Pyro-phosphate
2 Pi
5′
3′
A
T
C
G
G
C
T
A
OH
3′
C
5′

Antiparallel Elongation

- The antiparallel structure of the double helix affects replication
- DNA polymerases add nucleotides only to the free 3′ end of a growing strand; therefore, a new DNA strand can elongate only in the 5′ → 3′ direction

- Along one template strand of DNA, the DNA polymerase synthesizes a **leading strand** continuously, moving toward the replication fork

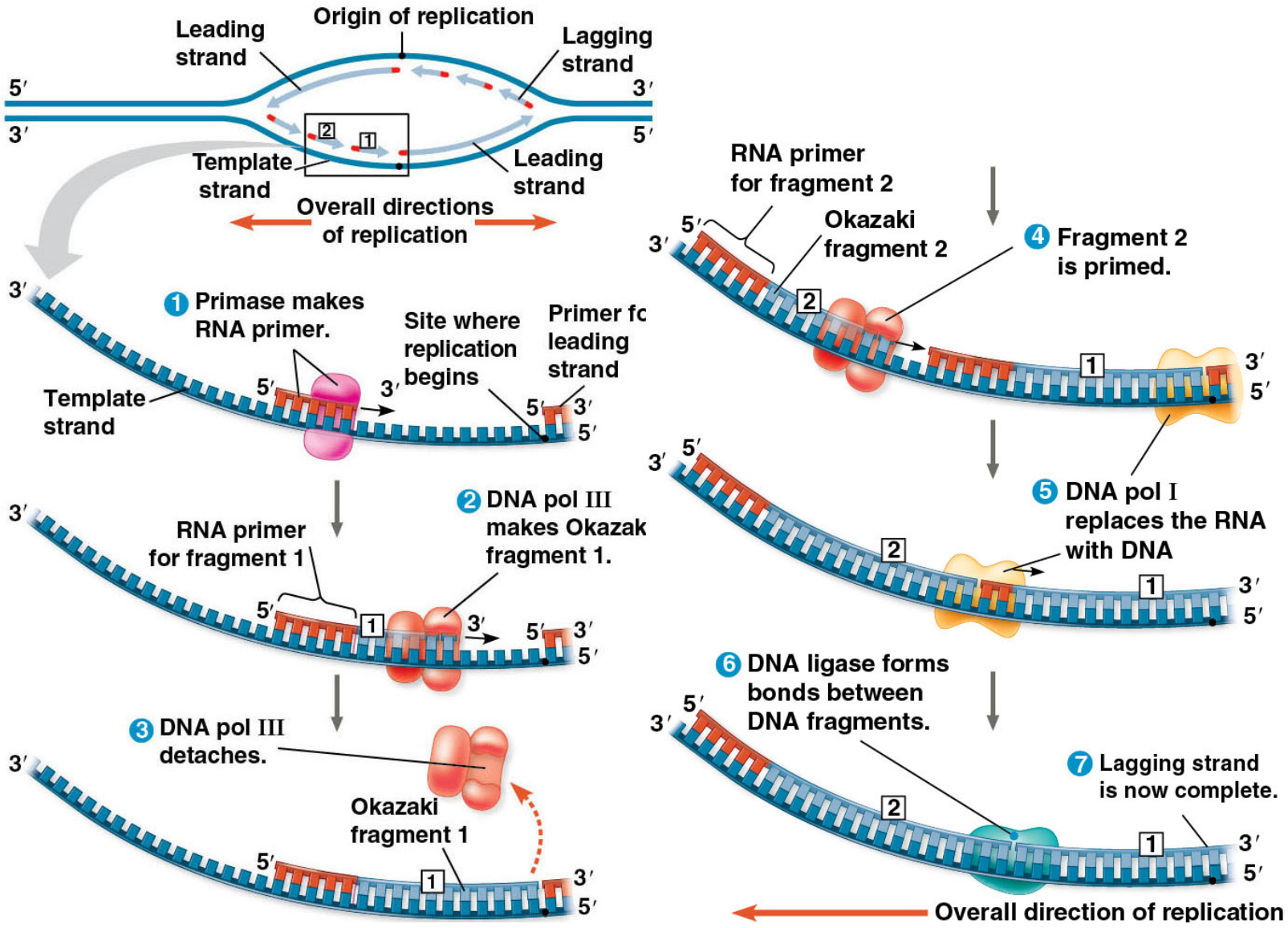
Figure 16.16


Leading strand
Origin of replication
Lagging strand
Template strand
5′
3′
Primer
3′
5′
Lagging strand
Leading strand
Overall directions of replication
1 DNA pol III starts to synthesize the leading strand.
Site where replication begins
Single-strand binding proteins
3′
5′
RNA primer
5′
3′
3′
Sliding clamp
Helicase
DNA pol III
Parental DNA
5′
3′
5′
2 Continuous elongation in the 5′ to 3′ direction
5′
3′
3′
5′

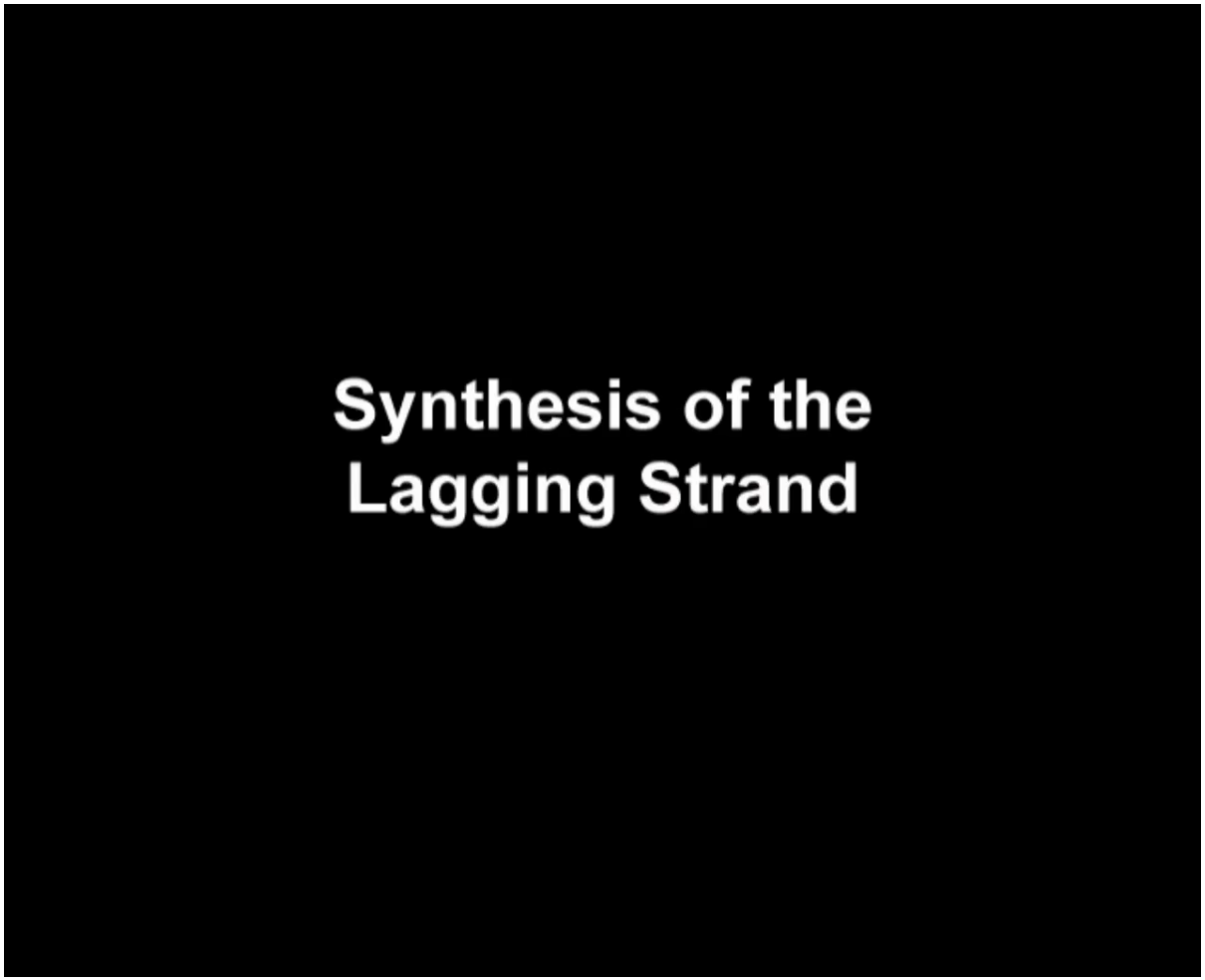
BioFlix® Animation: Synthesis of the Leading Strand

- To elongate the other new strand, called the **lagging strand**, DNA polymerase must work in the direction away from the replication fork
- The lagging strand is synthesized as a series of segments called **Okazaki fragments**, which are joined together by **DNA ligase**

Figure 16.17


Origin of replication
Leading strand
Lagging strand
5′
3′
3′
5′
2
1
Template strand
Leading strand
Overall directions of replication
1 Primase makes RNA primer.
Site where replication begins
Primer fo leading strand
Template strand
2 DNA pol III makes Okazak fragment 1.
RNA primer for fragment 1
3 DNA pol III detaches.
Okazaki fragment 1
RNA primer for fragment 2
Okazaki fragment 2
4 Fragment 2 is primed.
5 DNA pol I replaces the RNA with DNA
6 DNA ligase forms bonds between DNA fragments.
7 Lagging strand is now complete.
Overall direction of replication

BioFlix® Animation: Synthesis of the Lagging Strand



Animation: DNA Replication: An Overview

Animation: DNA Replication Review

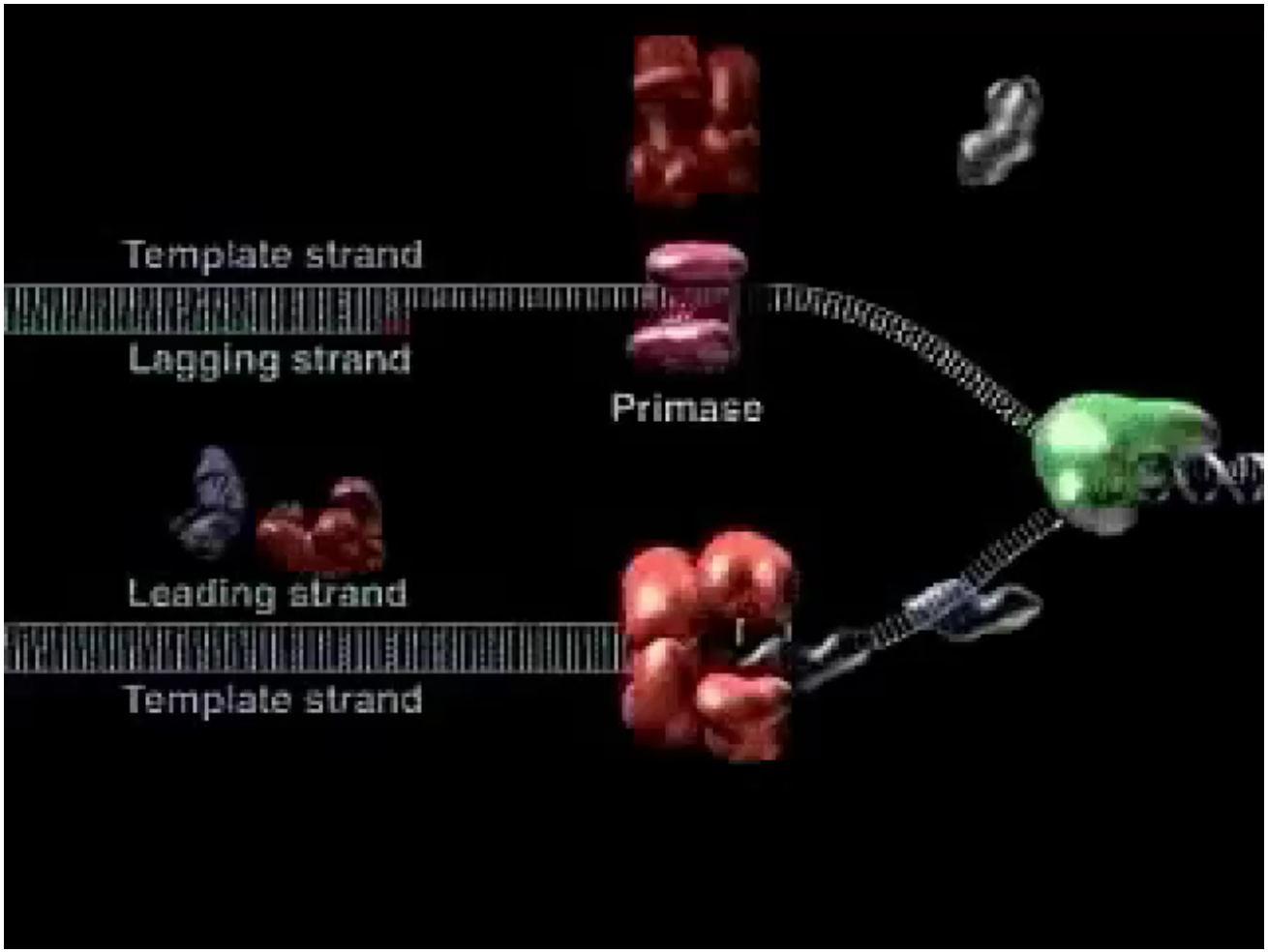


Figure 16.18

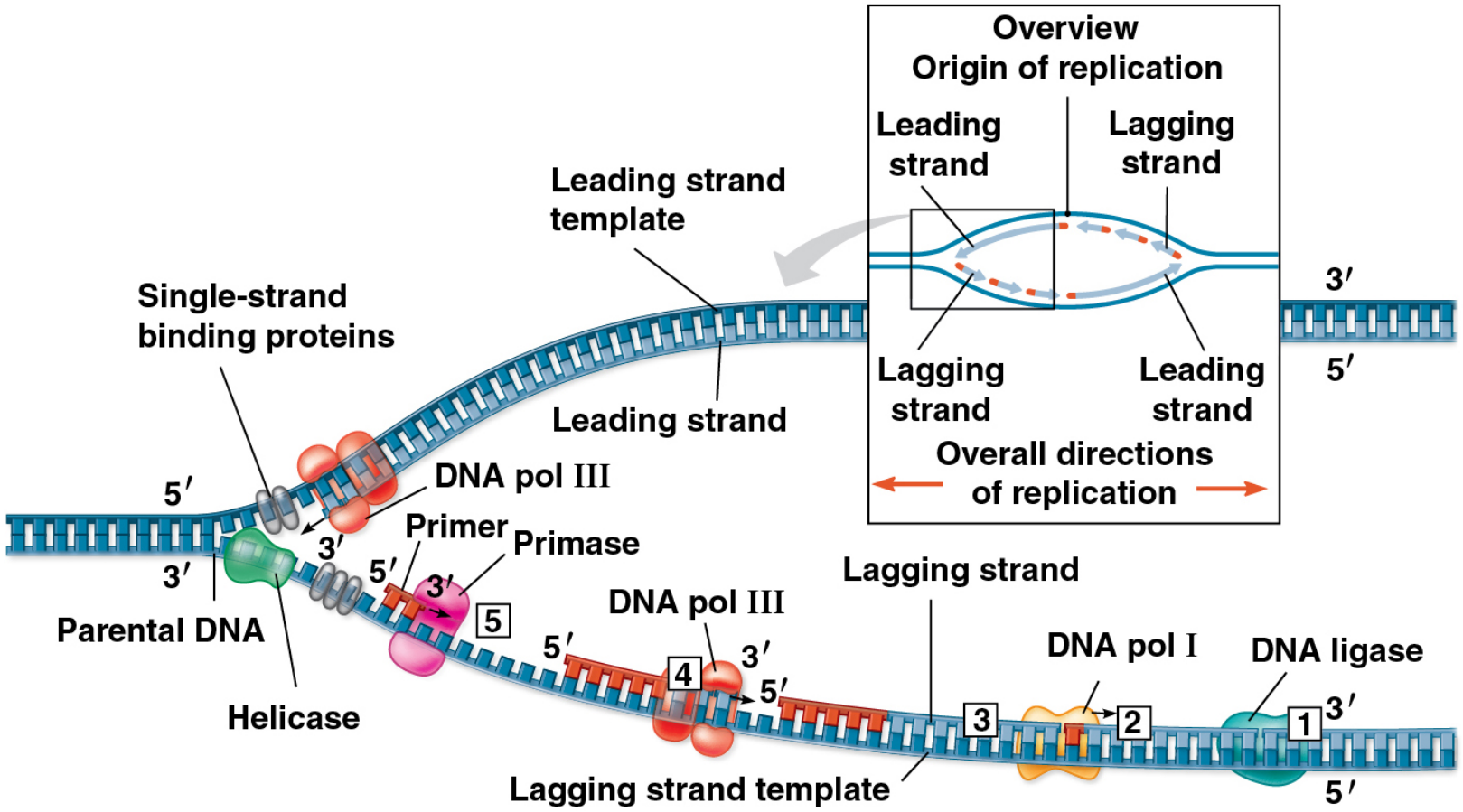
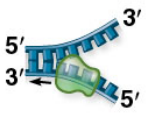
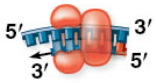
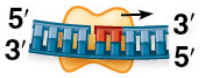

Overview
Origin of replication
Leading strand
Lagging strand
Lagging strand
Leading strand
Overall directions of replication
Leading strand template
Single-strand binding proteins
Leading strand
DNA pol III
Primer
Primase
Parental DNA
Helicase
DNA pol III
Lagging strand
DNA pol I
DNA ligase
Lagging strand template
5′
3′
1
2
3
4
5

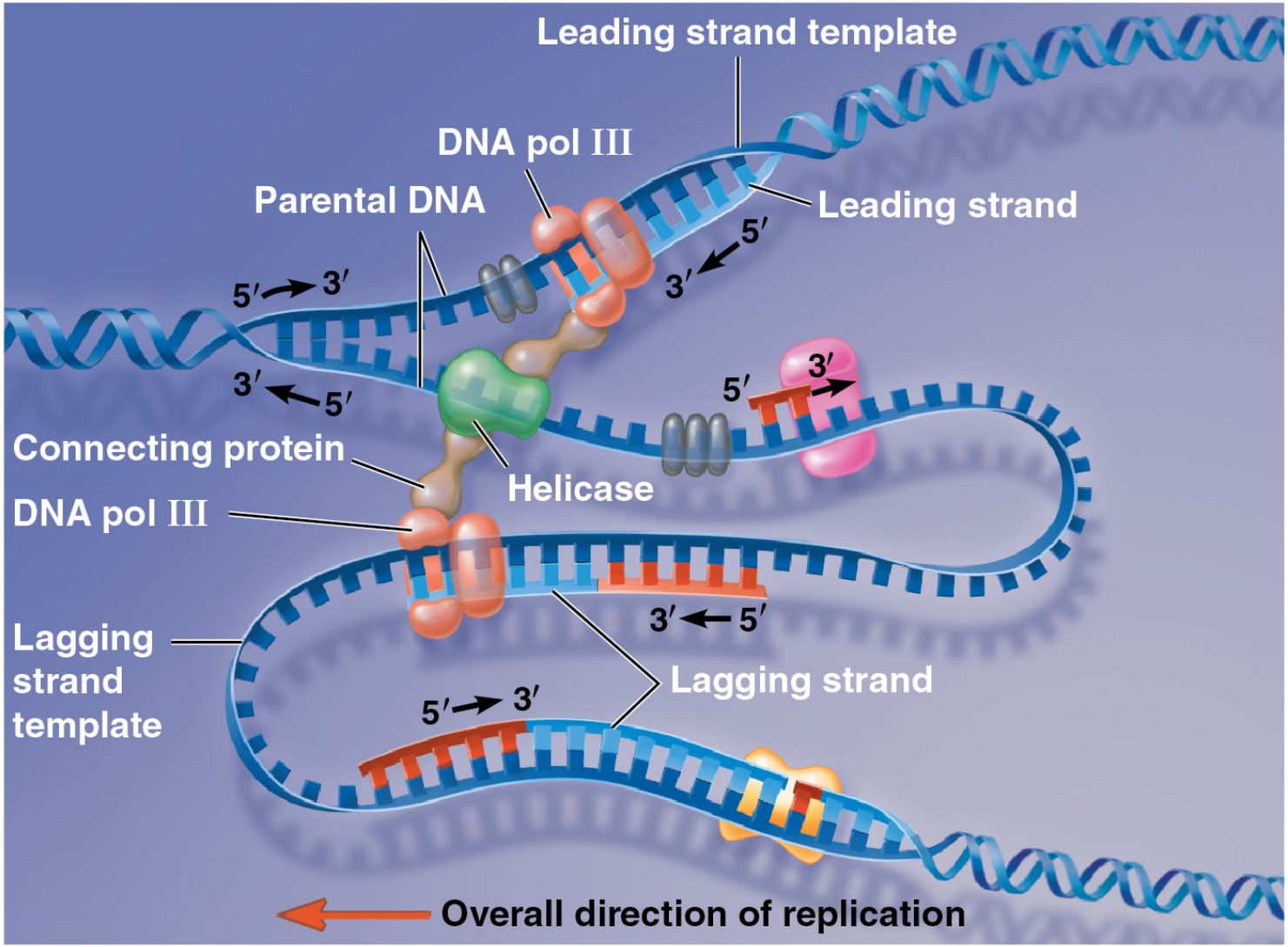
Table 16.1 Bacterial DNA Replication Proteins and Their Functions

Protein	Function
Helicase 	Unwinds parental double helix at replication forks
Single-strand binding protein 	Binds to and stabilizes single-stranded DNA until it is used as a template
Topoisomerase 	Relieves overwinding strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase 	Synthesizes an RNA primer at 5′ end of leading strand and at 5′ end of each Okazaki fragment of lagging strand
DNA pol III 	Using parental DNA as a template, synthesizes new DNA strand by adding nucleotides to an RNA primer or a pre-existing DNA strand
DNA pol I 	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides added to 3′ end of adjacent fragment
DNA ligase 	Joins Okazaki fragments of lagging strand; on leading strand, joins 3′ end of DNA that replaces primer to rest of leading strand DNA

The DNA Replication Complex

- The proteins that participate in DNA replication form a large complex, a “DNA replication machine”
- The DNA replication machine may be stationary during the replication process
- Recent studies support a model in which DNA polymerase molecules “reel in” parental DNA and extrude newly made daughter DNA molecules
- The exact mechanism is not yet resolved

Figure 16.19

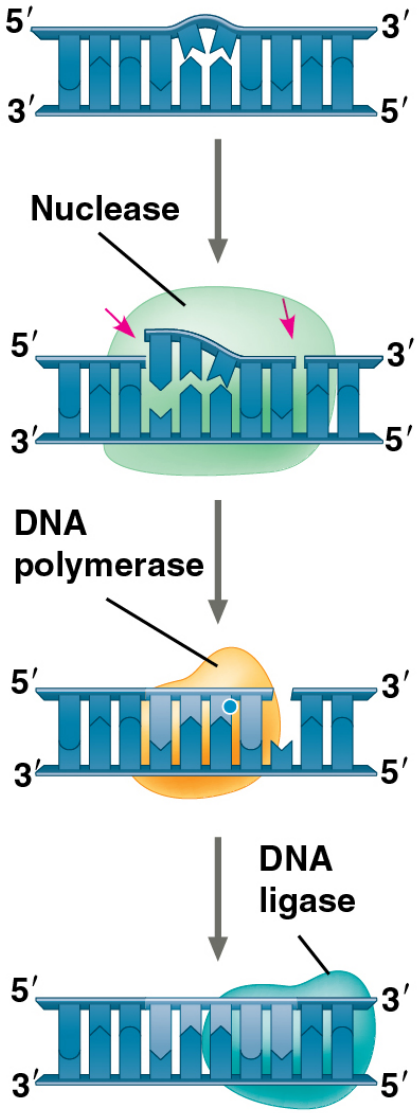

Leading strand template
DNA pol III
Parental DNA
Leading strand
5′
3′
5′ → 3′
3′ ← 5′
5′
3′
Connecting protein
Helicase
DNA pol III
3′ ← 5′
Lagging strand template
Lagging strand
5′ → 3′
Overall direction of replication

Proofreading and Repairing DNA

- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- In **mismatch repair** of DNA, repair enzymes replace incorrectly paired nucleotides that have evaded the proofreading process

- DNA can be damaged by exposure to harmful chemical or physical agents such as cigarette smoke and X-rays; it can also undergo spontaneous changes
- In **nucleotide excision repair**, a **nuclease** cuts out and replaces damaged stretches of DNA

Figure 16.20



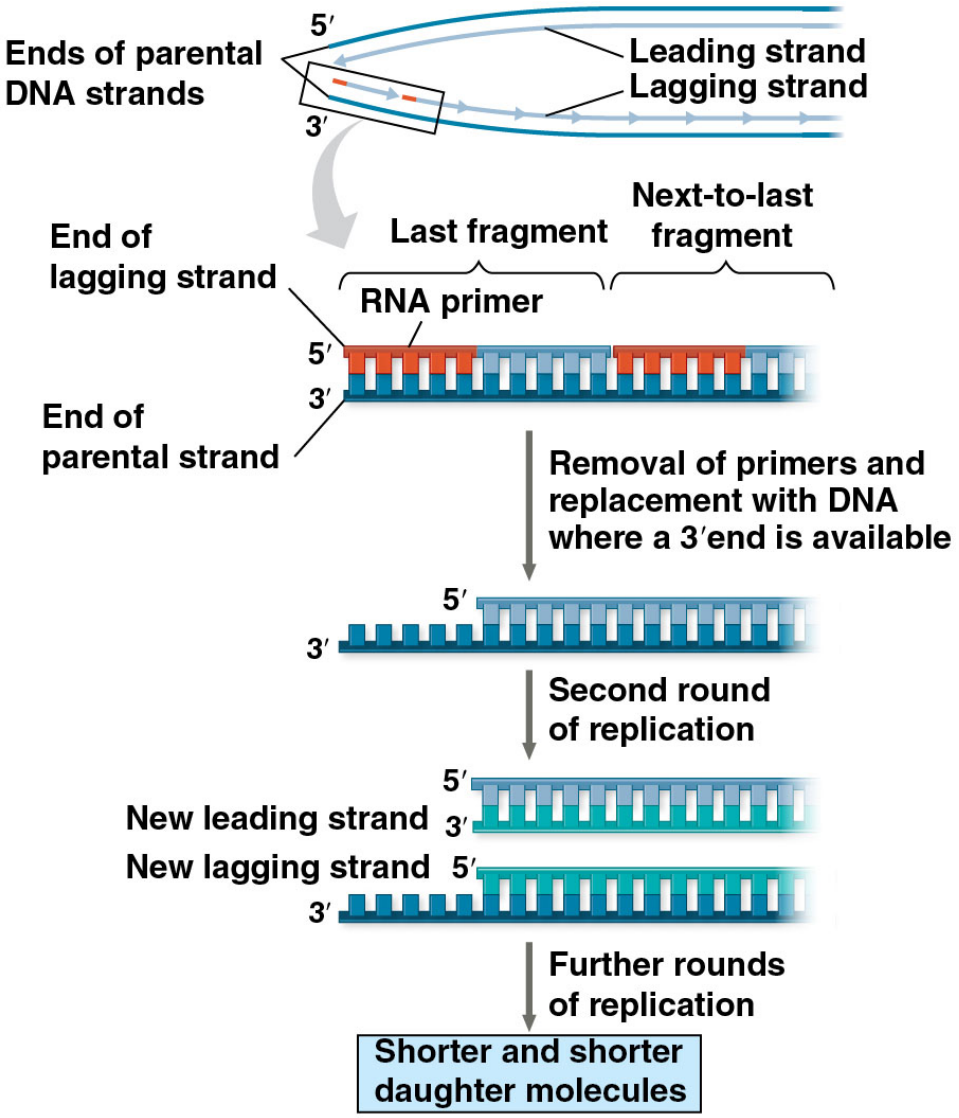
Evolutionary Significance of Altered DNA Nucleotides

- The error rate after proofreading and repair is low but not zero
- Sequence changes may become permanent and can be passed on to the next generation
- These changes (mutations) are the source of the genetic variation upon which natural selection operates and are ultimately responsible for the appearance of new species

Replicating the Ends of DNA Molecules

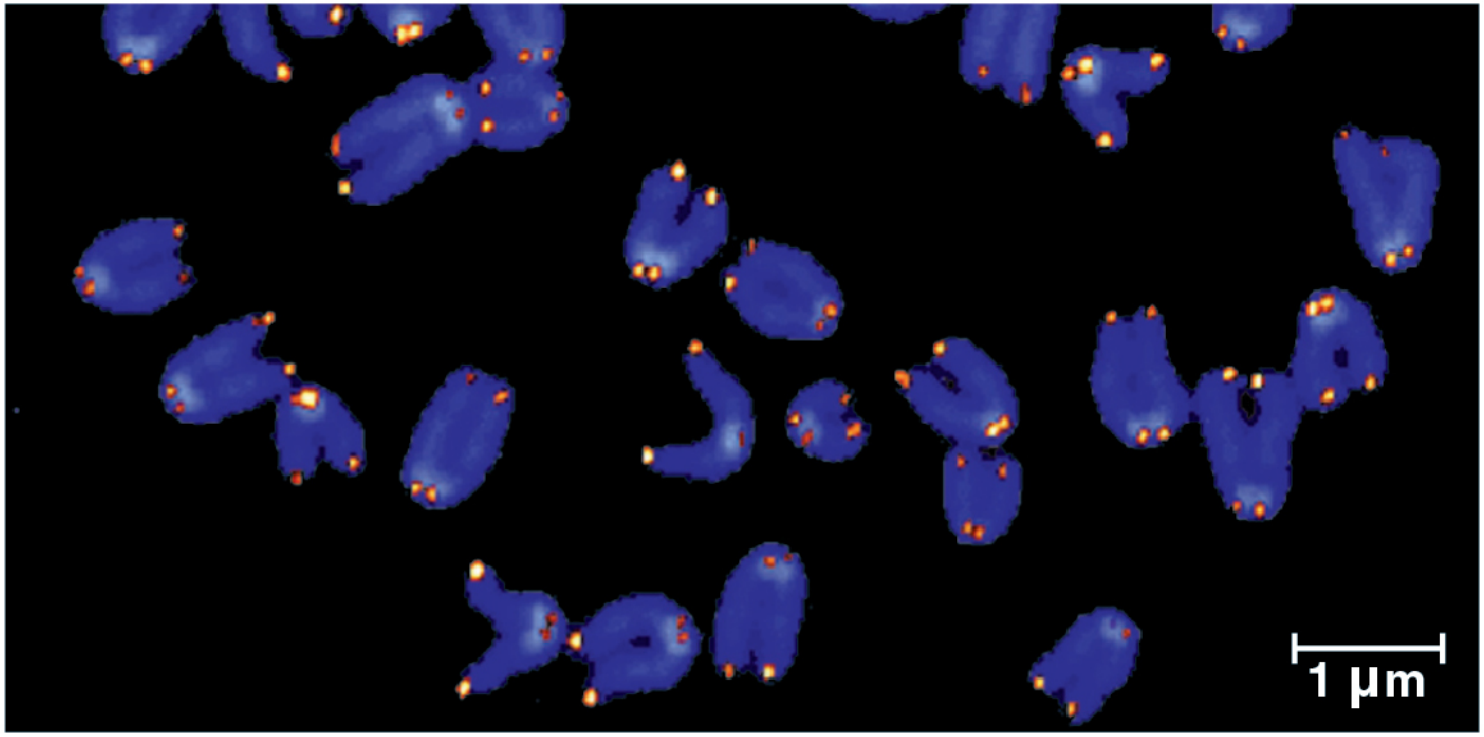
- For linear DNA, the usual replication machinery cannot complete the 5′ ends of daughter DNA strands
- There is no 3′ end of a preexisting polynucleotide for DNA polymerase to add on to
- Thus, repeated rounds of replication produce shorter DNA molecules with uneven ends
- This is not a problem for prokaryotes, most of which have circular chromosomes

Figure 16.21


Ends of parental DNA strands
5′
3′
Leading strand
Lagging strand
End of lagging strand
Last fragment
Next-to-last fragment
RNA primer
5′
3′
End of parental strand
Removal of primers and replacement with DNA where a 3′end is available
5′
3′
Second round of replication
5′
New leading strand
3′
New lagging strand
5′
3′
Further rounds of replication
Shorter and shorter daughter molecules

- Eukaryotic chromosomal DNA molecules have special nucleotide sequences at their ends called **telomeres**
- Telomeres do not prevent the shortening of DNA molecules, but they do postpone the erosion of genes near the ends of DNA molecules
- It has been proposed that the shortening of telomeres is connected to aging

Figure 16.22



- If chromosomes of germ cells became shorter in every cell cycle, essential genes would eventually be missing from the gametes they produce
- An enzyme called telomerase catalyzes the lengthening of telomeres in germ cells

- The shortening of telomeres might protect cells from cancerous growth by limiting the number of cell divisions
- There is evidence of telomerase activity in cancer cells, which may allow cancer cells to persist

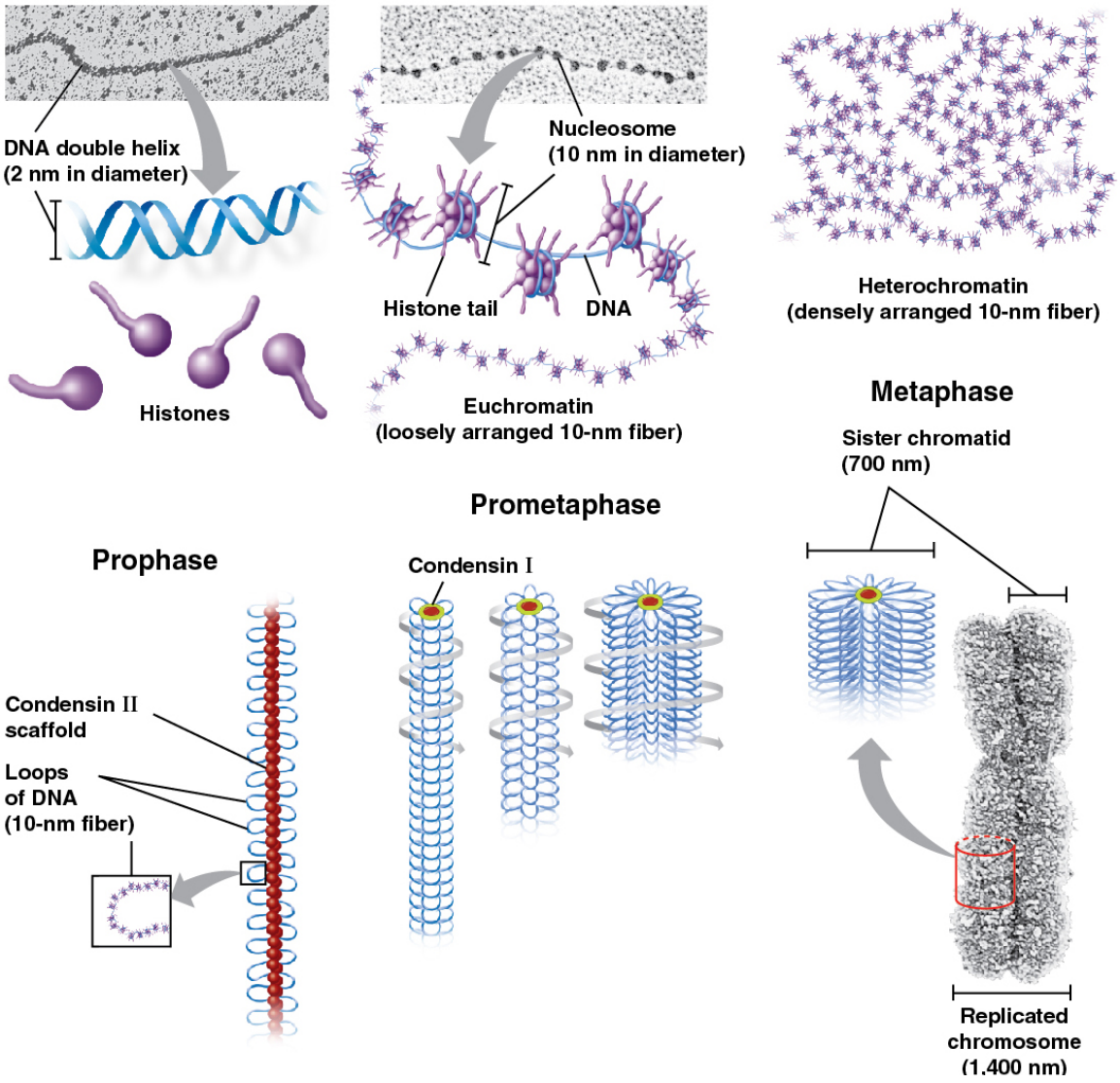
CONCEPT 16.3: A chromosome consists of a DNA molecule packed together with proteins

- The bacterial chromosome is a double-stranded, circular DNA molecule associated with a small amount of protein
- Eukaryotic chromosomes have linear DNA molecules associated with a large amount of protein
- In a bacterium, the DNA is “supercoiled” and found in a region of the cell called the nucleoid

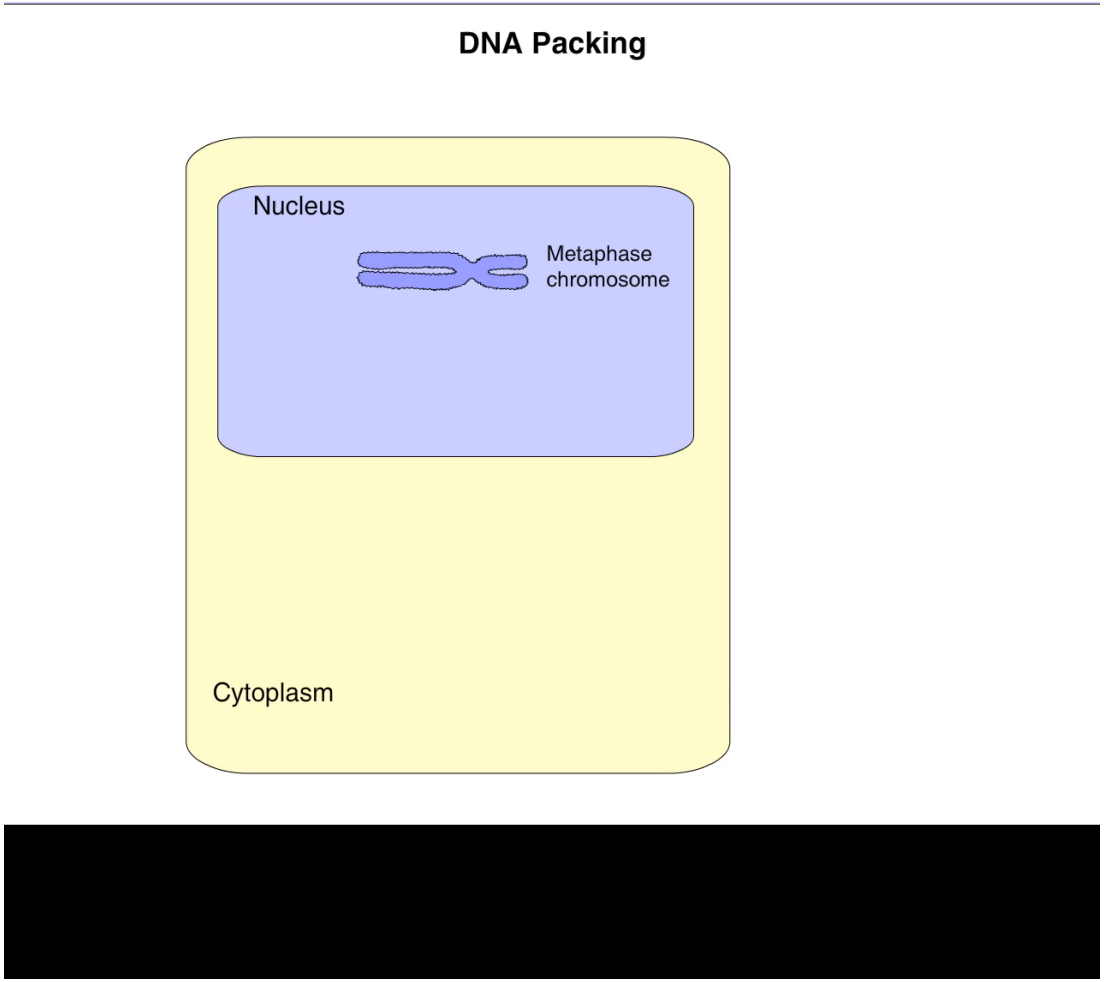
- In the eukaryotic cell, DNA is precisely combined with proteins in a complex called **chromatin**
- Chromosomes fit into the nucleus through an elaborate, multilevel system of packing
- Proteins called **histones** are responsible for the main level of DNA packing in interphase chromatin

- In a 10-nm chromatin fiber, the unfolded chromatin resembles beads on a string, with each “bead” being a **nucleosome**
- A nucleosome is composed of DNA wound twice around a core of eight histones, two each of the four main histone types
- The amino end of each histone (the histone tail) extends outward from the nucleosome and is involved in regulation of gene expression

Figure 16.23


DNA double helix
(2 nm in diameter)
Histones
Nucleosome
(10 nm in diameter)
Histone tail
DNA
Euchromatin
(loosely arranged 10-nm fiber)
Heterochromatin
(densely arranged 10-nm fiber)
Prophase
Condensin II
scaffold
Loops
of DNA
(10-nm fiber)
Prometaphase
Condensin I
Metaphase
Sister chromatid
(700 nm)
Replicated
chromosome
(1,400 nm)

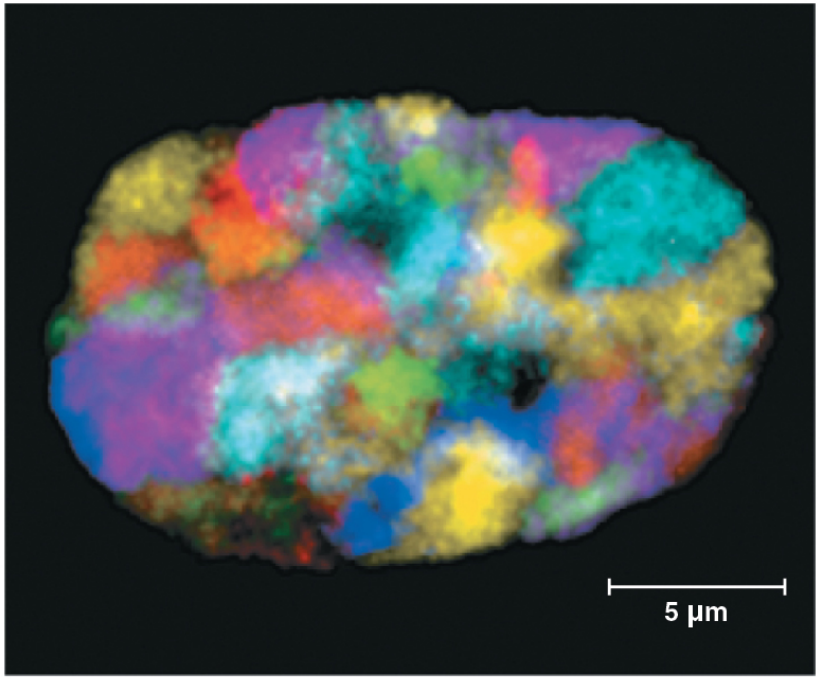
Animation: DNA Packing

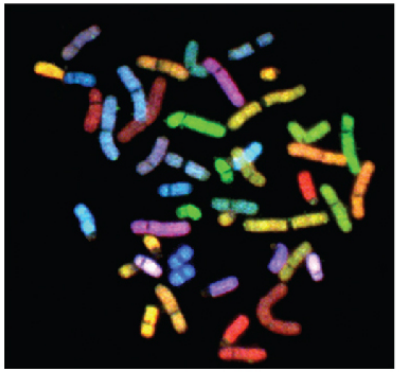


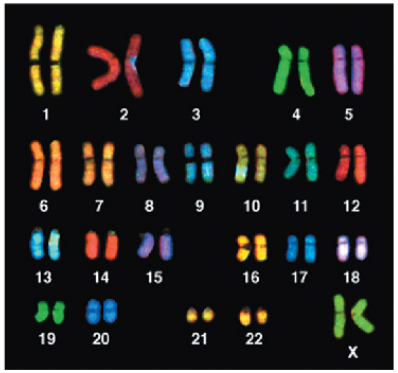
- Most chromatin is loosely packed in the nucleus during interphase and condenses prior to mitosis
- Loosely packed chromatin is called **euchromatin**
- During interphase a few regions of chromatin (centromeres and telomeres) are highly condensed into **heterochromatin**
- Dense packing of the heterochromatin makes it difficult for the cell to express genetic information coded in these regions

- Interphase chromosomes occupy specific restricted regions in the nucleus, and the fibers of different chromosomes do not become entangled
- Chromatin undergoes changes in packing during the cell cycle
- As the cell prepares for mitosis, the chromatin is organized into loops and coils, eventually condensing into short, thick metaphase chromosomes

Figure 16.24







CONCEPT 16.3: A chromosome consists of a DNA molecule packed together with proteins

- Histones can undergo chemical modifications that result in changes in chromatin condensation
- These changes can also have multiple effects on gene expression

Figure 16.UN01a

Source of DNA	Base Percentage			
	Adenine	Guanine	Cytosine	Thymine
Sea urchin	32.8	17.7	17.3	32.1
Salmon	29.7	20.8	20.4	29.1
Wheat	28.1	21.8	22.7	
E. coli	24.7	26.0		
Human	30.4			30.1
Ox	29.0			
Average %				

Figure 16.UN01b

Figure 16.UN02

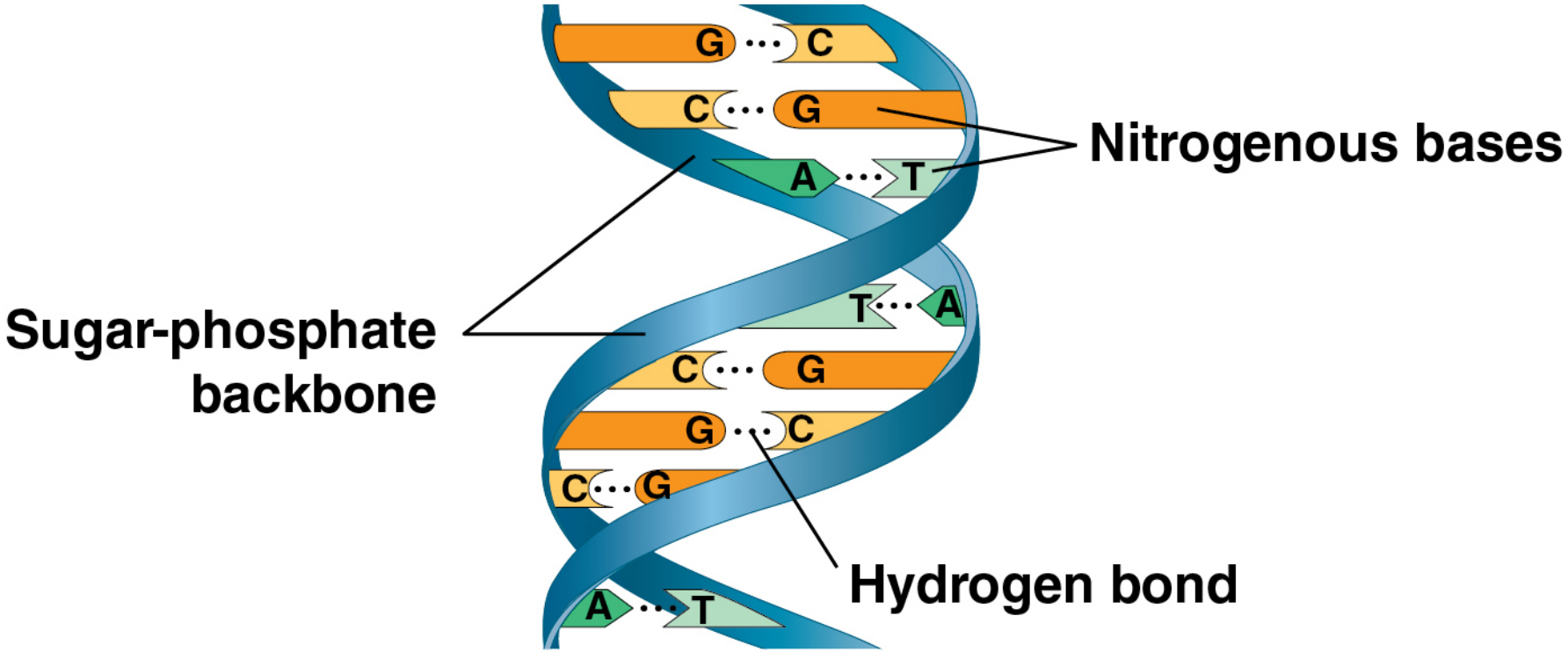


Figure 16.UN03

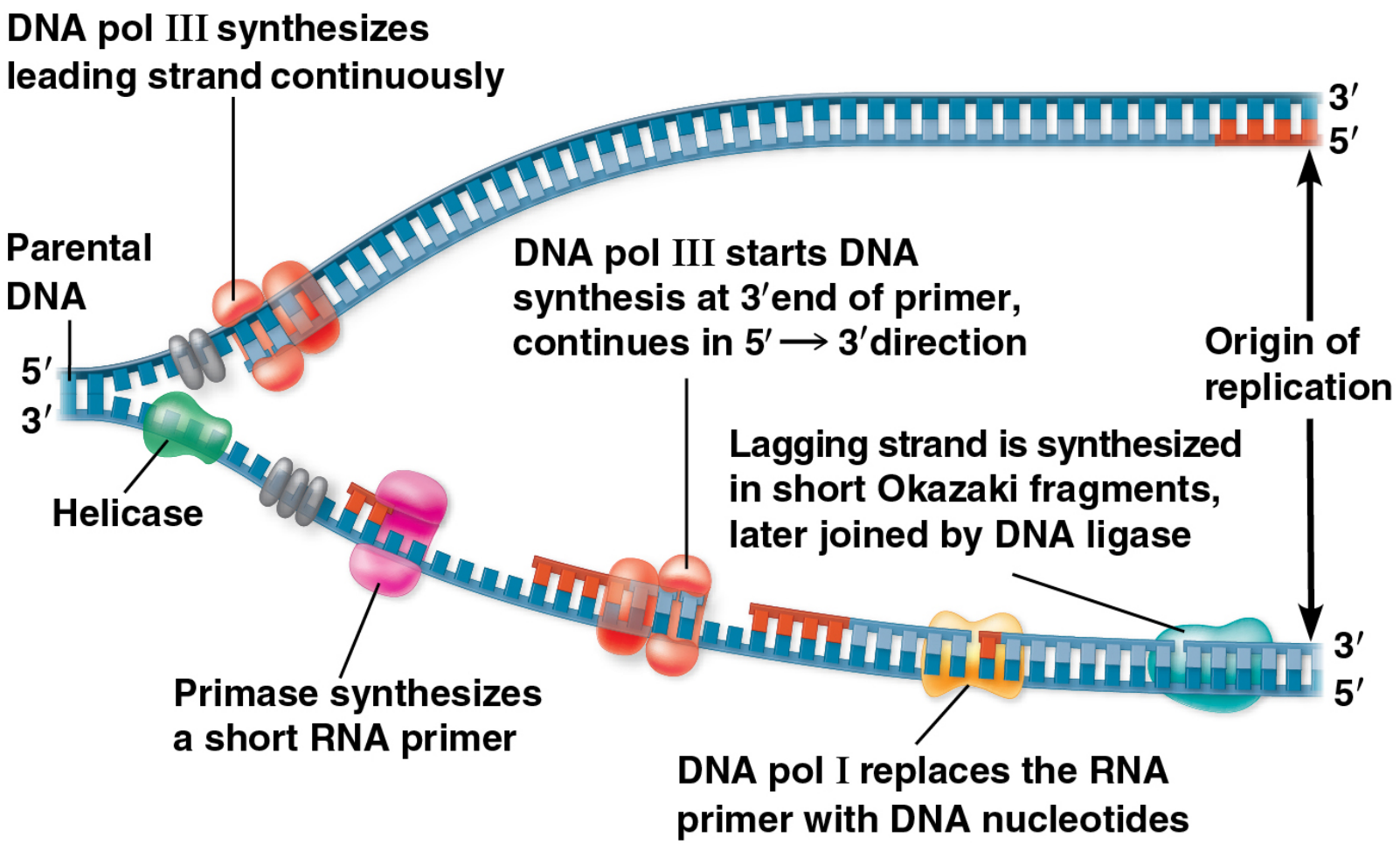


Figure 16.UN04

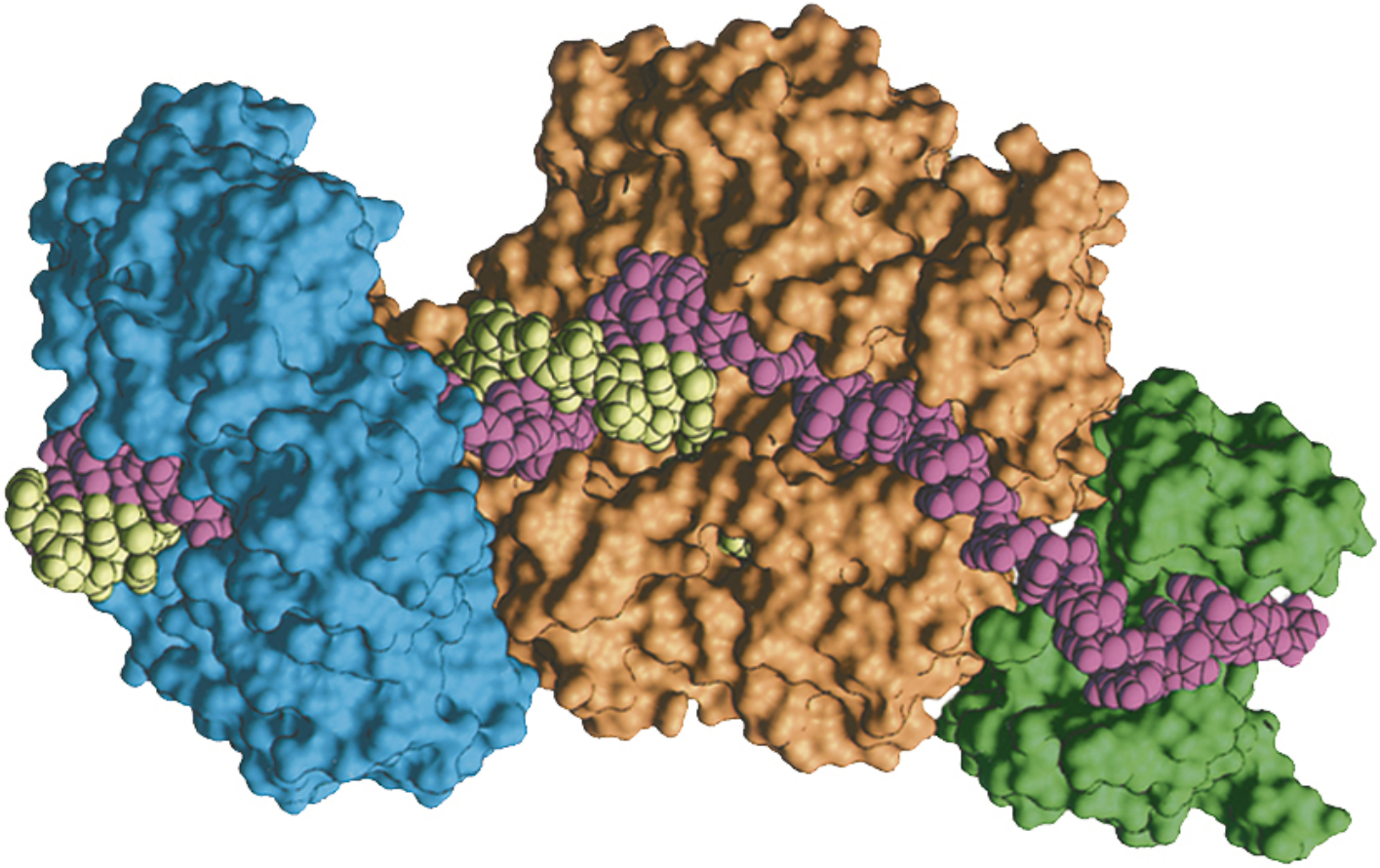


Figure 16.UN05