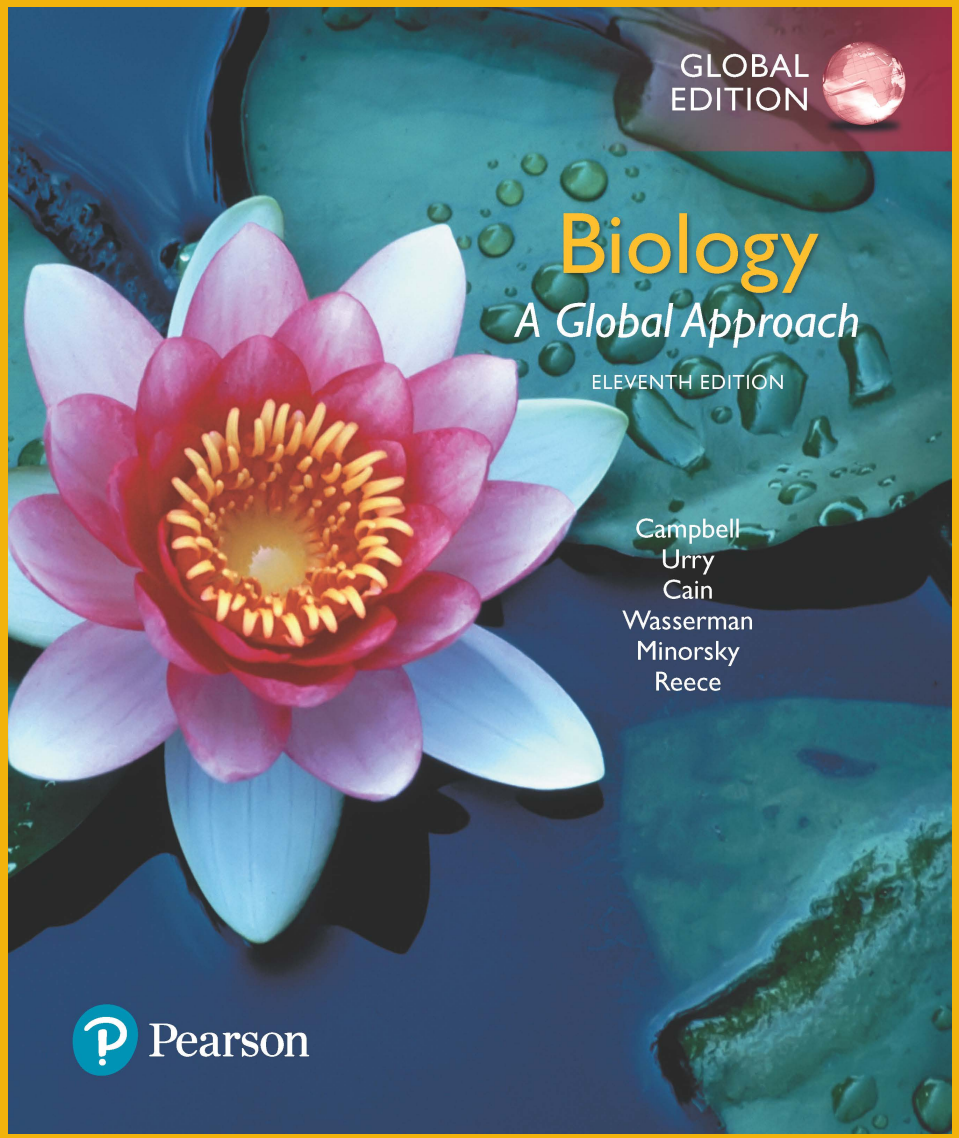


Chapter 16

Nucleic Acids and Inheritance

Lecture Presentations by
Nicole Tunbridge and
Kathleen Fitzpatrick

ife's Operating Instructions

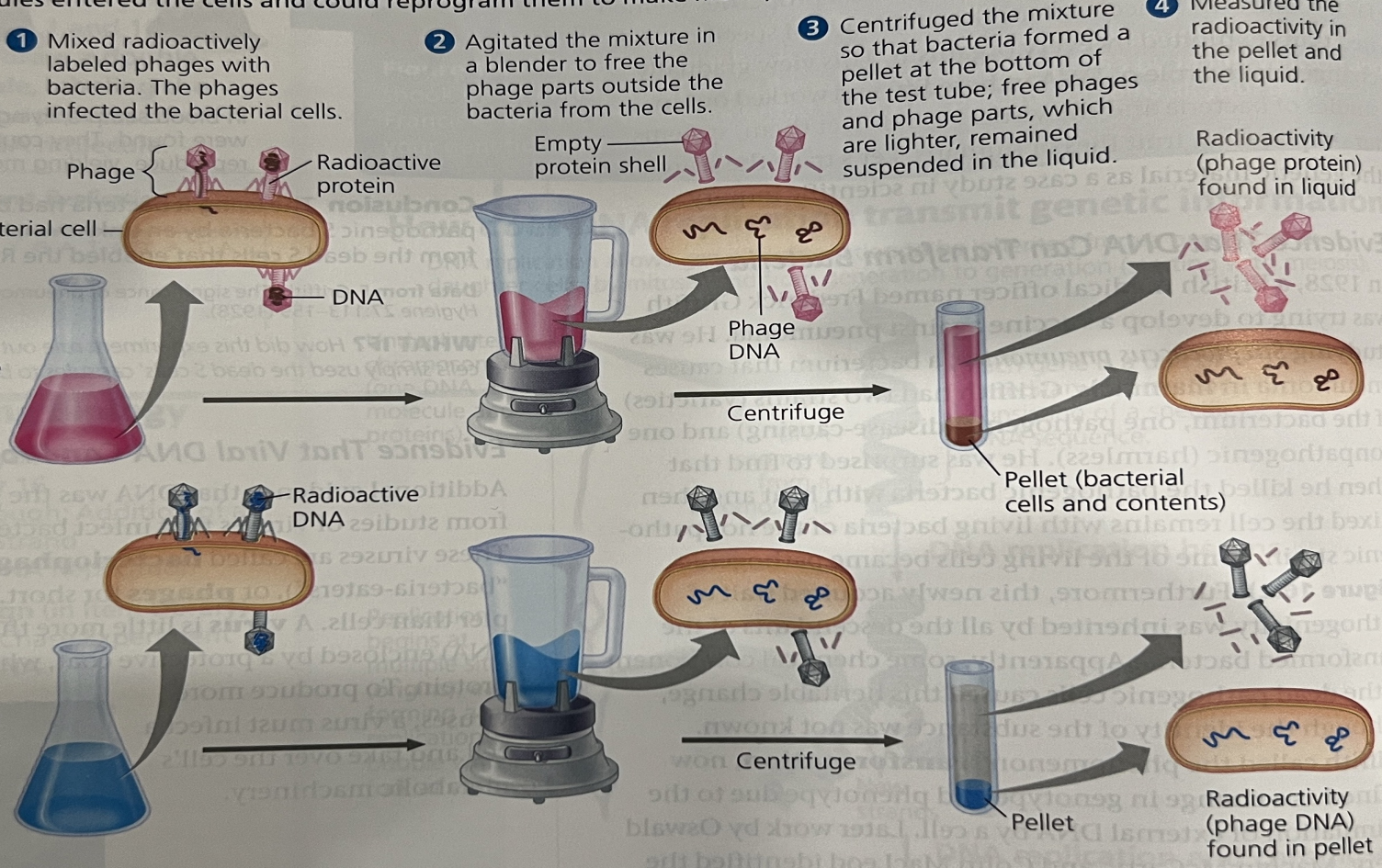
- In 1953, James Watson and Francis Crick introduced an elegant double-helical model for the structure of deoxyribonucleic acid, or DNA
- Hereditary information is encoded in DNA and reproduced in all cells of the body
- This DNA program directs the development of biochemical, anatomical, physiological, and (to some extent) behavioral traits

- DNA is copied during **DNA replication**, and cells can repair their DNA

▼ Figure 16.4 Inquiry

Is protein or DNA the genetic material of phage T2?

Experiment Alfred Hershey and Martha Chase used radioactive sulfur and phosphorus to trace the fates of protein and DNA, respectively, of T2 phages that infected bacterial cells. They wanted to see which of these molecules entered the cells and could reprogram them to make more phages.



Results When proteins were labeled (batch 1), radioactivity remained outside the cells, but when DNA was labeled (batch 2), radioactivity was found inside the cells. Cells containing radioactive phage DNA released new phages with some radioactive phosphorus.

Conclusion Phage DNA entered bacterial cells, but phage proteins did not. Hershey and Chase concluded that DNA, not protein, functions as the genetic material of phage T2.

Data from A. D. Hershey and M. Chase, Independent functions of viral protein and nucleic acid in growth of bacteriophage, *Journal of General Physiology* 36:39–56 (1952).

WHAT IF? *How would the results have differed if proteins carried the genetic information?*

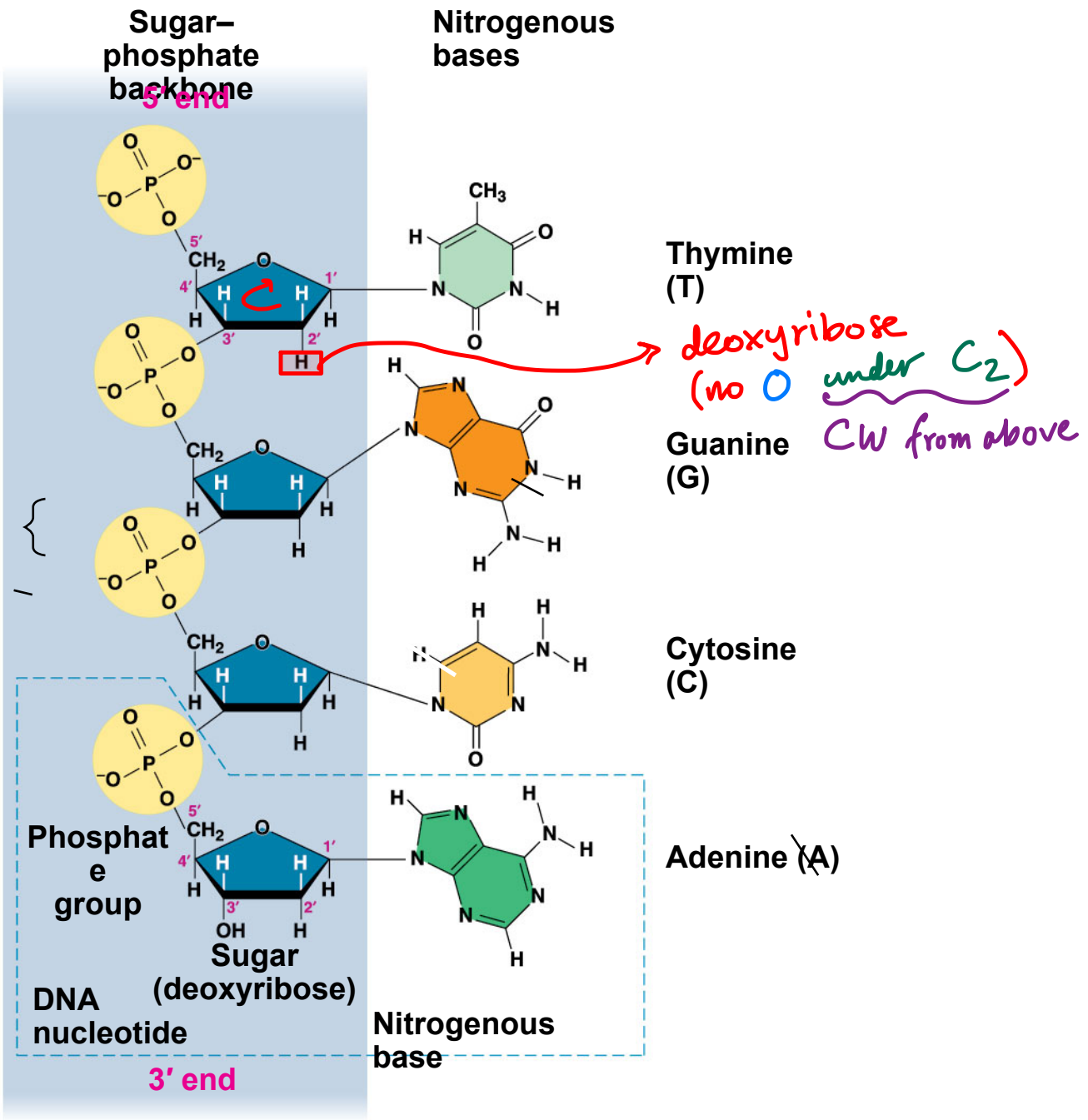
Mastering Biology Animation: The Hershey-Chase Experiment

dditional 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 diversity 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

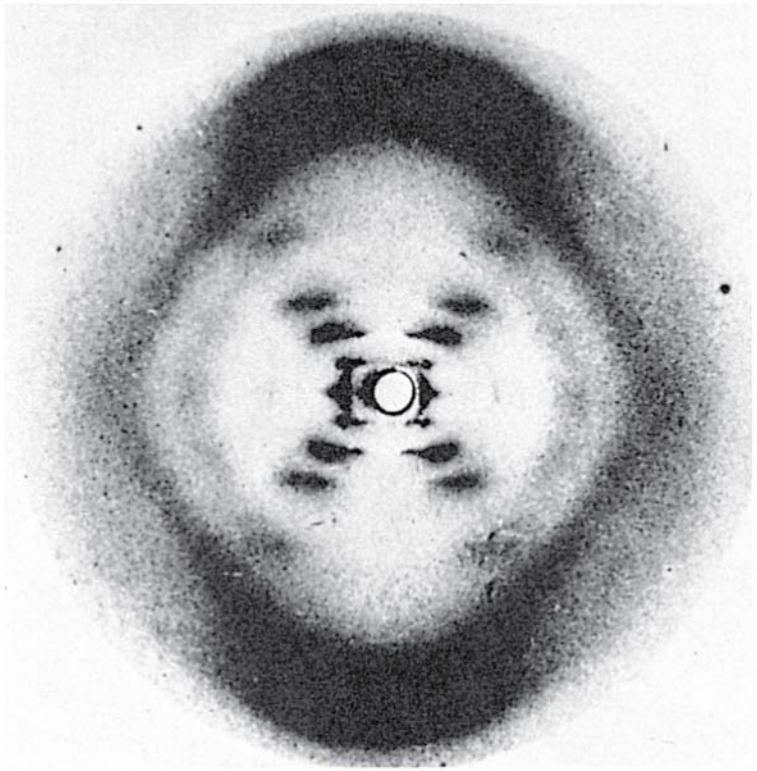
Figure 16.5



uilding a Structural Model of DNA: *Scientific Inquiry*

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

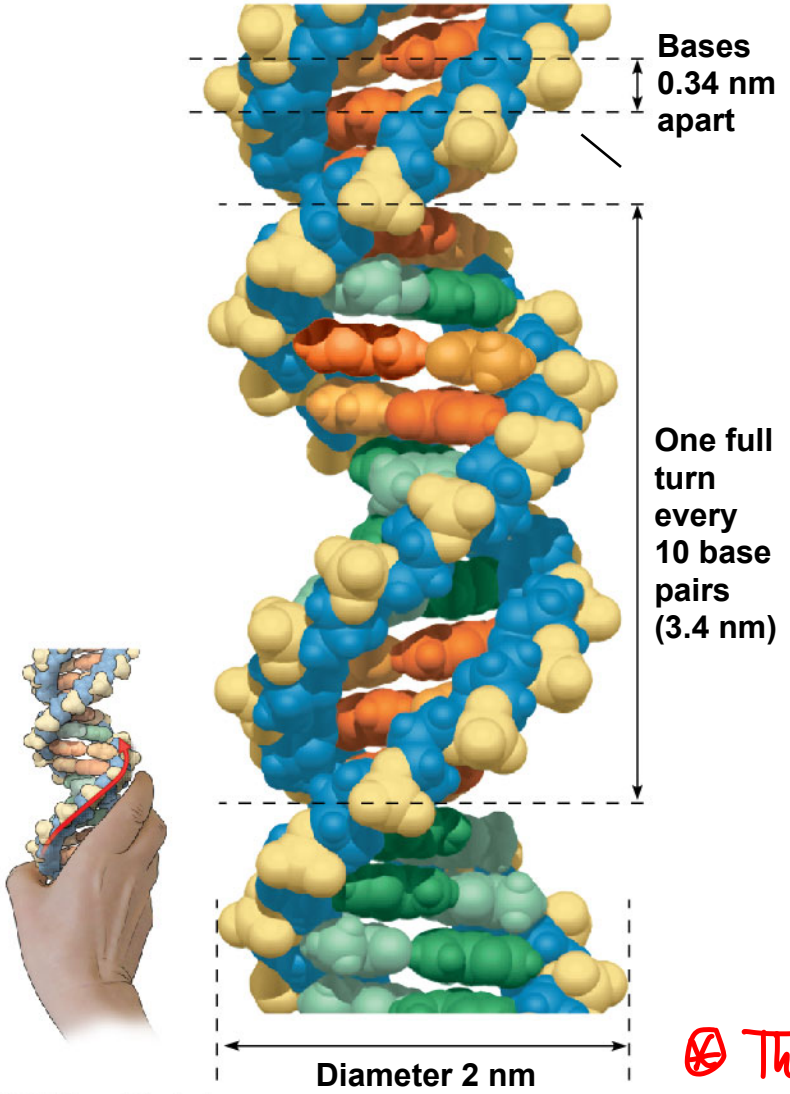
Figure 16.6b

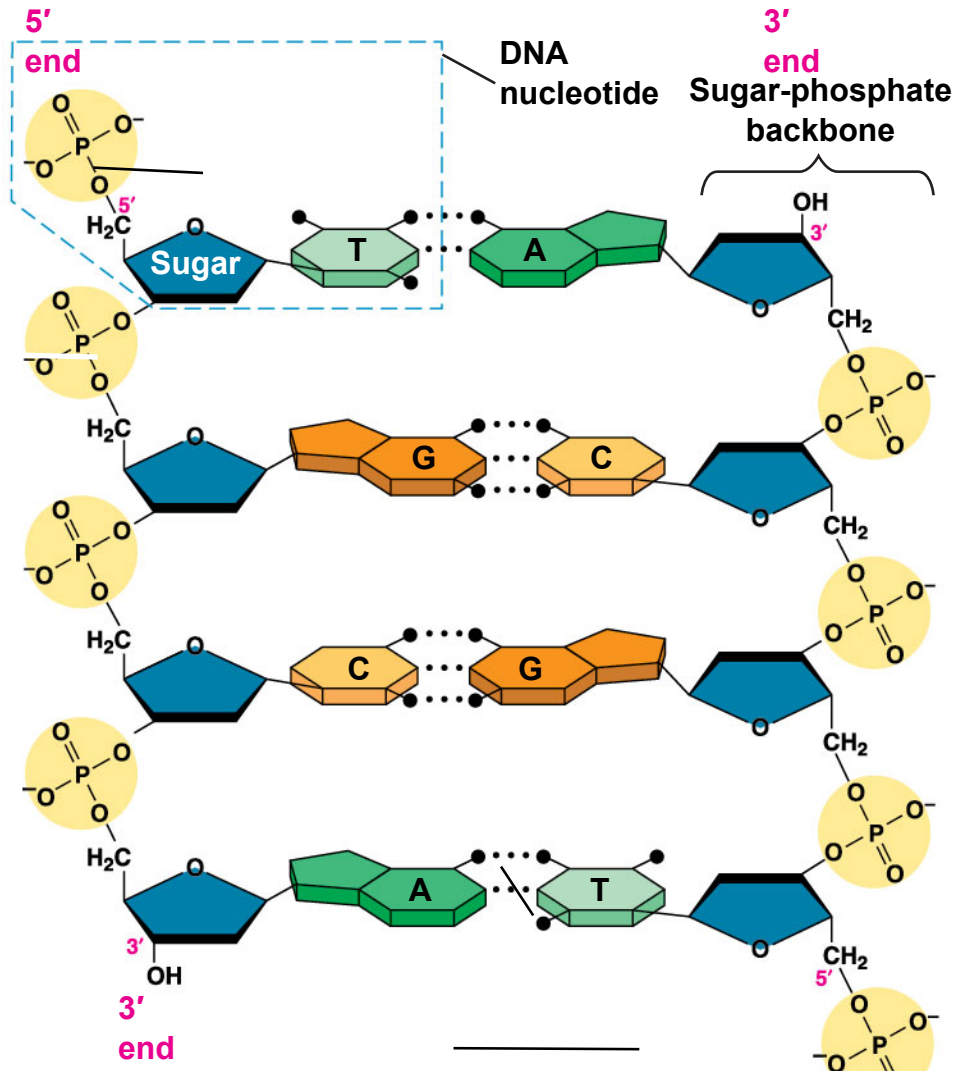


- Franklin's X-ray crystallographic images of DNA enabled Watson to deduce that DNA was helical
- The X-ray images also enabled Watson to deduce the width of the helix and the spacing of the nitrogenous bases
- The pattern in the photo suggested that the DNA molecule was made up of two strands, forming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

Figure 16.7a

Structural Images





The DNA double-helix is right-handed [you can follow the sugar–phosphate backbone only with your RH]

Figure 16.7aa

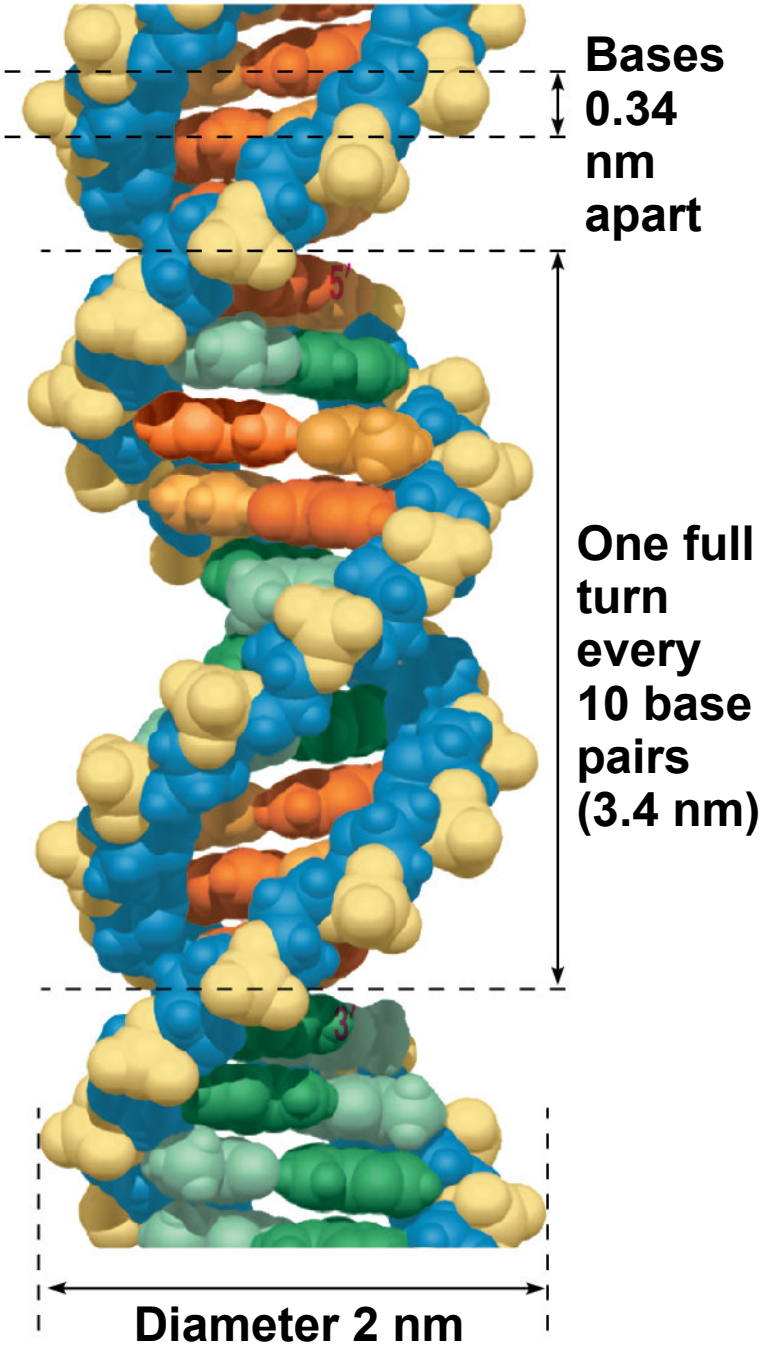
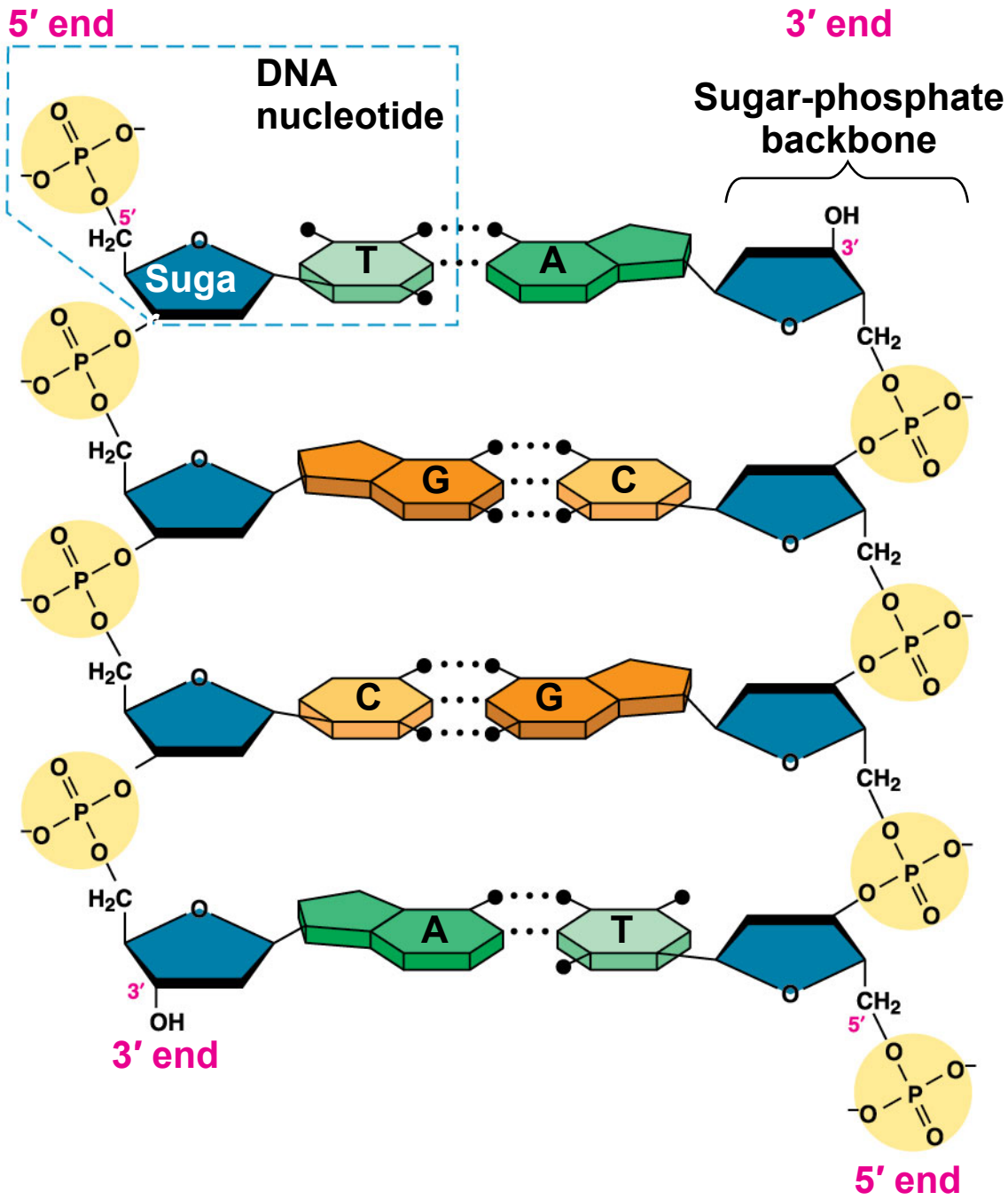


Figure 16.7ab

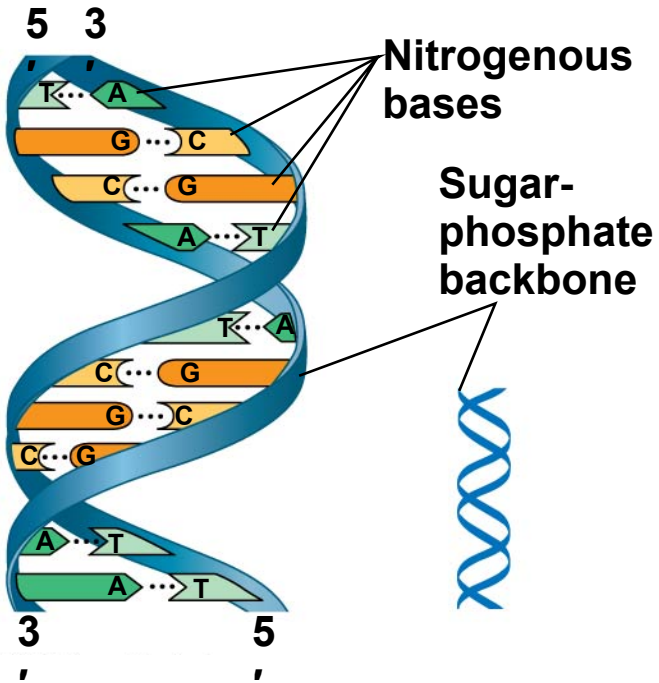


A ⋯ T
2 H-bonds

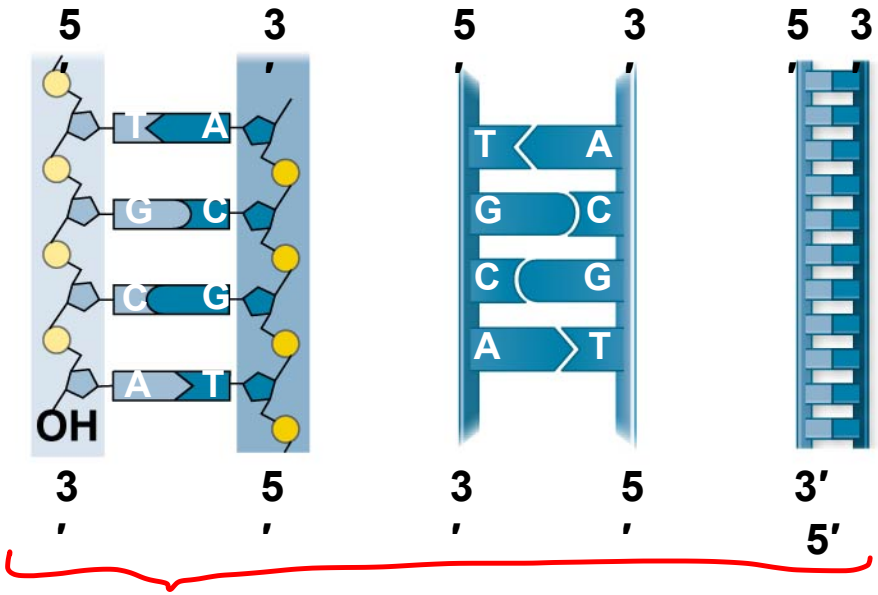
C ⋯ G
3 H-bonds

Figure 16.7b

Simplified Images



Ribbon models [3D structure]



Flattened ladder models

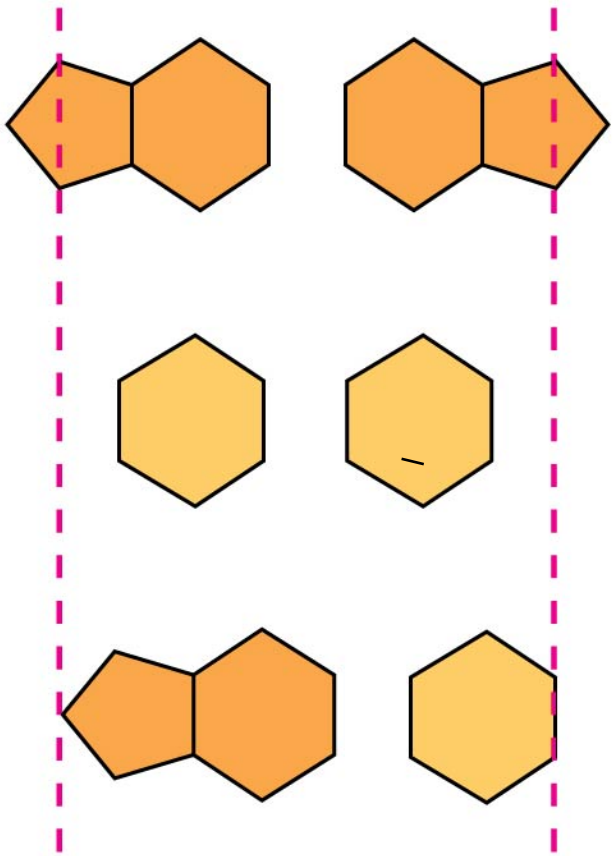
1. Backbones → Rails of the ladder
2. Base Pairs → Rungs of the ladder

Straight lines [simplicity].

- 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)

- 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.UN02



Purine + purine: too wide

Pyrimidine + pyrimidine: too narrow

Purine + pyrimidine: width consistent with X-ray data

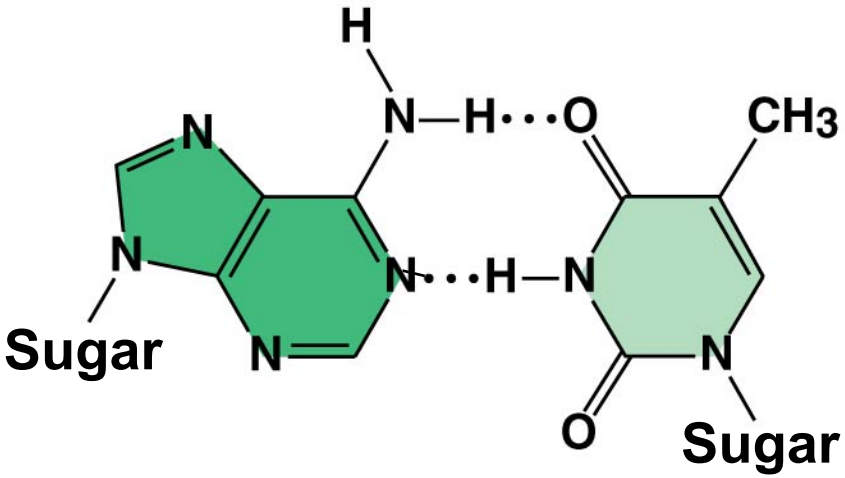
- Watson and Crick reasoned that the pairing was more specific, dictated by the base structures
- They determined that adenine (A) paired only with thymine (T), and guanine (G) paired only with cytosine (C)
- The Watson-Crick model explains Chargaff's rules: in any organism the amount of A = T, and the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

⊛ The base-pairing rule dictates the complementary pairing of N.Bases but not the N.Base sequence along the DNA molecules;

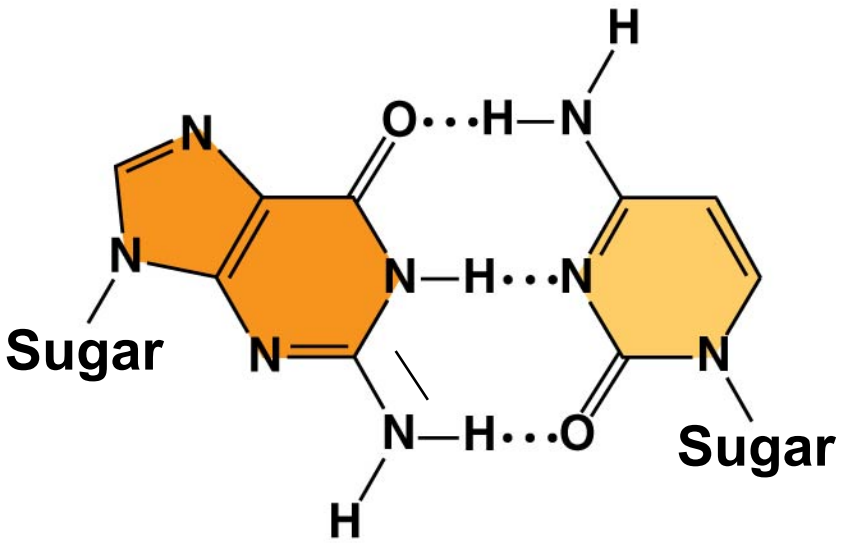
⇒ countless sequences can be obtained from these 4 bases.

Figure 16.8

⊛ The structure of DNA suggests its Replication Mechanism.
[see concept 16.2].



Adenine (A) Thymine (T)



Guanine (G) Cytosine (C)

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

- The relationship between structure and function is manifest in the double helix
- Watson and Crick noted that the specific base pairing suggested a possible copying mechanism for genetic material

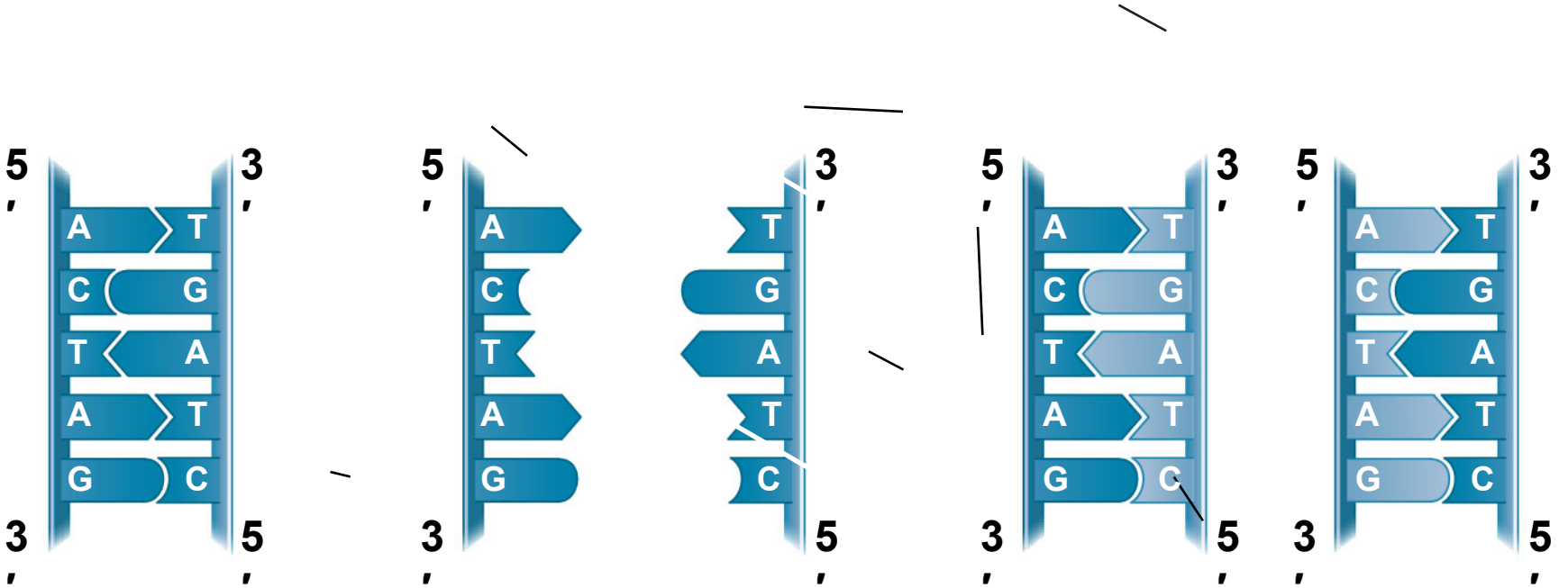
⊛ Hereditary information in DNA are responsible for development of Biochemical, Anatomical, physiological, and to some extent behavioral traits.

⊛ Nucleic Acids — unique from other "polymers" molecules — are able to dictate their own replication from monomers. nucleotides because of the special complementary bases' model.

he 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
- In DNA replication, the parent molecule unwinds, and two new daughter strands are built based on base-pairing rules

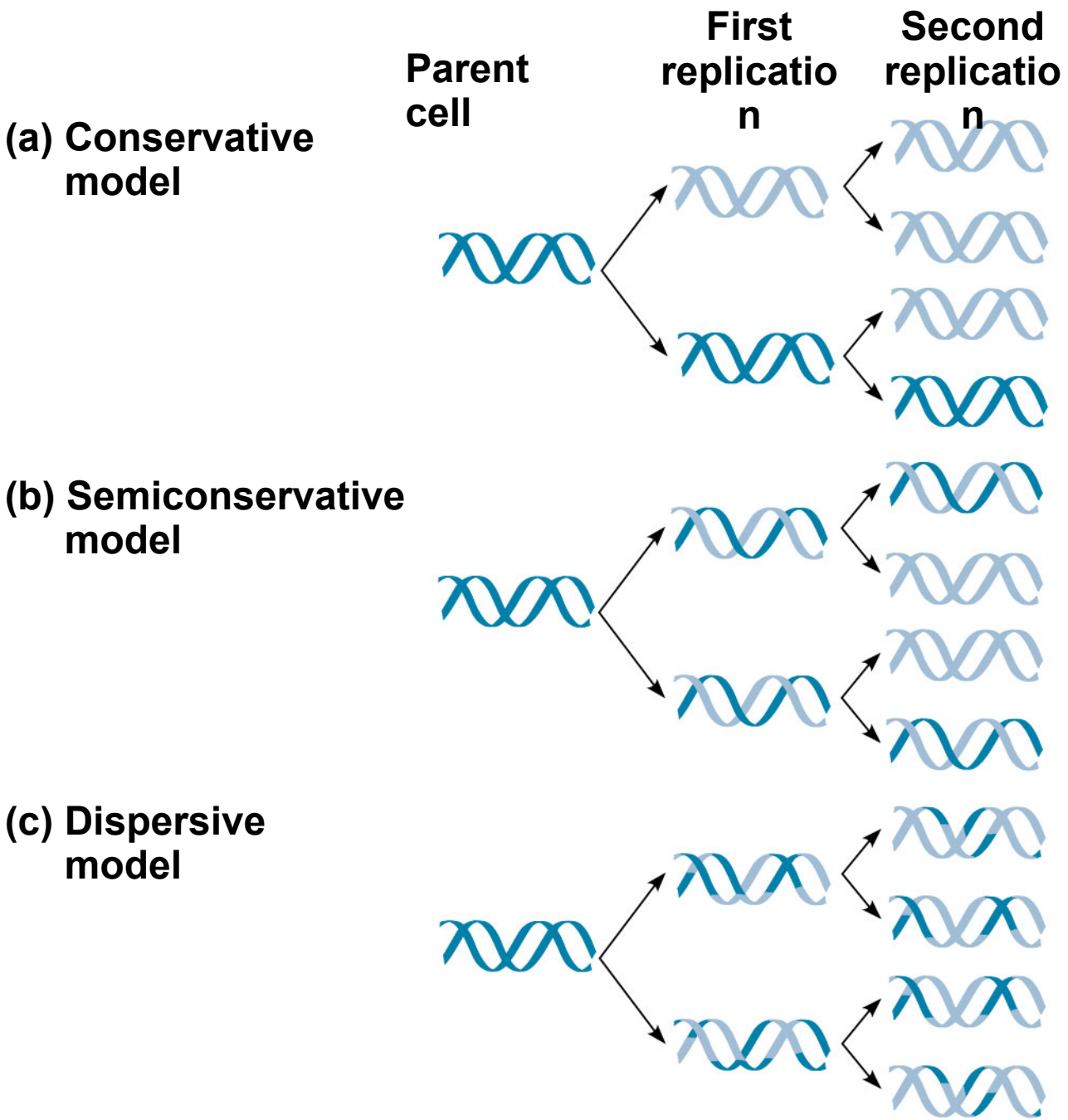
Figure 16.9_3



- 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)

⊛ The semiconservative model was later supported by further experiments by M. Meselson & F. Stahl distinguishing it from the other 2 models.

Figure 16.10



NA Replication: *A Closer Look*

- The copying of DNA is remarkable in its speed and accuracy e.g. E. coli ⇒ 4.6 Million pairs (one chromosome) fully replicated in < 1 hr.
- More than a dozen enzymes and other proteins participate in DNA replication

For human Somatic (diploid [2n]) cells :

- 46 DNA molecules (chromosomes)
- ≈ 6 billion pairs (distributed on the 46 chromosomes), > 1000 times of E. coli's.
- Replication techniques are fundamentally similar for both Prokaryotes & Eukaryotes (we have more info. about its replication) with some differences.

Getting Started

Specific sequence

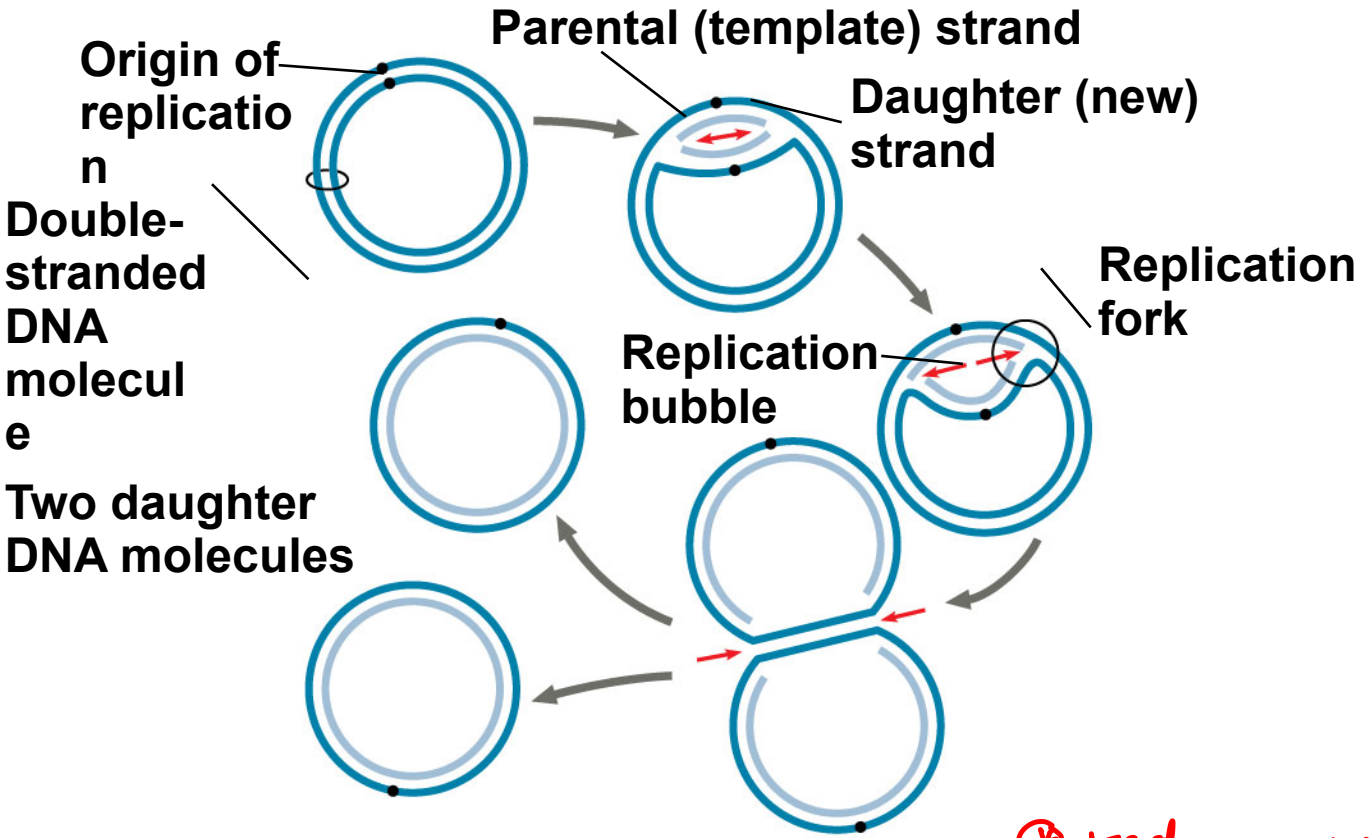
- Replication begins at particular sites called **origins of replication**, where the two DNA strands are separated, opening up a replication "bubble"
- A eukaryotic chromosome may have hundreds or even thousands of origins of replication → speeding up the process.
- Replication proceeds in both directions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

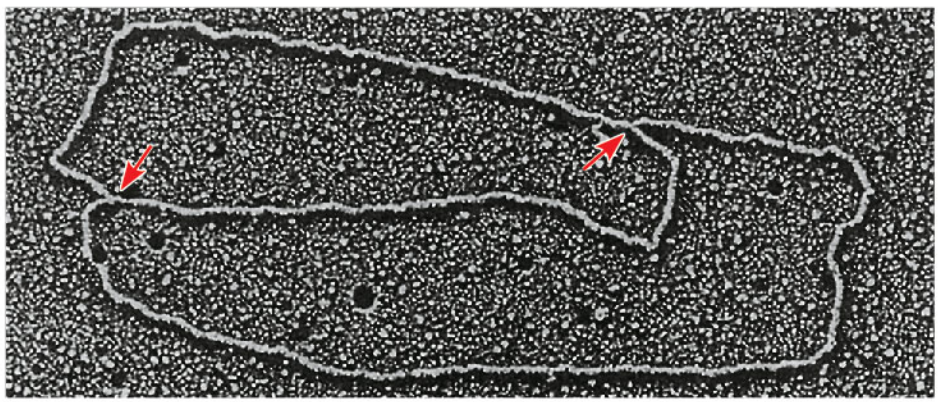
⊗ E. coli (like other bacteria) has a single circular DNA molecule with a single origin of replication

Figure 16.12a

(a) Origin of replication in an *E. coli* cell

[circular DNA]

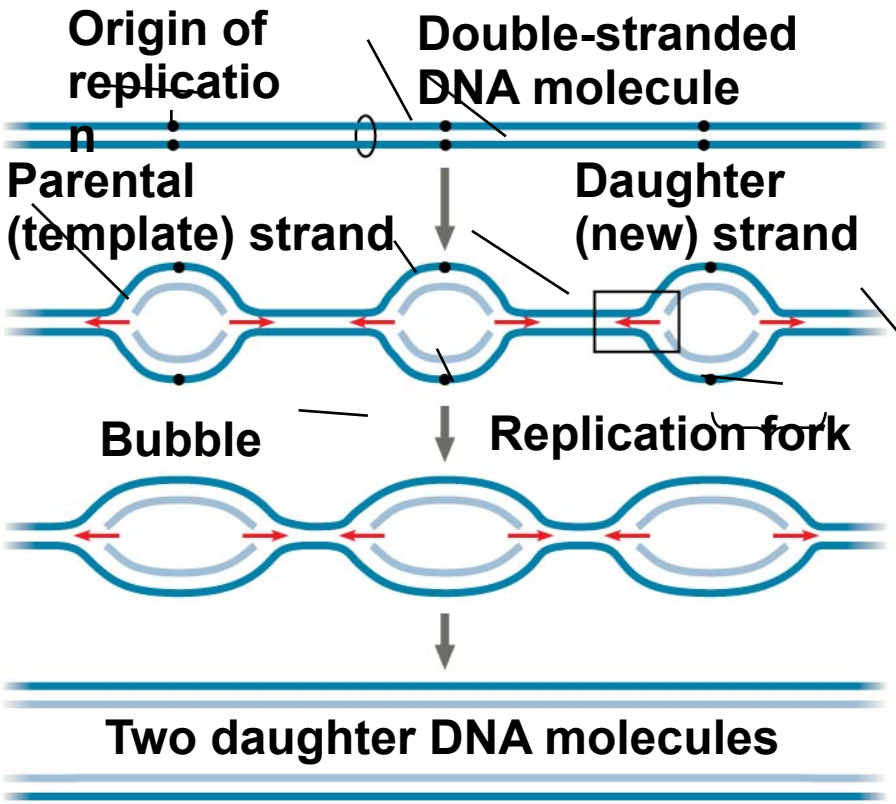




* red arrows indicate replication forks = 2 for a single bubble.

Figure 16.12b

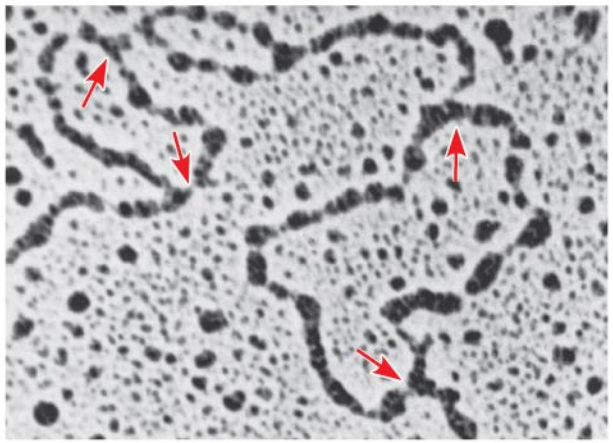
(b) Origins of replication in a eukaryotic cell [linear DNA]



In both directions

multiple bubbles fuse together

⊕ this process occurs in the S-phase of the Interphase of a eukaryote's cycle.



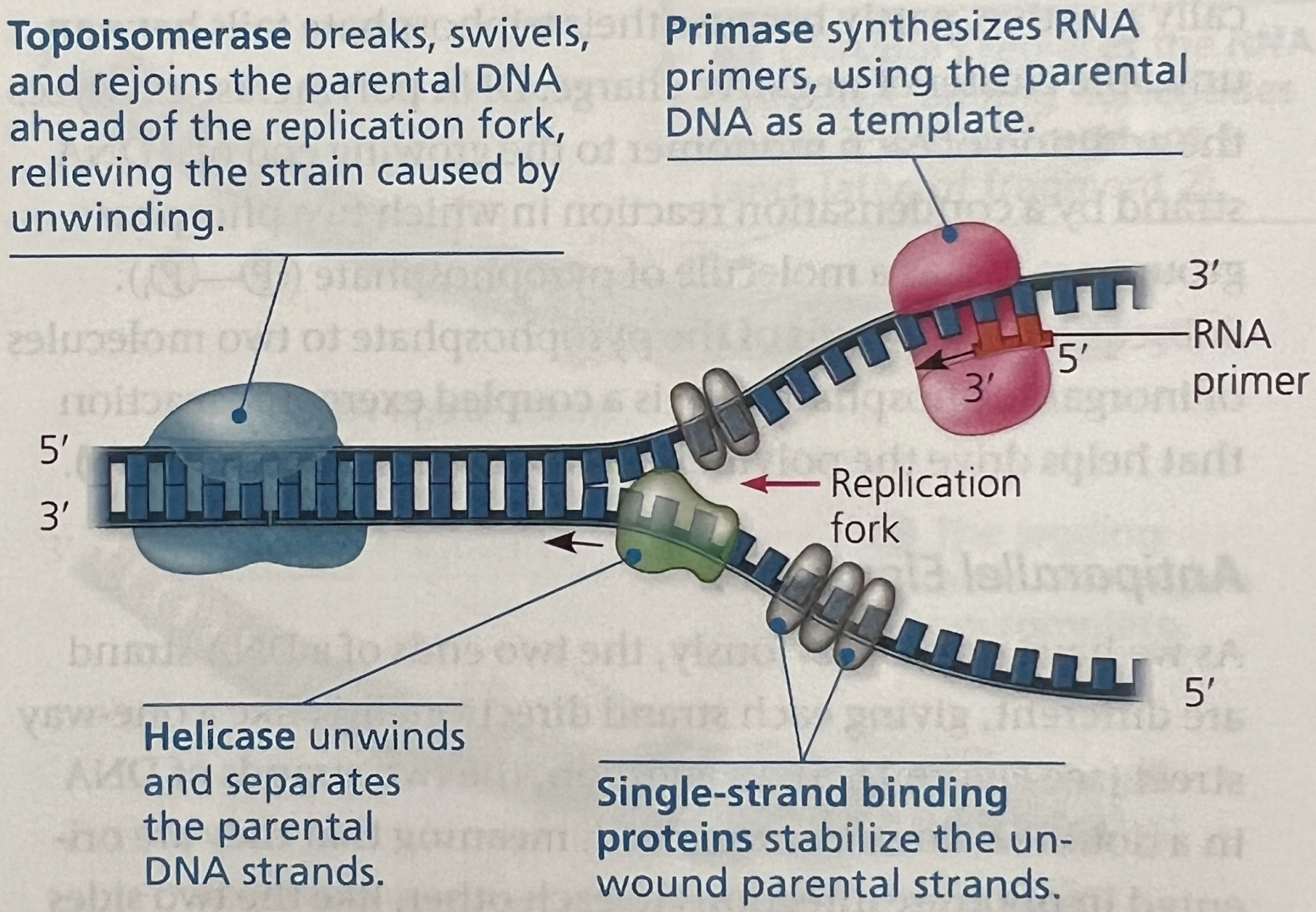
many Rep. forks

2 for every Bubble.

- At the end of each replication bubble is a **replication fork**, a Y-shaped region where new DNA strands are elongating
- **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

caused by helicases.

▼ Figure 16.14 Some of the proteins involved in the initiation of DNA replication. The same proteins function at both replication forks in a replication bubble. For simplicity, only the left-hand fork is shown, and the DNA bases are drawn much larger in relation to the proteins than they are in reality.



ynthesizing a New DNA Strand

- DNA polymerases require a primer to which they can add nucleotides
- The initial nucleotide strand is a short RNA **primer**
- This is synthesized by the enzyme **primase**

[not DNA sequence]

DNA enzymes are incapable of starting the process ⇒ they can add nucleotides to already existing chains.

- Primase can start an RNA chain from scratch and adds RNA nucleotides one at a time using the parental DNA as a template
- The primer is short (5–10 nucleotides long), and the 3′ end serves as the starting point for the new DNA strand

- 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

[faster elongation in Bacteria].

DNA Polymerases in replication:

- Prokaryotes (E. coli) mainly:
  1. DNA Polymerase III [which adds Nucleotides to an RNA primer]
  2. DNA Polymerase I
- Eukaryotes

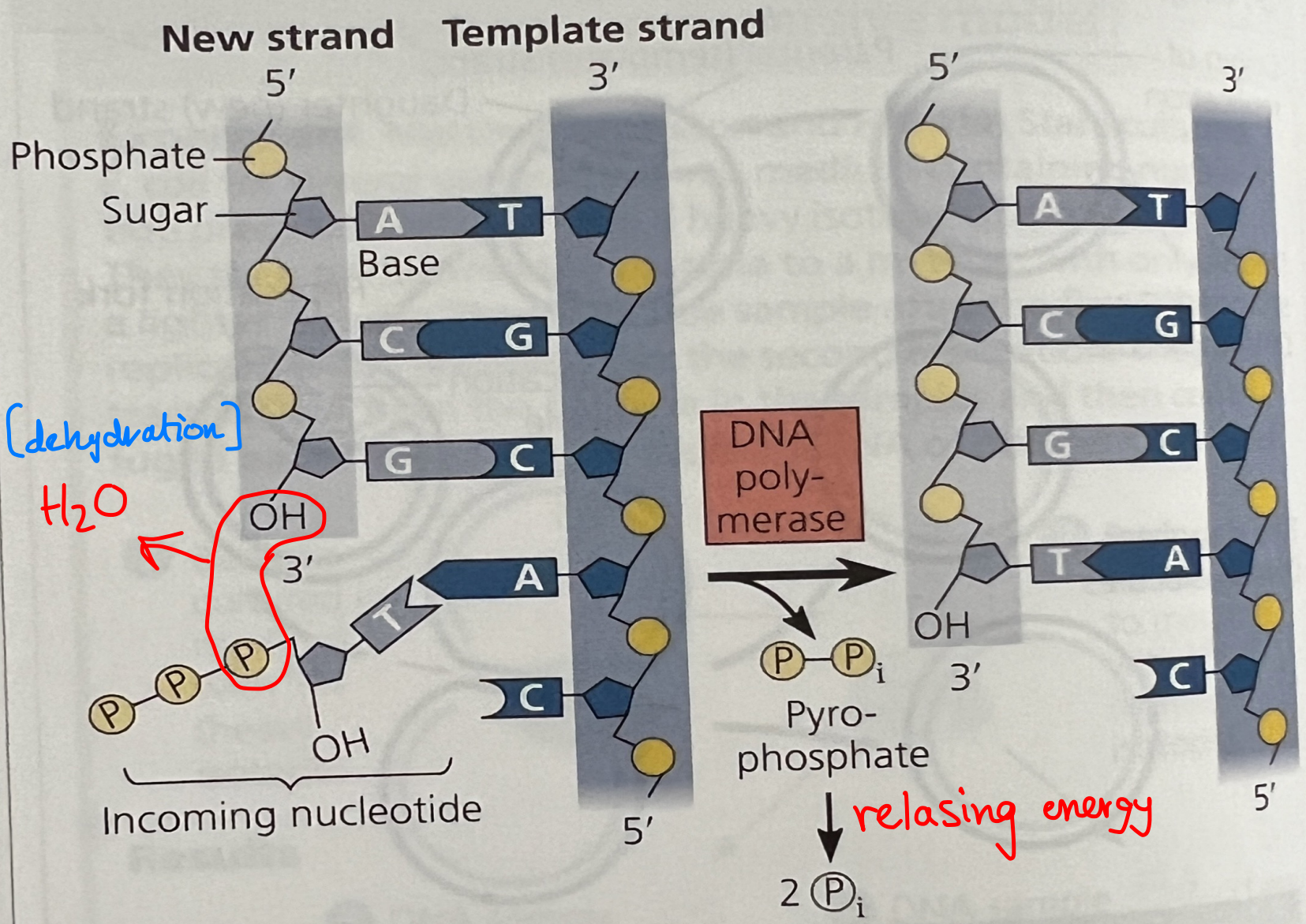
at least 11 DNA Polymerases discovered so far.

- 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

Catalyzed by DNA polymerase

hydrolysis of Pyrophosphate releases Energy to drive the Polymerization of nucleotides into the DNA.

▼ Figure 16.15 Addition of a nucleotide to a DNA strand.
DNA polymerase catalyzes addition of a nucleotide to the 3′ end of a growing DNA strand, with the release of two phosphates.



ntiparallel 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′ to 3′ direction

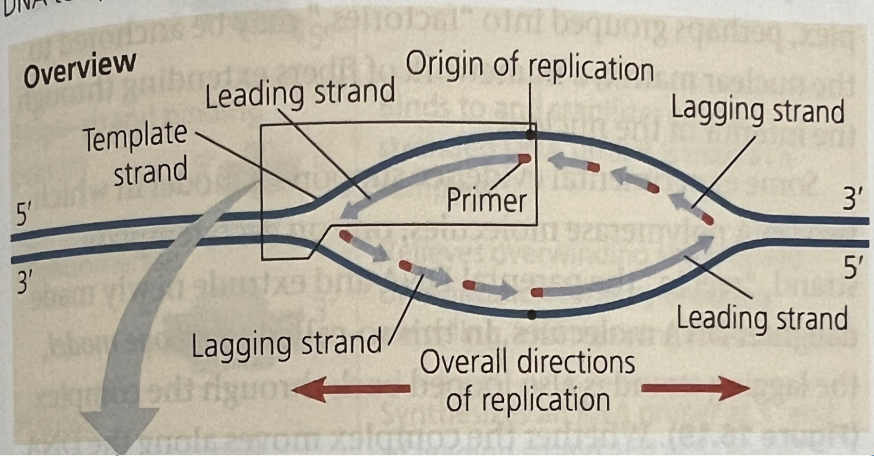
due to their structure

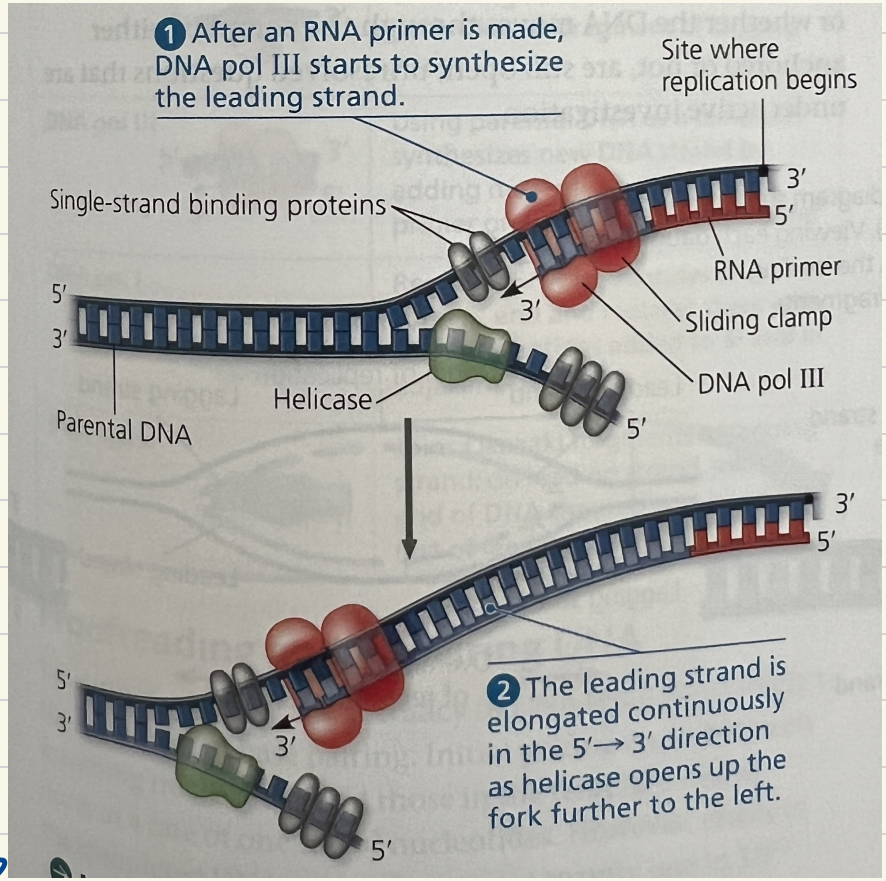
Strand	Template direction	New strand direction	Continuous?	RNA Primers
Leading	3′ → 5′	5′ → 3′	✓	1
Lagging	5′ → 3′	3′ → 5′	X	more than 1

The Leading Strand

[template is 3' → 5' ⟺ Continuous Synthesis]

Figure 16.16 Synthesis of the leading strand during DNA replication. This diagram focuses on the left replication fork shown in the overview box. DNA polymerase III (DNA pol III), shaped like a cupped hand, is shown closely associated with a protein called the "sliding clamp" that encircles the newly synthesized double helix like a doughnut. The sliding clamp moves DNA pol III along the DNA template strand.

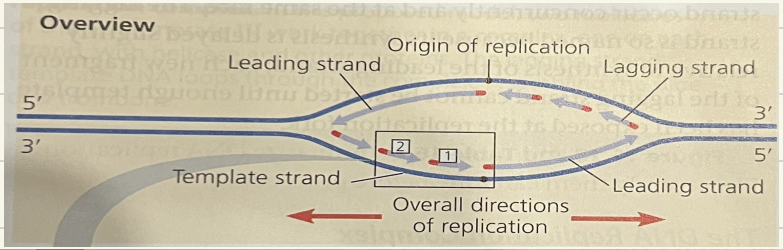


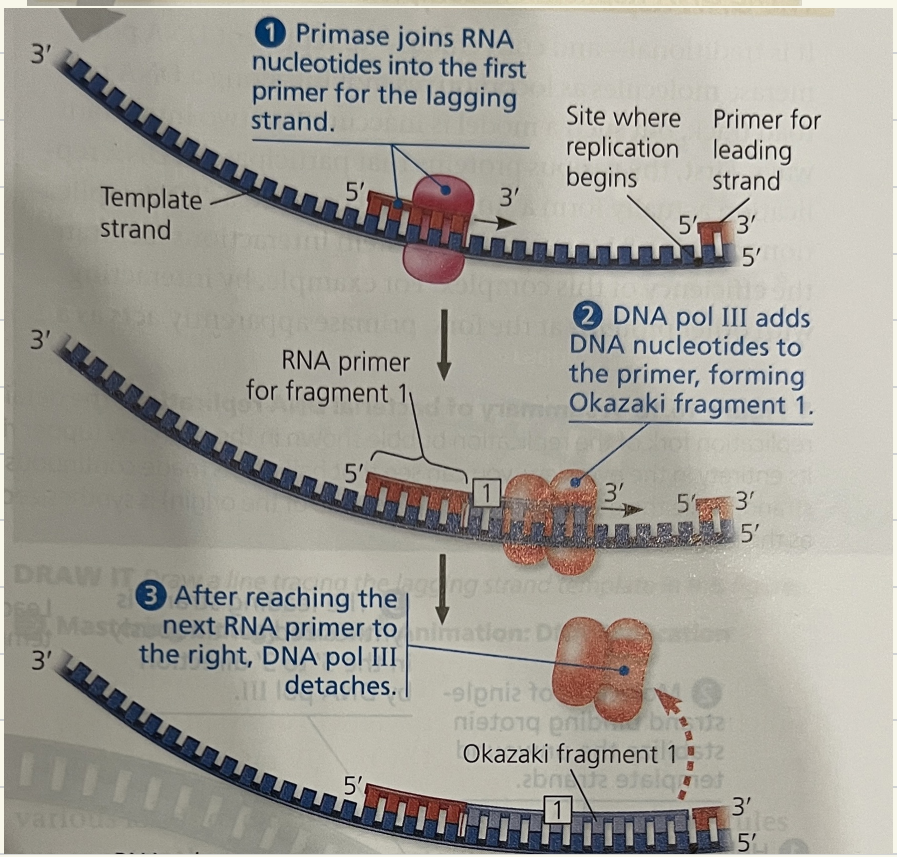


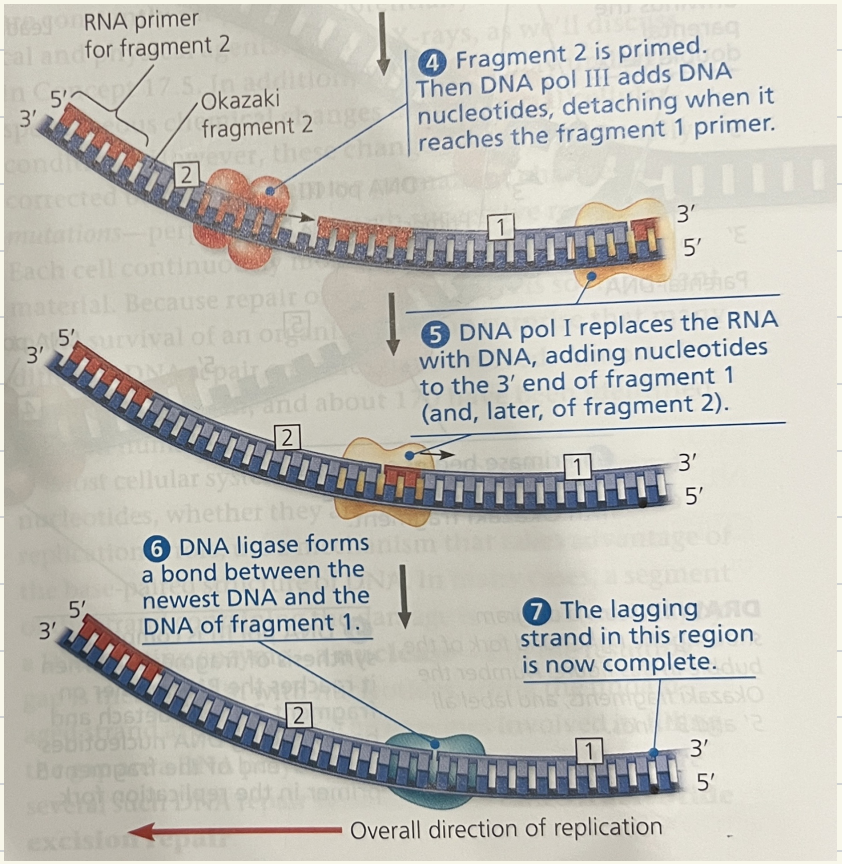
- To elongate the other new strand, called the **lagging strand**, DNA polymerase must work in the direction away from the replication fork [Against Helicase].
- The lagging strand is synthesized as a series of segments called **Okazaki fragments**, which are joined together by **DNA ligase**

The Lagging Strand

[template is 5′→3′ ⇔ discontinuous synthesis (Okazaki fragments)]







Notes on 16.2:

- Both strands are synthesized simultaneously and at the same rate.
- The lagging strand "Lags" behind the leading because it needs new template to be exposed for Primase to place a new primer.
- Proteins (responsible for replication) form a single large "Replication Complex".
- Protein—Protein interactions facilitate the replication efficiency of the Complex.
- Primase acts as a molecular brake — slowing down and coordinating the process.
- The moving part is not always the replication complex; Sometimes the Complex is anchored in the nuclear matrix and DNA passes through it to be synthesized

[primer placement and rate of synthesis on Both Strands].

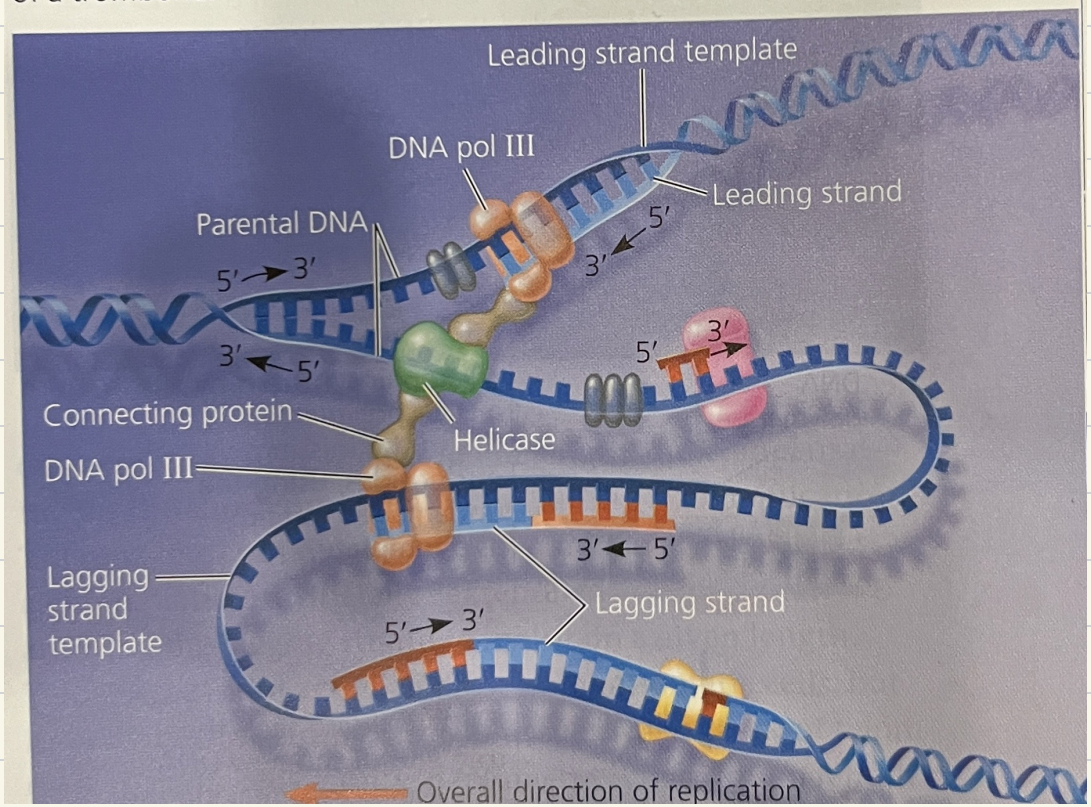
[multiple copies of the Complex can function together forming "factories"].

he 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

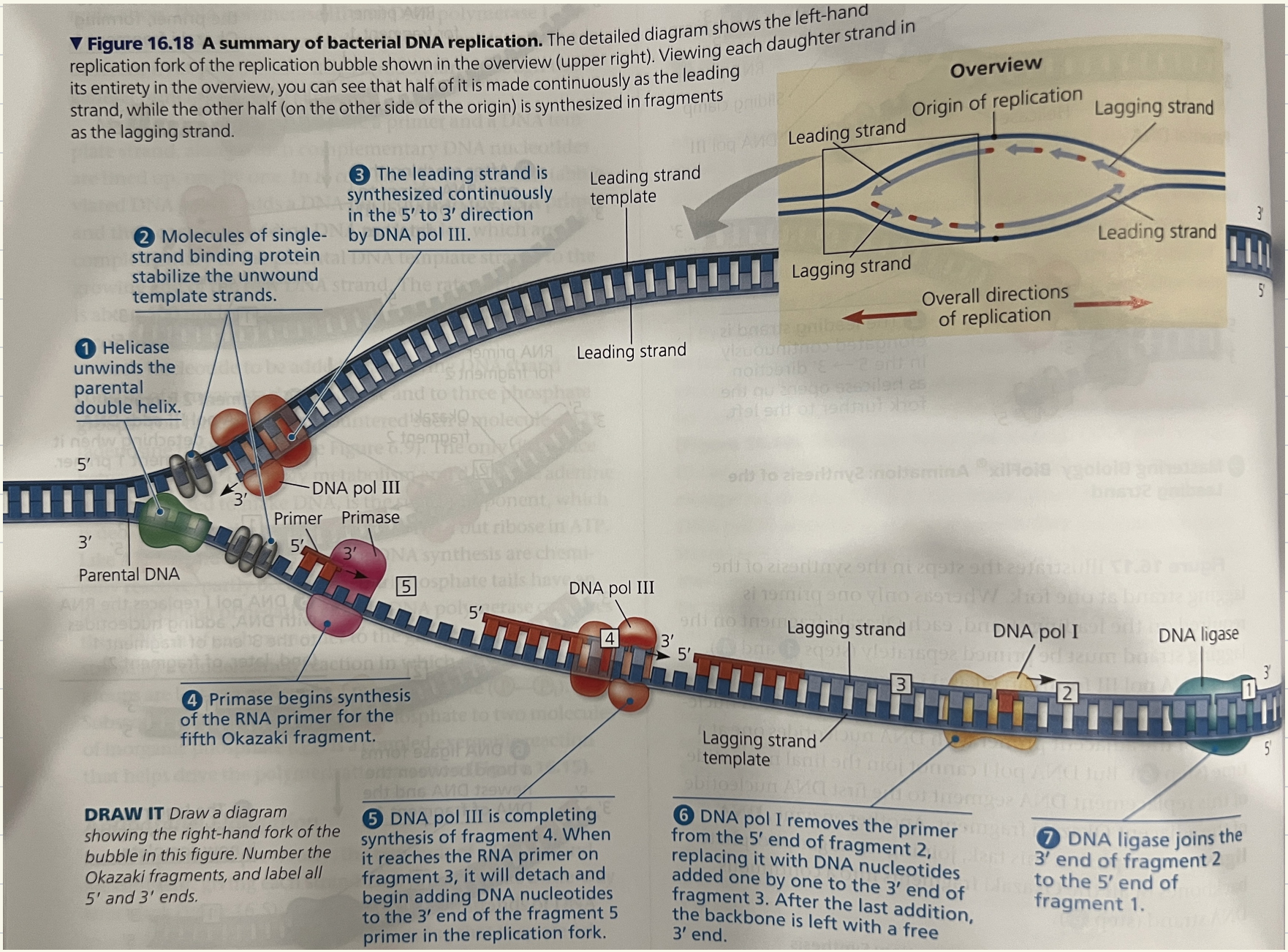
The Trombone Model

▼ Figure 16.19 The "trombone" model of the DNA replication complex. In this proposed model, two molecules of DNA polymerase III work together in a complex, one on each strand, with helicase and other proteins. The lagging strand template DNA loops through the complex, resembling the slide of a trombone.



Whether the DNA "slides" through protein complexes or proteins slide along DNA is still unsolved.

Overall Picture



▼ **Figure 16.18 A summary of bacterial DNA replication.** The detailed diagram shows the left-hand replication fork of the replication bubble shown in the overview (upper right). Viewing each daughter strand in its entirety in the overview, you can see that half of it is made continuously as the leading strand, while the other half (on the other side of the origin) is synthesized in fragments as the lagging strand.

Table 16.1a

Table 16.1 Bacterial DNA Replication Proteins and Their Functions

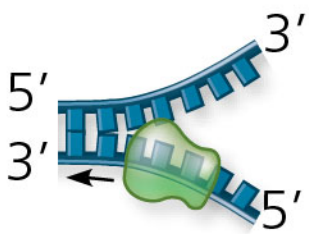
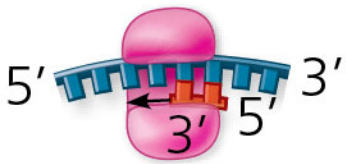
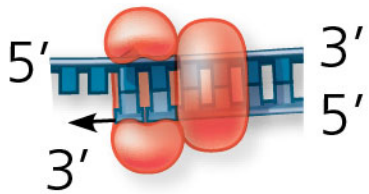
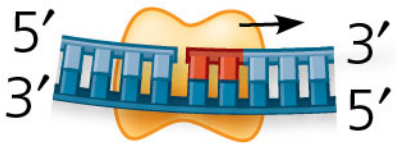
Protein	Function
Helicase 	Unwinds parental double helix at replication forks
Single-strand binding protein 	Binds to and stabilizes single-stranded DNA until it is used as a template
Topoisomerase 	Relieves overwinding strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase 	Synthesizes an RNA primer at 5′ end of leading strand and at 5′ end of each Okazaki fragment of lagging strand

Table 16.1 Bacterial DNA Replication Proteins and Their Functions

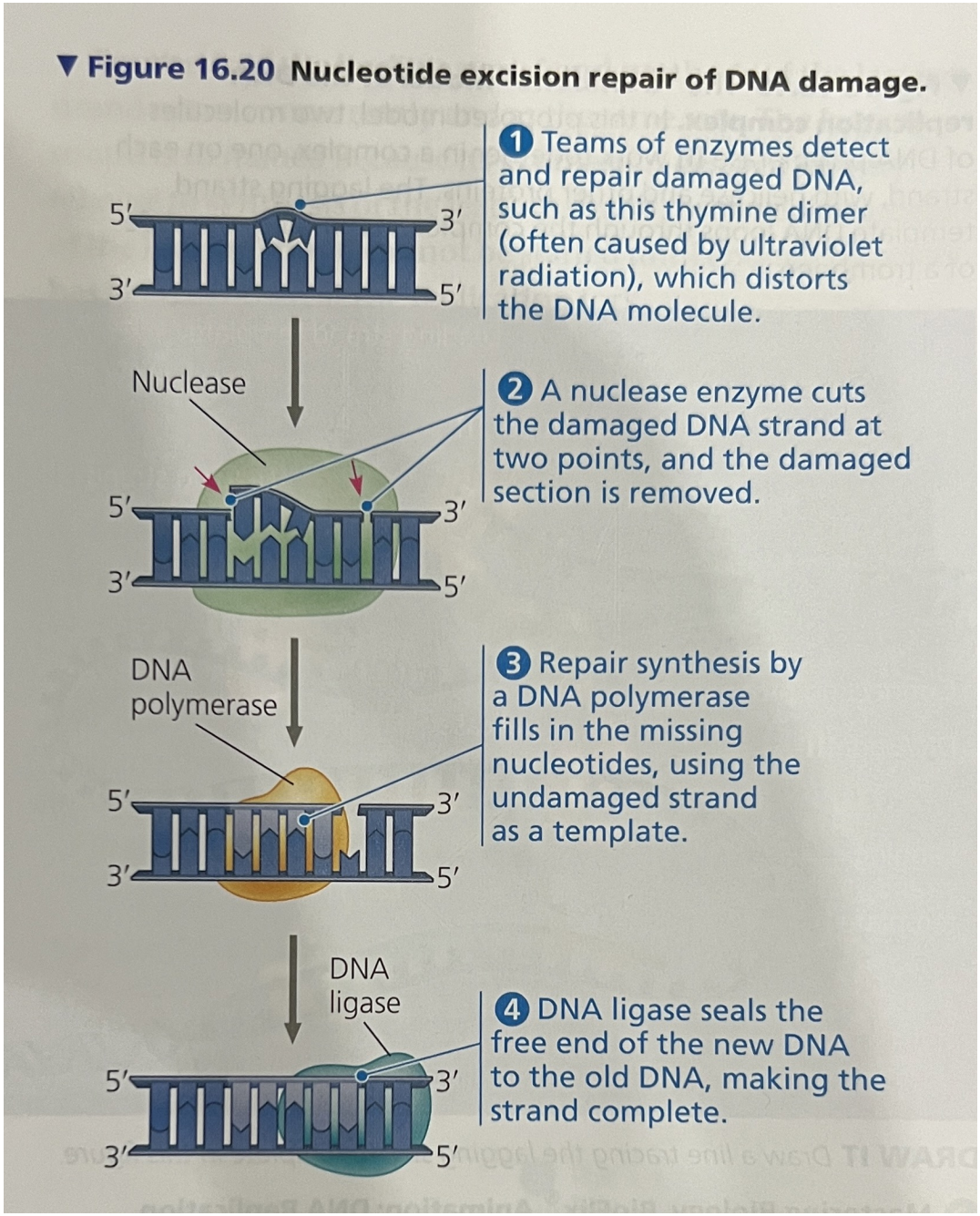
Protein	Function
DNA pol III 	Using parental DNA as a template, synthesizes new DNA strand by adding nucleotides to an RNA primer or a pre-existing DNA strand
DNA pol I 	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides added to 3′ end of adjacent fragment
DNA ligase 	Joins Okazaki fragments of lagging strand; on leading strand, joins 3′ end of DNA that replaces primer to rest of leading strand DNA

roofreading and Repairing DNA

lowering error from 1 in 10^5 to 1 in 10^{10}

- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- In **mismatch repair** of DNA, repair enzymes (other than Polymerases) correct errors in base pairing [if not corrected by proofreading]
- 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 16.19_1



→ often due to UV radiation [skin cells] especially thymine dimers [covalently bonded thymines] can be caused by UV radiation causing buckling and interference with replication.

Notes on Repairing DNA:

- Defects in Mismatch repair enzymes can cause certain errors to accumulate in DNA faster than normal e.g: Colon Cancer.
- Chemical changes to a DNA molecule are usually corrected before they become permanent changes.
- Mutations are permanent changes in DNA that are passes through successive replications.
- Almost 100 known repair enzymes exist in E-coli & 170 〃 〃 〃 〃 〃 Humans.
- Nucleotide Excision Repair takes advantage of complementary base-pairing
- Xeroderma Pigmentosum (XP) is caused by an inherited defect in Nucleotide excision repair enzyme which leaves skin mutations uncorrected; Children with XP are subject to developing Skin Cancer by age 10!