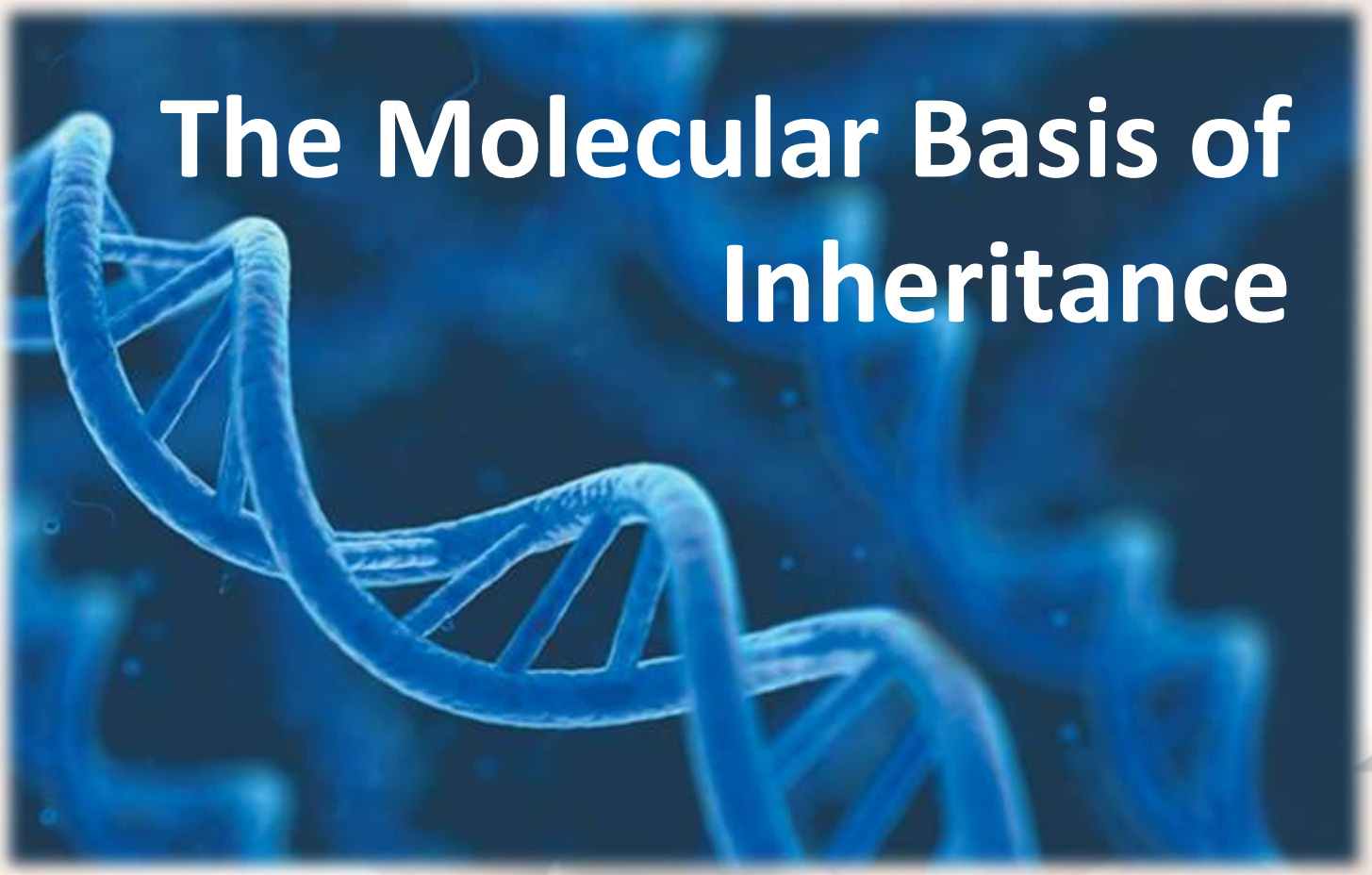
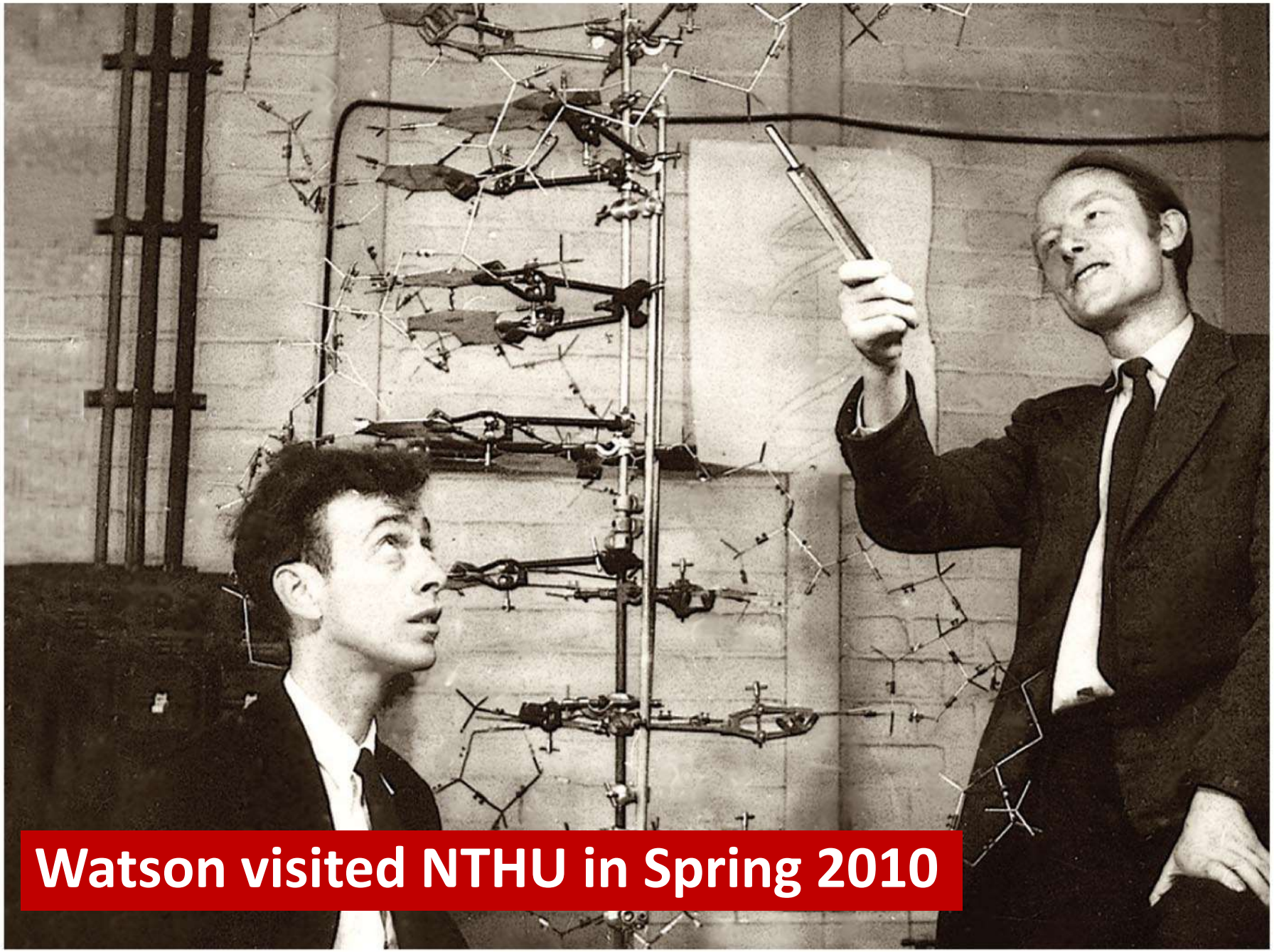
Chapter 16



Modified by YJ Chuang @ NTHU-DMS

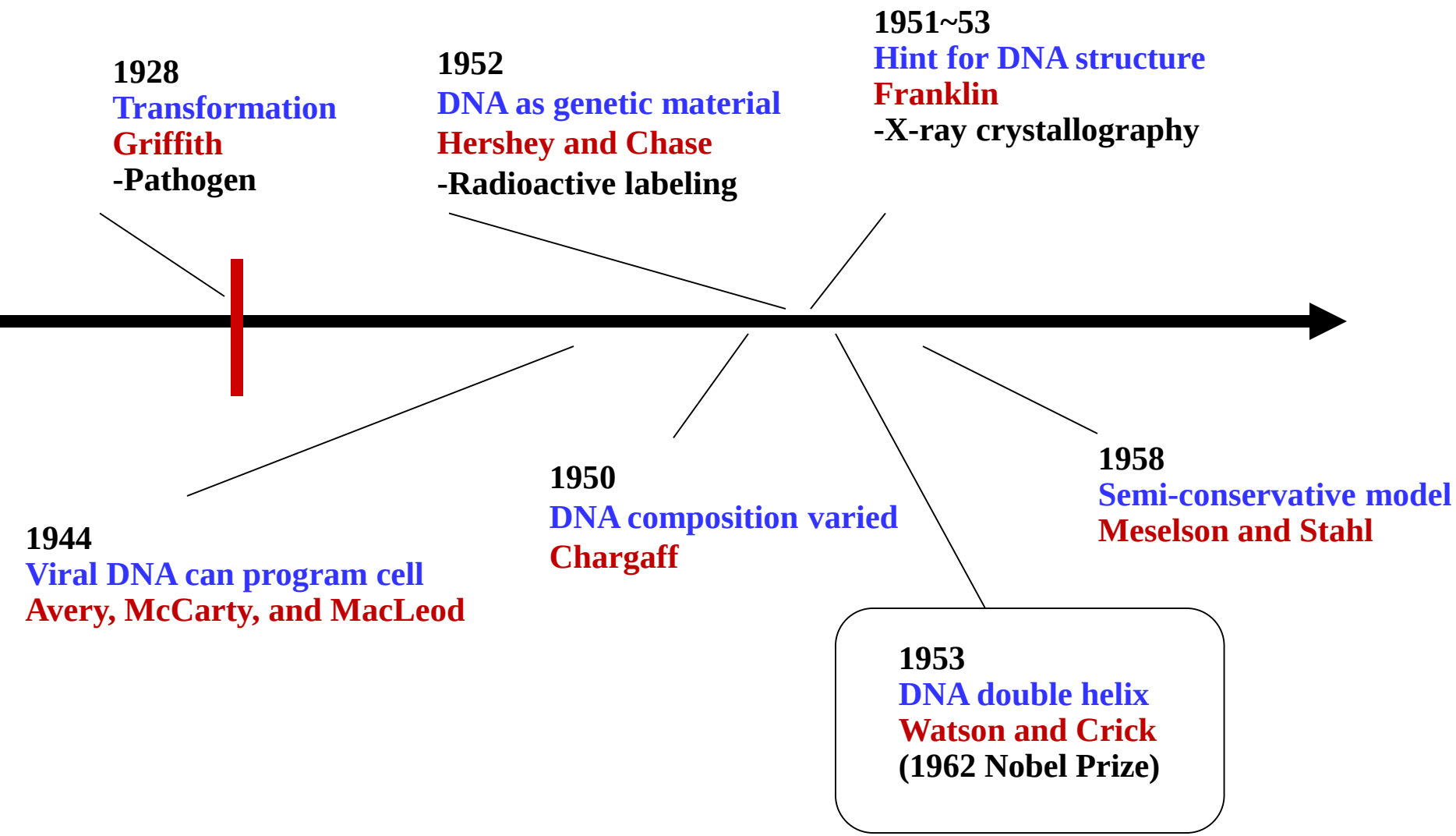
Overview: Life's Operation Instruction

- 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 nearly all cells of the body
- This DNA program directs the development of biochemical, anatomical, physiological, and (to some extent) behavioral traits



Timeline

關於DNA知識的累積與其連貫性



Concept 16.1: DNA is the genetic material

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

  (Phenotypes → Chromosome → Molecules)

- **Let us revisit the question :**

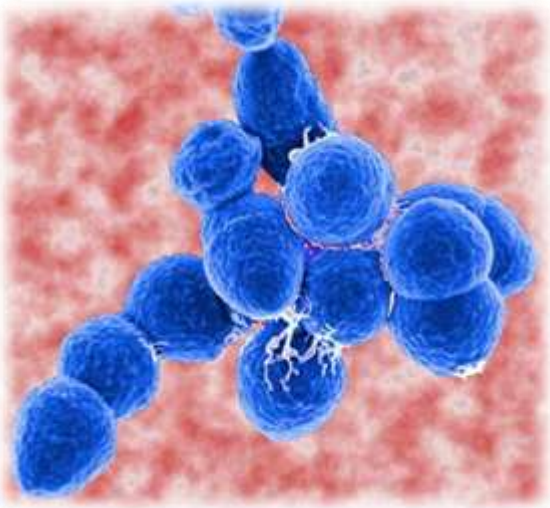
  What is the molecules of inheritance?

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 **key factor** in determining the genetic material was choosing appropriate experimental organisms (模式生物/研究系統)
- The role of DNA in heredity was first discovered by studying bacteria and the viruses that infect them

Evidence That DNA Can Transform Bacteria

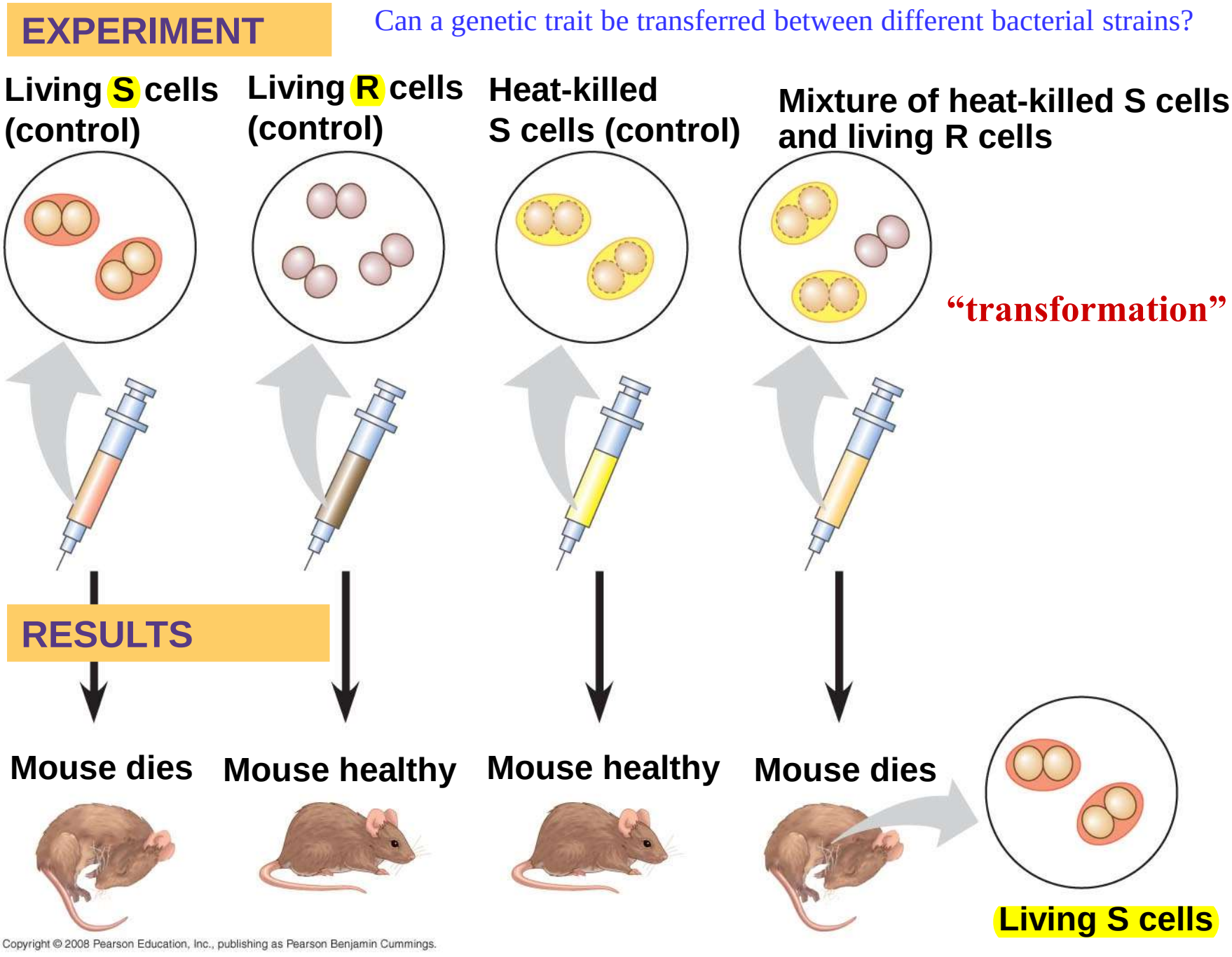
- The discovery of the genetic role of DNA began with research by Frederick Griffith in 1928
- Griffith worked with two strains of a bacterium (*Streptococcus pneumoniae* 肺炎鏈球菌), one pathogenic and one harmless



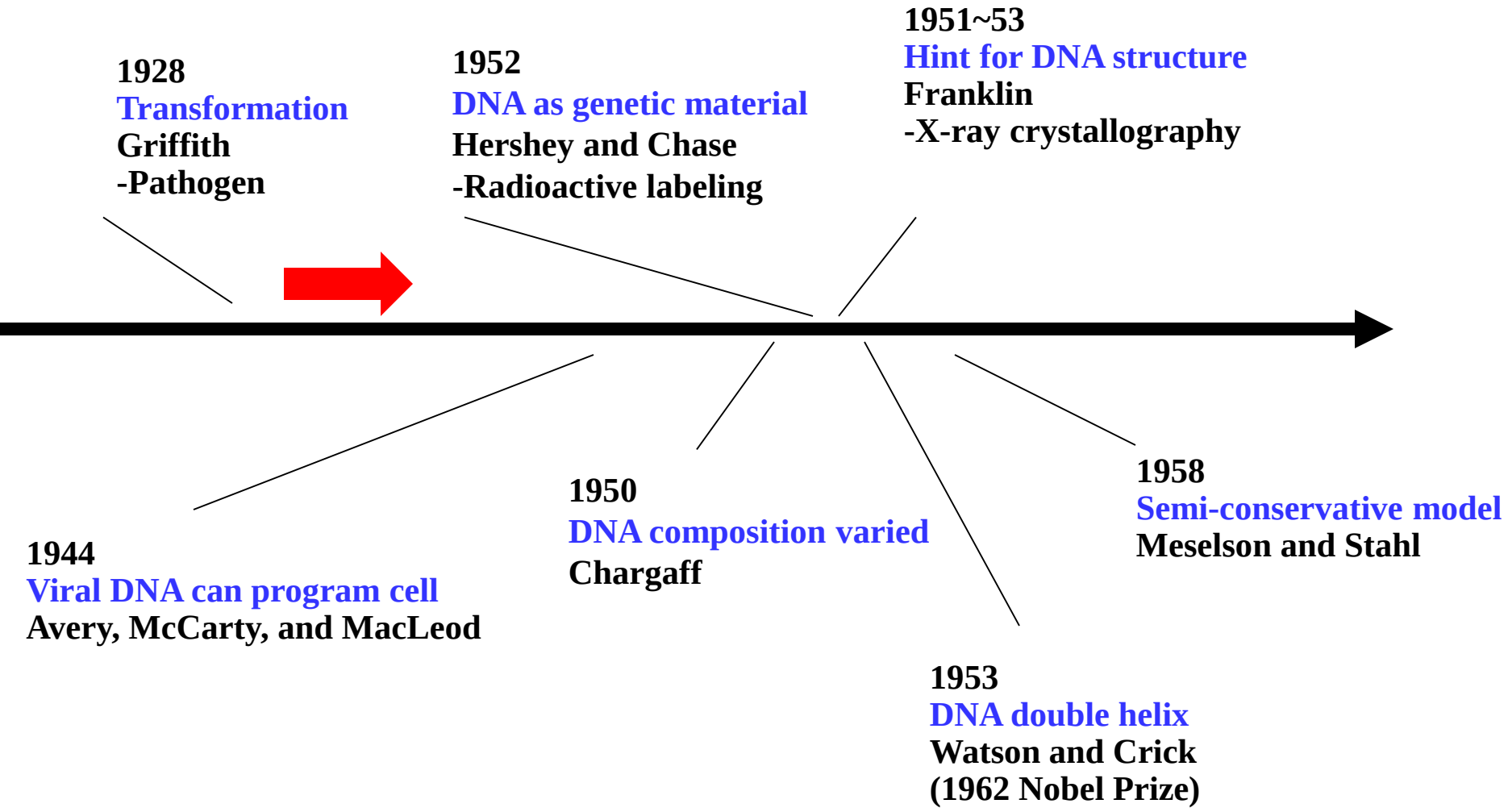
Transformation

- When he mixed **heat-killed** remains of the pathogenic strain with living cells of the harmless strain, some living cells became pathogenic (致病力的獲得; How?)
- He called this phenomenon "**transformation**", now defined as a change in genotype and phenotype due to **assimilation of foreign DNA**

Fig. 16-2



Timeline


1928
Transformation
Griffith
-Pathogen
1952
DNA as genetic material
Hershey and Chase
-Radioactive labeling
1951~53
Hint for DNA structure
Franklin
-X-ray crystallography
1944
Viral DNA can program cell
Avery, McCarty, and MacLeod
1950
DNA composition varied
Chargaff
1953
DNA double helix
Watson and Crick
(1962 Nobel Prize)
1958
Semi-conservative model
Meselson and Stahl

DNA as the transforming substance

- In 1944, Oswald Avery, Maclyn McCarty, and Colin MacLeod announced that the transforming substance was **DNA**
  - Their conclusion was based on experimental evidence that only DNA worked in transforming harmless bacteria into pathogenic bacteria

What is the evidences to support this?

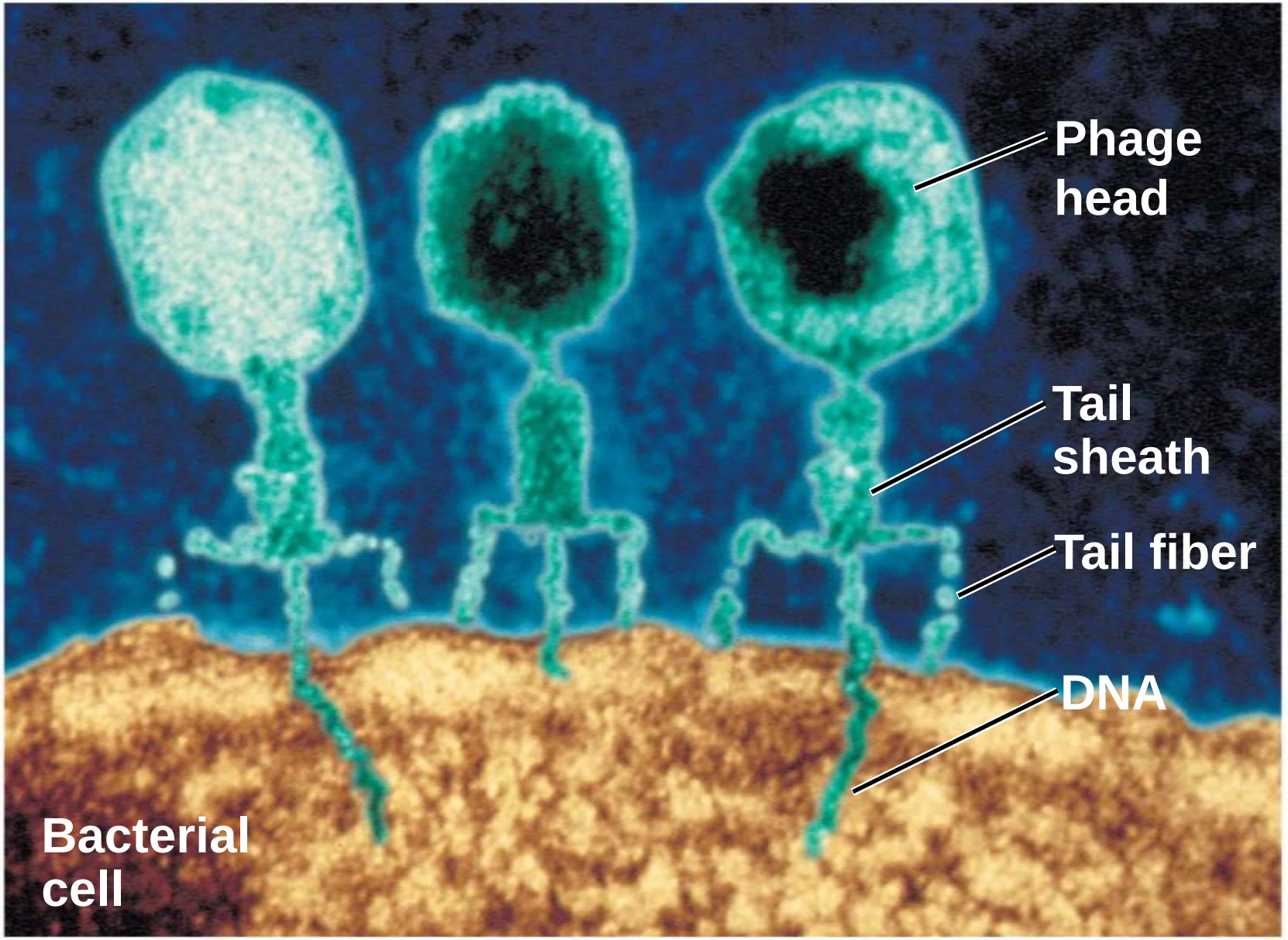
可被重複驗證的實驗證據!

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, called **bacteriophages** (or **phages**; 噬菌體), are widely used in molecular genetics research

Image next page: Bacteriophages (Phages)

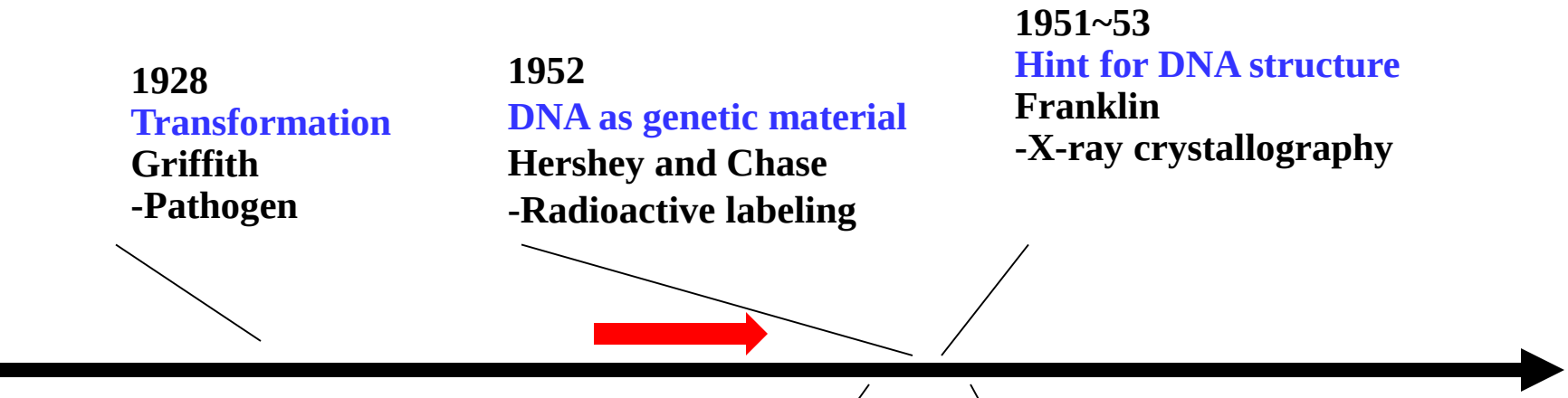
Fig. 16-3



PLAY

Animation: Phage T2 Reproductive Cycle

Timeline



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Hershey-Chase Experiment : Is protein or DNA the genetic material of phage T2?

- In 1952, Alfred Hershey and Martha Chase performed experiments showing that DNA is the genetic material of a phage known as T2
  - To determine the source of genetic material in the phage, 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

Fig. 16-4-1

Is protein or DNA the genetic material of phage T2?

EXPERIMENT

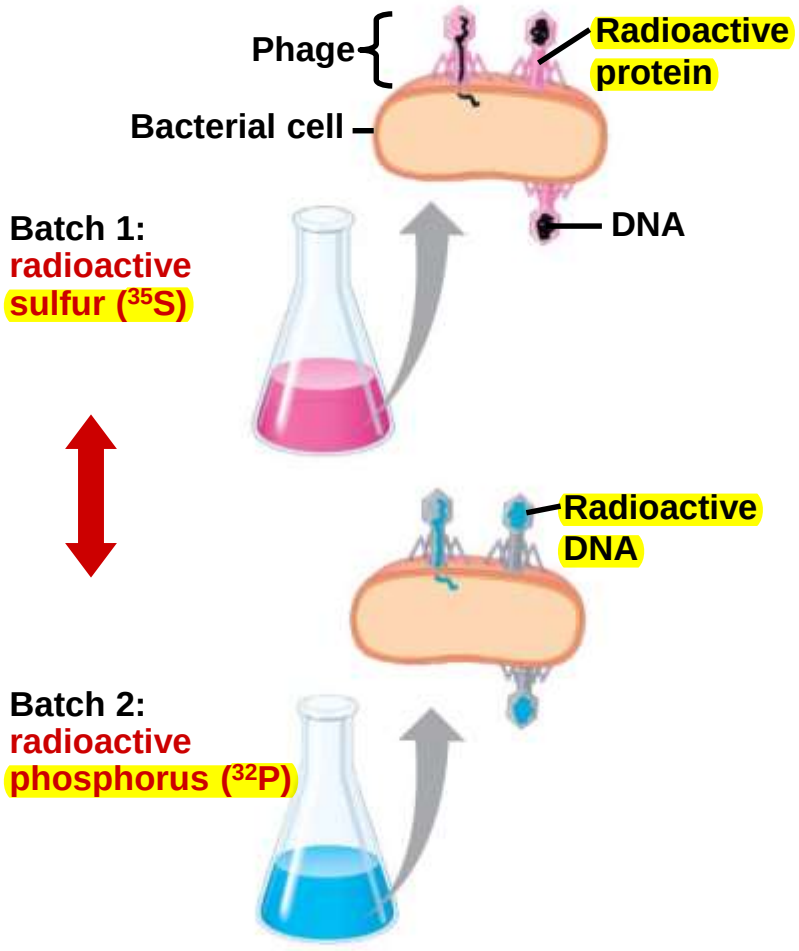


Fig. 16-4-2

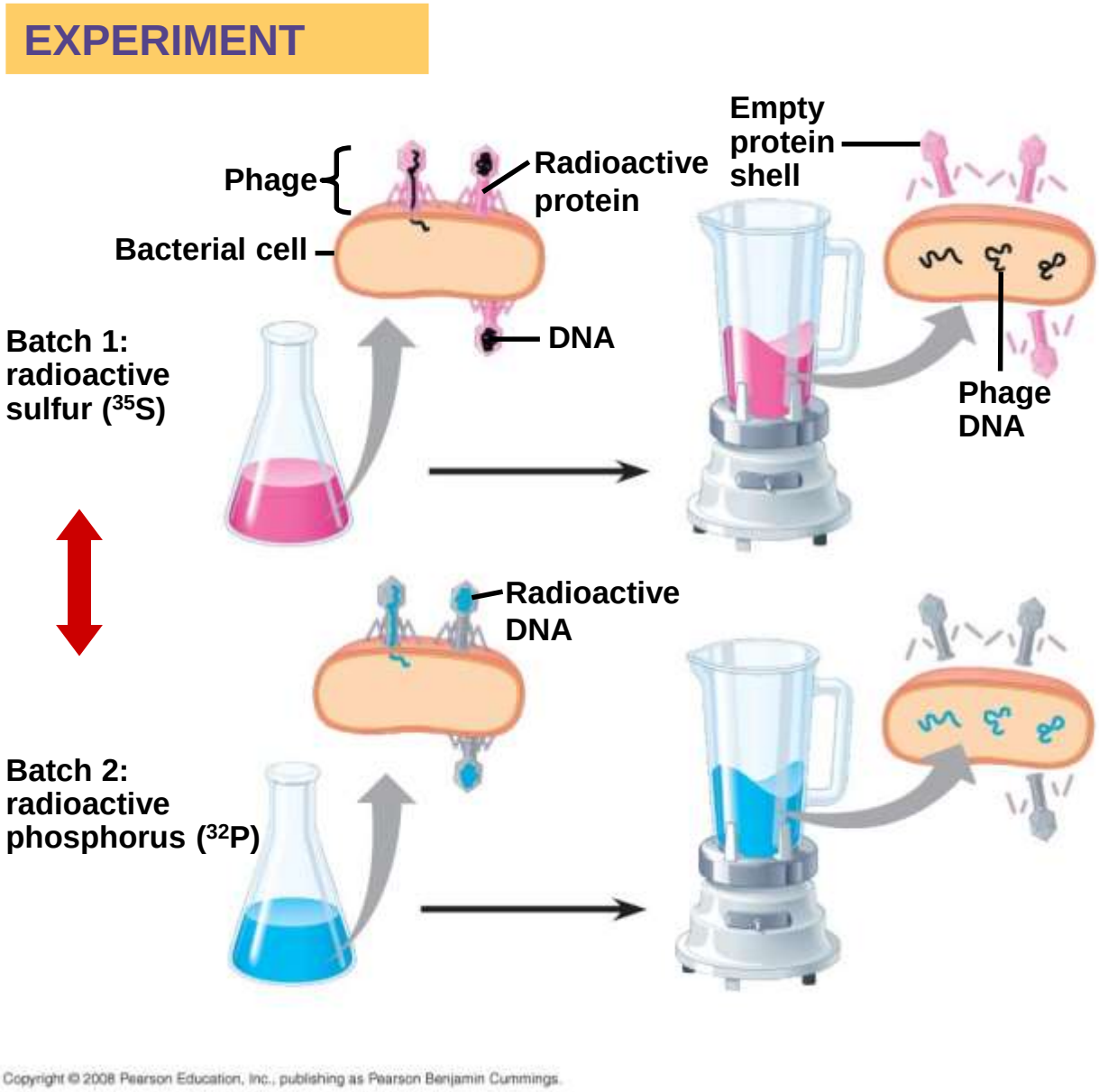
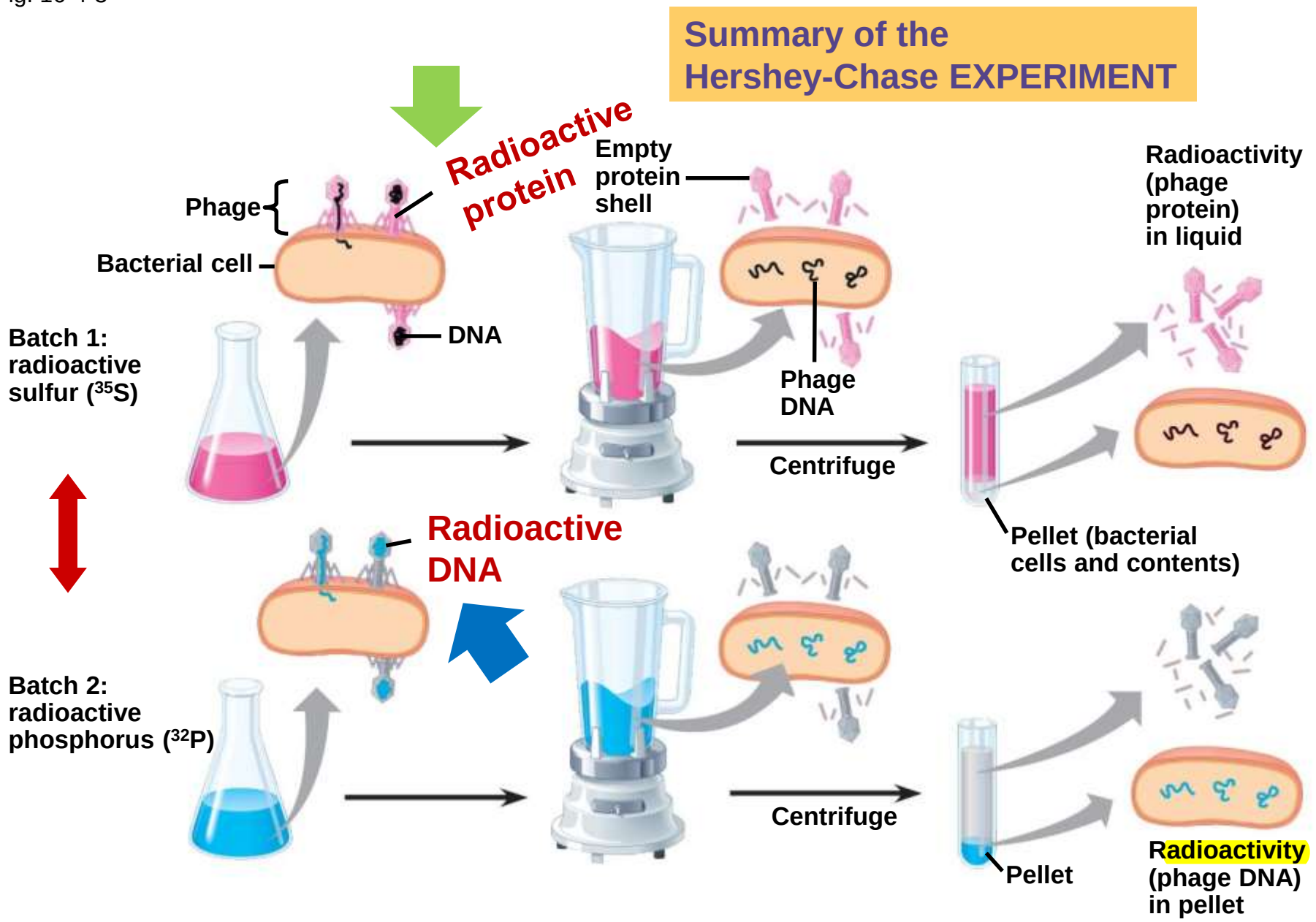


Fig. 16-4-3



Answer: The genetic material of Phage T2 is the DNA.

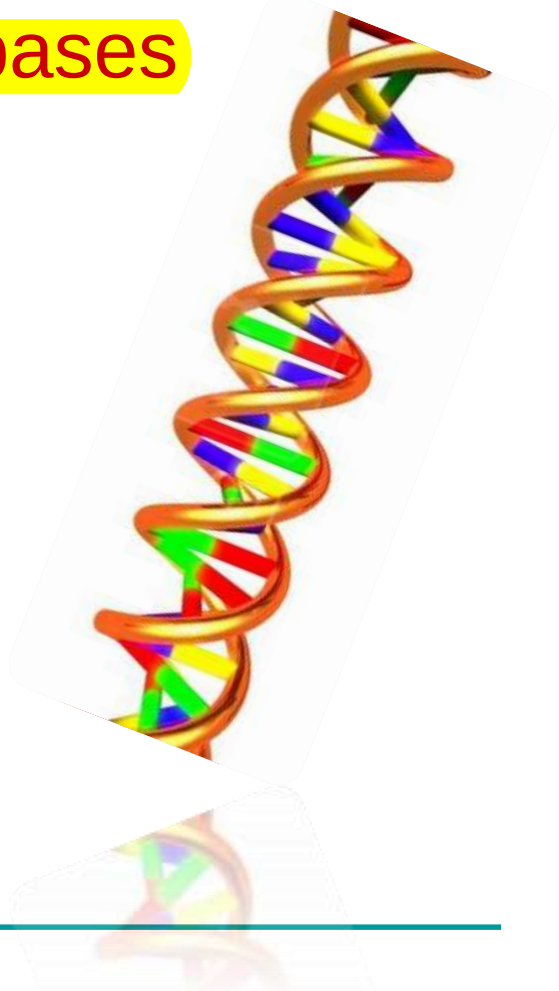
Additional evidence to support DNA Is the Genetic Material

- It was known that DNA is a polymer of nucleotides, each consisting of a nitrogenous base, a sugar, and a phosphate group
- 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

Ratio of DNA nucleotide

- ***Chargaff's rules*** state that in any species there is an equal number of A and T bases, and an equal number of G and C bases

Total number of A	=	**Total number of T**
Total number of G	=	**Total number of C**



Building a Structural Model of DNA: *Scientific Inquiry*

- After most biologists became convinced that DNA was the genetic material, the challenge was to determine: how its **structure** accounts for its role?

- 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

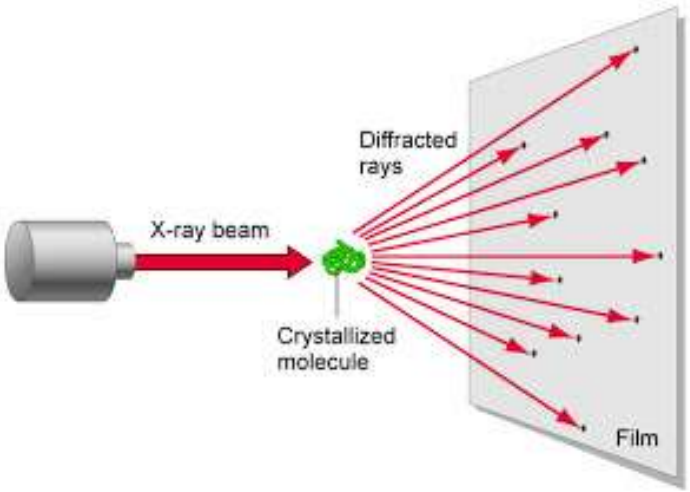
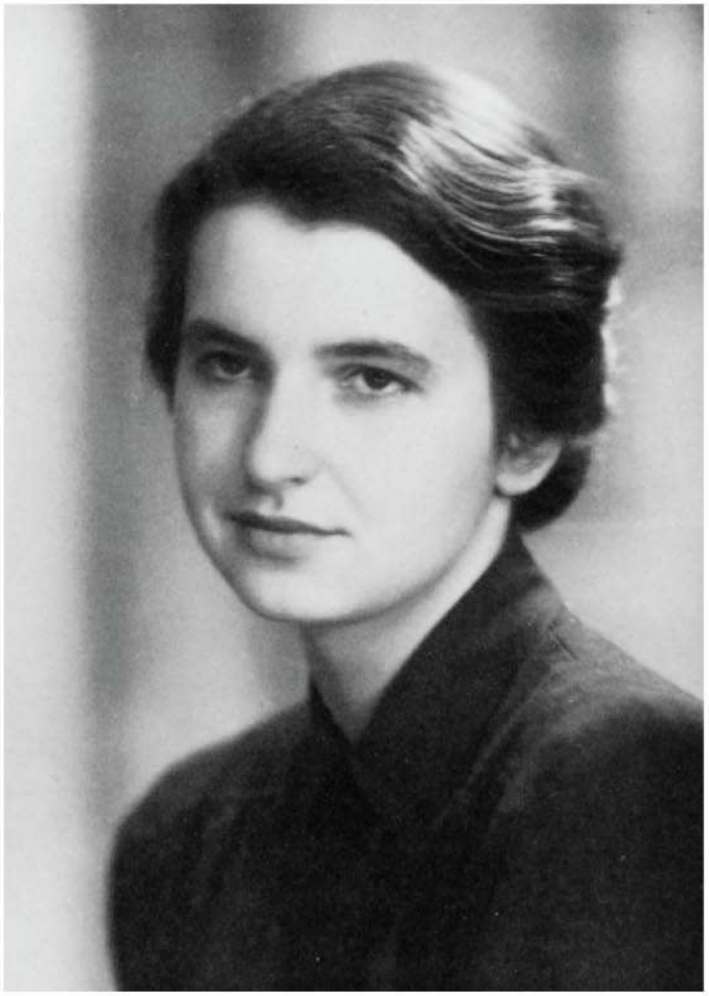
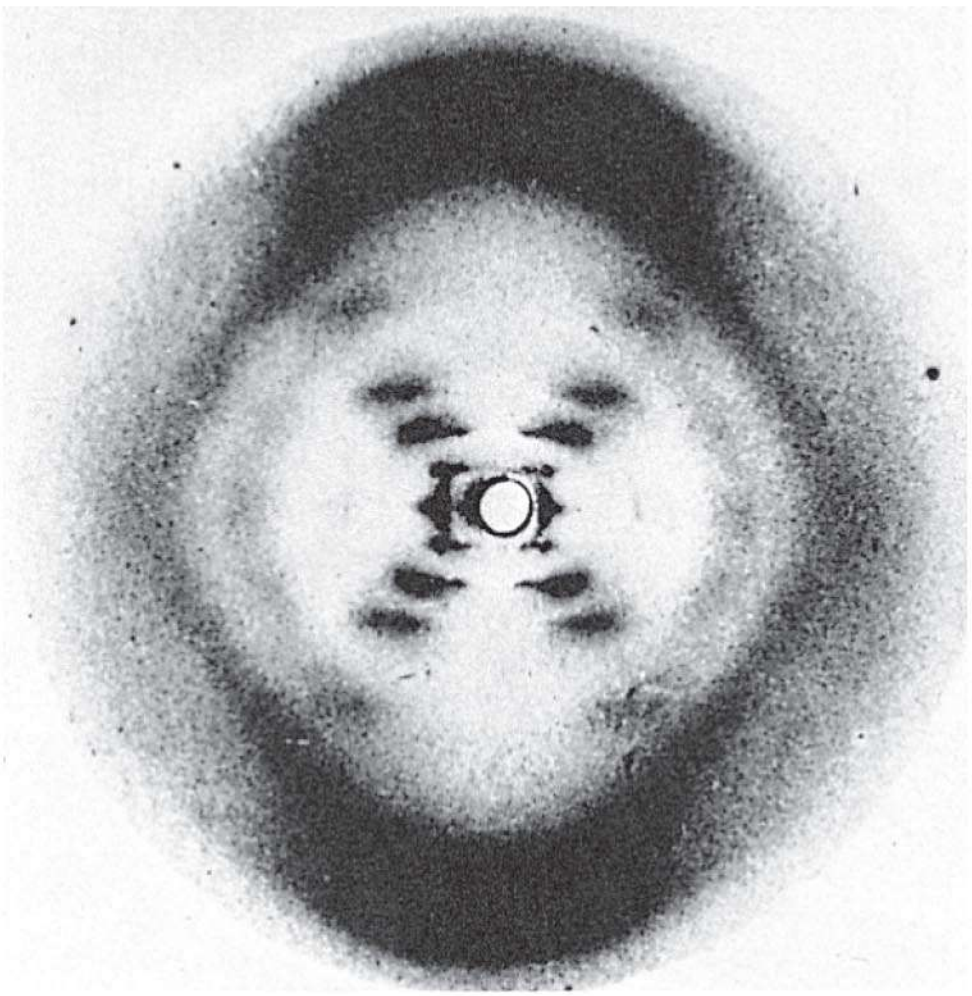


Fig. 16-6

Rosalind Franklin and her X-ray diffraction photo of DNA



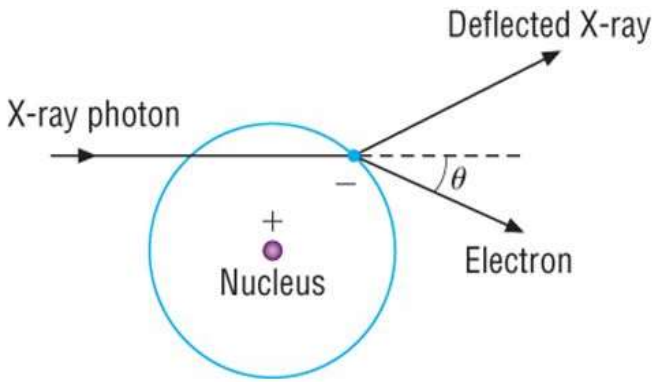
(a) Rosalind Franklin



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

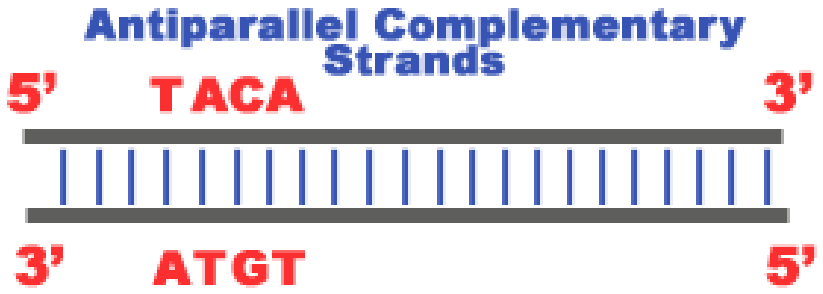
Interpretation of Franklin's experimental results

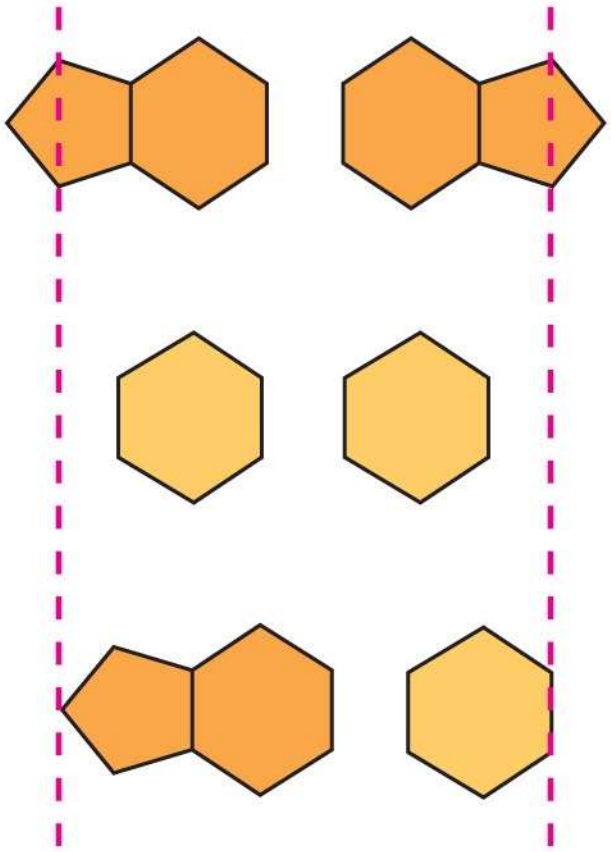
- Franklin's X-ray crystallographic images of DNA enabled Watson to deduce that DNA was helical, the X-ray images also enabled Watson to deduce the width of the helix and the spacing of the nitrogenous bases
- The **width** suggested that the DNA molecule was made up of two strands, forming a **double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



DNA Structure

- Franklin had concluded that there were two antiparallel sugar-phosphate backbones, with the nitrogenous bases paired in the molecule's interior





Purine + purine: too wide

(A or G)

Pyrimidine + pyrimidine: too narrow

(T or C)

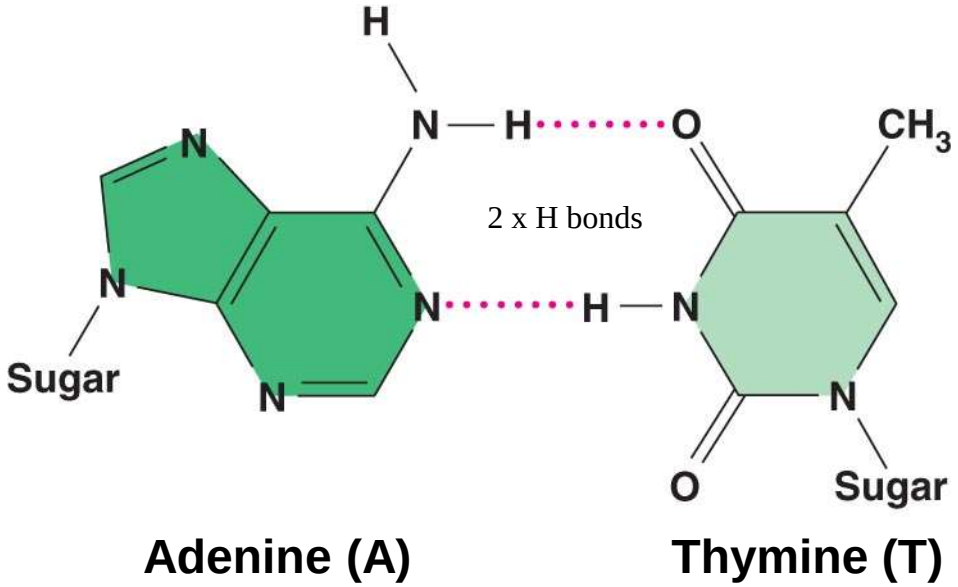
Purine + pyrimidine: width consistent with X-ray data

(A-T or G-C)

Pairing a purine (A or G) with a pyrimidine (T or C) resulted in a uniform width consistent with the X-ray;

The Watson-Crick model explains Chargaff's rules: in any organism the amount of A = T, and the amount of G = C

Fig. 16-8



Base pairing in DNA

A to T

G to C

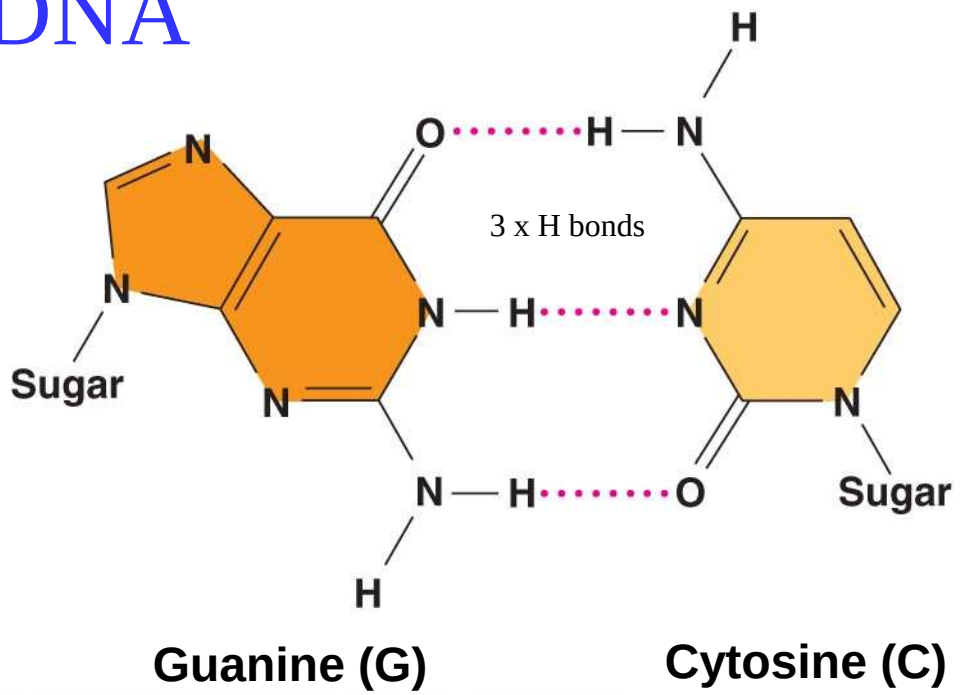
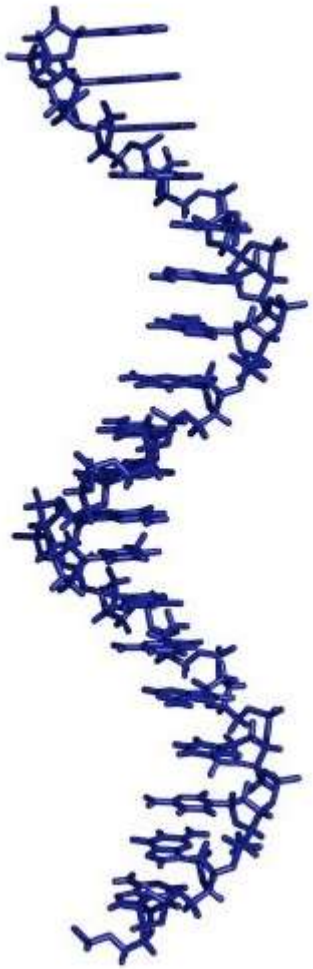
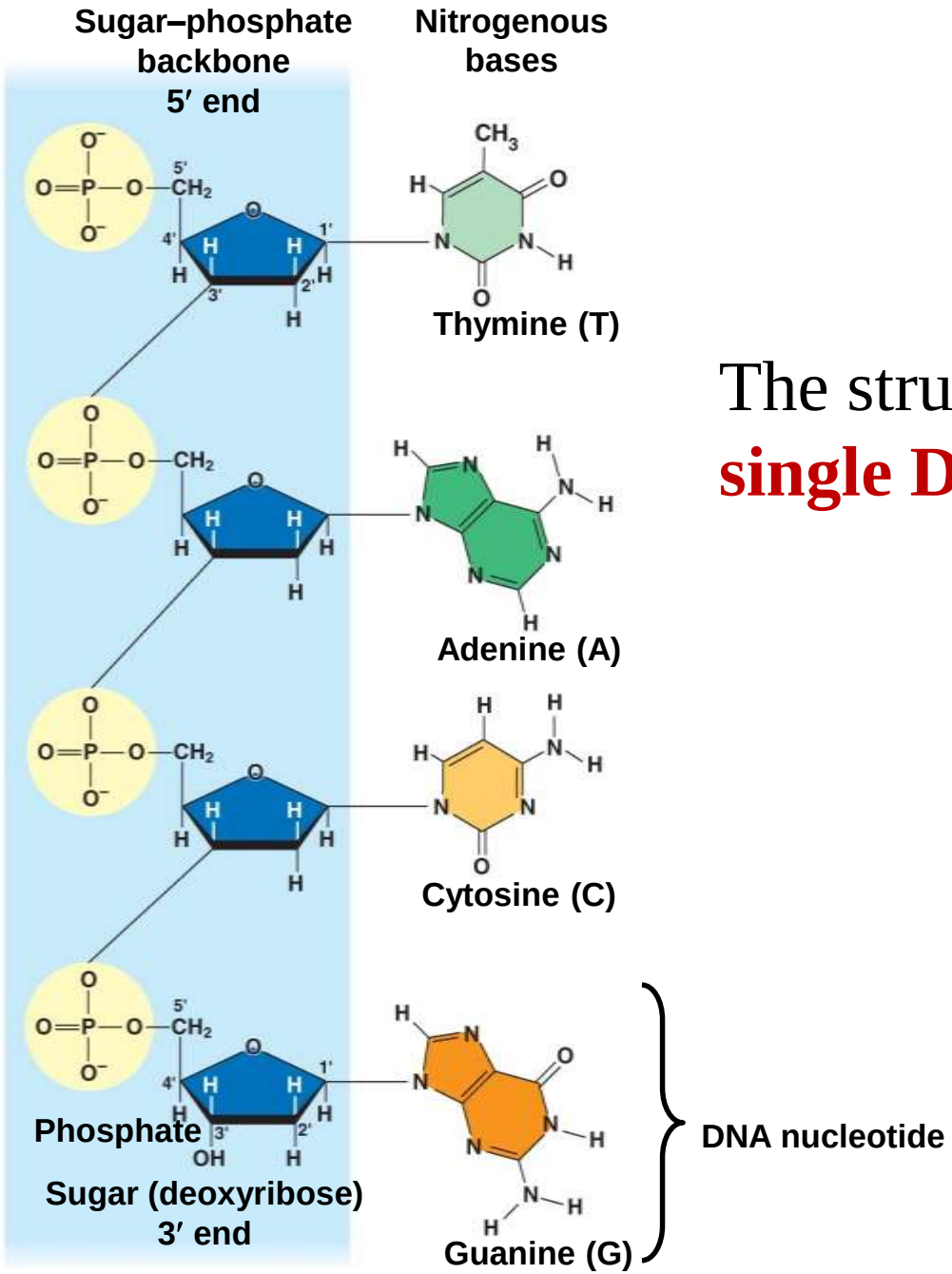


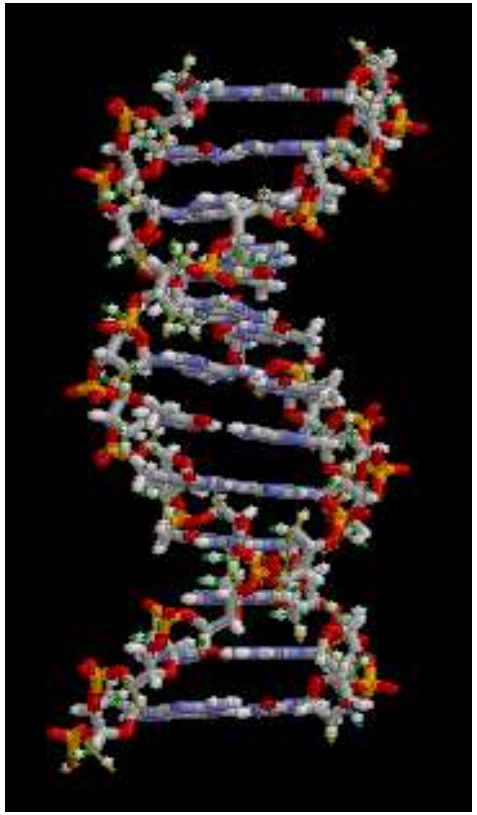
Fig. 16-5





The structure of a **single DNA strand**

Summary : **The structure of DNA**



Watson and Crick built models of a **double helix** to conform to the X-rays and chemistry of DNA.

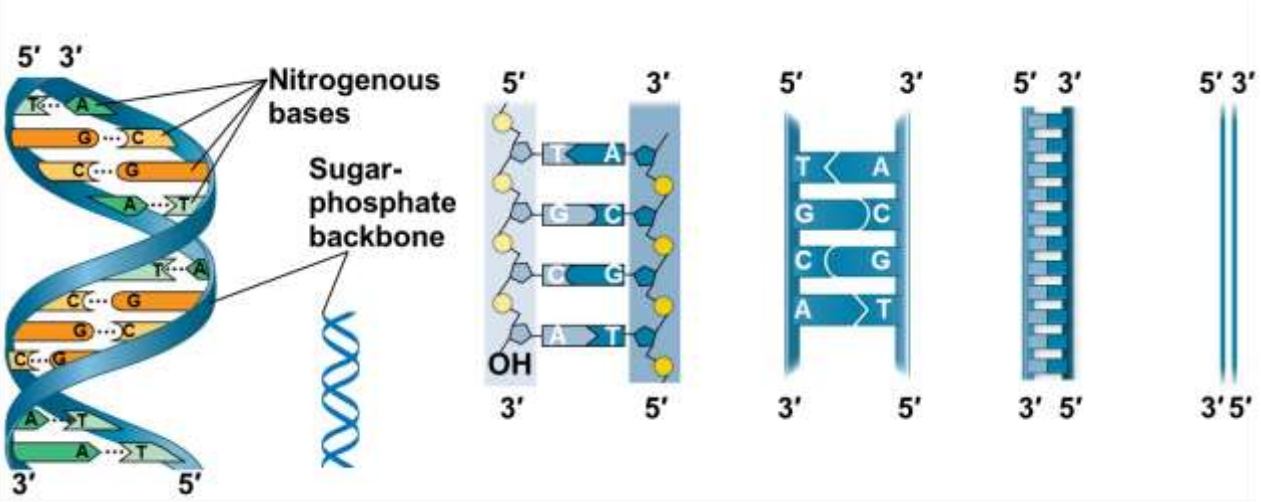


Figure 16.7 (11th Ed)

Concept 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.**

Genetic molecule? ➔ What structure? ➔ How does DNA replicate?

逐步深入的問題

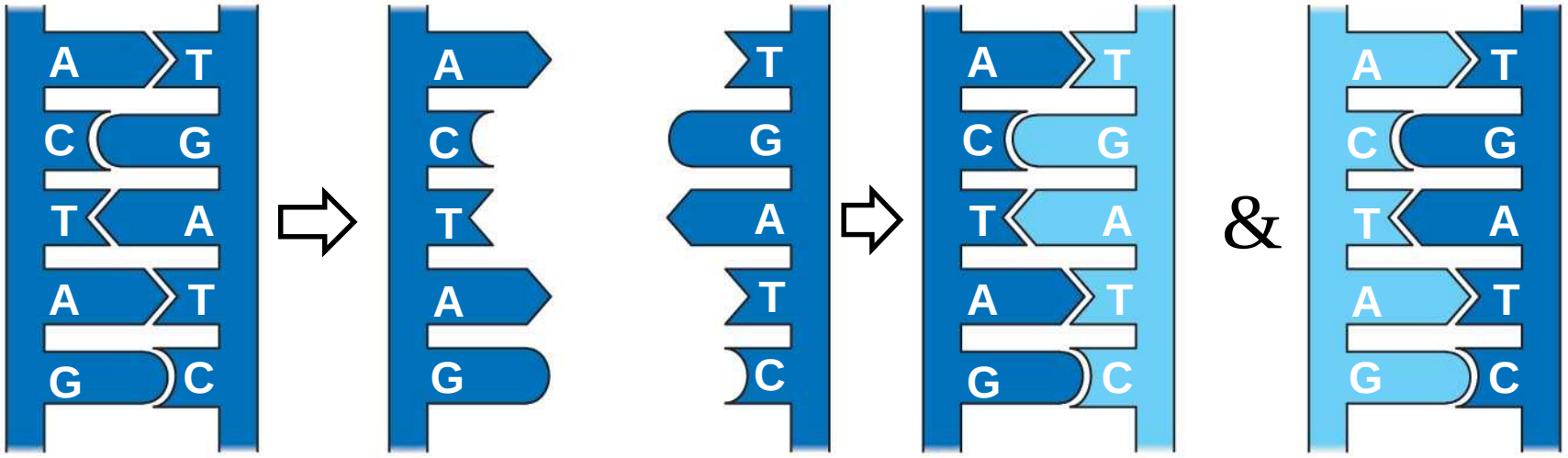
The Basic Principle: Base Pairing to a Template Strand

- Since the two strands of DNA are **complementary (互補)**, 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**

A model for DNA replication on next page

Fig. 16-9-3

A model for DNA replication: the basic concept



(a) Parent molecule

(b) Separation of strands

(c) "Daughter" DNA molecules, each consisting of one parental strand and one new strand

DNA replication

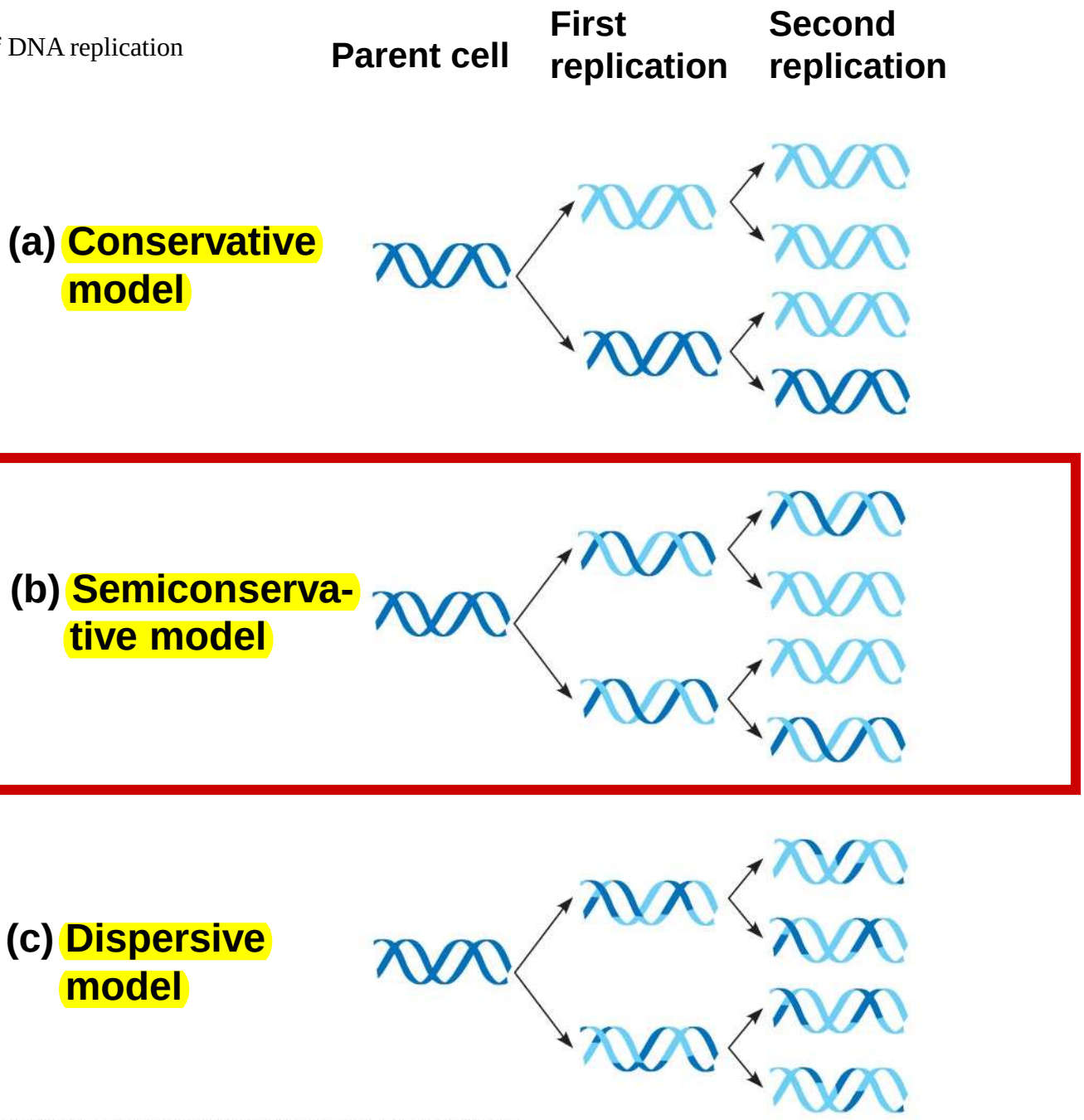
- Watson and Crick's **semiconservative model** of replication predicts that **when a double helix replicates, each daughter molecule will have one old strand** (derived or "conserved" from the parent molecule) and one newly made strand

以假說 (hypothesis)預測實驗結果

- Competing models were the conservative model (the two parent strands rejoin) and the dispersive model (each strand is a mix of old and new)

Fig. 16-10

Three alternative models of DNA replication



Experiments to prove the Semiconservative Model

- Experiments by Matthew Meselson and Franklin Stahl supported the semiconservative model
- They labeled the nucleotides of the **old** strands with a **heavy isotope** of nitrogen, while any **new** nucleotides were labeled with a **lighter isotope**

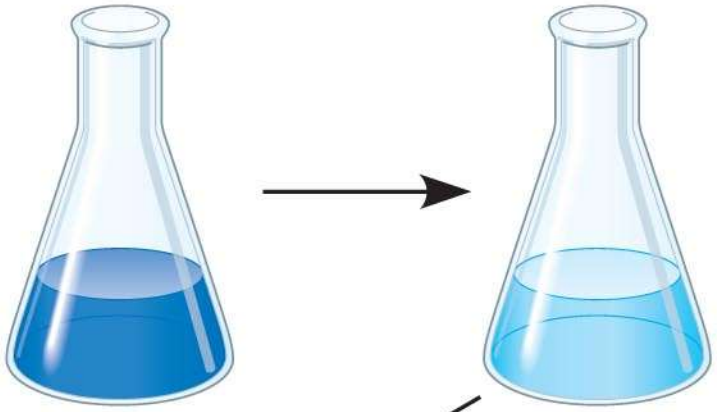
實驗方法與設計

Fig. 16-11a

Meselson and Stahl Experiment

EXPERIMENT

1 Bacteria cultured in medium containing ^{15}N

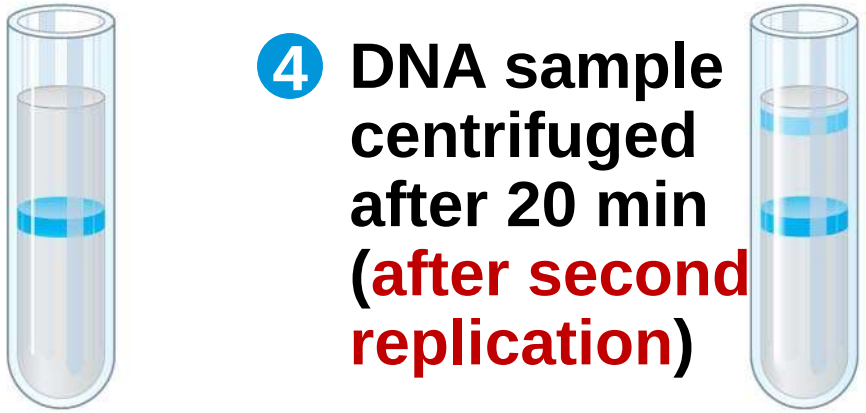


2 Bacteria transferred to medium containing ^{14}N

RESULTS

3 DNA sample centrifuged after 20 min (after first application)

4 DNA sample centrifuged after 20 min (after second replication)



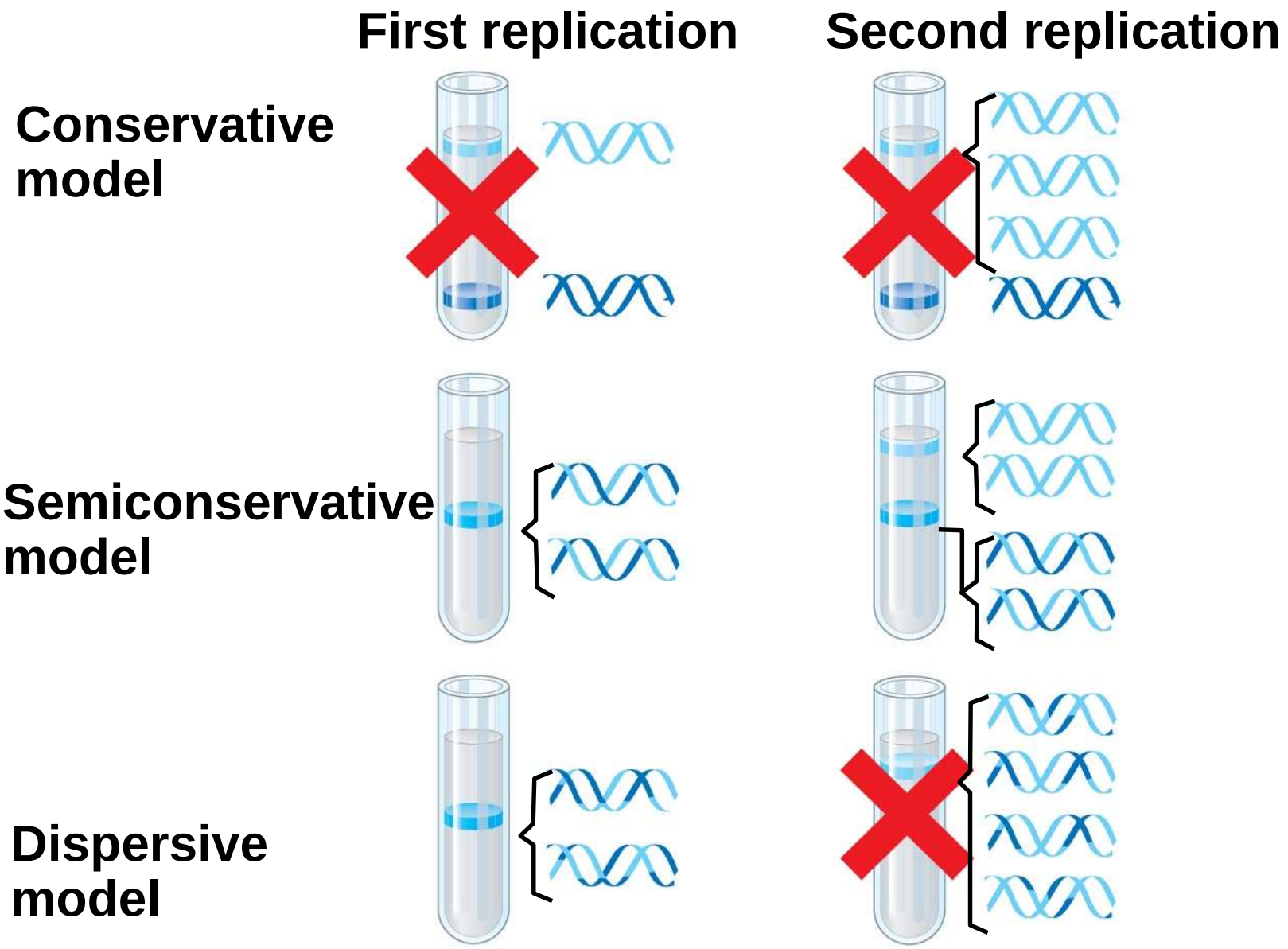
Less dense

More dense

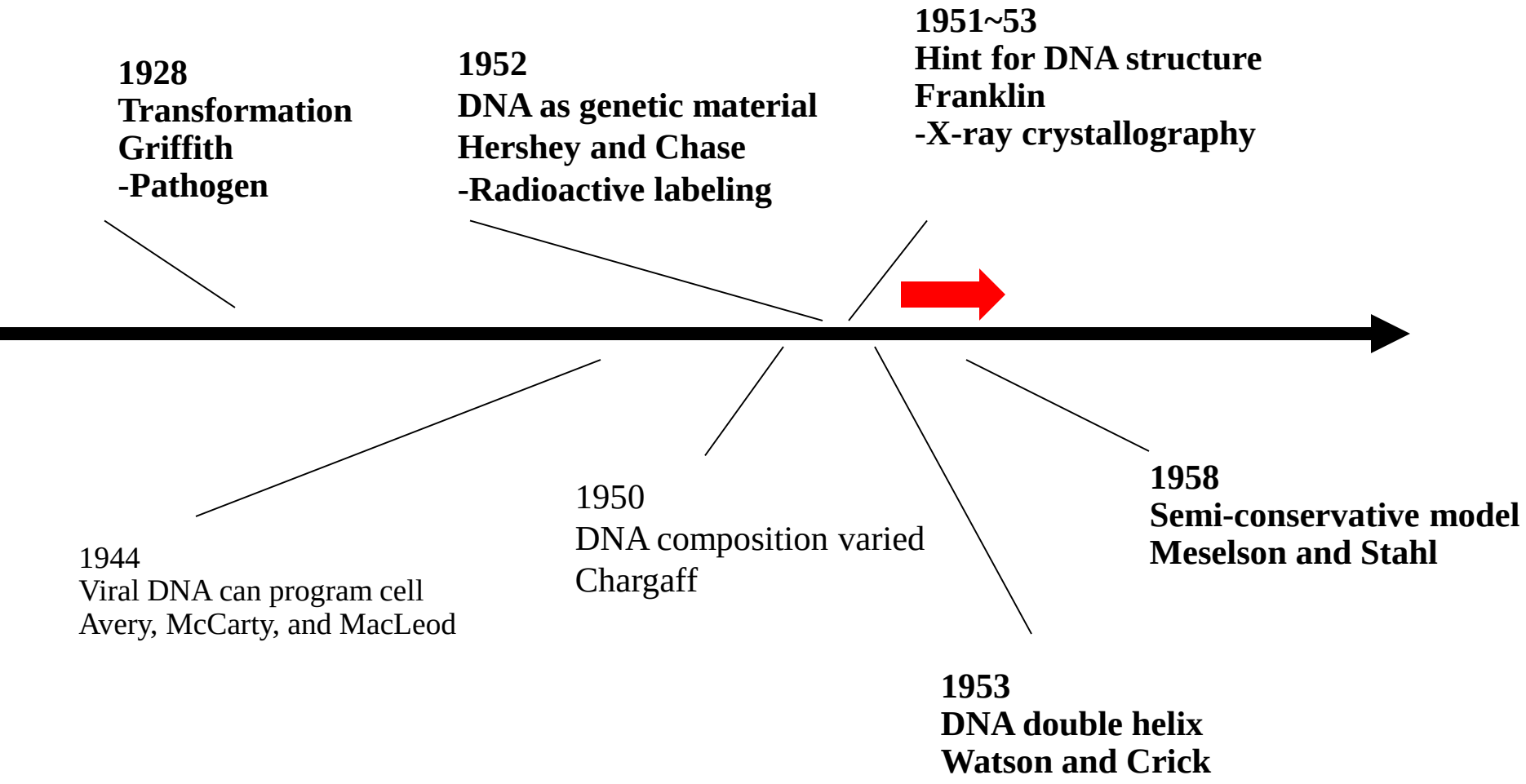
- The first replication produced a band of hybrid DNA, eliminating the conservative model
- A second replication produced both light and hybrid DNA, eliminating the dispersive model and supporting the semiconservative model

Fig. 16-11b

CONCLUSION



Timeline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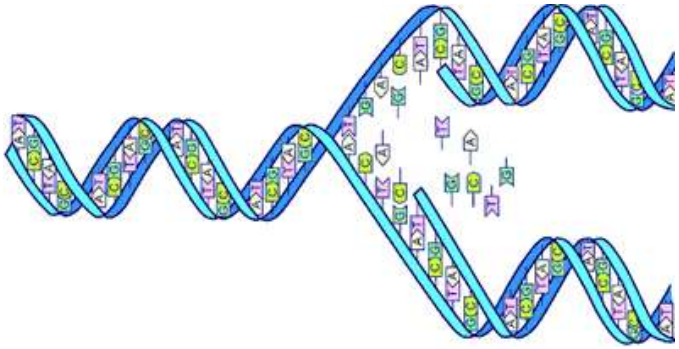
DNA Replication: *A Closer Look*

- The copying of DNA is remarkable in its speed and accuracy
- More than a dozen enzymes and other proteins participate in DNA replication



Preview:
Speed of DNA Replication
Bacteria (i.e. *E Coli*) about 500 nucleotides per second per origin of replication
Eukaryotes about 50 base pairs per second per origin of replication

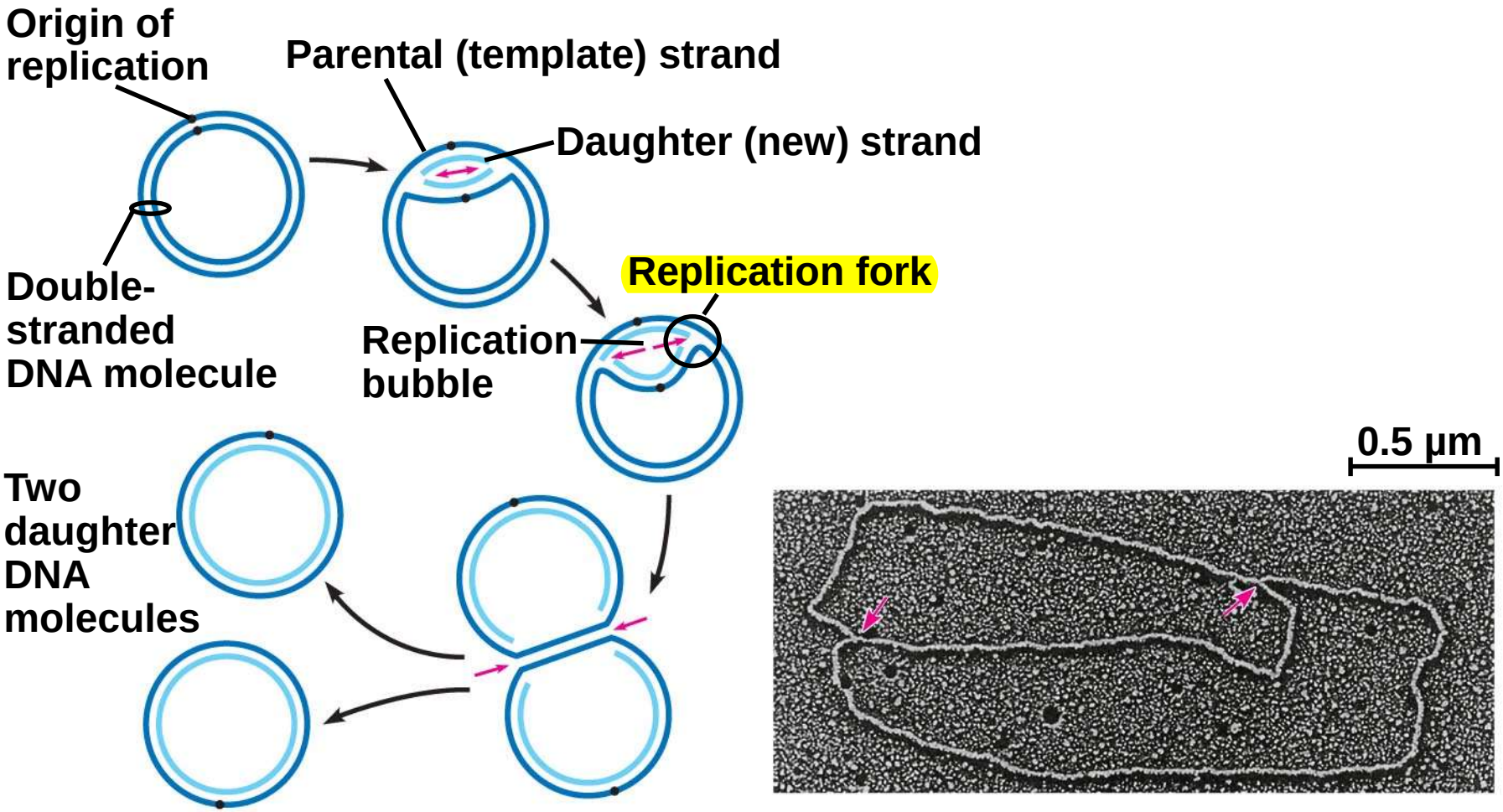
Getting Started – origins of replication

- Replication begins at special 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

Illustrations on next two pages

Fig. 16-12a

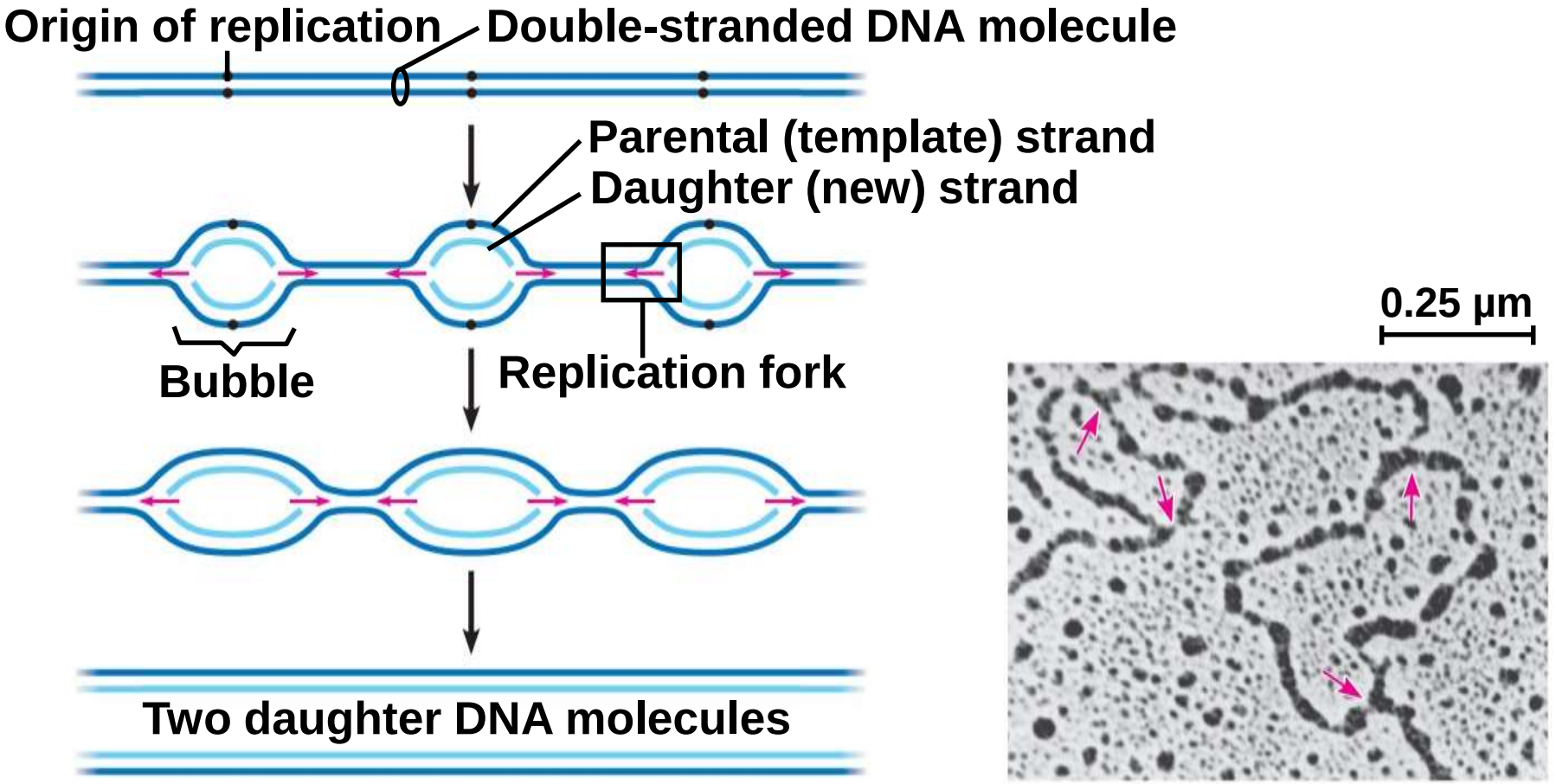
Origins of replication in *E. coli*



(a) Origins of replication in *E. coli*

Fig. 16-12b

Origins of replication in eukaryotes



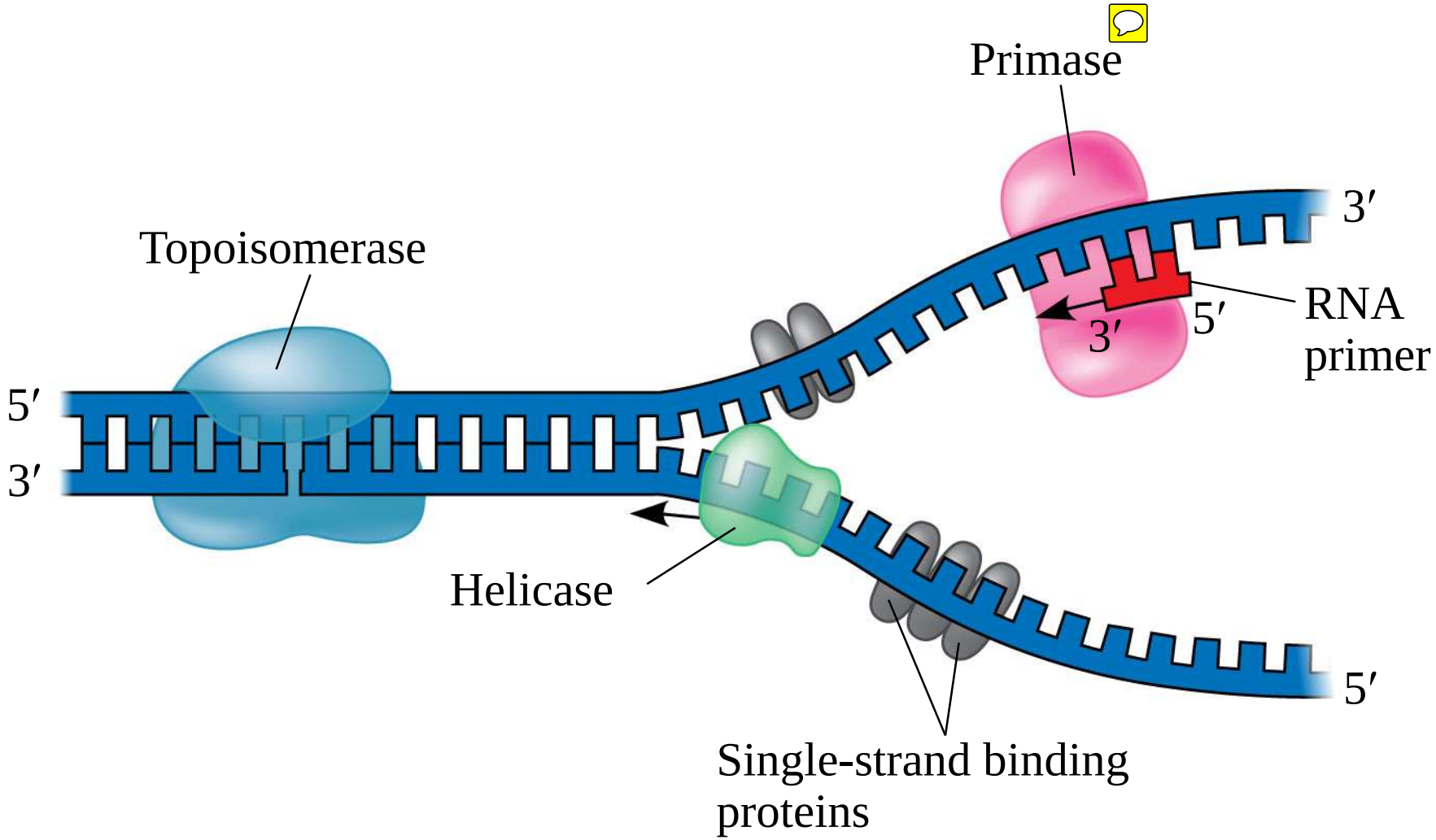
(b) Origins of replication in eukaryotes

重要專有名詞

- 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 protein** binds to and stabilizes single-stranded DNA until it can be used as a template
- **Topoisomerase** corrects "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands

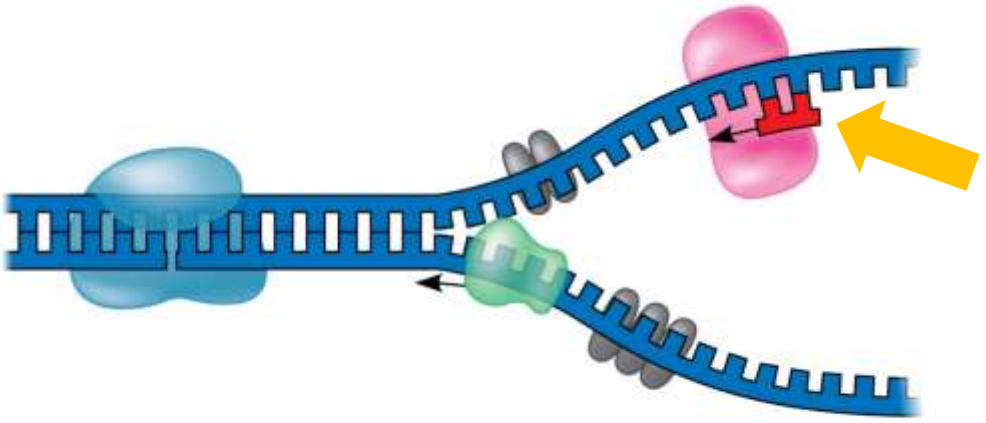
Figure 16.13

Some of the proteins involved in the initiation of DNA replication.



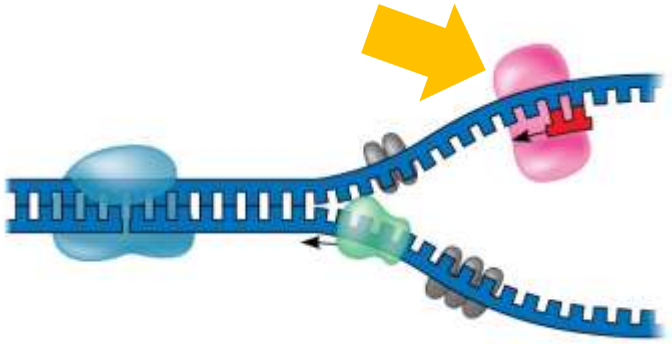
RNA Primer

- DNA polymerases **cannot** initiate synthesis of a polynucleotide; they can only add nucleotides to the 3′ end
- The initial nucleotide strand is a short RNA **primer**



Primase

- An enzyme called **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



DNA polymerases *Synthesizing a New DNA Strand*

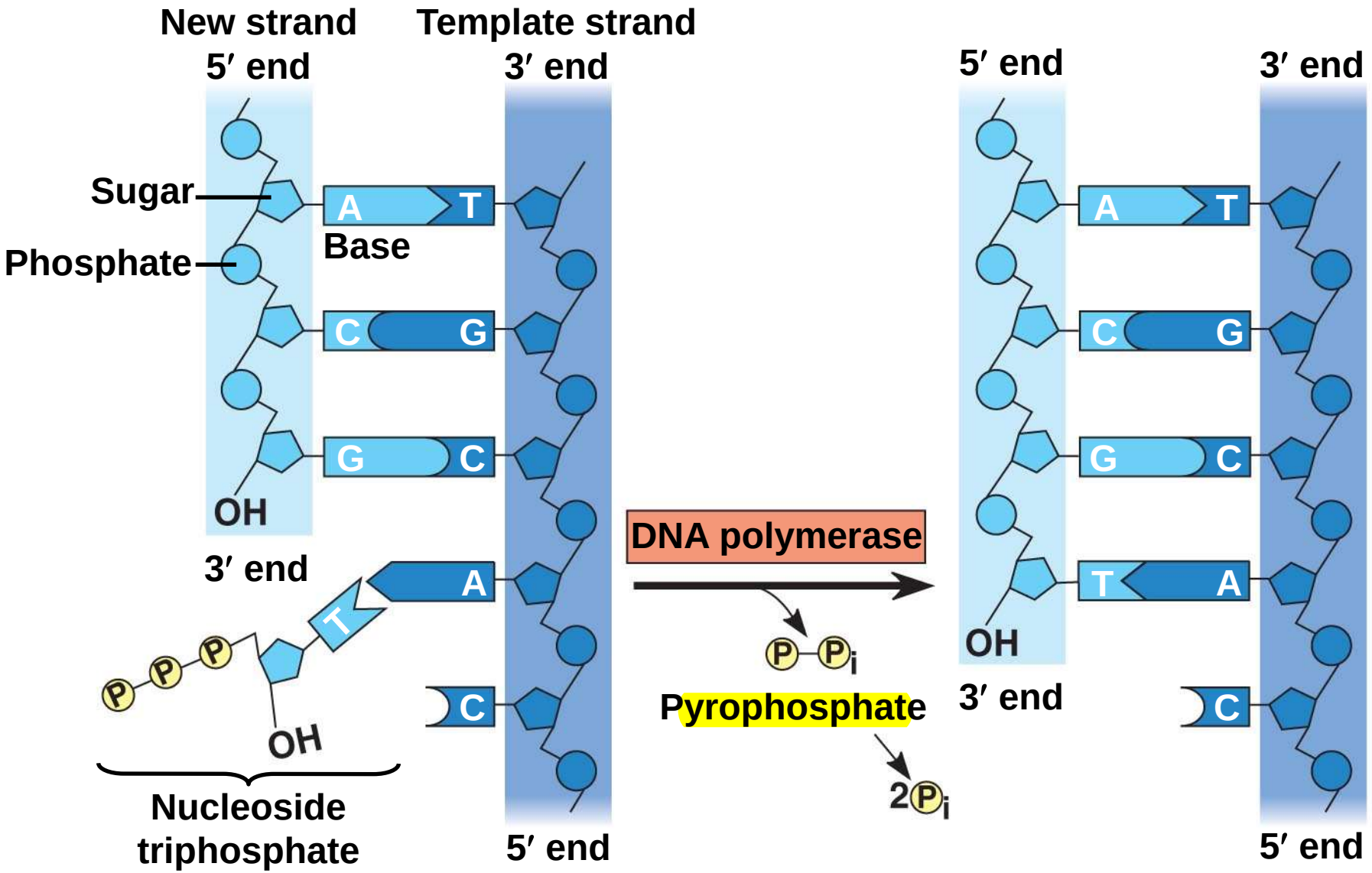
- Enzymes called **DNA polymerases** catalyze the elongation of new DNA at a replication fork
- Most DNA polymerases require a primer and a DNA template strand
- The rate of elongation is about 500 nucleotides per second in bacteria and 50 per second in human cells

dATP and ATP

- 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 of dATP joins the DNA strand, it loses two phosphate groups as a molecule of pyrophosphate

Fig. 16-14

Incorporation of a nucleotide into a DNA strand



Antiparallel Elongation

- The antiparallel structure of the double helix (two strands oriented in opposite directions) 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**

Leading stand

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

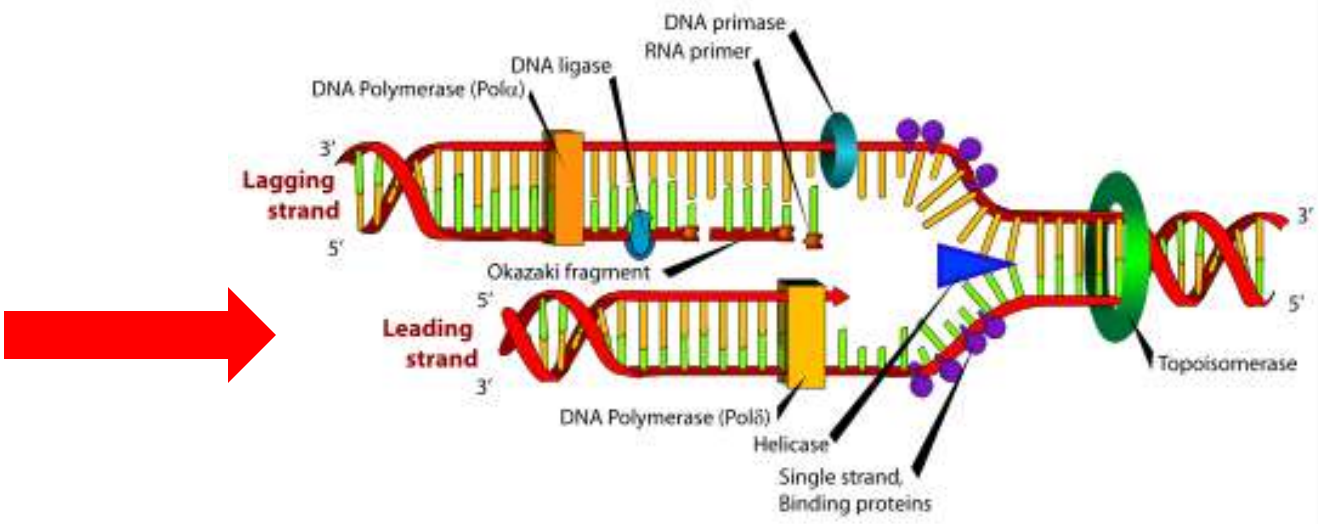


Fig. 16-15a

Synthesis of the leading strand during DNA replication

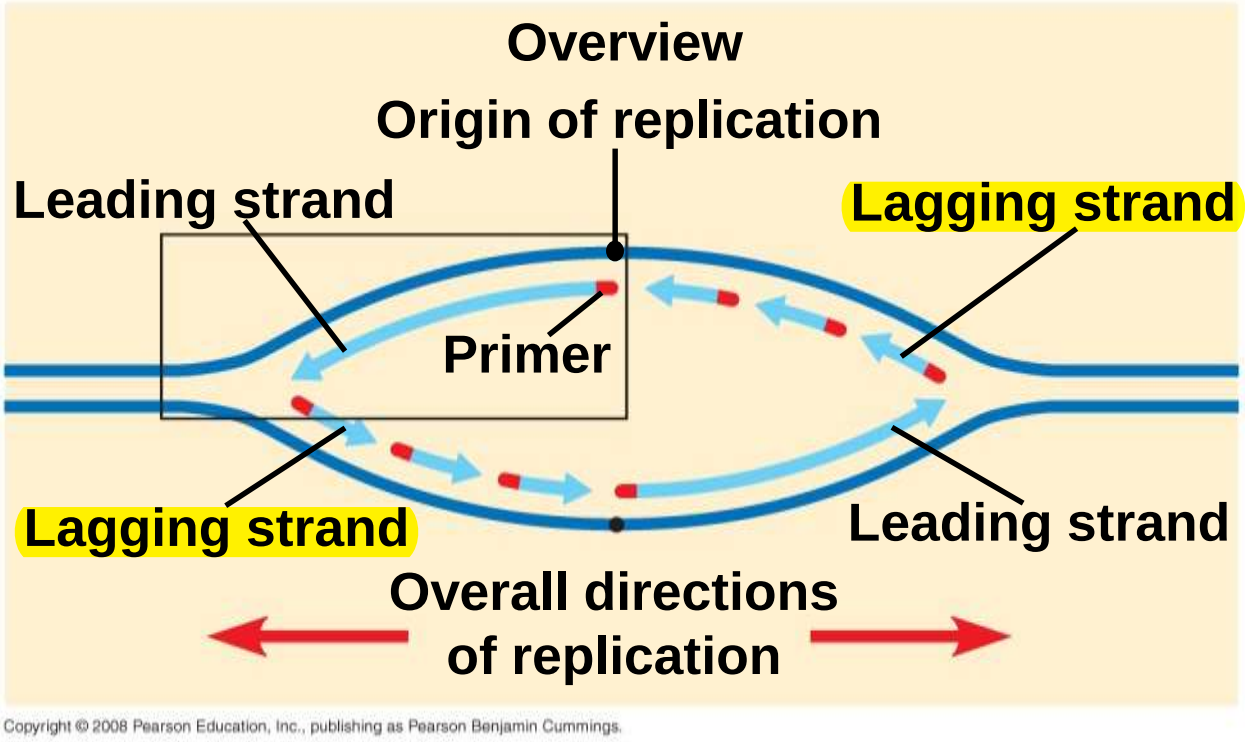
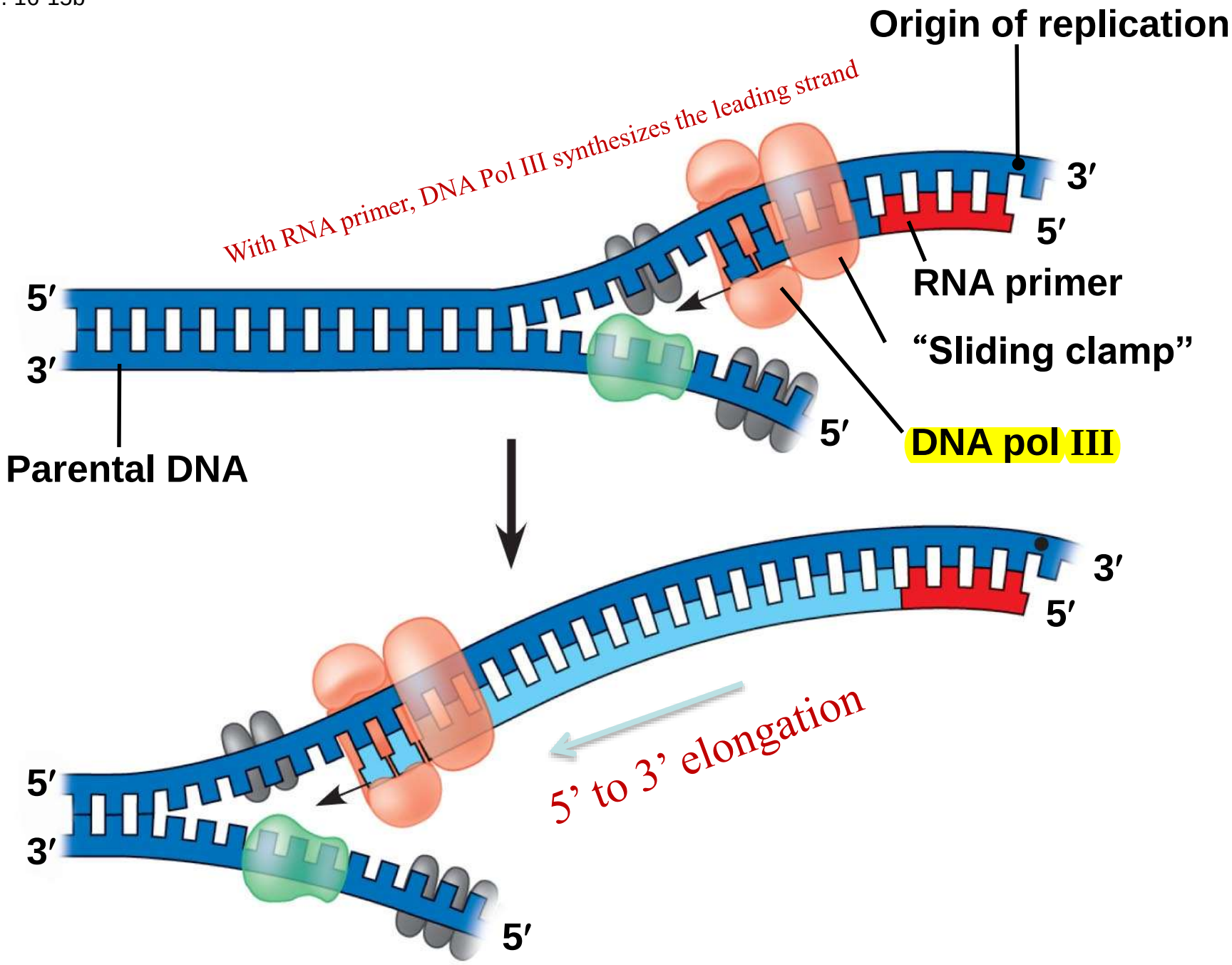


Fig. 16-15b


Origin of replication
With RNA primer, DNA Pol III synthesizes the leading strand
3′
5′
RNA primer
“Sliding clamp”
5′
3′
Parental DNA
5′
DNA pol III
3′
5′
5′ to 3’ elongation
5′
3′
5′

Lagging 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** (岡崎令治 discovered in 1968), which are joined together by **DNA ligase**

Fig. 16-16a

Synthesis of the lagging strand

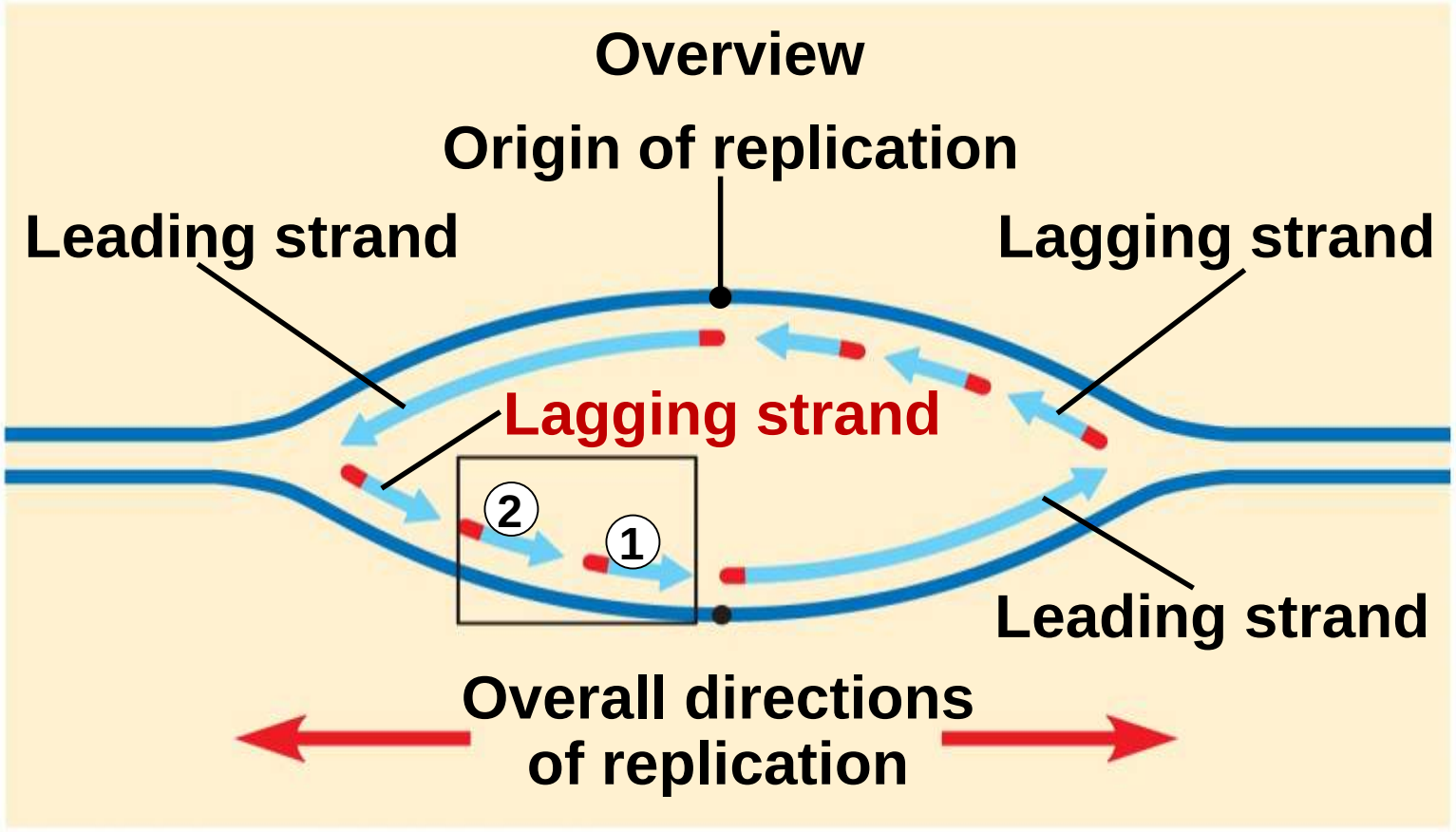


Figure 16.16b-1

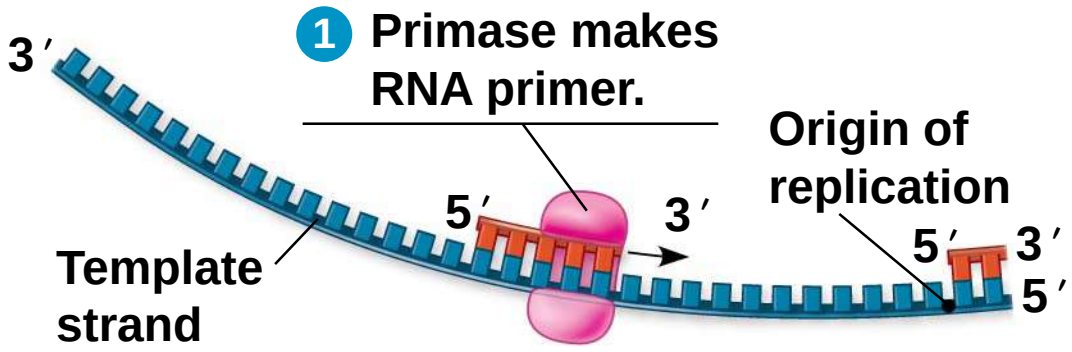


Figure 16.16b-2

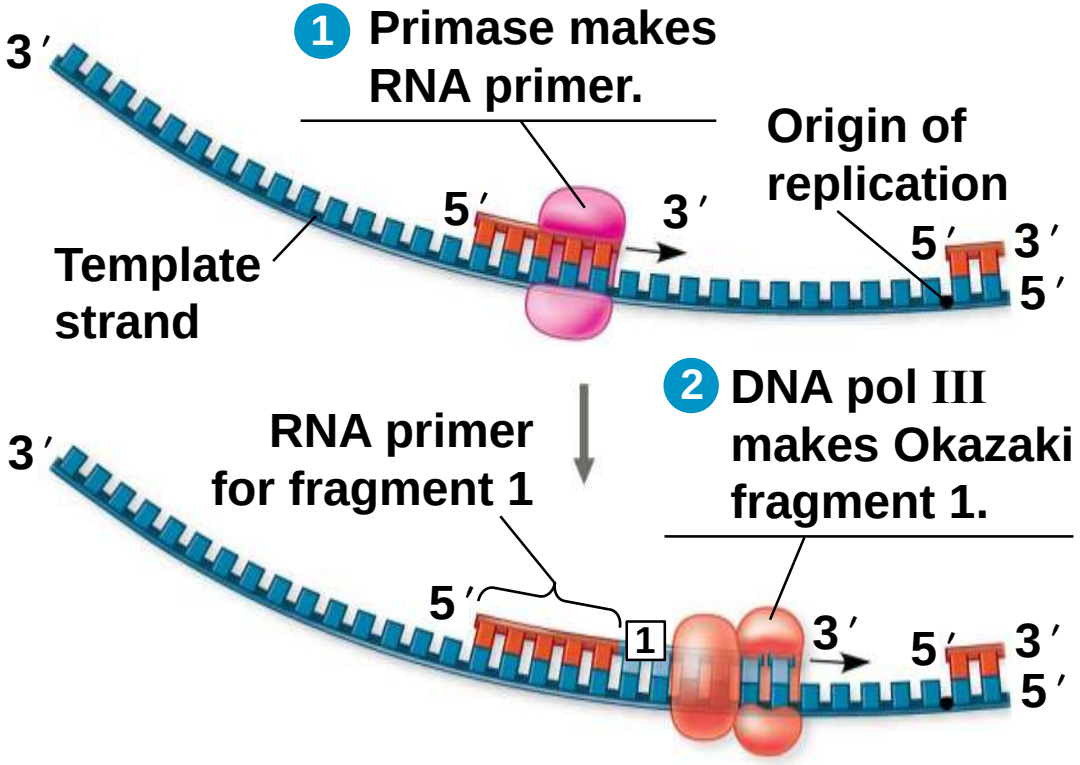


Figure 16.16b-3

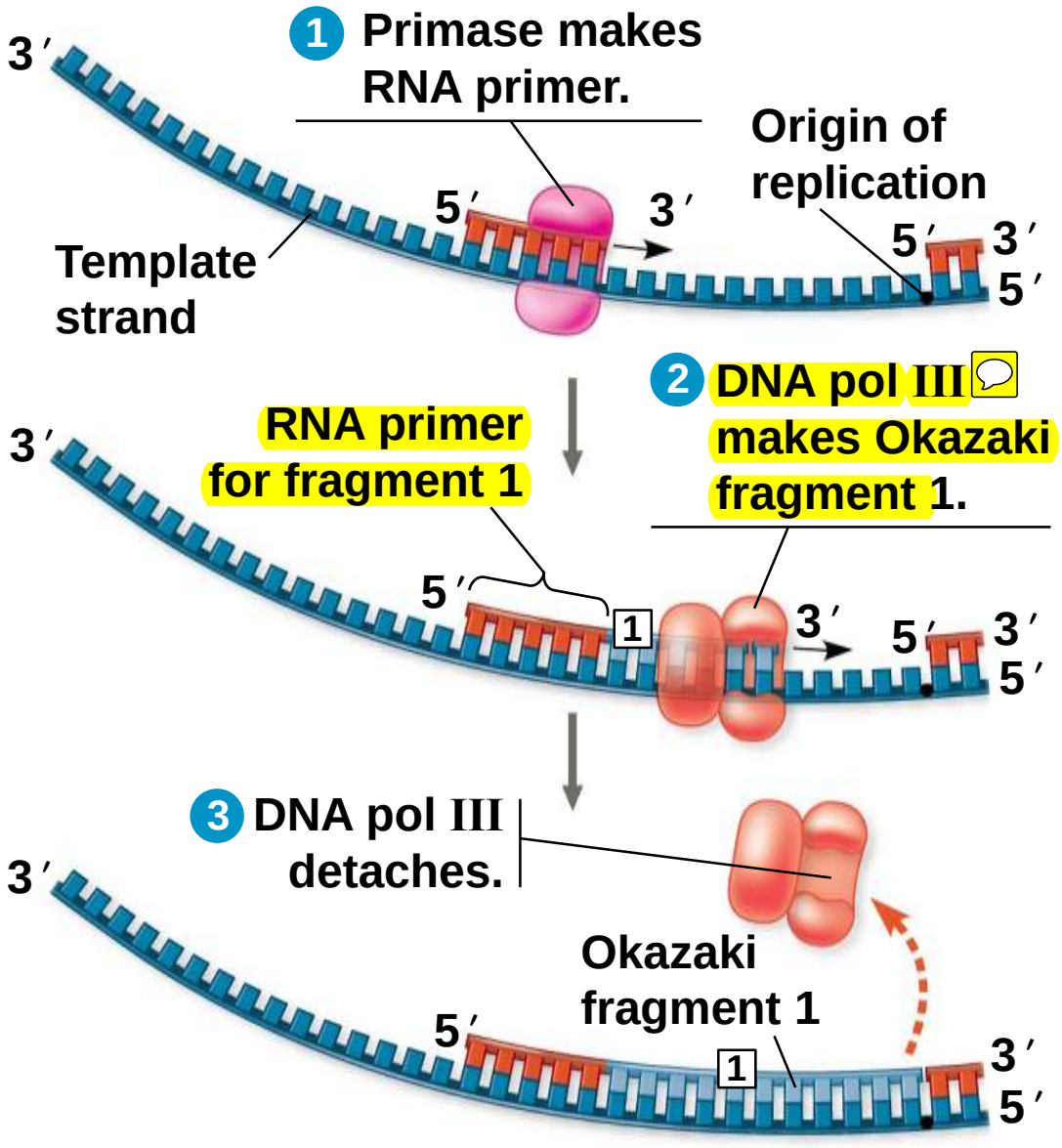


Figure 16.16c-1

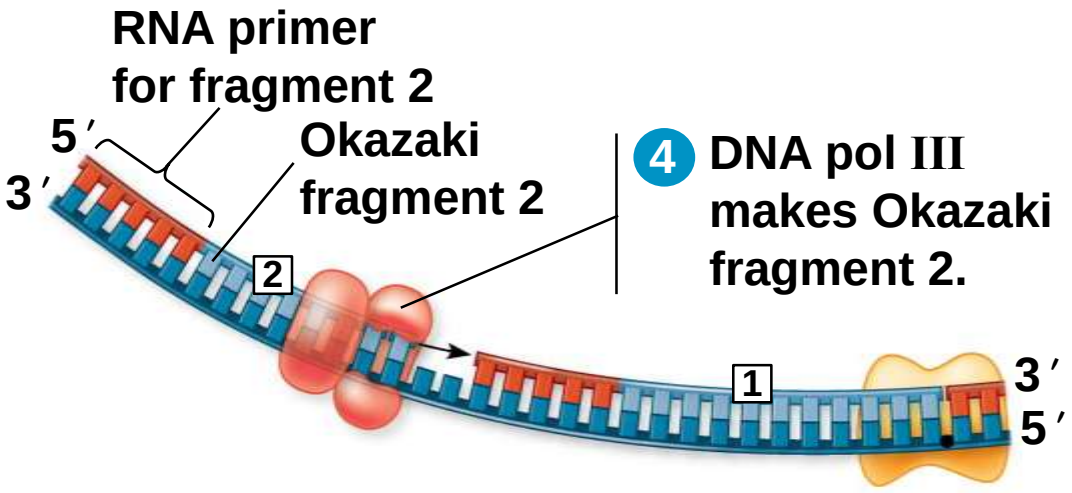


Figure 16.16c-2

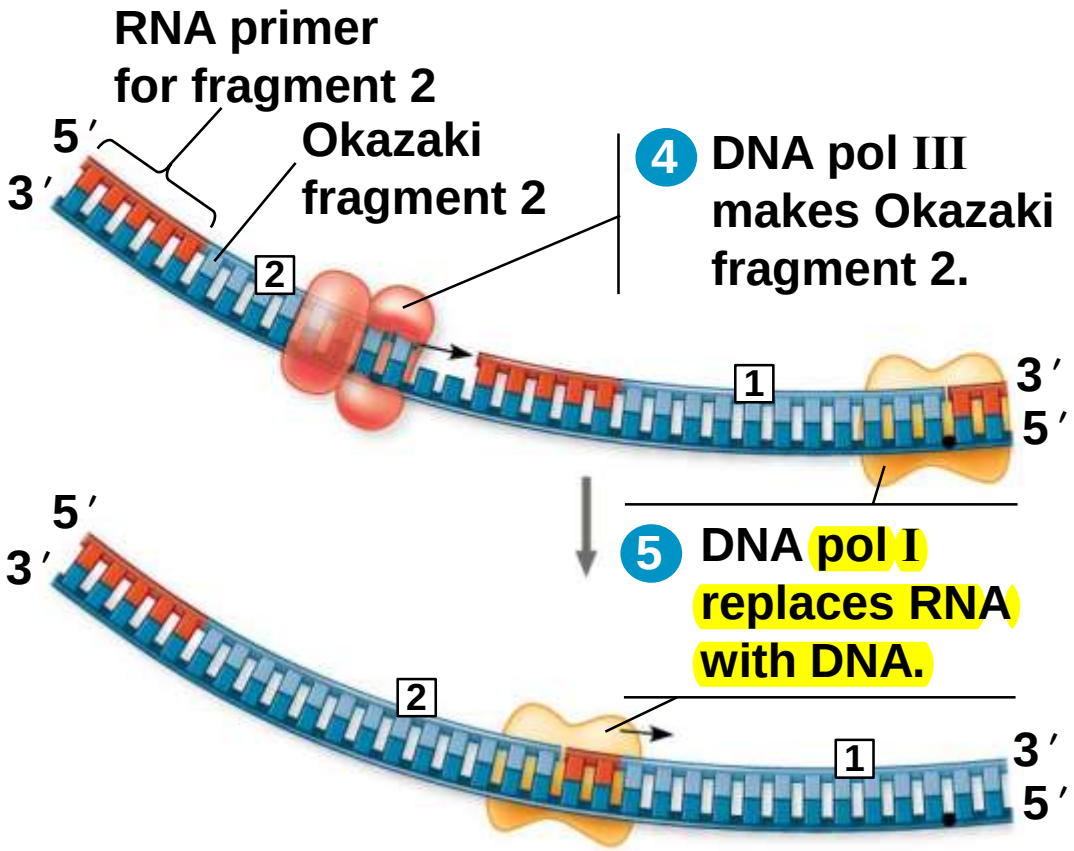
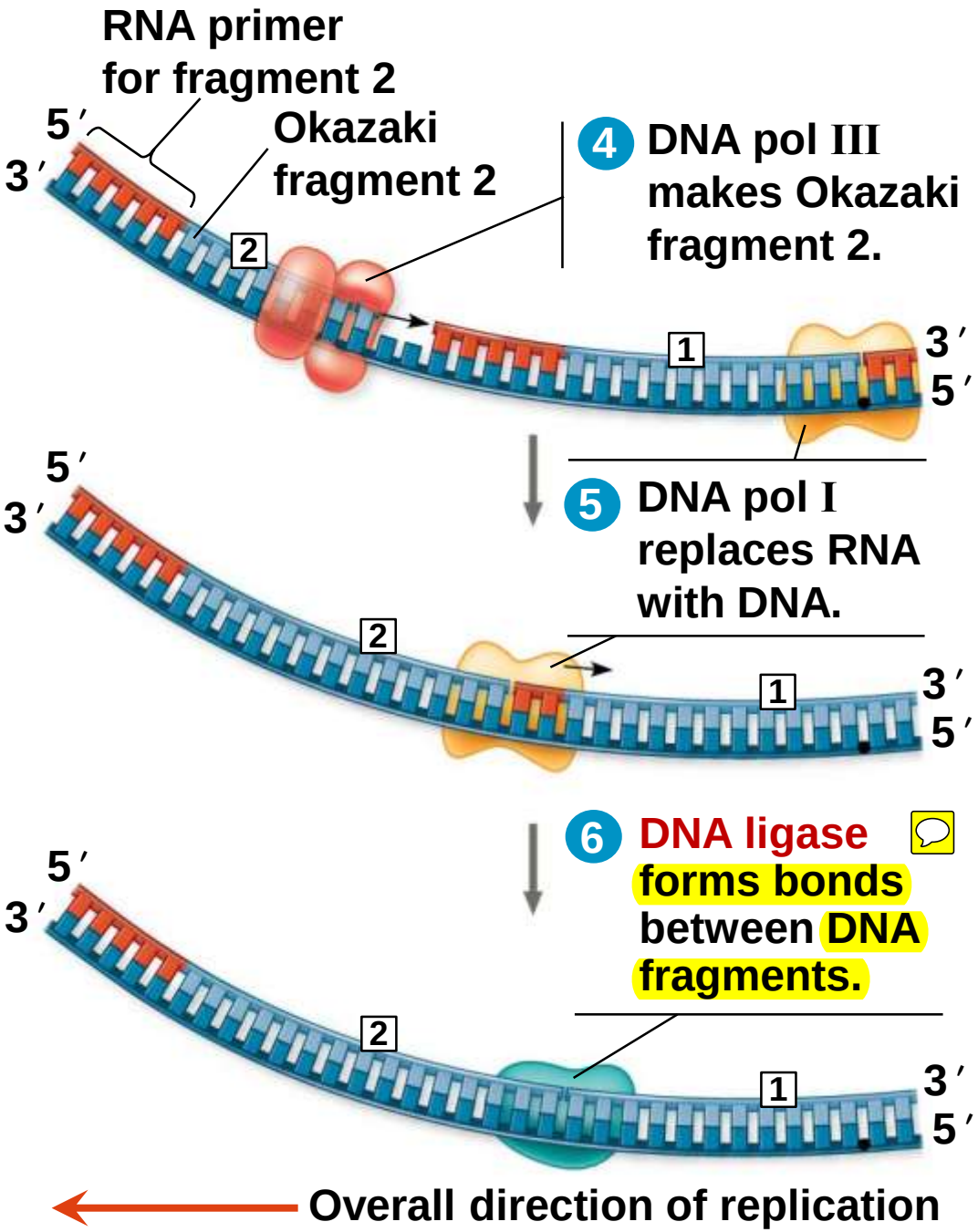


Figure 16.16c-3



Summary
重要
專有名詞

Protein	Function
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be 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 of each Okazaki fragment ofl agging strand
DNA pol III	Using parental DNA as a template, synthesizes new DNA strand by covalently adding nucleotides to the 3′ end of a pre-existing DNA strand or RNA primer
DNA pol I	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides
DNA ligase	Joins 3′ end of DNA that replaces primer to rest of leading strand and joins Okazaki fragments of lagging strand

Figure 16.17

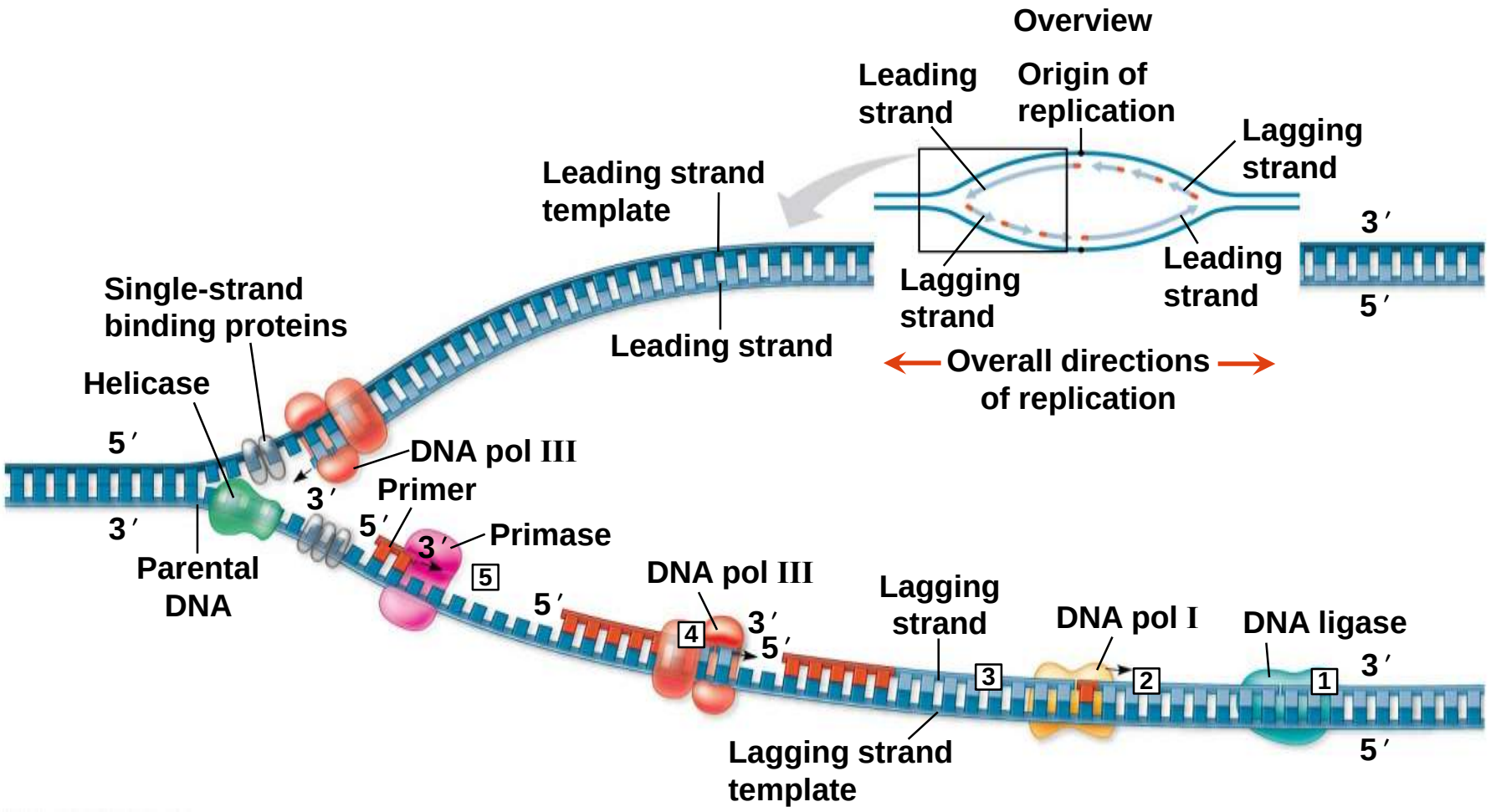


Figure 16.17a

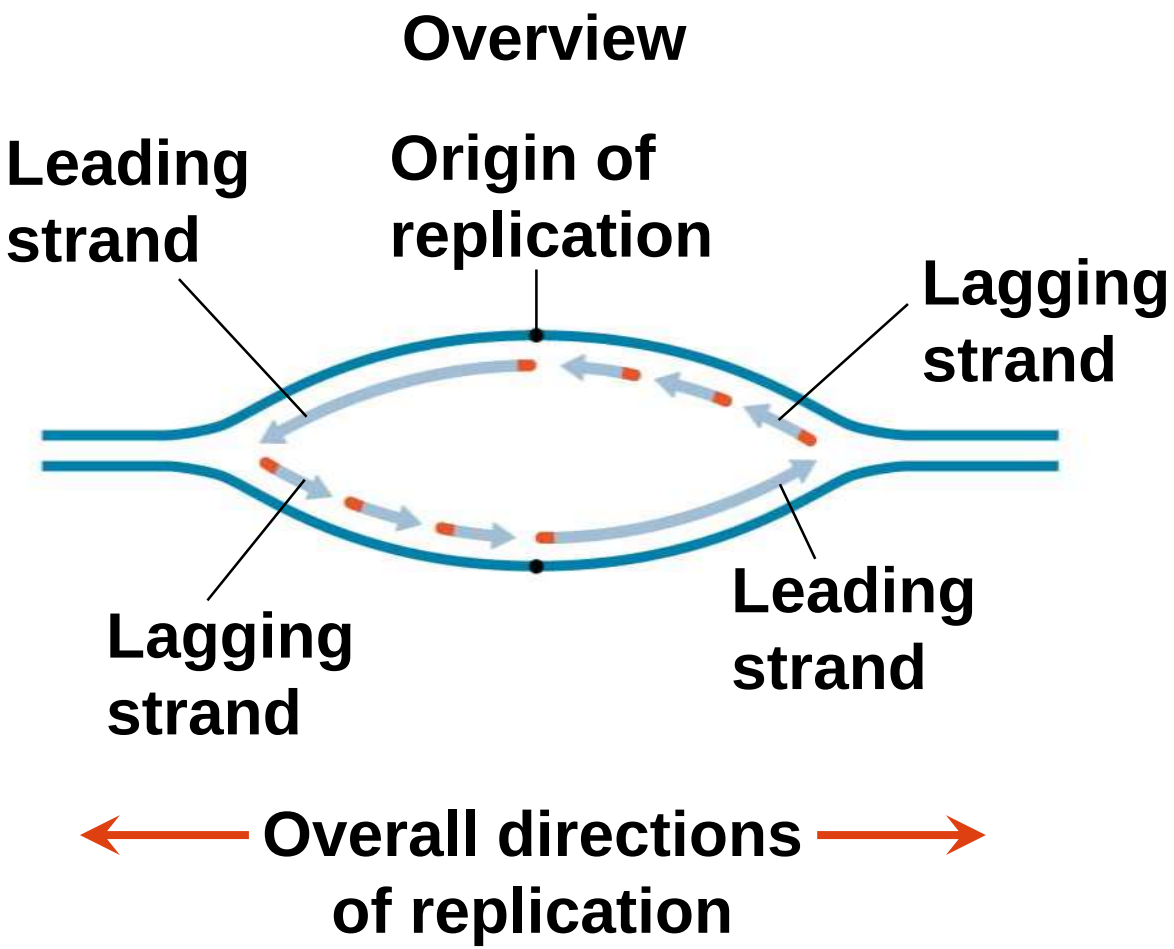


Figure 16.17b

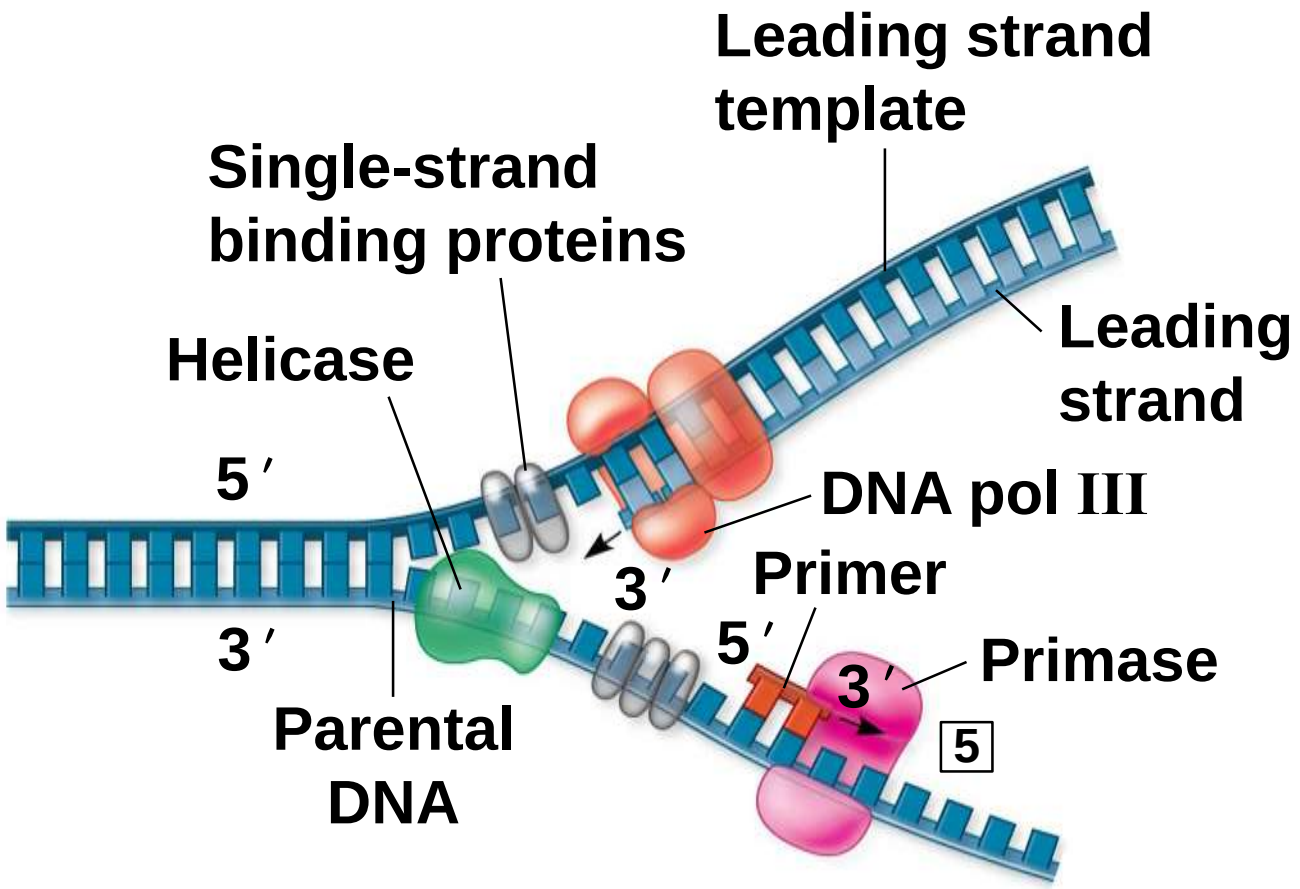
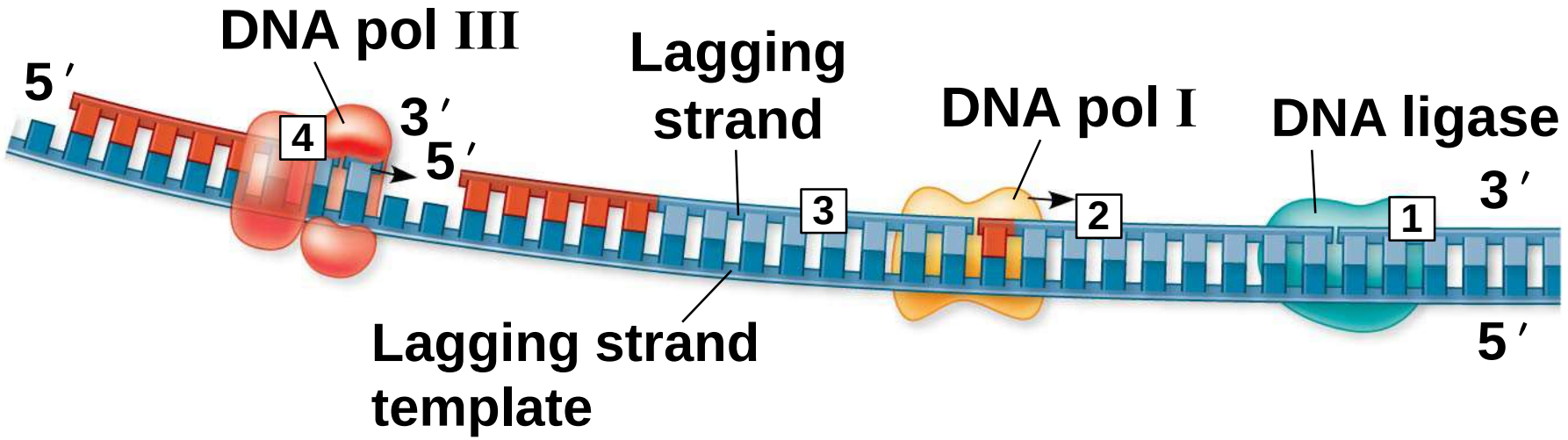


Figure 16.17c

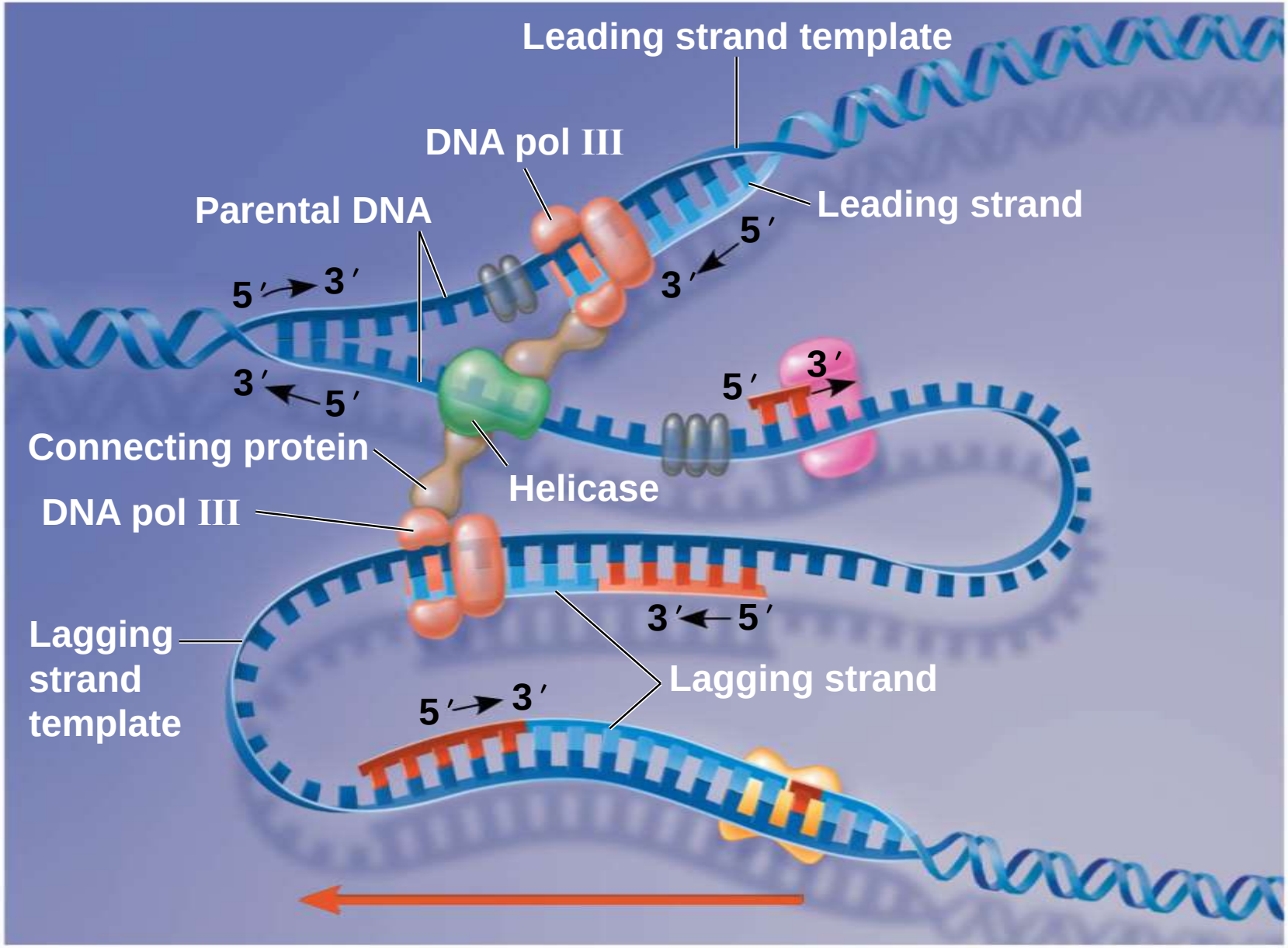


The DNA Replication Complex

- The proteins that participate in DNA replication form a large complex, a "DNA replication machine"
- The DNA replication machine is probably 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

Figure 16.18

A current model of the DNA replication complex.



Thinking question: Genotoxic drugs and cancer

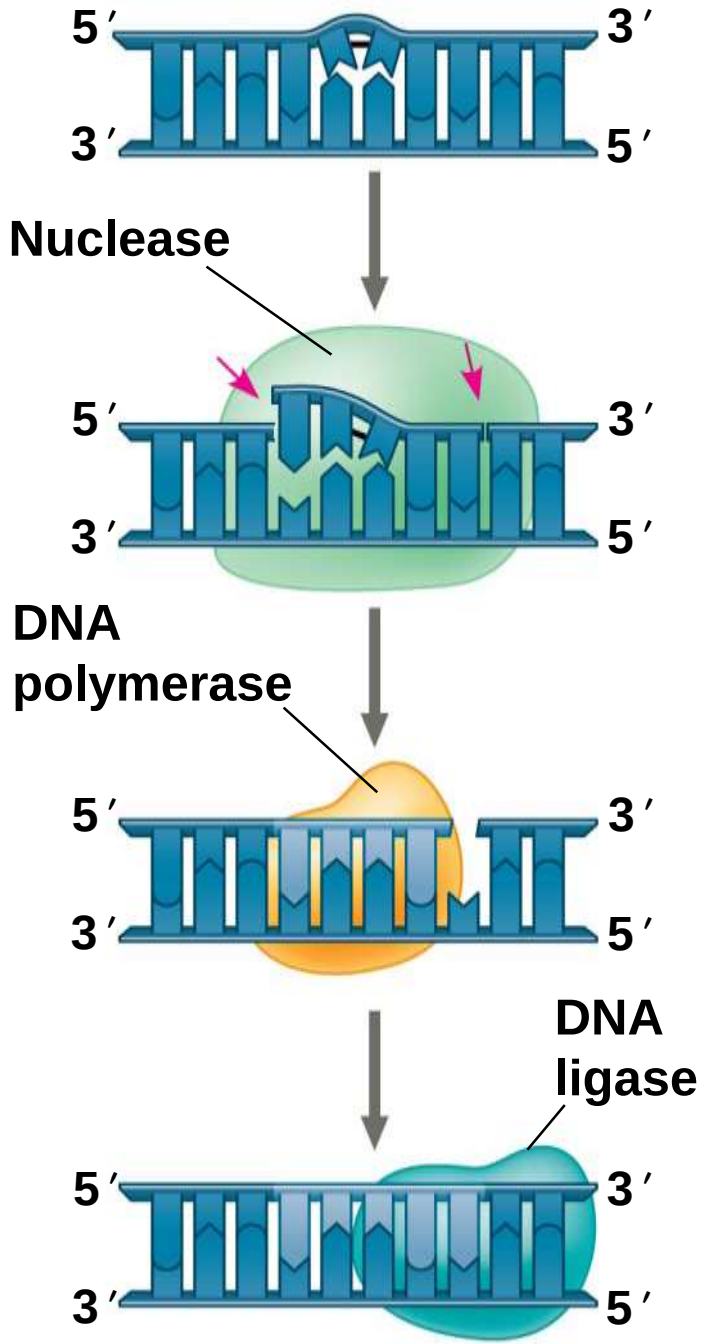
- Why chemicals that could disrupt DNA replication may be good candidate for anti-cancer (chemotherapy) drug?
  - **Cisplatin (阿樂癌):** DNA cross-links
  - **Paclitaxel (太平洋紫杉醇):** Mitotic inhibitor
  - **Etoposide (醫百幸):** Topoisomerase II inhibitor
  - **Peplomycin (培洛霉素):** Inhibit DNA metabolism
  - **Irinotecan (抗癌妥):** Topoisomerase I inhibitor
  - **Gemcitabine (健擇):** Compound nucleotides, which compete with dNTPs during DNA replication

Proofreading and Repairing DNA

- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- In **mismatch repair** of DNA, repair enzymes correct errors in base pairing
- DNA can be damaged by chemicals, radioactive emissions, X-rays, UV light, and certain molecules (in cigarette smoke for example)
- In **nucleotide excision repair**, a **nuclease** cuts out and replaces damaged stretches of DNA

Figure 16.19-3

Nucleotide excision repair of DNA damage.



Replicating the Ends of DNA Molecules

- Limitations of DNA polymerase create problems for the linear DNA of eukaryotic chromosomes
- The usual replication machinery provides no way to complete the 5′ ends, so repeated rounds of replication produce **shorter** DNA molecules

Figure 16.20a

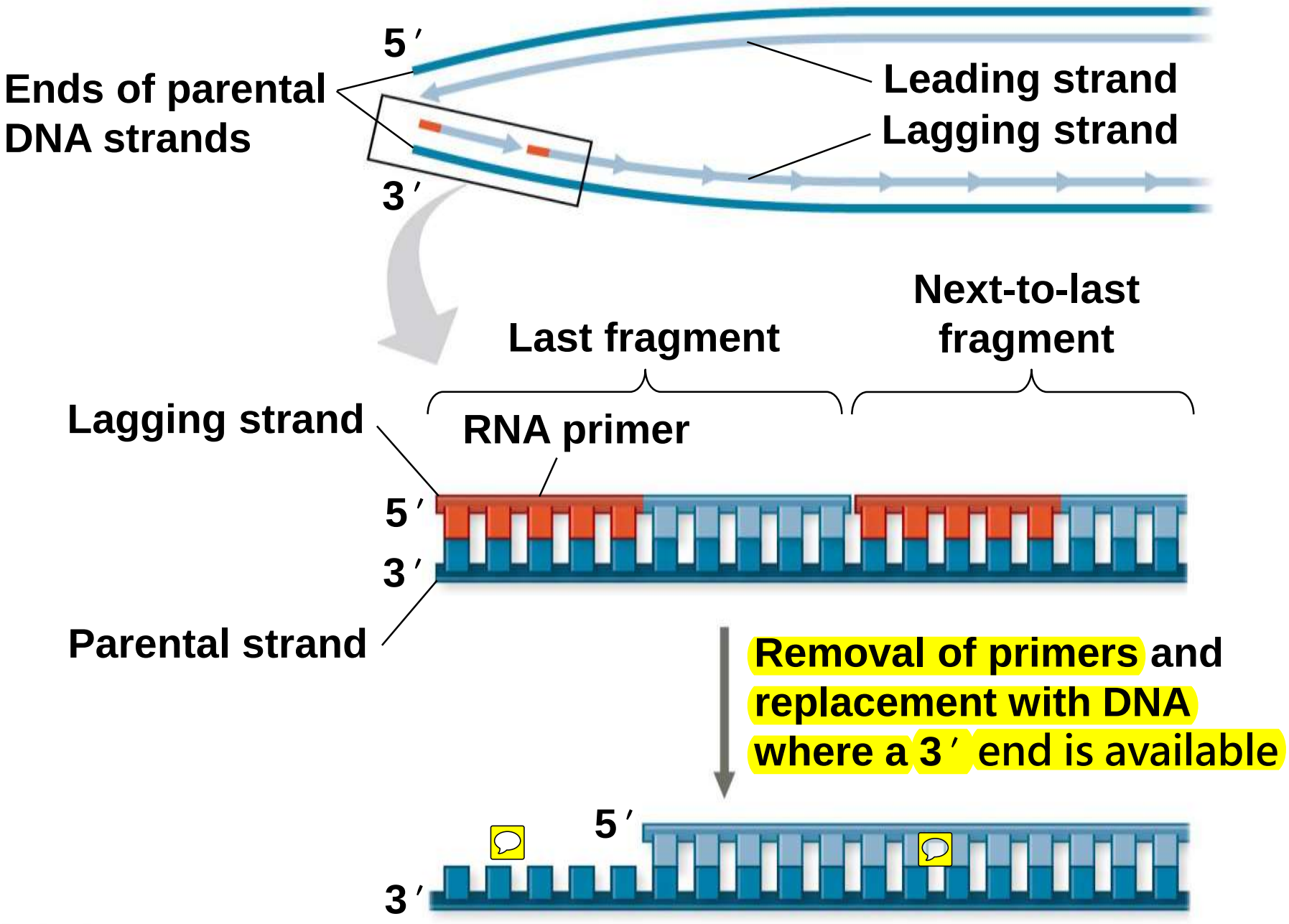
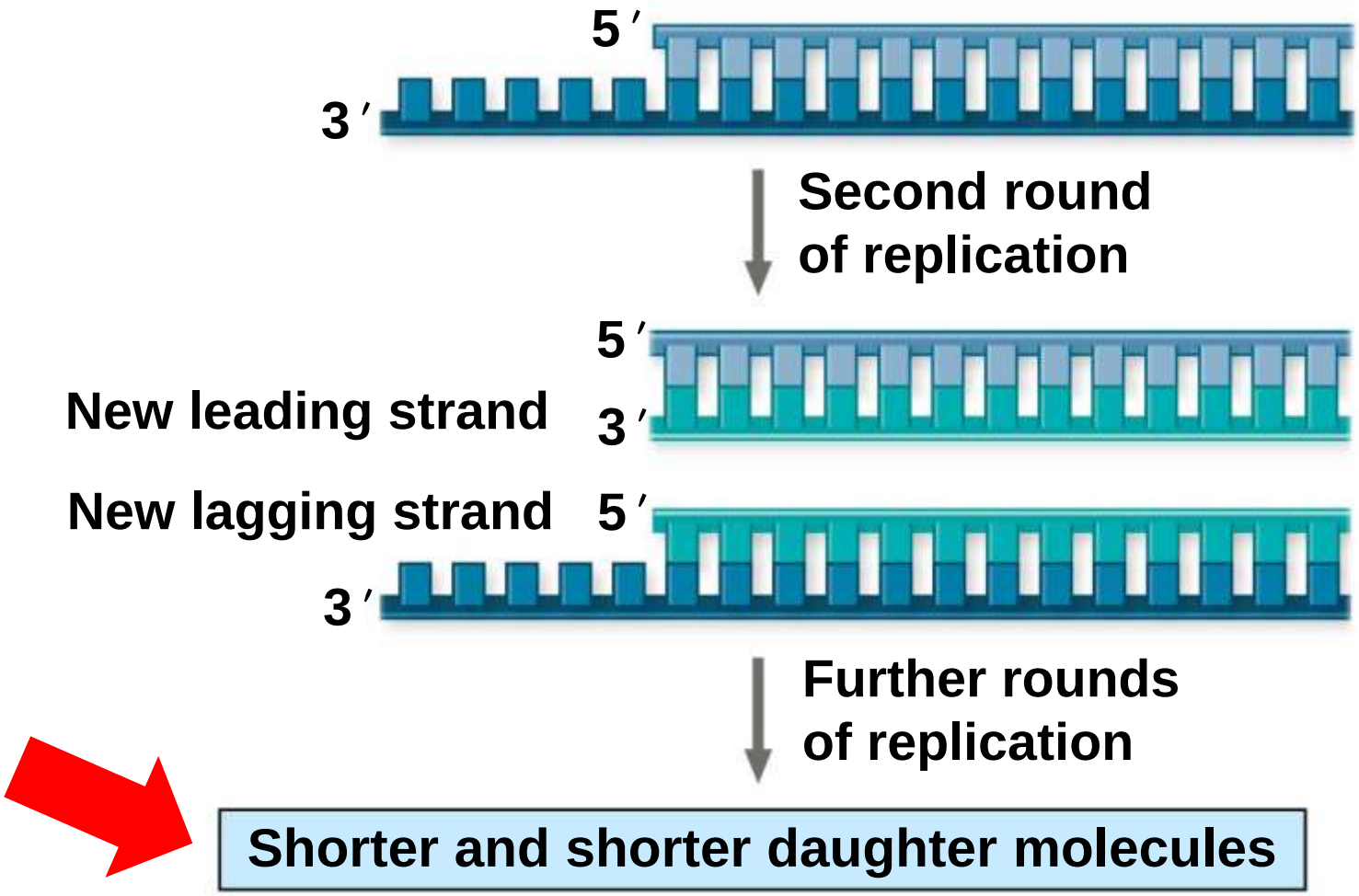


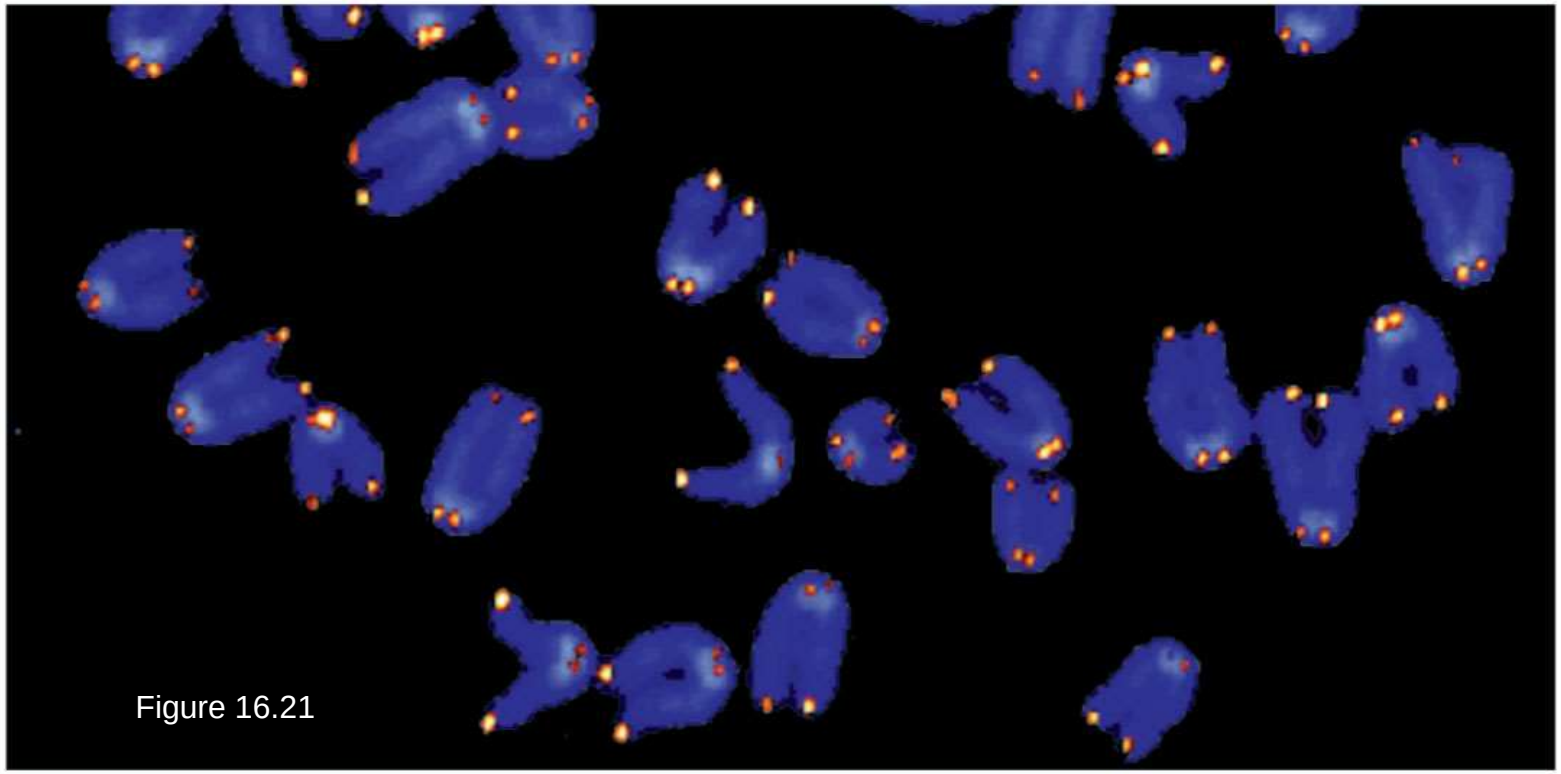
Figure 16.20b



Telomeres 端粒

- Eukaryotic chromosomal DNA molecules have at their ends nucleotide sequences 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

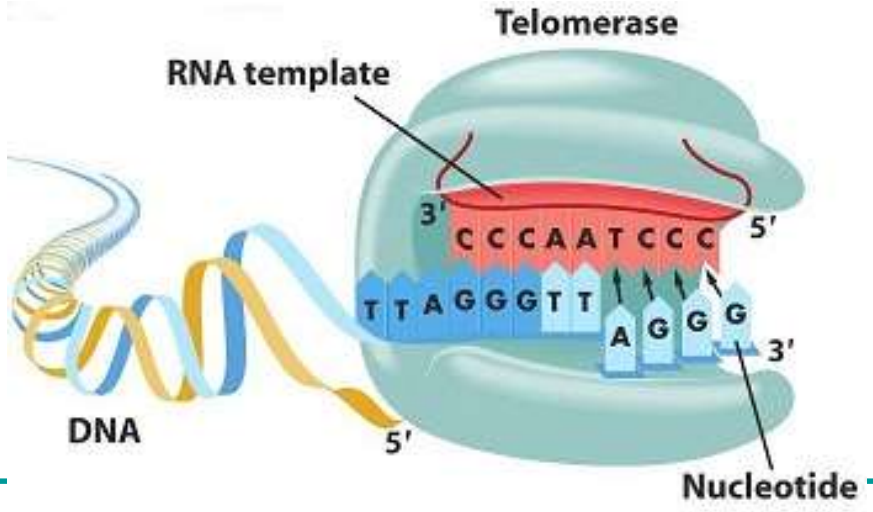
Telomeres
染色體尾端 又稱 端粒 (telomere)



Telomeres are stained orange in these mouse chromosome.

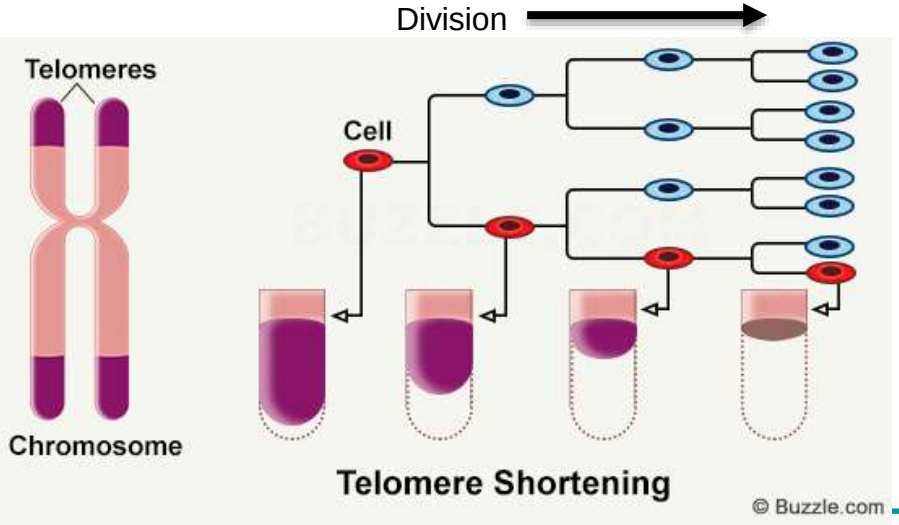
Telomerase 端粒酶

- 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



Protective function of telomeres

- 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



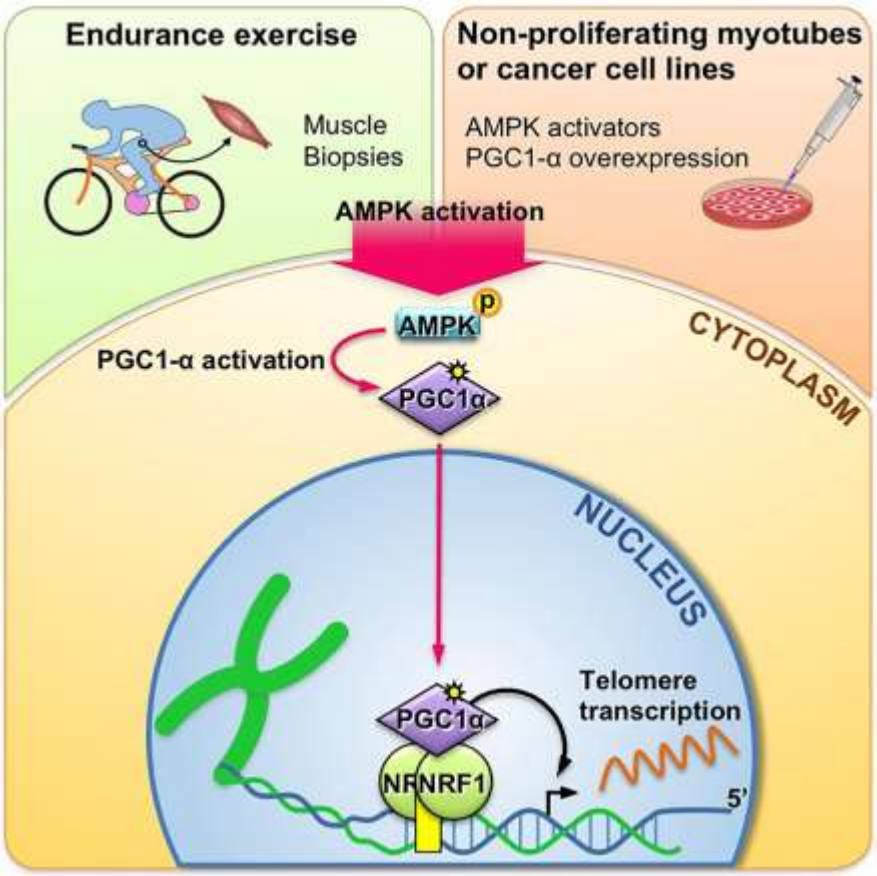
Exercise makes you younger at the cellular level: the telomere effect 運動抗老?

- **Nuclear respiratory factor 1 and endurance exercise promote human telomere transcription.** Sci Adv. 2016 Jul 27;2(7):e1600031
- **Physical activity and telomere length in U.S. men and women: An NHANES investigation.** Preventive Medicine 100 (2017) 145–151

- Physical activity was significantly and meaningfully associated with telomere length in U.S. men and women - high levels of physical activity tend to have longer telomeres, accounting for years of reduced cellular.

常見耐力鍛煉:

健走 (時速 ~6公里，雙手需擺動)
跑步 (時速 8公里+，持續20分鐘+)
跳舞 游泳 自行車 爬樓梯
網球 籃球 足球 羽毛球



思考題: Anti-Aging vs. Exercise

- "Although recent findings on the link of exercise and telomere length is compelling, the study didn't actually measure whether the 45 minutes of endurance exercise led to longer telomeres."
- NRF1 is also part of the pathway that's activated during **starvation**; some studies have indeed hinted that a fasting diet may help cells stay biologically young and not divide as frequently.

1. How to design an experiment to directly measure endurance exercise effect to telomere length?
2. What may happen on telomere length if one combine endurance exercise and fasting diet?

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**

Chromatin and Histones

- **Chromatin** is a complex of DNA and protein, and is found in the nucleus of eukaryotic cells
- **Histones** are proteins that are responsible for the first level of DNA packing in chromatin
  - Histones can undergo chemical modifications that result in changes in chromatin organization

PLAY Animation: DNA Packing

Figure 16.22

Chromatin Packing in a Eukaryotic Chromosome

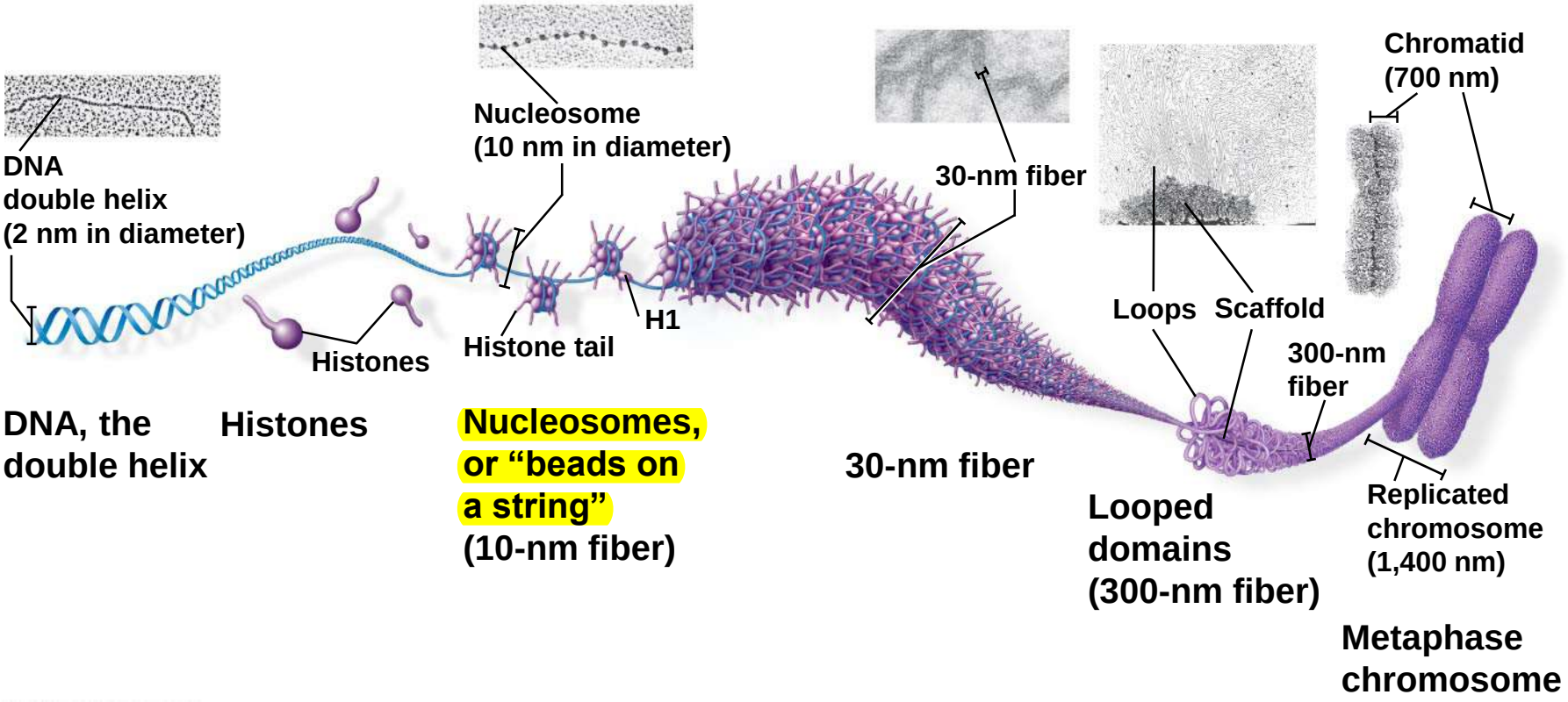
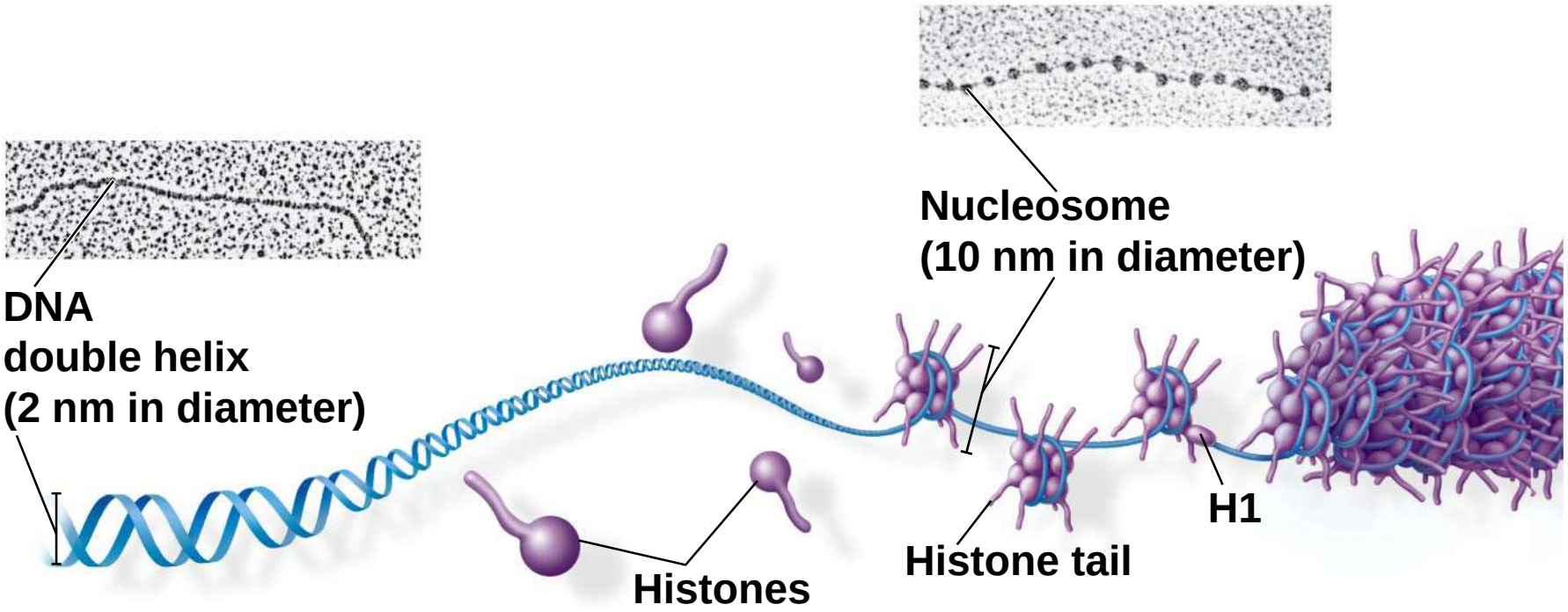


Figure 16.22a



DNA, the double helix

Histones

Nucleosomes, or “beads on a string” (10-nm fiber)

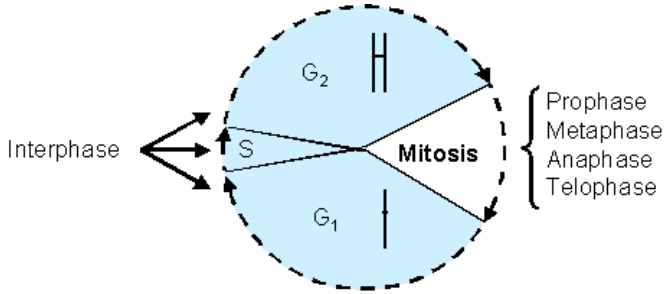
Chromatin is organized into fibers

- **2-nm DNA double helix**
- **10-nm fiber**
  - DNA winds around histones to form **nucleosome** "beads"
  - Nucleosomes are strung together like beads on a string by linker DNA
- **30-nm fiber**
  - Interactions between nucleosomes cause the thin fiber to coil or fold into this thicker fiber

- **300-nm fiber**
  - The 30-nm fiber forms **looped domains** that attach to proteins
- **700-nm Metaphase chromosome**
  - The looped domains coil further
  - The width of a chromatid is 700 nm

Euchromatin and heterochromatin

- 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**
    - It is difficult for the cell to express genetic information coded in these condensed regions

You should now be able to:

- Describe the contributions of the following people: Griffith; Avery, McCary, and MacLeod; Hershey and Chase; Chargaff; Watson and Crick; Franklin; Meselson and Stahl
- Describe the structure of DNA
- Describe the process of DNA replication; include the following terms: antiparallel structure, DNA polymerase, leading strand, lagging strand, Okazaki fragments, DNA ligase, primer, primase, helicase, topoisomerase, single-strand binding proteins
- Describe the function of telomeres
- Compare a bacterial chromosome and a eukaryotic chromosome