CAMPBELL BIOLOGY IN FOCUS

URRY • CAIN • WASSERMAN • MINORSKY • REECE

13 The Molecular Basis of Inheritance

Lecture Presentations by
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Simon Fraser University

SECOND EDITION

Overview: Life's Operating Instructions

- In 1953, James Watson and Francis Crick introduced an elegant double-helical model for the structure of deoxyribonucleic acid, or DNA
- DNA, the substance of inheritance, is the most celebrated molecule of our time
- Hereditary information is encoded in DNA and reproduced in all cells of the body (**DNA replication**)

Figure 13.1

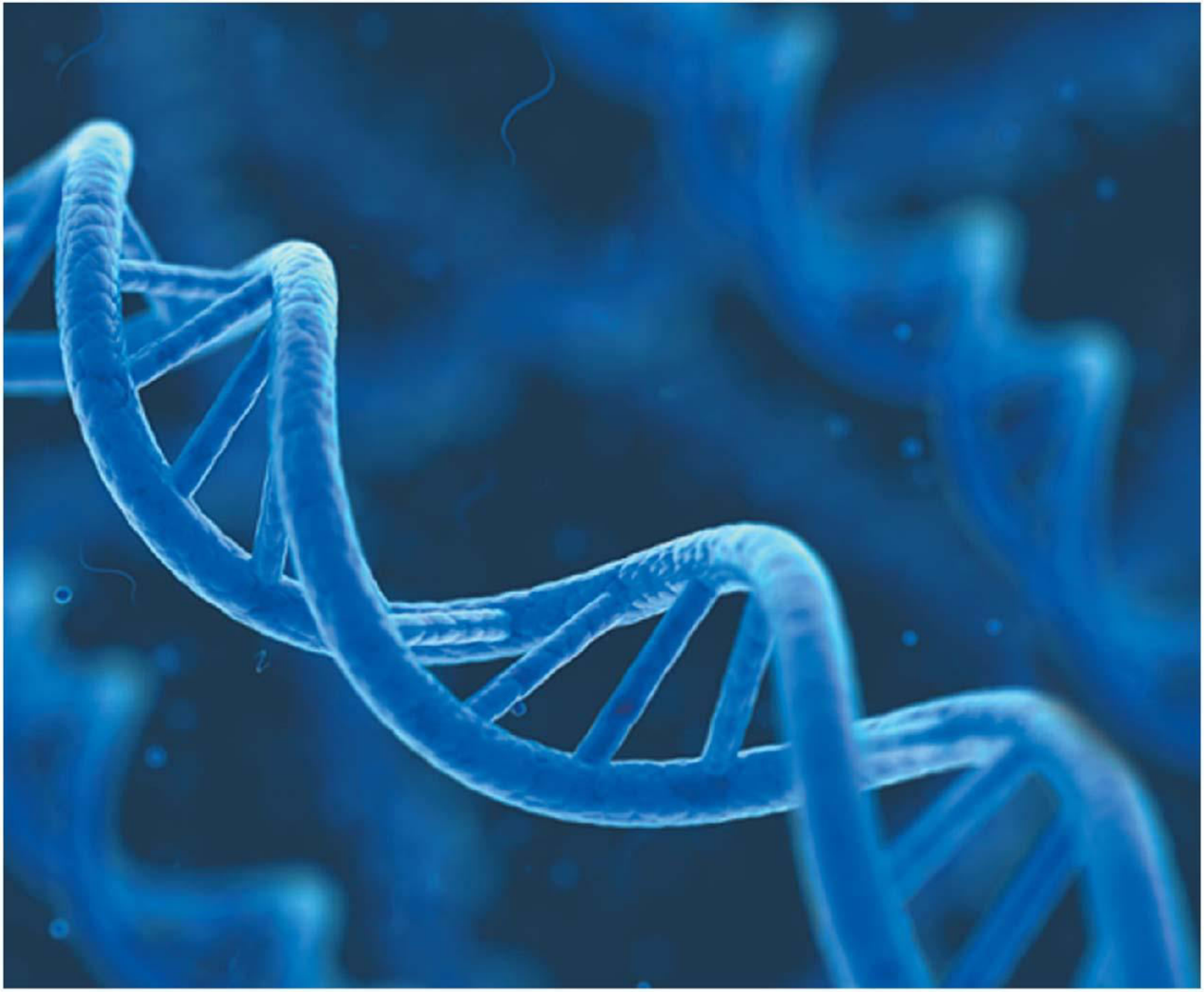
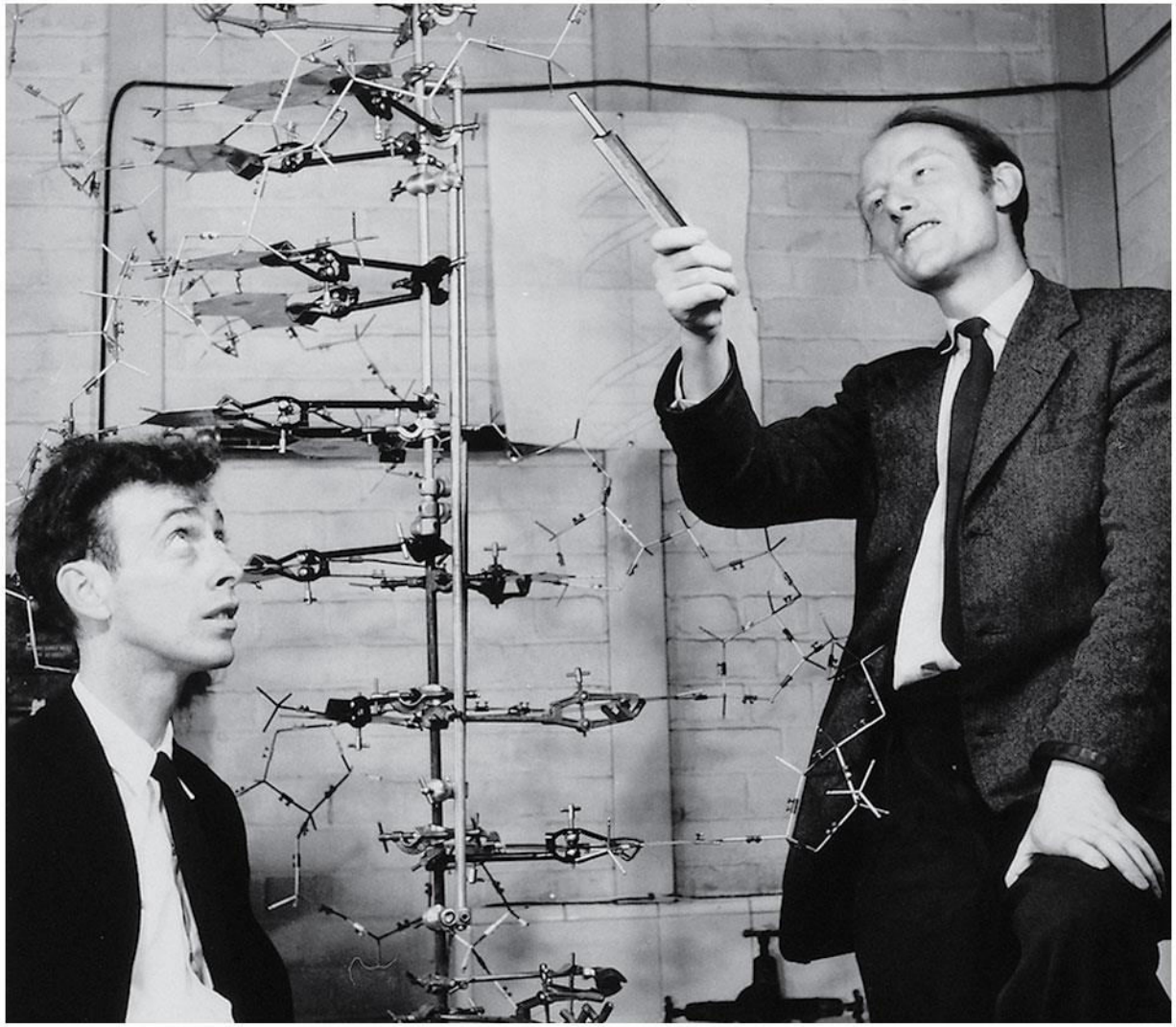


Figure 13.2



Concept 13.1: DNA is the genetic material

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

The Search for the Genetic Material: *Scientific Inquiry*

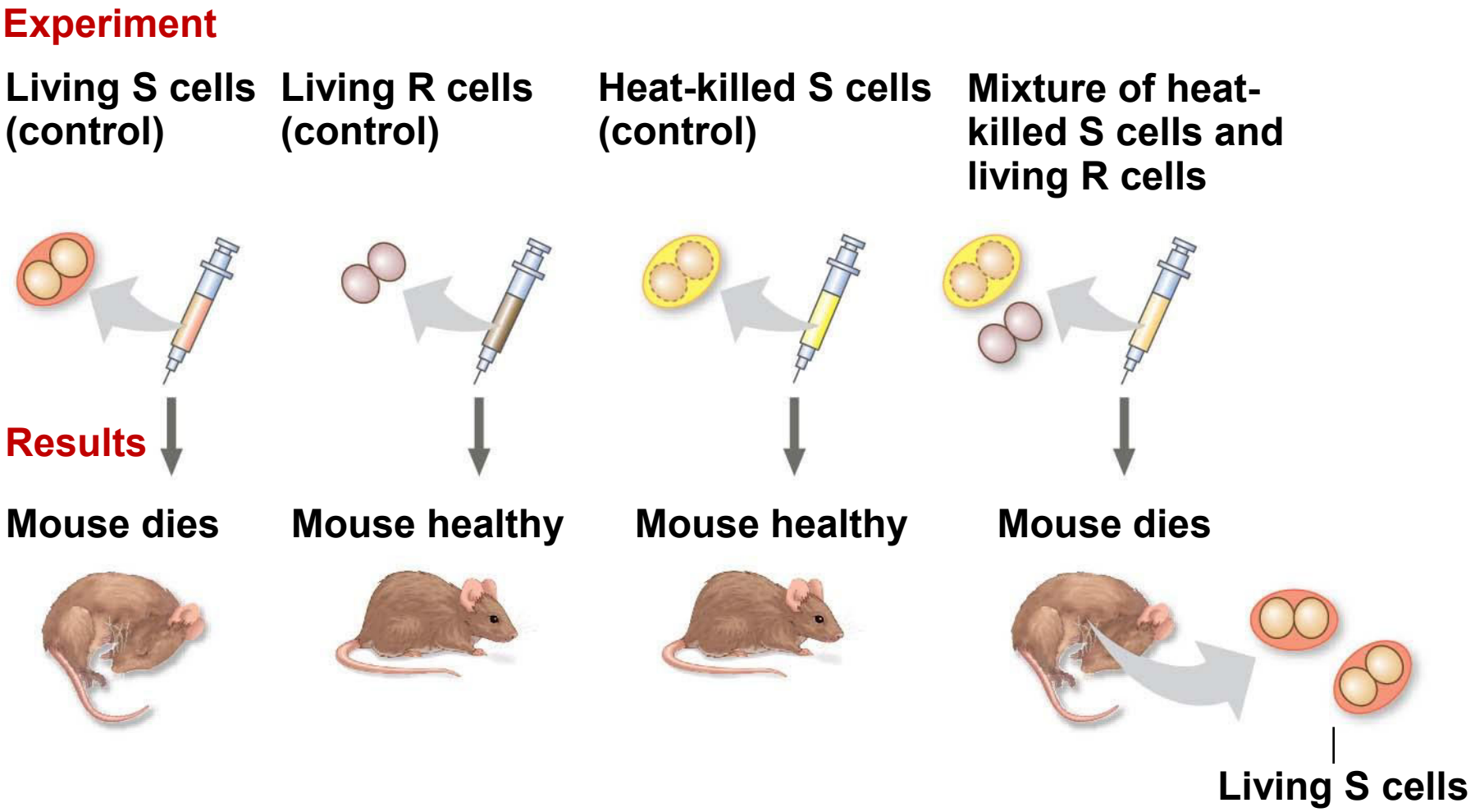
- When T. H. Morgan's group showed that genes are located on chromosomes, the two components of chromosomes—DNA and protein—became candidates for the genetic material
- The key factor in determining the genetic material was choosing appropriate experimental organisms
- The role of DNA in heredity was worked out 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 13.3

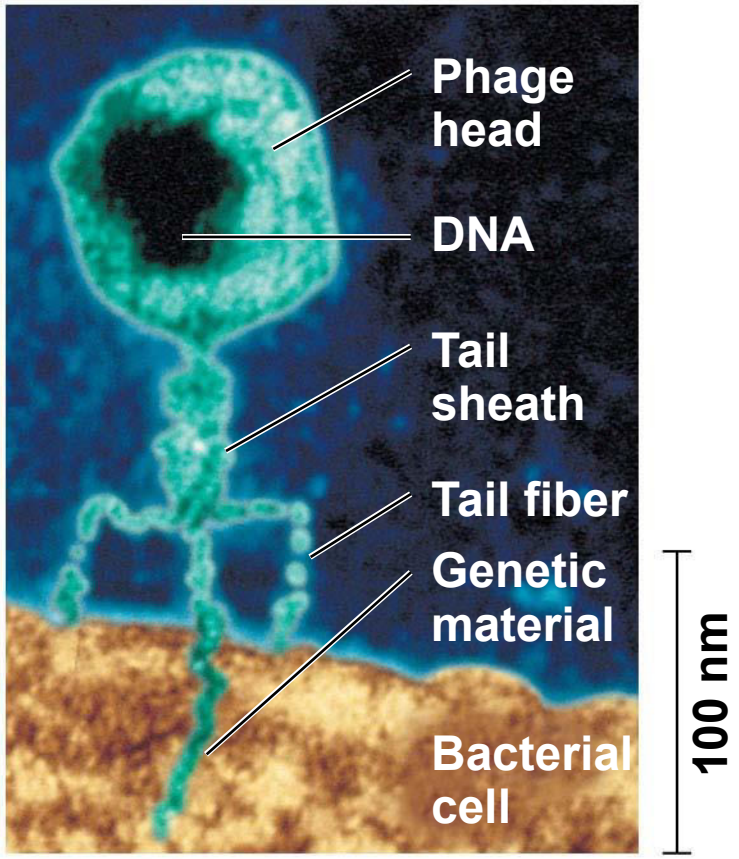


- Later work by Oswald Avery and others identified the transforming substance as DNA
- Many biologists remained skeptical, mainly because little was known about DNA and they thought proteins were better candidates for the genetic material

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**), are widely used in molecular genetics research
- A virus is DNA (or RNA) enclosed by a protective protein coat
- Viruses must infect cells and take over the cells' metabolic machinery in order to reproduce

Figure 13.4



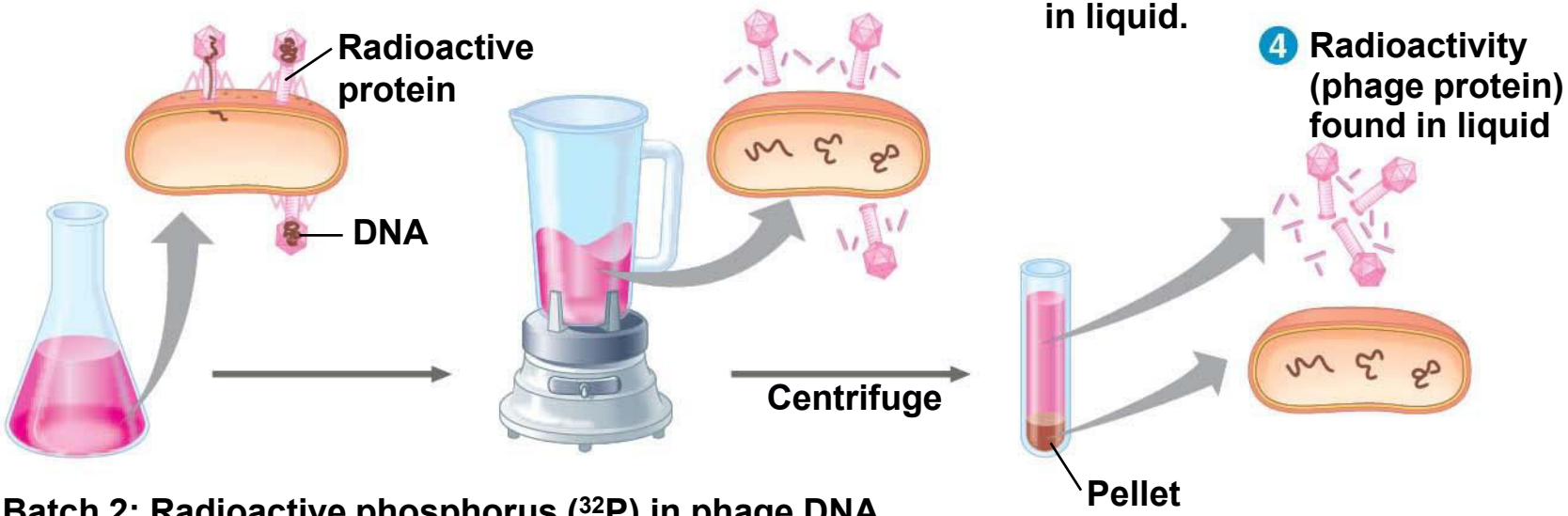
- In 1952, Alfred Hershey and Martha Chase showed that DNA is the genetic material of a phage known as T2
- To determine this, they designed an experiment showing that only the DNA of the T2 phage, and not the protein, enters an *E. coli* cell during infection
- They concluded that the injected DNA of the phage provides the genetic information

Figure 13.5

Experiment

Batch 1: Radioactive sulfur (^{35}S) in phage protein

1. Labeled phages infect cells.
2. Agitation frees outside phage parts from cells.
3. Centrifuged cells form a pellet. Free phages and phage parts remain in liquid.
4. Radioactivity (phage protein) found in liquid



Batch 2: Radioactive phosphorus (^{32}P) in phage DNA

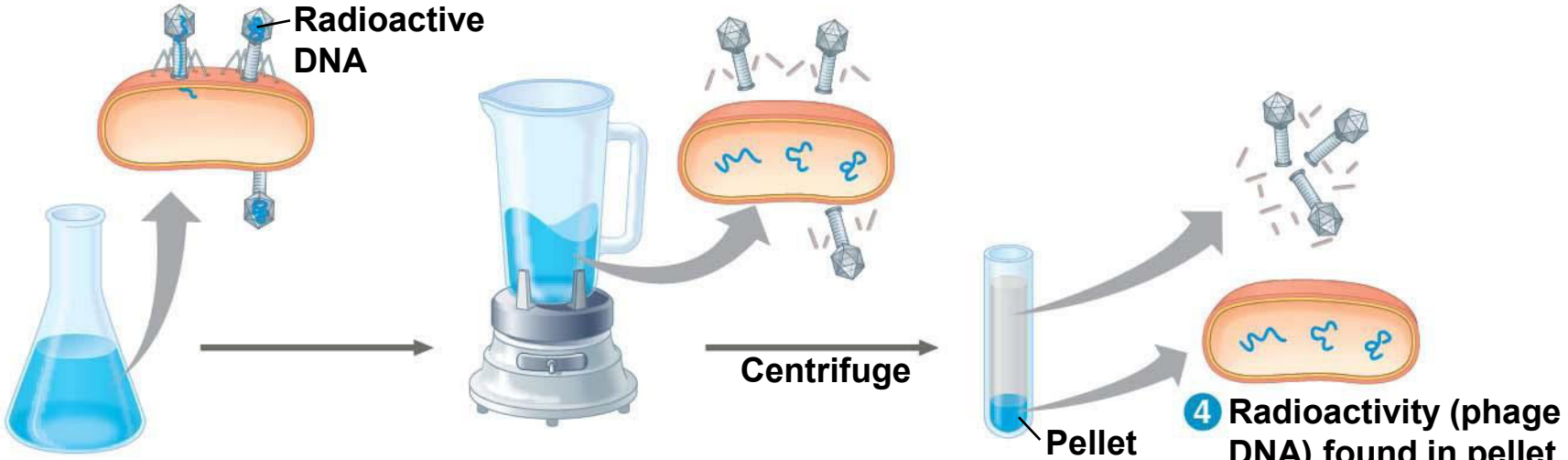


Figure 13.5-1

Experiment

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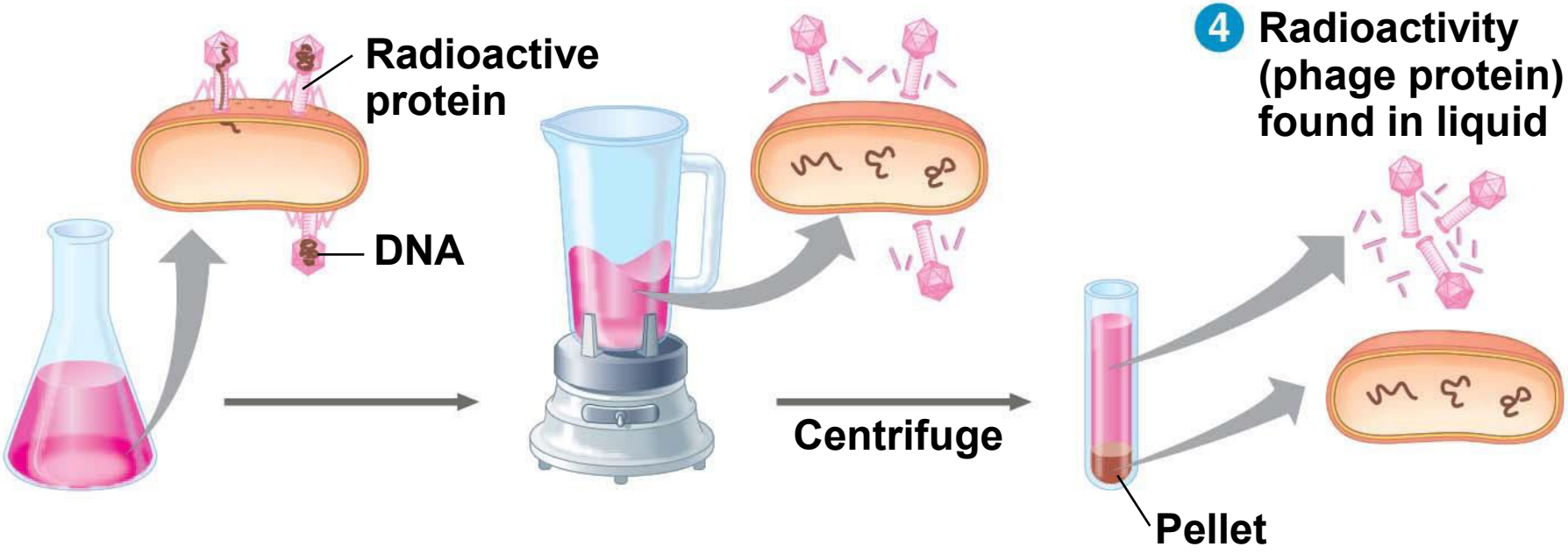
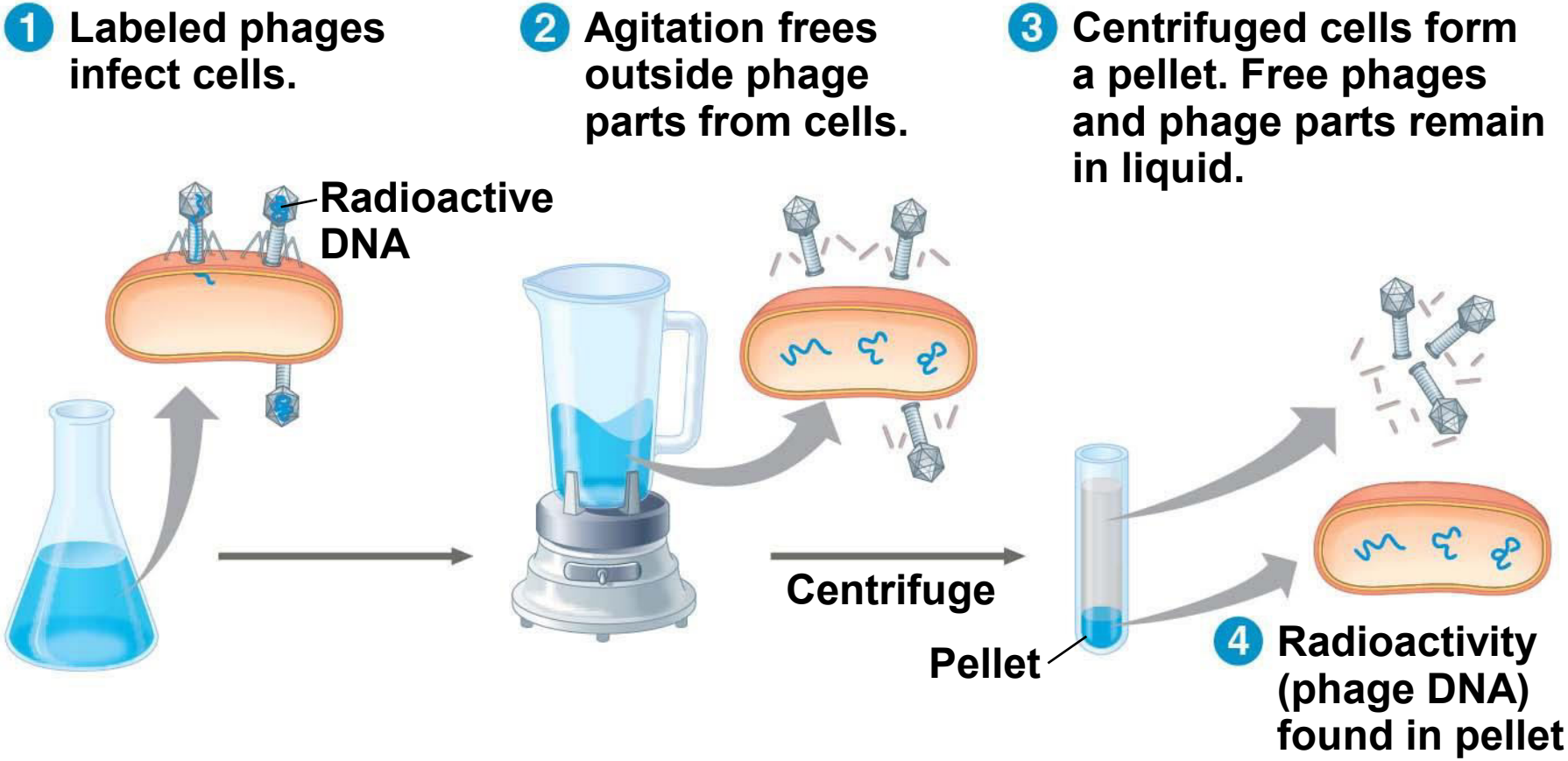


Figure 13.5-2

Experiment

Batch 2: Radioactive phosphorus (^{32}P) in phage DNA



Additional Evidence That 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

Figure 13.6

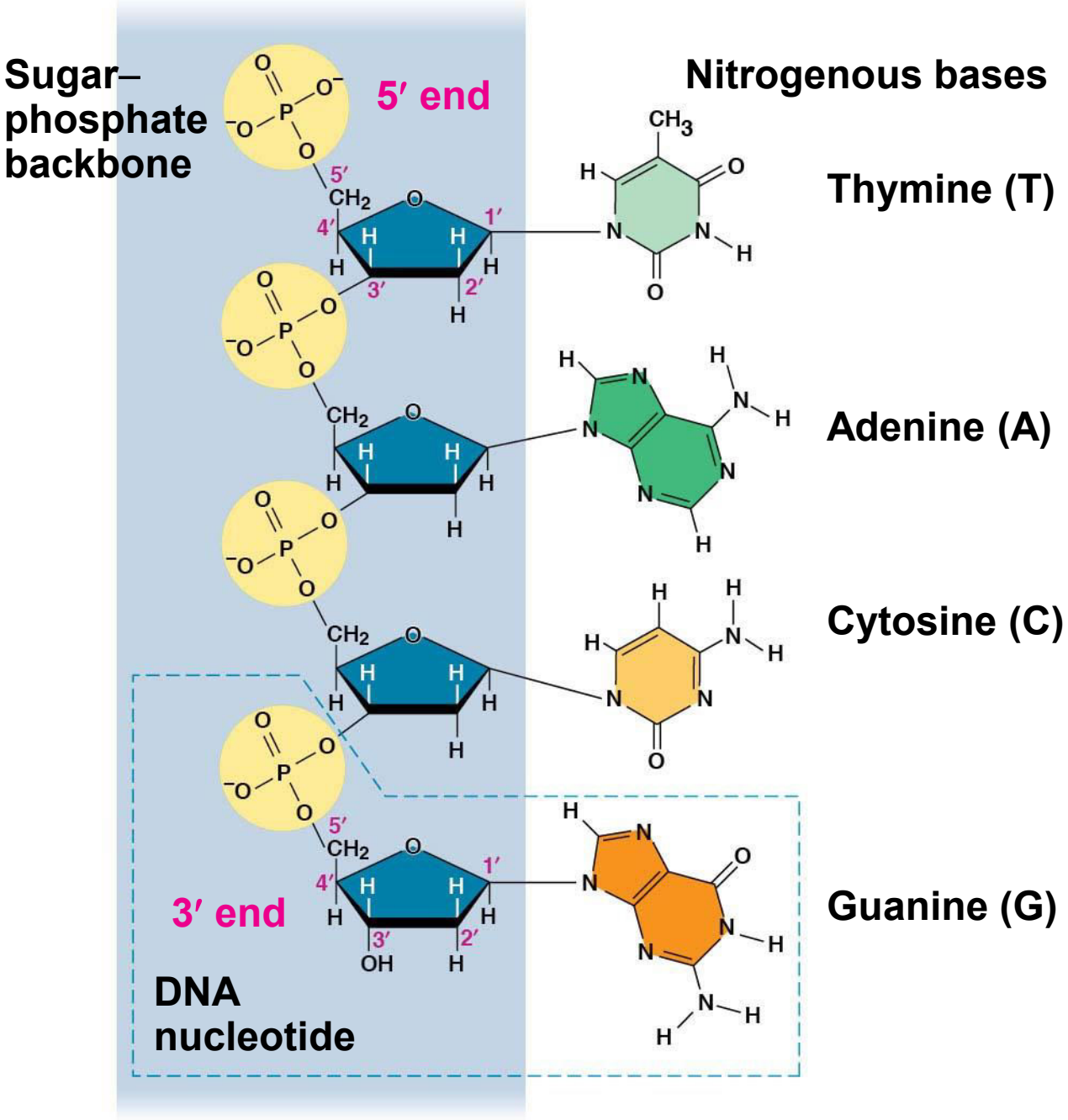
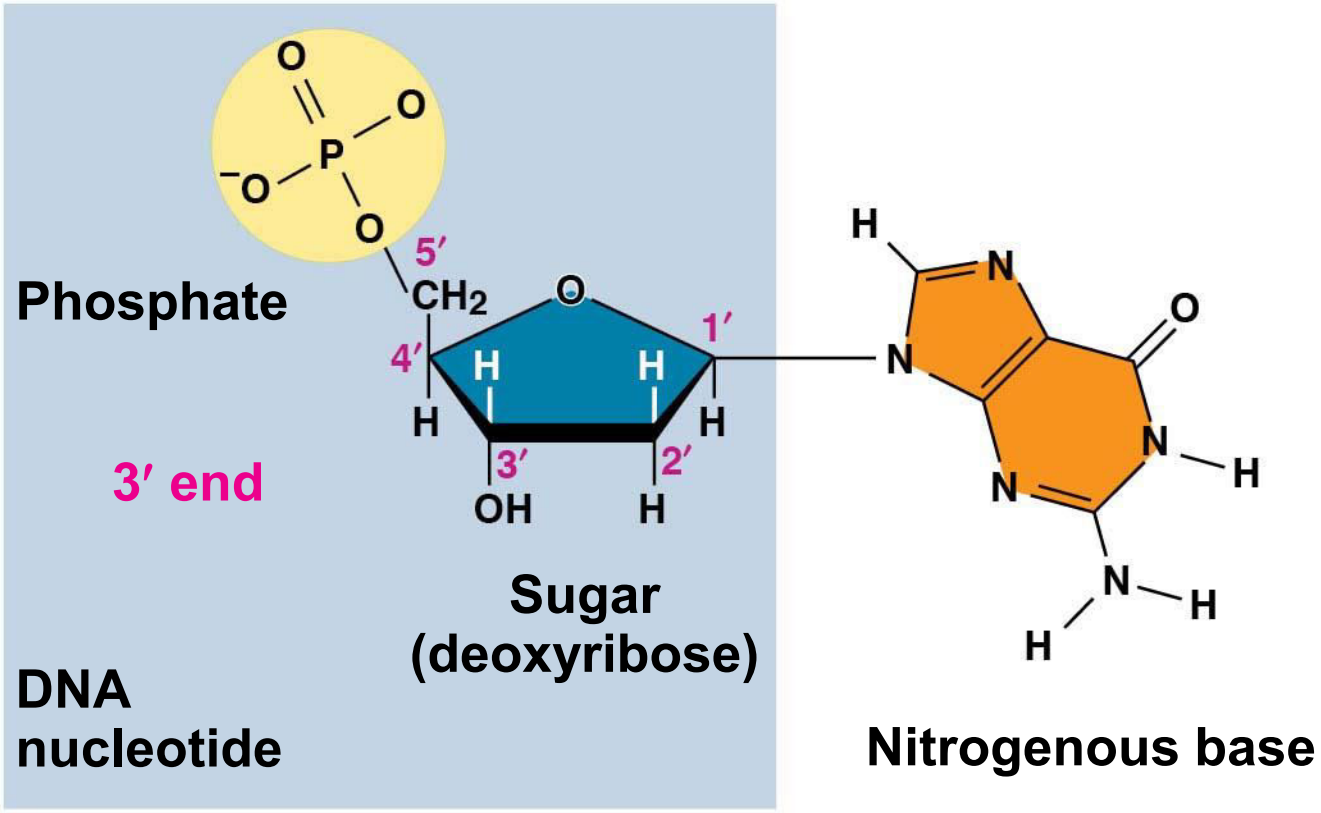


Figure 13.6-1

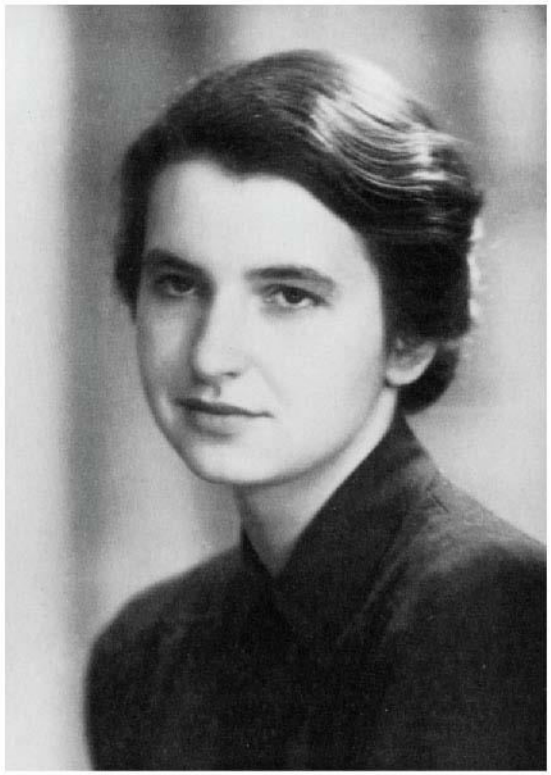


- Two findings became known as Chargaff's rules
  - The base composition of DNA varies between species
  - In any species the percentages of A and T bases are equal and the percentages of G and C bases are equal
- The basis for these rules was not understood until the discovery of the double helix

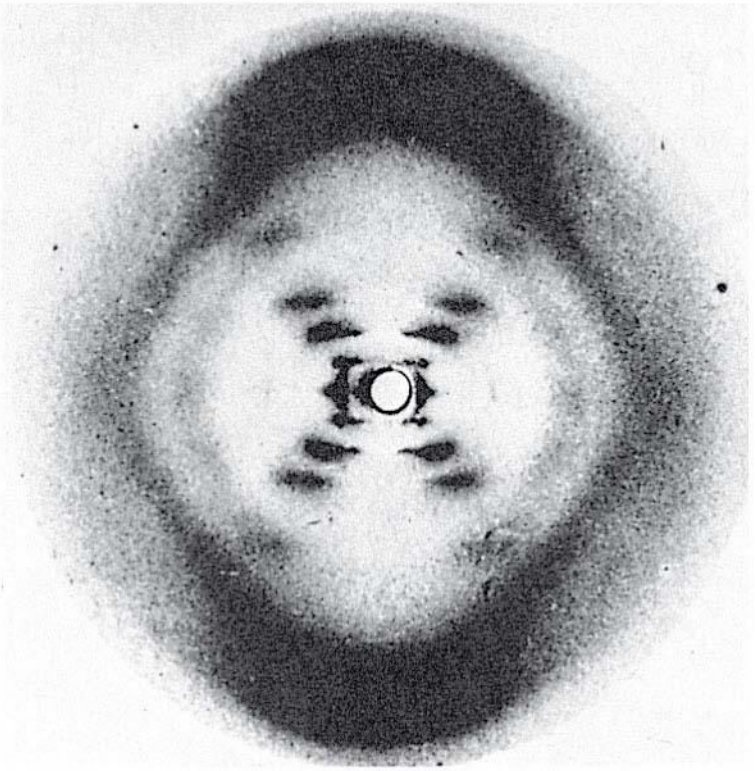
Building a Structural Model of DNA: *Scientific Inquiry*

- James Watson and Francis Crick were first to determine the structure of DNA
- 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 13.7



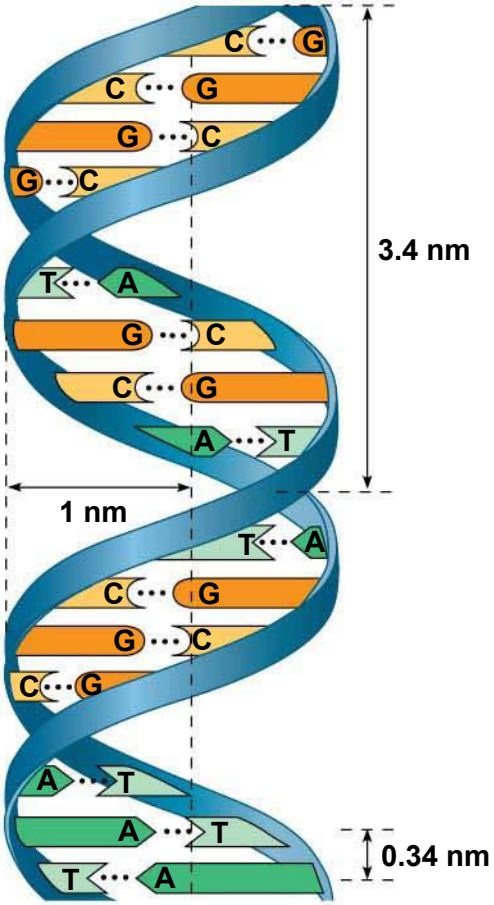
(a) Rosalind Franklin



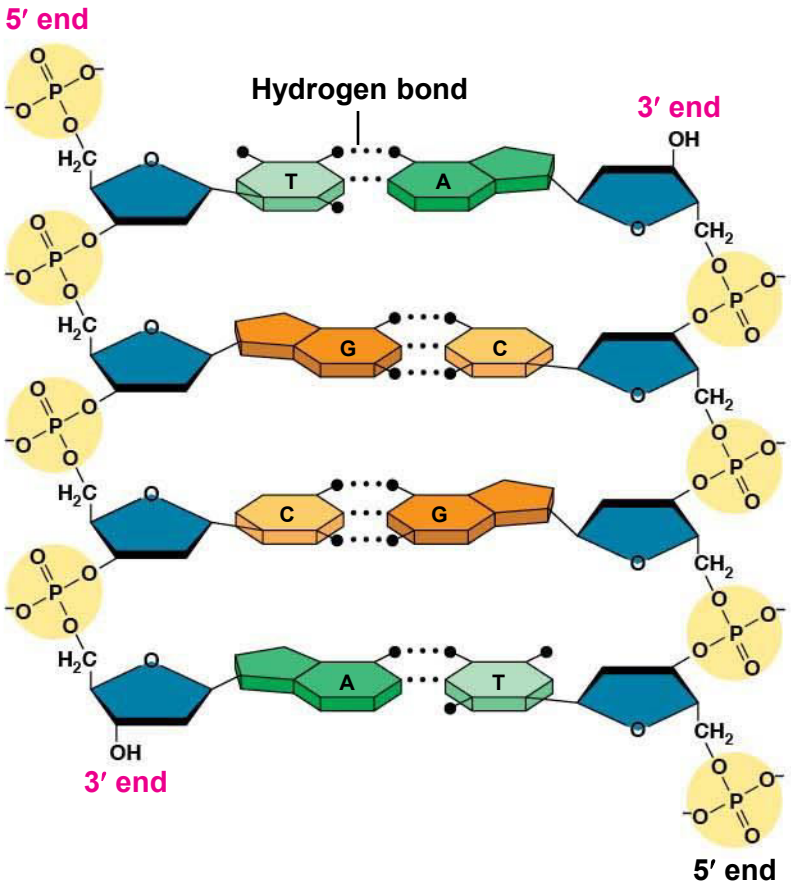
(b) Franklin's X-ray diffraction photograph of DNA

- 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**

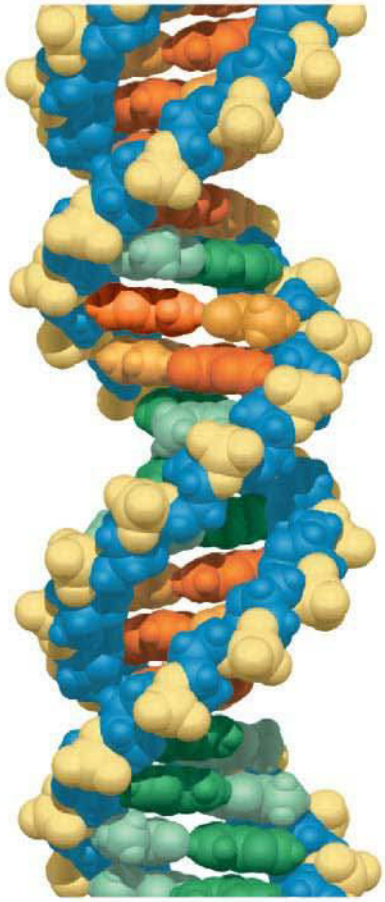
Figure 13.8



(a) Key features of DNA structure

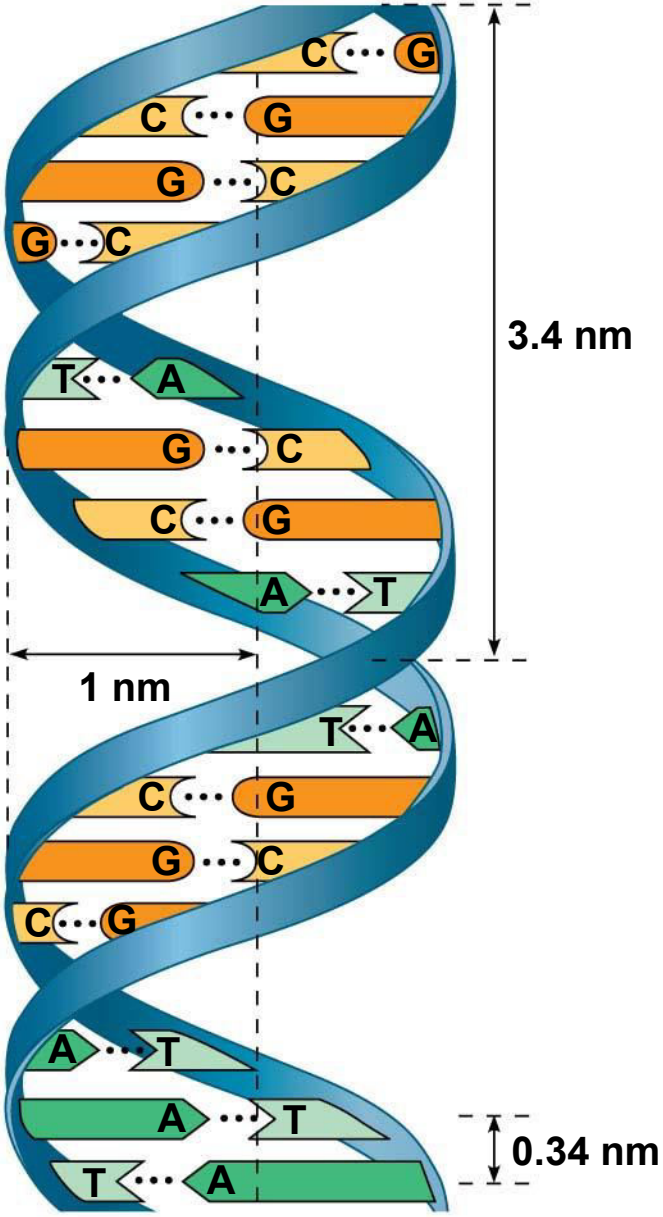


(b) Partial chemical structure



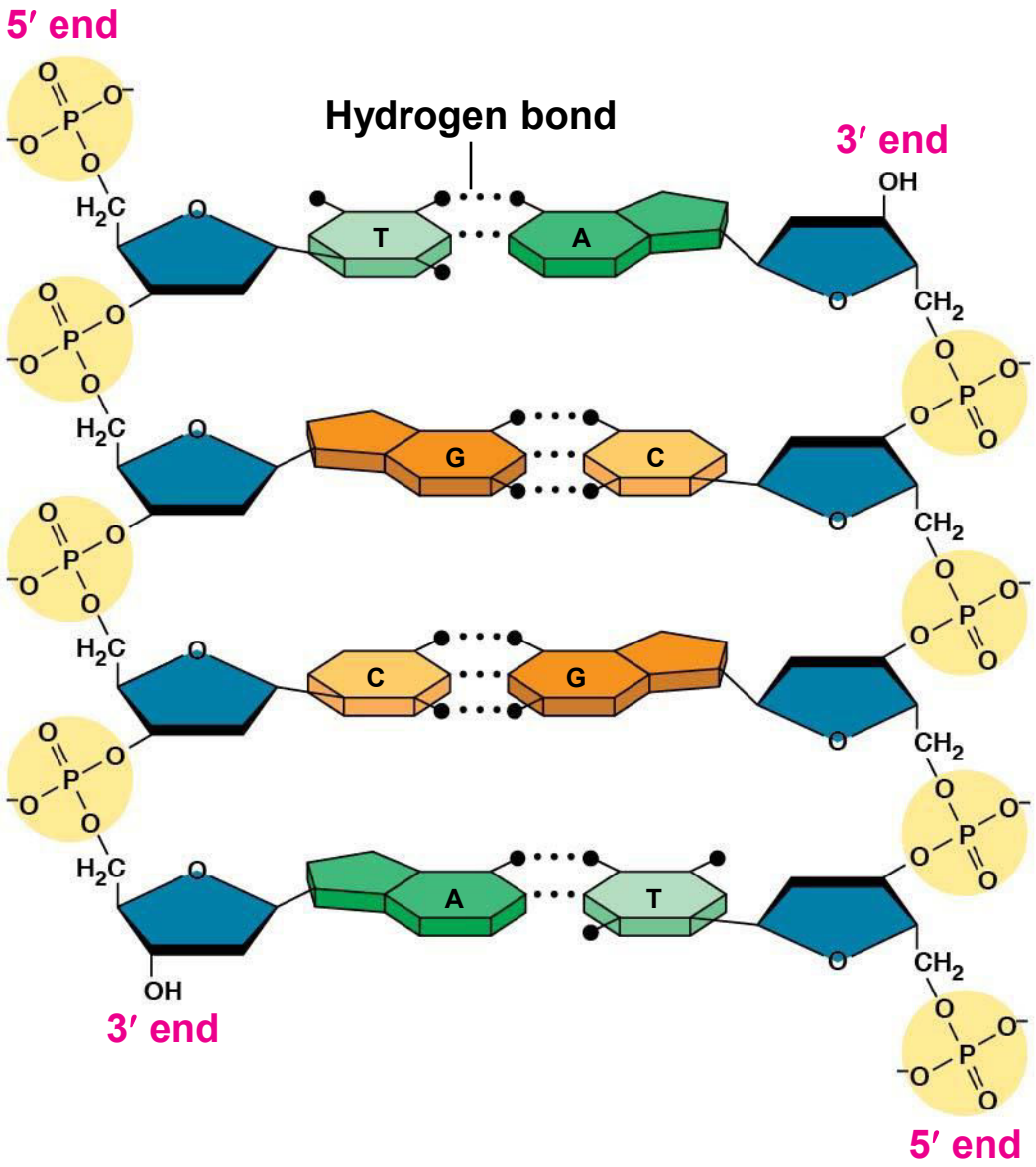
(c) Space-filling model

Figure 13.8-1



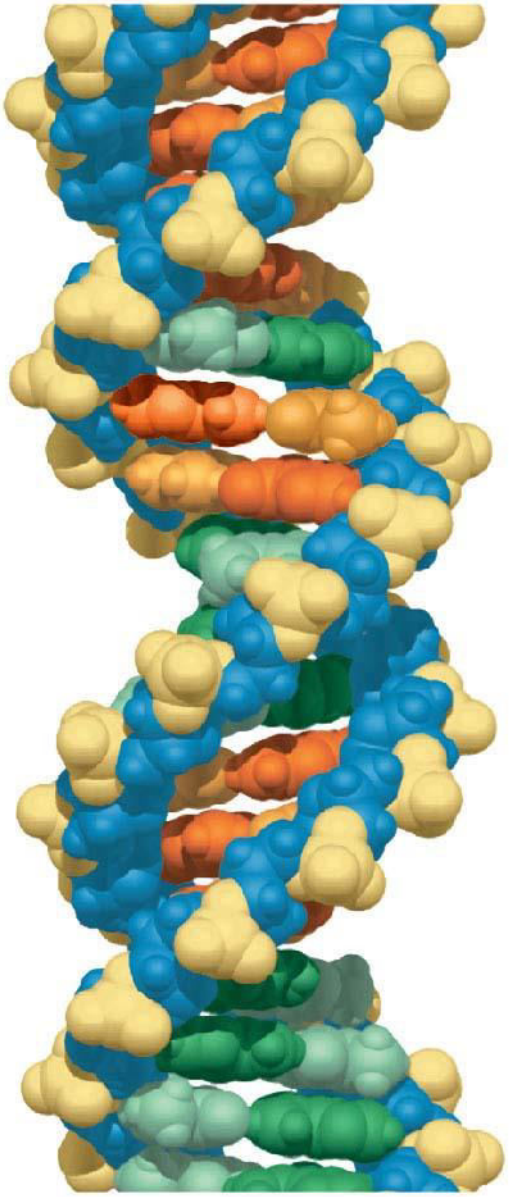
(a) Key features of DNA structure

Figure 13.8-2



(b) Partial chemical structure

Figure 13.8-3

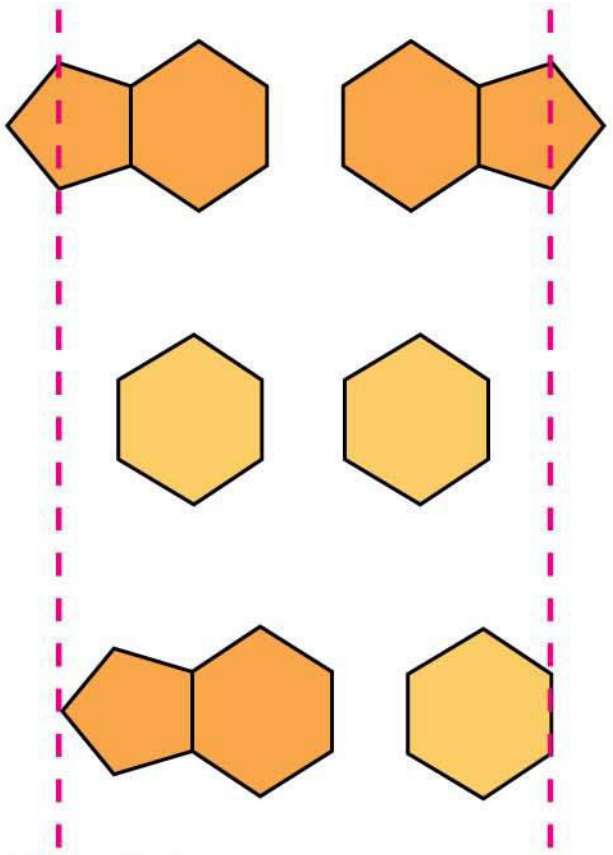


(c) Space-filling model

- Watson and Crick built models of a double helix to conform to the X-ray measurements and the 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 with a pyrimidine resulted in a uniform width consistent with the X-ray data

Figure 13.9



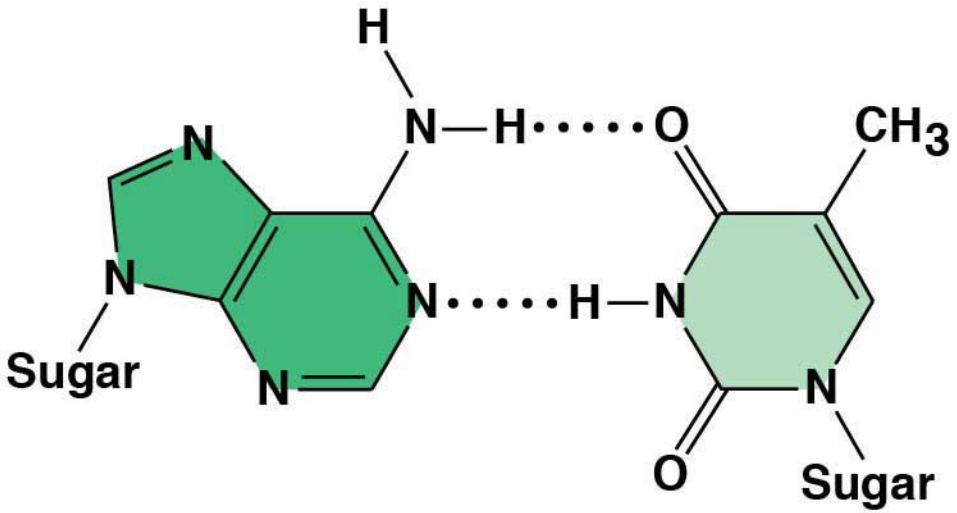
Purine + purine: too wide

Pyrimidine + pyrimidine: too narrow

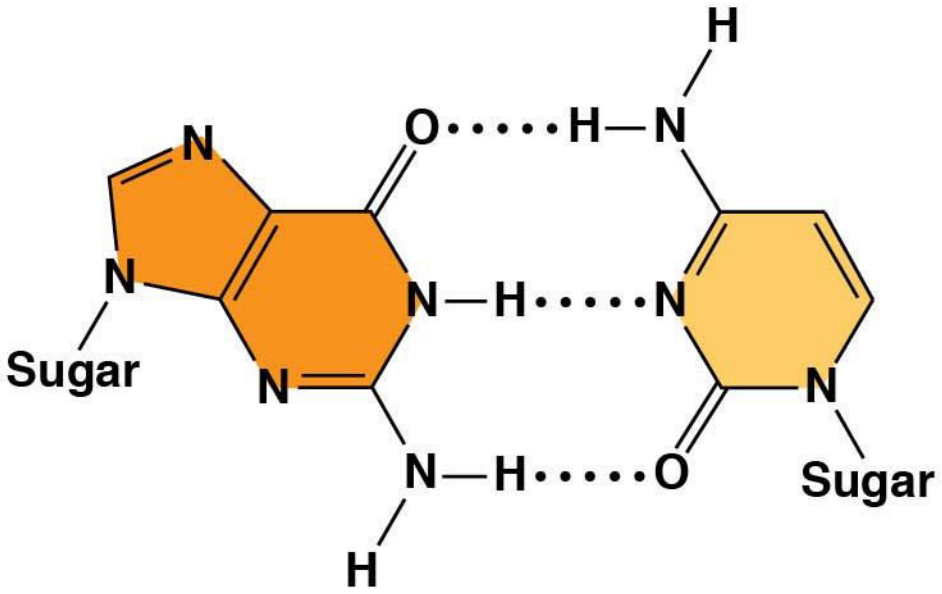
Purine + pyrimidine: width consistent with X-ray data

- 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 = the amount of T, and the amount of G = the amount of C

Figure 13.10



Adenine (A) Thymine (T)

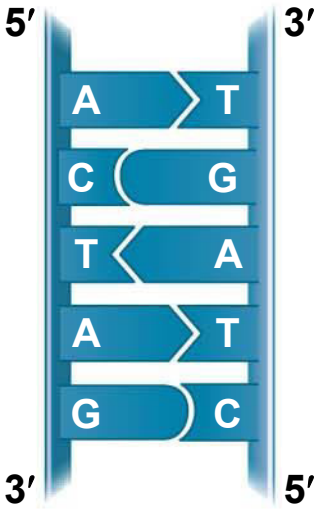


Guanine (G) Cytosine (C)

Concept 13.2: Many proteins work together in DNA replication and repair

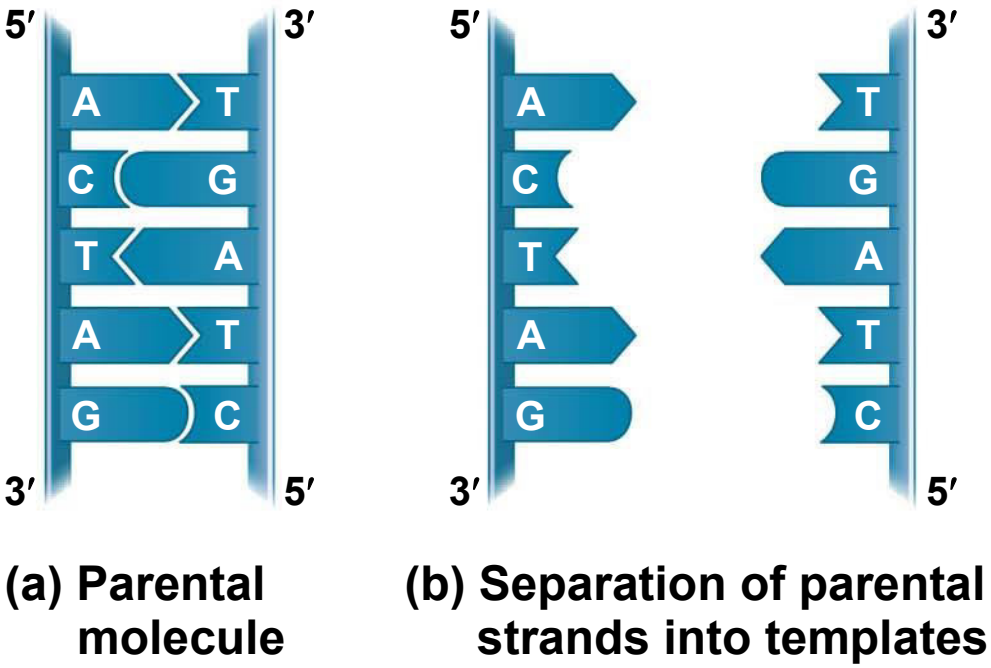
- The relationship between structure and function is manifest in the double helix
- Watson and Crick noted that the specific base pairing suggested a possible copying mechanism for genetic material

Figure 13.11-s1



(a) Parental molecule

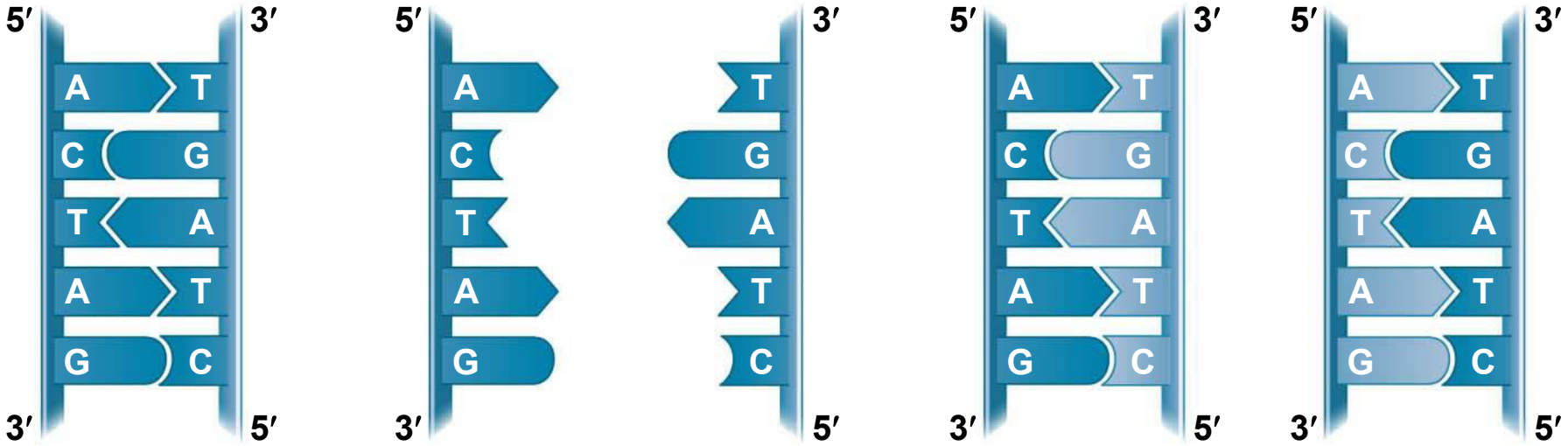
Figure 13.11-s2



(a) Parental molecule

(b) Separation of parental strands into templates

Figure 13.11-s3



(a) Parental molecule

(b) Separation of parental strands into templates

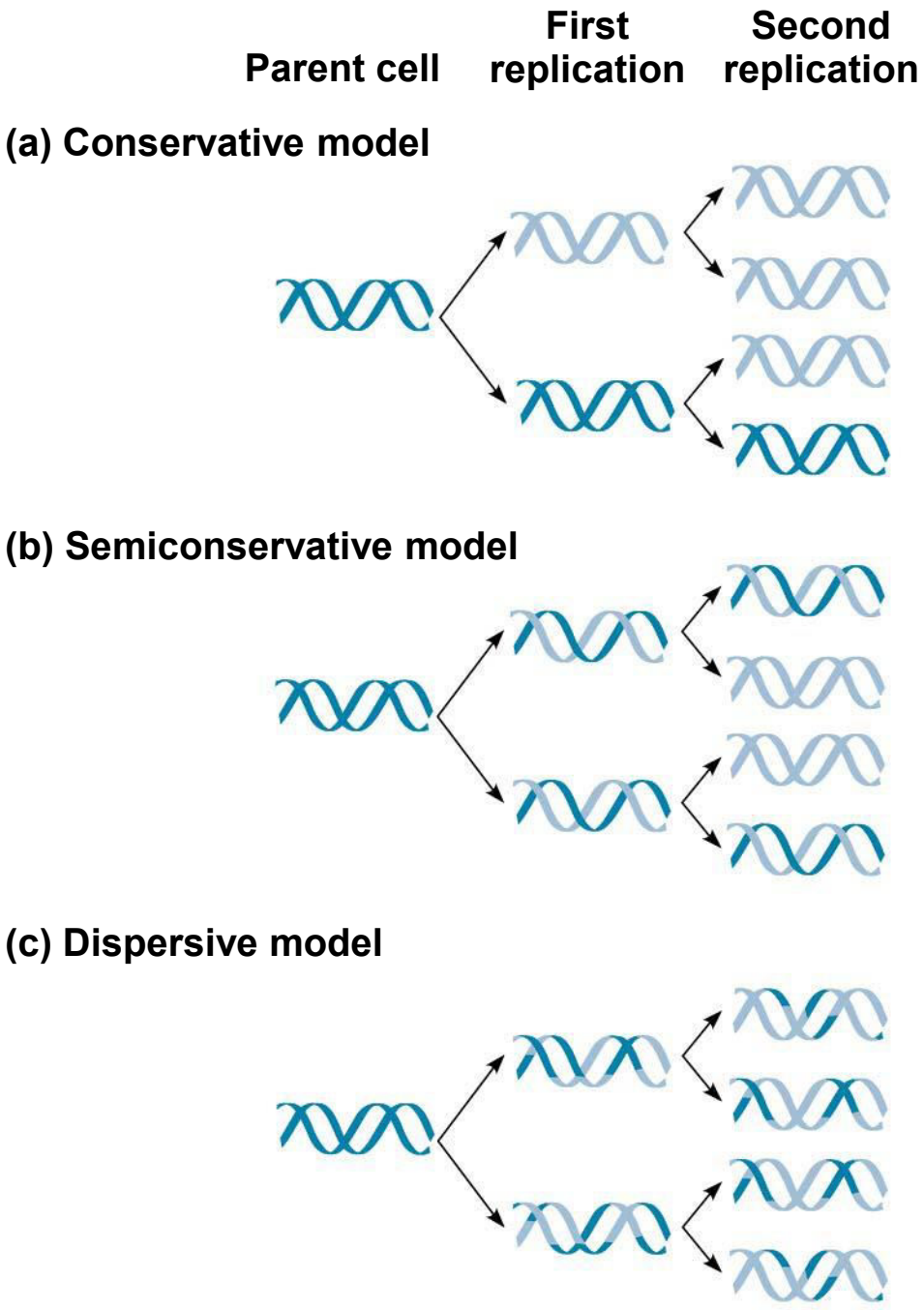
(c) Formation of new strands complementary to template strands

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

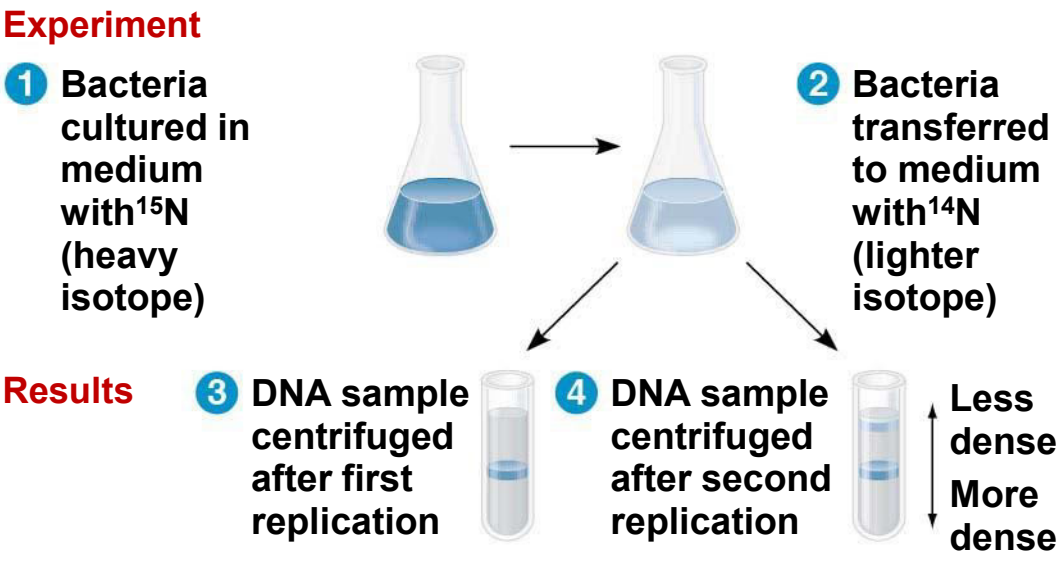
- Watson and Crick's **semiconservative model** of replication predicts that when a double helix replicates, each daughter molecule will have one old strand (derived or "conserved" from the parent molecule) and one newly made strand
- Competing models were the conservative model (the two parent strands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

Figure 13.12



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

Figure 13.13



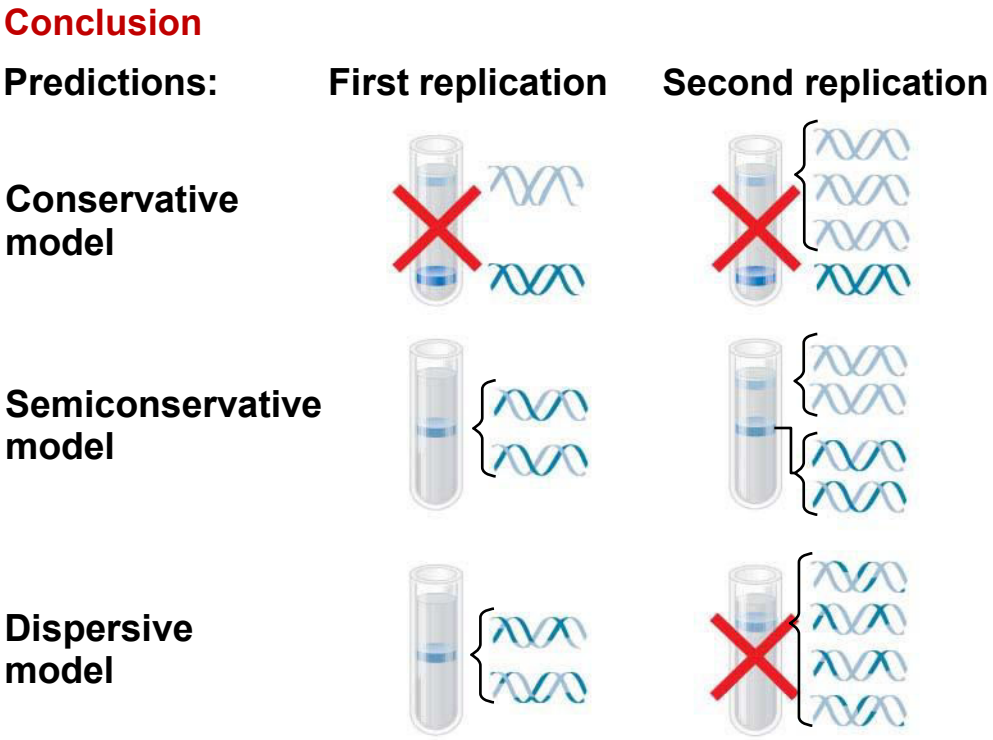
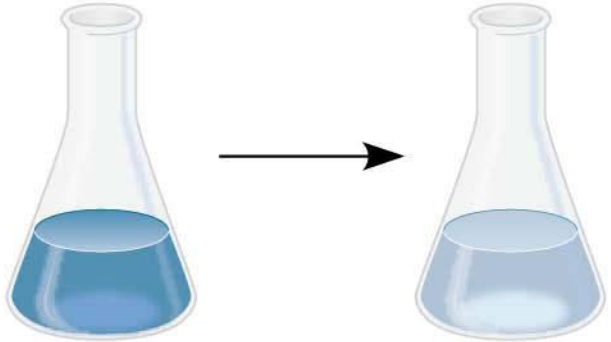


Figure 13.13-1

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

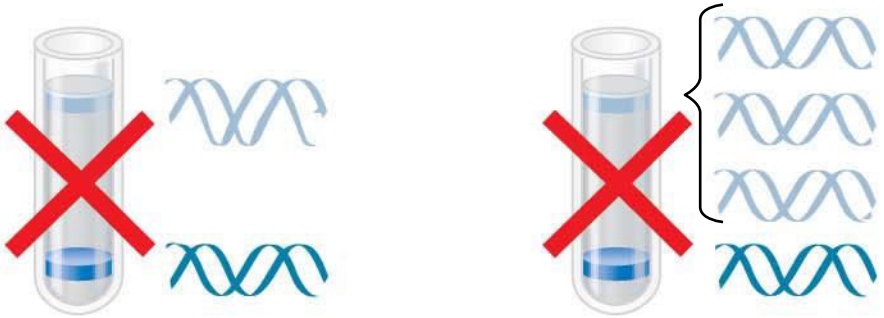
More dense

Figure 13.13-2

Conclusion

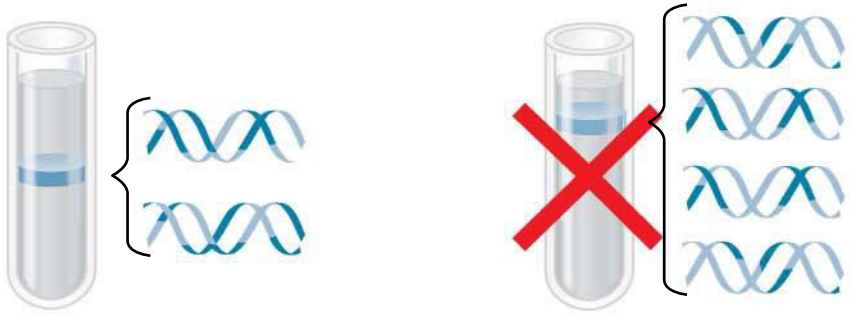
Predictions: **First replication** **Second replication**

Conservative model



Semiconservative model

Dispersive model



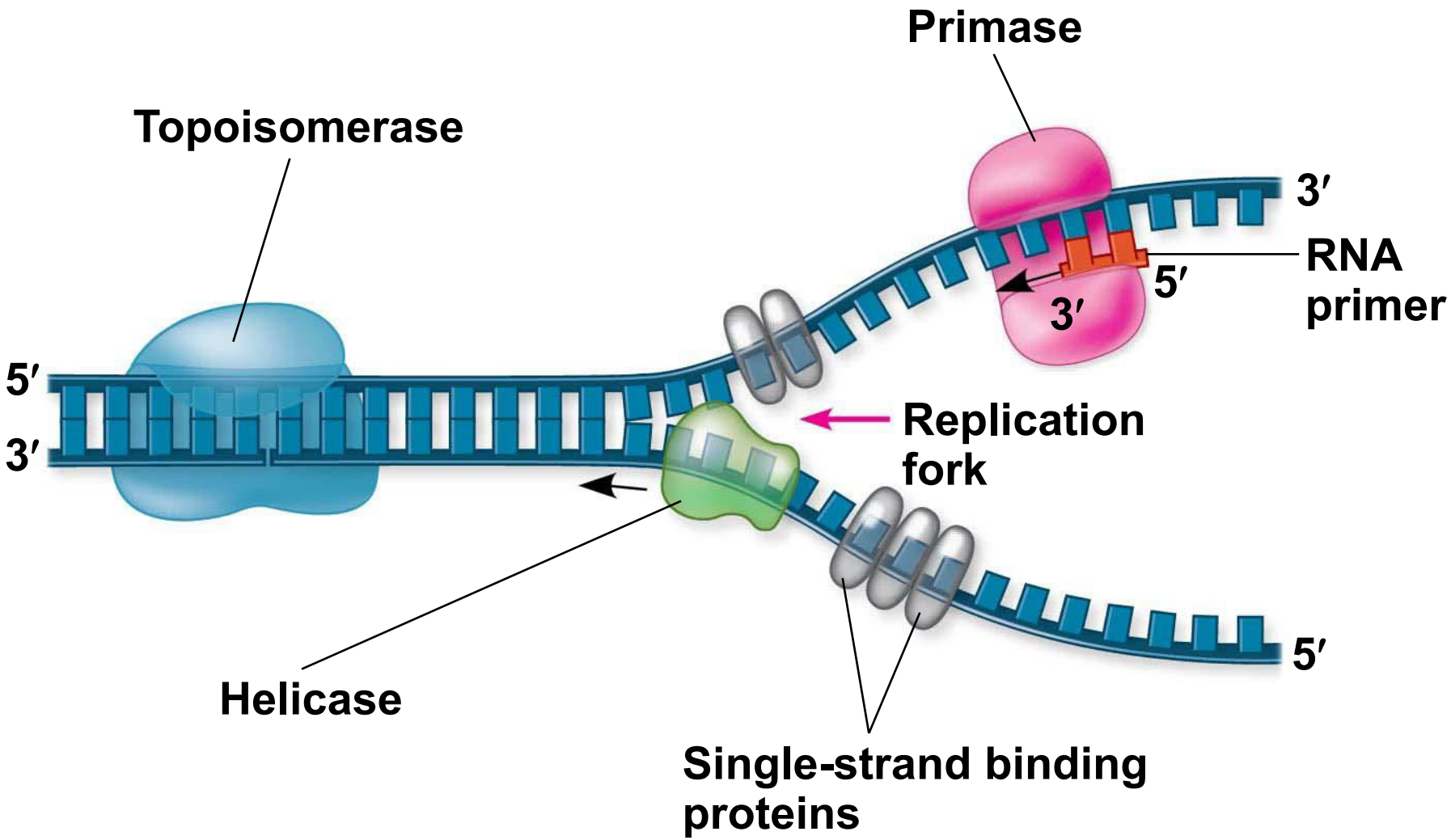
DNA Replication: *A Closer Look*

- The copying of DNA is remarkable in its speed and accuracy
- More than a dozen enzymes and other proteins participate in DNA replication
- Much more is known about how this “replication machine” works in bacteria than in eukaryotes
- Most of the process is similar between prokaryotes and eukaryotes

Getting Started

- Replication begins at sites called **origins of replication**, where the two DNA strands are separated, opening up a replication "bubble"
- At each end of a bubble is a **replication fork**, a Y-shaped region where the parental strands of DNA are being unwound

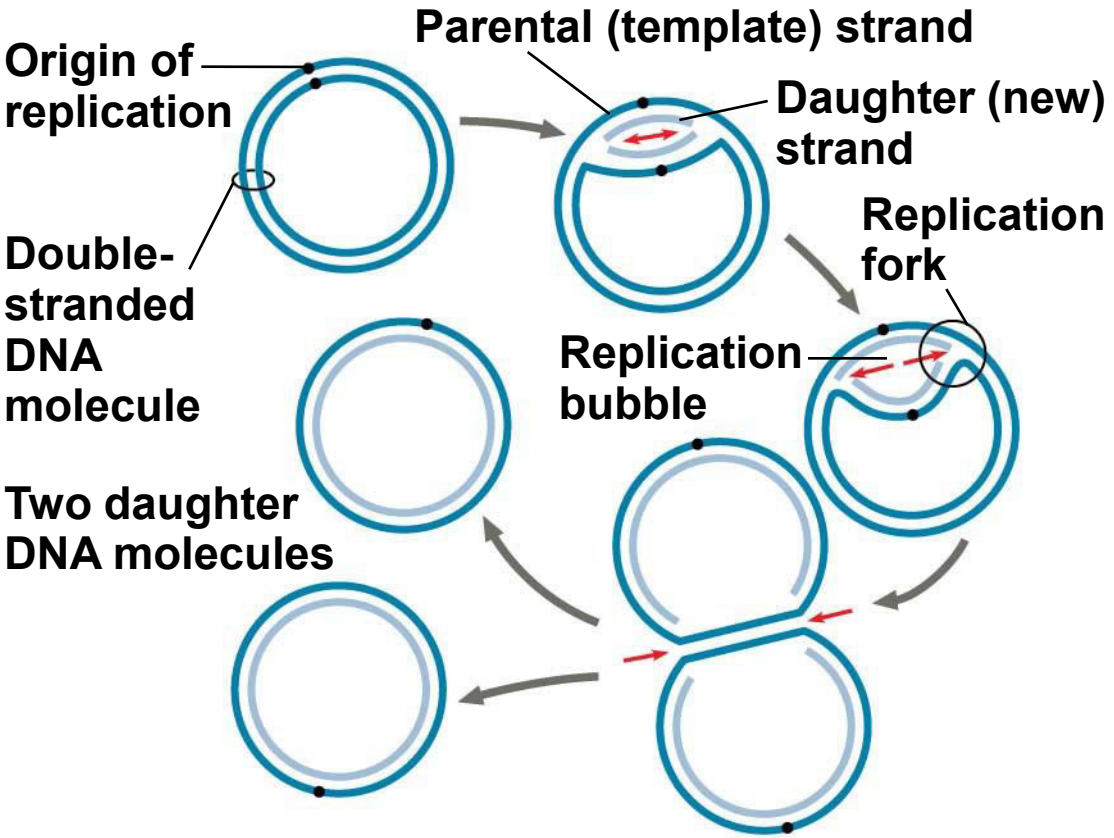
Figure 13.14



- **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 caused by tight twisting ahead of the replication fork by breaking, swiveling, and rejoining DNA strands

Figure 13.15-1

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



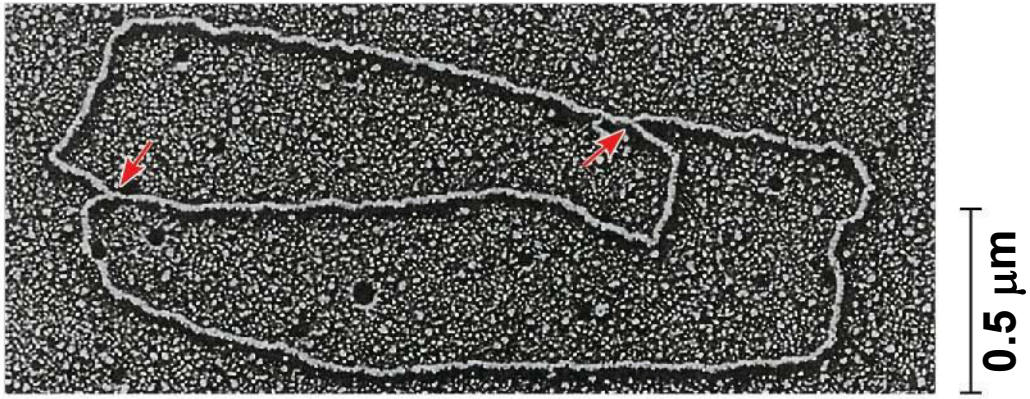
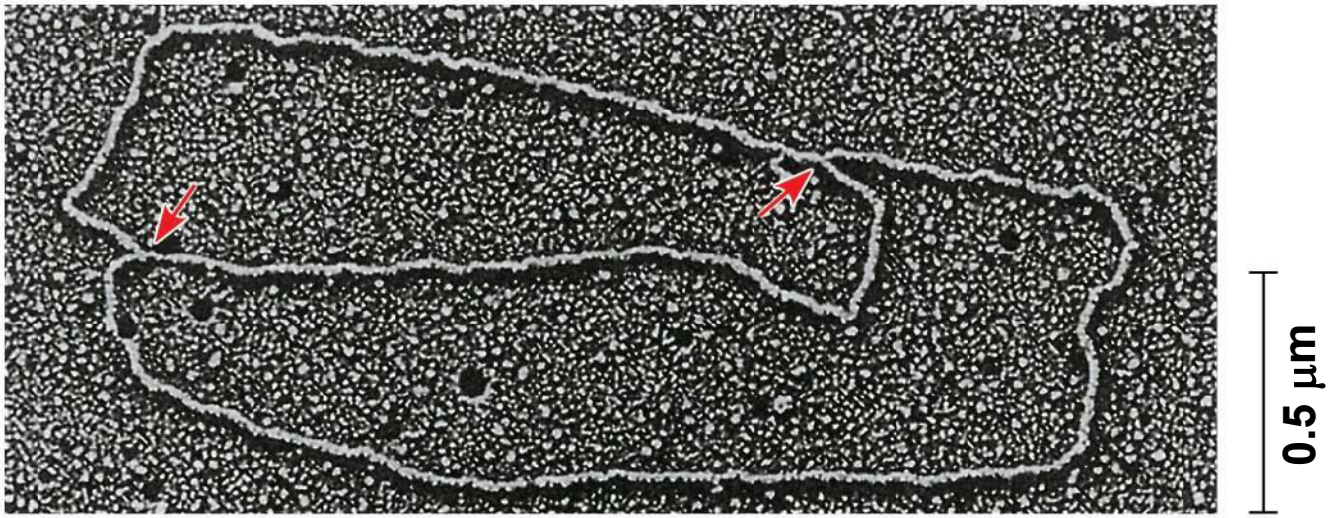


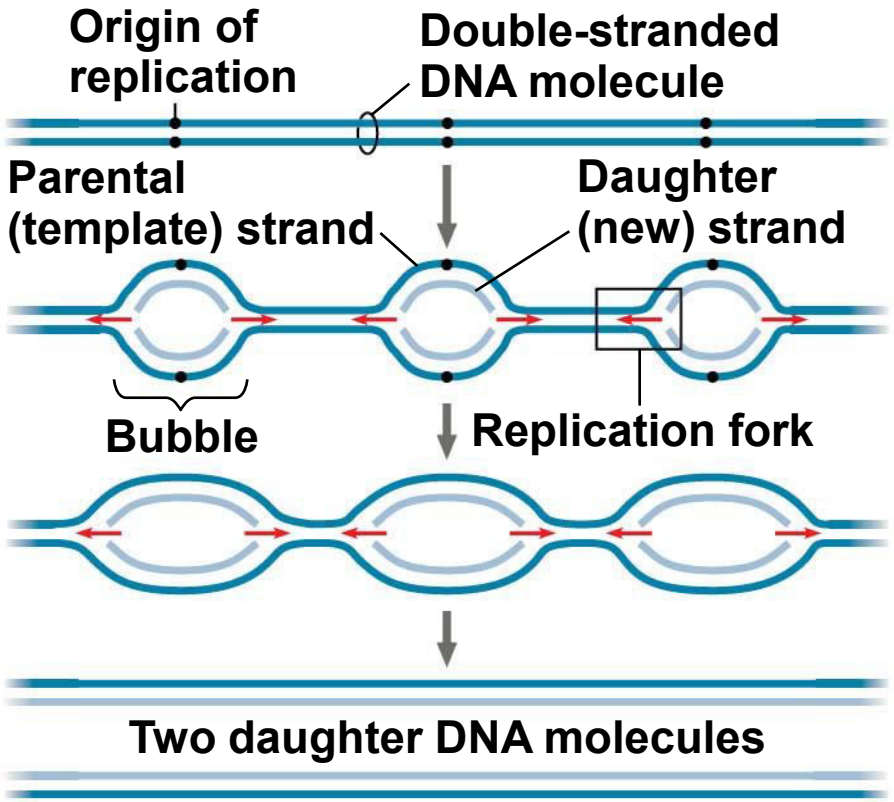
Figure 13.15-1a

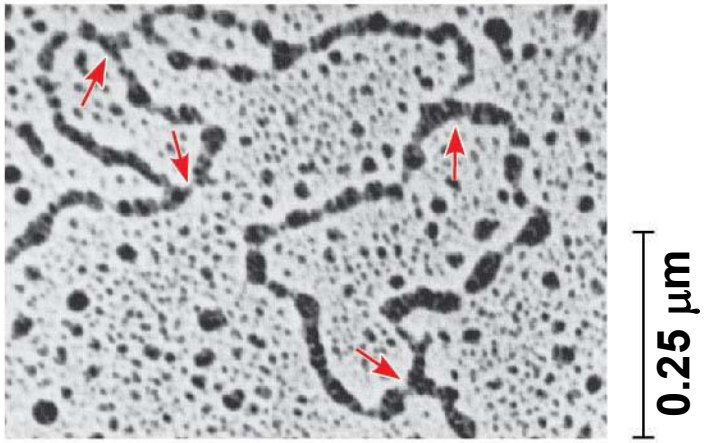


- Multiple replication bubbles form and eventually fuse, speeding up the copying of DNA

Figure 13.15-2

(b) Origins of replication in a eukaryotic cell



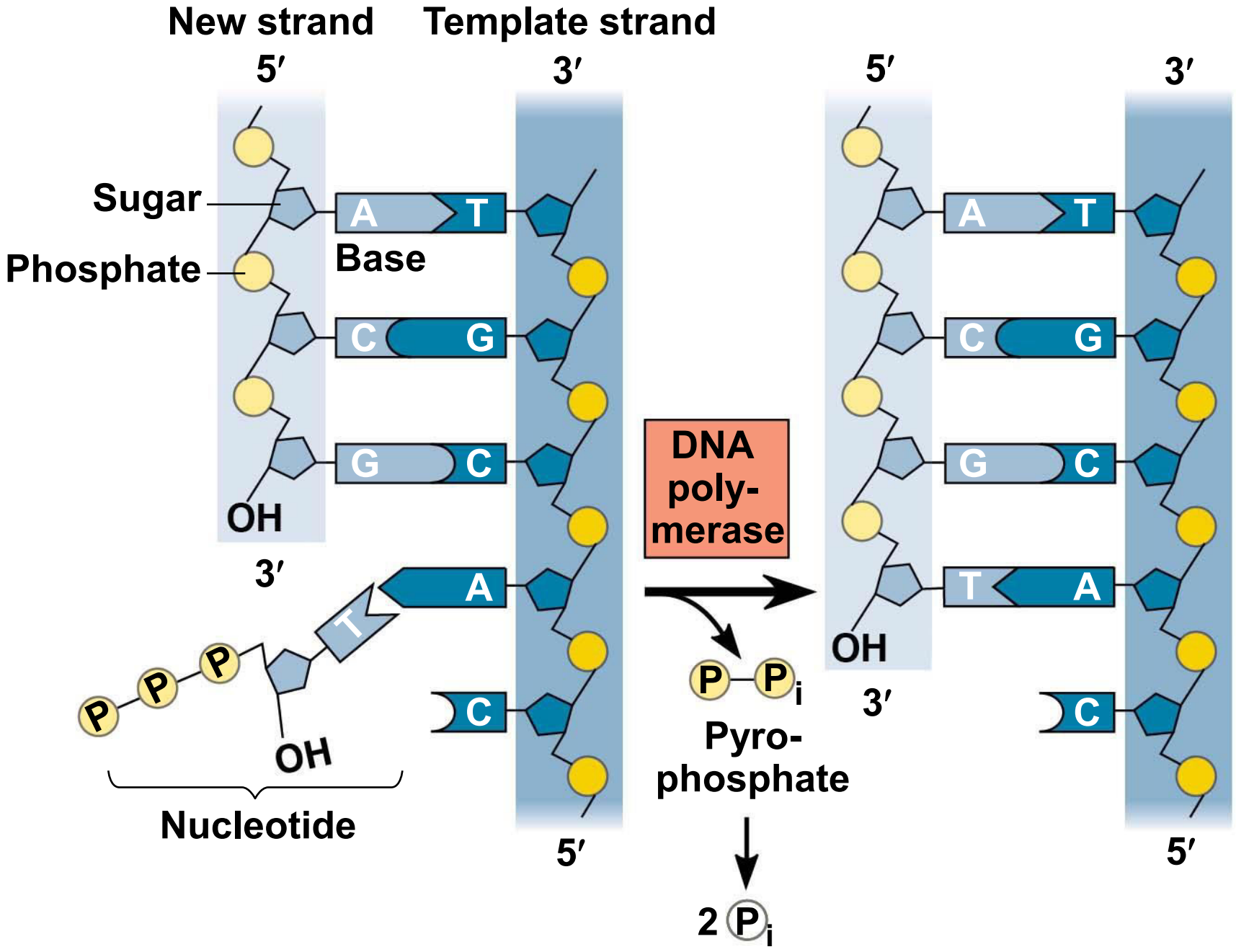


- The enzyme, **primase**, starts an RNA chain with a single RNA nucleotide and adds RNA nucleotides one at a time using the parental DNA as a template
- The primer is short (5–10 nucleotides long)
- The new DNA strand will start from the 3′ end of the RNA primer

- 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

- Each nucleotide that is added to a growing DNA consists of a sugar attached to a base and to three phosphate groups
- dATP is used to make DNA and is similar to the ATP of energy metabolism
- The difference is in the sugars: dATP has deoxyribose, while ATP has ribose
- As each monomer nucleotide joins the DNA strand, it loses two phosphate groups as a molecule of pyrophosphate

Figure 13.16



Antiparallel Elongation

- Newly replicated DNA strands must be formed antiparallel to the template strand
- 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

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

Figure 13.17

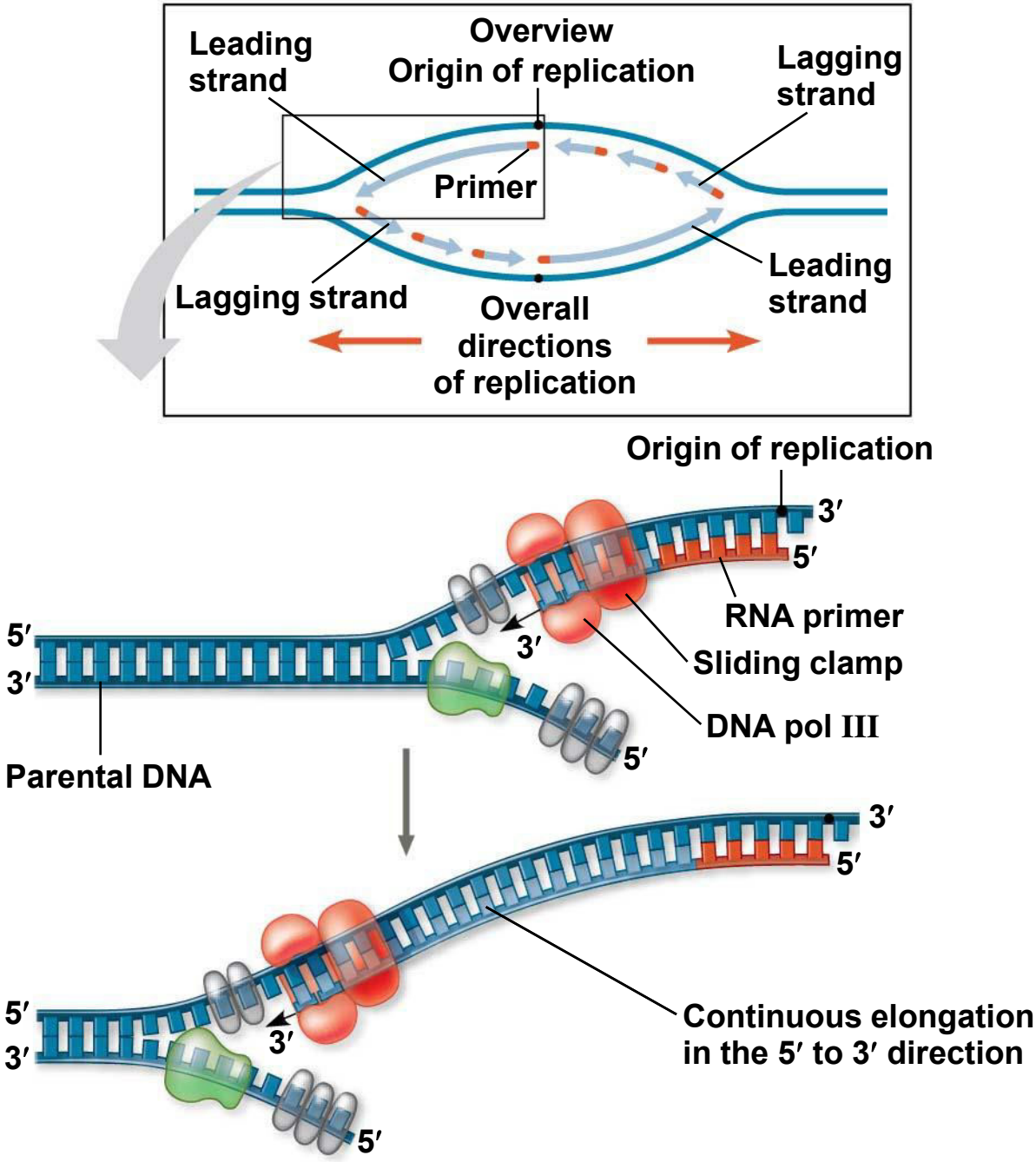


Figure 13.17-1

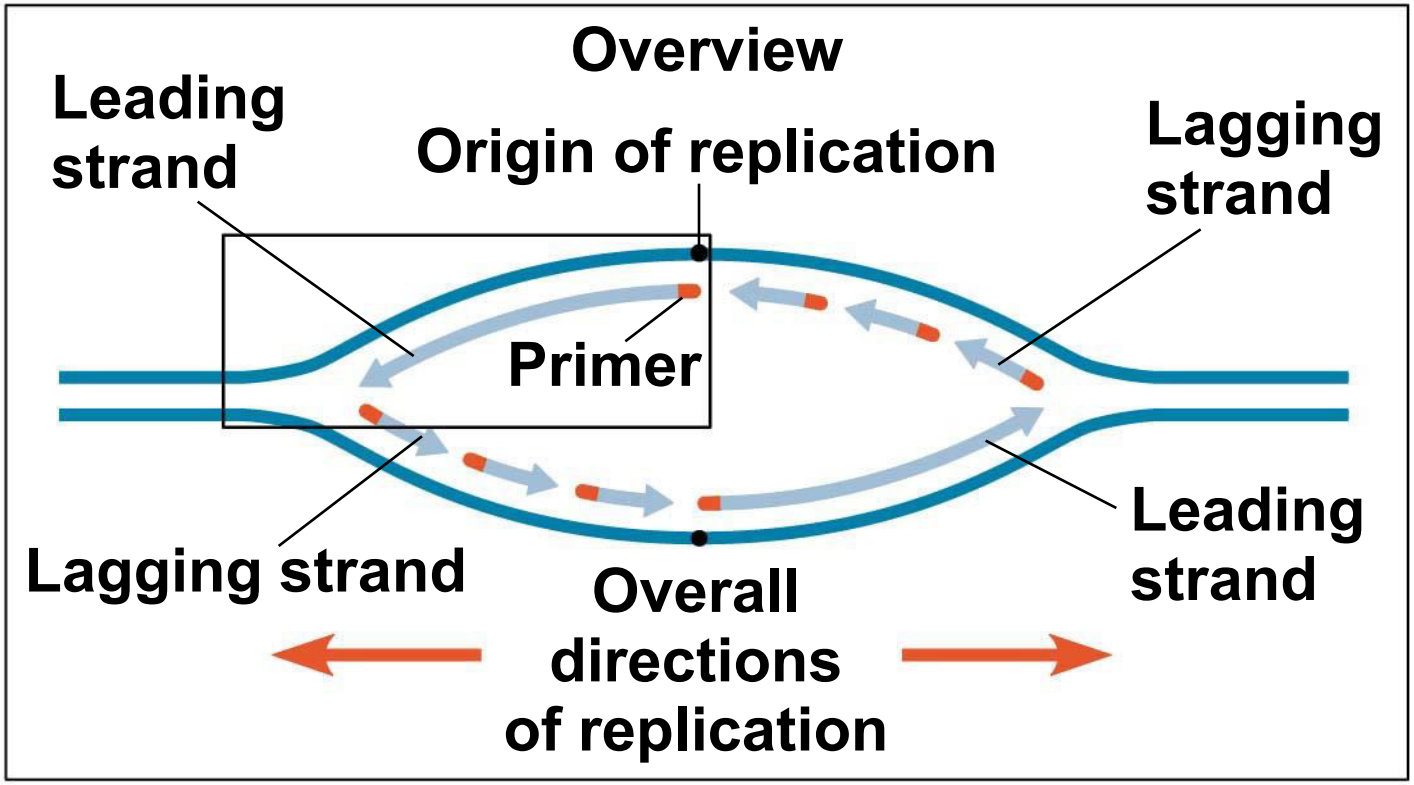
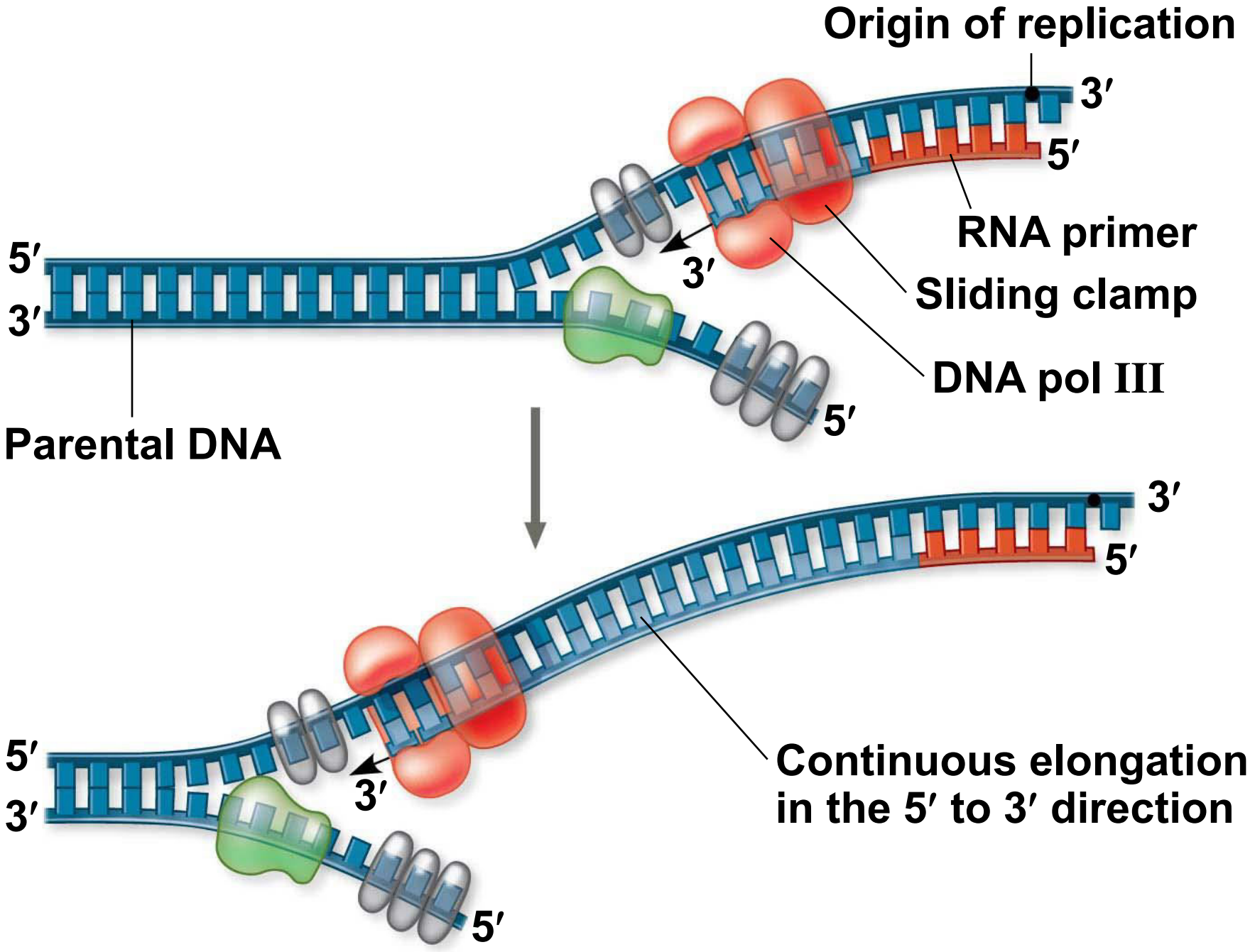


Figure 13.17-2



- To elongate the other new strand, 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**

- After formation of Okazaki fragments, DNA polymerase I removes the RNA primers and replaces the nucleotides with DNA
- The remaining gaps are joined together by **DNA ligase**

Figure 13.18

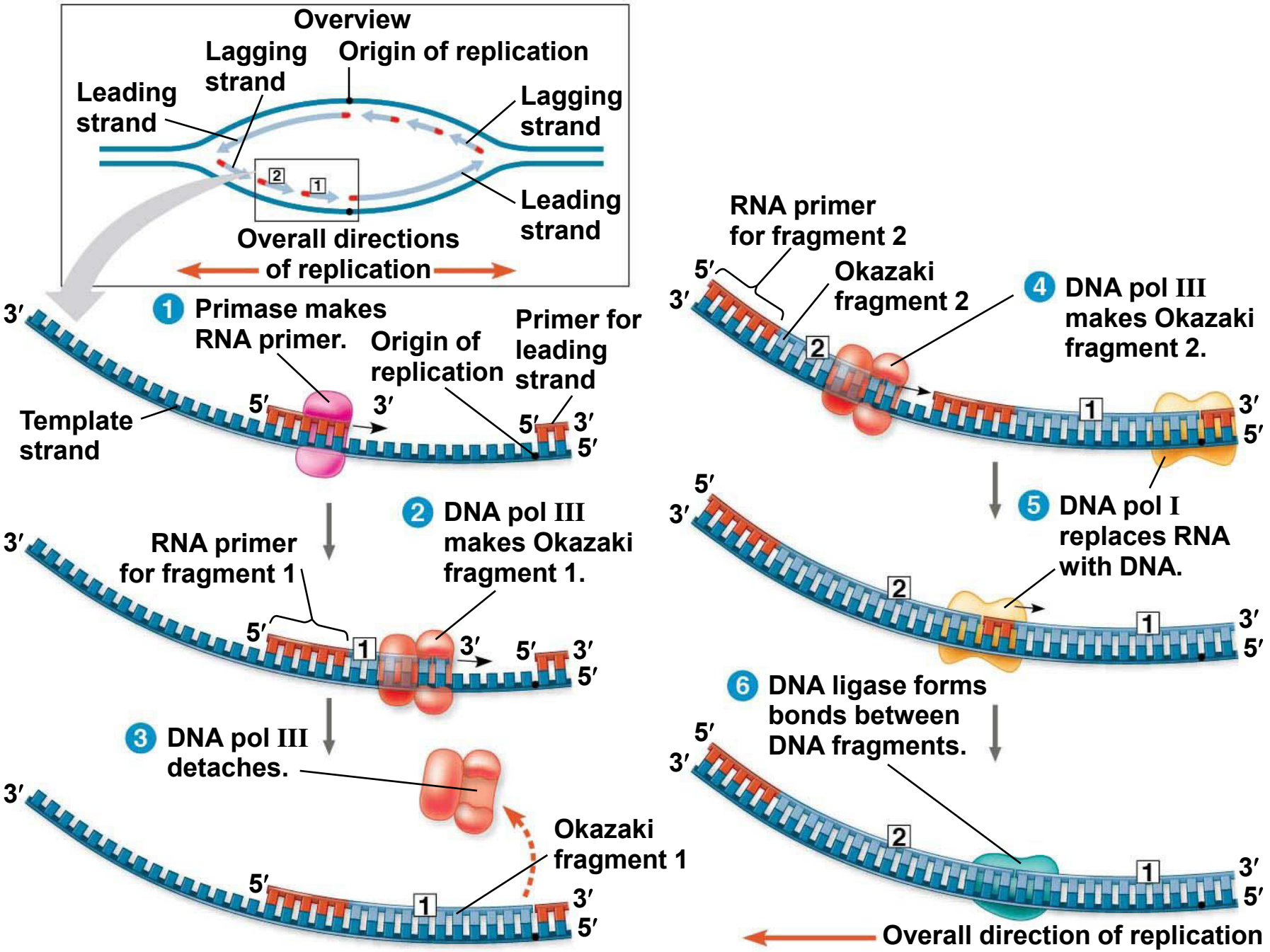
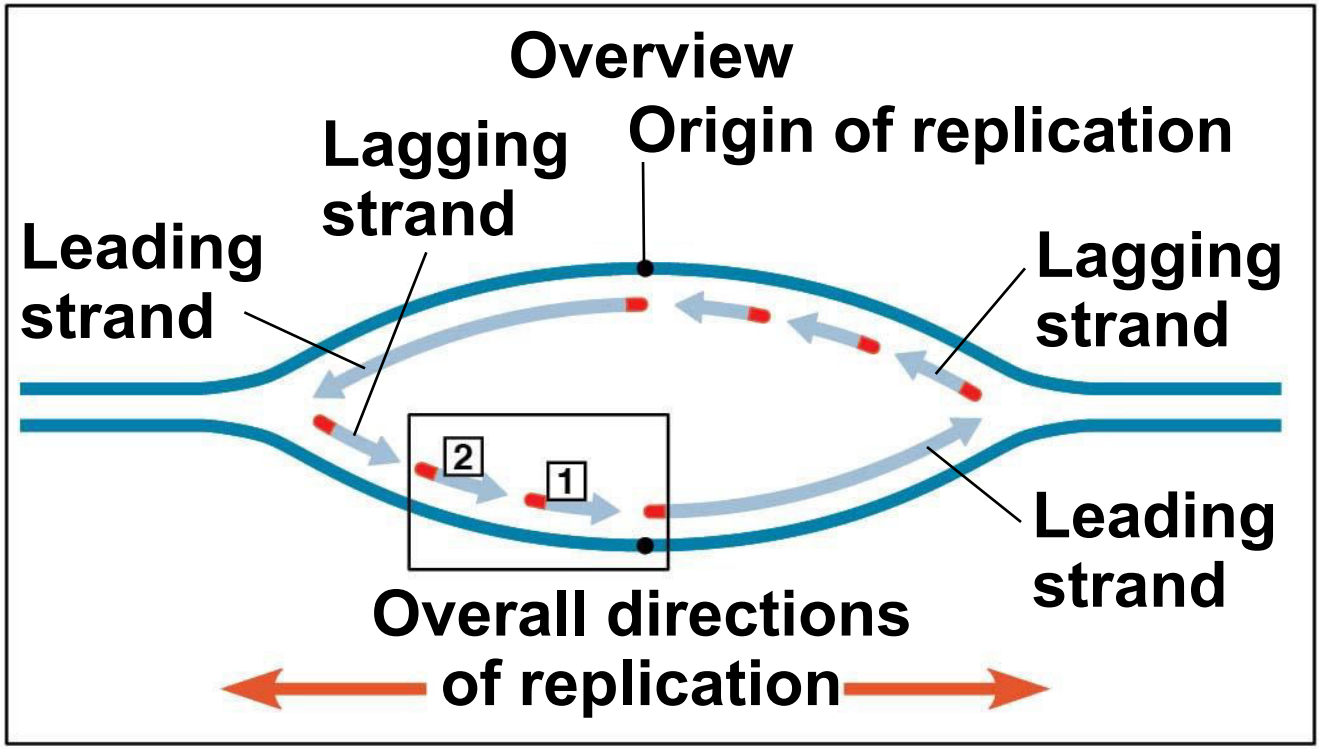


Figure 13.18-1



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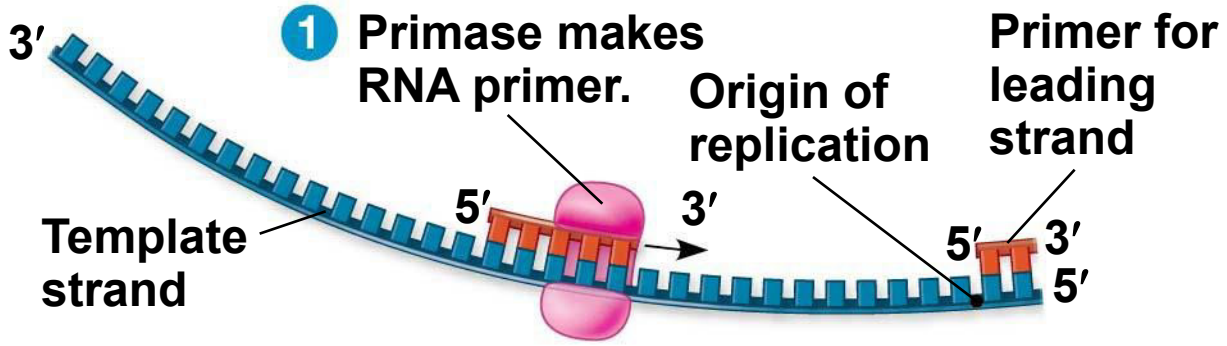

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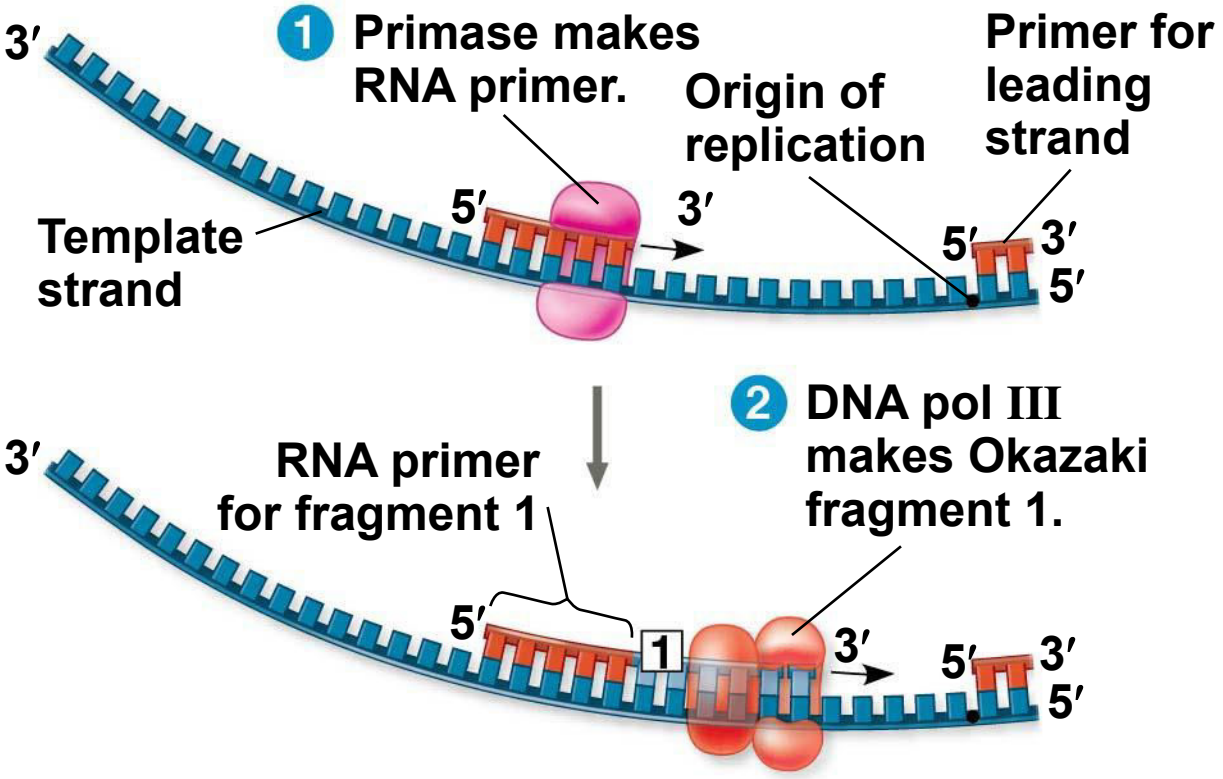

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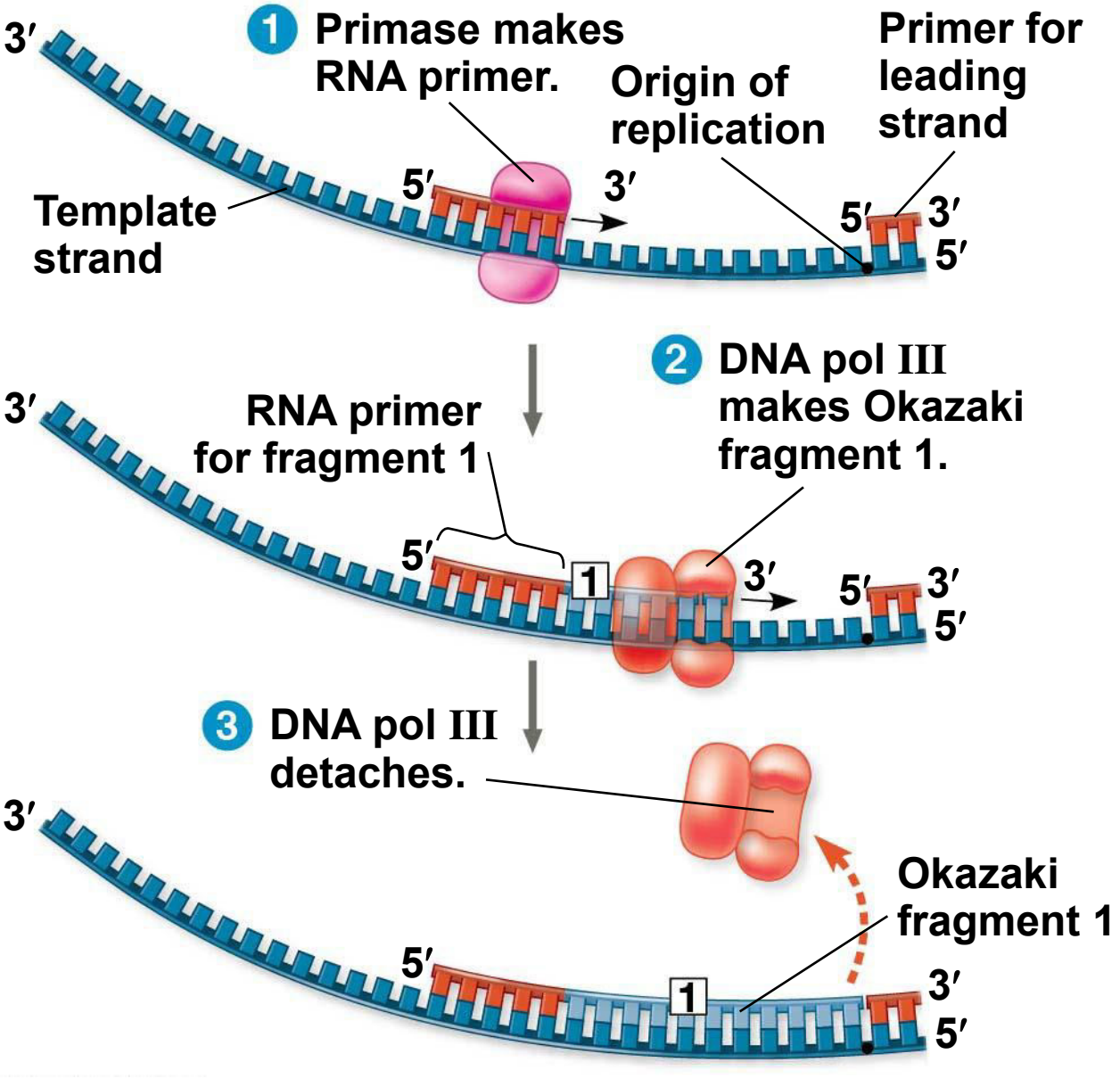
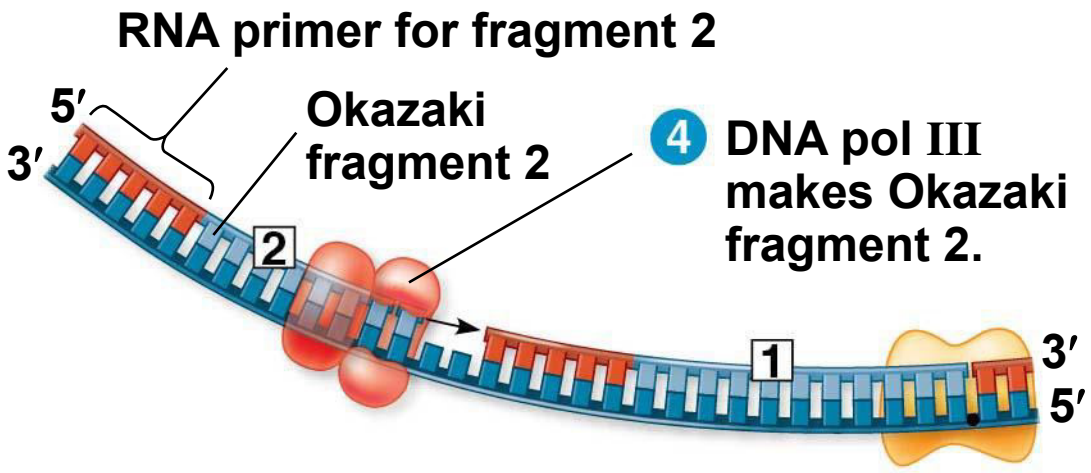


Figure 13.18-3-s1



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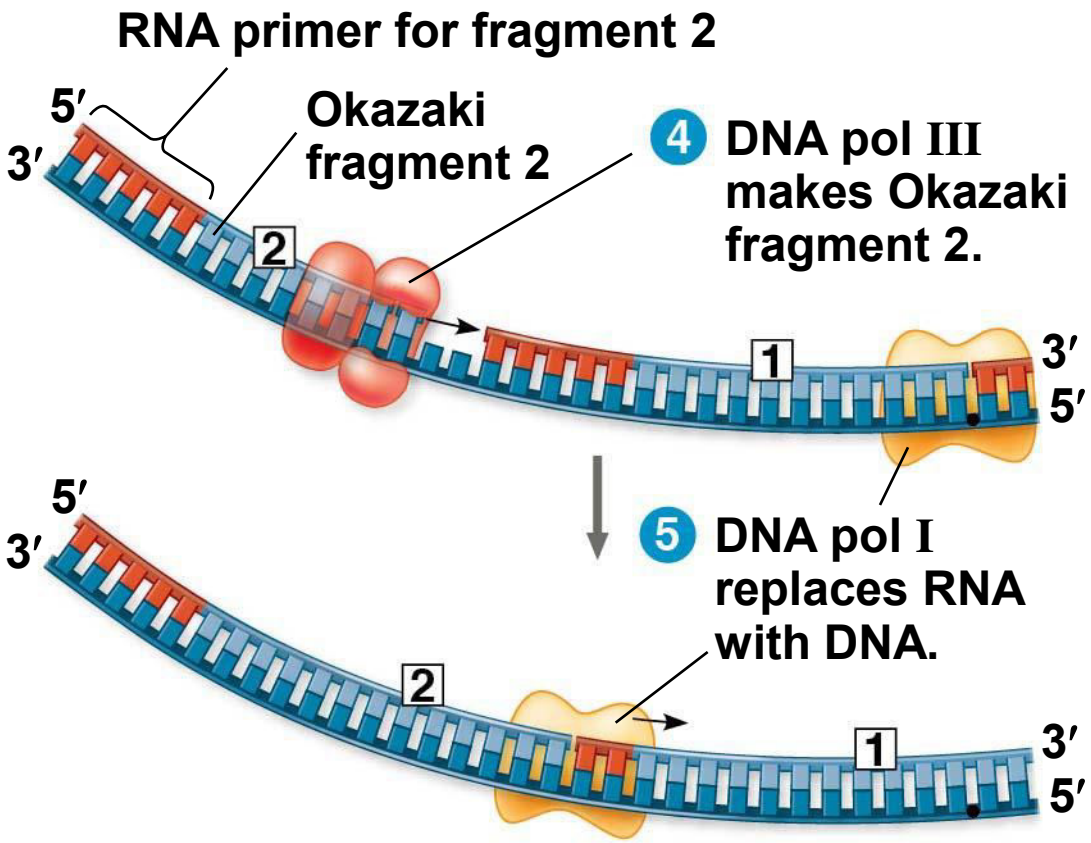

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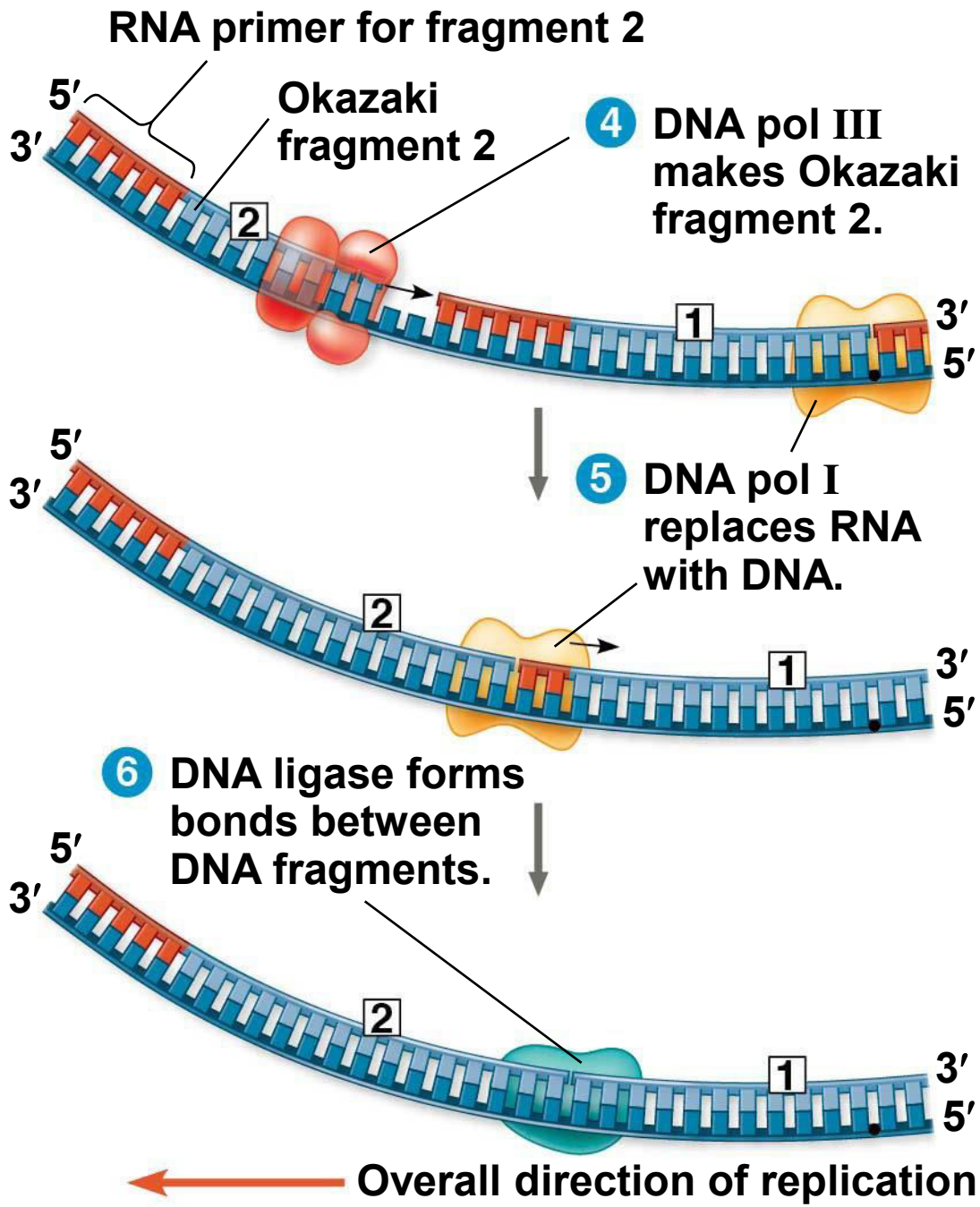
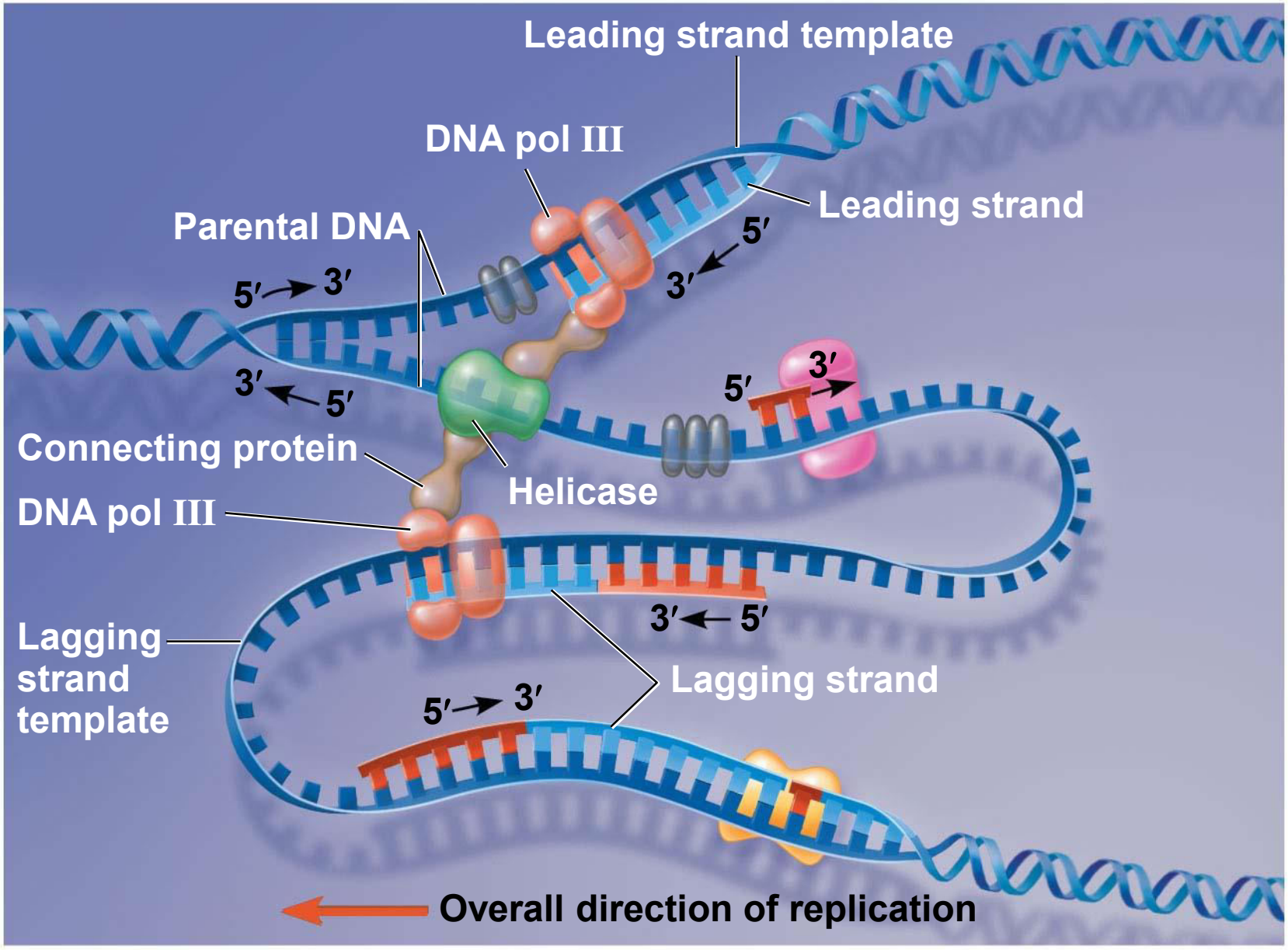


The DNA Replication Complex

- 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 two DNA polymerase molecules “reel in” parental DNA and “extrude” newly made daughter DNA molecules

Figure 13.20



Proofreading and Repairing DNA

- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- In **mismatch repair** of DNA, other enzymes correct errors in base pairing
- A hereditary defect in one such enzyme is associated with a form of colon cancer
- This defect allows cancer-causing errors to accumulate in DNA faster than normal

- 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 13.21-s1

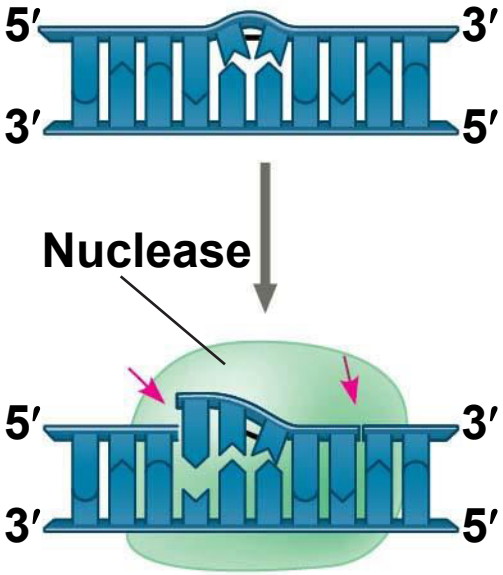


Figure 13.21-s2

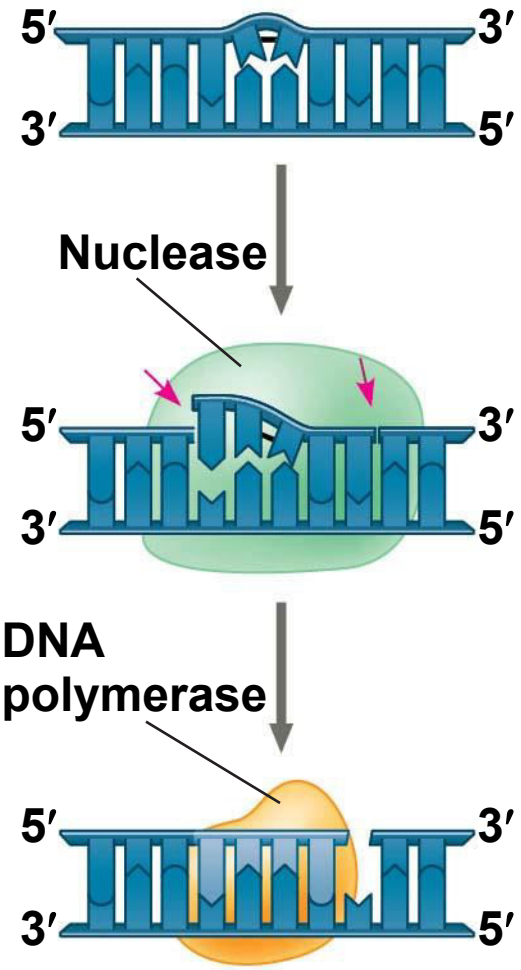
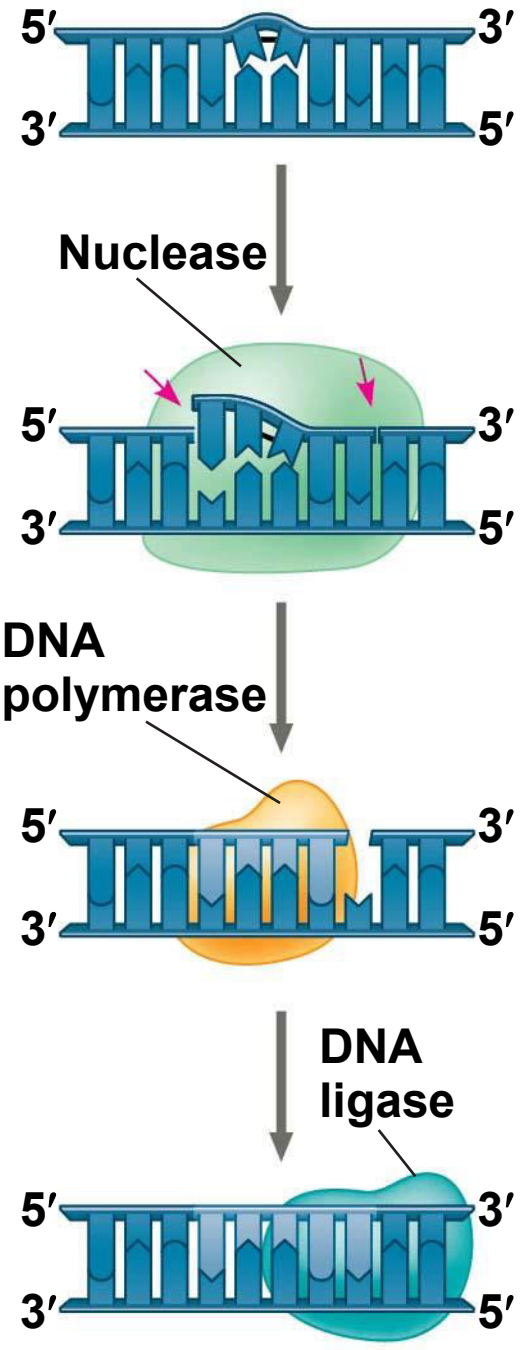


Figure 13.21-s3



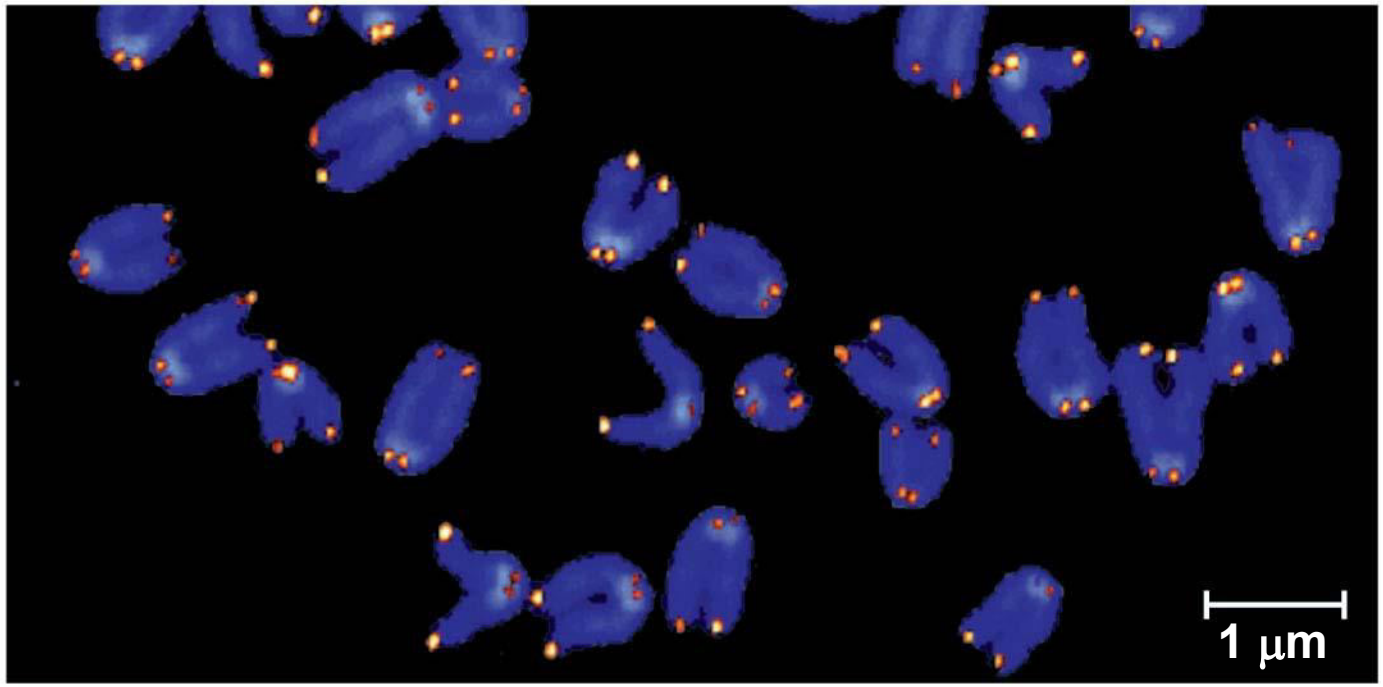
Evolutionary Significance of Altered DNA Nucleotides

- The error rate after proofreading 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

Replicating the Ends of DNA Molecules

- For linear DNA, the usual replication machinery cannot complete the 5′ ends of daughter strands
- Repeated rounds of replication produce shorter DNA molecules with uneven ends
- Eukaryotic chromosomal DNA molecules have special nucleotide sequences at their ends called telomeres

Figure 13.22



- Telomeres typically consist of multiple repetitions of one short nucleotide sequence
- Telomeres do not prevent the shortening of DNA molecules, but they do postpone it
- It has been proposed that the shortening of telomeres is connected to aging

- 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

- Telomerase is not active in most human somatic cells
- However, it does show inappropriate activity in some cancer cells
- Telomerase is currently under study as a target for cancer therapies

Concept 13.3: 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**, a complex of DNA and protein, is found in the nucleus of eukaryotic cells
- Chromosomes fit into the nucleus through an elaborate, multilevel system of packing
- Chromatin undergoes striking changes in the degree of packing during the course of the cell cycle

- Proteins called **histones** are responsible for the first level of DNA packing in chromatin
- Four types of histones are most common in chromatin: H2A, H2B, H3, and H4
- A **nucleosome** consists of DNA wound twice around a protein core of eight histones, two of each of the main histone types

Figure 13.23

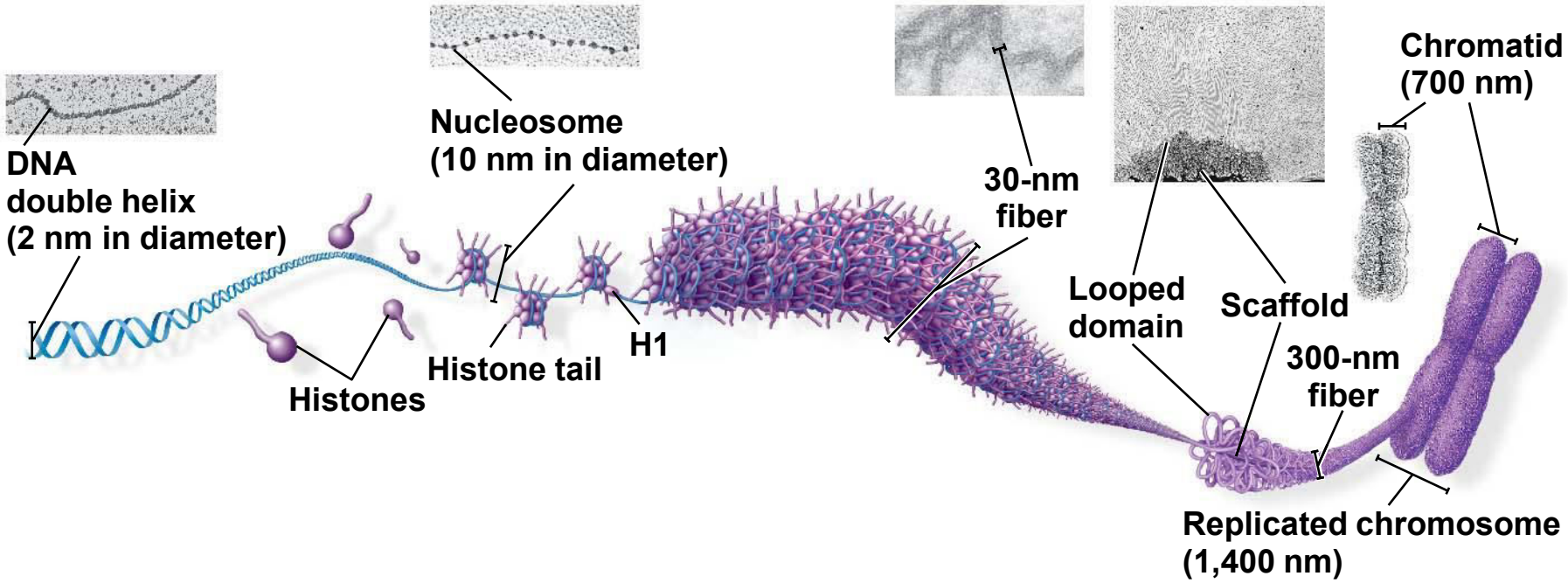


Figure 13.23-1

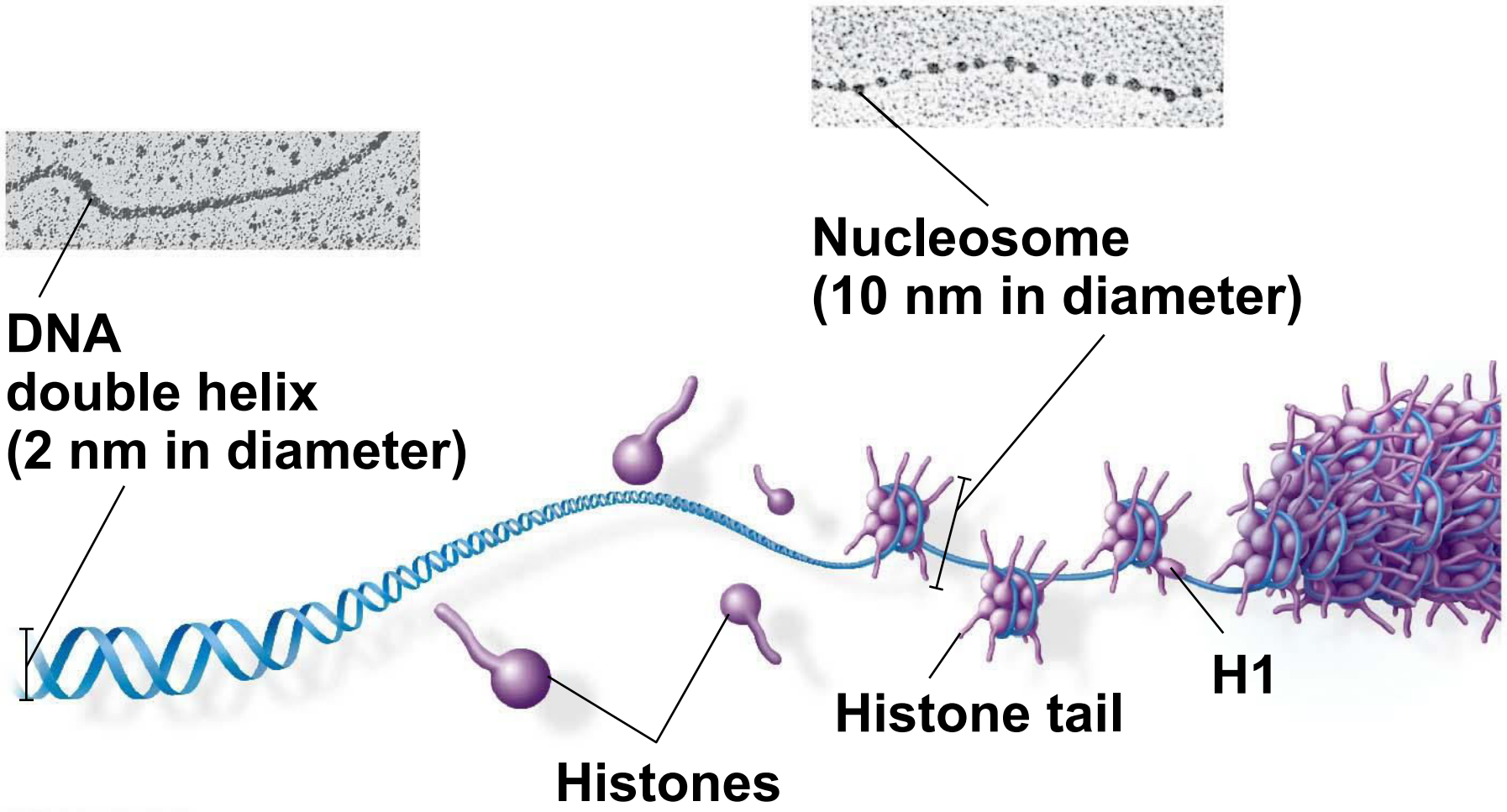
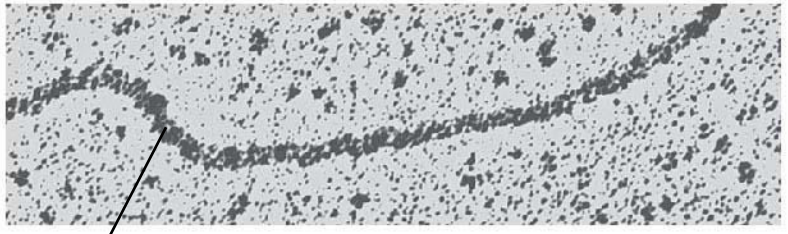
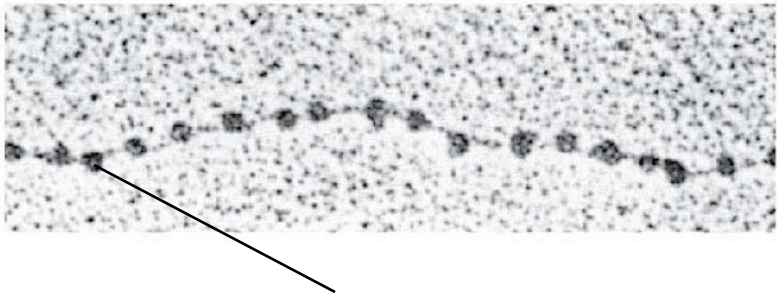


Figure 13.23-1a



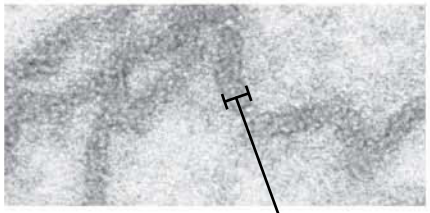
DNA
double helix
(2 nm in diameter)

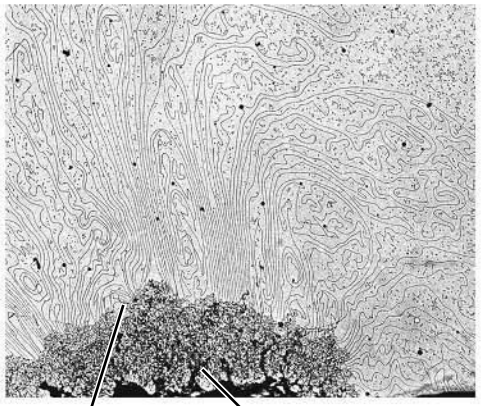
Figure 13.23-1b



Nucleosome
(10 nm in diameter)

Figure 13.23-2





Chromatid (700 nm)

30-nm fiber

Looped domain

Scaffold

300-nm fiber

Replicated chromosome (1,400 nm)

Figure 13.23-2a

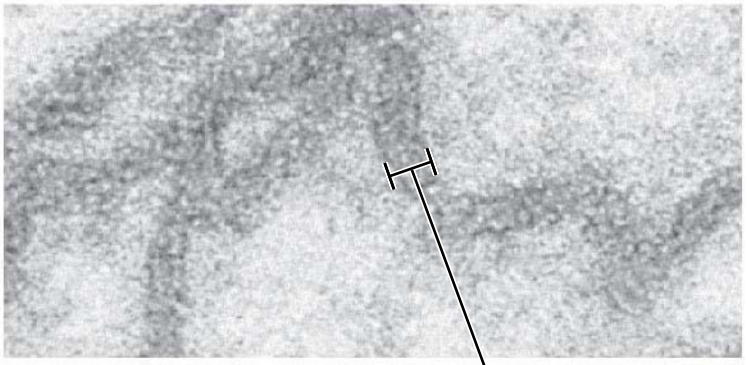


Figure 13.23-2b

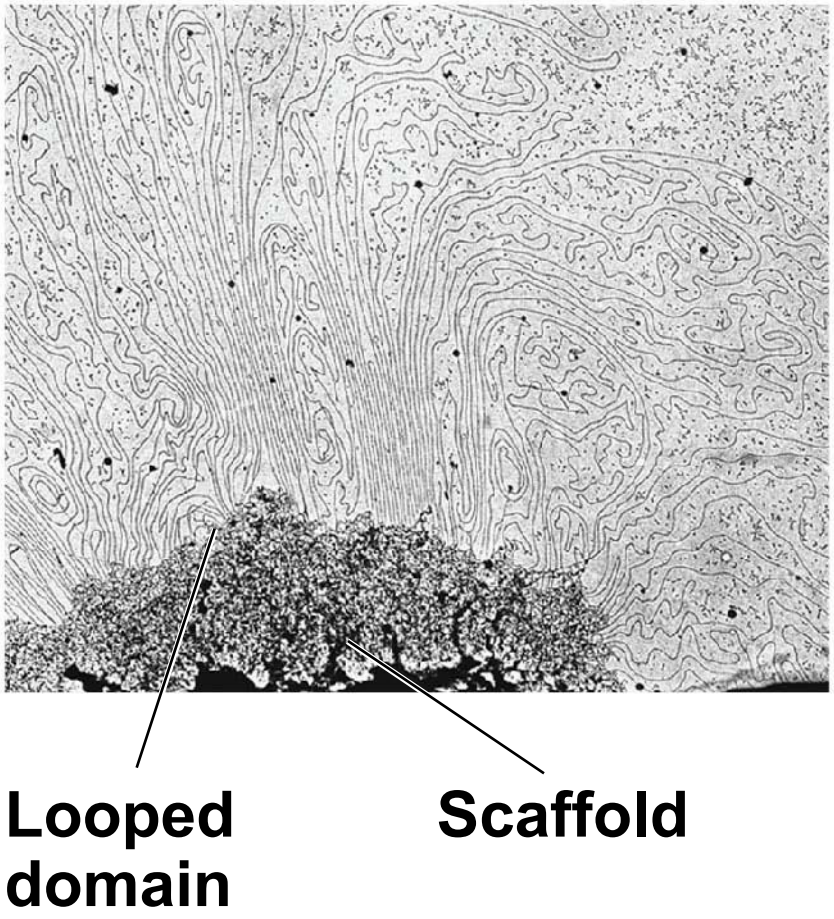
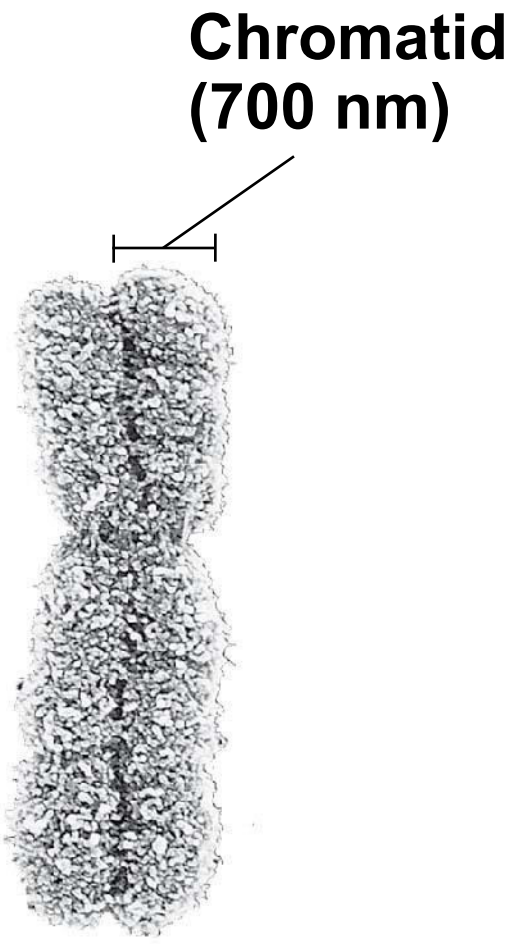


Figure 13.23-2c



- At interphase, most of the chromatin is compacted into a 30-nm fiber, which is folded further in some areas by looping
- Even during interphase, centromeres and some other parts of chromosomes are highly condensed, similar to metaphase chromosomes
- This condensed chromatin is called **heterochromatin**; the more dispersed, less compacted chromatin is called **euchromatin**

- Dense packing of the heterochromatin makes it largely inaccessible to the machinery responsible for transcribing genetic information
- Chromosomes are dynamic in structure; a condensed region may be loosened or modified as needed for various cell processes
- For example, histones can undergo chemical modifications that result in changes in chromatin organization

Concept 13.4: Understanding DNA structure and replication makes genetic engineering possible

- Complementary base pairing of DNA is the basis for **nucleic acid hybridization**, the base pairing of one strand of a nucleic acid to another, complementary sequence
- Nucleic acid hybridization forms the foundation of virtually every technique used in **genetic engineering**, the direct manipulation of genes for practical purposes

DNA Cloning: Making Multiple Copies of a Gene or Other DNA Segment

- To work directly with specific genes, scientists prepare well-defined segments of DNA in identical copies, a process called DNA cloning
- Most methods for cloning pieces of DNA in the laboratory share general features

- Many bacteria contain **plasmids**, small circular DNA molecules that replicate separately from the bacterial chromosome
- To clone pieces of DNA, researchers first obtain a plasmid and insert DNA from another source ("foreign DNA") into it
- The resulting plasmid is called **recombinant DNA**

Figure 13.24

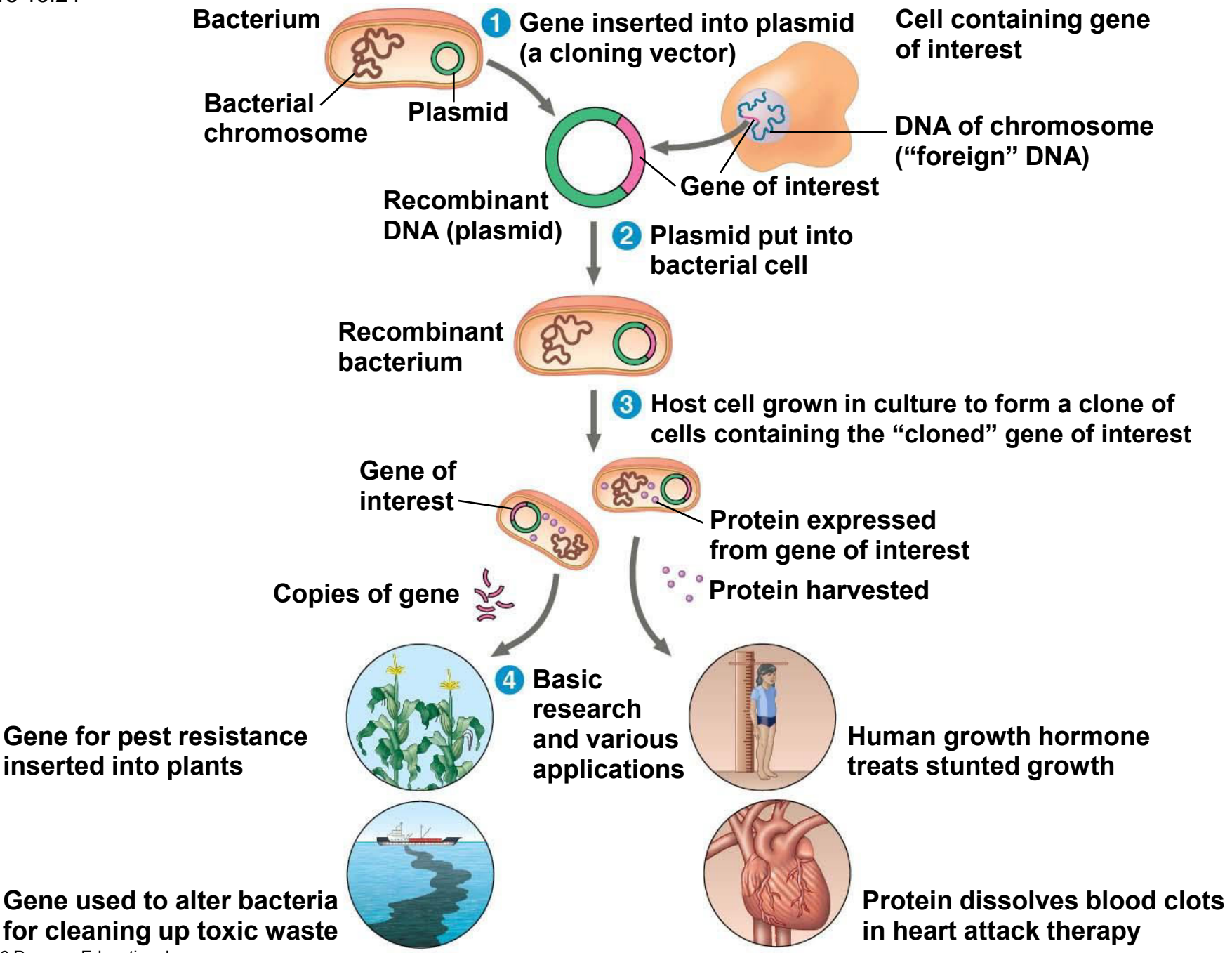


Figure 13.24-1

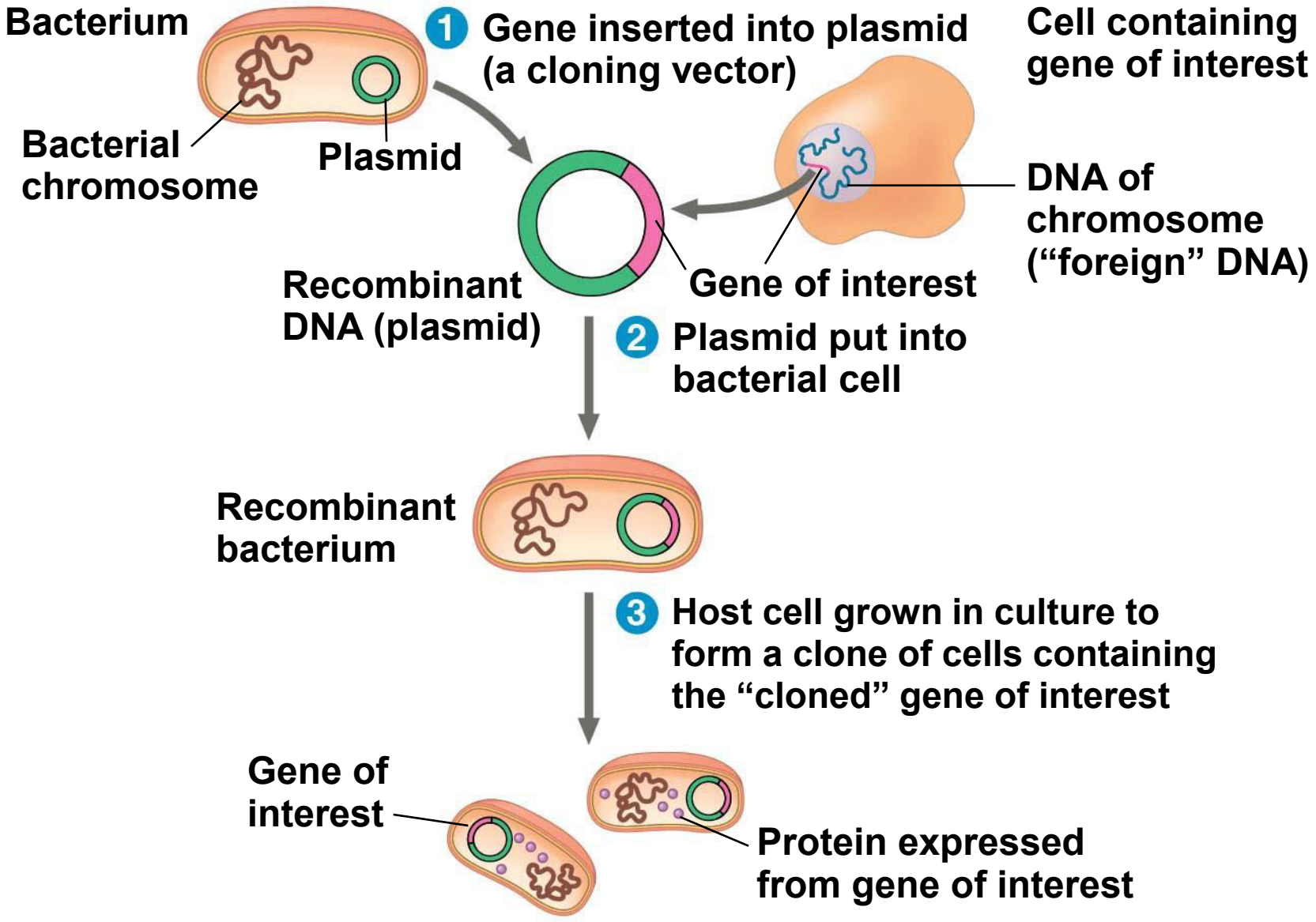
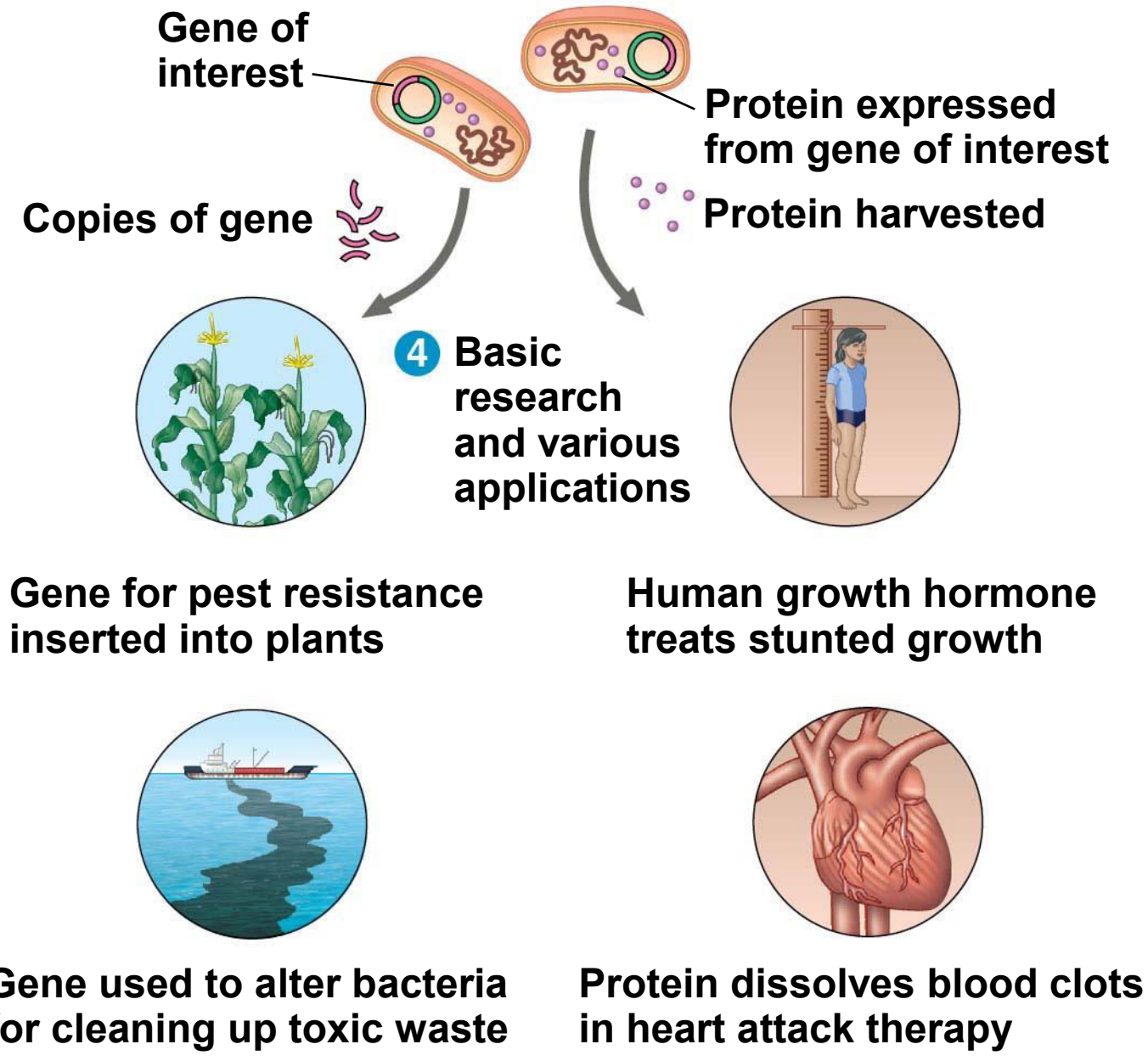


Figure 13.24-2



- The production of multiple copies of a single gene is called **gene cloning**
- The plasmid that carries the cloned DNA is called a **cloning vector**
- Gene cloning is used to make many copies of a gene and to produce a protein product
- The ability to amplify many copies of a gene is crucial for applications involving a single gene

Using Restriction Enzymes to Make Recombinant DNA

- Bacterial **restriction enzymes** cut DNA molecules at specific DNA sequences called **restriction sites**
- A restriction enzyme usually makes many cuts, yielding **restriction fragments**

Animation: Restriction Enzymes

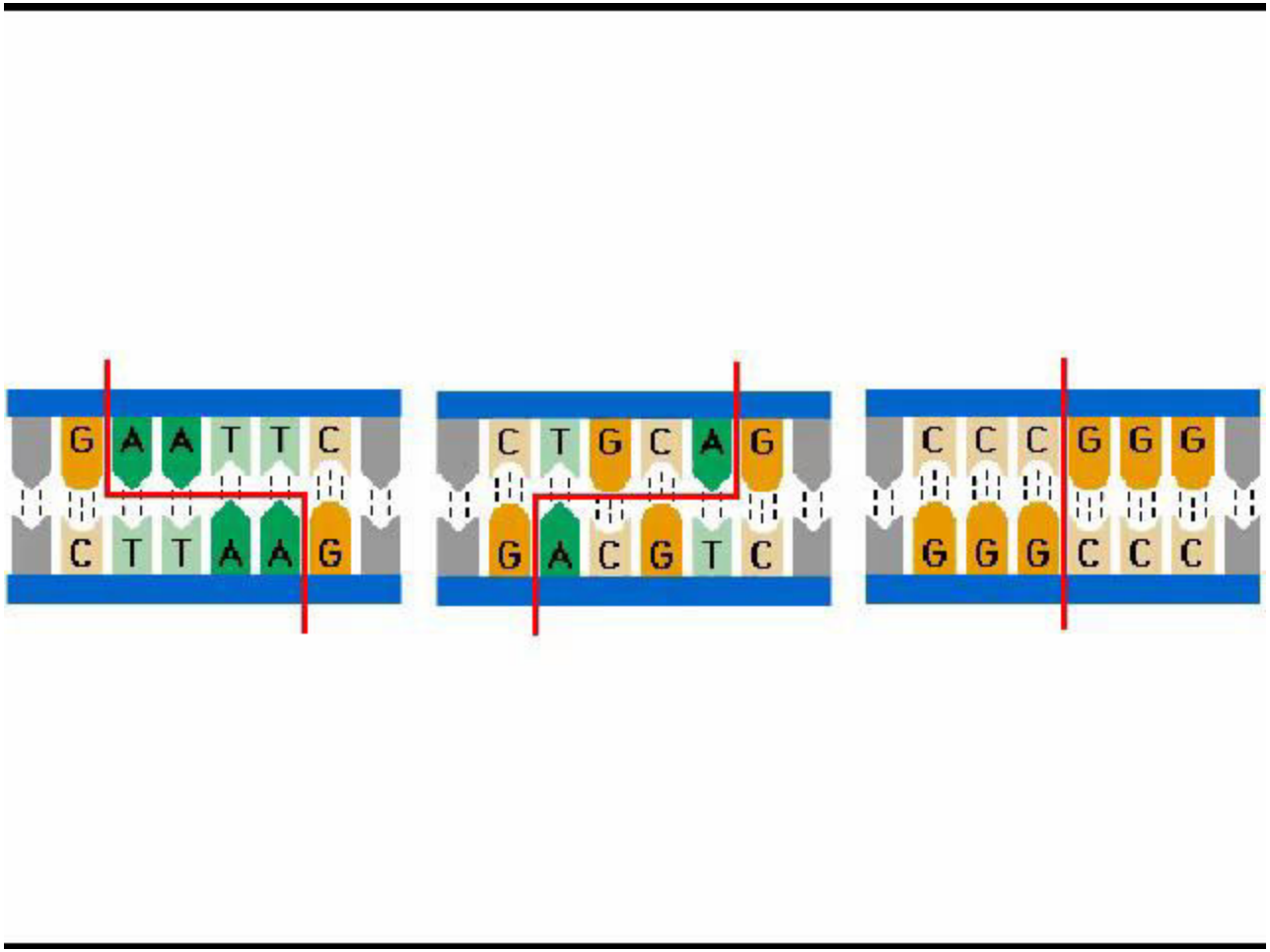


Figure 13.25

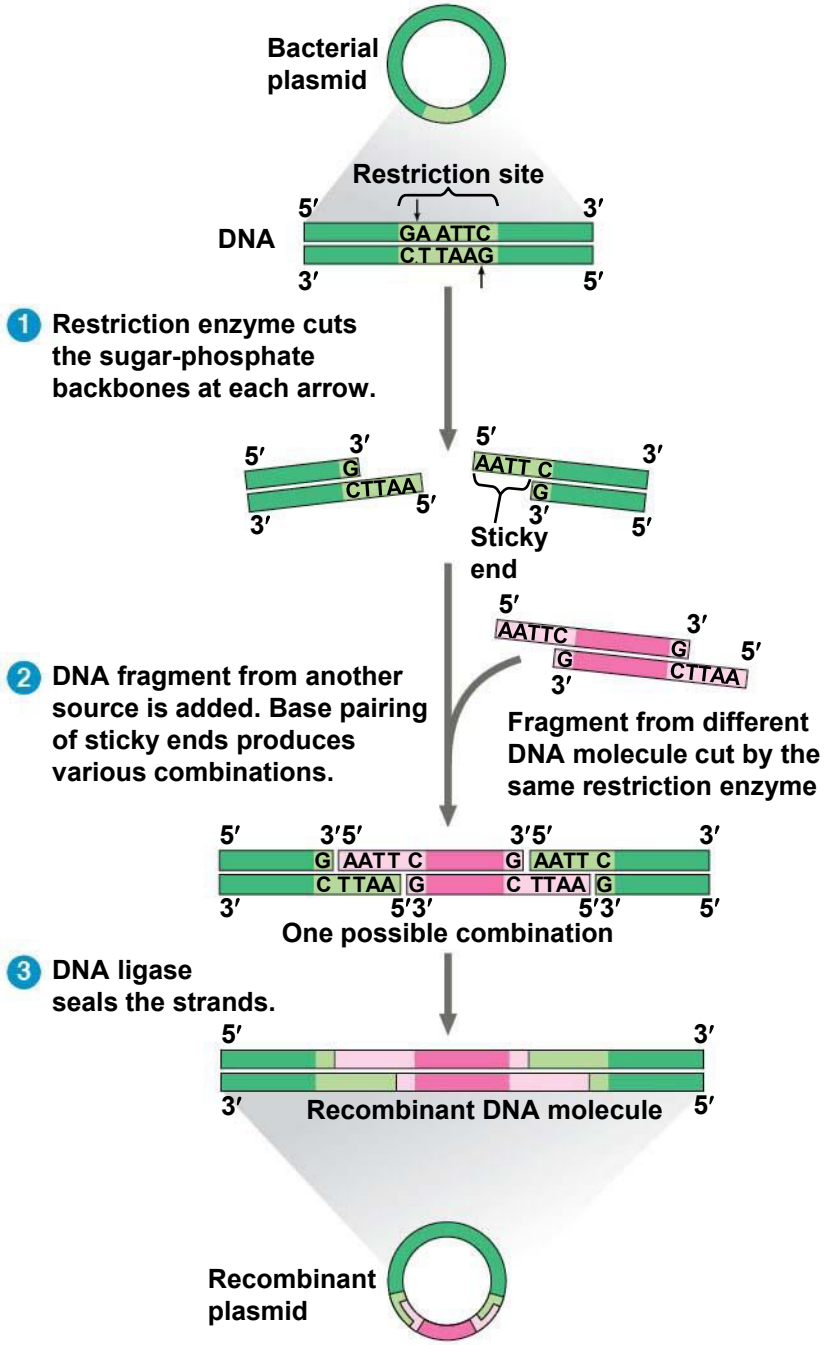


Figure 13.25-1

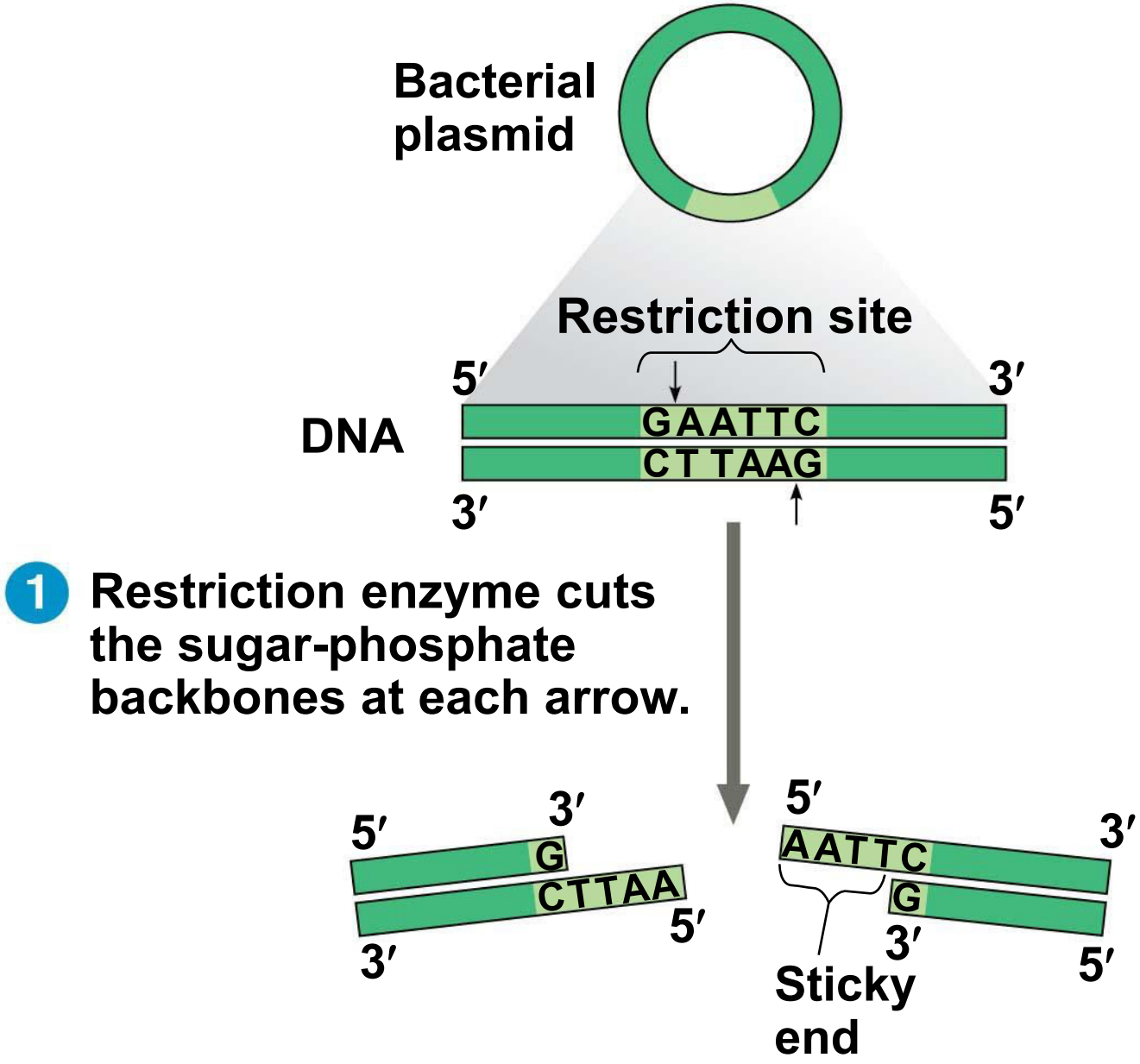


Figure 13.25-2

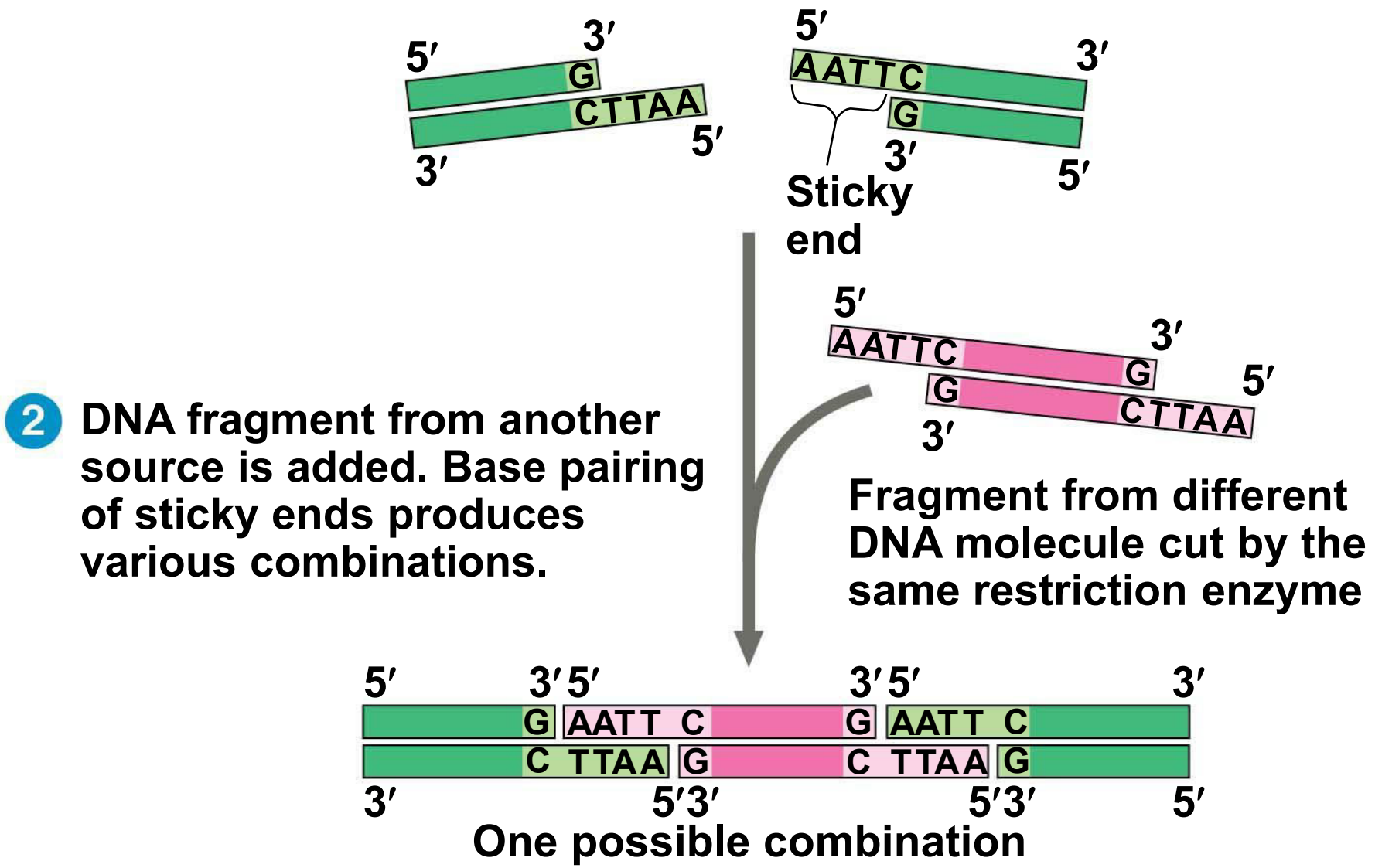
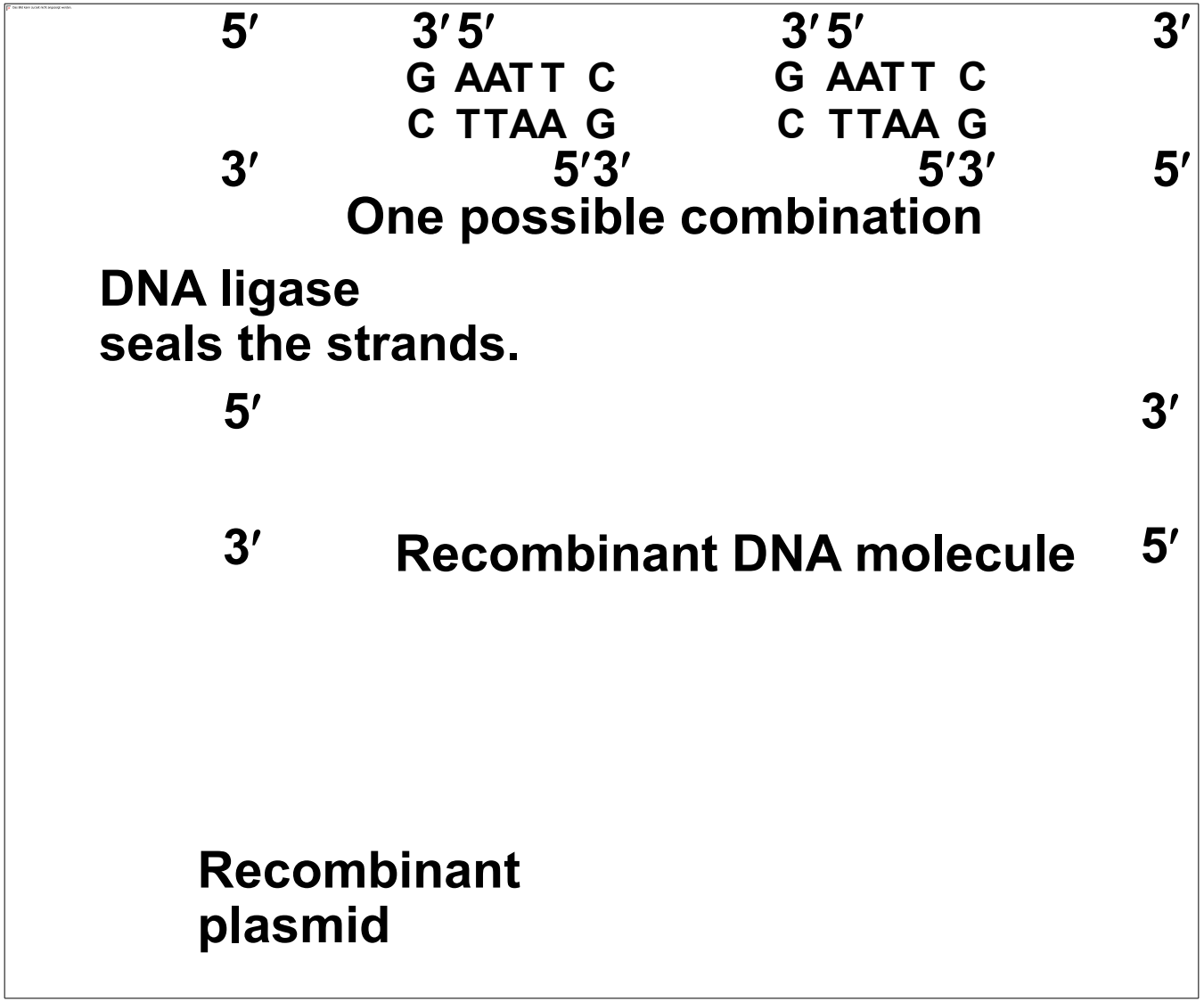


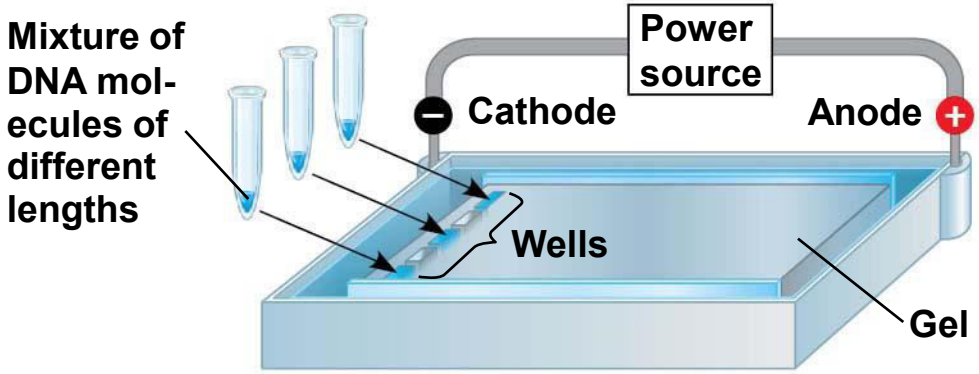
Figure 13.25-3



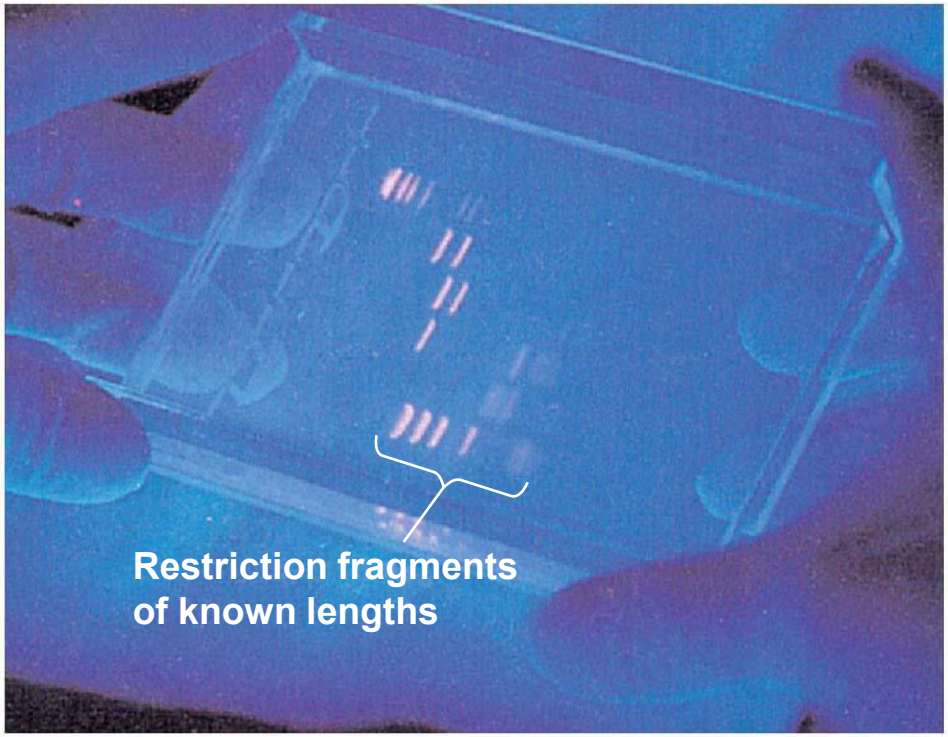
- The most useful restriction enzymes cleave the DNA in a staggered manner to produce **sticky ends**
- Sticky ends can bond with complementary sticky ends of other fragments
- DNA ligase can close the sugar-phosphate backbones of DNA strands

- To see the fragments produced by cutting DNA molecules with restriction enzymes, researchers use **gel electrophoresis**
- This technique separates a mixture of nucleic acid fragments based on length

Figure 13.26

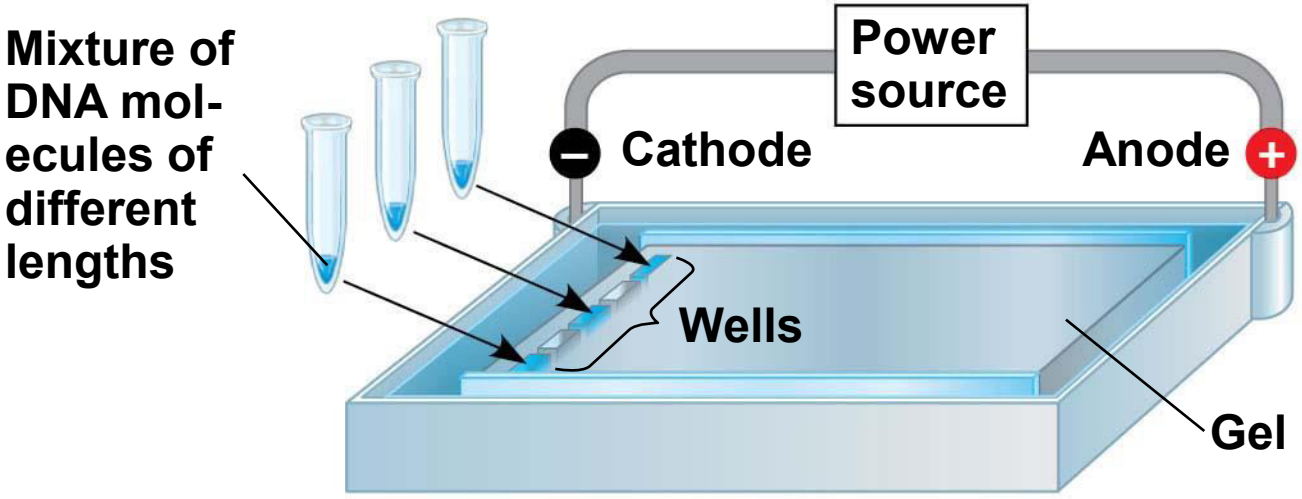


(a) Negatively charged DNA molecules will move toward the positive electrode.



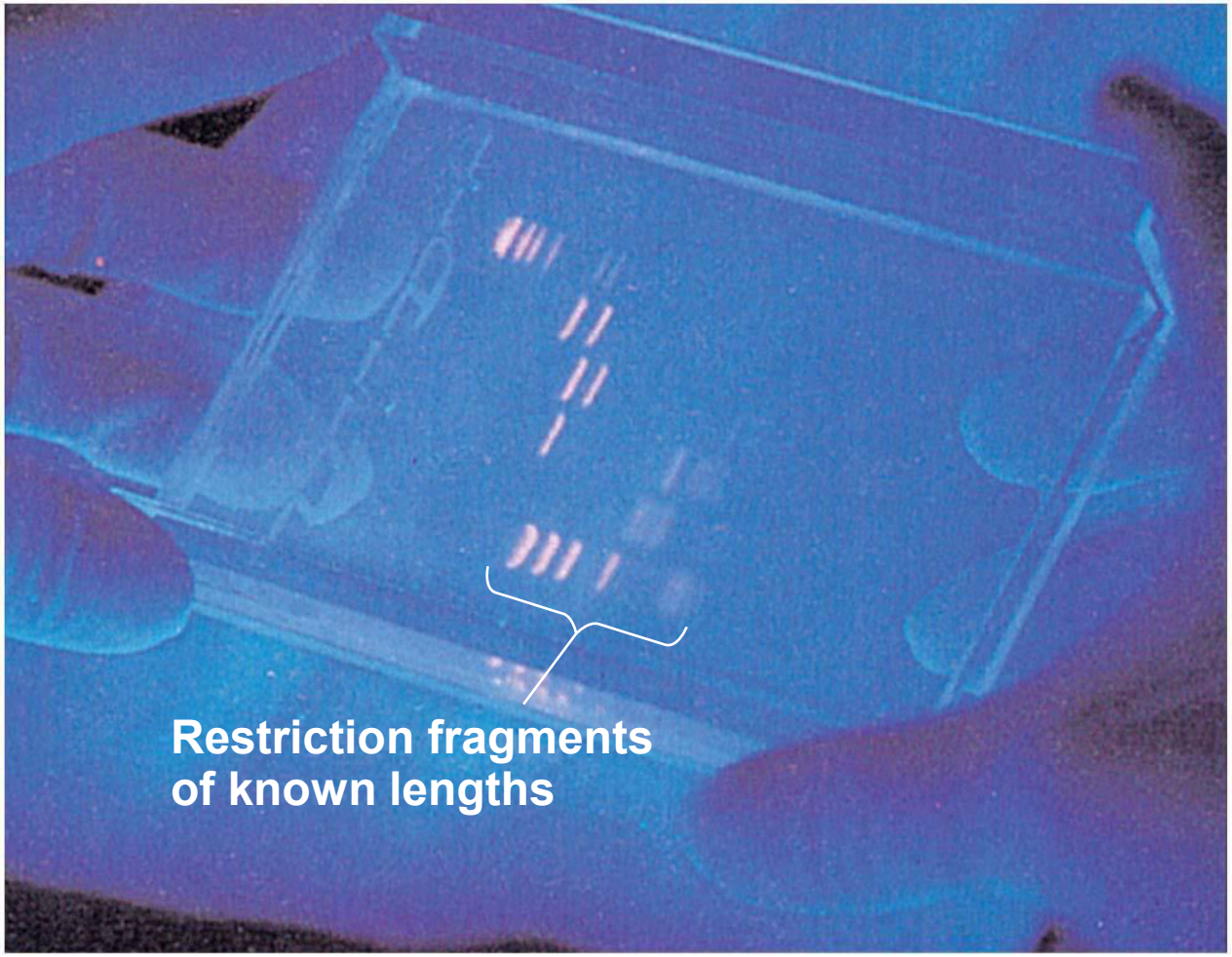
(b) Shorter molecules are slowed down less than longer ones, so they move faster through the gel.

Figure 13.26-1



(a) Negatively charged DNA molecules will move toward the positive electrode.

Figure 13.26-2



(b) Shorter molecules are slowed down less than longer ones, so they move faster through the gel.

Amplifying DNA *in Vitro*: The Polymerase Chain Reaction (PCR) and Its Use in Cloning

- The **polymerase chain reaction** (**PCR**) can produce many copies of a specific target segment of DNA
- A three-step cycle brings about a chain reaction that produces an exponentially growing population of identical DNA molecules
- The key to PCR is an unusual, heat-stable DNA polymerase called Taq polymerase.

Figure 13.27

Technique

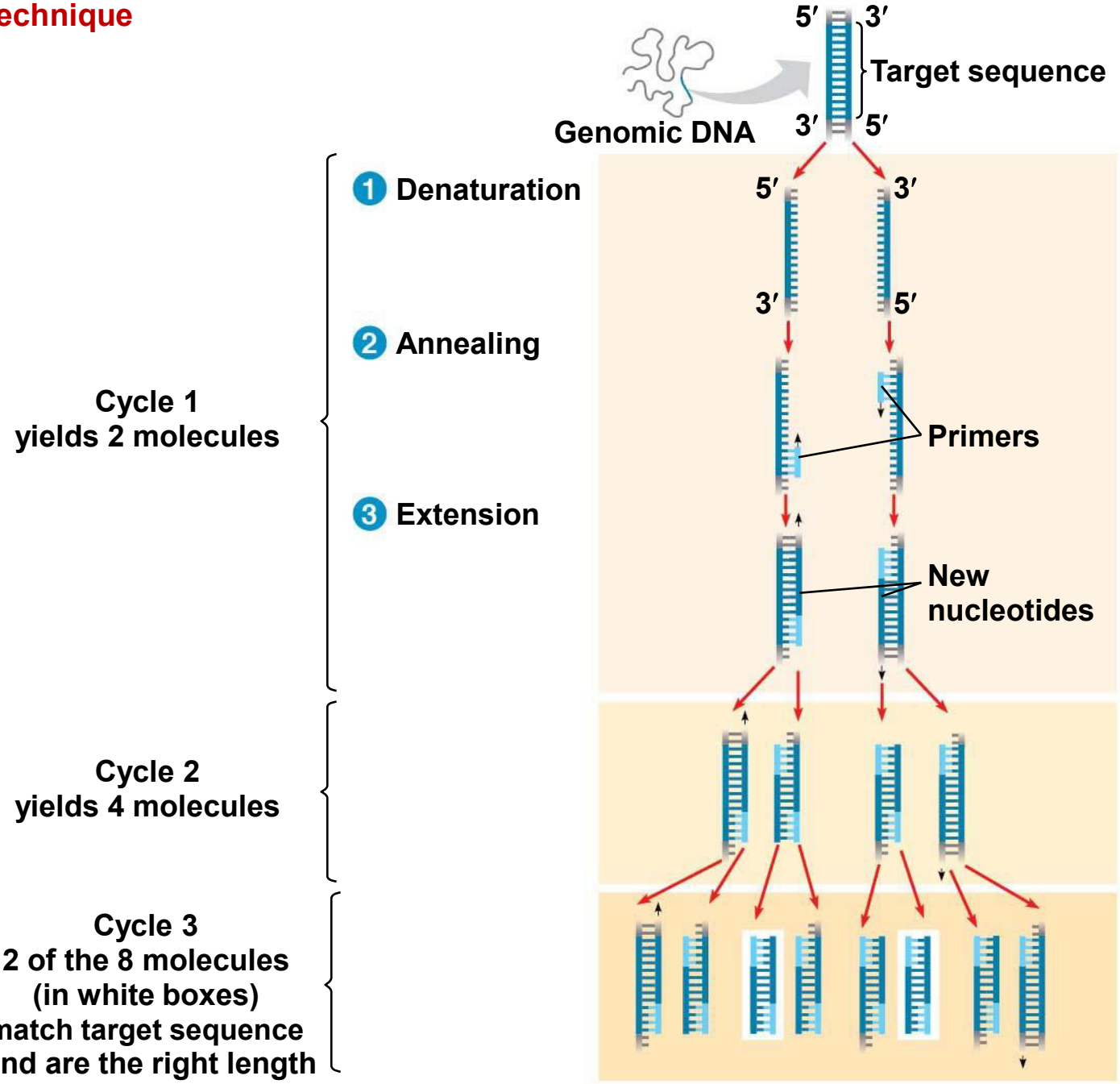


Figure 13.27-1

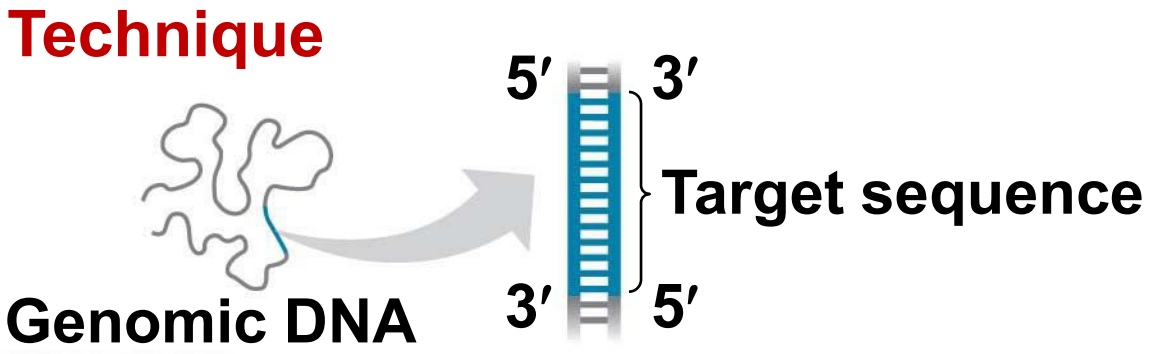


Figure 13.27-2-s1

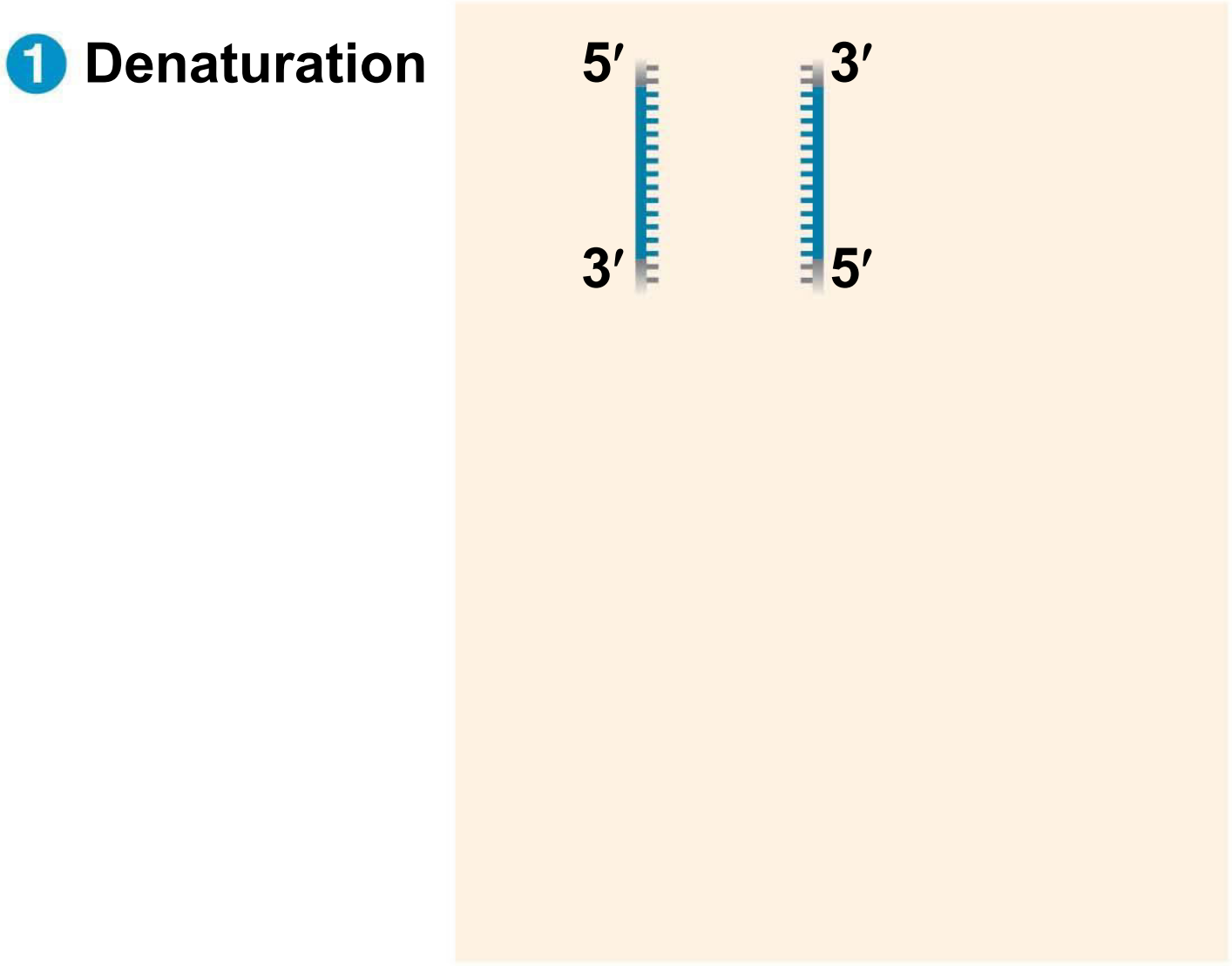


Figure 13.27-2-s2

1 Denaturation

2 Annealing

Cycle 1 yields 2 molecules

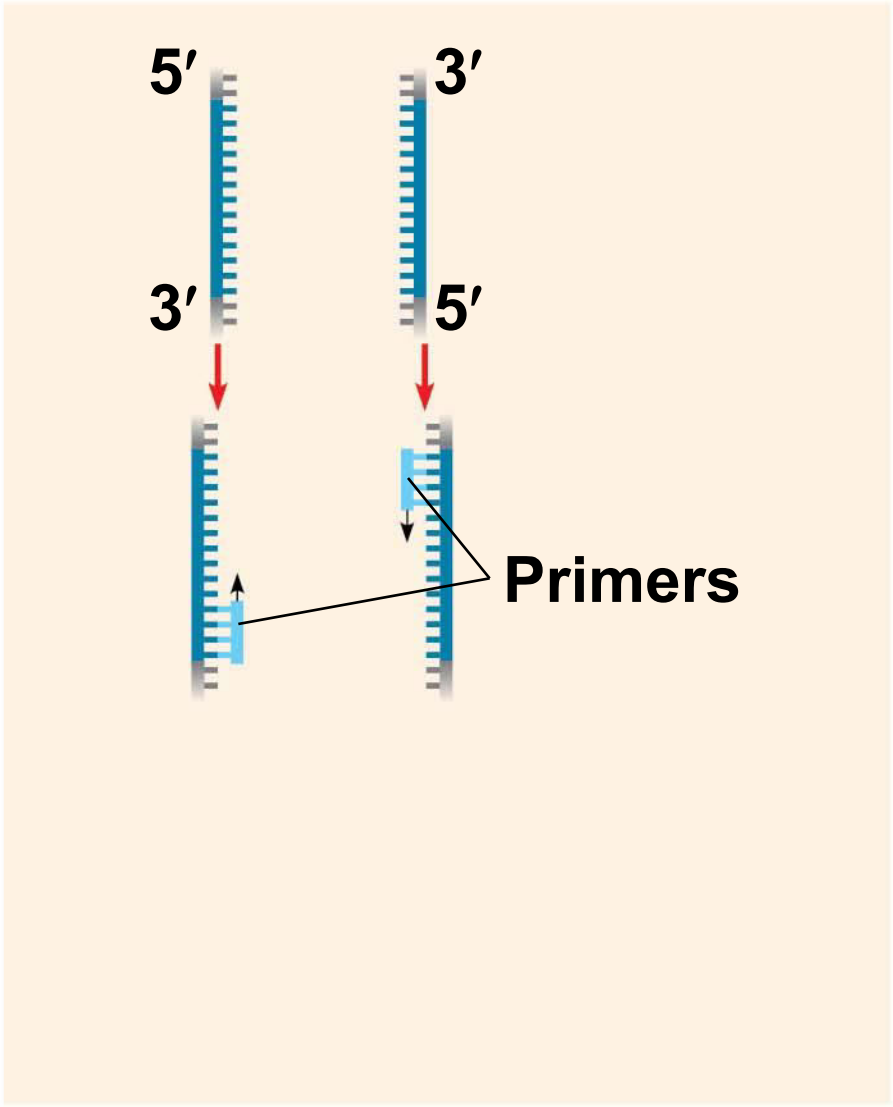


Figure 13.27-2-s3

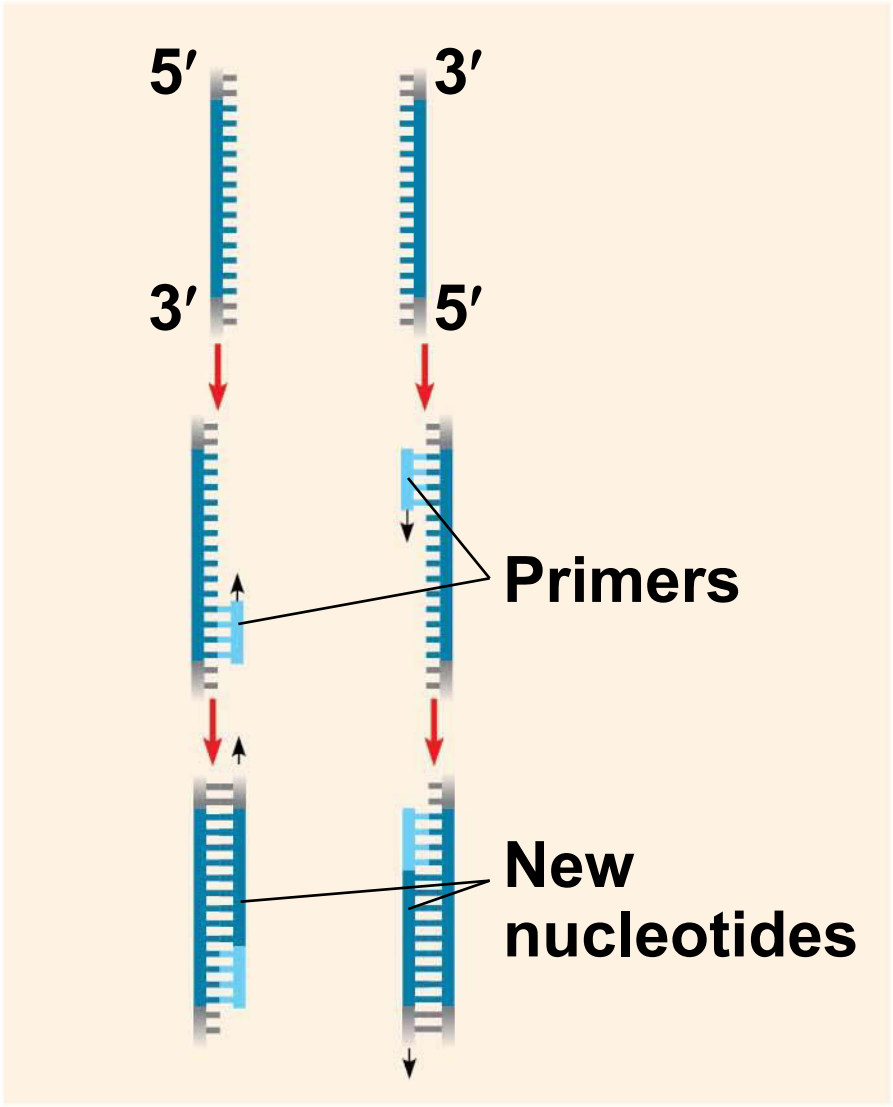
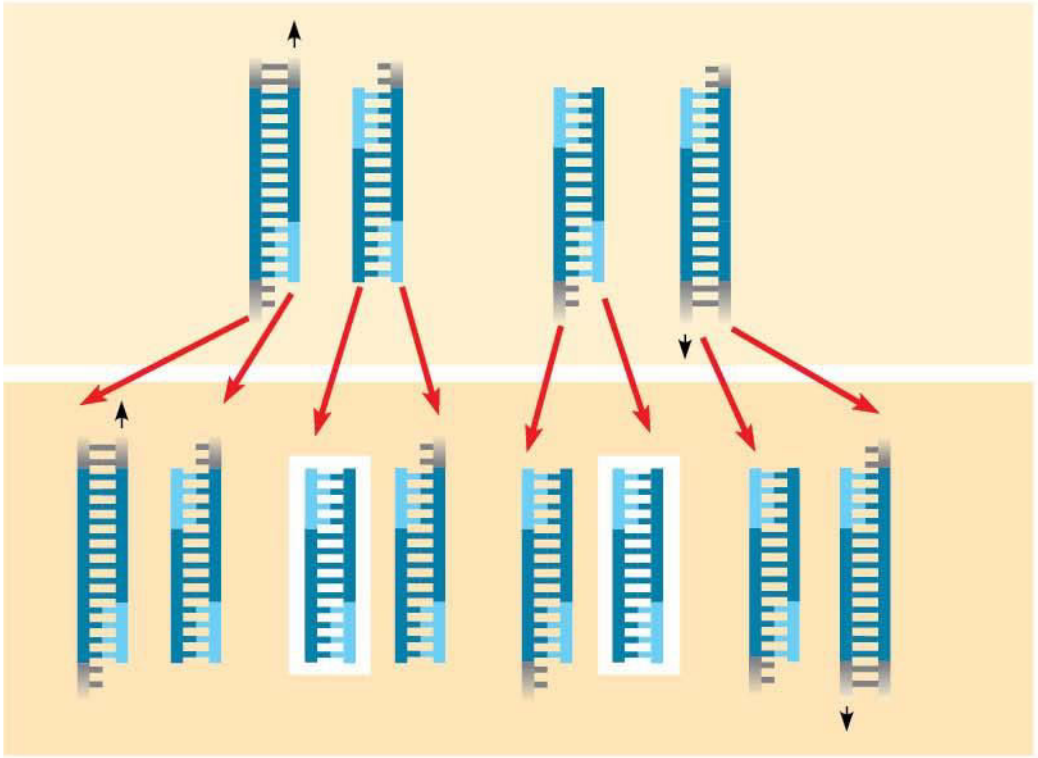


Figure 13.27-3

Cycle 2
yields 4 molecules

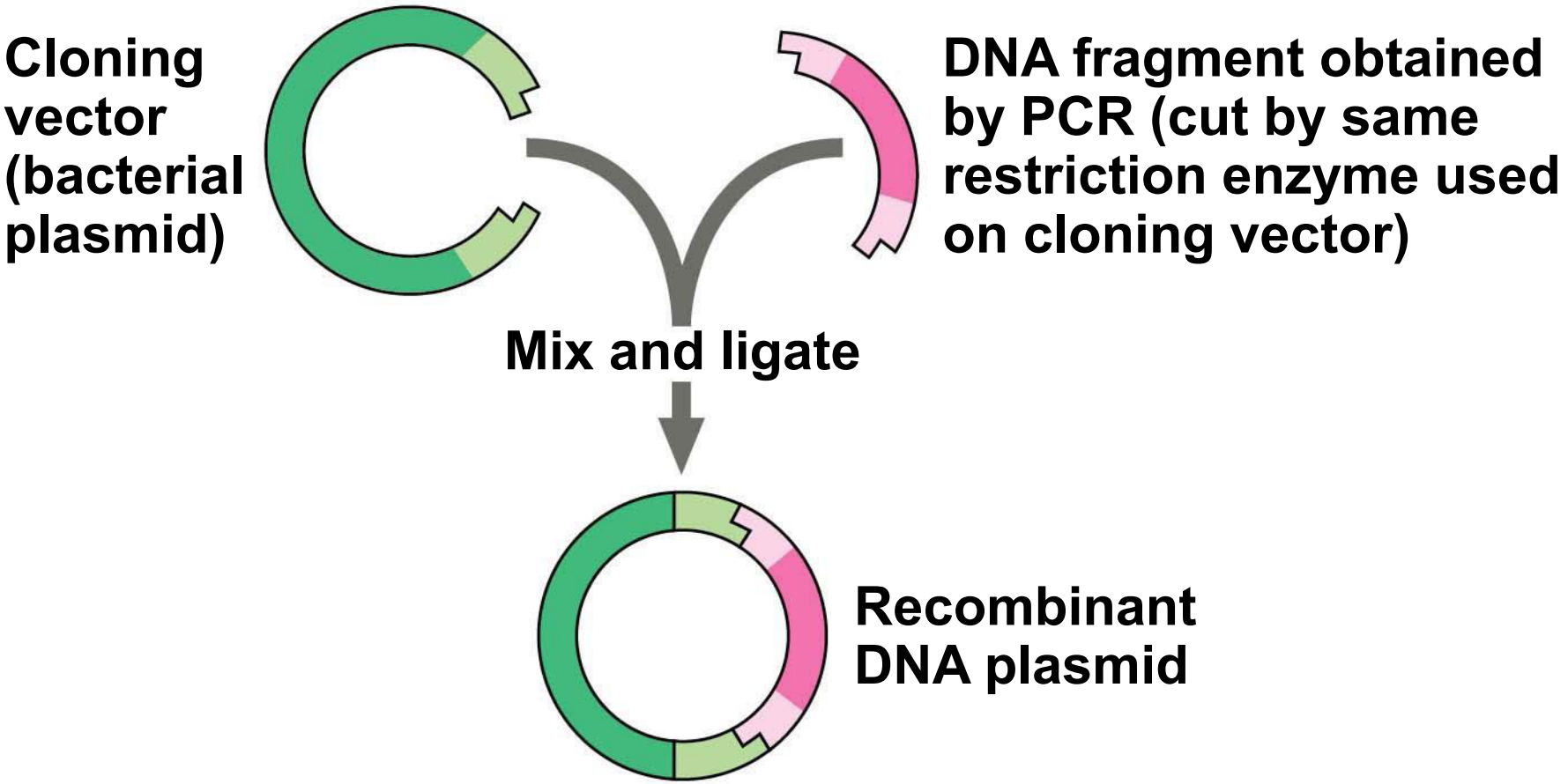
Cycle 3
2 of the 8 molecules
(in white boxes)
match target sequence
and are the right length



Results **After 30 more cycles, over 1 billion (10^9) molecules match the target sequence.**

- PCR amplification alone cannot substitute for gene cloning in cells
- Instead, PCR is used to provide the specific DNA fragment to be cloned
- PCR primers are synthesized to include a restriction site that matches the site in the cloning vector
- The fragment and vector are cut and ligated together

Figure 13.28



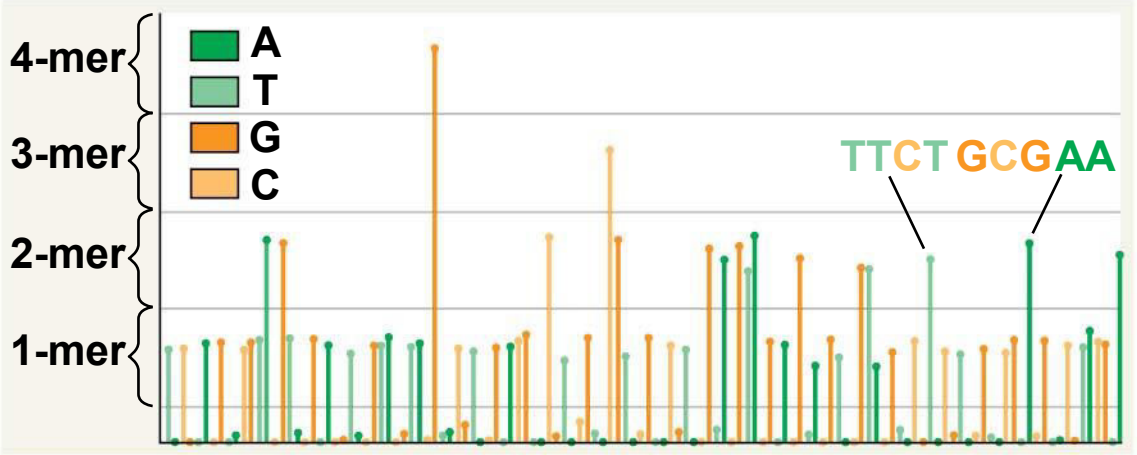
DNA Sequencing

- Once a gene is cloned, complementary base pairing can be exploited to determine the gene's complete nucleotide sequence
- This process is called **DNA sequencing**

- “Next-generation” sequencing techniques, developed in the last 15 years, are rapid and inexpensive
- They sequence by synthesizing the complementary strand of a single, immobilized template strand
- A chemical technique enables electronic monitors to identify which nucleotide is being added at each step

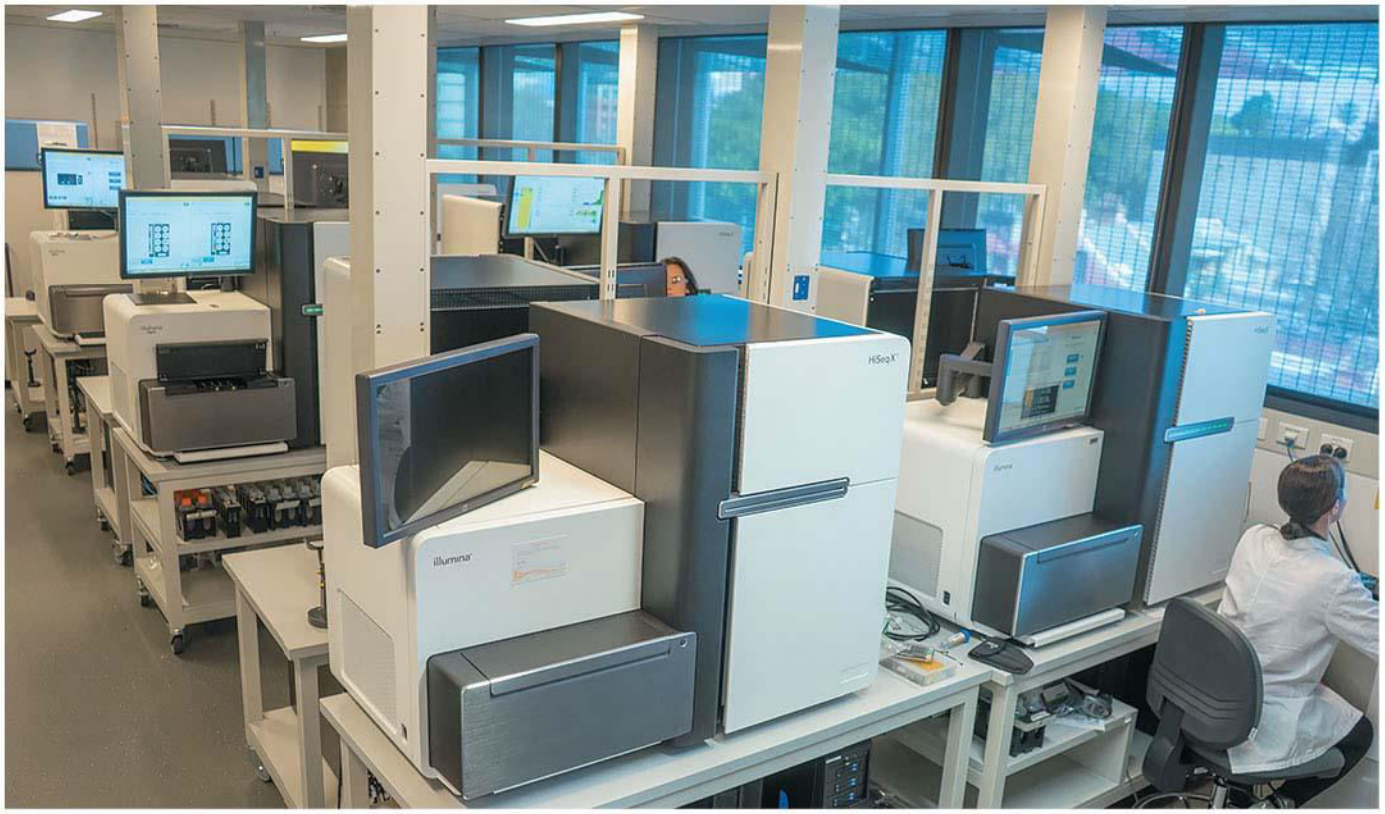
Figure 13.29

(a) Next-generation sequencing machines



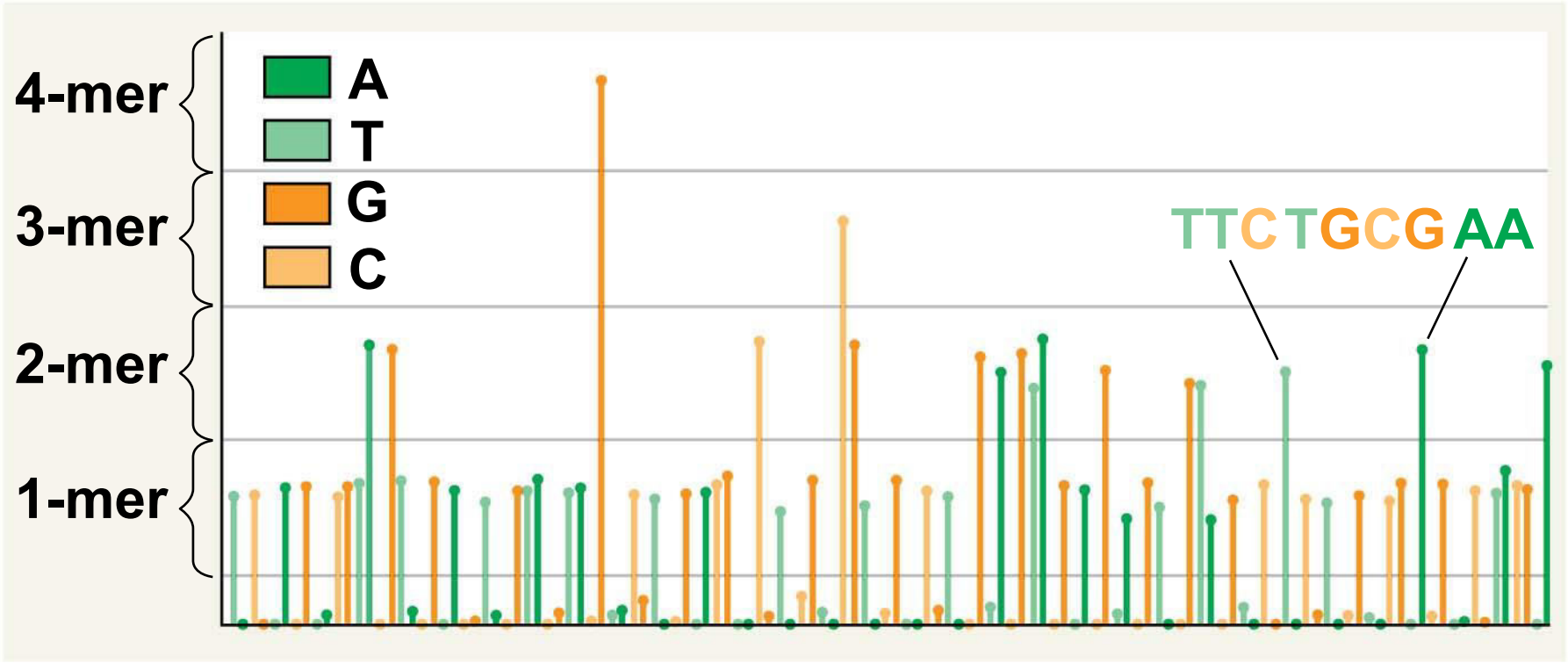
(b) A "flow-gram" from a next-generation sequencing machine

Figure 13.29-1



(a) Next-generation sequencing machines

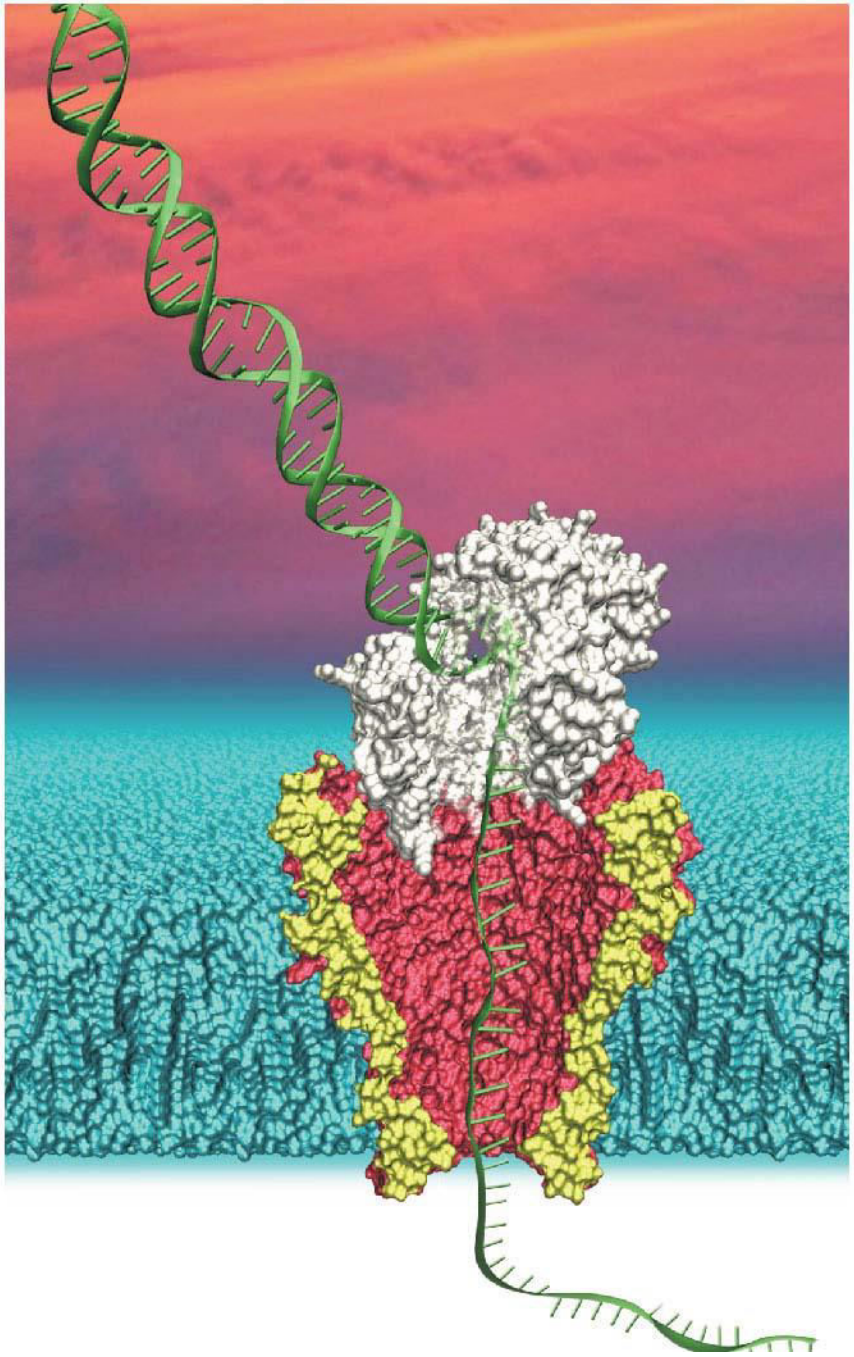
Figure 13.29-2



(b) A "flow-gram" from a next-generation sequencing machine

- Next-generation methods are being complemented or replaced by third-generation methods
- These newer techniques are faster and less expensive
- Several groups are working on “nanopore” methods, which involve moving a single DNA strand through a tiny pore in a membrane
- Nucleotides are identified by slight differences in the amount of time that they interrupt an electrical current across the pore

Figure 13.30



Editing Genes and Genomes

- Over the past five years, biologists have developed a powerful new technique called the CRISPR-Cas9 system
- Cas9 is a nuclease that cuts double-stranded DNA molecules as directed by a guide RNA that is complementary to the target gene
- Researchers have used this system to “knock out” (disable) a given gene in order to determine its function

- Researchers have also modified the CRISPR-Cas9 system to repair a gene that has a mutation
- In 2014 a group of researchers reported using this system to successfully correct a mutated gene in mice
- CRISPR technology is sparking widespread excitement among researchers and physicians

Figure 13.31

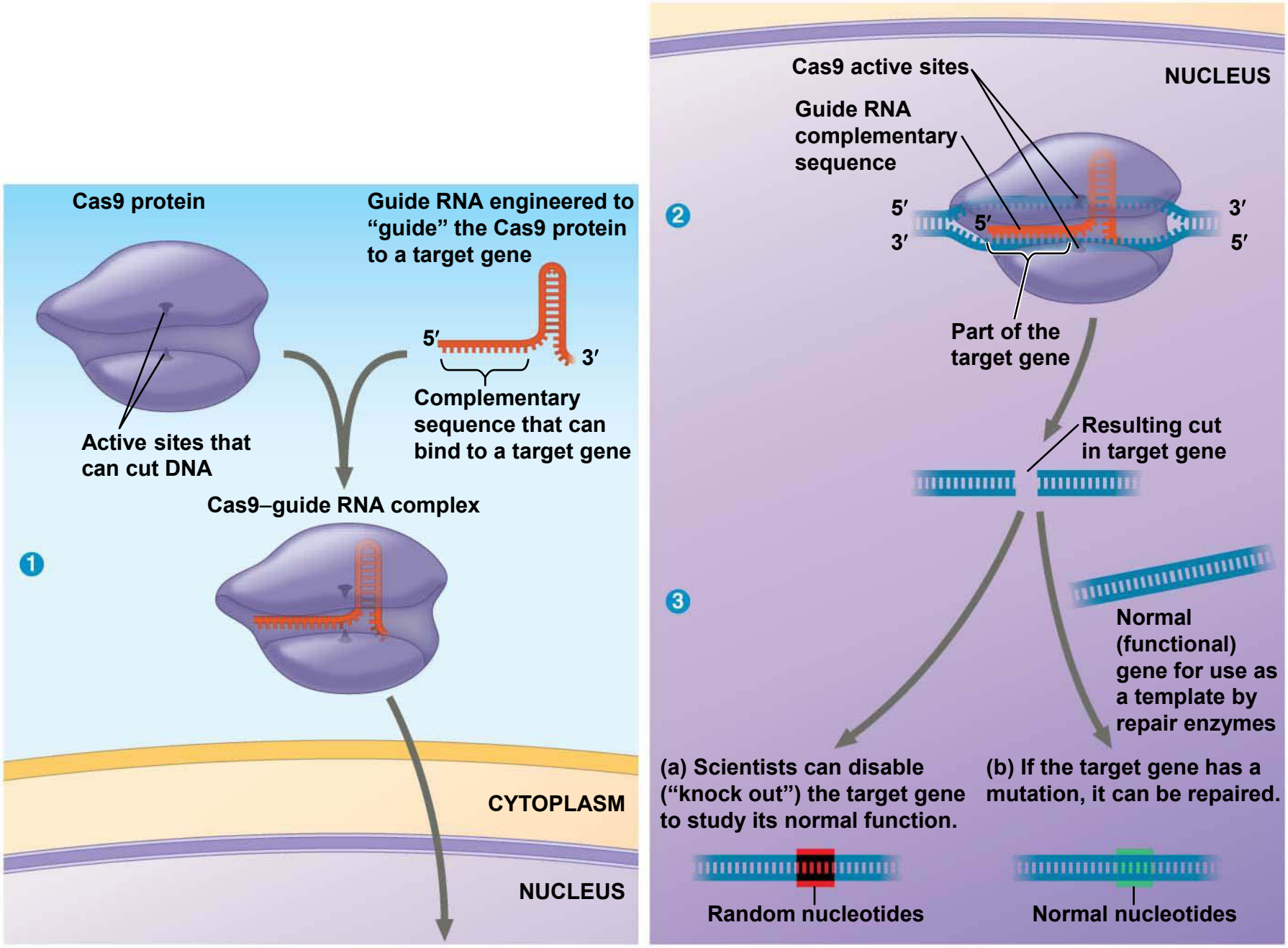


Figure 13.31-1

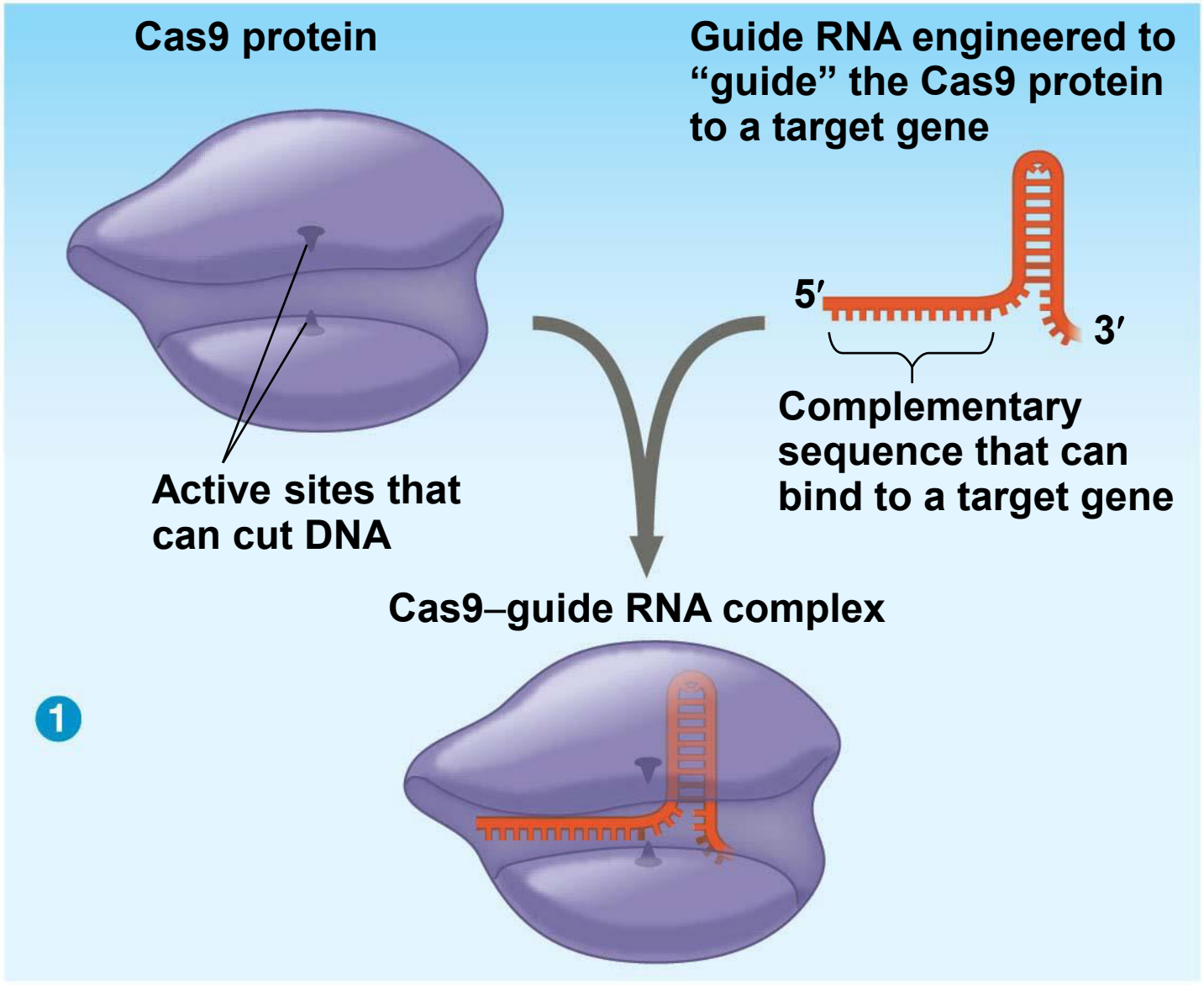


Figure 13.31-2

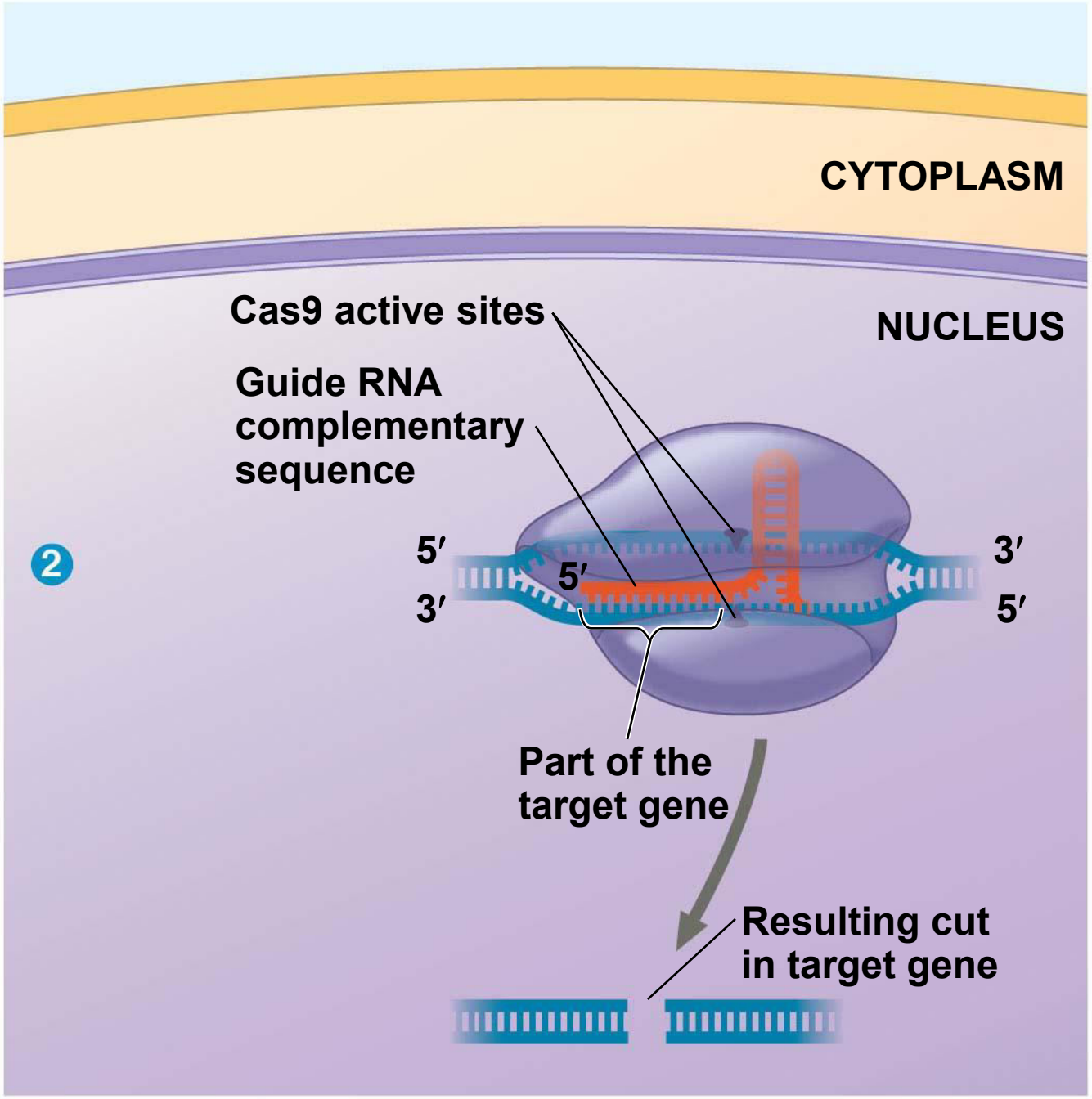


Figure 13.31-3

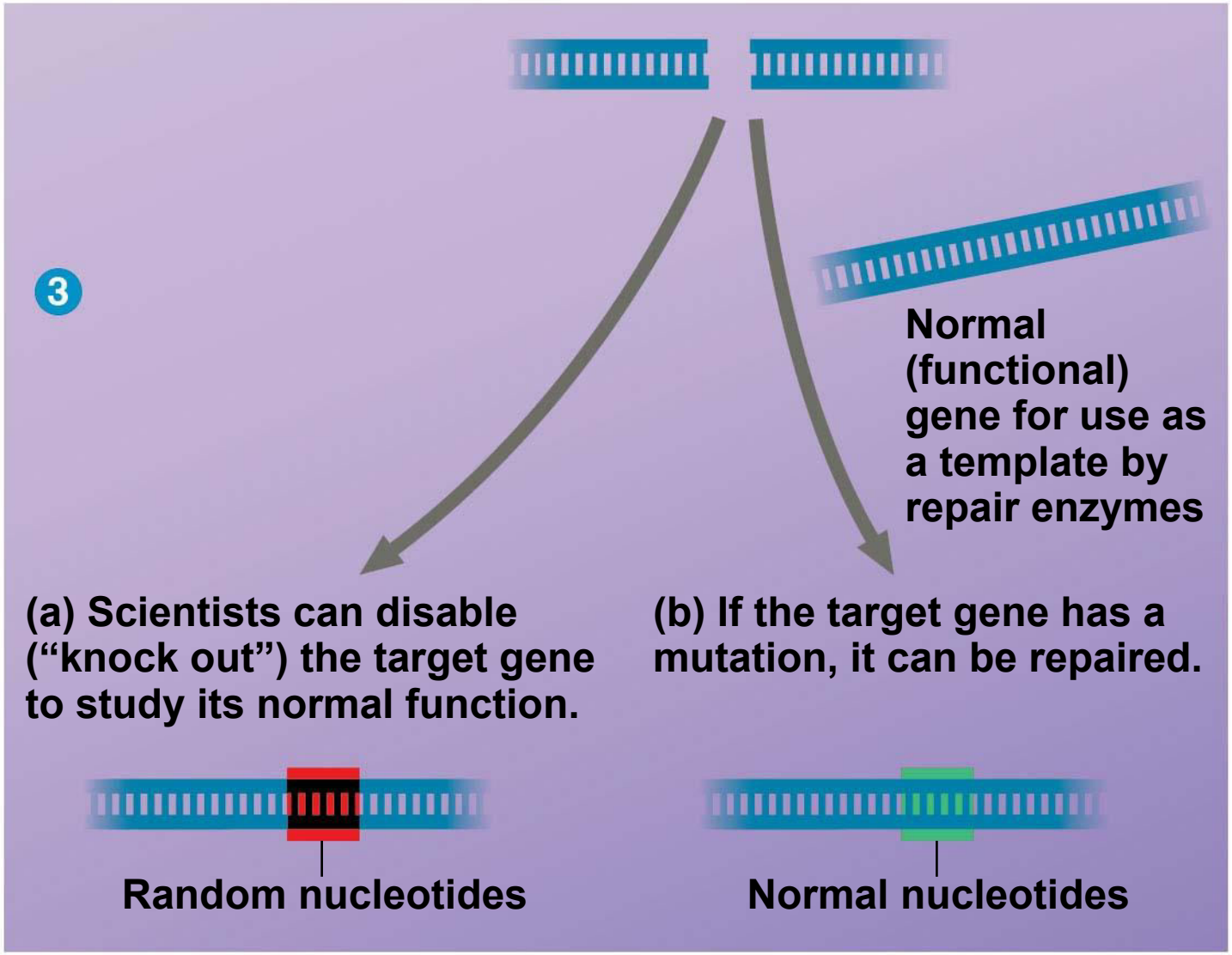


Figure 13.15

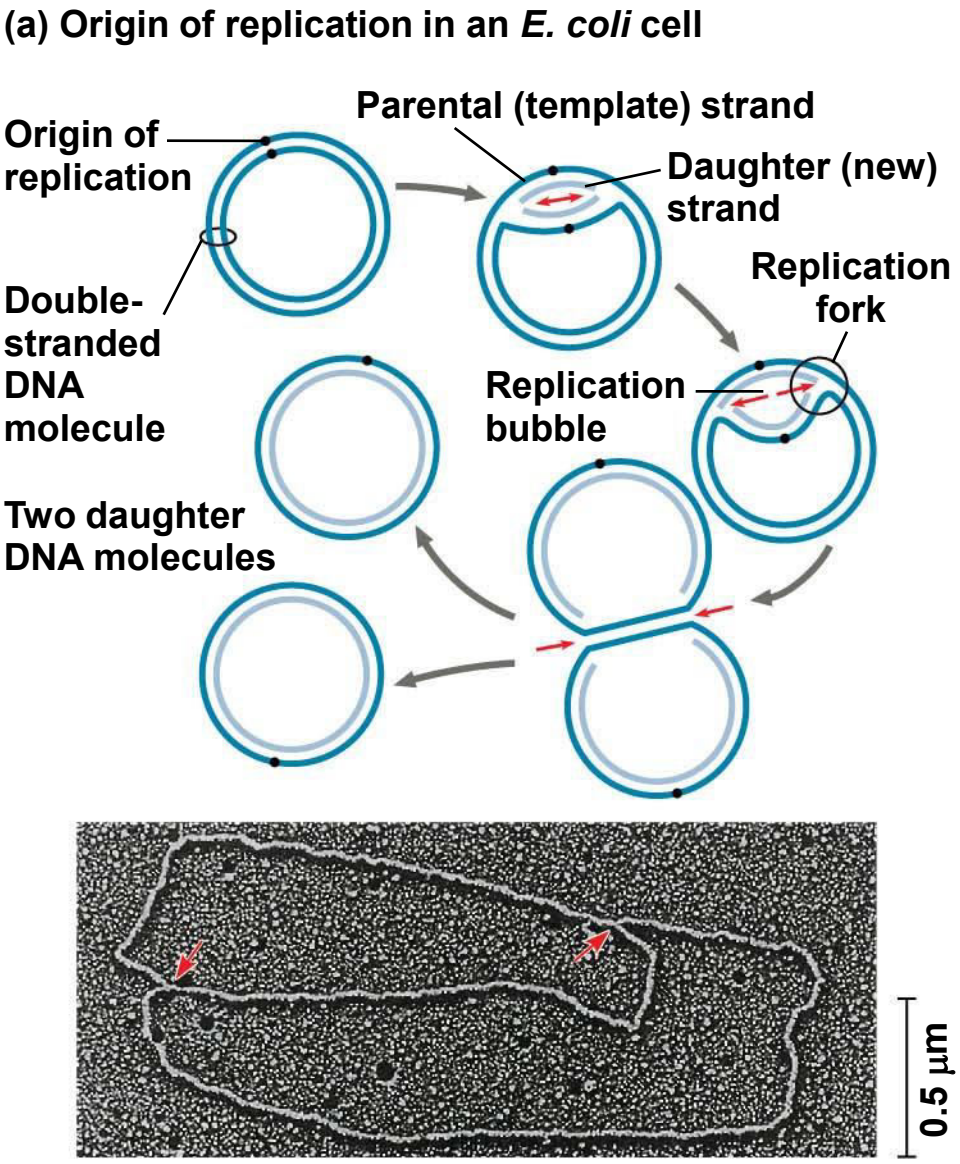


Figure 13.19

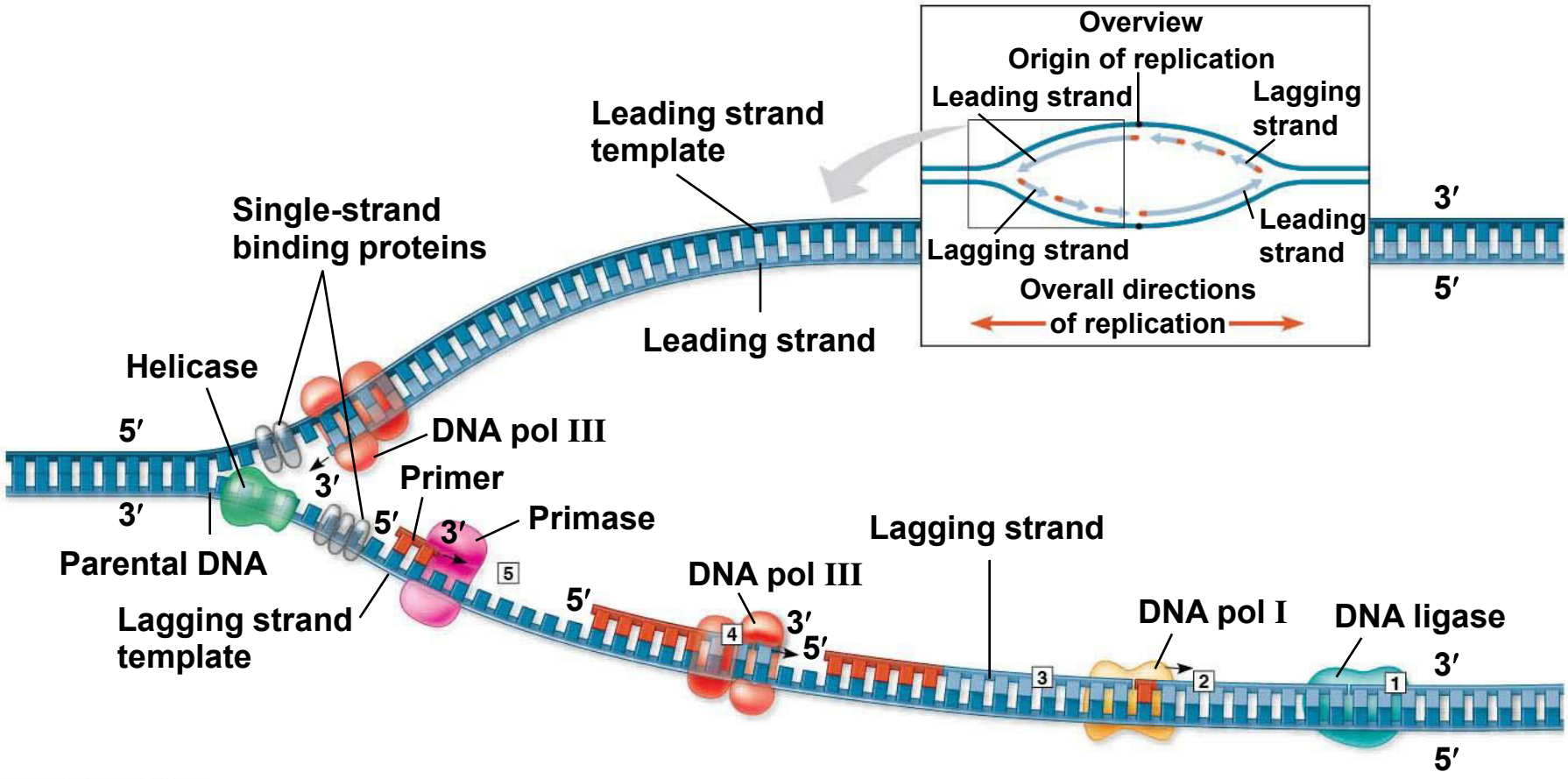


Figure 13.19-1

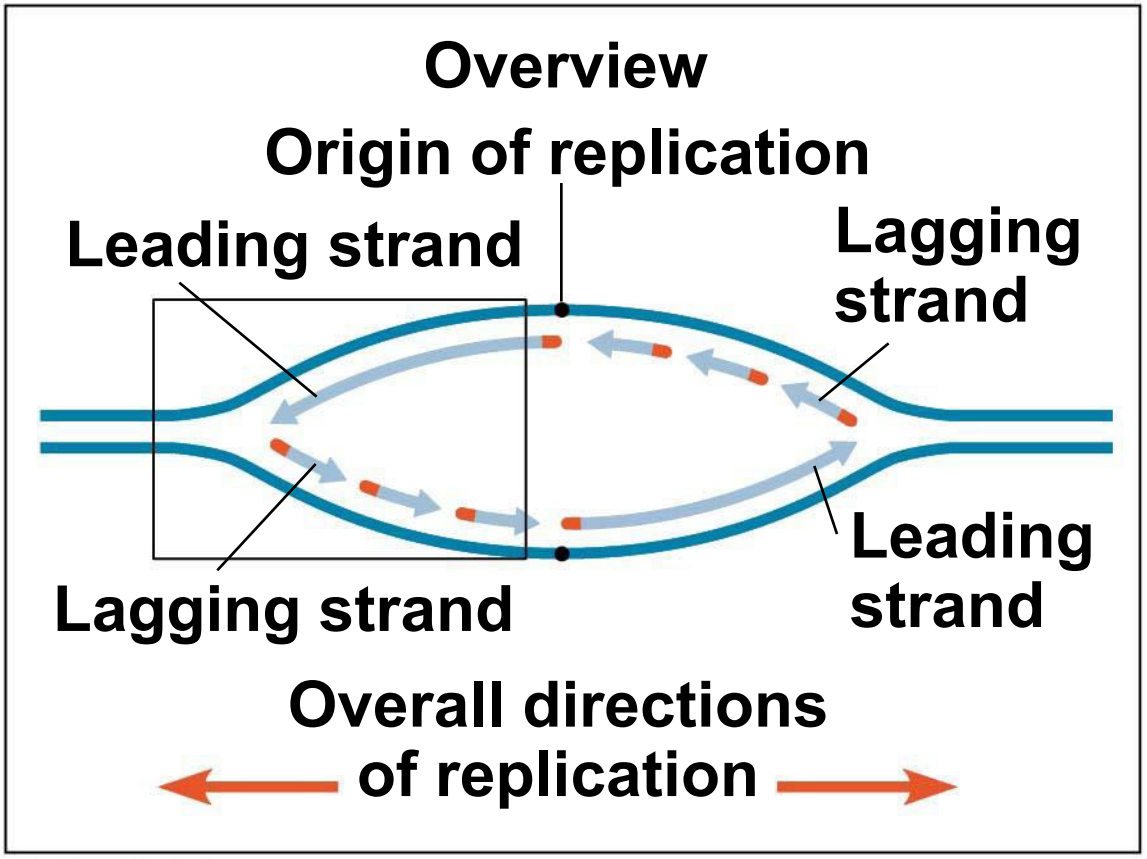


Figure 13.19-2

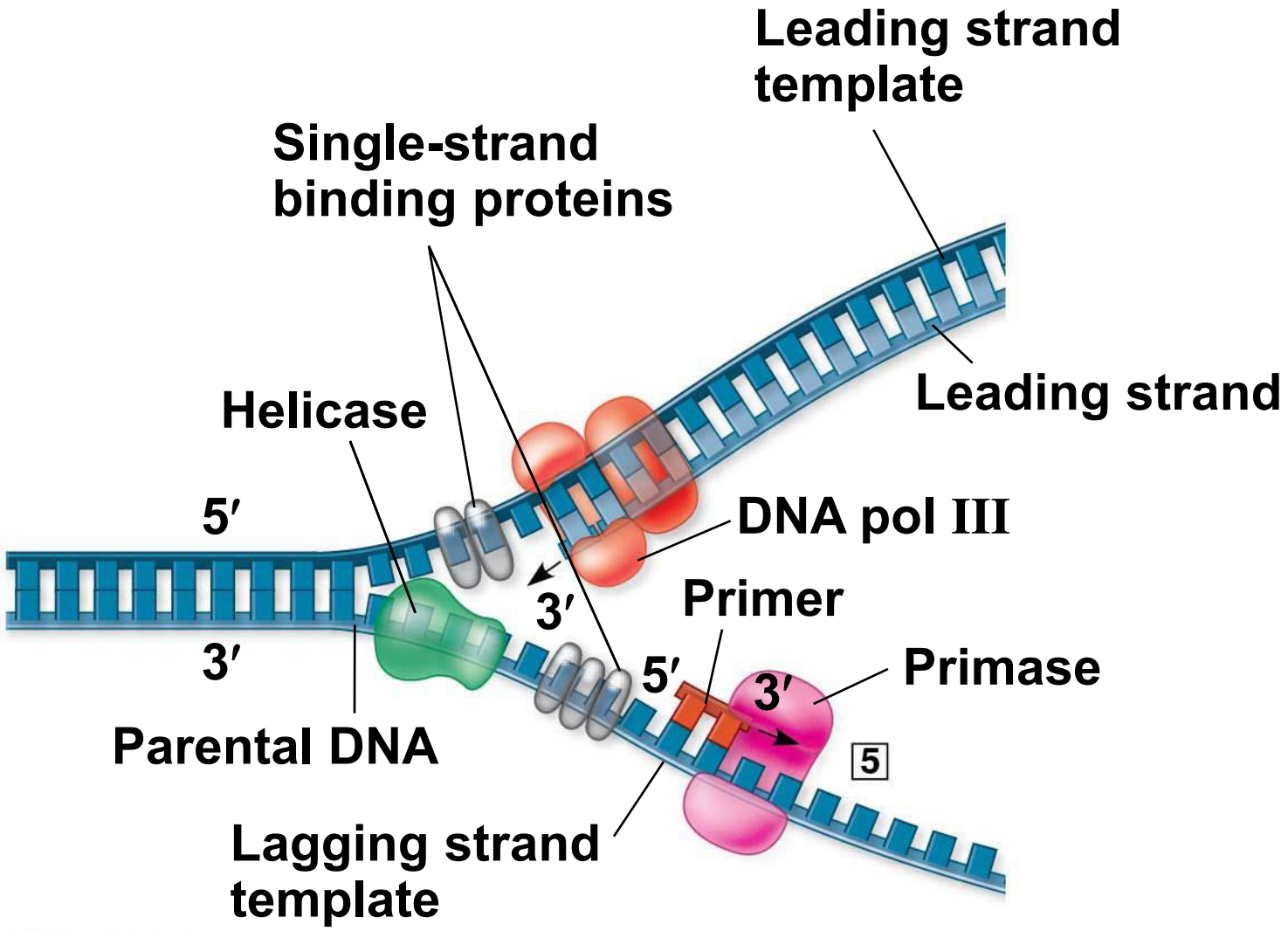


Figure 13.19-3

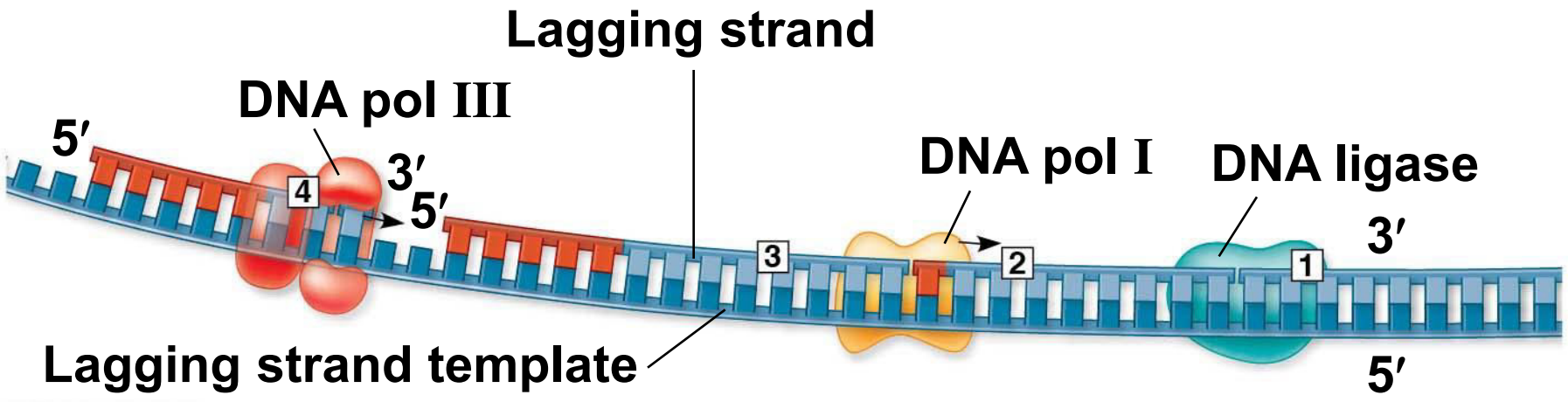


Figure 13.UN01-1

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 %				

Data from several papers by Chargaff, for example, E. Chargaff et al., Composition of the desoxypentose nucleic acids of four genera of sea-urchin, *Journal of Biological Chemistry* 195:155–160 (1952).

Figure 13.UN03

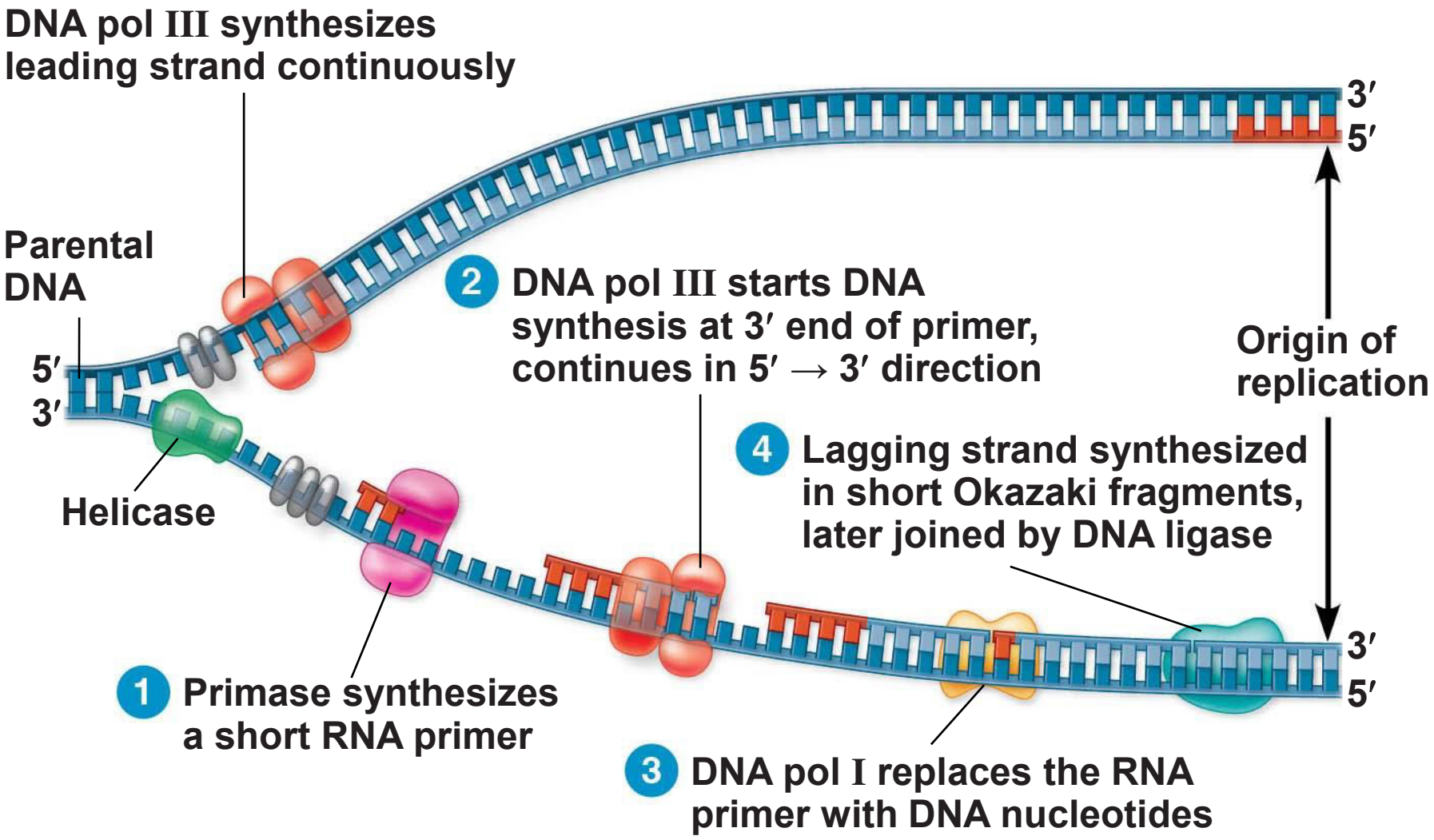


Figure 13.UN04

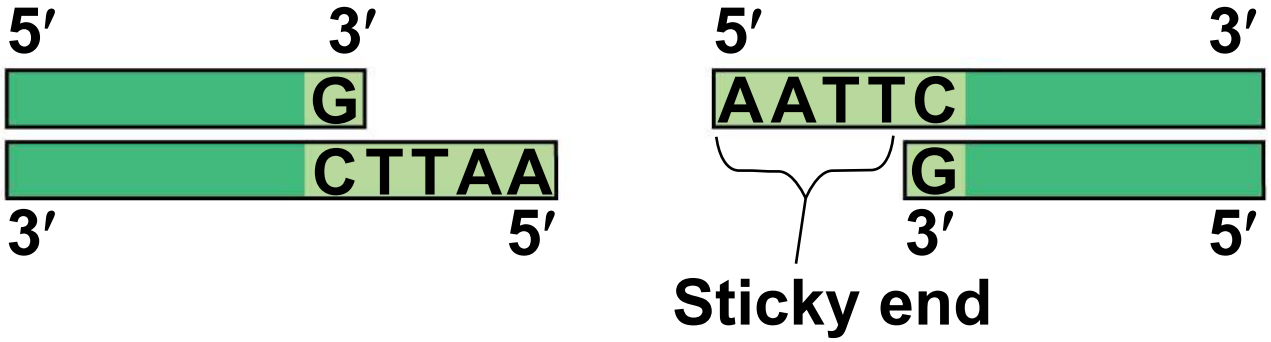


Figure 13.UN05

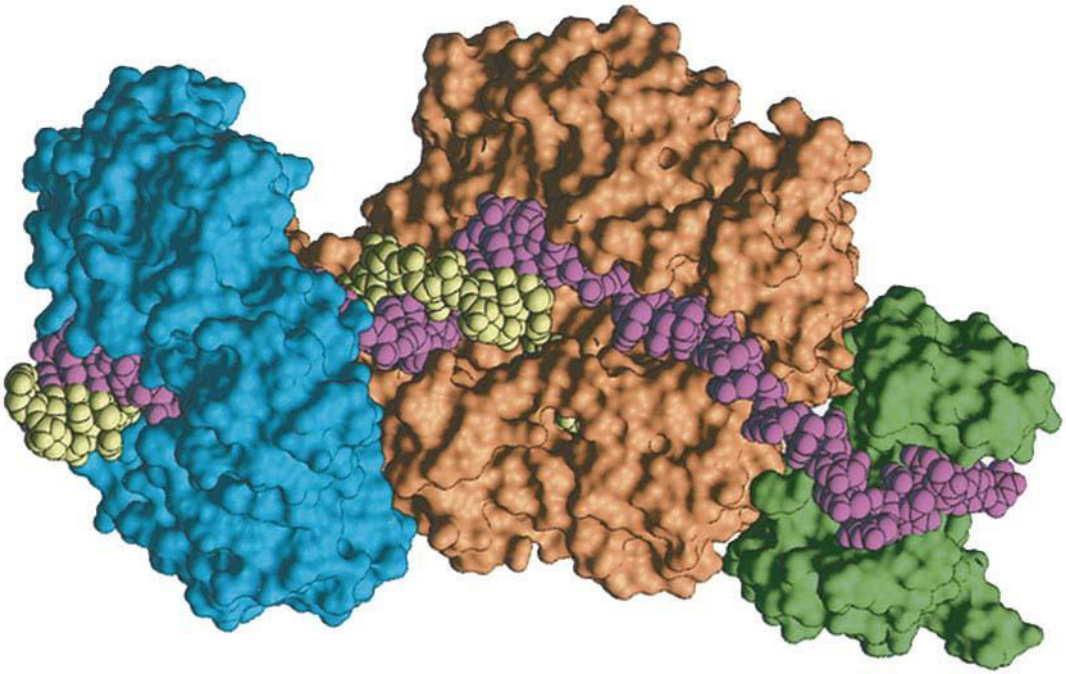


Figure 13.UN06