PowerPoint to accompany

Genetics: From Genes to Genomes

Fourth Edition

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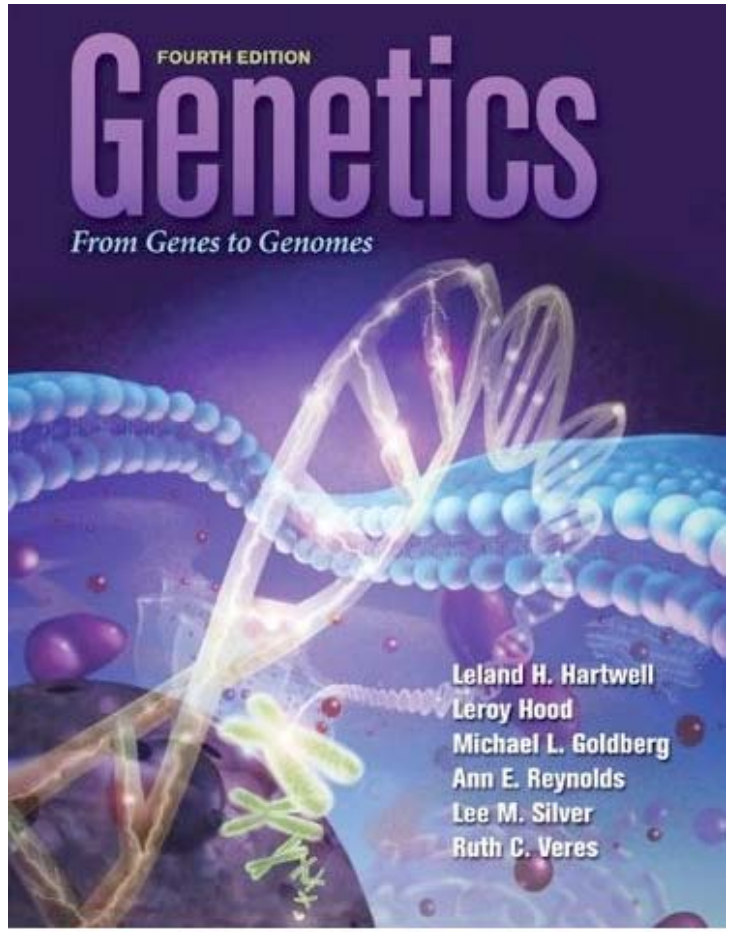

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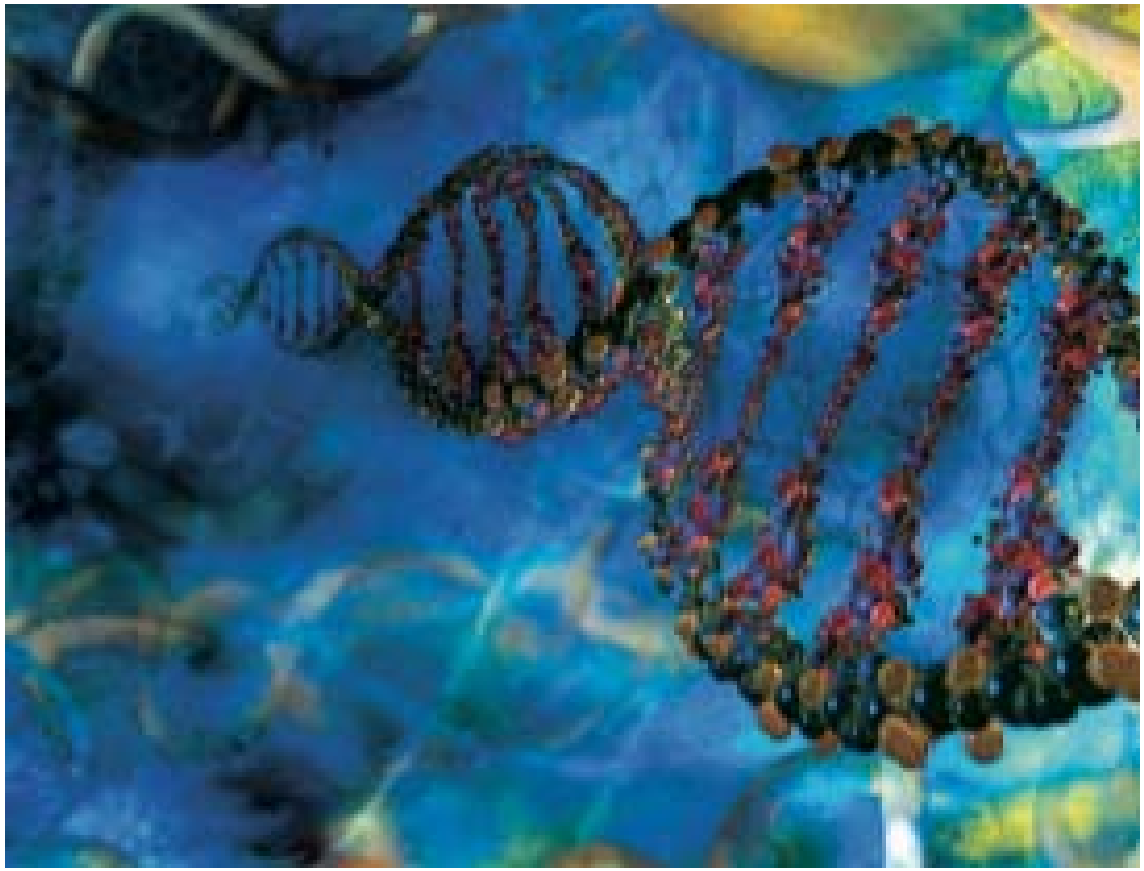
University of Georgia

DNA Structure, Replication, and Recombination



CHAPTER OUTLINE

- **DNA Structure, Replication, and Recombination**
- 6.1 Experimental Evidence for DNA as the Genetic Material
- 6.2 The Watson and Crick Double Helix Model of DNA
- 6.3 Genetic Information in DNA Base Sequence
- 6.4 DNA Replication
- 6.5 Recombination at the DNA Level

Two general themes to genes at the molecular levels

The genetic functions of DNA flow directly from its molecular structure

- **Knowledge of molecular structure of DNA makes it possible to understand biochemical processes of genetics**

All of the genetics functions of DNA depend on specialized proteins that "read" the information in DNA sequence

- **DNA itself is chemically inert**

Chemical studies located DNA in the chromosomes

F. Meischer (1869) extracted "nuclein" from nuclei of human white blood cells

- **Weakly acidic, phosphorus rich material**

Chemical analysis of nuclein revealed that its major component was deoxyribonucleic acid (DNA)

- **Contains deoxyribose, found in nucleus, and is acidic**

Staining of cells revealed that DNA localized almost exclusively within chromosomes

- **Schiff reagent – stains DNA red**

The chemical composition of DNA

DNA contains four kinds of nucleotides linked in a long chain

Phosphodiester bonds – covalent bonds joining adjacent nucleotides

Polymer – linked chain of subunits

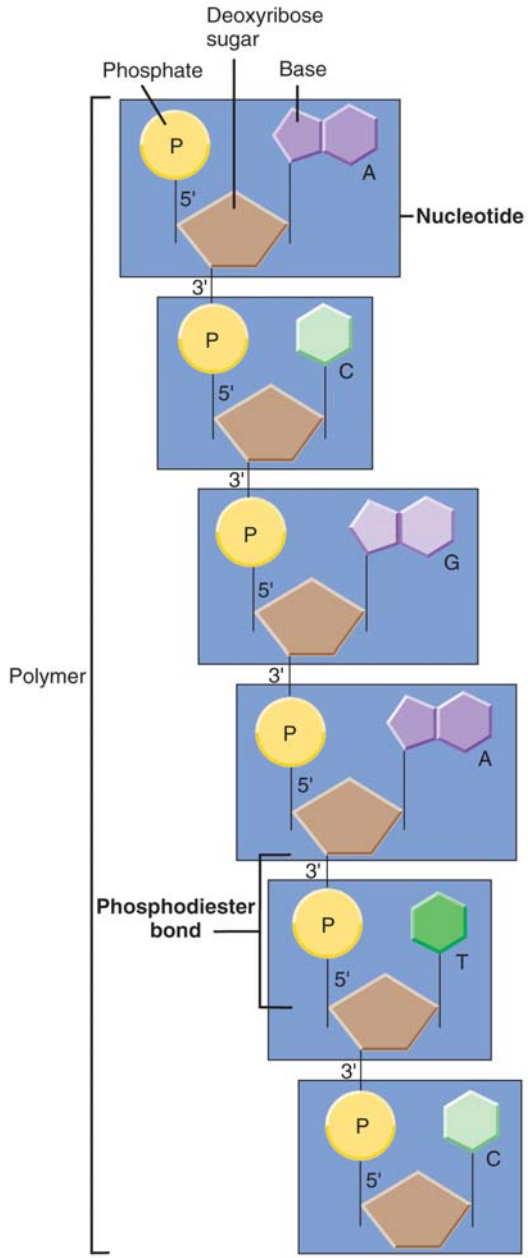


Fig. 6.2

Are genes composed of DNA or protein?

DNA is made of only four different subunits

- **Too simple to specify genetic complexity?**

Protein is made of 20 different subunits

- **More potential for creating different combinations?**
- **Chromosomes contain more protein than DNA**

Bacterial transformation implicates DNA as the substance of genes

F. Griffith (1928) did experiments with two strains of *Streptococcus pneumoniae*

- **Differ in colony morphology and biological activity**
- **Smooth (S) strain – virulent**
- **Rough (R) strain – nonvirulent**
- **R cells could be transformed by genetic material transferred from dead S cells**

Avery, MacLeod, and McCarty (1944) provided evidence that DNA is the "transforming principle" of S cells

Two forms of *S. pneumoniae* have different colony morphologies

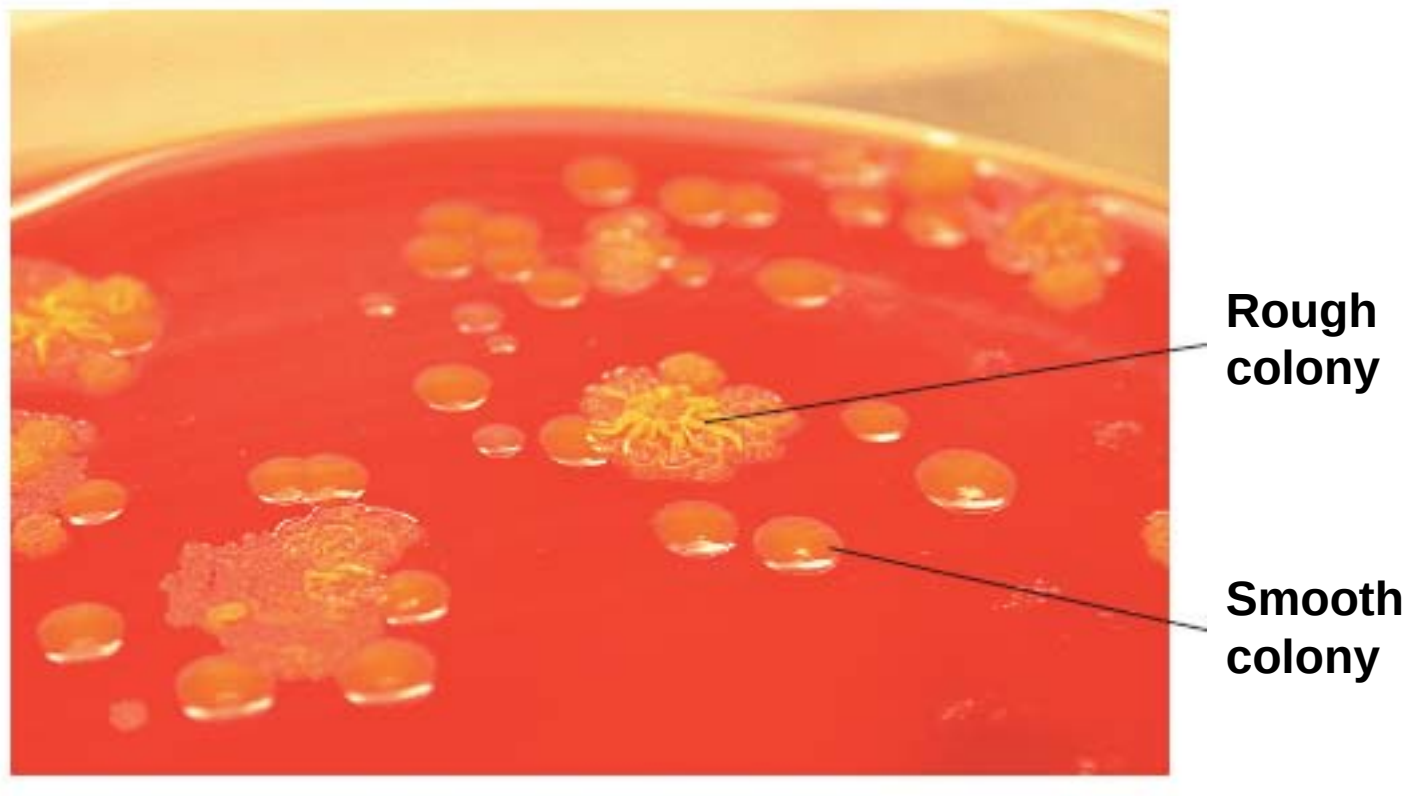


Fig. 6.3

The two forms of *S. pneumoniae* differ in their effects on mice

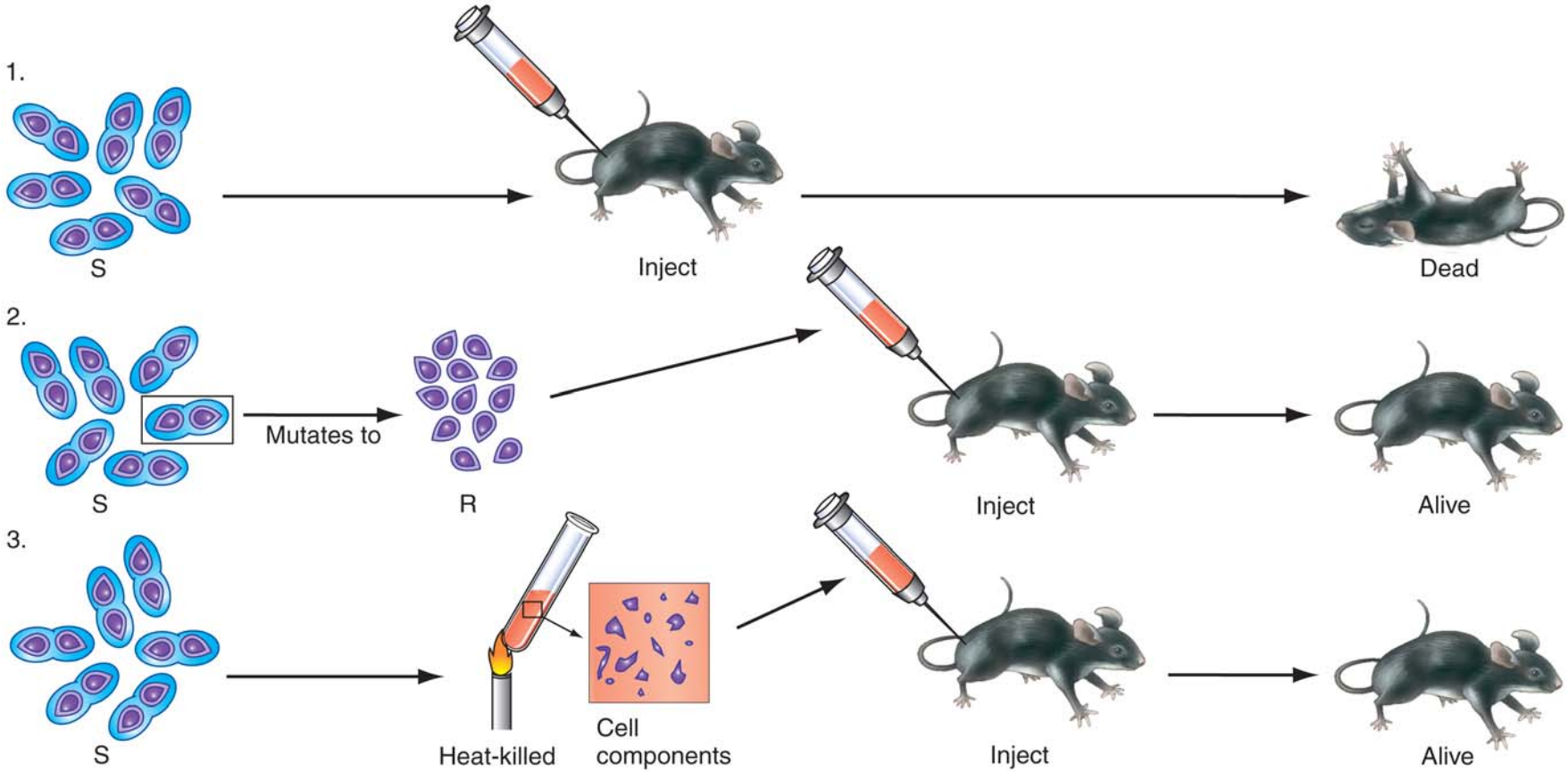


Fig. 6.4

Griffith's experiment that provided evidence of transformation

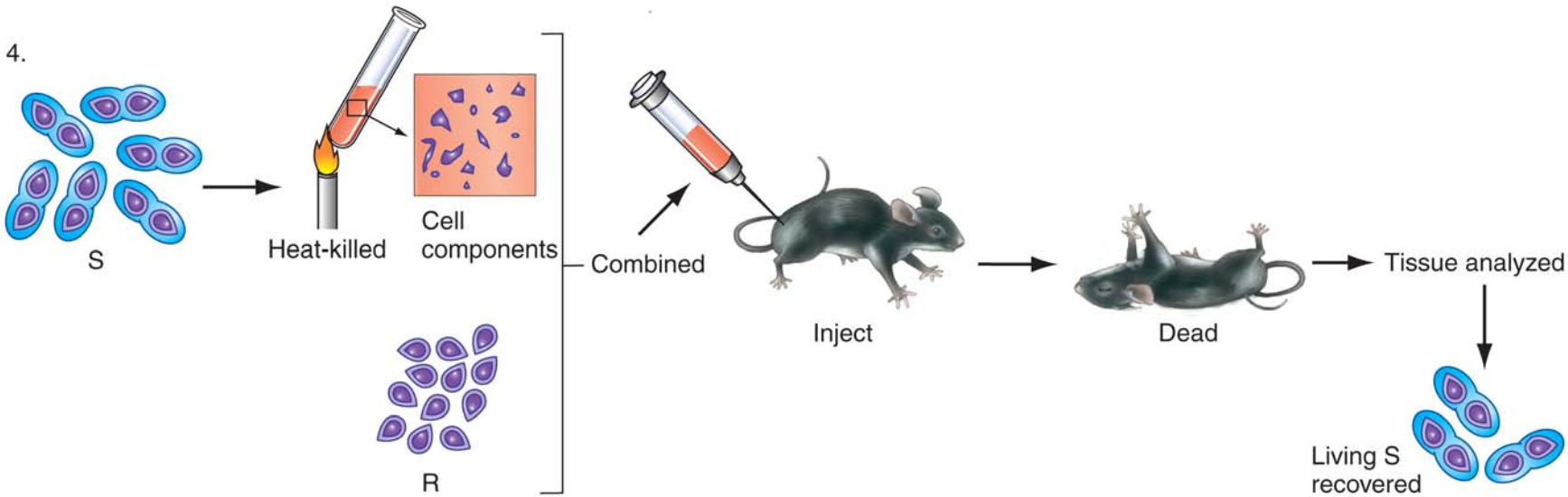


Fig. 6.4

Avery, MacLeod, and McCarty confirmed that DNA is the transforming principle

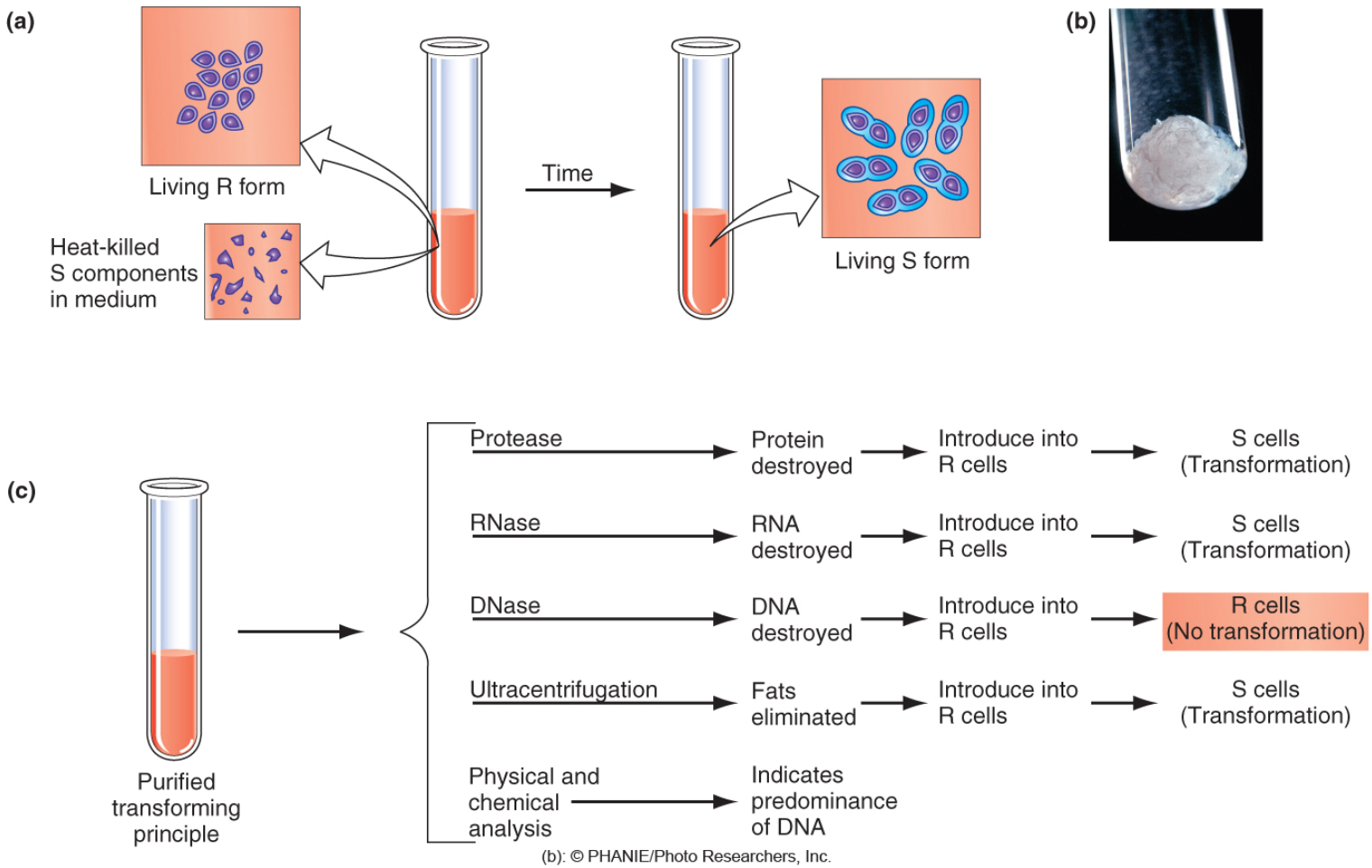


Fig. 6.5

A simple system to test whether protein or DNA is the genetic material

Bacteriophages (phages) are viruses that infect bacteria

Phage particles contain roughly equal amounts of protein and DNA

Contain very few genes but able to replicate themselves inside bacterial host

- **After infection, "ghost" of phage particle remains attached to outer surface of cell**
- **Phage genetic material "injected" into bacterial cell**

Structure and life cycle of bacteriophage T2

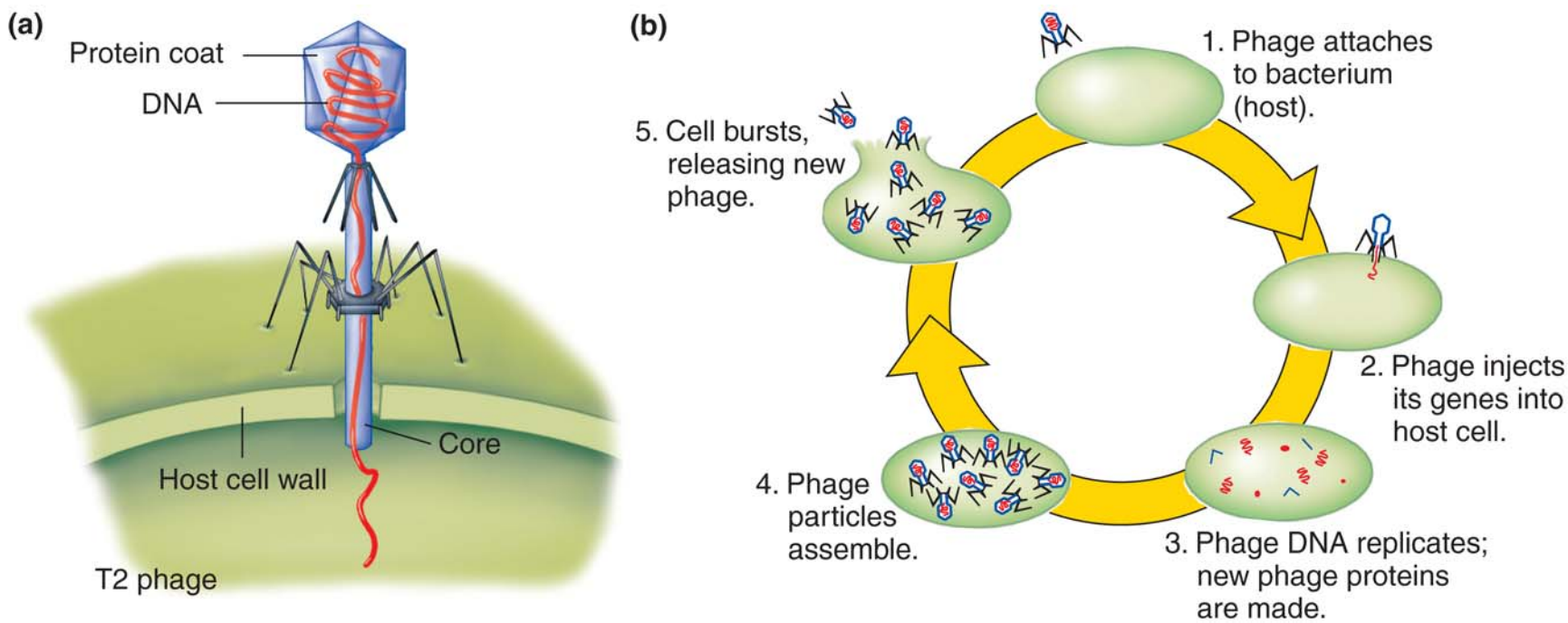


Fig. 6.6

The Hershey-Chase Waring blender experiment

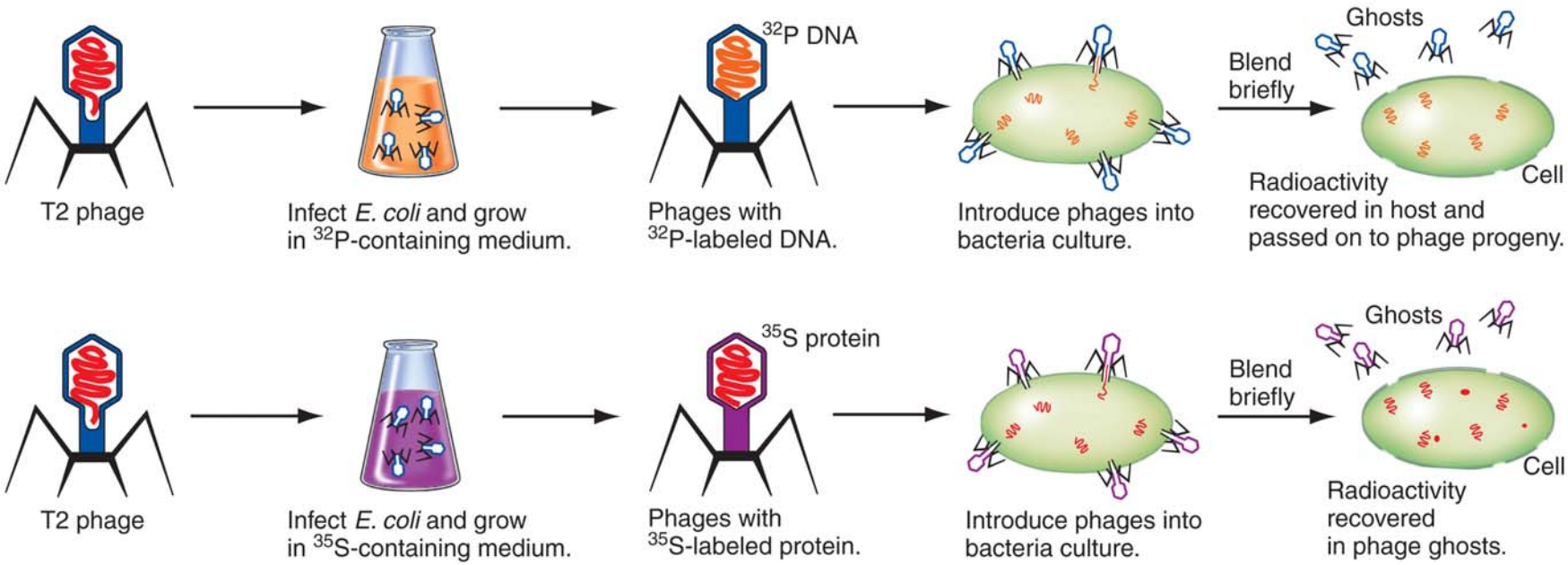


Fig. 6.7

The basis of the Watson-Crick double helix model of DNA

R. Franklin and M. Wilkins (1952) solved X-ray diffraction pattern of DNA (see Figure 6.8)

- **DNA is helical structure with 20 Å diameter**
- **Spacing between repeating units is 3.4 Å**
- **Helix undergoes a complete turn every 34 Å**

Detailed knowledge of chemical constituents of DNA

- **Four nitrogenous bases [guanine (G), adenine (A), cytosine (C), and thymine (T)], deoxyribose, phosphate**

E. Chargaff – base composition of DNA from many organisms

- **Ratios of bases: A:T ratio is 1:1, G:C ratio is 1:1**

A detailed look at DNA's chemical constituents

Deoxyribose **Phosphate** **Four nitrogenous bases**

Purines **Pyrimidine**

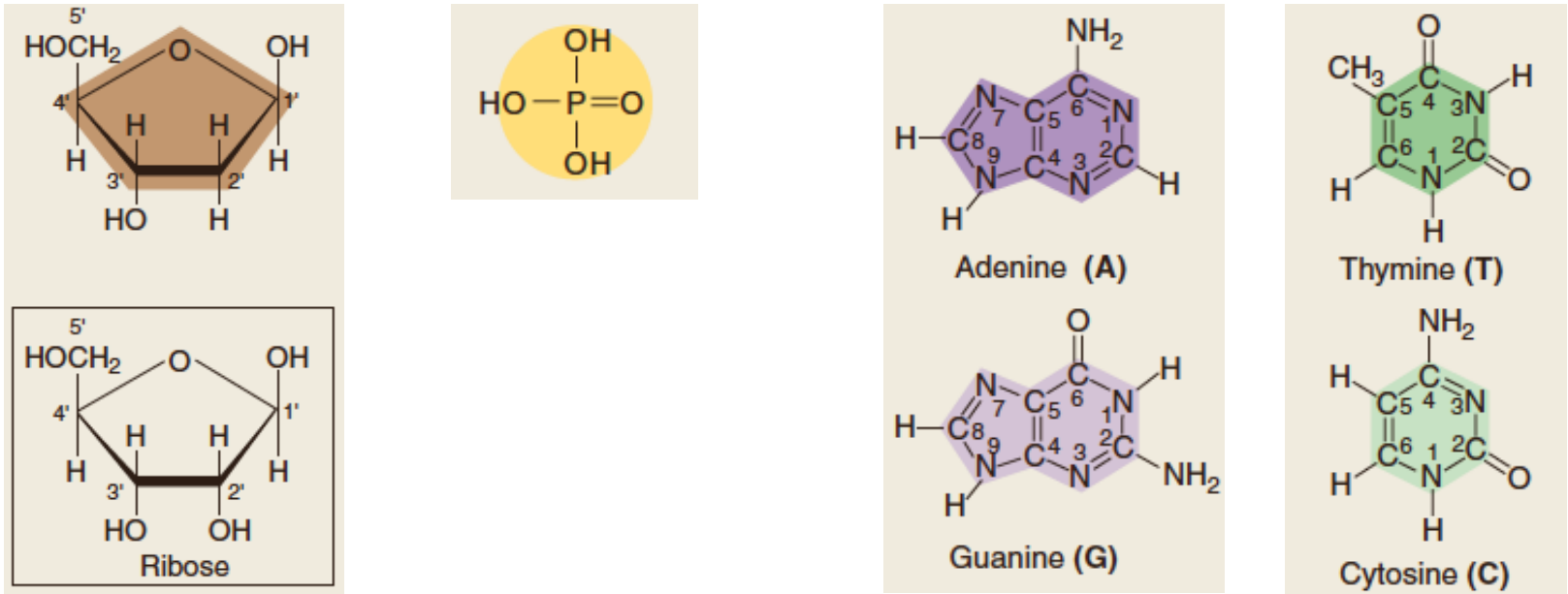


Fig. 6.9a

A detailed look at DNA's chemical constituents (cont)

Attachment of base to sugar

Addition of phosphate to nucleoside

Nucleoside

Purine nucleotide

Pyrimidine nucleotide

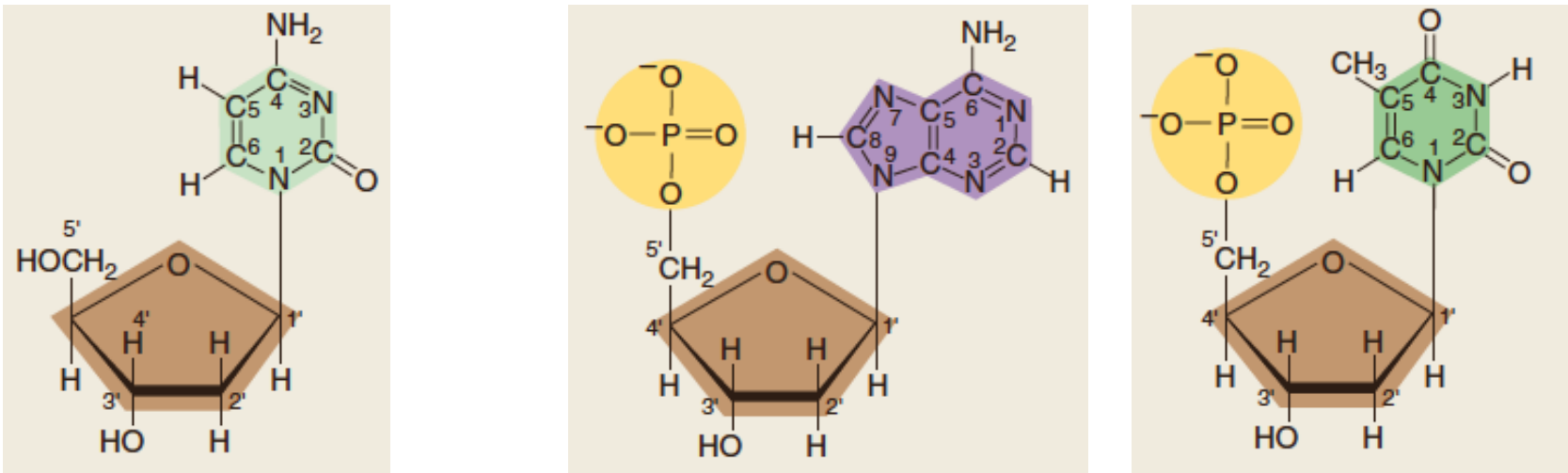


Fig. 6.9b

A detailed look at DNA's chemical constituents (cont)

Nucleotides linked in a directional chain

Phosphodiester bonds always form covalent link between 3' carbon of one nucleoside and 5' carbon of the next nucleoside

Note the 5'-to-3' polarity

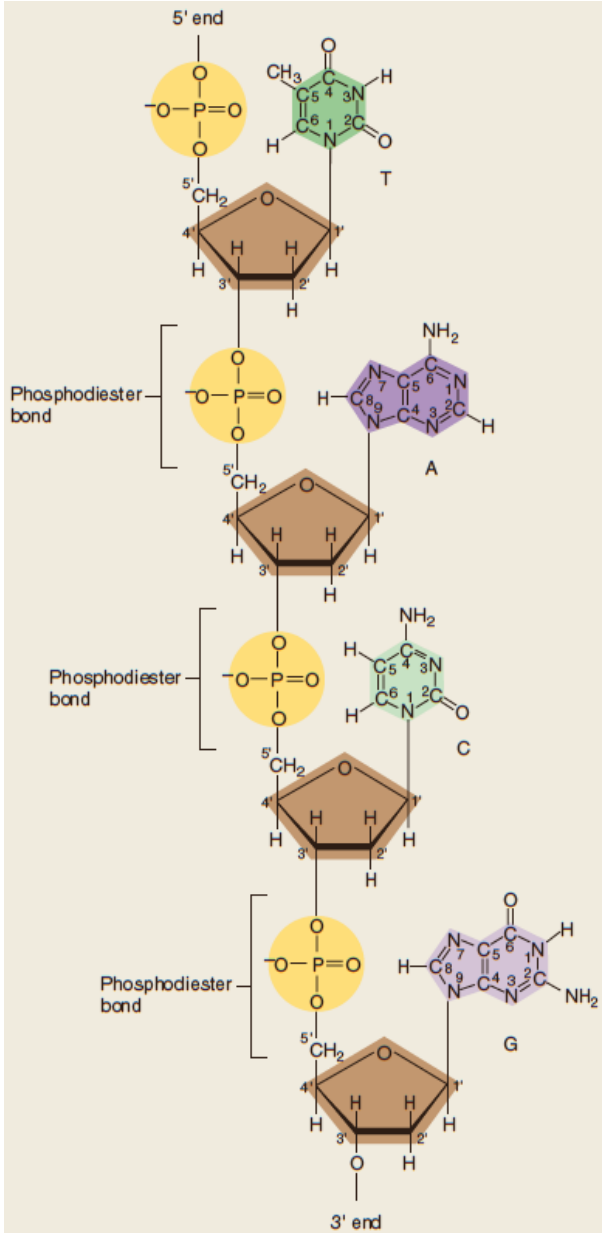


Fig. 6.9c

Chargaff's data on nucleotide base composition in the DNA of various organisms

In all organisms, ratios of A to T and G to C are roughly 1:1

	Percentage of Base in DNA				Ratios	
Organism	*A*	*T*	*G*	*C*	*A:T*	*G:C*
Staphylococcus afermentams	12.8	12.9	36.9	37.5	0.99	0.99
Escherichia coli	26.0	23.9	24.9	25.2	1.09	0.99
Yeast	31.3	32.9	18.7	17.1	0.95	1.09
*Caenorhabditis elegans**	31.2	29.1	19.3	20.5	1.07	0.96
*Arabadopsis thaliana**	29.1	29.7	20.5	20.7	0.98	0.99
Drosophila melanogaster	27.3	27.6	22.5	22.5	0.99	1.00
Honeybee	34.4	33.0	16.2	16.4	1.04	0.99
Mus musculus (mouse)	29.2	29.4	21.7	19.7	0.99	1.10
Human (liver)	30.7	31.2	19.3	18.8	0.98	1.03

Table 6.1

Complementary base pairing

Base pairs consist of hydrogen bonds (weak electrostatic bonds) between a purine and a pyrimidine (G with C, A with T)

Consistent with Chargaff's rules

Each base pair has ~ same shape

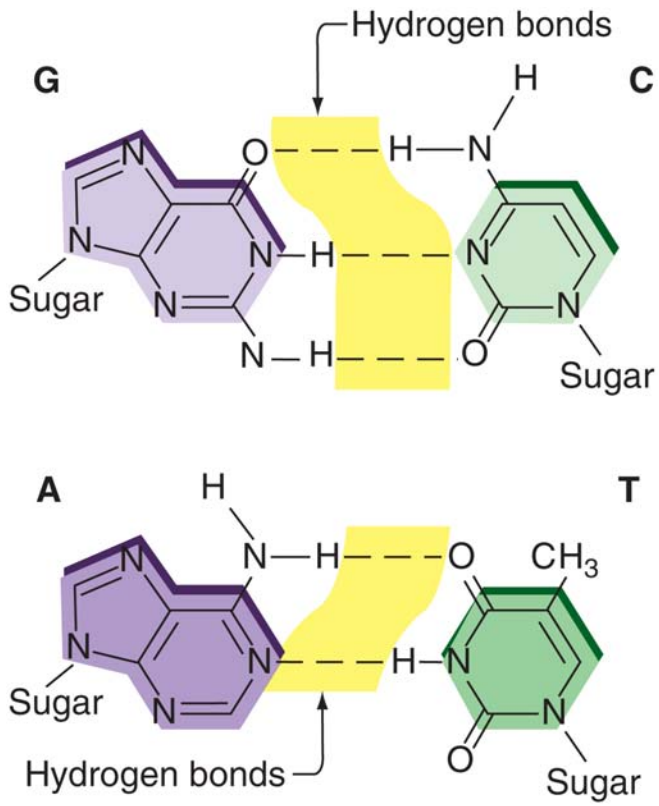


Fig. 6.10

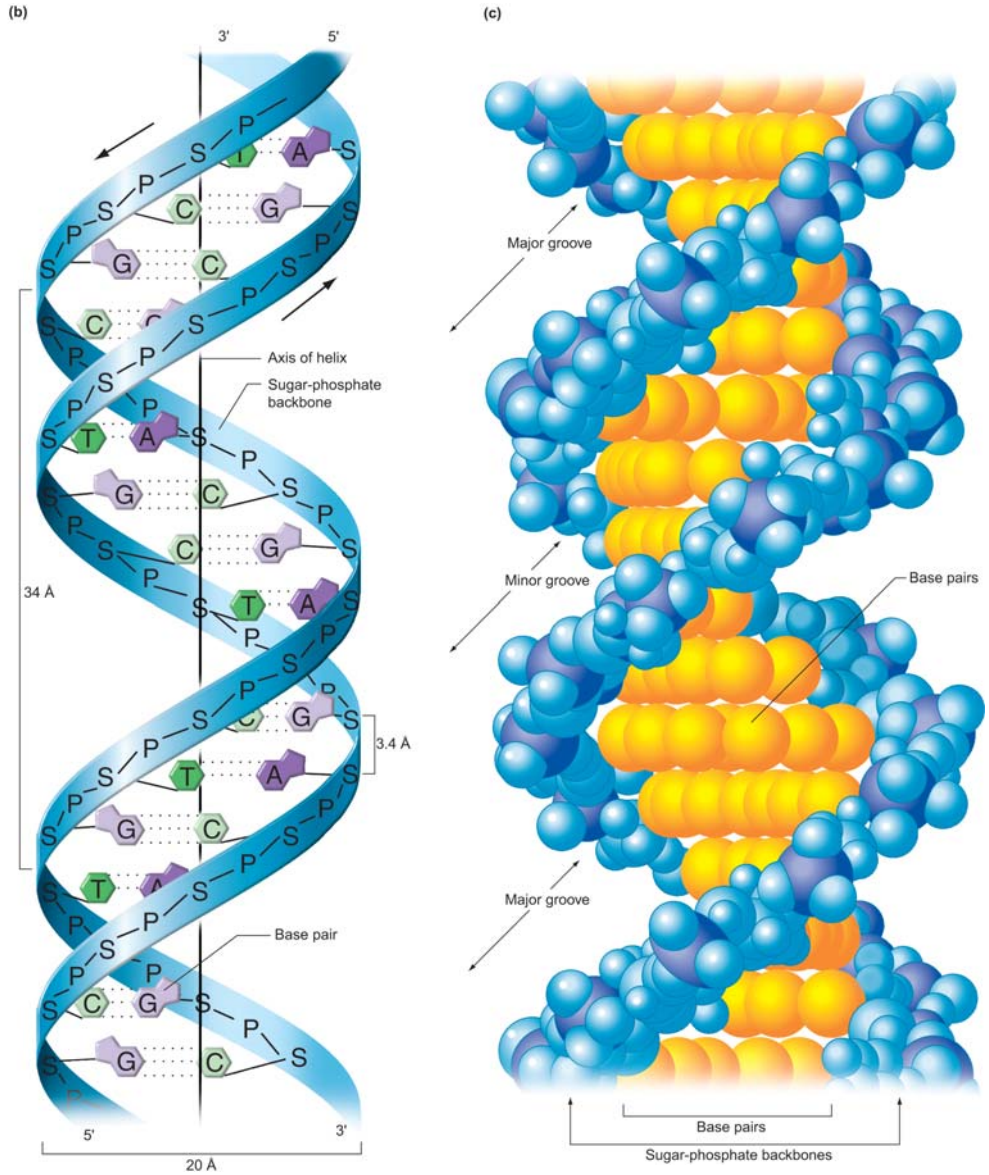
The double helix structure of DNA

Strands are antiparallel

Sugar – phosphate backbone on the outside

Base pairs in the middle

Two chains held together by H bonds between A-T and G-C base pairs



Feature Fig. 6.11

Z DNA is one variant of the double helix

B-form DNA forms right-handed helix and has a smooth backbone

Z-form DNA forms left-handed helix and has an irregular backbone

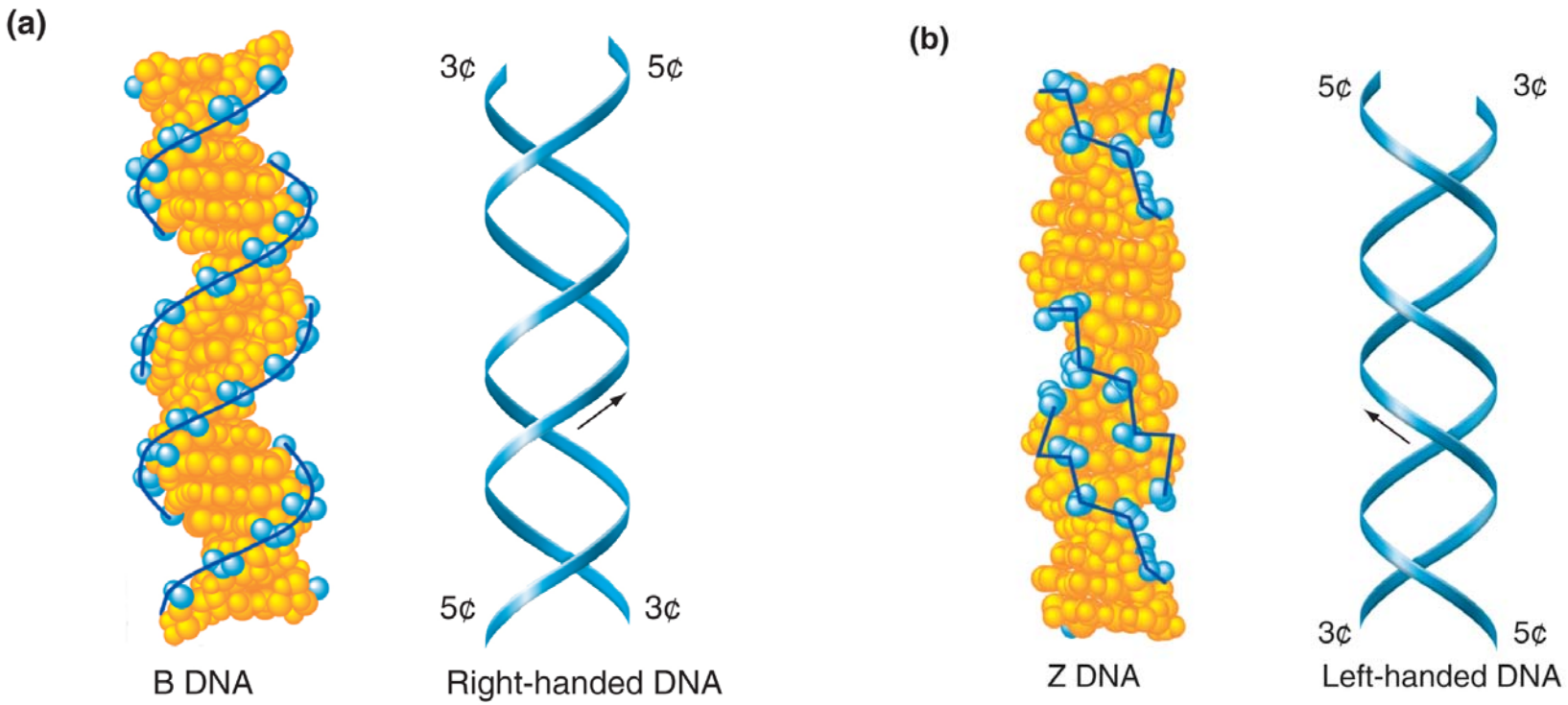


Fig. 6.12

Four questions about how DNA structure relates to genetic functions

How does the molecule carry information?

- **Base sequence**

How is that information is copied for transmission to future generations?

- **DNA replication**

What mechanisms allow the genetic information to change?

- **Recombination**
- **Mutations (chapter 7)**

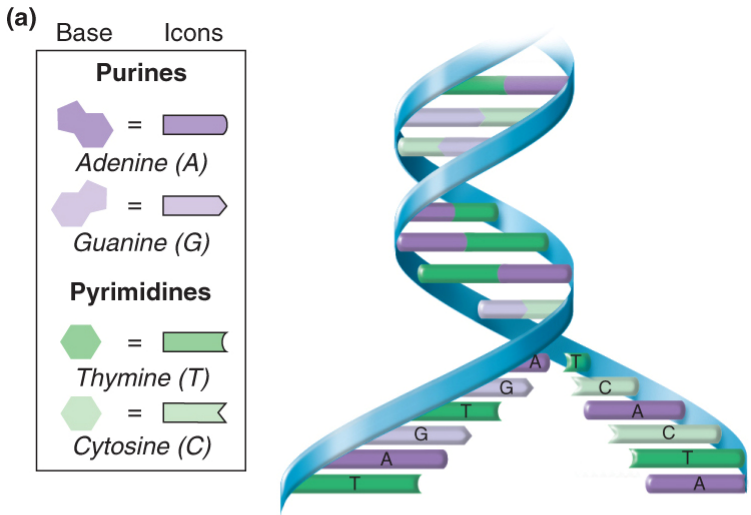
How does DNA-encoded information govern the expression of phenotype?

- **Gene functions (chapter 8)**

DNA stores information in the sequence of its bases

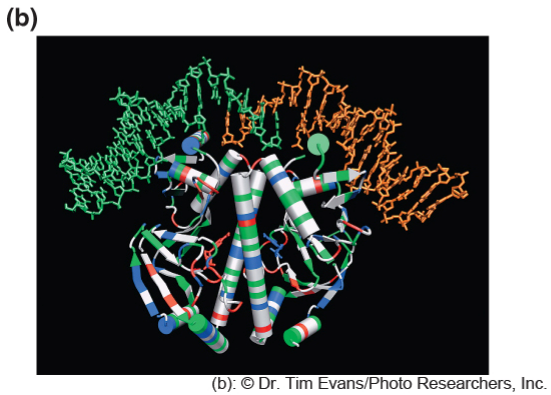
(a) Most genetic information is "read" from unwound DNA

e.g. synthesis of DNA or RNA



(b) Some genetic information is accessible within double-stranded DNA

e.g. DNA-binding proteins that regulate gene expression



(b): © Dr. Tim Evans/Photo Researchers, Inc.

Fig. 6.14

Chemical constituents of RNA

Three major chemical differences between RNA and DNA

Ribose sugar instead of deoxyribose

Uracil (U) instead of thymine (T)

Most RNAs are single stranded

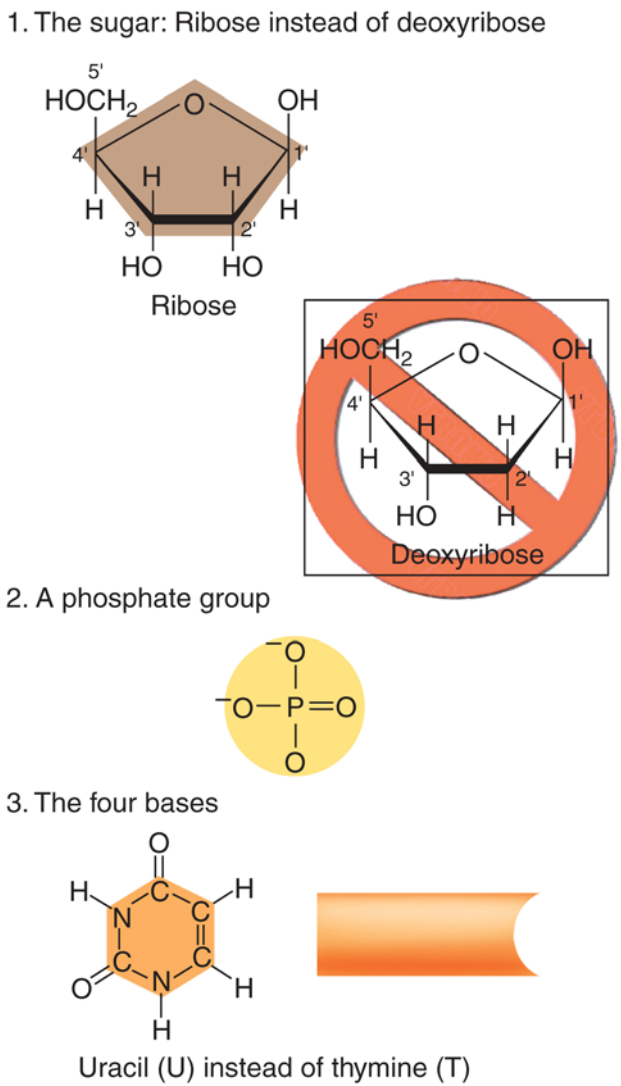


Fig. 6.15a

Complex folding pattern of RNA

Most RNAs are single stranded but can form base pairs within other parts of the same molecule

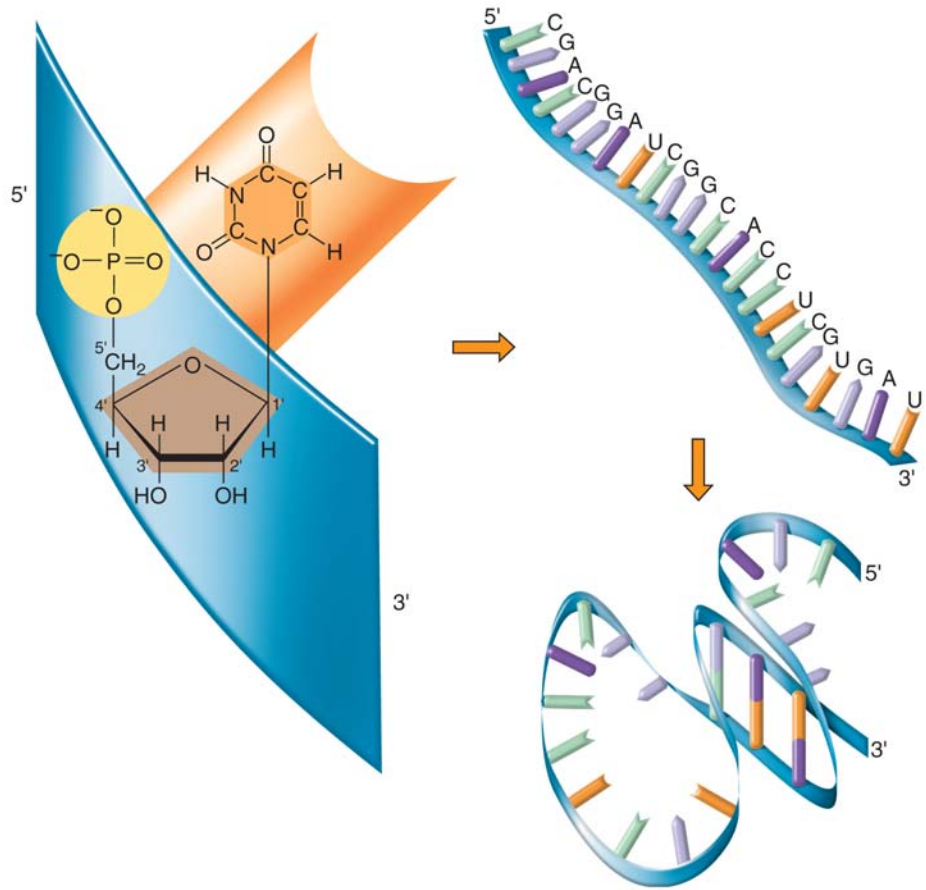


Fig. 6.15b, c

The model of DNA replication postulated by Watson and Crick

Unwinding of double helix exposes bases on each strand

Each strand can act as a template for synthesis of new strands

New strand forms by insertion of complementary base pair

Single double helix becomes two identical daughter double helices

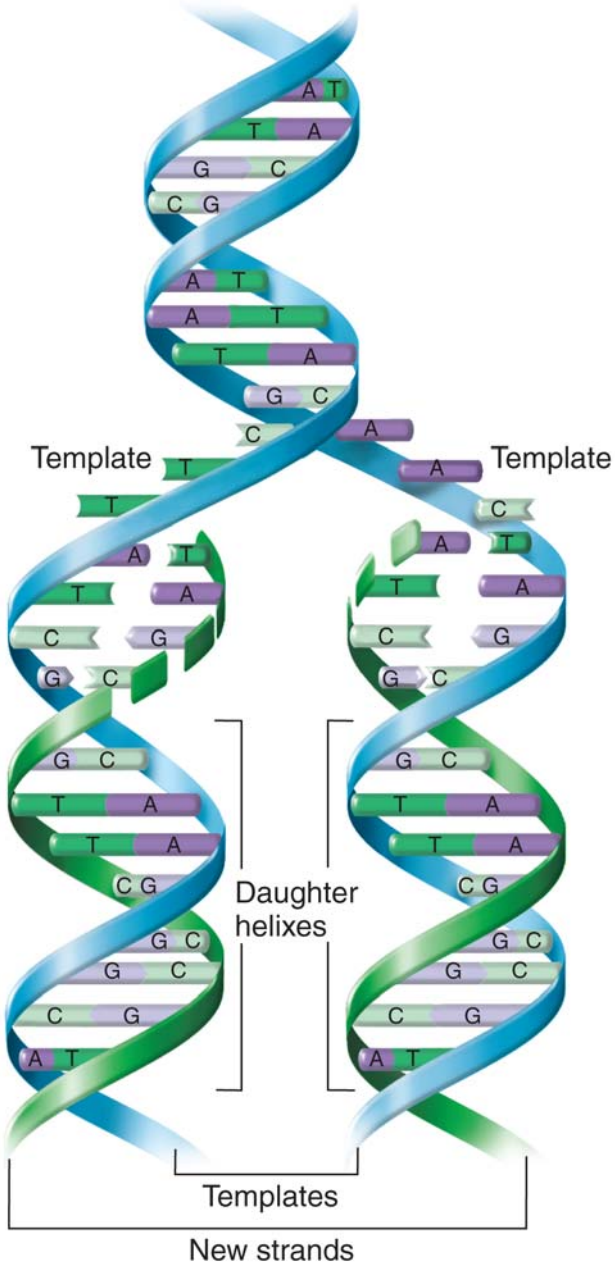


Fig. 6.16

Three possible models of DNA replication

Semiconservative – the Watson-Crick model

Conservative – parental double helix remains intact, both strands of daughter helices are newly synthesized

Dispersive – both strands of both daughter helices contain original and newly synthesized DNA

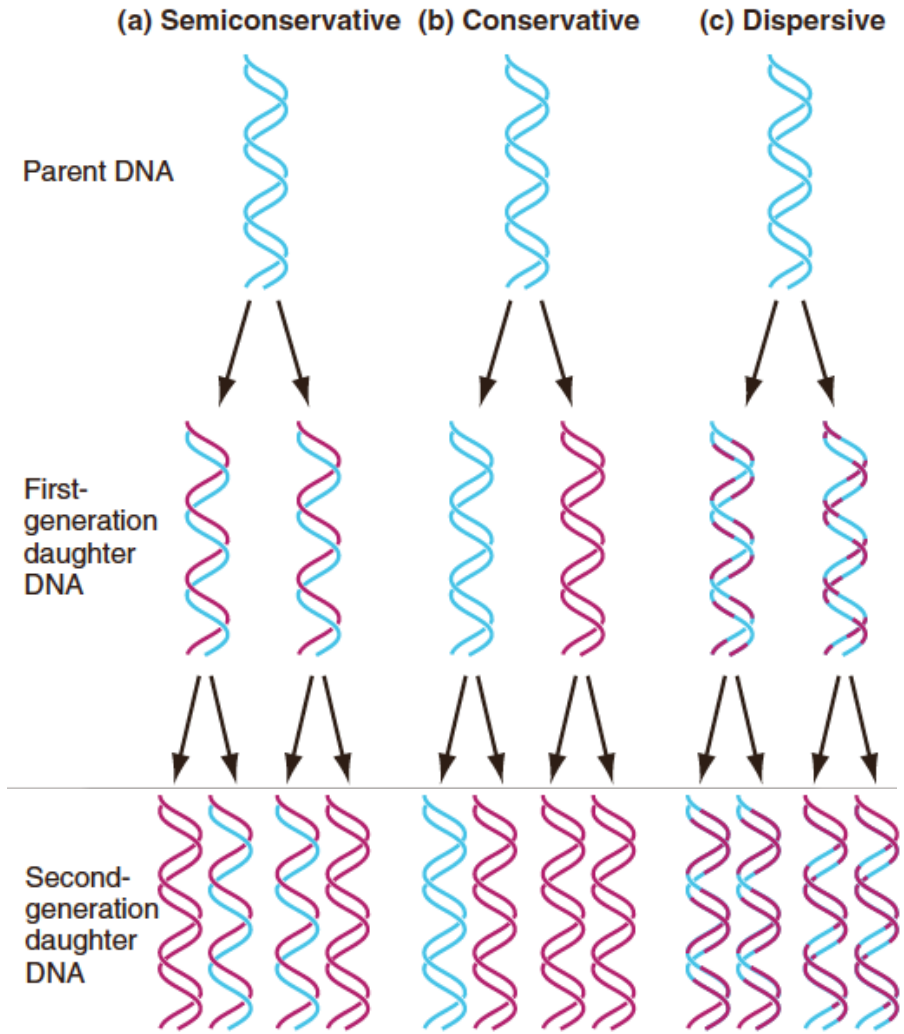


Fig. 6.17

The experimental approach to test the models of replication

M. Meselson and F. Stahl (1958) separated preexisting "parental" DNA from newly synthesized daughter DNA

- **Grew E. coli in media containing ^{15}N (heavy isotope) then switched to media containing ^{14}N (normal isotope)**

Purified DNA from cells and subjected it to equilibrium density gradient ultracentrifugation

- **Cesium chloride (CsCl) forms stable gradient with highest density at bottom of tube**
- **DNA forms a tight band at position where its density equals the CsCl density**

How the Meselson-Stahl experiment confirmed semiconservative replication

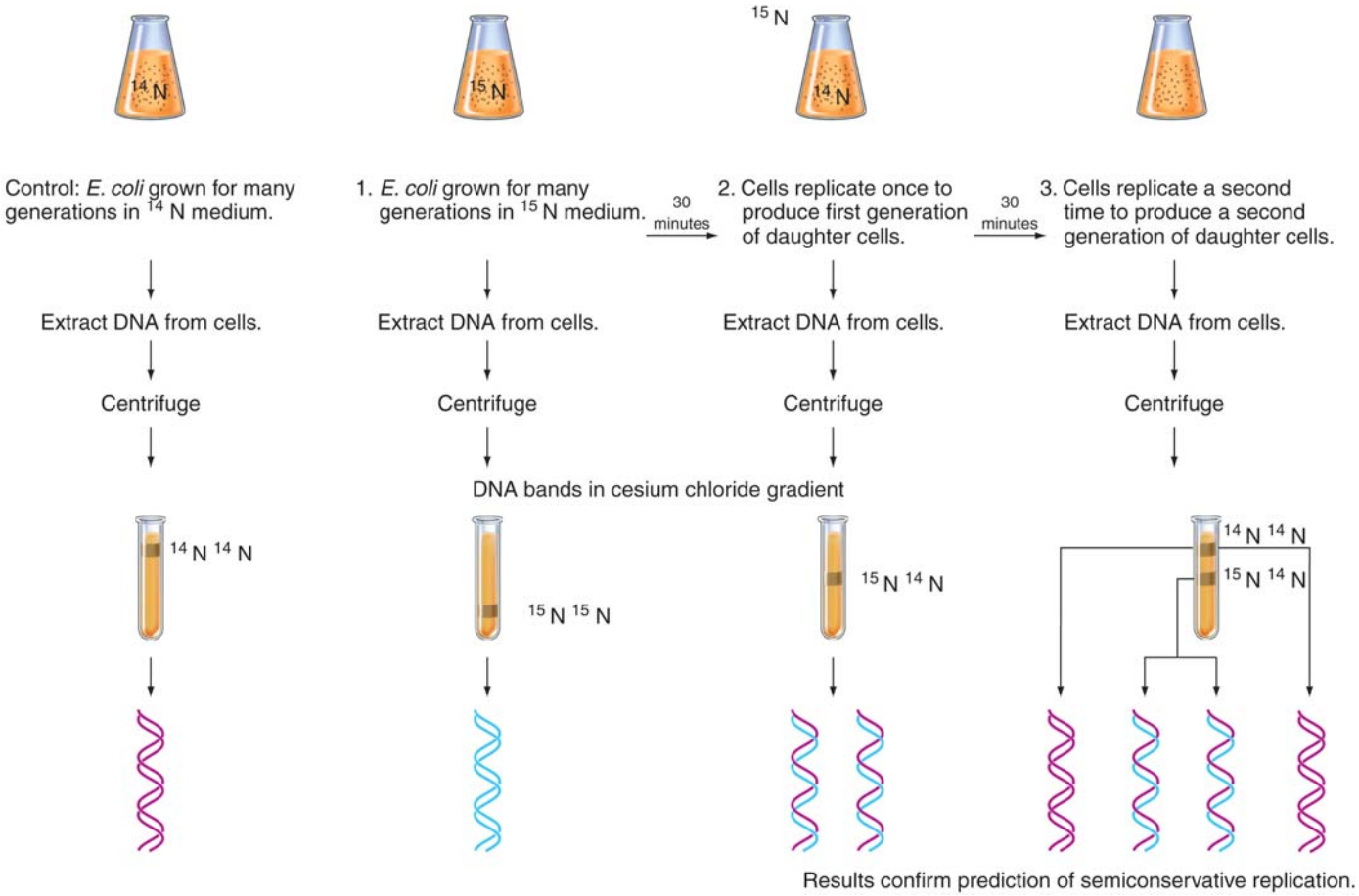


Fig. 6.18

The mechanism of DNA replication

A. Kornberg and others worked out the biochemical aspects of replication in E. coli

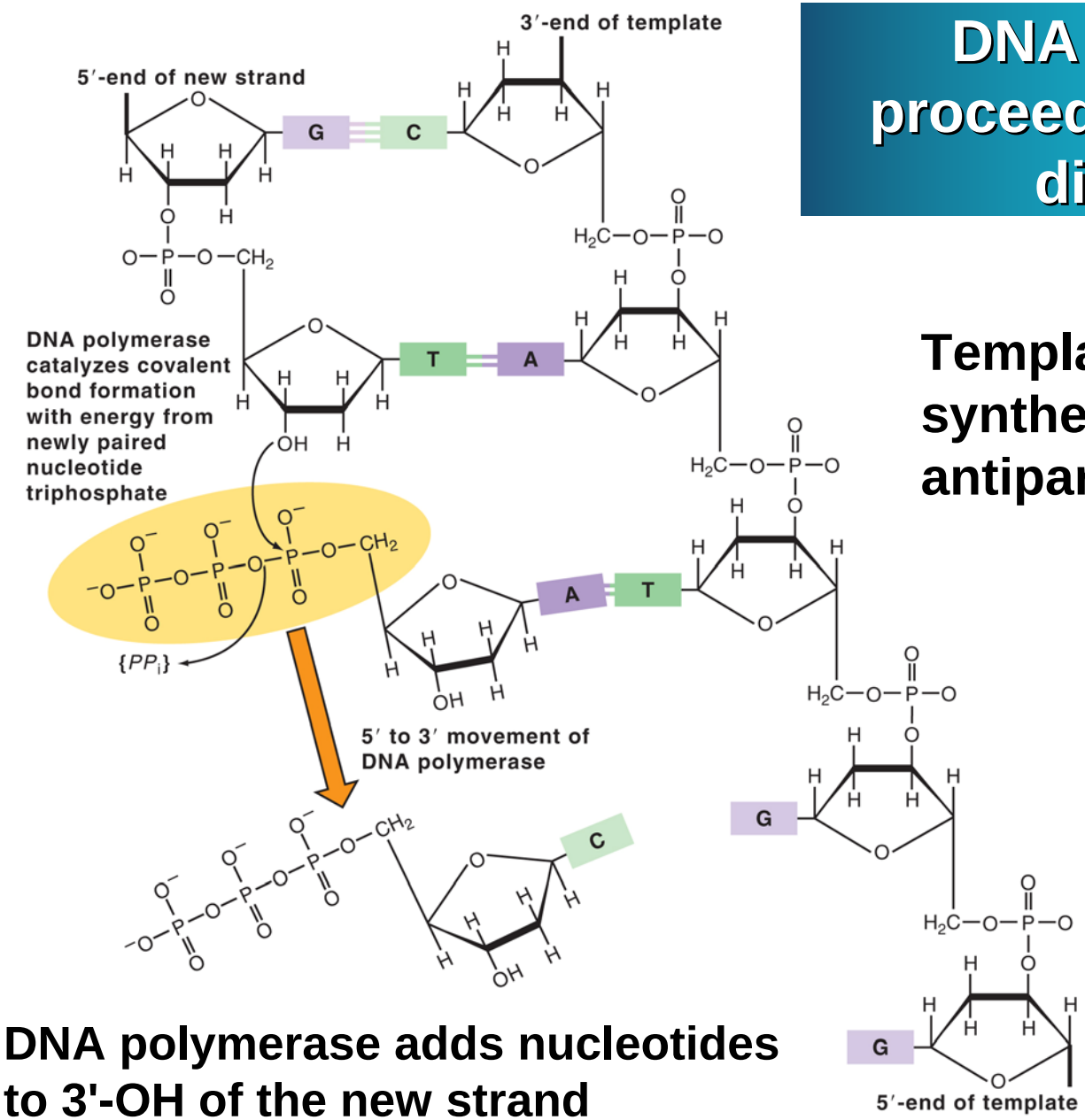
Energy for DNA synthesis comes from high-energy phosphate bonds associated with dNTPs

DNA polymerase (pol) catalyzes new phosphodiester bonds

Highly coordinated process has two stages

- **Initiation – proteins open up the double helix and prepare it for complementary base pairing**
- **Elongation – proteins connects the correct sequence of nucleotides on newly formed DNA stands**

DNA synthesis proceeds in a 5' to 3' direction



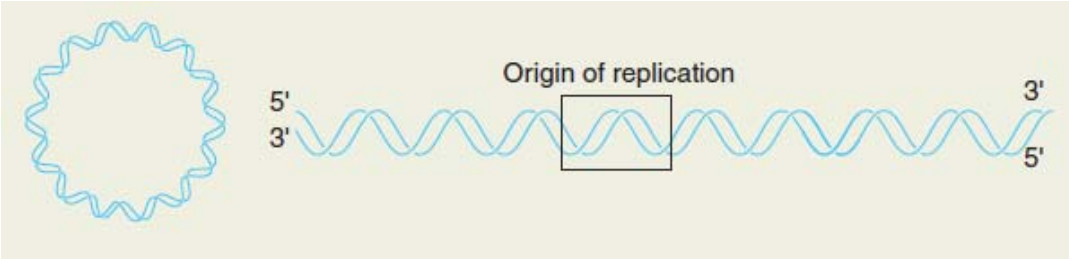
Template and newly synthesized strands are antiparallel

DNA polymerase adds nucleotides to 3'-OH of the new strand

Fig. 6.19

The mechanism of DNA replication: Initiation

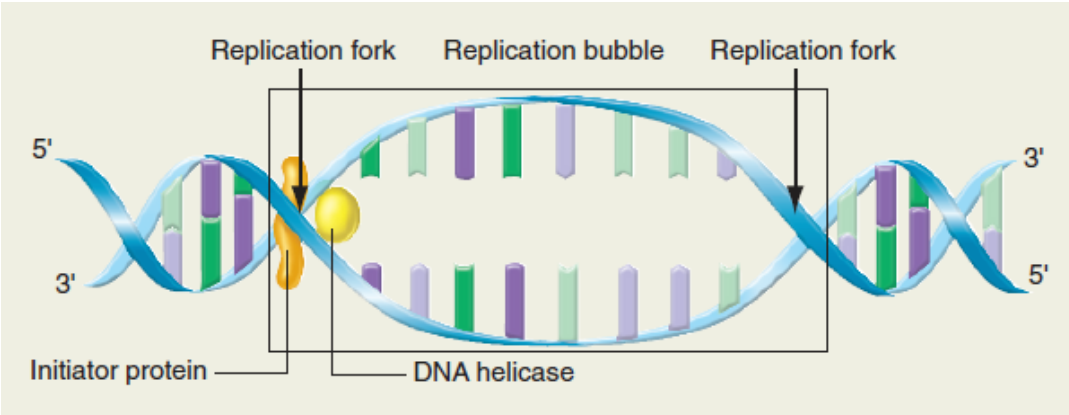
Initiation begins at the origin (Ori) of replication



Initiator protein binds to Ori

Helicase unwinds the helix

Two replication forks are formed



Feature Fig. 6.20a

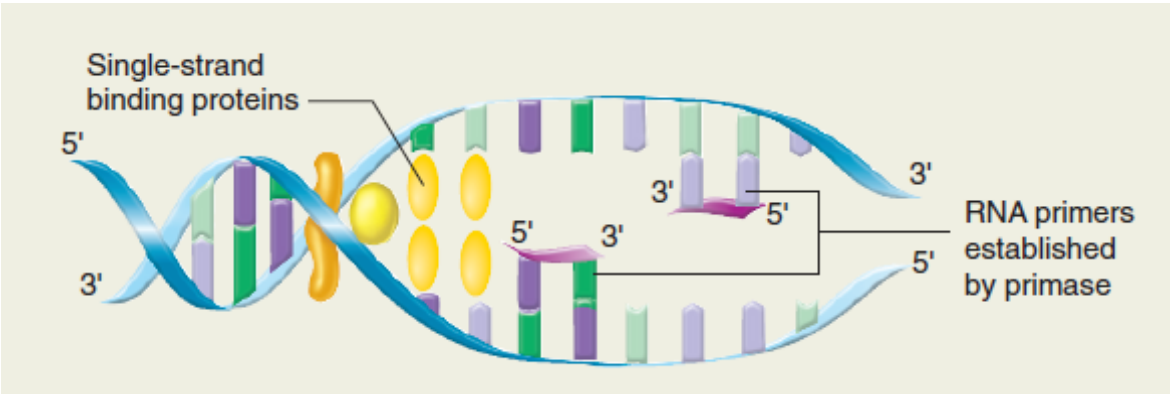
The mechanism of DNA replication: Initiation (cont)

Preparation of double helix for complementary base pairing

Single-strand binding proteins keep the DNA helix open

Primase synthesizes RNA primer

Primers are complementary and antiparallel to each template strand

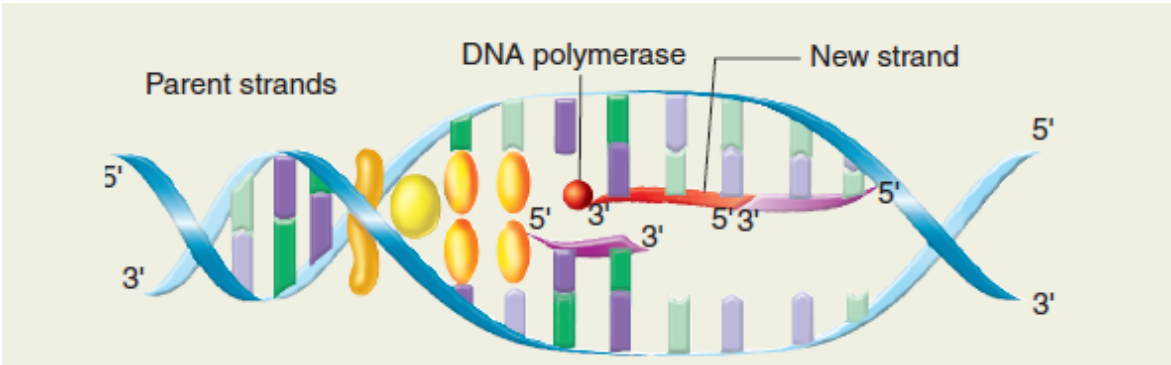


Feature Fig. 6.20a (cont)

The mechanism of DNA replication: Elongation

The correct nucleotide sequence is copied from template strand to newly synthesized strand of DNA

DNA polymerase III catalyzes phosphodiester bond formation between adjacent nucleotides (polymerization)



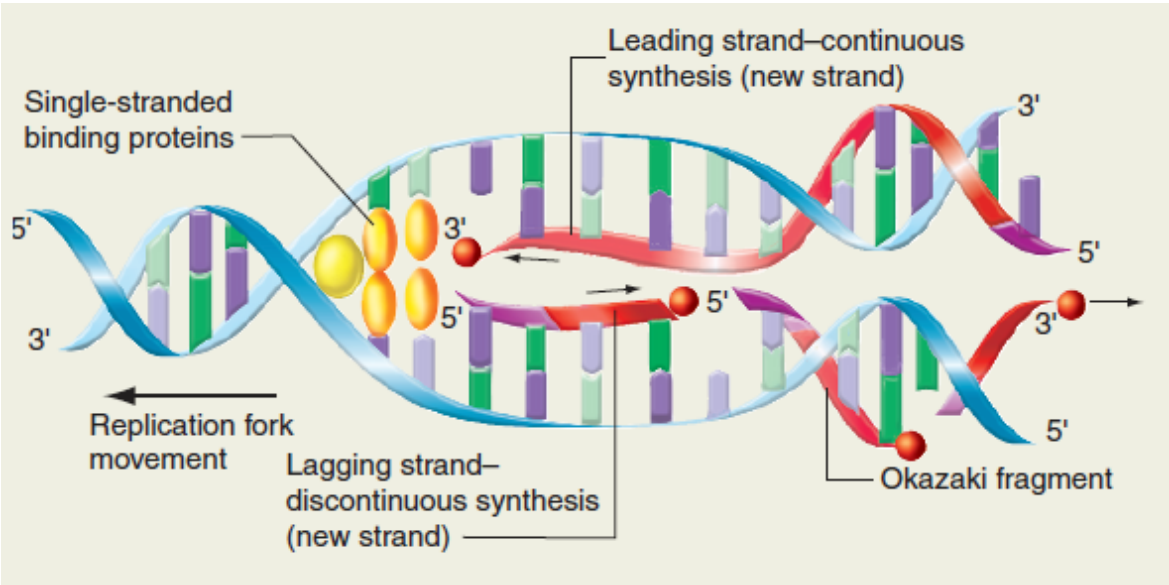
Feature Fig. 6.20b

The mechanism of DNA replication: Elongation (cont)

Leading strand has continuous synthesis

Lagging strand has discontinuous synthesis

Okazaki fragment – short DNA fragments on lagging strand

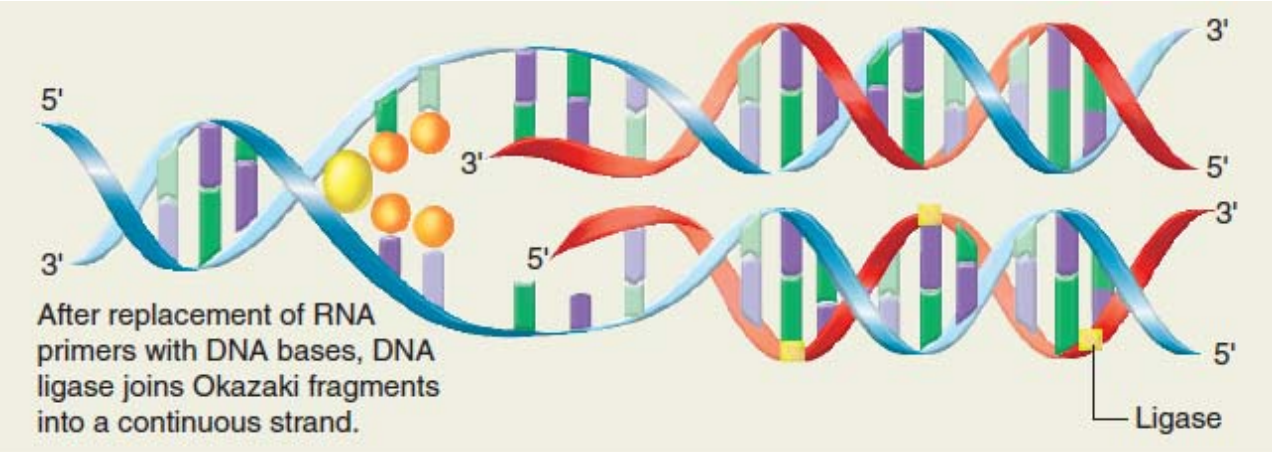


Feature Fig. 6.20b (cont)

The mechanism of DNA replication: Elongation (cont)

DNA polymerase I replaces RNA primer with DNA sequence

DNA ligase covalently joins successive Okazaki fragments together



Feature Fig. 6.20b (cont)

The bidirectional replication of a circular bacterial chromosome: An overview

Replication proceeds in two directions from a single Ori

Unwinding of DNA creates supercoiled DNA ahead of replication fork

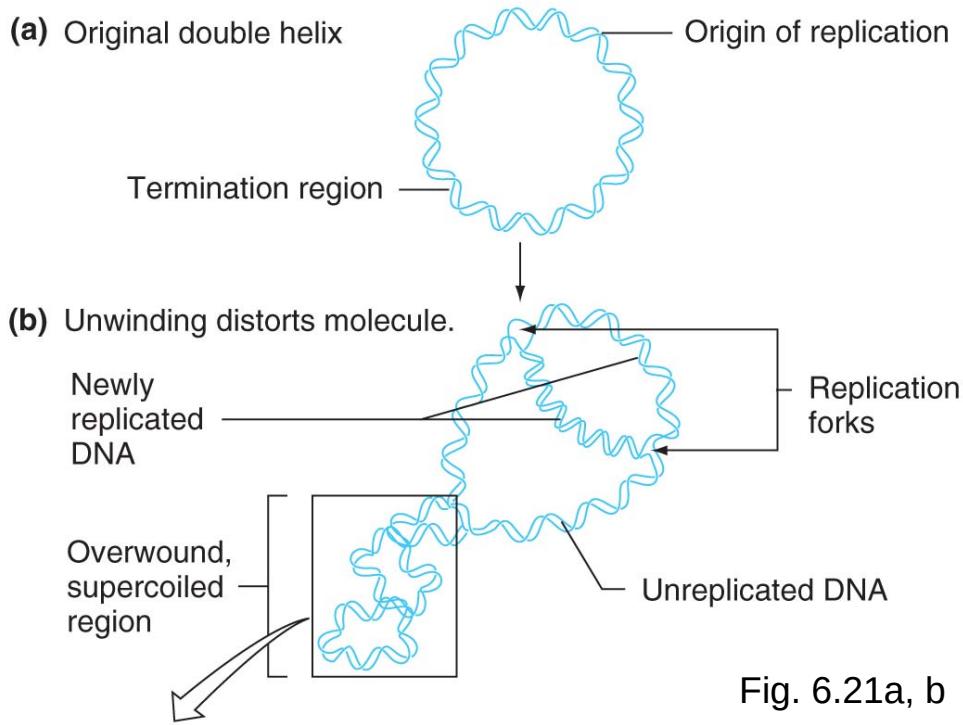


Fig. 6.21a, b

The bidirectional replication of a circular bacterial chromosome: An overview (cont)

DNA topoisomerases relax supercoils by cutting the sugar phosphate backbone bonds strands of DNA

Unwound broken strands then sealed by ligase

Synthesis continues bidirectionally until replication forks meet

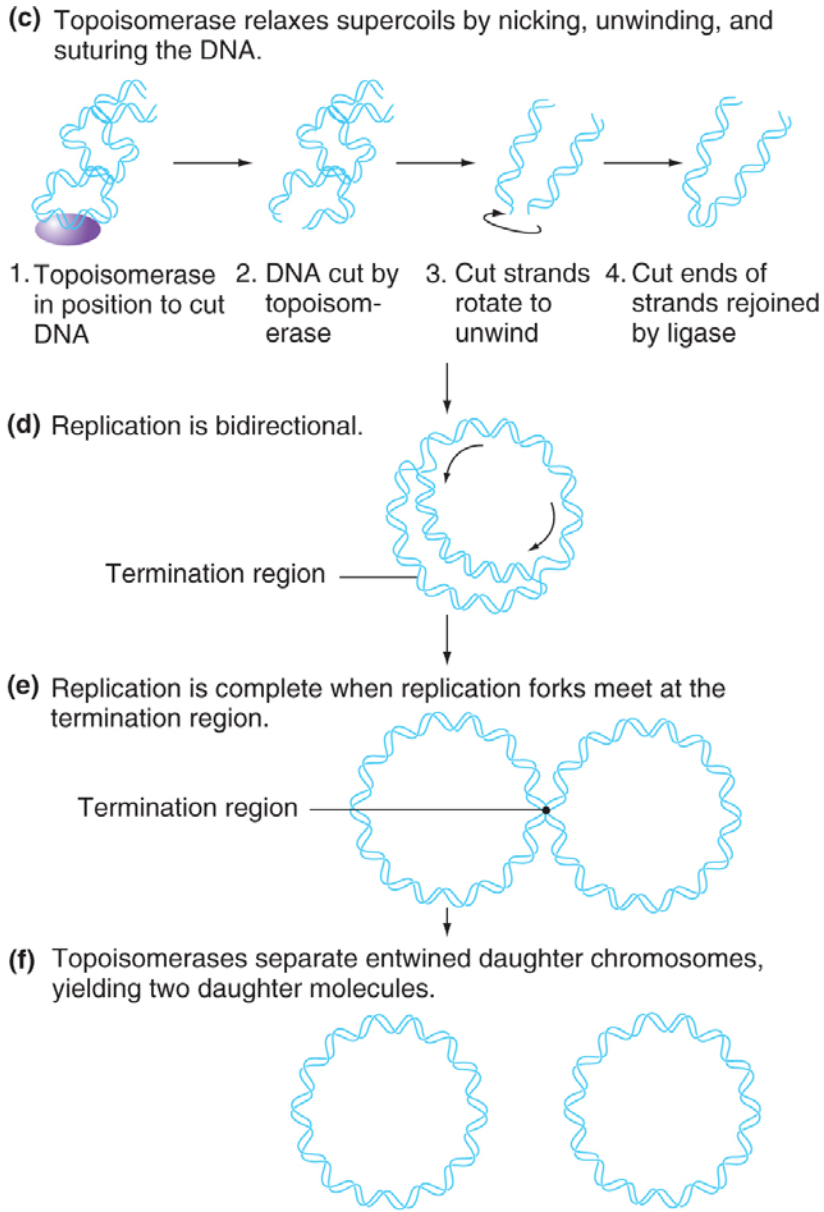


Fig. 6.21c-f

Cells must ensure accuracy of genetic information

Three ways to ensure fidelity of DNA information

Redundancy – either strand of the double helix can specify the sequence of the other strand

Precision of cellular replication machinery

- **DNA polymerase I and III have proofreading ability (more about this in Chapter 7)**

DNA repair enzymes (described in Chapter 7)

Recombination at the DNA level

New combinations of alleles are created by two types of events in meiosis

Independent assortment – each pair of homologous chromosomes segregates freely from the other (Chapter 4)

- **Creates new allele combinations for unlinked genes**

Crossing over – two homologous chromosomes exchange portions of DNA (Chapter 5)

- **Creates new allele combinations for linked genes**
- **Ensures proper chromosome segregation during meiosis**
  - **Mistakes can result in nondisjunction (described in Chapter 13)**

DNA molecules break and rejoin during recombination: The experimental evidence

M. Meselson and J. Weigle, co-infected E. coli with radio-labeled phage

Bacteriophage lambda with genetic markers grown on E. coli in media with heavy (^{13}C and ^{15}N) or light (^{12}C and ^{14}N) isotopes

Separated phage DNA on CsCl density gradient

Genetic recombinants had DNA with hybrid densities

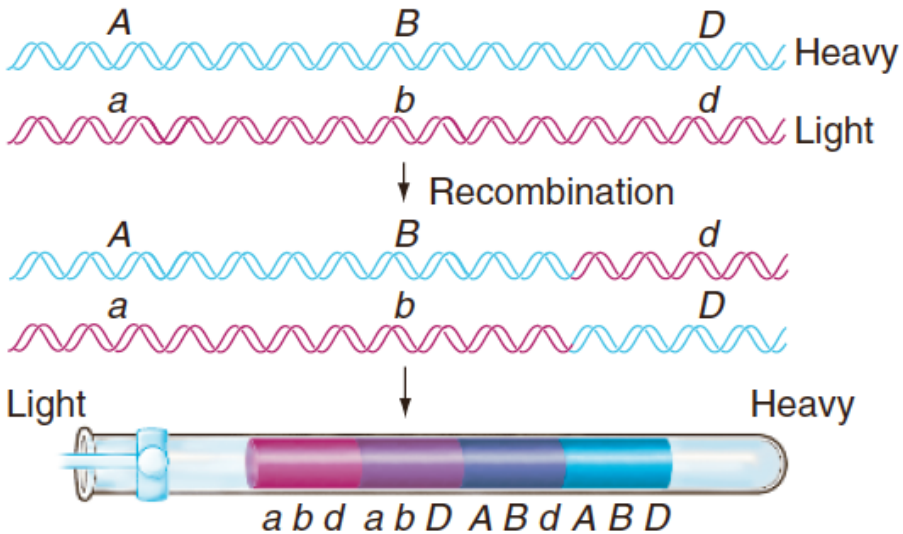


Fig. 6.22

Heteroduplex regions occur at sites of genetic exchange

Two strands of DNA don't break and rejoin at the same location

- **Breakpoints on each strand can be 100s-1000s bp apart**

Heteroduplex – region of DNA between breakpoints

- **One strand is maternal and other is paternal**
- **Strands can have mismatches**

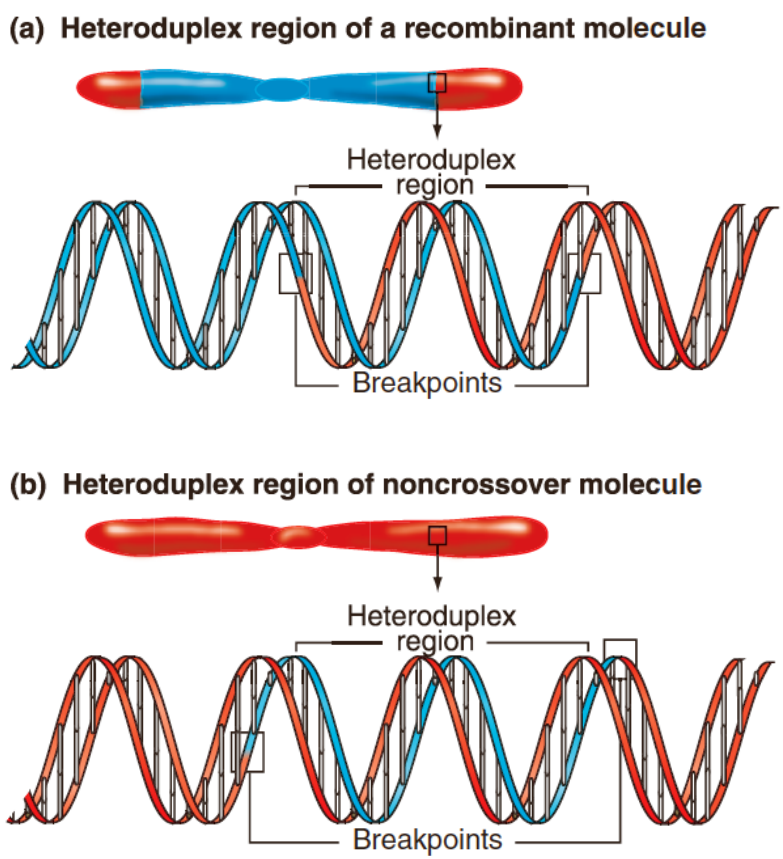


Fig. 6.23

Mismatches in heteroduplexes can be repaired

DNA repair enzymes eliminate mismatches

Either allele can be converted

Gene conversion – deviations from expected 2:2 segregation, e.g. 3:1 or 1:3

In yeast, gene conversion occurs 50:50 with and without crossing over of flanking markers

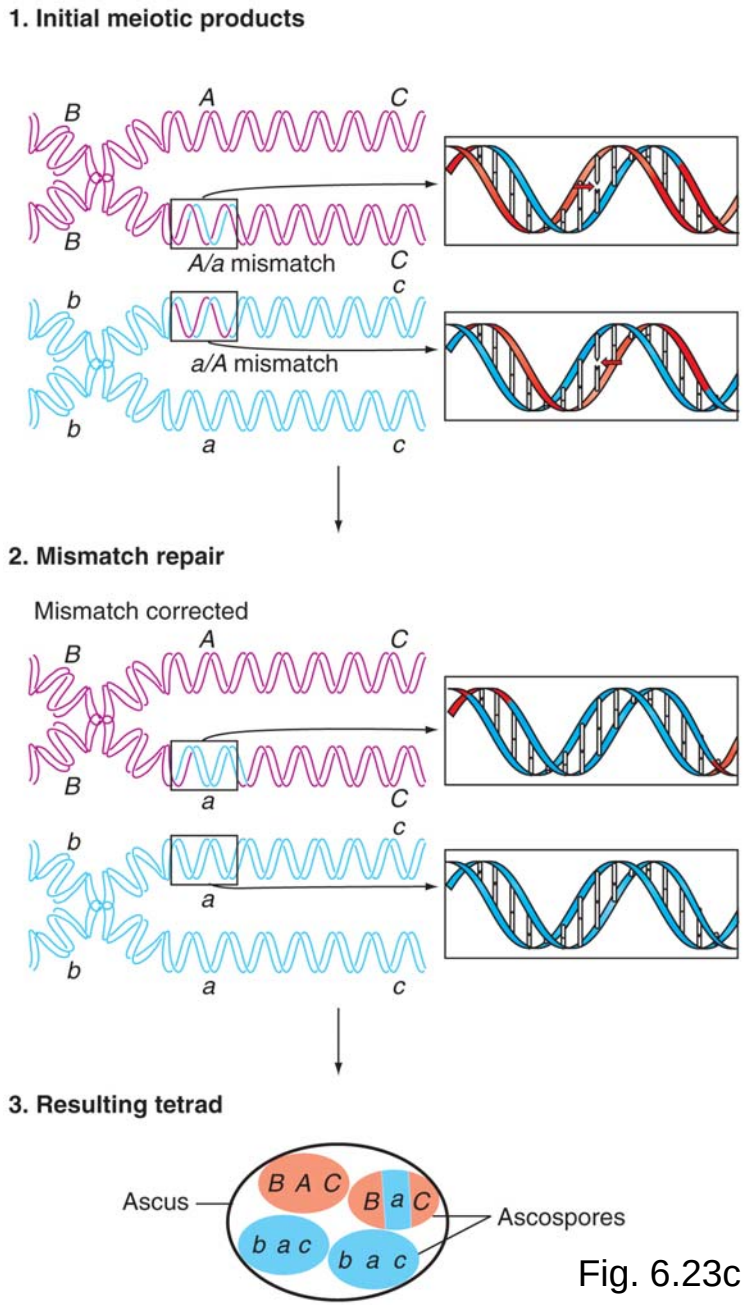


Fig. 6.23c

Experimental observations that led to development of a model of recombination

Tetrad analysis in yeast showed that only two of the four chromatids are recombinant

Recombination occurs only between homologous regions and is highly accurate

Crossover sites often associated with heteroduplex regions

Gene conversion can occur in absence of crossing over

- **Not all recombination leads to crossovers**

Double-strand-break repair model of meiotic recombination

Homologous chromosomes break, exchange DNA, and rejoin

Breakage and repair creates reciprocal products of recombination

Recombination events can occur anywhere along the DNA

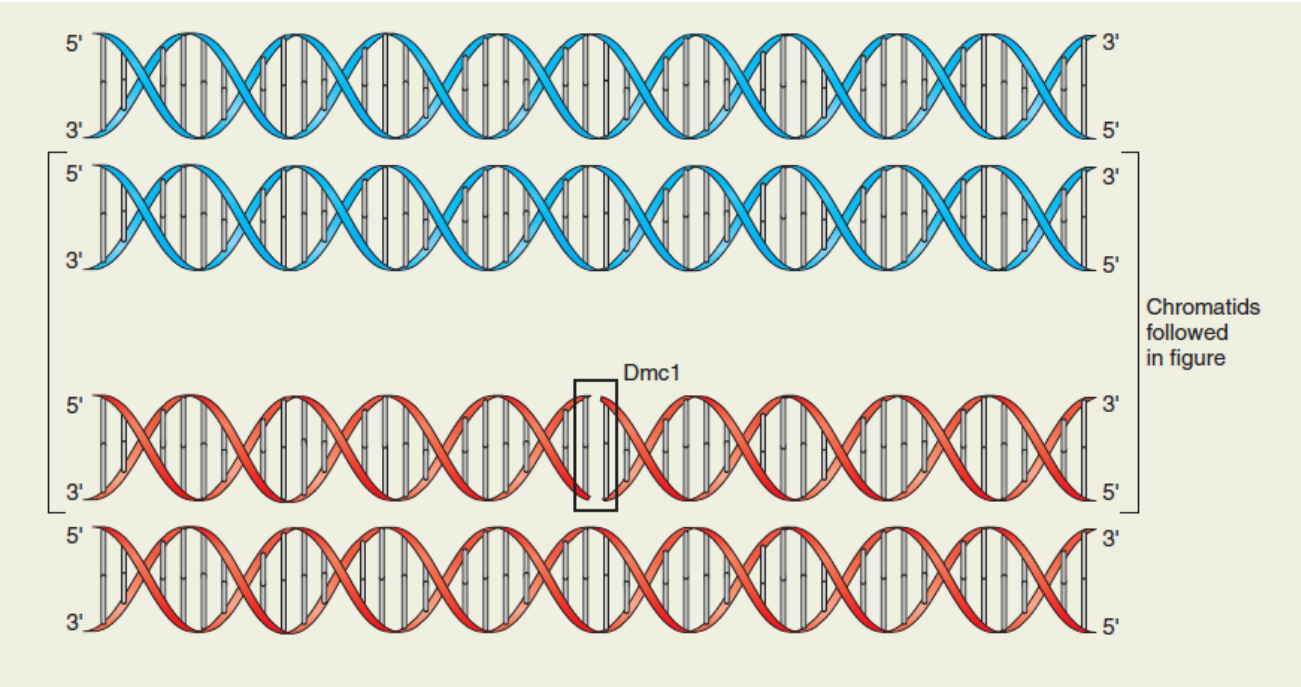
Precision in the exchange (no gain or loss of nucleotide pairs) prevents mutations from occurring

Gene conversion can give rise to an unequal yield of two different alleles

Step 1 in the model of recombination: Double-strand break formation

Dmc1 breaks phosphodiester bonds of both strands of one chromatid

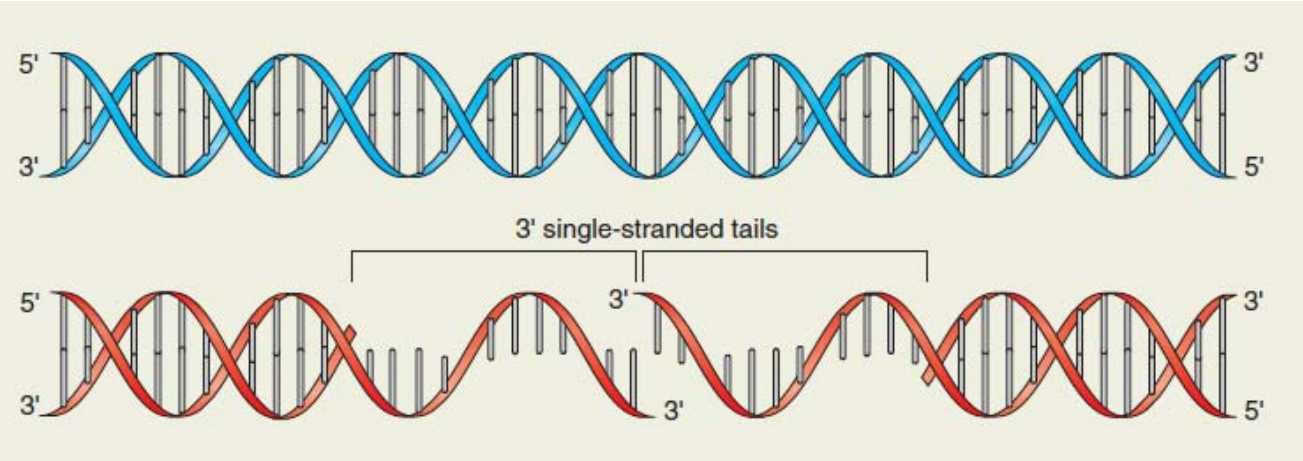
- **Spo11 in yeast is homologous to Dmc1 of multicellular eukaryotes**



Feature Fig. 6.24

Step 2 in the model of recombination: Resection

5' ends of each broken strand are degraded to create 3' single-stranded tails

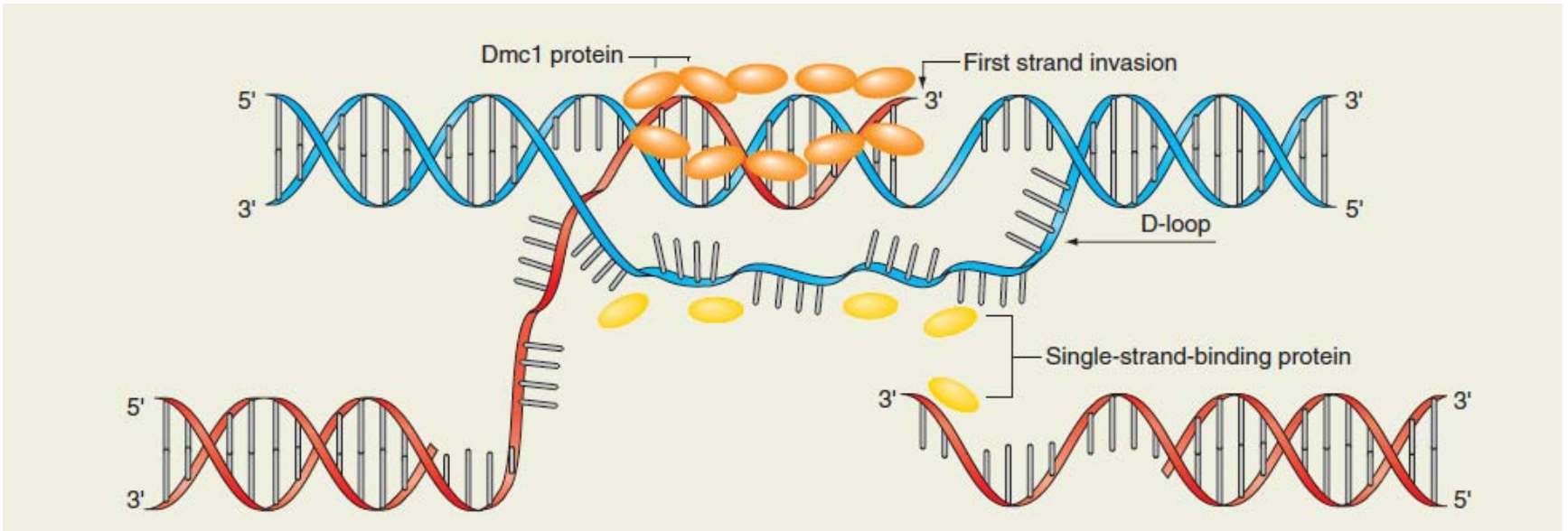


Feature Fig. 6.24 (cont)

Step 3 in the model of recombination: First strand invasion

One single-strand tail invades a non-sister chromatid and forms stable heteroduplex

Displacement loop (D-loop) from invaded chromatid is stabilized by single-strand binding protein

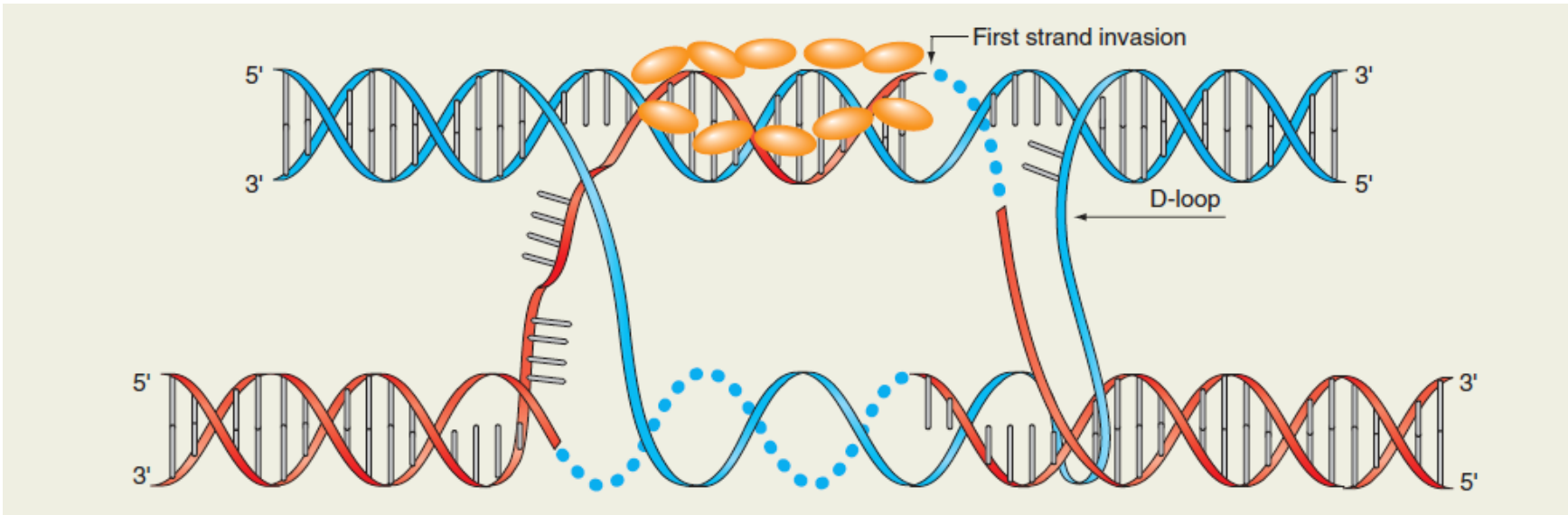


Feature Fig. 6.24 (cont)

Step 4 in the model of recombination: Formation of double Holliday junctions

D-loop enlarged by new DNA synthesis at 3'-end of invading strand

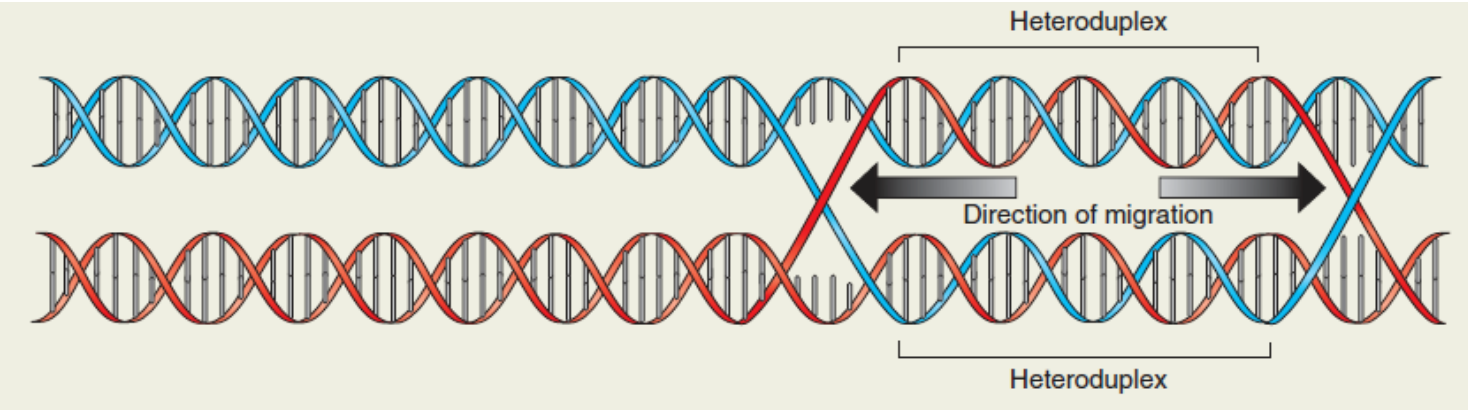
New DNA synthesis fills in gap in bottom strand using displaced strand as template



Feature Fig. 6.24 (cont)

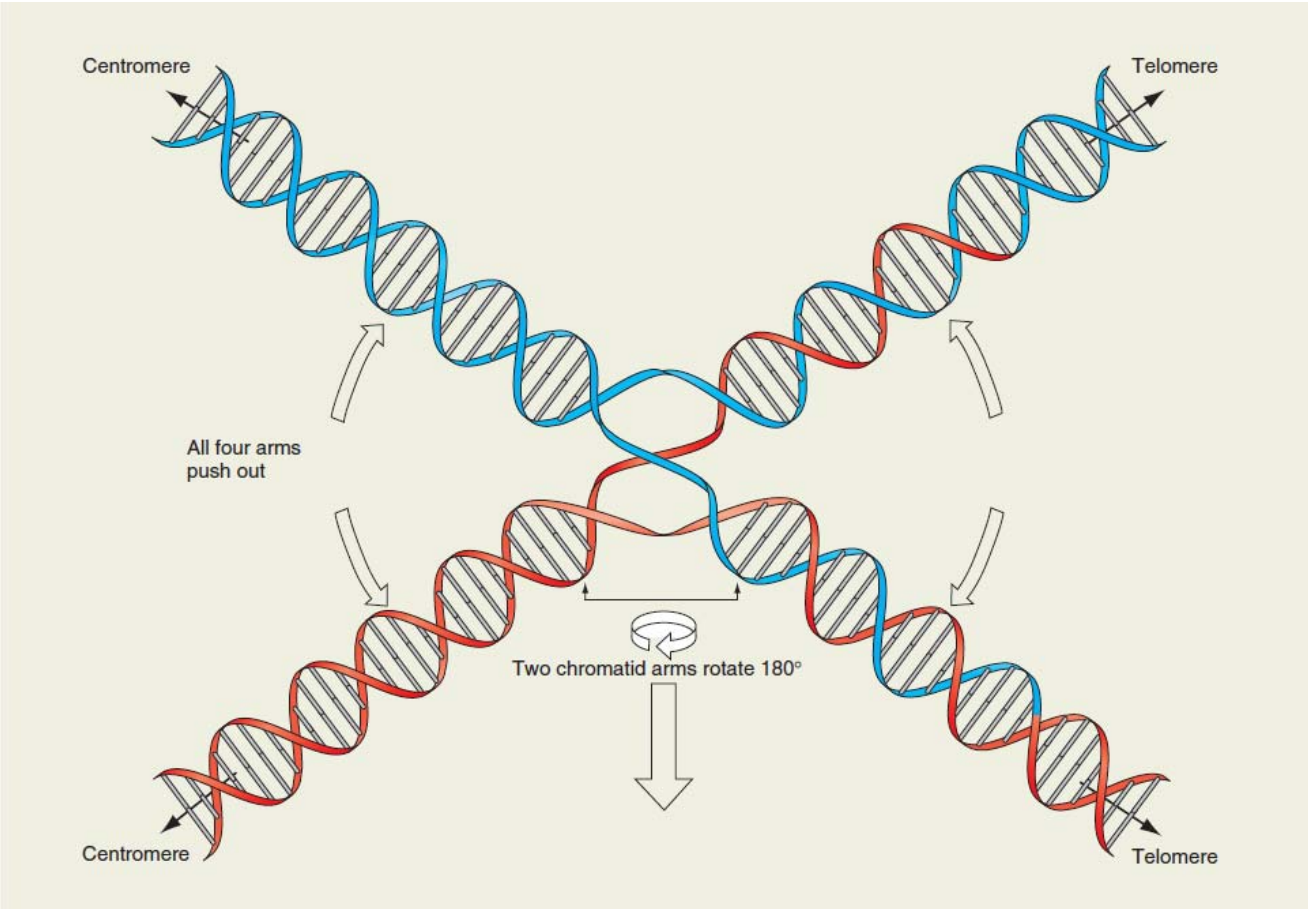
Step 5 in the model of recombination: Branch migration

Heteroduplex region of both DNA molecules is lengthened



Feature Fig. 6.24 (cont)

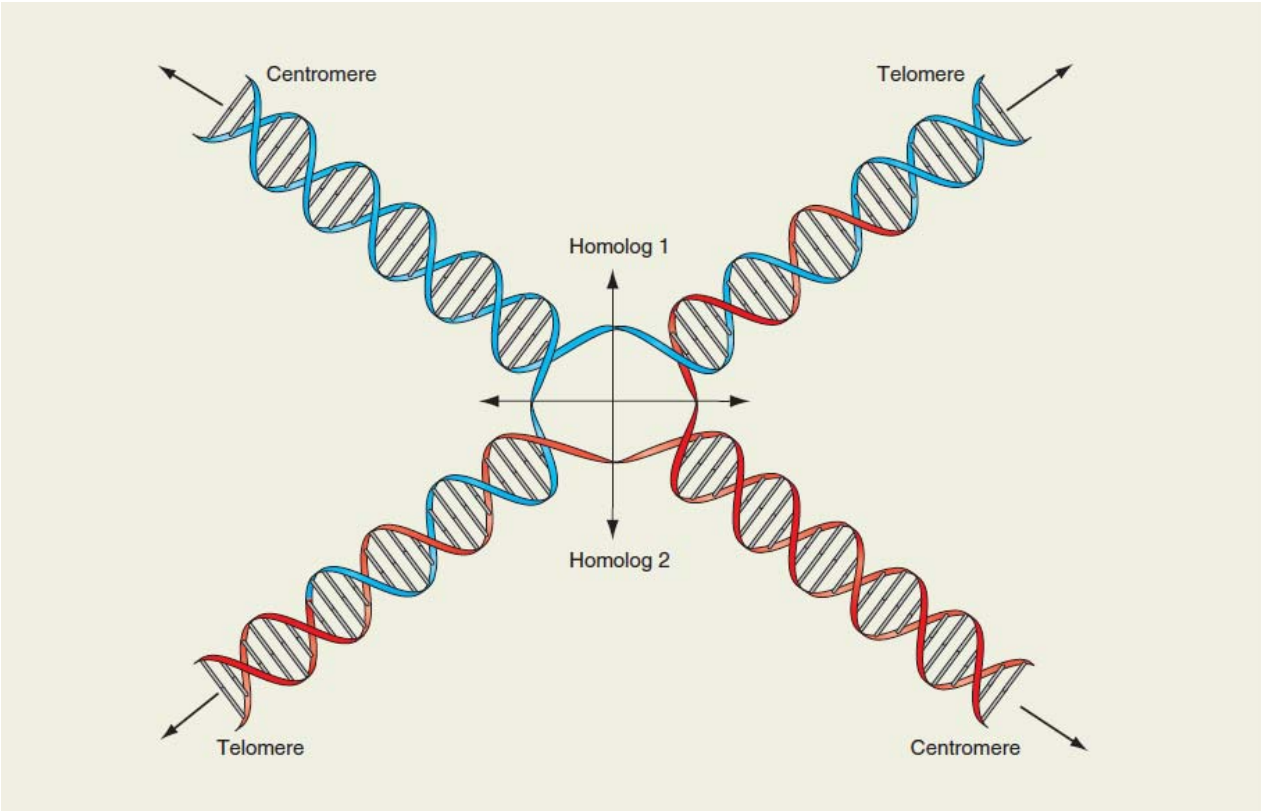
Step 6 in the model of recombination: The Holliday intermediate



Feature
Fig. 6.24
(cont)

Step 7 in the model of recombination: Alternative resolutions

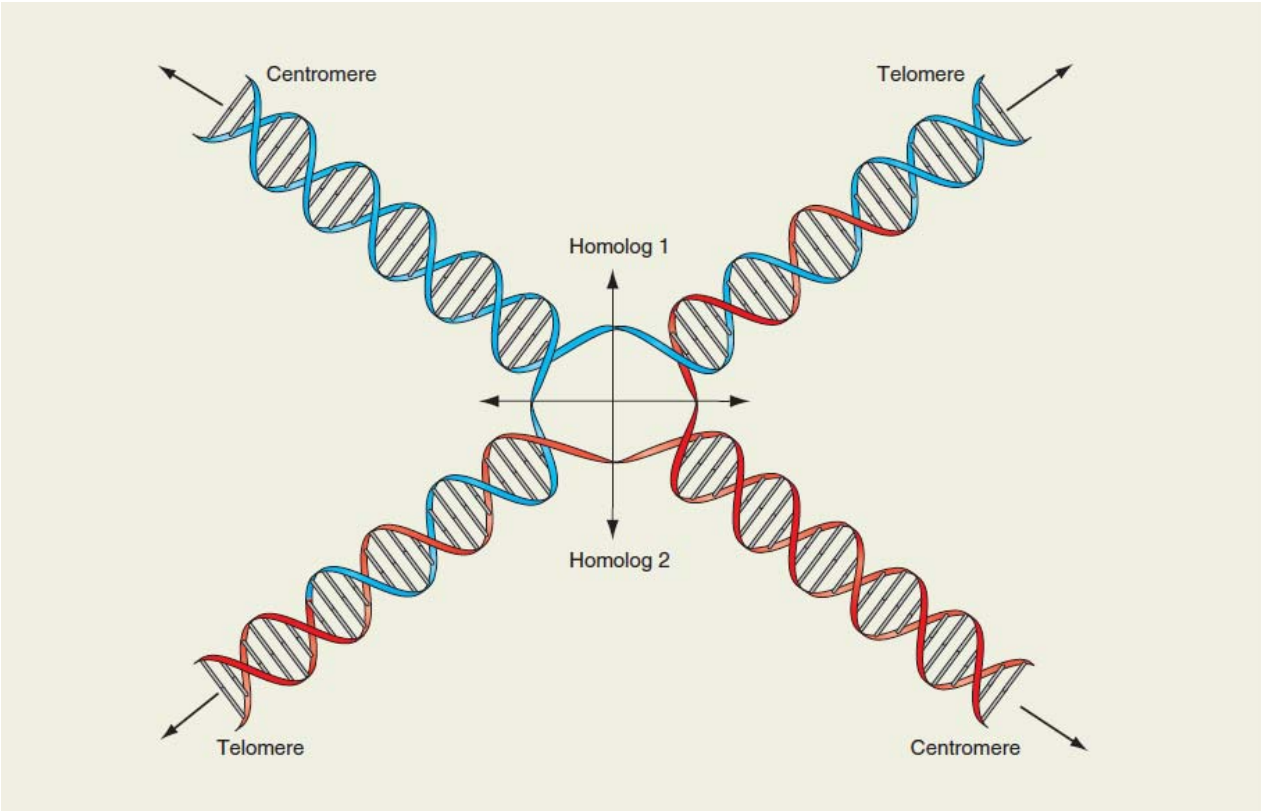
Cutting of Holliday junctions by endonucleases is equally likely in either vertical or horizontal plane



Feature
Fig. 6.24
(cont)

Step 7 in the model of recombination: Alternative resolutions

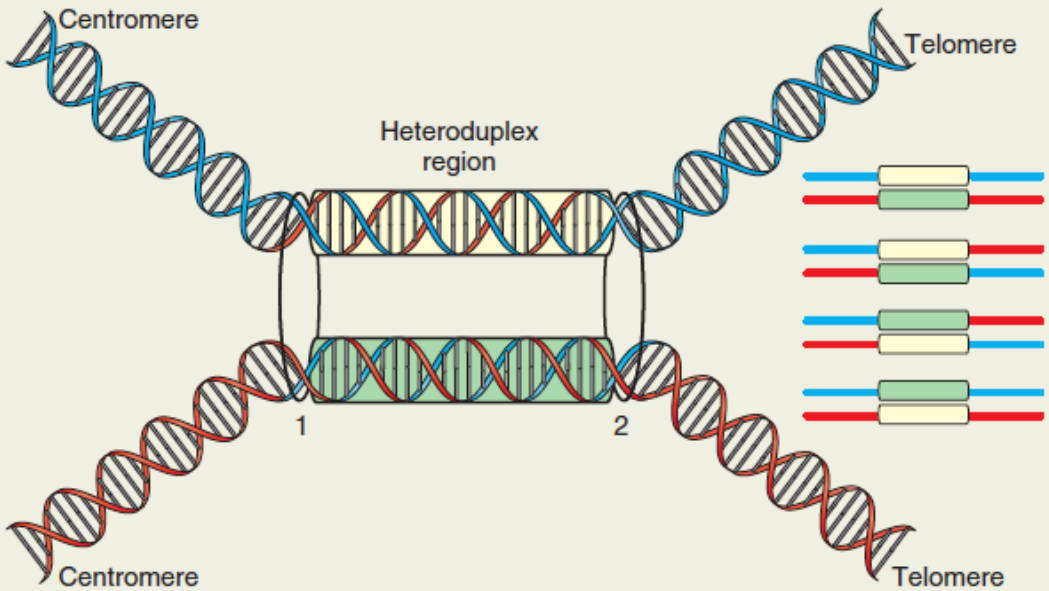
Cutting of Holliday junctions by endonucleases in either vertical or horizontal plane is equally likely



Feature
Fig. 6.24
(cont)

Step 8 in the model of recombination: Probability of crossover occurring

- **Non-crossover occurs when both junctions are resolved in same plane**
- **Crossover occurs with the two junctions are resolved in different planes**



Resolution of Intermediate 1	Resolution of Intermediate 2	RESULT
Horizontal	Horizontal	Aborted crossover
Horizontal	Vertical	Crossing over
Vertical	Horizontal	Crossing over
Vertical	Vertical	Aborted crossover

Feature Fig. 6.24 (cont)