

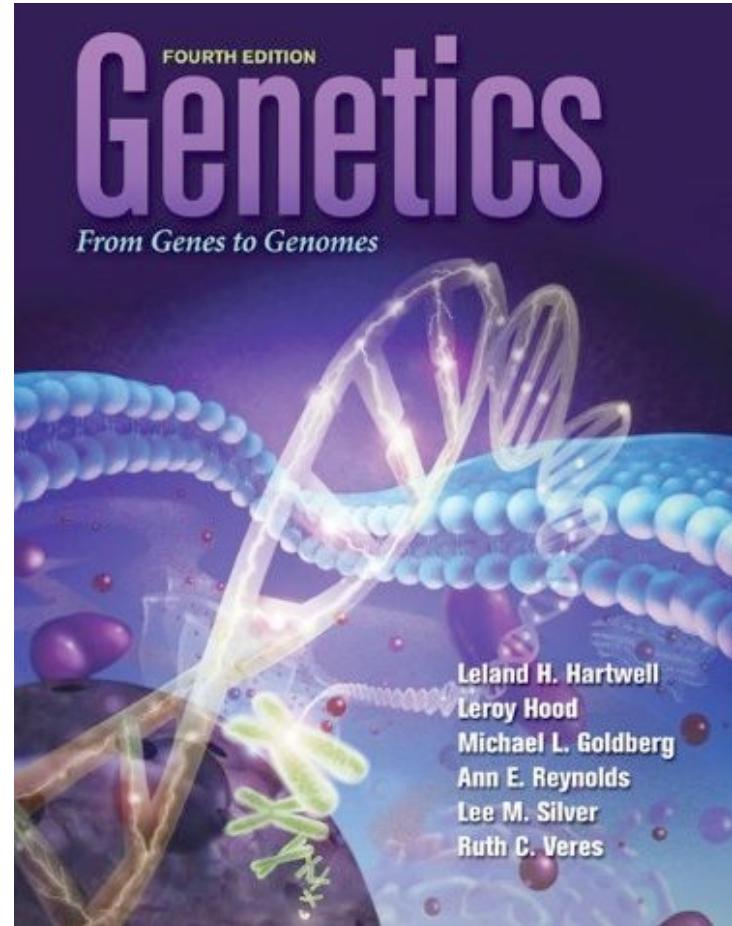
**PowerPoint to accompany**

# **Genetics: From Genes to Genomes**

## **Fourth Edition**

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## Anatomy and Function of a Gene: Dissection Through Mutation



## CHAPTER OUTLINE

### Anatomy and Function of a Gene: Dissection Through Mutation

- 7.1 Mutations: Primary Tools of Genetic Analysis
- 7.2 What Mutations Tell Us About Gene Structure
- 7.3 What Mutations Tell Us About Gene Function
- 7.4 A Comprehensive Example: Mutations That Affect Vision

# Mutations: Primary tools of genetic analysis

**Mutations are heritable changes in DNA base sequences**

**Forward mutation – changes wild-type allele to a different allele**

- e.g.  $A^+ \rightarrow a$  or  $b^+ \rightarrow B$

**Reverse mutation (reversion) – changes a mutant allele back to wild type**

- e.g.  $a \rightarrow A^+$  or  $B \rightarrow b^+$

**Forward mutation rate is usually greater than reversion rate**

# Classification of mutations by effect on DNA molecule

**Substitution** – replacement of a base by another base

- **Transition** – purine replaced by another purine, or pyrimidine replaced by another pyrimidine
- **Transversion** – purine replaced by a pyrimidine, or pyrimidine replaced by a purine

**Deletion** – block of 1 or more bp lost from DNA

**Insertion** – block of 1 or more bp added to DNA

**Inversion** – 180° rotation of a segment of DNA

**Reciprocal translocation** – parts of two nonhomologous chromosomes change places

# Mutations classified by their effect on DNA

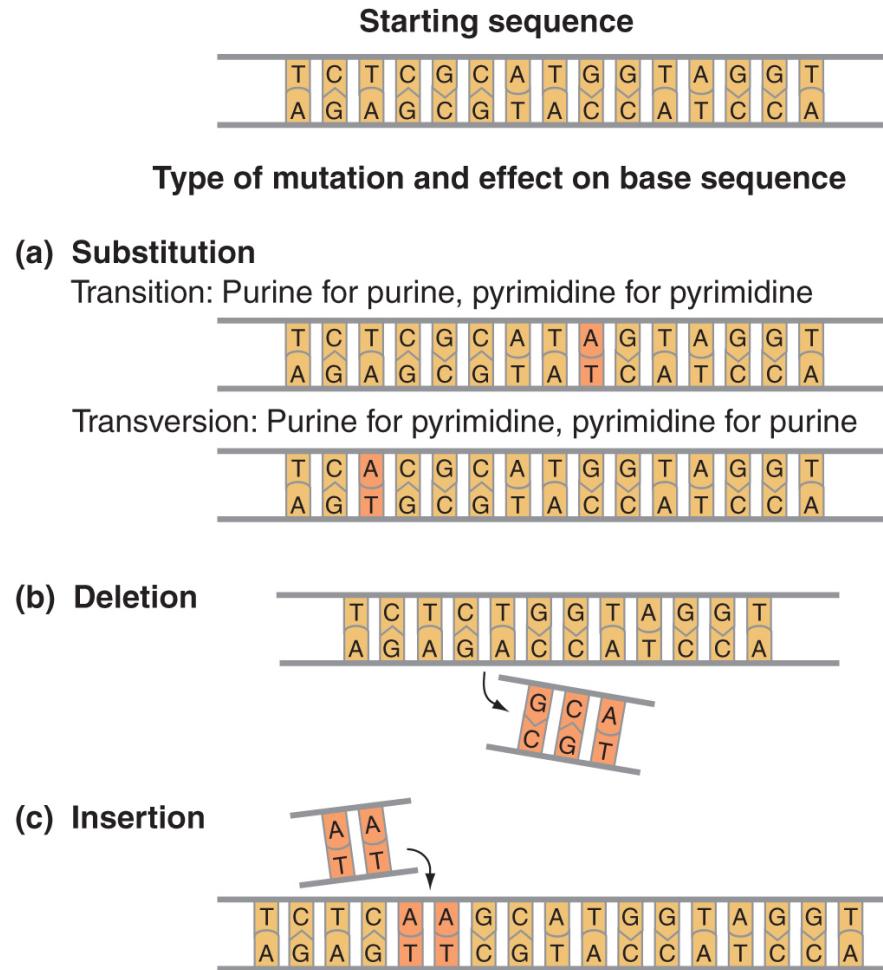


Fig. 7.2a - c

# Mutations classified by their effect on DNA (cont)

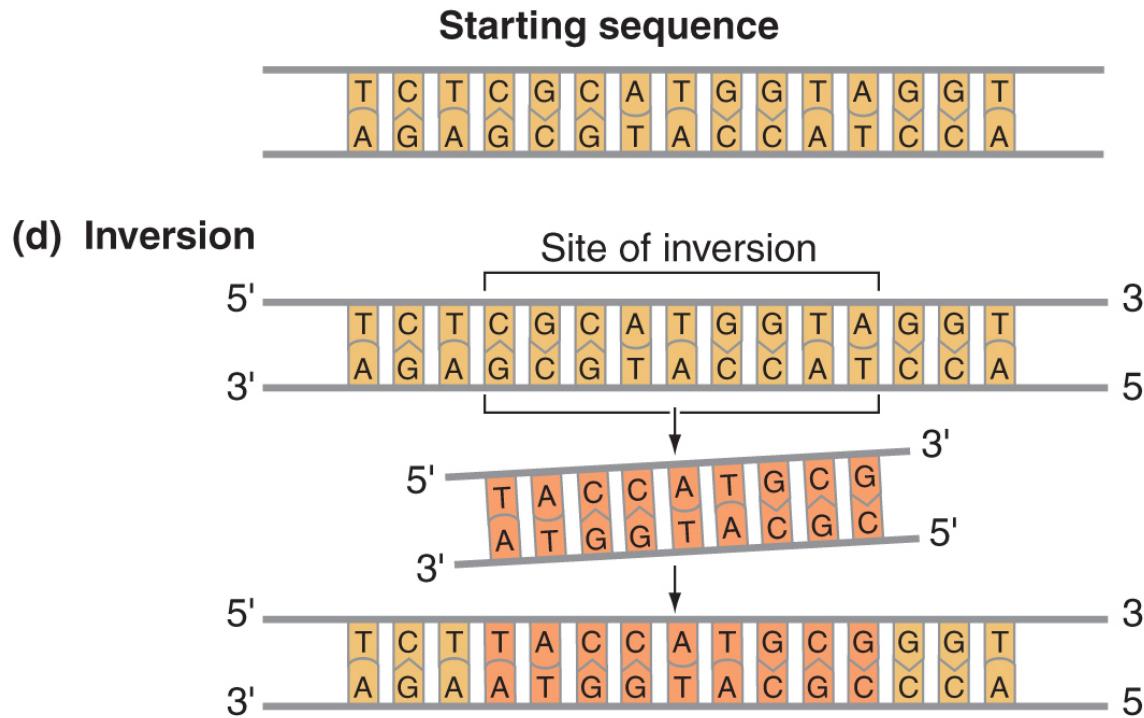


Fig. 7.2d

# Mutations classified by their effect on DNA (cont)

## (e) Reciprocal translocation

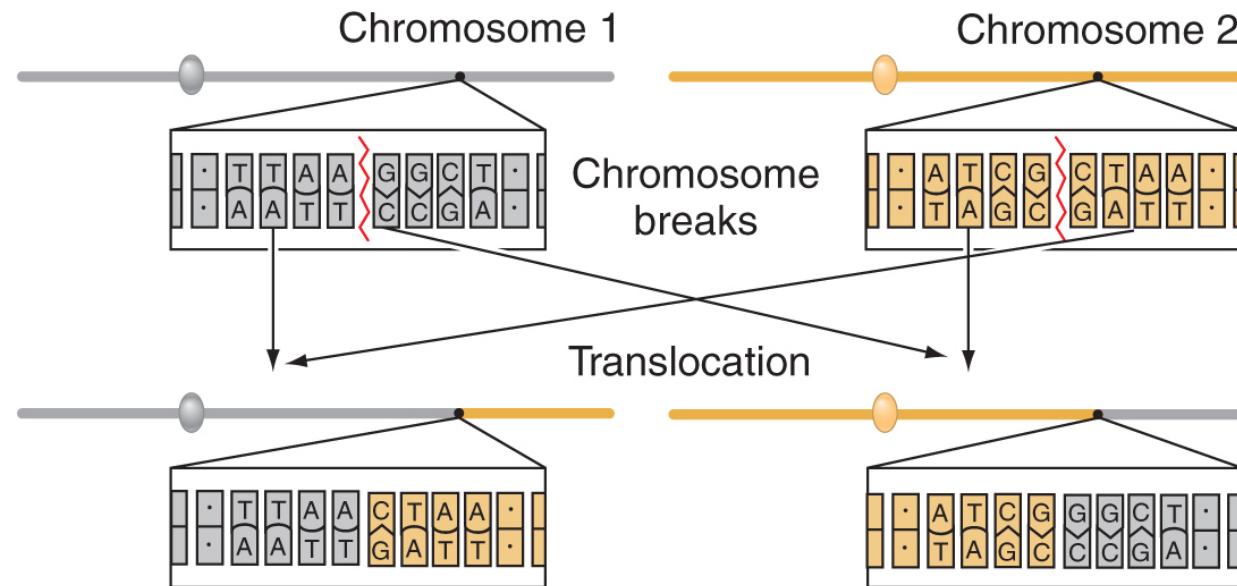


Fig. 7.2e

# Rates of spontaneous mutation

## Rates of recessive forward mutations at five coat color genes in mice

- 11 mutations per gene every  $10^6$  gametes

## Mutation rates in other organisms

- 2 – 12 mutations per gene every  $10^6$  gametes

Locus <sup>a</sup>	Number of gametes tested	Number of mutations	Mutation rate ( $\times 10^{-6}$ )
<i>a</i> <sup>-</sup> ( <i>albino</i> )	67,395	3	44.5
<i>b</i> <sup>-</sup> ( <i>brown</i> )	919,699	3	3.3
<i>c</i> <sup>-</sup> ( <i>nonagouti</i> )	150,391	5	33.2
<i>d</i> <sup>-</sup> ( <i>dilute</i> )	839,447	10	11.9
<i>In</i> <sup>-</sup> ( <i>leaden</i> )	243,444	4	16.4
	2,220,376	25	11.2 (average)

Fig. 7.3b

# Different genes, different mutation rates

Mutation rates are  $<10^{-9}$  to  $>10^{-3}$  per gene per gamete

- Differences in gene size
- Susceptibility of particular genes to various mutagenic mechanisms

Average mutation rate in gamete-producing eukaryotes is higher than that of prokaryotes

- Many cell divisions take place between zygote formation and meiosis in germ cells
  - More chance to accumulate mutations
- Can diploid organisms tolerate more mutations than haploid organisms?

# **Experimental evidence that mutations in bacteria occur spontaneously**

**S. Luria and M. Delbrück (1943) – fluctuation test**

**Examined origin of bacterial resistance to phage infection**

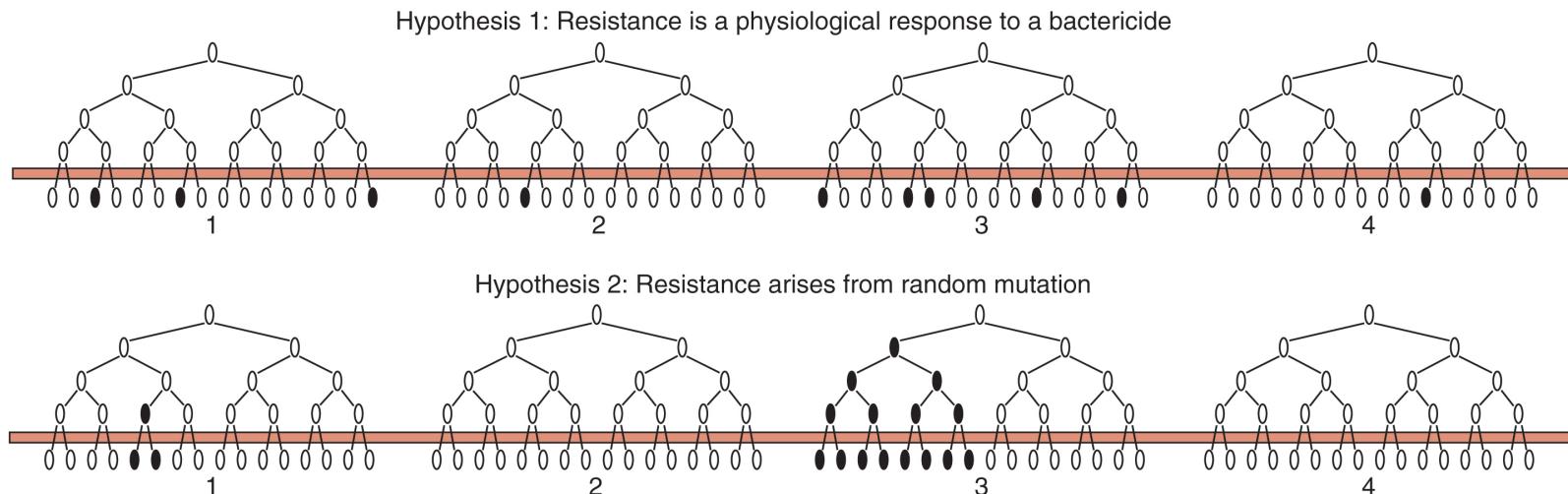
**Infected wild-type bacteria with phage**

**Majority of cells die, but a few cells can grow and divide**

- Had the cells altered biochemically?**
- Did the cells carry heritable mutations for resistance?**
- Did the mutations arise by chance or did they arise in response to the phage?**

# The Luria-Delbrück fluctuation experiment

## (a) Two hypotheses for the origin of bactericide resistance



## (b) Fluctuation test results

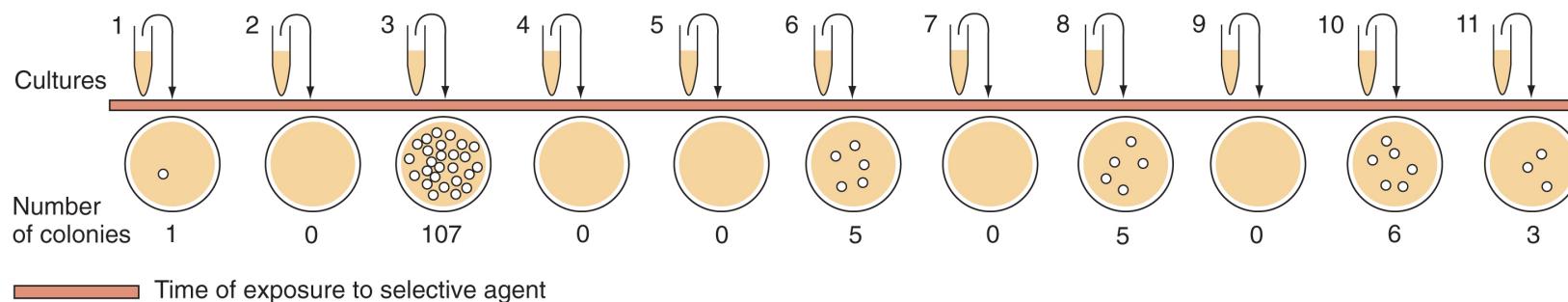
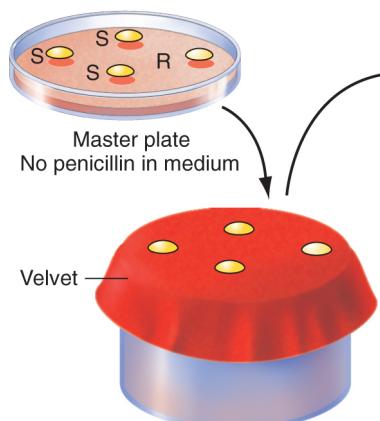


Fig. 7.4

# Replica plating verifies that bacterial resistance is the result of preexisting mutations

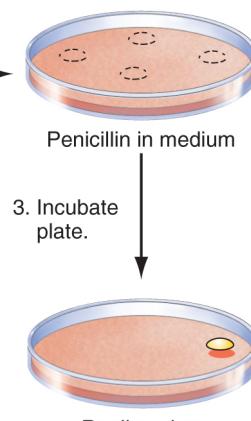
## (a) The replica plating technique

1. Invert master plate; pressing against velvet surface leaves an imprint of colonies. Save plate.



S = penicillin-sensitive bacteria  
R = penicillin-resistant bacteria

2. Invert second plate (replica plate); pressing against velvet surface picks up colony imprint.

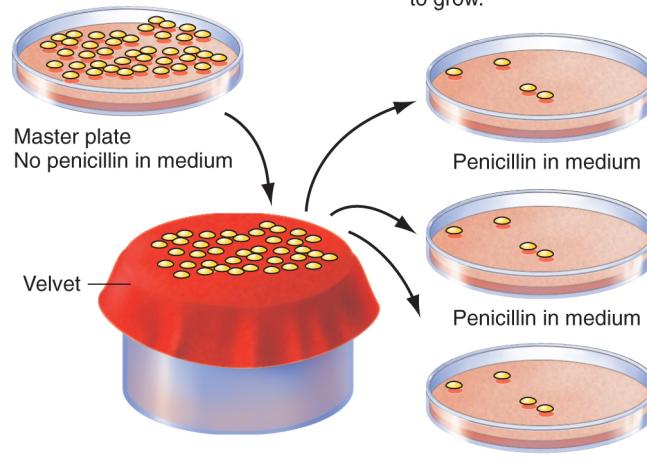


3. Incubate plate.
4. Only penicillin-resistant colonies grow. Compare with position of colonies on original plate.

## (b) Mutations occur prior to penicillin exposure

1.  $10^7$  colonies of penicillin-sensitive bacteria

Make three replica plates. Incubate to allow penicillin-resistant colonies to grow.



Penicillin-resistant colonies grow in the same position on all three plates.

Fig. 7.5

# Interpretation of Luria-Delbrück fluctuation test and replica plating

**Bacterial resistance arises from mutations that occurred before exposure to bactericide**

- Bactericide becomes a selective agent
- Kills nonresistant cells
- Allows survival of cells with pre-existing resistance

**Mutations occur as the result of random processes**

- Once such random changes occur, they usually remain stable

# How natural processes can change the information stored in DNA

## Depurination

- 1000/hr in every cell

## Deamination of C

- C changed to U
- Normal C-G → A-T after replication

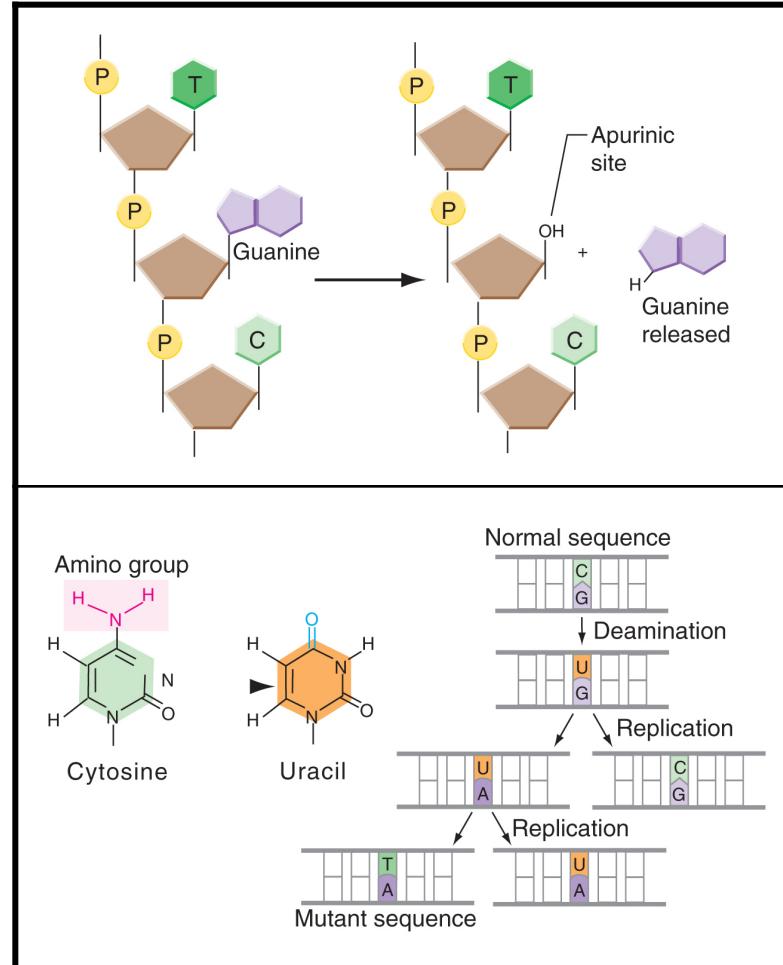
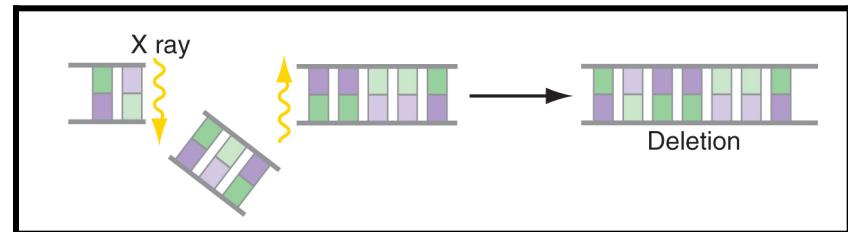


Fig. 7.6a, b

# How natural processes can change the information stored in DNA (cont)

X-rays break the sugar – phosphate backbone of DNA



Ultraviolet (UV) light causes adjacent thymines to form abnormal covalent bonds (thymine dimers)

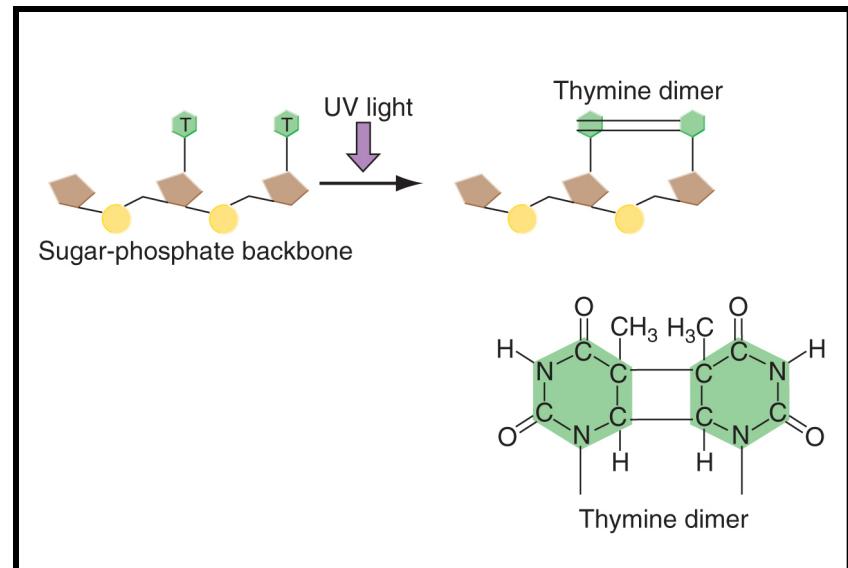


Fig. 7.6c, d

# How natural processes can change the information stored in DNA (cont)

Irradiation causes formation of free radicals (e.g. reactive oxygen) that can alter individual bases

- 8-oxodG mispairs with A
- Normal G-C → mutant T-A after replication

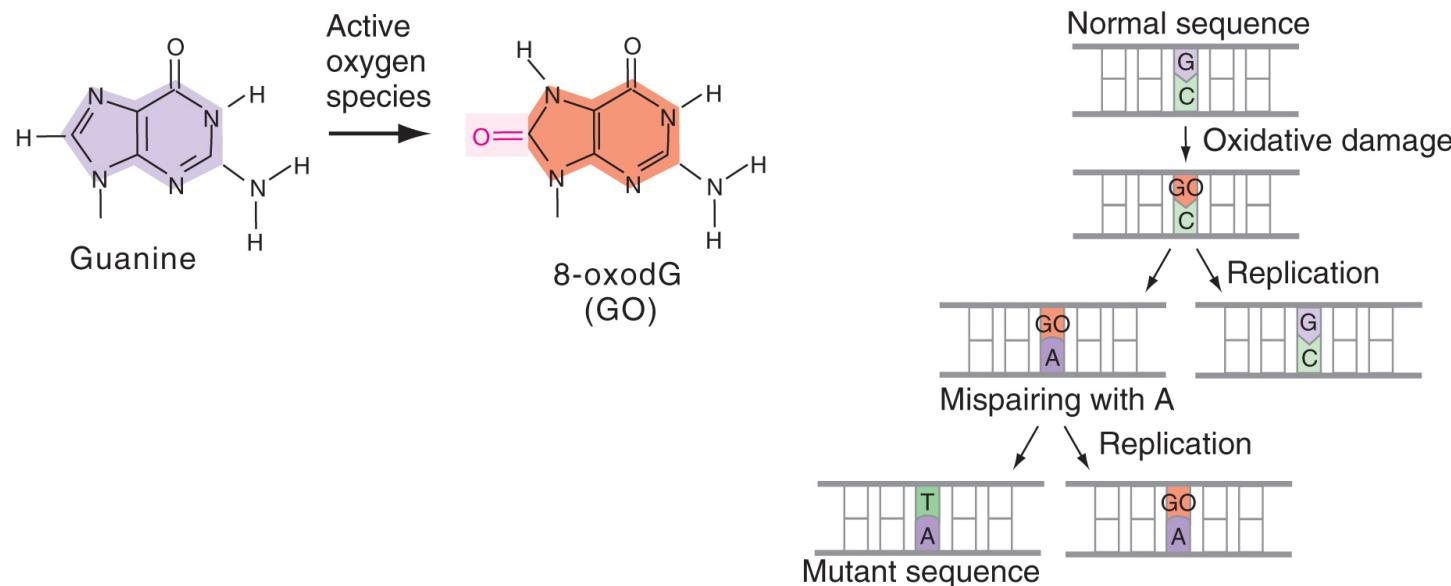


Fig. 7.6e

# Mistakes during DNA replication

Incorporation of incorrect bases by DNA polymerase is exceedingly rare ( $< 10^{-9}$  in bacteria and humans)

Two ways that replication machinery minimizes mistakes

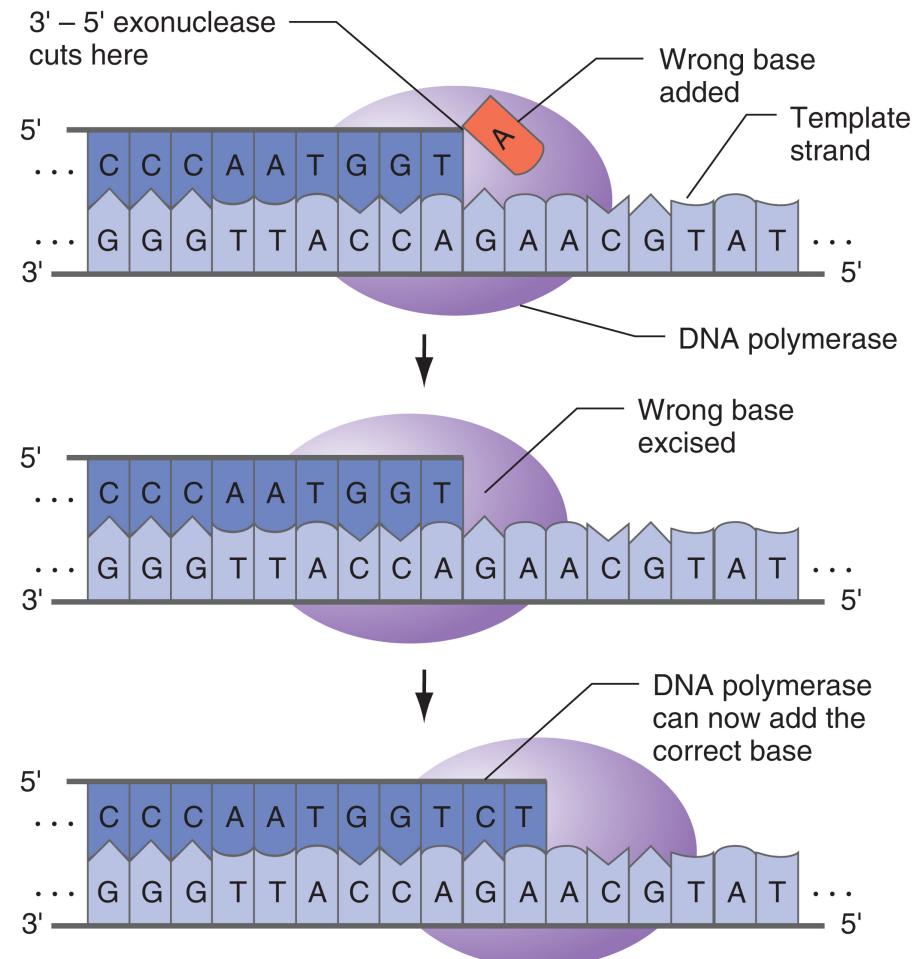
- **Proofreading** function of DNA polymerase (Fig 7.7)
  - 3'-to-5' exonuclease recognizes and excises mismatches
- **Methyl-directed mismatch repair** (later in this chapter)
  - Corrects errors in newly replicated DNA

# DNA polymerase's proofreading function

**Mispaired base is  
recognized and excised  
by 3'-to-5' exonuclease of  
DNA polymerase**

**Improves fidelity of  
replication 100-fold**

Fig. 7.7



# Unequal crossing-over can occur between homologous chromosomes

Pairing between homologs during meiosis can be out of register

Unequal crossing-over results in a deletion on one homolog and a duplication on the other homolog

## (a) Unequal crossing-over

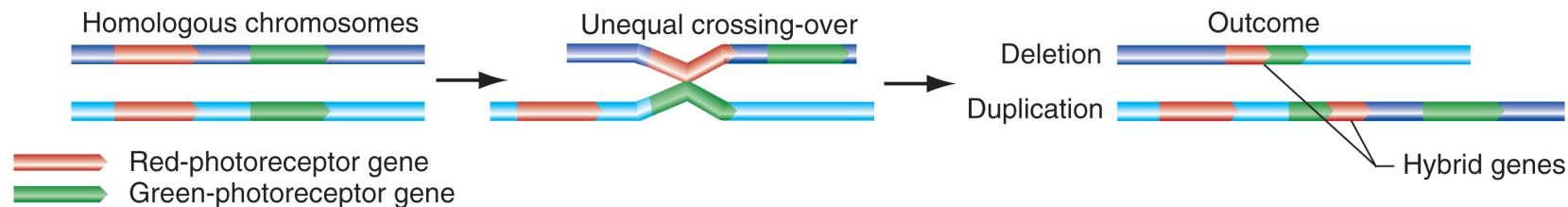


Fig. 7.8a

# Transposable elements (TEs) move around the genome

TEs can "jump" into a gene and disrupt its function

Two mechanisms of TE movement (transposition)

(b) Two mechanisms of TE movement

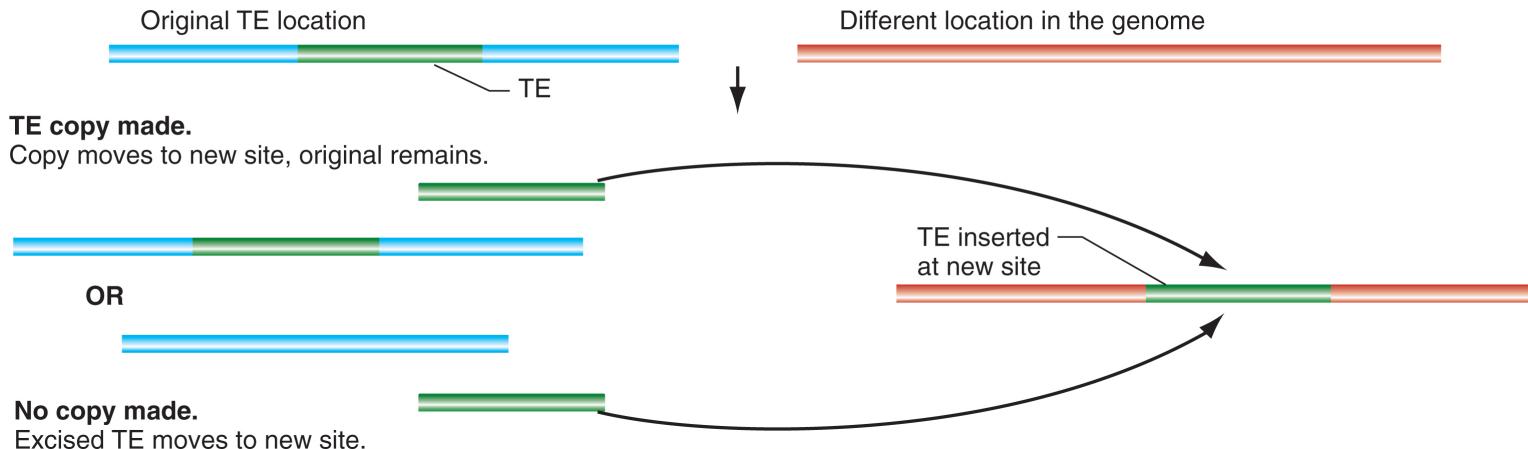


Fig. 7.8b

# Experimental evidence that mutagens induce mutations

H. J. Muller

X-ray dose above the naturally-occurring level causes increased mutation rate in *Drosophila*

- Exposed male *Drosophila* to X-rays
- Mating scheme (see Fig 7.9) used genetically marked "balancer" X chromosome
- Able to detect X-linked genes that are essential for viability

# Exposure to X-rays increases the mutation rate in *Drosophila*

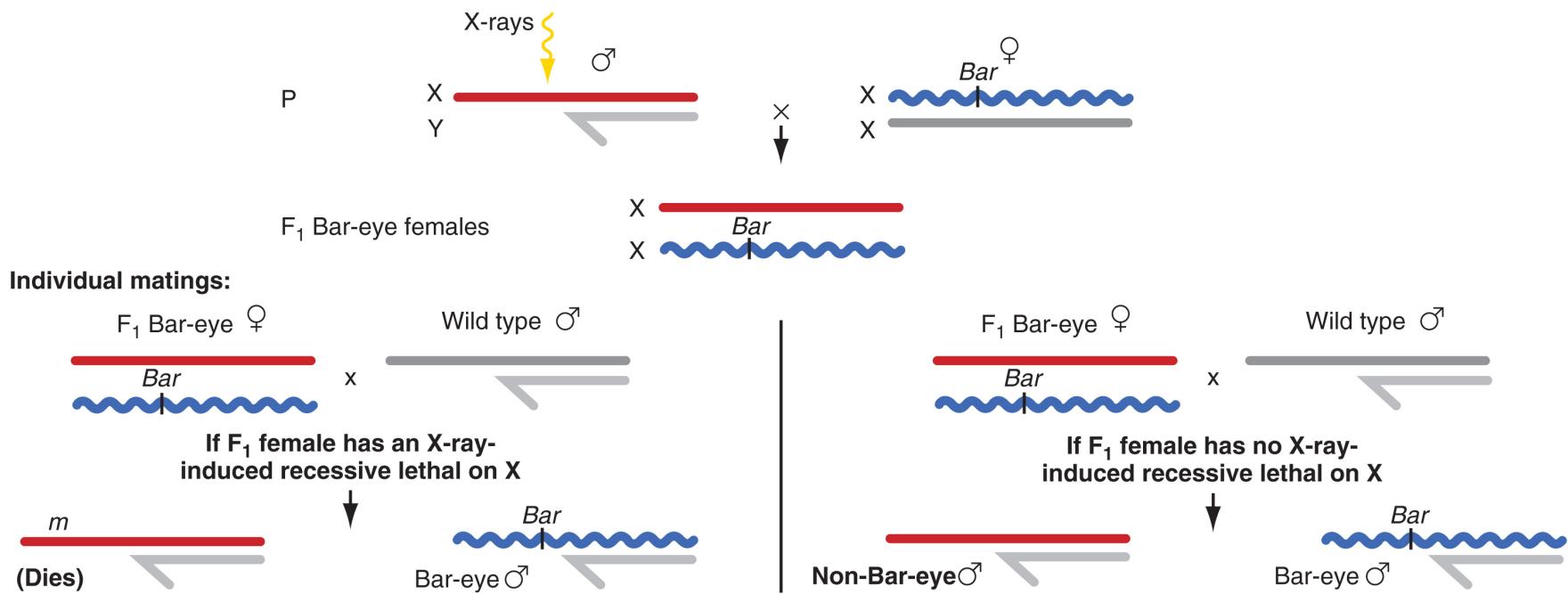


Fig. 7.9

# How mutagens alter DNA: Chemical action of mutagen

Replace a base: Base analogs - chemical structure almost identical to normal base

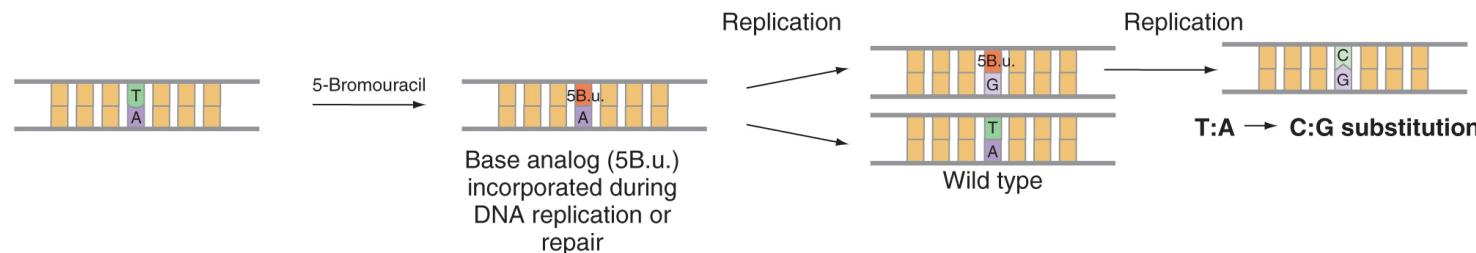
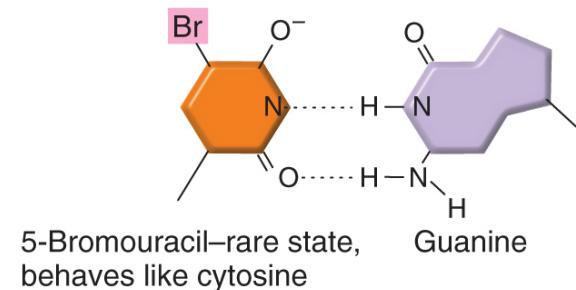
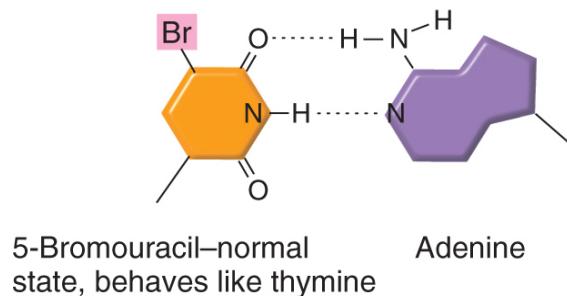


Fig. 7.10a

# How mutagens alter DNA: Chemical action of mutagen

**Alter base structure and properties: Hydroxylating agents add an –OH group**

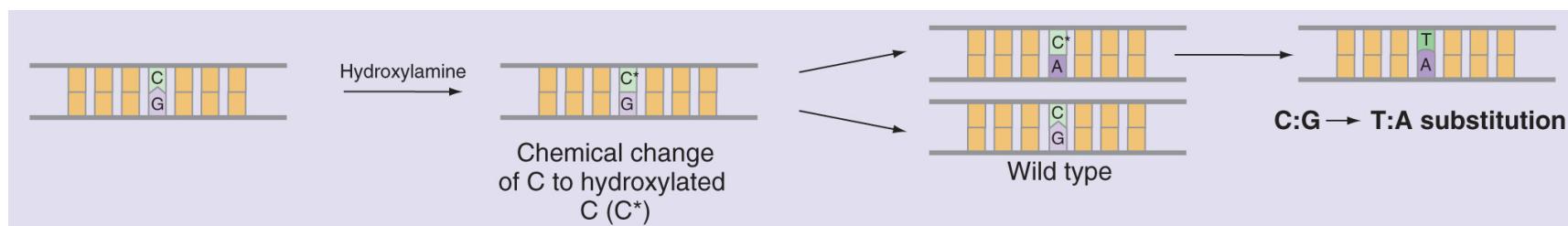
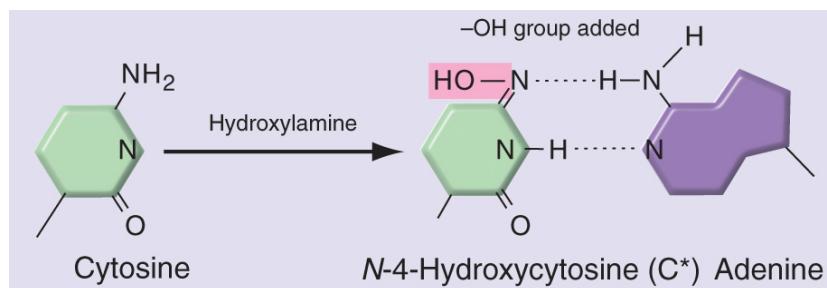


Fig. 7.10b

# How mutagens alter DNA: Chemical action of mutagen (cont)

**Alter base structure and properties (cont): Alkylating agents add ethyl or methyl groups**

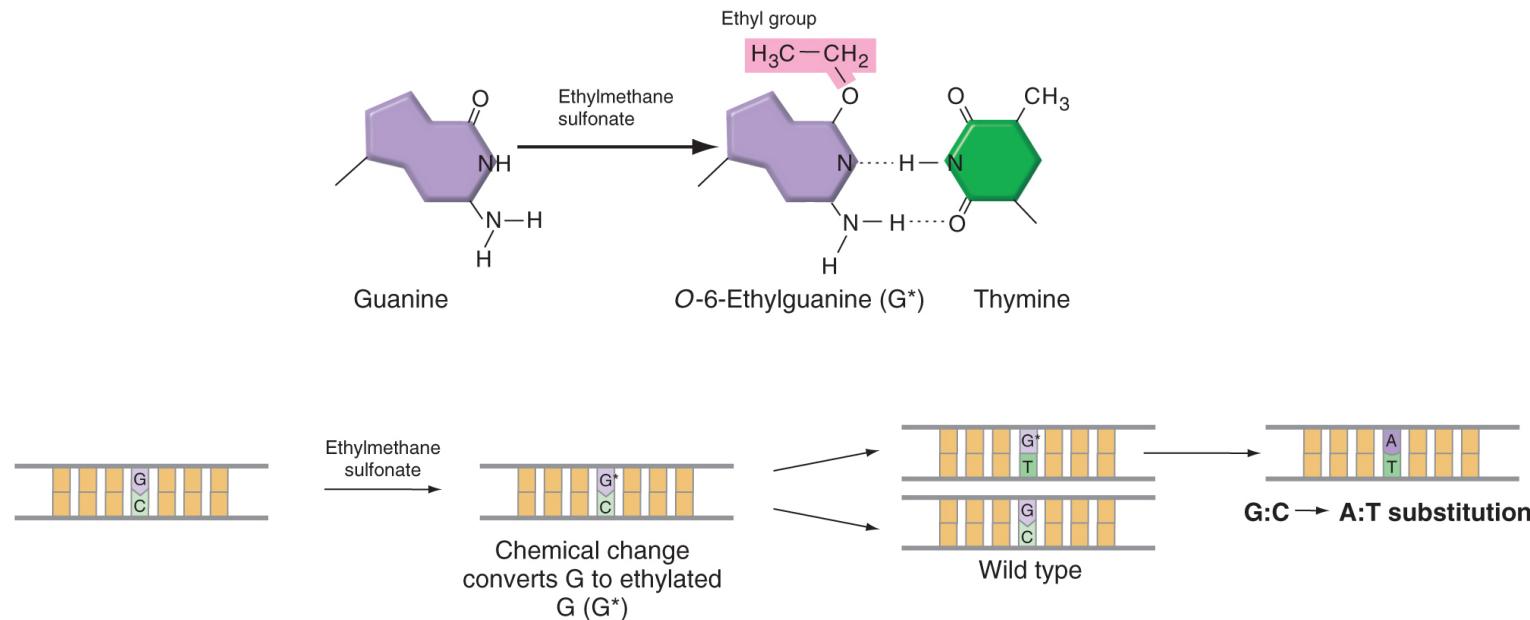


Fig. 7.10b

# How mutagens alter DNA: Chemical action of mutagen (cont)

**Alter base structure and properties (cont):  
Deaminating agents remove  
amine (-NH<sub>2</sub>) groups**

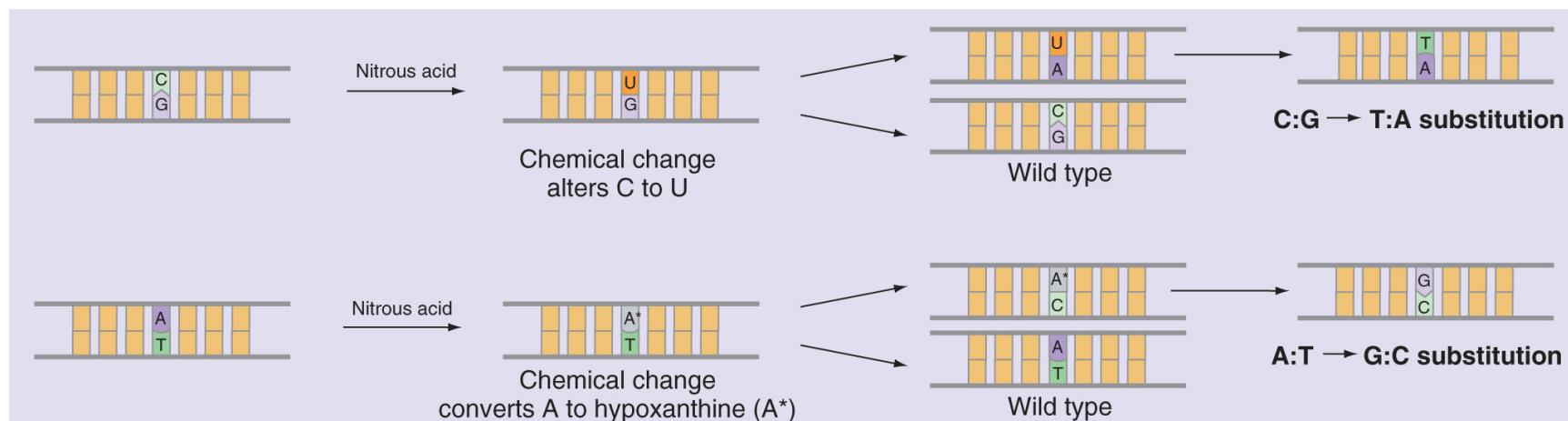
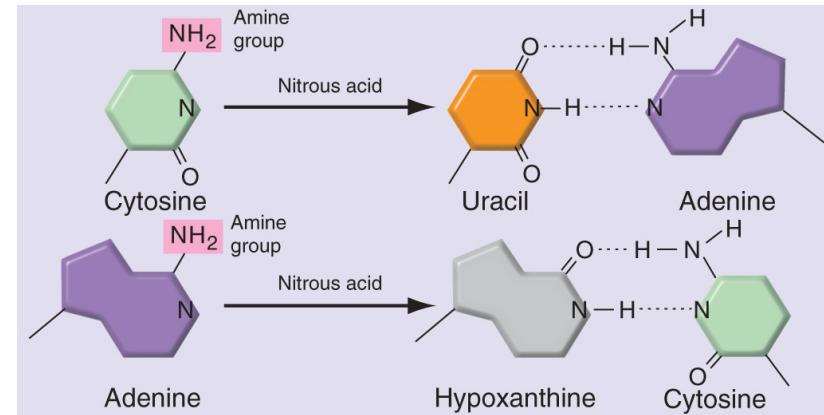


Fig. 7.10b

# How mutagens alter DNA: Chemical action of mutagen (cont.)

## Insert between bases: Intercalating agents

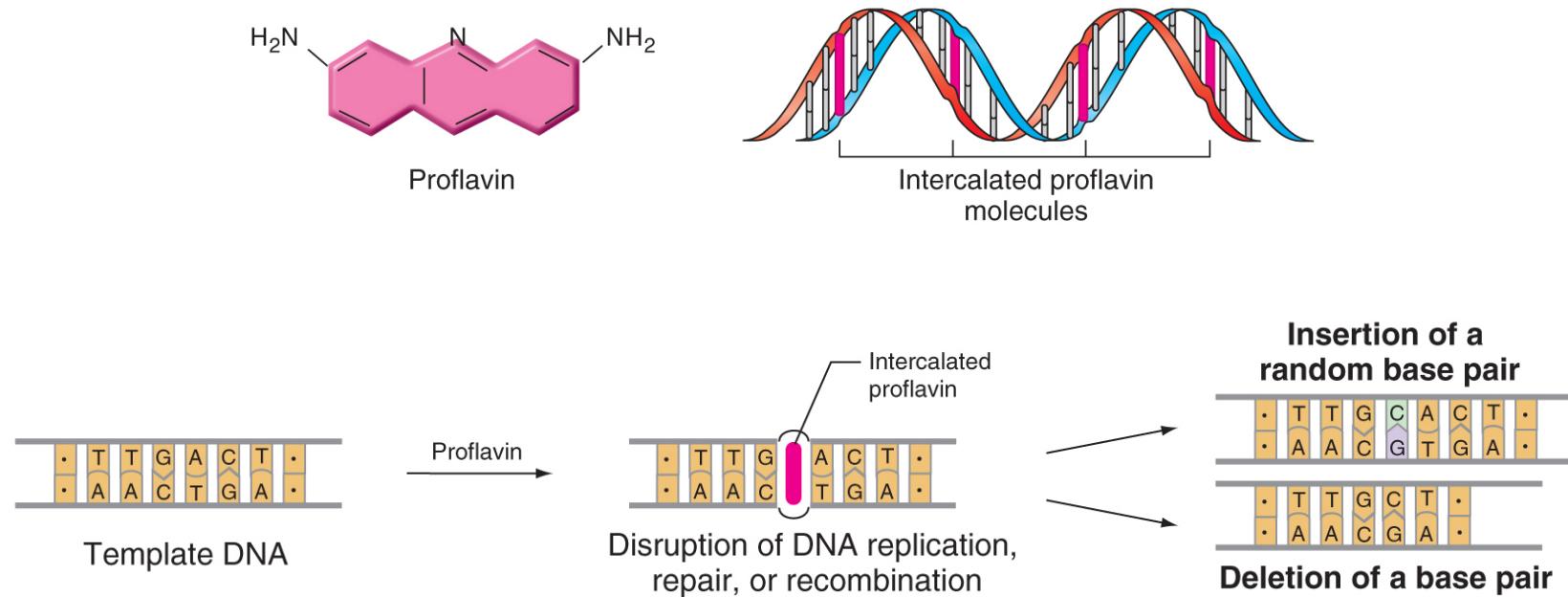


Fig. 7.10c

# DNA repair mechanisms that are very accurate

## Reversal of DNA base alterations

- **Alkyltransferase – removes alkyl groups**
- **Photolyase – splits covalent bond of thymine dimers**

## Homology-dependent repair of damaged bases or nucleotides

- **Base excision repair (Fig 7.11)**
- **Nucleotide excision repair (Fig 7.12)**

## Correction of DNA replication errors

- **Methyl-directed mismatch repair (Fig 7.13)**

# Base excision repair removes damaged bases

Different glycosylases cleave specific damaged bases

Particularly important for removing uracil (created by cytosine deamination) from DNA

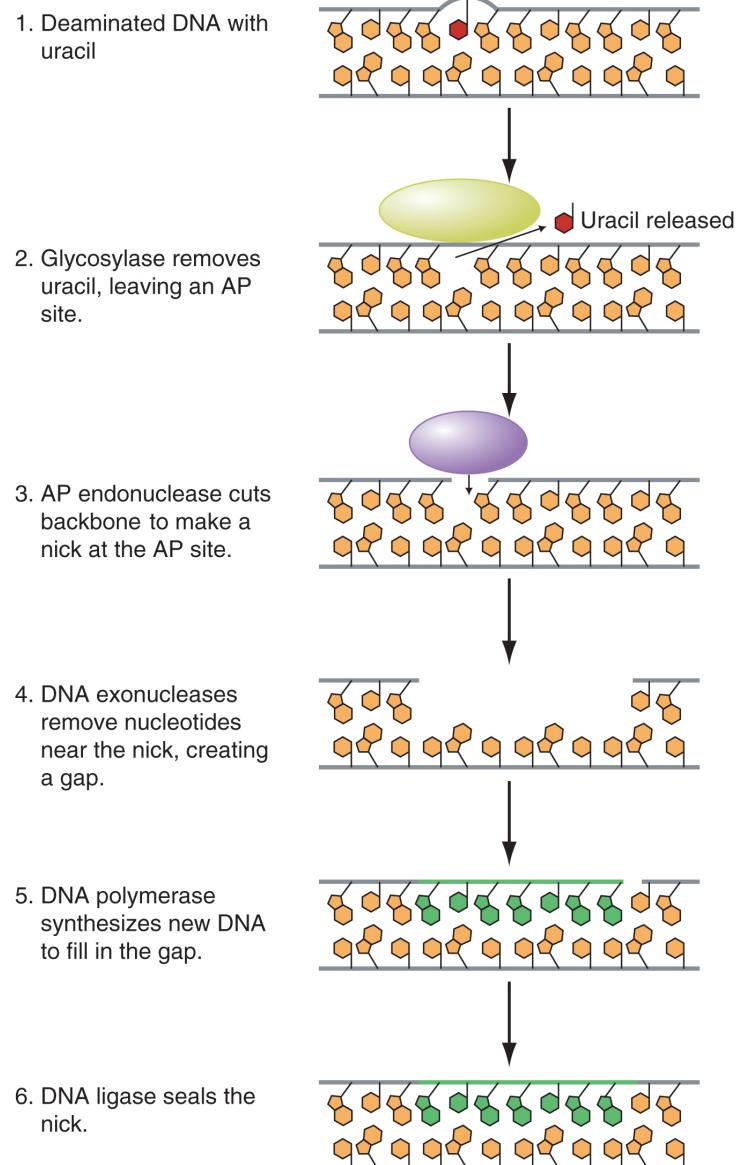


Fig. 7.11

# Nucleotide excision repair corrects damaged nucleotides

**UvrA – UvrB complex scans for distortions to double helix (e.g. thymine dimers)**

**UvrB – UvrC complex nicks the damaged DNA**

- **4 nt to one side of damage**
- **7 nt to the other side of damage**

1. Exposure to UV light
2. Thymine dimer forms.
3. UvrB and C endonucleases nick strand containing dimer.
4. Damaged fragment is released from DNA.
5. DNA polymerase fills in the gap with new DNA (*green*).
6. DNA ligase seals the repaired strand.

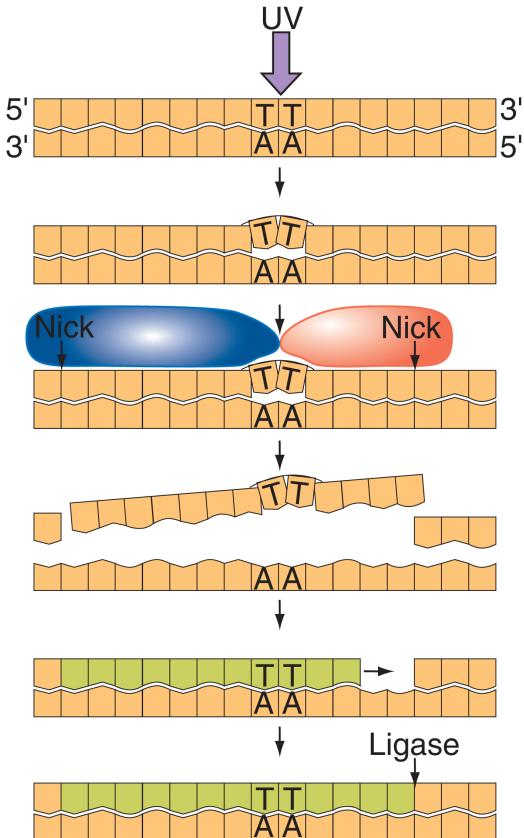


Fig. 7.12

# In bacteria, methyl-directed mismatch repair corrects mistakes in replication

## Parental DNA strand marked by adenine methylase

- Methyl group added to A in GATC sequence
- Newly-replicated DNA isn't yet methylated

## MutS and MutL bind to mismatched nucleotides

MutH nicks the unmethylated strand opposite the methylated GATC

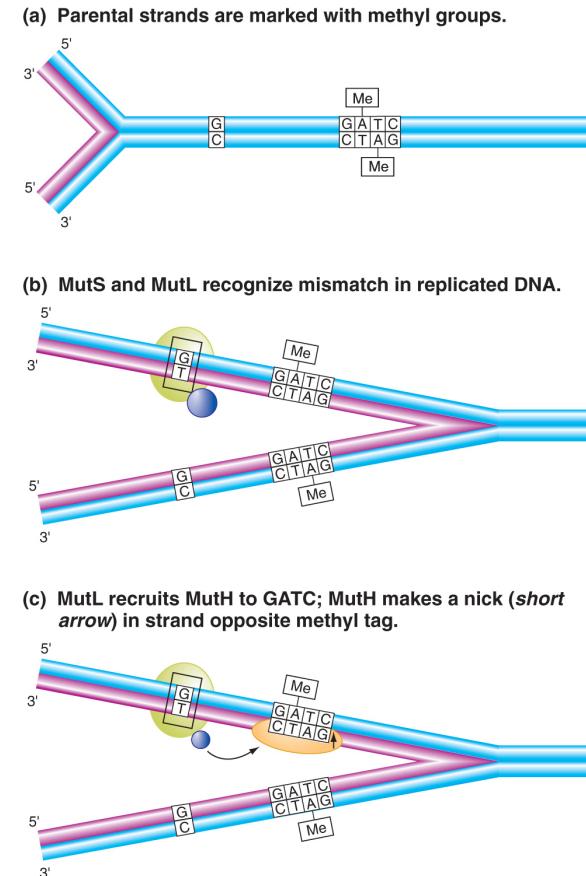


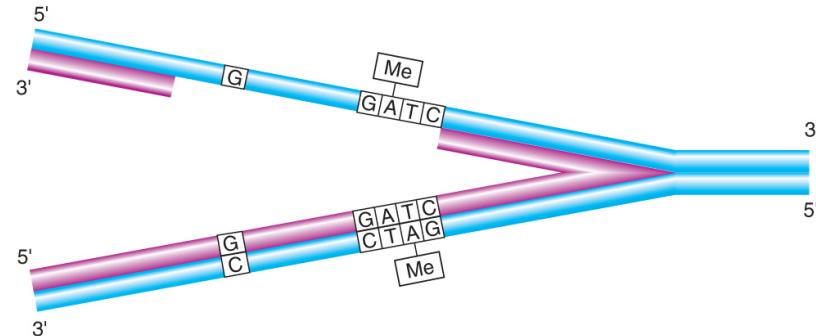
Fig. 7.13

# In bacteria, methyl-directed mismatch repair corrects mistakes in replication (cont)

**Gap made in unmethylated (new) strand by DNA exonucleases**

**Gap filled in by DNA polymerase using the methylated (old) strand as template**

(d) DNA exonucleases (*not shown*) excise DNA from unmethylated new strand.



(e) Repair and methylation of newly synthesized DNA strand

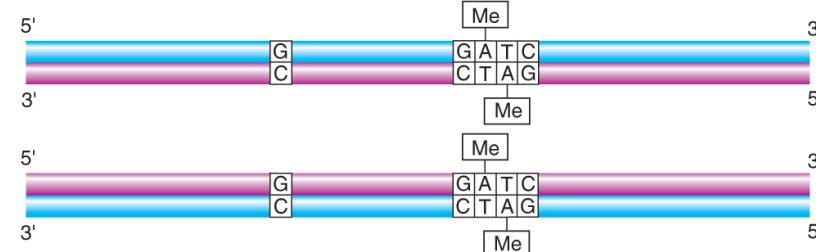


Fig. 7.13 (cont)

# DNA repair mechanisms that are error-prone

## SOS system – bacteria

- Used at replication forks that stalled because of unrepaired DNA damage
- "Sloppy" DNA polymerase used instead of normal polymerase
- Adds random nucleotides opposite damaged bases

## Nonhomologous end-joining (Fig 7.14)

- Deals with double-strand DNA breaks caused by X-rays or reactive oxygen

# Repair of double-strand breaks by nonhomologous end-joining

**Unrepaired double-strand breaks can lead to lethal chromosome rearrangements (e.g. deletions, inversions, translocations)**

**Resection step can lead to loss of DNA**

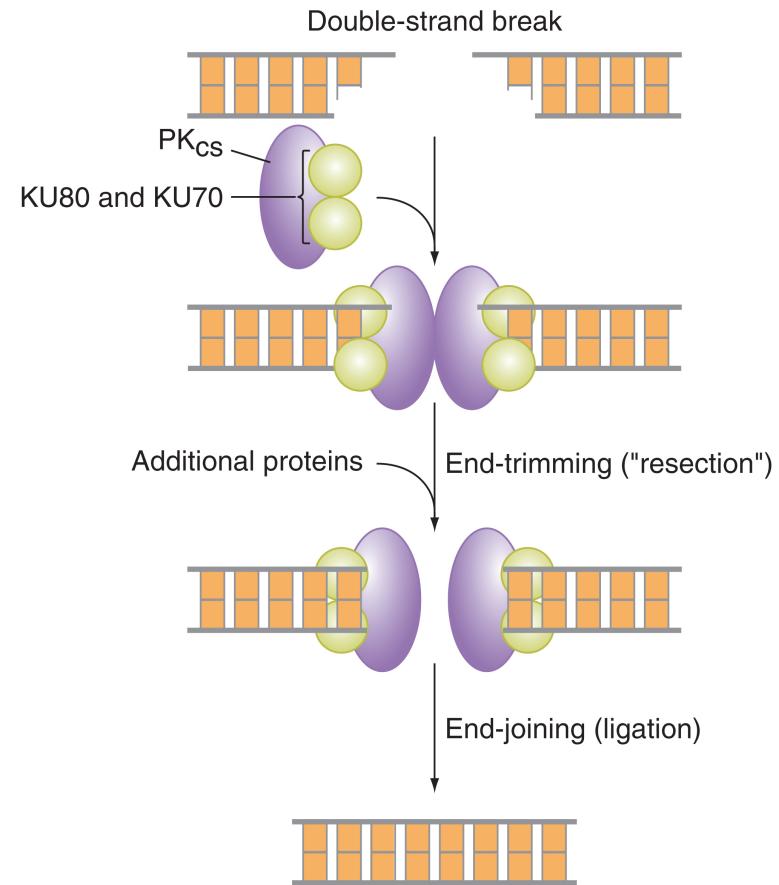


Fig. 7.14

# **Health consequences of mutations in genes encoding DNA repair enzymes**

**Xeroderma pigmentosum:**

**Mutations in one of seven genes encoding enzymes involved in nucleotide excision repair**



Fig. 7.15

**Hereditary forms of colorectal cancer (not shown):**

**Mutations in human homologs of bacterial genes (MutS and MutL) involved in mismatch repair**

# **Impact of unrepaired mutations**

**Germ line mutations – occur in gametes or in gamete precursor cells**

- Transmitted to next generation
- Provide raw material for natural selection

**Somatic mutations – occur in non-germ cells**

- Not transmitted to next generation of individuals
- Can affect survival of an individual
- Can lead to cancer

# The Ames test identifies potential carcinogens

Assay uses *his*<sup>-</sup> mutants in *S. typhimurium*

Detects mutations that cause *his*<sup>-</sup> to *his*<sup>+</sup> reversion

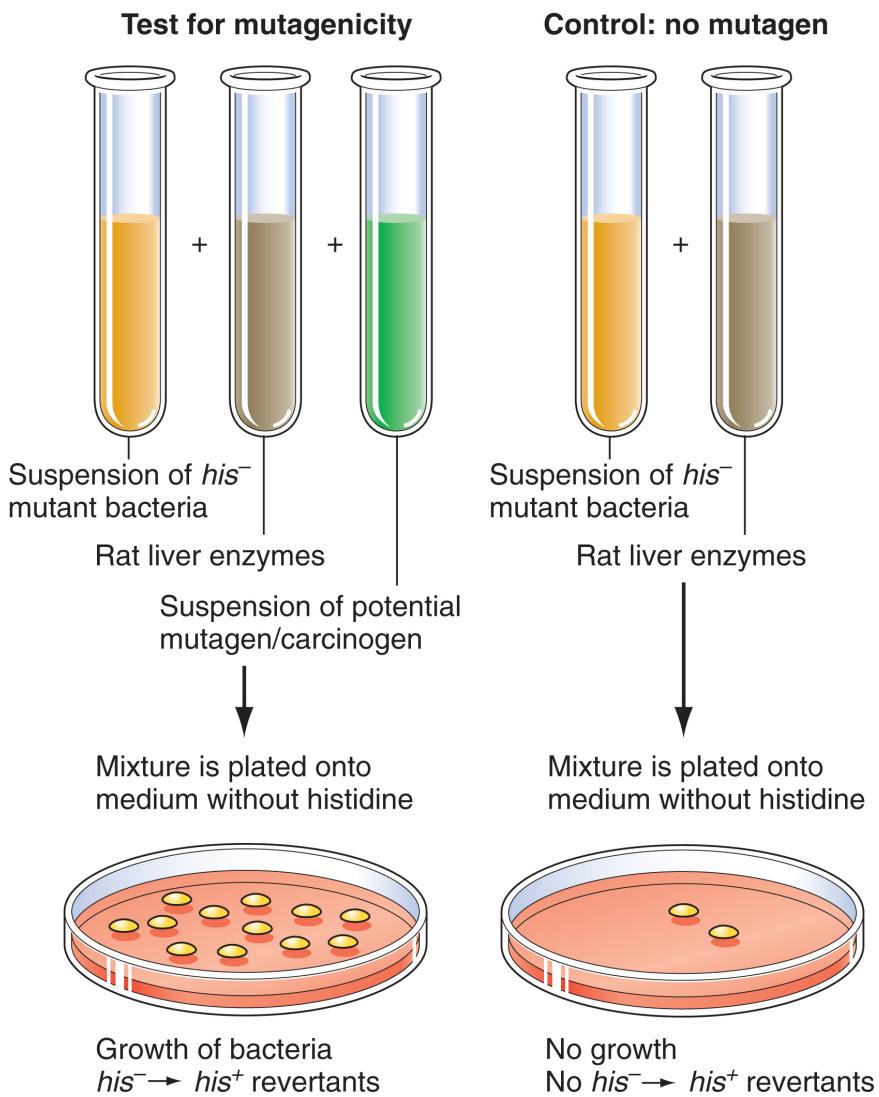


Fig. 7.16

# What mutations tell us about gene structure

## Complementation testing

- Reveals whether two mutations are in a single gene or in different genes
- "Complementation group" is synonymous with a gene

## Fine structure mapping

- Seymour Benzer used phage T4 mutants
- Experimental evidence that a gene is a linear sequence of nucleotide pairs
- Some regions of chromosomes have "hot spots" for mutations

# *Drosophila* eye color mutations produce a variety of phenotypes

Do these phenotypes result from allelic mutations or from mutations in different genes?

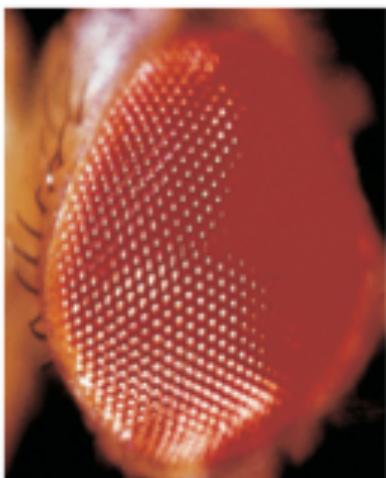


Fig. 7.17

# Complementation testing of *Drosophila* eye color mutations

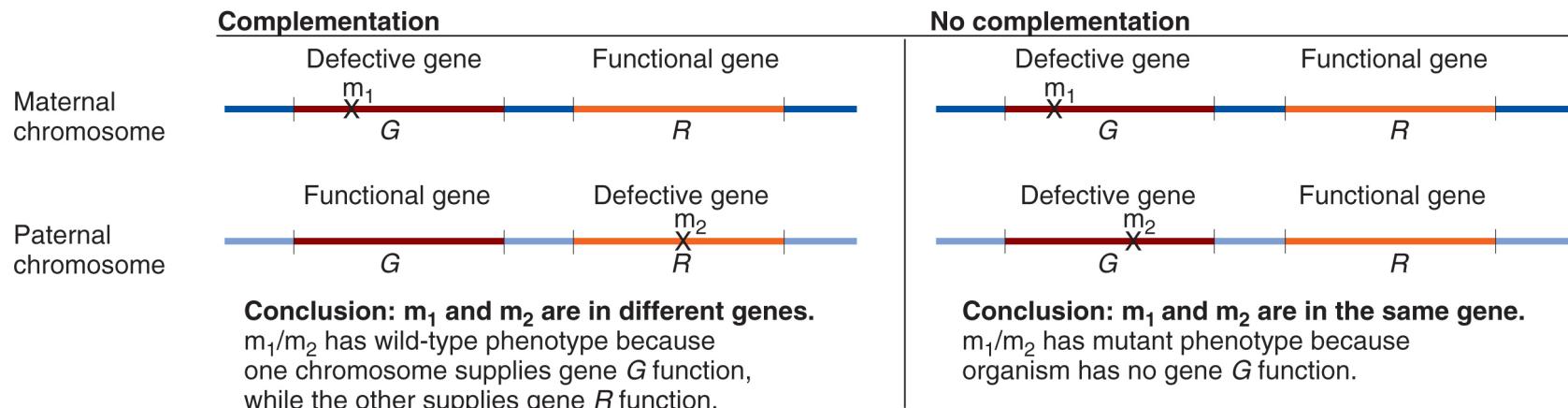


Fig. 7.18a

# A complementation table for X-linked eye color mutations in *Drosophila*

These results reveal five complementation groups (genes):

- Mutations in *white*, *cherry*, *coral*, *apricot*, and *buff* are allelic [(all affect the *white* (*w*) gene)]
- Mutations in *garnet*, *ruby*, *vermillion*, and *carnation* are not allelic with each other or with *white* mutations

Mutation	white	garnet	ruby	vermillion	cherry	coral	apricot	buff	carnation
white	-	+	+	+	-	-	-	-	+
garnet	-	+	+	+	+	+	+	+	+
ruby	-		+	+	+	+	+	+	+
vermillion			-	+	+	+	+	+	+
cherry				-	-	-	-	-	+
coral					-	-	-	-	+
apricot						-	-	-	+
buff							-	-	+
carnation								-	-

Fig 7.18b

# Fine structure mapping of phage T4 mutants

Seymour Benzer (mid 1950-1960s)

Can recombination take place between different mutations in the same gene (**intragenic recombination**)?

Phage T4 is a virus that infects E. coli

Advantages of phage T4 for fine structure mapping:

- Each phage can produce 100-1000 progeny in <1 hr
- Easy to produce large numbers of progeny to detect rare events
- Conditions allowed proliferation of only recombinant phages and death of parental phages

# How recombination within a gene could generate a wild-type allele

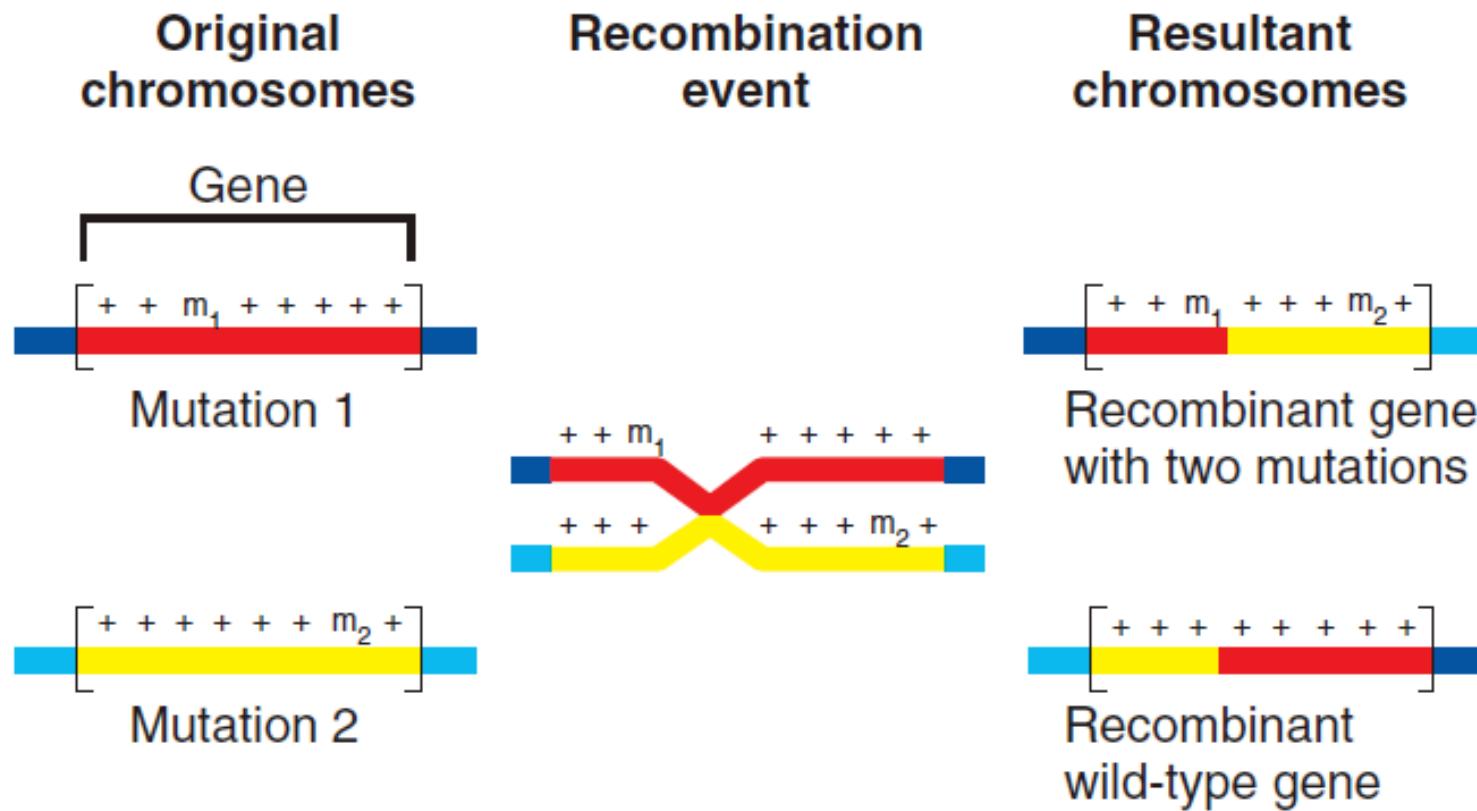
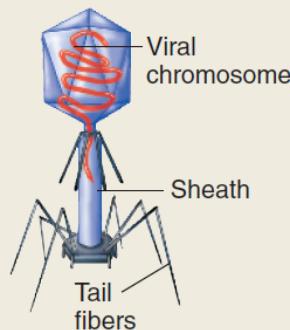
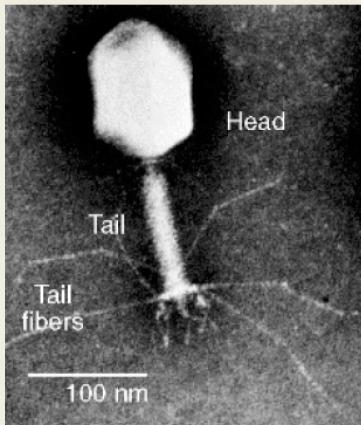


Fig 7.19

# Working with bacteriophage T4

(a.1)



(a.3)



(a.2)

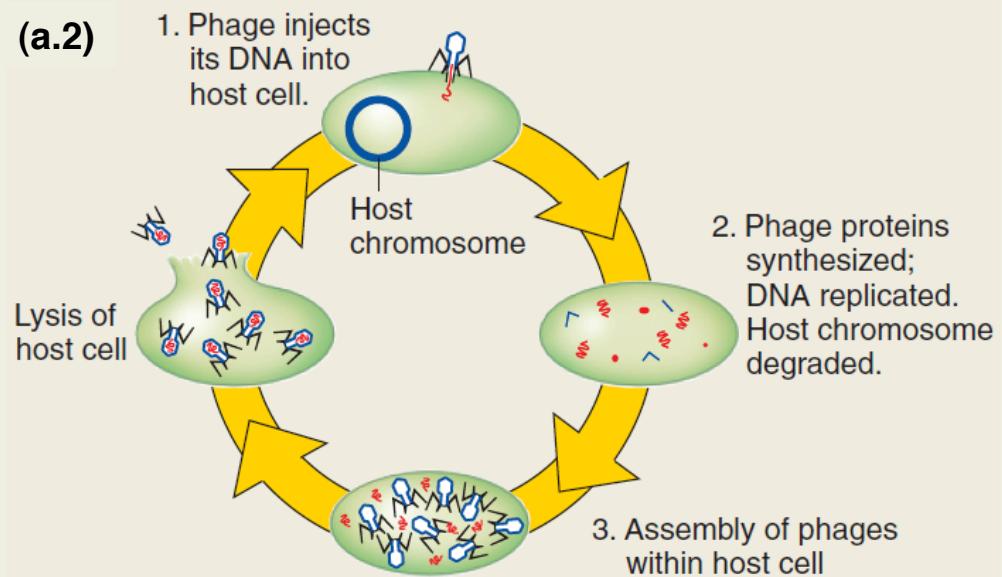


Fig 7.20a

# Counting bacteriophages by serial dilution

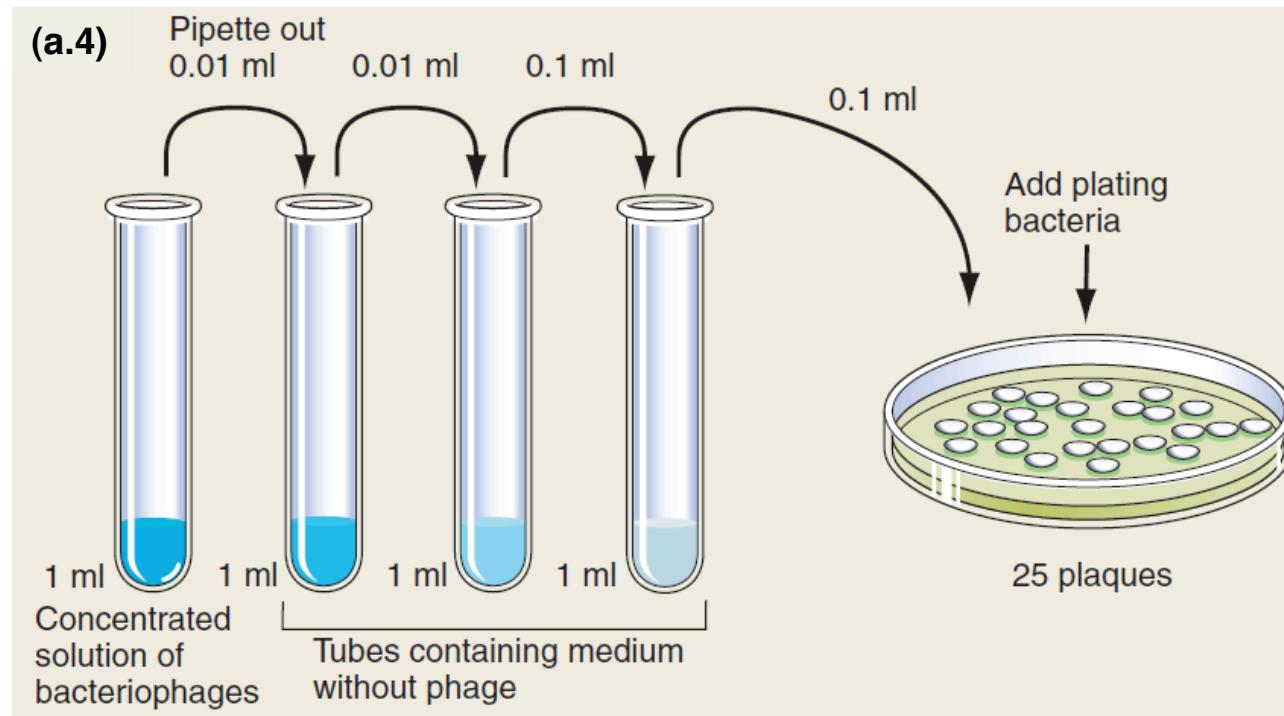
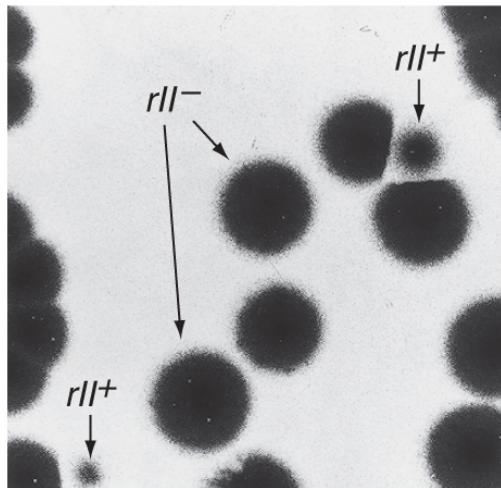


Fig 7.20a

# Phenotypic properties of $rII^-$ mutants of bacteriophage T4 (cont)

$rII^-$  mutants have an altered plaque morphology and altered host range

(b.1)



(b.2)

T4 strain	<i>E. coli</i> strain B	<i>E. coli</i> strain $K(\lambda)$
$rII^-$	Large, distinct	No plaques
	Small, fuzzy	Small, fuzzy

(b.1): © Seymour Benzer

Fig 7.20b

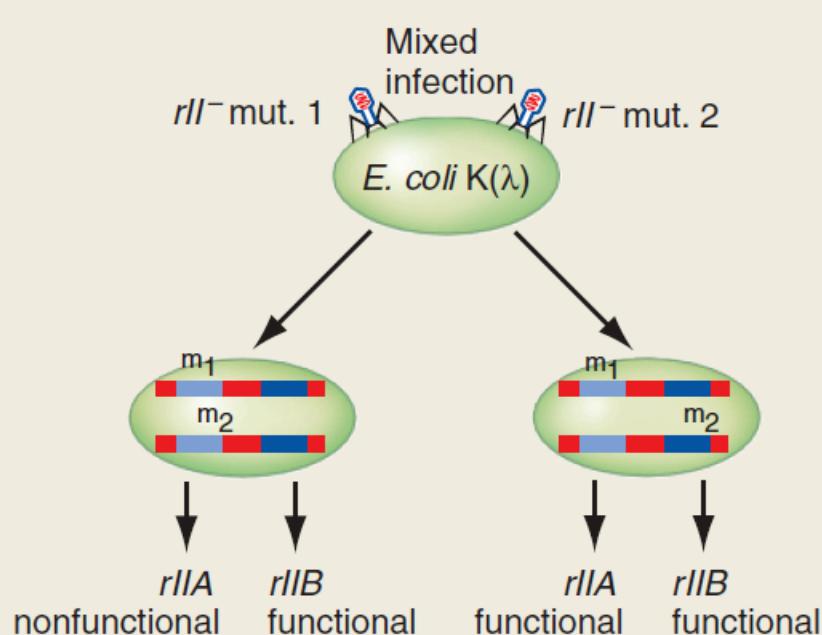
# **Benzer's experimental approach to fine structure mapping of the *rII* locus**

**Generated 1612 spontaneous point mutations and several deletions in *rII* locus**

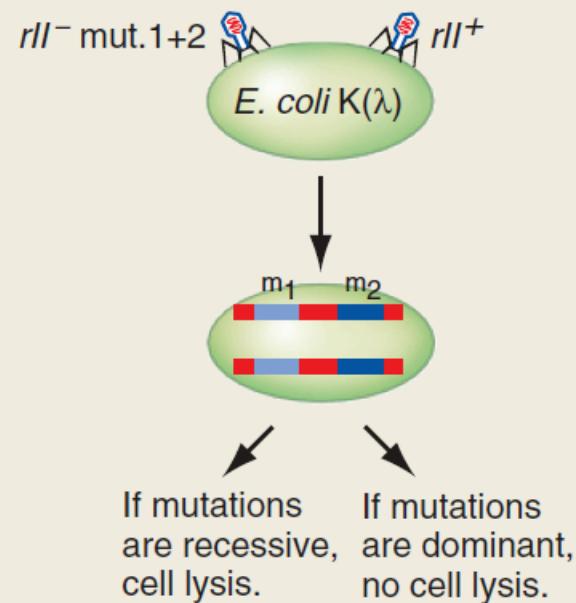
- Identified two complementation groups, *rIIA* and *rIIB* (Fig 7.20c)
- Mapped locations of deletions relative to each other using recombination (Fig 7.21a)
- Mapped locations of point mutations relative to the deletions (Fig 7.21a)
- Tested for recombination between all point mutations within the same complementation group (Fig 7.20d)

# A customized complementation test between $rII^-$ mutants of bacteriophage T4

## (c.1) Complementation test (*trans* configuration)



## (c.2) Control (*cis* configuration)



No complementation  
- no cell lysis  
- no phage progeny

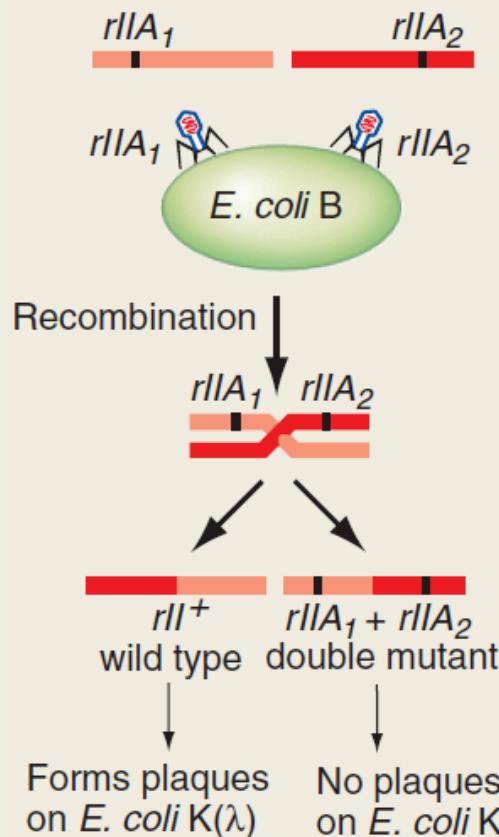
Complementation  
- cell lysis  
- phage progeny

■ Gene  $rIIA$  ■ Gene  $rIIB$

Fig 7.20c

# Detecting recombination between two allelic mutations

## (d.1) Recombination test



## (d.2) Control

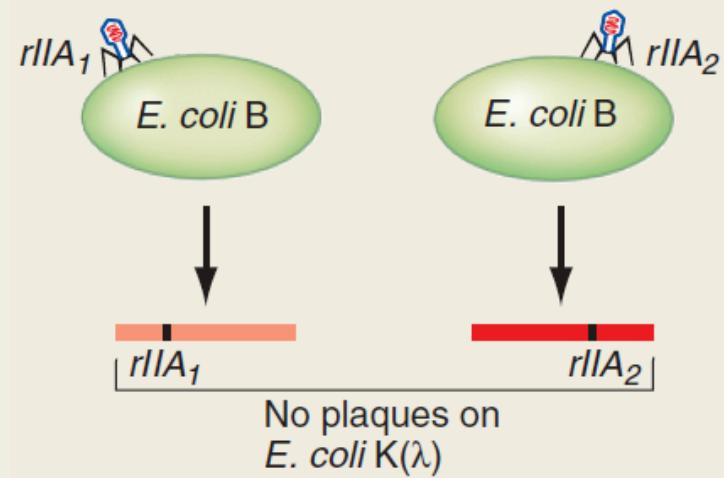


Fig 7.20d

# What mutations tell us about gene function

**Garrod (1902) – some human diseases result from "inborn errors of metabolism" (Fig 7.22)**

**Beadle and Tatum (1940s) – "the one gene, one enzyme" hypothesis (Fig 7.23)**

- *Neurospora crassa*, mutants in arginine (arg) synthesis
- Genetic dissection of a biochemical pathway

**Ingram (mid-1950s) – mutations in a gene can result in amino acid substitutions that disrupt the function of the encoded protein**

- Missense substitution in hemoglobin  $\beta$  causes sickle cell anemia

# Alkaptonuria: An inborn error of metabolism

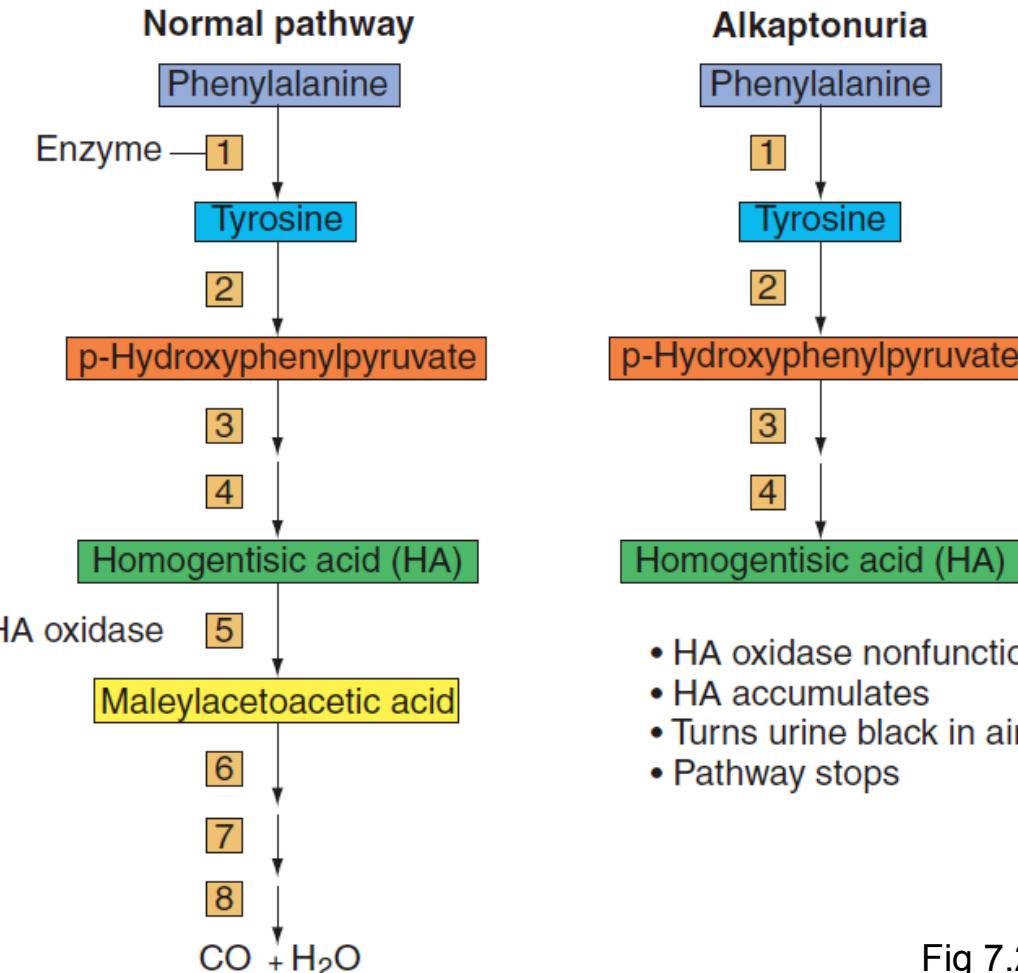


Fig 7.22

# Beadle and Tatum: The "one gene, one enzyme" hypothesis

Screened for X-ray induced mutations in *Neurospora* that disrupted synthesis of arginine (arg)

- **Prototroph** – wild-type strain that grows in minimal media without nutritional supplements
- **Auxotroph** – mutant strain that cannot grow in minimal media

Recombination analysis used to map mutations to four different regions of genome

Each region contained a different complementation group

- Four genes for arg biosynthesis – *ARG-E*, *ARG-F*, *ARG-G*, and *ARG-H*

# Experimental support for the “one gene, one enzyme” hypothesis

## Scheme used by Beadle and Tatum for isolation of *arg*<sup>-</sup> auxotrophs in *Neurospora*

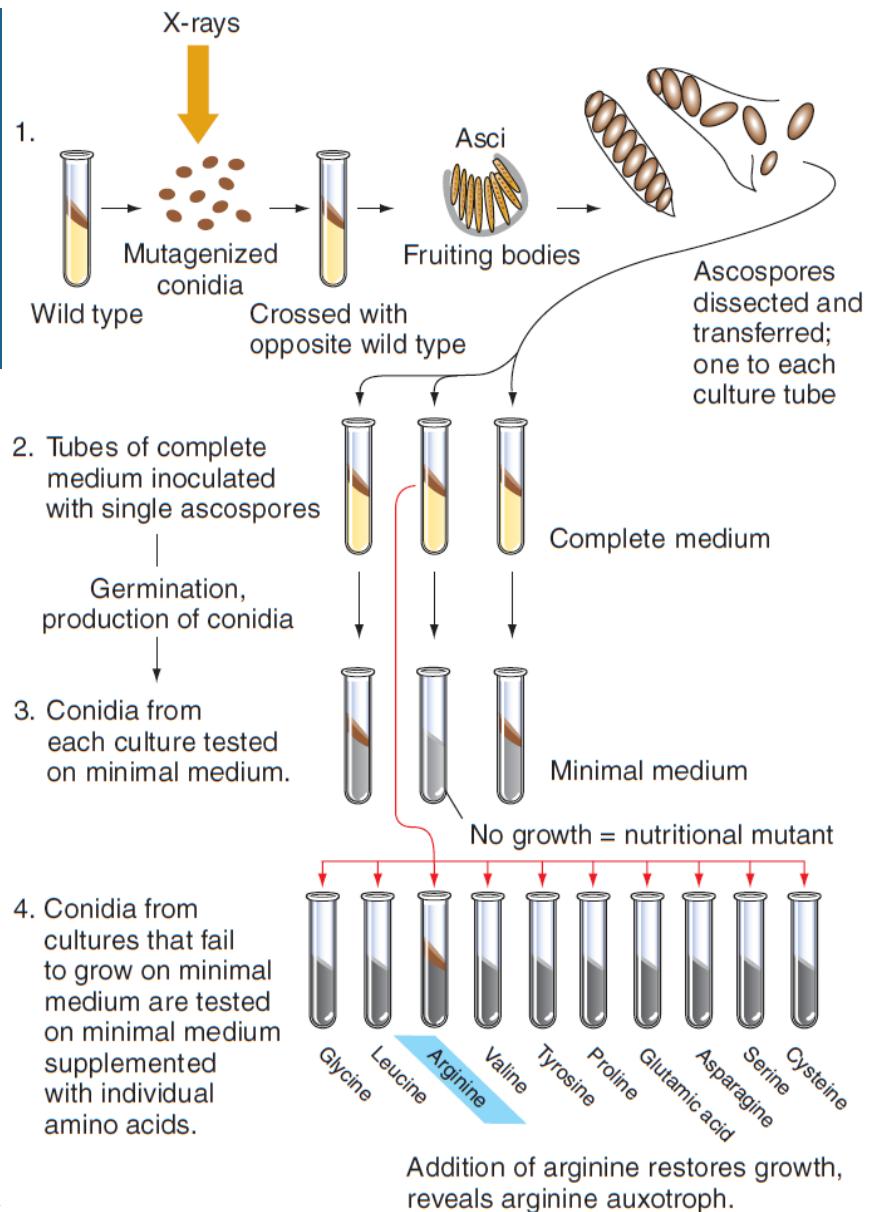


Fig 7.23a

# Experimental support for the “one gene, one enzyme” hypothesis

Growth response if nutrient is added to minimal medium

Mutant strain	Supplements				
	Nothing	Ornithine	Citrulline	Arginino-succinate	Arginine
Wildtype: <i>Arg</i> <sup>+</sup>	+	+	+	+	+
<i>Arg-E</i> <sup>-</sup>	-	+	+	+	+
<i>Arg-F</i> <sup>-</sup>	-	-	+	+	+
<i>Arg-G</i> <sup>-</sup>	-	-	-	+	+
<i>Arg-H</i> <sup>-</sup>	-	-	-	-	+

Inferred biochemical pathway

Each *ARG* gene encodes an enzyme needed to convert one intermediate to the next in the pathway

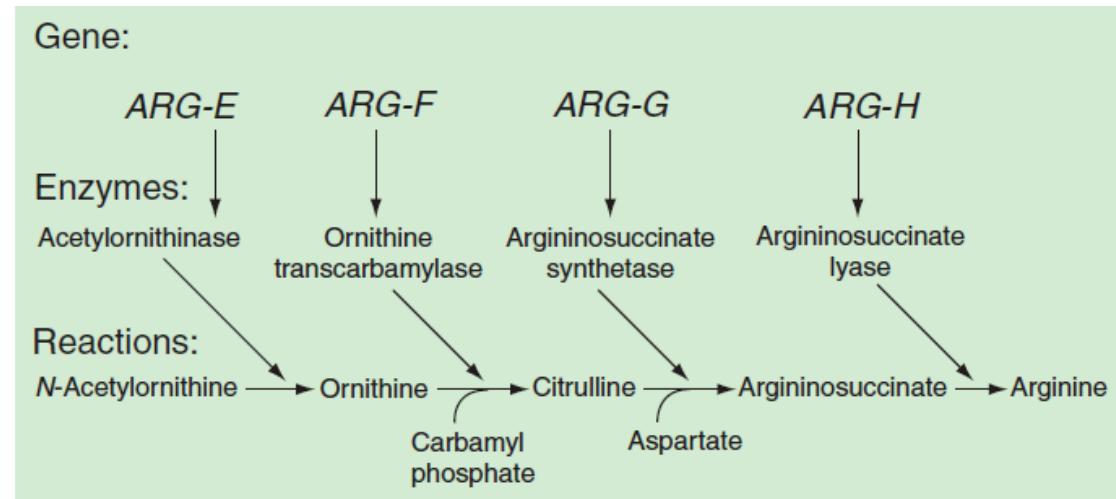


Fig 7.23b, c

# Proteins are chains of amino acids linked by peptide bonds

20 different amino acids

R group is the side chain that is unique to each amino acid

Four groups of amino acids based on R group properties  
(Fig 7.24b)

-COOH group and -NH<sub>2</sub> group of adjacent amino acids are joined in covalent peptide bond

Polypeptides have "N terminus" and "C terminus"

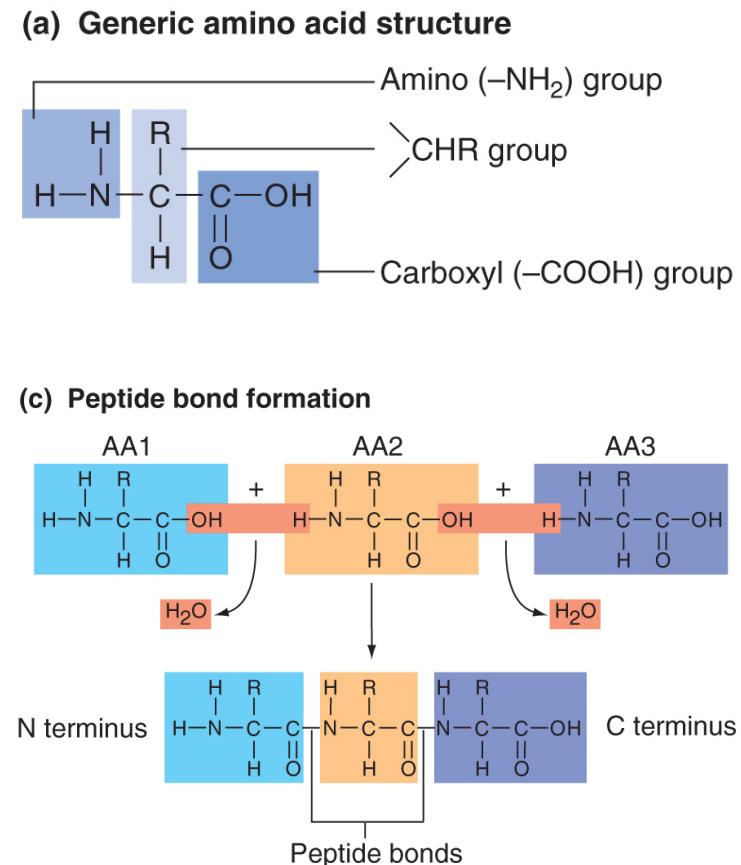


Fig 7.24a, c

# Amino acids with nonpolar R groups

R groups	Backbone	R groups	Backbone
Glycine (Gly) (G)	$\begin{array}{c} \text{H} \\   \\ \text{H} - \text{C} - \text{COOH} \\   \\ \text{NH}_2 \end{array}$	Proline (Pro) (P)	
Alanine (Ala) (A)	$\begin{array}{c} \text{H} \\   \\ \text{CH}_3 - \text{C} - \text{COOH} \\   \\ \text{NH}_2 \end{array}$	Phenylalanine (Phe) (F)	
Valine (Val) (V)	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array} \begin{array}{c} \text{H} \\   \\ \text{C} - \text{COOH} \\   \\ \text{NH}_2 \end{array}$	Tryptophan (Trp) (W)	
Leucine (Leu) (L)	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array} \begin{array}{c} \text{H} \\   \\ \text{CH}_2 - \text{C} - \text{COOH} \\   \\ \text{NH}_2 \end{array}$	Methionine (Met) (M)	$\begin{array}{c} \text{CH}_3 - \text{S} - \text{CH}_2 - \text{CH}_2 - \text{C} - \text{COOH} \\   \\ \text{NH}_2 \end{array}$
Isoleucine (Ile) (I)	$\begin{array}{c} \text{CH}_3 - \text{CH}_2 - \text{CH} \\   \\ \text{CH}_3 \end{array} \begin{array}{c} \text{H} \\   \\ \text{C} - \text{COOH} \\   \\ \text{NH}_2 \end{array}$		

Fig 7.24b

# Amino acids with uncharged R groups

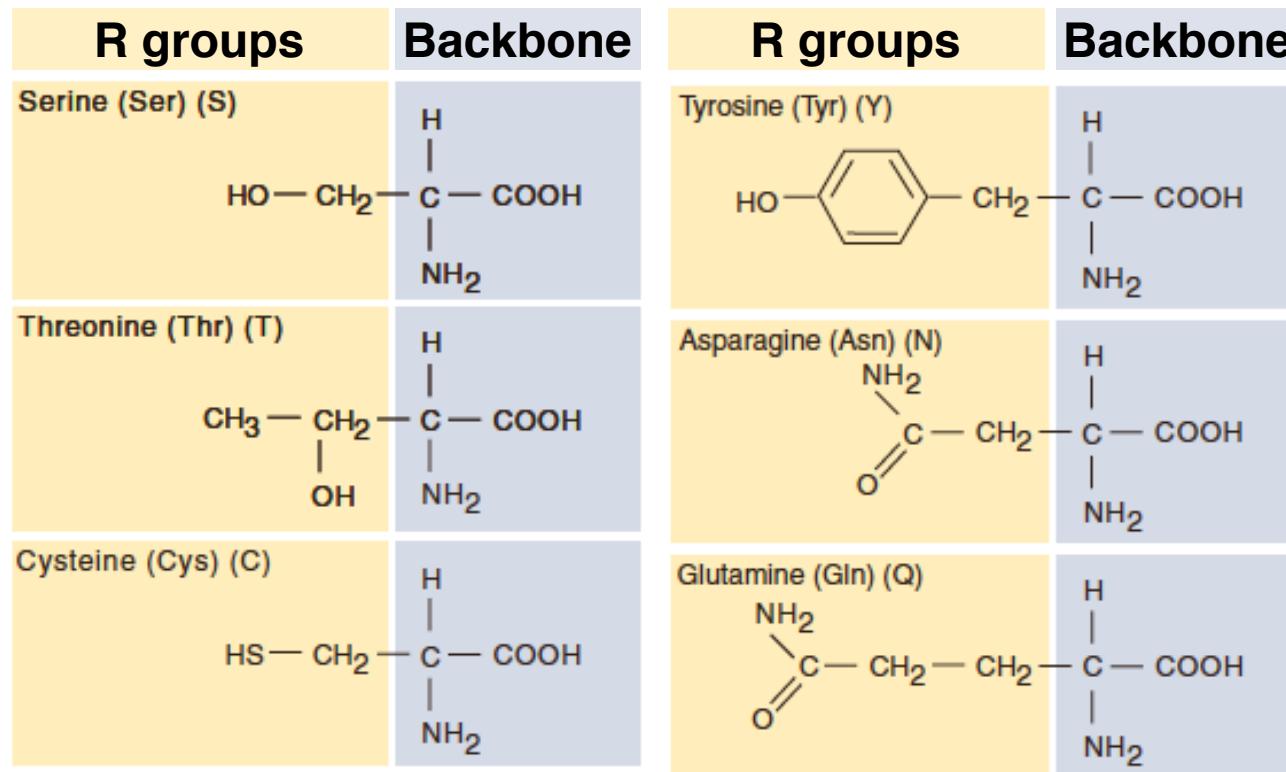


Fig 7.24b (cont)

# Amino acids with charged R groups

**Basic R group**

R groups	Backbone	R groups	Backbone
Lysine (Lys) (K)	H   C — COOH   NH <sub>2</sub>	Histidine (His) (H)	H   HC = C — CH <sub>2</sub> — C — COOH   N=C H NH
Arginine (Arg) (R)	H   C — COOH   NH <sub>2</sub>		

**Acidic R group**

R groups	Backbone	R groups	Backbone
Aspartic acid (Asp) (D)	H   C — CH <sub>2</sub> — C — COOH   NH <sub>2</sub>	Glutamic acid (Glu) (E)	H   HO C — CH <sub>2</sub> — CH <sub>2</sub> — C — COOH   NH <sub>2</sub>

Fig 7.24b (cont)

# The molecular basis of sickle-cell anemia

**Glu $\rightarrow$ Val substitution at sixth amino acid affects the three-dimensional structure of the hemoglobin  $\beta$  chain**

**Abnormal protein aggregates cause sickle shape of red blood cells**

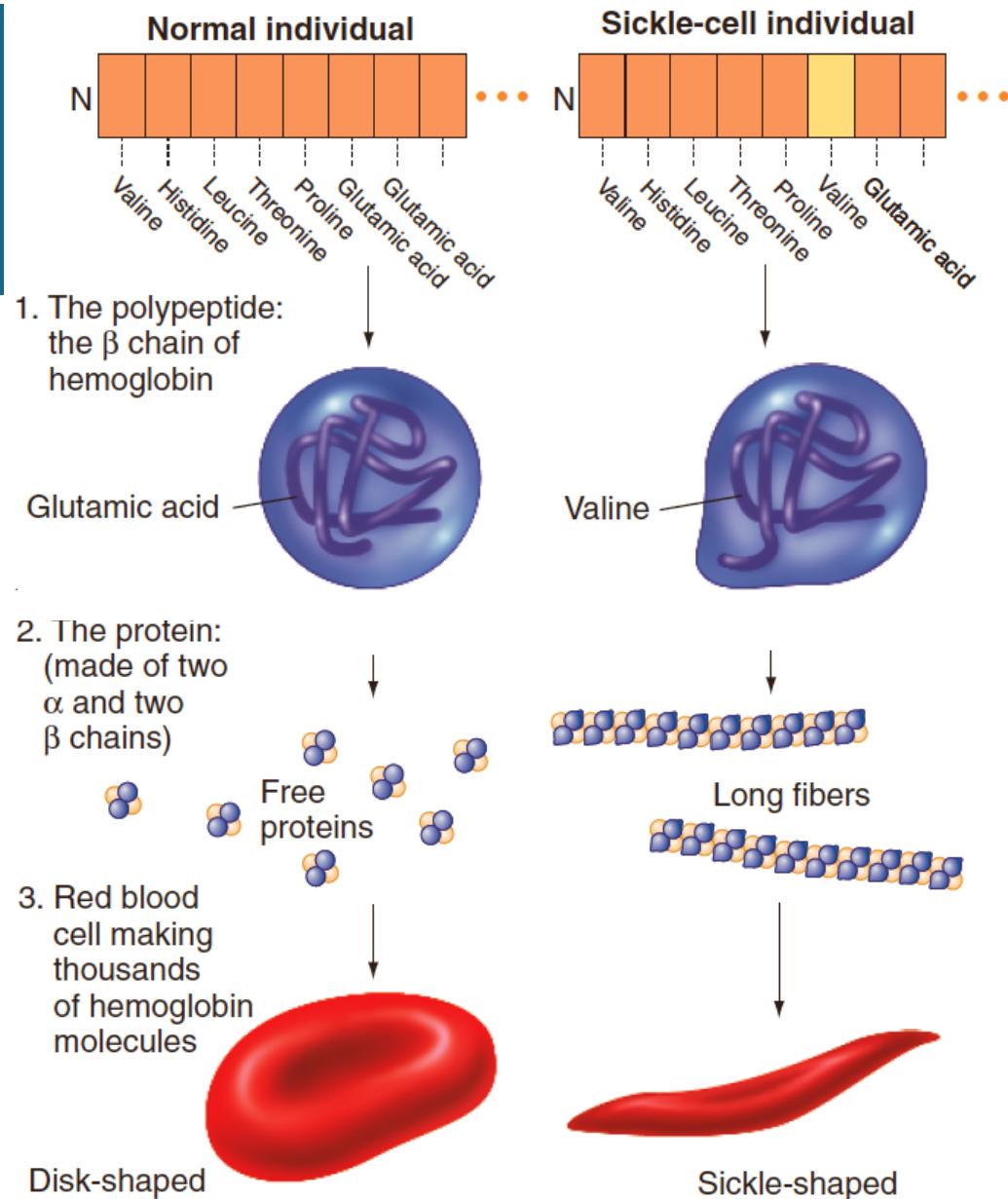


Fig 7.25a Disk-shaped

Sickle-shaped

# Sickle-cell anemia is pleiotropic

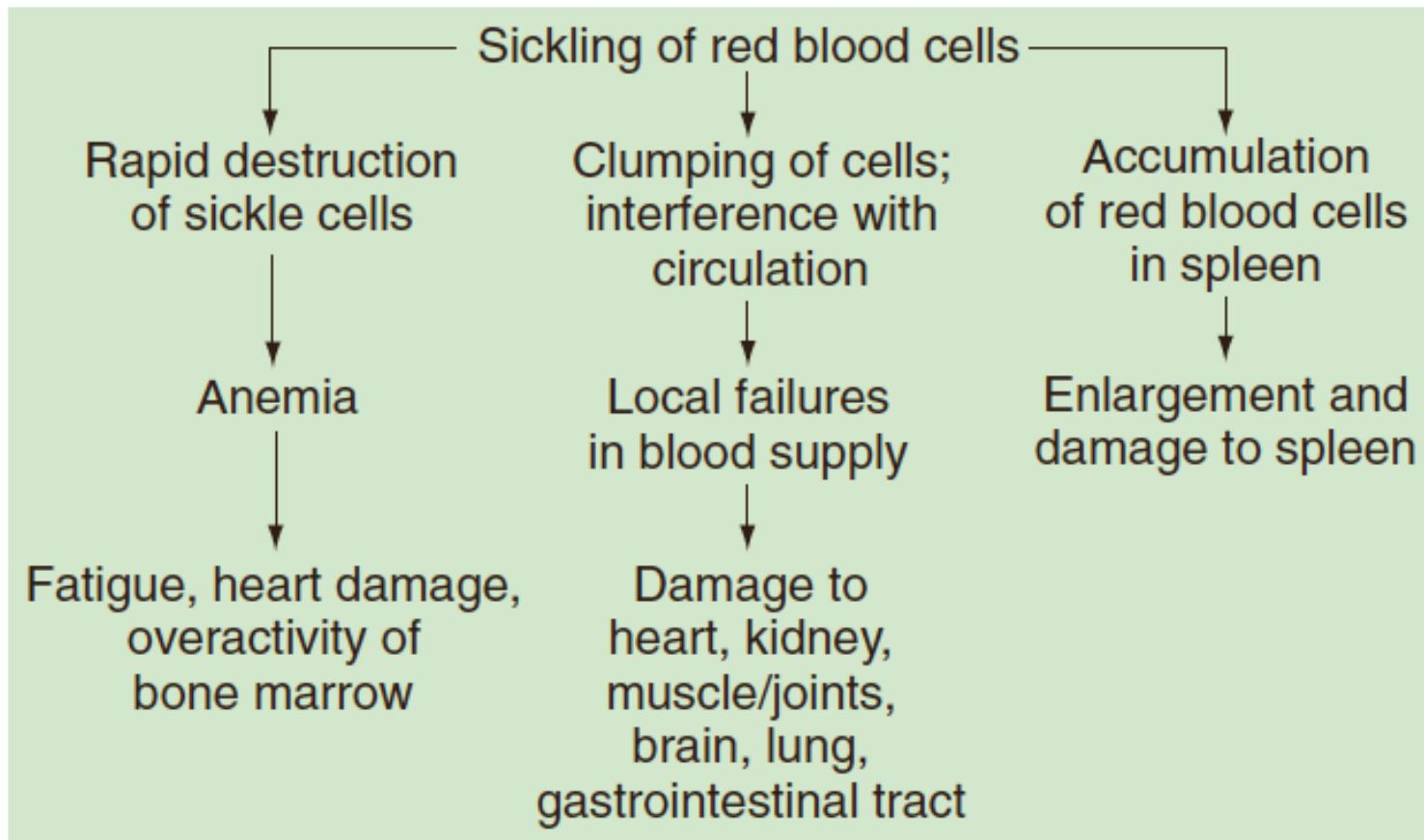


Fig 7.25b

# Levels of polypeptide structure

**Interactions that determine the three-dimensional conformation of a polypeptide**

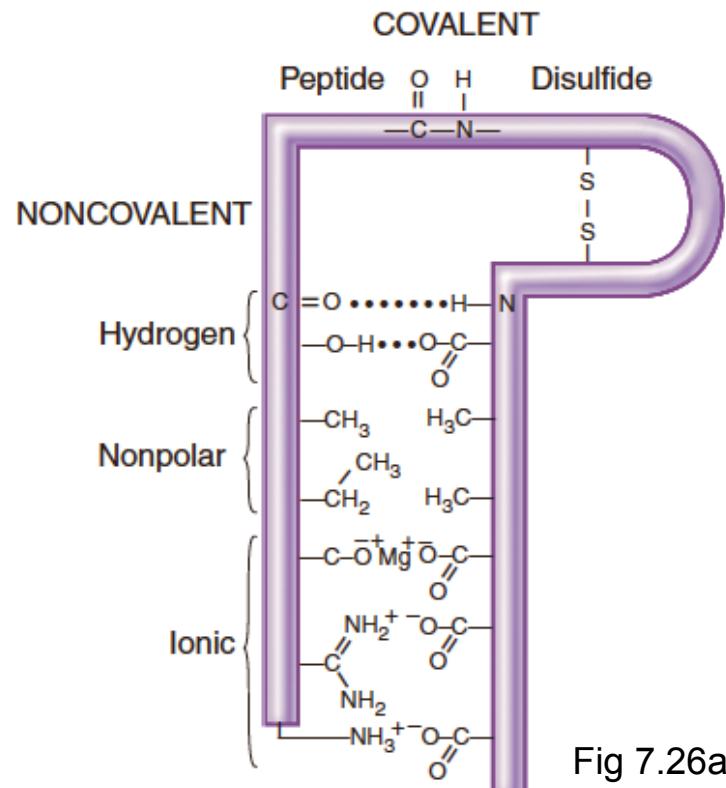
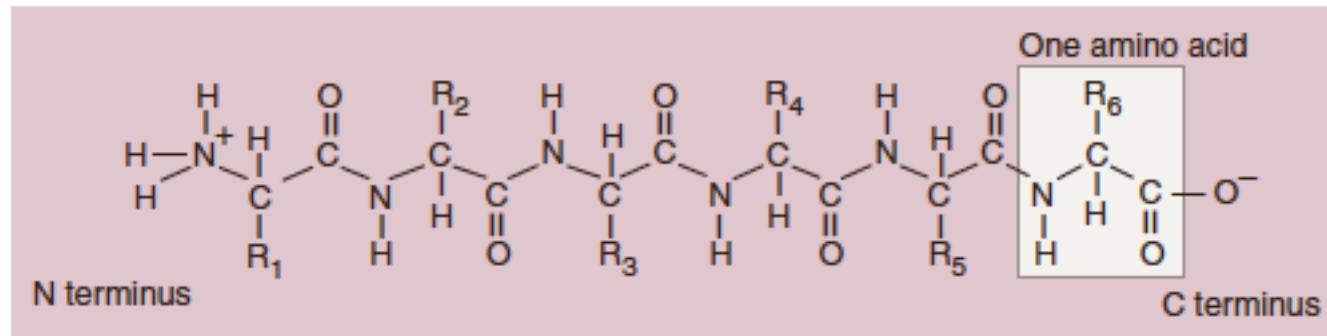


Fig 7.26a

# Levels of polypeptide structure (cont)

**1° structure** is the amino acid sequence



**2° structure** is the characteristic geometry of localized regions

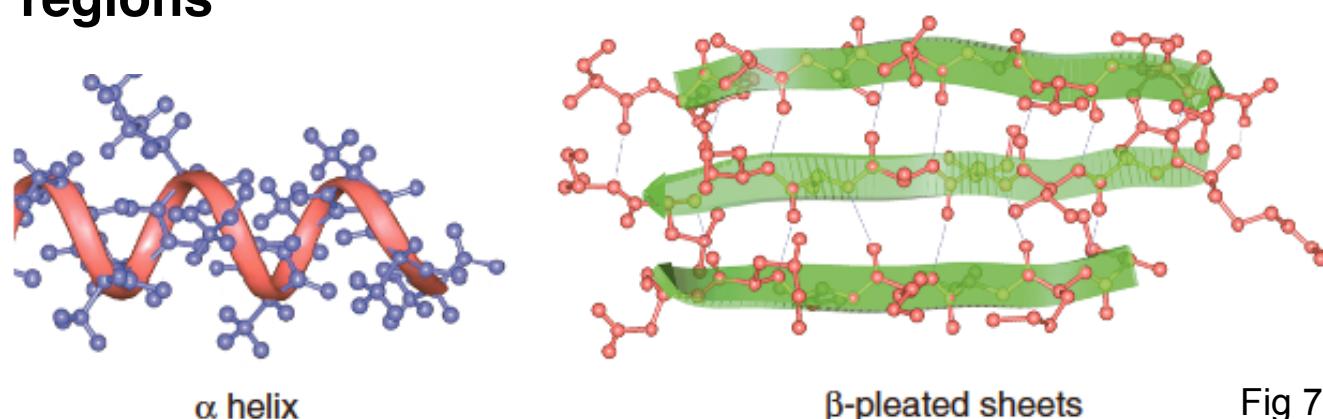
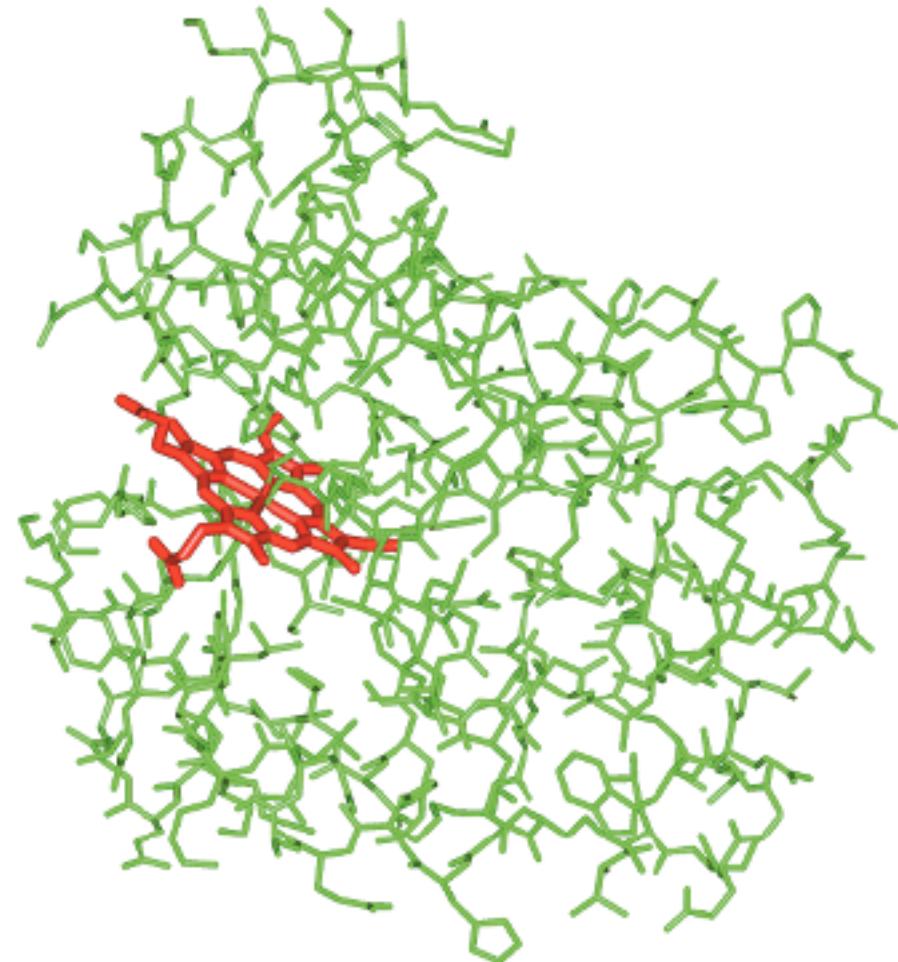


Fig 7.26b, c

# Levels of polypeptide structure (cont)

**3° structure** is the complete three-dimensional arrangement of a polypeptide

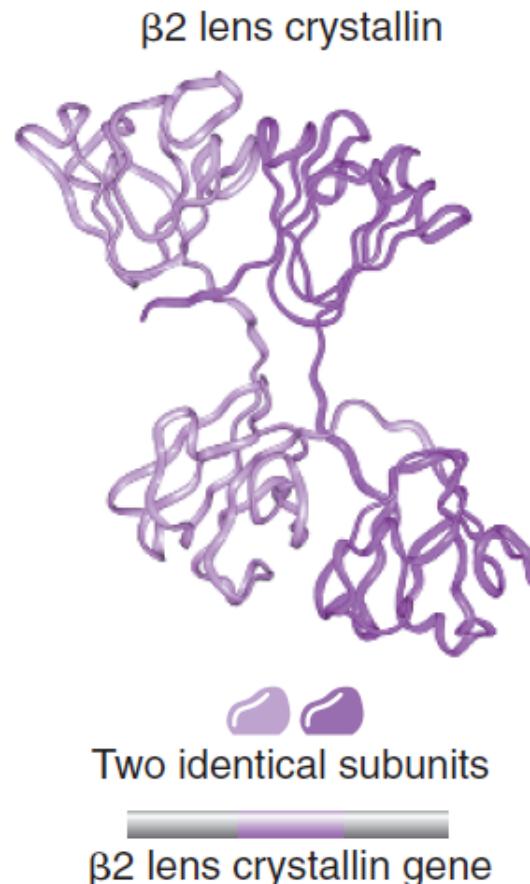


Myoglobin

Fig 7.26d

# Multimeric proteins are complexes of polypeptide subunits

## Identical subunits



## Non-identical subunits

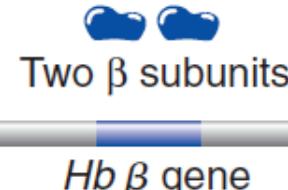
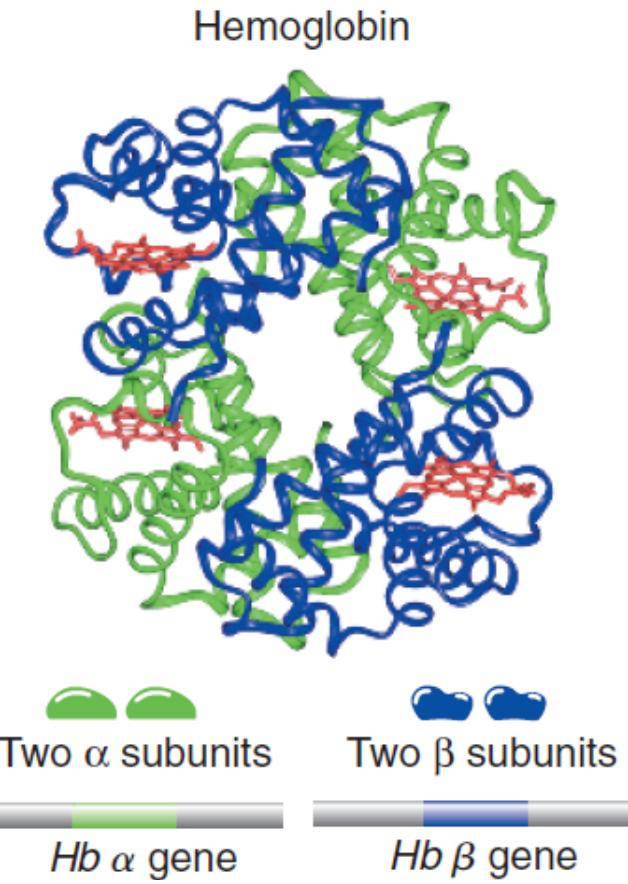
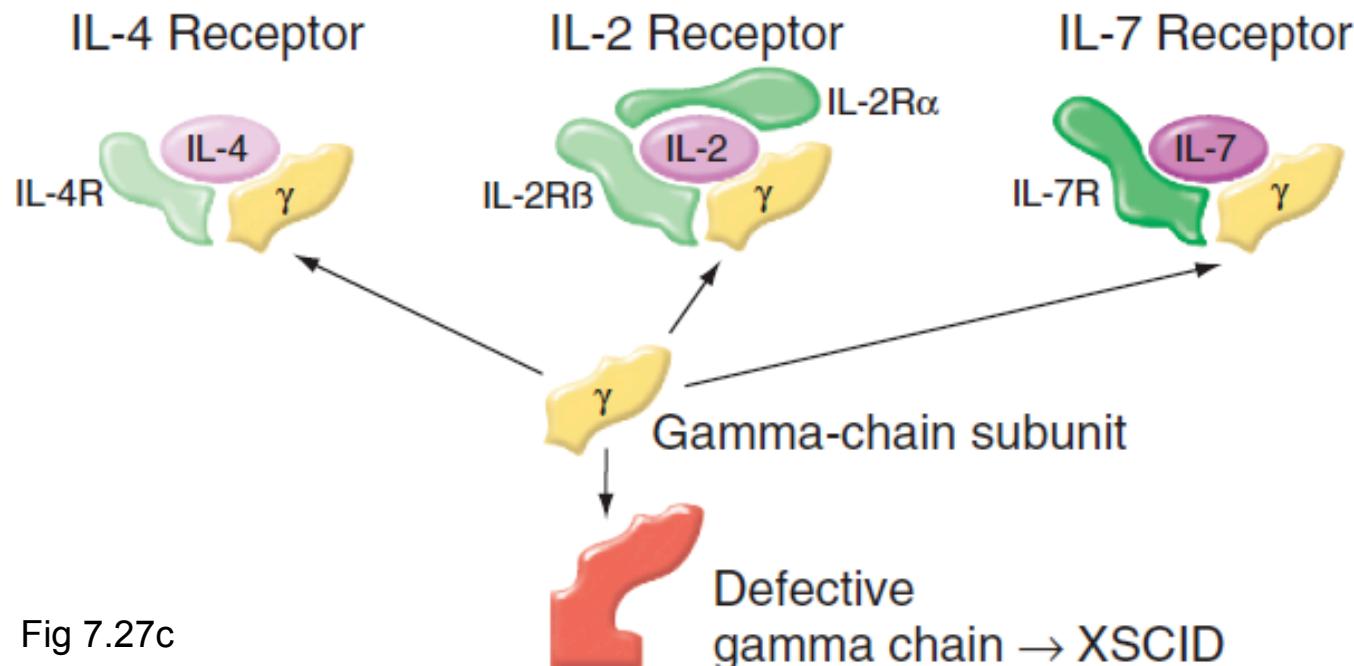


Fig 7.27a, b

# Multimeric proteins are complexes of polypeptide subunits (cont)

## One polypeptide in different proteins



# Multimeric proteins are complexes of polypeptide subunits (cont)

**Microtubules: large assemblies of subunits**

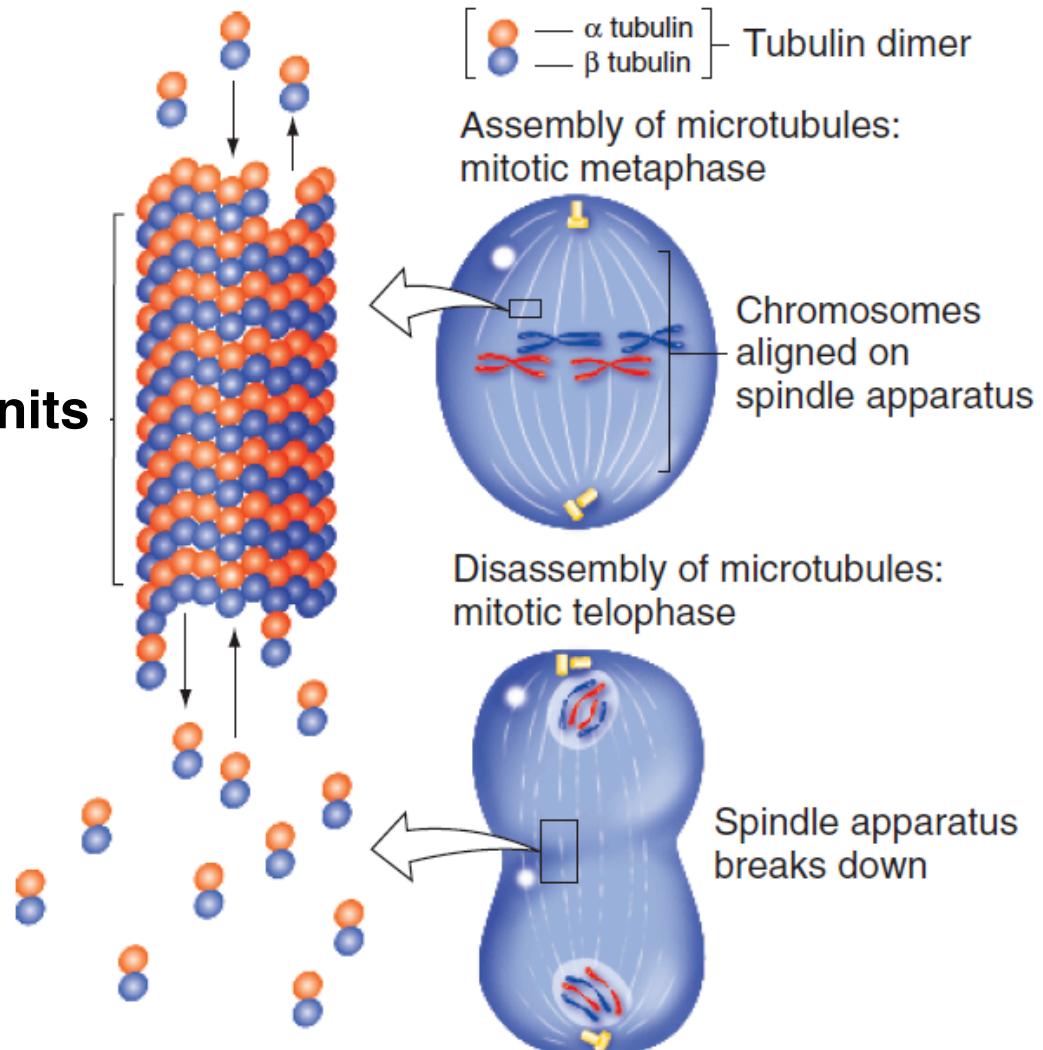


Fig 7.27d

# One gene, one polypeptide

"One gene, one enzyme" concept is not broad enough

- Not all proteins are enzymes
- Some proteins are multimeric and subunits are encoded by different genes

Complex pathways can be dissected through genetic analysis

Different mutations in a single gene can produce different phenotypes

- Different amino acid substitutions can have different effects on protein function
- Mutations can affect protein function by altering the amount of normal protein made

# Chapter 7 Questions